Mahendra Rai · Caue Ribeiro
Luiz Mattoso · Nelson Duran *Editors*

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Foreword

In 1970, Norman Borlaug won the Nobel Peace prize for his role in transforming agriculture in what became known as the "Green Revolution." The green revolution occurred after World War II and involved advances in plant breeding programs that selected for disease resistant, high yielding varieties of staple crops such as wheat, rice and maize. It also involved the development and increased use of synthetic pesticides, chemical fertilizers, and mechanization. Borlaug was known as "the man who saved a billion lives." Countries with impoverished populations that traditionally imported staple crops became self sufficient or even exporters themselves.

Borlaug recognized that while the green revolution ushered in the era of modern agriculture, further advances would be necessary to meet the ever-growing worldwide demand for food commodities. His prediction is proving true. According to a United Nations report, the world population surpassed seven billion in 2011 and is expected to increase almost 30 % by the year 2050. Nearly all of the population growth is projected to occur in developing nations where resources are already stretched to their limits. Maintaining the current level of agricultural production will be challenging enough for many countries but increasing crop yields by 30 % may be impossible without the help of new technologies.

Further complicating the issue is the fact that many of the intense farming practices introduced during the green revolution, while productive, are considered unsustainable over the long-term. For example, some estimates indicate that only 30 % of the chemicals applied by conventional means is actually utilized by the crop. Much of the fertilizer and pesticides applied in the field by conventional means is lost through various mechanisms including spray drift (wind), volatilization, leaching into ground water, and in water runoff from irrigation or rainfall. The poor efficiency of conventional field applications of agricultural chemicals is a growing concern due to the cost of the materials and their fate in the environment. Agriculture is one of the primary sources of pollution from chemicals found to have leached into groundwater. It is also a primary source of chemicals discovered in water runoff that enter into rivers, lakes, and estuaries. Fertilizer runoff containing nitrogen, potassium, and phosphorus can cause eutrophication of lakes, rivers, streams and estuaries. The impact of heavy pesticide use on fishes, birds, and other wildlife populations has been well documented. Environmentalists have called for a reduction in the use of agricultural chemicals in order to decrease pollution of our groundwater and waterways and to conserve resources. Many consumers also demand less chemical use in agriculture as a step toward reducing pesticide residue on farm produce.

Responding to these and other challenging issues in food and agriculture will require new, impactful technologies. Nanotechnology is one of the exciting new fields of research that holds great promise in addressing many of the pressing needs in the food and agriculture sectors. Nanomaterials typically have at least one dimension that is in the size range of 1–100 nm. The small size confers unique beneficial properties that cannot be matched by similar materials that have a larger size range. Several countries have recognized the potential impact of nanotechnology could have on their economies and investing heavily in research. In some cases, the research is done through initiatives such as with the National Nanotechnology Initiative that brings together a collaborative team of 20 departments and independent agencies in the USA. In most cases, however, nanotechnology research is accomplished at research centers, universities, and government laboratories scattered throughout the world. Periodic reviews that compile the research advances in particular fields are needed to determine the state of the technology and to spur further interest.

Although still in its infancy, the impact of nanotechnology is already being felt in diverse fields of science including medicine, physics, materials science, and agriculture. Products such as fuel cells, batteries, solar panels, sensors, and medical devices are beginning to enter the marketplace. In agriculture, nanoencapsulation technology is changing the way agricultural chemicals will be applied in the future. Conventional methods of applying agricultural chemicals in the field often result in overdosing in order to ensure that sufficient active agent is delivered to where it is needed. Environmental problems are created when the excess chemicals end up in waterways. Nanoencapsulation makes it possible to reduce chemical dosage because it can be applied more efficiently to a desired target area.

An example of nanoencapsulation has been demonstrated with starch microspheres that can encapsulate active agent within a nanoporous matrix. The microbeads are small enough to attach to the hairs on bees similar to pollen. Once they attach, they slowly release the active agent that repels the parasite, thus providing maximum protection using a minimal amount of active agent. Another example is the use of nanogels for the controlled release of insect pheromones that target specific pests. Nanoencapsulation is also being used to improve the efficiency of fertilizer applications and reduce the volume of chemicals polluting our ground water and waterways.

In food applications, nanosensors and nanobiosensors are being explored as a means of detecting food pathogens and improving food safety. Nanotechnology is also being used in food packaging as a means of prolonging shelf life of produce and decreasing bacterial counts. These are only a few examples of the use of nanomaterials in food and agriculture but, new nanomaterials and applications are being reported on a continual basis.

This publication is not meant to be a comprehensive treatise since the field is still evolving and new applications are being reported on a regular basis. However, it is intended to provide an invaluable resource on some of the current approaches and applications of nanotechnology in food and agriculture. It is also intended to provide a platform for establishing collaborations, formulating strategies, and spawning new ideas and approaches that will help resolve some of the most vexing challenges facing food and agriculture in a growing world.

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Gregory Glenn

Preface

In the twenty-first century, nanotechnology (NT) has been playing a crucial role in food and agriculture. It is a new science with emerging technologies in different fields in general and agriculture in particular. The nanoparticles have large surface area to the volume ratio, which provides better opportunity for interaction. The technology has a great potential to solve various issues, which have been a great problem so far. This technology may open up new avenues in agricultural production. The various emerging technologies include nanoagrochemicals (nanofertilizer, nanopesticides, herbicides), nanobiosensor, food processing and storage, food packaging and labeling, fruit preservation, food quality (nutritional supplement and nutritional drinks), plant growth promoters, crop improvement (nanoparticles-mediated gene transfer), role of nanocarriers in delivery of nitric oxide, nano-enhanced biotreatment for agricultural wastewater, etc.

These emerging nanotechnologies need to be evaluated for safety to the environment and living beings. The main issue is toxicity of the agrochemicals in soils and ultimately in food chains. The new and emerging technologies has a wide knowledge gap and understanding of the toxicity. Due to inadequate knowledge, it is much difficult to assess the risks posed by nanoparticles. There is a greater need to develop adequate risk management strategies.

This book has been divided into three parts: Part I incorporates emerging nanotechnologies, Part II deals with nanotechnologies used in detection, delivery, and treatment, Part III addresses the toxicity issues and acceptance of this technology by public.

The book on emerging nanotechnologies would be immensely useful for a diverse group of readers including physicists, chemists, microbiologists, biotechnologists, food technologists, agriculture engineers, nanotechnologists, lawyers and those who are interested in these newer technologies. The students should find this book useful.

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Part I
Emerging Nanotechnologies

Chapter 1
Nanotechnology in Foods

Jafarali K. Momin and B.H. Joshi

Abstract The advent of nanotechnology has opened up a whole universe of new possible applications in food industry. Some of these applications include: improved taste, flavor, color, texture and consistency of foodstuffs, better absorption, bioavailability of nutraceuticals and health supplements, food antimicrobials development, innovative food packaging materials with enhanced mechanical barrier and antimicrobial properties, nanosensors for traceability and monitoring food condition during transport and storage, as well as encapsulation of food components or additives. Bio-separation of proteins, rapid sampling of biological and chemical contaminants and smart delivery of nutrients, and nanoencapsulation of nutraceuticals are few more budding areas of nanotechnology for food sectors. Nanotechnology promises to revolutionize food products within as well as around. Regulatory systems must be capable of managing any risks associated with nanofoods as well as the use of nanotechnology in food industry for gaining confidence. In this chapter, status of nanotechnology applications in food industry is discussed.

Keywords Food packaging • Food safety • Nanofoods • Nanosensors • Nanotechnology • Nutraceuticals

1.1 Introduction

The term “nano” is derived from the Greek word meaning dwarf (Sangamithra and Thirupathi 2009). The concept of nanotechnology was given by Richard Feynman in 1959; however, the term “nanotechnology” was coined in 1974 by the late Norio Taniguchi (Warad and Dutta 2005). Nanoscience is defined as the study of phenomena and the manipulation of materials at the atomic, molecular, and macromolecular scales, where the properties differ from those at a larger scale (Mannino and Scampicchio 2007).

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Nanotechnology has been provisionally defined as relating to materials, systems, and processes which operate at a scale of 100 nanometers (nm) or less. The US National Nanotechnology Initiative has defined nanotechnology as “the understanding and control of matter at dimensions of roughly 1–100 nm, where unique phenomena enable novel applications; encompassing nanoscale science, engineering and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at nano scale” (Chen et al. 2006a). In 2009, the European Food Safety Authority (EFSA) defined nanomaterial as “any form of a material that has one or more dimensions in the nanoscale” and nanoparticle as “a discrete entity that has all three dimensions in the nanoscale” (FAO/WHO 2010).

The food and beverage sector is a global multitrillion dollar industry. Government, industry, and science have identified the potential of nanotechnology in the food and agriculture sectors and are investing significantly in its applications to food production (FAO/WHO 2010). Extensive research and development projects are ongoing with the ultimate goal of gaining competitive advantage and market share. For an industry where competition is intense and innovation is vital, nanotechnologies have emerged as a potential aid to advances in the production of improved quality food with functionalized properties. Advances in areas such as electronics, computing, data storage, communication, and integrated devices have an indirect impact on the food industries in the areas of food safety, authenticity, and waste management and its utilization (Cushen et al. 2012). In the coming near future, it will not be impossible to achieve food and nutritional security by implementing nanotechnologies in agricultural and food sector.

The prospect of nanotechnology in agricultural and food industries was first addressed by a US Department of Agriculture (USDA) road map published in September 2003 (Joseph and Morrison 2006). All the major food companies are constantly looking for ways to improve production efficiency, food quality, food safety, and food characteristics. A range of nanotechniques and materials are being developed in an endeavor to claim greater control over food characteristics, to enhance processing functionalities, such as flavor, texture, speed of processing, heat tolerance, shelf life, traceability, safety, bioavailability of nutrients, fortification of nutrients and bioactive ingredients, and cost-effective food analysis with major focus on functional foods as they offer the ability to control and manipulate the properties of substances close to molecular level (Chaudhry et al. 2008; Scrinis and Lyons 2007; Weiss et al. 2006; Momin et al. 2013). Nanotechnology offers considerable opportunities to develop innovative products and applications for agriculture, water treatment, food production, processing, preservation, and packaging. Application of nanotechnology may bring potential benefits to farmers, food industry, and consumers alike (FAO/WHO 2010). Nanotechnology, in fact, is a technology that has the potential to revolutionize the food industry in the coming future.

1.2 Nanotechnologies in the Food Industry

The foods which have been cultivated, produced, processed, or packaged using nanotechnology techniques or tools or to which manufactured nanomaterials, i.e., any material that is intentionally produced in the nanoscale to have specific properties or specific compositions, have been added are termed as nanofoods (Morris 2007). In reality, nanofood has been part of food processing for centuries, as naturally many food structures exist at the nanoscale (Shekhon 2010). The applications of nanotechnologies for the food and allied sector fall into the following main categories:

- Where nanosized, nano-encapsulated additives have been used
- Where food ingredients have been processed or formulated to form nanostructures
- Where engineered nanomaterials (ENMs)/manufactured nanomaterials defined as “any material that is intentionally produced in the nanoscale to have specific properties or a specific composition” have been incorporated to develop improved, active, or intelligent materials for food packaging or in food contact materials (FCMs) or surfaces
- Where nanotechnology-based devices and materials have been used, e.g., for nanofiltration (NF) and water treatment
- Where nano(bio)sensors have been used for food safety and traceability and contaminant detection
- Where applications of ENMs have been suggested for pesticides, veterinary drugs, and other agrochemicals to improve food production systems (Chaudhry et al. 2008; Momin et al. 2013)

Food industry, being a multi-technological manufacturing industry, involves a wide variety of raw materials, stringent biosafety regulations, and well-regulated technological process. Nanotechnology is moving out of the laboratory and into every sector of food production. Nanotechnology-based food and health food products, and food packaging materials, are already available to consumers in some countries. Estimates of commercially available nanotechnology-derived food products vary widely between 150–600 nanofoods and 400–500 nanofood packaging applications (Cientifica Report 2006; Daniells 2007; Helmut Kaiser Consultancy Group 2007a, b; Reynolds 2007; Momin et al. 2013).

Nanotechnology has the potential to address many of the industry’s current needs, and it is expected that nanotechnology-derived food products will be increasingly available to consumers worldwide in the coming future.

1.3 Nanofood Market

A report from a consulting firm Cientifica Report (2006) has estimated food applications of nanotechnologies at around $410 million (food processing, $100 million; food ingredients, $100 million; and food packaging, $210 million). According to this report, the existing applications are mainly for improved food packaging, with some applications for delivery systems for nutraceuticals. The USA is the leader in this business followed by Japan and China (Helmut Kaiser Consultancy 2004). There is a large potential for growth of the food sector in developing countries. As of now, more than 1,200 companies around the world are active in research and development. Among these, many of the world's leading food companies including H.J. Heinz, Nestlé, Hershey, Unilever, and Kraft are investing heavily in nanotechnology research and development (Joseph and Morrison 2006; Kuzma and Verhage 2006; Shelke 2006; Miller and Senjen 2008; Momin et al. 2013). At present, more than 180 applications are in different developing stages and a few of them are on the market already. Despite the infancy, this nanofoods sector is expected to surge from $2.6 billion today to $7.0 billion in 2015 and to $20.4 billion in 2020. By 2020, Asia with more than 30 % of the world population will be the biggest market for nanofoods wherein China is the leader (Helmut Kaiser Consultancy 2004).

1.4 Nanomaterials for Foods

Top-down approach and *bottom-up approach* (Table 1.1) are the two approaches to attain nanomaterials for food applications. The "top-down" approach involves physically machining materials to nanoscale by employing processes such as grinding, milling, etching, and lithography (Fig. 1.1). For example, dry-milling technology can be used to obtain wheat flour of fine size that has a high water-binding capacity. It is used to improve antioxidant activity in green tea powder as reducing size to 1,000 nm by dry milling, the high ratio of nutrient digestion and absorption resulted in an increase in the activity of an oxygen-eliminating enzyme. In contrast, self-assembly and self-organization are concepts derived from biology that have inspired the bottom-up food nanotechnology (Fig. 1.1). Bottom-up approaches build or grow larger structures atom by atom or molecule by molecule. These techniques include chemical synthesis, self-assembly, and positional assembly. The organization of casein micelles or starch and the folding of globular proteins and protein aggregates are examples of self-assembly structures that create stable entities (Sozer and Kokini 2009). Self-organization on the nanometer scale can be achieved by setting a balance between the different non-covalent forces (Acosta 2008; Sanguansri and Augustin 2006; Sozer and Kokini 2009; Meetoo 2011; Cushen et al. 2012; Momin et al. 2013).

Table 1.1 Size range of nanomaterials in the food sector

Structures	Diameter or length (nm)
DNA	12
Glucose	21–75
Liposome	30–10,000
LDH	40–300
Amylopectin	44–200
Casein micelle	60–100
PLA nanosphere	100–300
Zein	200
Cubosome	500
Nanosensors	<1,000

Source: Momin et al. (2013)

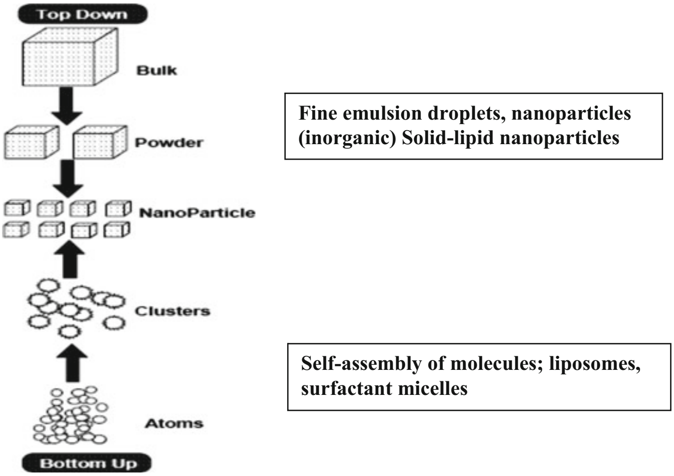

Fig. 1.1 Top-down and bottom-up approaches for nanomaterials manufacturing

Owing to the greater surface area of nanoparticles per mass unit, they are expected to be more biologically active than larger-sized particles of the same chemical composition. This offers several perspectives for functional food applications (Sozer and Kokini 2009).

1.5 Natural Self-Assembled Nanostructures in Foods

A key area of application of nanotechnology in food processing involves the development of nanostructures (also termed as nanotextures) in foodstuffs. Many natural foods have nanoscale components. Their properties are determined by their structure. These have been eaten safely for generations. In normal food processing, some of most important nutrients of food materials, i.e., proteins, starches, and fats, undergo structural changes at the nanometer and micrometer scales (Morris and Parker 2008). Milk proteins (e.g., native beta-lactoglobulin, 3.6 nm length) can undergo denaturation (via pressure, heat, pH, etc.) and reassemble to form larger structures, like fibrils or aggregates that assembled even larger gel networks (e.g., yogurt, dahi). Self-assembled nanotubes from hydrolyzed milk protein α-lactalbumin have been reported as a potential new carrier for nanoencapsulation of nutrients, supplements, and pharmaceuticals (Bugusu et al. 2009). Casein micelles practically may be explored as nanovehicles for entrapment, protection, and delivery of sensitive hydrophobic nutraceuticals within other food products (Semo et al. 2007).

A cow's udder is an excellent example of a nano-device synthesizing, assembling, and dispensing proteins and fat into an aqueous phase, where they later become building blocks for protein structures. Processes such as homogenization and fine milling do microstructural changes in food. Homogenized milk has a nanostructure of 100 nm sized droplets in it. In dairy industry, three basic micro-sized and nanosized structures (casein micelles, fat globules, whey proteins) are utilized to build all kinds of emulsions (butter), foams (ice cream and whipped cream), complex liquids (milk), plastic solids (cheese), and gel networks (yogurt). In fact, dairy technology is a mixture of a microtechnology and a nanotechnology that existed since the beginning of life on earth. Research into naturally occurring nanostructures in foods is mainly focused to improve the functional behavior of the food (Shekhon 2010).

1.6 Nanoencapsulation of Foods

Nanoencapsulation is the incorporation of ingredients in small vesicles or walled material with nano (or submicron) sizes (Surassmo et al. 2009). Nanoencapsulation in the form of micelles, liposomes, or biopolymer-based carrier systems has been used to develop delivery systems for additives and supplements for use in food and beverage products. Nanoencapsulation is the extension of microencapsulation, which has been used by the food industry for food ingredients and additives for many years. These nanomaterials offer several advantages such as preserving the ingredients and additives during processing and storage, masking unpleasant tastes and flavors, controlling the release of additives, better dispersion of water-insoluble food ingredients and additives, and improved uptake of the encapsulated nutrients and supplements (Chen et al. 2006a, b; Weiss et al. 2006; Momin et al. 2013). The protection of bioactive compounds, such as vitamins, antioxidants, proteins, and lipids as well as carbohydrates, may be achieved using this technique for the production of functional foods with enhanced functionality and stability. The improved uptake and bioavailability alone has opened up a vast area of application in food products that incorporate nanosized vitamins, nutraceuticals, antimicrobials, antioxidants, functional ingredients, etc. After food packaging, nanoencapsulation is currently the largest area of nanotechnology application in the food industry, and numbers of products based on nanocarrier technology are already available on the market. Nanoencapsulation can reduce the amount of active ingredients needed in formulation and so the cost (Huang et al. 2009). Table 1.2 lists the products reported with such nanomaterials.

1.6.1 Nanoencapsulation of Probiotics

According to the FAO/World Health Organization (WHO) (2010), probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host.” They can be added in fermented milk, yogurts, cheese, puddings, fruit-based drinks, etc. Nanoencapsulation can be used for designer probiotic bacterial preparations for delivery at specific parts of the gastrointestinal tract where they interact with specific receptors. These nanoencapsulated designer probiotic bacterial preparations may act as de novo vaccines, with the capability of modulating immune responses (Vidhyalakshmi et al. 2009).

1.7 Nanoemulsions for Foods

Nanoemulsion is an example of nanotechnology applications to an existing process to benefit the food industry. Nanoemulsions due to small droplet size possess unique rheological and textural properties to foodstuffs which is desirable in the food industry. Making nanoemulsions in food products can facilitate less fat use without compromising creaminess. Consumers will have healthier option from this technology. Low-fat nanostructured mayonnaise, spreads, and ice creams are few such examples (Chaudhry et al. 2008). Fat can be reduced from 16 to 1 % without compromising taste, texture, and quality by making nanoemulsions in ice cream (Hall 2002). A 2.5 % fat ice cream is commercially available worldwide from a recognized premium ice cream brand which claims to have no flavor defects due to the low fat content; however, no nanotechnology claim is made by the product. More choice of such low-fat ice cream is already available in the USA with many brands. As the size of the droplets in an emulsion is reduced, the less likely the emulsion will break down and separate. Hence, the need of certain stabilizers in a product may be reduced. In the coming time, nanoemulsions will revolutionize the production of spreads and mayonnaise, though they are in development stages (Cushen et al. 2012).

Table 1.2 Nanoencapsulates in food products

Nanoencapsulates	Characteristics	Products	References
Nanoemulsions	Oil in water emulsion, usually 50–500 nm	β-Carotene; α-tocopherol; nanoemulsion-based ice cream (Nestlé, Unilever)	Chu et al. (2007), Ribeiro et al. (2008), Tan and Nakajima (2005), Yuan et al. (2008), Joseph and Morrison (2006), and Miller and Senjen (2008)
Biopolymeric nanoparticles	Dense matrix network of sub-100 nm in which active molecules are dispersed throughout	β-Lactoglobulin; Nanoceuticals™ (RBC Life Sciences®); nano-calcium/magnesium (Mag-I-Cal.com, USA); nano-selenium-enriched Nanotea (Shenzhen Become Industry)	Bouwmeester (2007), Chaudhry et al. (2008), Chen and Subirade (2005), Sabliov and Astete (2008), Shelke (2006), Miller and Senjen (2008), and Zimet and Livney (2009)
Nanocapsules	Vesicles in which oil or liq. ingredients are confined within polymeric membrane of 20–100 nm	Nanocapsules of capsicum, oleoresin, eugenol, lysozymes, vitamins, phytosterols; "tip top" up bread with nano-fish oil (Nu-Mega), Kraft Foods-personalized flavors and colors	Choi et al. (2009a, b), Jafari et al. (2008), Joseph and Morrison (2006), McCall (2007), Semo et al. (2007), Shelke (2006), Surassmo et al. (2009), and Zhong et al. (2009)
Nanospheres	Solid colloidal particles enclosed by polymer matrix with several phases in suspension	Omega-3 fatty acids, whey protein nanospheres; citral flavor (key lime formulations) Chocola-chocolate chewing gum with nanococoa (Olala)	Shelke (2006), Subirade and Chen (2008), and Chen et al. (2006b)
Nanoliposomes	Polymeric aggregates of lipid bilayers esp. phospholipids such as egg or soy	Lactoferrin, nisin, phosvitin, enzymes, vitamins, antioxidants, coenzyme Q10	Bouwmeester (2007), Chen et al. (2006a), Liu and Park (2009), Malheiros et al. (2009), Ramon and Danino (2008), and Teixeira et al. (2008)
Nanocochleates	Cigar-shaped multilayered structure with spiral solid lipid bilayer	Bioral™ for nutrients (BioDelivery systems)	Chaudhry et al. (2008), Morris (2007), Sangamithra and Thirupathi (2009), Joseph and Morrison (2006), and Miller and Senjen (2008)

(continued)

Table 1.2 (continued)

Nanoencapsulates	Characteristics	Products	References
Nanoclusters		Slim shake chocolate, nanoceuticals (RBC Life Sciences); nanococoa (Royal Body Care)	Bouwmeester (2007), Chaudhry et al. (2008), Morris (2007), Shelke (2006), and Miller and Senjen (2008)
Nanomicelles	Sub-100 nm spherical particles formed spontaneously upon surfactant addition after critical micelle conc. has been reached	Limonene, carvacrol, lutein, eugenol, omega-3 fatty acids, whey proteins, essential oils; lycopene (BASF, Germany), NutiNano™ (Solgar, USA) NovaSOL (Aquanova®) for nutrients; Canola Activa Oil (Shemen Ind.)	Bouwmeester (2007), Chaudhry et al. (2008), Chen et al. (2006a), Garti and Aserin (2007), Gaysinsky et al. (2008), Joseph and Morrison (2006), Losso (2007), Zhang et al. (2008), and Miller and Senjen (2008)
Nanotube	20 nm in diameter and several μm in length	α-Lactoglobulin as gelling agent, delivery of nutrients and flavors	Bikker and Kruif (2006) and Ipsen and Otte (2007)
Nanofibers	100 nm fibers from polymer solutions	Vitamins, β-carotene, eugenol, antioxidants, flavors	Arecchi (2009) and Fernandez et al. (2009)

1.8 Nanotechnologies in Food Processing

1.8.1 Nanofrying

The US-based Oilfresh Corporation has marketed an OilFresh™ 1000 and 3000 series frying oil extender, a catalytic device for use in restaurant deep-frying machines. This nanotechnology-driven product reduces oil use in restaurants and fast-food shops by half due to its large surface area (Joseph and Morrison 2006; Pehanich 2006). It keeps frying oil fresh significantly longer, as well as it provides better taste, crisper deep-fried foods, better consistency of product, lower costs and greater profits, and substantial benefits to health and the environment (http://www.oilfresh.com/pdf/OilFresh%20News%20Release4NYShow%202-27-2006F.pdf).

1.8.2 Novel Foods

A Hungarian company has developed an ice gel for soft drinks or ice cream containing CO_2 bubbles of 1–10 nm in diameter for effervescence (http://files.nanobio-raise.org/Downloads/Nanotechnology-and-Food-fullweb.pdf). Nanotech spray is available in which 87 nm sized nano-droplets are utilized to enhance the uptake of vitamin B_{12} and other supplements for use in foods (Bouwmeester 2007). Kraft Foods' Nanotek Consortium has plans to incorporate the electronic tongue (which is a chemical change-based biosensor) into foods to release accurately controlled amounts of the suitable molecules for the development of personalized foods (Shelke 2006; Sozer and Kokini 2009).

1.8.3 Immobilization of Enzymes

Nanoporous media, nanofibers, carbon nanotubes, and magnetic nanoparticles are used for enzyme immobilization (Kim et al. 2008; Kosseva et al. 2009; Mao et al. 2006). Lipase immobilization on nanofibers and magnetic nanoparticles for soybean oil hydrolysis have already been reported (Li and Wu 2009; Lee et al. 2009; Wang et al. 2009).

1.8.4 Nanonutraceuticals

Nutraceutical may be defined as any substance that is a food or a part of a food and provides medical or health benefits, including the prevention and treatment of disease. Nanomaterials can be used as bioactives in functional foods and nutraceutical (Chau et al. 2007). Reducing the particle size of bioactives may improve the availability, delivery properties, and solubility and biological activity. These are because the biological activity of a substance depends on its ability to be transferred across intestinal membranes into the blood. In addition, the stability of such micronutrients during processing, storage, and distribution can be achieved by nanotechnology application (Chen et al. 2006a). Omega-3-fatty acids and certain beneficial probiotic bacteria species, lycopene, vitamin D_2, and β-carotene have demonstrated potential commercial success in research studies (El Sohaimy 2012). Maintaining the stability of nutraceuticals throughout the production process is a challenging task. The advent in nanotechnology will help to overcome this challenge for industry benefit (Cushen et al. 2012).

1.8.5 Nanofiltration

NF is a pressure-driven membrane process applied in the area between the separation capabilities of reverse osmosis (RO) membranes and ultrafiltration (UF) membranes. It separates ions from solutes such as small molecules of sugars and often removes the divalent ions (Ca, Mg, and ions of plating industry). The molecular weight cutoff of NF membranes is between 200 and 1,000 Da, and pore size ranges between 1 and 10 nm. The potential applications of NF in food industry and environmental protection are in desalination; milk, whey, and juice filtration; demineralization; color removal; concentration of products; waste water treatment; and water purification (Cuartas-Uribe et al. 2007; Diaz-Reinoso et al. 2009; Doyle 2006; Duke et al. 2008; Sangamithra and Thirupathi 2009; Tiwari et al. 2008; Kaul 2005).

1.8.6 Food Processing Equipment

Nanotechnology-based translucent insulation coatings called nanoinsulate PT have been reported for dairy processing tanks and pipes for reduced costs and extended life (Baruah and Dutta 2009; Pehanich 2006). Nanosilver has been incorporated into the inner surface of some domestic refrigerators to prevent microbial growth and to maintain a clean and hygienic environment in the fridge. Coatings that contain nanoparticles are used to create antimicrobial, scratch-resistant, antireflective, or corrosion-resistant surfaces. This involves the coating of nanoparticulate form of a metal, a metal oxide, or a film resin substance with nanoparticles. Examples of FCMs with nanocoating include antibacterial kitchenware, cutting boards, and teapots (FAO/WHO 2010).

1.9 Nanotechnologies in Food Packaging

Nano-enabled FCMs and food packaging make up the major share of the current and short-term predicted market for applications in the food segment (Chaudhry et al. 2008; Cientifica Report 2006). Most applications of nanotechnology in the food and agriculture sectors are currently at R&D or near-market stages. The contributing benefits to these developments include lightweight but strong packaging materials and prolonged shelf life of packaged foodstuffs and the likely low risk to the consumer attributable to the fixed or embedded nature of ENMs in plastic polymers. Many nanotechnology-derived FCMs are currently available worldwide. Nano-packaging applications as FCMs are anticipated to grow from a $66 million business in 2003 to over $360 million by 2008 (Scrinis and Lyons 2007). The main areas of nanotechnology application fall into the following broad categories:

- FCMs incorporating nanomaterial to improve packaging properties such as flexibility, gas barrier properties, temperature/moisture stability, light and flame resistance, transparency, and mechanical stability
- “Active” FCMs that incorporate nanoparticles with antimicrobial or oxygen scavenging properties

- "Intelligent" or "smart" food packaging incorporating nanosensors for sensing and signaling of microbial and biochemical changes, release of antimicrobials, antioxidants, enzymes, flavors, and nutraceuticals to extend shelf life
- Biodegradable polymer-nanomaterial composites by introduction of inorganic particles, such as clay into the biopolymeric matrix and can also be controlled with surfactants that are used for the modification of layered silicate (Alfadul and Elneshwy 2010; Sozer and Kokini 2009; Chaudhry et al. 2008; Miller and Senjen 2008; Brody 2007; Doyle 2006; Joseph and Morrison 2006; Lopez-Rubio et al. 2006).
- Edible nano coating materials that improve the storage life and quality of food products

1.9.1 Improved Packaging

Nanoparticle-reinforced materials termed as nanocomposites are polymers reinforced with small quantities (up to 5 % by weight) of nanosized particles and have been developed. These nanocomposites have high aspect ratios and are able to improve the properties and performance of the polymer. Polymer composites with nanoclay are among the first nanocomposites available in the market as improved materials for food packaging. Nanoclay with natural nanoscaled layer structure restricts the permeation of gases. Nanoclay–polymer composites have been made from a thermoplastic polymer reinforced with nanoparticles of clay which include polyamides (PA), nylons, polyolefins, polystyrene (PS), ethylene-vinyl acetate (EVA) copolymer, epoxy resins, polyurethane, polyimides, and polyethylene terephthalate (PET). Commercially, a number of nanoclay–polymer composites are available. Known applications of nanoclay in multilayer film packaging include bottles for beer, edible oils, and carbonated drinks and films (Chaudhry et al. 2008; Brody 2007). Examples of available nanoclay composites include Imperm® (from Nanocor® Inc.), Aegis® X (Honeywell), Durethan® KU2-2601 (Bayer AG), Plastic beer bottles, Miller Brewing Co. (USA), and Hite Brewery Co. Nanoparticle composites include DuPont Light Stabilizer 210, Durethan®, and Bayer's shaving (Scrinis and Lyons 2007; Chaudhry et al. 2008; Sozer and Kokini 2009; Miller and Senjen 2008). The use of nanocomposites in food contact material has been approved by the US Food and Drug Administration (USFDA) (Sozer and Kokini 2009).

1.9.2 Active Packaging

The nanomaterials commercially important are nanosilver and nanozinc oxide for antimicrobial action, nano-titanium dioxide for UV protection in transparent plastics, nano-titanium nitride for mechanical strength and as a processing aid, and nanosilica for surface coating in food packaging (Doyle 2006; Miller and Senjen

2008; Chaudhry et al. 2008). Kodak company is developing antimicrobial packaging for food products as "active packaging," which would absorb oxygen (Asadi and Mousavi 2006). Other companies include FresherLonger™ Miracle Food Storage Containers" and "FresherLonger™ Plastic Storage Bags" from Sharper Image® (USA), "Nano Silver Food Containers" from A-DO Korea, and "Nano Silver Baby Milk Bottle" from Baby Dream® Co. Ltd. (South Korea). Oxygen scavenging packaging using enzymes between polyethylene films have also been developed (Lopez-Rubio et al. 2006). An active packaging application could also be designed to stop microbial growth once the package is opened by the consumer and rewrapped with an active-film portion of the package (Brody 2007). Zinc oxide quantum dots were utilized as a powder, bound in a polystyrene film (ZnO-PS), or suspended in a polyvinylpyrrolidone gel (ZnO-PVP) as antimicrobial packaging against *Listeria monocytogenes*, *Salmonella enteritidis*, and *Escherichia coli* O157:H7 (Sun et al. 2009).

1.9.3 Smart/Intelligent Packaging

A multi-detection test—FoodExpertID—has been developed by bioMerieux for nano-surveillance response to food scares. Nanoscale radio-frequency identification tags (RFID) have been developed to track containers or individual food items (Baruah and Dutta 2009) and are being used in retailing chains (Joseph and Morrison 2006; Asadi and Mousavi 2006). The nanotech company pSiNutria is also developing nano-based tracking technologies, including an ingestible BioSilicon which could be placed in foods for monitoring purposes and pathogen detection but could also be eaten by consumers (Scrinis and Lyons 2007; Miller and Senjen 2008). The US company Oxonica Inc. has developed nano-barcodes (20–500 nm in diameter and 0.04–15 mm in length) to be used for individual items or pellets, which must be read with a modified microscope for anti-counterfeiting purposes (Miller and Senjen 2008; Warad and Dutta 2005). Engineered nanosensors are being developed by Kraft along with Rutgers University (USA) within packages to change color to warn the consumer if a food is beginning to spoil or has been contaminated by pathogens using electronic "noses" and "tongues" to "taste" or "smell" scents and flavors (Scrinis and Lyons 2007; Sozer and Kokini 2009; Joseph and Morrison 2006; Asadi and Mousavi 2006). Nestlé, British Airways, and Monoprix supermarkets are using chemical nanosensors that can detect color change (Pehanich 2006).

1.9.4 Edible Nanocoatings

A nanolaminate consists of two or more layers of material with nanometer dimensions that are physically or chemically bonded to each other (1–100 nm per layer, usually 5 nm). These could be used to encapsulate various hydrophilic, amphiphilic, or lipophilic substances, active functional agents such as antimicrobials, anti-browning agents, antioxidants, enzymes, flavors, and colors with enhanced moisture and gas barrier properties. These are developed from food-grade ingredients (proteins, polysaccharides, lipids). These functional agents would improve the storage life and quality of coated foods such as meats, cheese, fruit and vegetables, confectionery, bakery goods, and fast food (Weiss et al. 2006). The US company Sono-Tek Corp. announced in early 2007 that it has developed an edible antibacterial nanocoating which can be applied directly to bakery goods and is currently under testing with its clients (Miller and Senjen 2008).

1.10 Nanosensors in Food Safety and Analysis

Ensuring the food safety and its analysis is the need of time for any food industry. Nanosensors are devices consisting of an electronic data processing part and a sensing layer or part, which can translate a signal such as light or the presence of an organic substance or gas into an electronic signal structured at the nanometer scale (http://files.nanobio-raise.org/Downloads/Nanotechnology-and-Food-fullweb.pdf). Nanosensors are used for spoilage detection in food and food products. Nanosensors are used in the detection of adulterants, pathogens, toxins, toxic compounds, and harmful artificial colors and flavors or ingredients in the food products. Available nanosensor types and their potential applications in the food sector are summarized in Table 1.3.

Nanosensors may be used for pathogen detection in food and food products and reduce the time of detection. Such nanosensors could be incorporated in packaging material and would serve as "electronic tongue" or "noses" by detecting chemicals released during food spoilage (Garcia et al. 2006). Microfluidics devices are also known to detect pathogens with real time and high sensitivity. Microfluidic sensors are used to detect compounds of interest rapidly in only microliters of required sample volumes. Microfluidic sensors had widespread applications in medical, biological, and chemical analysis.

Table 1.3 Types of nanosensors with potential applications in foods sector

Types of nanosensors	Potential application	Reference
Array biosensors	Detection of food-borne contaminants	Bhattacharya et al. (2007) and Doyle (2006)
Electronic nose	Wine discrimination	Garcia et al. (2006) and Bhattacharya et al. (2007)
Carbon nanotube-based sensors	Measuring the levels of capsaicinoids in chili peppers	Baruah and Dutta (2009), Naja et al. (2009), Sozer and Kokini (2009), and Tang et al. (2009)
Microfluidic devices	Lab on a chip	Vo-Dinh et al. (2001), Baeummer (2004), and Mabeck and Malliaras (2006)
NEMS	Detection of pathogens	Ritter (2005) and Cane et al. (2006)

Laboratory-on-a-chip technology-type silicon-based microfluidic systems are well adopted in the market (Tay 2002). Nano-electromechanical system (NEMS) technology is already in use. NEM systems contain moving parts ranging from nano- to millimeter scale and serve as developing tools in food preservation. The US-based Polychromix has developed a digital transform spectrometer (DTS) that uses microelectromechanical systems (MEMS) technology for trans-fat content detection in foods (Ritter 2005). NEMS could be used in food quality-control devices as they are consist of advanced transducers for specific detection of chemical and biochemical signals. The use of so-called micro- and nanotechnologies (MNTs) has several advantages in the area of food technology, food safety and quality. The use of MNTs is particularly suitable to detect and monitor any adulteration in packaging and storage conditions (Cane et al. 2006). Nanocantilevers type biosensors are able to detect biological-binding interactions, such as between antigen and antibody, enzyme and substrate or cofactor, and receptor and ligand, through physical and/or electromechanical signaling (Hall 2002). Nanocantilevers type biosensors have the application of recognizing proteins and detecting pathogenic bacteria and viruses (Kumar 2006) and are well accepted in studies of molecular interactions and detection of contaminant chemicals, toxins, and antibiotic residues in food products (Ramirez Frometa 2006). The EU-funded BioFinger project has developed portable biosensor Bio-Nano and MicroElectroMechanical Systems (BioMEMS) using nanocantilevers for detection of biological entities, chemicals, and toxins (Joseph and Morrison 2006; Jain 2008). Detection of *E. coli*, an indicator of fecal pollution of water and food products, was carried out with the help of a cantilever coated with agarose (Sozer and Kokini 2009).

1.11 Toxicology and Safety Aspect of Nanomaterials

The incorporation of nanomaterials into foods presents a whole new array of risks for the public, workers in the food industry, and farmers because chemically they are more reactive than larger particles with greater access to our bodies than larger particles. They have enhanced toxicity due to greater bioavailability and even compromise our immune system response and may have pathological effects in the long term (Hoet et al. 2004; Miller and Senjen 2008; Chaudhry and Castle 2011).

In a recent review, Chaudhry et al. (2008) stated that these nanomaterials may translocate to the skin, brain, liver, etc. and may cause oxidative damage in cells. Many reports on nanoparticle uptake by endothelial cells, pulmonary epithelium, intestinal epithelium, alveolar macrophages, other macrophages, nerve cells, and other cells are now available, but no concrete conclusion comes out regarding the harmful effects on humans, plants, and environment (Chaudhry et al. 2008). They can translocate from the lungs to the blood, central nervous system (CNS) through nerve cells and have been implicated in Parkinson's and Alzheimer's disease and even can reenter in the food chain (Miller and Senjen 2008; Chau et al. 2007).

Long-term exposure can cause acute toxic response such as lesions in the kidneys and liver, granulomas, cancers, clots, etc. Nanoparticles can also be taken up by the broken or damaged skin or even by brain cells (Miller and Senjen 2008). Particles having a size below 70 nm can enter cell nuclei and even cause impairment of DNA replication and transcription (Chaudhry et al. 2008; Chaudhry and Castle 2011; Momin et al. 2013).

The current risk assessment approach used by the FAO/WHO and Codex is considered suitable for ENMs in food and agriculture, including the effects of ENM on animal health (FAO/WHO 2010).

1.12 Regulations for Nanotechnology in Food Applications

The European Union regulations for food and food packaging have recommended specific safety standards and testing procedures for introduction of new technology (Halliday 2007). Cushen et al. (2012) has given details about vertical and horizontal legislations for nanomaterials. In the USA, nanofoods and most of the food packaging are regulated by the USFDA (Badgley et al. 2007), while in Australia, nanofood additives and ingredients are regulated by Food Standards Australia New Zealand (FSANZ), under the Food Standards Code (Bowman and Hodge 2006). In India and China, food safety regulations are introduced recently but are not adequate for monitoring the safety of nanoparticles.

Existing laws are inadequate to assess risks posed by nano-based foods and packaging because: (1) toxicity risks remain very poorly understood (because of their unique properties); (2) they are not assessed as new chemicals according to many regulations; (3) current exposure and safety methods are not suitable for nanomaterials; and (4) many safety assessments use confidential industry studies (Chaudhry et al. 2008; Miller and Senjen 2008; Cushen et al. 2012; Chaudhry and Castle 2011).

Most recently, the FAO/WHO (2013) published a technical paper on state of the art on the initiatives and activities relevant to risk assessment and risk management of nanotechnologies in the food and agriculture sectors. The report has presented country-wise risk assessment and risk management of nanotechnologies in the food and agriculture sector which is very useful.

1.13 Websites on Nanotechnology

National Nanotechnology Initiative (NNI), National Science Foundation	www.nano.gov/
Project on Emerging Nanotechnologies (Woodrow Wilson Institute)	www.nanotechproject.org/

(continued)

Nanotechnology: small science, big deal (Science Museum, UK)	www.sciencemuseum.org.uk/antenna/nano/
Nanotech News	www.nanotechwire.com/
Nanotechnology Risk Resources	www.lafollette.wisc.edu/research/Nano/nanorisk/
IFST Information Statement on Nanotechnology	www.ifst.org/uploadedfiles/cms/store/ATTACHMENTS/Nanotechnology.pdf

1.14 Conclusion

Food sector had started innovation through nanotechnology. Potential results and applications are already being developed for food packaging and food safety. Food packaging industry is the first target for innovation to be the leader in the market as it is more accepted by people to have nanotechnology compared to within food. The development of innovative devices and techniques to detect microorganism and contaminants is also a promising application of food nanotechnology. Currently, though the applications of food nanotechnology are in the beginning stage and most are in the laboratories, soon the food will be modified and as per the personal need and with more attractive and nutritive form. The nutraceuticals and functional foods will be developed using nanotechnology that will have targeted delivery and more adsorbing and functional properties.

Social and ethical issues regarding nano-enabled food sector must be considered as the potential risks of nanomaterials to human health and environment are unknown. Safety aspect of nanotechnology-driven food industry must be ensured by its various testing. In order for nanotechnologies to be used to their full potential, consumers acceptance is a must. The public must be convinced by clear communication of the benefits of nanotechnologies for various purposes over existing technologies. Both benefits and risks should be acknowledged clearly. Governments should consider appropriate labeling and should also set down common regulations that will help to increase consumer acceptability.

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Chapter 2
Strategic Role of Nanotechnology in Fertilizers: Potential and Limitations

Emily Mastronardi, Phepafatso Tsae, Xueru Zhang, Carlos Monreal, and Maria C. DeRosa

Abstract The field of nanotechnology has seen tremendous growth over the past decade and has had a measurable impact on all facets of our society, from electronics to medicine. Nevertheless, nanotechnology applications in the agricultural sector are still relatively underdeveloped. Nanotechnology has the potential to provide solutions for fundamental agricultural problems caused by conventional fertilizer management. Through this chapter, we aim to highlight opportunities for the intervention of nanotechnologies in the area of fertilizers and plant nutrition and to provide a snapshot of the current state of nanotechnology in this area. This chapter will explore three themes in nanotechnology implementation for fertilizers: nanofertilizer inputs, nanoscale additives that influence plant growth and health, and nanoscale coatings/host materials for fertilizers. This chapter will also explore the potential directions that nanotechnology in fertilizers may take in the next 5–10 years as well as the potential pitfalls that should be examined and avoided.

2.1 Introduction

Agriculture today is faced with demands for greater efficiency in food production due to a growing population and a shrinking arable land base and water resources. Fertilizers are natural or synthetic products applied to soil–crop systems for satisfying the essential nutrient needs of the plants. Commercial fertilizers play a critical role in improving crop yields, yet inherent inefficiencies in conventional fertilizer management can lead to dire economic and environmental consequences. At least half of the fertilizer nitrogen applied to farmland is lost to water, air, and other processes, resulting in negative environmental impacts such as leached nitrates into

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marine ecosystems and the release of N-oxides into the atmosphere (Johnson and Raun 2003). Phosphorus use efficiency is equally dismal (Schroder et al. 2011) (<20 %), a great concern considering that it is a finite resource and that its runoff exacerbates eutrophication in aquatic ecosystems. The significant economic impact of inefficient fertilization also cannot be ignored. For example, farmers worldwide can improve their economic performance by approximately $4.7 billion annually by improving their nitrogen use efficiency by 20 % (Raun and Johnson 1999). New approaches and technologies need to be investigated in agriculture if global food production and demands are to be met in an environmentally and economically sustainable manner.

Nanotechnology encompasses a range of technologies related to the manipulation of matter at the length scale of 1–100 nm. Particles on the scale of less than 100 nm fall in a transitional zone between individual atoms or molecules and corresponding bulk material, which can lead to dramatic modifications in the physical and chemical properties of the material. Nanotechnology has already led to many innovations in fields as varied as medicine, material science, and electronics. Furthermore, nanotechnology is ubiquitous in our consumer products from textiles, to sports equipment, to electronics. Clear prospects exist for impacting agricultural productivity through the use of nanotechnology. Nanofertilizers are one potential output that could be a major innovation for agriculture; the large surface area and small size of the nanomaterials could allow for enhanced interaction and efficient uptake of nutrients for crop fertilization (DeRosa et al. 2010). The integration of nanotechnology in fertilizer products may improve release profiles and increase uptake efficiency, leading to significant economic and environmental benefits.

While nanotechnology may serve as an opportunity for the improvement of fertilizers, they may also be a source of concern. The increased surface area in nanomaterials can lead to increased reactivity and faster dissolution kinetics (Chahal et al. 2012); these factors might exacerbate inefficiency problems if nanofertilizer formulations are more easily dissolved and leached into the environment. The use of nanomaterials in fertilizers would constitute an intentional input of nanomaterials into the environment and could dramatically impact human and environmental exposure. Plants, particularly farmed crops, could serve as a potential pathway of nanoparticle bioaccumulation up the food chain. Thus, it is imperative that the risks and benefits of nanotechnology in fertilizers be critically evaluated.

This chapter provides a comprehensive review of the state of nanotechnology in agricultural products, specifically fertilizers and supplements. Examining patents and publications, three themes in nanotechnology implementation for fertilizers are explored: nanoscale fertilizer inputs, nanoscale additives, and nanoscale coatings/host materials for fertilizers. This chapter will also explore existing commercial products and the potential directions that nanotechnology in fertilizers and supplements may take over the next 5–10 years. An important goal of this chapter is to help bring focus to the application of nanotechnology and nanoscience in agriculture, especially for improving the use efficiency of essential fertilizer nutrients by crops and enhancing crop security for the long-term sustainability of agriculture and the environment.

2.1.1 Why Examine Nanotechnology in Fertilizers?

The extensive impact of nanotechnology in our society can already be felt by examining the widespread use of nanomaterials in consumer products. According to the Woodrow Wilson Project on Emerging Nanotechnologies, more than 1,600 consumer products currently on the market contain some form of nanotechnology; that number is double what was seen in 2008 (http://www.nanotechproject.org/inventories/consumer, accessed December 21, 2013). While the fields of nanoscience and nanotechnology have seen tremendous growth over the past decade, their applications to the agricultural sector are relatively undeveloped, particularly in comparison to other areas. For example, patent applications filed or papers published with the keywords "nano" and "fertilizer" have shown a steady increase over the past decade but are still relatively few when compared to those seen containing the keywords "nano" and "pharmaceutical" (https://scifinder.cas.org, accessed January 10, 2014) (see Fig. 2.1).

This matches trends observed in research funding. The investment from the US Department of Agriculture into the US National Nanotechnology Initiative's research budget rose from $0 in 2001 to over $11 million in 2013, clearly indicative of the increasing role that nanotechnology may play in agriculture. However, this investment is still significantly smaller than the investments from other sectors, such as the Department of Energy (over $350 million in 2013) (http://nanodashboard.nano.gov/, accessed January 20, 2014). While nanotechnology applications in agriculture have been somewhat slower to develop, industrial and academic interest in this field is growing. A series of reviews released over the past several years have focused on the prospects for nanotechnology in fertilizer and plant protection products suggesting an increased awareness of the field's potential (Gogos et al. 2012; Naderi and Danesh-Shahraki 2013; Ghormade et al. 2011; Hong et al. 2013; Nair et al. 2010). In contrast, public perception of all things "nano" is mixed. Nanotechnology has become something of a buzzword equated with innovation. Conversely, there is the sense in some members of the general public that anything and everything related to nanotechnology is dangerous. For example, reports on nanotechnology from the ETC Group and Friends of the Earth called for a complete ban on nanoscale formulations of agricultural inputs such as fertilizers and soil treatments, until an appropriate regulatory regime specifically designed to examine these products finds them safe (ETC Group 2004; Miller and Senjen 2008) Undoubtedly, a clearer picture of the prospective nanomaterials in fertilizer products and their properties will help inform the conversation that will need to take place between all stakeholders on this issue, from producers to regulators to consumers. As the field is relatively immature, there exists an opportunity to use some foresight and be prepared for the arrival of mass nanotechnology to fertilizer inputs, allowing industry, researchers, and regulators alike to anticipate upcoming developments.

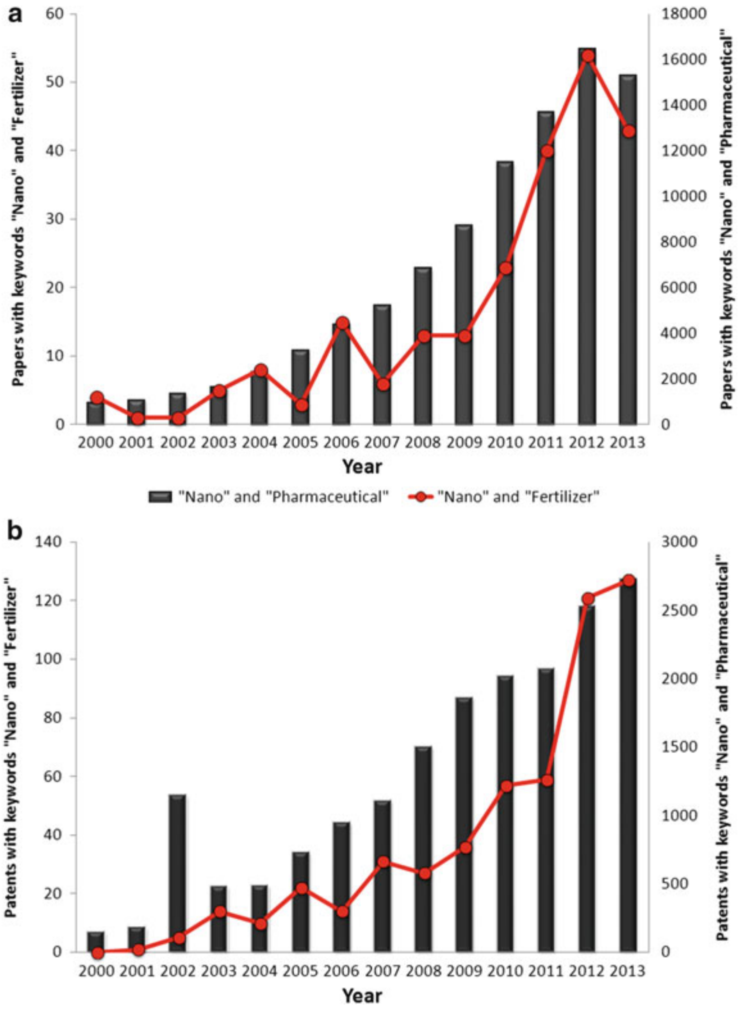

Fig. 2.1 Why look at nanotechnology in fertilizer inputs? Results from searches of the SciFinder database of papers (**a**) and patents (**b**) with the keywords listed (accessed January 3, 2014) show that the use of nanotechnology in fertilizers (*red lines*) is on the rise but still is far behind from what is seen in other applications, such as pharmaceuticals (*gray bars*)

2.1.2 Why Could Nanotechnology Be Useful in Fertilizer Products?

Nanometer scale structures are important in many facets of plant biology. Plant cell walls have pore diameters ranging from 5 to 20 nm (Fleischer et al. 1999). Plant roots, the nutrient gateway to the plant, are highly porous on the nanometer scale. Pores on the order of one to a few tens of nanometers in diameter, important for ionic and molecular transport processes, have been detected in roots (Carpita et al. 1979). Nanofertilizers may then experience improved uptake through these pores, or uptake could be facilitated by complexation with molecular transporters or root exudates, through the creation of new pores, or by exploitation of endocytosis or ion channels (Rico et al. 2011). Leaf surfaces are also nano- and microstructured surfaces, containing cuticular pores and stomata. A study on the penetration of two different sizes of water-suspended particles (43 nm or 1.1 μm diameter) into leaves of *Vicia faba* indicated that the nanosized particles (and not the larger particles) could penetrate the leaf interior through the stomatal pores (Eichert et al. 2008). A second study looking at pore diameters in a series of plant leaves found nanosized pores in both stomatous and astomatous leaf surfaces, although diameters varied widely. For astomatous leaf surfaces in *C. arabica*, the effective pore radius of cuticular pores was in the range of 2.0–2.4 nm (Eichert and Goldbach 2008). The stomatous leaf surfaces of *V. faba*, *P. cerasus*, and *C. arabica* had average pore radii ranging from 21.7 nm to >100 nm. Once within the plant, cell-to-cell transport within a plant could be facilitated by the plasmodesmata (Zambryski 2004). Plasmodesmata are nanoscale channels, 50–60 nm in diameter at the midpoint, that traverse plant cell walls, enabling cell-to-cell communication and transport. Nanoscale fertilizers could perhaps lead to more effective delivery of nutrients as their small size may allow them access to a variety of plant surfaces and transport channels. Indeed, single-walled carbon nanotubes were recently shown to penetrate the cell wall and cell membrane of intact tobacco plant cells and were shown to serve as “molecular transporters” by delivering a fluorescent dye cargo to the cells (Liu et al. 2009). Silica nanoparticles have been used to deliver cargo into plant cells as well (Torney et al. 2007). Alternatively, nanofertilizers could be more soluble or more reactive than their bulk counterparts. This has been observed, particularly in amorphous nanoparticles of poorly soluble drug compounds. These amorphous particles show faster dissolution kinetics and better bioavailability due to an increase in saturation solubility (Chahal et al. 2012). Consequently, preparation of nanosized formulations of fertilizer inputs could be perhaps expected to have a detrimental effect on fertilizer efficiency.

As a result of these apparent contradictions, there is a degree of uncertainty about what to expect in terms of the nature of the nanotechnology that can be employed for improving fertilizer products and the real impact that we can expect from these innovations. This chapter seeks to give a sense of what individual fertilizer products incorporating nanotechnology are moving through the pipeline by highlighting published papers, patents, and commercial products. Inputs such as pesticides are not included unless they are part of a formulation that is also considered a fertilizer. This chapter will also provide information about the toxicity and environmental effects of the nanomaterials described in the agricultural products. Finally, a brief look to future potential directions and pitfalls, and new opportunities for the use of nanotechnology in agricultural inputs will be provided.

2.2 Current Use of Nanotechnology in Fertilizers and Supplements

In this section, published papers, patents, and commercial products will be divided into one of three categories related to nanotechnology in agricultural inputs. Box 1 defines some key terminology in nanotechnology as it relates to fertilizers. It is important to note that the definitions for nano-object, nanoparticles, and nanomaterials appear to be somewhat relaxed when applied to fertilizer inputs. Several patents in particular describe materials with dimensions of less than 1,000 nm as "nano" providing they exhibit unique properties not recognized in micron- or larger-sized particles. It is debatable whether this is an accurate use of the term "nano"; however, these studies have been included nevertheless in this analysis. Nanomaterials can be realized using two different approaches: "bottom-up" or "top-down." Top-down approaches use physical or chemical processing to convert bulk materials into nanoscale ones. Examples of these processes include grinding, etching, and milling. Bottom-up nanotechnology relies on self-assembly and self-organization of smaller building blocks to create functional nanoscale materials. One example in this category could be the self-assembly of nanoscale liposomes from lipid molecules.

Box 1: Definitions in Nanotechnology

Nano-object: Materials with one, two, or three dimensions in the size range from 0.1 to 100 nm. In fertilizer applications, a looser definition appears to be in use. Materials with one, two, or three dimensions less than 1,000 nm that exhibit unique properties unseen in the bulk material have been termed "nano" in many fertilizer patents and publications.

Nanomaterial: Encompasses both "nano-objects" and "nano-structured materials" which are bulk materials that have important features on the nanometer length scale.

Nanoparticle: A material with all three dimensions in the nanoscale regime.

Granulation: The process of forming or crystallizing a material into small grains.

Shearing: The process of grinding or cutting of a material substance in which parallel internal surfaces slide past one another at high speeds.

Ball-milling: The process of grinding a material into a very fine powder using a cylindrical device filled with both the material to be processed and a grinding medium.

Emulsification: The process of forming a mixture of two immiscible (unblendable) liquids, yielding micro- or nano-sized droplets.

Before exploring the application of nanotechnology in fertilizer inputs in more detail, it may be worthwhile to take note of where around the world these innovations are originating. Sorting nanofertilizer patents from Fig. 2.1b based on country of filing indicates that about three quarters of these patents are of Chinese origin, with the USA and South Korea as two other major contributors in this area (see Fig. 2.2).

Current applications of nanotechnology in fertilizer and plant protection can be divided into three categories as shown in Fig. 2.3. Note that in many cases, these three categories have considerable overlap, and certain products may be best described as a combination of more than one category. The three categories of nanotechnologies for fertilizer inputs and plant protection are described below:

1. Nanoscale fertilizer inputs. This category describes examples of a nanosized reformulation of a fertilizer input. The fertilizer or supplement is reduced in size, using mechanical or chemical methods, down to the nanoscale. The input is typically in the form of nanoparticles but may also be in other forms.
2. Nanoscale additives. This category includes examples where the nanomaterials are added to bulk (>100 nm scale) product. These nanomaterials may be a supplement material added for an ancillary reason, such as water retention or pathogen control in plants or soils.
3. Nanoscale coatings or host materials for fertilizers. This category describes nano-thin films or nanoporous materials used for the controlled release of the nutrient input. These include, for example, zeolites, other clays, and thin polymer coatings.

As mentioned above, certain fertilizer input formulations may fall into more than one category. For example, a nanoscale fertilizer particle may also be incorporated into a nanoporous host material, yielding a final product that would fall into Categories 1 and 3.

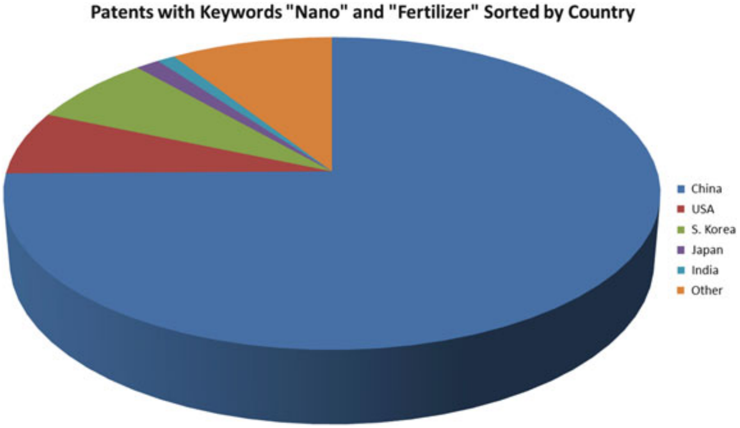

Fig. 2.2 Results from searches of the SciFinder database (accessed January 4, 2014) show that about 75 % of nanotechnology fertilizers patents are originating from China

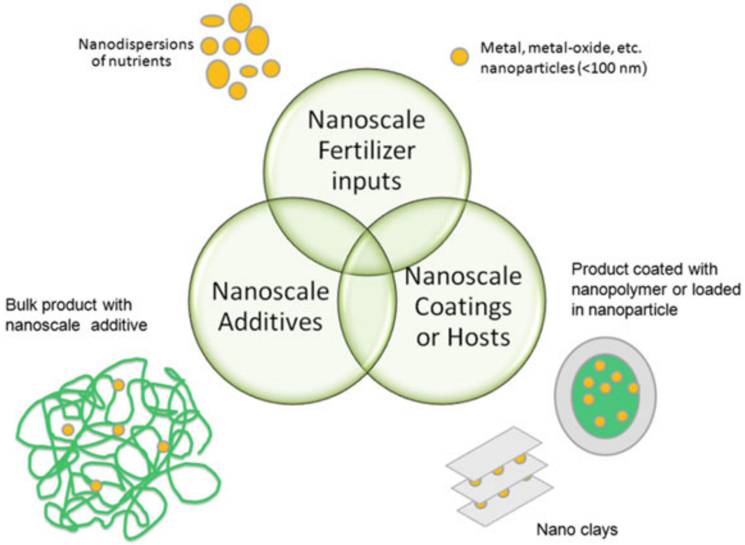

Fig. 2.3 The application of nanotechnology to fertilizer inputs can best be divided into three categories: nanoscale fertilizer inputs, nanoscale additives, and nanoscale coatings or host materials. These three categories do have some degree of overlap, meaning some products may fall into more than one category

2.2.1 *Nanoscale Fertilizer Inputs*

In this family, fertilizer inputs have been prepared in the form of particles or emulsions with nanoscale dimensions. Generally, the claim is made that reducing the size of the input leads to improved uptake and better overall release efficiency providing better efficacy with a lesser amount required. However, many patents and patent applications make these efficiency claims and further claims that their formulation lacks toxicity, but in most cases, little evidence is provided to corroborate these statements. Furthermore, many examples give minimal physical evidence for the size and monodispersity of their input particles (e.g., microscopy, dynamic light scattering, etc.).

Fertilizer nano-objects, including particles prepared from urea, ammonium salts, peat, and other traditional fertilizers, fall under this category. Notably, both chemical and organic-based fertilizers are represented in this category. For example, a peat/bacteria composite granulated to the nanoscale is claimed to lead to improved soil fertility over bulk fertilizer treatment (Wang 2008). Both chemical and physical approaches have been explored for the preparation of urea nanoparticles. A chemical process has been used to deposit urea on calcium cyanamide cores yielding a nanoparticle fertilizer formulation (Wan 2004). A nanoparticle formulation prepared by grinding a mixture of urea, bacteria, plant antibiotics, and an NPK composite fertilizer down to nanoscale dimensions has also recently been patented (Wang et al. 2008a). In some instances, a mixture of physical and chemical or biochemical methods is used to prepare the nanomaterial. For example, in a patent by He, top-down methods such as grinding and crushing are used to bring raw plant materials down to about 500 nm particles. Then, biochemical fermentation is used to give the final nanoscale product. This fertilizer is claimed to lead to improved yields and disease resistance (He 2008). In another example, ammonium humate, peat, and other additives are first ground down to micron size, then the mixture is exposed to biochemical reactions, followed by further grinding to yield their nanoscale product (Wu 2005).

One interesting group of fertilizer nanoparticles is prepared by incorporating the input into an emulsion that creates nanosized colloids or droplets. (Note that nanoemulsions could equally be classified under Category 3, "nanoscale host materials.") For example, a process has been patented where paper manufacturing sludge, phosphate, magnesium, and ammonium salts are mixed with cellulose to form nanoscale micelles. They also prepared nanoscale particles of similar composition using physical methods. Both are claimed to be efficient fertilizer treatments (Inada et al. 2007). Emulsification followed by polymer coating and high-speed shearing has been used to prepare nanoparticles of ammonium chloride, urea, and other components (Lin 2008). Other materials have also been used to form fertilizer nanoparticles. Pectin, a structural heteropolysaccharide contained in the primary cell walls of plants, has been used to prepare fertilizer nanoparticles (Nonomura 2006).

Micronutrients have also been incorporated into nanoparticle form in an effort to improve uptake. Several examples fall under Category 1, although in certain cases, these materials could also fall under Category 2 if they are described as nanoscale additives for a bulk NPK fertilizer. Zinc and selenium, for example, are nutrients that can be effectively provided to humans via micronutrient fertilization of crops (Bell and Dell 2008). A patent (He et al. 2009) and several publications have investigated the use of ZnO nanoparticles on a variety of crops such as cucumber (Zhao et al. 2013), peanuts (Prasad et al. 2012), sweet basil (El-Kereti et al. 2014), cabbage, cauliflower, tomato (Singh et al. 2013), and chickpea (Pandey et al. 2010). Figure 2.4 shows a TEM image of nano-ZnO applied to peanut seeds, resulting in greater seed germination, seedling vigor, and chlorophyll content, as well as increased stem and root growth. Overall, a higher crop yield was achieved, even at a 15× lower concentration than a chelated $ZnSO_4$ addition (Prasad et al. 2012). In another study, foliar application of ZnO combined with laser irradiation with red light led to enhanced yield compared to the nanoparticles alone (El-Kereti et al. 2014). This suggests that exploiting the unique electronic properties of nanoparticle nutrient formulations could be an effective strategy. Another study examining a variety of crops noted that nano-ZnO increased seed germination while a bulk form of ZnO used for comparison had a negative impact on germination. The nano-treatment increased pigments, protein and sugar contents, and nitrate reductase activities, and other antioxidant enzyme activities were increased (Singh et al. 2013). In a study on chickpeas exposed to nano-ZnO (20–30 nm), in addition to increased seed germination and root growth, higher levels of a plant growth hormone, indoleacetic acid (IAA), were observed (Pandey et al. 2010). Interestingly, while several studies have demonstrated the positive effects of nano-ZnO on

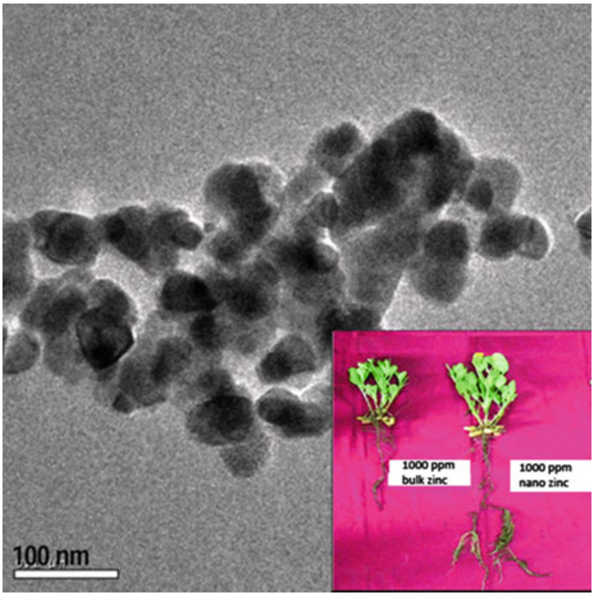

Fig. 2.4 TEM image of ZnO nanoparticles used in study on peanut plants. *Inset*: dramatic increase in root growth of peanut plant after nano-ZnO treatment (*right*: 1,000 ppm) after 110 days in comparison to bulk zinc (*left*: 1,000 ppm) over the same time period (Prasad et al. 2012). Reproduced with permission from Taylor and Francis

crop grain yields, other expected advantages of the nano-form of this nutrient have not been demonstrated. For example, a study on the uptake of zinc using a variety of Zn materials, including 40 nm ZnO, noted that the use of the nano-form did not lead to greater Zn content in roots compared to bulk Zn treatments (Watts-Williams et al. 2014). A second study examined the dissolution kinetics of nano-ZnO and bulk Zn as coating for a phosphate fertilizer and found that the kinetics of Zn dissolution and release were not affected by the form of Zn used (Milani et al. 2012). These data, in conjunction with the data found on IAA levels and enzyme activity after nano-ZnO application, appear to suggest that nutrient and physiological factors alone or combined help explain the effects on plant growth. Further research is warranted to determine the exact mechanisms by which micronutrient fertilizers affect plant growth and metabolism.

Selenium nanoparticles used as micronutrient fertilizers have been described in several patents and papers (Yu 2005b; Li 2007; Wu et al. 2008; Hu et al. 2008; Tong et al. 2008; Wei et al. 2012; Xuebin et al. 2009; Tian et al. 2012). In these studies or inventions, the selenium content in the specific crop was generally found to be increased when the nanoselenium was applied. For example, in the patent by Hu et al., Se particles milled down to approximately 400 nm in size were investigated as a foliar fertilizer for green tea (Yu 2005b). Higher selenium levels were found when compared to those exposed to selenium salts. Iron is another micronutrient that is being investigated in a nano-form. For example, a plant tonic comprised of nano-iron has been patented (Hong and Shim 2006). A 2004 patent describes nano-iron oxide mixed with peat and $CaCO_3$, leading to improved crop quality (Wu 2004a). Rare-earth element (REE) fertilizers have been applied as microelement fertilizers in Chinese agriculture since the 1980s (Wang et al. 2008b). REE fertilizers have been reported to improve nitrogen fixation efficiency and reduce water loss by plants (Brown et al. 1990). Several patents describe the use of nano-REE fertilizers (Wang et al. 2005a, b, c). For example, seed soaking or foliar treatment with REE (e.g., $La(OH)_3$, $Nd(OH)_3$, and $Ce(OH)_3$) nanoparticles is claimed to lead to increased yield and quality of crop, with less REE than that required to see effects with the bulk treatment.

Table 2.1 lists all the patents and patent applications whose inventions fall under this category, grouped in terms of their nano-content. In cases where more than one component of the formulation is described, the patent is listed under each nano-component.

2.2.2 Nanoscale Additives

In this category, a nanomaterial is included in crop rhizospheres not necessarily as the nutrient itself but perhaps as an additive to enhance plant growth, such as a binder or water retention material, or plant defense against soil pathogens. Note that while pesticides are not described in this chapter as a separate category, nanoscale additives to a fertilizer product used to provide pest resistance or antimicrobial properties have been included.

One of the first, widely cited, examples of the use of nanotechnology to improve crop yields investigated the effects of carbon nanotubes (CNTs) on the growth of tomato seedlings (Khodakovskaya et al. 2009; Biris and Khodakovskaya 2011). The nanotubes were found to penetrate the tomato seed coat and a dramatic increase in seed germination and growth was observed.[1] More recently a similar effect was demonstrated in chickpea using water-soluble carbon nanotubes (Tripathi et al. 2011). An increase in water absorption and retention was observed as a result of channels and capillaries created by the CNTs (see Fig. 2.5). Similar results were noted in mustard plants exposed to 30 nm diameter multiwalled CNTs (Mondal et al. 2011). In the root tissue exposed to CNT, dramatic uptake of black CNT was observed. In recent work examining the effects of CNTs in tomato (Khodakovskaya et al. 2013) and tobacco plants (Khodakovskaya et al. 2012), there are data to indicate that the CNTs may be involved in the upregulation of genes involved in a number of processes, such as water transport, cell division, and cell-wall extension. In this case, then, the CNTs themselves could be considered as a plant growth promoter or protector of crops under drought conditions. Furthermore, if carbon nanotubes are used as transporters for crop nutrients, these materials would also fall under Category 3. Several carbon-based nanomaterials have found applications in patents on nanofertilizer formulations (Biris and Khodakovskaya 2011; Lewis 2013; Liu and Wangquan 2012; Zhang and Chen 2012; Xie and Liu 2012; Li and Guan 2011; Zhang and Liu 2010).

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[1] Note that this paper has since been retracted for copyright reasons.

Table 2.1 Patents on inputs that fall into Category 1 (nanoscale inputs)

Nano-content	Patent title	Claims[a]	Ref.
Ammonium salts	Ammonium magnesium phosphate-containing nanocomposite and its manufacture	Paper manufacturing sludge is mixed with phosphate, magnesium, and ammonium salts. These materials are then prepared as nanoscale cellulose micelles or nanoscale particles (using physical methods)	Inada et al. (2007)
	Novel sustained-release nanosized fertilizer and production method thereof	Nanoscale fertilizer particles (urea, ammonium chloride, potassium chloride, etc.) are prepared by emulsification, coating with a polymer film and shearing down to the nanoscale. The fertilizer shows improved stability and slow-release properties. Cross-listed with Category 1 urea	Lin (2008)
	Nano-controlled-release fertilizer and its preparation	Ammonium humate and other additives are ground with peat brown coal to the micron scale and then, after further biochemical reactions, ground down once again to the submicron/nanoscale	Wu (2005)
	Nanosized active organic humate fertilizer and its preparation process	Ammonium humate and other additives are ball-milled, dried, and sieved to nanosize	Wu (2004b)
	Special sweet potato fertilizer and preparation method	Ammonium fertilizer preparation with cottonseed oil ground into nanopowder (no size information given) before mixing with ash. Claims of improved yield and quality	Liu et al. (2012b)
Fe	Method for preparing plant tonic comprising nano-iron aqueous solution	Nano-iron, mixed with other elements, kaolin and acrylic, yields a ceramic nanopowder used for improving plant yield	Hong and Shim (2006)
Humic acid	Method of producing granular organomineral nanofertilizers	Chemical and mechanical processes are used to form nanohumic dendritic substances of 40–100 nm. Claims of stimulating	Aleksandrovich (2002)

(continued)

Table 2.1 (continued)

Nano-content	Patent title	Claims[a]	Ref.
		growth, a protective effect against pathogenic microorganisms, and increased agrochemical efficiency	
Organic matter	A composition and a process for preparation of nano-bio-nutrient processed organic spray	Organic fertilizer is processed in such a manner that the final solution obtained is a nanomaterial (~20 nm). The fertilizer is claimed to require less frequent application and improves yield by 45 %	Anil and Ramana (2013)
Pectin	Compositions and methods for anti-transpiration in plant	A foliar or root additive-pectin composite, prepared as nanoparticles (25–50 nm), provides greater crop yields	Nonomura (2006)
Plant materials	Method for producing amino acid active fertilizer	Raw plant materials are crushed to 500 nm in size and mixed with water and fermenting enzymes. The resulting fertilizer is claimed to lead to higher yields and improved disease resistance	He (2008)
Rare earths (e.g., Nd_2O_3, La_2O_3, Ce_2O_3)	Application of nanometer rare-earth oxide for promoting plant growth	Seed or foliar treatment with nanoparticles of rare-earth oxide salts leads to higher yields with reduced usage of the salt. Could potentially be cross-listed in Category 2 as an additive to a bulk fertilizer	Wang et al. (2005a)
	Application of nanometer rare-earth hydroxide for promoting plant growth	Same as above except with rare-earth hydroxide salts	Wang et al. (2005b)
	Application of nanometer rare-earth precipitated salt for promoting plant growth	Same as above but with other rare-earth salts	Wang et al. (2005c)
Se	Cultivation technology for production of *Ziziphus jujuba* fruit rich in selenium	Selenium nanoparticles lead to higher yields of fruit and higher selenium content in the fruit. Could potentially be cross-listed in Category 2 as an additive to a bulk fertilizer	Yu (2005b)
	Selenium–potassium phosphate composite and applications thereof	Nanoparticles of the formula $K_3SeP_3O_{10} \cdot xH_2O$ are used to increase rice and tea yields. Could potentially be cross-	Li (2007)

(continued)

Table 2.1 (continued)

Nano-content	Patent title	Claims[a]	Ref.
		listed in Category 2 as an additive to a bulk fertilizer	
	Method for preparing nanoscale Se-rich green tea with antitumor activity	A foliar fertilizer of nanoscale selenium particles (less than 400 nm), prepared by milling, led to greater selenium content in green tea. Could potentially be cross-listed in Category 2 as an additive to a bulk fertilizer	Hu et al. (2008)
	Nanoselenium–amino acid foliar fertilizer and preparation method of the same	A nanoselenium–amino acid conjugate foliar fertilizer is prepared to achieve improved crop selenium absorption rate. Method describes the preparation of amino acid-coated selenium nanoparticles (average diameter 38 nm)	Wei et al. (2012)
	The preparation of a nano-long-acting selenium fertilizer	Natural selenium-rich carbonaceous, siliceous rock is pulverized into nanoparticles, heated and cooled, activated by alkaline water, and mixed with quartz sand	Xuebin et al. (2009)
	Method for improving quality of blueberry fresh fruit by using biological selenium nanometer fertilizer	Nanoselenium (size not specified) added to soil three to five times throughout blueberry growth cycle results in higher quality and a longer storage period	Tian et al. (2012)
Urea	Production process for blended high concentration sustained-release fertilizer	Nanosized urea particles are coated with a nanohumic acid-coating agent, creating a slow-release fertilizer. Cross-listed to Category 3 humic acids	Zhang and Yao (2005)
	Nanosized urea and its production process	Urea nanoparticles are prepared on a calcium cyanamide core using a chemical process	Wan (2004)
	Nanometer biofertilizer containing bacteria	Bacteria, urea, plant antibiotics, and NPK composite fertilizer are ground to the nanoscale and used to increase crop yields	Wang et al. (2008a)

(continued)

Table 2.1 (continued)

Nano-content	Patent title	Claims[a]	Ref.
	Novel sustained-release nanosized fertilizer and production method thereof	Cross-listed with Category 1 ammonium salts	Lin (2008)
Zn	Zinc oxide suspension as agricultural trace element fertilizer	A zinc oxide powder mixed with polymeric wetting agents and cellulose-based thickening agents is ground down to the size of 100–1,000 nm. When used as a trace element fertilizer, improved zinc supplementation is claimed. Could potentially be cross-listed in Category 2 as an additive to a bulk fertilizer	He et al. (2009)

[a] Description provided from patent information, however, there may not be evidence provided in the patent to corroborate the claims

Silicon dioxide (silica) is one nanomaterial that has been generating attention in both patents and research papers. Papers from Lin et al. have examined the effect of nanostructured silica treatments on growth in spruce and larch tree seedlings (Lin et al. 2004a, b). They found that nanostructured silica forms a protective film at the cell wall after absorption, which is thought to improve plant stress resistance. In their studies, seedling roots were soaked in solutions of nanostructured silica, although the size and morphology of the material were not described. At 500 μL of silica/L treatment, a statistically significant increase in height, main root length, root diameter, and number of lateral roots was found. This treatment also led to higher chlorophyll content than what was found in controls. Similar studies have looked at the effect of silica nanoparticles on maize (Suriyaprabha et al. 2012) and tomato (Siddiqui and Al-Whaibi 2014). Amorphous silica nanoparticles (20–40 nm by TEM) were compared to bulk silica treatments on the growth of maize, and the nano-treatment led to improved growth and greater silica accumulation. In the study on tomato, average 12 nm silica nanoparticles were utilized; however, large micron-sized particles are visible in the SEM images provided. Nevertheless, the authors noted greater seed germination, seed vigor index, and weight. Silica nanoparticles have also found their way into patented fertilizer formulations. For example, a Korean patent by Kim incorporated colloidal silica in the size range of 5–60 nm in a bulk NPK fertilizer as an additive for promoting plant propagation and increasing resistance to pathogenic bacteria (Kim 2007). Nanosilica has also been included in fertilizer treatments as a water and mineral adsorbent (Wei and Ji 2003; Zhang et al. 2005c; Chen 2002).

Nano-TiO_2 has been generating a considerable amount of research interest into its use as a fertilizer additive due to its photoactivity. Several papers have investigated the effect of nano-TiO_2 on spinach (Zheng et al. 2005; Yang et al. 2007). Spinach

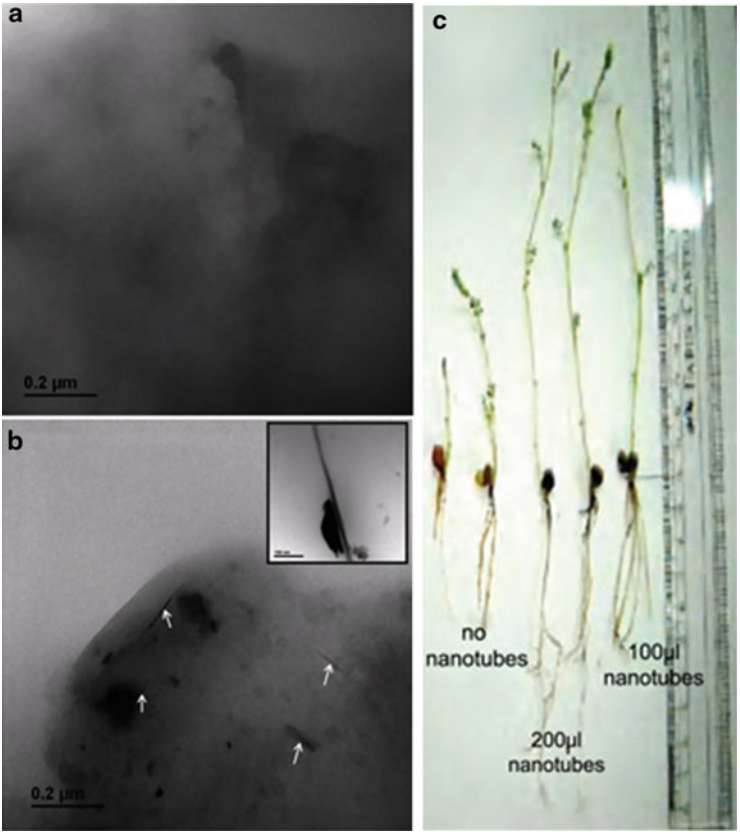

Fig. 2.5 TEM images of chickpea root tissue (**a**) without and (**b**) with exposure to CNTs. *White arrows mark* the carbon nanotubes. *Inset* in (**b**) shows a close-up image of a CNT within the tissue. (**c**) Comparison of plants after 10 days of growth. The plants exposed to CNTs showed greater root and shoot length as well as water uptake (Tripathi et al. 2011). Reproduced with permission from Springer

seeds soaked in 2.5 % nano-TiO_2 solutions under natural light illumination showed almost 3.5 times higher vigor indices compared to seeds treated with bulk TiO_2. Dry weight of the plants was 47 % higher in the nano-treated seeds than the bulk-treated seeds, and chlorophyll content increased by 28 %. These improvements are attributed to the photocatalytic effects of nano-TiO_2. More recently, the hypothesis was provided that nano-TiO_2 promotes photosynthesis and nitrogen metabolism within the plant (Yang et al. 2007). Similar photocatalytic effects have been claimed in patents. For example, particles of partially crystalline polymers such as polyethylene, polypropylene, etc., mixed with semiconductor nanoparticles, such as tin oxide, indium oxide, or indium–tin oxide (maximum diameter of 200 nm), are claimed to improve the efficiency of sunlight utilization by plants (Caro et al. 2006). Table 2.2 lists patents and patent applications whose inventions fall under category 2, grouped in terms of their nano-content.

Table 2.2 Patents on inputs that fall into Category 2 (nanoscale additives)

Nano-content	Patent title	Claim[a]	Ref.
Ag	Liquid complex fertilizer which contains nanosilver and allicin and preparation method thereof to provide antibacterial effects thus to increase crop production	Incorporating nanosilver with a fertilizer increases crop yields by reducing loss	Kim (2005)
	Nontoxic pesticides for crops containing nanosilver and growth-promoting material and use thereof	Nanosilver mixed with growth promoters and plant nutrient materials can be used as a fertilizer	Yoon (2005)
	Method for preparing silver nanoparticle and method for promoting seed germination and growth and development of seedling of cucumber with the silver nanoparticle	Biocompatible silver nanoparticles (20 nm) are prepared by using amino acids as reducing agents during synthesis. Claims that exposure to these silver nanoparticles improved cucumber seedling germination	Xia et al. (2013)
Au	Method for cultivating grape containing gold nanoparticles	Aqueous fertilizer containing gold nanoparticles and sulfur produces grapes containing gold nanoparticles. Claims of human health benefits	Um and Jong Tae (2010)
Al	Production process for mixing polymer of nano-subnano grade marsh dregs–gangue compound	Composite nanoparticles (50–200 nm) of kaolin, fermentation residue, Al_2O_3, and SiO_2 are prepared by acid digestion of the mixtures. The particles can be used as a water-retaining additive or as a controlled-release coating. Cross-listed in Category 2 Si and Category 3 kaolin	Zhang et al. (2005b)
Bentonite clay	Nanoscale biological/organic/inorganic compound fertilizer	A nanogranular bentonite clay binder mixed with a compound fertilizer and nitrogen-fixing bacteria imparts improved stress resistance and higher fertility. Could potentially be cross-listed under Category 3	Tan et al. (2008)

(continued)

Table 2.2 (continued)

Nano-content	Patent title	Claim[a]	Ref.
C	Method of using carbon nanotubes to affect seed germination and plant growth	A seed treatment with CNTs of 10–200 μg/mL leads to greater rate of seed germination, increased vegetative biomass, and increased water uptake in seeds	Biris and Khodakovskaya (2011)
	Carbon nanotube production method to stimulate soil microorganisms and plant growth produced from the emissions of internal combustion	Carbon nanotubes are produced from soot and are claimed to stimulate plant growth	Lewis (2013)
	Special fertilizer for rapeseed base fertilizer	Fermented organic fertilizer is mixed with nanocarbon and nano-phosphate powder. Nanocarbon and nano-phosphate serve as insecticides, allow for slow release, and improve soil structure. The yield and quality of the rapeseed is improved. Cross-listed as Category 2 phosphate	Liu and Wangquan (2012)
	Method for preparation of compound organic fertilizer containing nanocarbon and sulfate radical organic fertilizer	Nanocarbon (5–70 nm) mixed with organic fertilizer is claimed to treat plant diseases and decrease cadmium pollution in soil	Zhang and Chen (2012)
	Nanocarbon synergism compound fertilizer for tobacco and preparation method thereof	Nitrogen, phosphate, and potash are crushed into a powder, and nanocarbon (size not specified) is added. Claims that the compound fertilizer increases tobacco yield and reduces fertilizer loss	Xie and Liu (2012)
	Foliar fertilizer containing carbon nanoparticles for plants under stress conditions	Trace elements and nanocarbon applied in a foliar fertilizer improve leaf permeability and stress tolerance	Li and Guan (2011)
	Synergistic fertilizer containing nanometer carbon and rare earth and its preparation	NPK fertilizer containing nanocarbon (5–70 nm) and rare-earth nitrates (size not specified) claims to increase nitrogen use efficiency	Zhang and Liu (2010)

(continued)

Table 2.2 (continued)

Nano-content	Patent title	Claim[a]	Ref.
Ca	Nanopeat composite and its products and application	Iron oxide nanoparticles and calcium carbonate nanoparticles mixed with peat yield a fertilizer capable of improving crop yields. Cross-listed with Category 2 Fe.	Wu (2004a)
	Method for cultivating high-quality high-functionality fruit and vegetables	Selenium, calcium hydroxide, and iron oxide nanoparticles added to seedlings improve yield and mineral content of fruit and vegetables. Cross-listed in Category 2 Fe and Se	Kim (2011)
	Gardening fertilizer containing stevia extract and minerals and preparation method thereof by using fermented stevia extract as penetration accelerator for functional material	Stevia (a sweet herb and sugar substitute) is mixed with nanoparticles of Se, organo-Ca, rare-earth elements, and chitosan and pulverized to a size of 75–95 nm. When used as a seed-coating agent, improved root growth was noted. Cross-listed in Category 2 Se and rare earths	Lee et al. (2007)
Fe	Special fertilizer for spring corn base fertilizer	Nano-iron slag powder (no size information provided) used in the preparation of the fertilizer mixture. Claims of effective reduction of plant disease and pests in soil	Liu et al. (2012c)
	Special fertilizer for cotton base fertilizer	Completely fermented organic fertilizer is mixed with nano-iron ore tailing powder. Nano-iron powder acts as an insecticide, slows nutrient release, and improves the soil	Liu et al. (2012d)
	Complete plant growth medium comprised of naturally occurring zeolite coated with nanophase iron oxide and dosed with nutrients	A zeolite host covered with iron oxide nanoparticles (10–50 nm diameter) can be loaded with plant nutrients and has increased fertilizer use efficiency. Other benefits include water retention, odor suppression, and pest resistance. Cross-listed with Category 3 zeolites	Vempati (2008)

(continued)

Table 2.2 (continued)

Nano-content	Patent title	Claim[a]	Ref.
	Method for cultivating high-quality high-functionality fruit and vegetables	Cross-listed in Category 2 Ca and Se	Kim (2011)
	Nanopeat composite and its products and application	Cross-listed with Category 2 Ca	Wu (2004a)
Humic acid	Nanometer soil amendment and its application in field crops	Si nanoparticles mixed with humic acid are pulverized down to the nanoscale. The nanosilica lends a water- and mineral-adsorbing quality to the composite. Reduced fertilizer use and improved yields are claimed. Cross-listed in Category 2 Si	Wei and Ji (2003)
	Production of nanometer humic acids-polymer composite and its application in agriculture	Nanoscale particles of humic acid and calcium silicate are prepared by high shear and mixed with a starch–acrylonitrile copolymer. The composite can be used as a seed-coating agent and a fertilizer-coating agent for controlled release. Could potentially be cross-listed under Category 3	Zhang et al. (2003c)
Phosphate	Special fertilizer for rapeseed base fertilizer	Cross-listed as Category 2 C	Liu and Wangquan (2012)
Polymers	Method for preparing nanocomposite aquasorb with function of slow-release fertilizer	Cross-linked polymer nanoparticles mixed with attapulgite and humic acid improve water retention of soils and lead to a slow-release fertilizer. Cross-listed to Category 3 polymers and Category 3 palygorskite	Wang and Zhang (2007)
	Nanometer scale multifunctional sand-fixing water-loss reducer from weathered coal and waste plastics using microemulsification	Coal and polymers from waste plastics are emulsified to form nanoparticles that can act as an aquasorb	Zhang and Wang (2004)
Rare earths	Method for manufacturing nanoscale compound fertilizers by using nanomaterial and MgO-rich seawater	Kaolin, montmorillonite, and rare-earth nanoparticles mixed with MgO, N, P, and K lead to improved fertility, pest resistance, and disease resistance. Cross-listed to Category 3 kaolin and montmorillonite	Zuo (2007)

(continued)

Table 2.2 (continued)

Nano-content	Patent title	Claim[a]	Ref.
	Gardening fertilizer containing stevia extract and minerals and preparation method thereof by using fermented stevia extract as penetration accelerator for functional material	Cross-listed in Category 2 Se and Ca	Lee et al. (2007)
S	Preparation of seed-coating agent containing nanoparticles with low toxicity and high efficiency	Sulfur nanoparticles (as a microbicide) and silicon dioxide nanoparticles (as a dispersing agent) mixed with fertilizer lead to improved yields. Cross-listed in Category 2 Si	Ding and Wu (2005)
	Fertilizer and method of wheat treatment with this fertilizer	Sulfur nanoparticles (40–120 nm) dispersed in a liquid foliar fertilizer increase protein content of harvested wheat grain	Aleksandrovich et al. (2011)
Se	Gardening fertilizer containing stevia extract and minerals and preparation method thereof by using fermented stevia extract as penetration accelerator for functional material	Cross-listed in Category 2 Ca and rare earths	Lee et al. (2007)
	Method for cultivating high-quality high-functionality fruit and vegetables	Cross-listed under Category 2 Fe and Ca	Kim (2011)
	Specific nutrient fertilizers for honey peach rich in organic Se	Nanoselenium (size not specified) is mixed with potash and microbial fertilizers. Claims that it produces honey peaches from which selenium is more easily absorbed by humans	Bi et al. (2010a)
	Se-rich nutrient composition specific for strawberry	Strawberries are enriched in organic selenium when exposed to composite fertilizer containing nanoselenium (size not specified), plant nutrients, and organic and microbial fertilizers	Bi et al. (2010b)
	Preparation of selenium-rich Chinese cabbage using selenium nanoparticle containing nutrient	Composite fertilizer containing nanoselenium (size not specified), plant nutrients, and organic and microbial fertilizers. Claims of selenium-enriched cabbage	Bi et al. (2010c)

(continued)

Table 2.2 (continued)

Nano-content	Patent title	Claim[a]	Ref.
	Nanosized selenium-rich compound fertilizer for promoting longevity of house flowering plants	Urea fertilizer blend composed of nano-Se, nano-tourmaline, etc. crushed and mixed. No size information provided. Final product not nano (in the range of 500 nm–200 um). Claims of increased longevity of indoor flowering plants. Cross-listed to Category 2 tourmaline	Cheng and Cheng (2010)
Si	Products comprising an antimicrobial composition based on titanium dioxide nanoparticles	TiO_2, ZnO, SnO, ZrO_2, and SiO_2 nanoparticles modified with fatty acids are used as an antimicrobial treatment, with one of the example uses as a fertilizer additive. Cross-listed in Category 2 Ti, Zn, Sn, and Zr	Bignozzi et al. (2008)
	Agrochemical compositions containing agrochemicals absorbed on porous nanoparticles for controlled release	SiO_2 and TiO_2 nanoparticles are used as a carrier for fertilizer additives or pesticides due to their large surface area. Cross-listed in Category 2 Ti	Chen (2002)
	Production process for mixing polymer of nano-subnano grade marsh dregs–gangue compound	Cross-listed in Category 2 Al	Zhang et al. (2005c)
	Nanometer soil amendment and its application in field crops	Cross-listed in Category 2 humic acid	Wei and Ji (2003)
Sn	Biological organic compound liquid nanofertilizer and preparing process thereof	Cross-listed in Category 2 Fe and Al	Ni (2003)
	Products comprising an antimicrobial composition based on titanium dioxide nanoparticles	Cross-listed in Category 2 Ti, Zn, Zr, and Si	Bignozzi et al. (2008)
	Thermoplastics in growth accelerators, giving increased yields and quality of plants in agriculture	When partially crystalline polymers such as polyethylene and polypropylene are mixed with semiconductor nanoparticles of less than 200 nm (SnO, In_2O_3, or Sn-doped In_2O_3), the resultant fertilizer additive leads to improved sunlight utilization by plants	Caro et al. (2006)

(continued)

Table 2.2 (continued)

Nano-content	Patent title	Claim[a]	Ref.
Talc	Controlled-release fertilizer additive	Including a talc nanopowder and zeolites with a composite fertilizer leads to a more stable, slow-release fertilizer	Yang and Wang (2008)
Ti	The liquid composition for promoting plant growth, which includes nanoparticle titanium dioxide	Titanium dioxide nanoparticles, alone or mixed with other fertilizer, increases the efficiency of solar energy conversion in crops	Choi et al. (2003)
	Products comprising an anti-microbial composition based on titanium dioxide nanoparticles	Cross-listed in Category 2 Sn, Zn, Zr, and Si	Bignozzi et al. (2008)
	Agrochemical compositions containing agrochemicals absorbed on porous nanoparticles for controlled release	Cross-listed in Category 2 Si	Chen (2002)
	Liquid composition for promoting plant growth containing titanium dioxide nanoparticles	Colloidal titanium dioxide nanoparticles (3–100 nm, 95 % in the range of 15–25 nm) in water promote plant growth	Choi (2010)
Tourmaline	Nanosized selenium-rich compound fertilizer for promoting longevity of house flowering plants	Cross-listed to Category 2 Se	Cheng and Cheng (2010)
Zn	Products comprising an anti-microbial composition based on titanium dioxide nanoparticles	Cross-listed in Category 2 Ti, Sn, Zr, and Si.	Bignozzi et al. (2008)
Zr	Products comprising an anti-microbial composition based on titanium dioxide nanoparticles	Cross-listed in Category 2 Ti, Zn, Sn, and Si	Bignozzi et al. (2008)

[a] Description provided from patent information, however, there may not be evidence in the patent to corroborate the claims

2.2.3 *Nanoscale Films and Host Materials*

This category contains fertilizers and supplements that are encapsulated by nanoscale films or held in nanoscale pores or spaces within a host material. Clays finding applications in fertilizer products include those such as kaolinites, smectites, halloysites, and palygorskites. These vary in terms of their chemical composition, as well as their properties, such as surface area and surface charge. Nanoclays generally are used in other applications as supportive filling agents to form nanocomposite structures, improving the thermal stability and mechanical properties of a bulk material. In the case of these fertilizers, they are typically employed as a medium for the adsorption of the nutrient product. Within the nanosized interlayer space, fertilizers could be protected from decomposition by sunlight, heat, and microbes, minimizing fertilizer loss. Furthermore, strong adsorption within the clays would attenuate losses through leaching as well as allow for the slow release of the fertilizer. For example, in a study by Park et al., the intercalation of a magnesium–urea complex into the nanoscale interlayer space of montmorillonite clay was found to protect the urea from rapid degradation in soil, which could serve to improve nitrogen use efficiency (Park et al. 2004). Numerous patents have been filed exploiting the use of clays as nanoscale hosts for fertilizer products. Zeolites alone (Guo 2007; Yu 2005a; Wu and Wu 2010; Gai et al. 2011) or doped with nanoparticles (Vempati 2008) have been loaded with plant nutrients and found to increase fertilizer use efficiency. Similarly, the nanoscale pores and channels in palygorskite (also known as attapulgite) (Cao et al. 2007a, b, c, d), kaolin (Zhang et al. 2005b), and a Chinese clay known as Ximaxi (Li et al. 2002) have all been exploited for strong adsorption of fertilizers and the slow release of fertilizer from the matrix.

Fertilizer could also be coated on nanoparticles or housed in nanotubes. Metal nanoparticles, e.g., Ag, have been investigated as carriers for plant nutrients (Nilanjan 2013). Halloysite nanotubes deserve special mention separate from the discussion on the other clay materials. Halloysite nanotubes are hollow clay tubes formed by surface weathering of natural aluminosilicate minerals. The tubes have diameters that are typically less than 100 nm and lengths that range from about 500 nm to over 1.2 μm. They can be filled with any agent to allow for its extended release. A recent patent held by the company NaturalNano, Inc., has utilized these nanotubes as hosts for fertilizers (Price and Wagner 2008; http://www.naturalnano.com, accessed January 10, 2014). Hydroxyapatite (HA) nanoparticles have been investigated in patents (Wei et al. 2011); Kottegoda et al. 2011a, b; 2013) and papers (Kottegoda et al. 2011c) as a carrier for fertilizer (see Fig. 2.6). Urea-modified HA nanoparticles that were sequestered in the cavities of *Gliricidia sepium* wood exhibited much slower release profiles in soils of three different pH values (4.2, 5.2, 7) and were releasing >10 mg a day even by day 60, unlike the faster release that was noted for a conventional fertilizer. Control experiments with the conventional fertilizer housed within the wood would help to parse out the effect of the nanoparticle carriers on the release profile of the urea.

Nanotube fertilizer carriers have also been prepared using cochleate structures. Cochleate delivery vehicles are stable, nanoscale phospholipid-cation nanotubes. They have a multilayered structure consisting of a large, continuous, solid lipid bilayer sheet rolled up into a spiral. These structures provide their contents with improved solubility as well as protection from environmental conditions. When used in drug delivery, they deliver their contents to target cells through the fusion of the outer layer of the cochleate to the cell membrane (Gould-Fogerite et al. 2003). Small cochleate structures can possibly be taken in through the stomata of plants, allowing for improved delivery of fertilizer, pesticides, etc. Nanoscale cochleate

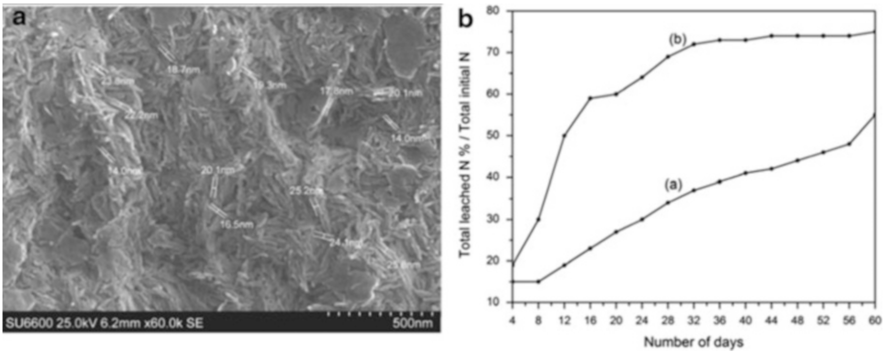

Fig. 2.6 Hydroxyapatite nanoparticles for urea delivery. (**a**) SEM image of urea-modified hydroxyapatite nanoparticles. (**b**) Comparison of %N released over 60 days for (**a**) the nanofertilizer and (**b**) a conventional fertilizer (Kottegoda et al. 2011c). Reproduced with permission from Indian Academy of Sciences

structures filled with commercial plant food have been tested on marigolds and compared to controls of the plant food alone. Larger foliage, more blooms, and more buds were noted in the cochleate-treated sample, suggesting improved delivery of the plant nutrients via foliage application (Yavitz 2006).

Other porous materials such as mesoporous silica and layered double hydroxides (LDHs) have been investigated for fertilizer delivery. The release of nitrogen via urea hydrolysis has been controlled through the incorporation of urease into nanoporous silica (Hossain et al. 2008). LDHs are a class of layered nanomaterials with positively charged crystalline inorganic layers (thickness of a few nm) and charge balancing anions located in the interlayer region. The anions located in the interlayer regions can be easily replaced, leading to an intense interest in the use of LDH intercalates for advanced applications such as controlled-release systems. A 2002 study examined the use of LDHs for the release of a plant growth regulator found that slow release of the compound could be controlled by pH (Hussein et al. 2002). Patents have described LDHs loaded with nitrate for use in fertilizers (Kottegoda et al. 2011a).

Also found within Category 3 are nanoscale polymer films, either wholly polymer based or composites with other materials such as humic acid cementing agents or clays. Several patents have been filed on nano-polymer fertilizer coatings, for example, those prepared from lignosulfonate particles (Zhang et al. 2003a; Du 2007), polyvinyl alcohol particles (Zhang et al. 2005a, e), polystyrene (Zhang et al. 2005d), polyolefin–starch conjugates (Zhang 2004), cellulose (Lin 2008), and polyelectrolytes (Li et al. 2010). Patents on polymer conjugates with zeolites (Barati 2010), palygorskite (Cai 2007), kaolin, and montmorillonite (Zhang et al. 2003b; Dong et al. 2006) have also been described. Several studies have been published related to the characterization of these clay–polymer nanocomposites and their slow-release properties (Liu et al. 2006; Zhang et al. 2006a, b). Mixtures of a poly(acrylic acid-*co*-acrylamide) polymer and kaolin nanopowder (40–100 nm in thickness) were studied as a coating for urea (Liang and Liu 2007). In another example, polystyrene mixed with nano-kaolin formed composites with 10–20 nm pores. When blended with urea and tested as seed coatings on winter wheat seeds, no adverse effects on germination were noted. Studies on the leaching of nitrogen from these composites showed that nitrogen release was slower than if the fertilizer was applied uncoated (as low as 56 % released in 40 days for the composite compared to 90 % from the fertilizer alone). Overall crop yield was seen to increase by up to 27 % compared to controls (Hussein et al. 2002).

All the patents and patent applications whose inventions fall under Category 3 can be found in Table 2.3, once again grouped in terms of their nano-content. Several examples are cross-listed to multiple categories.

2.2.4 Commercial Products from All Three Categories

Despite the relative infancy of the field of nanofertilizer inputs, there are several examples of products currently available on the market that claim some form of nanotechnology in their formulations (www.agro-genesis.com, accessed December 28, 2013; www.ngtech.com.au, accessed December 28, 2013; www.nafertino.com.tw/en, accessed January 11, 2014; www.stratbizworld.com, accessed January 11, 2014). In many cases, very little detail about the "nano" nature of the fertilizer product is provided. In some cases, however, it is clear that the term "nano" in these products is used very informally; no nanoparticles or nanomaterials appear to be incorporated in the product. For example, Nano-Gro (www.agronano.com, accessed December 21, 2013) contains nanomole amounts of iron, cobalt, magnesium, and manganese mixed with pharmaceutical grade sugar. The products, claimed to be "homeopathic plant medicine," are based on a concept that "a biologically active substance in very low concentrations triggers a high concentration response." Other products appear to contain nanomaterials, but the composition of the nanomaterials or the processes by which they are prepared are unclear. An example of this is the Chinese product "Silicon23 + Microbes NPK" (www.alibaba.com/product-detail/silicon-23-H4SiO4-Microbes-N-P_104977327.htm, accessed January 4, 2014). This fertilizer appears to be silica- or silicate-based nanoparticles. The Silicon23 + Microbes NPK fertilizer website states that it is a "microbes-silicate-based fertilizer containing a high content of essential elements and microbes using the latest nanotechnology." Calcium carbonate nanoparticles prepared by an ultrahigh-speed shearing technique called "nanotechnological tribodynamic activation" are the main component of a product named Lithovit high-yield fertilizer (www.lithovet.net, accessed January 4, 2014). The mechanism of action of this foliar fertilizer is to increase CO_2 levels in the leaves, leading to an increase in photosynthesis and higher crop yields. NanoNB is a fertilizer product from the UK company NanoVapor Technologies. They claim on their website that it is based on a blend of humified organic material and that it has plant growth stimulation properties. No other details are provided about the product. Their other

Table 2.3 Patents on inputs that fall into Category 3 (nanoscale coatings or hosts)

Nano-content	Patent title	Claim[a]	Ref
Bentonite	Special fertilizer for soybean	Preparation method made use of raw materials which included nano-parts by weight of bentonite. The powder coating involved grounding of raw materials after burning into nanopowder (no size information given)	Liu et al. (2012a)
Diatomite	Nano-diatomite and zeolite ceramic crystal powder	A plant growth medium can be prepared using diatomite and zeolites that has been ionized at high temperature and loaded with ammonia solution. Cross-listed to Category 3 zeolites	Yu (2005a)
Halloysite	A method for treating agricultural crops using materials associated with tubular carriers	Halloysite and other mineral-derived nanotubes can be used in controlled-release fertilizers for treating crops. Could also be cross-listed to Category 1	Price and Wagner (2008)
Hydroxyapatite	Phosphorus fertilizer comprising nano-hydroxyapatite and its preparation process	The nano-hydroxyapatite phosphorus fertilizer (grain diameter 20–50 nm) is prepared by precipitation or hydrothermal reactions from P and Ca base solutions. Claims that this fertilizer helps maintain soil P level, reducing its loss to water	Wei et al. (2011)
	Compositions for sustained release of agricultural macronutrients and process thereof	A nitrogen-containing macronutrient is adsorbed onto hydroxyapatite phosphate nanoparticles and intercalated within a nanoclay. This nanocomposite allows for slow release of the macronutrient upon contact with acidic soil (cross-listed with Category 3 layered double hydroxides)	Kottegoda et al. (2011a)
	Cellulose-based sustained-release macronutrient composition for fertilizer application	A nitrogen-containing macronutrient is adsorbed onto hydroxyapatite phosphate nanoparticles and encapsulated in wood cavities. The wood is coated with cellulose-modified	Kottegoda et al. (2011b)

(continued)

Table 2.3 (continued)

Nano-content	Patent title	Claim[a]	Ref
		hydroxyapatite phosphate nanoparticles, allowing for the slow release of the macronutrient to the soil	
	Composition and method for sustained release of agricultural macronutrients	Hydroxyapatite nanoparticles (Rods with 20–40 nm diameter) have a nitrogen-containing macronutrient adsorbed on their surface, which will slowly release after application to aqueous and terrestrial environments. No claims on efficacy	Kottegoda et al. (2013)
Humic Acid	Production process for blended high concentration sustained-release fertilizer	Cross-listed to Category 1 urea	Zhang and Yao (2005)
Kaolin	Compound fertilizer specific for peanut and preparation method thereof	Traditional fertilizer containing nano-kaolin and ground into a nanopowder (no size information provided)	Yang et al. (2012)
	Production process for mixing polymer of nano-subnano grade marsh dregs–gangue compound	Composite nanoparticles (50–200 nm) of kaolin, fermentation residue, Al_2O_3, and SiO_2 are prepared by acid digestion of the mixtures. The particles can be used as a water-retaining additive or as a controlled-release coating. Cross-listed in Category 2 Si and Category 2 Al	Zhang et al. (2005c)
Layered double hydroxides	Fertilizer, soil treatment agent, soil treatment method, and soil-less medium	Layered nanomaterials loaded with nitrate can be used as a controlled-release fertilizer	Gillman and Noble (2001)
	Compositions for sustained release of agricultural macronutrients and process thereof	Cross-listed to Category 3 hydroxyapatite	Kottegoda et al. (2011a)
Metal nanoparticles	Plant nutrient-coated nanoparticles and methods for their preparation and use	Metal nanoparticles (e.g., Ag) are coated with nutrients (e.g., B), facilitating transport and in some cases treating a nutrient deficiency	Nilanjan (2013)

(continued)

Table 2.3 (continued)

Nano-content	Patent title	Claim[a]	Ref
Montmorillonite	Method for manufacturing nanoscale compound fertilizers by using nanomaterial and MgO-rich seawater	Cross-listed to Category 3 kaolin and Category 2 rare earths	Zuo (2007)
	Technical process for producing clay nanoparticle–polyester blended polymer as binder for encapsulating fertilizer	Nanoscale emulsions are prepared by shearing aqueous dispersions of kaolin or montmorillonite clay with dodecylbenzenesulfonate surfactants and are used to encapsulate fertilizer. Cross-listed to Category 3 polymers and plastics	Zhang et al. (2003b)
Organosilane-coated magnetic nanoparticles	Agrochemical microcapsules adapted to rupture in a magnetic field	Microcapsules encapsulating agricultural chemicals have organosilane-coated magnetic nanoparticles covalently bound inside their shells. Application of a magnetic field ruptures the microcapsules, allowing for controlled release	Boday et al. (2013)
Palygorskite (attapulgite)	Palygorskite material-based sustained-release composite/potash/phosphorus/nitrogen fertilizer	A composite fertilizer, potash fertilizer, phosphate fertilizer, or urea fertilizer that is embedded in the nanoscale pores of a palygorskite clay yields a controlled-release fertilizer	Cao et al. (2007a, b, c, d)
	Method for preparing nanocomposite aquasorb with function of slow-release fertilizer	Cross-listed to Category 3 polymers and plastics and to Category 2 polymer nanoparticles	Wang and Zhang (2007)
	Controlled release of low-cost environmentally friendly nitrogenous fertilizer	Urea held within the nanoscale cavities of a nanoporous attapulgite–polyacrylamide conjugate yields a fertilizer with reduced nitrogen loss. Cross-listed to Category 3 polymers and plastics	Cai (2007)
Polymers and plastics	Production of sustained-/controlled-release fertilizer used for greenhouse tomato	NPK fertilizer covered with a nanoscale thin film composite of polyvinyl alcohol, foam plastic, and a humic acid cementing agent in the form of nanoscale granules yield a slow-release fertilizer	Zhang et al. (2005a)

(continued)

Table 2.3 (continued)

Nano-content	Patent title	Claim[a]	Ref
	Technical process for producing clay nanoparticle–polyester blended polymer as binder for encapsulating fertilizer	Nanoscale emulsions are prepared by shearing aqueous dispersions of kaolin or montmorillonite clay with dodecylbenzenesulfonate surfactants and are used to encapsulate fertilizer	Zhang et al. (2003b)
	Method for preparing nanocomposite aquasorb with function of slow-release fertilizer	Cross-listed to Category 3 palygorskite and Category 2 polymer nanoparticles	Wang and Zhang (2007)
	Controlled release of low-cost environmentally friendly nitrogenous fertilizer	Cross-listed to Category 3 palygorskite	Cai (2007)
	Preparation of polymeric mixture from waste polystyrene foam and useful for fertilizer adhesive or capsules	Polystyrene foam is emulsified with a granulating adhesive and sheared to form nanoparticles for fertilizer delivery	Zhang et al. (2005d)
	Manufacture of nano-olefin–starch blend as fertilizer packaging film adhesive or granulating binder	A fertilizer nanocoating is prepared by high-speed shearing of a nanoscale polymer blend cross-linked with starch	Zhang et al. (2004)
	Production technique of coating cement for nano-sulfonated lignin mixture fertilizer	Nanoscale particles of a lignosulfonate polymer are generated by high-speed grinding and then are used as a coating agent to prepare a slow-release composite fertilizer	Zhang et al. (2003a)
	Method for manufacturing fertilizer integument cementing agent by using papermaking black liquor	Lignosulfonate polymer nanoparticles are prepared by high-speed shearing and sprayed on the surface of a compound fertilizer to form a nanoscale polymer coating for slow-release applications	Du (2007)
	Methods to produce polymer nanoparticles and formulations of agricultural active ingredients	A polyelectrolyte-containing polymer and agricultural active compound are incorporated into a nanoparticle (examples given with sizes ranging from 4 to 400 nm). The active compound can be released based on an external stimulus	Li et al. (2010)

(continued)

Table 2.3 (continued)

Nano-content	Patent title	Claim[a]	Ref
	Novel sustained-release nanosized fertilizer and production method thereof	A nanofertilizer is produced by microemulsification, high-speed shearing, and "particle-guiding and variable speed technique" and is covered in a "release-sustaining nano-film" of cellulose by cementation. The release rate and time of the nanofertilizer can be altered based on the growth cycle of the crop	Lin (2008)
	Nanocomposite superabsorbent containing fertilizer nutrients used in agriculture	Using granularization, extrusion, and pelletization, "nanocomposite superabsorbent polymer" particles containing fertilizer can be isolated. This fertilizer helps with slow release and water retention. (Cross-listed with Category 3 zeolites)	Barati (2010)
Si	Special rapeseed fertilizer and preparation method of special rapeseed fertilizer	Quartz sand was sintered after adding oil of wintergreen, ground into nanopowder, and then mixed with ash to form a fertilizer carrier. No size information given. Claims of improved efficiency	Liu and Wangquan (2012)
Ximaxi (Chinese clay)	Preparation of Ximaxi controlled-release fertilizer	A composite fertilizer is granulated to form spheres and is coated with a nanoscale clay film to improve fertilizer efficiency	Li et al. (2002)
Zeolites	Long-acting additive for composite fertilizer	When urease inhibitors and nitrification inhibitors adsorbed onto nano-zeolite are used as a fertilizer additive, nutrient loss is minimized	Guo (2007)
	Complete plant growth medium comprised of naturally occurring zeolite coated with nanophase iron oxide and dosed with nutrients	Cross-listed to Category 2 Fe	Vempati (2008)
	Nano-diatomite and zeolite ceramic crystal powder	Cross-listed to Category 3 diatomite	Yu (2005a)

(continued)

Table 2.3 (continued)

Nano-content	Patent title	Claim[a]	Ref
	Nitrogen fertilizer-specific functional slow-release agent	Zeolite and bentonite are used to create a slow-release fertilizer that can reduce the amount of fertilizer applied by 20–30 %	Wu and Wu (2010)
	Nanocomposite superabsorbent containing fertilizer nutrients used in agriculture	Cross-listed with Category 3 polymers	Barati (2010)
	Granular fertilizer coated with mineral micro-/nanopowder	Micro-nano mineral powder is adsorbed onto the surfaces of granules of organic material, resulting in an "environmentally friendly, slow-release fertilizer that improves crop yield"	Gai et al. (2011)

[a] Description provided from patent information, however, there may not be evidence provided in the patent to corroborate the claims

commercial products are related to a physical method of dispersing nanosized surfactant particles for odor reduction and toxic vapor removal (www.setonresourcecenter.com/msdshazcom/htdocs/msds/n/nanovapor/nano-products%20summary%20industrial.pdf, accessed January 9, 2014).

Nanoemulsions are already on the market in the area of pesticide encapsulation and are also available in fertilizer products. One of the world's largest agrochemical companies, Syngenta, uses nanoencapsulation in their pesticide products and one of their growth-regulating products, Primo MAXX® (www.syngentaprofessionalproducts.com, accessed January 4, 2014). NanoGreen is a product from Global Biobased International Ltd. (www.nanogreensciences.com, accessed January 11, 2014). The product is a foliar fertilizer made of colloidal micelles/emulsions, 1–4 nm in diameter, containing alkylamines, amino acids, hydrogenated corn oil, nonionic surfactants, plant-based fatty acids, and organic alcohols. The product is taken up into the plant cells via the stomata of the leaves and is claimed to accelerate the rate of photosynthesis in the plant. As a result, the plant is found to be more resistant to disease and pests, and increases in crop yield and quality are observed. A series of Swedish fertilizer products, Skorda OP Nano Plant Nutrition, is also available commercially and comes in blends customized for certain crops, such as oil palm, wheat, and cotton. It is described as a "nanocarrier" loaded with enzymes, vitamins, secondary elements, microelements, and L-amino acids of vegetable origin (http://2lbersatu.com.my/skorda/about.html, accessed January 11, 2014). A commercially available supplement product that incorporates nanotechnology is Geohumus (www.geohumus.com, accessed December 28, 2013). Geohumus is a water-retaining hybrid (inorganic–organic-polymeric) material. The MSDS lists it as pulverized volcanic rock with special additives.

2.3 Potential Fate and Effects of Nanotechnology in Fertilizer Inputs

As the applications of nanotechnology become more and more ubiquitous, the toxicity and the environmental impact of these novel materials must be addressed. In the case where nanomaterials will be intentionally applied, as in the case with nanofertilizers, it is particularly important to understand their fate and effects. As farm crops serve as a potential pathway for nanoparticle transport and a route of bioaccumulation into the food chain, studies on the effects of nanoparticles on plants are needed. Information on whether nanoparticles will bioaccumulate in plants up through the food chain and end up in higher-level organisms, however, is limited. A recent review examined potential uptake, translocation, and biotransformation pathways for nanoparticles in plant systems as well as the positive and negative effects observed in a variety of food crops (Rico et al. 2011). In contrast to the studies showing that nanoparticles can be used to boost plant growth, there are a number of studies that report on the negative impacts of nanoparticles on higher plants. Recent studies on silver nanoparticle-amended sand have demonstrated a disruption in the growth of wheat plants (Dimkpa et al. 2013). Phytotoxicity studies of nanoscale alumina (nano-Al_2O_3) powders indicated that uncoated alumina particles could inhibit root elongation in crops such as corn, cucumber, soybean, cabbage, and carrot, while coated particles showed less of an effect (Yang and Watts 2005). High concentration of nanosized iron oxide particles inhibited *Zea mays* growth (Răcuciu and Creangă 2007). In another study, the effects of five types of nanoparticles (multiwalled carbon nanotubes, aluminum, aluminum oxide, zinc, and zinc oxide) on seed germination and root growth of radish, rapeseed, ryegrass, lettuce, corn, and cucumber were examined (Lin and Xing 2007). At concentrations of 2,000 mg/L, nano-Zn was found to inhibit seed germination in ryegrass, while nano-ZnO negatively affected corn. The same concentrations of those two nanoparticles terminated root elongation in all tested plant species. Nano-Al_2O_3 was shown to have a modest effect on root growth for corn. Nano-Al promoted root growth in radish and rapeseed but significantly retarded growth in ryegrass and lettuce. Recently, the effects of colloidal suspensions of clay or titanium dioxide nanoparticles on hydroponic maize seedlings were investigated. It was found that nanoparticle accumulation at the root surface led to rapid partial inhibition of cell-wall pore size, leaf growth, water transport, and transpiration (Asli and Neumann 2009). Unlike carbon nanotube studies presented in Sect. 2.2, negative effects on root elongation were observed in tomato, cabbage, carrot, and lettuce exposed to carbon nanotubes (Canas et al. 2008). Another study looked at the uptake and translocation of zinc oxide nanoparticles in a hydroponic ryegrass system. ZnO nanoparticles (20 ± 5 nm) were found in the endodermal and vascular cells of the ryegrass root. Ryegrass exposed to the nanoparticles had significantly reduced biomass, shrunken root tips, and collapsed root epidermal and cortical cells (Lin and Xing 2008). In other cases, no effect could be observed despite nanoparticle accumulation. For example, pumpkin plants (*Cucurbita maxima*) were chosen for a study on the uptake, translocation, and the accumulation of iron oxide nanoparticles within the plant tissues (Zhu et al. 2008). Using magnetization studies and microscopy, the authors determined that nanoparticles were transported to and accumulated in the leaves and other plant tissues, with no visible impact. Interestingly, pumpkin plants grown in sand and soil with irrigation using the iron oxide particle suspensions showed significantly less uptake and accumulation of the particles. This suggests that there is a difference in the bioavailability of the nanoparticles in sand or soil versus in aqueous solution. It is important to mention that visual indicators of toxicity, while valuable, are not always very sensitive, and whenever possible proteomic, genomic, and metabolic studies are warranted (Rico et al. 2011).

Potential impact of nanofertilizers and nanomaterials as plant additives on the health of soil microbial environments also warrants scrutiny. A 2009 study examined the effect of silica, palladium, gold, and copper nanoparticles on the germination of lettuce seeds, as well as their impact on soil microorganisms. Overall, no significant influence on the microbial communities was noted (Shah and Belozerova 2009). In another study, the structural diversity of a soil bacterial community was altered by gold nanorods, TiO_2 nanoparticles, and a number of polymer nanoparticles (Nogueira et al. 2012). Nano-CuO and magnetite were shown to negatively affect certain soil bacterial groups, particularly in a sandy loam soil (Frenk et al. 2013). Ag nanoparticles were shown to decrease mycorrhizal colonization of *Helianthus annuus* (Dubchak et al. 2010). Further study on the effects of nanoparticles and potential nanofertilizers on the soil microbiome is critical to any evaluation of the risks and benefits of nanotechnology in agriculture.

2.4 Limitations of Nanotechnology and Future Avenues of Research in Fertilizer Inputs

As nanotechnology continues to grow and develop as a field of research, more opportunities for the incorporation of nanomaterials into fertilizer inputs will emerge. The relatively slow progress of nanotechnology in fertilizer formulations, however, may at least partly be explained by lower levels of research funding, the lack of clarity on regulations, and the perceptions on innovation in the fertilizer industry. The trajectory of nanotechnology in the pharmaceutical industry may be a useful point of reference to help inform predictions on nanotechnology applications in fertilizer treatments. Many parallels can be drawn between the challenges of drug delivery and fertilizer delivery. For example, both need to function in complex biological systems and both have a requirement that the formulations be biocompatible, biodegradable, and nontoxic. One challenge however would relate to the value proposition for innovative drugs versus fertilizers. Increasing the cost of fertilizers due to the use of designer polymers as nanocoatings, for example, is less likely to be tolerated by the industry and by producers.

One major weakness in the current literature relating to nanotechnology in fertilizers is the dearth of overall standardization in the field. For example, many studies examining the effects of a particular nanofertilizer lack even the most basic physical characterization of the formulation. Differences in size, monodispersity, and surface chemistry can lead to dramatic physical and chemical changes in a nanomaterial; this could lead to seemingly contradictory effects from the same type of nanomaterial. At a minimum, basic physical characterization by microscopy (SEM, TEM, or AFM) to confirm size and monodispersity should be required in all nanofertilizer research. Furthermore, a standardization is required around the definition of "nano." Many formulations claiming to be "nano" are in fact more truly on the submicron or micron scale. In order to move forward with credibility, it is recommended that the universal definition of nanoscale (1–100 nm) be employed in fertilizer applications. Our review also indicates gaps in information associated with the patented products. For example, little information is available to indicate whether the nanomaterials were added to leaves or soil, whether the data used for registration was obtained from greenhouse or field studies, or from soil or soil-less media. Rigorous science-based processes for product development and performance evaluation are required to avoid misconstruing the real impact that nanotechnology may have in agriculture.

A major challenge for nanotechnology in fertilizer inputs and nanoscale coatings or host materials is to help synchronize the release of the essential fertilizer nutrients with the temporal and spatial demands by crops at the farm level. A "smart" nanofertilizer should prevent the nutrients from prematurely interacting with soil, water, and microorganisms and release nutrients only when they can be directly internalized by the plant. Agrium's Environmentally Smart Nitrogen (ESN) product is a polymer-coated (non-nanotechnology-based) product that releases nitrogen at changing rates depending on soil water and temperature (www.smartnitrogen.com, accessed January 4, 2014). More sophisticated release systems may require the incorporation of nanodevices, such as nanosensors, of chemical or biological origin within the fertilizer. Polymer capsules built from nanoscale polyelectrolyte layers have already been reported (Li et al. 2010) that can be used in triggered release systems for drug delivery (De Geest et al. 2007) suggesting that similar systems for fertilizer release may not be far behind. The incorporation of molecular recognition agents such as antibodies (Jongeijk and Verheesen 2011), or aptamers (Mastronardi et al. 2014; Sultan and DeRosa 2011; Zhang et al. 2013) to aid in the specificity of the fertilizer nutrients, could be transformative in this regard.

2.5 Conclusions

The goal of this chapter was to provide some insight into what impact nanotechnology might have on fertilizer inputs over the short- and long-term. The findings presented here indicate that nanotechnology is already beginning to have an impact.

Three main themes were explored in this chapter: nanoscale fertilizer input objects, nanoscale additives, and nanoscale coatings and hosts. While the majority of the examples within these categories are still relatively early in their development (at the stage of patents and research papers), several examples of commercial products incorporating nanotechnology are already on the market. It is still unclear whether the presented and discussed nanotechnologies for use in agriculture will have any negative long-term impacts on human health or the environment; thus further study into the impact that the intentional introduction of nanomaterials may have is warranted. It is difficult to predict the extent to which nanotechnology will affect fertilizers in the future; however, it is clear that there presently exists a unique opportunity to have some foresight of what is coming. For researchers, this insight may allow them to contribute to this emerging field in order to help it fully realize its potential. For regulators, this could allow for the measured and proactive analysis of any potential hazards of any new technologies in an effort to minimize and manage the risks in order to maximize its potential benefits.

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Chapter 3
Nano-fertilizers for Balanced Crop Nutrition

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Abstract Fertilizers play a pivotal role in improving the productivity across the spectrum of crops. The nutrient use efficiencies of conventional fertilizers hardly exceed 30–35 %, 18–20 %, and 35–40 % for N, P, and K which remained constant for the past several decades. Nano-fertilizers intended to improve the nutrient use efficiencies by exploiting unique properties of nanoparticles. The nano-fertilizers are synthesized by fortifying nutrients singly or in combinations onto the adsorbents with nano-dimension. Both physical (top-down) and chemical (bottom-up) approaches are used to produce nanomaterials, and the targeted nutrients are loaded as it is for cationic nutrients (NH_4^+, K^+, Ca^{2+}, Mg^{2+}) and after surface modification for anionic nutrients (NO_3^-, PO_4^{2-}, SO_4^{2-}). Nano-fertilizers are known to release nutrients slowly and steadily for more than 30 days which may assist in improving the nutrient use efficiency without any associated ill-effects. Since the nano-fertilizers are designed to deliver slowly over a long period of time, the loss of nutrients is substantially reduced vis-a-vis environmental safety. The work done on nano-fertilizers is very limited across the globe, but the reported literature clearly demonstrated that these customized fertilizers have a potential role to play in sustaining farm productivity. This chapter focuses on synthesis and characteristics of macro- and micronutrient carrying nano-fertilizers and their application in achieving balanced crop nutrition.

3.1 Introduction

Fertilizers are inevitable factor in improving soil fertility and productivity of crops regardless of the nature of cropping sequence or environmental conditions. It has been unequivocally demonstrated that one third of crop productivity is dictated by fertilizers besides influencing use efficiencies of other agri-inputs. In the past four decades, nutrient use efficiency (NUE) of crops remained constant despite our

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relentless efforts. The nutrients that are left in the soil may enter into the aquatic environment causing eutrophication. In addition to the low nutrient efficiencies, agriculture in developing countries including India is facing a problem of low organic matter, imbalanced fertilization, and low fertilizer response that eventually caused crop yield stagnation (Biswas and Sharma 2008). The fertilizer response ratio in the irrigated areas of the country has decreased drastically. It has been reported that 27 kg NPK ha^{-1} was required to produce one ton of grain in 1970, while the same level of production can be achieved by 109 kg NPK ha^{-1} in 2008. The optimal NPK fertilizer ratio of 4:2:1 is ideal for crop productivity, while the current ratio is being maintained at 6.7:3.1:1 in India due to the excessive use of nitrogenous fertilizers. In order to achieve a target of 300 million tons of food grains and to feed the burgeoning population of 1.4 billion in the year 2025, the country will require 45 Mt of nutrients as against a current consumption level of 23 Mt. The extent of multi-nutrient deficiencies is alarmingly increasing year by year which is closely associated with a crop loss of nearly 25–30 %. The extent of nutrient deficiencies in the country is of the order of 90, 80, 50, 41, 49, and 33 % for N, P, K, S, Zn, and B, respectively. Thus, from all sources, the country will be required to arrange for the supply of about 40–45 Mt of nutrients by 2025 (Subramanian and Tarafdar 2009).

Nanotechnology deals with particles measuring a dimension of one-billionth of a meter or one-millionth of a millimeter. This enables atom-by-atom manipulation, and thus processes or products evolved from nanotechnology are very precise and hardly possible to achieve through conventional methods. This fascinating field of science has been exploited widely in engineering, health, electronics, and material sciences, and agricultural scientists have begun to use it as a tool to improve the input use efficiencies by integrating nanotechnological approaches in the conventional production system. In this context, there would be greater importance of the information about how to increase the NUE of fertilizers by nanotechnology in the coming years.

In this chapter, current status of understanding of nano-fertilizer formulations and its associated effects on crop production systems has been narrated.

3.1.1 Nano-fertilizers

Nano-fertilizers are nutrient carriers of nano-dimensions ranging from 30 to 40 nm (10^{-9} m or one-billionth of a meter) and capable of holding bountiful of nutrient ions due to their high surface area and release it slowly and steadily that commensurate with crop demand. Subramanian et al. (2008) reported that nano-fertilizers and nanocomposites can be used to control the release of nutrients from the fertilizer granules so as to improve the NUE while preventing the nutrient ions from either getting fixed or lost in the environment. Nano-fertilizers have high use efficiency and can be delivered in a timely manner to a rhizospheric target. There are slow-release and super sorbent nitrogenous and phosphatic fertilizers. Some new-generation fertilizers have applications to crop production on long-duration human missions to space exploration (Lal 2008). Recently, Subramanian and Sharmila Rahale (2013) have monitored the nutrient release pattern of nano-fertilizer formulations carrying fertilizer nitrogen. The data have shown that the nano-clay-based fertilizer formulations (zeolite and montmorillonite with a dimension of 30–40 nm) are capable of releasing the nutrients particularly N for a longer period of time ($>$1,000 h) than conventional fertilizers ($<$500 h). Subramanian and Tarafdar (2009) suggested that clay particles are adsorptive sites carrying reservoir of nutrient ions. Major portion of nutrient fixation occurs in the broken edges of the clay particles. Zero valence nanoparticles adsorb onto the clay lattice, thereby preventing fixation of nutrient ions. Further, nanoparticles prevent the freely mobile nutrient ions to get precipitated. These two processes assist in promoting the labile pool of nutrients that can be readily utilized by plants. Fertilizer particles can be coated with nano-membranes that facilitate in slow and steady release of nutrients. This process helps to reduce loss of nutrients while improving fertilizer use efficiency of crops.

3.2 Synthesis of Nano-fertilizers

Nano-fertilizers are synthesized by top-down (physical) or bottom-up (chemical) approaches. Top-down approach is a commonly used method. In top-down approach, the adsorbent or substrate used for synthesis of nano-fertilizers such as zeolite or any other carrier is ball milled for several hours to achieve nano-dimension. Usually, natural zeolite measures a range of 1,000–3,000 nm, and grinding using high-energy ball mill reduced the size of the particles. Manikandan and Subramanian (2014) reported that the ball milling of zeolite at 1, 2, 4, and 6 h had reduced the dimension 1,078, 475, 398, 357, and 203, respectively. The size reduction closely coincided with the increase in the surface area of 41, 55, 72, 83, and 110 $m^2\ g^{-1}$. Such phenomenal increase in the surface area provides extensive surface area for nutrient adsorption and desorption. Despite the physical method of nanoparticle synthesis is very simple, the product is heterogeneous and particles often get agglomerated. To prevent agglomeration, stabilizing agents such as polymers or surfactants are used.

The studies on slow-release fertilizers (SRFs) based on zeolites are limited to nutrients, which can be loaded in cationic forms such as NH_4^+ and K^+. However, if the nutrients are in anionic forms such as SO_4^{2-}, NO_3^-, and PO_4^{3-}, the loading is negligible on unmodified zeolites. Therefore, it is imperative that the material should have adequate affinity for anions so that the anionic nutrients can be efficiently loaded for its use as SRFs. Anionic properties can easily be imparted on the zeolitic surface using the concept of surface modification using surfactant. Surface modification facilitates the loading of anion into the zeolite's surface by the anion exchange process. Haggerty and Bowman (1994) reported that surfactant-modified zeolite (SMZ), a type of inexpensive anion exchanger has been shown to remove anionic contaminants from water. Hexadecyltrimethylammonium bromide (HDTMABr), a cationic surfactant, was used for surface modification of zeolite, and results showed that at HDTMABr loading maximum of 200 mmol kg^{-1}, corresponding to 200 % of the zeolite's effective cation exchange capacity, a surfactant bilayer would form and the surface was reversed to positive (Li and Bowman 1997).

Li et al. (1998) revealed that SMZ has been studied extensively in the last 15 years due to its high capacity of sorption and retention of oxyanions. The surfactant molecules (HDTMABr) form bilayers on zeolite external surfaces with the lower layer held by electrostatic interaction between the negatively charged zeolite surface and the positively charged surfactant head groups, while the upper layer is bound to the lower layer by hydrophobic forces between the surfactant tail groups in both layers (Bowman 2003). Surface modified zeolite showed that positive results have been reported on the retention of chromate (Krishna et al. 2001) and phosphate (Bansiwal et al. 2006). Li and Zhang (2010) reported that the loading capacity of sulfate compared to nitrate on SMZ may be attributed to the charge effect of the anions. Each HDTMABr molecule contributes one positive charge, which needs only one negative charge to balance. Sulfate is divalent and thus needs two HDTMABr molecules to neutralize. Meanwhile, the HDTMABr surface configuration is not rigid because of the surfactant tail–tail interaction. Thus, bridging two HDTMABr molecules with one sulfate may be less favored compared to 1:1 neutralization of HDTMABr by nitrate.

3.3 Characterization of Nano-fertilizers

Synthesized nano-fertilizers are to be characterized using particle size analyzer (PSA), zeta analyzer, Fourier transform infrared spectroscopy (FTI-IR), Raman spectroscopy, X-ray diffraction (XRD), scanning electron microscope (SEM), energy dispersive X-ray spectroscopy (EDAX), transmission electron microscope (TEM), and atomic force microscope (AFM) to confirm the size, shape, charge distribution, functional groups, elemental composition, surfactant attachment, and sulfate attachment. The synthesized nano-fertilizers have been characterized using the set of equipments listed above. Extensive studies had been undertaken to characterize nitrogenous (Subramanian and Sharmila Rahale 2013; Mohanraj 2013; Manikandan and Subramanian 2014), phosphatic (Bansiwal et al. 2006; Adhikari 2011; Behnassi et al. 2011), potassic (Subramanian and Sharmila Rahale 2012), sulfatic (Selva Preetha et al. 2014; Thirunavukkarasu 2014), and zinc (Subramanian and Sharmila Rahale 2012) fertilizers.

3.4 Nano-fertilizer Research

Nano-fertilizer is quite innovative, and recent publications strongly supported that there is a lot to be done before the technology reaches the farm gate. Almost all essential nutrients have been attempted to be delivered through nano-adsorbents. In most cases, clays and other aluminum silicates have been used as effective adsorbents to deliver nutrients. It has been unequivocally demonstrated that the size reduction by physical or chemical methods increased the surface mass ratio; thereby, bountiful of nutrient ions get adsorbed and desorbed slowly and steadily for an extended period of time (Table 3.1). This table summarizes the literature review on nano-fertilizer formulations published in the recent past. Despite these literatures are in support of nano-fertilizer technology, still the data are yet to establish whether nutrient release is adjusted or regulated in accordance with the crop demand.

3.5 Mode of Entry

Plant cell wall acts as a barrier for easy entry of any external agents including nanoparticles into the plant cells. The sieving properties are determined by pore diameter of cell wall ranging from 5 to 20 nm (Fleischer et al. 1999). Hence, only nanoparticles with diameter less than the pore diameter of the cell wall could easily pass through and reach the plasma membrane. There is also a chance for enlargement of pores or induction of new cell wall pores upon interaction with engineered nanoparticles which in turn enhance nanoparticle uptake. Further internalization occurs during endocytosis with the help of a cavity-like structure that forms around the nanoparticles by plasma membrane. They may also cross the membrane using embedded transport carrier proteins or through ion channels. In the cytoplasm, the nanoparticles are applied on leaf surfaces; they enter through the stomatal openings or through the base of the trichomes and then translocated to various tissues. However, accumulation of nanoparticles on photosynthetic surface causes foliar heating which results in alterations of gas exchange due to stomatal obstructions that produce changes in various physiological and cellular functions of plants (Fernandez and Eichert 2009).

3.5.1 Nanocapsules Enter Plants Through Stomata Orifices and Prevent Infection

The crop protection agents (CPAs) are nano-encapsulated, and the resulted polymer nanocapsules are sprayed onto the leaf tissue. These nanocapsules enter the plant through the stomata orifices. The nanocapsule's chemical bonds of the polymer

Table 3.1 Synthesis, characteristics, and nutrient release from nano-fertilizers/formulations

Nutrients	Adsorbent	Approach	Size	Nutrient release (h)	References
N	Zeolite	Physical	25–30 nm	1,200	Subramanian and Sharmila Rahale (2013)
	Montmorillonite	Physical	35–40 nm	400	
	Zeolite	Chemical	200 nm	–	Komarneni (2010)
	Surface cross-linked superabsorbents (hydrogels)	Chemical	40–80 nm	672	Liu et al. (2006)
	Zeolite	Physical	420 μm	16	Li (2003)
	Hydroxyapatite nanoparticles + *Gliricidia sepium*	Biological	19–25 nm	1,440	Kottegoda et al. (2011)
	Zeolite	Physical	60 nm	1,176	Selva Preetha (2011)
	Zeolite	Chemical	7–10 nm	480	Mohanraj (2013)
	Zeolite	Physical	87 nm	1,152	Manikandan and Subramanian (2014)
	Montmorillonite	Chemical	50 μm	240	Bortolin et al. (2013)
P	Zeolite	Physical	25–30 nm	1,104	Subramanian and Sharmila Rahale (2013)
	Montmorillonite, bentonite	Physical	35–40 nm	284	
	Zeolite	Physical	60 nm	1,000	Selva Preetha (2011)
	Zeolite	Chemical	2–3 μm	1,080	Bansiwal et al. (2006)
K	Zeolite	Physical	25–30 nm	1,176	Subramanian and Sharmila Rahale (2013)
	Montmorillonite, bentonite	Physical	35–40 nm	216	
NPK	Nano-coating of sulfur layer Chitosan	Chemical	100 nm 78 nm	–	Wilson et al. (2008)
Nanocomposite	Kaolinite	Chemical	30–80 nm	–	Xu-mei et al. (2006)
S	Zeolite	Physical	70–93 nm	816	Thirunavukkarasu (2014)
	Zeolite	Physical	420 μm	55	Li and Zhang (2010)
	Zeolite	Physical	60 nm	1,520	Selva Preetha et al. (2014)

(continued)

Table 3.1 (continued)

Nutrients	Adsorbent	Approach	Size	Nutrient release (h)	References
Zn	Zeolite	Physical	25–30	1,176	Subramanian and Sharmila Rahale (2013)
	Montmorillonite, bentonite	Physical	35–40 nm	312	
	Nano-Zn	Chemical	35 nm	–	Nair et al. (2010)
	Nano-ZnO	Chemical	20 nm	–	Mahajan et al. (2011)
B	Zeolite	Physical	60 nm	1,500	Selva Preetha (2011)

wall can be weakened or broken by a critical amount of stress enzymes present. Plant cell stress enzymes are activated by mechanical, thermal, chemical, or biological stress. This stress sensitizes the plant during an attack and infection from fungi and bacteria. These polymer-based CPA nanocapsules sprays are able to prevent this infection: in this case, the plant cell stress enzymes are the stimuli triggering the CPA release (Uzu et al. 2010).

3.5.2 *Microelements Enter Plant Through Root Hairs and Deliver Nutrients*

The step-by-step method includes the following: the microelements, such as Ca, Mg, Fe, S, and Zn, are encapsulated into microspheres, and these polymer microcapsules are, with time, incorporated and dissolved into the soil. Once close to the root, the chemical bonds of the microcapsule's wall polymer are broken down by the organic acids or phenolic substances from the root exudates. These root exudates are typically released to enhance plant feeding during the plant growth process and represent the stimuli activating CPA release (Corredor et al. 2009). The possibility of targeting the movement of nanoparticles to specific sites of an organism paves way for the use of nano-biotechnology in the treatment of plant diseases that affect specific parts of the plant. Different procedures have made use of nanoparticles in plants, such as the controlled release of bioactive substances in solid wood and plant transformation through bombardment with gold or tungsten particles coated with DNA. In recent years, a breakthrough has been made as a result of the work by Torney et al. (2007) who were able to control the intracellular release of substances into protoplasts using mesoporous silica nanoparticles. Despite these advances, the delivery of nanoparticles into plant tissues has been limited to methods involving bombardment, a methodology that does not allow massive application of particles in large number of plants, thus being less exploited in crop improvement programs till date (González-Melendi et al. 2008). Copper biomineralization with some wetland plants that transform copper into metallic nanoparticles at soil–root interface with the help of some endomycorrhizal fungi was reported that could reduce copper toxicity in the contaminated soils (Manceau et al. 2008).

Increased applications of engineered carbon-based nanomaterial increase concerns about their toxicity to humans and animals. Carbon nanomaterials, such as single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs), and carbon buckyballs, also found increased applications in the field of agriculture and food. This raised questions regarding the safety of using such nanomaterials with crops. Various studies showed contradictory results on the phytotoxicity of carbon nanomaterials in plants. The effects of functionalized SWCNTs (fCNTs, functionalized with poly-3-aminobenzenesulfonic for high dispersibility) and nonfunctionalized SWCNTs (CNTs) on root elongation of six different crop species (cabbage (*Brassica oleracea*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), onion (*Allium* sp.)) have been extensively studied (Buzea et al. 2007).

3.6 Nanocomposites

Nanocomposites have been developed in order to supply wide range of nutrients in desirable properties. These compounds are capable of regulating the inputs depending on the conditions of soil or requirement of crops. Zinc–aluminum layered double-hydroxide nanocomposites have been used for the controlled release of nutrients that regulate plant growth (Hossain et al. 2002). In soil niches, nanomaterials are porous and hydrated, and as such they control moisture retention, permeability, solute transport, and availability of plant nutrients in soils. These nanomaterials also control exchange reactions of dissolved inorganic and organic species between the soil solution and colloidal surfaces. The physicochemical properties in the surface of nanocomposites provide much of reactivity to soil biological and abiotic processes (Navrotsky 2004). Zhang et al. (2006) reported that results of measurements on nutrients of wheat plant showed that in treatments with the five kinds of bulk-blended SRFs, the contents of NPK absorbed by wheat in shooting period were higher than that of chemical fertilizer of equal amount of NPK, which was consistent with the rule of nutrients demanded by wheat. Liu et al. (2005a) reported that the organic material was intercalated in the layers of kaoline clays, and the natural kaoline exfoliated into nanometer-sized layers. The organic agent and clays formed nanocomposites through hydrogen bond combination. The SEM pictures of polystyrene–starch nano-subnanocomposites showed that many pores were present on the surface of film at sizes ranging from 10 to 20 nm. These nano-subnanocomposites were used as the cementing and coating materials of slow-/controlled-release fertilizer.

Liu et al. (2006) studied the nitrogen slow-release behavior of the superabsorbent nitrogen fertilizer (SSNF) in water and water retention capacity of the soils with SSNF. They reported that the surface cross-linked product not only had good slow-release property but also excellent soil moisture preservation capacity, which could effectively improve the utilization of fertilizer and water resources simultaneously. They also found that SSNF could be applied in agriculture and horticulture, especially in drought-prone areas where the availability of water is insufficient. Qiang et al. (2008) reported that the wheat grain yield and protein were improved in some degree, but protein content was increased insignificantly, and soluble sugar content was decreased by slow-/controlled-release fertilizer coated and felted by nanomaterials compared with NPK chemical fertilizer. It was effective to use slow-/controlled-release fertilizer coated by nanomaterials to improve wheat yield and quality.

3.6.1 Effect of Nanocomposites on Crop Growth

Liu et al. (2005a) reported that the kaoline nano-subnanocomposite was prepared by the methods of organic material intercalation under certain temperature and pressure. This compound was used as the cementing and coating material of slow-/controlled-release fertilizer because of its strong adsorption and thickness to macronutrients and organic C.

Liu et al. (2005b) reported that the addition of nano-subnanocomposites benefits the soil and raises the utilized efficiency of fertilizer because of its excellent characteristics. The physical adsorption and chemical combination occurred between nutrient elements and nanocomposites due to surface reaction and small-size reaction of nanocomposites. They formed the efficient multifunctional fertilizer, which heightened the adsorption of nutrient elements by plants, lowered the leaching in soil, and the fixation of fertilizer in the soil. Liu and Zhang (2005) reported that the nano-subnanocomposites significantly affected or controlled the structure and penetrability of the soil, increased the organic mineral granule of the soil, improved fertilizer storage and water holding capability in the soil, promoted action of microorganisms, regulated the ratio of C/N, enhanced the fertility of the soil, and so on. Improved yields have been claimed for fertilizers that are incorporated into cochleate nanotubes (rolled-up lipid bilayer sheets). The release of nitrogen by urea hydrolysis has been controlled through the insertion of urease enzymes into nanoporous silica (Hossain et al. 2008). Eberl (2008) tested the controlled-release fertilizers in greenhouse pot experiments with sorghum–sudan grass using NH_4-saturated zeolite and P-rock with a phosphate application rate of 340 mg kg^{-1} soil and zeolite/P-rock ratios ranging from 0 to 6. Total phosphate uptake and phosphate concentration measured for the grass were related linearly to the zeolite/P-rock ratio, and yields summed over four cuttings were as much as four times larger than control experiments. Sultan et al. (2009) reported that the development of functional nanoscale films and devices has the potential to produce significant gains in the NUE and crop production.

Nano-silica particles absorbed by roots have been shown to form films at the cell walls, which can enhance the plant's resistance to stress and lead to improved yields (DeRosa et al. 2010).

3.7 Conclusion and Future Trends

Nanotechnology is an emerging field of science being exploited to derive solutions to highly complicated unresolved issues in engineering and biological sciences. In agriculture, nanotechnology is least investigated, but the reported literature strongly suggests that nanoscience is expected to play a critical role in developing smart delivery systems. This will enable the plants to produce larger biomass utilizing the available nutrients in the rhizosphere without associated ill-effects in the environment. Regulated and sustained release of nutrients assists in improving the nutrient use efficiencies. Though nutrient release is regulated through physical and chemical processes, the biological significance of nutrient release is yet to be clearly understood. More research is needed to address the smart delivery of nutrients, nutrient interactions at the physiological and molecular levels, antagonistic and synergistic interactions among nutrients, and biosafety of nano-fertilizers besides long-term impact of nano-fertilizers on physical, chemical, and biological properties of soils is yet to be determined.

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Chapter 4
Nano-fertilizers and Their Smart Delivery System

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Abstract Outburst of world population in the past decade has forced the agricultural sector to increase crop productivity to satisfy the needs of billions of people especially in developing countries. Widespread existence of nutrient deficiency in soils has resulted in great economic loss for farmers and significant decreases in nutritional quality and overall quantity of grains for human beings and livestock. Use of large-scale application of chemical fertilizers to increase the crop productivity is not a suitable option for long run because the chemical fertilizers are considered as double-edged swords, which on the one hand increase the crop production but on the other hand disturb the soil mineral balance and decrease soil fertility. Large-scale application of chemical fertilizers results in an irreparable damage to the soil structure, mineral cycles, soil microbial flora, plants, and even more on the food chains across ecosystems leading to heritable mutations in future generations of consumers.

In recent years, nanotechnology has extended its relevance in plant science and agriculture. Advancement in nanotechnology has improved ways for large-scale production of nanoparticles of physiologically important metals, which are now used to improve fertilizer formulations for increased uptake in plant cells and by minimizing nutrient loss. Nanoparticles have high surface area, sorption capacity, and controlled-release kinetics to targeted sites making them "smart delivery system." Nanostructured fertilizers can increase the nutrient use efficiency through mechanisms such as targeted delivery, slow or controlled release. They could precisely release their active ingredients in responding to environmental triggers and biological demands. In recent lab scale investigations, it has been reported that nano-fertilizers can improve crop productivity by enhancing the rate of seed germination, seedling growth, photosynthetic activity, nitrogen metabolism, and carbohydrate and protein synthesis. However, as being an infant technology, the ethical and safety issues surrounding the use of nanoparticles in plant productivity are limitless and must be very carefully evaluated before adapting the use of the so-called nano-fertilizers in agricultural fields.

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In this chapter, we emphasize on the formulation and delivery of nano-fertilizers, their uptake, translocation, and fate in plants as well as their effect on plant physiology and metabolism. Ethical and safety issues regarding the use of nanotechnology in agriculture are also discussed.

4.1 Introduction

Nanomaterials are at the leading edge of rapidly developing field of nanotechnology. According to the National Nanotechnology Initiative (NNI), "Nanotechnology research and development is directed towards understanding and creating improved materials, devices and systems that exploit nanoscale properties" (Nanoscale Science Energy and Technology Subcommittee 2007). Nanotechnology is an emerging technology, which has revolutionary breakthrough in various fields such as electronics, energy, remediation, automobile, space technology, and life sciences. It has great potential in biological and medical applications such as gene and drug delivery, biosensing, diagnostic and tissue engineering (Borm et al. 2006; Oberdörster et al. 2005).

The term "nano" is adapted from the Greek word meaning "dwarf." The word "nano" means 10^{-9} or one billionth part of a meter. Particles with at least one dimension less than 100 nm are considered as "nanoparticles" (Thakkar et al. 2010). Nanoparticles have high surface area to volume ratio, nanometer regime, and unique properties, which makes them highly applicable. Nanotechnology provides new interdisciplinary venture into agriculture and food sciences by converging science and engineering. It promises significant contribution to agricultural research, which can lead to new avenues for solving numerous agricultural problems. Nanoparticles have potential applications in agriculture system, viz., detection of pollutants, plant diseases, pests, and pathogens; controlled delivery of pesticide, fertilizers, nutrients, and genetic material; and can act as nanoarchitects in formation and binding of soil structure (Ghormade et al. 2011). Nanoparticles can result in modification of plant gene expression and associated biological pathways which ultimately affect plant growth and development (Nair et al. 2010). Nanoparticles can have varied compositions, from being composed of metal oxide, ceramics, silicates, magnetic materials, quantum dots, lipid, polymers, and dendrimers to emulsions. Composition of nanoparticles plays a significant role in their application. For example, polymer-coated nanoparticles are used as agrochemical carrier due to its controlled-release ability, whereas metal nanoparticles show size-dependent properties such as magnetism, fluorescence, and photocatalytic degradation, which have application in sensor development, agrochemical degradation, and soil remediation (Ghormade et al. 2011).

Outburst of world population in the past decade has forced for higher agriculture productivity to satisfy the needs of billions of people especially in developing countries. Widespread existence of nutrient deficiency in soils causes both great economic losses for farmers and significant decreases in nutritional quality and overall quantity of grain for human beings and livestock. Application of fertilizers can enhance the crop productivity. However, the available nutrients present in the bulk chemical forms are not fully accessible to plants. In addition, the utilization of most of the macronutrient is very low due to their inversion to insoluble form in soil. Crop plants typically use less than half of the chemical fertilizers applied (Loomis and Connor 1992). The remaining minerals may leach down and become fixed in soil or contribute to air pollution. So the use of large-scale application of chemical fertilizers to increase the crop productivity is not a suitable option for long run as these are double-edged swords, which on one end increase the crop production but on the other end disturb the soil mineral balance and decrease soil fertility. Excess use of chemical fertilizers causes an irreparable damage to the soil structure, mineral cycles, soil microbial flora, plants, and even more on the food chains across ecosystems leading to heritable mutations in future generations of consumers.

Considering the abovementioned points, there is an urgent need to develop smart materials that can systematically release chemicals to specific targeted sites in plants which could be beneficial in controlling nutrition deficiency in agriculture. “Smart delivery system” means combination of specifically targeted, highly controlled, remotely regulated, and multifunctional characteristic to avoid biological barriers for successful targeting (Nair et al. 2010). Advancement in technology has improved ways for large-scale production of nanoparticles of physiologically important metals, which are now used as “smart delivery systems” in order to improve fertilizer formulation by minimizing nutrient loss and increased uptake in plant cell (Naderi and Danesh-Shahraki 2013). These “nano-fertilizers” have high surface area, sorption capacity, and controlled-release kinetics to targeted sites attributing them as smart delivery system. However, being an infant technology, the ethical and safety issues surrounding the use of nanoparticles in plant productivity are limitless and must be carefully evaluated before adapting the use of the so-called nano-fertilizers.

4.2 Plant Mineral Nutrients and Their Deficiency

Plants essentially require sunlight, water, CO_2, and many chemical elements for their growth and development. Among these components, chemical elements can be acquired by the plant from the soil either through roots or through aerial parts (Marschner 1995). Those acquired from the soil are called as mineral nutrients. Certain mineral nutrients in the gaseous form (NH_3, SO_2, etc.) enter the leaves through the stomata. Carbon, hydrogen, and oxygen are derived from CO_2 and H_2O and are not treated as mineral nutrients. Out of 16 essential elements for the growth of plants, those required in low concentrations are called as micronutrients (Fe, Cu, Zn, Mn, B, Mo, Ni, Na, Cl), and those required in high concentrations are called macronutrients (N, P, K, Mg, Ca, S, Si). After entering the plant cell, mineral nutrients need to be translocated to different locations for their metabolism. Table 4.1 summarizes the list of all macro- and micronutrients and their role and

Table 4.1 Role of different mineral nutrients and their deficiency symptoms

Mineral nutrient and its availability	Physiological role	Deficiency symptoms
Nitrogen: present mainly in organic form (98 %) in soil. Remaining 2 % inorganic part comprises of NH_4^+ (immobile) and NO_3^- (highly mobile) forms. Soil nitrogen is often lost when crops are harvested and plant material is removed from the soil	Basic component of proteins and genetic material and hence required by the plant in greatest amount	Yellowing of leaves (chlorosis) and subsequent falling
Potassium: present as cation (K^+)	Involved in maintaining the turgor pressure of plant cells, enhancing the disease resistance and activates enzymes involved in photosynthesis and respiration; affects the synthesis of simple sugars, starch, and proteins, translocation of carbohydrates, reduction of nitrates, normal cell division, and stomatal movements	Mottling leading to necrosis and higher susceptibility to diseases
Calcium: present as Ca^{2+} ions	Intracellular messenger in the cytosol, synthesis of new cell wall, cell division, controlling membrane structure and function	Deficiency is rare in nature, but if deficient, then causes poor development of root, necrosis and curling of leaves, fruit cracking, poor fruit storage, etc.
Magnesium: present as divalent cation (Mg^{2+}), constituent of chlorophyll molecule	Activation of enzymes, involved in various physiological and biochemical processes like photosynthesis and respiration	Chlorosis, mainly in older leaves
Phosphorous: present in organic and mineral P forms in soil. Plants obtain P as orthophosphorous anion (HPO_4^{2-} and $H_2PO_4^-$) which are present in less amount in soil	Important constituent of nucleic acid, phospholipid component of membranes and ATP	Necrotic spots, dwarf/ stunted growth, distinct purple color develops in leaves
Sulfur: present as sulfides (S^-), elemental sulfur (S^0), and sulfate (SO_4^{2-}) forms in soil of which SO_4^{2-} form is absorbed by plants	Component of amino acids and vitamin A	Decrease in leaf chlorophyll content, protein synthesis, leaf chlorosis

(continued)

Table 4.1 (continued)

Mineral nutrient and its availability	Physiological role	Deficiency symptoms
Sodium: present as Na^+ ion	Stimulates growth by affecting cell expansion and water balance of plants, replaces potassium (K^+) as solute, participates in C_4 and CAM pathways	Chlorosis, necrosis
Silicon: present as SiO_2 in soil	Deposited in the form of hydrated amorphous silica ($SiO_2 \cdot nH_2O$) mainly in endoplasmic reticulum, cell wall, intercellular spaces	Increases the susceptibility to lodging (falling over) and fungal infection
Chlorine: present as chlorine ion (Cl^-)	Required in photosynthesis, cell division	Rare, causes wilting of leaves and subsequent chlorosis and necrosis
Iron: present as Fe^{2+} (ferrous) and Fe^{3+} (ferric) ions	Involved in redox reactions, required for the synthesis of chloroplast-protein complexes in chloroplast	Intervenous chlorosis, whitening of leaves
Boron: present as boric acid (H_3BO_3) and borate ($H_2BO_3^-$)	Role in cell cycle regulation, nucleic acid synthesis, cell elongation, membrane function	Black necrosis of young leaves, loss of apical dominance (leading to increased branching)
Manganese: present as Mn^{2+} ions	Mn^{2+} activates many enzymes involved in Krebs cycle, involved in photosynthetic reactions	Intervenous chlorosis along with necrotic spots
Zinc: present as Zn^{2+} ions	Integral component of many enzymes (alcohol dehydrogenase, carbonic anhydrase, alkaline phosphatase, etc.), structural component of ribosomes, maintains integrity of biomembranes	Rosetting (stunted growth due to shortening of internodes), small leaves, severe deficiency causes death of shoot apices
Copper: present as Cu^{2+}	Bound with enzymes of redox reactions (plastocyanin)	Dark green leaves, necrotic spots arising from tip and extending toward margin
Molybdenum: present as MoO_4^- ions	Component of enzymes (nitrate reductase and nitrogenase) involved in nitrate assimilation and nitrogen fixation, thus causing nitrogen deficiency	Chlorosis, necrosis, premature flower abscission
Nickel: present predominantly as Ni^{2+}	Component of urease	Accumulation of urea in leaves and subsequent necrosis

deficiency symptoms in the plant system, respectively. Chemical fertilization is a fast way of providing necessary macro- and micronutrients to the plants.

4.3 Nutrient Availability to Plants

The mineral nutrients present in the soil must be in bioavailable form, so that the plant takes them up easily (Barber 1995). Availability of nutrients to the plants depends on their amount, nature and their association with other nutrients in the solid phase. It can also be explained as the capacity of soil-plant system to supply/absorb nutrients, which includes release of nutrients from solid phase to solution, their movement and absorption by the plant (Comerford 2005).

The concentration of mineral nutrients in soil solution varies and depends on a number of factors like soil moisture, soil depth, pH, cation exchange capacity, redox potential, quantity of organic matter, microbial activity, etc. (Marschner 1995). Presence of excess minerals in the soil can also hinder the plant growth by limiting the water availability and accumulation of heavy metals in the soil that can cause severe toxicity.

Soil and root structure are the major factors that affect availability of nutrients to the plant. Even in well-structured soils, the contact of root with soil varies and depends on many factors. For instance, maintenance of root respiration and soil bulk density for nutrient uptake are affected by soil aeration and fertility (Marschner 1995). Soil pH affects not only the nutrient availability from the soil but also the growth of plant roots which are involved in nutrient uptake. Weathering of rocks is favored by acidic pH which results in release of various ions such as K^+, Mg^{2+}, Ca^{2+}, and Mn^{2+} and increases the solubility of carbonates, sulfonates, and phosphates, thereby facilitating their availability to the roots. Rainfall and decomposition of organic matter are the major factors that lower the soil pH (Taiz and Zeiger 2010).

The proper growth and development of plant roots is an important factor that affects nutrient absorption. The uptake of nutrients through the root surface from soil takes place by either diffusion or mass (bulk) flow. Diffusion refers to the movement of nutrients down the concentration gradient and occurs due to the movement of individual molecules. Short-distance flow (lateral flow) of fluids in plants, i.e., cell to cell, or in the roots from soil, occurs through diffusion. With due course of time, depletion zones near the roots develop, and their shape primarily depends on the balance between different factors like uptake of nutrients by roots, their replenishment and the mobility of ions by diffusion. Diffusion coefficient is the measure of mobility of ions (Marschner 1995). Mass flow refers to movement of molecules together due to the pressure gradient. Long-distance flow (mediated by xylem and phloem) employs mass flow which depends on transpiration rates and amount of nutrients present in soil (Mengel and Kirkby 2001). The relative contribution of mass flow varies with factors like plant species, age of plant, and time of the day (Marschner 1995).

Considering the above facts about the role of nutrients in plant system, it becomes quite evident that concentration of different nutrients in soil varies from one location to the other, thus highlighting the need of fertilizers in agriculture.

4.4 Conventional Fertilizers Versus Nano-fertilizers

Conventional Fertilizers are generally applied on the crops by either spraying or broadcasting. However, one of the major factors that decide the mode of application is the final concentration of the fertilizers reaching to the plant. In practical scenario, very less concentration (much below to minimum desired concentration) reaches to the targeted site due to leaching of chemicals, drift, runoff, evaporation, hydrolysis by soil moisture, and photolytic and microbial degradation. It has been estimated that around 40–70 % of nitrogen, 80–90 % of phosphorus, and 50–90 % of potassium content of applied fertilizers are lost in the environment and could not reach the plant which causes sustainable and economic losses (Trenkel 1997; Ombódi and Saigusa 2000). These problems have initiated repeated use of fertilizer and pesticide which adversely affects the inherent nutrient balance of the soil. According to an estimate by International Fertilizer Industry Association, world fertilizer consumption sharply rebounded in 2009–2010 and 2010–2011 with growth rates of 5–6 % in both campaigns. World demand is projected to reach 192.8 Mt by 2016–2017 (Heffer and Prud'homme 2012). But the large-scale use of chemicals as fertilizers and pesticides has resulted in environmental pollution affecting normal flora and fauna. Tilman et al. (2002) reported that excess use of fertilizers and pesticide increases pathogen and pest resistance, reduces soil microflora, diminishes nitrogen fixation, contributes to bioaccumulation of pesticides, and destroys habitat for birds. Hence, it is very important to optimize the use of chemical fertilization to fulfill the crop nutrient requirements and to minimize the risk of environmental pollution. Accordingly, it can be favorable that other methods of fertilization be also tested and used to provide necessary nutrients for plant growth and yield production, while keeping the soil structure in good shape and the environment clean (Miransari 2011).

Nanotechnology has provided the feasibility of exploring nanoscale or nanostructured materials as fertilizer carrier or controlled-release vectors for building of the so-called smart fertilizers as new facilities to enhance the nutrient use efficiency and reduce the cost of environmental pollution (Chinnamuthu and Boopati 2009). A nano-fertilizer refers to a product in nanometer regime that delivers nutrients to crops. For example, encapsulation inside nanomaterials coated with a thin protective polymer film or in the form of particles or emulsions of nanoscale dimensions (DeRosa et al. 2010). Surface coatings of nanomaterials on fertilizer particles hold the material more strongly due to higher surface tension than the conventional surfaces and thus help in controlled release (Brady and Weil 1999). Delivery of agrochemical substance such as fertilizer supplying macro- and micronutrients to the plants is an important aspect of application of nanotechnology in agriculture. As mentioned in Table 4.2, nano-fertilizers show controlled release of agrochemicals,

Table 4.2 Comparison of nanotechnology-based formulations and conventional fertilizers applications (Cui et al. 2010)

S. no.	Properties	Nano-fertilizers-enabled technologies	Conventional technology
1.	Solubility and dispersion of mineral micronutrients	Nano-sized formulation of mineral micronutrients may improve solubility and dispersion of insoluble nutrients in soil, reduce soil absorption and fixation, and increase the bioavailability	Less bioavailability to plants due to large particle size and less solubility
2.	Nutrient uptake efficiency	Nanostructured formulation might increase fertilizer efficiency and uptake ratio of the soil nutrients in crop production and save fertilizer resource	Bulk composite is not available for roots and decrease efficiency
3.	Controlled-release modes	Both release rate and release pattern of nutrients for water-soluble fertilizers might be precisely controlled through encapsulation in envelope forms of semipermeable membranes coated by resin-polymer, waxes, and sulfur	Excess release of fertilizers may produce toxicity and destroy ecological balance of soil
4.	Effective duration of nutrient release	Nanostructured formulation can extend effective duration of nutrient supply of fertilizers into soil	Used by the plants at the time of delivery, the rest is converted into insoluble salts in the soil
5.	Loss rate of fertilizer nutrients	Nanostructured formulation can reduce loss rate of fertilizer nutrients into soil by leaching and/or leaking	High loss rate by leaching, rain off, and drift

site targeted delivery, reduction in toxicity, and enhanced nutrient utilization of delivered fertilizers (Cui et al. 2010). These attributes of nanoparticles are due to their high surface area to volume ratio, high solubility, and specific targeting due to small size, high mobility, and low toxicity (Sasson et al. 2007).

4.5 Nano-fertilizer Formulations and Their Smart Delivery Systems

The formulation of any nano-fertilizer should be in such a way that they possess all desired properties such as high solubility, stability, effectiveness, time-controlled release, enhanced targeted activity with effective concentration, and less eco-toxicity with safe, easy mode of delivery and disposal (Tsuji 2001; Boehm et al. 2003; Green and Beestman 2007; Torney et al. 2007). Nanoparticles have great potential to deliver nutrients to specific target sites in living systems. The loading of nutrients on the nanoparticles is usually done by (a) absorption on nanoparticles, (b) attachment on nanoparticles mediated by ligands, (c) encapsulation in nanoparticulate polymeric shell, (d) entrapment of polymeric nanoparticles, and (e) synthesis of nanoparticles composed of the nutrient itself.

Corradini et al. (2010) evaluated the interaction and stability of chitosan nanoparticles suspensions containing N, P, and K fertilizers which can be useful for agricultural applications. Similarly, Kottegoda et al. (2011) synthesized urea-modified hydroxyapatite (HA) nanoparticles for gradual release of nitrogen with the crop growth. These nano-fertilizers showed initially burst and subsequently slow release of nitrogen up to 60 days of plant growth compared to commercial fertilizer which shows release only up to 30 days. The large surface area of HA facilitates the large amount of urea attachment on the HA surface. Strong interaction between HA nanoparticles and urea contributes to the slow and controlled release of urea. Similarly, polymer-based mesoporous nanoparticles can also provide efficient carrier system to agrochemical compounds which improves the efficiency and economical utilization. Mesoporous silica nanoparticles (150 nm) have been reported to entrap urea. It has been observed that 15.5 % of urea was loaded inside the nanoparticles pores and demonstrated a controlled urea release profile in soil and water. The study revealed at least fivefold improvement in release period (Wanyika et al. 2012). Zinc solubility and dissolution kinetics of ZnO nanoparticles and bulk ZnO particles coated on macronutrient fertilizers (urea and monoammonium phosphate) have been compared by Milani et al. (2012). They reported that coated monoammonium phosphate granules show faster dissolution rate. The mode of fertilizer application influences their efficiency and impact on plant systems. The following methods can be used for nano-fertilizer delivery to plants:

4.5.1 In Vitro Methods

4.5.1.1 Aeroponics

This technique was first reported by Weathers and Zobel (1992). In this technique, roots of the plant are suspended in air and the nutrient solution is sprayed continuously. Through this method, the gaseous environment around the roots can be controlled. However, it requires a high level of nutrients to sustain rapid plant growth, so the use of aeroponics is not widespread.

4.5.1.2 Hydroponics

This method was first introduced by Gericke (1937) for dissolved inorganic salts. The method is also commonly known as “solution culture” as the plants are grown with their roots immersed in a liquid nutrient solution (without soil). Volumes of nutrient solution, maintenance of oxygen demands, and pH are factors that need attention while using this method of nutrient delivery. Supporting materials (sand, gravel, etc.) are also employed in certain commercial application. In this case, nutrient solution is flushed from one end and old solution is removed from the other end. The disadvantages with this method are frequent pathogen attack and high moisture rates which may cause over wilting of soil-based plants.

4.5.2 In Vivo Methods

4.5.2.1 Soil Application

Soil application is the most common method of nutrient supplement using chemical and organic fertilizers. The factors that need attention while choosing this method of fertilizer applications are how long the fertilizer will last in the soil, soil texture, soil salinity, and plant sensitivities to salts, salt content, and pH of the amendment. It is well known that negative soil particles affect the adsorption of mineral nutrients. The anion exchange capacity of most agricultural soils is small compared to cation exchange capacity. Among anions, nitrate remains mobile in the soil solution and is susceptible to leaching by water moving through the soil. Phosphate ions bind to soil particles containing aluminum or iron because the positively charged Fe^{2+}, Fe^{3+}, and Al^{3+} have OH^{-} group that exchanges with phosphate. As a result, phosphate can be tightly bound, and its mobility and availability in soil can limit plant growth (Taiz and Zeiger 2010).

4.5.2.2 Foliar Application

In this method, liquid fertilizers are directly sprayed onto leaves. It is generally used for the supply of trace elements. Foliar application can reduce the time lag between application and uptake by plant during the rapid growth phase. It can also circumvent the problem of restricted uptake of a nutrient from soil. Uptake of iron, manganese, and copper may be more efficient with this method as compared to soil application where they get adsorbed on soil particles and hence are less available to root system (Taiz and Zeiger 2010). As stomata and leaf epidermal cells are majorly involved in nutrient uptake, foliar application method can have agronomic advantage if used for nano-fertilizers. However, damage to the leaves must be minimized in such cases by standardization of application protocol. The shortcomings of this method include specific time (morning and evening) of spraying because the stomata open during these time periods only. Another disadvantage is the possibility of plant damage if correct concentration of chemical (fertilizer) is not applied.

4.6 Nano-fertilizers and Plant Growth

Although most of the recent studies have emphasized the adverse effects of nanoparticles on plants (Lin and Xing 2007; Lee et al. 2008; Barrena et al. 2009), a few studies have suggested that nanoparticles delivered at safe dose may help in promoting plant growth and overall yield (Zheng et al. 2005; Gao et al. 2006; López-Moreno et al. 2010). Multi-walled carbon nanotubes (MWCNTs) have been reported to have the ability to increase the seed germination and growth of tomato and to enhance the growth in tobacco cells (Khodakovskaya et al. 2009, 2012). Mondal et al. (2011) reported the enhancement of seed germination and plant growth using MWCNTs in mustard plant. On the basis of germination index and relative root elongation, they showed that oxidized MWCNTs were more effective at lower concentration than the non-oxidized MWCNTs. Sahandi et al. (2011) reported that nanosilver is better than silver nitrate in improving the seed yield and preventing leaf abscission in borage plant. The plant hormone, ethylene plays a key role in leaf abscission, and silver ions have been shown to inhibit ethylene by replacing copper ions from the receptors. Employing the foliar spray method, both nanosilver and silver nitrate were sprayed on different sets of plants, and it was observed that nanosilver was effective at a lower concentration than silver nitrate. Effect of biosynthesized silver nanoparticles on emergence of seedling and various plant growth parameters of many economically important plant species were studied by Namasivayam and Chitrakala (2011).

Mahajan et al. (2011) used the agar plate method to test the effect of ZnO nanoparticles on the growth of *Vigna radiata* and *Cicer arietinum*. Evidence of nanoparticles adsorbed on the root surface was provided using correlative light and scanning electron microscopy. Inductively coupled plasma/atomic emission spectroscopy (ICP-AES) studies revealed the absorption of ZnO nanoparticles by seedlings. Using the foliar spray method, Burman et al. (2013) studied the effect of ZnO nanoparticles on growth and antioxidant system of chickpea seedlings. They found that lower concentration (1.5 ppm) of ZnO nanoparticles has positive effect on chickpea seedling growth. Moreover, seedlings treated with ZnO nanoparticles showed improved biomass accumulation which may be due to lower reactive oxygen species (ROS) levels as evident from lower malondialdehyde (MDA) content. Similarly, Prasad et al. (2012) observed that treatment of nano zinc at lower concentration (1,000 ppm) had positive effects on plant, but it showed toxicity symptoms at higher concentration (2,000 ppm) pointing out their meticulous use. Further, during field experiments, they reported usage of 15 times lower dose of ZnO nanoparticles compared to the recommended dose of $ZnSO_4$ and recorded 29.5 % higher pod yield. Likewise, ZnO nanoparticles showed root elongation in *Glycine max* at 500 ppm concentration but reduction in size at higher concentration of ZnO (López-Moreno et al. 2010). A study aimed to investigate the effects of ZnO and CeO_2 nanoparticles (400 ppm) on *Cucumis sativus* fruit quality showed that both the tested nanoparticles resulted in increased starch content and could alter the carbohydrate pattern (Zhao et al. 2014).

Lu et al. (2002) showed the productive effect of mixture of SiO_2 and TiO_2 nanoparticles in *G. max* with increase in water and fertilizer uptake capacity and stimulation of nitrate reductase and antioxidant activity. Studies demonstrating the effect of nano-TiO_2 in promoting photosynthesis and growth in spinach have also been conducted in which an increase in photosynthetic processes under both visible and ultraviolet light has been reported due to the pivotal role of TiO_2 (Lei et al. 2007). Zheng et al. (2005) reported that TiO_2 nanoparticles have increased 73 % dry weight, threefold higher photosynthetic rate, and 45 % increment in chlorophyll a after seed treatment in spinach. As suggested the reason of increment in photosynthetic rate may be due to the increase in absorption of inorganic nutrients which enhanced the utilization of organic substance and quenching of oxygen-free radicals. Unlike most of the studies showing negative impact of nanoparticles at higher concentration, Mahmoodzadeh et al. (2013) reported that up to 2,000 ppm concentration of TiO_2 nanoparticles leads to an increased seed germination and seedling vigor in *Brassica napus*.

Shah and Belozerova (2009) studied the effect of different metal nanoparticles such as silicon (Si), palladium (Pd), gold (Au), and copper (Cu) on lettuce seed germination. They conferred that nanoparticles showed positive influence at different concentration range such as Pd and Au at lower concentration, Si and Cu at higher concentration, and Au and Cu in combined mixture. Likewise, in a field study, Quoc Buu et al. (2014) reported an increased seed germination rate in *G. max* as compared to control when treated with nanocrystalline powder of iron, cobalt, and copper at an extra low concentration. In addition, a marked increase was observed in the chlorophyll index, number of nodules, and crop yield. Arora et al. (2012) reported that foliar spray of gold on *Brassica juncea* plant in field experiments showed positive effect as it resulted in increased plant height, stem diameter, number of branches, number of pods, and seed yield. Interestingly, gold nanoparticles also improved the redox status of treated plants. Suriyaprabha et al. (2012) reported that treatment with SiO_2 nanoparticles in maize plants significantly enhanced the plant dry weight and also enhanced the levels of organic compounds such as proteins, chlorophyll, and phenols.

4.7 Uptake, Translocation, and Fate of Nano-fertilizers in Plants

The uptake and fate of nano-fertilizers in plant is a growing field of research interest. The uptake, translocation, and accumulation of nanoparticles depend on the plant species, age, growth environment, and the physicochemical property, functionalization, stability, and the mode of delivery of nanoparticles. Rico et al. (2011) proposed a schematic representation of the uptake, translocation, and biotransformation pathway of various nanoparticles along with possible modes of cellular uptake in plant system (Figs. 4.1 and 4.2).

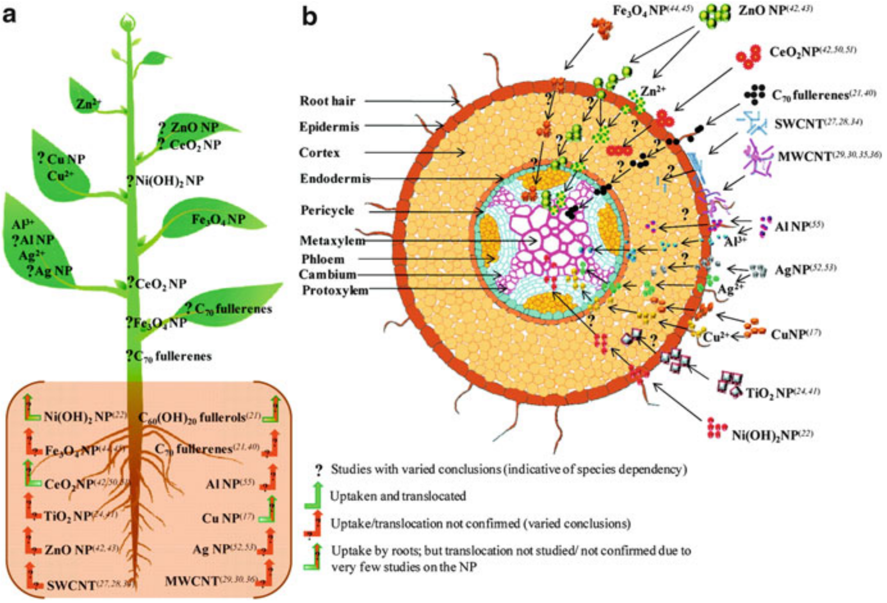

Fig. 4.1 Uptake, translocation, and biotransformation pathway of various nanoparticles in a plant system: (**a**) plant showing the selective uptake and translocation of nanoparticles; (**b**) transverse cross section of the root absorption zone showing the differential nanoparticle interaction on exposure. The superscripts depict the reference cited in the original paper. (Reproduced from Rico et al. (2011) with permission from American Chemical Society)

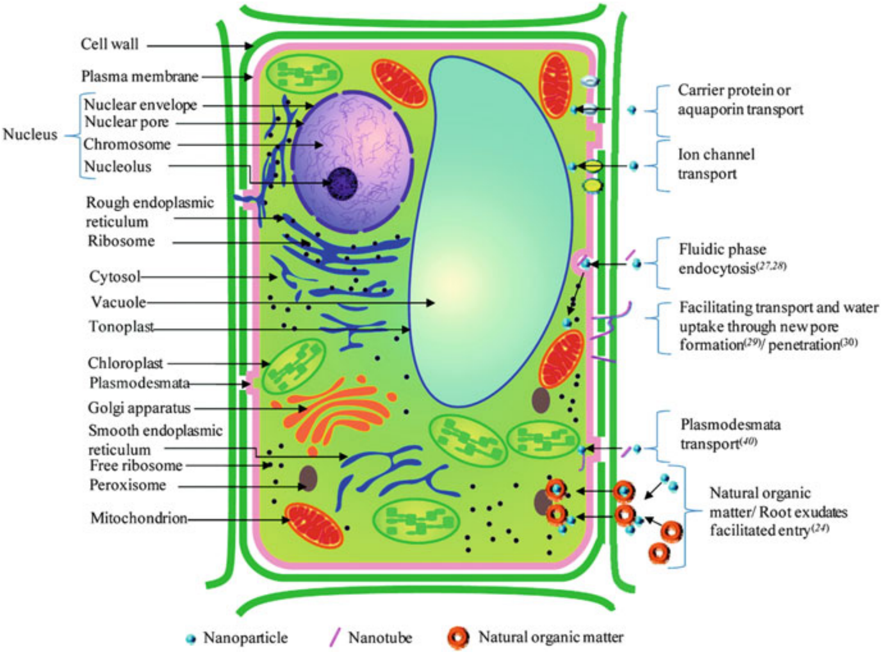

Fig. 4.2 Probable modes of cellular uptake of the nanoparticles in a plant cell. (Reproduced from Rico et al. (2011) with permission from American Chemical Society)

The entry of nanoparticles through the cell wall depends on the pore diameter of the cell wall (5–20 nm) (Fleischer et al. 1999). Hence, nanoparticles or nanoparticle aggregates with diameter less than the pore size of plant cell wall could easily enter through the cell wall and reach up to the plasma membrane (Moore 2006; Navarro et al. 2008). Functionalized nanoparticles facilitate the enlargement of pore size or induction of new cell wall pore to enhance the uptake of nanoparticles. Several reports have discussed the uptake of nanoparticles into plant cell via binding to carrier proteins through aquaporin, ion channels, or endocytosis (Nair et al. 2010). Further, nanoparticles can also be transported into the plant by forming complexes with membrane transporters or root exudates (Kurepa et al. 2010). Various other studies reported that nanoparticles could enter through stomata or the base of trichome in leaf (Eichert et al. 2008; Fernández and Eichert 2009; Uzu et al. 2010). Kurepa et al. (2010) reported uptake and translocation of TiO_2-alizarin red S complex in *Arabidopsis thaliana* seedling. They observed that mucilage released by roots develops pectin hydrogel complex around the root and found to be responsible for the entry of nanoparticle-dye complex. In a recent study carried out to understand the mechanism of nanoparticle uptake and translocation, fluorescently labeled monodispersed mesoporous silica nanoparticles were found to penetrate the roots via symplastic and apoplastic pathways and translocated via xylem tissue to the aerial parts of the plants including the stems and leaves (Sun et al. 2014). However, the exact mechanism of nanoparticle uptake by plants is yet to be elucidated.

After entering the cell, nanoparticles can transport apoplastically or symplastically. They may be transported via plasmodesmata from one cell to the other (Rico et al. 2011). In the cytoplasm, nanoparticles approach to different cytoplasmic organelles and interfere with different metabolic processes of the cell (Moore 2006). Furthermore, Larue et al. (2011) studied the uptake of TiO_2 nanoparticles in wheat and observed the nanoparticles in parenchyma and vascular tissues of the root.

Lin and Xing (2008) examined the cell internalization and upward translocation of ZnO nanoparticles in *Lolium perenne* (ryegrass). They showed that ZnO nanoparticles could enter the ryegrass root cells and move up to the vascular tissues. López-Moreno et al. (2010) studied the uptake and accumulation of ZnO nanoparticles in *G. max* seedling. They treated the seeds with ZnO nanoparticles in the concentration of 500–4,000 ppm and reported higher Zn uptake at 500 ppm. They proposed that at higher concentration nanoparticles get agglomerated which inhibits the nanoparticles entry into the seed through cell wall pores. Moreover, X-ray absorption spectroscopy of ZnO-treated seedlings revealed presence of Zn^{2+} ions instead of ZnO suggesting the role of roots in ZnO ionization on its surface. They also showed the presence of ZnO nanoparticles in apoplast, cytoplasm, and nuclei of the endodermal cell and vascular cylinder by high-magnification transmission electron microscopy.

In case of magnetite nanoparticles, Zhu et al. (2008) reported the presence of nanoparticles in root, stem, and leaves of *Cucurbita maxima* (pumpkin). They observed that the extent of nanoparticles uptake is affected by the type of growth medium. A higher uptake was achieved in hydroponic medium as compared to the plant grown in sand, whereas no uptake was observed in plants grown in soil which might be due to the adherence of magnetite nanoparticles to soil and sand grains. In contrast, no uptake was found in treated lima bean plants, showing that uptake of nanoparticles is also dependent on plant species. Wang et al. (2011) reported no uptake of magnetite nanoparticles in pumpkin plants because of the large size of nanoparticles. Effect of functionalization on uptake of nanoparticles was studied by Corredor et al. (2009) by applying carbon-coated iron nanoparticles on leaf of pumpkin plant. They observed presence of nanoparticles in epidermal cells but could not find nanoparticles near xylem. Lee et al. (2008) studied the uptake and translocation of copper nanoparticles in mung bean and wheat in agar growth medium. They reported that copper nanoparticles can cross the cell membrane and agglomerate in the cell. Unlike the conclusive studies on TiO_2 and ZnO nanoparticles, most of the uptake, translocation, and accumulation studies in plants are reported only up to the germination stage. Thus, the fate of nanoparticles in the plant system is largely unknown (Rico et al. 2011). The details of storage in plant system are yet to be elucidated.

4.8 Ethical and Safety Issues in Using Nano-fertilizers

Although nanotechnology has incredible potential to revolutionize many aspects of human life, the benefits may come with some price. One of the major questions faced by the world before accepting nanotechnology is whether the unknown risks of nanoparticles involving their environmental and health impact prevail over their potential benefits. The risks associated with the application of nanoparticles are yet to be evaluated before fully implementing this technology. This consideration has developed “nanotoxicology,” which is responsible for assessing toxicological potential as well as promoting safe design and use of nanoparticles (Oberdörster et al. 2005). A systematic and thorough quantitative analysis regarding the potential health impacts, environmental clearance, and safe disposal of nanoparticles can lead to improvements in designing further applications of nanotechnology (Meng et al. 2009).

Although no direct human disease has been linked to nanoparticles so far, early experimental studies indicate that nanoparticles could initiate adverse biological responses that can lead to toxicological outcomes (Nel et al. 2006). Nanoparticles which constitute a part of ultrafine particulate matter can enter in the human/animal system through oral, respiratory, or intradermal routes. Currently, there is a common assumption that the small size of nanoparticles allows them to easily enter tissues, cells, and organelles and interact with functional biomolecular structures (i.e., DNA, ribosomes) since the actual physical size of an engineered nanostructure is similar to many biological molecules (e.g., antibodies, proteins) and structures (e.g., viruses). A corollary is that the entry of the nanoparticles into vital biological systems could cause damage, which could subsequently cause harm to human health (Xia et al. 2009).

However, one of the most disgustful scenarios is the lack of concrete technical data on toxicological aspect of nanoparticles giving opportunity to both nanotechnology proponents and opponents to make contradictory, unscientific, and sweeping conclusions about the safety of nanoparticles. This atmosphere of uncertainty is precisely the feature of nanotechnology that causes cynics the greatest concern (Colvin 2003). Herein arises a need for proper physicochemical characterization and determination of appropriate exposure protocols and reliable methods for assessing nanoparticles outcome in the environment, their internalization, and their kinetics in living organisms. Once these issues are addressed, optimal experimental conditions could be established in order to identify if a particular nanoparticle poses a threat to human health (Thomas and Sayre 2005). Multidisciplinary research between materials scientists, environmentalists, and life scientists should overcome these limitations in identifying the true hazards of nanotechnology. Sadly, the risk assessments of nanotechnology are partly subjective and likely to be highly politicized.

Disastrously, no single scenario for describing risks and controls can be universally applied to conclude the outcome due to the heterogeneous and developmental nature of nanotechnology. Also, an absence of standardized methodologies and guidelines makes it difficult to compare the safety/toxicity assessments from different research groups (Dhawan et al. 2009). The ethical issues will be specific only for the knowledge base at a given time and for a specified product and its exposure scenario. Moreover, maintaining utmost specificity regarding design of experiments, alternative assessments are needed to take into consideration ethical, social, and political values that relate policies such as those involving nanotechnology (Schulte and Salamanca-Buentello 2007). Before interpreting toxicological data, it is thus essential to calculate and determine the expected concentrations of nanoparticles that may be exposed to the biological system or present in the ecosystem.

The use of nanotechnology in agriculture is significantly important as it directly affects humans (Bouwmeester et al. 2009). Nano-fertilizers enable nanoparticles to enter in the food chain allowing their distribution in every organism related to the food chain. As all substances, from arsenic to table salt, are toxic to plants, animals, or humans at some exposure level, this would not limit their use in various applications which are designed keeping in mind the critical exposure concentration. As discussed in most of the studies regarding the use of nanoparticles for promoting growth of plants with a focus on using lower concentrations of nanoparticles, it can be argued that it will pose insignificant health and environmental damage (Colvin 2003).

Many countries have identified the potential of nanotechnology in the food and agriculture sectors and are investing significantly in its applications to food production. However, owing to our limited knowledge of the human health effects of these applications, these countries recognize the need for early consideration of the food safety implications of nanotechnology. As suggested by the scientific committee of the European Food Security Authority (EFSA), “the risk assessment paradigm (hazard identification, hazard characterization, exposure assessment and risk characterization) is applicable for nanoparticles (EFSA Scientific Committee 2011). However, risk assessment of these nanoparticles in the food and feed area should consider the specific properties of the subject nanoparticles in addition to those common to the equivalent non-nanoforms.” It is most likely that different types of nanoparticles vary as to their toxicological properties. The available data on oral exposure to specific nanoparticles and any consequent toxicity are extremely limited; the majority of the available information on toxicity of nanoparticles is from in vitro studies or in vivo studies using other routes of exposure. The risk assessment of nanoparticles has to be performed on a case-by-case basis. Various parameters may be included in deciding the risk associated with the use of any particular nanoparticle in food and feed. These include physico-chemical characterization of nanoparticles, its stability in the food and feed, exposure scenario of the nanoparticles from food and feed, and toxicokinetics (absorption, distribution, metabolism/biotransformation, excretion/elimination) within the human and animal systems. The Nanotechnology Regulatory Science Research Plan of the US Food and Drug Administration (FDA) lays out a framework and implementation plan to provide coordinated leadership on regulatory science activities and issues related to FDA-regulated products that either contain nanoparticles or otherwise involve the application of nanotechnology to address key scientific gaps in knowledge, methods, or tools needed to make regulatory assessments of these products (Chaudhry and Castle 2011).

4.9 Conclusion

Widespread existence of nutrient deficiency in agricultural soils has resulted in significant decreases in crop productivity and great economic losses in agriculture. Although application of chemical fertilizers can enhance the crop productivity, their large-scale use is not a suitable option for long run. Moreover, the available nutrients present in the bulk chemical forms as delivered by conventional fertilizers are not fully accessible to plants. In addition, the utilization of most of the macronutrient is very low due to their inversion to insoluble form in soil. Delivery of agrochemical substance such as fertilizer supplying macro- and micronutrients to the plants is an important aspect of application of nanotechnology in agriculture. Nanoscale or nanostructured materials as fertilizer carrier or controlled-release vectors for building of the so-called smart fertilizers can enhance the nutrient use efficiency and reduce the cost of environmental pollution. Nano-fertilizers can precisely release their active ingredients in responding to environmental triggers and biological demands. Both in vitro and in vivo methods can be used for nano-fertilizer delivery to the plants. However, the uptake, translocation, and fate of nanoparticles in plant system are largely unknown resulting in the rise of various ethical and safety issues surrounding the use of nano-fertilizers in plant productivity. A systematic and thorough quantitative analysis regarding the potential health impacts, environmental clearance, and safe disposal of nanomaterials can lead to improvements in designing further applications of nano-fertilizers.

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Chapter 5
Nanotechnology Applied in Agriculture: Controlled Release of Agrochemicals

Fauze A. Aouada and Marcia R. de Moura

Abstract In the last few years, the use of nanotechnology to control environmental pollution has considerably increased. Several nanostructured or nanometric dimensional materials have been used as carrier vehicles in the controlled release of agrochemicals due to their biodegradability, low toxicity, low cost, high reproducibility, easy and fast preparation and characterization, water uptake, and reversible properties. The major advantages of these systems are the gradual, sustained, and controlled release over a long period of time, with the aim to improve agricultural and crop protection. In the case of agrochemicals, the scientific community also aims to obtain biodegradable matrices (biodegradable carrier vehicles) able to reduce the number and frequency of applications of nutrients and pesticides in the soil that would contribute to a decrease in environmental pollution and contamination by pollutants. Thus, it is evident that nanotechnology can play a decisive role in the agriculture field. However, only a few references (including papers and reviews) were found in the literature. This gap is most likely related to the short time that these nanomaterials have been studied for this specific application. With this in mind, the aim of this chapter is to collect reports about different polymeric, inorganic, and hybrid nanomatrices (nanocomposites) with great potential for applications as carrier vehicles in the controlled release of agrochemicals.

5.1 Introduction

The constant need to discover or improve scientific information has encouraged a great number of scientists across the world to divide or change their current research focuses. Due to its great potential, nanoscience (Leite et al. 2013; Munoz-Sandoval 2013; Newsome 2014a, b; Ruiz-Hitzky and Aranda 2014; Vilela Neto 2014) has become well-known in the scientific community.

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In the RS Policy document 19/04 (RS Policy document 2004), the terms nanoscience and nanotechnology (Daniel and Astruc 2004; Ferrari 2005; Whitesides 2005; Paul and Robeson 2008; Farokhzad and Langer 2009; Ferreira et al. 2013) were defined as "the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale" and "the design, characterisation, production and application of structures, devices and systems by controlling shape and size at nanometer scale," respectively. According to Duncan (2011), the nanotechnology term involves the synthesis or preparation, characterization, and material manipulations (including structures and devices) in this dimension scale. When the dimensions of one specific material are reduced from macro-dimensions to nano-dimensions, their physical and chemical properties are considerably improved. Additionally, according to the author, different segments of industry utilize concepts from nanotechnology to improve the properties of products, of the methodology of preparation, the application of these products, or for the preparation of novel products. Their uses are highlighted in agriculture (agrochemicals or vaccine delivery), food processing (encapsulation of flavor or odor enhancers), food packaging (pathogen, gas, or abuse sensors), and nutrient supplements (nutraceuticals with higher stabilities and bioavailabilities), among others; this is depicted in Fig. 5.1.

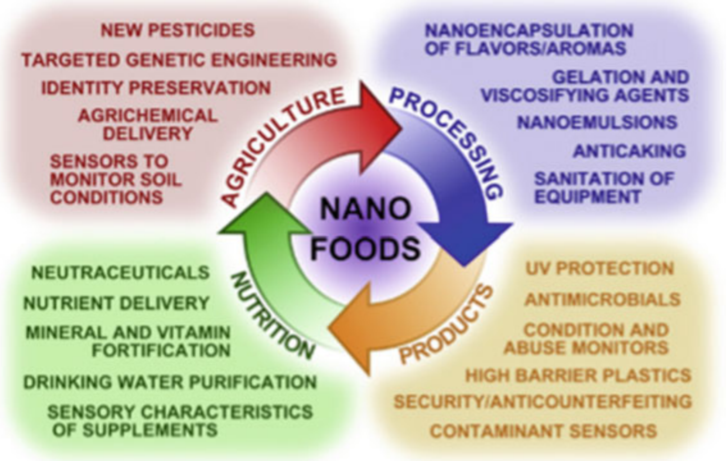

Fig. 5.1 Nanotechnology has applications in all areas within food science, from agriculture to food processing, security, packaging, nutrition, and neutraceuticals (Duncan 2011). Reproduced by permission of Elsevier

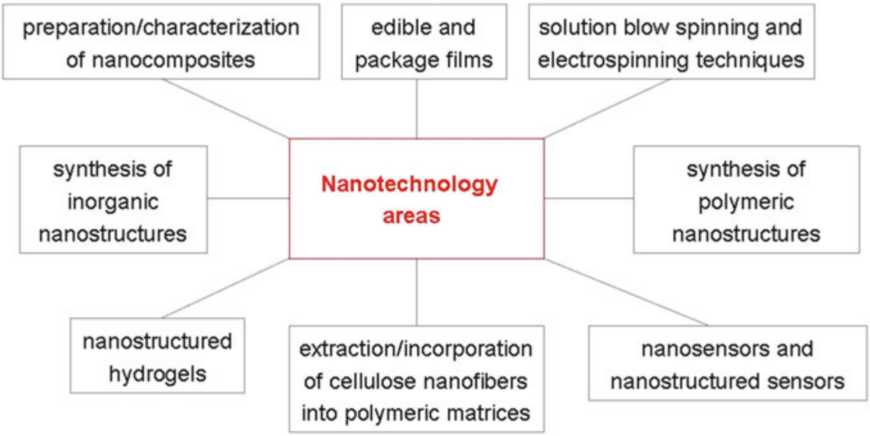

Fig. 5.2 Different nanomaterials or nanostructured materials used in the nanotechnology areas

Figure 5.2 shows the use of some nanomaterials or structured materials inside the nanotechnology areas, including in water and wastewater treatment (Table 5.1). In their research review paper, Ghormade et al. (2011) discussed some applications of nanotechnology in agriculture (Table 5.2) and in nano-biotechnology areas (Fig. 5.3).

The fast and constant growth of technological innovations leads to profound structural changes in the agricultural sector, with the aims of sustaining production, improving food security (Brooks 2014; Khoury et al. 2014; Kim et al. 2014), poverty reduction (Dorosh and Mellor 2013; Roe et al. 2013; Stewart 2013; Zhen et al. 2014), and improving public health (Akpor and Muchie 2011; Neff et al. 2011; Dimer et al. 2013; Fry et al. 2014). In this way, advances in science and technology could offer solutions for developing countries to innovate and add value to their current commodities production systems (Chen and Yada 2011). In the agricultural field, the use of nanotechnology offers advantages when compared to the use of materials on the macroscopic scale. Basically, the use of nanostructured materials focuses on improving the efficiency, productivity, crop production, and crop protection in the agricultural field (Khot et al. 2012). For instance, Pereira et al. (2014) showed that the use of nanoparticles as carriers of active chemicals in agriculture may increase the biological activity of the active substances and reduce the quantities required of the carrier vehicles, decreasing the contamination of hydric resources due to leaching. In their review work, Misra et al. (2013) highlighted the fact that nanotechnology may revolutionize modern agriculture. They presented a summary of nanotechnology applications utilized in agriculture, such as nanoparticles to deliver DNA to plants in genetic engineering; nanocapsules for the delivery of pesticides, fertilizers, and other agrochemicals more efficiently; nanocapsules to deliver vaccines; and nanosensors for monitoring soil/environmental conditions and crop growth, among others. In the same review work, the authors, based on the study conducted by Roco (2007), presented fundamental

Table 5.1 Current and potential applications of nanotechnology in water and wastewater treatment (adapted from Qu et al. 2013). Reproduced by permission of Elsevier

Applications	Representative nanomaterials	Desirable nanomaterials properties	Enabled technologies
Adsorption	Carbon nanotubes	High specific area, highly assessable adsorption sites, diverse contaminant–CNT interactions, tunable surface chemistry, easy reuse	Contaminant preconcentration/detection, adsorption of recalcitrant contaminants
	Nanoscale metal oxide	High specific surface area, short intraparticle diffusion distance, more adsorption sites, compressible without significant surface area reduction, easy reuse, some are superparamagnetic	Adsorptive media filters, slurry reactors
	Nanofibers with core–shell structure	Tailored shell surface chemistry for selective adsorption, reactive core for degradation, short internal diffusion distance	Reactive nano-adsorbents
Membranes and membrane processes	Nano-zeolites	Molecular sieve, hydrophilicity	High-permeability thin-film nanocomposite membranes
	Nano-Ag	Strong and wide-spectrum antimicrobial activity, low toxicity to humans	Anti-biofouling membranes
	Carbon nanotubes	Antimicrobial activity (unaligned carbon nanotubes)	Anti-biofouling membranes
		Small diameter, atomic smoothness of inner surface, tunable opening chemistry, high mechanical and chemical stability	Aligned carbon nanotube membranes
	Aquaporin	High permeability and selectivity	Aquaporin membranes
	Nano-TiO_2	Photocatalytic activity, hydrophilicity, high chemical stability	Reactive membranes, high-performance thin-film nanocomposite membranes
	Nano-magnetite	Tunable surface chemistry, superparamagnetic	Forward osmosis
Photocatalysis	Nano-TiO_2	Photocatalytic activity in UV and possibly visible light range, low human toxicity, high stability, low cost	Photocatalytic reactors, solar disinfection systems
	Fullerene derivatives	Photocatalytic activity in solar spectrum, high selectivity	Photocatalytic reactors, solar disinfection systems

(continued)

Table 5.1 (continued)

Applications	Representative nanomaterials	Desirable nanomaterials properties	Enabled technologies
Disinfection and microbial control	Nano-Ag	Strong and wide-spectrum antimicrobial activity, low toxicity to humans, ease of use	POU water disinfection, anti-biofouling surface
	Carbon nanotubes	Antimicrobial activity, fiber shape, conductivity	POU water disinfection, anti-biofouling surface
	Nano-TiO_2	Photocatalytic ROS generation, high chemical stability, low human toxicity and cost	POU to full-scale disinfection and decontamination
Sensing and monitoring	Quantum dots	Broad absorption spectrum, narrow, bright, and stable emission which scales with the particle size and chemical component	Optical detection
	Noble metal nanoparticles	Enhanced localized surface plasmon resonances, high conductivity	Optical and electrochemical detection
	Dye-doped silica nanoparticles	High sensitivity and stability, rich silica chemistry for easy conjugation	Optical detection
	Carbon nanotubes	Large surface area, high mechanical strength and chemical stability, excellent electronic properties	Electrochemical detection, sample preconcentration
	Magnetic nanoparticles	Tunable surface chemistry, superparamagnetism	Sample preconcentration and purification

Table 5.2 Some applications of nanotechnology in agriculture (adapted from Ghormade et al. 2011). Reproduced by permission of Elsevier

Application	Nanoparticles
Pesticide delivery	
Chemical	
Avermectin	Porous hollow silica (15 nm)
Ethiprole or phenylpyrazole	Poly-caprolactone (135 nm)
Gamma-cyhalothrin	Solid lipid (300 nm)
Tebuconazole/chlorothalonil	Polyvinylpyridine and polyvinylpyridine-costyrene (100 nm)
Biopesticides	
Plant origin: nanosilica for insect control *Artemisia arborescens* essential oil encapsulation	Nanosilica (3–5 nm) Solid lipid (200–294 nm)
Microorganisms: *Lagenidium giganteum* cells in emulsion	Silica (7–14 nm)
Microbial product: absorption of *Myrothecium verrucaria* enzyme complex	Chitosan/kaolin (250–350 nm)
Fertilizer delivery	
NPK controlled delivery	Nano-coating of sulfur (100 nm layer) Chitosan (78 nm)

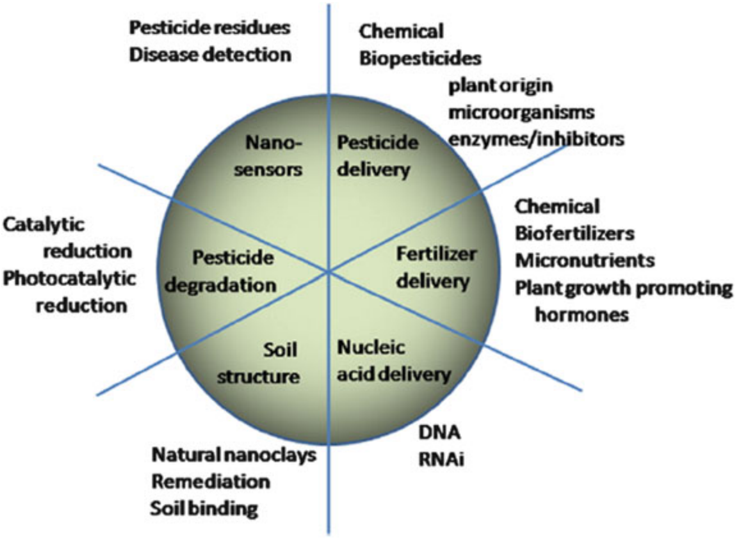

Fig. 5.3 Applications of nano-biotechnology in plant protection and nutrition (Ghormade et al. 2011). Reproduced by permission of Elsevier

characteristics of some nanoparticles: a large surface to volume ratio, chemically alterable physical properties, changes in the chemical and physical properties with respect to size and shape, and enhanced or delayed particle aggregation depending upon the type of surface modification type, among others.

5.2 Goals and Advantages of a Controlled Release System

Due to many advantages over traditional agrochemical application methods, several authors have used the concepts of controlled release (CR) systems (Aouada et al. 2009, 2010; Dubey et al. 2011; Bortolin et al. 2012; Ghazali et al. 2013; Carson et al. 2014; Cartmill et al. 2014; Wanyika 2014) to propose alternatives to combat environmental pollution (Chuxiang et al. 2014). The main objective of CR systems is to allow for the controlled delivery of an active compound over time and maintaining the activity through a desired period in the soil, resulting in the reductions of the quantities of the agrochemical used, manpower, and energy or combustives necessary to operate the application devices as well as in the increase of safety to humans who apply the chemicals (Aouada et al. 2011; Chevillard et al. 2012). Coupled with this objective, the use of nanotechnology has become an important strategy in environmental remediation efforts by providing solutions to environmental cleanup challenges (El-Temsah and Joner 2013). In addition, Sopeña et al. (2009) listed some advantages of CR in relation to conventional methods: (1) to reduce herbicide loss due to lixiviation, volatilization, drift, and degradation in soils; (2) to reduce phytotoxicity; and (3) to facilitate herbicide management and safer applications, reducing toxicity in humans. In the case of the

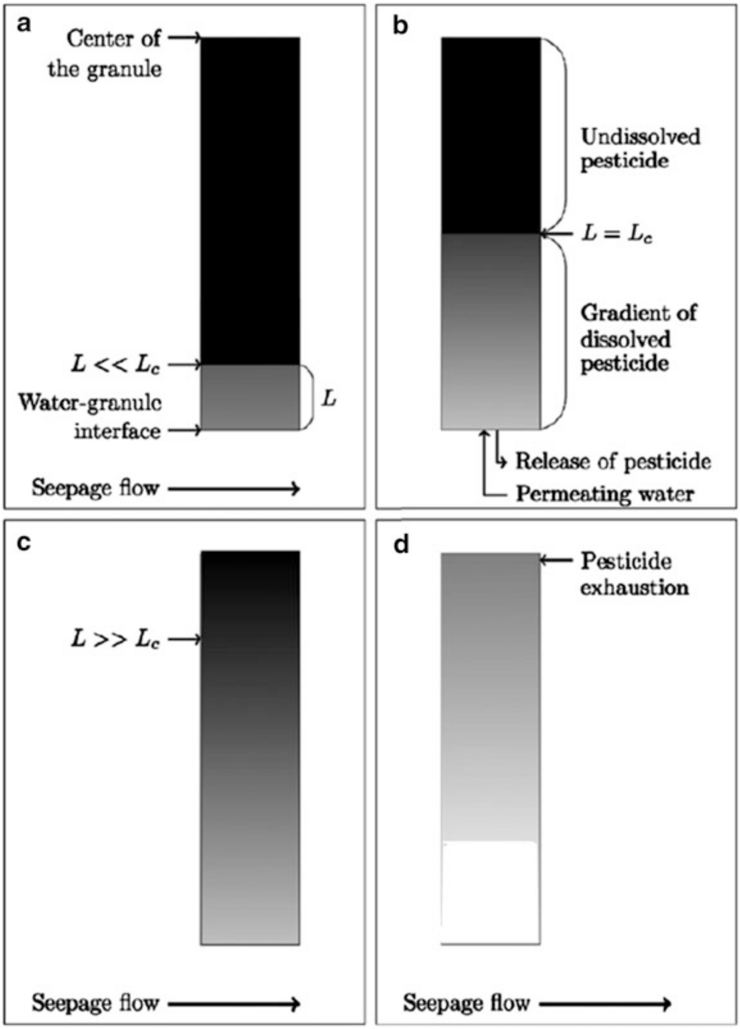

Fig. 5.4 Schematic stages of the release of a pesticide from a one-dimensional porous solid matrix. The inner part of the matrix is at the upper end of the rectangle, and the area of contact with water is at the bottom. *Black zones* denote the undissolved pesticide and *gray zones* the dissolved pesticide inside the porous matrix. L denotes the distance from the granule surface to the solubility front of the active ingredient within the granule, and L_c is the critical length at which the anomalous diffusion can occur. Case-II transport (**a**), anomalous diffusion (**b**), Fickian diffusion-controlled transport (**c**), and exhaustion (**d**) (Paradelo et al. 2012). Reproduced by permission of Elsevier

controlled release of fertilizers, an ideal system would be capable of controlling their delivery to coincide with the desired amount of the plant (Wu 2013) to avoid considerable losses due to leaching, evaporation, and other aspects of the weather. The formulations used in these technologies were obtained by the physical mixing or chemical binding of the active agent to the matrix (Kenawy and Sakran 2001).

According to Paradelo et al. (2012), the release process of actives from porous granules can be described by the Higuchi model. In this model, the diffusion of the solute occurs basically by three mechanisms: (1) diffusion within the granule; (2) solubilization of the active ingredient; and (3) movement of the solubility front inside the granule. Additionally, the authors correlated the release of a pesticide from a one-dimensional porous solid matrix with a mechanism of solute transport following the Ritger and Peppas model (1987), as depicted in Fig. 5.4.

5.3 Some Nanomaterials or Nanostructured Materials Used in CR Systems in Agriculture

5.3.1 Polymeric Matrices

Over the past few decades, several classes of nanostructured polymeric matrices were used to combat environmental pollution by acting more specifically as carrier vehicle in the CR systems of various agrochemicals. Beyond needing to be low cost, it is essential that these matrices be constituted from biodegradable and renewable sources. The controlled release formulations (or CRF) of agrochemicals have become an important mechanism of the reduction of pesticides in the environment. In the CRF, the formation of a chemical bond between a pesticide and the polymeric matrix can maximize the release process and consequently improve the safety, efficiency, and the economic cost for use in crop protection (Li-min et al. 2005).

Several authors have shown their contributions in this area. Corradini et al. (2010) studied the sorption capability of the NPK fertilizer into polymeric nanoparticles. The authors showed a full study of a chitosan nanoparticle stability that related the efficiency of incorporation of the fertilizers into the nanoparticles with the zeta potential and the average diameter of the particle. According to Subbarao et al. (2013), slow release fertilizer technology may contribute in the minimization of fertilizer loss because their indiscriminate use contributes to the degradation of the environment and soil. In this study, they observed that the coating of potash fertilizer by the deposition of polyacrylamide polymer decreased its releasing rate. A novel CR pesticide system based upon self-assembled nanocapsules with an aqueous core was developed by Sun et al. (2014). These core–shell polymeric nanocapsules were obtained by a photo-cross-linking reaction using azidobenzaldehyde (Az) and carboxymethyl chitosan (CMCS), as depicted in Fig. 5.5.

The authors observed that the encapsulation efficiency of methomyl pesticide-loaded cross-linked nanocapsules reached approximately 90 %, where the aqueous core was responsible in the accumulate of a fraction of the methomyl pesticide in its free state, and other fractions of the absorbed pesticide were located in the surface and the interior of the shell architectures. In relation to the release profiles of

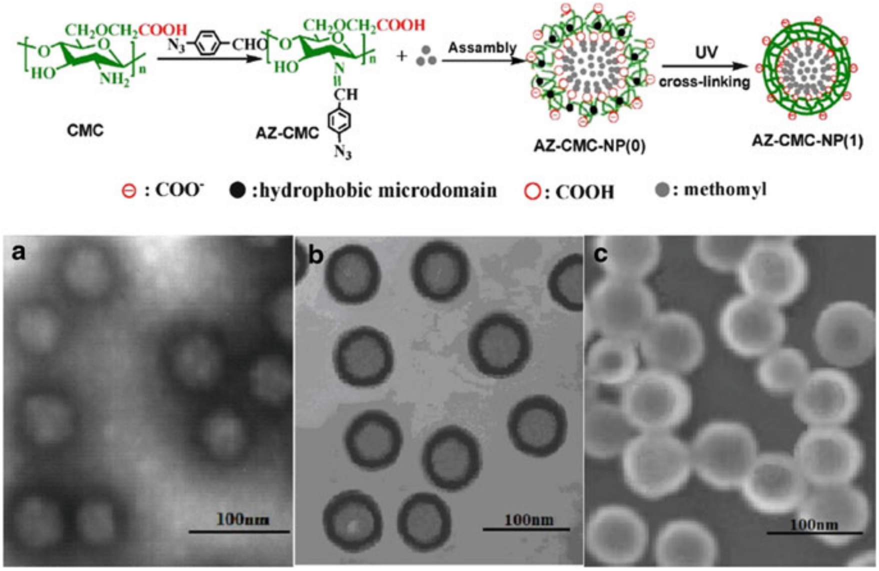

Fig. 5.5 Assembly process of methomyl-loaded shell cross-linked nanocapsules (*top*). TEM and SEM images of methomyl-loaded nanocapsules on the basis of Az-CMCS-3 with an Az substitution degree of 0.23 in an aqueous solution at pH 4.0: (**a**) TEM of Az-CMCS-NP(0)-3 sample before cross-linking, (**b**) TEM, and (**c**) SEM of Az-CMCS-NP(1)-3 sample after cross-linking (*bottom*) (Sun et al. 2014). Reproduced by permission of Elsevier

methomyl from the methomyl-loaded nanocapsules, the authors concluded that the pesticide desorption was controlled by cross-linking of the shell, as well as by the degree of cross-linking.

Other important agrochemical controlled release systems from polymeric matrices were reported by various researchers (Mishra et al. 2004; Xu et al. 2006; Abedi-Koupai et al. 2012; Li et al. 2012; Ma et al. 2013; Melaj and Daraio 2013; Saruchi et al. 2013; Yang et al. 2013; Zhao et al. 2013; Zhong et al. 2013; Su et al. 2014). Notably, these systems were not nanostructured polymeric matrices but were included due to the important concepts presented about the CR systems.

5.3.2 *Hybrid Matrices*

One of the pioneer studies utilizing hybrid matrices in the controlled release of pesticides was conducted by Gerstl et al. (1998). In this paper, the release of alachlor and atrazine pesticides from alginate and pectin polymeric matrices with or without sodium montmorillonite and kaolinite inorganic nanoclays was studied. It was observed that better results were achieved with the alginate-pectin blend. For hybrid matrices formed by a nanoclay and alginate, it was possible to conclude that the addition of the nanoclay to the alginate reduces the releasing of alachlor. The authors also concluded that the rate of release of the pesticides from the pectin polymer matrix was greater than from the alginate polymer matrix. In addition, considerable effects on the release of both pesticides were only observed for nanocomposites with montmorillonite clay.

Recently, Bortolin et al. (2013) obtained a novel nanocomposite based upon a PAAm/methyl cellulose/montmorillonite hydrogel and evaluated its application as a possible candidate to be used as a carrier vehicle in the controlled release of urea fertilizer. The results indicated that the release process was strongly dependent upon the hybrid nanocomposite hydrophilicity, the pH of the release medium, and a hydrolysis treatment. The results showed that the release of urea was more prolonged, approximately 192 times more slowly, for the hydrolyzed hydrogel when compared to the control (pure urea). Additionally, according to the authors, these nanocomposites were the first carrier vehicles to release 90 g of urea per gram of dry hydrolyzed hydrogel used.

Novel hybrid nanocomposites based upon wheat gluten and three types of commercial nanoclays (1 unmodified: sodium montmorillonite or HPS and 2 organically modified montmorillonites: Cloisite® 30B or C30B; Dellite 72T or D72T), using a bi-vis extrusion process, were developed by Chevillard et al. (2012). Thermal, structural, and morphological properties of these nanocomposites were characterized by differential scanning calorimetry, wide angle X-ray scattering analysis (WAXS), and transmission electron microscopy (TEM) techniques, respectively. The TEM results (Fig. 5.6) showed that the best intercalation/exfoliation degree was found for the WG-HPS-E hybrid nanocomposite (where E represents the presence of the pesticide ethofumesate). The absence of the HPS nanoclay diffraction peak observed in the WAXS technique supported this conclusion. However, the most important result about the controlled release of the ethofumesate pesticide from these hybrid nanocomposites is shown in Fig. 5.6. This figure shows that the quantity of ethofumesate pesticide released in water for three different temperatures from the wheat gluten-based hybrid nanocomposites followed the order WG-HPS-E > WG-E > WG-C30B-E > WG-D72T-E. This result is an important indication that the nanocomposite structures affected the pesticide release mechanism.

Jafari et al. (2014) developed a system for the determination of pesticides based upon polypyrrole/cloisite-Na^+ montmorillonite hybrid nanocomposites by an electropolymerization method at a constant anodic potential of 0.95 V vs Ag/AgCl using Ni–Cr wire, platinum electrode, and a pseudo Ag/AgCl electrode as the working, counter, and reference electrodes, respectively. SEM micrographs, presented in Fig. 5.7, show that the coated layer of the Ni–Cr wire (Fig. 5.7a) was approximately 32 μm. The polypyrrole (Ppy) film exhibited a dense morphology when compared to the Ppy/nanoclay film. Additionally, Ppy/nanoclay nanocomposites had a porous structure when compared to Ppy that was attributed to the insertion of Ppy into the clay galleries.

According to the authors, this highly porous structure would significantly increase the available surface area of the coated fiber and would enhance the extraction efficiency in solid-phase microextraction (SPME), which was confirmed

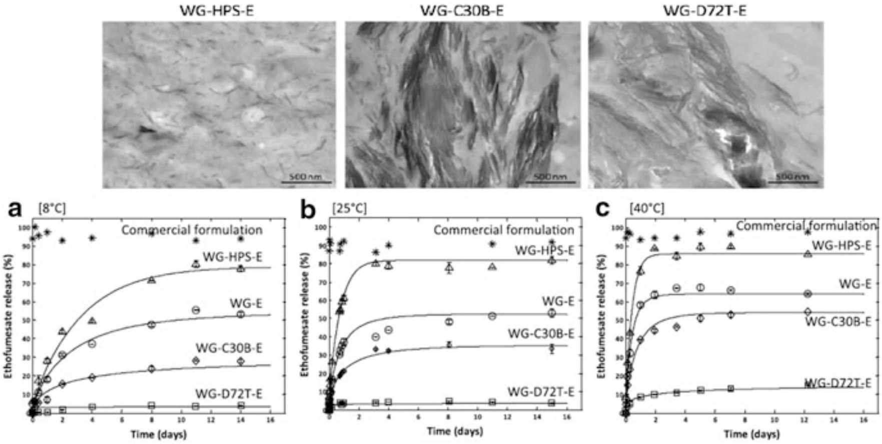

Fig. 5.6 TEM pictures of wheat gluten-based materials containing ethofumesate filled with unmodified MMT (WG-HPS-E) and organically modified MMMT (WG-C30B-E and WG-D72T-E) (*top*). Ethofumesate release kinetics obtained for the unfilled wheat gluten-based material (*white circle*); wheat gluten-based materials filled with HPS (*delta*), C30B (*diamond*), and D72T (*white square*); and commercial formation (*asterisk*) at 8 °C (**a**), 25 °C (**b**), and 40 °C. Symbols are experimental data points. *Error bars* represent standard deviation. *Dot lines* are fitting to the model. RMSE values were 0.04, 0.05, 0.10, and 0.51 at 8 °C; 0.05, 0.03, 0.04, and 0.39 at 25 °C; 0.04, 0.03, 0.05, and 0.15 at 40 °C for WG, WG-HPS, WG-C30B, WG-D72T, respectively (*bottom*) (Chevillard et al. 2012). Reproduced by permission of Elsevier

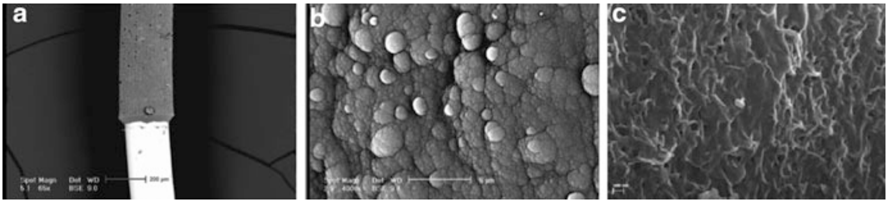

Fig. 5.7 Scanning electron micrographs of (**a**) uncoated and coated Ni–Cr wire, (**b**) Ppy, and (**c**) Ppy/nanoclay fibers (adapted from Jafari et al. 2014). Reproduced by permission of Elsevier

by detection of diazinon and fenthion pesticides through gas chromatography corona discharge ion mobility spectrometer (GC-CD-IMS) analysis. In these experiments, 5 real samples were analyzed, 2 of which were water (tap and river), 1 of which was fruit (apple), and 2 of which were vegetables (cucumber and lettuce), as shown in Table 5.3.

Dehaghi et al. (2014) studied chitosan–zinc oxide (CS–ZnO) nanoparticles as an absorbent vehicle for the removal of the pesticide permethrin from water. ZnO nanoparticles possessed an almost spherical morphology with a size of 58 nm. The authors investigated the influence of the amount of absorbent, agitation time, pesticide initial concentration, and pH on the sorption of the pesticide by CS–ZnO absorbents. The authors concluded that these novel absorbent materials may

Table 5.3 Analytical results of OPPs in real samples for SPME–GC-CD-IMS (adapted from Jafari et al. 2014). Reproduced by permission of Elsevier

Sample	Compound	Amount added ($\mu g\ L^{-1}$) or ($\mu g\ kg^{-1}$)[a]	Amount found ($\mu g\ L^{-1}$) or ($\mu g\ kg^{-1}$)[a]	Relative error[b] (%)	RR%[c]
Tap water	Diazinon	0.2	0.19 (7)[d]	5	98 (8)[d]
	Fenthion	0.2	0.19 (4)	5	97 (6)
River water	Diazinon	0.4	0.37 (6)	7.5	93 (5)
	Fenthion	0.4	0.38 (8)	5	95 (5)
Cucumber	Diazinon	2.5	2.18 (5)	12.8	87 (8)
	Fenthion	2.5	2.10 (4)	16	84 (7)
Lettuce	Diazinon	3.5	2.90 (7)	17.1	82 (5)
	Fenthion	3.5	2.80 (9)	20	80 (6)
Apple	Diazinon	5	3.90 (8)	22	78 (9)
	Fenthion	5	3.60 (11)	28	72 (7)

[a] $\mu g\ L^{-1}$ for tap and river water samples and $\mu g\ L^{-1}$ for cucumber, lettuce, and apple samples

[b] The relative errors is the absolute error divided by the actual measurement

[c] Relative recovery was calculated by analyzing real samples spiked with 0.2, 0.4 $\mu g\ L^{-1}$ for water and 2.5, 3.5 and 5 $\mu g\ L^{-1}$ for vegetable and apple samples

[d] Relative standard deviation, RSD (%)

be applied as an alternative biocompatible and eco-friendly strategy for pesticide removal, with a potential application in the water treatment process. Bin Hussein et al. (2009) had already developed a novel nanohybrid pesticide controlled release system from 4-(2,4-dichlorophenoxy)butyrate and a Zn–Al-layered double hydroxide inorganic interlayer by different methods.

5.3.3 *Inorganic Matrices*

Khan and Akhtar (2011) evaluated the potential of the application of a poly-*o*-toluidine Zr(IV) phosphate nanocomposite as a pesticide sensitive membrane electrode. The pesticide 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) was used in this study. Adsorption isotherm results of the nanocomposites with dimensions ranging between 42 and 100 nm were satisfactory. These electrodes presented a good degree of reproducibility up to the detection limit up to one month. Berahmand et al. (2012) studied, by factorial design, the application of silver nanoparticles on the growth of fodder maize (*Zea mays* L.). The authors concluded that the most efficient treatment was the treatment that combined the silver nanoparticles with a magnetic field, with results that achieved a yield of 74.5 tons per hectare, representing an improvement of 35 % in comparison to the control. Other important strategies of the controlled release of nitrogen and urea into the soil to improve the plant growth and the yield of tomatoes were reported by Kottegoda et al. (2011) and Zhu et al. (2012), respectively.

5.4 Conclusions

The controlled release system allows for the controlled delivery, over time, of various agrochemicals that maintain their activity through a desired time period in the soil while reducing the quantity of the agrochemical that was applied, the manpower, and the energy or combustive necessary to operate the application devices. The use of nanotechnology in the agriculture field provided advantages when compared to use of materials from the macroscopic scale, improving mainly crop productivity and protection. There is currently little existing information about the controlled release of agrochemicals from nanostructured materials. Our research group is focused on understanding the controlled release of NPK fertilizers and pesticides from hydrogel nanocomposites that are based on polysaccharide and silicate nanostructured-like zeolites and nanoclays.

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Chapter 6
Nanobiotechnology Strategies for Delivery of Antimicrobials in Agriculture and Food

Adriano Brandelli

Abstract The great advances in nanotechnology and materials science point to an important increase in the application of nanomaterials in agriculture and allied sciences in the next future. Microbial spoilage of crops and foods is associated with huge economical loses, and the utilization of nanoformulated antimicrobial substances arises as an interesting alternative to confront the damage caused by such microorganisms. Antimicrobial nanoparticles can find several applications in agriculture and food packaging, and the effectiveness of metallic nanoparticles including silver, nickel, iron, and zinc oxides has been demonstrated in many systems. Also, the entrapment of antimicrobial substances in different nanostructures may represent an alternative for delivery of these compounds. A diversity of nanovesicles has been developed for encapsulation of antimicrobial substances, including both natural and synthetic polymers as encapsulating material. Nanofibers may be interesting nanostructures for antimicrobial delivery, allowing different physical modes of antimicrobial loading, including direct adsorption on the nanofiber surface or the assembly of drug-loaded nanoparticles. Magnetic nanoparticles and nanotubes are also structures with potential application for entrapment of antimicrobials. This chapter presents the most important nanostructures as promising tools for antimicrobial delivery systems in agricultural and food applications.

6.1 Introduction

Contemporary technologies, such as biotechnology and nanotechnology, can play important role in agriculture by increasing production and improving the quality of crops and foods. In particular, nanotechnology is a quite novel technology that has the potential to create enormous changes in food and agricultural systems (Chen

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and Yada 2011; Mousavi and Rezaei 2011). Nanotechnology is generally related to materials, systems, and processes that operate in a size scale of 0.1–100 nm. This technology permits to take a look at the atomic and molecular level and to create structures in the nanometer range. Using nanotechnology, it is possible to regulate the catalysis of chemical reactions and to manipulate these nanoscale structures, allowing the preparation of nanoparticles with many specific applications (Sanguansri and Augustin 2006).

The term nanoparticle has been applied to a variety of structures within the nanometric scale and may also include the model of nanocapsules. Many nanoparticles have a solid core or matrix, mostly composed of metallic atoms. Because of their small size, nanoparticles show significant changes in their physical properties as compared with larger particles of bulk materials. This significant size reduction implies the emergence of quantum effects that lead to potentially useful phenomena such as Coulomb blockade, superparamagnetism, and surface plasmon resonance, among others. In addition, the increase in the surface area to volume ratio causes the appearance of surface effects related to the high number of surface atoms, as well as to a high specific area (Sharma et al. 2009). Many different materials are used for the manufacture of nanoparticles, including metal oxides, magnetic materials, ceramics, silicates, carbon nanotubes (CNTs), and synthetic and natural polymers (Faraji and Wipf 2009). The synthesis of nanoparticles with specific composition, size and of distinct properties has expanded the opportunity of their applications in numerous industries including agriculture and food.

Conventional techniques of nanoparticle synthesis that usually employ atomic, molecular, and particulate processing in vacuum or in a liquid medium are economically expensive and inefficient in terms of materials and energy use. The development of harmless and eco-friendly procedures for the synthesis of nanomaterials based on green chemistry and biological processes constitutes a growing demand. Therefore, several researchers have investigated biological processes that present excellent control on particle size using microorganisms (Korbekandi et al. 2009). In particular, the utilization of fungi for preparation of metallic nanoparticles has gained importance since they have some advantages as compared with other organisms. A better manipulation and control over crystal growth can be achieved due to slower fungi kinetics, fungal mycelia can resist flow pressure and agitation in bioreactors, and many enzymes secreted by fungi are capable of reducing metal ions, allowing a controlled synthesis of nanoparticles with well-defined size and shape (Kashyap et al. 2013).

Nanoparticles can serve as delivery systems to drugs, chemicals, or genes, which target particular parts of the organism to release their contents. Due to their small size, nanoparticles can enable effective penetration through cuticles and tissues, allowing slow and constant release of the active substances (Suri et al. 2007; Zhang et al. 2010a). Thus, nanotechnology creates new tools and materials for use in biological systems, which can be useful to both diagnosis and therapeutic purposes (Farokhzad and Langer 2006; Kuzma 2010). This convergence between nanotechnology and biology is called nanobiotechnology.

Several applications of nanobiotechnology in agriculture, food, and animal sciences have been proposed, including new tools for disease detection, enhancing the ability of plants to absorb nutrients, combat crop diseases, and effective systems for food processing, packaging, and storage (Azeredo 2009; Chen and Yada 2011; Kah and Hofmann 2014). Thus, nanotechnology may have many applications in all stages of production, processing, storage, packaging, and transport of agricultural products. Environmental benefits may be also expected with the use of nanotechnology in agriculture. Nanoparticles can offer the opportunity of more efficient and safe administration of pesticides, herbicides, and fertilizers by controlling precisely when and where they are released (Pérez-de-Luque and Rubiales 2009; Mousavi and Rezaei 2011). Smart sensors and smart delivery systems will help agriculture industry to combat plant pathogens, allowing lower doses to be used.

Considering the elevated loses caused by phytopathogens in crop production, the potential benefits of nanostructures to delivery of antimicrobials should be truly considered. Despite many advances in nanostructured antimicrobial delivery have been achieved in medicine and pharmaceutical sciences, applications in agriculture started and should result a great impact on microbial control in the near future. The most important nanostructures as hopeful tools for antimicrobial delivery systems in agricultural and food applications are discussed in this chapter.

6.2 Microbial Spoilage of Crops and Foods

Worldwide postharvest losses have been estimated at 50 %, and a large amount of such losses is related to fungal and bacterial infections. The most important factors that make plant products more susceptible to spoiling are the high water content in fruit and vegetables that allows pathogens to propagate more easily and wounding of such products, mainly during harvesting and transport, providing easier opportunity for microbial contamination (Spadaro and Gullino 2004; Saranraj and Geetha 2012). Food crops are attacked by numerous pests and diseases around the world, most of them are related to pathogenic fungal diseases. Filamentous fungi are robust organisms capable of growing on all kinds of foods, including cereals, vegetables, and fruits. They are important spoilage organisms in foods causing significant losses in the industry. Fungi are ubiquitous biological agents that are able to colonize different foods because of their potential to synthesize an assortment of hydrolytic enzymes. They cause numerous crop diseases, which are responsible for huge economic losses (Magro et al. 2006; Clark et al. 2012).

Fruits and vegetables are highly susceptible to fungal spoilage, both in the field and during postharvest storage. Fungal growth on fresh fruits and vegetables is responsible for food spoilage and numerous plant diseases. Significant genera comprise *Alternaria*, *Botrytis*, *Fusarium*, *Geotrichum*, *Penicillium*, *Pythium*, *Phytophthora*, *Rhizoctonia*, and *Sclerotinia* spp. (Spadaro and Gullino 2004). The vine plant and grapes may be affected by a series of diseases of which the most well known are grey rot (*Botrytis cinerea*), downy mildew (*Plasmopara viticola*), and powdery mildew (*Erysiphe necator*), which are generally prevented by phytochemical application. In addition, grapes may also bear saprophytic moulds (*Cladosporium* spp., *Aspergillus* spp., *Penicillium* spp.) responsible for several grape rots or mycotoxin production. However, these fungi do not have the ability to grow in wines, and their effect on wine quality is due to grape damage (Barata et al. 2012).

Grain crops are also susceptible to fungal contamination, being *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* the most frequent genera. In these products, moulds are responsible for off-flavor formation and contribute to heating and loss in dry matter in grains through the utilization of carbohydrates as an energy source, degradation of lipids and proteins, production of volatile metabolites, and production of allergenic compounds. This causes a reduction in the quality of animal feed and seed (Cabral et al. 2013). In addition, toxigenic fungi are a major problem in cereal crops as they produce a large number of toxic metabolites contaminating plants and food products. Many phytopathogenic and spoilage fungi also cause several potential carcinogenic and mutagenic diseases in humans and animals due to mycotoxin production. These secondary metabolites are produced by filamentous fungi as a natural protection. Mycotoxins are usually thermostable (above 100 °C) and thus can be transferred to food, even after microbial stabilization steps, such as heating and extrusion. Consequently, humans and animals are exposed to their toxic effects (Beretta et al. 2002; Oliveira et al. 2014). Inhibition of fungal growth in crops, fresh fruits, and vegetables is therefore necessary to reduce the risk to human and animal health. However, it is important to note that partial inhibition of fungal growth, such as reduction of fungal growth rate, could enhance mycotoxin production as a response of the mould to stress (Cabral et al. 2013).

Spoilage bacteria cause food crop deterioration and result in unpleasant odors, tastes, and textures. For example, these microorganisms can cause fruits and vegetables to become mushy or slimy or to develop a bad odor (Gram et al. 2002). Several types of bacteria are associated with the spoilage of fresh products, and most of them are pathogenic to the plant. For example, the gram-negative bacterium *Erwinia carotovora* is a common phytopathogen attacking virtually every vegetable type (Tournas 2005). *E. carotovora* is frequently associated with rot disorders in potatoes, carrots, tomatoes, and other vegetables, and infections often result in a complete loss of these crops. Infection by *Clavibacter michiganensis*, a gram-positive bacterium, causes bacterial wilt in diverse agriculturally important plants, including a variety of fruits and vegetables (Fatmi and Schaad 2002). Bacteria belonging to the genera *Pectobacterium* and *Dickeya* are causal agents of blackleg and tuber soft of potato. In seed potato production, these diseases are next in economic importance to bacterial wilt caused by *Ralstonia solanacearum* and before ring rot and common scab caused by *Clavibacter michiganensis* or *Streptomyces scabies*, respectively (Czajkowski et al. 2011). *Dickeya zeae* is the causal agent of devastating rice foot rot disease, which is a major concern of the agricultural industry in rice-growing regions (Zhou et al. 2011).

Despite continued efforts are devoted to control crop and food pathogens, microbiological safety threats prevail, and therefore, the investigation for methods to detect and control harmful microorganisms is constant. The most common preservation strategies applied in the food industry involve chemical or physical techniques. Several strategies have been used to extend the shelf life of vegetables and food, including heat treatments, infrared or microwave irradiation, using modified atmospheres during packaging, by adding chemical preservatives (e.g., sorbic, benzoic, and propionic acids), or emerging technologies such as pulsed electric field and high-pressure processing (Sun 2005). However, these methods only decrease microbial infections and often fail to completely eliminate microbial contaminants. Also, some fungi and bacteria are able to adapt to the presence of certain preservatives (Davidson and Harrison 2002). In addition, current consumer trends are increasingly demanding high-quality fresh vegetable products with extended shelf life and foods that are free of chemical pesticides. This last feature is an important aspect to consider when discussing the need for new preservation methods to inhibit the growth of undesirable contaminating microorganisms and is motivating the food industry toward a focus on natural preservation approaches (Cabral et al. 2013; Zheng et al. 2013). Biopreservation technologies are being favored to improve the safety, the nutrition value, and the organoleptic properties, in response to consumer demands. The fermentation by lactic acid bacteria has been used in food production to antagonize spoilage contaminants and is increasing in popularity due to their ability to enhance either the product quality or its nutritional profile (Gerez et al. 2013; Oliveira et al. 2014). In this context, the use of nanostructures for more efficient delivery of natural antimicrobials constitutes a real alternative to combat microbial spoilage and pathogenic microorganisms in crops and foods (Malheiros et al. 2010a; Gomes et al. 2011).

6.3 Nanostructures for Antimicrobial Delivery

Nanobiotechnology may represent a new paradigm in the development of nanostructured materials for antimicrobial delivery in agricultural sciences. A rising number of peer-reviewed publications are available on the investigation of nanopesticides, mainly devoted to insecticides (about 55 %) followed by fungicides (about 30 %). This greater proportion of insecticidal formulations may be associated with the limited water solubility of many conventional insecticides and with the possibility of using alternative ingredients that are less harmful to nontarget organisms and to the environment (Kah and Hofmann 2014). Some major nanostructures related to antimicrobial delivery and examples of agriculture and food applications are presented in the sequence.

6.3.1 Inorganic Antimicrobial Nanoparticles

Metallic nanoparticles can be used as antimicrobial agents or nanocarriers for active substances. Silver nanoparticles are known for their antimicrobial activity and have been used in applications to remove microorganisms in air filters, water, and medicine. The Ag^+ ions are effective in millimolar concentration while Ag nanoparticles in the nanomolar range, showing that they are much more effective in performing antimicrobial activities (Rai et al. 2009). Among the metallic nanoparticles, silver is considered the most promising nanomaterial with bactericidal and viricidal properties due to its wide-range efficacy, relatively low toxicity, ease of use, charge capacity, high surface to volume ratio, and adaptability to several substrates. Ag nanoparticles attach to the surface of cell membrane of these pathogenic bacteria and drastically disturb its proper function like respiration and permeability. The damage to the cell may be caused by strong interaction with sulfur- and phosphorous-containing molecules, such as proteins and DNA (Sharma et al. 2009; Prabhu and Poulose 2012). These interactions would prevent DNA or RNA synthesis, resulting in microbial death.

The potential use of Ag nanoparticles in biomedical applications has been extensively investigated. For example, the combination of the biocidal properties of Ag nanoparticles and the bacteriolytic activity of lysozyme was used to produce a colloidal suspension for coatings of medical instrumentation. These nanocomposites exhibit antimicrobial activity against several bacteria reducing the viability of *Acinetobacter baylyi*, *Bacillus anthracis*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* at least 1.5 log within 3 h (Eby et al. 2009).

Ag nanoparticles produced extracellularly by *Fusarium oxysporum* can be incorporated in several types of materials such as textiles. These textiles containing Ag nanoparticles are sterile and can be useful to prevent infection with pathogenic bacteria such as *S. aureus* (Durán et al. 2007). Ag nanoparticles synthesized by *Lecanicillium lecanii* were coated on bleached cotton fabrics, and the antibacterial activity of these materials was observed against *S. aureus* and *Escherichia coli* (Namasivayam and Avimanyu 2011). Extracellular synthesis of gold nanoparticles using *Rhizopus oryzae* was employed for the generation of nanogold bioconjugate structure. These nanostructures showed strong adsorption capacity and have been successfully utilized to obtain water free from pathogens and pesticides (Das et al. 2009). Silver nanoparticles and metal oxide nanoparticles have been also described for drinking water purification (Savage and Diallo 2005).

Although nanoparticles have been extensively studied for drug delivery and sustained release in biomedical sciences, similar applications in agriculture are still poorly investigated. Nanoparticle formulations of biopesticides have been proposed to reach a better spatial distribution of the pesticides on leaf surfaces, which would result a better efficiency (Liu et al. 2008). Solid and liquid formulations of silver, aluminum oxide, zinc oxide, and titanium dioxide nanoparticles were tested for the control of rice weevil and grasserie disease in silkworm (*Bombyx mori*) caused by *Sitophilus oryzae* and baculovirus BmNPV (*B. mori* nuclear polyhedrosis virus), respectively. After 7 days of exposure, 95 and 86 % mortality were observed with hydrophilic and hydrophobic formulations of Ag nanoformulations, and nearly 70 % of the insects were killed when the rice was treated with lipophilic formulations of Ag nanoparticles (Goswami et al. 2010). The antimicrobial activity of titanium dioxide has been recognized, and its application can suppress both bacterial and fungal crop pathogens. The use of nanoformulations of TiO_2–Zn could result in significant reduction of bacterial spot severity in tomatoes in both greenhouse and field trials (Paret et al. 2013). Copper nanoformulation has been also reported to suppress the growth of bacterial blight on pomegranate at concentrations 10,000-fold lower than that usually recommended for copper oxychloride (Mondal and Mani 2012). Formulations with calcium carbonate nanoparticles showed prolonged activity of validamycin, which can be explained by the sustained release of the antimicrobial over 14 days for control of *R. solani* (Quian et al. 2011).

Silver nanoparticles are the most studied nanostructures for inhibition of plant pathogens. The antifungal effect of double-encapsulated Ag nanoparticle solution against rose powdery mildew caused by *Sphaerotheca pannosa* var. *rosae* was investigated. The nanoparticle solution was diluted up to 10 ppm and sprayed at a large area infected by *S. pannosa* var. *rosae*. Two days after the spray, more than 95 % of rose powdery mildew faded out and did not return for a week (Kim et al. 2008). Min et al. (2009) studied the use of Ag nanoparticles as an alternative to pesticides for the control of sclerotia-forming phytopathogenic fungi. Ag nanoparticles, which have high surface area and high fraction of surface atoms, showed improved antimicrobial effect when compared to the bulk silver. A microscopic observation revealed that hyphae exposed to Ag nanoparticles were severely damaged, resulting in the separation of layers of hyphal wall and collapse of hyphae. The antifungal activity of Ag nanoparticles on *Colletotrichum gloeosporioides*, which causes anthracnose in a wide variety of fruits, was investigated by Aguilar-Méndez et al. (2010). A significant growth delay of *C. gloeosporioides* in the presence of Ag nanoparticles was noticed. The in vitro activity of Ag nanoparticles against 18 phytopathogenic fungi was recently demonstrated (Kim et al. 2012). Therefore, Ag nanoparticles could be an alternative fungicide to manage some plant diseases.

Efforts for the preparation of bionanoformulations of metallic nanoparticles with antifungal activity are gaining interest. The synthesis of silver nanoparticles with well-defined morphology and stability over several months can be achieved by several fungi, including *F. oxysporum*, *Trichoderma asperellum*, *Trichoderma viride*, and a number of *Aspergillus* spp. (Kashyap et al. 2013). However, their potential as antimicrobial agents to combat plant pathogens in the field needs to be further investigated.

In addition, metallic nanoparticles can be used as nanocarriers to antimicrobial agents. Nickel nanoparticles coated with a monolayer polyacrylic acid nanofilm were used to immobilize the antimicrobial peptide LL-37. This nanocomposite was effective to kill *E. coli* (Chen et al. 2009). Iron oxide magnetic nanoparticles coated with polymers, in particular with biopolymers such as polysaccharides, can be efficiently used to trap antimicrobials (Dias et al. 2011). Vancomycin-coated Au nanoparticles have an enhanced antibacterial activity against vancomycin-resistant enterococci and also showed an unexpected activity against *E. coli* (Gu et al. 2003). These studies suggest that metallic nanoparticles may serve as useful systems for delivery of antimicrobial compounds.

6.3.2 Lipid Nanocapsules–Liposomes

A few types of nanoparticles including liposomes, polymeric nanoparticles, and solid lipid nanoparticles (SLNs) have been widely investigated as antimicrobial drug delivery platforms (Zhang et al. 2010a; Brandelli 2012). Among these nanoparticles, liposomes and polymeric nanoparticles are the most studied systems in terms of nanoencapsulation of antimicrobial substances.

The term nanocapsule is generally reserved for structures composed of a thin external layer enclosing empty space inside. Encapsulation and controlled release methods are changing the use of drugs in medicine and offer a fascinating tool for delivery of active substances such as pesticides and fertilizers in agriculture (Pérez-de-Luque and Rubiales 2009). Encapsulation in nanoparticles may offer a potential solution to protect the active compound and to increase its efficacy and stability in practical applications. The active compound is protected from degradation or interaction with external agents and helps to improve solubility and penetration through tissues. In addition, it is possible to control the rupture of the nanocapsule to release the active compound, for example, by changing the external conditions such as the pH or temperature (Li et al. 2010). Thus, nanocapsules are an interesting system for controlled release of active substances once they reach certain sites with the required conditions (e.g., the alkaline environment of the stomach of some insects, in the case of insecticides).

Liposomes are spherical vesicles with a bilayer membrane structure formed by self-assembled amphiphilic lipid molecules dispersed in aqueous solution, with mean diameter sizes from nanometer to micrometer range. Their properties and functionalities depend on extrinsic and intrinsic parameters. The stability of liposomes and the release of their contents are controlled by factors such as temperature, pH, ionic strength, concentration and composition of the phospholipids, and the properties of the entrapped compound (Jesorka and Owar 2008; Zhang et al. 2010a). However, liposomes are only kinetically stable, i.e., they remain stable only for a limited time interval.

Liposomes can be prepared from either natural or synthetic lipids. Phosphatidylcholine, an electrically neutral phospholipid that contains fatty acyl chains of variable lengths and saturation degrees, is one of the most frequently used lipids in liposome manufacture. Cholesterol is incorporated into many liposome formulations to adjust membrane rigidity and stability. Regarding their structural properties, liposomes can be classified into multilamellar vesicles (MLVs), consisting of multiple phospholipid bilayer membranes, and unilamellar vesicles (ULVs), which have a single lipid bilayer. ULVs can be further classified into small unilamellar vesicles (SUVs) and large unilamellar vesicles (LUVs) depending on their size range (Jesorka and Owar 2008). The challenge in making liposomes for antimicrobial delivery is to achieve the formation of homogeneous structures with adequate properties and good encapsulation efficiency. Thus, several parameters must be considered when selecting the method for liposome preparation, including the physicochemical characteristics of the lipid ingredients and the substances to be enclosed within the liposomes, particle size, polydispersity, zeta potential, expected shelf life, batch-to-batch reproducibility, and the possibility for large-scale production of safe and efficient products (Sharma and Sharma 1997; Mozafari et al. 2008).

Liposomes, in particular ULVs, are not spontaneously formed. In general, an adequate energy input (sonication, extrusion, homogenization, shaking, or heating) is supplied to phospholipid suspension in aqueous environment (Jesorka and Owar 2008). The encapsulation of antimicrobial substances into liposomes is often achieved by the thin-film hydration method or by the reversed-phase method (Brandelli 2012). In the first method, a lipid film is hydrated with an aqueous buffer containing the antimicrobial substance, at a temperature above the phase transition temperature of lipids. The resulting population of MLVs is further processed by membrane extrusion or sonication to obtain SUVs of uniform size. In the reversed-phase method, a solution containing the antimicrobial substance is dropped into the lipid solution to form a water-in-oil emulsion. This emulsion is sonicated yielding reverse micelles. After the organic solvent is evaporated, a viscous organogel is formed, which is reverted to nanoliposomes after addition of ultrapure water (Mertins et al. 2005).

From the first description of the liposome structure in 1965, extensive studies on their fundamental properties including lipid polymorphisms, lipid–protein and lipid–drug interactions, and mechanisms of liposome disposition were developed, and the potential application of liposomes as a drug delivery vehicle was thoroughly recognized and started being transferred to practice in the 1980s. Liposomes were originally introduced to the cosmetic market by Dior in 1986, and subsequently, Doxil (doxorubicin liposomes) became the first liposome-based drug delivery system approved by the US Food and Drug Administration (FDA) in 1995 (Zhang et al. 2010a). Liposomes are the most extensively used antimicrobial drug delivery system. As the liposomes contain both lipid and aqueous phases, they can be used for the entrapment and delivery of hydrophilic, hydrophobic, and amphiphilic molecules. An important feature of liposomes is its lipid bilayer structure, which can directly fuse with cell membranes, releasing drug contents to the microbial membranes or the interior of the microorganism. In addition, the liposome surface can be easily modified with “stealth” materials to improve their stability. Polyethylene glycol (PEG) has been frequently conjugated to liposome surface to create a layer that prolongs the shelf life of liposomes in the bloodstream (Milla et al. 2012). The surface of liposomes can be also modified by the addition of targeting ligands such as antibodies or lectins, which can bind selectively to microorganisms or infected cells and then release the drug contents to exert its antimicrobial effect.

Liposomes have been made for drug delivery for many years and have been primarily used in pharmaceuticals and cosmetics, but applications in agriculture and food technology are also described. The use of liposomes in agriculture has been mainly associated as model membranes of plant organelles, leading to research in plant ageing, drying and freeze tolerance, and effects of toxins and pesticides (Taylor et al. 2005). However, the development of water-soluble liposomes may extend the potential use of antifungal drugs or antibiotics developed for other purposes for applications in agriculture. A new formulation embedding the broad-spectrum, water-insoluble, macrolide polyene antibiotic amphotericin B (AMB) in nanodisks composed of phospholipids and apolipoprotein A-I enhances antibiotic solubility and confers protection against environmental damage. The AMB-nanodisks were tested for efficacy against several phytopathogenic fungi in vitro and on infected living plants of chickpea and wheat. The results confirm that formulation of AMB increases its effectiveness against phytopathogenic fungi in vitro and showed some effect against fusarium wilt in chickpea, suggesting the possibility for its use on infected plants in the field (Pérez-de-Luque et al. 2011). In addition, applications in veterinary and animal science have been developed, increasing the efficiency of drug delivery in farm animals (Kuzma 2010). However, the main use of liposomes is associated with the food industry, especially for protection of substances such as enzymes, vitamins, and antimicrobials, until their release for improving the food quality (Mozafari et al. 2008; Malheiros et al. 2010a).

The antimicrobial peptide nisin, which is approved for food use in many countries, has been incorporated in nanometric liposomes, showing effective antimicrobial activity against important food pathogens (Malheiros et al. 2010a). A significant increase of the lag phases of *L. monocytogenes* and *E. coli* O157:H7 was observed by encapsulation of nisin and EDTA into liposomes, resulting in bacteriostatic inhibition of these pathogens for at least 48 h (Taylor et al. 2008). The addition of encapsulated nisin to fluid milk resulted in significant increase of the lag phase of *L. monocytogenes* Scott A incubated at 5 °C or 20 °C (Schmidt et al. 2009). Likewise, the inhibition of *L. monocytogenes* was achieved in either whole or skimmed fluid milk added with nanoencapsulated nisin and maintained at 7 °C or 30 °C (Malheiros et al. 2010b). The bacteriocin pediocin PA-1/AcH was also recently formulated in liposomes, and the antimicrobial activity against several strains of *Listeria* spp. was demonstrated (Mello et al. 2013). Although reports in real food systems are relatively scarce, these studies indicate that nanoparticles containing antimicrobials can be effective to control foodborne pathogens.

6.3.3 Polymeric Nanoparticles

Polymers have become the major constituents of nanoparticles used for drug delivery purposes. These nanoparticles can be formed by a polymeric membrane that encloses a liquid core, referred as nanocapsules. The antimicrobials can be dissolved in the inner core or adsorbed to the polymeric coat. Alternatively, the polymeric nanoparticles are formed by a polymeric matrix-type structure, called nanospheres. In this case, the antimicrobial can be retained or adsorbed to the structure (Vauthier and Bouchemal 2009; Brandelli 2012). Since polymeric capsules are maintained by strong covalent bonds, they are more robust and stable than liposomes, also in the dry form. The size and shape of the nanoparticles can be more efficiently controlled using polymers, and the polymers can be modified with different substances, adding new functional groups and properties.

Polymeric nanoparticles are primarily used to carry and deliver poorly water-soluble drugs because of the hydrophobic nature of the nanoparticle core. Major methods for preparation of polymeric nanoparticles are the solvent displacement method and the emulsion polymerization method. The solvent displacement method results in diblock polymeric nanoparticles consisting of a hydrophobic core and a hydrophilic shell (Vauthier and Bouchemal 2009). Several biodegradable polymers have been used to form the hydrophobic polymeric core of nanoparticles, including poly(lactic acid), poly(glycolic acid), poly(lactide-*co*-glycolide), poly (ε-caprolactone), and poly(cyanoacrylate), whereas PEG has been frequently used as a hydrophilic segment. Linear polymers such as polyacrylamide, poly(alkyl acrylates), poly(methyl methacrylate), and poly(ethyl cyanoacrylate) are used to make nanoparticles by the emulsion polymerization method (Zhang et al. 2010a).

Biocompatible and biodegradable polymers have been used extensively in biomedical and pharmaceutical sciences for controlled drug release. Polymeric nanoparticles have been explored to deliver various antimicrobial agents, and greatly enhanced therapeutic efficacy in treating many types of infectious diseases has been reported (Huh and Kwon 2011). Some distinctive properties of polymeric nanoparticles make them an attractive platform for antimicrobial drug delivery. Polymeric nanoparticles are structurally stable, and properties such as particle size, zeta potential, and drug release profiles can be accurately adjusted by selecting different polymer lengths, surfactants, and organic solvents during the synthesis. In addition, the surface of polymeric nanoparticles usually contains functional groups that can be chemically modified for targeted antimicrobial delivery. Polymeric nanoparticles have been functionalized with lectins, which are proteins that bind to carbohydrates present on most bacterial cell walls. The utilization of lectin-mediated drug targeting is based on the fact that most cell surface proteins, and many lipids in the plasma membrane, are glycosylated, and these glycans represent ligands for lectins (Gavrovic-Jankulovic and Prodanovic 2011). Poly(lactide-*co*-glycolic) nanoparticles incorporating rifampicin, isoniazid, or pyrazinamide were conjugated with the lectin wheat germ agglutinin (WGA) in order to reduce the dosage frequency of antituberculosis drugs. The WGA-modified nanoparticles were tested in animal models, showing potential for effective control of tuberculosis through the oral and aerosol routes (Sharma et al. 2004).

Among natural polymers, chitosan is the most studied for the development of nanocapsules. This polysaccharide is a linear polymer composed of D-glucosamine and *N*-acetyl-D-glucosamine residues linked in β-1,4-configuration, resulting in a rigid and unbranched structure. After cellulose, chitosan is the second most abundant natural polymer in nature and can be found mainly in crustaceans and to a minor extent in fungi and bacteria. This polysaccharide has been used for many biological applications because it is biocompatible (Agnihotri et al. 2004). Chitosan is recognized in agriculture for its antimicrobial properties, inhibiting fungal and bacterial pathogens and also stimulating defense responses in plants (Harish et al. 2007). However, chitosan has attracted particular attention as a biodegradable material for mucosal delivery systems, due to its adhesive properties, facilitating the transport of drugs across cellular membranes.

Gelatin is also an interesting polymeric material for development of nanoparticles for drug delivery. Gelatin is nontoxic, low cost, biocompatible, and highly biodegradable. In addition, it is easy to cross-link and to modify chemically, and its molecules contain both cationic and anionic groups, which guarantee functionality to encapsulate both acid and basic substances (Li et al. 2011; Karthikeyan et al. 2013). Therefore, gelatin encloses a great potential to be used for the preparation of antimicrobial delivery systems for application in food and agriculture.

6.3.4 Solid Lipid Nanoparticles

SLNs are another antimicrobial delivery system that has attracted attention as an efficient and a nontoxic alternative lipophilic colloidal carrier prepared either with physiological lipids or lipid molecules used as common pharmaceutical excipients (Almeida and Souto 2007). SLNs are essentially composed of lipids that are in solid phase at room temperature and surfactants for emulsification. Solid lipids utilized in SLN formulations include fatty acids (e.g., palmitic acid, decanoic acid, and behenic acid), triglycerides (e.g., trilaurin, trimyristin, and tripalmitin), steroids (e.g., cholesterol), partial glycerides (e.g., glyceryl monostearate and glyceryl behenate), and waxes (e.g., cetyl palmitate). Several types of surfactants are frequently used as emulsifiers to stabilize lipid dispersion, including soybean lecithin, phosphatidylcholine, poloxamer 188, sodium cholate, and sodium glycocholate (Zhang et al. 2010a). Several methods are available for preparing SLNs, including spray drying, high shear mixing, and ultra-sonication, but the high-pressure homogenization (HPH) and microemulsion-based techniques are the most important. Simple manufacturing techniques such as HPH make it possible to produce SLNs in a large-scale and reproducible manner. Moreover, unlike most polymeric nanoparticles, SLN production techniques do not need to employ potentially toxic organic solvents, which may also have deleterious effect on some active ingredients (Almeida and Souto 2007; Weiss et al. 2008).

Due to their occlusive properties, SLNs are a promising antimicrobial delivery system for topical applications. SLNs contain occlusive excipients that upon application can induce thin film formation that reduce water evaporation and extend residence time on the skin. The administration of azole antifungal drugs such as ketoconazole, miconazole, econazole, and oxiconazole is difficult since they are very water insoluble (Gupta and Cooper 2008). However, these lipophilic compounds can be efficiently encapsulated into SLNs. The combined small particle size and occlusive effect of SLNs can extend drug residence time on the epidermis and enhance drug penetration through the skin (Jain et al. 2010; Wavikar and Vavia 2013). These properties could be also useful to facilitate antimicrobial penetration through plant cuticles.

SLNs can be an interesting model for delivery of antimicrobials in food products, since food-grade lipids can be used in the formulation. SLNs for controlled release of nisin were prepared by HPH using Imwitor 90 as lipid base and poloxamer 188 and sodium deoxycholate as surfactant and cosurfactant, respectively. Platelet-shaped nanostructures with a mean diameter size of 119 nm were obtained, and the controlled release of nisin with effective antimicrobial activity was observed for up to 20 days (Prombutara et al. 2012).

6.3.5 Nanofibers

Nanofibers can be produced from diverse polymers specifically treated to form filaments with a diameter in the nanometer scale. Materials like polyurethane, poly (lactic acid), poly(lactic-*co*-glycolic acid), polyvinyl alcohol, silk fibroin, gelatin, and chitosan acetate are among the widely used polymeric substances used to develop nanofibers (Huang et al. 2003).

Several methods are used for production of ultrafine fibers, but the electrospinning method is the most effective and simple to produce nanofibers in quantity. In this method, an electrode is positioned in the polymer solution and the other is connected to a manifold, which is generally a metal tube or plate (static or rotating). The charge forms a Taylor cone at the tip of the needle and causes the acceleration of the polymer solution, the solvent evaporates and forms the nanofiber (Huang et al. 2003; Pham et al. 2006). The coaxial electrospinning is used to produce nanofibers with a core-shell structure. This method can be used to produce mixed nanofibers formed by a core of polyethylene oxide and a shell of chitosan (Pakravan et al. 2012) or other polymer combinations. Nanoemulsion nanofibers are formed from an emulsion prepared with an aqueous phase containing the hydrophilic polymer in an organic phase containing the polymer that makes the shell. An example of nanoemulsion nanofiber is the preparation of polyethylene oxide (core) in copolymer PEG-PLA (shell).

The quality and characteristics of the nanofiber depend on various factors such as the temperature, viscosity, surface tension of the solution, and electric field strength (Deitzel et al. 2001). Thicker fibers are often obtained with increasing polymer concentration. Under specific conditions, nanofibers with mean diameters of 20, 50, and 300 nm are obtained with polyvinylpyrrolidone concentrations of 4, 8, and 10 %, respectively. Also, the morphology of nanofibers can be altered according to the polymer concentration. Morphological differences of poly(lactic acid) nanofibers have been reported, where fibers prepared with 1 % PLA showed a smooth morphology, but a "bed-in string" morphology was observed by increasing PLA concentration to 3 %.

The nanofibers have a large diffusion potential for antimicrobials due to the high surface to volume ratio. Also, different drug-loading modes in the nanofiber surface can be obtained. Antimicrobials can be loaded on the surface of the nanofiber by simple physical adsorption generally provided by van der Waals interactions and hydrogen linkages. Alternatively, adsorption of the drug-loaded nanoparticles in the surface of the fibers can be done. An assembly as layer by layer on the cover allows a few nanometers by deposition of polyanions such as heparin (Yoo et al. 2009).

Another issue associated with the use of nanofibers is the possibility and use of surface modification methods to extend their applications (Yoo et al. 2009). Several methods of modifying synthetic polymers are used to improve the ability of drug diffusion from nanofibers. Wet chemical methods can be used, as illustrated by the treatment of PLA nanofibers with NaOH to increase the ability to bind Ca (which has been investigated for bone regeneration). Reaction of diamines with polyester nanofibers creates more loading capacity by charged surface adhesion. Plasma treatment is a technique that has been also used. For example, plasma treatment with oxygen or air can produce ammonium carboxyl or amino groups on the nanofiber surface. Another example is the treatment of PCL nanofibers with argon or air plasma to increase the number of carboxyl groups increasing the adhesion and proliferation of cells. The introduction of functional groups on the surface of nanofibers can be made by graft polymerization (copolymer). In general, UV or plasma treatment is used to generate free radicals for polymerization. One example is the development of modified polyurethane nanofibers 4-polyvinyl bromide hexylpiridinium, resulting in modified nanofibers with high antimicrobial activity against *S. aureus* and *E. coli*. Another way to obtain surface-modified nanofibers is the co-electrospinning, where the active agents are present in the polymer solution. The conjugation of the antimicrobial peptide (Ser-Glu-Glu)$_3$ terminally conjugated with polyethylene oxide resulted in a functionalized nanofiber with peptide orientation to the surface.

Application to compress for wound dressings has been a proposed utilization for nanofibers. Multifunctional nanofibers embedded with epidermal growth factor and antibiotics may provide antibacterial protection while stimulating healing (Schneider et al. 2008). Silk fibroin nanofiber incorporating the antimicrobial peptide cecropin B can reduce the counts of *S. aureus* and *E. coli* in more than 90 % (Bai et al. 2008). Surface modification of polyurethane nanofiber with quaternary ammonium resulted in a material with a highly effective antimicrobial activity, inhibiting both *S. aureus* and *E. coli* over 99.9 % (Yao et al. 2008).

Nanofibers may have also potential to delivery antimicrobials in food systems. Lysozyme, which is widely employed as a food preservative, has been encapsulated into nanofibers. Lysozyme was incorporated into poly(ε-caprolactone) and polyethylene oxide (90:10) nanofibers, and the release was 87 % over 12 days (Kim et al. 2007). Chitosan nanofibers were used to encapsulate lysozyme via cross-linked enzyme aggregates as used for continuous antimicrobial application. The nanofiber showed bacteriostatic effect after 10 cycles against *S. aureus*, *Bacillus cereus*, *Shigella flexneri*, and *Pseudomonas aeruginosa*, demonstrating that it is a promising material for antimicrobial delivery (Park et al. 2013). Bacteriocins have been also incorporated into electrospun nanofibers. The bacteriocin plantaricin 423 was in PEO nanofiber and maintained its antimicrobial activity after electrospinning inhibiting the growth of *Enterococcus faecium* and *Lactobacillus sakei* (Heunis et al. 2010).

Spasova et al. (2011) prepared nanofibrous mats containing chitosan and *T. viride* spores by electrospinning. It was observed that *T. viride* placed at 28 °C grows much faster and competes for space and nutrients against *Fusarium* sp. and *Alternaria* sp. In addition, *T. viride* produces extracellular hydrolytic enzymes, which directly attack the pathogens and destroy their cell walls. The spores incorporated into the nanofibrous mats are viable, and *T. viride* maintained its ability to inhibit the growth of phytopathogens tested.

6.3.6 Carbon Nanotubes

CNTs are a structure having potential for diffusion of antimicrobials. Two classes of CNTs are described, namely, single-walled nanotubes (SWCNTs), which consist of a single graphite sheet impeccably wrapped into a cylindrical tube with a diameter between 0.4 and 2.5 nm, and multiwalled carbon nanotubes (MWCNTs), which comprise more layers of graphite sheet with different diameters of up to 100 nm. The length of the tubes ranges from a few nanometers to a few micrometers. The unique structure of CNTs offers excellent physical and chemical properties that allow a wide range of applications (Zhang et al. 2010b). Their elevated loading capacity and ability to readily penetrate membranes represent a potential for antimicrobial delivery.

In a recent study, Chen et al. (2013) suggested that CNTs may selectively lyse the walls and membranes of human gut bacteria, depending not only on the length and surface functional groups of CNTs but also the shapes of the bacteria. The mechanism of antibacterial activity was associated with their diameter-dependent penetrating and length-dependent enfolding on the lysis of microbial walls and membranes, inducing release of intracellular components and loss of bacterial membrane potential, resulting in a complete destruction of bacteria. Thin and rigid SWCNTs show more effective wall/membrane penetration on spherical bacteria than MWCNTs. Long MWCNT may be covering around gut bacteria, increasing the area making contact with the bacterial wall. Thus, CNTs may be broad-spectrum antibacterial agents in the gut, and selective application of CNTs could reduce the potential hazard to probiotic bacteria.

SWCNTs dispersed with surfactants like sodium cholate displayed an extended antimicrobial effect and low toxicity to human cells (Dong et al. 2012). Nanocomposite films with embedded antimicrobial lysostaphin-carbon nanotube conjugates are effective to combat methicillin-resistant *S. aureus*, reducing viable counts in >99 % within 2 h (Pangule et al. 2010). This material may have important application for decontamination of surfaces, preventing the risk of staphylococci infection and biofouling of surfaces.

6.4 Conclusions

The use of nanobiotechnology in agriculture has started, and several tools are now available for development of this area. Many potential applications have been proposed, and many opportunities remain still unexplored. In the particular case of antimicrobial agents, a number of nanostructures could be useful to allow a more efficient delivery, allowing the combat of plant, food, and animal pathogens. The use of nanostructured antimicrobials could also reduce the toxicity of the chemicals used on the crops or food. Smart target-specific nanoparticles containing antimicrobial agents could be designed for use in living systems. The applied nanoencapsulated antimicrobial would be systemically distributed in the plant, avoiding phytotoxicity and/or detoxification problems. The system could be prepared to release the antimicrobial only when the nanoparticle reaches the target microorganism or just some time after application. This implies that lower doses of drugs would be necessary, since they would not be degraded by the plant and they would accumulate in the infection point due to the target delivery. Multiple ligands, such as proteins, carbohydrates, or antibodies, can be added to the surface to direct the nanoparticle toward the specific target (Suri et al. 2007).

The application of antifungal agents could be improved by encapsulation. In most cases, multiple fungicide applications are required to achieve an adequate control or significant grain yields (Salam et al. 2013). As suggested for herbicides (Pérez-de-Luque and Rubiales 2009), the nanostructured fungicide could be slowly released during the crop season, allowing a better control with a single application and with lower fungicide dose, also avoiding any further residual effect. Encapsulated fungicides could be also applied as seed coatings, preventing the multiple treatments needed for regular fungicides.

Nanoformulations may permit the utilization of antimicrobials that usually cannot be applied systemically (e.g., contact fungicides) to combat microbial plant pathogens, improving the effectiveness of each single treatment and reducing the drug amount to be delivered. In addition, substances with different modes of action could be used to develop antimicrobial nanoparticles, which could be applied separately and their contents released only after absorption. This strategy may be useful to achieve a synergistic effect, improving the efficacy of the treatment.

Nanobiotechnology can be also useful to provide plant resistance to pathogens through genetic transformation. A nanoparticle could be designed to transport DNA or RNA that stimulate defense responses in plants after being activated by an invading pathogen, allowing control before the crop was damaged (Pérez-de-Luque and Rubiales 2009). RNA encoding the activation of some resistance mechanisms, such as expression of defense enzymes or synthesis of antimicrobial compounds, could be used against pathogenic microorganisms. In addition, small interfering RNA (siRNA) has a huge potential to halt most diseases and may be used to disrupt the pathogenic processes of a specific microorganism (Rajam 2012). Despite some limitations, significant advances in the development of nanoparticles to transport siRNA have been described (Gao et al. 2010; Tam et al. 2013). This would permit an adequate siRNA delivery at infection points, targeting known genes of the microorganism involved in pathogenesis.

The application of nanotechnology in agriculture and food production may represent several benefits to improve productivity and safety against microbial contaminants. Intensive research has been devoted to improve delivery of nutrients, pesticides, herbicides, and food preservatives through nanotechnological approaches. However, some challenges need to be considered, in particular regarding risk assessments in the areas of health and environmental and socioeconomic impacts (Coles and Frewer 2013). Despite many natural nanomaterials have been safely used in traditional foods, nano-engineered materials may present specific health and/or environmental risks. A major point is associated with the poor scientific knowledge on the key factors needed for risk assessment, such as toxicity of nanoparticles, bioaccumulation, oral exposure, or the risks by ingestion. It seems therefore that additional efforts must be conducted to improve scientific research on nanotoxicology and to ensure adequate regulations for food products and crops produced using nanotechnology.

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Chapter 7
Nano-developments for Food Packaging and Labeling Applications

Yolanda Echegoyen

Abstract Nanotechnology is defined as the study and use of structures between 1 and 100 nm in length (at least in one dimension). Due to the different properties of nanosized materials compared to the bulk material, research in nanotechnology has increased exponentially in recent years. The food sector is no exception, and nanotechnology is present in different stages of the food chain, from agriculture to food processing, supplements, or food packaging. Among them, the most active area of food nanoscience research and development is food packaging.

Nanomaterials are used in packaging to improve the packaging barrier properties, to create active or intelligent packaging materials, or to enhance the properties of edible and biodegradable packaging materials. In addition to the packages itself, nanotechnology can also be used in labeling applications, like a nano-barcode. In the present chapter, all these applications and the commercialized products already in the market will be discussed.

The current legislation related to nano-developments in food packaging is at different stages in different countries, and there are concerns about the safety of the use of such nanosized materials for food packaging applications. The different studies concerning the possible migration of the nanomaterials used in the packaging to the food will be reviewed.

7.1 Introduction

Nanotechnology refers to the characterization, fabrication, and manipulation of structures, materials, and devices that have at least one dimension (or contains components with at least one dimension) in the nano-range, below 100 nm in length. Below this threshold, a particle presents chemical and physical properties very different from those of the bulk counterpart. For this reason, research in the

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nanotechnology field has increased drastically over the last two decades. Applications of nanosized materials include energy production, therapeutics and diagnostics, molecular computing, and structural materials. Among the food chain, there are many different areas in which nanotechnology can be present; nanotechnology will give new options for controlling and structuring foods adding greater functionality and value:

(a) *Agriculture*: nanocapsules for a more efficient delivery of agrochemicals as pesticides or fertilizers or for growth hormones delivery in a controlled manner (Nair et al. 2010); nanosensors for crop growth and soil conditions monitoring (Lal 2009) or for detection of animal and plant pathogens; or nanoparticles to deliver DNA to plants (Martin-Ortigosa et al. 2012).

(b) *Food processing* (Kampers 2009): nanoparticles and nanotubes can be used as gelation and viscosifying agents; meat's cholesterol can be replaced by a nanocapsule infusion of plant-based steroids; bioavailability of nutraceuticals in ingredients such as cooking oils can be improved using nanocapsules; flavor enhancers can be also nanoencapsulated; better availability and dispersion of nutrients can be achieved via nanoemulsions; or bind and remove chemicals or pathogens from food selectively by using nanoparticles.

(c) *Supplements* (Weiss 2009): cellulose nanocrystal composites can be used as drug carriers; the absorption of nutrients can be increased by nanosized powders; nutraceuticals nanoencapsulation for a better absorption and stability, or targeted delivery; nanodroplets of active molecules in vitamin sprays for better absorption; or coiled nanoparticles (nanocochleates) to deliver nutrients more efficiently to cells without affecting color or taste of food.

(d) And, of course, *food packaging applications* that will be explained in detail below.

7.2 Packaging Applications

Food packaging is used to maintain the safety and the quality of food products from production to consumers and to extend their shelf life by maintaining an adequate environment, avoiding spoilage by microorganisms, and keeping chemical contaminants, oxygen, moisture, light, etc., separated from food. In order to achieve that, packaging materials contribute both creating the physical protection and the physicochemical conditions appropriate for obtaining an adequate shelf life and maintaining food quality and safety. The packaging should prevent microbial contamination; keep the adequate moisture content depending on the type of food, and act as a barrier against permeation of gases like CO_2, O_2, H_2O, and other volatile compounds (flavors, taints, etc.). At the same time, the basic properties of packaging materials such as thermal, mechanical, and optical properties should be maintained (Singh and Singh 2005; Marsh and Bugusu 2007). Food packaging differs from other durable goods such as furniture, electronics, or home appliances because of its short shelf life and the necessary safety aspects.

Different materials are used for food packaging applications like plastic, paper and paperboard, metal, glass, or a combination of those materials, with different physical and chemical characteristics depending on the type of food. Lately, there's been a lot of effort in the development of new packaging materials that are able to keep the food quality, increase the shelf life, and at the same time have good mechanical properties and are easy to process.

One way to improve the packaging is by the addition of nanoparticles to improve characteristics such as the barrier properties to different gases, antimicrobial properties, and biodegradability, to incorporate sensors that can inform of the quality of the food, etc. Currently, the largest category of nanotechnology applications for the food sector is in food packaging materials: the global food and beverage packaging market with nanomaterials was US$4.13 billion in 2008 and has been predicted to grow to US$7.3 billion by 2014 (IR&P Inc. 2009), which represents an annual growth rate of 11.65 %.

7.2.1 *Packaging Materials with Improved Barrier Properties*

A critical issue for a food packaging material is permeability (Robertson 2012): no material is completely impermeable to water vapor, atmospheric gases, or different natural substances that can be contained within the food or in the packaging material itself. Also, different foods need different permeabilities through the packaging material; thus, fresh fruits and vegetables need a continual supply of oxygen for sustained cellular respiration and to avoid the spread of anaerobic bacteria. Plastic containers for carbonated beverages, on the other hand, must have high carbon dioxide and oxygen barriers to avoid decarbonation and oxidation of the beverage. For other products the issue is water vapor permeability. As we have seen, food products require sophisticated and different packaging functions. In addition, as the distance between producers and consumers is getting longer with the global market, the demands on the packaging industry are likely to rise.

Among the different materials used for food packaging, organic polymeric materials [polypropylene (PP), polyethylene (PE), polyethylene terephthalate (PET), polystyrene (PS), and polyvinyl chloride (PVC)] due to their low cost, ease of processing, light weight, and formability are attractive alternatives for the food packaging industry. The major problem is their inherent permeability to gases and other small molecules. It depends on their polarity and the position of the polymeric side chains, hydrogen bonding, molecular weight, cross-linking, crystallinity, and synthesis and processing methodology. No pure polymer possesses all the barrier and mechanical properties needed for every food packaging application, so usually polymer blends or complex multilayer films are used.

The option of using polymer nanocomposites as materials for food packaging is the last one proposed for solving the mentioned problems. They are created by dispersing inert nanoscale fillers throughout the polymeric matrix. Many different nanomaterials are used as fillers like silica nanoparticles (Ke et al. 2010; Bracho et al. 2012), clay nanoplatelets (Ku et al. 2004; Schuetz et al. 2011), organoclay (Ham et al. 2013; Gokkurt et al. 2013), graphene (Lee et al. 2013; Yousefi et al. 2013), polysaccharide nanocrystals (Lin et al. 2012), carbon nanotubes (Swain et al. 2013; Prashantha et al. 2009), cellulose-based nanomaterials (Floros et al. 2012; Sandquist 2013), chitosan nanoparticles (Chang et al. 2010), and other metal nanoparticles like ZnO_2 (Esthappan et al. 2013), colloidal Cu (Cárdenas et al. 2009), or Ti (Li et al. 2011).

The inclusion of nanofillers in the polymer matrix affects the barrier properties in two ways: by creating a tortuous path for gas diffusion and by causing changes to the polymer matrix itself at the interfacial regions (Choudalakis and Gotsis 2009). In the first way, due to the impermeability of the inorganic nanofillers, gas molecules can't follow a straight-line path (perpendicular to the film surface) but must diffuse around the nanoparticles. The result is a longer mean path for gas diffusion through the film in the presence of the nanofillers. It allows the manufacturer to accomplish larger effective film thickness with lower amounts of polymer.

In the second way, if the interactions between nanoparticles and polymer are favorable, the polymer strands close to each nanoparticle would be partially immobilized. As a result, the gas molecules have attenuated hopping rates between free volume holes or altered density and/or size of holes in the interfacial zones. This has been directly observed using positron annihilation lifetime spectroscopy (PALS) (Wang et al. 2007). Also, the presence of surfactants and other additives used to incorporate the nanofiller efficiently into the matrix can also modify the solubility or diffusivity of the gases. These effects in the interfacial regions are particularly important in polyolefins (Picard et al. 2007), which are polymer matrices that possess very high native gas permeability.

These mechanisms are the reasons why nanomaterials have been more successful than micromaterials as fillers for polymer composites. They have much higher aspect ratios, and the interfacial volume element is significantly greater in a polymer nanocomposite than in a polymer microcomposite from the same materials.

7.2.2 Active Packaging Materials

Active packaging relates to the incorporation of additives to the packaging systems with the purpose of maintaining or extending the shelf life and product quality. The active additives can be incorporated directly into the packaging matrix, attached to the interior of the packaging material, or introduced inside the package in separate containers such as sachets (Restuccia et al. 2010).

It provides dynamic, rather than the conventional passive, protection to the food inside and has an active role in food preservation, different from just providing an inert barrier from external conditions (Lim 2011). The additives release or absorb substances into or from the food and the surrounding environment (Brody 2001), thus promoting food preservation. Most used active packaging materials include substances that absorb ethylene, oxygen, carbon dioxide, moisture, flavors, or odors and other materials that release antioxidants, carbon dioxide, antimicrobial agents, or flavors (Vermeiren et al. 1999).

7.2.2.1 Oxygen Absorbers/Scavengers

Oxygen (O_2) is directly or indirectly responsible for the degradation of many foods. Direct oxidation reactions are responsible, for example, of rancidity of vegetable oils and browning of fruits. Food deterioration can also be produced by indirect action of O_2 due to food spoilage by aerobic microorganisms. Very low O_2 levels inside the packaging can be maintained by incorporating O_2 scavengers, which is useful for several applications.

Xiao-e et al. (2004) described the photocatalytic activity of nanocrystalline titania: UV illumination of nanocrystalline TiO_2/polymer films in the presence of excess organic hole scavengers resulted in the oxygen removal in a closed environment. According to the authors, it can be used for packaging many oxygen-sensitive products, but a major flaw is the UVA light requirement.

In another approach, different thermoplastic polymers (PET, PP, FEP, LLDPE, and nylon) have been infused with metal and metal oxide nanoparticles, and it has been discovered that films containing less than 1 wt% Pd and Pt nanoparticles were active as oxygen scavengers and reduced the oxygen flux by two orders of magnitude (Yu et al. 2004).

7.2.2.2 Antimicrobials

Different kinds of nanoparticles have been used to create antimicrobial food packaging. Among them, nanosilver is the most widespread (Rai et al. 2009), even with commercialized items already in the market. The antimicrobial properties of silver have been known since ancient times where wine and water were stored in silver vessels. Silver has many advantages over other antimicrobial agents. It has a broad spectrum and is toxic to numerous strains of fungi, bacteria, algae, and some viruses, with varying degrees of toxicity. In its elemental form, it is shelf stable for long periods of time. Silver can also be easily incorporated into many different materials such as plastics and textiles, making it especially useful for food packaging applications.

Despite the known antimicrobial properties of bulk silver, the mechanism of its activity is still unknown. There is also some controversy about the manner in which silver nanoparticles (AgNPs) are toxic to bacterial cells: some postulate that the activity comes from the Ag^+ ions detached from the surfaces of AgNPs which will act by the same mechanisms as conventional silver antimicrobials (Lok et al. 2007). However, some research shows that silver nanoparticles are more toxic than the equivalent amount of dissociated silver ion (Limbach et al. 2007). It has also been demonstrated that particles of different shapes, sizes, or other characteristics may behave differently, even in the same conditions (Eby et al. 2009). Morones et al. (2005) showed that the amount of silver ions released from silver nanoparticles in their experiment was too low to account completely for their toxicity.

Inorganic nanoparticles can be easily incorporated into the polymers to create antimicrobial nanocomposites (Althues et al. 2007), and that is one of the biggest advantages over molecular antimicrobials. AgNPs can be engineered to remain potent antimicrobial agents for long periods of time (Roe et al. 2008) due to their controlled release properties. And this makes nanosilver/polymer composites very attractive materials for food packaging. The antimicrobial activity of the silver nanocomposites is dependent on different factors that affect the Ag^+ release rate like the degree of polymer crystallinity, filler type (silver zeolites or silver nanoparticles), hydrophilicity of the matrix, and particle size.

Other nanomaterials have also been found to have antimicrobial properties, like TiO_2 (Hamal et al. 2010), MgO (Huang et al. 2005), Cu and CuO (Cárdenas et al. 2009), ZnO (Bajpai et al. 2010), Cd (Xie et al. 2011), chitosan (Lu et al. 2010), and carbon nanotubes (Kang et al. 2009).

7.2.3 Intelligent Packaging Materials

Intelligent packaging refers to those packages that allow the monitoring of the conditions and the quality of the content of the package from the production line to the consumer. Intelligent packaging can be related to the inclusion of smart labels in the package that give information about the physicochemical properties of the food or the interior of the package, like temperature, pH or different gases, chemical contaminants, and pathogens concentrations. Although a very promising area of research related to food packaging, most of the research on nanosensors or assays for analytes related to food is still in the early stages of development.

7.2.3.1 Monitoring Changes in pH

Martins et al. (2012) recently postulated the possible use of platinum nanoparticles as pH sensor for intelligent packaging. They were able to measure changes of pH by the difference in absorbance of a solution of Pt nanoparticles with sizes between 250 and 500 nm stabilized with thioglycolic acid and dispersed in water. The fraction of cluster population depended on the pH of the aqueous solution, and there was a memory effect in the response of the Pt nanoparticles to pH variations. This memory effect is not desirable for other applications but is an interesting feature for smart labels for food packaging. They suggest using an additional control sensor giving a reliable value of the actual pH, and if absorbance values read from the control and from the Pt nanoparticle sensor were different, it would be possible to conclude that the food in the package underwent a significant pH change.

7.2.3.2 Detection of Gases

However, the most promising use of nanosensors for food packaging applications is for the detection of gases. Excess of oxygen and moisture are the main causes of food spoilage. Normally, due to the necessity of the destruction of the package to analyze the vapor or gas content inside, packaged foods are tested randomly in processing facilities, typically one in every 300–400 (Mills 2005). This is costly and time consuming and yet does not ensure that unsampled packages meet the required standards (Luechinger et al. 2007). The use of noninvasive gas sensing methods based on nanoparticles permits to monitor the gas content of a package headspace easily during their whole life span.

Many studies have focused on the development of this kind of nano-based sensors with many different nanoparticles. One example of a sensor for moisture content (Luechinger et al. 2007) is based on copper nanoparticles with a carbon coating dispersed on a tensile film. The swelling of the polymer matrix in humid environments results in different degrees of separation between nanoparticles, which cause sensor strips to absorb or reflect different colors. Also, composite thin films with iron oxide nanoparticles have been used as conformable humidity sensors (Taccola et al. 2013). In this case, their sensitivity to humidity increased with increasing nanoparticle concentration.

Other authors (Lee et al. 2005; Mills 2005; Mills and Hazafy 2008) developed a photoactivated indicator ink based on nanosized TiO_2 or SnO_2 particles and a redox-active dye (methylene blue) for in-package oxygen detection. The detector changes the color (from white to blue or black) in response to even small amounts of oxygen. Quantification of the oxygen content is not possible with this sensor, but it would provide the customers an easy and visual method to detect possible fails in the seal integrity in modified atmosphere packages (MAPs). Another noninvasive method for measuring CO_2 content in MAPs has been developed by von Bültzingslöwen et al. (2002). It is based upon the lifetime analysis of luminescent dyes standardized by fluorophore-encapsulated polymer nanobeads with a wide detection range of 0.8–100 % and a resolution of 1 %.

Other gases related to food safety and quality can also be detected with nano-based sensors, like a series of electronic sensors with SnO nanobelts or $ZnO–TiO_2$ nanocomposites to detect volatile organic compounds (VOCs) like ethanol, acetone, or carbon monoxide (Comini et al. 2006a, b; Barreca et al. 2007); detection of gaseous amines (indicators of meat and fish spoilage) using fluorescence quenching of nanofibrils of perylene-based fluorophores (Che et al. 2008; Che and Zang 2009) or using conductance changes in composites of SnO_2 nanoparticles and TiO_2 nanorods (Zhang and Zhang 2008). In addition, ethylene gas (indicator of the fruit ripening) can be detected by $WO_3–SnO_2$ nanocomposites (Pimtong-Ngam et al. 2007) or nanocomposites with modified porous clay heterostructures (Srithammaraj et al. 2012).

This is a field that is growing; just during last year, many different ethanol sensors have been developed based on nanoparticles like p-Co_3O_4/n-TiO_2 heterojunctions (Liang et al. 2013), gold modified ZnO nanowires (Ramgir et al. 2013), WO_3 (Wang et al. 2013), CuO (Gopalakrishna et al. 2013), or nanocomposites (Jia and Wang 2013; Khan et al. 2013).

7.2.3.3 Detection of Other Organic Molecules

Nanosensors can also be used to detect adulterants or molecular contaminants in food. Some of the nanosensors are based on color changes that occur to metal nanoparticles solutions in contact with the analytes. For example, gold nanoparticles functionalized with cyanuric acid groups selectively bind to melamine, changing color from red to blue. Melamine is an adulterant used to artificially increase the measured protein content. The authors claim that this nanosensor can be used to precisely measure the melamine content in infant formula and raw milk at concentrations of 2.5 ppb with no more than the naked eye (Ai et al. 2009). Other approaches to the detection of melamine with gold nanoparticles with different modifications have been described by Cao et al. (2010) and Kuang et al. (2011) with slightly higher detection limits.

Other assays are based on changes in fluorescence, like the detection of gliadin (one of the primary food proteins that cause inflammation in patients with celiac disease) by enhanced fluorescence-linked immune-sorbent assay (EFLISA) using rhodamine-labeled antigliadin antibodies that are close to nanostructured silver island films (Staiano et al. 2009). Another fluorescence-based assay is able to determine cyanide in drinking water at 2 mM concentrations using gold nanoclusters (Liu et al. 2010).

Colorimetric detectors using metal nanoparticles have been developed for many other small molecules (Hong et al. 2009), proteins (Li et al. 2009), and metal ions (Wu et al. 2008; Wang et al. 2008).

Electrochemical detection is another approach for nano-based sensors with applications in the food industry. This method may be more useful for food because it avoids the matrix problem of absorption and light scattering from the different food components. Many electrochemical sensors are based on binding selective antibodies to a nanomaterial with conductive properties and monitoring the conductivity changes when the selected analyte binds to the antibodies. Glucose-sensitive enzymes coupled with gold nanoparticles can be used to measure glucose concentrations in beverages (Ozdemir et al. 2010). Many other examples of the use of nanomaterials for electrochemical detection of different biomolecules are provided by Wei et al. (2009) in a review of the topic.

7.2.4 *Edible Coatings*

Edible films are defined as a thin layer of material that can be ingested and act as a barrier to oxygen, solute movement, and moisture for the food. This material can be disposed as a continuous layer between food components or can be coating the whole food (Guilbert 1986). Edible films and coatings have many advantages over synthetic films, and for this reason, there has been many research in this area in the last years. Their main advantage over traditional synthetics is that they can be ingested along with the packaged products. If the films are eaten, packaging disposal problem is avoided, and even if the films are not eaten, as they are produced exclusively from renewable, edible ingredients can still contribute to the reduction of environmental pollution because they are supposed to degrade faster than polymeric materials (Bourtoom 2008).

The purposes of using coatings and films, depending on the food, are to hinder the migration of different components of the food or the ambient like moisture, oil, carbon dioxide, oxygen, and aromas, make the food more attractive, enhance its mechanical properties and integrity, and carry food additives or nutritional supplements (Kester and Fennema 1986; Krochta and Mulder-Johnston 1997). An edible film coating, acting as a barrier to oxygen, moisture, or aromas, can also help cut in packaging requirements and, therefore, waste. For example, a multilayer plastic package can be reduced to a single-component recyclable package if the barrier characteristics of an edible film permit to do so (Miller and Krochta 1997). The sensory characteristics of an edible film, which are of importance on its functionality, such as transparency, gloss, color sticking, and roughness can be selected depending on the purposes (Debeaufort et al. 1998). Edible films and coatings can also contain food additives to improve the general coating performance (i.e., strength, flexibility, or adherence); enhance the food texture, flavor, and color; and control microbial growth (Cuppet 1994).

In this area of food packaging, nanotechnology can be applied in two ways: On the one hand, the edible film can be a nanolaminate, manufactured as various nanolayers of diverse materials, added one at a time using the electrostatic attractions between the different layers. On the other hand, the edible film can be a nanocomposite, incorporating nanoparticles or emulsified nanodroplets containing active ingredients to enhance texture, appearance, or taste.

A nanolaminate is a really thin film with food grade composed of two or more layers with nanometer dimensions (1–100 nm per layer), where the individual laminates are physically or chemically bonded with each other. A very good control of the properties of the laminate can be obtained by a layer-by-layer (LBL) preparation of the coating (Weiss et al. 2006). This is the main advantage over conventional edible coatings used as barrier layers to increase shelf life (gums and waxes). The process of coating foods with nanolaminates consists in spraying or dipping layer by layer a series of solutions containing target compounds to the surface of the food (McClements et al. 2005). The electrodeposition technique can be used to coat very hydrophilic food systems such as precut vegetables and fruits adding antimicrobial agents or more vitamins (Vargas et al. 2008).

Nowadays, edible nanolaminates are fabricated from lipids, polysaccharides, and proteins. They are poor at protecting against moisture, although polysaccharide and protein-based films are good barriers against carbon dioxide and oxygen. On the other hand, lipid-based nanolaminates, although good at protecting food from moisture, have poor barrier to other gases and limited mechanical strength. Research is being done in identifying additives as polyols to improve them because neither lipids nor polysaccharides nor proteins deliver all of the desired properties in an edible coating. At present, coating foods that include nanolaminates involves either dipping the food into different solutions containing target substances or spraying the substance onto the food surface. Frequently, the formation of the nanolaminate is a result of the electrostatic attraction between compounds with opposite charges, and there are also various methods, which could cause adsorption. As a result, different nanolaminates might include various functional agents as antioxidants, antimicrobials, enzymes, anti-browning, colors, and flavors.

The second approach is the use of edible films with nanosized fillers. In this case, clay nanoparticles are the most important group of nanoparticles used to enhance the properties of edible films. The physical and mechanical properties of the pure polymer or conventional composites can be improved in a large scale when using a nanometer-sized dispersion of clay to create a polymer-clay nanocomposite (Rhim and Ng 2007). Nanoclay particles have been combined with both proteins (Shotornvit et al. 2009) and polysaccharides (Casariego et al. 2009; Tang et al. 2008b) to create enhanced edible films.

Also, films have been created by adding other nanoparticles, such as tripolyphosphate-chitosan (De Moura et al. 2009), microcrystalline cellulose (Bilbao-Sáinz et al. 2010), or silicon dioxide (Tang et al. 2009), to biopolymers. Using these nanoparticles, the moisture barrier properties have been improved and the microbial growth restricted. Rhim et al. (2006) studied different nanoparticles to improve the physical properties of chitosan-based films that also showed antimicrobial activity in a certain extent. The optical properties are affected to a greater or lesser extent depending on the type of nanoclay used; this has been observed in isolated whey protein-based films (Shotornvit et al. 2009) and gelatin-clay nanocomposites (Farahnaky et al. 2014) where light transmittance of the gelatin decreased with the inclusion of the nanoclay. The incorporation of other nanoparticles like porous silica-coated titania changed dramatically the appearance of whey protein-isolated films from a transparent appearance to opaque (Kadam et al. 2013).

Recently, the addition of bacterial cellulose nanocrystals to create a gelatin nanocomposite film (George and Siddaramaiah 2012) reduced the moisture affinity of gelatin, a very interesting property for edible packaging applications. The dynamic mechanical properties and degradation temperature of gelatin were also improved.

Other nanomaterials like carbon nanotubes have also been used as nanofillers in gelatin films. As studied by Ortiz-Zarama et al. (2014), the mechanical properties of gelatin were affected by the CNT addition, resulting in more elastic and harder films than the control film, but without affecting the tensile strength. The inclusion of CNT to the gelatin matrix modified appreciably the internal film morpho-structure, with no effect on its surface.

7.2.5 Biodegradable Packaging Materials

Due to their unique properties and wide range of applications, plastic has its own place in the market and our lives. Since the beginning of its mass production in the 1940s, the amount of produced plastic has been increasing steadily: more than 300 million tons were produced worldwide in 2010. The amount of plastic manufactured in only the first decade of the current century is similar to the total produced in the entire last century (Thompson et al. 2009). One of the consequences is that plastic packaging waste is an important part of municipal solid waste. There are increasing environmental concerns of the amount of plastic waste, which has resulted in the strengthening of various regulations to reduce it. Many of the oil-based polymers are virtually nonbiodegradable and, due to the complexity of the composites, are also difficult to recycle or reuse (Song et al. 2009). There are concerns about the use of nonbiodegradable packaging materials over exhausting natural resources, other environmental burdens, and also about food safety; this has led to an increasing demand for biodegradable packaging materials (biopolymers) created from renewable sources as an alternative to conventional plastics, especially for disposable applications and use in short-term packaging (Weber et al. 2002; Sorrentino et al. 2007; Siracusa et al. 2008).

Biodegradable polymers are polymers that are capable of being decomposed by bacteria or other living organisms. Biopolymers are obtained from renewable resources and are biodegradable at the end of their life. They are an environmentally friendly substitute for the use of conventional plastic materials that are nonbiodegradable and nonrenewable. They provide barrier to gas and solutes and, as the conventional polymers, can improve the quality of foods and extend their shelf life. However, there are concerns about the use of these materials related to their degradation rates under certain conditions, microbial growth potential, release of harmful compounds, or variation in their mechanical properties during storage. In fact, they have higher hydrophilicity and poor mechanical properties compared to the conventional polymers, and also poor processability, which is a major limitation for their industrial use (Cabedo et al. 2006). Carbohydrate and protein packaging films present good barrier properties against oxygen up to intermediate relative humidity and good mechanical properties; however, they have a hydrophilic nature, and so, their barrier properties to water vapor are low. Although currently many researchers are dealing with improving the biopolymer properties and there has been some success, their mechanical, physical, and thermal properties are not sufficient for the use of biopolymers in industrial applications.

Some of the problems of bio-based plastics, such as hydrophilicity, poor barrier, conductivity and inferior biocompatibility, narrow processing window, or low heat deflection temperatures, can be overcome by the use of bio-nanocomposites (Reddy et al. 2013). The rheological, thermal, mechanical, and barrier properties of the base biopolymers are improved due to the high surface area and high aspect ratio of the nanoparticles, as happened with the conventional polymers.

Nanocomposites for food packaging applications have to resist the stress of food processing (sometimes at high temperatures), storage, and transportation (Sinha Ray and Okamoto 2003; Thostenson et al. 2005). Recently, some biodegradable polymer nanocomposites with good properties for a wide range of applications have been prepared and characterized (Sinha Ray and Bousmina 2005). Layered silicate has been used as filler in biodegradable natural and synthetic polymers; this has increased their desirable properties (barrier and mechanical properties) while retaining their biodegradability in a comparatively economic way. Typical biodegradable polymers are those based on polylactic acid (PLA), polycaprolactone (PCL), polyhydroxyalkanoate (PHA), polyhydroxybutyrate (PHB), poly(butylene succinate) (PBS), starch or thermoplastic starch (TPS), and chemically modified cellulose. And the nanomaterials used to prepare the nanocomposites are different kinds of clays or organically modified nanoclays, natural biopolymers like chitosan, and different metals and metal oxides.

Biodegradability is one of the most controversial and interesting issues in the bio-nanocomposite materials. Biodegradation of biodegradable polymers may comprise many different processes like loss of mechanical properties, fragmentation, or at times degradation through the action of microorganisms such as algae, bacteria, and fungi. The degradation can be due to oxidation or to hydrolysis catalyzed by enzymes. The main advantage in the use of biopolymers is their biodegradability, so it is expected that the bio-nanocomposites retain the biodegradability rate.

Many authors have studied the degradability of different bio-nanocomposites with contradictory results. The first studies were made by Tetto et al. (1999), and they showed that PCL/clay nanocomposites showed improved biodegradability compared to pure PCL. Different posterior studies (Zhou and Xanthos 2008; Sinha Ray et al. 2003a) with a series of biodegradation tests for PLA/clay nanocomposites (using soil compost tests at 58 °C, respirometric test by measuring CO_2 evolution during biodegradation, and the molecular weight and residual weight with time) concluded that the biodegradability of PLA nanocomposite was significantly enhanced compared to neat PLA. Nieddu et al. (2009) also reported similar results of enhanced biodegradation (up to 10 times when measuring the lactic acid release or 22 times when measuring the weight change) of PLA-based nanocomposites using five different types of nanoclays and different concentrations of nanoclay using a melt intercalation method; it was found that the degradation rate depended on both variables. Paul et al. (2005) used phosphate buffer solution of PLA and PLA/organoclay nanocomposite films using three different types of organoclays, and they also found an improvement in biodegradability of the nanocomposites compared to the neat PLA. They also found that as the nanoclay used was more hydrophilic, the degradation was more pronounced. Fukushima et al. (2009) concluded that the higher biodegradability rates of the nanocomposites were due to the high relative hydrophilicity of the clays. This hydrophilicity allowed an easier permeability of water into the polymer matrix, thus activating the hydrolytic degradation process.

Also, a higher biodegradability rates have been observed for other bio-nanocomposite films compared to their biopolymer counterparts as for PHB (Maiti et al. 2007), soy protein-based nanocomposite (Sasmal et al. 2009), or nano-silica/starch/polyvinyl alcohol films (Tang et al. 2008a).

Other studies, however, present results that indicate a lower degradability rate for the nanocomposites, like for PBS/organoclay nanocomposite films after a soil compost test (Lee et al. 2002), PHB nanocomposite (Wu and Wu 2006), or PLA/chitosan/organically modified nanoclay (Sinha Ray et al. 2003b). They indicated that the lower degradability rate was due to the higher barrier properties of the nanocomposites. Other authors (Rhim et al. 2006; Someya et al. 2007; Song et al. 2009) suggest that the reduced biodegradability of nanocomposites of PBS and PHB is due not to the barrier properties but to the strong antimicrobial activity of the organoclay against food poisoning bacteria (especially Gram positive) and proposed that the antimicrobial action was due to the quaternary ammonium group in the modified organoclay. In this manner, the degradation of the polymer due to this type of bacteria was retarded, and the biodegradability rate was lower. They also suggested that the biodegradability rate could be controlled by modifying the amount of solvents used or by the incorporation of different types of organoclays modified with different types of surfactants.

According to the different research studies, nanoparticles can have two opposite effects on polymer nanocomposites: either degradation or stabilization depending on processing and environmental conditions (Kumar et al. 2009). Packaging industry can take advantage of this fact depending on the final use of the product.

7.3 Safety Concerns and Current Regulation

As we have seen, nanomaterials can serve for many new applications in the food industry, from stronger flavorings and colorings, and nutritional additives to enhanced materials with antibacterial or better barrier properties for food packaging. However, as nanomaterials have different properties compared to the bulk material, scientific concerns have been raised about the possible hazards to human health and the environment. The potential risks introduced by the use of nanomaterials for food packaging applications should be explored by the industry as a part of the development process.

Currently, there is a lack of regulation concerning nanomaterials in most of the countries. While this emerging technology is still in the research stage and benefits and risks are being discovered, there are already commercialized products with nanoparticles available in the market, generally with little or no information to the consumers; at present little is known about the extent of the use of nanomaterials in food packaging, food processing, or food products (Galland and Passoff 2011). A concerning fact, given that the human exposure to nanoparticles used in food industry is guaranteed.

Nanoparticles and other nanoscale materials have different physicochemical properties (persistence, reactivity, and bioavailability) than their larger-scale counterparts due to their much larger surface areas. Therefore, it is expected that the toxicity profile of a nanomaterial will differ from that of the bulk counterpart, thus making it difficult to draw any conclusions from known toxicity profiles; these changes in toxicity are still being discovered and are poorly characterized (Chaudry et al. 2008). In addition, many of the nanomaterials used for improving the food packaging properties are not considered food additives. For this reason, the toxicity of any material used for food packaging should be tested at the actual size used for a particular application, due to the certain human exposure and the potential environmental release.

The use of nanotechnology in food and food packaging applications has, therefore, given rise to concerns about the plausible ingestion of nanosized additives and ingredients through food and drinks and the possible hazards to consumer health. These concerns are based on many scientific studies showing the ability of free engineered nanoparticles (ENPs) to cross cellular barriers and the fact that exposure to some forms of nanoparticles induces the formation of oxyradicals and, as a consequence, produces oxidative damage to the cell (Li et al. 2003; Donaldson et al. 2004). Several studies demonstrated the toxicity of different nanoparticles to edible plants and their possible implications in the food chain. For example, nanosilver is proved to be hazardous to different kinds of cells [liver, zebrafish (Asharani et al. 2011)], and edible plants (Rico et al. 2011), producing reduced biomass in zucchini, cell wall disintegration in onion, and reduced germination and shoot length in flax or ryegrass. Different nanoparticles with diameters below 40 nm have been proved to be toxic to different crops (Rico et al. 2011), i.e., Cu nanoparticles are toxic to mung bean, wheat, or zucchini; Zn nanoparticles to radish, rape, ryegrass, lettuce, corn, and cucumber; ZnO nanoparticles to ryegrass, corn, radish, rape, lettuce, cucumber, zucchini, and soybean; CeO_2 nanoparticles to alfalfa, tomato, cucumber, maize, and soybean; Al_2O_3 nanoparticles to maize, cucumber, carrots, cabbage, and corn; and TiO_2 to maize. Other nanomaterials like single-walled nanotubes are toxic to rice and tomato, functionalized carbon nanotubes to lettuce, and multiwalled nanotubes to zucchini, lettuce, and rice (Rico et al. 2011).

Due to the safety concerns, governments in different countries have specific groups regulating the use of products containing nanoparticles for food contact applications. In the USA, the FDA (Food and Drug Administration) Nanotechnology Task Force was formed in August 2006, and their commitment is to determine regulatory approaches to facilitate the continued development of safe, effective, and innovative FDA-regulated products that contain nanotechnology materials. Their first report was published on July 25, 2007 (FDA 2007), where they decided not to adopt a precise definition for nanomaterials or nanotechnology, stating that these kinds of products did not necessarily present greater safety concerns than classes of products without nanoscale materials. In a report dated June 2011, the FDA stated that it "believes that evaluations of safety, effectiveness or public health impact of such products [containing nanomaterials] should consider the unique properties and behaviors that nanomaterials may exhibit" (FDA 2011); nevertheless, no specific guidelines for evaluating nanomaterials or products containing them were established, but rather the FDA affirmed that agencies will adhere to the Principles for Regulation and Oversight of Emerging Technologies. In their Draft Guidance for Industries in April 2012, it is stated that "there is debate regarding the appropriate testing to judge the safety of food substances where physical and chemical properties are manipulated by engineering particle size distributions in the nanometer range" (FDA 2012).

A scientific opinion was published in March 2009 by EFSA's (European Food Safety Association) Scientific Committee on nanoscience and nanotechnologies in relation to food and feed safety (EFSA 2009). In May 2011 followed a guidance document on how to assess potential risks related to certain food-related uses of nanotechnology (EFSA 2011a). However, a definition of nanomaterial is still being discussed by the European Commission after the recommendation for a definition of a "nanomaterial" for regulatory purposes (released in October 2011 (EFSA 2011b), after more than 2 years of debate) had been sharply criticized by both the chemical industry and environmental groups. The lack of a definition has undermined application of a range of EU rules, particularly the REACH (registration, evaluation, authorization, and restriction of chemicals) regulation and legislation covering cosmetics and foods. Regulation 450/29 on active and intelligent packaging states that "new technologies that engineer substances in particle size that exhibit chemical and physical properties that significantly differ from those at a larger scale, for example, nanoparticles, should be assessed on a case-by-case basis as regards their risk until more information is known about such new technology."

In Australia and New Zealand, FSANZ (Food Standards Australia and New Zealand) has been the agency in charge of the assessment of the capacity of the food existing legislation in those countries to handle any human health risks posed by nanotechnologies (Fletcher and Bartholomaeus 2011). The existing standards are used at the moment to evaluate new food substances and food packaging materials that use nanotechnologies or incorporate novel nanoscale materials. The requirements about particle size were strengthened in December 2008 in its Application Handbook. From then on, any industry must provide information on particle size, morphology, and size distribution, as well as any size-dependent properties. In those countries, there is also a lack of definition of nanotechnology or nanoscale materials.

In addition to all the regulatory issues, the acceptance of new technologies is dependent on consumer confidence. And this is particularly important in the food industry where consumer concern over health and safety in food products and food packaging is nowadays very high. The trust of the consumers in their governments and their ability to protect them from unknown hazards coming from the use of nanotechnology and nanomaterials will be of capital importance in the public acceptance of food or food-related products that incorporate or use nanomaterials. In this sense, openness on industry's part regarding the use of nanomaterials for food and food packaging applications is necessary to remove public fears about these materials. Nevertheless, a recent editorial article published in *Nature Nanotechnology* asserted that: "up to 400 companies around the world are researching possible applications of nanotechnology in food and food packaging – and many of them do not want their customers to know this" (Anonimous 2010).

7.4 Commercially Available Products with Nanomaterials for Food Packaging

Even with the lack of legislation, food and health products and food packaging materials incorporating nanomaterials or using nanotechnology are already available to consumers in some countries. Much research is being carried out about other products and applications, and some may reach the market soon. According to the current trend, it is expected that nanotechnology-derived food and food packaging products will be increasingly available to consumers worldwide in the next years. Below, there is a list of materials and containers that can be used for food packaging applications with nanosized components already in the market.

Materials with Increased Barrier Properties (Nanoclay) Due to the relative inexpensive manufacturing (Sánchez-García and Lagarón 2009), there are already various companies that have made them commercially available (Nanotechproject 2014): Nanocor™ (USA), with more than 40 issued patents, has a wide variety of polymer nanocomposites to be purchased in pellet form; there are other trademarked product lines like Durethan® (previously own by Bayer, now from LANXESS, Germany), Aegis™ (Honeywell polymers, Korea), Imperm® (ColorMatrix Corp., USA), NanoTuff™ (Nylon Corporation of America, USA), and NanoSeal™ (NanoPack Inc., USA). This kind of products has been used in the beverage industry for the fabrication of plastic beer bottles with high barriers to oxygen and carbon dioxide migration by Miller Brewing Company (specifically Miller Lite, Miller Genuine Draft, and Ice House brands), Hite Brewery, or Voridian. Also we can find a Nano flagon—Moon drunker from Top Nano Technology Co., Ltd. (Taiwan). Constantia Multifilm (USA) has in the market N-Coat, a clear laminate with, as they say, outstanding gas barrier properties that use a new, unique nano-based technology: an ultrathin coating of unknown composition applied on a polyester film.

Materials with Antibacterial Properties Among the commercial products with nanomaterials with antimicrobial properties, products with nanosilver are the most widespread. Many products are found in the American and Asian markets that claim to include silver in nanoform in their polymers such as BlueMoonGoods™ fresh box silver nanoparticle food storage containers

(BlueMoonGoods™, LLC, USA), Oso Fresh food storage containers and Nano Silver NS-315 Water Bottle (A-DO Global, Korea), FresherLonger™ miracle food storage containers and FresherLonger™ plastic storage bags (Sharper Image®, USA), Nano Silver Storage Box (Baoxianhe) (Quan Zhou Hu Zheng Nano Technology Co., Ltd.®, China), Nano Silver Baby Mug Cup (Baby Dream® Co., Ltd., Korea), and Nano Silver Salad Bowl (Changmin Chemicals, Korea). Companies like Daewoo (Korea) and Samsung (Korea) have in the market refrigerators with nanosilver embedded in the walls, and Haier YuHang (China) claims to have nanotechnology (iron nanoparticles) as insulating material in their refrigerators. Other commercially available products like the Nano Plastic Wrap from SongSing Nano Technology Co., Ltd. (Taiwan), claim the presence of zinc oxide nanoparticles with anti-mold properties that also help improving the material properties.

Carbon Nanomaterials Since 2004 the black-coated aluminum foil from Toppits (Melitta, Germany) is in the market. In this product, they claim to have carbon nanoparticles embedded in a glass matrix. This helps reducing the cooking time up to a 30 % by reaching 100 °C higher temperatures than their normal product.

7.5 Migration of Nanomaterials from Food Packaging

One of the safety concerns arisen from the use of nanocomposites for food packaging applications is the possible migration of the nanomaterials from the packaging to the food and their ingest by consumers or the release of those nanomaterials to the environment during washing or once they are discarded.

There is a theoretical study (Simon et al. 2008) on the potential migration of ENPs from packaging to food, where they evaluated the average distance traveled by nanoparticles inside a polymer matrix. Both the physicochemical properties of ENPs and polymers were taken into account. According to their results, only very small ENPs, with a radius around 1 nm, would be able to migrate through polymer matrices with low dynamic viscosity and no interaction with the nanoparticles. This would be the case of polyolefins (polypropylene and low- and high-density polyethylene) with silver nanocomposites. In the case of bigger ENPs or polymer matrices with higher dynamic viscosity like polyethylene terephthalate or polyethylene, no appreciable migration was predicted. Nevertheless, this study focused on the migration of the nanoparticles through the polymer, but didn't take into account the materials in which the nanoparticles are coating the polymer or nanocomposites with the nanoparticles homogeneously distributed in the polymer, including the surface in contact with the food or beverage.

Different authors have since then studied the migration of silver from commercially available food containers to various food simulants. Song et al. (2011) described the migration of ionic silver from polyethylene nanocomposite; migration of silver nanoparticles (seen by SEM-EDX) was found by Huang et al. (2011)

from polyethylene plastic bags and Echegoyen and Nerín (2013) from both polypropylene food containers and polyethylene plastic bags. The amount of silver migration in those studies was found to be higher in acidic simulants, by increasing temperature and time of contact and by microwave heating compared to a regular oven.

There is also a study that has determined the mineral migration (Si, Mg, Fe) from biodegradable starch/nanoclay nanocomposite films (Avella et al. 2005) to packaged vegetables. An insignificant increase in the levels of Mg or Fe was observed, but a consistent increase was observed for Si, which is the main component of nanoclay. Another work described the migration of nanosized layered double hydroxide platelets from polylactide nanocomposite films (Schmidt et al. 2011). These studies, however, deal only with biodegradable materials and not with the plastic nanocomposites containing nanoclay more likely to be used in food and drink containers, such as PE, PET, and PP.

7.6 Labeling Applications

Many areas of packaging and labeling will probably be revolutionized by the use of nanotechnology; previously impossible technical challenges to enhance products will be overcome by using nanoscale coatings, which are usually three orders of magnitude smaller than conventional inks particle size. Potential uses include invisible brand protection coding, smart labels showing changes in temperature or time, biohazard indicators for viruses or bacteria, or barrier treatments for papers. Nano-barcodes are also hypothesized to be feasible.

Radio frequency identification devices (RFID) could be used for similar objectives than the intelligent packaging with the advantage that the information on the product can be electronically transferred from the product to different devices like the refrigerator or a display in the shop. The consumers will be informed immediately of the status of a food product and when (or not) to consume it because RFID chips can detect a target molecule. The requirement of a silicon chip as a substrate for the high-frequency electronics could present a problem because it is not expected to obtain low-cost RFIDs for use with foods until at least a decade. Recently, some researchers (Jung et al. 2010) have discovered a low-cost printable transmitter that can be embedded in packaging in an invisible manner. It is based on a carbon-nanotube-infused ink for ink-jet printers. The thin-film transistors are made of ink; this is a key element in RFID tags that can now be printed on plastic or paper. They expect to replace the bar codes with this roll-to-roll technique.

Other researchers (Quin et al. 2007) have been studying how to use nanometer-sized disks of gold and nickel to encrypt information. These nanodisks can form a pattern much like a bar code, which means that each pattern would have a unique response to a stimulus, such as electromagnetic radiation or light, depending on what type of molecule (or molecules) is attached to the disks. Their small size would also allow them to be invisible to the naked eye and easily hidden in different materials or objects.

7.7 Conclusions

Nanotechnology is present in all steps of the food chain, and food contact materials including food packaging are no exception. Many nanocomposites with different nanomaterials (nanoclay, metallic and metal oxide nanoparticles, carbon nanotubes) are postulated as a way to improve the packaging characteristics: barrier to different gases, active packaging materials (oxygen scavenging, antimicrobial, etc.), edible and biodegradable materials, or smart packaging, in which sensors containing nanoparticles will inform the consumer about the properties of the food. Other area of food packaging in which nanomaterials can be present is in the use of invisible nano-labeling, with RFID chips or nano-barcodes. The scientific articles concerning the use of new nanomaterials and their applications for food contact materials are expected to continue increasing exponentially, as happened in the last decade. Many of these applications are still at an early stage of development, but the number of commercialized products containing nanomaterials will also grow in the coming years.

However, some studies have stated the toxicity of nanoparticles to different kinds of cells and crops, mainly because of the capacity of such tiny materials to penetrate cell walls. In recent works, some authors also have demonstrated the migration of nanoparticles from already commercialized food packages into food simulants. An interesting fact is that the migration was much higher when heating in a microwave oven, even for a very short period of time. In the current legislation for migration assays, the experiments are made in a regular oven, and maybe these results should be taken into account when preparing a new legislation for food contact materials containing nanoparticles.

Another concern is the presence of nanoparticles in many edible and biodegradable food packaging applications. In the first case, the nanoparticles will surely enter the gastrointestinal tract, and there are not many studies that describe their toxicity or if an accumulative effect can take place after many years of consumption. In the second case, after the degradation of the material, the nanoparticles will be released in the environment where, if no aggregation takes place, they will reach soils and waters used for food crops.

One of the factors to consider is that there is not in the world a specific legislation for nanomaterials for food or food contact materials. The development of the legislation is at different stages in the different countries, but the most important problem is the absence of a regulatory definition of nanotechnology or nanomaterial, without which regulation is very difficult. While in the European Union and in Australia and New Zealand, there are concerns about the presence of nanomaterials in food contact materials and no product will enter the market without a specific risk assessment, in Asian or US markets, there are many products available which include nanoparticles. In the current globalized market, those materials are expected to be found worldwide.

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Part II
Detection, Delivery and Treatment

Chapter 8
Strategic Role of Nanobiosensor in Food: Benefits and Bottlenecks

Semih Otles and Buket Yalcın

Abstract Nanotechnology has recently become one of the most exciting forefront fields in biosensors fabrication. Nanotechnology has been changing the area of biosensor for many kinds of fields as food. Nanobiosensor, an integration of molecular engineering, physical sciences, chemistry, biology and biotechnology, holds the possibility of manipulating and detecting molecules and atoms using nano-devices/machines, which have the potential for a wide range of both domestic and industrial applications. The role of nanobiosensors in food analysis and detection of chemical and biological compounds in food is an interesting and important area. Biosensors permit the detection of a wide spectrum of analyte in complex sample matrices and have denoted great promise in areas like food analysis. There has been a steadily growing use of biosensor technology for the detection of food contaminants such as food dyes, processing contaminants, veterinary drugs, marine toxins and mycotoxins. On the other hand, there are both some benefits and bottlenecks of nanobiosensors in food fields.

Keywords Food • Nanobiosensor

8.1 Introduction

Nanotechnology has recently become one of the most exciting forefront fields in biosensors fabrication. Nanotechnology could be defined as the creation of functional materials, systems and devices through control of matter at the 1–100 nm scale. By the way, nanotechnology is also a rapidly developing area of science and technology, which is usually interested in particles between 1 and 100 nm. This technology denotes great potential to provide new physical, chemical and biological features to materials in atomic or molecular scale (Otles and Yalcin 2013). A wide range of nanoscale materials of different compositions, sizes and shapes are now available. The enormous interest in nanomaterials is driven by their many desirable properties. Use of nanomaterials in biosensors permits the use of many

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new signal transduction technologies in their manufacture. Because of their size, nanoprobes, nanosensors and other nanosystems are revolutionizing the fields of biological and chemical analysis. Particularly, the ability to tailor the structure and size and thus the properties of nanomaterials presents excellent prospects for enhancing the performance of the bioanalytical assay and designing novel sensing systems (Viswanathan and Radecki 2008). Biosensors are used for a wide range of applications within biotechnology, engineering fields, chemistry, physical sciences, etc. There has been a steadily growing use of biosensor technology for the detection of food contaminants such as food dyes, processing contaminants, veterinary drugs, marine toxins and mycotoxins in the early 1990s. Other biosensing technologies such as piezoelectric and electrochemical have also been employed for the analysis of small-molecule contaminants (Huet et al. 2010). According to studies, more than 6,000 biosensor-related research papers were published between 1984 and 1997 (Luong et al. 2008), and the annual worldwide investment in biosensor research and development was estimated to be US$300 m (Weetall 1999). Amongst reported biosensor platforms, the optical biosensor appeared to be the most popular, and by the end of the twentieth century, surface plasmon resonance (SPR) optical biosensors had been greatly exploited with more than 75 % of associated research papers dealing with applications related to this technology platform (Homola et al. 1999). In a research of the 1999 commercial SPR-biosensor literature, it was reported that nearly 90 % of the 1,000 publications reported used Biacore instrumentation for research purposes. Other surveys performed in 2007 and 2008 reported 1,179 and 1,413 published scientific papers including the application of optical biosensors, respectively (Rich and Myszka 2008, 2010; Situ et al. 2010).

Some nanotechnological applications in the food industry are improving the bioavailability, absorption and uptake of supplements and nutrients in the body, producing new or improved tastes, and textures of foods, improving food-packaging materials, developing nanosensors that could give information about the spoilage or freshness of products during transportation, storage of food products. According to some studies, nanofood applications can be classified as (Bugusu and Bryant 2007; Otles and Yalcin 2013):

- Food quality and safety: nanosensors (nano-noses and nano-tongues) and antimicrobials (silver nanoparticles, metal oxides)
- Food packaging: biosensors, antimicrobials and nanocomposites (polymers, silicate nanoclays)
- Food processing: membrane separation systems, fractionation systems and encapsulation and delivery systems
- Food ingredients: nanoemulsions, bioactives, flavours, micronutrients and antimicrobials

Food preservation and production have become very important due to the need to store food for long periods, to prevent microbiological and chemical deterioration, pathogenic contamination and insect infestation. Food quality control is essential both for the food industry and for consumer protection. In the food industry, the quality of a product is evaluated through periodic microbiological and chemical analysis. These procedures conventionally use techniques as titration, spectrophotometry, electrophoresis, chromatography and others. These methods do not enable a rapid, easy monitoring, because they are complex analytical methods with expensive instrumentation, increasing the time of analysis, and also need well-trained operators. Currently, food analysis needs affordable and rapid methods to determine compounds that have not previously been monitored and to replace existing ones (Wagner and Guilbault 1994). An alternative to ease the analysis in routine of industrial products is the biosensors development. Biosensors are a subgroup of chemical sensors that integrate biological sensing elements with physical transducers where the interactions between target molecules and biological sensing elements are directly converted into an electronic signal (Viswanathan and Radecki 2008).

Applications of food nanotechnology might be defined under four parts. Systems (releasing of bioactive compounds and nutraceuticals, food processing and improving functional products, detecting pathogens and improving food safety and developing packaging systems) might affect product quality and shelf life in a positive way. In the last two groups, biosensors are playing an important role (Senturk et al. 2013).

8.2 Biosensors and Nanobiosensors

Biosensors are analytical devices combining a biological material, such as enzymes, cells, antibodies, etc., a biologically derived surface associated with a transducing system which can be piezoelectric, electrochemical, optical or magnetic. They have been applied to a wide range of analytical problems in food, medicine and the environment (Schaertel and Firstenberg-Eden 1988). A biosensor is an instrument combining a recognition element with a transducing device. Common to all such instruments is a support material, on which one of two affinity-pairing partners—the recognition element—is immobilized. The partners might be an antibody/antigen pair, an enzyme and its substrate, a receptor and its specific ligand or even living cells and an analyte that binds specifically to them. To detect the interaction of this pair, a biosensor uses a sensing transducer or device that converts the biological response into an electrical signal that is stored, quantified and amplified by a processor. There are many different kinds of biosensors that could be classified according to the transducing system or the biological recognition element used (Huet et al. 2010).

According to another definition, biosensors show a conceptually novel approach to on-site, real-time and simultaneous detection of multiple biohazardous agents. Samples are minimally processed, and they present rapid testing in the field setting with the option for post-analysis culture in the laboratory. Real-time detection of pathogenic contaminants is critical to the control and prevention of widespread damage from intentional or natural contamination. It offers immediate interactive information about the sample being tested, allowing decision makers to take corrective measures and to quickly recognize impending threats. Currently, no technology is available that offers a field-based real-time diagnosis of pathogenic contamination. These devices represent a promising device for food analysis due to the possibility to fulfil some demand that the classic methods of analysis do not attain (Venugopal 2002; Viswanathan and Radecki 2008).

A biosensor could be described as a transducer that incorporates a biological recognition component as the key functional element. It consists of three main components as the biorecognition element, the transducer and the signal display (Vo-Dinh and Cullum 2000). The interaction of the analyte with the biorecognition element is converted to a measurable signal by the transduction system. The signal is thus converted into a display. Biosensors are powerful tools for the analysis of biomolecular interactions in biochemical, clinical, food and environmental analyses (Hock et al. 2002). In the context of a medical setting, biosensors have the potential to offer real-time, rapid and accurate results in emergency and accident departments or at the physician's office (Conroy et al. 2009).

The "enzyme electrode" biosensor coupled glucose oxidase to an amperometric electrode for monitoring oxygen in blood (D'Orazio 2003). It was denoted that antibodies could be utilized in situ for the detection of a chemical carcinogen in a fibre optic-based immunosensor (Vo-Dinh et al. 1987). Substantially, the selectivity or specificity of the biosensor is dependent on the biorecognition element, which is capable of "sensing" the presence of an analyte (Vo-Dinh and Cullum 2000; Hock et al. 2002). Immunosensors utilizing antibody-based recognition elements have been developed on a wide range of transduction platforms for a multitude of analytes. The transducer element translates the selective recognition of the analyte into a quantifiable signal and, thus, has major influence on sensitivity (Jiang et al. 2008). Transduction approaches include electrochemical, piezoelectric and optical systems (Patel 2002; Conroy et al. 2009).

Biosensors are highly selective analytical tools, for the high selectivity of the biological recognition elements used, which have been applied in a sequence of disciplines which contain food technology, industry, environmental analysis, medicine and military. Recently, the fruit production economics allows a produce in one country and then ships it to different countries in the world (Rana et al. 2010). A food quality biosensor—analytical chemistry plays an important role—is a tool, which can transform the response into a detectable signal, frequently an electric signal, and respond to properties of food. This signal could have a known relation to the quality factor or could offer direct information about the quality factors to be measured. Nanobiosensors might be defined as biosensors, which are combined with nanotechnology by using several techniques (Otles and Yalcin 2012).

8.2.1 Nanobiosensor Usage in Food

Nanobiosensors could found many fields in food technology such as chemical, microbiological analysis of food, food quality control, new product development, enhancing the shelf life of a product, easier and faster analysis, characterization of food components as nanoscale, etc. There will be some samples about nanobiosensor usage in food.

The beta-lactam group is one of the most important families of antibiotic used in veterinary medicine in the treatment of septicaemia, pulmonary and urinary infections. The presence of penicillin residues in a food of animal origin like meat and milk might have several drawbacks: possible hypersensitivity reaction to the consumer, unfavourable microbiological effects in the dairy industry and antibiotic resistance. Maximum residue limits (MRL) were set at 4 μg l^{-1} for penicillin G, penethamate (penicillin G), amoxicillin and ampicillin (penicillin A) and at 30 μg l^{-1} for cloxacillin, oxacillin, dicloxacillin and nafcillin in milk (penicillin M) (EEC 508 and EEC 804, 1999). A biosensor-based immunoassay for the screening of penicillin residues in milk was developed. The Biacore biosensor which was used was based on SPR detection. One commercial antibody against ampicillin which had a much higher affinity for open beta-lactam ring than for closed ring was chosen. Two different pretreatments were tested prior to the biosensor assay to open the ring in order to increase the assay sensitivity. These two ways were enzymatic (penicillinase) and chemical pretreatments of the samples. Limits of detection for ampicillin in milk were 33 and 12.5 μg l^{-1} after enzymatic and chemical pretreatments, respectively. There were no cross-reactions with cephalosporins and other families of proteins or antibiotics in milk and in the buffer after both the pretreatments. Percentages of cross-reactivity with nine tested penicillins were frequently clearly higher in milk than in buffer. Furthermore, a lack of sensitivity after penicillinase pretreatment was observed. On the other hand, penicillin M and penicillin G might be detected below or at their respective MRLs (30 and 4 μg l^{-1} in milk), and amoxicillin and ampicillin might be detected only at about three MRLs after chemical pretreatment. Besides, the enzymatic pretreatment was much easier to perform and led to more stable results from the point of view (Guadin et al. 2001).

For the detection of veterinary drug residues in food, the most commonly used biological element is the antibody/antigen affinity pair, already widely used in the immunochemical screening of samples by other techniques. At this stage, the biosensor is called an immunosensor. For the detection of antibiotics, the immunosensors used frequently exploit optical or electrochemical transducer systems. The former may be amperometric, potentiometric or conductometric/capacitive. Potentiometric devices measure changes in ion concentration and pH when an antigen in a sample interacts with an antibody immobilized on an electrode. The potential difference between the electrode bearing the antibody and a reference electrode is a function of the concentration of analyte in the sample. An amperometric biosensor measures the current produced when an electroactive species is reduced or oxidized at an antibody-coated or antigen-coated electrode to which an analyte or antibody binds specifically. Capacitive or conductometric biosensors measure the alteration of the electrical conductivity in a solution at a constant voltage, caused by biochemical reactions that specifically consume or generate ions. As these transducers are generally non-specific and have a poor signal/noise ratio, they have been little used. In fact, they have not been used for the detection of antibiotics (Huet et al. 2010).

During the past four decades, there has been an increasing research on the development of lactate biosensors mainly because of the association of lactate with several severe clinical conditions. Elevated blood lactate concentration might predict multiple organ failure and death of patient with septic shock. In addition to the relevance in clinical diagnosis, the determination of lactate is very important in other areas such as food analysis and fermentation (Bakker et al. 1996; Sartain et al. 2006). The characterization and design of a lactate biosensor are described. The biosensor is developed through the immobilization of lactate oxidase (LOD) in a mucin- and albumin-composed hydrogel. The enzyme is then cross-linked with glutaraldehyde to the polymeric matrix and entrapped between two polycarbonate membranes. The hydrogen peroxide produced by the reaction of lactate and LOD is detected on a Pt electrode operated at 0.65 V versus Ag/AgCl. The performance of the biosensor was evaluated in matrixes with different amounts of albumin, mucin and glutaraldehyde. High reproducibility in the response was obtained when several biosensors were prepared with the same composition (Romero et al. 2008).

Few diseases have drawn more public and scientific attention than the transmissible spongiform encephalopathy (TSE). The infectious agent behind TSE/BSE (bovine spongiform encephalopathy) is a misshapen protein known as prion (PrPSC), typically found in the brain and spinal cord tissue (Prusiner 1982). The prion protein that causes the transmission of TSEs is formed by the post-conformational and translational change in the normal prion protein (Hope and Manson 1991; Prusiner 1991). Prions are highly stable proteins, and they are resistant to extreme conditions involved in food processing such as high temperature pasteurization, drying, freezing and radiation. An affinity-based biosensing technique was enhanced using an anti-transmissible spongiform encephalopathy monoclonal antibody to detect prion in 0.1 mol/l sodium phosphate buffer. Fluorescein isothiocyanate (FITC) labelled with a prion epitope (QYQRES) was used as a decoy for prions. Lowest detectable prion concentration was determined as 8 nmol/l in phosphate buffer. The biosensing scheme was used to probe the presence of prions in baby formula and gelatin. The gelatin interfered with the binding and the displacement reaction of the antibody, decoy and prion. Addition of sodium dodecyl sulphate (SDS) at 0.3 mg/ml to gelatin samples enabled prion detection in gelatin. The lowest detectable concentration of prion in gelatin was 0.5 nmol/l at 0.4 mg/ml gelatin. The baby formula samples produced light scattering, and the intrinsic peak of baby formula interfered with the dye peak. Serial dilutions of baby formula were done to reduce the interference. Addition of Triton X-100 at 0.454 mg/ml to the baby formula samples enabled the prion detection. The lowest detectable concentration of prion was measured as 2 nmol/l for baby formula. Gelatin, which is made from the beef bones and hides and pork skin, is used for the manufacturing of many kinds of products including food, cosmetics and drugs. The procedures used for gelatin production might reduce the infectiousness of BSE-contaminated raw material, but complete inactivation is not possible.

Similarly, in the case of baby formula, ingredients used in formulation are derived from the milk, produced by cattle. Thus, a sensitive detection technique is needed that could sense prion during the various processing steps of gelatin and that might be used as a quality measure to screen baby formula samples (Anand et al. 2005).

Ochratoxin A (OTA), a secondary fungal metabolite produced by various *Aspergillus* and *Penicillium* strains, is found to be one of the predominant contaminating mycotoxins in a wide range of food commodities like dried fruits, cereals, spices, cocoa, coffee beans, nut, wine, beer, etc. (Ahmed et al. 2007; Bennett and Klich 2003; Ghali et al. 2008, 2009; Imperato et al. 2011; Van Der Merwe et al. 1965). This toxin is highly nephrotoxic, hepatotoxic, mutagenic and teratogenic to most mammalian species (Duarte et al. 2010; Hussein and Brasel 2001). A reliable and fast sensing platform has been developed for the detection of mycotoxin OTA based on a target-induced structure-switching signalling aptamer. In the absence of a target, a fluorescein-labelled OTA aptamer hybridizes to a complementary DNA strand involving a quencher moiety, bringing the fluorophore and the quencher into close proximity for highly efficient fluorescence quenching. Upon OTA addition, a conformational change in the aptamer releases the quencher involving a DNA strand, generating a strong concentration-dependent fluorescent signal. Using this technique, the entire analysis and detection process of OTA could be completed within 1 min. Under optimized assay conditions, a wide linear detection range from 1 to 100 ng/ml was achieved with a detection limit down to 0.8 ng/ml. Additionally, the proposed assay system presented high selectivity for OTA against other mycotoxins (zearalenone and aflatoxin B_1) and limited interference from the structural analogue ochratoxin B. The biosensor was also applied to a non-contaminated corn material spiked with a dilution series of OTA, obtaining recoveries from 83 to 106 %. Utilization of the proposed biosensor for a quantitative determination of mycotoxins in food samples might offer significant improvements in the quality control of food safety through a rapid, simple and sensitive testing system for agricultural product monitoring (Chen et al. 2012).

Acetaldehyde has a common natural occurrence. It occurs in tobacco leaves and oak and is a natural component of broccoli, apples, grapefruit, coffee, lemons, mushrooms, onions, grapes, oranges, pears, pineapples, peaches, strawberries and raspberries; consumers might be exposed to acetaldehyde in heated milk, cheese, cooked chicken, cooked beef and rum. Wherever fermentation processes play a role in the production of beverages and food, the concentration of acetaldehyde rises considerably. Acetaldehyde is commonly found in alcoholic beverages as a result of the enzymatic oxidation of ethanol; it is more toxic than ethanol and plays an important role in the manifestation of alcohol intoxication. The concentration of acetaldehyde is a very important parameter because of its capacity of reacting with sulphur dioxide to form a very stable combination, therefore preventing it from exercising its antiseptic and antioxidant function. In addition to these, it is one of the earliest parameters to be noticeably affected when malfunction occurs during wine production. Due to its strong electrophilic properties, acetaldehyde is believed to induce biological changes as carcinogenesis and mutagenesis by reacting with DNA (Guru and Shetty 1990). Thus, an accurate and rapid assay for acetaldehyde determination is useful in food chemistry such as yoghurts, beer, wine, etc. Improved biosensors for acetaldehyde determination have been developed using a bienzymatic strategy, based on a mediator-modified carbon film electrode and co-immobilization of aldehyde dehydrogenase and NADH oxidase. Modification of the carbon film electrode with poly(neutral red) mediator resulted in a low-cost, reliable and sensitive NADH detector. Enzyme immobilization was performed using encapsulation cross-linking with glutaraldehyde or in a sol–gel matrix. The bienzymatic biosensors were characterized by studying the influence of cofactors, pH and applied potential. The glutaraldehyde and sol–gel biosensors indicated a linear response up to 100 and 60 μM, respectively, with detection limits of 3.3 and 2.6 μM, and sensitivities were 5.6 and 1.7 μA mM^{-1}. The optimized biosensors denoted good selectivity and good stability and have been tested for application for the determination of acetaldehyde in natural samples like wine (Ghica et al. 2007).

The nutritional significance of folate in the human diet and the forms in which it may exist in milk have been described in previous studies that formed the basis for the developments currently presented (Indyk 2010). Although supplementation of foods is currently achieved through the use of synthetic folic acid (pteroylmonoglutamic acid, PteGlu), the recently available (6S)-5-CH_3H_4-PteGlu calcium salt might become a more appropriate supplement (Houghton et al. 2006). National population strategies regarding the compulsory fortification of selected foods with folic acid are currently a controversial topic, and it seems unlikely that a common international policy will be adopted (Lawrence et al. 2009; Smith et al. 2008). An optical biosensor assay utilizing folate-binding protein was developed for a quantitation of the folate content in milk and milk-based adult and paediatric nutritional products. Samples extracted in reducing buffer were enzymatically deconjugated prior to biosensor analysis. The protein-binding assay was configured under inhibition conditions, utilizing a sensor surface functionalised with folic acid, and was subjected to single laboratory validation. Cross-reactivity of a folate-binding protein towards the dominant folate vitamers was indicated. The method was compared with both constituted high-performance liquid chromatographic and microbiological methods for a range of products. As a simple, rapid and automated technique, the method demonstrated to be an accurate and precise alternative to conventional techniques for application in routine compliance monitoring of milk-based nutritional products (Indyk 2011).

Recently, the fate and the occurrence of pharmaceutically active compounds in the environment has been recognized as one of the prevailing problems in chemistry and ecological risk assessment, as evidenced by their extensive coverage in the special issues published on emerging contaminants in soil, water, sludges and sediments (Barceló 2003, 2004), and those directly focused on the single subject of pharmaceuticals (Barceló and Petrovic 2007). There are several reviews on analysis (Kim and Carlson 2005; Díaz-Cruz and Barcelo 2005; Gross et al. 2006) and environmental risk (Heberer 2002; Boxall 2004). A recent review of the scientific literature appearing between 1998 and 2003 denoted that there were more than 350 papers on pharmaceutical residues (Stolker and Brinkman 2005). Among various pharmaceuticals, antibiotics belonging to the quinolones group, involving fluoroquinolones (FQs), are of particular environmental concern. Quinolones belong to the family of gyrase inhibitors. They denote a striking potency against enteric gram-negative bacilli, lesser activity against non-enteric gram-negative bacilli and staphylococci and usually marginal activity against anaerobes and streptococci. Quinolones have been used in veterinary and human medicine for over a decade; during this time, their entry into the environment has been continuous. In recent years, different reviews have covered, totally or partly, the subject of the analysis of quinolone residues in edible animal products (Carlucci 1998; Hernandez-Arteseros et al. 2002) or in the environment (Pico ve Andreu 2007).

Food products are complex mixtures consisting of naturally occurring compounds such as proteins, carbohydrates, lipids, vitamins, phenolic compounds, aromas and organic acids, as well as contaminants from agrochemical or veterinary treatments, packaging materials or technological processes. Quinolone analysis in those samples requires, additionally, a sensitive chromatographic method, an extraction procedure that offers suitable recoveries for the analytes and a clean-up step to remove some of the co-extracted compounds (Andreu et al. 2007). Thus, biosensors have demonstrated improved ability to monitor quinolones and other residues as cheaply and as quickly as possible and even to have the possibility of allowing on-site field monitoring. An electrochemical biosensor was described for the detection and the presumptive identification of quinolones and tetracycline in milk (Pellegrini et al. 2004). The measurement was based on the dioxide-production rate in relation to inhibition of microbial growth (*Escherichia coli* ATCC 11303). Sensitivity was satisfactory, considering that, for all tetracyclines and quinolones studied, it has been possible to detect residue concentrations equal to or below 25 lg/kg. MRLs for milk are all higher than this concentration. Besides, the most successful approach in this field has been the coupling of immunoassays with flow-through sensors, which makes possible the detection of biological interactions by an appropriate transducer in real time (Andreu et al. 2007).

The β-lactam antibiotics, including penicillins, are the most important antimicrobial substances used for mastitis treatment. Eventually, this is also the most usually occurring type of antibiotic residues in milk. Today, in addition to the traditional microbial inhibitor tests, sensitive and rapid immunoassays and receptor are used in residue control. Because of the limitations in throughput capacity of these tests, recent applications of automated biosensor technology in food analysis are of great interest. An SPR-based biosensor (Biacore) was used to design an inhibition assay to detect β-lactam antibiotics in milk. A microbial receptor protein with carboxypeptidase activity was used as a detection molecule. One advantage of using this receptor protein over antibodies that are more usually used is that only the active, intact β-lactam structure is recognized, whereas most antibodies detect both active and inactive forms. In the presence of β-lactam antibiotics, the formation of a stable complex between receptor protein and antibiotic inhibits the enzymatic activity of the protein. The decrease in enzymatic activity was measured using an antibody against the degraded penicillin G, and substrate in milk samples was quantitatively determined. The limit of detection of the assay for penicillin G was determined to be 2.6 μg kg^{-1} for antibiotic-free producer milk, which is below the European MRL of 4 μg kg^{-1}. The coefficient of variation at 4 μg kg^{-1} penicillin G ranged between 7.3 and 16 % on three different days (Gustavsson et al. 2002).

8.3 Benefits

Biosensors are an inexpensive, portable and suitable analytical tool, but a required sample preparation step prevents its use as a field device. One solution to this challenge is the development of bioanalytical microsystems, in which sample preparation and biosensing systems modules could be integrated on the same platform. Thus, they permit the design of easy-to-use and portable analytical tools. In addition, the combination of research in microfabrication, nanofabrication technology and material science will become an excellent resource for the development of suitable sample preparation steps, as extraction, concentration and isolation. It might also play a major role in the improvement of the transducer biorecognition element interface. Under environmental conditions, biosensors repeated use with complex sample matrices and long-term storage is a remaining challenge. Maybe the solution to this problem would be found with inexpensive microbiosensors designed for single use in order to avoid deterioration of the biosensor elements in complex matrices. Moreover, novel material research might help to enhance the situation. The researches about biosensors are also a faster and practical method to produce high-quality and controlled products.

Nano-sized bacterial pores might be used as a simple, fast, effective and selective way to detect the structure of peptides at the single molecule level. It is a promising candidate as a nanobiosensors' component designed to detect single molecules. Aerolysin also denoted nice performance according to its usage on single peptide molecule detection. According to this point, the foreground of bacterial nanopores in a nanobiosensor scope is quite broad with the possibility of finding some other kinds of bacteria that might play a role as an appropriate component of a nanobiosensor. In addition to this, food materials increased surface areas by nano-grinding (size reduction operations), so that these materials would be used as small as possible to get the same benefits from a macro or bigger size, and they are also more efficient. Mainly, biosensors could be an important alternative to the traditional methods for the detection of pathogens and toxins in food.

Nanosensors are not only useful for the quality control of flavours and freshness of products that consumers are able to purchase, but it also has the potential to reduce the frequency of food-borne illnesses and improve food safety. Such technology would obviously benefit consumers, food regulators and industry stakeholders. Packaging industry has been denoted great development because of nanosensors which are used for detection of microbiological degradation and toxic substances quickly and effectively and so prevent consumption of spoilage or contaminated products (Senturk et al. 2013; Qureshi et al. 2012; Duncan 2011).

8.4 Bottlenecks

The dilemma about food nanotechnology is still going on. There should be more studies about behaviour of food materials, while the nano size grinding. Moreover, there should be toxicological studies. Exposure to nanomaterials as a result of nanotechnologies being used in the food industry could take three main routes which are ingestion, dermal contact and inhalation. The concern is that nanoparticles with large reactive surfaces could cross biological barriers to reach those parts of the body. Studies denote the potential of nanomaterials to cause DNA mutation and cause major structural damage to mitochondria, even resulting in cell death. There is limited scientific evidence about the risks to people being exposed or the potential hazards that may exist to the newly developed nanotechnological products. Usually, the impact of nanoparticles on the body, such as nanotoxicity, depends on the properties like mass, particle size, surface properties, chemical composition and how the individual nanoparticles aggregate together. Even though there are various studies about toxicity of nanoparticles, it should be pointed out that the results are frequently obtained for only one size and type of nanoparticles. Furthermore, test animals are usually exposed to high concentrations under artificial conditions. This restricts the use of obtained data for risk assessment. Extrapolation from one size to another or from one type of nanoparticles to another is on the basis of present knowledge still impossible (Blasco and Pico 2011; Bouwmeester et al. 2008; Cushen et al. 2012; Senturk et al. 2013).

8.5 Conclusion

Future use of biosensors in the food area, the needs of the sector (imaging sensors, sensor networks to create, provide multi-analyte detection, product quality improvement, new product development, characterization of food components in the nanoscale) must meet. Food samples could give significant improvements in quality control of food safety through a rapid, simple and sensitive testing system for agricultural products monitoring, and also, there should be clearness about the nanoscale materials' toxicological effect on the food and food-related components.

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Chapter 9
Emerging Role of Nanocarriers in Delivery of Nitric Oxide for Sustainable Agriculture

Amedea B. Seabra, Mahendra Rai, and Nelson Durán

Abstract The endogenously found free radical nitric oxide (NO) has important roles in several aspects related to plant defense and growth. NO is a signaling messenger in animals and plants due to its particular chemistry, as uncharged and small molecule, relatively lipophilic. In recent year, important papers have been describing the advantages of using NO donors in agriculture. Indeed, administration of NO donors to plants is reported to stimulate plant greening and germination, control iron homeostasis, and improve plant tolerance to metal toxicity, salinity, drought stress, and high temperatures. Low molecular weight NO donors are known to be thermally and photochemically unstable, impairing their applications in agriculture. In this context, the combination of NO donors with nanomaterials has been emerging as a promising approach to optimize the beneficial effects of NO in plants. In spite that nanomaterials have been employed to carry agrochemicals in plants, the combination of NO donors and nanomaterials is yet not deeper explored in agriculture. In this scenario, this chapter highlights the advantages of applications of NO donors in plants, the uses of nanotechnology in agriculture, and the necessity to develop new strategies based on the combination of NO and nanomaterials in agriculture.

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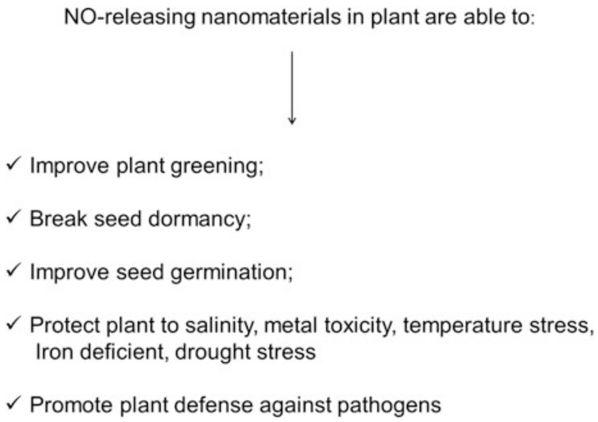
9.1 Introduction

Nitric oxide (NO) is an endogenous molecule responsible for controlling important physiological processes, such as control of blood pressure, immune responses, antioxidant activities, inhibition of platelet adhesion, promotion of wound healing, and neurotransmission, among others (Ignarro 2000; Seabra et al. 2004, 2007, 2008, 2010; Amadeu et al. 2007, 2008; Simplício et al. 2010). Besides humans and animals, NO is also an important signaling molecule in plants (Wendehenne and Hancock 2011). The actual role of NO in plants is mainly as a key regulator of cellular functions, similar to those observed in animals (Beligni and Lamattina 2000; Besson-Bard et al. 2008; Ferreira and Cataneo 2010). NO is a chemical messenger in plant biology and an important regulator of many physiological events, mainly in response to biotic and abiotic stress (Shi et al. 2012). Physiological processes such as pollen tube growth and senescence, induction of leaf cell death, wounding, root growth, seed germination, cell expansion, stomata closure, biotic and abiotic stresses were demonstrated to be depended to NO (Siddiqui et al. 2011; Baudouin 2011; Gupta et al. 2011c; Lin et al. 2011, 2012; Corpas et al. 2011). NO in plants is produced by hydroxylamine-mediated NO production and also by enzymes such as plasma membrane-bound nitrite reductase, cytosolic nitrate reductase, xanthine oxidoreductase, and nitric oxide synthase-like enzyme (Gupta et al. 2011a, b).

As discussed previously, the generation of NO has been extensively discussed; however, NO generation from polyamines, hydroxylamine, and especially arginine is still not fully elucidated, probably due to the lack of identification of appropriate genes, mutants, or proteins. Gene NIA1 (nitrate reductase [NADH]) has shown to be the most abundant source of NO, despite some functional redundancy with NIA2 (nitrate reductase 2; nitrate reductase (NADH)/nitrate reductase). Even when NIA2 is still functional, the *nia1* mutant exhibits reduced NO production. Probably in the other possible mechanisms, difficulties with lethality, functional redundancy or their activation under normoxia and hypoxia, could be an explanation for the fact that no generation mutants have been isolated so far (Mur et al. 2013).

Differently from biomedical applications, the utilization of NO donors in agriculture is a relatively new approach. Agricultural applications of these NO donors (e.g., small molecular weight NO donors), such as sodium nitroprusside (SNP) and *S*-nitrosothiols (RSNOs), have been used for improving plant defense and growth (Ederli et al. 2009; Seabra et al. 2013; Shi et al. 2014). In the case of the rice catalase mutant *nitric oxide excess 1* (*noe1*), higher levels of H_2O_2 induced the generation of NO, suggesting that NO acts as an important endogenous mediator in H_2O_2-induced leaf cell death and generation of reactive nitrogen species (RNS). Studies showed that RNS and reactive oxygen species (ROS) are important partners in plant leaf cell death. A recent review reported the progress on H_2O_2-induced leaf cell death and the interference of RNS and ROS signals in the plant hypersensitive response (HR), leaf senescence, and leaf cell death triggered by diverse environmental conditions (Wang et al. 2013).



NO-releasing nanomaterials in plant are able to:

- ✓ Improve plant greening;
- ✓ Break seed dormancy;
- ✓ Improve seed germination;
- ✓ Protect plant to salinity, metal toxicity, temperature stress, Iron deficient, drought stress
- ✓ Promote plant defense against pathogens

Scheme 9.1 Advantages of administration of exogenous NO-releasing nanomaterials in agriculture

In the past, *S*-nitrosylation has been the most studied NO-dependent regulatory mechanism. In summary, *S*-nitrosylation is a redox-based mechanism for cysteine residue modification and is being recognized as ubiquitous regulatory reaction comparable to phosphorylation. Then, it is emerging as a crucial mechanism for the transduction of NO bioactivity in plants and animals (Romero-Puertas et al. 2013). Recently, a mini-review reported an overview on *S*-nitrosylation of target proteins related to hormone networks in plants (París et al. 2013).

Till now, a few reports on the combination of NO donors with nanomaterials in agriculture have been available. In this context, the present chapter will discuss the importance of administration of exogenous NO donors in plants, the impact of nanotechnology in agriculture, and the necessity to develop new strategies based on NO-releasing nanomaterials in agriculture. Therefore, we hope that this chapter will open new perspectives in the field of nanotechnology, NO, and agriculture. Scheme 9.1 highlights important physiological processes in plant that can be modulated and improved by treating plants with NO-releasing nanomaterials.

9.2 Role of Exogenous NO Donors on Plants

NO donors are extensively used in animals in a great variety of biomedical applications (Seabra and Durán 2010, 2012; Carpenter and Schoenfisch 2012). However, in plants, its use is very restricted to few reports. Application of NO donors in plants might result in several advantages for agriculture, and it needs to be more explored in the near future. This section highlights the beneficial aspects of applications of exogenous NO donors in several aspects of plant growth and defense.

9.2.1 Exogenous NO Donors Acting on Dormancy and Germination of Seeds and Plant Greening

The delayed germination until optimal environmental conditions is named dormancy (Bewley 1997; Arc et al. 2013). Positive effects of application of NO-generating compounds such as nitrate, nitrite, sodium nitroprusside (SNP), and acidified nitrite were reported on dormancy release and germination of *Amaranthus retroflexus* L. In this case, NO scavenger inhibited the stimulatory effects of exogenous NO-generating compounds on germination of seeds (Liu et al. 2011). On the contrary, the break of deep dormancy of apple seeds upon treatment with NO donors (SNP or acidified nitrite) or HCN was reported (Gniazdowska et al. 2010). An increase of germination (over 60 %) after treatment of apple seed with NO donors and HCN was also reported. In this case, an improvement in the plant greening by increasing chlorophyll content in seedlings was reported (Gniazdowska et al. 2010). Similar result with the application of SNP in barley seedlings increased chlorophyll, and with NO scavenger decreased chlorophyll concentration by ca. 30 % (Zhang et al. 2006; Gniazdowska et al. 2010). These effects were related to the induction of ROS formation upon NO treatment, since ROS are important in the protection of embryo against pathogens (Gniazdowska et al. 2010; Seabra et al. 2013).

It is known that imposition of salt stress significantly reduced chlorophyll content, photosynthetic rate, transpiration rate, and altered different chlorophyll fluorescence attributes in plants of rice cultivars. The use of sodium nitroprusside (nitric oxide donor) on pre-sowing seed was effective in improving chlorophyll content, gas exchange, and chlorophyll fluorescence attributes (Habib et al. 2013).

Recently, results showed that the inhibitory effect of NO on the embryo germination was relieved by the sodium nitroprusside (SNP) pretreatment under light. However, the SNP pretreatment did not influence the inhibitory action of 2,5-norbornadiene (NBD) to embryo germination. The SNP pretreatment increased the chlorophyll content in leaves of seedlings and also ROS; however, this effect was canceled by 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO) treatment. Relationships between RNS-mediated (NO-dependent) dormancy removal and ROS accumulation in the embryo germination are clearly discussed (Ling et al. 2013).

9.2.2 Exogenous NO Donors on Plant Iron Deficiency

As iron is an important element for plant nutrition, its deficiency can cause metabolic disorders reducing chlorophyll concentration, impairing photosynthesis and plant respiration, and reducing plant growth (Ramirez et al. 2011). Administrations of NO donors, such as SNP, *S*-nitroso-*N*-acetylpenicillamine (SNAP), or gaseous NO on iron-deficient maize plants, prevented interveinal chlorosis

(Graziano et al. 2002). Recently, administration of SNP on the leaves of peanut (*Arachis hypogaea* Linn) iron-deficient plants enhanced plant growth and decreased leaf interveinal chlorosis (Zhang et al. 2012). Moreover, administration of NO donor increased the activities of root Fe III reductase and antioxidant enzymes and reduced malondialdehyde content (Zhang et al. 2012). Overall, NO might act as a signaling molecule in the regulation of physiological adaptive change in plant growth under iron-deficient conditions.

Recently, the relation between AtHO1 (encodes heme oxygenase-1 in *Arabidopsis*) on the catalysis of the cleavage of heme to biliverdin yielding iron and carbon monoxide (CO) was examined (Li et al. 2013). Indeed, NO is known to regulate iron homeostasis in plants. AtHO1-overexpressing plants generated high concentrations of NO; however, knockdown of AtHO1 expression decreased plant levels of NO. The NO scavenger 2-(4-carboxy-2-phenyl)-4,4,5,5-tetramethylinidazoline-1-oxyl-3-oxide (cPTIO) decreased AtHO1-induced ferric-chelate reductase (FCR) activity. In the case of iron-sufficient and iron-deficient conditions, application of SNP induced FCR activity in the *hy1* plants. The authors suggested that AtHO1 was involved in iron homeostasis in an NO-dependent manner (Li et al. 2013).

Recently, a novel insight into the effect of application of different concentrations of SNP on the nitrosative status and nitrate metabolism of mature and senescing *Medicago truncatula* plants showed that higher concentrations of SNP resulted in increased NO content, cellular damage levels, and augmentation of ROS concentration. Senescing in this plant demonstrated greater sensitivity to SNP-induced oxidative and nitrosative damage probably to iron deficiency. This fact suggests a developmental stage-dependent suppression in the plant's ability to survive with free oxygen and nitrogen radicals (Sivitz et al. 2012; Antoniou et al. 2013).

9.2.3 Exogenous NO Donors in Plants Tolerance to Salinity

Some interesting reports describing the beneficial effects of administration of NO donors on plants exposed to salinity stress were recently published. Corpas et al. (2011) reported the stimulation in the expression of plasma membrane H^+-ATPase of calluses of reed under the addition of high NaCl concentration and treatment with SNP. The authors observed an increase in the K^+ and Na^+ ratio, and the authors proposed that NO acts as a signal-inducing salt resistance (Corpas et al. 2011). Moreover, application of SNP enhanced seed germination in wheat and protected plant against mitochondrial oxidative damage caused by high salinity. The addition of NO donor also decreased the release rates of malondialdehyde (MDA), hydrogen peroxide, and superoxide anions by mitochondria (Zheng et al. 2009). A recent paper reported an increase in the Na^+ secretion per leaf of *Limonium bicolor* treated with high NaCl concentration combined with SNP for 20 days (Ding 2012).

Calcium chloride ($CaCl_2$) and/or SNP-acting mustard leaves improved the activities of nitrate reductase (NR) and carbonic anhydrase (CA), and also leaf chlorophyll (Chl) content, leaf ion concentration, and leaf relative water content (LRWC), in comparison with leaves treated with only NaCl. Increased levels of H_2O_2 and damages to membranes are caused by salinity stress, which led to lipoperoxidation.

Alleviation of salinity stress is achieved by enhanced antioxidant enzyme activities due to the presence of SNP and $CaCl_2$. This effect was eliminated in the presence of cPTIO, indicating that SNP in association with $CaCl_2$ improves plant tolerance to salinity stress by increasing antioxidative defense system, ionic homeostasis, and osmolyte accumulation (Khan et al. 2012).

Exogenous administration of SNP enhanced growth in seedlings of cucumber particularly leaves and roots under NaCl stress (e.g., increased plant height, stem thickness, fresh weight, and increased dry matter accumulation). Since the level of free polyamines and the activity of polyamine oxidase (PAO) in cucumber seedling leaves and roots initially increased under salinity stress, after the treatment, values of spermine + spermidine/putrescine (Spd + Spm)/Put also decreased under NaCl stress, in comparison with the control group. Improvement of plant tolerance to salinity was shown to be dependent to the high (Spd + Spm)/Put value and to the accumulation of Spm. The authors suggested that the mechanism by which NO enhanced the cucumber seedlings tolerance to salinity stress involves the regulation of the content and proportions of different types of free polyamines (Fan et al. 2013).

9.2.4 Exogenous NO Donors in Plants Tolerance to Metal Toxicity

Studies of metal toxicity in plants indicate that the plants have developed various extra- and intracellular defense mechanisms to fight against heavy metal toxicity, such as preventing the entry of heavy metals to root cells (e.g., mycorrhizal association). Or if enter the second line of defense is activated, which includes binding of metal ions with cell wall or plasma membrane, use of phytochelatins and metallothioneins or sequestration in vacuole. The multidisciplinary approach combining plant physiology, soil microbiology and biochemistry, genetic engineering, as well as agricultural and environmental engineering will help in optimization of plants for heavy metal detoxification (Dalvi and Bhalerao 2013; DalCorso et al. 2013).

Important metal ion chelators are phytochelatins, which can be found in plants and also in some fungi and invertebrates. Phytochelatins have key role in metal tolerance, and they received much attention in phytoremediation programs. Phytochelatins are oligomers of glutathione (GSH, characterized by the general structure (γ-Glu-Cys)n-Gly and synthesized by phytochelatin synthase, that is activated in the presence of metal ions). The phytochelatin complexes with ions are formed in the vacuole, thus decreasing the toxic effects of metals. Sulfur-deficient plants may switch to non-sulfur-based tolerance mechanisms, which often involve proline. However, phytochelatins appear to be essential for metal tolerance (Zagorchev et al. 2013).

Many studies are indicative of positive effects of NO donors on metal toxicity. They acted on alleviating manganese (Mn)-induced stress in rice leaves; however, the effect was reversed by the addition of a NO scavenger (Srivastava and Dubey 2012). Addition of SNP reversed the inhibitory effect of nickel on the growth of wheat (*Triticum aestivum* L.) in a dose-dependent manner through increased activities of antioxidant enzymes and the content of reduced ascorbate and glutathione (Wang et al. 2010). A similar paper reported the augmentation of antioxidant enzymes and suppression of lipid peroxidation due to administration of SNP to *Artemisia annua* stressed by boron and aluminum (Aftab et al. 2012).

Administration of SNP, a NO donor, efficiently alleviated copper toxicity effects. The Cu-induced NO in vascular bundles was associated primarily with the presence of the induced NADPH-diaphorase (NADPH-d) activity. These results suggested that NOS-like enzyme, but not the nitrate reductase, was the source of inducible NO generation in roots of *Vicia faba* under Cu stress (Zou et al. 2012).

9.2.5 Exogenous NO Donors in Plants Tolerance to Temperature Stress

It is known that plants possess a number of adaptive, avoidance, or acclimation mechanisms to survive with high-temperature environments. It is believed that the major tolerance mechanisms are those employing ion transporters, proteins, osmoprotectants, antioxidants, and factors involved in signaling cascades and transcriptional control. These mechanisms are activated to offset stress-induced biochemical and physiological alterations. Genetically, high temperature induced gene expression and metabolite synthesis that also substantially improve tolerance (Hasanuzzaman et al. 2013). SNP administrated in wheat (*T. aestivum* L.) stressed by high temperature increased activities of antioxidant enzymes and decreased lipid peroxidation (Bavita et al. 2012). Similar results were obtained by addition of the SNP or *S*-nitroso-*N*-acetylpenicillamine (SNAP) on the callus of reed under high temperature. An increase of activities of antioxidant enzymes was also found suggesting that NO may act as a signal molecule in defending plant against oxidative injury caused by heat stress (Siddiqui et al. 2011).

Exogenous NO (SNP) alleviated heat damage at the reaction center and electron transport from oxygen-evolving complex (OEC) to encoded peptides of *psbA* gene (D1 protein) in tall fescue (*Festuca arundinacea*). The results suggest that NO may improve the recovery process of photosystem II (PSII) by the upregulation of the transcription of PSII core protein, which leds a different strategy of the protective role of NO in plant PSII against heat stress. As an important strategy to protect plants against heat stress is probably based on the fact that NO could improve the recovery process of PSII by the upregulation of the transcriptions of genes encoding PSII core proteins (Chen et al. 2013).

9.2.6 Exogenous NO Donors in Plants Tolerance to Drought Stress

Application of NO donors might also enhance plant resistance to drought stress by decreasing water stress, enhancing stomatal closure, and reducing transpiration and ion leakage (García-Mata and Lamattina 2001). Addition of SNP on drought-stressed wheat seedlings increased activities of antioxidant enzymes, reduced oxidative damage, enhanced photosynthesis rate, and lowered water loss (Tan et al. 2008). These protective effects of SNP were inhibited by the addition of a NO scavenger. All these results show that administration of NO donors might have beneficial effects on plant growth and defense, mainly by increasing activities of antioxidants enzymes and decreasing damages induced by ROS. However, the majority of the papers are based on the use of free SNP.

NO and thidiazuron (TDZ) have been shown to extend the postharvest life of a range of flowers. Postharvest storage of flowers can be considered similar to drought stress due to water imbalance. NO plays an important role as signal molecule in plant growth and development. SNP and TDZ activities on vase life, flower diameter, relative water content, and electro leakage during postharvest of cut lilium flowers were studied. After treatment, the results showed that the use of TDZ increased flower longevity and water relative content. Treatment cut flower with SNP also increased water relative content and flower diameter and increased vase life (Kaviani and Mortazavi 2013).

Similar results were observed with NO donor (SNP) on vase life, flower diameter, relative water content, and electro leakage during postharvest of cut rose flowers (*Rosa hybrida* cv. ‘Sensiro’). Flowers were treated with SNP and then hold in the solution including a stabilizer such as 8-hydroxyquinoline (8-HQS). The results showed that treatment cut flower with SNP increased water relative content, flower diameter, and vase life (Talebi et al. 2013).

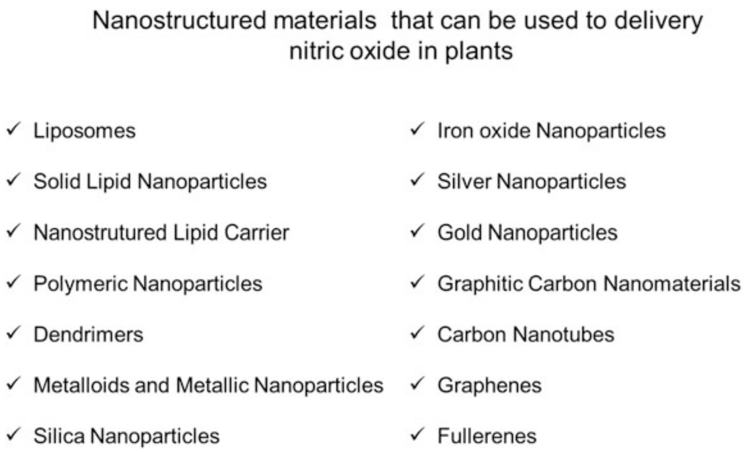
Taken together, the administration of exogenous NO donors in plant has several beneficial effects, as reported in this chapter. However, to the best of our knowledge, the combination of small molecular weight NO donors with nanomaterials has not been explored in agriculture. In contrast, combination of nanomaterials with NO donors has been extensively explored in biomedical applications (Seabra and Durán 2010, 2012; Seabra 2011). Therefore, the use of NO-releasing nanomaterials in agriculture might find important impacts.

9.3 Nanotechnology in Agriculture

The application of nanotechnology in agriculture is a relatively new approach, and it has been increasing in the last decade (Savithramma et al. 2012; Durán and Marcato 2013; Riccio and Schoenfisch 2013). Many advantages are possible to reach, such as efficient and safe delivery of fertilizers, pesticides, and herbicides. Nanoencapsulation of these agricultural defensives can lead to a better storage and controlled release of the agrochemicals directly to the target site of application (Ditta 2012; Grover et al. 2012). It is clear from the literature that these nanomaterials have a great potential to avoid degradation of the agrochemicals in the environment, reduce the doses to be applied, decrease costs, and control excess chemicals (Ditta 2012; Grover et al. 2012). Scheme 9.2 highlights some interesting nanostructure platforms that have a great potential to be used as vehicles to carry and to deliver NO in agriculture. This chapter will present and discuss each nanomaterial.

9.3.1 *Liposomes*

Many applications of liposomes have been described as model membranes of plant organelles (e.g., plant aging, drying, and freezing tolerance against toxins, pesticides, etc.) (Taylor et al. 2005). An interesting property of these lipid vesicles is to



Nanostructured materials that can be used to delivery nitric oxide in plants

✓ Liposomes
✓ Solid Lipid Nanoparticles
✓ Nanostrutured Lipid Carrier
✓ Polymeric Nanoparticles
✓ Dendrimers
✓ Metalloids and Metallic Nanoparticles
✓ Silica Nanoparticles
✓ Iron oxide Nanoparticles
✓ Silver Nanoparticles
✓ Gold Nanoparticles
✓ Graphitic Carbon Nanomaterials
✓ Carbon Nanotubes
✓ Graphenes
✓ Fullerenes

Scheme 9.2 Some interesting nanostructure platforms that have a great potential as vehicles to deliver NO in agriculture cross through the plant cuticle due to their amphiphilic properties (Wiesman et al. 2007). Huang et al. (2009) have reported that cationic liposomes are able to efficiently deliver NO to cells, which are readily incorporated into cellular membranes, suggesting its good potential for use in plants. The kinetics of NO release from liposome nanoparticles is well known. It is also known that encapsulation of NO into these nanoparticles was responsible to preserve NO bioactivity by avoiding its inactivation caused by hemoglobin scavenging (Huang et al. 2009). Koehler et al. (2008) described NO donor *S*-nitroso-*N*-acetylpenicillamine encapsulation within this vesicle.

NO donors have the advantages that the NO release can be photo-stimulated and dynamically modulated at the biological levels. Probably, this quality can be used in plants, since the rates of NO release from a nanomaterial can be induced and accelerated by light. Considering all these facts, it is possible to conclude that these materials can find practical applications in plant science.

9.3.2 Nanostructured Lipid Carrier and Solid Lipid Nanoparticles

Essential oil-loaded solid lipid nanoparticles (SLN) were useful for the formulations of nano-pesticides (Lai et al. 2006). Nanostructured lipid carriers (NLC) were prepared by combining hot homogenization and sonication with different ratios of corn oil (liquid lipid) and beeswax (solid lipid). Compared to SLN, the incorporation of corn oil gave a higher payload, slower release rate, and higher photo-protection for deltamethrin (Nguyen et al. 2012).

9.3.3 Polymeric Nanoparticles

There are several important polymers that are used as carriers for many activities in different areas. The following polymers are used in nanotechnology: chitosan that is a polysaccharide; polyesters, such as polylactic, poly(e-caprolactone), and poly (lactic acid-co-glycolic acid) (Marin et al. 2013); and dendrimers that is a polymer in which the atoms are arranged in many branches along a central backbone of carbon.

9.3.4 Chitosan Nanoparticles

The antimicrobial properties of chitosan are well known (Trotel-Aziz et al. 2006). One of the important profiles of these materials is their biodegradability and biocompatibility that enhance the transport of active polar compounds across epithelial surfaces in several biological applications (Zhukovskii 2008). Another important property is their mucoadhesivity, facilitating the transport of this active compound across cellular membranes. In special, this quality implies great potential for use in the agrochemical industry (Prashanth and Tharanathan 2007). Chitosan can be functionalized through diethylenetriamine and propionic aldehyde in order to modify the primary amine groups on this polymer. The diazeniumdiolates (chitosan/NO adducts) were prepared by suspending the chitosan derivatives in a sodium methoxide–methanol solution under NO gas. Diethylenetriamine-modified chitosan results in high NO loadings due to an increase of the nucleophilic sites (Gao et al. 2008). Therefore, the combination of NO and modified chitosan may find several applications in plants.

A combination of alginate/chitosan nanoparticles has been prepared as a vehicle to deliver *S*-nitrosoglutathione (GSNO) as the NO donor. Positive and negative surface charges on alginate/chitosan nanoparticles were obtained by changing alginate–chitosan ratios. Kinetics of NO release from encapsulated GSNO showed that nanoparticles decrease the rates of NO release, at physiological temperature, in comparison with free GSNO. An important observation in this system was that cytotoxicity for fibroblast V79 cells was not observed. These results showed the great potential of NO donor in plants (Seabra et al. 2012; Marcato et al. 2013).

A series of chitosans with different degrees of acetylation and molecular weights were reacted with gaseous nitric oxide (NO) to yield $[\text{NONO}]^-$ groups. Heterogeneous reaction of NO with NH_2 groups present in chitosan was shown to be dependent to the crystalline form of chitosan. Total NO release exhibited a bell-shaped distribution at different degrees of acetylation (Wan et al. 2010).

9.3.5 Polyester Nanoparticles

Fungicides and organic wood preservatives incorporated into wood products can reduce wood decay. This can be achieved by the use of biocide-containing polyester nanoparticles (Liu et al. 2002, 2003). In this way, nanoparticulate polyester formulations have an important potential as novel agrochemicals with high specificity and improved functions. The first tunable NO-releasing polymeric microparticles were reported by Parzuchowski et al. (2002). Encapsulated NO prodrug diethylenetriamine/NONOate into poly(lactic-co-glycolic) acid (PLGA) microparticles was responsive to protect the prodrug from dissociation under acidic conditions and to promote controlled release of NO (Yoo and Lee 2006). PLGA encapsulated with a ruthenium nitrosyl complex was found to be cytotoxic upon light irradiation.

Phototoxicity in presence of light suggests that cell death was due to NO release from the complex under light irradiation (Gomes et al. 2008). It is clear from the literature that this material presents an interesting system for carrying and locally delivering NO, under irradiation, with a great potential for its use in plants. Sunlight can enhance the rates of NO release from the material and promote a cytotoxic effect against plant parasites.

9.3.6 Dendrimers

Poly(etherhydroxylamine) (PEHAM) dendrimers were used in formulations for increasing the efficacy of many active agents in agriculture, and this was responsive to increase the solubility of active agents. As a consequence, several functions in plants were improved by enhancing water fastness of the active agent to plants or seeds or reducing enzymatic degradation of the active agent (Hayes et al. 2011).

Gene delivery in plants by dendrimers is another interesting platform. In this context, Pasupathy et al. (2008) reported the delivery of GFP-encoding plasmid DNA to turfgrass cells by using poly(amidoamine) dendrimers, which can directly perform gene delivery in a noninvasive fashion. Plant cells were transfected and the GFP genes were expressed, as observed by confocal fluorescence microscopy (Pasupathy et al. 2008). Spherical cationic dendrimers such as polyamidoamine (PAMAM) were designed to introduce plasmids and active molecules into plant cells. There are many different methods that are provided to genetically modify plants and to treat or to prevent plant diseases (Samuel et al. 2011).

Due to precise control over size and the ability to multifunctionalize their structures to enable targeting and tracking, dendrimers have become ubiquitous as drug delivery vehicles in the biomedical arena (Mintzer and Grinstaff 2011). Defined generations of branching and corresponding exponential increase in end group surface functionalities are inherent to their chain-growth synthesis. As such, their multifunctionality has been utilized to produce NO-releasing macromolecular scaffolds with large reservoirs of NO (Stasko and Schoenfisch 2006; Stasko et al. 2008). Stasko et al. (2008) functionalized generation 4 polyamidoamine (PAMAM) dendrimers with either *N*-acetyl-D,L-penicillamine or *N*-acetyl-L-cysteine to yield thiol-terminated dendrimers. Nitrosating these derivatives yielded *S*-nitrosothiol-modified dendrimers (G4-SNAP and G4-NACysNO). The loading was efficient and NO release kinetics varied based on the trigger (i.e., copper ions concentration, light).

In the same direction and focus, Benini et al. (2008) have hypothesized a similar platform, which could be used for local and controlled delivery of NO by using PAMAM dendrimers functionalized with nitrosyl ruthenium complexes. These NO-releasing dendrimers might find useful applications in agriculture due to their capability to load and later deliver high amounts of NO. This characteristic may be relevant to treat microbial infections in plants, since high doses of NO are known to present cytotoxic effects against fungi, viruses, and bacteria (Paradise et al. 2010).

9.3.7 Metalloids and Metallic Nanoparticles

Some metalloids, such as silicon, are semiconductors; hence, they can carry an electrical charge under special conditions. This property makes metalloids useful in computers and calculators. Metal nanoclusters exhibit unusual chemical and physical properties different from those of the bulk material or of the atoms and exhibited potential applications in heterogeneous catalysis, micro- and nanoelectronics, and optoelectronics devices. Due to the nanosize dimension, the presence of a large percentage of surface atoms occurred, and when nanoclusters are deposited on surfaces, their physical and chemical properties are strongly dependent not only on their particle size and chemical composition but also on the structure of the surface and that of the metal/substrate interface.

9.3.7.1 Silica Nanoparticles

Silica-based nanomaterials in the last years have been extensively studied due to the desirable aspects of silica, such as chemical stability, versatility, and biocompatibility (Slowing et al. 2008). Silica nanoparticles can be used as bioinsecticides in agriculture (Barik et al. 2008). These nanoparticles were successfully used as vehicle to deliver DNA and active chemicals into plant cells, as well as to trigger gene expression (Torney et al. 2007). Porous hollow silica nanoparticles exerted a shielding protection to pesticides from degradation by UV light, thus enhancing the photostability of the pesticides (Li et al. 2007).

Some reports showed evidence of possible use of silica as a NO-carrying and delivering material, which may also be applied in agriculture. For example, Shin et al. (2007) reported the advantages of NO-releasing silica nanoparticles over other nanoparticle systems. This is due to the diversity of NO release kinetics, scaffold nanoparticle size, and biocompatibility of silica. NO-release silica nanoparticles were demonstrated to be highly effective against *Pseudomonas aeruginosa* and nontoxic to human fibroblast cells. This fact showed the safe use of this nanomaterial to kill bacterial infections (Hetrick et al. 2008). Silica particles were functionalized with *N*-diazeniumdiolate NO donors against Gram-negative *P. aeruginosa* and Gram-positive *Staphylococcus aureus* biofilms as a function of particle size and shape. Smaller NO-releasing particles (14 nm) exhibited better NO delivery and enhanced bacterial killing compared to the larger (50 and 150 nm) particles (Slomberg et al. 2013).

In this scenario, based on the capacity of silica nanoparticles to tune NO storage and release, as well as their small particle size and biocompatibility, this material could be important in new NO-based applications in plants (Seabra et al. 2013).

9.3.7.2 Iron Oxide Nanoparticles

Many studies on iron oxide nanoparticles have been published as important materials for several biomedical applications, such as nuclear magnetic resonance imaging, hyperthermia, and drug delivery systems (Hadadd and Seabra 2012). Magnetic nanoparticles can also find important applications on plants. Small size carbon-coated magnetic iron oxide nanoparticles were shown to penetrate into plant tissues, and the bioferrofluid was able to spread out through the vascular system to plant after application of magnetic gradients. However, particles over 50 nm of diameter were not detected inside plant tissues (e.g., barrier imposed by cell walls and waxes) indicating that absorption of these nanoparticles depends on particle size (Gonzalez-Melendi et al. 2008). In some cases, this process occurred in plant tissues (e.g., *Cucurbita maxima*) grown in an aqueous medium containing the nanoparticles (Zhu et al. 2008). Then, plants have different responses to the same nanoparticles (Monica and Cremonini 2009).

Racuciu and Creanga (2007) using magnetic nanoparticles coated with tetramethyl ammonium hydroxide on the growth of *Zea mays* plant, in early ontogenetic stages, showed that these nanoparticles not only exert a chemical effect but also a magnetic influence on the enzymatic structures involved in different stages of photosynthesis. In this report, it was found that the growth was dose dependent; low concentration stimulated growth, while high one inhibited growth. Phytotoxicities of metal oxide nanoparticles, magnetite, aluminum oxide, silicone dioxide, and zinc oxide, were evaluated on the development of *Arabidopsis thaliana*. The most toxic was ZnO nanoparticles compared to magnetite, in seed germinations, root elongations, and number of leaves (Chang et al. 2010).

NO-releasing superparamagnetic iron oxide nanoparticles were able to spontaneously release controllable and therapeutic amounts of NO (Molina et al. 2013). This system might find important applications in plants and thus needs to be explored in more detail.

9.3.7.3 Silver Nanoparticles

Silver nanoparticles exert antimicrobial activities (Durán et al. 2010; Holtz et al. 2010). Several papers reported the toxic effects of silver nanoparticles in plants. Toxic effects of silver nanoparticles on the algae *Chlamydomonas reinhardtii* have been reported (Navarro et al. 2008). Growth of *Cucurbita pepo* was also found to be inhibited by these nanoparticles (Stampoulis et al. 2009). The phytotoxicity of silver nanoparticles depends on the plant. A more toxic effect on the plant (*Lemna minor*) was observed by increasing exposure time with nanoparticles, and in general, silver nanoparticles were found to be more phytotoxic in comparison with bulk silver (Eva et al. 2011). However, silver nanoparticles were reported to be less phytotoxic to *C. pepo*, compared to bulk silver (Musante and White 2012). Ultrahigh concentrations of silver nanoparticles (1 g/L), with average diameter of 20 nm, applied onto the roots of *Oryza sativa*, revealed the deposition of nanoparticles inside the root cells damaging cells and vacuoles by penetration through small pores of the cell walls. This reveals an important effect of silver nanoparticles inside cells related to periods of phytotoxicity (Mazumdar and Ahmed 2011). The environmental toxicity of silver nanoparticles at different particle sizes was evaluated using seed germination tests with ryegrass, barley, and flax exposed to different concentrations of metallic nanoparticles. Interestingly, no effect was observed for colloidal silver nanoparticles (5 and 20 nm) up to 10 and 20 mg L^{-1}, respectively (El-Temsah and Joner 2010). Similarly, *Phaseolus radiatus*, in the agar test, showed only 35 % inhibition in the seedling; however, in a soil test, no effects on shoots, roots, and seedlings were observed up to 150 mg/kg of soil. EC50 of silver nanoparticles on *P. radiatus* and *Sorghum bicolor* in the agar test gave values of 13 and 26 mg L^{-1}, respectively (Lee et al. 2012). Silver nanoparticles exhibited a broad spectrum of antimicrobial activities against plant diseases caused by fungal pathogens. However, high concentrations of silver nanoparticles damaged tested plants, such as exemplified in cucumber leaves and pansy flowers (Park et al. 2006). Silver nanoparticles can be used for control of sclerotium-forming plant pathogenic fungi (Min et al. 2009). It is known that silver nanoparticles interacted with fungal hyphae causing severe damage due to the separation of layers of hyphal wall and collapse of hyphae (Nair et al. 2010). Silver nanoparticles on *Raffaela* fungus also caused a detrimental effect. This fungus is important since it is responsible for causing the mortality of oak trees acting on conidial germination (Kim et al. 2009). Gerbera flower's lifetime was enhanced upon treating with silver nanoparticles, due to the inhibition of microbial growth and reduction of vascular blockage. This process led to an increase in water uptake by the plant and thereby maintaining the turgidity of the cells of flowers (Solgi et al. 2009; Liu et al. 2009a).

Indeed, silver nanoparticles can act as NO donor vehicles for several applications, and it was published as an invention that included at least one NO donor in combination with a second therapeutically active agent, e.g., silver nanoparticles, for antimicrobial and wound healing applications (Schoenfisch et al. 2009).

9.3.7.4 Gold Nanoparticles

Gold nanoparticles (with diameters of 3.5 nm) are able to penetrate into plant tissues through roots and move into the vascular system of tobacco plants (*Nicotiana xanthi*) producing leaf necrosis after long exposure of plants to these nanoparticles. This work showed the potential of gold nanoparticles to penetrate plant tissues through size-dependent mechanisms and translocation to cells and tissues, leading to biotoxicity upon long exposure (Sabo-Attwood et al. 2011).

Citrate-stabilized gold nanoparticles, in the presence of *S*-nitrosothiols, the –S–NO bond breaks, thereby releasing NO and modifying the gold nanoparticle surface with the corresponding thiol. This association allows for surface-controlled NO release that is proportional to the number of thiols bound to the gold nanoparticle surface (Taladriz-Blanco et al. 2013). Taken together, these results indicate that the combination of silver and gold nanoparticles with NO may find important applications in agriculture. However, so far, this strategy has not been used, and it opens new perspectives in this domain.

9.3.7.5 Graphitic Carbon Nanomaterials

Fullerenes, carbon nanotubes, and graphene represent a class of graphitic carbon nanomaterials with 0D, 1D, and 2D structure and no bulk counterpart. Their unique structure gives rise to special properties (Gogos et al. 2012).

9.3.7.6 Carbon Nanotubes

It was demonstrated that carbon nanotubes (CNTs) are able to penetrate thick seed coats and support water uptake inside tomato seeds, thus affecting their germination and growth rates. This effect was explained by assuming that the activated process of water uptake could be responsible for significantly faster germination rates and higher biomass production observed for plants that were exposed to CNTs (Khodakovskaya et al. 2009). CNTs enhanced root elongation in onion and cucumber, and nanotube sheets were formed by both functionalized CNTs and CNTs on cucumber root surfaces, due to their interaction with the root surface, but not entering into the roots. No effect on cabbage and carrot was found, but root elongation in lettuce was inhibited by CNTs. Tomatoes were found to be most sensitive to CNTs, with significant root length reduction (Canas et al. 2008). Positive effects on root growth and seed germination of six different crop species were found (Lin and Xing 2007; Nair et al. 2010). No effects on zucchini plants on seed germination and root elongation were observed, but a decrease in the biomass of plants occurred with a long period of exposition (Stampoulis et al. 2009). The ability of carbon nanomaterials to penetrate cell walls and cell membranes of intact plant cells from *Nicotiana tabacum* L. (BY-2 cells) through a fluid phase endocytosis was demonstrated (Samaj et al. 2004; Liu et al. 2009b). These results showed that CNT can be effectively absorbed by plants, indicating the promising uses of this approach to carry and deliver active molecules to plants. Also the CNTs can be associated with metallic nanoparticles leading to new nanostructured materials, which can act as NO donors in diverse applications, including in agriculture (Taladriz-Blanco et al. 2009). By reacting CNTs@poly(allylamine hydrochloride) with a bimetallic nanoparticle suspension, comprised of gold and silver, a CNT@AuAg hybrid colloid material was formed and reaction with a *S*-nitrosothiol solution (RSNO) resulted in the bond cleavage of S–N, due to the high affinity of gold for thiols, releasing free NO (Alvarez-Puebla et al. 2005; Taladriz-Blanco et al. 2009). Irradiation of a metal nitrosyl complex greatly accelerates the rate of NO release, by functionalization of the surface of multiwalled CNT with a Ru–NO complex (Collom et al. 2008). Therefore, this strategy can be considered as an interesting approach to introduce NO into plants, using CNTs and visible light.

9.3.7.7 Graphenes

Bacterial spot caused by *Xanthomonas perforans* is a major disease of tomatoes, leading to reduction in production by 10–50 %. Due to the appearance of resistance to copper-based bactericides, it developed DNA-directed silver (Ag) nanoparticles (NPs) grown on graphene oxide (GO) (Ag@dsDNA@GO). Low concentrations of Ag@dsDNA@GO led to efficient antibacterial capability in culture with significant advantages in improved stability, enhanced antibacterial activity, and stronger adsorption properties without any phytotoxicity (Ocsoy et al. 2013).

The effects of graphene on shoot growth, root, biomass, shape, cell death, and ROS of cabbage, tomato, red spinach, and lettuce were analyzed by Begum et al. (2011). The concentrations used in the study ranged from 500 to 2,000 mg L^{-1}, which is extremely high for biological systems. Combined morphological and physiological analyses indicated that after 20 days of exposure under experimental conditions, graphene significantly inhibited plant growth and biomass level, in a dose-dependent manner. Due to this, high graphenes oxide was also detected in ROS and cell death as well as visible symptoms of necrotic lesions. This effect was observed on cabbage, tomato, and red spinach mediated by oxidative stress necrosis. No effects were observed with lettuce seedlings under the same conditions (Fugetsu and Begum 2011; Jastrzebska et al. 2012).

Graphene at 0–80 mg L^{-1} effect on suspensions of *A. thaliana* (Columbia ecotype) T87 cells showed morphological changes and the adverse effects such as fragmented nuclei, membrane damage, and mitochondrial dysfunction. Analysis of intracellular ROS demonstrated that graphene induced a 3.3-fold increase in ROS, suggesting that ROS are key mediators in the cell death signaling pathway. Transmission electron microscopy study showed graphene entering into the cells by endocytosis. Then the authors suggested that graphene induced cell death in T87 cells through mitochondrial damage mediated by ROS (Begum and Fugetsu 2013). Again, this study is showing a toxic effect at high graphene concentration.

To assess biomass accumulation (physiological response), few-layer graphene materials were introduced into adequate media (50 mg L^{-1}) together with tomato seeds, and the germination and growth were followed (Khodakovskaya et al. 2011). It is interesting that the few-layer graphene carbon structures did not significantly affect plant growth rates, probably because of their inability to penetrate plant tissues.

Graphene oxide on wastewater microbial community was toxic in dose dependent, especially in concentrations over 50 mg L^{-1}. The authors suggested that possibly the ROS generation could be one of the responsible mechanisms for the toxic effect of GO (Ahmed and Rodrigues 2013).

It is worth to note that all of these studies showing toxicity have used extremely high concentration of graphene (0–2,000 mg L^{-1}). The biological effect must be studied at a range of concentration of 0–10 mg L^{-1}.

After an exhaustive literature search, as far as we know, no NO donors associated with graphenes were published. This data showed that if it works in lower concentration of the graphenes adduct with NO could be of great potential.

9.3.7.8 Fullerenes

No acute plant toxicity was observed upon the addition of 2–15 mg L^{-1} of fullerene nanoparticles (eastern cottonwood (*Populus deltoides*)) in terms of phenotype, water transpiration, and plant biomass in batch hydroponic studies (Ma and Wang 2010). Water-soluble fullerene $C_{70}(C(COOH)_2)_{4-8}$, on plant growth using the transgenic seedling lines expressing fluorescent makers, showed that retarded roots with shortened length and loss of root gravitropism for seedlings grown in the fullerene-containing medium. At a fullerene concentration of 5 mg L^{-1}, a 20 % exerted on root length an 9 % of hypocotyl elongation inhibition was found. Fluorescence studies revealed the abnormalities of root tips in hormone distribution, cell division, microtubule organization, and mitochondrial activity at higher concentrations (Liu et al. 2010). Apparently, fullerenes can be safely fused in plants due to its low toxicity.

9.4 Conclusions

Important works describe the positive impact of exogenous administration of small molecule NO donors in plants, as well as the advantages of nanomaterials in agriculture. However, the combination of NO donors with nanomaterials for agricultural purpose has not been explored. In this context, this chapter hopes to open new perspectives for the application of NO-releasing nanomaterials in plant.

This new approach may significantly enhance agriculture, since NO-releasing nanomaterials can impact plant physiology and pathology, especially seed germination, promotion of plant growth, and plant protection against pathogens and exogenous stresses. Moreover, nanomaterials and NO donors might economically improve agriculture, horticulture, as well as the energy sector, such as the production of biofuels. Finally, since depending on its concentration, NO-releasing nanomaterials might have toxic effects to humans and to the environment; hence, phytotoxicological investigations are required.

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Chapter 10
Nanoparticles-Based Delivery Systems in Plant Genetic Transformation

Mahendra Rai, Sunita Bansod, Manisha Bawaskar, Aniket Gade, Carolina Alves dos Santos, Amedea B. Seabra, and Nelson Duran

Abstract The production of transgenic plants is considered as a valuable tool in plant research and the technology is comprehensively useful in agricultural research. Gene transfer in plants is generally carried out by *Agrobacterium* sp., application of some chemicals, and physical techniques (electroporation, microprojectile, etc.). Now-a-days with better efficacy and stability, new methods for the gene transfer in plants are coming up. The advent of nanotechnology, the nanoparticles-based delivery systems for genetic transformation of plants, is coming in a big way.

In this chapter, we have discussed the novel nanotechnologies like nucleic acid-conjugated nanoparticles with their current status and future prospects in the development of gene transfer methods in plants. We have also highlighted the shortcomings of conventional techniques of gene transfer in plants and discussed the role of established nanotechnology and chemical-based strategy for surface

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modification of nanoparticles to improve efficacy, stability, and accuracy making it less time-consuming.

Keywords Bioconjugation • Biotransformation • Nanoparticles • Transgenic vehicle

10.1 Introduction

Genetic engineering is a powerful technique to create genetic modifications for the selected trait(s) and to allow transfer and integration of a gene of interest into the genome of host. DNA delivery is the most important aspect of gene transfer in plants to develop the transgenics. There are several methods used for gene transfer, but all of them have some or the other limitations. Small size of the nanoparticles ($1\ \text{nm} = 10^{-9}\ \text{m}$) allows them to bypass the cell barriers like cell wall and plasma membrane and deliver genes into the cell of living systems, and they can also be used as a transgenic vehicle for nucleic acid (Rai et al. 2012).

Nanoparticles-mediated gene transfer methods are new and have the potential to directly transfer DNA into the cells, achieving stable integration and rapid expression of the transgene. Nanoparticles are the particles at atomic or molecular level with dimension in nanoscale (Ball 2002; Roco 2003). The nanoparticles behave differently and exhibit different physiochemical properties as compared to their bulk counterpart (Nel et al. 2006). Nanoparticles are formed from a variety of materials, and their action depends upon their chemical composition, size, and shape (Brunner et al. 2006). On the basis of their origin, Roberto and Ruffini (2009) classified nanoparticles into three major types as: (1) natural nanoparticles; (2) incidental nanoparticles, also called as waste or anthropogenic particles, which are mainly formed due to the man-made activities; and (3) engineered nanoparticles or nanomaterials. The engineered nanomaterials can be classified into four types, viz., carbon based, metal based, dendrimers, and composite nanoparticles. Nowack and Bucheli (2007) have highlighted the role of modified nanomaterials for improving many sectors of the economy, such as consumer products, pharmaceutical materials, cosmetic products, transportation, energy, and agriculture.

The nanoparticles, which can be used as a vector for gene transfer, include calcium phosphate, carbon based, silica, gold, magnetite, strontium phosphate, magnesium phosphate, and manganese phosphate (Potrykus 1991). The nanoparticle-mediated gene transfer mechanism is reported as follows: initially, nanoparticles recognize and get adsorbed on the cell membrane, and later these nanoparticles are internalized by endocytosis. During this process, if DNA escapes out, before the fusion of endosome with lysosome, this escaped DNA successfully enters into the cell cytoplasm, where its degradation by nucleases can occur. However, for an efficient transfer, it must be protected from nucleases and enter into the nucleus. Generally, the DNA enters into the nucleus with the help of nuclear pore complexes (NPCs) which are large number of proteins, forming a channel. These proteins are buried into the phospholipid bilayer (Hayashimoto et al. 1990). NPCs can act as the semipermeable gate, which allows easy passage to small molecules; however, they restrict the movement of larger molecules. The molecules can overcome this barrier by having a nuclear localization sequence (NLS) which can be recognized by the protein named importins, which can be transported through the pores into the nucleus on the expense of energy (O'Neill et al. 1993). Actually, it is not clear how DNA alone or with nanoparticles is transported into the nucleus. One of the reasons may be that DNA-loaded nanoparticles may recognize the surface and get attached to the surface of the nuclear membrane, where the import in molecule can transfer the DNA into the nucleus. In this case, a nanoparticle protects DNA from nucleases until it gets into the nucleus. On this basis, several nanoparticles can be used to conjugate with nucleic acids for their proper transfer into the living cell (Ito et al. 1983), and various types of nanoparticles have been used as nucleic acid carriers (Ruzin and McCarthy 1986). The nanoparticles have some advantages over other methods as they are not subject to microbial attack, can be easily synthesized, are less toxic, and exhibit a good stability (Hoffmann-Tsay et al. 1994). Many scientists successfully adopted different nanoparticulate delivery carriers like polymeric nanoparticle, metallic nanoparticle, and quantum dots in gene therapy (Jun et al. 2008; Akhter et al. 2011). Nanoparticles play an important role as a gene carrier in biotransformation process, as well as protection of DNA damage from ultrasound as reported by Liu et al. (2008). Many scientists used green fluorescent protein (GFP) tagged with nanoparticles to demonstrate its effectiveness and reported that nanoparticles alone enter into the cytoplasm as seen in the green cytoplasmic content (Jun et al. 2008).

The GFP has been extensively studied in recent years, with various types and variants. GFP was first noticed in jellyfish, *Aequorea victoria*, in 1961. GFP is a compact, globular, acidic, 27-kDa molecule with stability at the greater range of pH from 5 to 9 (Chalfie et al. 1994). Santos et al. (2007) have reported the X-ray crystallographic analysis of the GFP revealing the presence of a β-barrel structure in which the fluorophore is buried in the protein interior. Amino acids Serine, tyrosine, and glycine form the fluorophore of GFP wild type, and these amino acids in recombinant GFP had two reactions, autocatalytic cyclization between the carbonyl of Tyr 66 and the amino group of Gly 67 and the carbonyl of Ser 65 and Tyr 66 amino group, giving rise to a covalent bond and oxygen-dependent slow step, where the single bond between the carbons Cα–Cβ Tyr66 results in the conjugated double bonds with fluorescent properties (Chalfie and Kain 1998). Recently, several mutants with emission at higher wavelength have been described with both enhanced fluorescence and rapid expression as compared to that of wild-type GFP, thereby increasing their sensitivity and applicability over wild-type GFP (Robey et al. 1998; Doi and Yanagawa 1999). Wild-type and mutant forms of GFP have been widely used as reporters of gene expression. Their small size, the limited or no effect upon cell physiology when expressed, and their stability coupled with their ability to maintain their intrinsic fluorescence when attached to other peptides or proteins have made them an essential part of molecular biology research.

Other methods are also available to monitor gene expression and distribution of proteins within cells. They made use of coding sequences for beta-galactosidase, luciferase, or bacterial luciferase for the formation of fused proteins (Chalfie et al. 1994). The requirement of exogenously adding of substrate or cofactors limits their application in living tissues.

Intracellular detection of GFP requires only irradiation by UV providing an easy means for the detection of gene expression and protein localization in living cells (eukaryotic and prokaryotic) (Chalfie et al. 1994; Dhandayuthapani et al. 1995; Bo Andersen et al. 1998). Another advantage of GFP is related to the fact that this does not interfere with cell growth and cell physiology. For the use of some proteins as a marker for gene transfer, the characteristics of the cell being studied and factors such as the utilization of fixed compounds should be taken into account. The use of fixed favor maintenance of the characteristics of cells for the study, however, can directly affect the fluorescence of the protein (Chalfie and Kain 1998). The choice of methods for fluorescence quantification is primordial to use GFP as a marker protein. Niedenthal et al. (1996) have reported the most common and fastest methods to determine GFP behavior in cells, which include methods such as epifluorescence microscopy, fluorometry, and flow cytometry.

Crystal structures of high-resolution GFP offer unprecedented opportunities for understanding and manipulating the relationship between the structure and function of protein spectroscopy. GFP has become a well-known marker system for gene expression and protein detection in intact target cells and organisms, opening new perspectives in physiological indicators, biosensors, and gene markers (Tsien 1998). New method of gene transformation in plants with nanoparticles is the new area in plant biotechnology (Fig. 10.1).

Plant tissue culture technique is an essential tool in agriculture and medicinal plant research. It depends on maintaining plant cells in controlled aseptic conditions on basal nutrient medium with appropriate hormonal concentration. The plant cell culture can be sustained as a callus (mass of undifferentiated cells) for an extended period of time or can be regenerated into whole plant. For the production of secondary plant metabolites and regeneration of plant with improved nutritional quality, higher yields, and tolerance against biotic and abiotic stresses, newer genes need to be introduced in the preexisting plants with normal physico- and phytochemical properties (Dodds and Roberts 1990; Bhojwani and Razdan 1996; Ohadi

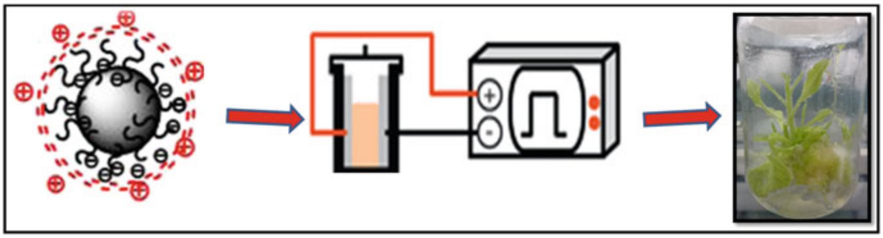

Fig. 10.1 The new method of transformation of plants with transgenes encapsulated in nanocarriers is relatively the new area in plant biotechnology

et al. 2011). Tissue culture is growing at a mature level in the area of gene transfer and development of transgenics (Murashige 1974; Kishinami and Widholm 1987; Datta et al. 1992; Jiang and Berg 1995; Husaini and Abdin 2008; Vieira and Camilo 2011; Cha et al. 2011). There are *physical methods* (electroporation, particle bombardment, microinjection, etc.), direct gene transfer techniques (protoplast fusion, transfection), gene transfer using plasma membrane destabilizing/precipitating agents (chemicals that facilitate the gene transfer through the cell membrane, e.g., polyethylene glycol 6000, polyvinyl alcohol, $NaNO_3$, DEAE, dextran, and DMSO) (Ito et al. 1983; Ruzin and McCarthy 1986; Hayashimoto et al. 1990; Potrykus 1991; O'Neill et al. 1993; Hoffmann-Tsay et al. 1994; De Rosa and La Rotonda 2009; Akhter et al. 2011), and natural means of gene transfer such as the use of *Agrobacterium* species (Jun et al. 2008). Although these conventional methods are effective in gene transfer, their efficiency is compromised over the speed in physical methods, whereas excessive use of chemicals in chemical methods damages the DNA and cells (Husaini and Abdin 2008).

The effective utilization of any crop species in plant biotechnology research depends on the development of effective and reproducible methods for regeneration. The success of gene transfer methods depends on the possibility to transform a cell or tissue, which can be regenerated into a complete plant. While for some species regeneration protocols have not been optimized, for others the regeneration protocols are optimized, but gene transfer methods have not been established or, if available, they are just suited for some genotypes. There is a need to overcome these genotype dependence concerning methods for regeneration and/or transformation in many species.

The main aim of this review is to highlight the investigation of successive transformation of genes by using nanoparticles.

A new era of novel nanotechnology was successfully adopted as main or adjuvant technologies in genetic transformation. Numerous recent reports are indicative of significant contributions of these novel technologies in gene transfer to the plant tissues and their culture techniques. In this chapter, we have discussed in detail about the nanoparticles as novel nanotechnology-based biotransformation systems for gene delivery in plants. Moreover, we will also describe in brief about commonly used gene transfer approaches and their limitations. Additionally, we will try to postulate innovative ideas on the footprints of established nanotechnology- and chemical-based strategy for improved efficiency, reproducibility, and accuracy of biotransformation.

10.2 Bioconjugation of Nanoparticles with Nucleic Acid

Bioconjugation is the chemical strategy to conjugate two biomolecules together. Understanding of bioconjugation process has enabled applications of biomolecules to numerous fields like medicine, agriculture, and materials. Functional groups on cross-linking reagents aid the reactive groups of biomolecules to couple (Hermanson 2008). Several modification and functionalization strategies provide the means to attach different modified biomolecules through their unique functional group. The functional groups found in biological systems provide a wide range of potential interactions with nanostructure surfaces, which can easily interfere with the structure and function of biomolecules. Most common types of bioconjugation reactions include coupling of lysine amino acid residues through amine-reactive succinimidyl esters, cysteine residues through sulfhydryl-reactive maleimide, tyrosine residues through electrophilic aromatic substitutions, and modification of the N- and C-terminus (Francis and Carrico 2010; Kalia and Raines 2010; Stephanopoulos and Francis 2011). These chemical reactions generally lack chemoselectivity and efficiency due to their overdependence on the presence of native amino acid residues, which are usually present in large quantities that may hinder selectivity. Need of the hour is to develop the chemical strategy that can efficiently attach synthetic molecules specifically to proteins. Conjugation of DNA with nanoparticles of calcium phosphate, carbon, silica, gold, magnetite, strontium phosphate, magnesium phosphate, and manganese phosphate has been used for DNA delivery, which have low toxicity and good storage capacity (Fukumori and Ichikawa 2006; Sokolova and Epple 2008).

Among bionano-conjugates, gold nanoparticles coupled with biomolecules attracted great attention in the last decade due to their ease of preparation and conjugation, biocompatibility, good tenability, high stability, etc. Many diverse simple synthetic methods are available to synthesize nanoparticles and conjugate with the biomolecules, which can be effectively used as smart delivery systems. Varied biomolecules may be synthetic or natural and they can be tailored to functionalize synthesized nanoparticles. Previous reports have demonstrated sequence-specific nucleotide interactions, which can affect conjugate behavior. In the synthesis of DNA nanoparticle conjugates, specific binding of the biomolecules with greater storage capacity and higher stability is a greater challenge.

10.2.1 Nanoparticles and Vectors

Now-a-days, nanoparticles differing in their elemental composition, size, and shape can be synthesized. Nanoparticles were tried for bioconjugation with the nucleic acids due to their unique electronic, optical, and catalytic properties (He et al. 2003; Murray et al. 2004; Masala and Seshadri 2004; Jason et al. 2004; Patil et al. 2005; Fukumori and Ichikawa 2006). Nanomaterials exhibit novel properties due to large surface area to volume ratio. He et al. (2003) reported the shielding of embedded DNA sequences by nanoparticle due to their small size with greater surface area and pore structure.

Genetic transformation allows the transfer of a foreign gene of interest (transgene) encoding the trait into the plant cell, in order to introduce a desired trait to a crop. Gene vectors play many important roles in genetic transformations which need to be constructed in order to transfer the gene of interest. Vector consists of a cassette which includes the genes of interest, flanked by the necessary controlling

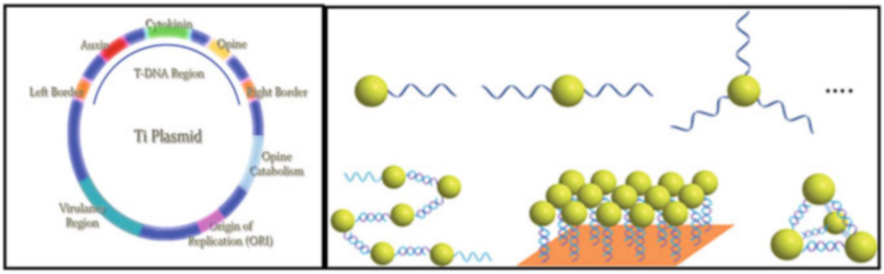

Fig. 10.2 Way of nucleic acid attached to nanoparticles

sequences, i.e., promoter, terminator, and marker gene, which allow efficient screening of the transformed and non-transformed. Binary vectors are the most commonly used plant transformation vectors due to their ability to replicate in both *E. coli* and *Agrobacterium tumefaciens* (Fig. 10.2).

For efficient plant transformation, three important steps are:

- Selection of appropriate plasmid
- Plasmid replication ability determining its copy number
- T-DNA region

The plant transformation vectors should have minimal heterologous sequences. These sequences are required for plant transformation and selection of transformants; they require all promoter and terminator sequences for plant expression of newly cloned genes. Generally the minimal selection vectors have either of the two plant selection genes: they have either *hptII*, encoding resistance to hygromycin, or *nptII* which encodes resistance to kanamycin. The selection gene is under the control of double-enhancer version of the CaMV35S promoter in both the cases. By subjecting to site-directed mutagenesis, interfering restriction sites are removed within the coding sequence by converting them to the silent changes. Kanamycin or chloramphenicol resistance marker allow a broad range of *Agrobacterium* or *E. coli* strains to be used in the transformation.

Various derivatives differing in the selectable marker, reporter, MCS, and other factors are available in the pCAMBIA series (www.cambia.org), pGreen series, and pPZP series. One of the derivatives is shown for each of the groups: empty vector pGreen, selection vector pPZP111, and reporter vector pCAMBIA1302. A similar variation is found in the derivatives of pBin19, which is considered to be a selection vector, and reporter vector pBI121. An empty version of pBin19 may be obtained by digesting pBin19 with *Cla*I and partially with *Sac*II, followed by recircularization. These vector groups have been successfully employed in many studies, which at a glance, account for two thirds of the recent publications in the area of plant transformation.

10.2.2 Synthesis of Nanoparticles

Nanoparticles can be synthesized by three processes, i.e., by physical, chemical, or biological route. Chemical route employs toxic chemicals and is energy intensive, thereby precluding biomedical applications. In physical method, narrow size distribution of the particles or monodispersity is often difficult to attain, whereas biological method (intracellular and extracellular) is more cost-effective and green method. For the application point of view, there is a need to develop green synthesis procedure for synthesis of metal nanoparticles. Biological synthesis of metal nanoparticles employs a greener approach effectively than physical and chemical methods. Biosynthesis includes challenging of the fungal filtrate with the respective salt; the biomolecules present in the fungal filtrate act as reducing agents, whereas chemical synthesis is achieved by using chemicals like sodium borohydride or trisodium citrate as a reducing agent (Lee et al. 2010). The biological route of metal nanoparticle synthesis has been demonstrated by exploiting bacteria such as *Rhodococcus* sp. (Ahmad et al. 2003a), *Thermomonospora* sp. (Ahmad et al. 2003b), *Shewanella algae* (Ogi et al. 2010), *Lactobacillus* strains (Prasad et al. 2007), and *Pseudomonas aeruginosa* (Deshmukh et al. 2012), while yeast species have included *Candida glabrata* (Dameron et al. 1989), *Schizosaccharomyces pombe* (Kowshik et al. 2003), filamentous fungi like *Aspergillus niger* (Gade et al. 2008), *Fusarium culmorum* (Bawaskar et al. 2010), and *Fusarium* sp. (Gaikwad et al. 2013a). Gade et al. (2014) recently have given green synthesis approach of silver nanoparticle synthesis by using fungi.

Fungi are more advantageous for the synthesis of nanoparticles compared with other organisms, particularly as they are relatively easy to isolate, grow, culture, and maintain in the lab, easy downstream processing of synthesized nanoparticles (Ingle et al. 2008), and they secrete large amounts of extracellular enzymes (Mandle et al. 2006). Moreover, nanoparticles with high monodispersity and dimensions can be obtained from microbial (Shahverdi et al. 2007) and fungal proteins (Mohanpuria et al. 2007; Birla et al. 2013; Gaikwad et al. 2013b; Gade et al. 2013) as it has a capacity of hydrolyzing metal ions quickly and through nonhazardous processes. There are many reports of mycosynthesis (synthesis by fungi) of metal nanoparticles (Birla et al. 2009; Gajbhiye et al. 2009; Bawaskar et al. 2010; Gade et al. 2010a, b, 2011; Raheman et al. 2011; Kumar et al. 2012; Dar et al. 2013; Gade et al. 2014). Many plants like *Carica papaya* (Mude et al. 2009), *Opuntia ficus-indica* (Gade et al. 2010a, b), *Murraya koenigii* (Bonde et al. 2012), *Hydrilla verticillata* (Sable et al. 2012), *Tagetes erecta* (Dhuldhaj et al. 2012), *Lawsonia inermis* (Gupta et al. 2013), and *Paederia foetida* (Madhavaraj et al. 2013), hop biomass in native and chemically modified form (Lopeza et al. 2005), and remnant water collected from soaked Bengal gram bean (Ghule et al. 2006) have been used for the synthesis of nanoparticles. However, alfalfa (Gardea-Torresdey et al. 2002, 2003), *Chilopsis linearis* (Rodriguez et al. 2007), and *Sesbania* seedlings (Sambrook and Russell 2001) have the potential of synthesis of gold nanoparticles inside living plant parts.

10.2.3 Isolation of the Plasmid and Coupling with the Nanoparticles

Isolation of plasmid DNA from *E. coli* and *A. tumefaciens* can be done by using an alkaline lysis method (Sambrook and Russell 2001).

The fractionalizing of nanoparticles approach, i.e., imparting functional groups to the surface of nanoparticles. The functional groups like carboxylic acids are often used for stabilizing the nanoparticles by electrostatic repulsion and making them more water soluble; these functionalized nanoparticles can be exploited for the conjugation of other molecules to the nanoparticles. Even other functional groups can be introduced on the surface of nanoparticles by their ligand molecules or a mixture of different ligands. The dodecanethiol monolayer of Au nanoparticles can be amended with bifunctional ligands by place-exchange reactions to introduce functional groups like bromide, ferrocene, hydroxyl, and carboxyl (Ingram et al. 1997; Templeton et al. 2000). Fractionalizing of nanoparticles approach can also be useful for phase transfer reaction by ligand exchange, during this incoming ligand already contains the desired functional group, as revealed for hydrophobic CdSe/ZnS quantum dots and ligands comprising, e.g., –COOH, –OH, –NH2 (Wuister et al. 2003; Hoshino et al. 2004; Susumu et al. 2007; Bansod et al. 2013), or CdTe and, for instance, a mixture of MPA and aminoethylthiol. Iron oxide nanoparticles modified with (di)mercaptosuccinic acid have been shown to exhibit both carboxylic and thiol functional groups (Wilhelm et al. 2003). Bifunctional groups can change the functional groups present on the surface of the nanoparticles. Reactions may harm the stability of the nanoparticles; to overcome this situation, generally mild reactions are preferred like the ones applied to the chemical modification of biomolecules (bioconjugation chemistry), and a large number of dysfunctional molecules are available commercially (Wilhelm et al. 2003; Hermanson 2008). The most common type of reaction found can be of carboxylic groups reacted with primary amines by means of a condensation reaction to yield amide bonds.

Protein fragment or peptides with known sequence of amino acid allow the rational design of ligand molecules that can be optimized to stabilize nanoparticles (Levy et al. 2004) or present various functional groups on the surface (Wang et al. 2005; Medintz et al. 2006; Garanger et al. 2008). Slocik et al. (2005) have reported the synthesis of gold nanoparticles by using peptides. Making use of the versatility of biological functionality of peptides, the nanoparticles uptake by cells can be specifically optimized by conjugating nanoparticles with the corresponding peptide; similar approach has been reported for Au nanoparticles (Nativo et al. 2008) or quantum dots (Pinaud et al. 2004). In this approach the ligand exchange with cysteine-containing peptides was performed to modulate reaction of immune system toward nanoparticles by peptide coating (Bastus et al. 2009). Peptide can also be modified by covalent conjugation chemistry, as reported for quantum dots with amino groups (Cai et al. 2006) or for iron oxide nanoparticles with amine groups (Josephson et al. 1999; Schellenberger et al. 2004). The quantum dots are also modified with streptavidin (available commercially) which can be

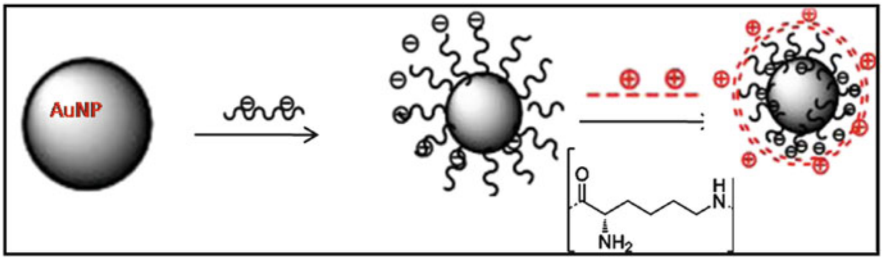

Fig. 10.3 Surface modification of DNA conjugated with nanoparticles with poly-L-lysine (PLL) that gives positive charge on nanoparticles—DNA complex and which attracts to negative charge of the cell wall of a plant cell

covalently conjugated to different biotinylated peptides (Chen and Gerion 2004; Lagerholm et al. 2004; Kim et al. 2008). Several approaches are available for the conjugation of biomolecules like proteins to nanoparticles, including enzymes or antibodies. Initially only "nonspecific" adsorption was employed: this can be achieved by incubating nanoparticles with the protein, which allows the adsorption of nanoparticles to the proteins by electrostatic attraction or by van der Waals forces, hydrogen bridges, thiol bonds (from cysteine residues), or amino groups modified by agents like bis-NHS for the coating on silica nanoparticles (Jana et al. 2007). Josephson et al. (1999, 2001) have demonstrated the covalent conjugation of magnetic iron oxide nanoparticles with peptides and oligonucleotides. There are several examples of nanoparticle-protein conjugation, which can be found in a number of reviews and research papers (Niemeyer 2001; Ghadiali and Stevens 2008).

Yu et al. (2012) reported a new method to use ZnS nanoparticles as a gene carrier for plant gene transfer. The ZnS nanoparticles with a diameter of around 3–5 nm were modified with positively charged poly-L-lysine (PLL) to bind negatively charged pBI121 plasmid DNA. The ZnS nanoparticles-mediated transformation of tobacco was carried out via ultrasound-assisted method. The ZnS nanoparticles-mediated stable gene expression in tobacco plant was observed for the first time. Cui et al. (2012) used polyethyleneimine (PEI)-modified magnetic nanoparticles as vectors to transfer genes to porcine somatic cells. The PEI-modified Fe_3O_4 magnetic nanoparticles are mixed with pEGFP-N1 in a certain mass ratio and incubated for 30 min at room temperature; this modified nanoparticles were employed to transfer the reporter gene into somatic cells, and also the gene delivery efficiency was examined (Hoshino et al. 2004) (Fig. 10.3).

10.2.4 Characterization of Nanoparticles (NPs)

Characterization of NPs is necessary to establish the understanding about synthesis and conjugation with the biomolecules. The surface characterization normally includes measuring the particle size, shape, surface charge, and magnetic properties. Transmission electron microscopy (TEM), scanning electron microscopy (SEM), and zeta potential measurement are commonly used to evaluate the size, shape, and charge on the nanoparticle (Verhaegh and Van 1994; Nyffenegger et al. 1993). Dynamic light scattering (DLS) technique is used to measure NP size in aqueous media while providing NP size, concentration, and relative dispersion (Van and Vrij 1993).

The comparative analysis study of prepared bioconjugates with different nanoparticles and DNA can be done by mixing them in a certain mass ratio and checked by performing:

(a) Mobility assay on agarose gel electrophoresis
(b) Spectrophotometer, TEM, and LM 20 analysis

10.2.5 Electron Microscopy

The SEM and TEM images of both nanoparticles and nanoparticle/DNA complexes can be analyzed to evaluate the surface characteristics, i.e., size and shape.

10.2.6 Zeta Potential and Size Measurement

The zeta potential measurement of a particle designates the overall charge that the particle acquires in a particular medium. The zeta potential value can be used to determine the colloidal stability of the NP and their conjugate. If the nanoparticles in suspension possess a large positive, i.e., greater than +30, or less negative charge, i.e., −30, zeta potential, they will repel each other and will avoid the NP agglomeration, whereas the zeta potential value between +30 and −30 will result in flocculation or coagulate loosely depending upon the charge. Therefore, surface modification can change the net charge on the particle surface in a particular medium and is important to improve the long-term stability of NPs. Cui et al. (2012) have reported that after conjugation with nucleic acid, the zeta potential of pure nanoparticles decreases due to the net negative charge present on the DNA molecule, which can neutralize the positive charge on the surface of metal nanoparticles (Cui et al. 2012).

10.2.7 Combination and Protection Analysis

Effectiveness of bioconjugated nanoparticles for gene transfer can be checked depending upon their capacity to protect the DNA from nuclease and sonication to demonstrate how DNA is protected by nanoparticle complex by physical and chemical agents by performing the following assays (Zou et al. 2009):

(a) Nuclease protection assays
(b) Sonication protection assays

He et al. (2003) used positively charged amino-modified bioconjugated silica nanoparticles for conjugation with DNA and protection of DNA from cleavage. Cui et al. (2012) have reported the use of PEI-modified magnetic nanoparticles for the binding with plasmid DNA. Moreover, the nanoparticle/DNA conjugate can protect the DNA against degradation by exonuclease or endonuclease by preventing the access to the cleavage sites. This can be demonstrated by agarose gel electrophoresis. The efficiency of gene delivery can be affected by the amount and charge to mass ratio of nanoparticle/DNA conjugate.

10.3 Biotransformation in Plant by Using Nanoparticles

Transport of nanoparticles in plants is being unraveled, and various studies are now ascertaining the role of different metal nanoparticles on plant growth and metabolism.

10.3.1 Mode of Entry, Transport, and Effects of Different Nanoparticles in Plants

The plant cell wall acts as a barrier, preventing the easy entry of any external agent including metal NPs into plant cells. The cell wall pore diameter ranging from 5 to 20 nm (Fleischer et al. 1999; Navarro et al. 2008) allows easy passage of the NPs having the diameter less than the pore diameter of the cell wall and enters the plasma membrane of the cell (Moore 2006; Navarro et al. 2008). There is also a possibility of pore size enlargement or an induction of new pores in the cell wall upon interaction with engineered NPs which in turn enhance their uptake. Internalization of NPs also occurs by endocytosis process, wherein a cavity-like structure is formed around the nanoparticles by a phospholipid bilayer. NPs may also cross the membrane using membrane-embedded transporter proteins or through ion channels. On entering the cytoplasm, NPs may interact with different cytoplasmic organelles and biomolecules, affecting the metabolic processes in the cytoplasm (Jia et al. 2005). When NPs are applied on the leaf surface, they can enter through stomatal openings or through the bases of trichomes (Eichert et al. 2008; Fernandez and Eichert 2009; Uzu et al. 2010). The NPs accumulated on photosynthetic surface can cause foliar heating resulting into the alterations to gas exchange. The stomatal obstruction caused by the NPs produces changes in various physiological and cellular functions of plants (Da Silva et al. 2006). Studies on the uptake and formation of nanoparticles within plants have also generated interest among the investigators on the use of plants as source for NP synthesis (Fig. 10.4).

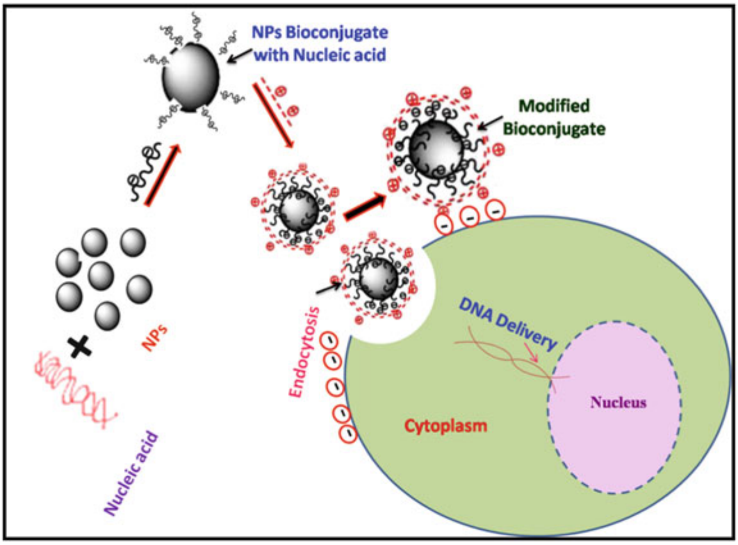

Fig. 10.4 Nanoparticles aggregates with diameter less than the pore diameter of the cell wall could easily pass through and reach the plasma membrane

10.3.2 Nanotechnology for Delivering Genetic Materials into Plants

The NPs which can carry the genetic material into cells efficiently and rapidly have potential applications in drug delivery, gene transfer in plants, and environmental monitoring. McKnight et al. (2003, 2004) have reported the integration of plasmid DNA with surface-modified carbon nanofibers in viable cells for controlled biochemical manipulations. The effective integration and delivery of plasmid DNA was confirmed from the gene expression, similar to the microinjection method of gene delivery (Neuhaus and Spangerberg 1990; Bolik and Koop 1991; Segura and Shea 2001). In plant, treated cells could be regenerated into whole plant and would allow the expression of the introduced trait. The DNA tethered on carbon nanofibers without allowing them to get integrated into the host genome will make it possible to transcribe some of the tethered genes without passing the modified trait to further generations. The use of fluorescent-labeled starch nanoparticles as plant transgenic vehicle was reported by Jun et al. (2008), in which authors reported the gene transfer with the help of NP-biomolecule conjugate. The conjugate was designed in such a way that it binds and shuttles genes across the cell barrier like the cell wall, cell membrane, and nuclear membrane of plant cells by inducing instantaneous pore channels with the help of ultrasound waves. Different genes can be integrated on the fluorescence-labeled nanoparticle at the same time, and their imaging with fluorescence microscope makes it possible to understand the movement and expression of exterior genes transferred. Increasing the porosity of the cell wall and cell membrane by a suitable agent helps in nanoparticle-mediated DNA transfer in regenerative calli and soft tissues. The ability of NPs to penetrate plant cell wall also helps precise manipulation of gene expression at the single-cell level by delivering DNA and its activators in a controlled fashion (McKnight et al. 2003). Honeycomb mesoporous silica nanoparticles (MSN) with 3-nm pores can transport DNA and chemicals into isolated plant cells and intact leaves. MSNs loaded with gene and its chemical inducers were capped with gold nanoparticles at the ends to protect the molecules from leaching out. Removal of gold nanoparticle cap enabled release of chemicals and triggered gene expression in plants under controlled conditions. Incubation of protoplast with fluorescently labeled MSNs revealed that surface modification of MSNs with triethylene glycol was necessary to penetrate the cells. The modification of MSNs with triethylene glycol also allowed adsorption of plasmid DNA on MSN surface. Plasmid DNA was released from the MSN upon its entry into the protoplasts; the plasmid DNA released allows expression of marker gene like GFP in the cell, which can be detected by fluorescence microscopy. This method can detect marker expression of 1,000-fold less than that required for the conventional delivery method. Efficiency of this delivery method has pronounced applications in various protoplast-based gene expression studies. Now-a-days, particle bombardment or gene gun is one of the popular methods to transfer DNA into intact plant cells (Klein et al. 1989; Deng et al. 2001). In particle bombardment method, the particles used are typically made of gold, due to their ability to adsorb DNA and nontoxic nature toward cells. MSNs are not suitable for gene transfer by particle bombardment method, since they are too light to be used for this method. This problem can be overcome by capping MSNs with gold nanoparticles, which will increase their momentum after acceleration by the gene gun. Plasmid DNA transferred by particle bombardment method using gold-capped MSNs was successfully demonstrated in intact tobacco and maize tissues. In particle bombardment method, simultaneous delivery of both DNA and effector molecules to the specific sites results in the expression of genes. Future studies include pore enlargement and multifunctionalization of MSNs to provide target-specific delivery of proteins, nucleotides, and chemicals in plant cells.

Some of the approaches, which can be used to improve nanoparticle-based gene delivery into plant cells, are given below:

1. The surface charge in protoplast and plasma membrane vary from −10 to −30 mV (Reid et al. 2002). Modifications to this surface charge can be done to enhance the adhesion of bioconjugate DNA, by introducing a positive charge over their surface. Wiesman et al. (2007) have demonstrated similar concept.
2. The porosity of protoplast is 0.1–5,000 nm (Berestovsky et al. 2001), and in plasma membrane, it varies between 1 and 50 nm (Berestovsky et al. 2001). (www.molecular-plant-biotechnology.info).
3. Stearylamine, triethylamine, and natural biodegradable polymers like chitosan can impart a positive charge on DNA conjugate nanomaterial surface (Li et al. 2011). By using the above approaches, mechanisms of nanoparticles-mediated gene transfer are presented in a simplified manner in Fig. 10.5.

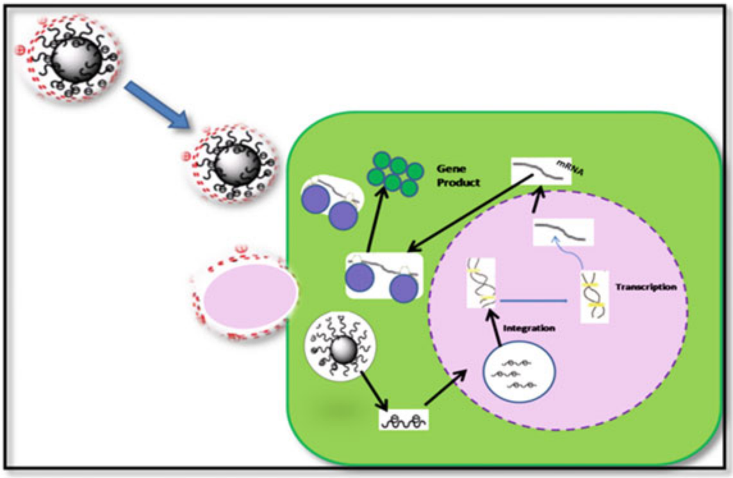

Fig. 10.5 Pictorial diagram of biotransformation of genes in modified nanoparticles and the influence of positively charged surface modification

4. The advancement in the nanotechnology may open the way for further development of efficient plant transformation system.

10.3.3 Conventional Biotransformation Technologies in Plants

Plant transformation methods and vectors have been developed with the time to enhance the efficiency and stable expression of transgene in plant transformation. Currently available plant transformation methods can be divided into two main groups: indirect and direct transformation method. The indirect method of plant transformation is based on the introduction of a plasmid carrying the gene constructs into the target plant cell (Shestibratov et al. 2007; Kakkar and Verma 2011 and www.biotechnology4u.com, Fig. 10.6).

The well-known example of indirect method includes the use of *A. tumefaciens*- or *A. rhizogenes*-based gene transfer in which their natural ability to infect and transfer genes is utilized precisely to transfer specific genes into the cells (www.biotechnology4u.com). Important events of T-DNA transfer and components involved in the *Agrobacterium*-mediated gene transfer are outlined in Fig. 10.3. The *Agrobacterium*-based DNA transformation method offers many unique advantages: (1) precise integration of genes with defined end, (2) transfer of desired DNA along with the marker gene, (3) high frequency of stable and intact gene transfer, and (4) ability to transfer long-stretch DNA (>150 kb) and reduced rate of transgene silencing (Tzfira and Citovsky 2003). Although, *Agrobacterium*-mediated gene transfer is a widely and commonly used method, it has several drawbacks

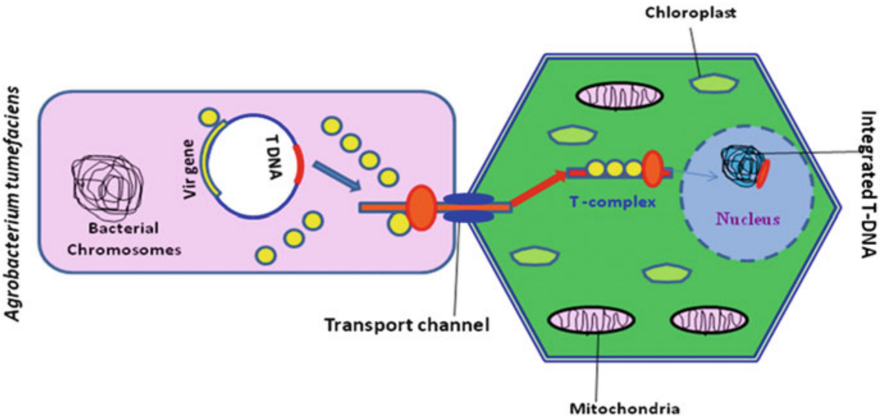

Fig. 10.6 *Agrobacterium*-mediated gene transfer in plants

(Tzfira and Citovsky 2003; Shrawat and Good 2011). These include (1) limitation to carry size base pair (<500 kb), (2) chances of transgene silencing, (3) poor gene transfer efficiency, and (4) effectiveness against dicot plants. To improve the efficiency of *Agrobacterium*-mediated gene transfer, newer modifications in indirect methods, e.g., application of acetosyringone, were applied to increase the host range (Verma and Mathur 2011). Further advances were made while developing transgenic plants lacking antibiotic marker genes, co-transformation of multiple T-DNAs, deployment of Ac-/Ds-based mobile genetic elements that helped in the elimination of marker genes in transgenic plants, and site-specific recombination strategies (Klee et al. 1987; Dafny-Yelin et al. 2008; Lacroix et al. 2008; Permiakova et al. 2009; Kakkar and Verma 2011).

10.3.4 Electroporation-Based Transformation in Plants

The transfer of DNA into cells by applying electric field is termed electroporation, which is one of the common methods for introducing genes into plant cells (Neumann et al. 1982). Gene transfer by electroporation so far has been worked out for many species, e.g., tobacco, rice, and wheat. The better results have been obtained for maize plant (Lurquin 1997). On applying electric current (pulses), the transient increase in porosity of the cell membrane allows DNA to easily transfer and enter into the cytoplasm. After sometime, the pores retain their porosity; as a result, DNA is unable to escape from the cell (Neumann et al. 1982; Chowrira et al. 1996; To et al. 1996; Lurquin 1997). Although electroporation seems to be an easy and effective method, its applications are limited to only a few species. In addition to that, the electric current applied may damage the gene leading to misleading codons and wrong translational end product (Gaertig et al. 1994; Rakoczy-trojanowska 2002; Uchida et al. 2002; Wechuck et al. 2002).

10.3.5 Temperature-Mediated Gene Transfer

Raising the temperature enhances gene transfer that is successfully adopted and confirmed to animal cell transfection process (Kenel et al. 2010). It seems from the literature that at a temperature above 37 °C, the gene transfer tendency increases and further increase up to 43 °C with a certain period of time provides greater transient transfection. This was confirmed by the work on interleukin-2 and swine growth hormone expressions using indirect ELISA (Dillen et al. 1997; Baron et al. 2001). Dillen et al. (1997) demonstrated the effect of temperature (15–29 °C) on *Agrobacterium*-mediated gene transfer in plants. Baron et al. (2001) reported the effect of temperature in biotransformation process with *A. tumefaciens* involved in cocultivation of *Phaseolus acutifolius* and further in *Nicotiana tabacum* biotransformation. In both the situations, the level of transient *uidA* expression decreased notably when the temperature was raised above 22 °C, further lowered down in temperature at 27 °C, and was undetectable at 29 °C (Baron et al. 2001; Iba 2002; Rakoczy-trojanowska 2002). Commonly used conventional methods of bio-transformation discussed here are summarized in Table 10.1 along with their advantages, mechanisms of gene transfer, and limitations.

10.3.6 Microinjection

In case of microinjection, DNA is directly injected into plant nucleus or cytoplasm, using a fine tip of 0.5–1.0 μm in diameter made up of glass needle or micropipette.

Table 10.1 Commonly used conventional methods of bio-transformation

Method	Advantages	Disadvantages
Viral	High efficient	Immunogenicity, carcinogenicity, a risk of recombination in case animal cells
Electroporation	Safe, easy, rather efficient	Need large amount DNA and has to be optimized for every cell type
Microinjection	Easy and highly efficient	Only one cell at a time can be transfected, no suitable for whole organism
Gene gun	Can delivered thousand of DNA copies, high transgen expression	Shallow penetration of DNA in tissue
Chemical method	Efficient	High toxicity
Nanoparticles	Highly efficient, size tuneable from 1 to 100 nm, possible to incorporate different functional groups on single particles, immunogenicity can be controlled	

This method is generally used to transfer genes into large cells such as oocytes and the early embryonic stage (Neuhaus et al. 1987; Holmberg and Bülow 1998; Baron et al. 2001; Iba 2002; Rakoczy-trojanowska 2002; Oard 1991; Casas et al. 1995).

10.3.7 Silicon Carbide-Mediated Transformation (SCMT)

In SCMT method, silicon carbide fibers are added to a suspension, containing both plant tissues and the plasmid DNA. It is then mixed rigorously so that DNA-coated fibers could penetrate through the cell wall (Neuhaus et al. 1987; Oard 1991; Casas et al. 1995; Holmberg and Bülow 1998; Rakoczy-trojanowska 2002; Asad et al. 2008; Sailaja et al. 2008). Although SCMT approach is an easy, fast, and economical method that can be applied to various plants, this methodology has disadvantages such as low gene transfer efficiency, can damage cells, and negatively influences their regeneration capability, and the precautions to be taken while dealing with fibers can lead to serious sicknesses (Asad et al. 2008; Sailaja et al. 2008). Moreover, the efficiency of SCMT method is affected by various variables like fiber size, vortexing time, shape of the vessels used, and the thickness of plant material (Asad et al. 2008).

10.3.8 Liposome-Mediated Gene Transfer

Liposomes are colloidal, vesicular structures consisting of one or more lipid bilayers that surround an equal number of aqueous compartments (Chen et al. 2011; Shirazi et al. 2011). Due to the presence of aqueous core and lipophilic vesicle structure, the plasmid/gene is expected to be encapsulated in an aqueous environment. Liposomal gene transfer technique has been successfully tried and adopted in gene delivery as the novel technique (Deshayes et al. 1985).

10.3.9 Gene-Loaded Liposomes Provide Numerous Advantages

Gene loaded Liposomes provides stability to genes, diminish or reduce the deletion in the DNA, while used along with the physical techniques, control release pattern of gene delivery, surface modification of liposome (positively charged) potentiate the penetration within a cell, show a higher degree of reproducibility, applicable to a wide range of cell types, and free from cellular toxicity. In this technique, the mechanism of DNA entry through the protoplast seems to be mediated by endocytosis process of liposome by the cells (Shirazi et al. 2011), which may involve the following steps: (a) adhesion of the protoplast to the lysosomal surface, (b) fusion of liposomes and protoplast after adhesion, (c) formation of endosome within the cytoplasm, and (d) release of plasmids inside the cell. The liposomal technique has been successfully used to deliver DNA into the protoplasts of a number of plant species (e.g., tobacco, petunia, carrot, etc.). Deshayes et al. (1985) developed positively charged liposomes bearing *E. coli* plasmid (pLGV23neo) having a kanamycin resistance gene, which were used in the development of transgenic tobacco. Protoplasts isolated from the leaves of transgenic tobacco were resistant to 100 mg/ml kanamycin.

10.3.10 Gene Gun (Particle Bombardment)

Gene gun is the gene transfer technology by applying mechanical force. The particle bombardment is the “biolistic particle delivery system” or a gene bombardment system, in which acceleration of the particles is achieved by using a pulse of helium. With the help of high pressure with helium, DNA can pass through cell membranes into the cytoplasm or even in the nucleus by evading the enzymatic degradation (Uchida et al. 2009). In this technique, DNA-coated gold particles are used and then transferred into target tissues or cells by using high pressure. DNA is delivered along with heavy metal particle into the target cell, and thus these particles must be nonreactive and nontoxic to the cell components cell. Naked DNA is mixed with these microparticles and then released within the cell with the help of gene gun. In Gene Gun method, DNA-coated tungsten particles were injected by gunpowder acceleration system, which transfers them through the plasma membrane into the nucleus, integrated, and ultimately resulted in stable gene expression. This method was used for transgene expression in plant cells initially and was further applied to mammalian cells and living tissues as well (Yang et al. 1990). However, the major drawback of this method was short-term and low-level expression of genes and shallow penetration ability of particles into the plant tissue. The loading of DNA onto the particles, the timing of delivery, and the velocity of acceleration were the important factors concerning the efficiency of this method. Moreover, the final distribution of DNA-coated particles is mainly influenced by the fine-tuning of the acceleration imparted by the particle bombardment method (Uchida et al. 2002). The efficiency of gene expression was dependent on the number of DNA-coated particles delivered to the nucleus and on the degree to which these particles are coated with the desired plasmid (Mehier-Humbert and Guy 2005).

10.4 Detection Method for Transformation

A transgenic plant is a plant in which one or more desired genes of foreign origin have been artificially transferred and inserted into the host genome, instead of the plant acquiring them naturally by crossbreeding or natural recombination events. The origin of transgene can be from the same genus, from a different genus within the same family, or even from a different kingdom (e.g., genetically modified Bt cotton, which produces the natural insecticide, contains a gene from a bacterium *Bacillus thuringiensis*). The development in the field of biotechnology is moving at a rapid pace, more transgenic varieties are emerging, and even the area under cultivation of transgenic is increasing day by day. Genetically modified crops can be identified either by detecting the insertion of a foreign gene at DNA level, or by the detection of mRNA transcribed from the newly inserted gene, or by the detection of a resulting protein, metabolite, or phenotype expressed by the foreign gene. The polymerase chain reaction (PCR method) helps in the detection of the inserted DNA, and immunological assays help in detecting the protein after their expression in the resultant phenotype. Although much progress in the development of genetic analysis methods is satisfactory, like those in PCR based, still other analytical technologies are emerging to provide solutions to current technical issues in the GM sample analysis. Emerging methods include the use of mass spectrometry, chromatography, near-infrared spectroscopy, microfabricated devices, and, in particular, DNA chip technology (microarrays) for GM sample analysis. The PCR-based method has been found to be more reliable for the detection of GM crop by the regulatory authorities.

10.4.1 Histochemical GUS Assay

GUS histochemical reporter gene assay is useful for the detection of transgenic plants in plant molecular study; GUS fusion constructs are previously described (Duchesne and Charest 1991; Sudan et al. 2006). GUS assay is available depending on the type of substrate used, and the best substrate currently available for GUS assay and demonstration of histochemical localization of β-glucuronidase activity in tissues and cells is 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc). This substrate works by giving a blue precipitate at the site of an enzyme activity. The product formed by glucuronidase action on X-Gluc is not colored itself. Instead, the indoxyl derivative produced by the enzymatic action must undergo an oxidative dimerization to form the insoluble and colored compound. Dimerization is enthused by atmospheric oxygen and can be also enhanced by using an oxidation catalyst such as a K+ ferricyanide/ferrocyanide mixture. Lacking a catalyst, the results are often good, but one can have concern about the possibility of apparent localization of glucuronidase. The procedure is as follows: (1) Take fresh leaves directly from control and transgenic tobacco plants, respectively. (2) Cut fresh leaf disks into quarters. (3) Transfer disks to 1 ml of histochemical reagent (X-Gluc) in 1.5-ml microfuge tubes. (4) Incubate at 37 °C for 1 h to overnight. (5) After incubation, rinse in 70 % ethanol for 5 min (Ohta et al. 1990; Vitha et al. 1995; Stangeland and Salehian 2002; Sun et al. 2006; Godoy-hernández et al. 2008).

10.4.2 PCR for Genomic DNA

PCR-based methods rely on the detection of the specific genes or DNA in the crop plant. There are several DNA-based methodologies for the detection of transgene; PCR-based method is the most common method used for commercial testing as well as by regulatory authorities for the detection of transgene. This method is based on amplification of a specific target DNA, allowing the million- or billion-fold copies of a particular gene by using a pair of oligonucleotide primers. The process involves extraction and purification of DNA, amplification of the specific DNA fragments by using a pair of primers, and confirmation of the amplified product. In principle, PCR is efficient in the detection of a single target gene in a complex DNA mixture (Seki et al. 1999).

10.4.3 Southern-Blot Analysis

Southern-blot analysis is the method routinely used for the detection of a specific gene in a DNA sample. To test for the presence of the transgene, gDNA can be isolated and purified by using standard protocol given by Sambrook and Russell (2001) followed by the digestion with restriction endonuclease like BamHI and XbaI. Then digested DNA can be purified using the phenol/chloroform method and concentrated by precipitation with ethanol (Sambrook and Russell 2001). The digested and purified DNA fragments can be separated by electrophoresis, and separated DNA fragments are transferred to a filter membrane. The detection of specific DNA fragment can be done by probe hybridization (Southern 1975).

10.4.4 Protein Extraction and Western-Blotting Analysis

Detection of the protein expressed by the transgene can be performed by Western-blot analysis. Western-blot analysis involves the extraction of the protein; the protein can be extracted from leaf tissue (100 mg) according to a protocol reported by Stoger et al. (1999). The standard protocol given by Towbin et al. (1979) included the extraction of proteins and their separation by the gel electrophoresis. The separated proteins are then transferred to a membrane, where detection of a target protein is done by using specific antibodies. Generally for the detection of target protein, primary antibodies are the polyclonal antibodies raised in rabbit against the N-terminal peptide of GUS reporter protein and secondary antibodies are raised in donkey against rabbit IgG. The membranes with the primary and secondary antibodies can be exposed to X-ray films, and the intensity of signals can be quantified by using the ImageJ program (Rybicki and von Wechmar 1982).

10.5 Conclusions and Future Prospects

It can be concluded that efficient transformation of plants with transgenes encapsulated in nanocarriers is relatively a new area in plant biotechnology. Genetic engineering is today's most important technique for fulfillment of the basic needs of the world's population. Till now, liposomes have been explored, and other nanotechnologies (nanoparticle, carbon nanotube, quantum dots, nanoemulsion, etc.) will definitely be used as they were successfully explored in animal biotechnology. Although nanotechnology-based biotransformation is relatively efficient, its combination with commonly applied approaches in transgenic plant development will surely improve in terms of efficiency and productivity, and the chances of transgene silencing can also be minimized. Nanoparticles-mediated gene transfer depends not only on the defined nanoparticle size and shape but also on surface functionalization of NPs, nucleic acid protection ability, and biocompatibility. All these parameters need to be fine-tuned for their use as carriers in gene transfer method. The mechanism of biotransformation in nanotechnology is still not clearly understood, but the possible role of endocytosis process as well as transcellular and paracellular transport of the nanocarriers-encapsulated transgene cannot be ruled out. Different kinds of nanoparticles can be loaded with nucleic acids (DNA or RNA), and cells appear to be quite indifferent to the chemical nature of these nanoparticles when it comes to an uptake by endocytosis.

On the ground of earlier reports that revealed the application of liposomal- and nanoparticle-based gene transfer, various mechanistic ideas can be postulated to improve the efficacy of this novel nonviral gene-protected biotransformation approach. Modification of physicochemical properties of nanocarriers, protoplast, and plasma membrane may significantly improve the liposomal- and nanoparticle-encapsulated gene into the plant cell and establish these gene transfer methods as sole process rather than adjuvant.

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Chapter 11
Perspectives in Nanocomposites for the Slow and Controlled Release of Agrochemicals: Fertilizers and Pesticides

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Abstract Agrochemical represented mainly by fertilizers and pesticides are vital inputs for agricultural production. However, their conventional application in field is poorly effective, with significant losses due mainly to volatilization and/or lixiviation of soluble agrochemicals. In some cases, the application exceeds two times the optimal quantity, meaning that other undesired consequences take part, such as environmental contamination or production of greenhouse gases. A considerable scientific effort has been made to develop viable systems for the controlled or slow delivery of agrochemicals, in order to adjust the nutrient availability in soil to minimal doses required for pest control or to levels needed by plants. Besides other technologies, the association of soluble materials containing fractions of minerals with very high surface area has shown to be an effective way for the optimization of agrochemical application, where the cation-exchange capacity (CEC) of minerals plays an important role. Then, the association of mineral structures (high CEC clays and layered double hydroxides, etc.) opens a new research field in the tailoring of nanocomposites, where the properties of minerals, polymers, and additives that are associated with agrochemicals (considered as the active moiety of the nanocomposites) can produce novel properties to the release control. Therefore, this chapter reviews the underlying principles in controlled or

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slow release of agrochemicals, the fundamentals of key technologies, and the current perspectives in the production of new materials, comparing their potential with conventional materials regularly produced.

11.1 Introduction

Estimates indicate that the world population will reach 9.5 billion by 2050 (Geohive 2012), and this population growth comes with the need to increase production of foods. Brazil has promoted a significant increase of national grain production due to the use of novel technologies in field, which make better use of natural resources, and this increase in productivity is supplying the growing domestic and worldwide demands. From 1992 to 2012, the Brazilian grain production increased by 143 %, while the planted area increased by only 32 %. This increase in the efficiency of grain production was accompanied by the consumption growth of agrochemicals such as fertilizers and pesticides. For example, the growth in fertilizer consumption was 217 % in the same period. Brazil ranks as the fourth largest consumer of fertilizers in the world, consuming approximately 29.1 million tons in 2013, which is equivalent to 6 % of worldwide fertilizer consumption. In the same year, the consumption of nitrogen fertilizers was 3.3 million tons, being only 33 % of this amount supplied by domestic production (ANDA 2013).

However, many agrochemicals applied to crops are not fully exploited by the agricultural production chain. Processes such as leaching or surface runoff, volatilization, immobilization, or even erosion may cause losses of some agrochemicals (Resende 2002; Benke et al. 2008). These losses cause directly and indirectly a decrease in productivity and quality of crops, in final profits, and also damage the environment. Agrochemical industries have been working to improve the efficiency of using inputs, by studying the best possible agronomic practices, from fertilization to prevent pest attack in order to benefit farmers, increasing the productivity, improving quality of food, and preserving the environment (Finck 1992). Various application technologies have also been explored to mitigate or even nullify these effects.

An alternative to enhance the efficiency of agrochemicals is the use of systems that could present a lower or controlled release of the desired compound. It should be noted that there is no official distinction between “slow-release system” and “controlled-release system.” Nevertheless, it is proposed that the term slow release is associated with the delay of the release mechanism, but not changing the mechanism itself, while in the case of controlled release, a change on the type of delivery mechanism would be responsible for delaying the nutrient release (Trenkel 2010).

The delay of initially available compounds or the extension of their continuous availability period. can occur by several mechanisms. The main strategy is to coat a conventional water-soluble agrochemical (fertilizer or pesticide) with an insoluble or poorly soluble protective layer that controls water penetration and, thus, the dissolution rate in order to synchronize the release and the consumption rates of the medium (Trenkel 2010). Among the most likely investigated systems are polymer composites, where agrochemicals are embedded within the matrix that hinders the diffusion of nutrients to the environment. In order to ensure the expected release reduction, it is usually necessary to have a large amount of host matrix (about 40 % or greater). However, formulations with only small amounts of active compounds are possible (Trenkel 2010). These limitations have motivated several studies on improving the performance of slow- or controlled-release systems based on polymeric composites or nanocomposites.

Therefore, some of the main recent advances in research concerning the application of nanocomposite materials in slow or controlled release of agrochemicals are reviewed in this chapter, emphasizing their application and prospects.

11.2 Controlled- or Slow-Release Systems Applied to Fertilizers

Plants require various nutrients for their growth and development, some in higher amounts (classified as macronutrients) and others in lower amounts (classified as micronutrients). The group of macronutrients is composed of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S). Carbon (C), hydrogen (H), and oxygen (O) are also considered macronutrients, but they are available in large quantities in the environment and are directly assimilated by plants. The group of micronutrients is composed of boron (B), chlorine (Cl), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) (Finck 1992). All these nutrients, if not available in sufficient amounts in soil, can be provided by fertilizers.

Fertilizer is any organic or inorganic material from natural or synthetic origin (except limestone) that is added to soil to supply one or more essential nutrients for normal growth and development of plants (Glossary of Soil Science Terms 2008). Nowadays, fertilizers are essential for obtaining high levels of agricultural productivity. Nitrogen stands out among the macronutrients that are mainly applied in soil management for different crops. It is required and applied in large quantities in the composition of various fertilizers. Data presented by the Brazilian Association for Promotion of Fertilizers (ANDA) in 2013 show that from 1990 to 2011, the consumption of nitrogen in Brazil increased nearly 275 %, from 0.8 to 3 million tons.

Despite nitrogen presents as one of the most abundant structural elements in living beings, it only ranks as the 17th element that composes the Earth's crust. In the diatomic molecular form (N_2), it is the major component of the atmosphere, but this state is a chemically inert compound that reacts only with great expenditure of energy. For this reason it was called diazo by Lavoisier, meaning “no life” (Mora and Lara 2000). According to Voroney and Derry (2008), approximately 8.7 % of the total nitrogen is present in the surface of the terrestrial system, being distributed as follows: (1) about 26 % is geological N–N embedded into rocks; (2) approximately 74 % is N_2, including over 99.9 % of atmospheric nitrogen; and (3) the remaining, about 0.03 %, is reactive (or fixed) nitrogen in air, water, and earth. The nitrogen in soil can be generally classified as organic or inorganic. The organic nitrogen occurs as slowly labile organic nitrogen, immobilized in the microbial biomass in residues of plants and animals or fixed in clays, and as a constituent of free amino acids or proteins, amino sugars, and other complexes (Victoria et al. 1992). With respect to the inorganic nitrogen in soil, it occurs as ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O), nitric oxide (NO), and elemental nitrogen (N_2). The last form is inert, except when used by nitrogen fixer microorganisms. From the soil fertility point of view, NH_4^+, NO_2^-, and NO_3^- are the most important forms, arising from the aerobic decomposition of organic matter in soil or from the addition of various commercial fertilizers. Also, it is noteworthy that N_2O and NO are toxic gases for environment (Tisdale et al. 1985).

Chemical or synthetic fertilizers are the most important sources of nitrogen used in large-scale cultivation of crops (Chien et al. 2009). Usually, nitrogen is used in combined forms, for instance, ammonium nitrate or other ammonium salts or nitrates (ammonium sulfate, calcium nitrate, potassium nitrate, etc.) and urea. Urea ($(NH_2)_2CO$) is the most common source of nitrogen, covering nearly half of the worldwide need for nitrogen-based fertilizers. It has been widely used due to its high nitrogen content and easy application, in dry granular form or as aqueous solutions (Saggar et al. 2013). Also known as carbamide, urea is an endogenous product from protein and amino acid catabolism. All industrial processes to produce urea involve the reaction of ammonia (NH_3) and carbon dioxide (CO_2), at elevated temperature and pressure to form ammonium carbamate, which is then dehydrated to form urea (Lewis 1997). The concentration of biuret (an impurity usually found in low concentrations) in urea deserves special attention due to its phytotoxicity (Mikkelsen 1990). Biuret levels of about 20 % can be tolerated in most fertilization programs. Citrus and other crops, including pineapple, are more sensitive to biuret in urea, applied by foliar spraying. In this way, a product containing less than 0.25 % of biuret is recommended for those crops (Albrigo 2002; Achor and Albrigo 2005).

When urea is added to the soil, it is first hydrolyzed to NH_4^+ by urease enzymes (Eq. (11.1)). This process depends on the number of active urease molecules and factors that affect their activity, such as urea concentration, organic matter content, temperature, humidity, and soil pH (Tabatabai and Bremner 1972; Wang et al. 2004). In a second step, the NH_4^+ is oxidized to NO_3^- by the nitrification process (Eqs. (11.2) and (11.3)) (Cartes et al. 2009):

$$CO(NH_2)_2 + 3H_2O \xrightarrow{\text{urease}} 2NH_4{}^+ + HCO_3{}^- + OH^-, \tag{11.1}$$

$$NH_4{}^+ + \frac{3}{2}O_2 \rightarrow NO_2{}^- + 2H^+ + H_2O, \tag{11.2}$$

$$NO_2{}^- + \frac{1}{2}O_2 \rightarrow NO_3{}^-. \tag{11.3}$$

Depending on conditions such as cation-exchange capacity (CEC), texture class (clay content), pH, and soil temperature, $NH_4{}^+$ can also be converted to NH_3, which is lost to the atmosphere (Byrnes 2000).

Thus, it is observed that not every compound applied to crops as fertilizer is absorbed by plants. Losses may occur through leaching, evaporation, fixing, or even by erosion (Aarnio and Martikainen 1995; Resende 2002). The average nitrogen content absorbed by crops, particularly in tropical regions, is between 50 and 70 % (Finck 1992; Mortvedt et al. 1999). It means that an average of 60 % of this nutrient is lost after application, causing part of investment in fertilization not to return as expected. The loss of nitrogen through volatilization of NH_3 to the atmosphere is the main problem responsible for the low efficiency of urea applied to the soil surface, which may lose up to 80 % of applied nitrogen (Tabatabai and Bremner 1972; Wang et al. 2004). In addition to the economic losses, there are also environmental damages because the excess of those nutrients in the soil can lead to a contamination of both water and atmosphere, depending on the applied material. Thus, the efficient use of agrochemicals is essential and strategic to obtain maximum crop yields and to minimize the economic and nutrient losses to the environment.

The nitrogen cycle, as for other elements, is normally in equilibrium. However, this cycle can be driven by agricultural or industrial activities or even by natural causes. This often results in undesirable accumulation of intermediates; many of them show certain toxicity. When the nitrogen cycle becomes unbalanced, there are often ecological problems. For example, excess $NH_4{}^+$ and oxidized nitrogen compounds promote algal blooms, which in conjunction with other problems cause oxygen depletion. The $NO_2{}^-$ and $NO_3{}^-$ ions, at certain levels, may be toxic to humans and have been associated with various diseases. Nitrogen compounds are also one of the causes of acid rain (Kuenen and Robertson 1988; Liu et al. 2013).

Another problem, originated from the imbalance of the nitrogen cycle in soil, is the release of gases such as N_2O and NO, which can also cause environmental problems. In the soil, these gases are produced mainly by denitrification process, that is, the conversion of $NO_3{}^-$ to N_2, but this process may produce NO and N_2O as intermediates (Eq. (11.4)), in the presence of specific enzymes. This process primarily occurs under anaerobic conditions, but can also occur in partially anaerobic conditions (anaerobic sites in the soil), which indicates that during the nitrification process, N_2O can also be formed (Bremner 1997; Kool et al. 2011).

$$\begin{array}{ccccccc} NO_3^- & \rightarrow & NO_2^- & \rightarrow & N_2O & \rightarrow & N_2. \\ & & \downarrow & & & & \\ & & NO & & & & \end{array} \tag{11.4}$$

The production of these gases is an important factor, considering the effect that they can have on atmospheric chemistry, effectively contributing to the greenhouse effect and ozone depletion (Snyder et al. 2009; Serrano-Silva et al. 2011). The atmospheric N_2O concentration is substantially lower than that of CO_2; however, the impact on the global warming on a mass base shall be 298 times higher than CO_2, prospecting for 100 years. The effective contribution of each substance to global warming must be balanced by the molecular weight, the mean residence time in the atmosphere, and the cumulative heating effect of each gas (USEPA 2011). Moreover, it is expected that N_2O emissions from agriculture, on a global basis, could increase by 35–60 % by 2030 (FAO 2003; IPCC 2007), considering the need for agricultural production to supply the growing global population demand. Thus, it is necessary to control the presence of nutrients in soil in order to minimize the environmental and economic damages caused by inappropriate use of fertilizers. However, this is not a simple challenge because not only nitrogen but also losses of other nutrients from agricultural sources mainly vary with the type of soil and climate, parameters that are beyond the control of any farmer (Cavigelli 2005).

Many strategies can be developed to enhance the use of nutrients in fertilizer, as well as to maximize their benefits to the soil–plant–atmosphere system. The concept of efficient use of fertilizer is the one that reflects the increase in production per unit of applied nutrient, whereas in contrast, the low efficiency means low productivity and low profits (Lopes and Guilherme 2000). In general, some changes in management can increase the efficiency in the use of nitrogen fertilizers, for instance, proper incorporation into the soil; split fertilization; controlled irrigation; adjustment of fertilizer based on tracking the nitrogen content in plant tissues; removal of factors that limit plant growth, such as grass weeds, which cause damage and competition, and pests; limitation of other nutrients; use of cover crops to fix the residual nitrogen in soil during postharvest periods; use of improved technologies in work with farmers; and finally optimization of application rate and timing of fertilizer, using specific forms of nitrogen compounds in individual cases to reduce losses (slow- or controlled-release fertilizers and nitrification inhibitors) (Lopes and Guilherme 2000; Cavigelli 2005; Ni et al. 2011). In relation to the use of materials for slow or controlled release, considerable scientific efforts have been made to develop new materials capable of increasing the efficiency of nitrogen fertilizers, especially for urea. This has been done through, for example, coating urea granules with polymers (Gagnon et al. 2012) or sulfur (Costa do Nascimento et al. 2013), use of urease inhibitors (Singh et al. 2013), etc. However, these products have a limited use due to the additional costs incurred in the manufacturing process, resulting in an increase of the final product cost and, consequently, the cost of production.

Due to the recorded losses during and after the agrochemical applications, various forms of management and technology are also being explored to mitigate or even nullify these effects. An alternative to increase the efficiency of using fertilizers is the application of sources which present a slower or controlled release of nutrients.

Conceptually controlled- and slow-release fertilizers, also called “smart fertilizers,” are prepared to release their nutrient content in a gradual fashion and, if possible, match its release with the nutritional requirements of a plant or to make its availability much longer than that of a reference product, such as high soluble fertilizers, which rapidly make their nutrients available (Hanafi et al. 2000; Trenkel 2010). There is no official distinction between the concept of controlled-release and slow-release fertilizers. Shaviv (2001) reported the following distinction: the term “controlled-release fertilizers” is acceptable when applied to fertilizers in which the factors that dominate the rate, type, and duration of release are well known and controllable, during the preparation thereof; “slow-release fertilizers” involve a slower nutrient release than usual, but the rate, type, and release duration are not well controlled. However, the distinction between the terms is not determinative because these definitions are still open and modifiable.

The significant advantages of these systems are that they can eliminate the use of parcels of fertilization of covering, reducing the labor, saving fuel, minimizing soil compaction and root damage, and preventing physical damage to the crop from the application process, as well as reducing environmental contamination (Shaviv 2001; Trenkel 2010). Figure 11.1 shows a comparison between a conventional system and a controlled release for a particular active ingredient.

For example, Zhao et al. (2013) evaluated the effects of applying controlled-release fertilizer on the production, ammonia volatilization, nitrogen use efficiency, and photosynthetic rate in maize cultivation (hybrid cultivar Zhengdan 958). They

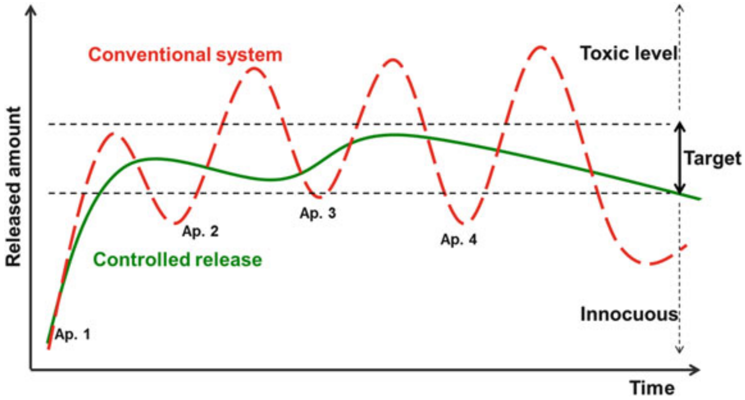

Fig. 11.1 Difference between the conventional model and controlled-release system, indicating different times of application for a compound

observed that the use of materials (coated urea with resin or sulfur) provided better results in all parameters than the commonly used fertilizers.

Gagnon et al. (2012), in a 3-year study (2008–2010) conducted in clay soil near the city of Quebec, Canada, compared the effect of polymer-coated urea, urea with nitrification inhibitor, dry urea, and urea/ammonium nitrate 32 %, on the yield of corn cultivation, accumulated N in the plant, and NO_3^- remaining at harvest soil. The authors observed that the polymer-coated urea had superior behavior for two of the three evaluated parameters, especially in wet years—suggesting that this material can be an alternative for farmers in the fertilization of corn crops in eastern Canada.

Li et al. (2014) investigated the benefits of slow-release fertilizer systems, in potassium sources, by means of a material based on treated coal ash. In that study, tests were made directly into the soil, where they observed that the slow-release system contributed significantly to the development of crops.

Controlled- or slow-release systems can be prepared by (a) chemical modification, usually organic nitrogen compounds, for example, condensed aldehydes with urea, which are much less soluble in water than urea; (b) change in the physical size of granular fertilizers, using much larger urea granules, usually called supergranules (the combination of larger particle size with a deeper application of fertilizer reduces loss by leaching and evaporation); (c) the use of inorganic compounds with low solubility, such as metal ammonium phosphates (e.g., magnesium ammonium phosphate ($MgNH_4PO_4$)), and partially acidified phosphate rocks; (d) use of physical barriers, in which the fertilizer may be formatted as tablets or coated granules (encapsulated) with hydrophobic polymers or minerals; or (e) as matrixes in which the active soluble material is dispersed into a continuous medium that restricts the nutrient dissolution.

Some products are already commercially available; for example, Basacote® and Osmocote® refer to the surface coating of conventional granules, which provide a solubilization barrier; however, when its rupture occurs, they lose their retention efficiency. Thus, many studies have been conducted to develop new methods to protect the agrochemicals, such as incorporation into a composite structure. Yang et al. (2013) synthesized a bio-polyurethane from corn straw, isocyanate, and diethylenetriamine, which was used to coat urea granules, in order to create a controlled-release fertilizer. In the same study, a new superabsorbent compound was also formulated from proteins of chicken feathers, acrylic acid, and *N*,*N*-′-methylenebisacrylamide and tested as outer granular coating for water retention. The obtained materials were effective in reducing N losses by leaching and increased retention capacity of water in soil when compared to the conventional fertilizer tested. Figure 11.2 shows images of these new fertilizer materials and cumulative nitrogen release rate of different coated fertilizers in water and soil.

Liang and Liu (2006) presented the preparation of a slow-release compound fertilizer based on double-coated urea granules with poly(acrylic acid) (outer coating) and polystyrene (inner coating). Tao et al. (2011) succeeded in preparing a controlled-release nitrogen fertilizer which was able to retain water. However, this was possible only with the triple-coated urea granule, being polyethylene as the inner coating, superabsorbent poly(acrylamide-*co*-acrylic acid) as the medium

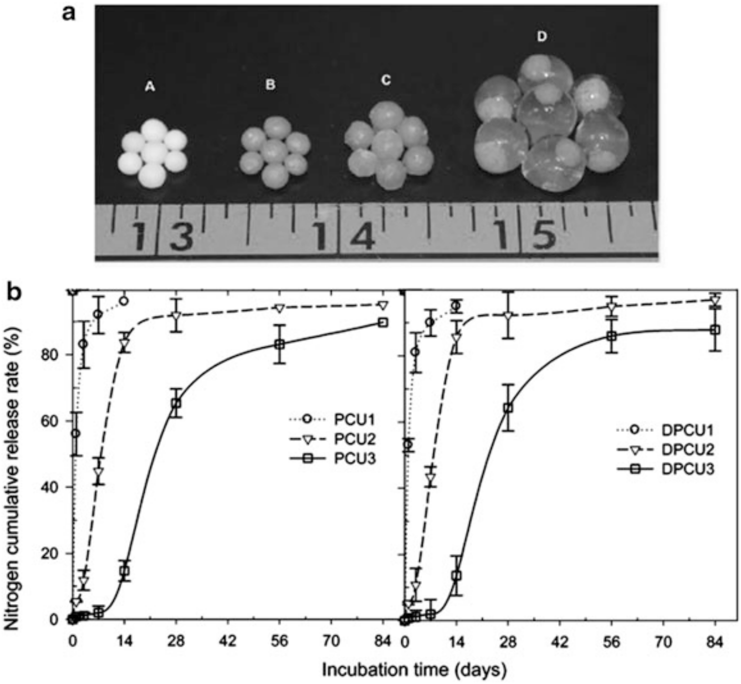

Fig. 11.2 (**a**) Images of fertilizer materials used in the experiments: urea (A), polymer-coated urea (B), dry double-layer polymer-coated urea (DPCU) (B), and swollen DPCU (D); (**b**) cumulative nitrogen release rate of different coated fertilizers at 25 °C in water (A) and soil (B) to double-layer polymer-coated urea (DPCU). Reprinted (adapted) with permission from Yang et al. (2013), copyright 2014 American Chemical Society

coating, and poly(butyl methacrylate) as the outer coating. Costa et al. (2013) studied urea granules coated with polyhydroxybutyrate and methyl cellulose under various conditions, in the presence of emulsifiers, showing that the tested polymers were effective as coating, leading to a reduction of the dissolution rate of urea in water.

Nitrogen losses can also be reduced using zeolite as an additive to fertilizers in order to control the retention and release of NH_4^+. Zeolites are hydrated crystalline aluminum silicate minerals of alkali or alkaline earth metals. They are structured in rigid three-dimensional crystalline lattices formed by SiO_4 and AlO_4 tetrahedrons, whose union of rings forms a system of channels, cavities, and pores (Ming and Mumpton 1989). This structural description is fairly comprehensive, allowing the formation of zeolite structures with channels (mesopores) of around 3 Å to 2 nm. Zeolites are naturally found in various forms (Ming and Mumpton 1989), but they can be easily synthesized with the same structures from aluminosilicate sources as well (Mignoni et al. 2008). Due to this mesoporous structure, zeolites generally have high surface area (above 100 $m^2\ g^{-1}$). However, the aluminosilicate structure is not neutral and requires counterbalancing cations, being the most common Na^+ and K^+ (Barrer 1981).

The presence of cations, associated with high surface areas, provides one of the most important properties of zeolites, that is, their high capacity of cation exchange (CCE), generally exceeding 2 meq g^{-1}. Thus, zeolites also may be used to retain exchangeable cations such as ammonium (NH_4^+) from the urea hydrolysis. As this retention has chemical character, i.e., the exchanged cation chemically adsorbed on the structure, it can be understood that the principle of zeolite action in ammonium retention consists in decreasing the element concentration in solution by cation exchange (Bartz and Jones 1983; Ferguson and Pepper 1987) and chemical fixation of the exchanged cation.

When zeolites adsorb NH_4^+, they can also contribute to reduce its losses from the soil, as shown by Mackown and Tucker (1985), who observed that the clinoptilolite zeolite structure decreased nitrification of NH_4^+ to NO_3^- up to 11 %. This decrease was a result of the retention of NH_4^+ by clinoptilolite in sites where nitrifying bacteria could not oxidize NH_4^+. He et al. (2002) obtained a significant reduction of losses by volatilization when urea (200 mgN kg^{-1}) was combined with clinoptilolite zeolite (15 g kg^{-1}). In Brazil, Bernardi et al. (2014) also observed a decrease in volatility losses and improved the fertilizer efficiency by mixing N-urea and a national zeolite named stilbite.

Clinoptilolite, in particular, is an interesting zeolite for retaining NH_4^+ because its structure shows a high affinity by this cation. As the zeolitic structural cavities (mesopores) present distinct volumes, it is common to present affinity with different cations, due to the volume of exchanged ion. Thus, zeolites with relatively large mesopores, as clinoptilolite, present a greater stability for bulky cations, such as NH_4^+, while other structures exhibit different affinities (McGilloway et al. 2003; Gruener et al. 2003; Rehakova et al. 2004). In Brazil, there are some natural zeolites; however, clinoptilolite is not abundant. There is a significant deposit of natural stilbite, located at Parnaíba Basin, Maranhão. The availability of mineral becomes, in this sense, a key topic for the viability of scaling up zeolite-based controlled-release technologies.

However, the cation-exchange behavior, in general, is shown by aluminosilicate, represented not only by zeolites, but especially by clay minerals. Clay minerals are basically crystalline hydrated aluminum silicates, structurally oriented as silicate lamellas bonded to aluminate lamellas. These lamellas are spatially arranged by stacks and separated by exchangeable ions and water molecules, forming a three-dimensional arrangement (Murray 2000). As observed in zeolites, the CEC in clay minerals is quite pronounced; however, the diversity of values is very large, ranging from around 10^{-3} meq g^{-1} (phyllites) to around 1 meq g^{-1} (montmorillonites and vermiculite). In nature, they are commonly identified as clays, which is the common term for mineral materials, consisting predominantly of clays, mixtures of quartz, organic material, and other contaminants in smaller proportions. There are about 40 clay minerals, separated into groups with basis on similarities in chemical composition and crystalline structure. The crystalline structures are classified into two types: structures 1:1 (kaolinite, serpentine) and structures 2:1 (talc–

pyrophyllite, mica, smectite, vermiculite, chlorite, attapulgite, sepiolite). Only a small number of clay minerals are components of industrial clays: kaolinite (kaolin), montmorillonite (bentonite), talc (talc), vermiculite (vermiculite), and chrysotile (asbestos).

These materials are being used more and more because of their abundance, their ease to handle, and their potential in various applications, including fertilizers. The interest in clay minerals is also because of some of their special behaviors such as swelling, adsorption, rheological and colloidal properties, and plasticity (Santos 1989; Konta 1995).

There are some examples of clay minerals used in conjunction with fertilizers. Bentonite avoids a major problem in the manufacture of fluid fertilizers, that is, the difficulty to obtain formulations with a high concentration of nutrients. Particularly, dispersed bentonite increases the viscosity of formulations with more than 12 % of K_2O, preventing the precipitation of crystals (Korndörfer and Datnoff 1995). Another example is a fertilizer produced in Canada, in which molten elemental sulfur is incorporated into bentonite, thereby obtaining a granulated fertilizer that facilitates the application of the product (Boswell et al. 1988; Saik 1995). There are still reports with rocks containing biotite or phlogopite, which show potential as nutrient source (Nascimento and Loureiro 2004). Thus, exploration of mineral diversity is important to discover new fertilizer sources and to design new methods of implementation and their availability.

However, the cation-exchange behavior in clays is quite different from that observed in zeolites. Since the structure behaves as a set of stacked nanometric lamellas, the ionic accessibility depends on its appropriate exfoliation. This phenomenon occurs spontaneously in aqueous suspensions, but in dry material, there is a tendency to re-agglomeration (Santos 1989; Konta 1995). Thus, applications of clay minerals where their high surface must be accessible are only possible after surface modification of clays, which exposes the clay nanostructure. This area has received much attention because it allows expanding the possibilities of using clays, creating new materials and new applications, mainly in nanocomposites production.

Studies have demonstrated that structure of clay minerals can be modified by pillarization (Luna and Schuchardt 1999; Pergher and Sprung 2005) or exfoliation phenomena (Murray 2000). Exfoliation takes places by a primary ion exchange (Na^+ or Ca^{2+}) by larger ions, resulting in a interlayer expansion. This process was demonstrated using NH_4^+, from the urea hydrolysis, which makes the process interesting for agriculture (Gardolinski et al. 2001). However, the most significant recent studies involve surface modification of clays with polymers or compatibilizers, allowing exfoliation of the clay within the polymer matrix.

A recent study conducted by Kim et al. (2011) proposed the intercalation of a large amount of complexed urea and magnesium in montmorillonite. The efficiency of the product was measured and confirmed by considerable suppression of the emission of both NH_3 and N_2O. It was also noted that these effects improved the nitrogen uptake by crops and, therefore, the productivity. Figure 11.3 illustrates the behaviors of intercalated urea molecules in soil. However, the authors studied the

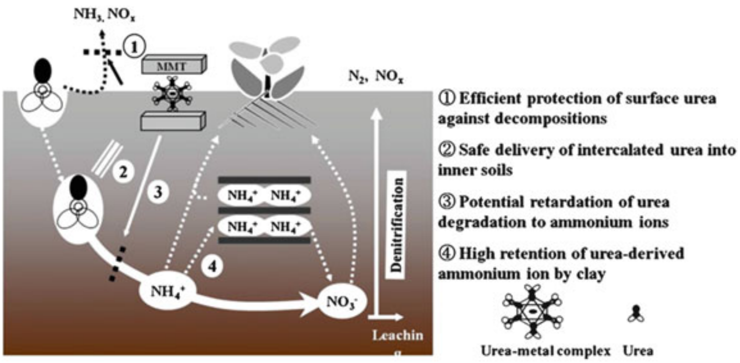

Fig. 11.3 An illustration for behaviors of intercalated urea molecules in soils. Reprinted (adapted) with permission from Kim et al. (2011), copyright 2014 Springer

material from an agronomic point of view, i.e., the material (prepared by physical mixing of the constituents and melting at 105 °C) was not studied in terms of final properties and their relations with the processing method. It is noteworthy that the agronomic data are motivating and allow one to suppose that more attention in the composite production stages could maximize the final results.

Pereira et al. (2012) demonstrated that nanocomposites produced from montmorillonite exfoliation into urea matrix can control the solubilization process of urea, delaying its release to environment. The results showed that it is possible to obtain a material processable by cold extrusion, with high N content and strength compatible to the final application. Microstructural analysis of the nanocomposites showed that the extrusion process generated two regions, one comprising the nanocomposite itself (montmorillonite and urea) and the other with urea granules. Thus, the authors attributed the release process not only to the clay mineral–urea interaction but also to the creation of barriers to free urea diffusion out of the granules. Scanning electron microscopy (SEM) images of these nanocomposites and a scheme of the proposed pathway for urea intercalation during extrusion process are depicted in Fig. 11.4.

Hydrogels have been studied as polymer matrixes for controlled-release application, specially because of their interesting properties in the release of water-soluble inputs. Hydrogels are materials formed by long, flexible polymer chains, which can be linked by covalent (chemical or cross-linked hydrogels) or physical interaction (physical hydrogels) (Peppas et al. 2000). Under specific conditions, these materials can absorb large quantities of water as well as a nutrient solution containing some active compound (Campese et al. 2007). The difference between chemical and physical hydrogels is in their chain formation: chemical hydrogels cannot be dissolved once they are formed, whereas physical hydrogels can be dissolved with external stimuli such as changes in temperature, pH, ionic strength, etc. Due to these properties, hydrogels are used in a wide range of sectors, strength in the manufacture of hygiene products (Singh et al. 2010), agriculture (Leone et al. 2008; Sorbara et al. 2009; Zhang et al. 2009), drug delivery (Hamidi

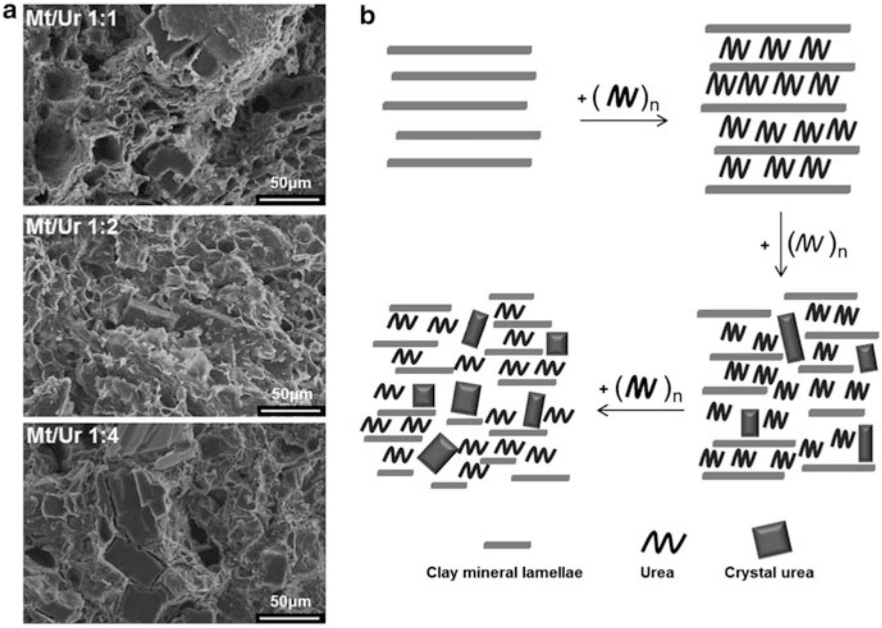

Fig. 11.4 (**a**) SEM images of the nanocomposites prepared in different weight ratios of the montmorillonite (Mt) and urea (Ur); (**b**) scheme of the proposed path for urea intercalation in extrusion process. Reprinted (adapted) with permission from Pereira et al. (2012), copyright 2014 American Chemical Society

et al. 2009), pharmaceutical industry (Kondaveeti et al. 2013), biomedical applications (Kaihara et al. 2008; Wang et al. 2013), tissue engineering and regeneration (Zhang et al. 2011), dressings (Sikareepaisan et al. 2011), barrier materials to regulate biological adhesions (Roy et al. 2010), and biosensors (Khimji et al. 2013), among many others.

Beyond the physical and chemical character, hydrogels can be classified as natural or synthetic. Natural hydrogels are composed of polymers from natural origin, with or without chemical modification (hyaluronic acid, alginate, starch, chitosan). Synthetic hydrogels are formed by polymerization of synthetic monomers, such as acrylamide, methacrylic acid, etc. Hydrogels can also be obtained by the combination of natural and synthetic polymers, such as polysaccharides, which in many cases can improve the properties of the final material (Enas 2013).

Hydrogels can be classified as homopolymers, copolymers, and interpenetrating polymer chains, depending on the preparation method and ionic charge. Homopolymer hydrogels are formed by a single type of hydrophilic monomer, which can present cross-linking depending on the monomer nature and polymerization technique (Takashi et al. 2007). Copolymer hydrogels are composed of two or more different kinds of monomers having at least one hydrophilic component, arranged in a random configuration (alternating or block), along the polymer chain network (Yang et al. 2002). Interpenetrated chain hydrogels or IPN (interpenetrating polymer network) is made up of two polymers, where one is cross-linked to the polymeric chain of the other, thereby getting their chains intertwined at the molecular level. Hydrogel may also be semi-IPN; in this case, the polymer is also formed by the combination of two different polymers, but one in cross-linked form and another linear (Yoshunari et al. 2005; Ma et al. 2007).

Over the last decades, natural hydrogels have been gradually replaced by semisynthetic hydrogels, which exhibit greater durability, high capacity to water absorption, higher mechanical strength, and biodegradability.

Within the various application areas, an aspect that has been highlighted is the application of hydrogels in agriculture. Some studies reported in literature show that hydrogels began to be studied conditioners from the 1980s (Willingham and Coffey 1981; Wallace 1987; Sayed et al. 1991). Thereafter, it was shown that the application of hydrogels optimizes water availability in soil, reduces nutrient losses by leaching and percolation, improves aeration and soil drainage (Henderson and Hensley 1986; Lamont and O'Connell 1987), increases the seedling budding index, and accelerates the root development and plant aerial part, leading to a significant increase in the final production per hectare (Nissen 1994).

Recently, several works started reporting hydrogels as carriers of nutrients. Guo et al. (2005), in a study about encapsulation of urea fertilizer in starch hydrogels, found that 40–70 % of nitrogen present in the hydrogel and urea capsule is released into the soil, so it can be absorbed by plants. Mikkelsen et al. (1993) studied the efficiency of hydrogels regarding the loss of nitrogen by leaching and concluded that the presence of the hydrogel reduced by 45 % the nutrient losses by leaching and increased the growth of a grass (test plant) by 40 % in comparison to grass planted in standard conditions. Bajpai and Giri (2003) studied the potential of controlled nutrients release in graft-polyacrylamide hydrogels without carboxymethyl cellulose chains. The authors observed that the release was highly dependent on the chemical structure of the hydrogel, pH, and temperature of swelling.

Despite the good performance of hydrogels in agriculture, their application is still limited to the final price and low biodegradability of the product, often preventing this material from using in scaled-up applications. In order to improve the potential of hydrogels in agriculture, various studies have been performed to obtain hydrogels combined to polysaccharides (Nie et al. 2004; Leone et al. 2008). Polysaccharides present a high number of hydroxyl and carboxylic groups that could improve the hydrophilic character of hydrogels; they also increase the hydrogel biodegradability because their structures have glycosidic groups, which facilitate biodegradation by bacterial or fungal attacks (Leone et al. 2008; Wallace 1987).

Another strategy for the modification of hydrogel properties is to design it as a clay-based composite or nanocomposite. As abovementioned, clay is widely present in soil and has high hydrophilicity, high cation-exchange ability, and, therefore, high affinity to the hydrophilic hydrogel chains. Thus, clay minerals generally can be properly incorporated into the polymeric network, during the hydrogel synthesis, allowing improvements on the mechanical properties, but also on sorption and desorption of nutrients (Yumei et al. 2009; Zhou et al. 2014). Furthermore, depending on the amount of clay mineral added to hydrogels, the cost of the material can be substantially reduced, making it competitive in the trading market.

Obtaining hydrogels modified by clay minerals results in materials called composite, defined as a material obtained from two or more constituents, with different physical and chemical characteristics, and that remain separated at a microscopic scale (Mitchell 2004), but the combination ensures the material properties associated to the interaction of its components (Lan and Pinnavaia 1994).

Clay minerals-based nanocomposite hydrogels have been studied for various applications, particularly in controlled sorption and desorption, since their presence causes more interaction of the gel with cationic groups into the swelling medium. The formation of nanostructured hydrogels with clay minerals has been studied by some authors, although they are still focused on modifying hydrogel properties, especially concerning increments on mechanical strength (Wu et al. 2000; Liu et al. 2006). However, some authors (Kasgoz and Durmus 2008; Yi and Zhang 2008; Li et al. 2009) have observed that the obtained composite hydrogels could have interesting ions adsorption properties and extended release. Recent studies show the properties of this class of nanocomposites for capturing ions in solution, especially for the removal of heavy metals and cationic dyes (Yi and Zhang 2008; Li et al. 2009; Zhang et al. 2014). However, these papers cited above involved small amounts of clay (up to 2.0 % by mass of hydrogel).

The choice of using hydrogels as a base of composites, as supporting material for controlled/slow release in agricultural applications, is because they are multifunctional materials, i.e., they contribute to keep both water and nutrient release rates in optimum ranges for development of crops. Therefore, modified hydrogels are suitable carrier systems for applications in controlled or slow release of agricultural inputs (Khare and Peppas 1995).

Mikkelsen et al. (1993) evaluated the effect of hydrogel with MnO, $MnSO_4 \cdot 4H_2O$, and $MnCl_2$ in a soybean crop and observed an increase of 89 % in the accumulation of the micronutrient Mn in the air portion of the crop. Ni et al. (2011) developed a superabsorbent material for slow/controlled release of urea, and it presented a good release behavior: after 30 days in soil, the material released about 70.0 % of the incorporated urea. However, the authors incorporated urea during the material synthesis in the proportion of urea–hydrogel (wt.%) of 3 g urea/g of hydrogel, but water absorption rate was relatively low (50–70 g/g product), and the high cost hampers current application of the material.

Bortolin et al. (2013) synthesized a novel series of hydrogels, composed of polyacrylamide, methylcellulose, and montmorillonite, in which the presence of the clay mineral resulted in some improvements of the materials' properties. Release tests of urea (adsorption–desorption) showed that the inclusion of montmorillonite in the hydrogel allowed the composite to release urea in a more controlled rate than the pure hydrogel and almost 200 times slower than conventional pure urea (Fig. 11.5). These data demonstrate the great potential of clay minerals, particularly montmorillonite, as the basis for new slower- or controlled-release fertilizers.

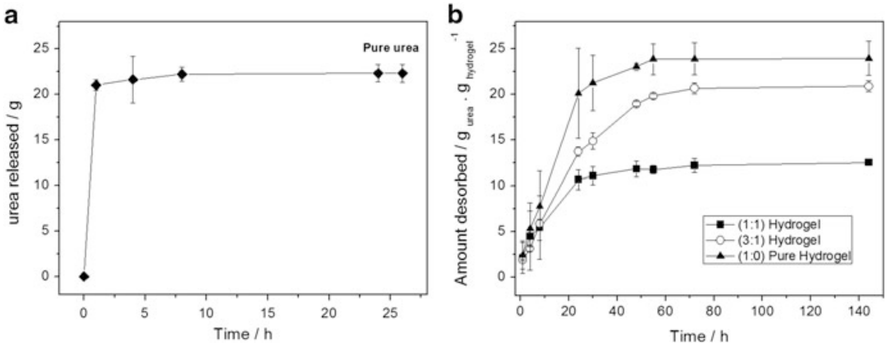

Fig. 11.5 Kinetic curves of controlled desorption of urea (mg/g) for (**a**) pure spherical urea and (**b**) different hydrogels at pH 9.0. Reprinted (adapted) with permission from Bortolin et al. (2013), copyright 2014 American Chemical Society

11.3 Controlled- or Slow-Release Systems Applied to Pesticides

The use of agricultural pesticides is an essential practice for the protection of crops against pests, diseases, and weeds because they compete with crops for water, light, nutrients, and space, thereby reducing crop production. Weeds can also host pests and diseases (Ratnadass et al. 2012). Pesticides are substances or mixture of substances intended to prevent, destroy, repel, or mitigate any pest. Agriculture will always have to deal with crop losses caused by these biotic stresses. In this way, during the last 50 years, the use of pesticides has contributed to improve the production and productivity of crop harvest, providing a better quality of crops and supplies and thus contributing to the promotion of human health (Soane et al. 2012).

According to Arias-Estévez et al. (2008), 60–70 % of pesticides used in agricultural fields do not reach the target surface, and these fractions are lost in the environment. Therefore, pesticides have become one of the most important organic pollutants found in water and soil, generating concerns about their effects to the environment and human life (Canle et al. 2001). The controlled release of agricultural pesticides could be a promising strategy to reduce the applied amount of these agrochemicals, reducing their impact on the environment and human health and reducing farming costs (Fernández-Pérez et al. 2004; Sopenã et al. 2007; Bessac and Hoyau 2013).

The application rates for conventional formulations of herbicides are generally higher than the minimum dosages required, allowing losses by degradation, leaching, volatilization, and adsorption (Muro-Suñé et al. 2005). In addition to the significant increase in costs of agrochemicals per application, super dosages are sources of environmental contamination. Pesticides have become one of the largest organic pollutants of water and soil, generating worries related to their effects on the environment and human life (Canle et al. 2001; Giacomazzi and Cochet 2004;

Torres 2007). In the middle of the last decade, research started being widespread focusing particularly on the controlled release of pesticides in both theoretical and experimental directions (Yan et al. 2013).

Encapsulation of pesticides by natural polymers (starch, for instance) has received much attention recently (El Bahri and Taverdet 2005; Chen et al. 2008; Jerobin et al. 2012). The use of starch is being encouraged because of its nontoxic characteristics, biodegradability, easy handling and processing, and low cost. When using these systems, the release is primarily controlled by diffusion processes: the starch granules applied to the soil absorb water and swell, and the encapsulated compound diffuses out of the starch matrix (Wienhold and Gish 1994; Giroto et al. 2014). In this type of release process, the release itself is difficult to be controlled, since the diffusion of the active compound is influenced only by the matrix properties and environmental conditions. Biopolymers produced using starch have attractive features, however, tend to present some limitations in certain aspects, such as weak mechanical resistance and hydrophilic degradation. In this last case, the water molecules can attack the hydrogen bonds of the starch structure, weakening its bonding strength, thus diminishing its functional properties that are essential for use in agriculture (Matsuda et al. 2013). Another important aspect is the affinity between the polymer matrix and the compound to be encapsulated, as the case of many pesticides and starch. The hydrophilic character of starch and the low water solubility of pesticides result in a poor chemical affinity between two components, making the pesticide encapsulation in starch matrix an unsuitable strategy.

In order to minimize or even nullify these drawbacks, modifications of starch are reported. The preparation of starch–clay composites has been widely explored (Cyras et al. 2008; Chivrac et al. 2010; Aouada et al. 2011; Ojijo and Ray 2013). The clay can be included into the matrix by intercalation as well as exfoliation processes. If the clay presents an attraction to the polymer matrix, it binds to hydrophobic sites of the starch molecule chains, reducing its hydrophilic character and leading to a more compatibility with the herbicide. In this way, aluminosilicates (clays) have become one of the most commonly reported materials in the preparation of starch-based biodegradable composites. The improvement on properties is proportional to the dispersion level of the clays within the polymer matrices. A homogeneous dispersion of clay can provoke an increase on mechanical properties and generate a physical barrier effect against the release of compounds or even to water absorption. Thus, the water molecules need to follow through a more tortuous path in the polymer–clay composite, reducing the diffusion rates of compounds within and outside the matrix, and thus to high barrier levels.

Céspedes et al. (2013) observed that the modification of alginate with kaolin and bentonite reduced the release rate of pesticides from the composites. Clays acted as adsorbents, slowing the release time of pesticides and reducing the amount of active compounds available for leaching or volatilization. Figure 11.6 shows that 89.78 % of technical grade product is dissolved in less than 6 days, whereas it takes at least 108 days to release 93.43 % of active compound from the alginate-based

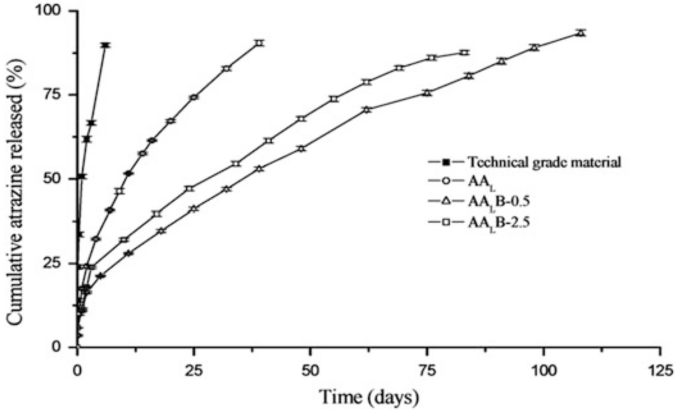

Fig. 11.6 Cumulative release of atrazine from granules into static water (error bars represent the standard deviation of three replicates). Reprinted (adapted) with permission from Fernández-Pérez et al. (2004), copyright 2014 American Chemical Society

controlled-release formulation AALB-0.5 (containing 1.2 % of technical grade atrazine (A), 1.4 % of sodium alginate (AL), and 5 % of acid-treated bentonite).

Singh et al. (2009) studied the incorporation of clays such as kaolin and bentonite as reinforcement to alginate and starch, intended for the controlled release of the fungicide Thiram®. The presence of both clays in the polymer delayed the fungicide release. The authors found out that the use of such composites as controlled-release systems can be very useful for safely handling of pesticides. In the study conducted by Chevillard et al. (2012), a slow release of ethofumesate was observed due to the use of hydrophobic montmorillonites as carrier matrixes. Such a behavior was explained by the higher affinity of ethofumesate with the composite in the presence of hydrophobic clay than of the hydrophilic montmorillonite. They observed that the release mechanisms were particularly governed by interactions between pesticides and montmorillonite. The clay acted at the component interface decreasing or even nullifying the chemical incompatibility.

Grillo et al. (2014) studied the controlled-release behavior of the pesticide Paraquat®, in hydrogels composed of poly(vinyl alcohol) cross-linked with glutaraldehyde. The authors observed that the pesticide release process was strongly dependent on the concentration of glutaraldehyde. The hydrogels exhibited a poor release capacity of pesticide when it was confined in highly cross-linked hydrogels. More recently, some works (Zhang et al. 2006; Kasgoz and Durmus 2008) described the influence of the addition of calcium montmorillonite on the controlled release of herbicides from a hydrogel based on carboxymethyl cellulose. They found out that the addition of montmorillonite significantly prolonged the herbicide release.

11.4 Conclusions and Perspectives

Studies on novel technologies for the production of composites intended for slow or controlled release of agrochemicals have been enormously disseminated. More effective applications of agrochemicals through composite materials are desired because they can reduce possible economic losses and principally environmental damage. However, more studies are still necessary to find the best materials to formulate highly efficient carrier composites and create cost-effective routes of preparation because the use of composite materials is still limited by the additional cost derived from the manufacturing process, which increases the final product cost and, consequently, the cost of crop production, and to establish improvements in the obtained material properties through a comparison of different modified compounds, depending on the application, as fertilizer or pesticides. Another point to be emphasized is related to the application of biodegradable carrier matrixes only. Any alternative to rationalize the application of agricultural inputs should not become a new environmental or economic problem. Therefore there is the need to incorporate or produce new compound, which, after use, can be easily biodegraded or incorporated into the soil.

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Chapter 12
Nano-enhanced Biological Treatment of Agricultural Wastewater

Yi An and Qi Dong

Abstract Agricultural wastewater is one of the main types in agricultural nonpoint source pollution and mostly produced by the abusement of fertilizers, pesticides, and plastic film. Characterized by high BOD and ammonia, agricultural wastewater is generally degraded by microbial technique, including natural treatment and anaerobic or aerobic techniques. However, long reaction period and remnant of a persistent organic cannot be solved appropriately. Recently, nano-materials have drawn much attention in in situ remediation of groundwater because of their small particle size and high specific surface area. Especially, it was found that nano-iron corrosion in water produced molecular hydrogen, which can be used as an electron donor for autotrophic microbes. And nitrate, perchlorate, trichlorethylene, and other pollutants could be removed in this process. In addition, a photocatalytic technology using nano-TiO_2 as photocatalyst was an efficient and safe method for antibiotic degradation, which was hardly observed in conventional microbial treatment technology. Also, some researchers developed novel methods using nanofiltration membrane combined with microbial technology for wastewater treatment. The results showed that the quality of effluent including microbiological indicators, heavy metals, and POPs is in full compliance with the requirements of drip irrigation.

12.1 Introduction

With the rapid development of agriculture, agricultural demands for water resources are increasing. However, due to the excessive application of fertilizers, pesticides, and other agrochemicals, a lot of agricultural wastewater containing nitrogen, phosphorus, benzene, chlorine, and other pollutants has been generated.

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That is, the generated agricultural wastewater has also exacerbated the contradiction between supply and demand of water resources.

Due to the complexity of the agroecosystem, traditional urban sewage treatment technologies, such as activated sludge process, anaerobic fermentation, and constructed wetland, are difficult to use in the agricultural system. In particular, for persistent organic pollutants such as pesticides and veterinary drugs, conventional water treatment technology is even more difficult to play a role.

In recent years, more and more attention has been paid to nano-materials due to their small surface area and high reactivity. However, because of the high cost of nanometer materials and high technical requirements, there are only a few applications in agro-environmental protection field.

In this chapter, we selected three kinds of nano-materials (nano-iron, nano-TiO_2, and nanofiltration) with higher technological maturity and good promotion prospects from numerous nanotechnologies and applied them for fertilizer-polluted groundwater, livestock wastewater, and irrigation water for greenhouse vegetables, respectively. The emphasis is placed on the nature, scope, and pollutant removal capabilities of these nano-materials.

With the development of industry and agriculture, nitrate (NO_3^-) detection frequency and concentration in groundwater is increasing in many countries and has become a very serious environmental issue. In the early 1960s, the USA and Europe had reported NO_3^- pollution due to chemical nitrogen fertilizer. Moreover, the results of continuous monitoring in Palestine between 1982 and 2004 revealed that the NO_3^- content of groundwater in the West Bank region showed an increasing trend (Anayah and Almasri 2009). Even in countries with the most developed economy and technology, such as the USA, NO_3^- pollution is serious. The US national water quality survey indicated that among the 1992 surveyed wells, 3 % contained NO_3^- content exceeding the standard limit, and the proportion was over 25 % in high-pollution risk areas (Nolan et al. 1997).

Numerous studies have shown that excessive use of agricultural nitrogen fertilizer is one of the main causes of the increase in the NO_3^- concentration in groundwater. As nitrogen cannot be completely absorbed by crops and because soil colloids cannot adsorb monovalent NO_3^- ions, when a large quantity of nitrogen fertilizer or manure is applied to farmland, the NO_3^- in the soil easily penetrates into groundwater through rainfall and irrigation. In some developing countries, such as China, India, and Brazil, which have very large increasing populations, there is a need to increase food production in the next 10 years by using more quantities of nitrogen fertilizer, which will definitely result in serious NO_3^- pollution of groundwater.

12.2 Microbial Treatment Technology for the Removal of NO_3^- from Groundwater

Many studies have developed various methods—physicochemical, chemical, and biological denitrification reduction methods—to remove NO_3^- from groundwater. In the physicochemical process, the NO_3^- is concentrated or spread, instead of being removed from the groundwater, whereas in the chemical reduction method, high concentrations of ammonia cannot be completely removed. In contrast, the microbial method is gradually becoming a popular groundwater pollution treatment technology because of its high-efficiency, low-cost, and complete degradation with no secondary pollution.

The biological denitrification method refers to the denitrification process in which NO_3^- or nitrite (NO_2^-) in water is transformed to nitride and nitrogen under hypoxic environment by facultative anaerobic bacteria with NO_3^- or NO_2^- as an electron acceptor, instead of oxygen. The denitrification process comprises the following steps: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$. This process is not only a major part of the nitrogen cycle in the ecosystem but also a main mechanism of wastewater denitrification. In the sewage treatment system, more than 50 genera and over 130 species of denitrifying bacteria exist, and many of them, including *Achromobacter*, *Bacillus*, *Brevibacterium*, *Enterobacter*, *Lactobacillus*, *Alcaligenes*, *Micrococcus*, *Pseudomonas*, *Spirillum*, etc., have been reported.

The main factors affecting the biological denitrification process include the oxygen concentration, nutrients supply, pH, and temperature. High oxygen concentrations can inhibit some or all of the steps of the denitrification process. Presence of sufficient nutrients is vital for the normal growth of bacteria; among the nutrients, C, H, O, N, S, and P elements are essential for cellular synthesis, while minerals and trace elements in groundwater, such as K, Na, Mg, Ca, Fe, Mn, Zn, Cu, and Co, are necessary for the growth of bacteria. The optimum pH value for the denitrification process is 7.0–8.0; at low pH, methane bacteria dominate, whereas at high pH, NO_2^- accumulation occurs. Furthermore, the effect of temperature on the denitrification process is very significant; low temperatures (0–5 °C) reduce the denitrification rate (except for some psychrophiles), whereas a rise in the temperature to 10 °C can result in a twofold increase in the denitrification rate.

The heterotrophic biological denitrification process requires the addition of organic carbon as one of the nutrients for the denitrifying bacteria. Methanol, ethanol, and acetic acid are the most frequently used organic carbon sources for the denitrification process. In 1979, the flowing sand bed technique was employed to study the removal of NO_3^- in a river and proved the feasibility of this method for the treatment of groundwater. Since then, various techniques, such as the one-way submerged membrane reactor, continuous flow membrane reactor, rotating bioreactors, upflow sludge blanket reactor, Dentropur biological denitrification process, and other groundwater denitrification reactors, have been developed for groundwater treatment. However, a disadvantage of heterotrophic biological denitrification technology is that it requires the addition of methanol, ethanol, glucose, acetate, and other organic substrates, which do not exist in groundwater. In addition, the fast growth of heterotrophic bacteria is more likely to cause clogging of groundwater aquifers, thus increasing residual bacteria and organic contaminants in the effluent.

On the other hand, autotrophic biological denitrification process can utilize gaseous hydrogen or sulfide, rather than organic carbon sources, to transform carbon dioxide, bicarbonate, and other compounds to cellular components. To date, there have been many reports on the sulfur and hydrogen autotrophic denitrification process. In the sulfur/limestone biological denitrification process, sulfur acts as an electron donor, while limestone provides alkalinity as well as acts as a biocarrier, which can effectively remove NO_3^- from groundwater. This process is economical and simple because it does not require organic substrates and uses less sulfur and limestone. However, the major disadvantage of the sulfur/limestone biological denitrification process is the production of sulfate; 7.54 mg of SO_4^{2-} is produced per mg of NO_3^- removed. Therefore, this method is suitable for removing NO_3^- from groundwater with low sulfate concentrations. Comparatively, hydrogen is an ideal electron donor because hydrogen itself or its oxidation product is not toxic, and autotrophic denitrification with hydrogen as the electron donor is a clean denitrification process. However, the application of this process is limited by low solubility, low efficiency, and explosibility of hydrogen.

12.3 Zero-Valent Iron Enhances Hydrogen Availability for Autotrophic Microorganisms Involved in Denitrification of Groundwater

In recent years, it has been proposed that hydrogen produced during corrosion of zero-valent iron in water could be utilized by denitrifying bacteria during the reduction of NO_3^- to nitrogen.

The iron-denitrifying bacteria composite system contains a variety of molecules, ions, and enzymes, which make the reaction more complex. From the theoretical point of view, the reaction mechanism of the system can be divided into main reactions and secondary reactions. Among them, the main reactions include ammonium (NH_4^+) generation, hydrogen evolution, and denitrification, as shown in Eqs. (12.1)–(12.3):

$$4Fe^0 + NO_3^- + 7H_2O \rightarrow 4Fe^{2+} + NH_4^+ + 10OH^- \tag{12.1}$$

$$Fe^0 + 2H_2O \rightarrow H_2 + Fe^{2+} + 2OH^- \tag{12.2}$$

$$\begin{aligned} &0.33NO_3^- + H_2 + 0.08CO_2 + 0.34H^+ \\ &\quad \rightarrow 0.015C_5H_7O_2N + 0.16N_2 + 1.11H_2O \end{aligned} \tag{12.3}$$

As can be noted in the equations, NH_4^+ is generated during the reaction between Fe^0 and NO_3^-, thus indicating that the amount of NH_4^+ should be decreased and the reaction probability between Fe^0 and NO_3^- should be inhibited, whereas the reaction between Fe^0 and H_2O should be promoted.

On the other hand, secondary reactions may include five secondary reactions as shown in Eqs. (12.4)–(12.8):

$$Fe^0 + NO_3^- + 2H^+ \rightarrow Fe^{2+} + H_2O + NO_2^- \tag{12.4}$$

$$3Fe^0 + NO_2^- + 8H^+ \rightarrow 3Fe^{2+} + NH_4^+ + 2H_2O \tag{12.5}$$

$$\begin{aligned} &NO_3^- + 2.82Fe^0 + 0.75Fe^{2+} + 2.25H_2O \\ &\rightarrow NH_4^+ + 1.19Fe_3O_4 + 0.50OH^- \end{aligned} \tag{12.6}$$

$$2NO_3^- + 2H_2 \rightarrow 2NO_2^- + 2H_2O \tag{12.7}$$

$$2NO_2^- + 3H_2 \rightarrow N_2 + 2H_2O + 2OH^- \tag{12.8}$$

Till et al. (1998) conducted experiments using a homemade bottleneck and verified that hydrogen denitrifying bacteria could use the hydrogen generated during iron corrosion for denitrification, during which NO_3^- is degraded into harmless nitrogen. However, they also observed dynamic competition between zero-valent iron and denitrifying bacteria, and the higher reactivity of iron was not conducive for the denitrification process. In addition, the results of column experiments showed that hydraulic retention time (HRT) was directly and inversely proportional to the NO_3^- removal rate and ammonia production, respectively. Subsequently, Kielemoes et al. (2000) studied the effect of NO_2^- on the composite system, and the results clearly showed that NO_2^- remarkably inhibited the corrosion process and increased the probability of the toxic byproduct, NO. Moreover, the addition of microorganisms was found to improve the production of hydrogen, which indicated that a substance in the microorganisms (possibly a certain bioenzyme) could catalyze iron corrosion.

Furthermore, Biswas and Bose (2005) simulated in situ remediation of NO_3^- contamination by permeable reactive barrier (PRB) composed of zero-valent iron and denitrifying bacteria and achieved improved denitrification rate as well as a decrease in NH_4^+ production by optimizing the iron type, concentration, HRT, and other parameters. Their results showed that more than 13 days of HRT were required for the denitrification process using a PRB barrier composed of 0.5 g of steel wool and 125 cm^3 of mixed sand. In addition, Jha and Bose (2005) considered that continuous corrosion of zero-valent iron increased the pH value, which inhibited the production of hydrogen and subsequently limited the denitrification efficiency of the composite system. Therefore, they introduced FeS_2 into the composite system, which reacted with OH^- in the alkaline solution to produce Fe $(OH)_2$ precipitate that was separated from the aqueous phase to maintain the pH of the solution, as shown in Eq. (12.9):

$$FeS_2 + 18OH^- \rightarrow Fe(OH)_2 + 2SO_4^{2-} + 14e + 8H_2O \tag{12.9}$$

The results showed that during the denitrification reaction, the solution pH increased from 7.55 to 9.14 with the addition of FeS_2, whereas the pH only increased from 7.55 to 7.73 without FeS_2 addition, and the NO_3^- removal rate was also enhanced.

12.4 Nano-iron Enhances Hydrogen Availability for Autotrophic Microorganisms Involved in the Denitrification of Groundwater

With the development of nanotechnology, nano-iron has been widely used in the field of environmental remediation because of its high specific surface area and reactivity. Instead of zero-valent iron, Shin and Cha (2008) used nano-iron together with denitrifying bacteria to remove NO_3^-. They found that the reaction rate was substantially improved, and the denitrification process took only 3 days to complete. Their experiments proved that the composite system exhibited more adaptability to different environment temperatures than the single-phase nano-iron system.

To observe the denitrification capacity and products of the composite system consisting of nano-iron and denitrifying bacteria, nanoscale zero-valent iron prepared by liquid-phase reduction and *Alcaligenes eutrophus* (a kind of hydrogen autotrophic denitrifying bacterium) were mixed with added NO_3^- solution, and the denitrification process was carried out in an anaerobic environment (An et al. 2009). As a control, only nano-iron or denitrifying bacteria were used.

As shown in Fig. 12.1, with time, the NO_3^- concentrations in the system with denitrifying bacteria decreased by about only 15 % with no NH_4^+ generation within 8 days. As no additional electron donor was added to the system, the denitrification process did not occur, and the slight decrease in the concentration of NO_3^- may be attributed to the adsorption of certain polysaccharides secreted by the bacteria. Furthermore, the addition of denitrifying bacteria into the system significantly affected NO_3^- removal. It only took 2 days to complete NO_3^- degradation by the system with nano-iron alone, with about 95 % of NO_3^- being converted to NH_4^+. On the other hand, the reaction time taken to remove NO_3^- from the system with added denitrifying bacteria increased from 2 to 8 days, with about only 33 % of NO_3^- being converted to NH_4^+, thus indicating that the denitrifying bacteria significantly decreased the proportion of NH_4^+ and nitrogen.

To study the denitrification process in the composite system, NO_3^-, NO_2^-, and NH_4^+ concentrations were determined (An et al. 2009), as shown in Fig. 12.2.

From Fig. 12.2, it can be observed that in the first 4 days, the NH_4^+ content of the system increased from 0 to 35 % and subsequently remained stable between 33 and 36 %. As described earlier, NH_4^+ was produced only during the chemical reaction between iron and NO_3^-, whereas the biological denitrification process converted NO_3^- into nitrogen gas rather than NH_4^+. Thus, according to the varying patterns, the denitrification process in the composite system can be divided into two stages:

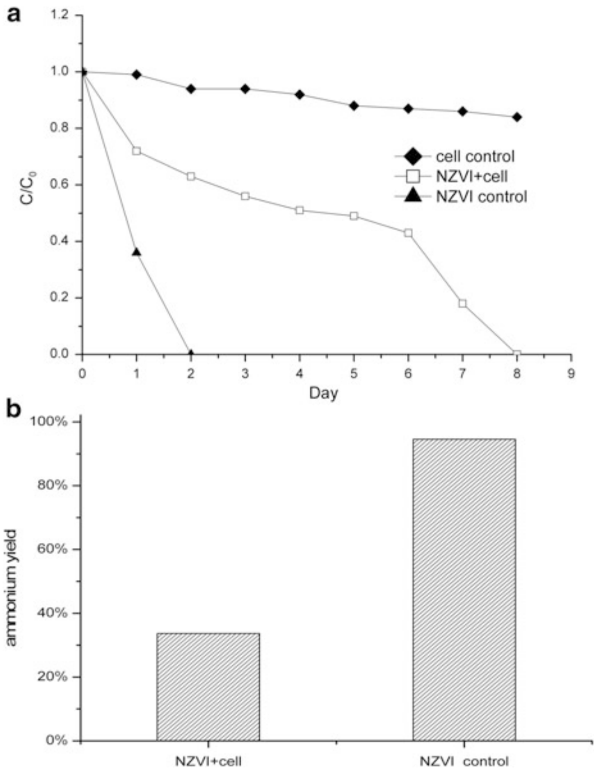


Fig. 12.1 Denitrification under different conditions: (**a**) NO_3^- concentration with time and (**b**) yield of NH_4^+ (An et al. 2009)

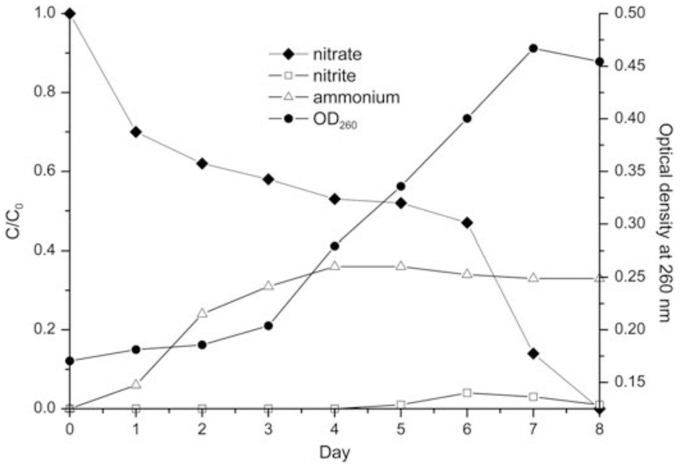


Fig. 12.2 Changes in the concentrations of NO_3^-, NO_2^-, NH_4^+, and OD_{260} vs. time in a nano-iron-denitrifying bacteria system (An et al. 2009)

in the first 4 days, about 53 % of NO_3^- was removed, of which 64 % was transformed into NH_4^+, indicating that during this period, chemical reduction played a major role in NO_3^- removal. However, evaluation of the proportion of NO_3^- that was not transformed to NH_4^+ might not be possible because of the sorption of nano-iron or denitrification by the bacteria. During the last 4 days, the residual NO_3^- was removed completely without an increase in NH_4^+ concentration. Meanwhile, a brief trend of NO_2^- accumulation (up to 22 %) was observed, with nano-iron playing a leading role in the denitrification process, and NO_2^- was not detected in the previous 4 days. These results indicated that the chemical reduction of nano-iron was not obvious in the last 4 days and that biological denitrification played a leading role in the NO_3^- removal process. The reason for the transition in the reaction mechanism could be the toxicity of nano-iron, which prevented the normal growth of the denitrifying bacteria; however, with increasing iron corrosion, the toxicity of nano-iron was decreased, the denitrifying bacteria gradually adapted to the new environment, and the denitrification process proceeded normally.

Many reports have shown that coating the appropriate proportion of another metal with a high reduction potential (e.g., Ni, Pd, Ag, Cu, etc.) to form a binary metal system can increase the number of active adsorption sites on the iron surface as well as the stability of the nanoscale zero-valent iron. In a previous study (An et al. 2010), nano-iron/Ni and nano-iron/Cu systems were used (Ni and Cu loading of about 5 %) with denitrifying bacteria to examine the NO_3^- removal rate and reaction products.

Figure 12.3 shows the effect of different catalysts on the reactivity of nano-iron. Under the same conditions, the reactivity of the nano-materials on NO_3^- reduction was as follows: nano-iron/Ni > nano-iron/Cu > nano-iron. This finding demonstrated that the introduction of Ni, Cu, and other metals could significantly improve the denitrification rate. Although no difference could be observed between Ni and Cu catalysts based on the denitrification rate curve, the catalytic mechanisms of these two metals were noted to be completely different with respect to NO_2^- and NH_4^+ concentration curves and products (An et al. 2010), as shown in Fig. 12.4.

From Fig. 12.4, it can be observed that although the introduction of Ni and Cu did not produce any effect on the NO_3^- removal rate, taking only 6 and 7 days to complete the degradation process, respectively, the NO_3^- degradation products were completely different. With regard to nano-iron/Ni bimetallic composite system, NO_2^- was not detectable throughout the denitrification process, but the proportion of ammonia was as high as 69 % after the reaction. On the other hand, with respect to nano-iron/Cu bimetallic composite system, the NO_2^- concentration increased throughout the reaction and remained at about 33 % even after the complete degradation of NO_3^-. At the end of the reaction, only 39 % of NO_3^- was converted to NH_4^+ in the nano-iron/Cu bimetallic composite system.

The addition of Ni was found to catalyze simultaneous reduction of NO_2^- and NO_3^- by nano-iron; therefore, NO_2^- accumulation did not occur throughout the denitrification process. Unlike the ammonium generation curve in nanoiron-bacteria system, that increased monotonously when Ni added. It indicated that Ni catalyzed

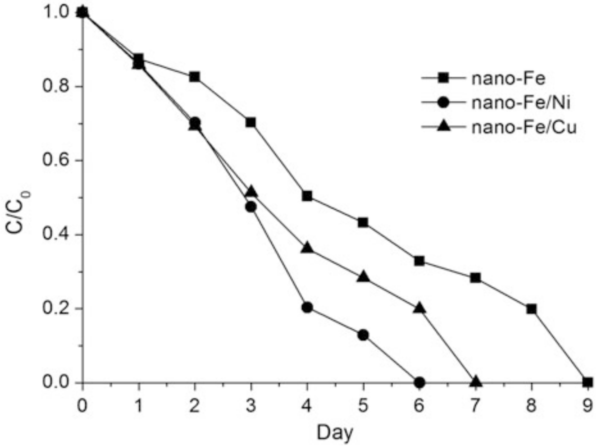


Fig. 12.3 The NO_3^- removal rates of three different nano-iron particles (An et al. 2010)

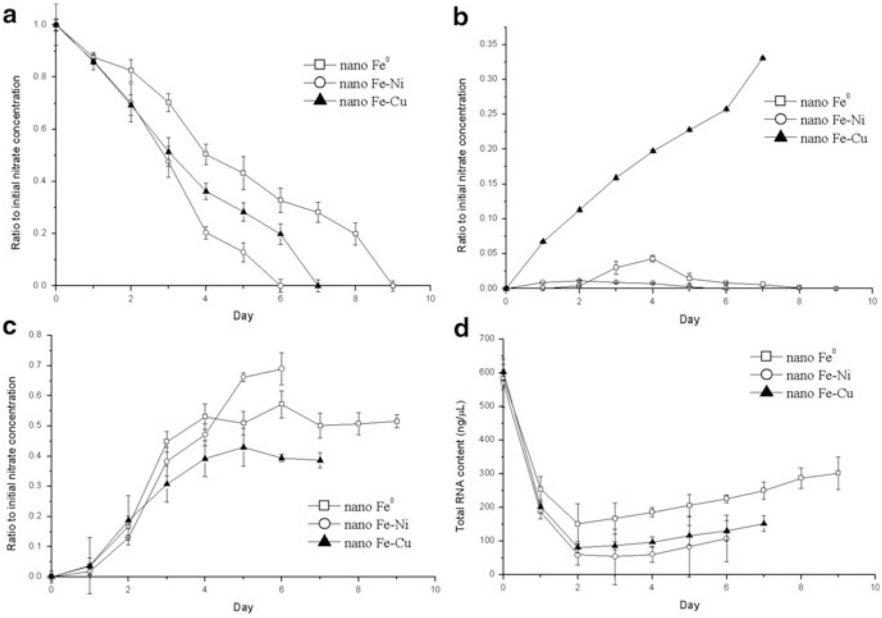


Fig. 12.4 Changes in the concentrations of (**a**) NO_3^-, (**b**) NO_2^-, (**c**) NH_4^+, and (**d**) bacteria contents in the composite systems composed of bimetallic nano-materials and denitrifying bacteria (An et al. 2010)

the chemical reduction process, in which nitrate converted into ammonium. However, despite the accelerated denitrification rate, the enhanced toxicity of the nitrogen products resulting from the introduction of Ni limits its use in the denitrification process.

Table 12.1 Product distribution of the denitrification process with the three nano-iron materials after complete removal of NO_3^-

	Initial concentration of NO_3^- (mg/L)	NO_2^- Concentration (mg/L)	NO_2^- Proportion (%)	NH_4^+ Concentration (mg/L)	NH_4^+ Proportion (%)
Nano-Fe^0	46.57	0.01	0.00	24.03	51.59
Nano-Fe/Ni	45.53	0.00	0.00	31.40	68.97
Nano-Fe/Cu	49.27	16.73	33.07	19.05	38.66

Unlike Ni, addition of Cu improved the NO_3^- removal rate and significantly increased the selectivity of NO_2^-; therefore, the rate of NO_2^- production was rapid in the nano-iron/Cu reaction system. On the other hand, the surface of the nano-iron/Cu exhibited poor capacity for NO_2^-; thus, the conversion of NO_2^- to NH_4^+ was relatively slow. In other words, Cu changed the selectivity of the products during NO_3^- reduction, as a result of which the denitrification products remained as NO_2^-, rather than being further converted to NH_4^+. The NO_2^- residue in the system with denitrifying bacteria was degraded into gaseous nitrogen, separated from the aqueous phase, thereby reducing the toxicity of the denitrification products in the composite system. The product distribution of the denitrification process in the three systems of nano-iron materials and denitrifying bacteria is shown in Table 12.1. After the reaction, the proportion of NH_4^+ in the three systems was as follows: nano-iron/Ni > nano-iron > nano-iron/Cu.

The results presented in Table 12.1 demonstrate that the Ni catalyst increased the proportion of NH_4^+ from 51.59 to 68.97 % and enhanced the toxicity of the products rather than decreasing it, thus indicating that Ni is not a suitable catalyst in the composite system. On the other hand, the Cu catalyst decreased the NH_4^+ concentration from 51.59 to 38.66 % with 33.07 % of NO_2^- production; the NO_2^- produced was removed completely in the next 2 days with no NH_4^+ generation (Fig. 12.4). Furthermore, microbial denitrification transformed the residual NO_2^- in the solution to nitrogen gas or nitrogen oxide, which was separated from the aqueous phase. Therefore, it can be concluded that Cu is a more suitable catalyst in the composite system for the denitrification process.

12.5 Combined Use of Anaerobic Microorganisms and Titanium Dioxide for the Removal of Antibiotics from Aquaculture Wastewater

12.5.1 Conventional Farming Wastewater Treatment Process

In recent years, to shorten the growth cycle of livestock farming, increase production, and reduce costs, large-scale livestock breeding industry has been rapidly developing worldwide. However, large-scale livestock farming not only produces large volumes of animal wastewater that can pollute the surface of water bodies, causing environmental problems such as eutrophication, but also deteriorates the groundwater quality and ecosystem diversity and even endangers human health. The characteristics of livestock wastewater are high chemical oxygen demand (COD), suspended solids (SS), and ammonia nitrogen (NH_3-N) content; good biodegradability and precipitation performance; variation in water quality and quantity; presence of pathogens; and fetid nature. To control the direct discharge of livestock wastewater into the environment, various livestock wastewater treatment technologies are being employed, which can be broadly divided into physicochemical technology and biological technology.

12.5.2 Physicochemical Technology

The common physicochemical methods include absorption, magnetic flocculation, electrochemical oxidation, and Fenton oxidation. The basis for adsorption is selection of the adsorption medium; currently, zeolite is used as the adsorption medium. In a previous study, increases in the uptake of enrofloxacin onto natural zeolite were obtained by decreasing the pH and increasing the temperature. Also, increasing the ammonia concentration from 50 to 200 mg/L resulted in 50 % increase in the adsorption of enrofloxacin (Ötker and Akmehmet-Balcıoğlu 2005). Furthermore, wastewater can be treated by employing magnetic flocculation separation using magnetic seed and flocculants. This process is straightforward with good settleability and a short processing cycle, but can generate a large amount of chemical sludge. Fenton oxidation is effective in removing higher COD and color (Lee and Shoda 2008) and can be used as an advanced treatment of livestock wastewater, despite the requirement of a large amount of Fe^{2+} dosage and low H_2O_2 removal efficiency. Thus, these physicochemical treatment methods can remove COD, NH_3-N, and the color of livestock wastewater and can be applied to livestock wastewater as pretreatments or advanced treatments. Nevertheless, further research on this technology is still required because of the lack of engineering knowledge.

12.5.3 Biological Technology

Biological treatment technology is commonly used for livestock wastewater treatment and includes anaerobic treatment, aerobic treatment, and anaerobic–aerobic treatment. Anaerobic treatment is suitable for livestock wastewater containing high concentrations of organic matter. The common anaerobic treatment methods include the use of anaerobic baffled reactor (ABR), upflow anaerobic sludge blanket (UASB), microbial fuel cell (MFC), etc.

A study from Wu et al. (2013) was designed regarding orthogonal experiments to operate the four-compartment ABR with a hydraulic retention time (HRT) = 24 h, influent COD ~ 4,600 mg/L, and temperature = 35 ± 1 °C and discovered the optimal parameters with pH = 7, C/N = 44, and 2-bromoethanesulfonate (BES) concentration = 20 mmol/L to achieve the high acetic acid accumulation of effluent. Dias et al. (2014) presented a broad evaluation of one particular system (UASB reactor, three shallow ponds, and a coarse rock filter in series) in Brazil treating sewage from 250 inhabitants over a period of 10 years. They found that even with short HRTs in each pond (2–6 days), the results endorse the good capacity for organic matter and ammonia removal and excellent removal of coliforms, helminth eggs, and sulfides.

It can be observed that the COD volumetric loading of ABR is adequately high, and the HRT is sufficiently long to treat wastewater containing high concentrations of organic matter. This new high-rate anaerobic reactor was developed by McCarty and Bachmaann in 1982, and has good adaptability to shock load. The characteristics of UASB include engineering expertise, steady operation, good results, high efficiency, cost-effectiveness, and small size. On the other hand, the MFC can convert chemical energy into electricity while achieving sewage treatment and has become the focus of current studies. The MFC can remove organics at a high rate and is particularly suitable for wastewater with high concentrations of organic matter. Anaerobic treatment technology is widely applied for the treatment of wastewater with high concentrations of organics, such as pharmaceutical, chemical, and farming wastewater, with advantages of good COD removal efficiency and utilizing small area. However, the effluent formed after anaerobic treatment does not meet the quality standards, and often, further treatment is required if odor is emitted from the non-closed reactor.

The common livestock wastewater aerobic treatment technologies include sequencing batch reactor (SBR), sequencing batch biofilm reactor (SBBR), biofilm, biological filter, moving bed biofilm reactor (MBBR), membrane bioreactor (MBR), and anoxic/oxic (A/O) method.

Effects of dilution rates, glucose supplementation, and HRTs on the efficiency and performance of the SBR system with electroplating wastewater (EPWW) solutions containing CN^- and Zn^{2+} were conducted by Sirianuntapiboon (2013). The system showed that the highest COD, BOD_5, TN, TKN, CN^-, and Zn^{2+} removal efficiencies were 91 ± 2, 71 ± 6, 48.6 ± 1.0, 56.9 ± 5.3, 62.4 ± 3.0, and 88.0 ± 0.6 %, respectively, at the 1:5 diluted EPWW solution containing 100 mg/L glucose (BOD_5 to TN ratio of 100:10) and HRT of 10 days (organic loading of 0.008 kg BOD_5/m^3 day, CN^- loading of 0.86 g/m^3 day, and Zn^{2+} loading of 1.2 g/m^3 day). Lotito et al. (2012) address the characterization of the sludge produced from sequencing batch biofilter granular reactor (SBBGR) in terms of some physical properties (settling properties, dewaterability, rheology). The results show that such sludge is characterized by good settling and dewatering properties, adding a new advantage for the full-scale application of SBBGR technology. Janus (2014) developed an integrated model for MBR reactor, which was composed of three interconnected submodels: the activated sludge model (ASM) extended with soluble and bound biopolymer kinetics, the membrane fouling model, and the interface model relating cake back-transport rate to air-scour intensity and specific cake resistance to concentration of extracellular polymeric substances (EPS). Simulation methods of a system dynamics model for activated sludge wastewater treatment plants were developed by Park et al. (2014). A wastewater treatment plant in South Korea was chosen for these analyses. The inflow rate beyond which aeration tank size needs to be increased was calculated while satisfying the effluent water quality requirement. The corresponding sizes of aeration tank were also calculated for different activated sludge return rates under the maximum inflow rate which does not require expansion of the aeration tank.

SBR has good removal characteristics with respect to decarbonization and denitrification, with COD and NH_3-N removal rates being more than 90 %. Furthermore, the reactor can be controlled automatically with less area and a variety of technologies such as ICEAS, CAST, DAT–IAT, etc., which are commonly used in the livestock wastewater treatment process. On the other hand, SBBR is theoretically characterized by high biomass, less area, good carbonization and denitrification performance, good aerobic granular sludge settling properties, the ability to withstand high organic loading, and better decarbonization and denitrification; however, practical application of this technology requires further study. Biofilter has good sludge settling properties, high biomass, high organic load capacity, and good decarbonization and denitrification performance and is suitable for livestock wastewater treatment; nevertheless, further research regarding its practical application is needed. Moreover, biofilter needs an appropriate filler with good denitrification characteristics. The MBBR technology, a combination of both traditional and biological technologies with the advantage of fluidized oxidation, is a new and efficient method for sewage disposal. On the other hand, MBR has a longer sludge age, higher sludge concentration, and good decarbonization and denitrification performance, but faces a tough problem of membrane fouling that limits its further development and implementation. Nevertheless, studies have shown that the addition of a suitable carrier and development of granular sludge are effective ways to decrease MBR membrane fouling. Lastly, the A/O process is simple and less equipped, and no external carbon source is needed.

12.5.4 Antibiotics Pollution in Aquaculture Wastewater

As natural, synthetic, or semisynthetic compounds that are resistant to microbial activity, antibiotics are widely used as antimicrobial agents to treat infectious diseases in humans and animals. In 2002, the quantity of antibiotics used annually worldwide was reported to be about 10–20 million tons. Livestock industry is an important source of antibiotic contamination. Among the many uses of antibiotics, about half of the total amount of antibiotics is employed in livestock. In many countries around the world, antibiotics are widely used for the prevention and treatment of animal diseases or added to the feed as a growth promoter. Currently, in almost all regions around the world, antibiotics are being used to achieve high production and economic efficiency. Antibiotics misuse in aquaculture animals may induce the production of drug-resistant genes, and a vast majority of oral antibiotics are directly excreted from the body of the livestock without absorption or metabolism. As animal waste contains large amounts of nutrients, such as N, P, etc., which are required for plant growth and generally used as fertilizer in agriculture, antibiotics may migrate to the soil and surface water, ultimately get absorbed by crops, and enter the food chain, posing a potential threat to animals and humans.

Currently, swine wastewater treatment technology mainly focuses on the removal of N, P, COD, and other conventional pollutants and pays less attention to the removal of antibiotics, heavy metals, and other trace contaminants. To date, there are no qualified indicators to determine the concentrations of antibiotics in wastewater. As even low concentrations of antibiotics in wastewater can pose a potential threat to the environment, the development of technologies to effectively treat and remove antibiotics from wastewater is gradually becoming a hot topic.

Zhou et al. (2006) used ABR to remove chlortetracycline and achieved a removal rate of only 25–31 %. Furthermore, the removal rate of sulfamethoxazole (SMX) by using the SBR was reported to be 66–91 %, and the removal performance and sludge retention time were noted to have a direct relationship. Owing to the differences in the structure and properties of antibiotics, their migration and transformation behaviors in biological treatment are different. Recently, studies on the degradation behavior of antibiotics in the activated sludge process were carried out, which showed that some lipophilic antibiotics are likely to get adsorbed onto the solid phase of the activated sludge, despite being subjected to degradation. Furthermore, it was reported that reduction of some antibiotics such as tetracycline (TC) may be mainly based on the adsorption behavior, instead of biodegradation. In addition, some studies demonstrated that biological treatment process cannot achieve complete removal of most of the antibiotics.

12.6 Nano-titanium Dioxide Photocatalytic Technology

Photocatalytic technology, especially that using nano-titanium dioxide (TiO_2), has been reported to show good performance in removing recalcitrant organic pollutants, has advantages such as low cost, high stability, suitable band gap, etc., and has become the most widely used technique. In 1972, electromotive force was observed when placing TiO_2 under xenon lamp irradiation with electrolysis of water to generate hydrogen, indicating that TiO_2 converts light energy into chemical energy stored in hydrogen. TiO_2 is characterized by chemical stability, nontoxic nature, rich source, high quantum efficiency, and catalytic efficacy. Currently, the major applications of TiO_2 include solar cell materials, photosensitive materials, and sensors. In addition, TiO_2 is also used in water purification, sterilization, deodorization, decontamination, defogging, and other environmental fields.

Photocatalytic performance of TiO_2 depends on its semiconductor structure. According to the energy band theory, the low energy band structure of the semiconductor material consists of a valence band with low energy and a conduction band with high energy, with a band gap existing between the valence and conduction bands. TiO_2 is a semiconductor with a band gap of 3.2 eV, and only under UV irradiation (<385 nm), the electrons of the valence band absorb the energy of the photon and jump into the conduction band to form electron–hole pairs. The photogenerated electrons and holes adsorbed onto the surface of the semiconductor transfer the charged species, i.e., the hole-captured substances adsorbed onto the surface of the electronic semiconductor or electrons of the solvents, so that pollutants are activated and oxidized. An electron acceptor is reduced by receiving the electrons, and a heterogeneous catalysis occurs through such continuous charge transfer. However, UV radiation only occupies 4 % of sunlight, whereas visible light occupies about 43 % of sunlight, thus making this technology less efficient. In recent years, numerous studies have been carried out on photogenerated carriers to reduce the recombination rate, expand the excitation wavelength range, etc., including semiconductor doping, surface sensitization, noble metal deposition, nonmetal doping, and so on.

The main methods of TiO_2 doping include metal doping, nonmetal doping, and co-doping, and among them, metal doping is classified into transition metal doping and rare-earth metal doping. Transition metal doping decreases the energy gap of the photocatalyst, increases the electron–hole pair recombination, and subsequently enhances the photocatalytic performance. In contrast, some of the more common nonmetal doping methods use doping elements such as N, S, C, H, halogens, etc. Asah doped small amounts of N (0.75 %) into the lattice, instead of oxygen, and achieved the activity under visible light, and was the first to prepare TiNx-doped TiO_2 with visible excitation property. Chen reported that black TiO_2 nanocrystals, which introduce chaos on the TiO_2 crystal surface through hydrogenation, can effectively utilize energy in the infrared region. In recent years, there has been a gradual development of halogen doping. Yu carried out hydrolysis of titanium tetraiso-alcohol in NF_4–H_2O solution to prepare a nanocrystalline catalyst with high catalytic activity. The results obtained showed that F doping increased anatase crystallinity and suppressed the generation of brookite and transition from anatase to rutile with increasing doping dosage of F. In addition, the catalytic activity of this photocatalyst on acetone in air was higher than that of P-25, the width of the band gap was significantly decreased, and a strong absorption performance was noted in the UV–vis range.

12.7 Photocatalytic Degradation of Antibiotics by Nano-TiO_2

Numerous studies have shown that unlike conventional water treatment technologies, advanced oxidation technologies can remove organic pollutants with chemical stability and low biodegradability in a short period of time and are considered as very promising treatment methods. Photocatalytic oxidation technology can effectively and rapidly remove trace organic pollutants in mild environment with no secondary pollution. In recent years, many scholars have conducted studies on photocatalytic degradation of a variety of antibiotics, such as amoxicillin (AMX), ampicillin (AMP), cloxacillin (CLX), SMX, TC, chloramphenicol, sulfamethazine (SMZ), and other sulfa drugs. In addition, investigations on the conditions of photocatalytic degradation, intermediate generation, degradation pathway, degradation dynamics, and other aspects have drawn much attention.

In the photocatalytic antibiotic degradation process, many environmental factors affect the degradation efficiency, including the use of catalysts and their concentrations, wavelength of light used, irradiation time, pH, H_2O_2, initial contamination concentration, and other active parameters. Furthermore, the photocatalytic degradation process produces a variety of plasma, whose status and quantity are affected by the pH value, which, in turn, significantly influence the degradation performance. The molecule of antibiotics such sulfonamides, TCs, and fluoroquinolones contains acid and alkali dissociation groups that can be remarkably disturbed. These dissociating groups occur in different forms in the solution at different pH, resulting in significantly different chemical reactivities. However, the effect of solution pH on the photochemical behavior of different types of antibiotics is varied, because different forms of the antibiotic molecule have different absorption spectra and photolysis quantum yield. As a result, a difference in the rate constant of photolysis occurs, indicating that the photochemical behavior of antibiotics is influenced by the pH-affected status and chemical properties of the antibiotics.

Photocatalyst has an integral role in the photocatalytic reaction. Often, previous studies have explored the effect of the concentration of photocatalyst on the efficiency of photocatalytic antibiotics degradation and tried to determine the optimal concentration. Numerous studies have reported an optimal TiO_2 concentration and observed that the degradation rate of pollutants increased with the increasing TiO_2 concentration within the optimal value and decreased over the optimal value.

Auxiliary oxidants such as H_2O_2 can promote photocatalytic oxidation rate and have two functions during the photocatalytic oxidation process: (1) they can promote oxidation degradation as good oxidants, and (2) they can receive photogenerated electrons from the valence band to form hydroxyl radicals with strong oxidizing ability. Thus, auxiliary oxidants not only increase the oxidative capacity of photocatalytic degradation but also promote the reaction process. Similar to aeration process, increasing the oxygen content in the reaction solution promotes production of oxidizing substances, subsequently enhancing the oxidation effects.

12.8 Photocatalytic Reactor

Currently, in the field of photocatalysis, there has been much focus on the preparation of materials and improvements, and less attention has been given to the engineering applications of photocatalytic technology, which has limited the transition of this technology from the laboratory to engineering applications. Photocatalytic reactors are mainly classified into floating and fixed reactors. According to the status of the photocatalyst, the TiO_2 photocatalytic reactors are generally divided into floating and fixed reactors. The floating photocatalytic reactor is generally cylindrical and filled with TiO_2 powder, and the pollutants and TiO_2 powder are mixed by gases or stirring. The characteristics of this reactor include large surface area, good mass transfer effects, and low degradation rate. However, light penetration decreases with the increasing concentration of TiO_2 powder, and the separation of TiO_2 powder from the solution is difficult. As a result, TiO_2 is generally recycled by filtration and centrifugation, which confer high operational complexity and cost. An et al. (2004) designed a new type of floating photocatalytic reactor, in which gas distribution was achieved by using porous platen. The reactor promoted separation of photogenerated electrons and holes by impressed voltage to improve the photocatalytic efficiency. Within 120 min of operation, 500 mL of 0.25 mmol/L quinoline was degraded in the reactor, with a degradation rate of 93 %. Fixed photocatalytic reactors, on the other hand, are more commonly applied, in which TiO_2 is fixed on the surface of the substrate in a certain manner. Mansilla et al. (2007) coated TiO_2 on the surface of a glass and achieved a degradation of 18 mg/L of flumequine under UV light irradiation in 20 min, with the degradation rate reaching 80 %.

Recently, researchers tried to combine the advantages of floating and fixed reactors by employing magnetic TiO_2 photocatalyst and designed a batch reactor, known as a magnetic separation photocatalytic reactor, characterized by recoverability of the photocatalyst. In this novel reactor, the TiO_2 powder is suspended in the system and separated as well as recycled by a strong magnetic field after the treatment process, thus overcoming disposal problems. The steps involved in this reactor are as follows: during the treatment, the coil is not energized, and the catalyst is evenly dispersed, having full contact with the organic matter in the solution under aeration. After the treatment, the coil is energized and a strong magnetic field is produced around the coil. As a result, the magnetic photocatalyst is deposited at the bottom of the container, the treated solution is discharged through the outlet, and the photocatalyst is left behind for the next batch of degradation.

In our experiments, two different magnetic carriers were prepared by burst calcining and ultrasonic mixing, and the TiO_2 layer was loaded by the sol–gel method to obtain photocatalysts with magnetic carrier (MT) and a porous material (MCT), respectively. Subsequently, their morphologies, structures, and photocatalytic abilities were investigated by employing characterization methods. Scanning electron microscopy (SEM) revealed that the MT surface resembled that of a honeycomb with a diameter of 1.5 μm (Fig. 12.5), whereas the MCT surface was a piled structure with a specific surface area (BET) of 111.14 m^2/g, which was 47 % larger than that of MT. Furthermore, X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS) results showed that Fe and Ti were in the form of Fe_3O_4 and TiO_2, respectively.

Figure 12.6 shows the adsorption and TC degradation curve of MT and MCT under darkness and UV excitation, respectively. The adsorption capacity of MT was significantly lower than that of MCT owing to its larger surface area, which was consistent with the results of SEM and BET. Under the UV lamp, TC was degraded by MT and MCT within 8 h with a removal rate of 90.0 and 88.7 %, respectively. At the initial stage of the reaction, the degradation rate of TC by MT was higher than that by MCT, and the removal rate at 1 h was 43 %, which was 10 % higher than that exhibited by MCT, possibly because of the higher content of Ti (15.9 %). After 3 h, the removal rates of TC presented by both MT and MCT decreased, and the decrease was more prominent with respect to MT. TC was adsorbed onto the surface of MCT because of its larger specific surface area, and the removal rate of TC exhibited by MCT at the adsorption stage was 51.7 %, whereas that presented by MT was only 26.9 %. Furthermore, the close contact between TC and TiO_2 was more conducive for the catalytic degradation under UV light. In addition, the overall removal efficiency of TC by MCT (including adsorption and photocatalytic removal) was higher than that by MT (26.1 %). Subsequently, the photocatalytic rate constants were calculated from the results: $MCT = 0.2629\ h^{-1}$ ($R^2 = 0.95$) and $MT = 0.2156\ h^{-1}$ ($R^2 = 0.97$). It was noted that both the absorption capacity and photocatalytic activity of MCT were higher than those of MT.

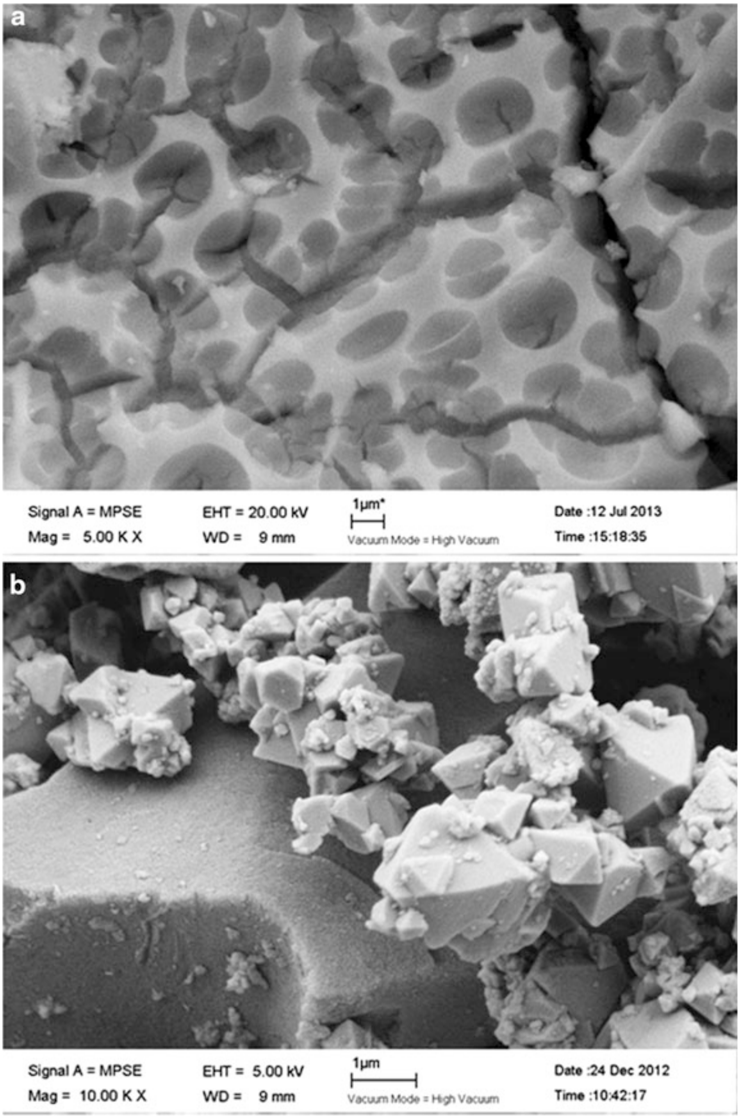
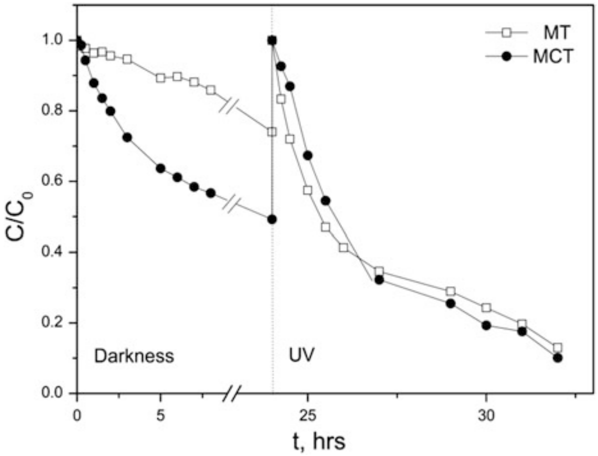


Fig. 12.5 SEM of photocatalysts: (**a**) MT and (**b**) MCT

12.9 Use of Anaerobic Microorganisms and TiO_2 to Remove Antibiotics from Aquaculture Wastewater

Biochemical-enhanced photocatalytic oxidation processes have achieved good results with respect to exhaustive treatment of wastewater, especially in removing substances that are toxic to microorganisms. In addition, the biochemical-enhanced

Fig. 12.6 Adsorption and TC degradation curve of MT and MCT under *darkness* and UV excitation



photocatalytic oxidation processes have also exhibited many advantages. The role of photocatalytic oxidation in the removal of chroma and that of biological method in COD removal has provided a complementary advantage to these novel processes. Hess et al. (1998) treated TNT wastewater using a photocatalysis biochemical method, and their results showed that the mineralization rate was 14 % when 100 mg/L of TNT wastewater was treated by using the biochemical method alone, which increased to 23 and 32 % after catalytic pretreatment for 2 and 6 h, respectively.

It is well known that conventional biological treatment is not ideal for antibiotics removal from livestock wastewater, because it often leaves a small amount of antibiotic residues in the treated water and requires a secondary purification step for the recycling of treated water. To overcome this drawback, a method combining photocatalytic oxidation and biodegradation was developed, which presented more advantages. Photocatalysis transforms the residual antibiotics to intermediate products that can be utilized or decomposed by microorganisms, which significantly improves the removal efficiency. Thus, when compared with the traditional methods, a combined biological photocatalysis method can overcome the shortcomings of these methods and has a broader development and application potential for livestock wastewater treatment in the future.

However, the combined use of photocatalytic oxidation and biological treatment methods for the removal of antibiotics from livestock wastewater has many issues that need further investigation. First, the mechanism of photocatalytic oxidation with biodegradation is not yet well understood, and numerous parameters such as the effects of combination mode or order on treatment performance and those of photocatalytic oxidation stages on the growth and distribution of microorganisms need to be examined. Second, the photocatalyst load and development method should be improved, so that the photocatalysts are cost-effective as well as practically durable and possess good catalytic properties.

12.10 Microbial Nanofiltration Technology for Exhaustive Treatment of Drip Irrigation Water

12.10.1 Agricultural Wastewater Reuse Treatment Technology

The lack of freshwater resources worldwide has become an indisputable fact. A United Nations report has pointed out that 12 billion people across 80 nations face a shortage in water resources. With the development of sewage purification technology, many countries are beginning to consider purification of sewage for supplying drinking water to the public.

In 1962, Japan started recycling of sewage, and it has been with scale in 70 years. With constant updating of sewage recycling technology, regeneration and costs have continued to decrease, and the water quality has continued to improve. As a result, sewage purification technology has gradually become an important measure to alleviate the shortage of water resources. At the beginning of the 1990s, a nationwide survey research was conducted in Japan, and a process design for wastewater reuse was developed, which thoroughly investigated the feasibility, operation, and application of wastewater reuse technology. Subsequently, this technology was widely applied in the arid areas of Japan. As a result, in recent years, water consumption in Japan has been decreasing, thus achieving initial success in water conservation. Similarly, it has been reported that in the USA, the city sewage recycling dosage is 2.6×10^6 m^3/day and that 62 % of the reclaimed water is used for agricultural irrigation. Furthermore, Israel has more than 200 sewage treatment projects with a capacity from 27 to 2×10^5 m^3/day, and 100 % rural sewage and 72 % city wastewater are being treated and reused, of which 75 % is used for agricultural irrigation (20 % of total irrigation), and the rest is used for groundwater recharge, industry and municipal constructions, and other purposes. In Japan, during the mid-1980s, the amount of city sewage reached 6.3×10^7 m^3/day, and the regenerated water was used for water reuse system, farmland irrigation, and river or city supplies. In addition, sewage reuse is also very common in Russia and Western Europe.

The three levels of sewage treatment, which is composed of chemical coagulation, sedimentation, filtration, and disinfection, have been shown to be effective in achieving sterilization, removal of particles, turbidity reduction, increased cleanliness of recycled water, and esthetic sense. Furthermore, exhaustive treatment technologies such as activated carbon adsorption, oxidation, ultrafiltration (UF), and reverse osmosis (RO) can produce water with a better quality than the traditional drinking water. Currently, in California, USA, there are more than 50 level 3 sewage treatment facilities with the most strict water quality control criteria (e.g., 0 % pathogens in the recycled water).

12.10.2 Drip Irrigation Water Quality Requirements

To meet the needs of agricultural irrigation and ensure national food security, drip irrigation technology has been popularized and used in most of the areas in China. Drip irrigation has various advantages such as water and energy conservation, requirement of less time and labor, and prevention of plant diseases and insect pests and has been adopted by many farmers. However, the drip irrigation system is expensive and requires strict daily maintenance, especially the selection of water source based on stringent standards.

Drip irrigation is an irrigation method carried out based on the crop water requirement by using low-pressure pipe system and capillary emitter to add water and necessary crop nutrients through uniform and slow drips into the crop root zone soil. This method saves water; does not destroy soil structure; maintains suitable level of water, fertilizer, gas, and heat in the internal soil for crop growth; decreases evaporation loss; does not produce surface runoff; and almost does not cause any deep percolation. The main characteristics of drip irrigation are as follows: (1) limited irrigation rate of only 2–12 L/h—the irrigation cycle is short, utilizing limited water at frequent intervals—(2) low working pressure, (3) ability to accurately control the amount of irrigation, (4) reduction of unnecessary evaporation, (5) no wastage of water, and (6) control by automatic management.

However, the presence of high quantities of sediments, weeds, suspended solids, and chemical precipitates in water may directly lead to clogging of the drip irrigation pipe and dripper, and long-term accumulation may cause collapse of the drip irrigation system, resulting in unnecessary economic losses. Similarly, very high quantities of suspended solids in water may cause soil pore clogging, reduce soil permeability, and make it difficult for the root system of the plants to get sufficient oxygen, thus resulting in a slow plant growth. Hence, before being used in the drip irrigation system, the water is usually filtered to ensure that it is clear. In addition, application of drip irrigation to soil with high salt content or use of saline water for drip irrigation may result in salt accumulation at the humid zone edge. If this area encounters rain, then these salts may get washed into the crop root zone causing salt damage and requiring drip irrigation to be carried out continuously. Furthermore, drip irrigation, or drip irrigation with saline water, should not be carried out in the absence of sufficient irrigation conditions, in places without adequate rainfall, or in high-salinity soil.

12.11 Nanofiltration Technology and Its Development

Nanofiltration (NF) is a pressure-driven membrane separation process, whose separation range lies between UF and RO. The membrane used for NF has a pore size of a few nanometers, and a novel membrane developed for ultralow-pressure RO separation technology is known as ultralow-pressure RO membrane. In general, the NF membrane has five characteristics: separation range between RO and UF, aperture of >1 nm (in general, 1–2 nm), suitable for separating 200–1,000 molecules with relative molecular weight, membrane surface with negative charge, and monovalent ion removal rate of <90 % and divalent and polyvalent ion removal rate of >90 %.

The sieving effect and charge repulsion are the primary separation mechanism of the NF membrane, and the membrane retains organic pollutants and multivalent salts in water. The important mechanical principle underlying separation using the NF membrane is the electrical effect—an electrostatic effect emerges between the negatively charged groups in the NF membrane and electrolyte ions with different charge strengths, resulting in varied ion retention rates. In diverse systems containing different valence ions, the NF membrane selectivity for different ions is not the same due to the Donnan effect, and the proportion of different ions passing through the NF membrane is not similar; multivalent ions can be prevented from passing through the NF membrane, which can maintain high desalting performance under low pressure. Typically, the NF membrane is negatively charged under neutral and alkaline conditions and positively charged under acidic conditions. The charge on the surface of the NF membrane has a significant role in the separation of ions. In general, the negatively charged NF membrane is composed of polymer-containing sulfonic acid group ($-SO_3H$) or carboxyl group (–COOH) or a polymer film into which the negatively charged group has been introduced. On the other hand, the positively charged NF membrane is composed of materials mainly containing amino group ($-NH_2$), and colloidal particles and bacterial toxins in water get adsorbed onto the surface of the membrane. Moreover, the positively charged NF membrane can be used to separate positively charged amino acids, proteins, and other substances and can be employed for clean production during cathodic electrophoretic painting process because the positively charged NF membrane exerts repulsive force on the particles with similar electric property. The interception of neutral molecules (such as glucose and other organic molecules) by positively or negatively charged NF membrane is caused by the molecular sieve effect of nanoscale micropores, and the interception rate is associated with the molecular particle size, operating pressure, concentration, and molecular characteristics. On the other hand, the separation of inorganic ions and charged organic substances by the NF membrane is affected not only by the particle size but also by the chemical potential and potential gradient. The mass transfer process is significantly affected by the surface charge and the Donnan effect among the charged molecules (ions), and if the charge on the surface of the ions and NF membrane is higher, then the removal rate of the contaminants will also be higher. Therefore, different charged NF membranes exhibit varied interception capacities for different valence ions. Furthermore, the ion selectivity of negatively charged NF membrane increases with the increasing pH, indicating that pH affects the removal of pollutants.

NF membranes are widely used, particularly in the field of water treatment, because of its stability, nontoxicity, and energy-efficient nature. In recent years, basic research and practical engineering studies on NF technology have achieved rapid development, resulting in broader prospects for the application of NF technology.

In advanced water treatment, to achieve separation and purification, selectively permeable membrane is used as a separation medium, and driving forces are applied on both sides. As a result, the components of the raw material on one side of the membrane get selectively penetrated into the other side. However, during the penetration process, the membrane resistance increases and the membrane flux declines. As a pressure-driven process, NF faces the problems of fouling and purge control in practical applications similar to RO and UF. The two main reasons for membrane flux decline are as follows: (1) concentration polarization effects—the interception of some solvents through the membrane results in accumulation of components on the surface of the membrane, forming a high concentration layer on the membrane surface, namely, the concentration polarization layer. This layer decreases the permeability of water and further reduces the permeation flux because of higher osmotic pressure. Nevertheless, this effect is reversible by reducing the feed concentration or improving the hydrodynamic fluid conditions of the feed liquid, such as increasing the flow rate, using turbulence promoter, or designing more rational flowing structure, to decrease the concentration polarization effect and restore the separation performance of the membrane. (2) Solvent adsorption and particles deposition—a high concentration of solvent is deposited to form a gel layer, and the suspended particles migrate to the surface of the membrane with sediments, resulting in clogging of the membrane pores. This gel layer reduces the hydraulic permeability and permeation flux, promoting the formation of long-term and irreversible contamination. Unlike pollution, concentration polarization is often an important factor in the formation of membrane fouling. Studies have shown that factors affecting the fouling of NF membrane include properties of the pollutants, inflow conditions, membrane surface properties, and hydraulic conditions, and the contribution of these factors on membrane fouling is interrelated.

Although numerous studies have been carried out to develop methods to reduce membrane fouling, this pollution is still inevitable. Some of the physical methods to reduce membrane fouling include washing the contaminated membrane, lowering the operating pressure, increasing the circulation of the liquid material, flushing the surface of the membrane, etc. In addition, chemical cleaning methods, such as acid and alkali solution cleaning, surfactants cleaning, enzymes cleaning, changing the surface properties of the membrane (e.g., transforming the membrane to hydrophilic membrane), etc., are also employed to reduce membrane fouling.

12.12 Membrane Bioreactor

Traditional aerobic biological treatment methods such as the activated sludge process have been widely used for a long time in sewage and agricultural wastewater treatment. However, as the solid–liquid separation of water and microorganisms is based on gravity sedimentation, these methods face the following limitations: (1) as the liquid separation efficiency in the sedimentation tank is not high, the concentration of the sludge in the aeration tanks is not maintained at a high level, resulting in low volumetric loading and large area; (2) the treated effluent is nonideal and unstable; (3) low oxygen transfer efficiency and high energy consumption; (4) high sludge yield; and (5) complex management operations. Thus, it is difficult for conventional biological treatment processes to meet the increasingly stringent standards for effluent discharge and wastewater reuse.

A new water treatment technique, known as MBR, exploiting NF membrane separation technology, instead of traditional gravity sedimentation tank, has attracted wide interest among various researchers. MBR was first used in the microbial fermentation industry, and its application in the field of wastewater treatment began in the 1960s in the USA. However, at that time, owing to limited membrane production techniques, the membrane life was short with low water permeability, thus hindering its practical application. In the late 1970s, Japanese researchers vigorously developed the membrane separation technology, which resulted in practical applications of MBR. In the 1980s, many studies on MBR were carried out around the world. Japan's Ministry of Construction developed a large-scale research program called "Aqua Renaissance '90," which mainly focused on membrane development, research on membrane reactor, etc. Similarly, France, the USA, Australia, and other countries also devoted much effort to develop MBR, making the research on MBR more comprehensive and exhaustive, and laid the foundation for further application of this technology in the 1990s.

MBR processes generally consist of biological reactors and membrane components. According to the position of the membrane components, the MBR processes can be divided into segregated and integrated modes. Furthermore, based on the presence of oxygen, the bioreactors can be divided into aerobic and anaerobic MBRs. The membrane of the segregated-mode MBR generally employs a pressurized pattern. A mixture enters into the bioreactor and passes across the membrane after pressurization through a pump under pressure. Subsequently, solid and macromolecular substances are intercepted by the membrane and flow back to the bioreactor with the concentrated liquid. On the other hand, the integrated-mode bioreactor is characterized by stable operation, it is easy to manage and clean, and its membrane can be easily changed. However, under normal conditions, to reduce the deposition of contaminants on the membrane surface, the flow rate of water supplied by the circulation pump is maintained at a high level, which results in higher power consumption.

In the integrated-mode MBR combined technology, the membrane components are placed into the reactor, and a vacuum pump or another suction pump is used to obtain the filtrate. To reduce contamination of membrane surface and extend the operating cycle, general pump suction is applied intermittently. When compared with the segregated-mode bioreactor, the most important feature of the integrated-mode bioreactor is its low operation costs; however, stability, operation management, and cleaning are difficult to achieve. Currently, applications of segregated- and integrated-mode MBR technology are rarely reported.

It has been realized that the design and operation of the MBR technology are the key for its successful applications in water treatment. To meet the needs of different processes, achieve further improvement in the water quality of the effluent, and accomplish a better utilization of water resources, MBR is often combined with other traditional methods. For example, MBR + A/O process can improve the removal of organic matter and NH_3-N from sewage; MBR + aerobic/anoxic/oxic (A/A/O) process can strengthen the performance of nitrogen and phosphorus removal; MBR + PAC (powdered activated carbon) process can reduce membrane fouling during operation; and MBR + efficient strain process can be used for the treatment of refractory organic wastewater.

Cleaning is the last step in membrane technology applications. Reasonable membrane cleaning can not only restore membrane flux effectively and improve the efficiency of the system but can also extend the life of the membrane and reduce investment costs as well as operation expenses. The cleaning methods applied to MBR membrane include physical cleaning, chemical cleaning, and a combination of both. Physical cleaning includes aeration, water rinse with a high velocity, backwashing, ultrasonic cleaning, etc. Among them, aeration and backwashing can be easily and automatically controlled and are more common. In addition, shortening or extending the pumping period is also effective in reducing membrane fouling. As a new technology developed in recent years, ultrasonic cleaning produces excessive disturbance, strong shock waves, and microjets on the membrane, accelerating the separation of contaminants on the surface of the membrane. However, further studies are needed to determine whether ultrasonic cleaning produces any harmful effects on the membrane. In the past, MBR studies basically used activated sludge and pressurized flat membrane, and the types of wastewater were mainly sewage and fecal sewage. Currently, MBR technology is being successfully applied in waterways sewage treatment, fecal sewage treatment, landfill leachate treatment, and other wastewater treatments.

12.13 Combined Use of Microorganisms and NF for Exhaustive Drip Irrigation Water Treatment

The Israel Peres Peace Center had developed MBRs with new NF membrane technology as the core and a supporting reclaimed water irrigation technology model. Among them, the NF membrane products are the core of the MBR components. When compared with the membrane flux and NaCl interception of the current commercial NF membranes such as polypiperazine amide, cellulose acetate, and NTR-7450 sulfonated polysulfone, those of the new NF membrane are 48 and 1.8 times higher, reaching values of 2,358 $L/m^2/h$ and 31 %, respectively, at the same pressure (10 bar). Thus, application of MBR can significantly improve the efficiency of sewage treatment.

Table 12.2 Swine wastewater and effluent characteristics in various processing stages (Zhang et al. 2011)

	pH	SS	COD	NH_4^+–N	K	Na	Ca	Mg
Raw water	7.25–8.9	386–3,306	60–7,722	823–900	572.93–674.9	364–470	177.3–260.2	97–105
Anaerobic effluent	6.82–8.5	154–75	305–2,266	740–810	300.8–462.8	338–402.2	87.8–116.4	63.12–100
Effluent of ecological pond	6.68–9.3	28–624	164–1,960	62–685	227.93–398.3	394–540	119.6	84.86–109.84

In our experiment, we applied the methods presented in existing works for the treatment of wastewater from a piggery in Tianjin. The characteristics of the swine wastewater and effluent are shown in Table 12.2. The NF membrane model used was ESNA1-4040 (Nitto Denko) aromatic polyamide, the water production was 7.4 m^3/day, the desalination rate was 85–95 %, and the effective membrane area was 7.9 m^2.

We employed nano-MgO-activated carbon NF membrane to remove various pollutants from swine wastewater (Zhang et al. 2011). The farm ecological pond water was diluted 30-fold and poured into the raw water tank. The vacuum pump was mounted onto the mixing tank. The water was only initially added to the nano-MgO, and subsequent addition of water was not required. The mixer was operated to stir the water at 80 rpm for 5 min, and then, the water was allowed to stand for 20 min. Then, by using the vacuum pump, the water was continuously mixed and passed through the sand filtration column, activated carbon filtration column, and NF membrane component (Fig. 12.7). Simultaneously, the metering pump was operated to pump KH_2PO_4 into the NF membrane pipe at a flow rate of 2 L/h. The dosages of nano-MgO and KH_2PO_4 were 0.1 kg and 0.05 mol/L, respectively.

The removal of NH_3-N by the combination process (Fig. 12.8) and the KH_2PO_4 index showed a similar trend, and the concentration of organic matter and NH_3-N in water gradually decreased after subjecting to nano-MgO, filtration, and NF. In particular, after filtration through the NF membrane, the concentration of organic matter and NH_3-N decreased by 85–90 and 70–85 %, respectively, and remained in a stable concentration range, and the water reached the quality of irrigation water. However, with respect to water treated by nano-MgO and filtration, the concentration of NH_3-N and KH_2PO_4 index showed a gradual increasing trend. This finding suggested that with the decrease in nano-MgO consumption and medium adsorption, the pollutants removal efficiency of the NF membrane pretreatment process decreased. It should be noted that after the quantity of the processed water reached 1 m^3, the organics removal capacity of nano-MgO was stable at about 40 %. These findings (An et al. 2011), along with prior screening test results, indicate that nano-MgO removes organic matter by adsorption and oxidation.

As mentioned earlier, the use of nano-MgO increased water turbidity; however, after the two-stage filtration, the turbidity of water decreased to about 1 NTU, and after filtration using NF membrane, no turbidity could be detected in the effluent.

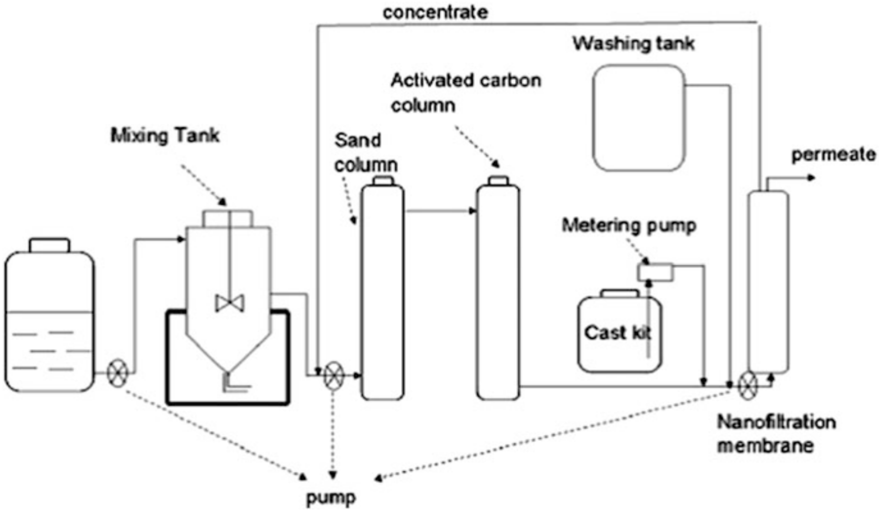


Fig. 12.7 Nano-MgO-activated carbon purification process (Zhang et al. 2011)

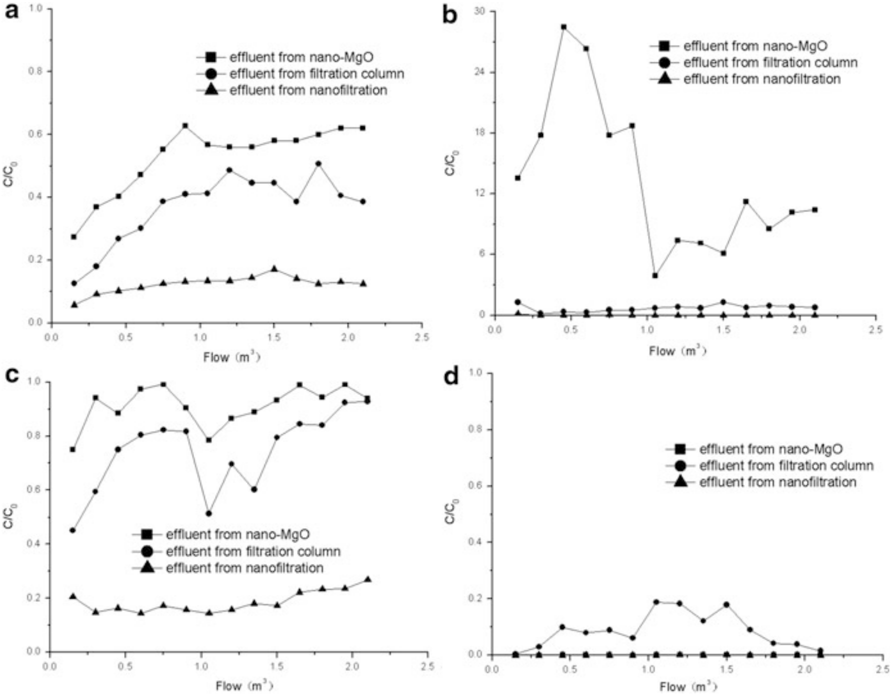


Fig. 12.8 Pollutant removal performance of the combined membrane technique. (**a**) KH_2PO_4 index, (**b**) turbidity, (**c**) ammonia, and (**d**) colony counts (An et al. 2011)

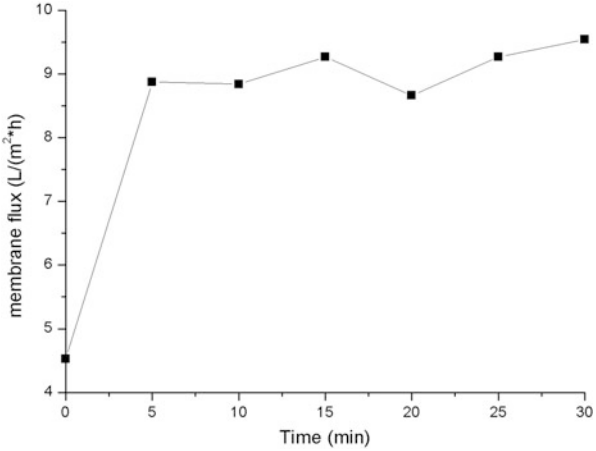


Fig. 12.9 Hydraulic cleaning for NF flux recovery

Furthermore, the changes in the total number of bacteria showed a surprising trend. The total bacteria removal rate of nano-MgO and NF membrane was >99.99 %, whereas that of the filter column was 80–98 %, which may be owing to the microorganisms in the filter using nano-MgO organic compounds for growth and metabolism. Therefore, it can be concluded that nano-MgO does not have any bactericidal effect and that NF membrane could intercept bacteria. In addition, according to the results obtained, by regulating the level of KH_2PO_4, the pH of water could be maintained at about 7, thus meeting the standard of irrigation water.

For cleaning, we used the physical method of hydraulic cleaning as follows: the outlet of the NF membrane permeation liquid was closed, and the liquid was used for washing. As a result of closing of the outlet, the pressure of the inner membrane filtrate increased and caused the liquid to reverse-flow through the roll film at a high speed, thus removing the dirt deposited on it.

From Fig. 12.9, it can be observed that after hydraulic cleaning for 5 min, the membrane flux increased from 4.53 to 8.87 L/(m^2 h) and that with the increase in the period of cleaning, the membrane flux slightly increased. These findings indicate that through hydraulic cleaning, the pollutants can be effectively removed from the NF membrane surface, and the processing ability of the NF membrane can be restored. At the same time, the results also suggest that we can apply “batch processing mode for 1 h and cleaning for 5 min” to NF membrane components to effectively prevent membrane pore blockage and maintain high membrane flux.

12.14 Conclusion

In this chapter, the applications of three kinds of nano-materials (nano-iron, nano-TiO_2, and nanofiltration) for the treatments of fertilizer-polluted groundwater, livestock wastewater, and irrigation water for greenhouse vegetables, respectively, were described. Based on the discussion above, some conclusions could be drawn:

1. Nitrate could be removed from groundwater using a nano-iron–bacteria denitrifying system with little toxic by-products generation under anaerobic conditions.
2. Biochemical-enhanced photocatalytic oxidation processes have achieved good results with respect to exhaustive treatment of wastewater containing antibiotics.
3. The quality of effluent from the combined use of microorganisms and nanofiltration is in full compliance with the requirements of drip irrigation.

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Part III
Toxicity Issues and Public Perception

Chapter 13
Nanoecotoxicology: The State of the Art

Hudson C. Polonini and Roberta Brayner

Abstract Manufactured nanomaterials are used in many commercially available consumer products, such as cosmetics, textiles, and paints. Due to the increasing production volumes, environmental exposure to these materials is evident. Here, we will discuss the toxicological impact of some nanomaterials, such as ZnO, TiO_2, and $BaTiO_3$ nanoparticles on aquatic microorganisms by giving some examples. It is clear that the physicochemical properties as well as the structure and morphology of nanomaterials have a high influence on toxicity. We will emphasize the importance of nanomaterial characterization before biological tests.

13.1 Introduction

The study, production, and usage of nanomaterials are currently a fact. These materials have gone through a very fast development, and, as a consequence, a vast amount of products can be found in the market with a "nano" prefix in their names, symbolizing that they contain a "whole new technology" for consumers—whether this is true or only a marketing strategy. However, this rapid development and spread of nanomaterials in the contemporary world can put them in contact with living organisms in situations not yet fully understood. By living organisms, we mean every single organism contained in the environment (Handy et al. 2008; Cullen et al. 2011).

Until now, some studies have shown that nanoscale particles can lead to unexpected and different ecotoxicological effects caused by the source material, even

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when it is relatively inert (Handy et al. 2008; Durán et al. 2014). A recent research indicates that nanomaterials have different toxicity profiles compared to large particles, due to their small size and high reactivity (Brayner et al. 2010). Some studies suggest that nanomaterials, for their small size, may have a higher permeability through the skin, mucous membranes, and cell membranes, what may magnify their toxic effect. A classic example is gold, which is virtually an inert metal, but becomes reactive in the nanoparticle form (Paschoalino et al. 2010). However, one may consider that this is a relatively new subject, and then it is still uncertain whether the environmental impact of materials is inversely proportional to their size—thus the ecotoxicity profiles cannot be generalized, which makes the study of the materials on a case-by-case basis necessary.

For humans (and consequently for other organisms), the exposure to these nanomaterials can occur directly or indirectly (Nel et al. 2006). Humankind can be exposed to these materials since their synthesis and production of derived products (occupational exposure) until their end use (consumer exposure), also including their removal and subsequent accumulation in the environment (environmental exposure). The main routes of entry of nanomaterials into the human body are the skin, gastrointestinal and respiratory tracts, either by the use of topical creams or oral medications or by contact with water, air, and contaminated soil (Hagens et al. 2007; Mihranyan et al. 2012).

The nanoparticles can be found in nature in two main forms. They can be immobilized in/at a larger size material (composite, functionalized nanoparticles, part of the surface of a micronized material, etc.) or be free nanoparticles, capable of mobility in the environment and in the human body (Hansen et al. 2008). The latter is the most worrying from a toxicological point of view.

The complexity of nanoparticle effect lies, in part, in their ability to bind and to interact with biological material, changing its surface characteristics depending on the environment in which they are into. The latest scientific knowledge on the interaction mechanisms of cells and nanoparticles has indicated that many cells readily internalize the nanoparticles through either active or passive mechanisms. At intracellular level, however, the mechanisms and pathways are more difficult to understand. Even the same material particles may have completely different behavior due to slight differences in surface coating, drug load, or size. This is one of the main distinctions between classical toxicology and nanotoxicology. Moreover, the filing of bioassays involving nanomaterials is still in development and in general has not yet been internationally validated (Elsaesser and Howard 2012).

This chapter aims to discuss the toxicological impact of some nanomaterials, such as ZnO, TiO_2, and $BaTiO_3$ nanoparticles. We will emphasize the importance of nanomaterial characterization before biological tests.

13.2 Ecotoxicology at Large

The exposure of nanomaterials in different environmental compartments (water, soil, and air) may result in their increased bioavailability and accumulation along food chains (Oberdorster et al. 2004; Ramsden et al. 2009). Likewise, they can reach the human body through air, water, and soil; nanomaterials are likely to interact with other living cells, causing effects unknown in their entirety. In this sense, the three basic strategies for screening the toxicity profile of nanomaterials, according to Oberdörster et al. (2005a) are:

- Physicochemical characterization (size, surface area, shape, solubility, aggregation)
- Elucidation of the biological studies from in vitro effects
- Confirmation of the effects through in vivo studies

These three points were formulated from the point of view of the potential effects of nanomaterials in humans. But when an entire ecosystem is taken into consideration, the question becomes broader and more complex. Despite a growing body of information about the toxic effects of nanomaterials on humans by direct or indirect exposure, related environmental impact studies were just in the beginning (Kahru and Dubourguier 2010). The trend, however, is that this assessment increasingly becomes a global concern and is required by regulatory agencies.

The ecotoxicology itself can be comprehended as a science whose core is to study the contaminants and their effects on components of the biosphere, including humans (Newman and Zhao 2008). It was René Truhaut who first mentioned the term "ecotoxicology" in 1959, defining it as "the branch of toxicology concerned with the study of toxic effects caused by natural or synthetic pollutants for components of animals (including humans) vegetable and microbial ecosystems, in an integral context" (Truhaut 1977). The ecotoxicological research has been developed rapidly due to the environment pollution induced by the rapid industrial development of that era, permeated by major industrial accidents. Policies have been developed since then, and ecotoxicology has become a significant part of the environmental and ecological risk required by these new laws.

Ecotoxicological tests have been developed greatly for aquatic environments. In this context, Blaise (1998) classified the development stages of aquatic toxicity tests in terms of decades: (1) "the dark ages" until the 1950s, (2) the decade of fish studies in the 1960s, (3) the 1970s regulatory decade, (4) the ecotoxicological decade in the 1980s, and (5) the decade of microbiotests in the 1990s. More recently, Kahru and Dubourguier (2010) designated the decade 2010–2020 as the "era of (eco)toxicogenomics and ecotoxicology."

Despite the growing understanding that synthetic nanomaterials should be evaluated for their potential environmental hazard prior to their use in products and their inevitable release into the environment, there is currently little data on this. The first few studies were initiated in the 1990s, primarily evaluating the pulmonary impact of ultrafine particles (Oberdörster et al. 2005b). There was then a lag of 10 years, until the number of nanotoxicological research works began to increase exponentially, mostly focused on human health (Royal Society 2004). In relation to the environmental impact, the number of studies is still small and does not reflect the substantial number of new applications developed for these materials (Kahru and Dubourguier 2010).

In this context, ecotoxicology has gradually established itself as a multidisciplinary field of research essential to elucidate the real impact of these new materials in the environment.

13.3 Aquatic Nanoecotoxicology as a Major Data Source

Aquatic toxicity tests are widely used because these ecosystems are the main enclosures of contaminants, whether coming from direct release into water bodies through discharge of effluents or released into the air or deposited in soils (Kendall et al. 2001).

The aquatic environment is complex and diverse. It comprises several types of ecosystems, among which are rivers, lakes, estuaries, seas, and oceans (Rang et al. 1995). They are open and dynamic systems capable of undergoing continuous changes in their chemical composition. For example, in freshwater, calcium, magnesium, and carbonate ions are the most abundant, but sodium, potassium, phosphorus, iron, sulfur, and silicon compounds are also present. There are also non-conserved components, which include dissolved O_2, CO_2, and N_2 gases; nutrients such as phosphate and nitrate; dissolved organic compounds such as amino acids and humic substances; trace elements such as copper, zinc, chromium, molybdenum, vanadium, manganese, tin, iron, nickel, cobalt, and selenium; and particulate materials such as sand, clay, colloid, nonliving tissues, and excreta (Rang et al. 1995; Ravera 2004).

Among the biochemical and physiological effects caused by toxic agents on aquatic organisms, we can mention: change in cell membrane permeability; interference in adenosine 5′-triphosphate (ATP) production; reversible or irreversible inhibition of enzymes; disturbances in carbohydrate metabolism; respiratory process disorders by inhibiting electron transport and oxidative phosphorylation; lipid metabolism disturbances, which may result in liver abnormalities; and change in the structure or activity of enzymes that participate in regulatory processes, affecting the synthesis and release of hormones (Costa et al. 2008).

The toxicity tests can be classified into acute and chronic, depending on the duration and final responses that are measured. The acute toxicity tests are used to measure the effects of toxic agents on aquatic species during a short period of time relative to the life of the test organism. They aim to estimate the dose or concentration of a toxic agent that could produce a measurable response in a specific test organism or population in a relatively short period of time, usually 24–96 h. The toxic effects measured in acute toxicity tests include any response displayed by a test organism or population resulting from a chemical stimulus. Typically, the effect measured in acute toxicity studies with aquatic organisms is lethality or some other body event that precedes it, for example, the state of immobility (Costa et al. 2008).

Chronic toxicity tests, in turn, are conducted to measure the effects of chemicals on water for a period that may cover part or all of the test organism species life cycle. The fact that a chemical does not produce toxins for aquatic organisms in acute toxicity test effects does not indicate that it is not toxic to them. Chronic toxicity tests allow us to evaluate the possible toxic effects of chemicals under conditions of prolonged sublethal concentration exposure, i.e., concentrations that permit the survival of organisms, but that affect their biological functions, such as reproduction, egg development, growth, and maturation, among others (Kendall et al. 2001; Ronco et al. 2004).

Toxicity tests can be further classified into static, semi-static, and dynamic, according to the method of addition of the chemicals solutions. The static tests are performed without the renewal of the test solutions and are recommended for samples that do not cause oxygen depletion, which are not volatile and are stable in aqueous medium. On the other hand, unstable or volatile toxic substances have their concentrations reduced throughout the test, contributing to the underestimation of the result. In such cases, the semi-static tests, in which the solutions are renewed periodically, are recommended. The long-term chronic toxicity tests are usually performed in dynamic mode. In dynamic testing, the test solutions are continuously renewed (APHA 1998; Adams and Rowland 2003; ABNT 2004).

13.4 Characterization of Materials for Ecotoxicology Is Essential

The acquired experience in ecotoxicology throughout the years allows the affirmation that not only the size influences the reactivity of nanomaterials but also a wide range of other properties that must be evaluated when conducting toxicological tests, and physico-chemistry is paramount when trying to understand the fate and behavior of the particles in the environment (Brayner et al. 2010). For Jiang et al. (2008), for example, until the relationship between the characteristics of nanoparticles and toxicity is fully understood, it will be necessary to ensure that all potentially significant characteristics are measured.

Since the determination of all these features is virtually impossible, some authors have identified a number of key properties that must be addressed in toxicology studies, which are size, dispersion state, surface charge, shape, chemical composition, surface area, and surface chemistry (Oberdörster et al. 2005a, b; Powers et al. 2006). These factors play important roles in the uptake and distribution of the particles within live organisms (Brayner et al. 2010). If not determined, the possible toxic effects could not be easily attributed to a certain property of the nanomaterial or even the nanomaterial itself because impurities and other components can account for such effects (Sayes and Warheit 2009).

Furthermore, it is essential that the material characterization is performed in biorelevant media for the toxicity tests intended (Jiang et al. 2009), i.e., those in which the ecotoxicological experiments will be conducted. This is because potential physical and chemical changes (e.g., agglomeration/aggregation and change in surface charge) may occur with the particles in different solutions, which have a direct impact on the toxicological responses (Powers et al. 2007).

Here, we draw attention to the importance of establishing the dispersion state of nanomaterials. This is needed because certain particles are extremely reactive in aqueous medium, which changes their size and shape compared to the dry powder. The dry nanomaterials can take two forms: aggregates (strong links between primary particles) and clusters/agglomerates (controlled by weaker forces such as van der Waals). The state of the nanoparticles, aggregate or agglomerate, may be controlled during synthesis (Tsantilis and Pratsinis 2004; Jiang et al. 2007). After dispersing nanomaterials in solution/suspension, they can remain as singlets or form agglomerates or remain as aggregates, surrounded by an electrical double layer. Typically, when agglomerated nanoparticles are added to a liquid, they can be separated to overcome the attraction of weak forces through various methods such as the use of ultrasound. However, the aggregated nanoparticles cannot be separated.

In order to illustrate the importance of such determinations mentioned, below we cite some studies on selected nanomaterials.

13.5 Nanoecotoxicology of Selected Nanomaterials

13.5.1 Titanium Dioxide (TiO_2)

Potential applications of TiO_2 include, but are not limited to, (1) solar cells (Usui et al. 2004); (2) food additives (Lomer et al. 2002); (3) paints, cosmetics, and sunscreens (Serpone et al. 2007); (4) photocatalysts (Carp et al. 2004); and (5) photocatalytic water purification (Hagfeldt and Gratzel 1995).

Without photoactivation, nano-TiO_2 is considered chemically inert (Bernard et al. 1990; Lindenschmidt et al. 1990), but it becomes highly reactive under UV irradiation and produces reactive oxygen species (ROS) (Reeves et al. 2008), which leads to strong antibacterial activity (Sunada et al. 2003).

Among the nanomaterials, the production of nanoparticulate TiO_2 is the highest in the world, an order of magnitude greater than the next most widely produced nanomaterial, ZnO (Miller et al. 2012). Thus, TiO_2 may reach its highest concentrations in surface waters and pose a significant threat to aquatic ecosystems (Gottschalk et al. 2009, 2010).

TiO_2 is a photocatalyst capable of producing highly oxidizing ROS, and studies have shown that it still exhibits a photocatalytic and antibacterial activity in the absence of UV illumination (Sayes et al. 2006). In a general manner, absorption of a photon with sufficient energy (3.2 eV for anatase) is the condition required for photochemical reactions to proceed at the photocatalyst surface (Czili and Horvath 2008). According to Miller et al. (2012), "when TiO_2 reaches an electronically excited state, an electron (e^-) is promoted from the valence band to the conduction band, generating a hole in the valence band (h^+). The resulting electron–hole pair can then recombine or migrate to the surface of the particle and may react with H_2O or OH^- to form $OH^\bullet$ or can directly oxidize adsorbed species. The electrons may also react with adsorbed molecular oxygen to form $O_2^{-\bullet}$ ions."

Some reports have dealt with the toxicity profile of nano-TiO_2, showing that it is toxic to *Escherichia coli* via ROS production, under sunlight (Wei et al. 1994); it can cause inflammatory responses in mammalian cells (Peters et al. 2004) or even be genotoxic (Carinci et al. 2003). On the other hand, some studies concluded that there were very low or even absent effects of TiO_2 nanoparticles (Rehn et al. 2003; Peters et al. 2004). These contrasting conclusions arised from different dispersion states in these experiments encouraged Planchon et al. (2013) to perform a comprehensive study coupling toxicity assessments with physicochemical studies, using *E. coli*. Specifically, they aimed to explore the influence of the dispersion state that nanoparticles would undergo in natural water on their potential toxicity to the microorganism.

The authors used Seine River water (SRW) (pH between 7.8 and 8.2) as a natural environmental medium to quantify the ecotoxicological impact of the three types of manufactured titanium dioxide (TiO_2) and also in synthetic waters at pH = 5.0 and 8.0. The three nanoparticles were different, going from 100 % rutile (R) to a rutile/anatase mixture (M and P25). R type were rod shaped, while M were rutile rods and anatase spheres mixture, and P25 were an assemblage of anatase spheres and rutile polygons (anatase spheres are smaller than rutile and thus P25 contained the largest particles). They found the isoelectric points of the three materials to be between 6.0 and 6.2, close to that of bare TiO_2 surface (and thus the particles were not covered by any stabilizing oxide or organic layer). All particles were negatively charged in SRW and pH 8.0 water and positively in pH 5.0 water, the zeta potential being very limited in amplitude, all below 15 mV (for colloidal stability, it must be at least 25 mV). This instability accounted for the aggregated state of TiO_2 nanoparticles in the media used.

The impacts of the nanoparticles on *E. coli* survival were measured as function of concentration and time. TiO_2 nanoparticle toxicity was found to start at 10 ppm (the toxicity was found to be lower at pH 5 compared to Seine water and pH 8.0 water) and to depend slightly on the TiO_2 mineralogical phases, increasing from pure rutile to assemblages of the rutile and anatase phases. The lethal effect was effective from 1-h contact and did not amplify with time. Regarding composition, P25 turned out to be more toxic than M and R types. The proportion of the rutile phase affected the aggregation state of the nanoparticles and also their toxicity. Indeed, the bacterial survival is all the more reduced when cells were exposed to small aggregates, so size increased and toxicity decreased with increasing rutile proportion. The influence of the aggregate size was not unexpected, since the reactivity of nanoparticles is driven by their specific surface area, which is reduced by aggregation. To assess the nanoparticles dispersion state and their interactions with bacteria, cryogenic transmission electron microscopy (TEM) and zeta potential measurements were performed. A higher nanoparticle aggregate sorption on cells was observed in pH 5 water, compared to SRW, i.e., the observed toxicity was not directly linked to the particles sorption onto the cell surfaces.

Another comprehensive study was conducted by Miller et al. (2012) toward the effects of TiO_2 nanoparticles on phytoplankton (0.2–200-μm single or clustered cells), the dominant primary producers in marine ecosystems (Behrenfeld et al. 2006), as they are the base of oceanic food webs and a dominant component of the global carbon cycle, as well as other biogeochemical cycles. Also, the marine and estuarine ecosystems are expected to be the destination of most industrially discharged nanomaterials (Musee et al. 2011).

Significant suppression of population growth occurred for three of the species used, under UV light (UVA averaged 4.5 W m^{-2} and UVB 4.1 W m^{-2}, levels comparable to UV intensities near the ocean surface, <1 m depth in coastal waters). In one species, *Isochrysis galbana*, toxicity was evident at the lowest concentration tested, 1 mg L^{-1}, indicating a no-effect concentration (NOEC) <1 mg L^{-1}. In the other two species affected, *Thalassiosira pseudonana* and *Dunaliella tertiolecta*, significant toxicity was evident at 3 mg L^{-1}, although a slight depression of growth rates was seen for *D. tertiolecta* at 1 mg L^{-1}. No significant effect on growth rates of any species was seen in the blocked UV treatment except in the case of *I. galbana* at the highest TiO_2 concentration tested, 7 mg L^{-1}. No significant effect of nano-TiO_2 on growth rate was seen in any treatment for the diatom *Skeletonema costatum*. Also in this study, scanning electron microscopy (SEM) revealed that TiO_2 nanoparticles were adhering to the surfaces of phytoplankton cells as aggregates with sizes between 10 and 100 nm.

Chen et al. (2012) investigated the toxicological effects of nanometer titanium dioxide (21 nm, anatase-to-rutile mixture) on a unicellular green alga *Chlamydomonas reinhardtii*, by investigating the changes of the physiology and cyto-ultrastructure of this species under treatment. There was no significant difference between the control and the treatment group with nano-TiO_2 during an 8-h exposure. After a 24-h exposure, there was significant reduction in the treated groups and the control. 10, 20, and 100 mg L^{-1} nano-TiO_2 inhibited growth significantly, and the cell density of those treated groups (10, 20, and 100 mg L^{-1} TiO_2) reduced gradually during the experiment, which showed that cell growth was inhibited completely by high TiO_2 dosage. The photosynthetic activity (Fv/Fm, a ratio of variable to maximum fluorescence, indicating photosynthesis activity) of *C. reinhardtii* decreased sharply with high TiO_2 concentration (>1 mg L^{-1}). SEM micrographs of *C. reinhardtii* treated with nano-TiO_2 indicated that TiO_2 NPs had assembled on the cell wall surface. The content of soluble protein in algae cells increased after 24-h treatment with TiO_2 at 0.1, 1, and 10 mg L^{-1}, while it decreased in cells treated with TiO_2 at concentrations of 20 and 100 L^{-1}. After

72-h treatment, the protein content in 0.1, 1, and 10 mg L^{-1} treatment groups increased, while no significant changes were observed in groups exposed to 20 and 100 mg L^{-1} TiO_2 concentration. There were no significant changes for chlorophyll *a* in different levels of treatment groups and exposure times, while for chlorophyll *b* and carotenoids content, there were obvious decreases after treatment, and then there were significant increases in treated groups after 3 and 4 days of exposure. The chlorophyll *a*:*b* ratio increased when the concentration of nano-TiO_2 increased after 24-h treatment. The malondialdehyde (MDA) content in all treatment groups (0.1, 1, 10, 20, and 100 L^{-1}) increased after 4 h of treatment, compared to that of the control group. They observed cell aggregation in TiO_2 treatment groups with SEM images and light microscopy, as well as evidence of TiO_2 nanomaterial absorbance on some cell surfaces in the high concentration treatment groups. The TEM images of high dosage groups also supported NP aggregation on the cell surface. This could prevent cellular exchange with the external milieu, for example, by sequestering nutrients or altering pH or redox potential.

Ji et al. (2011) also showed that large aggregates of TiO_2 nanoparticles could entrap the algal cells and consequently reduce the light and nutrient available to the algal cells, inhibiting their growth. Aruoja et al. (2009) also showed that large nano-TiO_2 aggregates could entrap algal cells, while the cultures with bulk TiO_2 always contained free algal cells. Huang et al. (2005), for their turn, indicated that *Pseudokirchneriella subcapitata* also adsorbed nano-TiO_2 nanoparticles onto their surface, being capable of carrying 2.3 times their own weight in TiO_2 particles, and the kinetics and the extent of nano-TiO_2 adsorption on algae were extremely dependent on pH (in this case, the maximum adsorption occurred at pH 5.5).

Song et al. (2012) examined the toxic effects of TiO_2 nanoparticles on aquatic plants, evaluating the macrogrowth and microresponse of *Lemna minor* L. (duckweed) exposed to several concentrations of TiO_2. Duckweed is a widespread, free-floating aquatic macrophyte, a source of food for waterfowl and a shelter for small aquatic invertebrates. They used 10-nm particles, in near rhabditiform shape. The particle diameter of the nanoparticles in the culture medium decreased with their increasing concentration in the media. Zeta potentials of TiO_2 nanoparticles and bulk TiO_2 in the media were both negative. The zeta potential absolute values of bulk TiO_2 were higher than those of nanoparticles in the same concentration. The stability of culture media-added bulk TiO_2 was higher than that added with the same concentration of TiO_2 nanoparticles. However, both nanoparticles and bulk TiO_2 affected the growth of *L. minor*, but the effect of bulk TiO_2 was not as obvious as that of TiO_2 nanoparticles, which increased *L. minor* growth in low concentrations but inhibited it in high concentrations.

L. minor cells also accumulated more ROS when the plant was exposed to nanoparticles than when exposed to the same concentration of bulk TiO_2. The plant cells increased antioxidant defense enzyme (POD, SOD, and CAT) activity to eliminate the accumulated ROS in plant cells when the TiO_2 nanoparticle concentration was lower than 200 mg L^{-1} in the culture media. The SOD activity decreased when the TiO_2 nanoparticle concentration was higher than 200 mg L^{-1} and the plant cell encountered serious damage from 500 mg L^{-1} TiO_2 nanoparticle concentration in the culture media. Therefore, *L. minor* was more sensitive to TiO_2 nanoparticles than to bulk TiO_2.

Dalai et al. (2013) focused on the cytotoxicity potential of TiO_2 nanoparticles toward a dominant freshwater crustacean, *Ceriodaphnia dubia*, in a lake water system. The studies were carried out under a 16:8-h light and dark photoperiod. Commercial dry titanium (IV) oxide nanopowder (99.7 % anatase, particle size: <25 nm) was used. The photoperiod toxicity experiments showed a concentration-dependent reduction in viability until 16 mg L^{-1} (viability 20 ± 10 %). However, at 32 mg L^{-1}, a slightly higher organism viability was noted (30 ± 10 %) as compared to 16 mg L^{-1} ($p > 0.05$), which further increased until 65 ± 10 % ($p < 0.05$) at 64 mg L^{-1}. The increased survival of daphnids at higher concentrations could be attributed to the reduced nanoparticle toxicity owing to its rapid aggregation at higher concentrations. A 50 % reduction in viable (mobile) organisms was recorded around 8 mg L^{-1} [$EC_{50} = 8.26$ mg L^{-1}, photoperiod]. Under dark conditions, the maximum toxicity was observed at 32 mg L^{-1}, where nearly 50 % (±10 %) reduction in viability was noted [$EC_{50} = 27.45$ mg L^{-1}, dark]. As observed for photoperiod experiments, in dark conditions, an increase in viability was observed at 64 mg L^{-1} concentrations, showing nearly 80 % (±10 %) survival ($p > 0.05$). This work is relevant because it showed reactivity of TiO_2 nanoparticles both in the photoperiod and in dark conditions. They concluded that although aggregation may limit nanoparticle mobility in the environment, it may facilitate ingestion or adhesion by aquatic organisms.

The authors also cite works on daphnids for comparison: Lovern and Klaper (2006) reported concentration-dependent mortality of *Daphnia magna* on exposure to filtered TiO_2 NPs (~30 nm), with an LC_{50} of 5.5 mg L^{-1}; Warheit et al. (2007) and Zhu et al. (2010) found EC_{50} greater than 100 mg L^{-1} for TiO_2 (100–140 nm) for *D. magna* after 48 h of exposure; and Amiano et al. (2012) showed the EC_{50} value of 3.4 mg L^{-1} TiO_2 after exposure to 0.56 mW cm^{-2} UVA radiation using river water as the matrix.

Cardinale et al. (2012) examined how TiO_2 nanoparticles impacted the growth and metabolism of three species of freshwater green algae (*Scenedesmus quadricauda*, *Chlamydomonas moewusii*, and *Chlorella vulgaris*). They exposed the cultures to five concentrations of TiO_2 (0, 50, 100, 200, and 300 ppm) and measured impacts on species population growth rates, as well as on metabolic rates of gross primary production (GPP) and respiration (R). Population growth rates were reduced by nano-TiO_2, but the reduction mechanisms differed among species. For *Chlamydomonas*, nano-TiO_2 reduced both GPP and R, but effects on GPP were stronger, and then carbon was respired more quickly than it was fixed, leading to reduced growth. In *C. vulgaris*, the TiO_2 stimulated both GPP and R, but, because R was more stimulated than GPP, carbon loss exceeded fixation, leading to reduced growth. For *Scenedesmus*, nano-TiO_2 had no significant impact on R, but reduced GPP. This pattern also caused carbon loss to exceed fixation.

Basniwal et al. (2014) also reported TiO_2 nanoparticle toxicity to *Chlorella* sp. Nanoparticle suspensions were prepared in various concentrations from 0.03 to 0.12 g L^{-1}. They observed that with the increase of the nanoparticle concentrations, the growth of algae decreases simultaneously. 86 % growth was shown in the presence of TiO_2, compared to the control.

Clément et al. (2013) used commercial 15-, 32-, and 25-nm anatase nanoparticles for assessing the TiO_2 nanoparticle toxicity. For the 15- and 32-nm anatase particles, the calculated average particle sizes were, respectively, 16.3 ± 1.9 and 32.6 ± 3.8 nm. In the case of anatase, the results indicated that the smaller grain size, the higher the specific surface area. There was a formation of aggregates in the different particle suspensions (concentrations, 0.001–1,000 mg L^{-1}). They used *C. vulgaris* as test organisms, and the average toxicity obtained for a particle concentration of 100 mg L^{-1} was 5.70 ± 0.20 % for anatase (25 nm). They also used *D. magna*, and for these microcrustaceans, the mobility was more inhibited in the presence of TiO_2 nanoparticles compared to the micrometric particles. EC_{50} values for 15-, 25-, and 32-nm anatase were 1.3, 3.15, and 3.44 mg L^{-1}, respectively. The authors observed that the crystalline form of TiO_2 induced various toxic responses. Anatase nanoparticles were more toxic than rutile compared to *D. magna*.

Manzo et al. (2013) evaluated the growth rate alterations of marine chlorophyte *D. tertiolecta* derived from the exposure to ZnO nanoparticles. The hydrodynamic diameters of nano-ZnO and bulk ZnO particles, at 10 mg L^{-1} in seawater, were 900 ± 200 and 1,300 ± 200 nm, respectively. In the next 3 h, the mean size of the nano-ZnO aggregates increased to 1,500 ± 300 nm and up to a final detectable size of around 2,600 ± 700 nm on the third day of observation. The growth of the algal population was clearly affected by the presence of nano-ZnO. The effects were not significant at Zn concentrations below 0.08 mg L^{-1} (NOEC), while the LOEC was obtained at 0.40 mg L^{-1}. The EC_{50} was measured at 1.94 (0.78–2.31) mg L^{-1}.

Bulk ZnO presented as less toxic than nano-ZnO: the effects were not significant at Zn concentrations below 0.8 mg L^{-1} (NOEC), while LOEC was obtained at 2.41 mg L^{-1}; the EC_{50} value was found at 3.57 (2.77–4.80) mg L^{-1}. The nano-ZnO and bulk ZnO aggregates showed very similar sizes, with nano-ZnO smaller than bulk ZnO, and the polydispersity of the first system being much higher, i.e., nano-ZnO had a wider particle size distribution centered at smaller dimensions. Thus, the observed toxicity could not be attributed to a mechanical injury caused by large aggregates onto the algal cells, but rather to the presence of smaller particles that may have had an enhanced surface reactivity with respect to larger aggregates.

On the other hand, the enhanced solid–solid interfaces among nanoparticles can result in increased surface energy transfer and reactivity. This is a critical issue to be addressed when dealing with nanomaterials, and it is worth being further investigated in the future/near future. Since the main differences in the designed experiments relied on the original primary particle size (i.e., nano-ZnO against bulk ZnO), the observed differences in the toxic effects are most likely to be attributed just to this factor. Their results on the growth rate suggested that in their case, the toxic action was likely to be exerted through a mechanical injury and hindrance to diffusion processes as well (pristine size of the dispersed particles did affect the overall toxicity).

13.5.2 Zinc Oxide

Zinc oxide (ZnO) nanoparticles have unique optical, catalytic, semiconducting, piezoelectric, and magnetic properties, all reasons why it has been widely produced and technologically applied (Li et al. 2011a, b).

Tang et al. (2014) explored the toxicity of ZnO nanoparticles (nano-ZnO) and Zn^{2+} to *Anabaena* sp., cyanobacteria. Results showed that nano-ZnO and Zn^{2+} could inhibit *Anabaena* sp. growth with the EC_{50} (concentration for 50 % of maximal effect) of 0.74 ± 0.01 and 0.3 ± 0.01 mg L^{-1}, respectively. Nano-ZnO had more damage to the cell membrane than Zn^{2+} did, which could be proven by the malondialdehyde content in *Anabaena* sp. cells.

Brayner et al. (2006) reported studies of biocidal effects and cellular internalization of ZnO nanoparticles on *E. coli* bacteria. They used synthesized ZnO nanoparticles, in diethylene glycol (DEG) medium by forced hydrolysis of ionic Zn^{2+} salts. Particle size and shape were controlled by the addition of small molecules and macromolecules such as tri-*n*-octylphosphine oxide, sodium dodecyl sulfate, polyoxyethylene stearyl ether, and bovine serum albumin.

The presence of these nanoparticles at a concentration between 10^{-2} and 3.0×10^{-3} M caused 100 % inhibition of bacterial growth. Concentrations between 3.0×10^{-3} and 1.5×10^{-3} M inhibited bacterial growth by 85 %. For concentrations between 1.5×10^{-3} and 10^{-3} M, an increase of *E. coli* colonies was observed. Cellular internalization of these nanoparticles was observed. One prediction made by the authors through these results was that this increase was metabolism-dependent, because bacteria can metabolize Zn^{2+} as an oligoelement, which showed that ZnO nanoparticles were not toxic for *E. coli* at the concentrations used.

Brayner et al. (2010) also evaluated the ZnO nanoparticles toxicity to *Anabaena flos-aquae* and the euglenoid *Euglena gracilis*. The nanoparticles were synthesized in DEG medium by forced hydrolysis of zinc acetate, the general procedure involving addition of zinc acetate to 80 mL of polyol and H_2O to reach a final concentration between 0.06 and 0.63 mol L^{-1}. The hydrolysis ratio was varied from 10 to 80, and protective agents such as TOPO and Brij-76 were added to zinc acetate and polyol solution with concentrations between 10^{-2} and 10^{-1} M to control particle size and shape. For ZnO prepared without protective agent addition, spherical submicrometer-sized nanoparticles were observed. After the increase of the hydrolysis ratio from 10 to 30, nanorods were formed ($30 < \text{length} < 100$ nm). At $H = 300$, particles with crown morphology were also observed. For ZnO prepared with TOPO, using $H = 2$, very small spherical nanoparticles were obtained ($d = 2.0 \pm 0.4$ nm). At $H = 10$, spherical nanoparticles with narrow size distribution were observed ($d = 15.0 \pm 0.7$ nm). For $30 < H < 70$, nanorods were also formed, and the length varied from $30 < \text{length} < 200$ nm. Finally, for ZnO prepared with Brij-76, for $H = 2$, 30, and 70, agglomerates were obtained by coalescence of spherical nanoparticles. The sizes of these agglomerates were 100 ± 10 nm for $H = 2$, 150 ± 10 nm for $H = 30$, and 300 ± 25 nm for $H = 70$. On the other hand, for $H = 10$, nanocubes and nanorods forming nanobelts were formed.

The zeta potential of ZnO nanoparticles with varying pH of the medium was measured. The zero point charge for ZnO nanoparticles was observed at pH 9.15. The ZnO and ZnO-TOPO nanoparticles were positively charged between pH 6 and 8 and negatively charged between pH 10 and 12. At acidic pH, partial dissolution of all ZnO nanoparticles was observed, and zeta potential was negative. After the addition of ZnO, ZnO-TOPO, and ZnO-Brij-76 nanoparticles ($H = 10$), a progressive decrease of photosynthetic activity was observed in the first 10 days for both microorganisms, caused by stress, originated by the addition of the nanoparticles.

For *A. flos-aquae*, after 10 days of incubation, the presence of ZnO-TOPO nanoparticles caused cellular death. For *E. gracilis*, after 10 days of incubation, the decrease of photosynthetic activity was followed in all cases by cellular death. A live/dead test was conducted. For *A. flos-aquae*, 75 % cell survival was observed after contact with ZnO and ZnO-Brij-76 nanoparticles, and only 25 % cell survival was observed after contact with ZnO-TOPO nanoparticles. For *E. gracilis*, the percentage of cell survival was near 10 % for all cases. For *A. flos-aquae* after contact with ZnO and ZnO-Brij-76 nanoparticles, aggregates of spherical nanoparticles were observed around the cell wall composed of the polysaccharides, and after contact with ZnO-TOPO, the cell wall was damaged, and the intracellular content had leaked out. Internalization of some spherical aggregates of nanoparticles was also observed. In the case of *E. gracilis*, vesicles filled by spherical ZnO nanoparticles internalized by endocytosis were observed; ZnO-TOPO spherical nanoparticles were found in the cytoplasm, and with ZnO-Brij-76, the cell wall was damaged, and the intracellular content had leaked out. But in all cases, no nanoparticle agglomeration was observed.

The ability of ZnO to form aggregates and to dissolve was also reported by Franklin et al. (2007), using 30-nm particles. Particle characterization using TEM and dynamic light scattering techniques showed that particle aggregation is significant in a freshwater system, resulting in flocks ranging from several hundred nanometers to several microns. Chemical investigations using equilibrium dialysis demonstrated rapid dissolution of ZnO nanoparticles in a freshwater medium (pH 7.6), with saturation solubility in the milligram per liter range, similar to that of bulk ZnO. They also evaluated the toxicity, using *P. subcapitata* freshwater alga that revealed comparable toxicity for nanoparticulate ZnO, bulk ZnO, and $ZnCl_2$, with a 72-h IC_{50} value near 60 μg Zn L^{-1}, attributable solely to dissolved zinc.

Li et al. (2011a, b) also showed that the dissolved Zn is a key factor for nano-ZnO toxicity. The authors investigated the effect of five commonly used aqueous media with various chemical properties on the toxicity of nano-ZnO to *E. coli*, including ultrapure water, 0.85 % NaCl, phosphate buffered saline (PBS), minimal Davis (MD), and Luria–Bertani (LB). They concluded that the toxicity of nano-ZnO is mainly due to the free zinc ions and labile zinc complexes. The toxicity of nano-ZnO in the five media deceased as follows: ultrapure water > NaCl > MD > LB > PBS. The generation of precipitates ($Zn_3(PO_4)_2$ in PBS) and zinc complexes (of zinc with citrate and amino acids in MD and LB, respectively) dramatically decreased the concentration of Zn^{2+} ions, resulting in the lower toxicity in these media. Additionally, the isotonic and rich nutrient conditions improved the tolerance of *E. coli* to toxicants. According to the authors, the effect of water chemistry on the physicochemical properties of nanoparticles should receive more attention in future nanotoxicity evaluations.

Bai et al. (2010), in turn, evaluated the ZnO nanoparticle (nano-ZnO, 30 nm) properties in a water suspension (E3 medium) and then used a zebrafish 96-h post fertilization embryo–larval test to assess the toxicity of nano-ZnO suspension. As in the other studies, nano-ZnO was found to readily form aggregates with different sizes: small aggregates (142.4–517.7 nm) were suspended in E3 medium, but large aggregates (>1 μm) quickly deposited on the bottom of 24-well plates; nano-ZnO was partially dissolved to Zn species. The embryo toxicity test revealed that nano-ZnO killed zebrafish embryos (50 and 100 mg L^{-1}), retarded the embryo hatching (1–25 mg L^{-1}), reduced the body length of larvae, and caused tail malformation after the 96 h of exposure. The dissolved zinc only partially contributed to the toxicity of nano-ZnO.

13.5.3 Barium Titanate ($BaTiO_3$)

Barium titanate ($BaTiO_3$, BT) is a perovskite-like oxide traditionally known to be an important semiconductor/ferroelectric material with remarkable dielectric constant and therefore with high employability in the electroceramics field (Ciofani et al. 2010a). Nanosized barium titanate (BT), in turn, has been recently gaining application in biomedicine, as a biological nanocarrier for proteins (Ciofani et al. 2010a), an uptake enhancer of low-molecular-weight drugs such as doxorubicin (Ciofani et al. 2010b), a biomarker for imaging probes (Hsieh et al. 2010), and as bone graft material (Ball et al. 2014).

Polonini et al. (2014) performed ecotoxicological tests on three aquatic microorganisms: *C. vulgaris*, *A. flos-aquae*, and *E. gracilis*. Two materials (micro- and nanosized) were used. They were identified by XRD as tetragonal $BaTiO_3$ (BT MP) and cubic $BaTiO_3$ (BT NP), with average crystallite sizes of 172.0 ± 102.4 Å (with 0.19 ± 0.06 % of micro strain) and 60.0 ± 16.7 Å (0.10 ± 0.05 %), respectively. Using SEM and TEM, the diameter of the particles could be estimated as 170 nm (BT MP) and 60 nm (BT NP), and the BT MP presented some degree of polydispersion. The BET surface area was calculated as 3.24 m^2 g^{-1} for BT MP and 16.60 m^2 g^{-1} for BT NP; the total pore volumes were 0.006 and 0.07 cm^3 g^{-1}, respectively, and the mean pore diameters were 7.34 and 17.46 nm, respectively. Both materials were negative and easily aggregated (unstable) in all aqueous media studied, and the release rate of Ba^{2+} ions did not exceed 1.5 %. BT presented a statistically significant toxic effect on *C. vulgaris* growth from the lowest concentration tested (1 μg mL^{-1}) that seemed to be mediated by an induced oxidative stress. For *A. flos-aquae*, BT had a low toxic effect on the growth, but both particles affected cell viability from the lowest concentration tested, which was caused by indirect effect on oxidative stress in cells. For *E. gracilis*, BT showed a statistically significant toxic effect on cell growth and viability from the lowest concentration tested (1 μg mL^{-1}), related to the effect of endocytosed particles in such an amount that led to a rupture of their membranes—the effect was more pronounced for BT MP. However, size did not seem to be an issue in BT particle toxicity, since micro- and nanoparticles produced significant effects on algae growth—although the growth inhibition was more pronounced with the nanomaterial.

13.6 Conclusions

The toxicity of nanomaterials is still a new topic, with more studies needed. Its complexity relies on the fact that several factors influence the toxicity profile, mainly the size and shape of the particles, but also the hydrodynamic behavior, crystalline phase, and surface charge, among others. Some studies show greater toxic profile of nanomaterials compared to the bulk form, while others do not find this association. A case-by-case approach is necessary in the current phase, as the studies shown here permitted to affirm that no obvious trend in the effects of these materials on aquatic organisms could be inferred.

13.7 Future Trends

The present decade, and also the next ones, will be remarkable for studies on the impact of nanoparticle into the environment. To obtain a full understanding of all possible effects on the different living organisms, powerful and robust laboratory assays are needed, although there is lack of guidelines to perform that. The safe future tends to claim for high-throughput bioassays and computational (QSAR) models in lieu of the conventional toxicity tests, in order to handle the vast diversity and complexity of these materials.

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Chapter 14
Uptake and Accumulation of Engineered Nanomaterials and Their Phytotoxicity to Agricultural Crops

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Abstract Rapidly expanding world population and dwindling arable land around the world demand innovative technologies to drastically enhance the global crop yield in the near future. The advancement in nanotechnology provides some possibility to achieve this goal. However, the application of nanomaterial-containing fertilizers and other agricultural products also carries environmental and health risks such as the accumulation of nanomaterial residues in edible tissues, which leads to potential phytotoxicity to agricultural crops and disturbance to the ecosystem. These environmental and health risks need to be well understood before the application of nanotechnology in agriculture can be fully embraced. This chapter presents a summary on the available information concerning the uptake, transport, and accumulation of engineered nanomaterials (ENMs) by agricultural crops and their potential toxicity to these crops. This chapter also discusses the modifications of the fate and transport of coexisting environmental chemicals by ENMs and potential correlations between the unique properties of ENMs with their fate and impact in agricultural systems to shed light on further beneficial applications of ENMs in agriculture.

14.1 Introduction

Rapid advancement of nanotechnology provides unprecedented opportunities to revolutionize many current technologies used for water treatment, environmental remediation, agriculture, medicine, and manufacturing. In the agricultural industry,

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engineered nanomaterials (ENMs), defined as man-made materials with their size less than 100 nm in at least two dimensions (Bradford et al. 2009), have been developed and incorporated into fertilizers, nanosensors, and pathogen-combating formulae (Knot et al. 2012). The broad potential applications of ENMs are derived from several appealing features of ENMs: their very small size and large specific surface area, manipulative surface chemistry, and generation of reactive oxygen species. Even though the size and properties associated with it (e.g., large specific surface area) are the predominant parameters distinguishing ENMs from other man-made materials, other physicochemical properties of ENMs such as their composition, shape, surface coating, and charge also heavily affect their environmental stability and biological compatibility. Therefore, while ENMs is often applied in the literature as a general term, it should be understood that ENMs contain a diverse group of materials within unique physicochemical properties and consequently highly different environmental impacts.

While these diverse features lend ENMs more versatile applications, there have also been concerns about their environmental health and safety impacts. In terms of the interactions of ENMs with plants, numerous previous researches have demonstrated that ENMs can affect plant growth, which plays important ecological and economical roles. There also have been reports showing that ENMs could accumulate in plant tissues, providing a potential pathway for the exposure of ENMs to humans through food consumption. These interactions complicate the beneficial applications of ENMs as fertilizers, nanosensors, and other agriculture-enhancing products and have important implications for food safety. Therefore, the understanding of the interactions of ENMs with agricultural crops is of critical importance. This chapter will start with a brief introduction on ENMs, followed by a discussion on their physiological and genetic effects on agricultural crops and their uptake and accumulation by agricultural crops. The chapter will then continue to examine the alteration on the fate and transport of coexisting environmental chemicals by ENMs in agricultural systems. The chapter will be closed with a future perspective section highlighting important future research needs on the interactions of ENMs with agricultural crops and a short conclusion. The primary objective of this chapter is to present a comprehensive review on the interactions of the four most commonly encountered ENMs with agricultural crops, summarizing their potential toxicity to plants and accumulation in plant tissues in the hope to draw insights on the possible relationship between their fate and impact in agricultural systems and their unique properties. The second objective is to evaluate the interactions of ENMs with other co-contaminants in agricultural systems to obtain a more comprehensive understanding on the impacts of ENMs in agricultural systems.

14.2 Engineered Nanomaterials

As mentioned above, ENMs refer to artificial materials with their size smaller than 100 nm in at least two dimensions. Within the nanoscale, a reduction of the size will lead to exponential increase of specific surface area, and from the environmental health point of view, ENMs smaller than 20–30 nm in diameter are the ones of most concern (Auffan et al. 2009). For some metallic NMs such as silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnONPs), the increase of specific surface area results in significant increase of the dissolution rate and markedly change the interactions of ENMs with surrounding environment (Liu and Hurt 2010; Reed et al. 2012). The altered dissolution rate could either be a boon or a doom to the agricultural industry. For instance, the rapid dissolution rate of ZnONPs has been explored to increase the Zn releasing efficiency of Zn fertilizer (Milani et al. 2012), but a separate study found that rapid dissolution rate of Zn ion was directly associated with the enhanced toxicity of ZnONPs (Miao et al. 2010).

ENMs with similar size but different shape exhibit considerably different optical properties due to the variations of collective oscillations of a nanoparticle's free electrons into resonance, defined as localized surface plasmon resonance (Nehl and Hafner 2008). The variation on optical property and other properties such as physical contact angles with biological systems has direct consequences on their toxicity to plants. Our previous studies showed that plate-shaped AgNPs are more hazardous to lettuce growth than wire-shaped AgNPs at the same concentrations (Fig. 14.1). Lettuce exposed to plate-shaped AgNPs also showed smaller and less healthy roots. Due to the *van der Waals* forces, naked ENMs often attract to each other and aggregate in liquid solutions. As a result, these materials are typically passivated with different materials to electrostatically or sterically stabilize them in liquid solutions. Numerous studies have indicated that surface coating materials dictate the surface chemistry and interactions of ENMs with biological systems (Albanese et al. 2012). Evidences are also abundant that these physicochemical

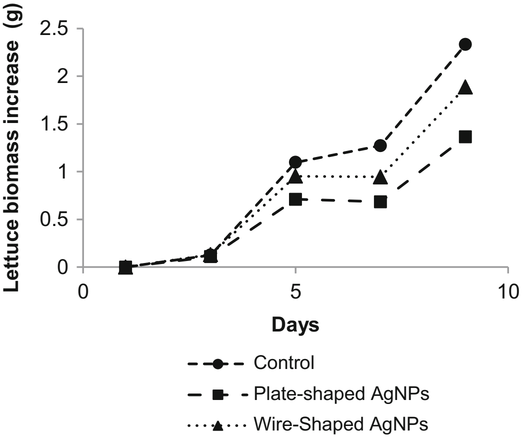

Fig. 14.1 Accumulative biomass of lettuce plants grown hydroponically in solutions containing different shapes of AgNPs. The AgNPs were coated with PVP. The reported values are the average of six replicates, $n = 6$

properties of ENMs have a pronounced impact on the stability of ENMs in the environment and consequently their toxicity to the biological systems (Yang et al. 2009; Simon-Deckers et al. 2009). Previous studies including our own research indicated that surface coating and surface charge strongly affect the phytotoxicity of ENMs. According to our preliminary data, polyvinylpyrrolidone or PVP-coated AgNPs appeared more toxic to plants than citrate-coated AgNPs, possibly because that PVP-coated AgNPs are more stable in solutions than citrate-coated AgNPs (Tejamaya et al. 2012).

The USEPA currently organizes ENMs into four categories according to their composition and structures: carbonaceous NMs, metallic NMs, dendrimers, and composites. Carbonaceous NMs are the most abundant ENMs and primarily consist of fullerenes and nanotubes. Metallic NMs include both elemental metallic NMs (e.g., Au, Ag) and metal oxide NMs (e.g., TiO_2, CeO_2, ZnO). Dendrimers are spheroid or globular nanostructures used in different industries deriving mainly from its large voids inside and versatile functional groups on the surface. Nanocomposites are combinations of different NMs or NMs with larger, bulk-type materials. These materials are often added to auto parts and packaging materials to enhance mechanical and thermal properties. ENMs with the most agricultural relevance are primarily from the first two categories: carbonaceous NMs and metallic NMs. The chapter will focus on four ENMs with the most agriculture significance: multi-walled carbon nanotubes (MWCNTs), AgNPs, titanium dioxide nanoparticles (TiO_2NPs), and cerium oxide nanoparticles (CeO_2NPs).

14.3 Impact of Engineered Nanomaterials on Agricultural Crops

The impact of ENMs on agricultural crops was affected by a suite of factors such as the properties and concentrations of ENMs, plant species, presence of dispersants (e.g., surfactants or natural organic matters), and growth media. As a result, the reported impact of ENMs on agricultural crops varied dramatically from their enhancive impact to plant growth to inconsequential impact and inhibitive effect (Ma et al. 2010a, b; Remedios et al. 2012). More details on the phyto-effect of ENMs will be presented below. Again, due to the large body of information on the interactions of ENMs with plants, the discussion will focus only on the four ENMs most relevant to agricultural business.

14.3.1 Phyto-Effect of Multi-Walled Carbon Nanotubes

The impact of CNTs on plants varied from whether the CNT contained only one layer of graphene sheet (SWCNTs) or more than one layer of the graphene sheet

(MWCNTs). SWCNTs are still very expensive to synthesize and are less relevant to current discussion. As for the interactions of MWCNTs with plants, the literature generally reports positive effect of MWCNTs on plants. Miralles et al. (2012) found that industrial grade MWCNTs up to 2,560 mg/L of MWCNTs with 640 mg/L of catalyst impurities (Al_2O_3) did not display any negative effect on wheat and alfalfa; instead, they improved the growth of both species. Both Raman mapping and transmission electron microscopic images revealed that the root of alfalfa and wheat were not damaged in the presence of this high concentration of MWCNTs. Interestingly, these authors noticed that the impurities in MWCNTs boosted the beneficial impact of MWCNTs. Similarly, exposure of mustard plant seeds to low levels of MWCNTs resulted in higher germination rate and seedling growth due to the improved moisture content in mustard plant seeds and enhanced water uptake by the seedlings (Mondal et al. 2011). These authors further showed that MWCNTs with high oxygen content on the surface were more effective in stimulating mustard seed germination and seedling growth. For instance, the authors reported that MWCNTs with high content of oxygen could achieve the same stimulative effect on mustard growth at one-tenth of the concentration of unfunctionalized MWCNTs, but the mechanisms were not provided. A later study provided some insights on the observed phenomenon in which the authors showed that functionalized MWCNTs with high oxygen content on CNT surface were better dispersed in liquid solution and their dispersion state correlated well with the expression of water channel protein: aquaporin (Villagarcia et al. 2012).

In contrast, Begum and Fugetsu (2012a) reported that after 15 days of hydroponic exposure to 1,000 mg/L of MWCNTs, the growth of red spinach was strongly inhibited. The root and leaf morphology was also adversely affected and the induction of reactive oxygen species (ROS) was suggested as a main mechanism for MWCNTs phytotoxicity. In a separate study, these authors greatly expanded the plant species to determine the differential responses of different plant species to MWCNTs. Their results showed that after exposure to 1,000 and 2,000 mg/L of MWCNTs, the growth of red spinach and lettuce was drastically inhibited and the root membrane integrity severely damaged (Begum et al. 2012b). Rice and cucumber were also affected but the extent of impact was less severe compared to spinach and lettuce. Chili and soybean displayed no signs of toxicity after exposure to these high concentrations of MWCNTs for 15 days. Clearly, the phyto-effect of MWCNTs is species dependent.

In summary, the literature reported seemingly contradictory results concerning the impact of MWCNTs on agricultural crops. However, these contradictions could be reconciled if the results are examined closely. Most negative reports concerning the phyto-effect of MWCNTs came from studies with high concentrations of MWCNTs, while the enhancive observations derived primarily from studies applied low concentrations of MWCNTs (<100 mg/L). Tiwari et al. (2013) demonstrated that the beneficial effect of MWCNTs on the growth and water content of maize was only observed at 20 mg/L or lower concentrations of MWCNTs and beneficial impact diminished at higher concentrations. Plant species undoubtedly play a role in their response to MWCNTs, yet how the crop morphology and anatomy affect the plant responses to MWCNTs is not clear.

14.3.2 Phyto-Effect of Silver Nanoparticles

While AgNPs have been frequently applied as antimicrobial agents in agricultural practices, their impact on agricultural crops is not extensively evaluated. Musante and White (2010) compared the phytotoxicity of AgNPs and bulk Ag to squash and found that AgNPs decreased the biomass accumulation and transpiration of squash by 66–84 % compared with bulk Ag. The authors noticed that Ag ion concentration was 4.4–10 times higher in AgNPs solution than bulk Ag solution, but the role of ionic Ag was not explicitly investigated. A later study indicated that AgNPs are phytotoxic to Italian ryegrass and the observed toxicities such as the inhibited root hair development, vacuolated and collapsed cortical cells, and broken root caps were not observed when plants were exposed to the same concentrations of ionic Ag, suggesting that AgNPs exerted unique toxic effects on plant growth (Yin et al. 2011). Lee et al. (2012) reported that AgNPs are toxic to mugbean and sorghum in agar medium, but their phytotoxicity to both plant species was greatly reduced in the soil medium at the same concentrations. Mechanistic investigations suggested that the underlying mechanisms of AgNPs phytotoxicity to these two species differed in different media. The authors attributed the phytotoxicity of AgNPs to the release of ion in agar media, while in the soil medium, the ion release became less important, possibly due to the adsorption of ion and nanoparticles by soil particles. Another study showed that AgNPs-treated water hyssop (a herb) did not display any morphological abnormalities; however, the anatomical structure of root and shoot tissues was affected (Krishnaraj et al. 2012). These authors also noticed heightened expressions of some antioxidant enzyme activities such as the peroxidase and catalase activities, but the induction of these oxidative stress regulation proteins was not different from the effect of $AgNO_3$. The observation was supported by another study which showed that both AgNPs and Ag ions induced the expression of redox regulation enzymes in salad rocket; however, some proteins related to the endoplasmic reticulum and vacuole were uniquely altered by AgNPs, providing further evidence that phytotoxicity of AgNPs cannot be fully explained by the release of ions (Vannini et al. 2013). In conclusion, the debate on the nature of AgNPs toxicity to plants appeared to favor that AgNPs exert their unique effect on plant growth and metabolism, and even though ionic release may explain part of the observed toxicity, the release of Ag ion cannot fully account for the observed toxicity of AgNPs. It is important to indicate that all these studies were conducted through root exposure. The only foliar exposure of AgNPs to plants showed that foliar exposure of lettuce to up to 1,000 mg/L AgNPs did not result in any toxicity on lettuce growth (Larue et al. 2014). The authors found that some AgNPs were internalized in the leaf tissues and washing did not effectively remove Ag content.

While AgNPs may be generally perceived as toxic due to their antimicrobial property, recent two studies with nonagricultural crops may strike a positive note on the applications of AgNPs to agriculture. One study showed that while AgNPs at high concentrations exerted toxic effect on poplar and *Arabidopsis* growth, at a narrow concentration range, AgNPs stimulated the growth of these two plant species as indicated by their higher root elongation rate, transpiration rate, as well as higher biomass (Wang et al. 2013a, b). Another study, which is probably more relevant to the agricultural industry, showed that when horseshoe pelargonium was sprayed with different concentrations of AgNPs and stored in the dark, AgNPs at 20–60 mg/L improved petal longevity of the flower and enhanced the defense enzyme activities of the flower plant (Hatami and Ghorbanpour 2014). APX activity was highest at 40 mg/L AgNP-treated plants; SOD and CAT activities and chlorophyll content were also higher, demonstrating some potential beneficial applications of AgNPs in the storage of agricultural products.

14.3.3 Phyto-Effect of Titanium Dioxide Nanoparticles

As with other ENMs, both positive and negative impacts of TiO_2NPs on plant development were reported. Frazier et al (2013) showed that TiO_2NPs significantly inhibited the seed germination rate, root elongation, and biomass accumulation of tobacco plants and the plant responses to TiO_2NPs exposure are concentration dependent. In addition to the impact on plant physiology, very low concentrations of TiO_2NPs (0.1 % of TiO_2) could significantly alter plant RNA (~20–22 nt) expressions related to plant development regulations and plant tolerance to abiotic stresses. When wheat plants were planted and grown in soils conditioned with TiO_2NPs for 2 months, wheat biomass was reduced by 13 % compared with plants grown in soil without TiO_2NPs (Du et al. 2011). In this study, the authors also demonstrated that the soil enzyme activities were significantly reduced. Ghosh et al. (2010) reported that TiO_2NPs caused chromosomal aberrations in onion cells and damage to the DNAs in both onion and tobacco root cells as revealed by different DNA fragmentation techniques. The genotoxic effect of TiO_2NPs corroborated well with the reduced root elongation. These researchers also detected increased concentration of MDA in onion roots when treated with 4 mM of TiO_2NPs, and they ascribed TiO_2NPs impact on plant root membrane integrity as a possible mechanism for TiO_2NPs genotoxicity and cytotoxicity. Other researchers also reported adverse effect of TiO_2NP suspension on corn leaf development and transpiration, but attributed the toxic effect of TiO_2NPs to their physical adsorption on plant root surface and consequently the reduction of hydraulic conductivity due to the blockage of root cell wall pores (Asli and Neumann 2009). A further examination showed that the diameter of maize root cell wall pores was reduced from 6.6 to 3.0 nm, which could block cell membrane proteins such as signaling receptors and membrane uptake channels.

In contrast to these abovementioned studies, Mohammadi et al. (2013) showed that treatment of chickpea seeds with low levels of TiO_2NPs (<10 mg/L) did not display any negative effect on seed germination and seedling morphology of these plants. Instead, exposure to TiO_2NPs strengthened root membrane integrity (e.g., lower electrolyte leakage and low membrane lipid oxidation) of both cold-sensitive and cold-resistant chickpea seedlings during cold treatment. Jaberzadeth et al. (2013) showed that foliar application of low concentrations of TiO_2NPs to wheat increased almost all agrometic traits such as plant biomass and yield under water-deficit stress conditions. Wheat grains exposed to TiO_2NPs also had higher gluten and starch content. These studies supported several earlier studies which showed that foliar treatment of spinach plants with TiO_2NPs is beneficial for plants in terms of the overall growth and improvement of plant photosynthesis (Zheng et al. 2005). The three principal aspects of photosynthesis that are affected under foliar treatment with TiO_2NPs are the light collection aspects of the photochemical reactions (Hong et al. 2005; Yang et al. 2007), the enzyme that initiates the biochemical reactions (Gao et al. 2006), and the protection systems that prevent radicals and lipid peroxidation from inhibiting photosynthesis (Ma et al. 2008). Both nano-anatase (4–6 nm mean grain size) and nano-rutile TiO_2 enhanced the chlorophyll content of sprayed spinach leaves, the capacity to absorb light, the hydrolysis of water to provide electrons to the photochemical system, and the activity of the electron transport carriers. Collectively, these effects lead to increased efficiency in the production of the energy storage compounds (i.e., ATP and NADPH) needed to drive the synthesis of carbohydrates and other macromolecules. These studies showing the beneficial effect of TiO_2NPs on photosynthesis, however, are short-term studies, and TiO_2NPs were applied only once. Whether TiO_2NPs-enhanced photosynthesis after one-time foliar application will be important or not in the long term is not known.

14.3.4 Phyto-Effect of Cerium Oxide Nanoparticles

Compared with other ENMs, the reported impact of CeO_2NPs on plants is generally benign even though anecdotal reports on the toxicity of CeO_2NPs to plants at very high concentrations can be spotted in the literature. Ma et al. (2010a, b) investigated the potential CeO_2NP phytotoxicity and found that the seed germination of seven different species (e.g., radish, canola, tomato, wheat, lettuce, cabbage, cucumber) was completely unaffected by 2,000 mg/L of CeO_2NP suspension. Subsequent investigation showed that root elongation of these plant species was also largely unaffected. Only lettuce root growth was suppressed by 34 % at this concentration. Similarly, CeO_2NPs at 2,000–4,000 mg/L had no overt toxicity on soybean, although the authors reported genotoxicity as measured by random amplified polymorphic DNA assay (Lopez-Moreno et al. 2010a). In a following study, the same research group reported the effects of 0–4,000 mg/L CeO_2NP exposure on alfalfa, corn, cucumber, and lettuce growth (Lopez-Moreno et al. 2010b).

The germination and root elongation of several of the species were enhanced at lower concentrations but were significantly inhibited (20–30 %) at 2,000 and 4,000 mg/L. However, the shoot elongation was enhanced in nearly all cases.

Birbaum et al. (2010) showed that exposure of CeO_2NPs to corn grown in soil (50 mL of 10 μg/mL CeO_2NPs per day through irrigation) for 14 days resulted in no observable toxicity. The authors also included an aerial exposure on leaves, and similarly corn growth was not affected. Wang et al. (2012) grew tomato in the presence of CeO_2NP-amended (0.1–10 mg/L) irrigation water throughout the lifetime of this plant and reported either no impact or slight enhancements in plant growth and yield. A recent study demonstrated that CeO_2NPs had a modest impact on soybean growth in soil but showed a detrimental impact on the nitrogen fixation bacterial community on soybean roots (Priester et al. 2012).

Taken together, the impact of ENMs on agricultural crops is highly diverse and is strongly dependent upon the composition and properties of ENMs, the applied concentrations of ENMs, the plant species, as well as the growing conditions. One important observation of the checked literature studies is that most of the toxicity studies were performed with very high concentrations highly unexpected in the real environmental conditions in the foreseeable future and in a very short term (from days to a few weeks). A most likely scenario we will face is that crops will grow in an environment with low levels of ENMs for many generations; however, the long-term impact of ENMs on plant growth at environmentally relevant concentrations is still elusive. Our lab has germinated tomato seeds collected from the mother plants grown in the presence or absence of 10 mg/L of CeO_2NPs throughout their life cycle and then grew the seedlings again hydroponically in the presence and absence of 10 mg/L of CeO_2NPs to obtain some insights on the impact of CeO_2NPs on the second-generation seedlings. Our study showed that while CeO_2NPs stimulated the growth of tomato seedlings developed from unexposed seeds, seedlings developed from seeds collected from CeO_2NPs-treated mother plants had smaller biomass, lower water transpiration, and much higher hydrogen peroxide in their roots (Wang et al. 2013a, b). Interestingly, the second-generation seedlings grown from treated mother plant seeds developed significantly more root hair, irrespective of their exposure to CeO_2NPs or not in the second generation. We also grew the second-generation seedlings in suspensions containing different types of ENMs and observed the root elongation and biomass collection. Our results indicated that except for seedlings exposed to raw MWCNTs, all second-generation seedlings developed with seeds from CeO_2NPs-treated mother plants displayed slower root elongation (Fig. 14.2). Plant biomass development demonstrated similar trend as root elongation (Fig. 14.3). The only exception is that treated second-generation tomato seedlings had slightly higher biomass than untreated seedlings 10 days after their growth in suspensions containing 10 mg/L of C_{60} fullerene. These results clearly showed that the long-term impact of ENMs on plant development varied with their short-term impact and the long- term, multigenerational impact of ENMs on plant development should be further investigated.

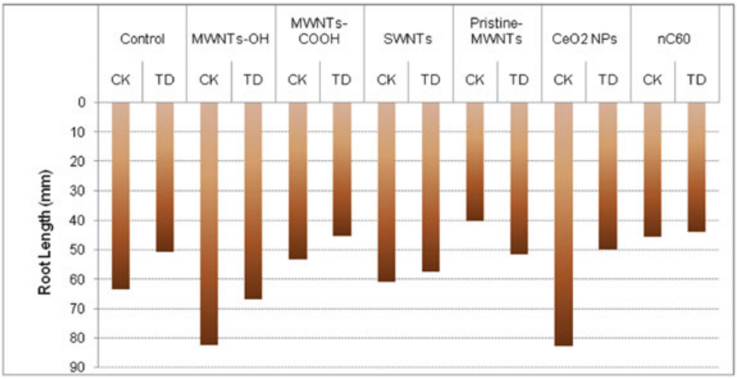

Fig. 14.2 Root elongation of second-generation tomato seedlings 10 days after their growth in suspensions containing different types of ENMs. The reported values are the average of five replicates. CK, second-generation tomato seedlings developed from seeds of unexposed mother plant; TD, second-generation tomato seedlings developed with seeds from 10 mg/L of CeO_2NPs-treated mother plants; Control, suspension containing no ENMs; MWNTs-OH, OH functionalized multi-walled carbon nanotubes; MWNTs-COOH, COOH functionalized multi-walled carbon nanotubes; SWNTs, single-walled carbon nanotubes; Pristine-WWNTs, unfunctionalized multi-walled carbon nanotubes, nC_{60}, fullerene

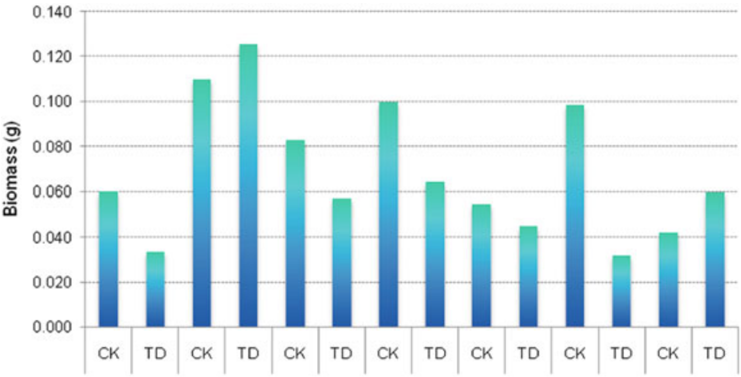

Fig. 14.3 Fresh biomass of second-generation tomato seedlings 10 days after their growth in suspensions containing different types of ENMs. The reported values are the average of five replicates. CK, second-generation tomato seedlings developed from seeds of unexposed mother plant; TD, second-generation tomato seedlings developed with seeds from 10 mg/L of CeO_2NPs-treated mother plants; Control, suspension containing no ENMs; MWNTs-OH, OH functionalized multi-walled carbon nanotubes; MWNTs-COOH, COOH functionalized multi-walled carbon nanotubes; SWNTs, single-walled carbon nanotubes; Pristine-WWNTs, unfunctionalized multi-walled carbon nanotubes, nC_{60}, fullerene

14.4 Uptake and Accumulation of Engineered Nanomaterials by Agricultural Crops

In addition to the impact of ENMs on the physiological health of agricultural crops, their potential accumulation in agricultural crops, especially edible tissues, is an important consideration because the accumulation of ENMs in these tissues could become an important pathway for humans exposed to these ENMs. Current knowledge on plant uptake and accumulation of ENMs is limited. The following section will present a summary of the uptake and accumulation of ENMs by agricultural crops, focusing again on four of the most agriculturally relevant ENMs.

14.4.1 Uptake and Accumulation of Multi-Walled Carbon Nanotubes

Lin et al. (2009) investigated the uptake and translocation of carbonaceous nanomaterials by rice plants (*Oryza sativa*) and found that fullerene nC_{70} could be easily taken up by plant roots and transported to plant shoots, but similar phenomenon was not observed for MWCNTs. With a two-photon excitation microscope, another group of researchers demonstrated that MWCNTs are primarily adsorbed on root surface as individual and aggregated CNTs even though one or both ends can pierce through root cap cell walls (Wild and Jones 2009). Miralles et al. (2012) exposed wheat and alfalfa to 2,560 mg/L of MWCNTs and used electron microscopic techniques to examine the root tissues for possible internalization, and the results showed no internalization of MWCNT. The accompanying two-dimensional Raman mapping analysis confirmed that MWCNTs are predominantly adsorbed onto the root surfaces of these plants without significant uptake and translocation. However, using Fe_3O_4-functionalized MWCNTs, they did detect CNTs in the epidermis of one wheat root tip, suggesting that internalization was possible but unusual. Some recent studies also showed that MWCNTs could be taken up by plant roots and transported to shoots. Researchers used photothermal and photoacoustic techniques to confirm the presence of MWCNTs in tomato leaves and fruits following their root exposure (Khodakovskaya et al. 2011). Other researchers also showed that CNTs entered into plant tissues through roots and lined up with plant vascular tissues in roots and stems to enhance plant water uptake (Tripathi et al. 2011). However, these studies are largely qualitative. Using ^{14}C-radiolabeled MWCNTs, Larue et al. (2012a) demonstrated that MWCNTs indeed can be taken up by wheat root and transported to leaves; however, the quantity of MWCNTs accumulated in leaves is extremely small (0.0005 % of the total MWCNTs added to the system) which did not induce any modifications on plant photosynthetic activity or oxidative stress responses, suggesting that while MWCNTs can be potentially taken up by crops, the actual accumulation in plant tissues and potential transfer to food chain via crop consumption is very low.

14.4.2 Uptake and Accumulation of Silver Nanoparticles

Stampoulis et al. (2009) reported that the uptake of AgNPs by zucchini (*Cucurbita pepo*) was on average 4.7 times higher in plants exposed to 10–1,000 mg/L of AgNPs than to their corresponding bulk materials. The authors suggested that higher dissolution rate of AgNPs than bulk Ag might be responsible for the greater Ag concentration in AgNPs-treated zucchini. A separate study that involved some of the same researchers also showed that AgNPs were taken up by soybean roots and translocated to stems and leaves and the Ag content was two times higher in AgNPs-treated soybeans than Ag bulk-treated soybeans (Torre-Roche et al. 2013a). However, a detailed study on the forms of Ag inside the plants was not evaluated in both studies. Lee et al. (2012) reported that AgNPs as the particulate form were taken up by both mug bean and sorghum. AgNPs in the plant roots increased with exposure concentration of AgNPs, but shoot concentration of Ag appeared unaffected by the dosing concentration. They also noticed that growing media played a significant role in the extent of accumulation of AgNPs by these two species, with soil greatly reducing the accumulation of AgNPs when compared to agar media. When AgNPs were exposed to rice seedlings, it was noticed that some AgNPs deposited on the root surface and some penetrated into plant cells (Mazumdar and Ahmed 2011). AgNPs which have successfully penetrated through cell membranes are predominantly smaller than 25 nm, and the penetration resulted in damaged cell walls and vacuoles, suggesting that the physical entrance through the cell wall could be one of the mechanisms for AgNPs internalization. Our previous study with *Arabidopsis* also indicated that there is a size limitation on the potential uptake and accumulation of AgNPs. In that study, we found that only AgNPs smaller than 40 nm were successfully internalized in *Arabidopsis* root cells and some appeared to reach the vascular tissues for possible transport to the shoots (Geisler-Lee et al. 2013).

Haverkamp and Marshall (2009) also investigated the uptake of Ag ion complexes and AgNPs by *Brassica juncea* plants. However, they found no uptake and accumulation of AgNPs by *Brassica*. Ionic Ag was readily taken up by *Brassica* plant roots, and within plant tissues, ionic Ag was reduced to AgNPs. The reduction of ionic Ag to zero valent AgNPs in plant tissues appeared common in nature and provided an opportunity for the biosynthesis of AgNPs (Harris and Bali 2008). The biosynthesis of AgNPs is beyond the scope of this chapter, but the literatures on the biosynthesis of AgNPs suggested that accumulation and storage of AgNPs in plant tissues may naturally occur and some accumulation of AgNPs in plant tissues may not necessarily prohibit the application of AgNPs in agricultural practices.

14.4.3 Uptake and Accumulation of Titanium Dioxide Nanoparticles

Larue et al. (2012b) used microparticle-induced X-ray emission coupled with Rutherford backscattering spectroscopy to quantify absorbed titanium and micro X-ray fluorescence (uXRF) based on synchrotron radiation to determine the Ti distribution in the roots and leaves of two agricultural crops: wheat and rapeseed. They reported that both crop species could uptake and translocate TiO_2NPs to leaves after root exposure in hydroponic solutions and higher Ti content was observed in rapeseed seedlings than wheat seedlings. Distribution of Ti in root cross sections depended upon NP agglomeration state. These authors further investigated the impact of TiO_2NPs' size and crystal structure impact on their uptake and accumulation by wheat (Larue et al. 2012c) and reported that TiO_2NPs smaller than 36 nm were taken up by wheat roots and translocated to aboveground tissues. TiO_2NPs larger than 36 nm but smaller than 140 nm still accumulated in wheat root parenchyma but do not reach stele and do not translocate. NPs above 140 nm did not associate with wheat roots, likely precipitated to the bottom of growing containers. Both crystal phases of TiO_2NPs (anatase and rutile) were taken up by wheat, and they maintained their phase structure in plant tissues. Jacob et al. (2013) reported that exposure of wheat and beans to nutrient solution containing TiO_2NPs as a single source of Ti significantly increased root Ti sorption and uptake. However, upward translocation was not reported for these two crops. The aggregation state of Ti was not reported, but it is likely that they aggregated to sizes larger than the reported threshold sizes by Larue et al. (2012a, b, c). Interestingly, these researchers noticed that the presence of phosphorus significantly decreased the Ti uptake by plants even though the mechanisms were not discussed.

Servin et al. (2012) also investigated the uptake and translocation of TiO_2NPs by cucumber with similar techniques as employed by Larue and colleagues, and their study confirmed the transport of Ti from roots to leaves, and, in particular, they found high concentrations of Ti in leaf trichomes, suggesting that trichomes are a possible sink or excretory system for Ti. In consistent with the reports from other groups, they found no biotransformation of Ti in plant tissues. Noticeably, they reported that anatase TiO_2NPs were primarily associated with root tissues and rutile TiO_2NPs were primarily associated with leaf tissues and trichome structure, suggesting that rutile TiO_2NPs might be easier to transport from roots to shoots than anatase TiO_2NPs. In a subsequent study by the same group, however, the authors reported that both crystal phases of TiO_2NPs could be transported from roots to shoots. Using synchrotron-based techniques, these authors verified the accumulation of TiO_2NPs in cucumber fruits for the first time for both crystal phases, suggesting a possible pathway of TiO_2NPs transfer from soil into food chain (Servin et al. 2013). Once again, they confirmed that TiO_2NPs in plant tissues did not undergo any chemical reactions. These authors employed FTIR spectroscopy to analyze the cucumber fruits and found that fruit macromolecules such as the amide, lignin, and carbohydrates were modified by TiO_2NPs, suggesting that TiO_2NPs could affect the metabolic processes of plants.

14.4.4 Uptake and Accumulation of Cerium Oxide Nanoparticles

Zhang et al. (2011) reported that CeO_2NPs exposed to cucumber hydroponically for 14 days are mostly loosely bound to the root surface and more than 85 % of the NPs could be washed off with deionized water. Translocation of the particles to shoot tissue was measurable but insignificant. Smaller nanoparticles (7 nm) were found at substantially higher amounts than larger particles (25 nm) in plant tissues. Schwabe et al. (2013) also observed some translocation from pumpkin roots to shoots after 8-day exposure to 100 mg/L CeO_2NPs. However, no cerium was detected in wheat shoots with similar exposure scenario. Interestingly, the association of cerium with the roots of both plant species was reduced in the presence of natural organic matter.

Birbaum et al. (2010) were the first to report on CeO_2NP exposure to terrestrial plants (corn) under soil conditions. The authors reported that after 14-day exposure with the CeO_2NPs in the irrigation water (50 mL of 10 μg/mL per day) resulted in no detection of ceria in the leaves or sap of corn plants. In contrast, Wang et al. (2012) grew tomato from seeds to the maturity of the plants in the presence of CeO_2NP-amended (0.1–10 mg/L) irrigation water and reported the detection of ceria in all plant tissues, including tomato fruits, suggesting translocation. Interestingly, the authors also noticed high concentrations of ceria in tomato seeds irrigated with 10 mg/L CeO_2NP-containing solution than controls. The observation that cerium may cross the phloem membrane and accumulate in fruits was supported by a recent publication which reported that CeO_2NPs are bioaccumulated in all tissues of cucumbers including their roots, stems, leaves, and fruits (Zhao et al. 2013). However, the translocation from root to stem only represented a small fraction of Ce associated with plant roots (1.44 and 1.79 % in plants treated with 400 and 800 mg/kg CeO_2NPs). Once ceria has reached the stem tissue, the upward transport to the leaves became much easier (e.g., 37 % in plants treated with 400 mg/kg of CeO_2NPs). It was found that CeO_2NPs were mainly localized in the vasculature of the leaf vein suggesting that the transport of CeO_2NPs was with the water flow. In an earlier study, the authors also demonstrated that the uptake and accumulation of CeO_2NPs was heavily affected by the properties of CeO_2NPs and the properties of growing media (Zhao et al. 2012). After 1 month of growth in soil conditioned with CeO_2NPs of different surface properties, corn roots accumulated significantly greater quantities of alginate-coated CeO_2NPs than uncoated particles. These authors also noticed that soils with high organic matter generally enhanced the association of CeO_2NPs with roots but reduced the translocation to shoots, regardless of the surface properties of CeO_2NPs. The effect of soil organic matter was more significant on uncoated CeO_2NPs than alginate-coated CeO_2NPs.

Following the exposure of 0–4,000 mg/L CeO_2NP to alfalfa, corn, cucumber, and lettuce growth for 7 days, ICP-OES analysis confirmed the ceria presence within the seedlings tissue of these four plants (Lopez-Moreno et al. 2010b). After dilute acid rinsing, XAS confirmed that the oxidation state was unaltered in the root tissues of these four plant species. In a separate study in which 2,000 mg/L CeO_2NP was exposed to cucumber for 3 weeks, minimal root to shoot translocation was observed (Zhang et al. 2012). However, soft X-ray scanning transmission microscopy (STXM) and near edge X-ray absorption fine structure (XANES) analysis did show measurable accumulation and biotransformation of CeO_2NPs to $CePO_4$ in roots and cerium carboxylates in shoot tissue. The authors hypothesized that root exudate-mediated dissolution of nanoparticles precedes ion uptake, subsequently followed by in planta reduction to nanoceria and/or biotransformed products. The hypothesis, however, was not scientifically confirmed.

14.5 Engineered Nanomaterials Mitigated Uptake of Environmental Chemicals by Agricultural Crops

Once released into the environment, ENMs closely interact with the coexisting environmental chemicals and may substantially alter their fate and uptake by agricultural crops. The first evidence that ENMs may change the uptake of environmental pollutants was provided by a study conducted in our lab in which we reported that the presence of nC_{60} fullerene significantly enhanced the uptake of trichloroethylene by some wetland plants (Ma and Wang 2010). Recent work with agricultural crops grown in vermiculite confirmed that fullerene exposure significantly increased the accumulation of pesticide residues by cucumber in both root and shoot tissues (Torre-Roche et al. 2012). The presence of fullerene also increased the root accumulation of pesticides by soybean and tomato by 30–65 %, but the shoot accumulation of pesticides by soybean was reduced by 48 % by the presence of fullerene. The shoot accumulation of pesticides by tomato was unaltered by fullerene, demonstrating the variances between different plant species. A similar work with AgNPs was conducted and the results showed that AgNPs suppressed the accumulation of these pesticides by soybean plants by up to 40 % and the effect was significantly higher for AgNPs than bulk Ag (Torre-Roche et al. 2013a). In a subsequent study with actual soil containing aged pesticides, the same group of researchers demonstrated that nC_{60} fullerene again displayed species-specific impact on the accumulation of weathered pesticides by four agricultural crops (cucumber, soybean, corn, and tomato); however, the presence of MWCNTs consistently suppressed the uptake and accumulation of aged pesticides by all four crops (Torre-Roche et al. 2013b). Clearly, the nature of nanoparticles and the properties of growth media as well as the plant species all play important roles in the altered accumulation of organic compounds by plants.

In addition to the mediation of uptake and accumulation of coexisting environmental organic compounds, Servin et al. (2012) showed that TiO_2NPs increased the nitrogen content in cucumber root by 51.2 % compared with control cucumber root, but the nitrogen content in leaf tissues are comparable between control and TiO_2NPs-treated plants. These authors also reported that TiO_2NPs drastically modified the levels of macro- and micronutrients in cucumber fruits (Servin et al. 2013). For example, treatment of cucumber with 500 mg/L of TiO_2NPs increased the potassium content by 35 % and phosphorous content by 34 % in cucumber seedlings. Jacob et al. (2013) also reported that plant uptake of micronutrients such as Mn and Mg in nutrient solution coexisted with TiO_2NPs was altered due to the presence of TiO_2NPs.

14.6 Future Perspectives

With the continuing exploration and applications of ENMs in agricultural industry and many other industries in society, it is reasonable to anticipate that the chances for agricultural crops to encounter ENMs will continually increase in the future. However, ENMs in the actual environment is not expected to reach the levels used in most lab studies in the foreseeable future. But these ENMs will linger in the environment for a long time and affect not only the earlier stages of agricultural crops (e.g., seed germination, root elongation) but will likely affect the whole growth stages of these crops and even many generations of plant development if the sources are not removed. Therefore, it remains essential to understand how environmentally relevant concentrations of ENMs will affect the long-term growth of agricultural crops and what are the subsequent implications for our food security.

Uptake and accumulation of ENMs by agricultural crops, especially the edible tissues, is always a concern which needs to be explicitly addressed. Current technologies are insufficient to tackle the challenges raised by ENMs. For example, most microscopic imaging techniques only allow qualitative investigation on a tiny fraction of plant tissues, which are often not representative of the whole plant tissues. In addition, these techniques have high detection limits, requiring the exposure of plants to unrealistically high concentrations. While tissue extraction techniques such as ICP-MS or ICP-OES allow quantitative determination of metallic ENMs, detailed information on the state and localization of ENMs in plant tissues is lost after the acid extraction. For nonmetallic ENMs such as CNTs, quantitative determination on plant tissues is still elusive. A recent publication concerning the analysis of CNTs in plant root tissues with microwave-assisted temperature measurement is a welcome development for CNT analysis (Irin et al. 2012); however, a long way is still ahead. Techniques or a combination of techniques which allow mass analysis of ENMs in plant tissues at relatively low concentrations and at the same time retain the chemical and physical state are needed.

Based on the available information, most uptake and accumulation studies reported in the literature were conducted in hydroponic systems with young seedlings. However, it is clear from a few limited studies that the uptake and accumulation of ENMs by plants is directly affected by the properties of growing media such as the nature and quantity of natural organic matter, moisture, and pH; the plant species (e.g., monocot vs. dicot); and, most importantly, the physicochemical properties of ENMs. The unique correlation of these parameters with the uptake and accumulation of ENMs by agricultural crops has not been established, yet it is critically important to predict the potential accumulation of ENMs by food crops. One important aspect which is not covered in this chapter is the bioaccessibility of ENMs in plant tissues. It should be understood that the consumption of food crops containing ENMs will not necessarily result in similar bioavailability of ENMs as the consumption of food crops containing equivalent amount of ions or other chemical forms of the same material. The extent of absorption of ENMs by the membranes in digestive systems and their bioavailability and health risks following consumption of ENM-containing crops need further investigation.

As has been shown, the impact of ENMs is not limited to their direct effect on crop growth and their potential accumulation and bioaugmentation in the food chain. ENMs interact closely with environmental pollutants and alter their plant uptake and accumulation in agricultural systems. Limited study has demonstrated that ENMs with different properties displayed different effects on the accumulation of coexisting environmental pollutants by plants, but studies have not been performed to evaluate the role of the physicochemical properties of coexisting contaminants in this process. In addition, all of the previous studies limited to evaluating the alteration of fate and transport of organic compounds by ENMs and the impact of ENMs on the uptake and transport of heavy metals have not been assessed. In addition to the modification on the uptake and accumulation of co-present environmental chemicals, ENMs are able to affect the accumulation of some macro- and micronutrients which directly affect the nutritional values of agricultural products. The extent of effect, however, is not known. The issues raised above all hold some importance to the applications and implications of ENMs in agricultural industry and warrant further investigation in the future.

14.7 Conclusion

In closing, we reviewed the available literature concerning the interactions of several agriculturally important ENMs with crops and presented our assessment on this important issue. Based on the available information, it is clear that ENMs interfere with crop development at both physiological and molecular levels. The interface could be both enhancive and inhibitive for the same ENMs, and the exposure concentration appeared to be critically important. The safe threshold exposure concentration has not been established. Growing conditions, exposure methods, and plant species also affect the interactions of ENMs with agricultural crops. It appeared that natural soil reduced the negative (maybe positive as well) effect of ENMs on agricultural crops and foliar exposure seemed to be less detrimental to plants than root exposure. It could be argued that the differential responses of foliar and root exposure may just be an age issue of plants. When plants are ready to be foliar treated, they have grown stronger and more effective to defend the hazardous effects of ENMs compared with most root exposure studies: plants were exposed to ENMs at the beginning stage of their life cycle (e.g., seed germination stage). Further investigation is needed to confirm this hypothesis. In addition, the investigations on the ENM impact of crop development are predominantly based on short-term studies, leaving the long-term impact of ENMs at environmentally relevant concentrations still unaddressed.

The uptake and accumulation of ENMs by agricultural crops appeared to be certain. But the extent of accumulation and the subsequent distribution and stability of ENMs in plant tissues after plant uptake appeared to vary greatly between different ENMs. Even though MWCNTs can be taken up by plant roots and translocated to shoots, the upward transport and the actual accumulation of MWCNTs in aboveground tissues seem to be very limited and are unlikely to cause any serious concern on food safety. AgNPs uptake and accumulation are still controversial, and more mechanistic study to distinguish AgNPs vs. Ag ion uptake needs to be conducted. One thing seems to be certain that irrespective of the exposure form of Ag, many plants are capable of storing AgNPs in their tissues. Plant uptake, transport, and accumulation of TiO_2NPs are broadly reported and could be a concern considering that several studies confirmed their detection in the edible tissues. The uptake and accumulation seemed to be mainly governed by their size, and both crystal phases of TiO_2NPs can be accumulated by agricultural crops. The upward transport of these two different crystal structures may differ, but more studies seem to be needed. CeO_2NPs can also be taken up and accumulated in plant tissues in most conditions, but the fate (e.g., maintain the nanoparticle structure or undergo chemical reactions) following their uptake is not fully understood. The mechanisms regarding the entrance of ENMs in plant tissues are still poorly understood.

ENMs also affect the agricultural systems through their alteration of the uptake and accumulation of coexisting environmental chemicals and necessary macronutrients and micronutrients. Studies on these areas are severely lacking. In summary, ENMs have profound impact on agricultural crops. Even though some insights have been obtained, substantial efforts are needed to understand the full implications of ENMs on agricultural health.

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