Green Energy and Technology

Volodymyr Ivanov
Viktor Stabnikov

# Construction Biotechnology

## Biogeochemistry, Microbiology and Biotechnology of Construction Materials and Processes

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*The co-authors would like to dedicate this book to Elena Stabnikova, who is our major support in science and life*

# Preface

Biotechnology is a scientific discipline and an area of engineering on industrial manufacturing and practical applications of microorganisms and their products such as proteins, nucleic acids, polysaccharides, storage compounds, and low molecular weight metabolites. Food, Medical, Veterinary, Agricultural, and Environmental Biotechnologies differ in their areas of applications. A new biotechnological discipline, Construction Biotechnology, arose during the last decade.

Two major directions in Construction Biotechnology are (Fig. 1):

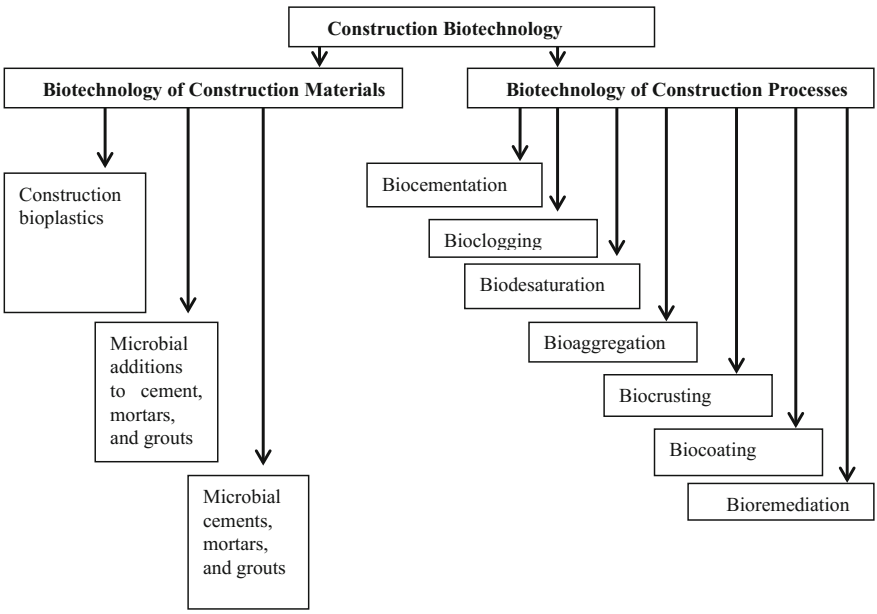


**Fig. 1** Directions of construction biotechnology

(1) The industrial production of the construction materials, for example cement admixtures or bioplastic, by microorganisms and (2) *in situ* applications of microorganisms or their products in the construction process. The aim of this book is to show the current trends and new potential directions for the further development of Construction Biotechnology.

Construction Biotechnology is a new interdisciplinary area involving applications of environmental and industrial microbiology and biotechnology in civil engineering. The topics covered in this book are as follows: biotechnological production of new construction materials such as biotechnological admixtures to cement, construction biocomposites, construction bioplastics, self-healing concrete, as well as such construction-related processes as biocementation, biogrouting, bioclogging, biosealing, soil surface fixation, biocoating of construction material surface, biotechnologies of green building and green city, microbiology and biosafety of construction environment, prevention of biocorrosion, biodeterioration and biofouling in civil engineering. Biomediated precipitations of calcium, magnesium, and iron compounds as carbonates, phosphates, sulfides, and silicate minerals in soil or fractured rocks, as well as on surface of materials, for their clogging, strengthening or coating are considered from geotechnical, chemical, and microbiological points of view. Some basic microbiological knowledge that can be useful for civil engineers to perform construction biogeochemical processes is also given in the book. The design principles and considerations for different field implementations are discussed from practical point of view. The book can be used as a textbook for graduate and senior undergraduate students in biotechnology, civil engineering, and environmental engineering, as well as a reference book for the researchers and practitioners who are working in new interdisciplinary area of Construction Biotechnology.

Singapore, Singapore and Kiev, Ukraine — Volodymyr Ivanov
Viktor Stabnikov

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# Contents

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# Chapter 1
# Basics of Microbiology for Civil and Environmental Engineers

## 1.1 References for the Chapter

This chapter is given almost without references considering for its use as an introduction for the engineers starting their work in the interdisciplinary area of Construction Biotechnology. The major references that can be used additionally to this text are (Ivanov et al. 2012; Ivanov 2015; Madigan et al. 2014).

## 1.2 Microorganisms

Microorganisms are organisms that are not visible without aid of microscope, i.e., with size less than 70–100 μm. Microorganisms play an important role in the natural or engineered protection of environment from chemical or biological pollution and they can restore polluted or degraded environment. Microorganisms or their products can be also used in the construction to improve the mechanical properties of the ground and building materials. At the same time, the engineers have to design the technologies against microorganisms causing diseases human, animals, and plants and preventing microbially induced deterioration or corrosion. Therefore, both civil and environmental engineering includes many engineering solutions that are based on the relevant knowledge of microbiology. However, this knowledge must be not a general biological science but has to be tightly connected with the engineering problems.

Civil and environmental engineers working in Construction Biotechnology have to understand such essential topics as diversity and functions of microorganisms in environment, the principles of microbial genetics and molecular biology, the major points of public health microbiology, as well as the essential topics for civil engineering processes such as biodeterioration, biocorrosion, bioclogging, and biocementation.



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## 1.3 Groups of Microorganisms

There are five kingdoms of living creatures on Earth: *Bacteria*, *Archaea*, *Fungi, Plants,* and *Animals.* All *Bacteria, Archaea,* and *Fungi* are microorganisms which cells are not visible under microscope. However, the cells aggregates, colonies of cells, and fruit bodies of *Bacteria, Archaea,* and *Fungi* are visible without microscope. Some groups of *Plants* and *Animals* are also microorganisms; they are mainly the groups of unicellular *Algae* and *Protozoa.*

## 1.4 Cells of Microorganisms

All living organisms are composed of cells. Two major types of cells are prokaryotes, which cells have no true nucleus, and eukaryotes, which cells have true nucleus and other organelles. *Bacteria* and *Archaea* are prokaryotic cells that are relatively simple in structure. *Fungi, Plants,* and *Animals* are composed of eukaryotic cells that are more complex than prokaryotic cells. Viruses are also important for public health aspects of Construction Biotechnology. They are not organisms but just the particles assembled from biopolymers. They are parasites of host cell, which are capable of multiplication and self-assembling as the new virus particles inside host cell. The typical size of a virus is about 0.1 μm.

*Bacteria* are microorganisms with the typical cell size about 1–2 μm. Bacteria are most active in their biogeochemical activity in the range of temperature from 0 to 100 °C, pH from 2 to 10, salinity from 0 to 300 g NaCl/L, and are capable to use energy of either organic and inorganic substances or light energy. The most common shapes of bacterium are spherical or rod-shaped cell but there are also curved and helix-like cells. This is a major group of microorganisms that are used in Construction Biotechnology for production of construction materials and for in situ bioclogging and biocementation. Bacteria with thick and rigid cell wall ("Gram-positive" bacteria, i.e., cells staining positively by Gram) are living mainly in terrestrial environment where osmotic pressure is changing quickly and significantly because of rainfall or drying. Bacteria with thin and elastic cell wall ("Gram-negative" bacteria, i.e., cells staining negatively by Gram) are living mainly in aquatic environment or animal and plant tissues, where osmotic pressure is stable. Curved and helix-like cells are living mainly in viscous sites like bottom sediments in the lakes and ocean or mucus of animals and human.

Cells of *Archaea* are very similar to that of *Bacteria* by size and shapes but they are living mainly in extreme environments such as the places with one of such factors as high temperature (above 50 °C), high salinity (above 100 g/L), strict anaerobic conditions (redox-potential below −250 mV), or low pH (below pH 3).

*Fungi* are microorganisms with the typical cell diameter in the range 10–20 μm that assimilate organic substances by adsorption. Fungi are active degraders of polymers. They produce often allergic and toxic spores and metabolites, and can

cause biodeterioration, biofouling, biocorrosion of construction materials and can be a reason of "sick building syndrome."

*Algae* are microorganisms with the typical cell size in the range from 10 to 50 μm that assimilate carbon dioxide as carbon source using light energy. Algae can be used in Construction Biotechnology for the immobilization of the paricles on soil surface and sealing of the sun-exposed surfaces of the ponds and channels.

*Protozoa* are unicellular (i.e., organism of one cell) animals that digest the particles of organic food including bacteria using engulfing. The typical cell size is in the range of 20–50 μm. Many protozoa cause diseases, which is important for the public health aspects of Construction Biotechnology.

## 1.5 Microbial Populations and Communities

Microbial cells of one origin are combined into a population. Usually populations are used for industrial biotechnological production of construction materials. Populations are combined into a microbial community living in specified biotope, for example in lake, groundwater, soil, or bioreactor. The community can be dispersed in biotope as microbial suspension or can be aggregated and attached to surface, for example as a microbial biofilm. The microbial communities are often used for bioaggregation, bioclogging, and biocementation of soil.

## 1.6 Phenotypic Classifications and Identification of Microorganisms

All studied microorganisms are classified (grouped) by their functional properties and structure of organism using phenotypic classification. First step in the study is isolation of a strain from the visible colony of cells grown on solid medium. A collection of strains with similar properties is defined conventionally as a species. The higher unit of the relatedness between microorganisms is *genus* (*genera* pl.). The names of species are conventionally given in *Latin*. Both the *genus* and the *species* are included in the name, for example, *Bacillus subtilis* (where the species is *subtilis* and the genus is *Bacillus*), *Escherichia coli* (*coli* being a species from the genus *Escherichia*), or *Pseudomonas aeruginosa* (where the species is *aeruginosa* and the genus is *Pseudomonas*). It would be incorrect to write "*Bacillus Subtilis*," "Bacillus Subtilis," or "bacillus subtilis." After the first mention of a full name (e.g., *Bacillus subtilis*) in a report, the researcher may abbreviate the genus, e.g., *B. subtilis*. "*Bacillus* sp." means that the species was not defined, whereas "*Bacillus* spp." indicates numerous species from the genus *Bacillus*. The higher levels of classification are family, then order, and finally kingdom.

The major phenotypic characteristics that are used in phenotypic classification are shown below

- cell structure characteristics such as cell size, shape, inclusions, membrane structures, and typical cell aggregates;
- physiological characteristics such as type of biological energy production, relation to oxygen, pH, temperature, and specific enzymatic activities;
- ecological characteristics such as optimal conditions, types of habitats, colonial structures, and interrelationships with other organisms;
- chemical characteristics such as chemical composition of cell wall, membrane lipids, and presence of specific pigments.

Therefore, phenotypic classification and identification of microorganisms require high level of professional microbiological knowledge and are time-consuming and expensive approaches.

## 1.7 Phylogenetic (Genotyping) Classification and Identification of Prokaryotes

Much faster and simpler is phylogenetic (genotyping) identification of prokaryotes, which are major bioagents in Construction Biotechnology. These classification and identification are performed through DNA sequencing. Therefore, there is often no need in cultivation of microorganisms because instead of cell growth the in-tube amplification of specific DNA from the natural sample can be used.

There are many phylogenetic divisions of bacteria, based on a comparison of 16S rRNA gene sequences. One of the most important of these groups for Construction Biotechnology is *Proteobacteria* containing about 7000 known 16S rRNA gene sequences. The division of *Proteobacteria*, which comprises the majority of prokaryotes with Gram-negative cell walls, includes the following subdivisions:

- Alpha subdivision
- Beta subdivision
- Gamma subdivision
- Delta subdivision
- Epsilon subdivision and some other small groups.

Identification using phylogenetic classification is much simpler and does not require professional microbiological knowledge as shown in Table 1.1.

A priori theoretical selection of the group of chemotrophic bacteria for selected Construction Biotechnology process using modern phylogenetic classification of prokaryotes is impossible because it is based mainly on the comparisons of one gene of 16S rRNA (Bergey's Manual of Systematic Bacteriology 2001, 2005). This classification has weak connection with the physiological grouping of chemotrophic

**Table 1.1** Identification steps using phenotypic and genotypic classifications

| Identification step | Identification using phenotypic classification | Identification using genotypic classification |
|---|---|---|
| 1 | Isolation of strain from environment | Isolation of gene from environment |
| 2 | Amplification of cells | Amplification of gene |
| 3 | Study of cell characteristics | Study of gene sequence (sequencing) |
| 4 | Deposition of strain in cell collection | Deposition of the sequence in digital database |
| 5 | Identification of a new strain by comparison with the reference strains | Identification of a new sequence by comparison with the reference sequences |

prokaryotes and cannot be used as a practical tool in the biodesign of needed biotechnology. There are also no logical connections between the groups, which complicates understanding and learning of microbiology. There are no strict and clear correlations between the groups of prokaryotes classified by the comparison of 16S rRNA gene and the groups classified by their physiological properties.

## 1.8 Physiological Classification of Prokaryotes Using Periodic Table

Therefore, for understanding of prokaryotic diversity and for theoretical selection of needed microorganisms we proposed physiological classification of prokaryotes shown below in Table 1.2. Details can be found in the reviews (Ivanov 2015; Ivanov et al. 2012). Physiological classification of all chemotrophic (i.e., using

**Table 1.2** The periodic table of physiological classification of chemotrophic prokaryotes. Some conventional genera related to the groups are shown inside the cells

| Place of origin | Type of energy generation | | |
|---|---|---|---|
| | Fermenting prokaryotes (obligate anaerobes) | Anaerobically respirating prokaryotes (obligate or facultative anaerobes) | Respirating prokaryotes (obligate aerobes) |
| Prokaryotes of aquatic origin (*Gracilicutes*) | *Bacteroides*<br>*Prevotella*<br>*Ruminobacter* | *Desulfobacter*<br>*Geobacter*<br>*Wolinella* | *Pseudomonas*<br>*Acinetobacter*<br>*Nitrosomonas* |
| Prokaryotes of terrestrial origin (*Firmicutes*) | *Clostridium,*<br>*Peptococcus*<br>*Eubacterium* | *Desulfotomaculum*<br>*Desulfitobacterium*<br>*Deferribacter* | *Bacillus*<br>*Arthrobacter*<br>*Streptomyces* |
| Prokaryotes originating from extreme environments (*Archaea*) | *Desulfurococcus*<br>*Thermosphaera*<br>*Pyrodictium* | *Methanobacterium*<br>*Thermococcus*<br>*Haloarcula* | *Picrophilus*<br>*Ferroplasma*<br>*Sulfolobus* |

chemical energy) prokaryotes can be shown in three evolutionary periods of fermenting, anoxic respiring, and aerobic respiring organisms that exist in three lines of aquatic, terrestrial, and extreme environment origins (Table 1.2).

## 1.9 Three Types of Chemotrophic Energy Generation

Three major types of biological energy generation from chemical compounds are the results of evolution of earth's atmosphere from an anaerobic to an aerobic one. Therefore, physiological diversity of chemotrophic prokaryotes can be shown in three evolutionary periods related to fermenting organisms that are obtaining energy using intramolecular oxidation–reduction, anaerobically respiring organisms that are obtaining energy using oxidation of substances without oxygen, and aerobically respiring organisms that are obtaining energy using oxidation of substances by oxygen. There are also intermediate groups, for example, microaerophilic or facultative anaerobic prokaryotes, between these groups.

## 1.10 Three Sources of Origin of Prokaryotes

These microbial groups can be found in three parallel semi-independent phylogenetic lines

- organisms of aquatic origin;
- organisms of terrestrial origin;
- organisms originated from extreme environment.

These lines have low frequency of genetic exchanges between organisms due to life in relatively separated aquatic, terrestrial and extreme environments. Prokaryotes of aquatic, terrestrial, and extreme environments are in the following lines:

- Gram-negative bacteria (*Gracilicutes*), cells with thin and elastic wall living in environment with a stable osmotic pressure such as fresh water, sea water, or tissues of macroorganisms;
- Gram-positive bacteria (*Firmicutes*), cells with a rigid and thick cell wall living in environment with changeable osmotic pressure such as soil and surfaces of plant and animals tissues exposed to sun;
- *Archaea*, cells with cells wall, cytoplasmic membrane and some molecular-biological properties different from bacterial cell because life at some extreme conditions such as high temperature or salinity, and low oxidation–reduction potential or pH.

## 1.11 Nine Physiological Groups of Chemotrophic Prokaryotes

This physiological classification is based just on two features: (1) relation to oxygen, which is connected to the type of energy generation and (2) type of cell wall. Three periods (columns) in Table 1.1 are as follows: (1) fermenting anaerobes; (2) anaerobic respiring prokaryotes that produce energy by anaerobic oxidation of chemical substances using such electron acceptors as nitrate ($NO_3^-$), nitrite ($NO_2^-$), ferric ($Fe^{3+}$), sulfate ($SO_4^{2-}$), sulfur (S), or carbon dioxide ($CO_2$); (3) aerobes producing energy through oxidation of different electron donors by oxygen. There known are also intermediate physiological groups, for example, facultative anaerobes using either anaerobic or aerobic respiration, depending on conditions. Microaerophilic bacteria is intermediate group that is adapted to low concentration of oxygen and generation of energy due to oxidation using one electron transfer from electron donors to oxygen. So, there are a total of nine physiological groups (or 12 groups if account intermediary groups of microaerophlic and facultative anaerobic prokaryotes) in the periodic table of chemotrophic prokaryotes.

## 1.12 Additional Periodic Table of Phototrophic Prokaryotes

There are also related groups of phototrophic prokaryotes that are using products of fermentation, anoxic respiration, or aerobic respiration as electron donors (Ivanov 2015). Every physiological group of phototrophic prokaryotes depends in nature on related group of chemotrophic prokaryotes because final products of one type of energy generation are used for other type of energy generation as shown in Fig. 1.1 below.

## 1.13 Classification and Identification of Fungi

Classification and identification of fungi is based on their morphology and diversity of their sexual reproduction. There are five major groups of fungi

- *Oomycetes*, water molds that are not used in Construction Biotechnology;
- *Zygomycetes*, molds producing numerous spores that are allergic and often toxic and may be a reason of a sick building syndrome due to growth of molds on indoor surfaces in the atmosphere of moist indoor air;
- *Ascomycetes*, unicellular fungi (yeasts) and multicellular fungi, which also can produce allergic and toxic spores; some ascomycetes can produce exopolysaccharides that can be used as cement admixtures in Construction Biotechnology;

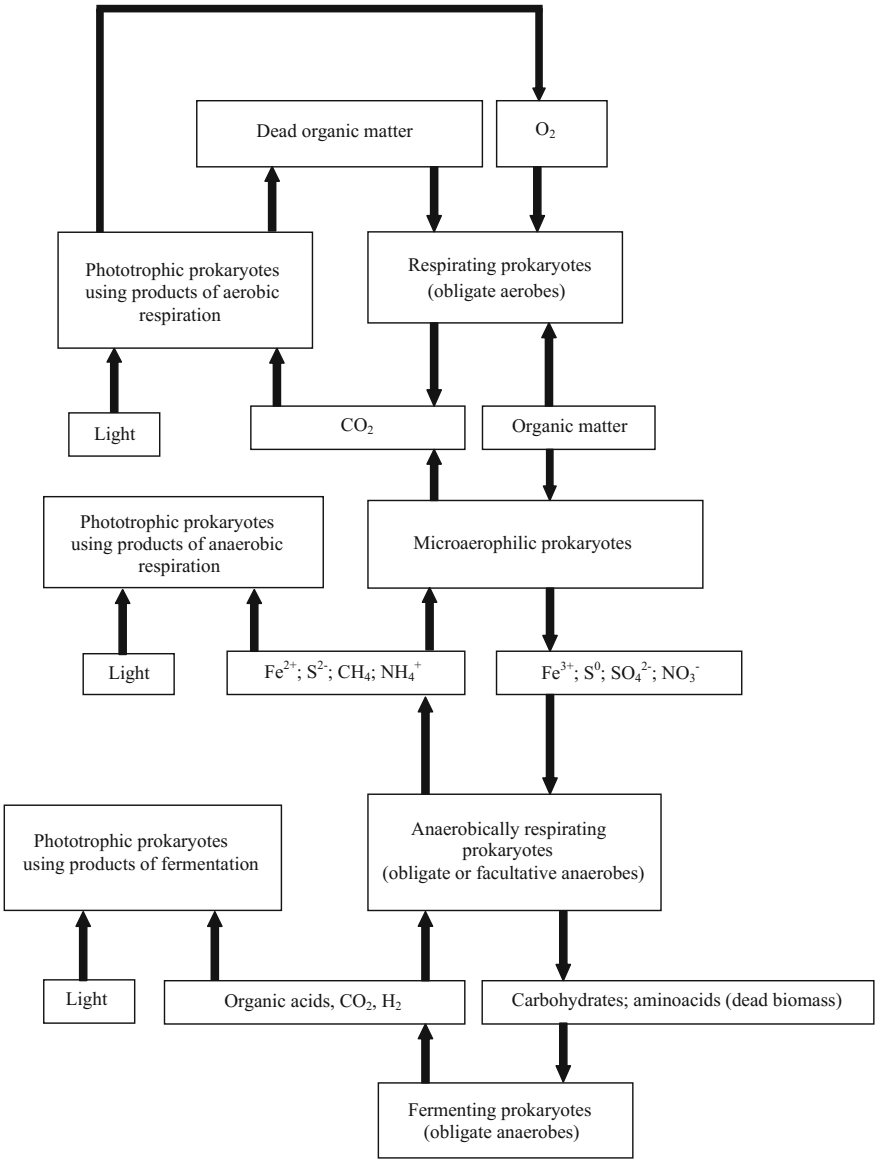


**Fig. 1.1** Interactions between different physiological groups of chemotrophic and phototrophic prokaryotes in nature

- *Basidiomycetes*, causing biodegradation and biodeterioration of wooden constructions due to active destruction of cellulose and lignin;
- *Deuteromycetes* are active biodegraders of xenobiotics.

## 1.14 Classification and Identification of Algae

Classification of algae is based on their morphology, type of photopigments, cell wall structure, and type of carbon reserve material

- *Chlorophyta* (green algae);
- *Chrysophita* (golden-brown algae);
- *Euglenophyta* (cell with flagella);
- *pyrrophyta* (dinoflagellates);
- *Rhodophyta* (red algae);
- *Phaeophyta* (brown algae).

Algae remove nutrients from water and are active microorganisms in waste stabilization ponds but some algae are fast growing in polluted water and produce toxic compounds, an example is "red tide" in polluted coastal areas due to overgrowth of toxic dinoflagellates. Diversity of algae indicates water quality. Some algae are commercially used for production of biodiesel, pigments, and unsaturated fatty acids so construction of algae ponds using biogrouting could be an important part of Construction Biotechnology.

## 1.15 Classification and Identification of Protozoa

Classification of protozoa is based on their morphology and mechanism of motility:

- amoebas move by means of false feet;
- flagellates move by means of flagella;
- ciliates use cilia for locomotion;
- some protozoa have no means of locomotion.

Protozoa form cysts under adverse environmental conditions, which are resistant to desiccation, starvation, high temperatures, and disinfection, so pollution of aquatic environment, especially the sources of drinking water with the cysts of some protozoa, is important environmental problem.

## 1.16 Enzymes as the Catalysts of Biochemical Reactions

Application of microorganisms in Construction Biotechnology is due to their fast and diverse biochemical reactions. All biochemical reactions in cell are catalyzed by protein molecules called enzymes. Each enzyme increases the rate of one specific biochemical reaction. An enzyme decreases the energy of activation of a reaction due by stereochemical arrangement of substrate inside the 3D-structure of protein, which is favorable for initiation of the reaction. Typically, the

enzyme-catalyzed biochemical reaction is going several thousand or even million times faster than the same chemical reaction without enzyme.

The trivial name of an enzyme includes the type of substrate, the type of catalyzed reaction, and ends with "ase." For example, an enzyme catalyzing the oxidation of alcohol by removal of hydrogen has trivial name alcohol dehydrogenase. To identify thousands of enzymes, the enzyme classification (EC) numbers and names are used. Six classes of enzymes are described below.

1. Oxidoreductases catalyze oxidation–reduction reactions. Oxidoreductases are important enzymes for microbial oxidation of organic substances.
2. Transferases catalyze transfers of different chemical groups. They are most important in biosynthesis of cellular substances and regulation of cellular processes.
3. Hydrolases catalyze hydrolysis reactions where a molecule is split into two or more smaller molecules by the addition of water. This type of enzymes is important for example in microbially induced carbonate precipitation performed due to enzymatic hydrolysis of urea.
4. Lyases catalyze the cleavage of C–C, C–O, C–S, and C–N bonds by means other than hydrolysis or oxidation. These enzymes catalyze some reactions of biodegradation of organic compounds.
5. Isomerases catalyze rearrangements of isomers, for example optical isomers.
6. Ligases catalyze the reactions of biosynthesis where two molecules are joined.

The EC number of an enzyme includes four digits. For example, the EC number of oxidative–reduction enzyme catalase is EC 1.11.1.6, and for hydrolyzing enzyme urease the number is EC 3.5.1.5. In scientific and technical literature, it is required to provide not only trivial names of enzymes but also their EC numbers and names.

Extracellular enzymes, separated from cells, and intracellular enzymes, extracted from cells and separated or not from cell debris and cellular components, can be used in Construction Biotechnology.

## 1.17 Velocity of Biochemical Reaction

The velocity of biochemical reaction is measured as the conversion of 1 μmole of substrate or formation of 1 μmole of product per minute at optimal conditions. The rate of biochemical reaction can be given also as mol of substrate $g^{-1}$ of biomass $s^{-1}$ or mol of substrate $L^{-1}$ $min^{1}$ (mM/min). Empirical units of enzymatic activity, that can be easily measured, are also used in the engineering practice. These units are for example, the rate the change of optical density, pH, electric conductivity, viscosity of solution, or volume of produced gas.

## 1.18 Control of the Enzymatic Reaction Rate

The rate of a biochemical reaction is controlled by:

1. The content of enzyme in cell or concentration of enzyme in the solution/suspension ($X_e$), mol of enzyme $g^{-1}$ of biomass or g of enzyme $L^{-1}$. The content of intracellular enzyme in cell or extracellular enzyme in solution/suspension is changed by the turning on (induction of enzyme synthesis in cell) or the turning off (repression of enzyme synthesis in cell) of the relative gene(s) of enzyme in cell.
2. The substrate concentration affects the rate of enzymatic reaction usually according to the Michaelis–Menten equation

$$V = V_{max}S/(S + K_s),$$

where $V$ and $V_{max}$ are current and maximum velocities, respectively; $S$ is the concentration of substrate; $K_s$ is the constant of half-saturation for substrate.

Different inhibitors ($I$) decrease enzymatic velocity. There are noncompetitive and competitive inhibitors of enzyme activity. Nonspecific enzyme inhibitors such as acids, bases, alcohols, and heavy metals change the structure of the active center of enzyme and the secondary, tertiary and quaternary structure of enzymes. This noncompetitive inhibition of enzymatic activity can be described by the following equation:

$$V = V_{max}/(1 + I/K_i)],$$

where $V$ and $V_{max}$ are current and maximum velocities, respectively; $K_i$ is the inhibition constant describing the affinity of the inhibitor for the enzyme; $I$ is concentration of inhibitor.

Specific inhibitors are the competitors of substrate for active center of enzyme. Competitive inhibition of enzymatic activity can be described by the equation

$$V = V_{max}S/[S + K_s/(1 + I/K_i)],$$

where the symbols are the same as in the paragraph above.

## 1.19 The Role of Enzyme Kinetics in Engineering

These kinetic equations for enzymatic activity are important in engineering because very often the whole rate of cellular metabolism depends on the rate of the slowest biochemical reaction. In this case, the rate of the whole cell metabolism, cellular biodegradation of energy source, cellular growth can be described by the equation similar to the equation of the enzyme kinetics. For example, the specific rate of

exponential growth of microbial population ($\mu$) limited by the concentration of one nutrient ($S$) can be described by the equation similar to Michaelis–Menten equation

$$\mu = \mu_{\max} S/(S + K_s).$$

However, there are many other known models describing $\mu$ as a function of $S$. For example, a double limitation of $\mu$ by electron donor and oxygen as electron acceptor is typical for the cases when the initial step of catabolism is catalyzed by enzyme oxidase or oxygenase incorporating atom(s) of oxygen into a carbon molecule or energy source. Well-known kinetic equations describing the effects of pH, temperature, inhibitors, or products on enzymatic activity often can be used in the modeling of growth rate and biochemical activities of cells, population, and even microbial communities.

## 1.20 Induction, Repression and Feed-Back Control of Enzymatic Activity

The effective control mechanism that regulates the content of enzyme in the cell is also the turning on or off of the production of enzyme in cell. The appearance of new substrate in medium may induce the synthesis of the enzyme needed for biochemical transformation of this substrate (induction of synthesis). Excessive concentration of product of enzymatic reaction will repress the synthesis of this enzyme (feed-back control). The substrate, which is metabolized faster or with higher efficiency, can repress the synthesis of the enzymes catalyzing transformation of another substrate. This effect, called catabolite repression, is often revealed at the level of cell metabolism when glucose is present in medium. In this case, enzymes oxidizing glucose are synthesized, but the synthesis of enzymes for oxidation of another substrate is repressed. However, the synthesis of these enzymes will start when glucose gets consumed from the medium. This mechanism is important for cultivation and application of ground improving microorganisms because biodegradation of slowly degraded organic compounds in soil can be repressed by the addition of glucose or some other rapidly metabolized carbohydrates.

## 1.21 The Types of Biogeochemical Reactions

Many biochemical reactions performed by microorganisms in the hydrosphere on the depth from the surface to 11 km and in the lithosphere on the depth from surface to 3 km, but especially in soil (it is about 10–50 cm of the upper part of lithosphere), are essential for many natural geological processes and for the engineering activities. These reactions are specified as biogeochemical reactions. Generally, the types of the biogeochemical reactions are as follows:

- Biogeochemical cycling of carbon compounds on Earth through their cyclic oxidation–reduction and synthesis- biodegradation.
- Biogeochemical cycling of nitrogen, sulfur, iron, phosphorus, and other elements on Earth through their oxidation–reduction or dissolution-precipitation-volatilization.
- Purification of water on Earth by the removal of organics, nutrients, and heavy metals.
- Transformation of chemical and light energy into biomass.
- Accumulation of microbial metabolites in geological scale, for example, accumulation and deposit of elemental sulfur and sulfide of metals, iron (hydr) oxides, and oil.
- Evolution of the atmosphere on Earth from anaerobic to aerobic one and protection of terrestrial forms of life from UV radiation due to oxygenic photosynthesis of prokaryotes.
- Biogeochemical cycling of greenhouse gases in atmosphere, such as $CO_2$, $CH_4$, $H_2S$, $N_2O$, that absorb and emit of infrared radiation emit by Earth and affects average temperature on Earth surface.

Many of these biogeochemical reactions are used in Construction Biotechnology for production of construction materials and performance of construction processes.

## 1.22 Biogeochemical Reactions that Can be Used for Production of Construction Materials and for Construction Processes in Situ

Different types of biogeochemical reactions and processes that can be used in Constriction Biotechnology to produce construction materials and perform construction processes in situ are shown in Table 1.3.

## 1.23 Biosafety

In relation to biosafety, all microorganisms can be conventionally differentiated as

– saprophytic microbes feeding by dead organic matter,
– pathogenic microbes that can grow in or on animal or plant tissue and can cause diseases of the plants, animals, and human,
– opportunistic microbes that are normally harmless to humans but have the potential to be pathogenic in immunocompromised individuals or after penetration through the skin.

**Table 1.3** The types of biogeochemical reactions used to produce construction materials and perform construction processes in situ

| Physiological group of microorganisms | Biogeochemical reactions | Essential conditions for reaction/process | Potential applications |
|---|---|---|---|
| Exopolysaccharide-producing aerobic bacteria | Industrial large-scale biosynthesis of crude exopolysaccharides from carbohydrates, ethanol or other organic raw materials | Supply of air, carbon source, and other nutrients sources, aseptic production | Cement admixtures |
| Exopolysaccharide-producing fungi | Industrial large-scale biosynthesis of crude exopolysaccharides from carbohydrates, ethanol or other organic raw materials | Supply of air, carbon source, and other nutrients sources, aseptic production | Cement admixtures |
| Aerobic polyhydroxyalkanoates-accumulating bacteria | Industrial large-scale biosynthesis of crude bioplastic polyhydroxyalkanoates from organic waste materials (organic acids, lipids) | Supply of air, carbon source, and other nutrients sources | Construction biodegradable bioplastic for temporarily constructions |
| Anaerobic lactic acid bacteria | Industrial large-scale biosynthesis of bioplastic polylactic acid from polysaccharides | Supply of carbon source, and other nutrients sources | Construction bioplastic fibers for special construction purposes |
| Anaerobic and aerobic urease-producing bacteria | Formation of undissolved carbonates of metals (Ca, Fe, Mg, Al) in soil due to increase of pH and release of $CO_2$ during hydrolysis of urea | Presence of urea and dissolved metal salt | To enhance stability for retaining walls, embankments, and dams; to increase bearing capacity of foundations; to reduce hydraulic conductivity in retaining walls, embankments, dams, tunnels, channels |

(continued)

**Table 1.3** (continued)

| Physiological group of microorganisms | Biogeochemical reactions | Essential conditions for reaction/process | Potential applications |
|---|---|---|---|
| Strictly anaerobic iron-reducing bacteria | Production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil | Anaerobic conditions during Fe(III) reduction that are changed for aerobic conditions for Fe(II) oxidation; presence of ferric minerals | To diminish hydraulic conductivity of soil in retaining walls, embankments, dams, tunnels, channels |
| Strictly anaerobic sulfate-reducing bacteria | Production of undissolved sulfides of metals (Ca, Fe, Mg, Al) in porous soil. | Anaerobic conditions; presence of sulfate and organic electron donor in soil | To strengthen storage levies and barriers of sulfate-containing wastes |
| Exopolysaccharide-producing anaerobic and aerobic bacteria | Production of clogging exopolysaccharides from the medium supplied into soil or cracks of the rocks | Supply of carbon source as well as electron donor and acceptor | To diminish hydraulic conductivity of soil in retaining walls, embankments, dams, tunnels, channels |
| Phosphatase-producing aerobic and anaerobic bacteria | Formation of undissolved phosphates of metals (Ca, Fe, Mg, Al) in soil due to release of phosphate during hydrolysis of polyphosphates, cyclic phosphates, and phospho-organics | Supply of phosphate-containing compounds | To diminish hydraulic conductivity of soil in retaining walls, embankments, dams, tunnels, channels |
| Anaerobic nitrate-reducing (denitrifying) bacteria | Production of clogging microbubbles of biogas from the medium supplied into porous soil or cracks of the rocks | Supply of organic or inorganic electron donors and nitrate as electron acceptor | Desaturation of water-saturated soil for mitigation of liquefaction and clogging of the pores |
| Phototrophic microorganisms such as cyanobacteria and algae alone or in association with fungi | Microbial biofilm binding particles together | Sunlight and suitable osmotic pressure/water content as well as presence of mineral nutrients | To diminish wind and water erosion of soil; for dust suppression |

The diseases caused by the transfer of pathogenic microorganisms from the environment or from one macroorganism to another are called as infectious disease, which are conventionally differentiated as air-borne, water-borne, soil-borne, vector-borne (through insects), and food-borne infectious diseases. The ability to cause infectious disease depends on the abilities of microorganisms to produce toxins, killing cells of plants, animals, and human, and to form microstructures for specific adherence of microbial cells to the cells of plant, animals, and human, and protection from the reaction of these cells.

## 1.24 Biosafety in Construction Biotechnology

Biosafety in Construction Biotechnology are the rules and methods aiming to prevent outbreaks of the infectious diseases during the production of construction biomaterials or application of bioprocesses in construction. These rules are as follows:

- working with not identified culture treat it as potential pathogen, i.e., disinfect work and spill areas, equipment after use and waste materials, professional care about personal hygiene to avoid potential infection;
- use of pure isolated cultures of microorganisms instead of enrichment cultures;
- use of non-infective microbial products instead of live microbial cells if possible;
- identification of all pure cultures used in the industrial process;
- test or at least studying of these cultures as potential pathogens or opportunistic pathogens using WHO Classification of Infective Microorganisms by Risk Group and data of scientific literature from PubMed;
- all manipulations with microorganisms or their live biomass in laboratory, in industry, and in the construction area must be carried out under the supervision of a professional microbiologist or biotechnologist who knows and understands professionally the biosafety rules and methods, and can monitor microbiology of the process.

Therefore, the best option for application of bioprocesses in Construction Biotechnology is industrial production of biosafe grouts and cements, which are not containing live cells of microorganisms, so that their application in the field can be performed by civil or environmental engineer without supervision of microbiologist.

To diminish the risk of pathogenic bacteria accumulation and release during non-aseptic biotechnological process the following selective conditions can be used:

- an application of carbon sources, which are used in nature by saprophytic microorganisms, such as cellulose-containing agricultural wastes and starch-containing, acidic, vegetable-processing waste;
- the conditions, which are suitable for the growth of autolithotrophic bacteria for which carbon dioxide is used as a carbon source and inorganic substances ($NH_4^+$, $Fe^{2+}$, S) are used as electron donor;
- the conditions that are suitable for application of anaerobically respiring bacteria with $SO_4^{2-}$ or $Fe^{3+}$ as electron acceptors;
- an application of solution with low concentration of carbon source for preferable growth of oligotrophic microorganisms in soil.

## 1.25 Disinfection in Construction Biotechnology

Pathogens can be removed from materials, surfaces, equipment, soil, air, and water using following methods:

– bulk or membrane filtration for air and aerosol disinfection;
– UV and X-ray disinfection of air and aerosol, solid surfaces and microbial biofilms, and water;
– chemical disinfection of water and soil by oxidants such as chlorine, chlorine dioxide, ozone, and hydrogen peroxide;
– chemical disinfection of water and soil by organic solvents;
– chemical disinfection of water and soil by surfactants;
– chemical disinfection of surfaces by salts of heavy metals;
– thermal disinfection of materials, water, and soil;
– disinfection of soil using acidification or alkalinization.

Efficiency of disinfection must be monitored by professional microbiologist or biotechnologist.

## 1.26 Theoretical Screening of Microorganisms for Construction Biotechnology

The group of chemotrophic prokaryotes is the most suitable one for the industrial production of construction materials as well as for soil bioaggregation, bioclogging, and biocementation because of their smallest cell size, typically from 0.5 to 2 μm, ability to grow inside soil, and big physiological diversity.

Phototrophic prokaryotes, mainly cyanobacteria, grow only on soil surface because light penetrates a few millimeters into soil. These bacteria can produce rigid crust on surface of soil or sediment, which diminishes soil infiltration rate and improves slope stability. Cyanobacteria can also create millimeter-scale laminated

calcium carbonate build-ups called stromatolites, which are formed in shallow marine environment, due to the sequence of sedimentation, growth of biofilm, production of a layer of exopolymers, and lithification of sediments by the precipitation of microcrystalline carbonate (Reid et al. 2000; Dupraz et al. 2009).

Group of *Archaea* could be excluded from the consideration as the bioagents of soil bioaggregation, bioclogging, and biocementation, because all *Archaea* are living in extreme environments such as the biotops with extremely high temperature, extremely low redox-potential, or extremely high salinity that are not compatible with the majority of the conditions on the construction site.

Psychrophiles are microorganisms adapted to live at low temperature, minimum for growth is −12 °C due to production of cryoprotectants (Bakermans 2012). They can be found in all three phylogenetic lines. These microorganisms could be useful in the cases when the bioprocess must be performed at low temperature.

## 1.27 Use of Anaerobic Fermenting Bacteria in Construction Biotechnology

Anaerobic fermenting bacteria may be involved in cementation of soil particles under the presence of calcium, magnesium, or ferrous ions. This cementation can be due to the increase in pH caused by ammonification (release of ammonia from amino acids) of dead biomass protein and increase of carbonate concentration due to carbon dioxide production in soil (Castanier et al. 1999, 2000; Hammes and Verstraete 2002). The insoluble carbonates and hydroxides of metals could precipitate thus binding the soil particles and clogging soil pores. So, the hydraulic conductivity of soil or fractured rocks can be diminished during fermentation due to precipitation of carbonates and production of biogas. However, fermenting anaerobic bacteria can diminish the soil pH due to formation of organic acids during fermentation of carbohydrates that not only preventing precipitation of carbonates but even dissolving present carbonates and hydroxides that are binding soil particles or plugging soil pores. Anaerobic bacteria cannot clog soil pores by the synthesis of extracellular polymers because they are not able to produce big quantity of slime (Atmaca et al. 1996) due to their low efficiency of biological energy production in fermentation.

## 1.28 Use of Anaerobically Respiring (Anoxic) Bacteria in Construction Biotechnology

Organic acids, hydrogen, and alcohols, which are produced by anaerobic fermenting bacteria from polysaccharides and monosaccharides, can be used as donors of electrons by anaerobic respiring bacteria. One example is the group of

iron-reducing bacteria, which are using products of fermentation as electron donors to produce dissolved Fe(II) ions by reduction of insoluble Fe(III) compounds (Lovley et al. 2004; Weber et al. 2006). Microbial reduction of Fe(III) is used in environmental biotechnology for treatment of groundwater and wastewater (Fredrickson and Gorby 1996; Ivanov et al. 2004, 2005; Stabnikov and Ivanov 2006) and could be used hypothetically for soil cementation because iron-reducing bacteria could produce $Fe^{2+}$ in situ from cheap sources of Fe(III) and products of anaerobic fermentation of organic wastes (Ivanov et al. 2004, 2005; Stabnikov and Ivanov 2006). Ions of ferrous can be oxidized chemically or biologically. Products of this oxidation are insoluble ferric hydroxides and ferric carbonates, which could clog the soil pores and bind the soil particles altogether. Chelated ferrous ions can be oxidized by "iron-oxidizing" bacteria. A set of experiments have been done on soil bioclogging using *Leptothrix discophora* or indigenous "iron-oxidizing" bacteria (Weaver et al. 2011).

Another example of anaerobically respiring bacteria, which could be used in bioclogging and biocementation, are sulfate-reducing bacteria. These bacteria produce dihydrogen sulfide using organic acids, hydrogen, or alcohols as electron donors and sulfate as electron acceptor. Sulfide reacts with iron and other metal cations to form insoluble suphides of metals, which clogs the soil pores and binds the soil particles. However, the soil compaction created by the formation of sulfides is unstable because they can be chemically or biologically oxidized to sulphuric acid or sulfates under aerobic conditions. There is known a case where a thousand houses built on excavated non-weathered mudstone sediments have been damaged by microbially induced heaves of foundations (Yamanaka et al. 2002). The mechanism of this damage was as follows: (1) sulfate-reducing bacteria in the mudstone reduced sulfate to hydrogen sulfide; (2) the mudstone sediments under the houses became permeable to air due to gradual drying; (3) dihydrogen sulfide was oxidized by sulfide-oxidizing bacteria to sulphuric acid; (4) acid dissolved calcium carbonate binding the particles of mudstone sediments. Additional negative impact of activity of sulfate-reducing bacteria is the increased corrosion and release of toxic and bad smelling dihydrogen sulfide.

Denitrification process, which is bioreduction of nitrate ($NO_3^-$) and nitrite ($NO_2^-$) to nitrogen gas, could be used for the soil desaturation and bioclogging because bacteria produce big volume of dinitrogen gas during denitrification in situ.

## 1.29 Use of Facultative Anaerobic and Microaerophilic Bacteria in Construction Biotechnology

Facultative anaerobic bacteria could be considered as the most suitable bioagents for soil bioclogging and biocementation because many species are able to produce big quantity of exopolysaccharides, which usually promote formation of cell aggregates, and can grow under either aerobic or anaerobic conditions. Last

property of facultative anaerobic bacteria is most essential for biotreatment of soil in situ where supply of oxygen is limited by the soil porosity and both aerobic and anaerobic microzones coexist in soil. There are, for example, bacteria from the genera *Alcaligenes*, *Enterobacter, Staphylococcus, Streptococcus*, *Rhodococcus*, corynebacteria (*Gordonia, Nocardioides*), gliding bacteria (*Myxococcus, Flexibacter, Cytophaga*) and oligotrophic bacteria (*Caulobacter*) (Wingender et al. 1999; Jones et al. 2004; Ivanov and Tay 2006a, b, c).

Microaerophilic bacteria could be used for the biobinding of soil particles because many strains of microaerophilic bacteria are combined in filaments (Beccari and Ramadori 1996; Seviour and Blackall 2007) or joined by sheath (Mulder and Deinema 1992) and these filamentous structures can also bind the soil particles. The filamentous bacteria from the genera *Beggiatoa*, *Haliscomenobacter*, *Microthrix*, *Nocardia*, *Sphaerotilus*, and *Thiothrix* are common in aerobic tanks of wastewater treatment plants (Beccari and Ramadori 1996; Seviour and Blackall 2007) and can be probably used for biobinding of soil particles.

## 1.30 Use of Aerobic Bacteria in Construction Biotechnology

Aerobic bacteria could be suitable for soil bioclogging, biocementation, and biobinding of soil particles because many species are able to produce big quantity of slime, form chains and filaments, increase pH, and oxidize different organic and inorganic substances. Cells of many *Actinomycetes*, a group of Gram-positive bacteria, typical soil inhabitants, form particles—binding mycelium and produce particles-binding slime in soil (Wu et al. 1997; Jones et al. 2004; Dworkin et al. 2006). These bacteria are the most prospective for the aerobic soil bioclogging, biocementation, and biobinding. The examples of direct involvement of both facultative anaerobic and aerobic bacteria may be the cementation of soil particles under presence of calcium, magnesium, or ferrous ions due to increase of pH caused by ammonification and carbon dioxide production in soil with added urea (Kucharski et al. 2005). The insoluble carbonates and hydroxides of metals are precipitating at high pH thus binding the soil particles and clogging the soil pores. Such bacterial groups as gliding bacteria, oligotrophic bacteria, and nitrifying bacteria could be most active in the formation of polysaccharides, which are binding the soil particles. Aerobic sulfide-, sulfur-, and ammonium—oxidizing bacteria produce sulphuric or nitric acids could hypothetically compact the soil particles due to dissolution of minerals, change of zeta-potential of colloid particles and precipitation of colloidal silica at low pH.

## 1.31 Use of Anaerobic Bacteria in Construction Biotechnology

The use of anaerobic bacteria can be complicated by the presence of oxygen in the upper layer of soil and sensitivity of anaerobic bacteria to oxygen. Alternatively, if aerobic bacteria are used for soil clogging or cementation, a major technological problem is the air supply into soil. If the rate of oxygen supply into soil by aeration and diffusion is not sufficient, there will be formation of anaerobic layer or zones, where aerobic bacteria will not be active. Therefore, from the technological and biological points of view, the most suitable physiological groups for the soil bioclogging and biocementation in situ are facultative anaerobic bacteria, which are active under both aerobic and anaerobic conditions. Depending on the site conditions of a real soil treatment project, a technique to alter the anaerobic and aerobic conditions in situ can be implemented to ensure the sequence of anaerobic and aerobic biogeochemical processes facilitating soil bioclogging or biocementation.

Another assumption from the general consideration of physiological diversity of prokaryotes is that the most suitable bacteria for soil bioclogging or biocementation are bacteria with Gram-positive type of cell wall because these bacteria are most resistant to the changes of osmotic pressure, which is the typical condition for soil on construction or reclamation sites.

## 1.32 The Major Groups of *Bacteria* Suitable for Construction Biotechnology Processes

The major groups of prokaryotes important for Construction Biotechnology are as follows:

- Anaerobic Gram-negative and Gram-positive fermenting bacteria, for example bacteria of the genus *Clostridium,* that are performing such stages of anaerobic digestion of organic wastes as the hydrolysis of biopolymers and fermentation of monomers. Organic acids produced by these bacteria can be used for the dissolution of limestone or dolomite producing calcium and magnesium salts, which are major components of biocement.
- Sulfate-reducing bacteria that are using organic compounds as electron donor and sulfate or elemental sulfur as electron acceptor. Dihydrogen sulfide is the toxic product of this process but it precipitates almost all metals as insoluble sulfides.
- Denitrifying bacteria, for example, *Pseudomonas denitrificans* or *Paracoccus denitrificans*, that reduce nitrate to nitrogen gas using organic and inorganic electron donors. They can be used for desaturation of water-saturated sand, porous soil, and fractured rocks under anoxic conditions, and bioclogging using production of exopolysaccharides in situ.

- Iron-reducing bacteria that can reduce different Fe(III) compounds producing soluble ferrous ions that can be oxidized in aerobic conditions producing insoluble ferric hydroxides. They can be used for bioclogging of porous soil.
- Facultative anaerobic bacteria producing slime or having urease activity. They can be used in bioaggregation, bioclogging, and biocementation of soil.
- The growth of microaerophilic filamentous bacteria in poor aerated soil may form a biofilm.
- Aerobic heterotrophic Gram-negative bacteria are most active in production of exoplysaccharides, which can be used as admixtures.
- Aerobic heterotrophic Gram-positive bacteria, for example from the genus *Bacillus,* are most important in ground improvement. Some of them form spores. *Bacillus* spp. are dominating bacteria in the aerobic treatment of wastewater or solid waste, which are rich in such polymers as starch or protein.
- Actinomycetes are aerobic heterotrophic Gram-positive filamentous bacteria which are active degraders of natural biopolymers and are active in the formation of biofilm.
- Nitrifying bacteria produce nitric acid which can effect precipitation of silicates and is strong corrosive agent.
- Sulphur-oxidizing chemilitotrophic bacteria oxidize reduce sulfur compounds and form sulphuric acid which is strong corrosive agent and can affect precipitation of silicates and calcium sulfate.
- Iron-oxidizing bacteria are capable to oxidize Fe(II) in acid or neutral environments. The precipitation of biogenic iron hydroxide can be used for the bioclogging.
- Cyanobacteria are oxygenic (producing oxygen) prokaryotic phototrophs (using light energy) and algae are eukaryotic phototrophic organisms that carry out oxygenic photosynthesis with water serving as the electron donor. Phototrophs are used for the soil bioaggregation and formation of soil crust.

# Chapter 2
# Basics of Biotechnology for Civil and Environmental Engineers

## 2.1 References for the Chapter

This chapter is given without references considering its use as an introduction for the engineers starting work in the interdisciplinary area of Construction Biotechnology. The major references that can be used additionally to this text are (Ivanov 2015; Madigan et al. 2014; Wang et al. 2010; Bhattacharyya and Banerjee 2008; Fulekar 2010).

### *2.1.1 Biotechnology*

Biotechnology is a scientific and engineering knowledge on the use of microorganisms or their products in large-scale industrial processes. Depending on the area of application, the microbial biotechnologies are differentiated as

- Food Biotechnology, which was started in ancient times from the production of bread, wine, beer, cheese, yogurt, pickled vegetables, fermented souses, etc.;
- Medical and Veterinary Biotechnologies, deals with industrial production of antibiotics, vaccines, and other pharmaceuticals, as well as with microbial probiotics—live microorganisms for enhancement of physiological activities of human or animals;
- Environmental Biotechnology, maintains clean and sustainable environment using the microbial processes in water, wastewater, polluted soil, and polluted air;
- There are also developing areas of Agricultural (biofertilizers), Mining (bioleaching of metals), and Energy (production of biofuels) Biotechnologies;
- Construction Biotechnology, described in this book, is a new discipline developing in two directions: (1) biotechnological production of construction materials, and (2) biotechnological construction processes in situ.



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## 2.2 Applicability of Construction Biotechnology

Biotechnology can be applied for the production of construction materials due to four reasons:

- low cost due to use of mining or organic wastes as raw materials;
- lower cost in comparison with the products of chemical industry due to simpler and less energy consuming technology;
- lower toxicity of biomaterials than chemical materials;
- sustainability of the biotechnological production.

Construction Biotechnology is usually applied in geotechnical engineering for bioaggregation, bioclogging, and biocementation of porous soil or fractured rocks in situ by the same reasons but there are important additionally features such as:

- low viscosity of biogrouting and biocementing solutions and deep penetration of this solution into porous soil or fractured rocks;
- ability to control rate of biochemical reactions in situ by the concentration or activity of biomass or enzyme;
- ability for self-multiplication (proliferation) of microbial cells in situ;
- better public acceptance of biotreatment of environment rather than chemical treatment.

However, a combination of biotechnological, chemical, and mechanical treatments may be more efficient than a single type of treatment. So, it is a common rule that a combination of biological and chemical materials, and technologies and proper mechanical optimization ensures the development of the most effective technology. There is almost no example of application-only bioprocess without chemical or mechanical processes in Construction Biotechnology.

## 2.3 Bioprocesses Used in Construction Biotechnology

The following bioprocesses are mainly used in Construction Biotechnology:

1) Exponential growth of biomass $X$

$$dX/dt = \mu X = Y(dS/dt)$$

where $\mu$ is specific growth rate and $Y$ is growth yield; $\mu = \Delta \ln X/\Delta t$ for the time interval time $\Delta t$.

2) Linear growth or decay of biomass

$$dX/dt = k$$

where $k$ is the rate of growth or decay.

3) Primary biosynthesis of metabolite $P$ (production of the substance $P$ depends on biomass)

$$dP/dt = f(X).$$

4) Secondary biosynthesis of metabolite $P$ (production of substance $P$ is independent of biomass)

$$dP/dt = f(t).$$

5) Enzymatic hydrolysis, which is decay of oligomer or polymer by the addition of molecule of water between monomer units (M):

$$(\mathrm{M})n + n\mathrm{H}_2O \rightarrow n\mathrm{M}.$$

6) Coupled oxidation/reduction of two substances, $S_1$ and $S_2$, with the formation of products, $P_1$ and $P_2$:

$$S_1 - n\mathrm{e}^- \rightarrow P_1$$
$$S_2 + n\mathrm{e}^- \rightarrow P_2.$$

This oxidation–reduction can be coupled with the microbial growth:

$$dS_1/dt = Y dX/dt$$

but can be also independent of the growth rate:

$$dS_1/dt = f(t).$$

## 2.4 The Stages of Biotechnological Process

Any biotechnology includes:

- A preliminary step (upstream processes)
- A cultivation or biogeochemical activity step (core process)
- A posttreatment step (downstream processes)
- Process monitoring and control.

## 2.5 Upstream Processes in Construction Biotechnology

Upstream processes include:

- Pretreatment of raw materials
- Preparation of a medium for cultivation
- Selection, isolation, and collection of microbial strains
- Preparation of inoculum.

## 2.6 Upstream: Pretreatment of Raw Materials

Pretreatment of raw materials in Construction Biotechnology includes:

- Crushing, grinding, sieving, and homogenization of the particles;
- Homogenization (mechanical or by ultrasound) of suspended hydrophobic substances;
- Chemical oxidation of hydrophobic substances by hydrogen peroxide or ozone for better dissolution and oxidation;
- Chemical treatment with alkali or acids to hydrolyze and dissolve nutrients for faster assimilation;
- Chemical treatment with alkali or acids to disinfect raw materials;
- Preliminary washing by surfactants to clean up surface from hydrophobic substances;
- Thermal pretreatment of raw materials for disinfection or faster assimilation;
- Freezing pretreatment of raw biomass for disinfection or faster assimilation after killing of cells by ice crystals.

## 2.7 Upstream: Preparation of a Medium for Cultivation

A medium (pl. *media*) is an artificial environment for the cultivation of microorganisms in the form of solution, suspension, or solid matter. A medium in Construction Biotechnology can be a waste that is undergoing biological treatment. Preparation of the medium includes:

- Mixing
- Addition of concentrated nutrients
- Addition of microelements
- pH and oxidation–reduction potential (ORP) adjustment.

There may be also:

- Addition of indicators of medium quality (pH, ORP, or sterilization quality indicators)
- Sonication of suspension
- Thermal pretreatment of the medium
- Sterilization of the medium
- Conservation of the medium.

To store the medium without deterioration, it can be cooled, frozen, dried, pasteurized, or sterilized.

## 2.8 Upstream: Components of Medium

The major elements of microbial biomass are C, H, O, and N. Carbon content in biomass is usually 50 % (w/w). The average content of dry matter of bacterial cells is as follows: protein, 55 %; RNA, 15 %; polysaccharides, 10 %; lipids, 5 %; DNA, 5 %; and monomers and inorganic ions, 10 %. The water content in the cells is 70–80 %.

The average content of major elements in biomass can be shown by the empirical formula $CH_{1.8}O_{0.5}N_{0.2}$. The sources of C, H, and O are usually carbohydrates (empirical formula $CH_2O$) such as disaccharide saccharose and lactose (empirical formula $C_{12}H_{22}O_{11}$), polysaccharides starch or cellulose (empirical formula of monomer unit is $C_6H_{10}O_5$), alcohols (mainly ethanol $C_2H_5OH$), and organic acids (mainly acetic acid $CH_3COOH$). The sources of N are usually inorganic compounds: salts of ammonium, nitrate, or urea. There is also need for growth of other macronutrients of biomass (P, S, K, Na, Mg, Ca, Fe) and micronutrients (Cr, Co, Cu, Mn, Mo, Ni, Se, W, V, Zn), which are supplied as the salts or as extracts from meat, microbial biomass, and plants. Iron salts, which are very important for life activity of microorganisms, are unstable at neutral pH and form iron hydroxides so ferric or ferrous ions in the medium must be chelated, usually by organic acids, to be protected from oxidation of ferrous ions or hydrolysis of ferrous and ferric ions. Some microorganisms, adapted to live in rich environment and called auxotrophic strains, require an addition to the medium the growth factors, which are organic compounds such as vitamins, amino acids, and nucleosides. The simplest and most economical way to supply all the necessary nutrients for growth is to use digests or extracts of natural ingredients such as meat broth, hydrolyzed soya beans, casein, or a cheapest organic material—biomass of activated sludge of municipal wastewater treatment plants. This rich medium must be supplemented with the source of energy, usually carbohydrate, because the components of the medium extracted from animal or plant biomass are used for the production of a new microbial biomass but not for the production of biological forms of energy used by microbes for the life activities.

In the majority cases, medium is used as a solution or suspension. pH of the medium is adjusted to optimum for growth and biochemical activity value. If the

pH is changed during cultivation due to accumulation of acid or alkaline, pH buffering substances are introduced into medium or titrants such as NaOH or HCl are added automatically to maintain optimum pH during cultivation. Common pH buffers are a mixture of $H_2PO_4^-/HPO_4^{2-}$ salts, $Na_2CO_3/NaHCO_3$ salts, and $CaCO_3$.

If the aeration rate and the content of surfactants (proteins, polysaccharides, and salts of long-chain fatty acids) are high, the formation of foam in bioreactor can destroy cultivation of microorganisms, so the antifoaming substances have to be added to the medium at the beginning or during cultivation.

In the cases of solid-phase fermentation, a medium as a mixture of solid substances with optimum content of water for microbial growth and activity, usually it is within the range from 50 to 80 % (w/w).

The calculation of the medium components can be made using balance of elements. Roughly, the growth yields of aerobic and anaerobic growth using carbohydrates as the sources of carbon and energy are 0.5 and 0.1 g of dry microbial biomass/g of consumed carbohydrate, respectively.

## 2.9 Upstream: Isolation and Selection of Microbial Strain (Pure Culture) for Bioprocess

Unicellular microorganisms can be isolated from nature as a strain (the cells of one colony) or a clone (the cells originating from one cell). Isolation of a pure culture (microbial strain) is usually performed by spreading diluted microbial suspension on a Petri dish with a semisolid medium to produce from 10 to 50 colonies on the dish after several days of cultivation. The cells of one colony are picked up for the next round of cultivation on a semisolid or liquid medium. Some advanced methods can also be used for the isolation of a pure microbial culture:

- Mechanical separation of cells by micromanipulator;
- Sorting of cells or microbeads with immobilized cell using a flow cytometer;
- Magnetic or immunomagnetic separation of cells;
- Cell chromatography.

## 2.10 Upstream: Acquiring of Microbial Strain from Collection

Known and useful microbial strains are stored in the culture collections, which are the centers for deposition and storage of strains for further study and use. Deposition of the commercially important strain in the national collection is a mandatory condition for the strain patenting and protection of the patent rights. The universities and national collections contain also type strains to serve as the

standards of the characteristics attributed to a particular species. The best-known national culture collections are, for example, American Type Culture Collection (ATCC) and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). These collections are a nonprofit bioresource centers that provides biological products, technical services, and educational programs to industry, government, and academic organizations around the world. Their functions are to acquire, authenticate, preserve, develop, and distribute biological materials, information, technology, intellectual property, and standards for the advancement, validation, and application of scientific knowledge. A strain is identified by its assigned number in a microbial collection and the name of the species. For example, name "*Bacillus subtilis* ATCC 6633" refers to a strain of the species *Bacillus subtilis* that is stored under number 6333 in the ATCC.

However, the microbial strain acquired from the culture collection cannot be adapted to the conditions of the real bioprocess. Therefore, an isolation of a strain from the site of application or from the site with the conditions similar to the conditions in the site of application could be often a better option than acquiring the strain from the culture collection.

## 2.11 Upstream: Selection of an Enrichment Culture

The autoselection of an enrichment culture refers to the autoselection of a microbial community with one or several dominant strains that are preferably growing in the bioreactor as a result of the selection pressure, i.e., of selective conditions of medium and cultivation for these strains. Enrichment culture can be used in Construction Biotechnology for the performance of large-scale construction bioprocesses in situ. Selection pressure for the production of an enrichment culture is as follows: (1) source(s) of energy; (2) source of carbon; (3) source of nitrogen; (4) source of phosphorus; (5) temperature; (6) pH; (7) presence of a specific antibiotic in the medium; (8) concentration of dissolved oxygen or oxidation–reduction potential (ORP); (9) osmotic pressure of the medium; (10) spectrum and intensity of light; (11) settling rate, etc.

The mechanisms for autoselection of an enrichment culture are as follows:

1. Faster or more efficient growth of one or several strains (positive growth-related autoselection).
2. Faster or more efficient biochemical functions of one or several strains (positive metabolic autoselection).
3. Slower or less efficient growth of one or several strains (negative growth-related autoselection).
4. Slower or less efficient biochemical functions (negative metabolic autoselection).
5. Better survival under harmful conditions (positive survival-related autoselection).

6. Weaker resistance to some environmental factors (negative survival-related autoselection).
7. Stronger or more specific adherence of cells to surface (positive or negative cell adherence-related autoselection).

The selected properties of an enrichment culture can be genetically unstable and may disappear after several generations of cells in case when the selection pressure is absent. This instability is known for cell surface hydrophobicity, which can be significantly increased using the retention of cells on a hydrophobic carrier over several cell generations. Conversely, cell surface hydrophobicity can decrease for several cell generations if there is no selection pressure (retention on hydrophobic carrier) in the medium (Stabnikova 2000). This feature is important in case of microbial construction processes on hydrophobic surface.

## 2.12 Upstream: Selection of an Ecosystem

Main property of ecosystem is either physical boundary such as air–water or water–soil interphases or chemical boundary such as steep gradient of concentrations separating this ecosystem from other parts of environment. The selection of an artificial microbial ecosystem is similar to the selection of an enrichment culture, but (1) it must be within system separated by the boundary; and (2) there may be several changed selective factors (selection pressures) ensuring dominance of several microbial communities with different, even alternative, physiological functions. For example, the aerobic and anaerobic microbial communities can simultaneously exist in a selected artificial microbial ecosystem due to the presence of both aerobic and anaerobic conditions in the ecosystem. The external and self-developed internal selective pressure is a major factor of self-formation of an artificial microbial ecosystem. The selection mechanisms for an artificial ecosystem can be positive or negative interactions such as commensalistic, mutualistic, amensalistic, antagonistic, or parasitic relationships between the microbial communities of ecosystem.

## 2.13 Upstream: Construction of Genetically Engineered Microorganisms

Microorganisms that are suitable for the production of construction materials or construction processes in situ can be isolated from the natural environment. However, their ability can be modified and amplified by artificial alteration of the genetic (inherited) properties of these microorganisms. Natural recombination of the genes occurs during DNA replication and cell reproduction. It includes the breakage and rejoining of chromosomal DNA molecules and plasmids

(self-replicating mini-chromosomes containing several genes). Recombinant DNA techniques and genetic engineering can create new, artificial combinations of genes and increase the number of desired genes in the cell. Genetic engineering of recombinant microbial strains for Construction Biotechnology involves typically the following steps:

- DNA is extracted from a cell and is cut into small sequences by specific enzymes.
- Small sequences of DNA are introduced into DNA vector (either a virus or a plasmid).
- A vector is transferred into the cell and self-replicated to produce multiple copies of the introduced genes.
- Cells with newly acquired genes are selected based on their specified activity, e.g., the production of defined enzymes, and the stability of the acquired genes.

The main problem in an application of recombinant strains is maintaining stability of the plasmids in these strains. Other technological and public concerns include the risk of the application and release of genetically modified microorganisms into the environment.

## 2.14 Upstream: Preparation of Inoculum

The organisms used to start the core process are called the *inoculum* by microbiologists, or sometime as the "seeds" by engineers. The inoculum could be a suspended, frozen, dried, or cooled biomass of microorganisms. Cultivation of the inoculum is performed usually in batch process mode. The volume of inoculum for microbial batch cultivation in the bioreactor must be about 5–20 % of the bioreactor volume. However, for application of microorganisms in the construction process the dosage of inoculum depends on the process conditions and properties of microorganisms and could be as small as few grams of biomass per 1 $m^3$ of soil/particles. Inoculum for biotreatment of soil can be selected using following microbiological and molecular biological methods:

- Obtaining and testing of the microbial strains from national collections of microorganisms, for example American Type Culture Collection (ATCC, USA) or German Collection of Microorganisms and Cell Cultures (DSMZ, Germany).
- Isolation, identification, and testing of wild strains from natural sites with environmental conditions close to the conditions that are needed for the biotreatment, for example, with high salinity, high or low temperature, aerobic or anaerobic conditions, alkaline or acid pH. However, many bacteria are pathogenic (causing diseases) for human, animal, and plants. Therefore, biosafety of biotechnological process is always an important issue and only nonpathogenic isolated strains of bacteria can be used for Construction Biotechnology applications.

- The biomass of the isolated and identified strain can be produced in bioreactor and used as a starter culture for nonaseptic environmental processes for faster start-up and increased biosafety (Ivanov et al. 2006b; Ivanov and Tay 2006a, b).
- Autoselection in continuous culture, selection of the mutants, and construction of the recombinant microbial strains from wild strains for the biotreatment. However, there are many restrictions on the applications of recombinant microbial strains so they can be used mainly for industrial production of such construction materials as polysaccharides or bioplastic.
- Selection and testing of suspended enrichment cultures using such selective conditions (selection pressure) as source of energy, carbon, nitrogen and phosphorus, temperature, pH, salinity (osmotic pressure), concentration of heavy metals, concentration of dissolved oxygen, and spectrum and intensity of light (for photosynthetic microorganisms). Some autoselected features of the enrichment culture can be genetically unstable and could disappear after several generations when the selection pressure will be absent.
- Selection and testing of aggregated enrichment cultures, such as flocs, biofilms, and granules using such selective pressure as settling rate of microbial aggregates and adhesion of cells to solid surface. An example is the formation of bacterial cells aggregates that cannot penetrate inside sand, settled onto the surface of sand and formed calcite crust (Stabnikov et al. 2011; Chu et al. 2012a).

## 2.15 Core Biotechnological Process: Batch Cultivation of Microorganisms in Bioreactor

The volume of bioreactor varies from 100 mL in laboratory to several thousand cubic meters in industry. The most useful aseptic bioreactors in industrial biotechnology are 50–100 $m^3$ aerobic and anaerobic tanks with complete mixing and the devices for the bioprocess monitoring and control. Cultivation in the bioreactors of bigger volume is performed in nonaseptic conditions, i.e., without sterilization of medium and equipment.

For cultivation of microorganisms in industrial biotechnological plant, bioreactor is cleaned, washed, filled with medium, sterilized in the case of aseptic cultivation, the temperature, aeration rate, stirring rate, and monitoring system are adjustment to the required parameters. Then, suspension of inoculum for microbial batch cultivation is introduced into the bioreactor in a dosage of 5–20 % of the bioreactor volume.

The system of microbial cultivation with gas exchange only is called as batch culture and the system with gas and liquid exchange is called as continuous culture. The following phases can be separated in batch culture (Fig. 2.1): lag phase (adaptation of the cells), log phase (exponential growth), stationary phase (absence of the growth), and death phase.

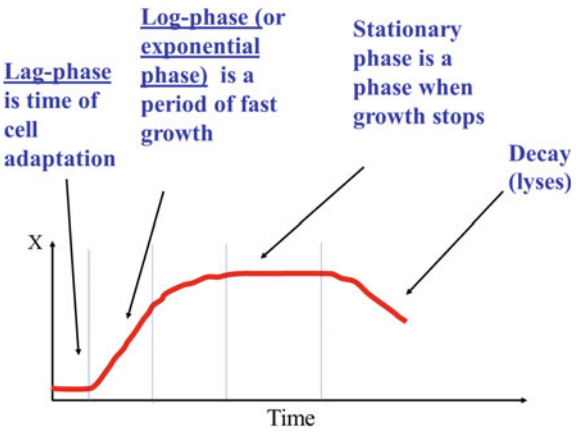


**Fig. 2.1** Phases of growth in batch culture

To minimize duration of lag phase, the conditions in bioreactor must be close to optimal values and the inoculum must be in the state of the fast growth. The rate of exponential growth of microbial biomass diminished after short period of maximum growth rate due to exhaustion of nutrients or accumulation of products inhibiting growth. Stationary phase can be short or long, depending on the properties of microbial strain and conditions of cultivation. Gram-negative bacteria usually dying after stationary phase, but gram-positive bacteria and fungi usually produce anabiotic forms of cells like endo- and exospores and cysts that can survive long-term starvation, presence of toxic compounds, low activity of water/high osmotic pressure, drying, and high temperature.

## 2.16 Core Biotechnological Process: Batch Cultivation of Introduced Microorganisms in Soil

Growth of microorganisms introduced as suspension into porous soil also can be considered as a batch cultivation process with the similar specific phases, but there are following features of this cultivation:

- Inoculum cannot be bigger than 0.1–1.0 % w/v (=1–10 kg of dry biomass/$m^3$ of soil) because of economic reasons. Therefore, lag phase can be relatively long.
- Conditions in soil—temperature, pH, and content of heavy metals—are not optimal for microbial growth, so biomass growth rate and biogeochemical activity of microorganisms in soil will be significantly lower than in industrial or laboratory bioreactor.
- Introduction of microorganisms in soil will be accompanied with adsorption of cells on the soil particles. Slow injection of bacterial suspension into the bottom part of the soil column will give the highest concentration of bacterial cells in the bottom part (Fig. 2.2a) because of the cells settling.

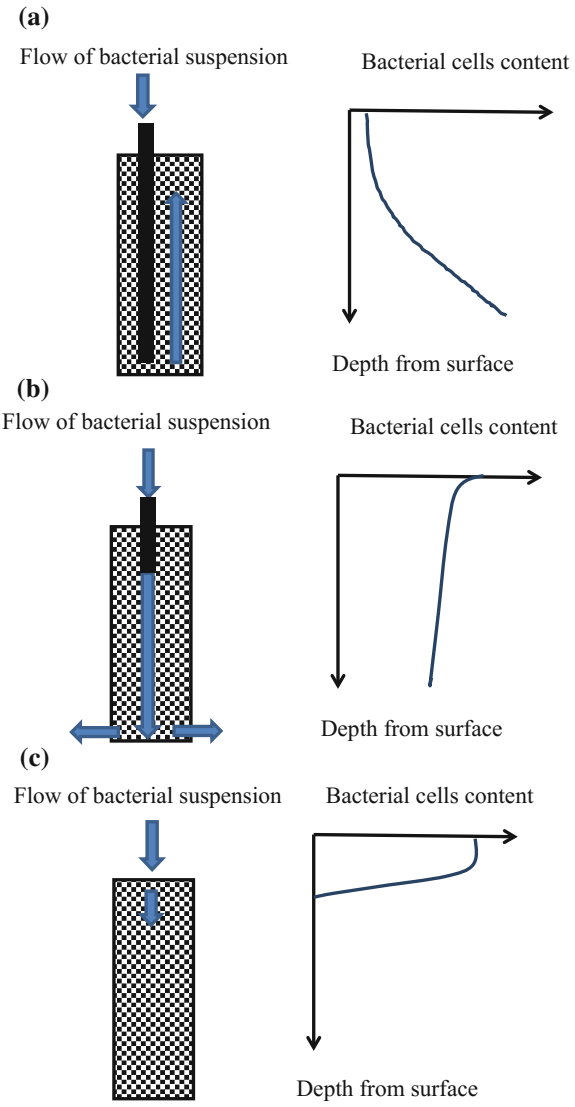


**Fig. 2.2** Effect of injection on the distribution of adhered bacterial cells in soil column

An injection of suspension into the upper part of the soil column will give almost even distribution of bacterial cells in the soil column (Fig. 2.2b) because of fast flow under gravity and fast distribution of bacterial suspension in the volume of the pores. A spraying of small volume of bacterial suspension on the soil surface will produce a surface layer with high concentration of adhered bacterial cells (Fig. 2.2c) because liquid will be delayed in a surface layer of porous soil by the capillary forces.

- Usually, laboratory or industrially grown microorganisms released into soil die; so the cell death rate, cell survivability, or decrease of the biogeochemical activity of cells must be evaluated and accounted in the design of the bioprocess in soil. A rule of the thumb is that applied microorganisms must have some reasonable limits of lifetime or limit their biogeochemical activity in the treated and surrounding areas. This short lifetime can prevent accumulation or spread of unwanted microorganisms in the environment.

## 2.17 Core Biotechnological Process: Batch Cultivation of Indigenous Microorganisms in Soil

In some cases, when soil is rich with indigenous microorganisms having needed biogeochemical function, for example urease activity, soil biotreatment can be performed only by indigenous microorganisms, without preparation and supply of microbial inoculum (Burbank et al. 2011, 2012a, b; Weaver et al. 2011). To enhance the needed biogeochemical function of indigenous microorganisms, soil can be amended with the related reagent. For example, to enhance urease activity of indigenous microorganisms before the biotreatment, urea can be added to soil (Burbank et al. 2011).

However, if microorganisms, used in construction bioprocess, are indigenous it does not mean that they are safe for human, animals, and plants because nonselective conditions of the soil bioprocess, especially in the case of application of nutrients-rich medium, can enhance the proliferation of pathogens or opportunistic pathogens in soil. Additionally, it may take several weeks for ureolytic indigenous groundwater microorganisms to grow and become ureolytically active (Tobler et al. 2011). So, application of known safe strain grown in industrial bioreactors is more preferable than use of unknown indigenous microorganisms.

## 2.18 Core Biotechnological Process: Continuous Cultivation of Microorganisms in Bioreactor

Microbial continuous culture is open for exchange by gasses and liquids. The bioreactors, which are used in continuous cultivation are as follows:

- Complete mixing bioreactor. The most common type of this reactor is a chemostat, where the dilution rate ($D$) is maintained constant. $D = F/V$, where $F$ is a flow rate and $V$ is a working volume of the bioreactor. If $D <$ the maximum of specific growth rate ($\mu_{max}$) the stability of the system is maintained due to feedback interactions between the specific growth rate ($\mu$), the concentration of

the substrate ($S$), and the biomass concentration ($X$): increase of $X$ decreases $S$, and then $\mu$, which decreases $X$ at a constant $D$.

- Plug-flow system, whose parameters are constant in time but changed along the length of the reactor; one long plug-flow bioreactor can be replaced by a series of complete mixing reactors connected consecutively.
- Bioreactors with the retention of biomass. In Fixed Biofilm Reactor (FBR), microbial biomass is attached to the carrier/support material with a big specific surface inside FBR. In Membrane Bioreactor (MBR) biomass is retained using membrane filtration inside or outside of MBR and removal from MBR only solution but not biomass. Semi-continuous and Sequencing Batch Reactor (SBR), which is continuous cultivation with the periodical addition of nutrients and removal of suspension. Very often SBR is used for selection of fast settling cell aggregates and retention of biomass aggregates inside SBR due to sedimentation. A sequencing batch reactor retains biomass due to the periodic settling of the biomass followed by removal of the liquid. It sustains a semi-continuous cultivation with the periods of the biomass settling, removal of the liquid (effluent), and addition of fresh medium (influent).

The retention or recycling of biomass in all types of the bioreactors during continuous cultivation ensures the following properties:

- The flow rate through bioreactor can be higher than the specific growth rate of microorganisms so that slow-growing microorganisms can be maintained continuously in the bioreactor.
- The concentration of biomass and the rate of biogeochemical reactions in the bioreactor can be higher than in the chemostat.

## 2.19 Core Biotechnological Process: Continuous Cultivation of Microorganisms in Soil

Growth of microorganisms, during continuous or semi-continuous introduction of inoculum and medium into porous soil and removal or dispersion of effluent in surrounding environment. The porous soil or fractured rocks cannot be considered as complete mixing bioreactor, but conventionally can be considered as a plug-flow bioreactor or fixed biofilm bioreactor. The boundaries of this natural "bioreactor" are determined by the steep gradient of physical properties of soil such as hydraulic conductivity, compressive strength, porosity or chemical concentrations of oxygen, ferrous ions, hydrogen sulfide, values of pH, and oxidation–reduction potential separating for example of aerobic and anaerobic zones in soil and the layers with different properties.

Adsorption of bacterial cells and formation of biofilm on the surface of soil particles or rock fractures are most important stages of this continuous cultivation. Adsorption of bacterial cells depends on the method of injection and velocity of the

medium, which affects the cells distribution pattern in soil. Same as in batch cultivation, an injection of bacterial suspension into the bottom part of the soil column will give the highest concentration of bacterial cells in the bottom part (Fig. 2.2a) in case if the flow velocity is lower than settling velocity of cells. There can be sorption/desorption equilibrium described by Freundlich equation:

$$Q = KC^n$$

where: $K$ and $n$ are the coefficients characterizing adsorption, $Q$ is a mass of adsorbed cells per unit mass of adsorbent (adsorption capacity); $X$ is the concentration of bacterial cells in fluid. Adsorption capacity varies from one to several thousand bacterial cells/mg of soil and depends on physicochemical properties of soil particles and cell surface. Parameters of adsorption $K$ and $n$ can be determined from the slope and intercept on the linear graph derived from the Freundlich equation:

$$\log Q = \log K + n \log X.$$

An injection of suspension into the upper part of the soil column will give the almost even distribution of bacterial cells in the soil column (Fig. 2.2b) in case when downward flow is high and there is no equilibrium between cell sorption and desorption. A spraying of bacterial suspension on the soil surface will produce a surface layer with high concentration of adhered bacterial cells (Fig. 2.2c) because the liquid medium and the bacterial suspension will be delayed in surface layer of the porous soil by capillary forces. The patterns of the biogeochemical activities of cells are complicated by the medium flow, which can be from the bottom or from the top of soil, i.e., as a parallel flow or a counterflow with the flow of bacterial suspension. Continuous flow of the medium through soil increases risk of accumulation or spread of unwanted microorganisms in the environment.

Porous space of soil or fractured rocks contains diverse microenvironments during microbial cultivation. These microenvironments are characterized by the microgradients of chemical concentrations in the micrometer scale, and that are formed due to microbial activity in biofilm. The typical scale of such microbial microenvironments as cell aggregates, biofilms, or microbial mats is between 0.1 and 100 mm. Microbial cells are concentrated on a liquid–solid interphase or a liquid–gas interphase because the concentrations of the nutrients are higher there than in the bulk of liquid. Due to the presence of different microenvironments, the physiological groups of microorganism living there could be also very different, for example anaerobic, facultative anaerobic, microaerophilic, and aerobic microorganisms growing together in one treated volume of soil or fractured rocks. Presence of microenvironments in soil enhances the opportunities for the engineering bioprocesses in soil.

## 2.20 Downstream Processes

Typical downstream processes include

- Separation and concentration of biomass and products from the culture liquid
- Drying/dewatering of the biomass
- Packing/disposal of secondary waste.

## 2.21 Downstream: Separation and Concentration of Biomass

Simplest way for biomass separation and concentration is sedimentation. The sedimentation velocity ($V$) of cells and cell aggregates with diameter $D$ and density dp can be described by Stokes' law:

$$V = D^2\left(d_\mathrm{p} - d_\mathrm{l}\right)/18\,\mu g$$

where $d_\mathrm{l}$ is the liquid density, $\mu$ is the viscosity of the liquid, and $g$ is the gravitational acceleration.

Because of the small size of bacterial cells and their density of 1.04–1.10, which is close to the density of water, their sedimentation rate by gravity settling is too low to be used in practice. Therefore, bacterial cells are separated from culture liquid, and are concentrated by three ways:

- centrifugation with acceleration (centrifugal force) > $5000 \times g$;
- membrane filtration with diameter of pores below 0.2 μm;
- bacterial cells aggregation and settling of the aggregates by gravity.

## 2.22 Downstream: Aggregates of Cells

A multicellular aggregate is formed and separated from its surrounding environment due to:

- Aggregation resulting from hydrophobic forces, electrostatic interactions, or salt bridges.
- A loose polysaccharide or inorganic matrix (iron hydroxide, for example) combining the cells by mechanical embedding, chemical bonds, hydrogen bonds, electrostatic forces, or hydrophobic interactions.
- Formation of mycelia, which is a net of branched cell filaments.
- A polysaccharide matrix with a filamentous frame.

- Coverage by a common sheath of organic (polysaccharides, proteins) or inorganic origin (iron hydroxide, silica, calcium carbonate); a common sheath can also be made from the dead cells of an aggregate (the "skin" of a microbial aggregate).

Bacterial cells can be aggregated by adjustment of pH to 3–5 to minimize zeta potential of cell surface but cells can be inactivated at low pH. Another way for production of bacterial cells aggregates is selection of fast settling flocks or granules with the sizes ranging from 50 μm to several mm using retention of self-formed cell aggregates in enrichment culture. Settling an aggregate of cells for 20–30 min and retaining or recycling of the settling aggregate in a bioreactor is a method for the selection of flocs. Intensive aeration for mechanical compaction of the aggregate by air bubbles, settling of the aggregate for 2 min, and retention of this settling aggregate in the bioreactor is a method for selection of granules. A simple method for selection of cellular aggregates is using the largest colonies grown in semisolid medium in a Petri dish. The largest colonies originated from not one bacterial cell but from an aggregate of several cells.

Cell aggregates are most suitable for the biotreatment of soil surface for soil bioaggregation and formation of soil crust but not for biotreatment of the bulk of soil for its bioclogging and biocementation.

## 2.23 Downstream: Separation and Concentration of Products

Different methods are used for separation and concentration of microbial products from the culture liquid. Depending on the product and its application there may be used:

- precipitation of exopolysaccharides (cement admixtures) and enzymes by ethanol or salts;
- Reverse Osmosis (RO) filtration for concentration of biopolymers;
- Adsorption on Granulated Activated Carbon, aluminum oxide, and hydrophobic sorbents;
- Flotation—concentration in foam formed by the gas microbubbles;
- Evaporation of volatile substances.

Separation and concentration of intracellular enzymes and intracellular accumulated bioplastic polyhydroxyalkanoates (PHAs) requires preliminary disruption of cells using chemical or mechanical treatments of microbial biomass.

## 2.24 Downstream: Drying, Mixing and Packing of Biotechnological Products

Drying of cells requires a soft regime to avoid inactivation of cells or enzymes. Best but the most expensive way is freeze-drying, but soft regime of spray drying at temperature of air below 50–60 °C could be also suitable for some microbial biomass and products. The very important point for practical applications is that dry biomass can be easily suspended as the separated cells or it forms in suspension the cell aggregates.

Dry biomass or microbial product has to be mixed with chemicals to produce ready to use construction material. Calcium chloride must be used for the mixing in hydrated form to avoid overheating of biomass or enzyme. In the mixtures with cement, the particles of biomass or microbial product must be encapsulated, coated with insoluble polymer to protect biosubstances from inactivation under high pH of cement.

All dry biomaterials are highly hygroscopic substances, attracting and holding water, so they must be sealed from air. Shelf-life of biomaterials is usually few months, but the spores of gram-positive bacteria can be stored for years.

# Chapter 3
# Biotechnological Admixtures for Cement and Mortars

## 3.1 The Types of Biopolymers

Biopolymers are the major components of cell, typically consisting 90 % of dry cell matter. Oligomers combine few monomers. Biopolymer can combine several hundred or thousands of monomers forming chain, branching chain, globular, or layered structure. The major cell monomers are monosaccharides, amino acids, and nucleotides, which are combined by covalent and other chemical bonds forming such polymers as polysaccharides, proteins, ribonucleic and deoxynucleic acids, respectively. Storage compound, "bacterial fat" polyhydroxybutirate (PHB), is polyester of the molecules of hydroxybutiric acid. Lipids also could be considered as the physico-chemical polymers due to aggregation of lipid molecules by the hydrophobic forces forming cellular membranes and lipid granules containing thousands of molecules of lipids. Microbial cells produce also extracellular polymers, mainly exopolysaccharides, producing under excess of carbon and energy sources and serving for cells as a store of carbon and energy and a protector of cells from drying, heavy metals, and antibiotics.

## 3.2 Structural and Metabolically Active Biopolymers

Biopolymers are performing different functions in a cell:

- Cell metabolism (a network of all biochemical reactions) is performed due to enzymatic activity of proteins (Pr), which are synthesized by ribosomal ribonucleic acids (rRNA) using the information in the messenger ribonucleic acids (mRNA), which is transcribed from deoxyribonucleic acid (DNA) of chromosome. These compounds are active as solution or suspension in intracellular cellular gel.



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- Store of materials and energy in the cells is in the form of glycogen or polyhydroxybutirate granules in prokaryotic cells, starch, and lipids granules in plant and animal cells, and polyphosphate granules in bacterial cell. Extracellular polysaccharides are attached to a cell or concentrated nearby cell. Their main functions are to protect cell from drying and toxic compounds as well as attach cell to solid surface and to other cells. Metabolically active store biopolymers are fast biodegradable substances, for exemption of PHB and lipid because of their hydrophobicity and insolubility in water.
- Structural polymers such as rigid peptidoglycan of bacterial cell wall, chitin of fungal cell wall, cellulose and hemicellulose of plant and fungal cell walls, and intercellular pectin of plants are slow biodegradable substances, which are relatively stable being cell construction materials.

All biopolymers, but especially the storage and structural biopolymers, can be used in construction.

## 3.3 Historical Use of Biopolymers in Construction

Animal biomaterials such as blood, urine, eggs, milk, lard, as well as plant biopolymers such as wood, straw, bark, cactus juice, flour have been used as admixtures from ancient times to improve properties of mortars and plasters. Straw and cattle dung were used and are used even at the present time in rural construction as the composite biomaterials to improve construction properties of clay. Probably, the Aztecs used fermented juice of nopal cactus (*Opuntia ficusindica*) to improve plasticity and water absorption capacity of lime mortar and earthen plasters due to the presence of cellulose fibers, gel polysaccharides, and fermentation products. Extracts of this nopal cactus and water hyacinth are proposed even in our days to enhance viscosity of cement-based materials (León-Martínez et al. 2014; Sathya et al. 2013). The cactus mucilage is a heteropolysaccharide, which as an admixture prevents moisture transport to the interior of lime mortars, increased the plasticity and resistance of Portland cement, and increases the setting times as well as decrease the hydration rate of cement-based materials in the hardened state (Ramírez-Arellanes et al. 2012; Cano-Barrita and Leon-Martínez 2016).

Sticky rice-lime mortar, composite building material containing amylopectin as the major organic component, was developed in ancient China and extensively used in urban constructions (Yang et al. 2010). The amylopectin in the lime mortar was found to act as an inhibitor of the growth of the calcium carbonate crystals and lead to a compact structure with the enhanced performance compared to single-component lime mortar (Yang et al. 2010).

Currently, chemical derivates of plant biopolymers, for example carboxymethylcellulose, carboxymethylcellulose sulfate, or such industrial waste as lignosulfonates

are often used as cement and mortar admixtures for set retarding and increase of plasticity of self-consolidated concrete (Plank 2004; Yuan et al. 2013; Bezerra 2016; Cano-Barrita and Leon-Martínez 2016; Vazquez and Pique 2016).

## 3.4 The Bioadmixtures for Cement

Annual production of cement is several billion tons because it is the universal construction binder producing high compressive strength. Other inorganic binders such as structural gypsum, hydraulic lime, silicates are using in much smaller quantities in the mortars (a composite of a binder and sand). The mechanical property of the mortar or concrete mixture can be changed by an addition of the polymer admixture. The admixtures can accelerate or retard the hydration process and decrease or increase viscosity of the cement paste (Vazquez and Pique 2016). Up to 80 % of concrete is produced with one or more admixtures in some countries (Mehta and Monteiro 2006). Chemical and biological admixtures, mainly microbial extracellular polysaccharides, are using in cement- and gypsum-based materials for dispersing/thickening effects, viscosity enhancement, water retention, set acceleration and retardation, air entrainment, defoaming, hydrophobization, adhesion, and film forming (Plank 2003) to improve such properties of the material as plasticity, water retention, adhesion, shrinkage reduction, flow ability, and stability. The global market of admixtures is estimated at the level of US$15 billion with the share of more than 500 different biological and biodegradable admixtures about 13 % (Plank 2004). Plant and microbially produced polysaccharides are most used as the bioadmixtures.

## 3.5 Applications of Microbial Polysaccharides as Bioadmixtures

The advantage of microbial admixtures is that the biosynthesis rate of the microbial biopolymers is significantly higher, by 2–4 orders of magnitude than that of the plants, and these substances can be produced in industrial scale on biotechnological factories. The major application of microbial biopolymers in construction industry is an addition to concrete and dry-mix mortars. The examples of microbial admixtures that are used in concrete are protein hydrolysates and welan gum; and in case of dry-mix mortar these admixtures are succinoglycan and xanthan gum. The market share of microbial biopolymers is expected to increase because of technological advances and the growing trend to use natural or biodegradable products in building materials (Plank 2004; Ramesh et al. 2010). These microbial products of biotechnological industry are mainly viscosity-enhancing admixtures used to achieve high resistance to segregation of concrete. These biotechnological admixtures are usually microbial polysaccharides as shown in Table 3.1.

**Table 3.1** Microbial polysaccharides as the major biotechnological admixtures used in building materials (based on Plank 2003, 2004; Mun 2007; Fytili and Zabaniotou 2008; Pacheco-Torgal and Jalali 2011; Pei et al. 2013; Isik and Ozkul 2014; Bezerra 2016; Cano-Barrita and Leon-Martínez 2016; Vazquez and Pique 2016)

| Admixture | Function, applications, and dosage | Estimated price, US$ |
|---|---|---|
| Xanthan gum | Set retarder for self-consolidated concrete, floor screeds, paints, 0.2–0.5 %. Extracellular polysaccharide produced by bacterial plant pathogen *Xantomonas campestris* from molasses or hydrolyzed starch. Xanthan is widely used in many industries, including food industry | 2000–5000 |
| Welan gum | Set retarder for self-consolidated concrete controlling segregation on highly fluid mixtures in very dosages from 0.01 to 0.1 % by weight of cement without affecting the final fluidity of the mixture. Anionic extracellular polysaccharide produced by *Alcaligenes* sp. from molasses | 2000–5000 |
| Diutan gum | Viscosity-Modifying Agent (VMA) for cementitious materials controlling flow, segregation, sedimentation, and bleed. Diutan develops a higher degree of pseudoplasticity than other polymeric rheology modifiers. Alkaline solutions containing diutan gum have better stability than those of xanthan gum | 1500–2000 |
| Scleroglucan | Thermostable viscosifier (no loss of viscosity at 90 °C for 500 days), similar to xanthan gum | 2000–5000 |
| Succinoglycan | High shear-thinning behavior with temperature-induced viscosity breakback for self-leveling compounds, soil stabilization, 1–15 g/L of water | 2000–5000 |
| Curdlan gum | Viscosifier (thickener), set retarder for self-consolidated concrete, up to 10 g/L of water; can absorb water about 100 times | 1000–10000 |
| Polyaspartic acid | Biodegradable dispersant, inhibitor of corrosion in concrete, air-entraining agent for concrete or mortar, set retarder for gypsum | 1000–10000 |
| Sodium alginate | Stabilizer, thickener, and emulsifier | 2000–10000 |
| Carrageenan | Foam for protecting freshly poured concrete from premature drying during highway construction | 2000–10000 |
| Dextran | Admixture to Portland cement, self-leveling grouts, fresh or saltwater oil well cement slurries, microfine cements improving flow resistance (rheology-modified additive) | 3000–9000 |
| Pullulan | Viscosifier (thickener), set retarder for self-consolidated concrete | 5000–20000 |
| Sewage sludge | Viscosifier (thickener), set retarder for self-consolidated concrete<br>Production of sintered lightweight aggregated for nonstructural concrete (clay: sewage sludge ratio is from 1:1 to 1:3 by mass)<br>Co-combustion of sewage sludge in cement manufacturing (5 % of the clinker production capacity) | 0 |
| Bacterial cell walls | Microstructured filler for concrete increasing compressive strengths of concrete by 15 % and decreasing its porosity; 0.03–3.3 % by mass | 2000–10000 |

## 3.6 Effect of Biopolymers on Cement Hydration

The admixtures affecting the first hours of cement hydration during the dormant period, when cement has no strength yet and can be manipulated (Vazquez and Pique 2016). During this period, newly formed calcium silicate hydrate and calcium aluminate hydrate form a shell around the clinker grain of cement reducing diffusion of water to anhydrous cement surface (Odler 1998). Water transfer to the unsaturated surface is driven most significantly by capillary suction, which depends on fluid viscosity, so the changes in viscosity affect hydration of the clinker grains. Therefore, an admixture with high molecular weight will increase viscosity and the water-retention capacity of cement paste. It could also be a result of the filling of the cement pores with biopolymer (Vazquez and Pique 2016). That is why water-soluble polysaccharides are strong water reducers, set retarders, and water retention agents for concrete and cement mortars. Hydrophilic polysaccharides, added to the cement paste to the content 3–5 % as the water-retention agents can increase mechanical strength of the hardened material because they keep constant the water/cement ratio, which can be reduced without admixtures due to evaporation, filtration, and water loss. However, hydration delay, depending on the concentration of hydrophilic groups of the admixture, is an undesired secondary effect induced by some water-retention biopolymers (Vazquez and Pique 2016).

## 3.7 Microbial Polysaccharides as Viscosity-Modifying Admixtures

Self-compacting cement grouts, which are able to flow and consolidate under their own weight without any compaction, require viscosity-modifying admixture. These cement grouts are used for ground treatment, repair of concrete, reduction of rock or soil permeability, rock and oil well grouting because of the ability to penetrate voids and cracks (Vazquez and Pique 2016). The mode of action of viscosity modifiers depends on shear force and concentration of biopolymer. At low rates of shear and above a critical polymer concentration, the long intertwined and entangled chains of biopolymer will increase the viscosity. However, at high shear rate, the intertwined chains would break and align along the direction of the flow, decreasing the viscosity. It is known for cellulose-based viscosity modifiers and polysaccharides of bacterial cell walls (Saric-Coric et al. 2003; Pei et al. 2015).

It was shown in several papers that welan gun affects the hydration retardation of the tricalcium aluminate hindering its reaction with water and allowing longer concrete workability but the results with diutan gum and xanthan gum were not that promising (Bezerra 2016). It was shown also that the inclusion of biopolymers does not increase the compressive strength of a concrete but can affect other properties such as durability, low permeability, and high modulus of elasticity (Bezerra 2016).

## 3.8 Pseudoplasticity of Microbial Polysaccharides

The viscosity-modifying agents (VMA) with anionic charges, such as microbial exopolysaccharides welan, diutan, or xanthan bind the positively charged cement particles and, as a result, increase the viscosity (Vazquez and Pique 2016). For example, for the cement-based grout water-to-binder ratio 0.40 and the dosages of either diutan gum or welan gum from 0.02 to 0.08 % by mass of binder exhibited high apparent viscosity values at low shear rates which were attributed to the entanglement and intertwining of biopolymer chains at low shear rate and association of water between adjacent chains. For a given dosage, diutan gum showed a high apparent viscosity than welan, which could be attributed to the molecular weight and to the long-side chain of diutan gum leading to greater entanglement and intertwining (Sonebi 2006). However, diutan gum is exhibited high pseudoplasticity, which is characterized by viscosity, which increases and decreases in response to the removal and application of shear. Pseudoplastic fluids can flow but can also stabilizing setting components. Destruction of the bonds between the polymer chains resulting in a reduction in the viscosity is also known for chemically produced cellulose-based viscosity modifiers agents (Saric-Coric et al. 2003; Pei et al. 2015). Addition of sodium gluconate, which a monomer, has approximately same effect as anionic polymers: adsorption of gluconate ions on the surface of cement particles and formation of calcium gluconate shell on surface reduce hydration, improving dispersion of the particles, and their flowability so that lower w/c ratio can be used for the defined level of concrete strength.

## 3.9 Biotechnological Water and Permeability Reducers

Microbial polysaccharides, which are viscosity–modifying admixtures, are also water reducers, i.e., substances that allow diminish water-to-cement (w/c) ratio. Lower w/c increases mechanical strength of concrete but cannot be lower than some level, because w/c ratio determines also flowability of cement paste. So, an addition of water reducer, which is usually anionic polymer, to cement paste increases the dispersion of cement particles due to adsorption of the surface and forming a stronger repulsive force at the surface—liquid interface of the cement particles. This improves the flowability of cement-based material. Therefore, a lower w/c ratio can be used, which increases mechanical strength of concrete (Vazquez and Pique 2016).

Major water reducers are lignosulfonates, which are wastes of the paper production. They used in dosages from 0.1 to 0.3 % by weight of cement (Darweesh 2016). However, when anionic microbial polysaccharides are used as VMAs, they can also disperse cement particles if viscosity is low, and this dispersion will produce water reducing effect.

Permeability reducers are very fine and inert powders that are added in sufficient amounts to the fresh cement and/or concrete to reduce the residual pore volume. These are very fine particulate materials or air-entraining substances such as lignosulphonates and anionic surfactants (Darweesh 2016). So, it could be expected that good permeability reducer will be sewage sludge biomass, which is biomass of bacterial cells with size about 1–2 μm. However, addition of fine particles could be of benefit only in the concrete mix with low cement content but in the cement-rich mixes the effect could be the reverse, because the addition of fine particles could increase the water requirement leading to a less dense and lower strength concrete (Darweesh 2016).

## 3.10 Industrial Biotechnology Wastes as Admixture

Wastes of industrial biotechnology such as biomass after production of antibiotics, alcohol fermentation, and other types of microbial biomass contains different biopolymers and could be used as cement admixtures (Bolobova and Kondashchenko 2000; Martirena et al. 2016). Probably, anaerobic sewage sludge and excessive activated sludge of municipal wastewater treatment plants, which are mainly biomass of microorganisms, can be used also as cement admixtures. The objections for these applications can be high contents of heavy metals in sewage sludge and persistent organic compounds, which are adsorbed on the surface of microbial cells.

Our experiments with the addition of pure linear (xanthan, DNA), branched (amylopectin), or globular (albumin) biopolymers to Portland cement showed that even small addition of 0.1 % (w/w) of these hydrophilic biopolymers changed the strength of concrete. It was higher with an addition of biopolymers than in control after three days but was lower than in control after seven days, probably because a highly hydrophilic biopolymer on the surface or in the capillaries of cement grains hindered their hydration. These biopolymers are major components of sewage sludge and activated sludge wastes, so several million tons of these materials are available to be used as the cement admixtures.

## 3.11 Biotechnological Production of Polysaccharide Admixtures

Microbial extracellular polysaccharides are using in cement-based materials for dispersing, thickening, water retention, set retardation, air entrainment, and film formation. The market of microbial admixtures is estimated at the level about US$2 billion (Plank 2004). The major producers are Gram-negative bacteria from the genera *Xanthomonas* and *Alcaligenes*, but the spectrum of industrially available producers is permanently increasing (Table 3.2). The most commonly used

**Table 3.2** Production of the major biotechnological admixtures used in building materials (based on Plank 2003, 2004; Mun 2007; Fytili and Zabaniotou 2008; Pacheco-Torgal and Jalali 2011; Pei et al. 2013; Kaur et al. 2014; Cano-Barrita and Leon-Martínez 2016)

| Admixture | Chemical type | Biotechnological process |
|---|---|---|
| Sodium gluconate | Organic salt | Biooxidation of glucose with production of gluconic acid by fungi *Aspergillus niger* or the yeast-like *Aureobasidium pullulans* |
| Xanthan gum | Anionic Polysaccharide | Biosynthesis by bacteria *Xanthomonas campestris.* Xanthan is produced mainly by Cargill, Staley (Tate and Lyle), CP Kelco, and Danisco |
| Welan gum | Anionic polysaccharide | Biosynthesis by bacteria *Alcaligenes* sp. |
| Diutan gum | Anionic heteropolysaccharide | Biosynthesis by bacteria of *Sphingomonas* sp. |
| Scleroglucan | Polysaccharide | Biosynthesis by fungi from the genera *Sclerotium, Corticium, Sclerotinia*, and *Stromatinia* |
| Succinoglycan | Polysaccharide | Biosynthesis by bacteria *Rhizobium meliloti* or *Agrobacterium tumefaciens* |
| Curdlan gum | Polysaccharide | Biosynthesis by bacteria *Agrobacterium* sp. or *Alcaligenes faecalis* |
| Polyaspartic acid | Polyanionic polyaminoacid | Chemical synthesis |
| Sodium alginate | Anionic polysaccharide | Biosynthesis by *Pseudomonas aeruginosa* or *Azotobacter vinelandii*. Extraction from brown seaweeds |
| Carrageenan | Linear sulfated polysaccharide | Extraction from plants or red seaweeds |
| Dextran | Polysaccharide | Microbial synthesis by *Leuconostoc mesenteroides* or *Streptococcus mutans* |
| Pullulan | Polysaccharide | Biosynthesis by fungi *Auerobasidium pullulans* |
| Sewage sludge | Mixture of biopolymers | Waste biomass of municipal wastewater treatment plants |
| Sewage sludge ash | Incineration ash of sewage sludge | Waste of incineration plants |
| Bacterial cell walls | Structural polysaccharides and proteins | Aerobic cultivation of bacteria *Bacillus subtilis* could be produced from sewage sludge |

polymers in cement-based materials from microbial production are welan, diutan, and xanthan gums (Cano-Barrita and Leon-Martínez 2016) (Fig. 3.1).

Promising exopolysaccharide ethapolan can be produced from ethanol, vegetable oil, and other organic substances using bacteria from the genus *Acinetobacter* (Pirog et al. 2003).

All microbial polysaccharides are producing by conventional biotechnology including the following steps: (1) preparation of microbial inoculum; (2) aseptic cultivation in fermenters, (3) separation of bacterial biomass if it is possible from

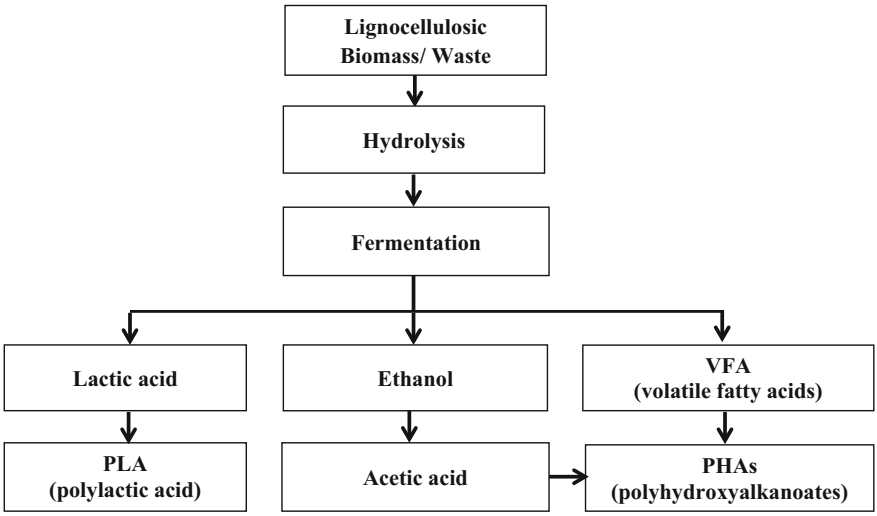


**Fig. 3.1** Schematics of biotechnological production of PLA and PHA bioplastics at a lignocellulosic biorefinery

viscous medium after cultivation; (4) concentration of polysaccharides, usually by precipitation after addition of ethanol; (5) separation and drying of the precipitated polysaccharide. All these steps are relatively expensive, so the cost of product could be at the levels ranging from US$2000 to US$20000 per 1 metric ton.

## 3.12 Low Cost Biotechnological Admixtures

The ways to produce cheap admixtures could be either nonaseptic cultivation on organic cheap materials or extraction of biopolymers from dry sewage sludge or dewatered dry sewage sludge of municipal wastewater treatment plants. Sewage sludge is used in cement industry (Mun 2007; Fytili and Zabaniotou 2008). Up to 10 % of dry sewage sludge can be added to concrete for use in certain very specific applications (Valls et al. 2004). In majority cases, it is considered just as a way for disposal of sewage sludge.

However, this organic waste is mainly biomass of anaerobic bacteria and archaea growing in an anaerobic digester of activated sludge. This material contains various biopolymers such as linear and branched polysaccharides, globular proteins and rRNA, and linear chains of DNA and mRNA. Our experiments with the addition of pure linear (xanthan, DNA), branched (amylopectin), or globular (albumin) biopolymers to Portland cement showed that even addition of 0.1 % of these hydrophilic biopolymers changed the strength of concrete. It was higher with an addition of biopolymers than in control after 3 days but was lower than in control

after 7 days, probably because a thin layer of the hydrophilic biopolymer on the cement grain hindered its hydration. So, it could be possible to expect thickening, water retention, set retardation, and air entrainment effects in concrete using admixtures of biopolymers extracted from sewage sludge.

## 3.13 Biotechnological Production of Biopolymers on Biorefineries

Production of polysaccharide admixtures could be more sustainable if it will be performed on the biorefinery. Biorefinery is a facility for industrial production of different chemical products, first of all a fuel, based on biotransformation of million tons of renewable biomass sources (Kamm et al. 2005). This raw material could be biomass of plants like starch of corn, sorghum and other crops, oils of terrestrial plants and aquatic algae, cellulose and hemicellulose of trees and grasses. The common products of any fermentation on biorefinery or pyrolytic transformation of wood to fuel—ethanol, acetic acid, and hydrogen—are the most useful substances for the production of biocements, biogrouts, bioplastics, and polysaccharide admixtures. Ethanol is one of the best sources of carbon and energy for the production of polysaccharide admixtures.

# Chapter 4
# Construction Biotechnological Plastics

## 4.1 Bio-Based and Biodegradable Plastics

Historically, biopolymers of timber were the first construction materials that were replaced later for stronger and durable concrete and bricks. Petrochemical plastics are also important construction materials at present. The building and construction market consumes about 23 % of produced plastics (Storz and Vorlop 2013). However, concrete, bricks, and construction plastics consume big quantity of energy for their production and remain as not usable construction wastes consuming land for disposal.

Therefore, current trend on the production and use of bio-based or biodegradable materials, biopolymers, and bioplastics in construction industry is permanently increasing (Plank 2004; Ramesh et al. 2010; Pacheco-Torgal and Labrincha 2013a, b). Three basic groups of these materials are (modified from La Rosa 2016)

- Biodegradable petrochemical polymers;
- Nonbiodegradable bio-based polymers;
- Biodegradable biotechnologically produced biopolymers.

The use of degradable and nondegradable bioplastics such the composites of starch, lignin, lignosulfonates, and natural cellulose-containing fibers with thermoplastics can increase environmental and economic sustainability of construction industry because the composite bio-based plastics are made mainly from the renewable sources. However, the production of starch-based biodegraded composites consumes substances, which can be used as a food or feed. The lignin-based plastic composites, for example ARBOFORM® and ARBOBLEND® (Nagele et al. 2002), are not biodegradable at all, so they must be incinerated or landfilled after use.



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## 4.2 Biotechnologically Produced Biodegradable Bioplastics

Use of biodegradable plastics in construction could diminish expenses for the demolition and disposal of the construction wastes. It can also increase both environmental and economic sustainability of construction industry because the biotechnologically produced and biodegradable plastics are low embodied energy building materials, they are made from the renewable sources, and they can be finally composted to produce soil fertilizer instead of landfilling or incineration.

Most available types of the biotechnologically produced biodegradable plastics (further bioplastics) for construction industry are polyhydroxyalkanoates (PHAs), especially poly-3-hydroxybutyrate (PHB) with monomer formula (–OCH($CH_3$)–$CH_2$–C(O)–) and polyhydroxyvalerate (PHV) with monomer formula (–OCH($CH_2CH_3$)–$CH_2$–C(O)–). The content of PHAs in dry biomass of some microorganisms can be up to 80 % (w/w). These PHAs can be extracted from the bacterial biomass and used in practice as bioplastic. Its melting temperature is 160–180 °C and tensile strength is 24–40 MPa. Elasticity of the PHAs, which is expressed as elongation at break, is from 3 to 142 % and depends on the content of PHV in PHAs. These mechanical properties are comparable with the properties of petroleum-based thermoplastics (Braunegg et al. 1998; Castilho et al. 2009; Sudesh et al. 2000; Sudesh and Abe 2010; Volova 2004; DeMarco 2005; Khanna and Srivastava 2005; Lenz and Marchessault 2005).

Another type of available bioplastic for construction industry is polylactic acid/poly-L-lactid (PLA or PLLA), which is manufactured by chemical polymerization of lactic acid produced by anaerobic lactic acid bacteria from mono- or oligosaccharides. PLA/PLLA is applicable in many industries and medicine (Ebnesajjad 2012). However, PLA/PLLA is a pure biotechnological and chemical product, so it is more expensive material than crude PHAs and polypropylene (PP) or polyvinylchloride (PVC) but cheaper than pure PHAs.

Currently, use of biotechnologically produced biodegradable bioplastics in construction is just 2500 tons while agriculture and horticulture consumes 84,000 tons, catering requires 101,000 tons; automotive applications consumes 130,000 tons, bottles production and packaging materials are the major consumers of bio-based plastics: 450,000 and 510,000, respectively (European Bioplastics, Institute of bioplastics and Biocomposites; http://en.european-bioplastics.org; Gkaidatzis 2014). The reason of low scale applications of bio-based plastic in construction is high cost of the bioplastics available on the market.

## 4.3 Biotechnological Production of Biodegradable Bioplastics for Construction

Use of biodegradable bioplastics in construction industry can diminish

- the cost of excavation of temporarily constructions because many biodegradable constructions can be left in soil;
- the disposal cost of the construction wastes;
- the consumption of nonrenewable sources, thus increasing environmental and economic sustainability.

Meanwhile, the cost of bioplastics produced aseptically in fermenters is usually several times higher than the cost of petrochemical-based plastics, so the reduction of the bioplastic production costs using cheap raw materials and technological innovations is still essential for the bioplastic industry and applications. Most available types of bioplastics for construction industry are polyhydroxyalkanoates (PHAs), which are polyesters that can be accumulated up to 80 % of dry bacterial biomass as a storage compound. Accumulated PHAs can be extracted from bacterial biomass, and can be used in practice as bioplastic (Lowell and Rohwedder 1974; Braunegg et al. 1998; Sudesh et al. 2000; Volova 2004; DeMarco 2005; Khanna and Srivastava 2005; Lenz and Marchessault 2005; Castilho et al. 2009; Sudesh and Abe 2010).

The following options for raw materials, biotechnology of production, and applications of bioplastic can help to diminish the cost of the bioplastic PHAs.

1. Use of cheap raw materials (Serafim et al. 2008) such as organic fraction of municipal solid wastes, liquid wastes of municipal wastewater treatment plants, food-processing waste, or agricultural wastes such as unbaled straw; corn cobs, stalks and leaves (corn stover); silage effluent; horticulture residuals; farm yard manure; coconut fronds; husks and shells; coffee hulls and husks; cotton (stalks); nut shells; rice hull, husk, straw, and stalks; and sugarcane bagasse. Globally, 140 billion metric tons of biomass is generated annually from agriculture, which is equivalent to approximately 50 billion tons of oil. So, biomass wastes have attractive potentials for large-scale industries and community-level enterprises (UNEP 2009).
2. Batch or continuous nonaseptic cultivation for biosynthesis of bioplastic by mixed bacterial culture (Yu et al. 1999; Lu 2007; Salehizadeh and Van Loosdrecht 2004).
3. Production of crude bioplastic for construction industry and agriculture avoiding its concentration and extraction of bioplastic using chemical treatment, filtration, centrifugation, or flotation.

## 4.4 Cost-Efficient Production of PHAs

The production of biodegradable plastics can provide many benefits to both biotechnological and construction industries and to environment. However, the following options for raw materials, biotechnology of production, and applications of bioplastic can help to diminish the embodied energy and cost of the bioplastics. Aseptic cultivation of microorganisms requires thermal sterilization of all materials and equipment and is energy-consuming process. So, batch or continuous nonaseptic cultivation of mixed bacterial culture accumulating PHAs or lactic acid is cost-effective solution in the production of construction bioplastic. Third point, which is also essential for the cost-efficient production of PHAs for construction industry, is avoiding of its extraction from microbial biomass using chemical treatment, filtration, centrifugation, or flotation.

For the biosynthesis of PHAs under nonaseptic conditions, organic wastes can be converted to organic acids through acidogenic fermentation of organics, and then organic acids can be converted to PHAs (Yu 2006). Most typical material balance of acidogenic fermentation is as follows (Madigan et al. 2014):

$$\mathrm{C_6H_{12}O_6 + 0.82H_2O \rightarrow 1.13CH_3COOH + 0.35C_2H_5COOH + 0.26C_3H_7COOH + 1.67CO_2 + 2.47H_2} \tag{4.1}$$

where $C_6H_{12}O_6$ is a monomer of cellulose, $CH_3COOH$, $C_2H_5COOH$, and $C_3H_7COOH$ are acetic, propionic, and butyric acids, respectively.

The pH of this process must be controlled by titrant, typically NaOH, or using addition of protein-containing waste because the pH drops below 5.5 during acidogenic fermentation of carbohydrate-rich wastes (Barlaz et al. 2010), meanwhile optimal pH for acidogens is above 6.0 (Moosbrugger et al. 1993). As shown in our experiments, $CaCO_3$ cannot be used to maintain optimal pH of acidogenesis because concentrations of $Ca^{2+}$ above 0.1 M inhibited acidogenic fermentation.

Batch biosynthesis of bioplastic is simpler but less productive than continuous process, which productivity can be about 1 kg of PHAs/day·$m^3$ of the bioreactor (Ben Rebah et al. 2009). Production of PHAs can be done as semi-continuous cultivation of a mixed culture using a feast–famine cycle comprising a feast phase, and a famine phase in one bioreactor. This cycling process promotes not only accumulation of PHAs in biomass but also selection of PHAs-producing microorganisms (Beun et al. 2006; van Loosdrecht et al. 1997, 2008).

## 4.5 Crude PHAs Composite Material

All known methods of PHAs extraction suffer from a high cost and/or environmental pollution. Therefore, crude bioplastic produced without extraction of PHAs from microbial biomass could be used for construction applications. The major advantage of PHAs for construction applications is biodegradability of bioplastic to carbon dioxide and water for about 1.5 months in anaerobic sewage, 1.5 years in soil, and 6.5 years in seawater (Mergaert et al. 1992; Reddy et al. 2003; Castilho et al. 2009). Dead bacterial biomass with PHAs contains also polysaccharides of cell wall, proteins, polynucleotides, and phospholipids, whose content is about 15, 50, 25, and 10 % of dry biomass without PHAs, respectively, and biodegradation rate is higher than that of PHAs. Therefore, from the point of view of biodegradability there is no need to extract PHAs from biomass but to use dry biomass with PHAs as a crude nanocomposite material for construction purposes. Such nanocomposites should be more flexible and faster biodegradable than extracted PHAs. Sustainability of this biodegradable construction materials is due to: (1) production of bioplastic from renewable sources or even from organic wastes; (2) fast biodegradability of this material under the conditions of landfill or composting, so negative effect of construction waste on environment will be minimized.

One area of applications of such nanocomposite bioplastic from bacterial biomass containing PHAs is the production and use of biodegradable construction materials, which do not require removal and incineration after temporary application. Biodegradable bioplastic foam could be used for insulation walls and partitions, construction of nonstructural (internal) elements such as separating walls and partitions, and for the temporarily constructions that can be landfilled for fast degradation. Other examples of potential application of crude nanocomposite from bacterial biomass and PHAs are construction silts and dust fences that can be landfilled for fast biodegradation or composted as biomass. Biodegradable plastics could be also useful for vertical drains, geotextile, geomembranes, and soil stabilization mats. These materials are used temporarily for soft soil stabilization, filtration, and drainage (Ogbobe et al. 1998), so biodegradability of the material can eliminate the cost of extraction and disposal of the temporal objects.

## 4.6 Biotechnological Production of Polylactic Acid

Biodegradable bioplastic polylactic acid/poly-L-lactid (PLA or PLLA) is produced by chemical polymerization of lactic acid that is synthesized by anaerobic lactic acid bacteria from any source of saccharides, for example from starch of corn or potato, or lactose of whey, or sucrose of the sugar beet or cane. This combined bio- and chemical technology produces a pure bioplastic, which is relatively expensive material that can be applied in construction industry only in special cases, for example, reinforcing fibers, nanocomposites, or biodegradable resin compositions

(Tokiwa and Tsuchiya 2003; Huda et al. 2006; John and Thomas 2008; Bajpai et al. 2013; Faludi et al. 2013; Saba et al. 2015). Price for PLA in 2009 was about 1.9 Euro/kg, while prices for PHAs were 2.4–4.0 Euro/kg, and for PP and PVC they were 1.0–1.1 Euro/kg (Sin et al. 2012). Therefore, PLA can be used for the production of more expensive or more environmentally friendly materials than petrochemical plastics or crude PHAs.

In the case when lactose of whey, saccharose of molasses or glucose of chemically or enzymatically hydrolyzed starch, agro-industrial, or wood processing residues are the major components of feedstock for PLA bioplastic production; these substances can be used for the homofermentative lactic acid fermentation by *Lactobacillus acidophilus, L. amylophilus, L. bulgaricus, L. helveticus,* and *L. salivarius* (Koutinas et al. 2014). Yield of lactic acid could be from 129 to 215 g $L^{-1}$ with the productivity up to 8 g $L^{-1}h^{-1}$, and the cost of 0.47 Euro $kg^{-1}$, especially effective in a continuous system combining lactic acid fermentations with an electrodialysis membrane unit for lactic acid separation (Koutinas et al. 2014). This pure lactic acid further chemically transformed via direct condensation or via its cyclic lactide form to PLA (Sin et al. 2012).

The worldwide biotechnological production of L-lactic acid was about 275,000 tons in 2006 with average annual growth of 10 % (DeJong et al. 2006) and the primary came from large-scale production of PLA, new effective biotechnologies of lactic acid fermentation, and use of inexpensive fermentative substrates, such as dairy products, food and agro-industrial wastes, glycerol, and algal biomass (Abdel-Rahman et al. 2013; Hofvendahl and Hahn–Hägerdal 2000). 136,000 metric tons of pure PLA is producing annually from corn-based sugar raw material as Ingeo plastics and fibers by Nature Works LCC (former Cargill Dow LLC) on the plants in USA and Thailand and about 140,000 tons annually are produced by Zhejiang Hisun Biomaterials Co. in China, and PURAC Biochem on the plants in The Netherlands, USA, Spain, Brasil, Thailand. The manufacturing cost of lactic acid monomer must be less than $0.55/kg because the selling price of PLA should decrease from its present price of $2.20/kg (Golden et al. 2015).

## 4.7 Biorefinery as a Facility Producing Bioplastics for Construction Industry

An option of bioplastic production is conversion of carbohydrates to organic acids and hydrogen by acidogenic fermentative bacteria on biorefinery (Surendra et al. 2015). Then produced organic acids and hydrogen can be transformed by aerobic PHAs-accumulating bacteria to PHAs and cell biomass. Third option is the production of fuel ethanol by yeast and oxidation of a portion of ethanol by microaerophilic bacteria to acetic acid, which then will be transformed to PHAs by aerobic PHAs-accumulating bacteria (Fig. 4.1).

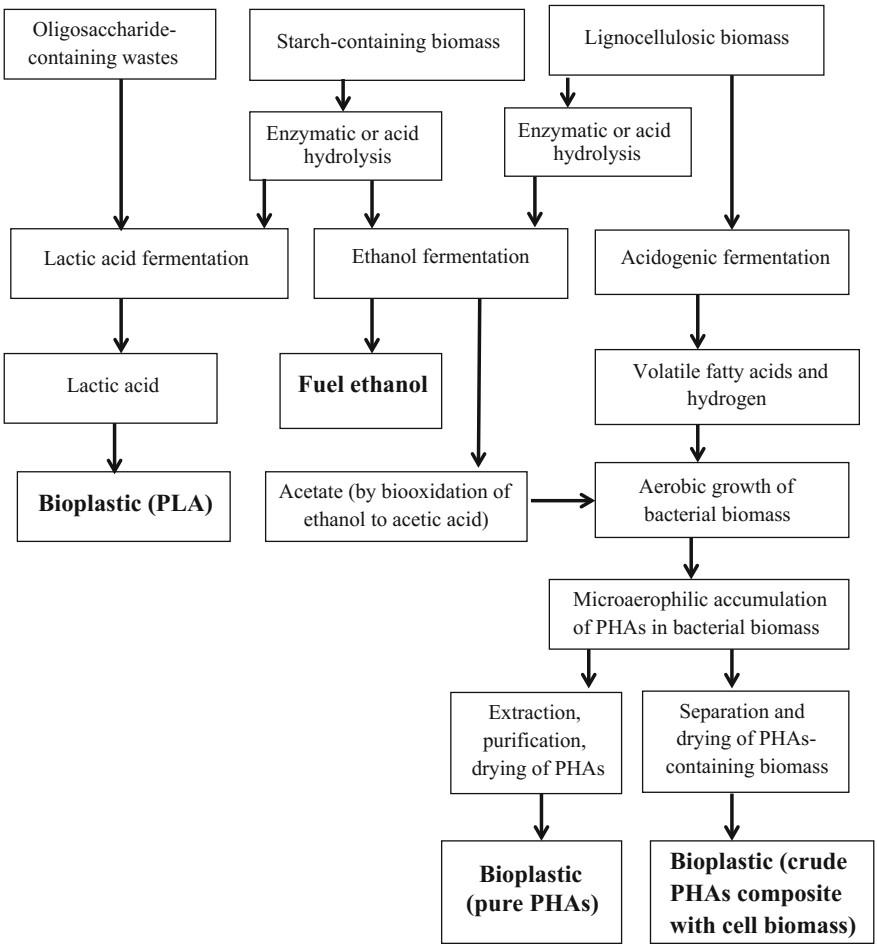


**Fig. 4.1** Schematics of biotechnological production of bioplastics on biorefinery

Bioplastics are entirely compatible with the production of fuel ethanol and diesel on the facilities using hydrolysis or pyrolysis of lignocellulosic biomass (Yu and Chen 2008; Snell and Peoples 2009). Biorefineries are the most suitable plants for the production of bioplastics (Pervaiz and Sain 2006; Bohlmann 2007; Ivanov and Christopher 2016). Biofuel by-products can be used for production of bioplastic, for example, xylose remaining after ethanol fermentation of sugarcane bagasse hydrolysate (Silva et al. 2014) or lignin remaining after processing of wood can be used in PLA-lignin composites (Spiridon et al. 2015). Proof-of-concept technology for production of PHAs on biorefinery has been demonstrated (Snell and Peoples 2009).

Production of bioplastics can be more sustainable if it will be performed on the biorefinery, which is a facility for industrial production of different chemical

products, first of all a fuel based on biotransformation of million tons of renewable biomass sources (Kamm et al. 2005). This raw material could be biomass of plants like starch of corn, sorghum and other crops, oils of terrestrial plants and aquatic algae, cellulose and hemicellulose of trees and grasses. The common products of any fermentation on biorefinery or pyrolytic transformation of wood to fuel—ethanol, acetic acid and hydrogen—are the most useful substances for the production of biocements, biogrouts, bioplastics, and polysaccharide admixtures. Acetic acid is neutralized by $Ca(OH)_2$ with the formation of calcium acetate as a major component of biocement. Sodium acetate and hydrogen are the best sources of carbon and energy for the production of PHAs. Ethanol is one of the best sources of carbon and energy for the production of polysaccharide admixtures. Another green solution for the bioplastic production is the utilization of hydrogen producing by hydrolysis of water by photoelectricity (Yu 2014).

Use of cheap raw materials, such as cellulose- and starch-containing biomass on biorefinery, is the most essential factor for production of construction biomaterials. Globally, 140 billion metric tons of biomass is generated every year from agriculture, which is equivalent to approximately 50 billion tons of oil. So, as a raw material, biomass has attractive potential for biorefineries of large-scale and community-level enterprises (UNEP 2009). This biomass, which can be used for the production of bioplastic on the biorefineries, is as follows:

- the wood processing waste, forestry waste, short rotation poplar containing mainly cellulose, hemicellulose, and lignin (Christopher 2012, 2013; Golden et al. 2015); about 131 million $m^3$ of wood residues are generated in the world including 40 mln in Europe, 27 mln in East Asia, 22 mln in USA and Canada, and 14 mln in Brasil (Koutinas et al. 2014);
- the agricultural waste such as unbaled straw; corn cobs, stalks, and leaves (corn stover); silage effluent; horticulture residuals; farm yard manure; coconut fronds, husks, and shells; coffee hulls and husks; cotton (stalks), nut shells; rice hull, husk, straw, and stalks, sugarcane bagasse; perennial grasses (Golden et al. 2015);
- the food-processing wastes such as molasses, whey, different starch-containing waste, potato peels, vegetable oil-containing wastes (Arancon et al. 2013; Girotto et al. 2015);
- the waste of pulp and paper factories (Bajpa 2013; Christopher 2013).

Meanwhile, the biorefinery producing bioethanol or biodiesel (Christopher 2013; Christopher and Kumar 2015), can easily accommodate biotechnological line for the bioplastic production because their major function is valorization of lignocellulosic waste through biotechnological production of biofuel, organic acid, biopolymers, and even substances for food and pharmaceutical industries (Christopher 2012, 2013; Liguori et al. 2013). Combining biofuel and bioplastic productions can reduce capital and operating costs.

Production of bioplastic from waste lignocellulosic biomass on biorefinery, which is close to the feedstock and do not use petroleum, could reduce transportation-associated and petroleum-associated carbon footprint.

PHAs can be also produced on wood pyrolysis refinery. Water-soluble fraction of the wood pyrolysis refinery includes from 5 to 8 % of acetic acid. So, this acetic acid after separation from other compounds can be biotransformed to PHAs as shown in the sections below. 1000 kg of cellulose-containing biomass can give about 30 kg of acetic acid, so about 20–25 kg of PHAs.

## 4.8 PHAs Production from Municipal Solid and Liquid Wastes

A considerable economic and environmental need exists for the further development of degradable plastic polyhydroxyalkanoates (PHAs), which are produced by bacteria. However, the production cost of this bioplastic, manufactured using conventional technologies, is several times higher than that of petrochemical-based plastics. This is a major obstacle for the industrial production of PHA bioplastic for nonmedical use. The most suitable methods for the significant reduction in bioplastic production costs are as follows (Stabnikova et al. 2010; Ivanov et al. 2015a; Ivanov and Christopher 2016): (1) The organic fraction of municipal solid waste treated by acidogenic fermentation or liquid after anaerobic digestion of excessive activated sludge of municipal wastewater treatment plants can be used as the raw materials; (2) nonaseptic cultivation using mixed bacterial culture can significantly reduce the production cost; (3) biotechnology of bacterial cultivation should ensure selection of PHA-accumulating strains; (4) applications of PHA-containing material in both construction industry and agriculture do not require expensive extraction of PHAs from bacterial biomass. The implementation of the above findings in the current manufacturing process of PHA-containing bioplastic would significantly reduce production costs, thereby rendering PHA-containing bioplastic an economically viable and environmentally friendly alternative to petrochemical-based plastics (Ivanov et al. 2015; Ivanov and Christopher 2016). Production of PHAs from the municipal wastes was shown by anaerobic digestion of municipal biosolid and biotransformation of the supernatant with soluble organic compounds was to PHAs using bacteria *Bacillus licheniformis* (Sangkharak and Prasertsan 2013). The concentrations of biomass and PHAs were 42 and 37 g/L, respectively.

The resultant polymers were poly-3-hydroxybutyrate-*co*-3-hydroxyvalerate [P(3HB-*co*-3HV)] and poly-3-hydroxybutyrate-*co*-4-hydroxybutyrate [P(3HB-*co*-4HB)], respectively. So, it is additional prove that the PHA production stage can be integrated in the solid municipal waste treatment.

## 4.9 Municipal Solid Wastes (MSW) as a Resource for Bioplastics Production

Approximately 250 million tons of MSWs are generated in USA and only 85 million tons of these materials are recycled or composted (US EPA 2011). About 55 % of MSW is deposited in landfills and about 13 % is incinerated (Barlaz et al. 2010). Landfills are a significant source of greenhouse gas emissions and require a lot of land. Incineration of MSW generates air pollutants as well as and a lot of ash requiring landfilling as hazardous waste. Meanwhile, such components of MSW as paper and paperboard (16.2 % of MSW in USA), wood (8.2 % of MSW in USA), yard trimmings (8.6 % of MSW in USA), and food scraps (20.5 % in MSW in USA) contain biodegradable organic matter (US EPA 2011). So, about 53 % of MSW can be used for biotransformation to value-added products instead of landfilling or incineration. Ideally, all this organic matter can be utilized.

An effective way to avoid the construction of new incineration plants and reduce area of landfills is shredding of MSW following the automatic hydroextraction and hydroseparation of shredded MSW, and then the conversion of the different fractions to value-added products (Rahman et al. 2014). Cost-efficient waste-to-resource technological solution, especially for the coastal areas, could be automated seawater-based separation of MSW with the production of settled fraction (stones, metals, glass, and rigid plastic), floated fraction (plastics), and suspended fraction (dissolved and suspended biodegradable organics), all of which could be transformed to value-added products. To cut off the expenses for freshwater, separation of MSW in the coastal areas could be performed using gravitational separation in seawater with the production of three fractions: floated fraction (plastics), settled fraction (stones, metals, glass, and rigid plastic), and suspended fraction, which is represented by dissolved and suspended biodegradable organics (Cesaro et al. 2011).

The organic fraction of MSW is commonly planned to be used for anaerobic digestion that is producing methane for heating or generation of electricity (Kelleher 2007). Organic wastes can generate methane in quantity from 60 to 220 L of methane/kg of dry waste when the content of anaerobically biodegradable components such as cellulose, hemicellulose, and starch changed from 55 to 95 %. There is approximately 0.11 kg of organic carbon per dry kilogram of MSW (Barlaz et al. 2010), so the content of biodegradable carbohydrates is $0.11 \times 30/12 = 0.275$ kg/kg of dry MSW. The problem of methane production from MSW is that methanogens are very sensitive to many components of water-extractable fraction of MSW such as heavy metals, pesticides, surfactants, ammonium, oils, fats, and sulfates (Ivanov et al. 2002; O'Flaherty et al. 2010; Mudhoo and Kumar 2013). Additionally, cellulose, hemicellulose, and starch are dominating compounds in the organic fraction of MSW. Therefore, methanogenesis from this fraction will be inhibited without pH control because the major products of anaerobic digestion of these compounds are organic acids decreasing pH to about 5, whereas optimum pH for majority of methanogens is about 7. That is why optimal biotransformation of the organic fraction of MSW could not be the

production of methane but other value-added products, first of all construction bioplastics.

Water-saving process could be shredding of MSW, separation of the organic fraction of MSW, and performance of acidogenic fermentation in seawater. The complication of anaerobic acidogenic fermentation of the organic fraction of MSW in seawater is sulfate bioreduction that produces toxic, corrosive, and bad-smelling dihydrogen sulfide. It could be possible to diminish sulfate reduction using Fe(III) compounds such as iron ore to stimulate iron bioreduction that is competing with sulfate bioreduction (Stabnikov and Ivanov 2006). Halophilic bacteria, which can grow in concentrated seawater, have such advantage that intracellular PHA granules can be isolated by cost-effective osmotic cell lysis using distilled or freshwater. This process can significantly reduce the cost of PHA extraction from bacterial biomass (Selvakumar et al. 2011).

## 4.10 Use of Non-carbohydrates for PHA Accumulation

Agricultural, food-processing, and biodiesel production wastes containing palm oil (Gumel et al. 2012; Din et al. 2013; Sudesh 2013), seeds (Preethi et al. 2012), fats and waste cooking oil, as well as, glycerol after fat hydrolysis or biodiesel production (Palmeri et al. 2012) can also be used for the low-cost production of PHAs. The sources of N, P, S, Fe, and microelements can be also supplied as the components of organic wastes. The ratio of the major inorganic nutrients and the sources of N and P to organic carbon should be in the range from 75:5:1 to 125:5:1 (Braun et al. 2010). Typical C:N:P ratio for anaerobic acidogenic fermentation is 100:5:1. Nitrogen could be assimilated from amines, nitrates, and ammonium. Phosphorus is usually supplied from nucleotides and orthophosphates. Suitable sources of inorganic nutrients could be reject water of municipal wastewater treatment plants (see below) and the mixtures of food-processing and agricultural wastes containing N, P, S, Fe, and microelements. However, cost of mineral components is a small contribution to the overall cost of PHA production. The most important factor is a cost of carbon and energy source(s).

## 4.11 Acidogenic Fermentation as First Step of Bioplastic Production of PHAs

A cost-effective option for production of bioplastic is conversion of carbohydrates to organic acids and hydrogen by acidogenic fermentative bacteria on biorefinery (Surendra et al. 2015). Then produced organic acids and hydrogen are transformed by aerobic PHAs-accumulating bacteria to PHAs and cell biomass. Third option is the production of fuel ethanol by yeast and oxidation of a portion of ethanol by

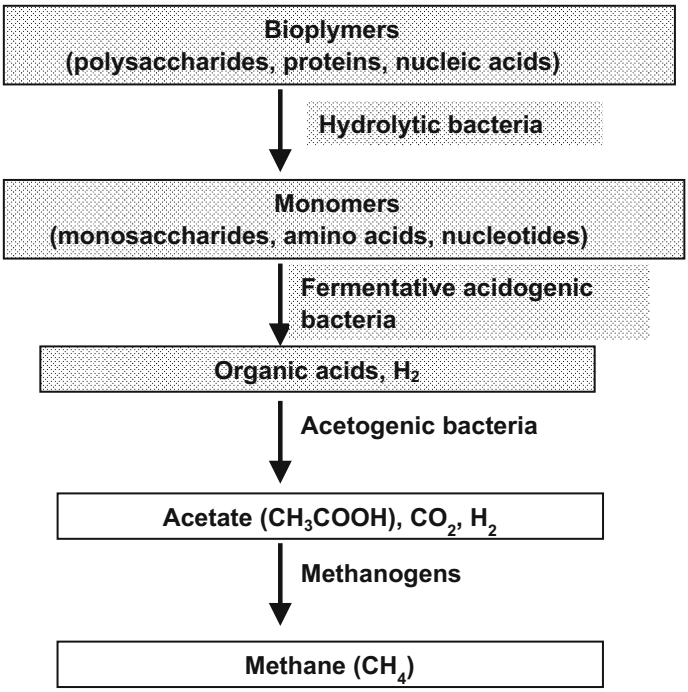


**Fig. 4.2** The microbial groups and substances of anaerobic digestion of biopolymers. The sequence of the acidogenic fermentation processes shown with *dark pattern filling*

microaerophilic bacteria to acetic acid, which then transformed to PHAs by aerobic PHAs-accumulating bacteria.

Lignocellulosic or starch-containing materials can be converted to PAHs using the anaerobic acidogenic fermentation to organic acids and nonaseptic biosynthesis of PHAs from these organic acids (Yu 2006; Lynd et al. 2002). These steps are shown in Fig. 4.2. Produced organic acids can be used for nonaseptic cultivation of mixed culture to ensure selective conditions for growth of PHAs–accumulating bacteria.

The most typical material balance of acidogenic fermentation of carbohydrates is shown in the Eq. 4.1. In one real example, batch acidogenic fermentation of maize silage in a solid-state fermentation, were produced 3.3 g/L of acetic acid, 0.3 g/L of propionic acid, 0.4 g/L of isobutyric acid, 4.0 g/L of n-butyric acid, 0.1 g/L of iso-valeric acid, 0.2 g/L of n-valeric acid, 2.8 g/L of caproic acid, and 0.2 g/L of lactic acid. Produced gas contained 35 % of hydrogen and 69 % of carbon dioxide (Strauber et al. 2012).

However, pH can drop below 5.5 during acidogenic fermentation (Barlaz et al. 2010), meanwhile optimal pH for acidogens is above 6.0 (Moosbrugger et al. 1993). To maintain optimal pH during acidogenic fermentation of lignocellulosic

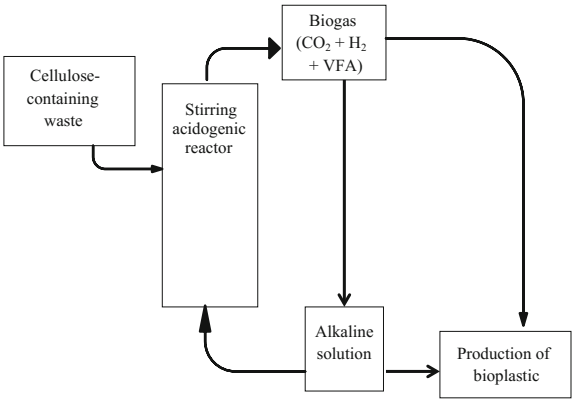


**Fig. 4.3** pH maintenance during acidogenesis through the recycle of biogas and adsorption of VFA

biomass pH must be controlled automatically by titration with alkali or using co-digestion with the wastes with high content of protein (Ahring et al. 1992; Macias-Corral et al. 2008). Biogas produced in the bioreactor for acidogenic fermentation removes VFA from liquid and must be treated by alkali to separate VFA salts (Fig. 4.3). Gas after removal of VFA is used to remove new portions of VFA from acidogenic reactor while liquid containing VFA salts is used for the biosynthesis of PHAs (Fig. 4.3).

If a source of protein-containing waste is available, mixing of carbohydrate- and protein-containing waste could balance concentration of protons results during anaerobic digestion of carbohydrates producing acids and ammonification of protein producing hydroxide ions.

The yield of VFA in grams of chemical oxygen demand (COD) per grams of volatile solids (VS = approximately g of organic matter) from of source-sorted organic MSWs was 0.27 g COD/g VS at psychrophilic conditions (14–22 °C) and 0.5–0.9 g COD/g TVS at mesophilic (25–30 °C) and thermophilic conditions (45–55 °C). VFA could reach 15 % of the soluble COD at psychrophilic conditions and the hydraulic retention time (HRT) 4–4.5 days and 90 % of soluble COD at mesophilic conditions and HRT 1–2 days in the continuously stirred tank reactor (Bolzonella et al. 2005). The organic loading rate for the anaerobic acidogenic reactor fermenting the organic fraction of municipal solid wastes could be between 20 and 90 kg of organics/$m^3$/day with the first-order kinetic constant ($K_h$) for hydrolysis of solid organic wastes about 0.1–0.5 1/day (Bolzonella et al. 2005).

The requested HRT to achieve a given final concentration of fed substrate is $(S_0 - S)/S\,K_h$, where $S_0$ is the concentration of the influent substrate (as COD), g/L; $S$ is the concentration of the effluent substrate (as COD), g/L; and $K_h$ is the first-order reaction constant, 1/day. So the volume of the anaerobic reactor for digestion of OFMSW from a population of 100,000 inhabitants, produced 200 g organic wastes/inhabitant/day, has to be about 40–65 $m^3$ (Bolzonella et al. 2005). Therefore, anaerobic digestion of the organic fraction of MSW to organic acids and

hydrogen can be effectively used to produce nutrients for the production of PHA-containing bioplastic.

If the biorefinery uses starch or hydrolyzed cellulose for fermentation of ethanol as biofuel, part of this ethanol can be easily converted to acetate (Lin and Tanaka 2006). Another source of acetate can be pyrolysis of lignocellusic biomass producing fuel and a lot of pyroligneous acids, especially acetic acid from the acetyl groups of hemicelluloses (Yaman 2004).

## 4.12 Transformation of Volatile Fatty Acids to Bioplastic

Using VFAs from acidogenic fermentation or acetic acid from pyrolysis of lignocellulosic biomass or from partial oxidation of fuel ethanol bioplastic PHAs can be accumulated in cells of pure cultures of the species from genera *Acinetobacter, Alcaligenes, Alcanivorax, Azotobacter, Bacillus, Burkholderia, Delftia, Klebsiella, Marinobacter, Pseudomonas, Ralstonia, Rhisobium,* or mixed bacterial cultures using batch, semi-continuous or continuous nonaseptic cultivation (Ben Rebah et al. 2009; Beun et al. 2006; van Loosdrecht et al. 2008).

Aseptic cultivation of selected or recombinant strains of bacteria requires thermal sterilization of materials and equipment. Cost of aseptic cultivation is several times higher than the cost of nonaseptic cultivation. Therefore, nonaseptic cultivation of mixed microbial culture, which is able to accumulate PHAs in biomass, could be low-cost technology for industrial production of PHAs. The major points of using a mixed bacterial culture for PHA production were considered in several patents, reviews, and papers and are shown below: (a) Activated sludge, a well-known mixed culture, is able to store PHAs as the carbon and energy storage material under unsteady conditions arising from an intermittent feeding regime and variation in the presence of an electron acceptor; (b) activated sludge accumulates PHAs to around 20 % of dry weight under anaerobic conditions, but the content can be increased to 62 % in a microaerophilic–aerobic sludge process.

Activated sludge for the production of PHAs can be from municipal wastewater treatment plant (Mokhtarani et al. 2012) or from food industrial wastewater treatment process (Buathong et al. 2012). In the last case, PHA production and accumulation in activated sludge added with acetate were 1 g/L and consisted 49 % of dry sludge weight. Oxygen management is crucial for the process because excessive aeration rate allows high biomass growth yield but decreases PHA yield; (c) the content of accumulated PHAs in a mixed culture is usually lower that in a pure culture, which can accumulate PHAs up to 80 % of cell dry weight. However, the merits of the PHA production in a mixed culture are an enhanced economy, a simpler process control, no requirement of aseptic processing, and use of wastes.

PHA production by activated sludge treating municipal wastewaters or pulp and paper mill effluents is affected by their nutrient composition, in particular carbon and nitrogen sources, sludge retention time, pH, temperature, etc., with typical values between 20 and 30 % PHA by weight. To stimulate accumulation of PHA in

activated sludge, a feast/famine strategy (aerobic dynamic feeding) with alternating high and low organic load is normally applied (Dias et al. 2006; Albuquerque et al. 2007; Bengtsson et al. 2008; Arcos-Hernandes et al. 2013; Ivanov et al. 2015a). These dynamic conditions generate unbalanced cell growth and favor production of intracellular storage polymers (such as PHAs) during the high-carbon load phase.

Acclimation and optimization of PHA production is normally carried out as a two-step process: (1) selection of PHA-accumulating cultures under feast/famine conditions; (2) batch PHA accumulation using selected cultures and substrate (wastewaters). However, as mixed cultures do not store carbohydrates as PHAs, carbohydrate-rich substrates such as biomass hydrolysates, pre-hydrolysates, pulping effluents need to be first converted to VFA using anaerobic acidogenic fermentation. As mixed cultures can store VFA as PHAs, this additional step will further increase the PHA yield reaching levels of around 70 % by weight, which is competitive with PHA production using pure cultures. However, the use of pure cultures (wild and recombinant) and pure substrates for PHA production is inherently expensive. As the substrate cost accounts for about 40 % of the total PHA production costs, the use of mixed cultures (as in the activated sludge) and mixed organic matter contained in the biorefinery effluents with a negative cost is expected to diminish additionally the cost barriers to a widespread use of the PHA bioplastics.

Major technical challenges associated with the PHAs biosynthesis using cellulosic biomass waste include

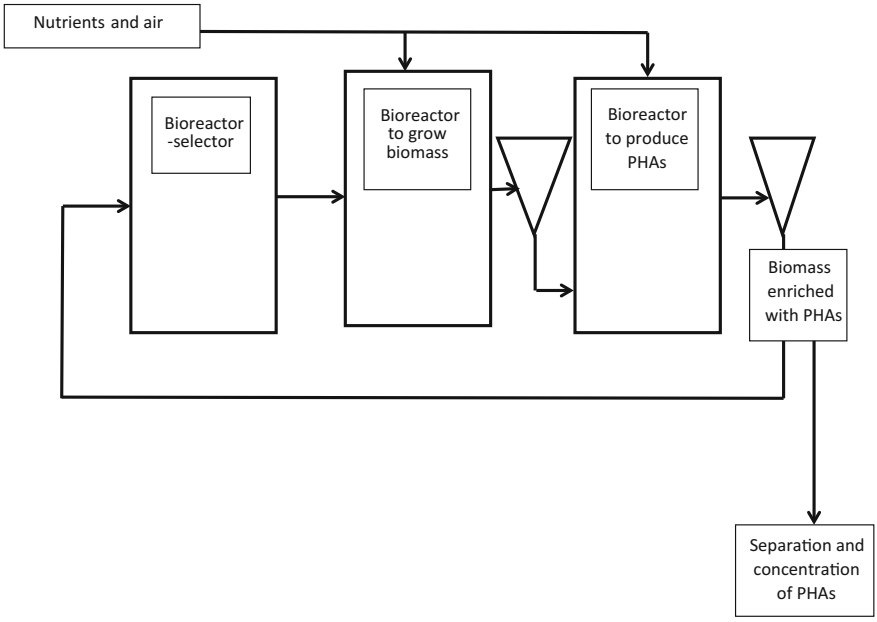


**Fig. 4.4** Continuous production of PHAs from VFA with selection of PHAs-accumulating cells

- complete utilization of all fermentable sugars including pentose sugars such as xylose and arabinose;
- incompatibility of the pH for enzymatic hydrolysis of cellulosic biomass to fermentable sugars (acidic) with the pH for optimal neutral or slightly alkaline pH for PHA production;
- stability of PHAs synthesis in continuous nonaseptic system by mixed bacterial culture. This stability can be maintained using recycling and bioreactor–selector for starvation ensuring preference for cells that accumulated PHAs (Fig. 4.4).
- PHAs recovery from a complex solid mixture of PHA-containing cells, residual biomass containing lignin, and undigested fibers (Wang et al. 2014).

## 4.13 PHAs Recovery

In the convenient biotechnology, PHAs are extracted from cells after their accumulation in bacterial biomass. Microbial cells can accumulate up to 80 % PHA of their dry cell weight. Due to the solid state of the PHA particles, PHA recovery from microbial biomass is challenging and requires dissolution of either the PHA polymer or the non-PHA biomass. The former method employs organic solvents such as chloroform and dichloromethane (chlorinated hydrocarbons). The extracted PHA is then precipitated with methanol to give very pure and intact PHA macromolecules. The PHA extraction method, however, suffers from some major drawbacks such as use of large quantities of organic solvent and associated recovery costs, lengthy extraction, and filtration procedures. The alternative method for PHA recovery is by centrifugation which separates the solubilized non-PHA cell biomass (proteins, nucleic acids, lipids, peptidoglycans) from the high-density PHA granules which remain in the solid phase. For dissolution of the cell organelles and fragment, both nonselective (alkali, alkaline hypochlorite) and selective (anionic surfactants, proteases) have been used (Madkour et al. 2013). However, all methods of extraction suffer from high cost, environmental pollution, and increase of embodied energy. Therefore, the best option for the construction applications could be use of crude composite bioplastic, which is dry biomass with the granules of PHAs inside cells.

## 4.14 Cost of Bioplastics Production

PLA is producing in industrial scale about 136,000 metric tons per year by NatureWorks, Cargill-PTT Global Chemical (Golden et al. 2015), it will be produced also in Germany, Russia, India, and other countries. Global demand for PLA is about 50 million tons/year. However, the selling price of PLA is about $2.20/kg (Golden et al. 2015), which could be too high for the construction applications. So,

the technology lactic acid fermentation must be enhanced or cheaper raw materials must be used for the production of PLA as a construction material. The production cost of PHA varied by different evaluations from 1.5 to 6 Euro/kg, while the market price of petrochemical plastic polyethylene terephthalate (PET) is 1.3 Euro/kg (Dacosta et al. 2015). However, use of nonaseptic production from cheap raw materials using mixed bacterial culture altogether with the removal of extraction stage from technology could significantly decrease the production cost of crude composite PHAs as the construction material to the competitive level of petrochemical plastics.

Currently, PHAs are produced on pilot scale 50–500,000 tons with the cost from 1.5 to 13 Euro $kg^{-1}$ by Mitsubishi Gas Chemical Company Inc. (Japan), Telles (US), PHB Industrial Company (Brazil), Biomer Inc. (Germany), Tianan Biologic, P&G (US), Ningbo (China), Lianyi Biotech (China), Kaneka Corporation (Japan) (Koutinas et al. 2014). The biorefinery technology and the utilization of waste and by-product streams are most essential factors in order to develop cost-competitive bioplastics (Koutinas et al. 2014). Biorefinery materials, organic fraction of municipal solid waste, or liquid waste ("reject water") of municipal wastewater treatment plants can be used as the raw materials for the bioplastic production (Ivanov et al. 2015a) but the incorporation of the biotechnological line for the production of value-added materials on these municipal plants is still a problem. Hypothetically, biotechnological production of bioplastic on municipal solid or liquid waste treatment facilities as a public–private partnership can reduce waste treatment costs and generate revenue for the waste treatment facilities. However, no one example is known at present.

## 4.15 Biodegradability of Biotechnologically Produced Bioplastics

Important property of PHAs for construction applications is its biodegradability to carbon dioxide and water for about 1.5 months in anaerobic sewage, 1.5 years in soil, and 6.5 years in seawater (Mergaert et al. 1992; Reddy et al. 2003; Castilho et al. 2009). Dead bacterial biomass with PHAs contains also polysaccharides of cell wall, proteins, polynucleotides, and phospholipids, which content is about 15, 50, 25, and 10 % of dry biomass without PHAs, respectively, and biodegradation rate is higher than that of PHAs. Therefore, from the point of view of biodegradability of bioplastic construction wastes there is no sense to extract PHAs from biomass but to use dry biomass containing PHAs as a crude nanocomposite consisting of the granules of PHAs and interlayers of cellular biopolymers. Such nanocomposites should be more flexible and better biodegradable than extracted PHAs.

Biodegradability of PLA and PLLA is soil and water is going even faster and can be done in soil or compost for several weeks (Fukushima et al. 2000) but the

bars pf PLA with nanocomposites can be degraded with the rate up to 50 % a year (Shogren et al. 2003). An addition of nanocomposites such as montmorillonites (Fukushima et al. 2000), starch or poly(hydroxyester-ether) (Shogren et al. 2003), or just a source of protein (Yang et al. 2015) facilitated biodegradation of PLA. PLA can be mixed with PHAs to improve mechanical stiffness and increase of the film stretchability but this mixing decreased biodegradation rate of both bioplastics, which can be increased by an addition of cellulose nanocrystals (Arrieta et al. 2014).

## 4.16 Environmental Impacts of Bioplastics

Eco-efficient construction and building materials is one of the most important aspects in construction industry (Pacheco-Torgal and Labrincha 2013a, b). Major environmental impact classification factors for production of materials are as follows (La Rosa 2016):

1. Acidification Potential expressed as $SO_2$ equivalent;
2. Aquatic Toxicity Potential;
3. Human Toxicity Potential is calculated in kg of toxic materials released in air, water, and soil;
4. Eutrophication Potential for aquatic ecosystems in kg of nitrogen, phosphorus, and chemical oxygen demand expressed as phosphate equivalents.
5. Global Warming Potential (GWP) of greenhouse gases $CO_2$, $N_2O$, $CH_4$, and volatile organic compounds expressed as $CO_2$ equivalent.
6. Nonrenewable/Abiotic Resource Depletion Potential is calculated for fossil fuels, metals and minerals by dividing the quantity of resource used by the estimated total world reserves of that resource.
7. Ozone Depletion Potential represents the potential of depletion of the ozone layer due to the emissions of chlorofluorocarbon compounds and other halogenated hydrocarbons.
8. Photochemical Oxidants Creation Potential. Photochemical smog is caused by the degradation of VOCs and nitrogen and is expressed in kg of ethylene.

PHAs is eco-friendly bioplastic that can be produced from lignocellulosic biorefinery as value-added coproduct. Life cycle assessment (LCA), performed with the greenhouse gas (GHG) emissions and fossil energy requirement per kg of bioplastics produced, showed that PHAs bioplastic contributes the GHG emissions 0.49 kg $CO_2$/kg, while petrochemical plastics contribute 2–3 kg $CO_2$/kg. The fossil energy requirement per kg of bioplastic is 44 MJ while for the petrochemical plastics this parameter is 78–88 MJ/kg (Yu and Chen 2008).

Another example of LCA, made according to ISO 14040 and ISO 14044 standards and supported by EcoInvent 2.2 database showed that the greenhouse gas emissions (GHG) and the nonrenewable energy use (NREU) for PHB production

were 1.97 kg $CO_2$ eq/kg of PHB and 109 MJ/kg of PHB, respectively (Dacosta et al. 2015), while for production of petrochemical plastic PET the total GHG emissions were 2.15 kg $CO_2$ eq/kg and NREU 69 MJ/kg. However, steam for sterilization of the facility of PHB production presented a 15 % share of the total GHG emissions and a 12 % of the share of the total NREU, the sources of N and P presented a 20 % share of the total GHG emissions and a 15 % of the share of the total NREU, and materials for PHB extraction (NaOCl and SDS) from biomass presented a 25 % share of the total GHG emissions and a 26 % of the share of the total NREU (Dacosta et al. 2015). So, nonaseptic production of PHB using waste sources of N and by mixed bacterial culture without extraction of PHB from bacterial biomass will be characterized by the greenhouse gas emissions (GHG) 0.9 kg $CO_2$ eq/kg of crude PHB (46 % of GHG emissions for pure PHB) and the nonrenewable energy use (NREU) 51 MJ/kg of PHB, which are 42 and 74 % of the respective values for PET. So, partial replacement of petrochemical plastics with bioplastic PHAs mitigates global warming.

Using the raw data from the pilot plant to form the eco-inventory, PHAs has a clear disadvantage to fossil competitors, showing an aggregated ecological footprint of 10.4 $m^2$/kg of PHAs compared to 1.7 $m^2$ kg of polypropylene. The major contribution to this large footprint comes from the use of electricity used in the fermentation step (Narodoslawsky et al. 2015).

Embodied energy of pure PHB is 80 MJ/kg (Dicker et al. 2014). Considering a linear correlation between embodied energy and the GHG emissions, embodied energy of crude PHAs is about 46 % of the value of pure PHB, i.e. about 37 MJ/kg of crude PHAs. It is higher than embodied energy of timber (9.4 MJ/kg) but significantly lower than embodied energy of petrochemical plastics (70–160 MJ/kg) (Hammond and Jones 2008). So, crude PHB and generally PHAs can be considered as low embodied energy construction material.

Polylactide (PLA) NatureWorks™ produced by Cargill Dow LLC has the gross fossil energy requirement 54 MJ/kg and GHG emissions 1.8 kg $CO_2$ eq/kg (Vink et al. 2003; La Rosa 2016). Embodied energy of pure PLA is about 80 MJ/kg (Dicker et al. 2014), which is comparable with the values of petrochemical plastics. Only with regard to land use biopolymers clearly show higher ecological impact. Annual land use for PHAs and PLA is about 2.5–3 $km^2$/kt of biopolymers (La Rosa 2016).

## 4.17 Applicability of Crude PHAs

Potential market of bioplastics includes packaging materials, catering products, consumer electronics, medical materials, agriculture and horticulture (biodegradable mulch foil), toys, textiles. Past and present industrial production of PHA-containing bioplastic is mainly for medical applications such as skin substitutes, heart valves, vascular grafts, scaffolds, bone graft substitutes, and drug delivery microspheres because of PHA biocompatibility and biodegradability (Chen

et al. 2005). Application of PHA-containing bioplastic for the manufacturing of low-volume and high-cost biomedical items is becoming a reality, but even for these applications, there must be developed more efficient and economical processes for the PHA production, isolation, purification, and improvement in PHA material properties (Keshavarz and Roy 2010).

However, PHA-containing bioplastic, which is produced by nonaseptic cultivation of mixed bacterial cultures from waste materials, cannot be used for biomedical, food packaging, or catering applications because chemical and physical properties of PHA-containing bioplastic are not controlled in nonaseptic cultivation, and there may be a lot of pollutants in the product that were released from MSW and microorganisms.

The type of application depends also on the mechanical properties of bioplastic. PHA mechanical properties depend very significantly on the chain length of the monomer. For example, PHB is stiffer and more brittle than for polypropylene, but copolymerization with hydroxyvalerate (PHB-co-PHV) makes bioplastic much more flexible (Castilho et al. 2009). This copolymer can be used for packaging material like films and bottles (Ben Rebah et al. 2009). However, PHB applications are limited by its thermal degradation during molding and stiffness of bioplastic. For aseptic cultivation of genetically modified strains of PHA producers is it possible to select specific medium and conditions for the production of PHAs with the desired mechanical properties, but for nonaseptic cultivation of mixed culture, the chemical content of accumulated PHAs will be determined mainly by the spectrum of fatty acids in the medium.

There are two potential problems in the applications of crude PHA-containing nanocomposite bioplastic. First problem is the high temperature of PHA melting, which is in the range 160–180 °C (Castilho et al. 2009; Sudesh et al. 2000; Sudesh and Abe 2010; Volova 2004). Melting temperature of PHB is close to its thermal decomposition temperature $T_d$—10 % (Chen and Lo 2012). Thermal decomposition temperatures of proteins, polysaccharides, and polynucleotides are also close to this value, i.e., all biopolymers have poor thermal stability at the temperature of PHA melting. Natural antioxidants, which present in biomass, can reduce the rate of thermal destruction of biopolymers (Giner et al. 2012). Protein itself can be considered as the thermoplastic material but with addition of plasticizers, which inhibit the formation of cross-linking that can result in the formation of the thermoset material from extruded protein (Verbeek and van den Berg 2009). Therefore, the molding of composite crude PHA-containing bioplastic must be as short as possible to diminish the thermal decomposition of PHAs and other biopolymers of bacterial biomass.

## 4.18 The Applications of PHAs in Construction

Due to biodegradability of PHAs, their major application is in the temporarily constructions. The bioplastic materials can be used as the sealants and insulants, solid foam (Willke and Vorlop 2004), silt and dust fences, for construction of nonstructural (internal) elements such as separating walls and partitions, and manufacturing of the biocomposite packaging films. The applications of PHAs bioplastic from bacterial biomass could be the production and use of biodegradable construction materials, which can diminish area of land used for the landfilling because they are degraded in soil or in the landfill (Pilla 2011). For example, biodegradable bioplastic foam can be used for insulation of the walls and partitions in the temporarily constructions. Bioplastics can be used also as the sealants and insulants replacing petrochemical plastics in construction industry (Willke and Vorlop 2004). Other examples of potential application of crude nanocomposite from bacterial biomass containing PHAs are silt and dust fences that can be landfilled for fast biodegradation or even left in construction ground for degradation. Other potential applications where the biocomposites can be applied are formwork, scaffolding, walls and wallboards, decorative panels, cubicle walls, temporarily shelters, i.e., all temporarily construction elements, which are dismantled after temporarily use and the waste is usually dumped in landfills but in case of bio-based material it can be composted and reused as soil conditioner (Pilla 2011).

There could be a big market for biodegradable bioplastic foam construction material, which does not require incineration after demolition. Plastic foam and foam insulators that are used in the construction industry produce hazardous non-biodegradable waste after demolition of buildings or temporary constructions. The PHA-containing bioplastic foam would be an innovative and environmentally green, sustainable construction material that would degrade quickly being landfilled or could alternatively be composted. Other potential applications of crude nanocomposite from bacterial biomass containing PHAs include construction silt and dust fences that can be landfilled for fast biodegradation or composted as biomass. Sustainability of biodegradable construction materials is due to (1) the production of bioplastic from renewable sources or even from organic waste and (2) fast biodegradability of this material under landfill conditions or composting to minimize the negative environmental impact of construction waste. In addition, biodegradable bioplastic foam construction material would be highly marketable because it does not require incineration after demolition.

One potential application is the replacement of dark plastic mulch, which is currently in bioremediation of polluted soil to suppress reduce water evaporation from soil and warm soil for faster bioremediation. Nonbiodegradable plastic mulch requires labor-intensive removal from the field for disposal or energy-consuming and environmentally unfriendly recycling of the used film. The advantage of utilizing film from nanocomposite bioplastic from bacterial biomass containing PHAs is that it can provide all of the benefits of traditional plastic mulch but can be left in

place for natural biodegradation on the field. Another application of nanocomposite bioplastic from bacterial biomass with PHAs is manufacturing of slow-release fertilizers for bioremediation using bioplastic coating or embedding of fertilizers in bioplastic granules, bars, or films. For example, fertilizer embedded in bioplastic bars can be used on the site of bioremediation as time-released fertilizers for 0.5–2-years' time period of the bioplastic degradation.

## 4.19 The Applications of PLA in Construction

PLA is a pure biotechnological/chemical product so it is more expensive material than crude PHAs and polypropylene (PP) or polyvinylchloride (PVC) but cheaper than pure PHAs. The synthetic fibers are widely used to reinforce plaster or concrete (Balaguru 1994; Zeiml et al. 2006; Suchomel and Marsche 2013) because natural plant fibers have variable and changeable mechanical properties (Suchomel and Marsche 2013). So, the bioplastics can be used as fiber reinforced polymer composites consisting of the reinforcing fibers embedded in a bioplastic matrix.

PLA can be used for the manufacturing of composite biodegradable fibers for reinforcement of the construction materials (Huda et al. 2006; John and Thomas 2008; Bajpai et al. 2013; Faludi et al. 2013; Saba et al. 2015), the PLA biocomposite sheets can be used for construction and packaging, the biodegradable resin composition for the construction molds (Tokiwa and Tsuchiya 2003), and for the production of environmentally friendly flooring material, which can be recycled or rapidly decomposed upon the discarding (Ko et al. 2014).

PLA also has been used in the manufacturing of wood biocomposites (Patachia and Croitoru 2016). Probably, in this case PLA can increase the life cycle of wood constructions only if it will be loaded altogether with fungicide or bactericide, which is releasing and protecting wood only under conditions when biodegradation of PLA will begin.

Pure PLA and PHAs bioplastics cannot be used in construction industry because of their brittleness. Elongation at failure for PLA and PHB is about 2–6 %, while for PP and PET is 28–320 % (Peelman et al. 2013). So, natural fibers and plasticizers have to be used altogether with PLA and PHAs in the composites. Natural fiber-reinforced bioplastics can be strong and rigid composite material but at the same time these composites will be more biodegradable, elastic, and strong than pure bioplastic. Same bio-based fiber and particles that are used for the reinforcement of petrochemical plastics—wood, bark, straw, coconut, kenaf, flax, jute, hemp, or the particles of lignin and lignosulfonate—can be used as the composite material for bioplastics. Probably, different type of the biodegradable textile can be used on the construction sites as silt fence, sediment trap, drainage pipe, biodegradable cover, and erosion control blanket, or as a geotextile for the temporarily slope stabilization. These materials do not require post-construction extraction and can be remained in soil for biodegradation after construction.

## 4.20 Advantages of Construction Biodegradable Bioplastics

The use of bioplastics instead of petrochemical plastics can:

- increase energy building efficiency because of low embodied energy of crude bioplastic;
- diminish cost of the extraction and disposal of the construction wastes because bioplastic materials can be left in soil for their biodegradation in situ;
- diminish area of land used for the landfilling of the construction waste;
- do not require incineration of bioplastic construction after demolition because of its biodegradability;
- bioplastic construction waste can be used for composting and soil fertilization;
- make sustainable construction material from the renewable organic sources.

It is important that biodegradable plastic composites can be composted when their compostability has been verified by standards, for example the European Standards EN 13432 and EN14995, US Standards ASTM D6400 and ASTM 5338, and international standard ISO 14855. The key requirements are usually as follows: there are limits for toxic volatile and nonvolatile organic compounds, heavy metals, and fluorine; breakdown at least 90 % of material to $CO_2$, water, and minerals for 6 months; physical decomposition of at least 90 % of material to tiny pieces less than 2 × 2 mm for at least 12 weeks; there must be no negative effect of this compost on seeds germination and plant biomass growth.

## 4.21 Composite and Blended Bioplastic Materials

Blending of bioplastic PLA and PHAs with petrochemical plastics could reduce brittleness and increase durability of bioplastic composites so such composites can also find their applications in construction industry. Bioplastics and petrochemical plastics are not alternatives. They can be blended forming better quality. For example, poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV) blend with some amount of poly(ethylene succinate) (PES) improved the elongation at break significantly while still keeping considerably high tensile strength and Young's modulus (Miao et al. 2008).

PLA can be used for the manufacturing of composite biodegradable fibers for reinforcement of the construction materials (Huda et al. 2006; John and Thomas 2008; Bajpai et al. 2013; Faludi et al. 2013; Saba et al. 2015), the biocomposite sheets for construction and packaging, the biodegradable resin composition for the construction molds (Tokiwa and Tsuchiya 2003), and for the production of environmentally friendly flooring material, which can be recycled or rapidly decomposed upon the discarding (Ko et al. 2014).

PLA can be used also for the manufacturing of bionanocomposites with the significantly improved and new value-added properties in comparison with conventional PLA composites. For example, addition of 5 % (v/v) clay to PLA improved the tensile strength, break elongation, scratch resistance, and other mechanical properties (Ray 2012). PLA nanocomposite containing 0.15 % (w/w) multi-walled carbon nanotubes and 6 % (w/w) of the plasticizer polyethylene glycol (PEG) have the tensile and flexural strengths up to 43.8 and 81.4 MPa that are suitable levels for many industrial applications including construction industry (Maizatulnisa et al. 2013).

The problem with the brittleness of biocement could be solved using biomimetic approach (Sarikaya 1994; Mayer and Sarikaya 2002) using composite strengthening through combination of mineral and organic nano- and microparticles. By the analogy with nanomaterials in cement (Pacheco-Torgal and Jalali 2011), applications of composite micro- and nanomaterials can also be useful to increase strength and ductility of biocement. Theoretically, ductile biocement could be made as a bioinspired material (Pacheco-Torgal and Labrincha 2013a, b), with the 3D-composite structure of hierarchically arranged nano- and micrometric units (Imai and Oaki 2010), or just simply with the layers or inclusions, where inorganic crystals of calcium carbonate (calcite, aragonite, and vaterate), calcium phosphate (hydroxyapatite), and oxides of Si and Fe create the hardness and the organic components such as proteins and polysaccharides ensure flexibility of the biocemented structure. This property is well known from the structure of the natural biominerals such as bones, shells, and corals as well as artificial engineering composite materials (Mayer and Sarikaya 2002; Yao et al. 2011). However, the cost of micro- and nanocomposites could be too high to be suitable for construction practice.

The bioplastics can be used also for the manufacturing of bionanocomposites with the significantly improved and new value-added properties in comparison with conventional PLA composites. For example, addition of 5 % (v/v) clay to PLA improved the tensile strength, break elongation, scratch resistance, and other mechanical properties (Ray 2012). PLA nanocomposite containing 0.15 % (w/w) multi-walled carbon nanotubes and 6 % (w/w) of the plasticizer polyethylene glycol (PEG) have the tensile and flexural strengths up to 43.8 MPa and 81.4 MPa that are suitable levels for many industrial applications including construction industry (Maizatulnisa et al. 2013).

Very promising is the production of construction composites with the biomimetic structure of bone. The porous poly(3-hydroxybutyrate-co-hydroxyvalerate) PHBV scaffold was presoaked in a saturated $Ca(OH)_2$ solution for 7 days and incubated in 100 mM $CaCl_2$/Tris and $Na_2HPO_4$/Tris solutions alternatively for up to seven cycles. Thus a novel three-dimensional composite scaffold material consisting of apatite biomimetically deposited on a PHBV matrix was constructed and characterized. The nano-sized needle-like apatite crystals were deposited preferably at the sites of micropores in scaffold walls. This highly porous biodegradable organic/inorganic composite is expected to be used as an artificial bone (Huang et al. 2008). The composites of natural fibers with biodegradable resins and

recycled petrochemical thermoplastics have the market opportunities for many industrial sectors including construction (La Rosa 2016).

Construction flexible and rigid foams are produced mainly from poly(urethane), poly(ethylene), poly(propylene), poly(acrylonitrile butadiene styrene), but construction foam from biodegradable bioplastics can also gain the market share because of their low $CO_2$ footprint (Javadi et al. 2011; Chevali and Kandare 2016). These foams could be used as sealants, thermal barriers, and flooring.

# Chapter 5
# Biogeochemical Basis of Construction Bioprocesses

## 5.1 The Functions of Microorganisms in Hydrosphere and Lithosphere

Microorganisms live in the hydrosphere (seawater, freshwater, and groundwater), lithosphere (soil), and survive in the atmosphere. The bacterial mass on Earth is about $10 \times 10^{12}$ tons, which is about 30,000 times bigger than mass of human population.

The major biotope of microorganisms in the lithosphere is soil, from the surface layer of the lithosphere to a depth of 0.3 m. The biotopes of microorganisms in the hydrosphere are the surface, bulk and bottom of the oceans, rivers, lakes, and groundwater reservoirs.

The functions of microorganisms in biosphere are as follow:

- biogeochemical cycling of carbon, oxygen, nitrogen, phosphorus, and other elements performing such cyclic chemical changes of compounds as oxidation–reduction or synthesis-biodegradation;
- mediation of physical changes of chemical compounds such as volatilization, dissolution,
- and precipitation;
- assimilation of photo- and chemical energy in biosphere accompanying with production of microbial biomass and products of its decay;
- specific accumulation of metabolites and biomass-derived products, for example, oil, sulfide ores of metals, elemental sulfur deposits;
- evolution of atmosphere and biosphere, for example, accumulation of oxygen by cyanobacteria changing atmosphere from anaerobic to aerobic one creating the conditions for new form of life and origin of terrestrial life due to protection from UV light;
- colonization and transformation of abiogenic ("no life") surfaces, for example, formation of fertile soil by the biochemical transformation of volcanic lava surface;



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– positive or negative interactions of microorganisms with the plants and animals, for example, nitrogen-fixing symbiosis with plants, utilization of cellulose by the ruminant animals, and infectious diseases of plants, animals, and human.

## 5.2 The Biogeochemical Carbon Cycle

Major pathways of the biogeochemical cycle of carbon include the assimilation of $CO_2$ from the atmosphere and oxidation of organic carbon to $CO_2$. Photosynthesis can be oxygenic ("generating oxygen"):

$$CO_2 + H_2O + \text{energy of light} \rightarrow CH_2O(\text{organic matter}) + O_2, \tag{5.1}$$

or anoxygenic ("not generating oxygen") photosynthesis, for example:

$$CO_2 + 2H_2S + \text{energy of light} \rightarrow CH_2O(\text{organic matter}) + 2S + H_2O, \tag{5.2}$$

$$CO_2 + 4Fe^{2+} + 3H_2O + \text{energy of light} \rightarrow CH_2O(\text{organic matter}) + 4Fe(OH)^{2+}. \tag{5.3}$$

Photosynthetic microorganisms—cyanobacteria, photosynthetic *Archaea*, and microalgae—produce their biomass from $CO_2$ of air. Inorganic compounds, such as the sources of N, P, S, Fe, and others necessary for biomass synthesis must be supplied from environment. Cyanobacteria are often assimilated nitrogen from nitrogen gas of air.

Microorganisms oxidize organic matter to $CO_2$ using fermentation, anoxic oxidation, or aerobic oxidation, for example:

– acidogenic fermentation of carbohydrate

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CO_2 + 4H_2 + 2CH_3COOH, \tag{5.4}$$

– anoxic oxidation of carbohydrate by nitrate, sulfate or iron oxide:

$$5C_6H_{12}O_6 + 24NO_3^- \rightarrow 30CO_2 + 24N_2 + 18H_2O + 24OH^-, \tag{5.5}$$

$$C_6H_{12}O_6 + 3SO_4^{2-} \rightarrow 6CO_2 + 3H_2S + 6OH^-, \tag{5.6}$$

$$C_6H_{12}O_6 + 24Fe^{3+} + 6H_2O \rightarrow 6CO_2 + 24Fe^{2+} + 24H^+, \quad (5.7)$$

– aerobic oxidation (oxidation by oxygen)

oxidation of carbohydrate

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O, \quad (5.8)$$

oxidation of calcium or magnesium salts of organic acids

$$Ca(CH_3COO)_2 + 4O_2 \rightarrow CaCO_3 \downarrow + 3CO_2 \uparrow + 3H_2O. \quad (5.9)$$

## 5.3 Applications of the Biogeochemical Carbon Cycle in Construction Bioprocesses

Microbial oxygenic photosynthesis can be used in construction biotechnology for the formation of soil crust to control dust release, to stop sand dunes movement, and to immobilize atmospheric dispersion of soil surface pollutants, for example, radioactive substances.

Microbial anoxygenic photosynthesis could be used in construction biotechnology for the removal of $H_2S$ from water or from biorestoration of marble historical sculptures or constructions deteriorated by the black precipitate of CaS produced by bacterial sulfate reduction of sulfur oxides from polluted air:

Acidogenic fermentation could be used in construction biotechnology for the production of organic (mainly acetic) acid and dissolution of calcium carbonate, magnesium carbonate, or iron (hydr)oxide with the formation of soluble salts of Ca, Mg, and Fe, for example:

$$C_6H_{12}O_6 + H_2O + CaCO_3 \rightarrow 3CO_2 + 4H_2 + Ca(CH_3COO)_2. \quad (5.10)$$

Our experiments on the production of biocement from limestone and agricultural wastes showed that maximum concentration of dissolved calcium after acidogenic fermentation was about 0.1 M, however in some other experiments made after that (Chu and Wen 2015) concentration of dissolved calcium was up to 0.4 M.

So, high concentrations $Ca^{2+}$ inhibited acidogenic fermentation. However, it could be that selected halotolerant or halophilic culture of acidogenic *Bacteria* or *Archaea* will be able for the production of more concentrated solution of calcium acetate.

Calcium salts of organic acids can be used further for bioaggregation, bioclogging, and biocementation of porous soil or fractured rocks. For example, there could be urease-induced calcium carbonate precipitation:

$$Ca(CH_3COO)_2 + (NH_2)CO\,(\text{urea}) + 2H_2O + \text{urease} \rightarrow CaCO_3 \downarrow + 2CH_3COO(NH_4), \tag{5.11}$$

or bioclogging/biocementation caused by aerobic oxidation of salt:

$$Ca(CH_3COO)_2 + 4O_2 \rightarrow CaCO_3 \downarrow + 3CO_2 + 3H_2O. \tag{5.12}$$

## 5.4 The Biogeochemical Nitrogen Cycle

Nitrogen can occur in a variety of oxidation states such as ammonium $NH_4^+$ and organic amines R–$NH_2$ (oxidation state of N is −3), hydrazines $R_2N$–$NR_2$ (oxidation state of N is −2), hydroxyl amine $R_2NOH$ (oxidation state of N is −1), nitrogen gas $N_2$ (oxidation state of N is −0), nitroso-group R–N = O (oxidation state of N is +1), nitrite $NO_2^-$ (oxidation state of N is +3), nitrate $NO_3^-$ (oxidation state of N is +5). The microbiological processes of the nitrogen cycle in soil include mainly transformations shown in the Table 5.1.

## 5.5 Applications of the Biogeochemical Nitrogen Cycle in Construction Bioprocesses

Nitrification produces nitric acid, so without addition of the titrant such as NaOH, KOH, $Ca(OH)_2$, or without presence of pH buffer, such as $CaCO_3$, $Fe(OH)_3$, $Fe_2(CO_3)_3$, or the mixture of $H_2PO_4^-$ and $HPO_4^{2-}$ pH will drop to the value that inhibits and finally stops activity of nitrifying bacteria. Oxidation of ammonium to nitric acid is one of the important factors of biocorrosion of metals and biodeterioration of concrete and marble.

However, decaying protein-containing wastes forming ammonium can be used for production of dissolved calcium nitrate:

$$CaCO_3 + 2NH_4^+ + 4O_2 \rightarrow Ca(NO_3)_2 + CO_2 \uparrow + 3H_2O + 2H^+ \tag{5.22}$$

Heterotrophic denitrification is performed by denitrifying bacteria under anaerobic conditions and can be used for soil desaturation through the replacement of pore water by gas in the pores. This is an effective way to mitigate soil liquefaction during earthquake. It is also considered as a way to precipitate Ca because of production of hydroxide ions but our data showed that denitrification inhibited when concentration of dissolved calcium is above 0.05 M.

Autotrophic oxidation of ferrous ions (Eq. 5.18) can produce such clogging material as insoluble ferric hydroxide $Fe(OH)_3$ but this reaction is accompanying with acidification. So, most probably that this process will require additionally urea hydrolysis to maintain neutral pH.

**Table 5.1** Biogeochemical transformations of nitrogen

| Initial/final oxidation state of nitrogen | Process trivial name | Biogeochemical reaction | |
|---|---|---|---|
| −3/−3 | Decarboxylation (a stage of decay of dead organic matter) | $R(NH)_2COOH$ (aminoacid) → $R–NH_2 + CO_2$ | (5.13) |
| −3/−3 | Ammonification (a stage of decay of dead organic matter) | $R-NH_2 + H_2O + H^+ \rightarrow NH_4^+ + ROH$ | (5.14) |
| −3/+3 | First step of nitrification | $NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+$ | (5.15) |
| +3/+5 | Second step of nitrification | $NO_2^- + 0.5O_2 \rightarrow NO_3^-$ | (5.16) |
| +3 and −3/0 | Anammox process | $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$ | (5.17) |
| +5/0 | Autotrophic denitrification | $10Fe^{2+} + 2NO_3{}^- + 24H_2O \rightarrow N_2 + 10Fe(OH)_3 + 18H^+$ | (5.18) |
| | | $5S + 6NO_3^- + 2H_2O \rightarrow 3N_2 + 5SO_4^{2-} + 4H^+$ | (5.19) |
| +5/0 | Heterotrophic denitrification | $5CH_3COOH + 8NO_3^- \rightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^-$ | (5.20) |
| 0/−3 | Nitrogen fixation | $4N_2 + 6CH_3COOH + 12H_2O \rightarrow 8NH_3 + 12CO_2 + 12H_2$ | (5.21) |

## 5.6 The Biogeochemical Phosphorus Cycle

The main processes of the phosphorus cycle are as follows:

- biomineralization of organic phosphorus—containing compounds by hydrolysis with the production of orthophosphates $H_2PO_4^-$, $HPO_4^{2-}$ and $PO_4^{3-}$;
- bioassimilation of phosphate as a component of nucleic acids and phospholipids;
- bioprecipitation of phosphates controlled by pH and by the presence of $Ca^{2+}$, $Mg^{2+}$, $Fe^{2+}$ and $Fe^{3+}$, and $Al^{3+}$ ions that are such insoluble compounds as $Ca_{10}(PO_4)_6(OH)_2$ (hydroxyapatite), $FeHPO_4$; $Fe_3(PO_4)_2 \cdot 8H_2O$, $AlPO_4 \cdot 2H_2O$;
- biosolubilization of phosphates from the complexes with Ca, Mg, Fe, and Al due to the bioproduction of acids;
- bioaccumulation of intracellular granules of polyphosphate in bacterial cells under aerobic conditions and biological release of phosphate from the cells under anaerobic conditions.

Theoretically, bioprecipitation of Ca, Mg, Fe, and Al phosphates could be important application of the biogeochemical phosphorus cycle in construction bioprocesses and there are known several papers on this topic. Most interesting could be formation of cementing composite materials with 3D structure similar to the bone material ("bone cement"). However, their application in civil and environmental engineering could be limited by the high costs of phosphate compounds.

## 5.7 The Biogeochemical Sulfur Cycle

Sulfur can occur in a variety of oxidation states, such as sulfide/sulphhydryl ($S^{2-}$), organic sulfur (RSH), elemental sulfur (S), sulfite $SO_3^{2-}$, and sulfate $SO_4^{2-}$. The microbiological processes of the sulfur cycle in soil include mainly transformations shown in Table 5.2.

**Table 5.2** Biogeochemical transformations of sulfur

| Initial/final oxidation state of sulfur | Process trivial name | Biogeochemical reaction | |
|---|---|---|---|
| +6/−2 | Sulfate reduction | $SO_4^{2-} + CH_3COOH \rightarrow H_2S + 2CO_2 + 2OH^-$ | (5.23) |
| −2/−2 | Precipitation of sulfide ions by metal ($Ca^{2+}$, $Fe^{2+}$ as example) | $H_2S + Ca^{2+} \rightarrow CaS\downarrow$<br>$H_2S + Fe^{2+} \rightarrow FeS\downarrow$ | (5.24)<br>(5.25) |

(continued)

**Table 5.2** (continued)

| Initial/final oxidation state of sulfur | Process trivial name | Biogeochemical reaction | |
|---|---|---|---|
| −2/0 | Microaerophilic oxidation of sulfide<br>Phototrophic oxidation of sulfide | $H_2S + 0.5O_2 \rightarrow S + H_2O$<br>(sulfur is accumulated in the cells of filamentous bacteria) | (5.26) |
| | | $2H_2S + CO_2 + \text{light} \rightarrow 2S + CH_2O + H_2O$<br>(requires anaerobic conditions; sulfur is accumulated inside cell of phototrophic bacteria) | (5.27) |
| 0/+6 | Aerobic biooxidation of sulfur | $S + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$ | (5.28) |
| 0/+6 | Anaerobic biooxidation of sulfur | $5S + 6NO_3^- + 2H_2O \rightarrow 3N_2 + 5SO_4^{2-} + 4H^+$ | (5.28) |

## 5.8 Applications of the Biogeochemical Sulfur Cycle in Construction Bioprocesses

Aerobic oxidation of sulfides (Eq. 5.23) or sulfur (Eq. 5.25) by acidophilic sulfur-oxidizing *Bacteria* or *Archaea* is accompanied by acidification of the medium due to the formation of sulfuric acid. This acidification is a cause of biocorrosion, the production of acid mine drainage, and leaching of heavy metals from sulfides. So, prevention of biocorrosion caused by microbial oxidation of sulfides is an important task and several billion dollars are spent at present to diminish corrosion of the wells, pipelines, equipment caused by oxidation of $H_2S$.

Sulfate bioreduction (Eq. 5.23) is accompanied by precipitation of sulfides of almost all metals and increase of pH (Eqs. 5.24 and 5.25). So, theoretically this process could be used for the bioclogging and probably biocementation of the porous soil but it is practically unapplicable because of the production of toxic, bad smelling, and corrosive $H_2S$.

## 5.9 The Biogeochemical Iron Cycle

The biogeochemical cycle of iron includes the oxidation of ferrous ions ($Fe^{2+}$) and reduction of ferric ions ($Fe^{3+}$) as well as their hydrolysis and precipitation of ferrous or ferric hydroxides. Ferrous ions are stable in solution at low pH but quickly chemically oxidized by oxygen of air at neutral pH.

$$4Fe^{2+} + O_2 + 10H_2O \rightarrow 4Fe(OH)_3 + 8H^+. \tag{5.29}$$

However, ferrous ions in the majority cases are present in solution as the chelates of organic acids so atom of Fe(II) cannot be oxidized by oxygen of air at neutral

**Fig. 5.1** Iron hydroxide precipitate formed in a natural stream due to the oxidation of ferrous chelates by neutrophilic iron-oxidizing bacteria

pH. That is why a big physiological group of so-called neutrophilic iron-oxidizing bacteria are oxidizing the protecting organic envelope from fatty acids, humic, and fulvic acids. Usually, these bacteria are microaerophilic producers of free oxygen radicals which are degrading protecting organic envelope and after that ferrous ions are oxidized by oxygen of air. Brown precipitated of ferric hydroxide produced by these bacteria can be found everywhere in nature where Fe(II) solution meets oxygen (Figs. 5.1 and 5.2.)

The biooxidation of stable ions of $Fe^{2+}$ under low pH is performed by acidophilic iron-oxidizing bacteria:

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O. \tag{5.30}$$

The reduction of $Fe^{3+}$ under anaerobic conditions is performed by iron-reducing bacteria:

$$8Fe^{3+} + CH_3COOH + 2H_2O \rightarrow 8Fe^{2+} + 2CO_2 + 8H^+. \tag{5.31}$$

Probably, the cheapest way to produce soluble $Fe^{2+}$ is the reaction of hematite iron ore ($Fe_2O_3$) with the products of acidogenic fermentation, mainly with acetic acid:

**Fig. 5.2** Iron hydroxide precipitate formed in the Dead Sea (site is shown by *arrow*) due to the oxidation of ferrous chelates by neutrophilic iron-oxidizing bacteria

$$4Fe_2O_3 + CH_3COOH + 6H_2O \rightarrow 8Fe^{2+} + 2CO_2 + 16OH^-. \quad (5.32)$$

Ferric oxide, $Fe_2O_3$, is the major component of hematite iron ore and lateritic red topical soils. Fe(III) of iron oxide can be reduced by iron-reducing bacteria to dissolved ferrous ions or colloidal ferrous hydroxides using hydrogen or organic carbon under strictly anaerobic conditions (Lovley 2000; Francis et al. 2000). This dissolved ferrous, which is produced by iron-reducing bacteria, can be used in different chemical or environmental engineering applications (Ivanov et al. 2014c), which commercial use in USA is protected by US Patent 7393452 (Tay et al. 2008). The applications of microbial reduction of iron in the treatment of fat-containing wastewater (Ivanov et al. 2002), removal of phosphate during anaerobic digestion of activated sludge (Stabnikov et al. 2004) or from reject water (Guo et al. 2009; Ivanov et al. 2005, 2009), the combination of microbial reduction and microbial oxidation of iron for the treatment of food-processing wastewater (Ivanov et al. 2004; Stabnikova et al. 2010), treatment of sucralose production wastewater and many other applications (Ivanov et al. 2014c) were reported. The source of iron oxide could be hematite iron mine tailings, which are voluminous wastes that occupy land and pollute air with their dust. For example, the Godli mine (India)

generates 0.6 million tons of tailings per year with an average grade of 50 % Fe. Magnetic fraction of tailing contains more than 62 % of Fe, mostly hematite. Distribution by size of hematite granules in this fraction is as follows: 64.6 % less than 12 μm; 27.4 % from 12 to 35 μm; 8 % from 35 to 45 μm (Brown and Rai 2002). Totally, tailings from iron primary extraction processes only in India are estimated as 11 million tons per year (Pappua et al. 2007). As an inexpensive raw material iron ore tailings were proposed to be used as value-added product in some technologies, for example, in the production of ceramics (Wagh et al. 2002; Liu et al. 2010), in civil engineering constructions for road construction, building materials, and brick making (Yellishetty et al. 2008) or in the pigments production (Hedin 2003) but an effective way could be also the production of biogrout through acidogenic fermentation of cellulose (Stabnikov and Ivanov 2016b).

The simplest way for the production of ferric-based grout is just chemical dissolution of iron ore in acetic or chloric acids:

$$Fe_2O_3 + 6CH_3COOH \rightarrow 2Fe(CH_3COO)_3 + 3H_2O. \quad (5.33)$$

$$Fe_2O_3 + 6HCl \rightarrow 2FeCl_3 + 3H_2O. \quad (5.34)$$

At approximately pH above 5 ferric and ferrous ions are precipitated as hydroxides:

$$Fe^{2+} + 2H_2O \leftrightarrow Fe(OH)_2 + 2H^+. \quad (5.35)$$

$$Fe^{3+} + 3H_2O \leftrightarrow Fe(OH)_3 + 3H^+. \quad (5.36)$$

## 5.10 Applications of the Biogeochemical Iron Cycle in Construction Bioprocesses

Iron-based biogrout can be produced by bacteria that are reducing ferric oxide of hematite iron ore (Eq. 5.32) in association with bacteria performing acidogenic fermentation of cellulose (Eq. 5.4). Concentration of produced dissolved ferrous ions was proportional to the quantity of the iron ore particles, i.e., to their surface because both stoichiometry and kinetics of Fe(III) bioreduction are based on the biochemical processes on the surface (Stabnikov and Ivanov 2016b). However, precipitation of ferric hydroxide in the pores of sand requires increase of the pH and can be supported by urease-producing bacteria. The hydraulic conductivity of sand after treatment with the iron-based biogrout decreased from $7 \times 10^{-4}$ m/s to $1.4 \times 10^{-6}$ m/s at the final content of Fe in biocemented sand 4.5 % (w/w) and efficiency of dissolved iron precipitation 99 % (Stabnikov and Ivanov 2016b). This

bio-induced precipitation of ferric hydroxide could be used in practice for the sealing of the agricultural channels, the aquaculture and wastewater treatment ponds, the landfill sites, the dams and retaining walls, and the tunneling space before or after excavation.

Practical application of iron-reducing bacteria for production of soluble iron-based biogrout from iron ore should be done with a cheap electron donor. Organic wastes can be such donors to produce dissolved ferrous salts of organic acids during acidogenic fermentation. Acidogenic fermentation is widely used in biotechnology to convert organic wastes into volatile fatty acids (VFA), mainly acetate (up to 58 % of total VFA), propionate, butyrate, valerate, and lactate (in particular) (Traverso et al. 2000; Bengtsson et al. 2008; Arroja et al. 2012). Organic matter is degraded to VFA by hydrolytic and acidogenic bacteria in an acidogenic reactor. There are many bacterial species able to hydrolyse polysaccharides, including cellulose and hemicellulose, and then to ferment them to organic acids, hydrogen, and carbon dioxide as the end products. The most typical material balance of this process can be shown by the following equation of acidogenic fermentation in the rumen (molar ratios of VFA were taken from Madigan et al. 2014):

$$C_6H_{12}O_6 + 0.82H_2O \rightarrow 1.13CH_3COOH + 0.35C_2H_5COOH + 0.26C_3H_7COOH + 1.67CO_2 + 2.47H_2, \quad (5.37)$$

where $C_6H_{12}O_6$ is a monomer of cellulose, $CH_3COOH$, $C_2H_5COOH$, $C_3H_7COOH$ are acetic, propionic and butyric acids, respectively. These VFA can be used for bioreduction of Fe(III) producing soluble ferric and ferrous salts from the mixture of calcium carbonate for neutralization of organic acids, iron ore, and iron-reducing bacteria.

Both reduction of $Fe^{3+}$ in anaerobic medium (Eq. 5.31) and formation of iron hydroxides (Eqs. 5.35 and 5.36) require neutralization of the pH. This could be done by two ways: (1) addition of $CaCO_3$, which produces soluble ions of $Ca^{2+}$ as shown by Eq. 5.38 and (2) addition of urea and urease-producing bacteria that produce hydroxide ions according to the Eq. 5.39:

$$CaCO_3 + 2H^+ \rightarrow Ca^{2+} + CO_2 + H_2O, \quad (5.38)$$

$$(NH_2)_2CO + 3H_2O \rightarrow 2NH_4^+ + CO_2 + 2OH^-. \quad (5.39)$$

Clay is one of the most used construction materials. So, aerobic or anaerobic biotransformations of iron in clay—bioreduction, biooxidation, sulfate reduction, acidification, alkalinization, formation of biocomposites, formation of nanopores, and nanochannels—could be a way for improvement of clay as the construction material. However, there were no such studies.

## 5.11 The Biogeochemical Cycle of Calcium

The main processes of the calcium biogeochemical cycle are as follows:

- bioaccumulation of organic-bound calcium. For example, content of calcium accumulated as calcium dipicolinate (calcium salt/chelate of pyridine-2, 6-dicarboxylic acid) in the bacterial spores can be up to 20 % of dry biomass. This compound protects spores and their DNA from heat denaturation. Calcium ions form also the salt bridges between the protein increasing thermal stability of cell proteins. Proteins of thermophilic microorganisms contain more calcium than proteins of mesophiles, so the medium for cultivation of thermophiles must contain dissolved calcium salts.
- biomineralization of organic calcium—containing compounds by hydrolysis of the organic component with the production of dissolved ions of $Ca^{2+}$ and salts such as $CaCl_2$, $Ca(NO_3)_2$; $Ca(HCOO)_2$; $Ca(CH_3COO)_2$, $Ca(HCO_3)_2$, and partially dissolved alkali $Ca(OH)_2$. Calcium ions are released to medium in big quantities during the germination of bacterial spores;
- calcium-based homeostasis: the equilibrium between dissolved and precipitated calcium plays one of the major roles in the maintenance of the stable concentration of $CO_2$ and pH in gaseous and liquid biosystems:

$$CaCO_3 \downarrow + CO_2 \uparrow + H_2O \leftrightarrow Ca(HCO_3)_2 \leftrightarrow Ca^{2+} + 2OH^- + CO_2; \qquad (5.40)$$

- biosolubilization of calcium from the undissolved salts and chelates of calcium phosphates, calcium carbonate, and calcium sulfide due to the bioproduction of organic and inorganic acids;
- bioprecipitation of calcium carbonate and phosphates at the pH above 7 and presence of carbonate or phosphate ions with the formation of mainly such insoluble compounds as $CaCO_3$ in mineral forms of calcite, vaterite, or aragonite, and $Ca_{10}(PO_4)_6(OH)_2$ as hydroxyapatite. Inside organism, this precipitation is going as the formation of the composite material of shells, pearls, and bones.

## 5.12 Applications of the Biogeochemical Cycle of Calcium in Construction Bioprocesses

Bioprecipitations of Ca as calcium carbonate or phosphate are the most important application of the biogeochemical calcium cycle in construction bioprocesses. There are known hundreds of the papers on this topic shown in the following chapters on bioaggregation, biocementation, bioclogging, and biocoating, which are

performing mainly due to precipitation of calcium carbonate minerals from dissolved calcium salt, carbonate and increase of the pH due to urea hydrolysis or biooxidation of organics. Most interesting process could be the formation of cementing composite materials with 3D structure similar to the pearl or bone structures. However, there are still no data on the production and application of such biocomposites in civil and environmental engineering.

## 5.13 The Biogeochemical Cycle of Magnesium

The main processes of the magnesium biogeochemical cycle are similar to the calcium biogeochemical cycle, for exemption that many magnesium salts are more soluble that calcium ones:

- bioaccumulation of organic-bound magnesium, which is the essential element for many enzymatic activities. An important point is the interaction of calcium and magnesium ions so their molar ratio significantly affects the growth rate, yield and other physiological parameters of organisms.
- biomineralization of organic magnesium–containing compounds by hydrolysis of the organic component with the production of dissolved ions of $Mg^{2+}$.
- biosolubilization of magnesium ions from the undissolved salts and chelates of magnesium phosphates, carbonate, and sulfide due to the bioproduction of organic and inorganic acids;
- bioprecipitation of magnesium carbonate at the pH above 7 and the presence of carbonate or phosphate ions with the formation of low soluble compound $MgCO_3$. At the pH above 9 and the presence of ammonium and phosphate ions, magnesium can precipitate in the form of struvite $Mg(NH_4)PO_4$. Presence of $Mg^{2+}$ ions significantly affects precipitation and crystallization of calcium carbonates.

## 5.14 Applications of the Biogeochemical Cycle of Magnesium in Construction Bioprocesses

Precipitation of struvite could be used for the clogging of the porous soil, especially if it will be combined with the precipitation of calcium carbonate, considering that a mixture of calcium and magnesium dissolved salts will be produced from the cheap dolomite powder (a mixture of $CaCO_3$ + $MgCO_3$) or from limestone containing magnesium carbonate. Probably, a mixture of dissolved calcium and magnesium salts, produced from dolomite, could be used for bioclogging in same way as it was shown for calcium salts.

## 5.15 The Biogeochemical Cycle of Silicon

Silica shell of some marine organisms—dinoflagellates, radiolarians, sponges—is the best known case of direct involvement of silicon in bioprocesses. Silicon does not include into organic compounds and exists in environment in form of silica ($SiO_2$), silicates ($SiO_4^{4-}$), cyclic, and single chain silicates $nSiO_3^{2-}$, and the sheet-forming silicates $nSiO_{2.5}^{-}$ and weak silicic acid $H_4SiO_4$:

$$SiO_2 \downarrow + 4OH^- \leftrightarrow SiO_4^{4-} + 2H_2O. \tag{5.41}$$

$$SiO_4^{4-} + 4H^+ \leftrightarrow SiO_2 \downarrow + 2H_2O. \tag{5.42}$$

So, dissolution of silica can be performed due to addition of alkali and precipitation of silica from silicate can be due to addition or formation in situ of inorganic or organic acids.

Some atoms of silicon in the chain and sheet-forming silicates are substituted by aluminum. Aluminum can form also aluminosilicate ($Al_2SiO_5$), which is the major component of clay. Silicon compounds are not biologically active themselves but silicon interacting with the cycles of Ca forming calcium aluminosilicate $CaAl_2Si_2O_8$ and components of Portland cement such as $CaO \cdot Ca_2SiO_4$ and $Ca_2SiO_4$. Particles of different types of clays are active adsorbents of many ions and organic compounds so they are able to interact with the biogeochemical cycles of carbon, nitrogen, sulfur, phosphorus, calcium, and magnesium due to their adsorption capacity.

## 5.16 Applications of the Biogeochemical Cycle of Silicon in Construction Bioprocesses

The major construction materials—glass, bricks, Portland cement—are made of silica, calcium and magnesium oxides, aluminosilicates, and calcium silicates. However, there are not known yet the biotechnologies for the formation of solid aluminosilicates or calcium-magnesium silicates from limestone, dolomite, and clay. Hypothetically, the clogging and cementation of porous soil could be possible due to precipitation of silica from silicate because of microbial production of inorganic or organic acids in situ (Eq. 5.42) however, it could be slow and probably not effective process.

# Chapter 6
# Biotechnological Improvement of Construction Ground and Construction Materials

## 6.1 The Stages of Biotechnological Improvement of Ground

Any microbial biotechnology of soil improvement includes following stages:

1. Preparation for the biotreatment of soil: preparation of medium, equipment, and microbial inoculum for the biotreatment of soil; preparation of soil for the treatment; cultivation and storage of inoculum. Growth of bioagent in a medium designed using stoichiometrical equation of microbial growth that includes consumption of C, H, O sources for the synthesis of biomass with the empirical formula $CH_{1.8}O_{0.5}N_{0.2}$ and other essential elements such as N, P, S, K, Na, Mg, Ca, and Fe and microcomponents containing Cr, Co, Cu, Mn, Mo, Ni, Se, W, V, and Zn that are required in quantities of several mg/L. In some cases, organic growth factors such as vitamins, amino acids, and nucleosides are required for the growth and activity of some microbial strains.

Biotreatment can be performed by indigenous microorganisms of soil, without preparation and supply of microbial inoculum (Burbank et al. 2011, 2012 a, b) but it bears a risk of the propagation in soil of pathogenic or opportunistic microorganisms.

Preparation of soil or particles before biotechnological treatment can include the following steps: (1) crushing, grinding, sieving, homogenization of treated soil, solid particles, or soft clay; (2) chemical pretreatment of soil or particles with alkali, acids, salt solution, or surfactants; (3) excavation, transportation, storage and supply of soil or particles in the bioreactor in the case of the offsite biotreatment.

2. Production of biocement or biogrout.

The cultivation of microorganisms is performed at suitable conditions, usually at optimal temperature, pH, osmotic pressure, and concentration of gases (oxygen, carbon dioxide, hydrogen), on a semisolid or liquid medium containing all



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necessary substances for the growth of the strain. Microbial biomass for biotreatment is stored and used as a liquid suspension, frozen suspension, concentrated paste, dry (usually freeze-dried) biomass. The medium for microbial cultivation and the treatment medium for soil/particles can be mixed together or used separately in the form of solution, suspension, or slurry by the mixing of chemical reagents and agricultural fertilizers. Different kind of wastes or residuals such as mining and agricultural residuals, organic fraction of municipal solid wastes, sewage sludge, and reject water of municipal wastewater treatment plants can be used as a medium

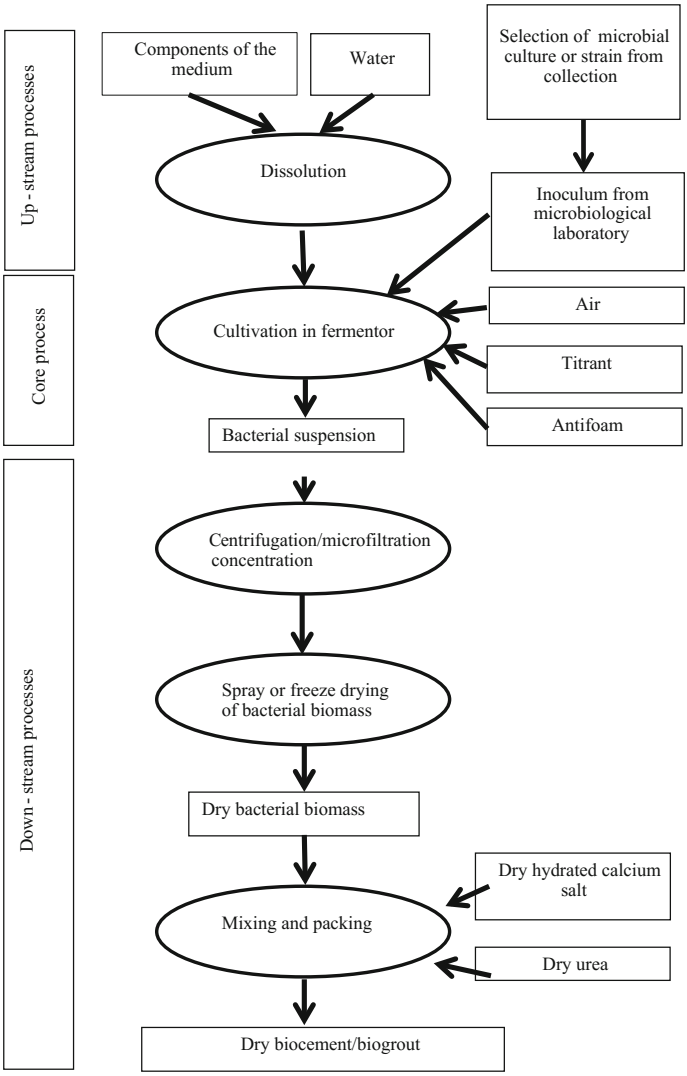



**Fig. 6.1** The stages of biocement/biogrout production

to reduce the cost of large-scale biogeotechnical work. For long-term storage of these media prior to microbial cultivation or biotreatment, they can be acidified, cooled, frozen, dried, pasteurized, or sterilized. Finally, liquid or dry bioagent is supplied into soil, separately or mixed with the reagents. This mixture can be prepaid as the commercial product is in the form of dry biocement or biogrout, which can be used for soil biotreatment (Fig. 6.1).

3. Biotreatment of soil as bioaggregation, biocrusting, biocoating, bioclogging, biocementation, biodesaturation, bioencapsulation, or bioremediation of soil.
4. Posttreatment: waste treatment, disposal, and reuse. Application of microorganisms in construction process can be performed without downstream processes but usually there could be such downstream step as a detoxication of polluted air and water after biocementation of soil or particles. To avoid pollution of atmosphere, the toxic components of air must be absorbed, chemically or biotechnologically inactivated, or incinerated.

All these processes must be monitored and controlled using mechanical, chemical, or biological tools.

## 6.2 The Types of Construction Biotechnological Processes

The classification of the construction-related biotechnological processes is based on the final goal of the biotreatment. This classification and related mechanisms of biotechnological ground improvement are shown in Fig. 6.2.

The major types of construction biotechnological processes are as shown below.

1. **Bioaggregation** of soil or particles is a process to increase the size of fine particles so that water and wind soil erosions, sand movement, as well as, dust emission will be reduced (Bang et al. 2011; Stabnikov et al. 2013a).
2. **Biocrusting** of soil surface is a process to form mineral or organic crust onto soil surface so that erosion, dust emission, and water infiltration will be reduced (Stabnikov et al. 2011; Chu et al. 2012a).
3. **Biocoating** of solid surface is a process to form a layer on solid surface so that aesthetics or colonization of surface will be enhanced.
4. **Bioclogging** of soil or porous matrix is a process to fill in the pores and channels in soil/matrix so that hydraulic conductivity of soil or fractured rocks will be significantly reduced (Ivanov et al. 2014a).
5. **Biocementation** of porous soil or fractured rocks is a process to significantly increase their strength (Ghosh et al. 2005; Mitchell and Santamarina 2005; Whiffin et al. 2007; Ivanov and Chu 2008; De Muynck et al. 2008 a, b, 2010, 2013; Sarda et al. 2009; van der Ruyt and van der Zon 2009; Achal et al. 2010a, b; Ivanov 2015; Van Tittelboom et al. 2010; van Paassen et al. 2010; Harkes et al. 2010; Chu et al. 2012a; Dhami et al. 2012; Li and Qu 2012; DeJong et al. 2010, 2013; Dosier 2013; Raut et al. 2014).

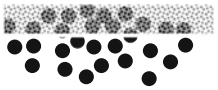
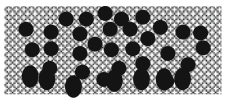
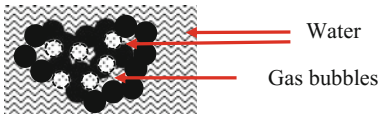
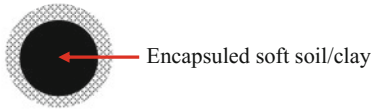
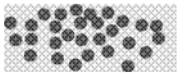
| Type of biotreatment process | Soil particles or construction material (dark color) after biotreatment forming binding material/biocement (grey color) |
|---|---|
| Bioaggregation of soil: increase of soil particles size so that soil erosion and dust emission will be reduced |  |
| Biocrusting: formation of crust on soil surface so that wind and water erosions, dust emission, and water infiltration will be reduced |  |
| Biocoating: formation of a layer on the solid surface so that colonization or aesthetics, or corrosion protection of surface will be enhanced |  |
| Bioclogging: filling the pores and channels in soil of fissured rock so that hydraulic conductivity of soil or fissured rock will be significantly reduced |  |
| Biocementation: binding of the soil particles that significantly increase strength of soil |  |
| Partial biodesaturation of saturated soil: production of biogas bubbles *in situ* to reduce saturation and liquefaction potential of soil |  |
| Bioencapsulation: increase of the strength of soft clayey soil, saturated loose soil, quick sand, muck soil of the drained swampland. |  |
| Bioremediation: biodegradation or bioimmobilization of the soil pollutants before construction process |  |

**Fig. 6.2** Types of the biotreatment process

6. **Biodesaturation** of soil is a process to decrease saturation and as a result the liquefaction potential of soil through the biogas production in situ (Chu et al. 2009a; Rebata-Landa and Santamarina 2012; Chu et al. 2013b; He et al. 2013).
7. **Bioencapsulation** of clay/soil/particles is a process to increase strength of soft clayey soil through the formation of a strong shell around a piece of soft material (Ivanov et al. 2014b).

8. **Bioremediation** of soil is a process to remove pollutants from soil or immobilize pollutant in soil before construction (Warren et al. 2001; Fujita et al. 2004; Mitchell and Ferris 2005).
9. **Sanitation of soil** is a process to kill microbial pathogens or insects in soil prior to excavation.

All these types of ground improvement often use the same biogeochemical reactions. Therefore, the final results depend mainly not on the mechanism of ground improvement but on the quantity of added raw materials or quantity of binding biomaterials added or produced in soil.

## 6.3 Bioaggregation to Control Wind Soil Erosion and Dust Emission

This process is aiming to increase size of soil aggregates so that soil erosion and dust emission will be reduced. Such processes as the wind erosion of fertile soil, dune movement in sand deserts, dust storms in arid and semiarid regions, as well as, emission and dispersion of agricultural, construction, transportation, or mining dusts create a lot of problems and dangers for human life, environment, and infrastructure. Dust is also a carrying agent for soil–associated pollutants such as chemicals, viruses, and microorganisms. So, the release of the polluted dust in atmosphere can cause the deposition of these pollutants to ecosystems located downwind and negatively affect human health and environment (Boreson et al. 2004; Falkovich et al. 2004; Griffin et al. 2007; Raisi et al. 2010). Dust storms originated in desert areas affect many countries in Middle and East Asia and North Africa (Lee et al. 2006; Ci and Yang 2010).

## 6.4 Dust Control Technologies

There are many known biological, chemical, and mechanical technologies to prevent and control wind erosion of soil, wind dispersion of dust, desertification due to wind-blown sand movement, as well as, the release of dust from agriculture, construction, or transportation on unpaved roads. One conventional, but limited by agricultural conditions, way is the use of vegetation as the land surface cover to reduce wind erosion of soil (Ci and Yang 2010). Wind erosion of soil and dust formation could be prevented by the compaction of the soil surface layer using treatment with inorganic compounds such as sodium chloride, calcium chloride, magnesium chloride, lime, or fly ash (Petry and Little 2002; Santoni et al. 2005). Many patents have been issued on the reagents to control erosion of soil and dust suppression. However, application of binding reagents could negatively affect plants (Goodrich and Jacobi 2012) and quality of surface and underground waters

due to the solubility of the reagents in water and their dispersion in environment with runoff.

Another approach to suppress dust dispersion is the use of organic compounds such as an asphalt emulsion (Santoni et al. 2005), lignin and lignosuphonates (Gargulak and Lebo 1999), surfactants (Copeland et al. 2009), fly ash and polyacrylamide (Yang and Tang 2012), starch ethers, polyacrylonitrile, polyvinyl alcohol, urea-formaldehyde and their copolymers, polyacrylamide copolymers, carboxymethyl cellulose, polyvinyl acetate, epoxyresins and many others (Nij et al. 2003; Santoni et al. 2005; US Army 2005; Orts et al. 2007; Yang et al. 2007a, b; Naemi and Ghorbanalizadeh 2010; Zandieh and Yasrobi 2010). These dust suppressors change the physical properties of the soil surface by wetting or binding the fine particles. These suppressors are used in construction industry, mining, at the loading/unloading points, transportation on unpaved roads, and using of airfields. However, organic fixing reagents are relatively expensive for large-scale application and could be environmentally harmful due to their toxicity or ability to cause eutrophication of water.

## 6.5 Biotechnological Methods for Dust and Wind Erosion Control

Alternative way for the dust suppression with chemical reagents can be an application of biomediated cementation/aggregation of the soil particles (Ivanov and Chu 2008; Bang et al. 2011; Chu et al. 2012a, b; DeJong et al. 2010, 2011, 2013). Biocementation is based on the precipitation or crystallization of insoluble compounds in porous soil using enzymatic activity of microorganisms (Ivanov and Chu 2008; Ivanov 2015). The most popular technology of biocementation is crystallization of calcite ($CaCO_3$) from the solution of calcium chloride and urea mediated by enzyme urease or biomass of urease-producing bacteria (Ivanov 2015). Enzymatic hydrolysis of urea by urease increases the pH and releases carbonate following with calcium carbonate crystallization on the surface of soil particles and their binding according to the following biochemical reaction:

$$(NH_2)_2CO + 2H_2O + CaCl_2 \rightarrow CaCO_3 \downarrow + 2NH_4^+ + 2Cl^- \qquad (6.1)$$

However, it is important to know that chemical precipitation of calcium carbonate in sand did not produce effect of sand cementation (Chu et al. 2012b).

Even small level of sand dust biocementation can prevent its dispersion. There is known experimental attempts to study the effects of biocementation on sand immobilization (Bang et al. 2011) but the published results are not applicable for sand dust suppression because of the following reasons: (1) the object of study was not fine sand or sand dust but big sand particles with the sizes from 0.075 to 4.76 mm; (2) the effect of biocementation on the sand dust formation in the wind

tunnel was studied only by the mass losses of sand; (3) the content of precipitated calcium in sand was not measured; (4) the effects of some biotreatments were measured after simultaneous application of urease-producing bacteria, calcium chloride and urea to sand, which can cause chemical precipitation of calcium carbonate due to the high pH of medium after cultivation of urease-producing bacteria. Meanwhile, only crystallization of calcite on sand surface mediated by urease activity is important for the sand grains binding because chemical precipitation of $CaCO_3$ did not bind the sand grains (Chu et al. 2012b).

## 6.6 Biotechnological Control of Air-Born Movement of Sand Dust and Dust-Associated Chemical and Bacteriological Pollutants

One study examined the effects of small dosage, calcium-based biomediated aggregation of fine sand on the air-born movement of sand dust and dust-associated chemical and bacteriological pollutants (Stabnikov et al. 2013a). The bioaggregation reagent was a solution of calcium chloride and urea sprayed over the sand surface, which was preliminary treated with the suspension of urease-producing bacteria. Quantity of calcium used for the sand dust suppression was 15.6 g Ca/$m^2$. After biotreatment of the fine sand, the release of the sand dust and its artificial pollutants to atmosphere decreased in comparison with control by 99.8 % for dust, 92.7 % for phenantherene, 94.4 % for led nitrate, and 99.8 % for bacterial cells of *Bacillus megaterium*. This immobilization of dust and dust pollutants was due to bioaggregation of the fine sand particles. The size of 90 % of the sand dust particles increased from 29 μm in control to 181 μm after bioaggregation. Bioaggregation treatment of the soil surface could be a useful method to prevent the dispersion of dust and the dust-associated chemical and bacteriological pollutants in water, air, and soil (Stabnikov et al. 2013a). Probably, the same technology could be used to immobilize radioactive pollution of soil surface after nuclear power plant accident, explosion of radioactively "dirty" bomb, or nuclear weapons.

## 6.7 Biocrusting

This process is aiming to form crust on soil surface so that soil erosion, dust emission, water infiltration, and the leaching from polluted soil will be reduced. It is performed by three different mechanisms

1. formation of soil crust by filamentous and photosynthetic microorganisms binding the particles of soil. There could be hyphas (cellular "threads") of mycelial fungi, actinomycetes, and filamentous phototrophic and heterotrophic bacteria;

2. biocrusting by an addition of bacterial polymers to soil surface or by the production of bacterial exopolymers in situ;
3. biocrusting using biocementation of soil particles.

## 6.8 Formation of Soil Crust by Filamentous and Photosynthetic Microorganisms

The soil surface's crust is created due to physical or biological (biotic) processes. Non-biotic formation of soil crust is due to raindrops or run off breaking soil aggregates, then physical redeposition of fine soil particles, clogging soil pores with these particles, and drying these particles on soil surface forming sealing crust where salts, lime, and silica are also deposited at surface as water evaporates (Belnap et al. 2001; Jeffery et al. 2010). Physical crust of soil is often impenetrable for germinating seeds of plants. Soils especially susceptible to physical crusting are those with low content of organic matter and high content of silt (Belnap et al. 2001).

Biotic crusting is due to filamentous and slime-producing microorganisms binding the soil particles together. Microbial crust at soil surface is formed mainly by such phototrophic microorganisms as cyanobacteria and algae alone or in symbiosis with mycelial fungi, which are chemotrophic organisms consuming organic compounds (Fig. 6.3).

In last case, the phototrophic and chemotrophic microorganisms form symbiotic organisms called lichens, where the major function of the fungal component is to extract inorganic nutrients from soil, while the photosynthetic cyanobacteria or algae are producing photosynthetic organic compounds, which are used also by fungi. Filamentous fungi and cyanobacteria weave through soil particles gluing them together (Belnap et al. 2001; Belnap 2003). Microbially formed crust creates and maintains fertility in infertile desert soils fixing carbon and nitrogen from air and capture nutrient-rich dust but they are vulnerable to climate change and grazing livestock and their recovery is very long (Belnap 2003). So, artificial inoculation of soil surface by cyanobacteria could be useful for the recovery of desert soil crust. Cyanobacteria with urease activity can participate in the artificial formation of calcium carbonate minerals from calcium salts and these minerals can bind particles forming the soil crust (Ariyanti et al. 2011, 2012).

There are different successional stages in the development of biological soil crust: (1) cyanobacterial crust where lichen and moss coverages <20 %, (2) lichen crust where lichen coverage >20 % but moss coverage <20 %, semi-moss crust where moss coverage >20 % but <75 %), and moss crust (moss coverage >75 %). Cyanobacterial and microalgal biomass content decreased in moss crust but the content of nitrogen-fixing cyanobacteria and heterotrophic microbes increased in the semi-moss and moss crusts (Lan et al. 2013). There must be different hydraulic conductivities, water retention capacity and strength of soil crust at these successional stages.

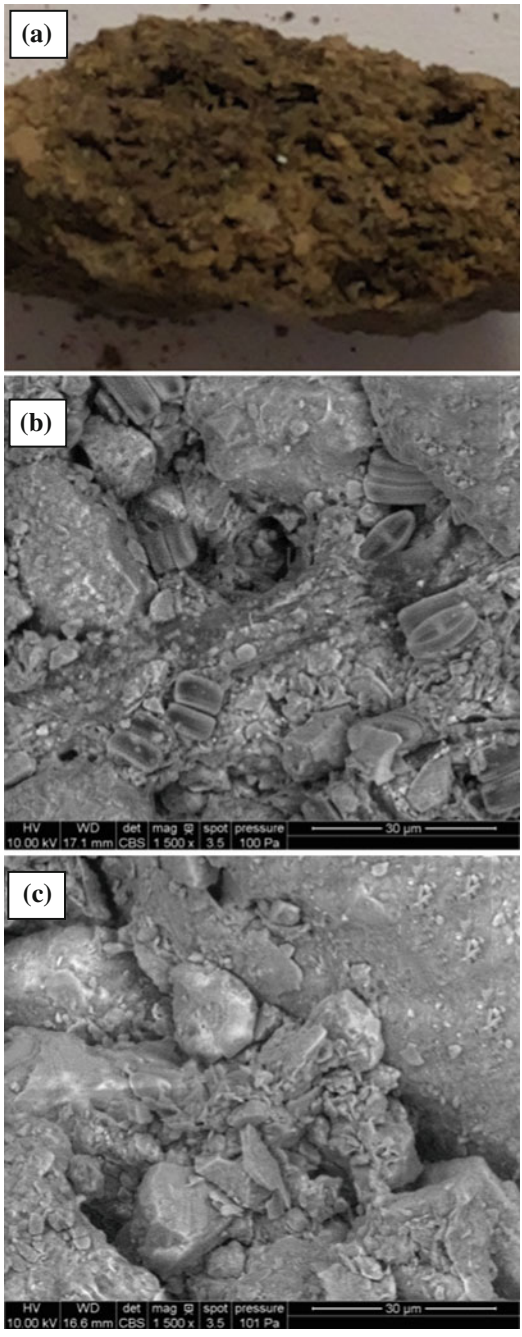



**Fig. 6.3** The soil crust (**a**) with algae cells (**b**) and fine particles (**c**) binding sand grains

## 6.9 Biocrusting Using Microbial Polysaccharides

The important role of microbial polysaccharides in soil particles aggregation is well known (Chenu 1993). A lot of gel-forming water-insoluble microbial polysaccharides are produced in industry (Sutherland 1990) and some of them, for example, xanthan as well as its or co-polymers or composites can be used for soil particles binding and crust formation to reduce wind and water erosion of soil (Yang et al. 2007a, b). However, big scale geotechnical sand fixation using addition of microbial products could be not feasible because of the high cost involved.

Production of bacterial exopolymers in situ can be used to reduce cost of soil particles binding in the soil crust formation. However, the production of bacterial polysaccharides by heterotrophic microorganisms requires addition of organic matter up to 60 tons/ha. Therefore, production of exopolysaccharides by phototrophic microorganisms remains most economical way for the bioenhanced formation of soil crust.

## 6.10 Biocrusting Using Calcium-Based Biocementation

Calcium-based formation of soil crust is based on the precipitation or crystallization of insoluble compounds in porous soil using enzymatic activity of microorganisms (Ivanov and Chu 2008; Ivanov 2015; DeJong et al. 2010, 2011, 2013). Chemical precipitation of calcium carbonate in sand by mixing of calcium chloride and sodium bicarbonate did not produce calcite crystals and sand cementation (Chu et al. 2012b).

To form soil crust a defined volume of the biomass of urease-producing bacteria and then a solution of calcium chloride and urea must be sprayed one time over soil surface (Stabnikov et al. 2013a). This volume (V, $m^3$ of solutions/$m^2$ of soil surface) can be calculated using the values of porosity (P, $m^3$ of pores/$m^3$ of soil), thickness of crust (H, m)

$$V = P \cdot H \, m^3 \text{ of solutions}/m^2 \text{ of soil surface} \tag{6.2}$$

For example for the formation of 1 cm soil crust in soil with porosity 0.5 the volume of one spraying must be $0.5 \times 0.01 = 0.005$ $m^3$ of solution/$m^2$ of soil surface.

Number of the treatments (N) can be determined from the required precipitation of calcium carbonate (CC, moles/$m^3$ of soil) and concentration of calcium ions in the treatment solution (C, moles/$m^3$ of solution). For example for the formation of 1 cm soil crust containing 10 % (w/v) $CaCO_3$ (=1000 mol/1 $m^3$ of soil) and the number of the treatments with 0.5 M $CaCl_2$ and 1 M of urea solution must be

$$\begin{aligned} N &= \left(1\,m^2 \times 0.01\,m \times 1000\ \text{moles}/m^3\ \text{of soil}\right)/\left(0.005\,m^3 \text{of solution}\right. \\ &\quad \left.\times 500\ \text{moles}/m^3\ \text{of solution}/m^2 \text{of soil surface}\right) \\ &= 4\ \text{treatments}/m^2\ \text{of soil surface} \end{aligned} \tag{6.3}$$

Formation of thin crust can be used for the sealing of aquacultural ponds or reservoirs in sandy soil (Stabnikov et al. 2011; Chu et al. 2012a; Ivanov and Stabnikov 2016). These ponds could be used for outdoor commercial aquaculture, such as fish, shrimp, and mollusk production (Biao et al. 2007), for large-scale cultivation of algae (Lebeau and Robert 2003), for biofuel production in desert coastal area, or as water collecting reservoirs. It is suitable for many other geotechnical engineering applications, where the soil crust must be with the thickness from 1 to 10 cm with hydraulic permeability lower than $10^{-8}$ $ms^{-1}$ and strength above 0.1 MPa. These applications can be the formation of the bottom layer and slopes of aquaculture ponds or channels, and the slopes and top of the levies. Formation of water-impermeable crust on the surface of the bottom and slopes of aquaculture ponds or channels and the slopes and top of the levies is possible through calcium-based biocementation (Stabnikov et al. 2011).

Many of other biogeochemical reactions shown in the Chap. 5 can be used for the formation of thin or thick soil crust.

## 6.11 Bioclogging

Clogging of porous soil or fractured rocks is performed usually by the chemical grouts to fill in the soil voids to diminish water flow (Karol 2003). Chemical grouts are solution or suspension of sodium silicate, acrylates, acrylamides, and polyurethanes, which are expensive and often toxic construction materials.

Bioclogging is a grouting using microbial biopolymers or microbially mediated precipitation of inorganic compounds in situ for water flow control. Different possible microbial processes that can lead potentially to bioclogging are summarized in Table 6.1. These include formation of impermeable layer of algal and cyanobacterial biomass; production of slime in soil by aerobic and facultative anaerobic heterotrophic bacteria, oligotrophic microaerophilic bacteria and nitrifying bacteria; production of undissolved sulfides of metals by sulfate-reducing bacteria; formation of undissolved carbonates of metals by ammonifying bacteria; and production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil by iron-reducing bacteria. Not all of these processes have been tested in the laboratory and in the field.

**Table 6.1** Microbial processes that can lead potentially to bioclogging

| Physiological group of microorganisms | Mechanism of bioclogging | Essential conditions for bioclogging | Potential geotechnical applications |
|---|---|---|---|
| Algae and cyanobacteria | Formation of impermeable layer of biomass | Light penetration and presence of nutrients | Reduction of water infiltration into slopes |
| Aerobic and facultative anaerobic heterotrophic slime-producing bacteria | Production of slime in soil from organic compounds | Presence of oxygen and medium with ratio of C:N > 20 | Cover for soil erosion control and slope protection, and prevention of slope failure |
| Oligotrophic microaerophilic bacteria | Production of slime in soil from organic matter | Low concentration oxygen and medium with low concentration of carbon source | To reduce drain channel erosion |
| Nitrifying bacteria | Production of slime in soil using oxidation of ammonium and assimilation of $CO_2$ | Presence of ammonium and oxygen in soil | To reduce drain channel erosion |
| Sulfate-reducing bacteria | Production of undissolved sulfides of metals | Anaerobic conditions; presence of sulfate and carbon source in soil | To form grout curtains to reduce the migration of heavy metals and organic pollutants |
| Ammonifying and urease-producing bacteria | Formation of undissolved carbonates of metals (Ca, Mg, Fe) in soil due to increase of pH and release of $CO_2$ during ammonification or urea hydrolysis | Presence of amino acids or urea and dissolved metal salt | To prevent piping of earth dams and dikes |
| Iron-reducing bacteria | Production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil | Anaerobic conditions changed for aerobic conditions; presence of ferric minerals | To prevent piping of earth dams and dikes |
| Nitrate-reducing (denitrifying) bacteria | Production of $N_2$ in the pores and diminishing of hydraulic conductivity because of this | Anaerobic conditions and presence of electron donors for bioreduction of nitrate | To diminish seepage from landfills, polluted sites, channels. It is required immobilization of the nitrogen gas bubbles to ensure stability of the clogging |

## 6.12 Biocementation of Soil

Biocementation is a process aiming to significantly increase strength of porous soil. Chemical cementation (or chemical grouting) is used to fill in the sand voids with fluid grouts to produce sandstone like masses to carry loads. The chemicals that are used to bind soil particles include suspension of ultrafine cement, acrylates, acrylamides, and polyurethanes. These materials can be viscous, expensive, and toxic. Microorganisms can bind the soil particles together into a hard rock due to biomediated crystallization of calcium and magnesium carbonates and phosphates (DeJong et al. 2006) or ferric hydroxides (Ivanov and Chu 2008). The unconfined compressive strength of bio-sandstone depends on the content of precipitated calcium carbonate and regime of treatment and could be up to 6–12 Mpa (Rong et al. 2012a, b) or even up to 30 MPa (van Der Star et al. 2009).

Example of sand cementation in nature is the formation of red sandstone cemented by ferrihydrite or formation of sandstone cemented by calcium carbonate crystals. Iron hydroxides, depending on its crystallization, can be also an important cementing agent in soils (Dniker et al. 2003). Drying of soil samples containing iron hydroxide can produce irreversible soil hardening and cementation. In areas of soil with high pH or redox potential the iron hydroxide is precipitated forming cemented concretions or nodules. Biological cementation with iron hydroxides can be detected on the roots of all wetland plants where Fe(II), produced by iron-reducing bacteria, reacts with oxygen released by the roots (Johnson-Green and Crowder 1991; Weiss et al. 2005). To form ferric hydrates by oxidation and hydrolysis of Fe (II), iron (III) must be preliminary reduced by iron-reducing bacteria. Oxidation of ferrous ions and chelates in soil is performed chemically or catalyzed by neutrophlic or acidophilic iron-oxidizing bacteria.

Another example of natural cementation is precipitation of silica dioxide from sodium silicate, which fills in the pores and glues the soil particles together. Aggregation of silica dioxide depends on pH (Knoblich and Gerber 2001), so microbially mediated decrease of pH due to oxidation or fermentation could affect precipitation and cementation of silica dioxide.

## 6.13 Biodesaturation of Water-Saturated Cohesionless Soil

Earthquake is one of the most devastating types of geohazards on earth causing great economic losses including damages to infrastructures and properties. Many of the damages were related to soil liquefaction—a phenomenon whereby the saturated relatively loose soil substantially loses strength and stiffness due to generation of excess pore water pressure causing that soil behaves as a viscous liquid. Such

soil loses the bearing capacity so shallow foundations can settle down or shift aside causing building and infrastructure damages.

Conventional ground improvement for mitigation of liquefaction-induced damages is the ground densification using vibro replacement stone columns, vibro compaction, compaction grouting, and deep dynamic compaction methods. However, these methods are energy-consuming, expensive, and dynamic compaction cannot be used in the city areas.

Recent fundamental studies in soil mechanics showed that inclusion of gas bubbles in saturated sand can reduce its susceptibility for liquefaction substantially (Xia and Hu 1991; Yang et al. 2004; Yegian et al. 2007; Eseller-Bayat et al. 2012). It has been demonstrated that the liquefaction resistance of saturated sand can be significantly increased when the sand is slightly desaturated with some voids displaced by nitrogen gas produced by denitrifying bacteria (Rebata-Landa 2007; Chu et al. 2009a, 2011; Rebata-Landa and Santamarina 2012; He et al. 2013). Biodesaturation of soil is a process aiming to change such properties of soil as saturation and liquefaction potential through biogas production in situ.

The microbially induced partial desaturation in loose saturated sands can decrease excess pore water pressure and increase the bearing capacity and shear strength of the soil, which is beneficial in foundation design and roadway construction (Seagren and Aydilek 2010). The liquefaction resistance of saturated sand significantly increased when the sand is slightly desaturated with some voids displaced by gas (Yegian et al. 2007; He et al. 2013). Even small decrease in the degree of sand saturation to 99–97 % increases resistance of water-saturated sand to liquefaction by 30–40 % (Xia and Hu 1991; Yang et al. 2003), while reduction of the sand saturation to 90 % can increase resistance of water-saturated sand to liquefaction twice (Chaney 1978; Yoshimi et al. 1989).

Microbiological production of nitrogen gas in the depth of saturated soil in situ was proposed to introduce smaller and more stable gas bubbles in saturated soil (Rebata-Landa 2007; He et al. 2013). This method is an introduction of nitrogen gas bubbles into soil using biochemical reduction of nitrate (denitrification) in situ. It is most suitable approach because nitrogen gas is a chemically inert substance (Rebata-Landa and Santamarina 2006; Seagren and Aydilek 2010). The using of denitrifying bacteria to generate nitrogen gas in situ (He et al. 2013) has two major advantages over the other gas-introducing methods: (1) the gas bubbles generated by denitrifying bacteria are tinny and thus the bubbles are more stable underground; and (2) nitrogen gas is inert and very low in solubility.

## 6.14 Bioencapsulation of Soft Soil

Formation of thin layer of inorganic minerals or composite bioprecipitated materials on solid surface (biocoating) or around solid particle (bioencapsulation) can be used in many areas of civil, geotechnical, and environmental engineering. For example,

it can be used for the coating of concrete coastal engineering objects to improve their aesthetics or to create calcium carbonate surface optimal for the colonization by marine or freshwater epibiota: cyanobacteria, algae, and shellfishes. Biocoating can be used to make solid fill from soft marine clay or to avoid diffusion of toxic pollutants from clay. Soft marina clay can be transformed to the solid matter using bacterial encapsulation with calcium carbonate crystals (Ivanov et al. 2014b).

Layers of peat in the subsoil lead to differential settlements of roads, railways and foundations. Soft and highly compressive peat can be transformed to the solid matter if bacterially induced precipitation of calcite in peat will reach 16 % (w/w) by weight (Canakci et al. 2015). Soft organic soil also can be solidified if the content of $CaCO_3$ will be up to 20 % (Sidik et al. 2014). Another biotechnology to solidify soft peat is biosilicification process that involves the addition of a cationic surfactant, a sodium meta silicate solution, and molasses to stimulate microbial acidogenic ("acid-producing") fermentation that are hardening the peat to unconfined compressive strength 1 MPa (Hamer et al. 2009).

## 6.15 Bioimmobilization of the Pollutants in Soil

Binding of the particles of the polluted soil using MICP can also diminish the release of pollutants from soil (James et al. 2000). For example, an indigenous calcifying bacterial strain *Kocuria flava* CR1 removed from solution of Cu 1000 mg $L^{-1}$ 97 % of copper so that MICP-based bioremediation of the copper-contaminated site could be a viable, environmental friendly biotechnology (Achal et al. 2011a, b). Other heavy metals such as As(III) (Achal et al. 2012), Cr (VI) (Achal et al. 2013) as well as Pb, Sn, Co, Zn, and Cd also can be immobilizated in polluted media by urease-producing bacteria due to increase of pH and production of carbonate during urea hydrolysis (Li et al. 2013).

There are several patents on consolidation of polluted soil particles and such soil pollutants as $Hg^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Ni^{2+}$, $Pb^{2+}$ due to generation of carbonates and precipitation of calcium using urease-producing bacteria (Patent application CN1923720A, 2007; CN 102139278 A, 2011). Biocementation of particulate material that can be used for immobilization of the pollutants, was claimed in the Patent Application US 20130196419 A1 (Crooker et al. 2013). Li et al. (2015) described formation of insoluble compounds of nickel, copper, lead, cobalt, zinc, cadmium, and calcium by *Terrabacter tumescens* for both heavy metals removal and biogrouting geotechnical applications. Strontium, radioactive pollutant of soil and groundwater, can be co-precipitated with calcite using MICP (Mitchell and Ferris 2005).

We demonstrated that the particles of lava (lava of Mount Fuji, Japan was used), which are slowly covered by microbial biofilm, can be biocoated by neutral calcium carbonate layer and this coating makes the lava particles more suitable to be used as fertile soil for the plant cultivation.

## 6.16 Sanitation of Soil

Sanitation is aiming to kill microbial pathogens and helminth eggs in soil prior excavation. Eggs of parasitic helminth are present in soil contaminated by human feces, so excavation works with this soil can contaminate workers and other people nearby excavation area by different species of parasitic worms. Soil polluted even hundreds years ago with the horse manure, animal corpses after outbreaks of infections, or just with organic wastes of farming or agriculture can contain spores of *Clostridium tetani*, *Clostridium botulinum*, *Clostridium perfringens*, *Bacillus anthracis* causing deadly diseases *tetanus*, *botulism*, *gas gangrene,* and *anthrax*. For example, 290,000 persons in Asia, Africa, and South America died of tetanus in 2006 (Baumgardner 2012). The list of soil-related pathogens includes also *Bacillus cereus* caused *gastroenteritis*, *Listeria monocytogenes causing gastroenteritis* and *meningitis*. In wet tropical soil is very common presence of pathogenic bacteria *Burkholderia pseudomallei causing melioidosis*, and many other pathogens. *Blastomycosis* is caused by fungi *Blastomyces dermatitidis* occurs usually in soil associated with waterways and animal excreta. *Coccidioides*, a fungi causing pneumonia is associated with alkaline and salinic sandy soils (Baumgardner 2012). Spores of the molds, for example from genera *Aspergillus* and *Penicillium* are causing different diseases. All these bacterial and fungal infections may occur by inhalation of soil dust, inoculation into wounds, ingestion of soil particles or soil-contaminated food.

So, excavated soil polluted with pathogenic bacteria, fungi, or parasitic helminths must be either disposed on the landfill or treated on site using disinfection or bioremediation methods. No dust or polluted air could be dispersed from the construction site. Site workers must be protected from the polluted soil and dust.

Currently, there are no biomethods for the removal of specific pathogens from polluted soil. However, for thousand years Chinese farmers used one of the most effective methods to remove pathogens from rice paddy soil just keeping level of water above soil surface to create anaerobic conditions for bacterial sulfate reduction and production of toxic $H_2S$ in soil which kills all pathogens. Hypothetically, aeration, bioacidification, bioalkalinization, and bioproduction of $H_2S$ or other toxic metabolites in bacteriologically polluted soil can be used for ecological and cheap treatment of soil polluted with pathogens.

## 6.17 Comparison of the Different Mechanisms of Ground Improvement

Biotreatment of porous soil and fractured rocks could be performed by upward and downward injection, percolation by gravity, spraying of the soil surface with solutions, treatment of soil slurry in the stirring, aerated, or rotating reactors. Advantages and limitations of these methods are shown in the Table 6.2.

**Table 6.2** Ground improvement methods using microbial biogeotechnologies

| Method | | Description/mechanisms | Typical applications | Advantages | Limitations |
|---|---|---|---|---|---|
| Spraying methods | A. Soil crust formation | A process to aggregate soil particles and form solid soil crust due to microbial formation of insoluble minerals or biofilm in situ | Reduction of wind and water erosion of soil: dust control, water erosion control, pollution control | Low cost and technologically simple application | The method may not be applicable when porosity of soil is too high and crust cannot be formed |
| | B. Bioclogging and biocementation of upper layer of soil | A process to form solid layer of porous soil due to microbial formation of insoluble minerals in situ | Bioclogging and biocementation of upper layer of soil to reduce its permeability and water erosion, and to strengthen soil | Low cost and technologically simple application | The thickness of the layer cannot be strictly determined |
| Injection methods | A. Bioclogging and biocementation of the bulk of porous soil | A process to form solid and impermeable porous soil due to microbial formation of insoluble minerals in situ | Bioclogging and biocementation of the bulk of porous soil to reduce its permeability and to strengthen soil | Deep penetration, low cost and technologically simple application | Possible production of ammonium and high pH in situ |
| | B. Bioclogging of the fissured rocks | A process to form impermeable rocks due to microbial formation of insoluble minerals in the rock fissures | Bioclogging of the fissured rocks to reduce their permeability: tunneling works | Deep penetration, low cost and technologically simple application | Possible production of ammonium and high pH in situ |
| | C. Biodesaturation of porous soil | A process to reduce water saturation of soil due to microbial formation of gas from solution | Replacement of water by gas in water-saturated soil: mitigation of soil liquefaction | Low cost and technologically simple application | Sequential treatment of soil for biodesaturation and fixation of gas bubbles in situ using biocementation |

# Chapter 7
# Biocementation and Biocements

## 7.1 Calcium-Based Microbial Cementation in Nature

Formation of calcium carbonate by soil or aquatic bacteria that are producing carbonate ions and increasing pH of microenvironment is a common and well-known process (Boquet et al. 1973; Castanier et al. 1999; Cacchio et al. 2003; Brehm et al. 2006; Wright and Oren 2005). This can be shown by equations for chemotrophic production of bicarbonate ions or phototrophic alkalinization during synthesis of organic matter with empirical formula $\langle CH_2O \rangle$:

$$\langle CH_2O \rangle + O_2 \rightarrow HCO_3^- + H^+, \tag{7.1}$$

$$HCO_3^- + H_2O + \text{energy of light} \rightarrow \langle CH_2O \rangle + O_2 + OH^-. \tag{7.2}$$

There are known microbially induced sedimentary structures such as stromatolites and mats, calcium-cemented layered aggregates produced by the biofilms of cyanobacteria, algae, and lichens (Noffke 2003).

Urea is released to the environment as final product of nitrogen metabolism of mammals. Urea is hydrolysing in nature due to the enzyme urease, which is produced by many soil microorganisms. Urease-dependent calcium carbonate precipitation in soil is performed by aerobic and anaerobic chemotrophic microorganisms:

$$Ca^{2+} + (NH_2)_2CO + 2H_2O \rightarrow CaCO_3 \downarrow + 2NH_4^+. \tag{7.3}$$

## 7.2 Calcium-Based Cementation in Macroorganisms

Macroorganisms produce different structures such as mollusc and turtle shells, pearls, coral reefs, bones and teeth, where calcium carbonate—$CaCO_3$—minerals aragonite and calcite or calcium phosphate mineral hydroxyapatite—



V. Ivanov and V. Stabnikov, *Construction Biotechnology*,
Green Energy and Technology, DOI 10.1007/978-981-10-1445-1_7

$Ca_{10}(PO_4)_6(OH)_2$—are playing major roles in the strength of these structures. However, the artificial structures from these minerals are brittle. Therefore, the natural structures are 3D or layered organo-mineral composites containing a mineral matrix embedded, covered, bound with elastic proteins and glycoproteins. Organics of the bones include mainly collagen and small portion of glycoproteins. Organic matrix of the sea shells and pearls includes chitin, silk fibroin, and soluble proteins. There are two types of soluble proteins: (1) protein conchiolin that contains mainly aminoacids with hydrophobic and positively charged side chain (glycine, valine, leucine, lysine, arginine) which can be used for the binding with hydrophobic surface and with negatively charged ions, and (2) proteins enriched with aspartic and glutamic aminoacids that both contain negatively charged carboxylic group in side chain, which can be used for the binding with positively charged calcium ions.

## 7.3 Urease-Dependent, Calcium-Based Microbial Cementation (MICP) in Engineering

Geomicrobiological process of urease-dependent calcium carbonate crystallization and precipitation in soil is actively studied (Fujita et al. 2000; DeJong et al. 2006; Burbank et al. 2012; Chu et al. 2012a, b, c; Cacchio et al. 2015; Kim et al. 2016). It is used in practice for aggregation, clogging, and strengthening of soil (Whiffin et al. 2007; Burbank et al. 2011; DeJong et al. 2013; Cheng and Cord-Ruwisch 2014; Gurbuz et al. 2015; Sotoudehfar et al. 2016) or for immobilization of the soil pollutants (Warren et al. 2001; Gadd and Pan 2016).

It is called often as microbially induced calcium carbonate precipitation (MICCP) or microbially induced calcite precipitation (MICP). Actually, both terms are not scientifically correct because not only calcite and not only precipitation are the basic features of this process. There are many other chemotrophic and phototrophic types of "microbially induced calcium carbonate precipitations" but it is not mentioned in the terms of MICP or MICCP that it is urease-induced process. "Urease-dependent calcium carbonate crystallization on solid surface," which is more correct but not convenient for use term, includes the following processes: (1) adhesion of cells of urease-producing bacteria (UPB) or enzyme urease on the solid surface (Fig. 7.1a); (2) creation of a microgradient of concentration of bicarbonate/carbonate and pH in the site of cell attachment due to hydrolysis of urea by urease of UPB; (3) formation of calcium carbonate crystals such as calcite, vaterite, or aragonite nearby urease-producing cells (Fig. 7.1a); (4) precipitation of these crystals and their adhesion to the solid surface; (5) binding of solid surfaces due to adhesion between calcium carbonate crystals (Fig. 7.1b).

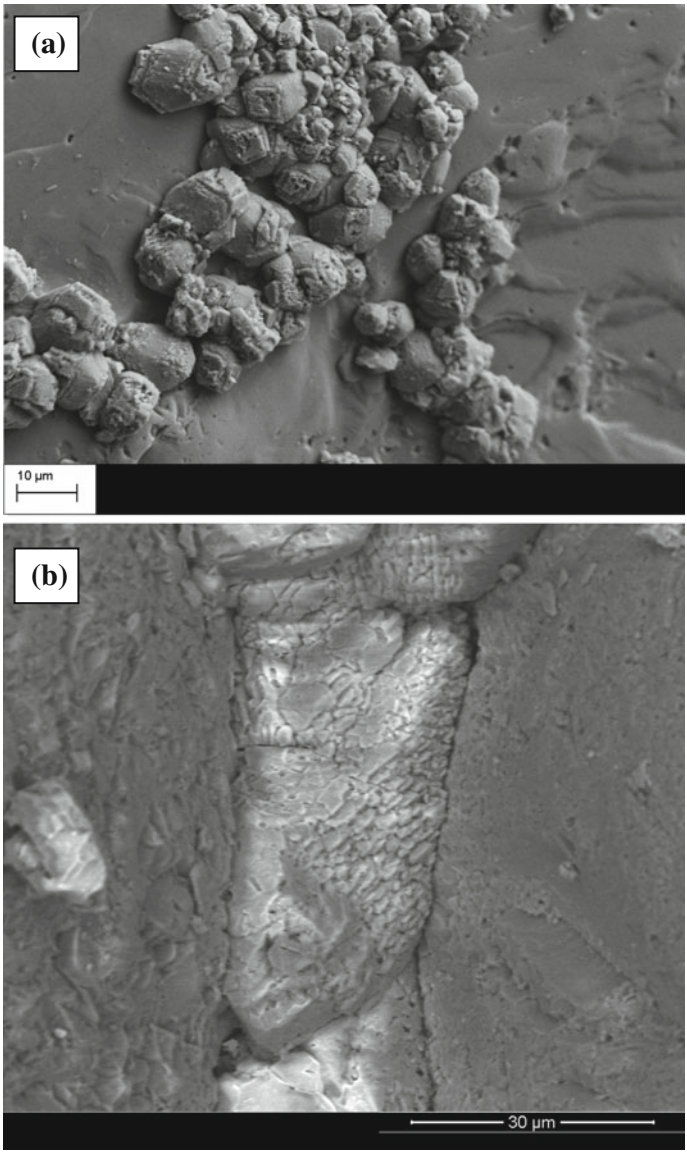



**Fig. 7.1** Attachment of bacterial cells and crystals to sand surface (**a**) and binding of sand grains by calcite crystals in the contact zone (**b**)

## 7.4 Biochemistry of MICP

The biogeochemical reactions of this biocementation process are as follows:

$$(NH_2)_2CO + 2H_2O(+\text{Enzyme urease}) \rightarrow CO_2 \uparrow + 2NH_4{}^+ + 2OH^-, \quad (7.4)$$

$$CO_2 + H_2O(+\text{Enzyme carbonic anhydrase}) \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3{}^- \leftrightarrow 2H^+ + CO_3{}^{2-}, \quad (7.5)$$

$$CaCl_2 + 2NH_4^+ + 2OH^- + 2H^+ + CO_3{}^{2-} \leftrightarrow CaCO_3 \downarrow + 2NH_4{}^+ + 2Cl^- + 2H_2O, \quad (7.6)$$

In total:

$$CaCl_2 + (NH_2)_2CO + 2H_2O + (\text{urease and carbonic anhydrase}) \rightarrow CaCO_3 \downarrow + 2NH_4Cl. \quad (7.7)$$

Enzyme urease (EC 3.5.1.5) is produced by a wide range of microorganisms because urea is a final product of nitrogen metabolism of human and animals and plays a role of nitrogen source for many microorganisms in nature. Another enzyme with important role in MICCP is carbonic anhydrase (EC 4.2.1.1) catalyzing the reversible hydration of $CO_2$ (Dhami et al. 2014a, b; Li et al. 2011).

## 7.5 Use of Urease for MICP

Some researchers considered urea-producing bacteria just as a stock of urease that can be replaced by enzyme itself (Nemati et al. 2005; Handley-Sidhu et al. 2012; Yasuhara et al. 2012; Neupane et al. 2015). For example, use of urease with the activity of 2950 U/g in concentration 1–2 g/L (it is approximately 3 mM of hydrolyzed urea $min^{-1}$, which is typical urease activity in MICP) ensured 70 % of calcium precipitation in sand column (Neupane et al. 2013; Putra et al. 2015). However, use of enzyme could be too expensive for large-scale application and enzyme will be more sensitive to high pH and osmotic pressure than urease-active cells. Also, the surface of bacterial cell plays a major role in adsorption of cell-connected urease activity on solid surface. Therefore, bacterial cells are not just a stock of enzyme but also a point of crystallization due to high urease activity on the site of cell adsorption. Adsorption of enzyme molecules cannot create such density of urease activity on solid surface.

## 7.6 Bacteria Used in MICP

In majority of the biocementation research, the Gram-positive bacterial species *Sporosarcina pasteurii* (former *Bacillus pasteurii*), especially the strain *S. pasteurii* ATCC11859 (DSMZ33, LMG 7130) (Achal et al. 2009a, b; Dupraz et al. 2009; Mortensen and DeJong 2011; Tobler et al. 2014; Whiffin et al. 2007) is used because of its high urease activity and ability to grow at pH above 8.5 and at high concentration of calcium, at least at 0.75 M $Ca^{2+}$. Last property is especially important for MICP. Other physiologically similar species using for biocementation are the representatives of the genus *Bacillus* (Wong 2015). There can be used *B. cereus* (Castanier et al. 2000); *B. megaterium* (Bang et al. 2001a, b; Dhami et al. 2014a, b), *B. sphaericus* (Hammes et al. 2003; De Muynck et al. 2008a, b; Wang et al. 2012), *B. pseudofirmus* (Jonkers et al. 2010), *B. subtilis* (Reddy et al. 2010), *Bacillus pumilus* (Daskalakis et al. 2015), *B. alkalinitrilicus* (Wiktor and Jonkers 2011), *B. licheniformis* (Vahabi et al. 2014), *B. lentus* (Sarda et al. 2009), *Idiomarina insulisalsae* (Oliveira et al. 2015), and not identified species (Achal et al. 2010a, b; Stabnikov et al. 2011, 2013b; Hammes et al. 2003; Lisdiyanti et al. 2011). The strain *Bacillus* sp. VS1 (Chu et al. 2012a, b, c, 2013a, b; Ivanov et al. 2015a, b; Stabnikov et al. 2011, 2013a, b) was isolated from sand in Singapore. Batch cultivation of this strain under aseptic conditions was characterized with maximum specific growth rate of 0.1 $h^{-1}$ and maximum urease activity of 8.3 ± 0.1 hydrolyzed urea mM/min. 16S rRNA gene sequence of this strain with GenBank accession number JF896459 has 99 % identity with the 16S rRNA gene sequence of *Bacillus* sp. CPB 2 with GenBank accession number AF548874, isolated and used for microbial precipitation of carbonate in Ghent University, Belgium (Hammes et al. 2003). Combination of urease-dependent cementation of *S. pasteurii* and heterotrophic precipitation of calcium carbonate by non-ureolytic species of *B. subtilis* could enhance MICP (Gat et al. 2014) but it cold be only at conditions favorable for bacterial growth, i.e., presence of oxygen and the nutrients.

It is well known that some halotolerant species of genus *Staphylococcus* exhibited high urease activity (Jin et al. 2004; Christians et al. 1991). Halotolerant urease-producing strain of Gram-positive bacteria of *Staphylococcus succinus* was isolated from water of the Dead Sea with salinity 34 % (Stabnikov et al. 2013b). However, the strains of *S. succinis* are often hemolytic and toxigenic ones (Zell et al. 2008) and were associated with some infectious diseases (Novakova et al. 2006; Taponen et al. 2008). Therefore, the isolated strain was not used for biocementation studies and applications.

## 7.7 Comparison of the Strains

There is big diversity of urease activity not only between the special of urease-producing bacteria (UPB) but also between the strains of one species, for example most popular for MICP species of *S. pasteurii* (Achal et al. 2009a, b,

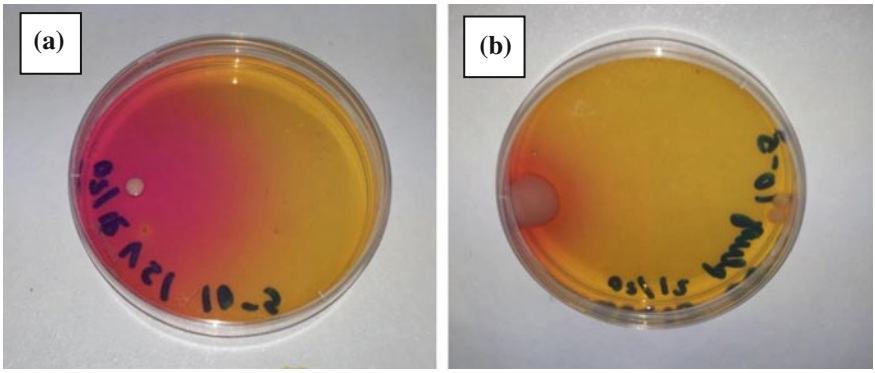



**Fig. 7.2** Bacterial colonies with high (**a**) or low (**b**) urease activity. *Red color* indicates increase of pH above 8.2 due to hydrolysis of urea and diffusion of $NH_4^+$ and $OH^-$ ions in agar medium. Agar medium is colorless at pH below 6.8

2010a, b). Simple selection of the strains by their urease activity can be made using visualization of color of pH indicator phenol red around individual 1–2 colonies on the dish (Fig. 7.2).

Detailed comparison of the cultures is done by their urease activity. This activity is usually defined as amount of ammonium produced from 1 M solution of urea per minute. Amount of ammonium produced from urea can be easily determined using electric conductometer showing linear correlation between the molar concentrations of $NH_4^+$ and the changes of electric conductivity of solutions in mS/cm. Urease activity in soil or in the biocementation system, where the baseline electric conductivity is high, can be determined by the direct measurement of produced ammonium concentration.

Other engineering parameters for the comparison are maximum specific growth rate ($\mu_{max}$) determined for exponential phase of batch culture by the increase of biomass ($\Delta X$) for a period of time $\Delta t$:

$$\mu_{max} = \Delta \ln X / \Delta t, \tag{7.8}$$

and growth yield $Y$ determined by the changes of biomass and concentration of carbon and energy ($\Delta S$):

$$Y = \Delta X / \Delta S. \tag{7.9}$$

Dry weight of bacterial biomass ($X$) is determined after centrifugation, resuspension of biomass in water, second centrifugation, and drying at 105 °C to a constant weight.

Third type of the parameters for the strains comparison are the parameters of biocemented/bioclogged soil after application of the strain such as unconfined compressed strength, hydraulic conductivity, and others. For example, the hydraulic

conductivity of the bioclogged sand ($P$) in the sand columns treated with different microbial strains can be measured using constant head method and calculated as follows:

$$P = V/t \cdot S\,(\mathrm{m/s}), \tag{7.10}$$

where $V$ is a volume of liquid, $t$ is time of the liquid passed through the sand at water head 20 cm, $S$ is a cross-sectional area of the treated sand. Accuracy of all measurements must be taken into account in the comparison of the strains.

## 7.8 Selection of Enrichment Culture of UPB

Proper urease-producing bacteria (UPB) for the bioclogging of soil must be: (1) a nonpathogenic strain; (2) with cells of low aggregation ability for deeper penetration into porous soil or fractured rocks; (3) with cells that are able to adhere to the treated surface; (4) with cells that can produce enzyme urease, which is active at pH 8.5–9.2 in the medium with high concentration of salts (DeJong et al. 2006; Stabnikov et al. 2013a, b).

UPB are present in almost all terrestrial and aquatic sites, where urea is supplied as a final product of nitrogen metabolism of mammals. UPB start up biotransformation of urea nitrogen in environment. That is why there is a significant positive correlation between urease activity and nitrogen removal efficiency in lake water (Liang et al. 2003). Activated sludge of municipal wastewater treatment plants (MWWTPs), which is mainly bacterial biomass, also contains a lot of UPB (Al-Thawadi and Cord-Ruwisch 2012a, b). An enrichment culture of UPB can be obtained by the enrichment of soil samples with urea (Al-Thawadi 2013; Stabnikov et al. 2013a, b). So, it is relatively easy to select a halophilic and alkaliphilic enrichment culture of UPB.

## 7.9 Pure or Enrichment Cultures Must Be Used?

There are different opinions on the application of pure or enrichment cultures for microbial precipitation of calcium carbonate. One point is the different urease activity of such cultures. For example, an application of pure culture of *B. sphaericus* was more effective in decreasing the permeation properties of mortar and concrete compared to the treatment with ureolytic enrichment culture (De Muynck et al. 2008). An application of co-culture of ureolytic and non-ureolytic bacteria led to accelerated microbial-induced precipitation of $CaCO_3$, probably due to creation of additional nucleation sites by adhered non-ureolytic bacterial cells (Gat et al. 2014).

It was shown in our previous publications that newly prepared enrichment culture of UPB from the soil samples decreased urease activity with each culture transfer (Chu et al. 2014a, b; Stabnikov et al. 2013a, b). Urease activity of UPB in newly prepared enrichment culture decreased in 20 times after 5 transfers into fresh medium. However, enrichment culture of UPB selected during four years of experimental work showed relatively slow decrease of urease activity. Thus, average urease activity of enrichment culture was about 3.7 mM hydrolyzed urea/min in 2009 and 1.9 mM hydrolyzed urea/min in 2013. This activity was relatively stable during storage of the culture liquid at 4 °C. The reasons of instability of urease activity in pure culture and stability in enrichment culture are not clear. In every case, bacterial suspension must be examined for urease activity before any MICP application.

## 7.10 Biodiversity in Enrichment Culture

In our experiments, the enrichment culture of UPB was selected and used during 4 years of laboratory and pilot scale applications of bioclogging and biocementation. This enrichment culture was produced under nonaseptic conditions using a portion of the previous liquid culture as an inoculum for new set of batch cultivation. Typical medium for cultivation includes Tryptic Soya Broth DIFCO™, 20 g; urea, 20 g; NaCl, 20 g, $NiCl_2 \cdot 6H_20$, 24 mg, tap water 1 L, pH 8.2. Such procedure was repeated every month. Biomass of UPB was used for bioclogging and biocementation of sand or fractured rocks. Typical solution for biocementation or bioclogging contains $CaCl_2$, 0.75–1 M, and urea, 1.5–2 M. The final pH of culture liquid was in the range from 8.8 to 9.2. These conditions for cultivation and biocementation were selective for alkaliphilic and halotolerant bacteria. Application of this enrichment culture for biocementation/bioclogging of sand showed decrease of hydraulic conductivity of sand to $3.8 \times 10^{-7}$ m/s and increase of unconfined strength up to 1400 kPa at the content of calcium carbonate of 9 % (w/v). So, enrichment culture was suitable for biocementation/bioclogging of sand from the technical point of view but its biosafety was unknown.

Isolation of the pure cultures from enrichment culture was done on Petri dishes and identification of colonies was done by the sequencing of 16S rRNA gene. This microbiological analysis showed that enrichment culture of UPB contained at least four strains—VS8, VS1, VS21, and VS17—which were differentiated by the shape, color, shining, and pattern of the edge of the colonies grown on the surface of solidified medium in the Petri dishes. The shares of the different colonies were 55, 30, 10, and 5 % of the colonies for the strains VS8, VS1, VS21, and VS17, respectively.

Major culture was Y*aniella* sp./*Enteractinococcus* sp. strain VS8 (GenBank accession number KT 182991 for the sequence of 16S rRNA gene) with cells looking like micrococci. This dominating strain VS8 was from the family *Micrococcaceae* and close to the species *Enteractinococcus* sp. YIM 101632

(identity was 98 %) of newly proposed genus *Enteractinococcus* (Cao et al. 2012), Y*aniella* sp. YIM 100590 (identity was 96 %), *Actinomycetales* bacterium SSCS15 (identity was 95 %), and *Yaniella flava* strain YIM 70178 (identity was 95 %). The strain Y*aniella* sp. VS8 has Gram-positive, spherical, nonmotile cells with average diameter about 1 μm. This strain grows well at pH 6.5–11.0 in the medium with the content of NaCl 5 %. The strain has urease activity up to 25 mM hydrolyzed urea/min. It is important for the industrial process that bacteria of the genus Y*aniella* belong to Risk group 1 and there were no references in PubMed database (25 million citations for biomedical literature) on the potential pathogenicity of species from genus *Yaniella*. It is known that some strains from the genus *Yaniella* are halotolerant (Chen et al. 2010; Dhanjal et al. 2011; Li et al. 2004). For example, the halophilic (growth was in the medium with up to 20 % (w/v) NaCl), and facultatively alkaliphilic (growth was in the medium at pH from 6.0 to 10.5) strain *Yaniella soli* sp. *nov*. was isolated from forest soil in China (Chen et al. 2010).

The second strain was *Bacillu*s sp. strain VS1 isolated by Stabnikov et al. (2011) and used previously in our laboratory studies for bioclogging and biocementation. The third urease-positive strain VS17 was a representative of the genus *Staphylococcus.* fourth strain VS21 was from genus *Bacillus* but did not show urease activity and but had a high growth rate.

## 7.11 Presence of the Potential Pathogens in Enrichment Culture of UPB

The third urease-positive strain VS17 was close to *Staphylococcus* sp. R-25657 (identity 100 %), and *Staphylococcus* sp. ZWS13 (identity 99 %), *Staphylococcus* sp. JY04 (identity 99 %), *Staphylococcus cohnii* subsp. *urealyticus* strain CK27 (identity 99 %). Staphylococci are always living on the human skin but some strains of *Staphylococcus* are pathogens causing different infectious diseases of humans and other mammals. *Staphylococcus cohnii* subsp. *urealyticus* strain CK27 (ATCC 49330), which is a close relative to the strain VS17, was isolated from the human skin (Kloos and Wolfshohl 1991) and has biosafety level 2.

Fourth strain VS21 was close to *Bacillus thuringiensis serovar kurstaki* strain HD1 (identity was 99 %); *Bacillus thuringiensis serovar galleriae* strain HD-29 (identity was 99 %); *B. cereus* 03BB102 (identity was 99 %), and *Bacillus anthracis* strain IHB B 15126 (identity was 99 %). The strain *Bacillus thuringiensis serovar kurstaki* strain HD1 is entomopathogenic strain, which is an environmentally compatible biopesticide used worldwide (Day et al. 2014). *Bacillus thuringiensis serovar galleriae* strain HD-29 is also a strain of commercial biopesticide (Zhu et al. 2015).

The species of *B. cereus* incudes the opportunistic human pathogens, and *B. cereus* strain 03BB102, which is close to the strain VS21, was isolated from the blood of patient, who died as a result of a severe pneumonia probably to be caused by these bacteria (Hoffmaster et al. 2006). At the same time, some species can induce precipitation of calcium carbonate (Han et al. 2013). Species of *B. anthracis* includes pathogens of mammals that cause infectious disease like anthrax and is considered as a biological warfare.

These experimental data showed that enrichment culture of UPB may contain bacteria that are opportunistic or even real pathogens from genera *Staphylococcus* and *Bacillus*. Even non-ureolytic halophilic and alkaliphilic bacterial strains could be present in UPB enrichment culture because it is growing in rich medium. Therefore, only pure and biologically safe bacterial cultures have to be used in bioclogging and biocementation experiments and practice.

## 7.12 Use of Enrichment Culture of Indigenous Microorganisms with Urease Activity In Situ

Enrichment cultures with urease activity that were selected in laboratory from the soil samples or in situ as a culture of indigenous soil microorganisms were proposed to be used for calcium carbonate precipitation in soil (Burbank et al. 2011, 2012; Hammes et al. 2003). An application of enrichment microbial culture in comparison with pure strain of UPB allows a cheaper production of the bioagent with urease activity.

Selection of UPB is going in the medium with high concentration of salts and at high pH (Stabnikov et al. 2013a, b). This selectivity gives the opportunity to obtain culture liquid with high urease activity even in the case when batch or continuous cultivation in organic rich medium is performed under nonaseptic conditions (Cheng and Cord-Ruwisch 2013a, b).

However, it is well known that a lot of urease-active bacteria belong to opportunistic pathogens such as *Proteus vulgaris*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and even to pathogens such as *Helicobacter pylori* that can infect the human stomach (Stabnikov et al. 2013a, b). Also, there are known a lot of halotolerant and alkaliphilic strains of pathogenic species. For example, some halotolerant species of the genus *Staphylococcus* exhibited high urease activity (Christians et al. 1991; Jin et al. 2004). Halophilic strain of *S. succinus*, isolated from water of Dead Sea with salinity about 35 %, possessed urease activity (Stabnikov et al. 2013a, b). Meanwhile, the strains of *S. succinus* could be hemolytic and toxigenic ones (Zell et al. 2008), and are associated with some infectious diseases (Novakova et al. 2006; Taponen et al. 2008). So, it is not safe to stimulate growth of indigenous urease-producing soil bacteria because, the high pH and salinity do not prevent growth of urealytic and non-urealytic pathogenic bacteria.

## 7.13 Biosafety of MICP Using Pure Culture

A major point for the use of pure bacterial culture for MICP is still biosafety requirements for cultivation of cells, production and storage of biocement, injection into or spraying onto soil, persistence of introduced microorganisms in soil, effect of introduced microorganisms on human and environment, fate of these microorganisms in environment. It requires many expensive studies that most probably, not only indigenous UPB and enrichment cultures of UPB but also live cells of selected and relatively safe pure cultures of UPB cannot be used for large-scale geotechnical and construction applications because of the risk of not predicted negative consequences for the environment. Additionally, large-scale application of live bacteria to the geoenvironment requires a lot of formal approvals and epidemiological tests. Another complication of biosafety issue during bioclogging of soil is that practical applications of this microbial geotechnical process usually are performed by the experts in civil and environmental engineers but not in microbiology and biotechnology. Therefore, to increase the biosafety of the biocementation/bioclogging process new technology of MICP, where the dead but urease-active cells of UPB are used, was developed.

## 7.14 MICP Using Dead but Urease-Active Bacterial Cells

The best way to ensure biosafety of microbial introduction into environment is killing of live bacterial cells so that their urease activity remains. An application of enzyme urease for large-scale bioclogging of sand or other porous materials is also technologically possible (Nemati and Voordouw 2003; Nemati et al. 2005), but the use of enzyme could be too expensive to be used in practice. Additionally, the bacterial cells with urease activity are needed because they attach to the surface of bioclogged material and form crystallization centers for the formation of calcium carbonate minerals (Stocks-Fischer et al. 1999; Gat et al. 2014). Therefore, we tested different methods for killing bacterial cells but retain their urease activity, which is needed for biocementation/bioclogging.

Microbiological study of UPB enrichment culture showed that best approach is as follows: (1) to use pure culture of *Yaniella* sp. VS8 for bioclogging of sand because cells of *Yaniella* sp. VS8 are almost twice smaller than cells of *Bacillus* sp. VS1 and do not form big aggregates of cells in solution of calcium chloride, so they could penetrate deeper into fine sand and fractured rocks; (2) most practical approach could be to find a method to kill cells of *Yaniella* sp. VS8 before bioclogging/biocementation procedure but at the same time to retain urease activity of dead cells at the acceptable level for bioclogging, i.e., at least 0.1 mM hydrolyzed urea/min but preferably 1–5 mM of hydrolyzed urea/min.

Treatment of bacterial cells in the culture liquid of strain *Yaniella* sp. VS8 was done with the aim to kill cells but retain urease activity of cell. Therefore, the

methods that are disrupting cell membrane but do not destroy significantly hydrolytic activities of enzymes have been tested: (1) ultrasound sonication at 750 W, 20 MHz, amplitude 50 % during 30, 60, and 300 s in ice water bath; (2) incubation of bacterial cells for 60 min with ethanol at final concentrations of 25, 50, and 70 % (v/v), and (3) incubation of bacterial cells in 0.5 % (w/v) solution of surfactant sodium dodecyl sulfate (SDS) for 10, 30, and 960 min.

The best results were obtained in the case when 0.5 % solution of SDS was used for 16 h. SDS is an anionic surfactant inhibiting growth and killing many Gram-positive bacteria (Flahaut et al. 1996) and Gram-negative bacteria (Adamowicz et al. 1991; Woldringh and Van Iterson 1972) through dissolution of cell membrane (Hansen et al. 2011; Kramer et al. 1984). Even 0.5 h of the treatment with SDS decreased live bacterial cells concentration from $5.2 \times 10^8$ to $1.0 \times 10^1$. Overnight incubation in 0.5 % SDS solution for 16 h killed all cells but decreased urease activity from 4.9 to 3.2 mM of hydrolyzed urea/min. Average urease activity for long time incubation of these dead cells and not-treated bacterial cells in 1 M solution of urea were almost the same.

SEM showed disruption of the treated dead cells of *Yaniella* sp. VS8 so that only the remainders of cell wall are visible (Fig. 7.3).

Cells of *Bacillus* sp. VS1 were much more resistant to all types of the treatments than cells of *Yaniella* sp. VS8 and did not consider further for use as dead but urease-active cells.

Hence, the most applicable method to kill cells but remain their urease activity was an incubation of bacterial suspension in 0.5 % solution of SDS for 0.5–1 day. This technology simplifies considerably the procedure of biocementation/bioclogging because there is no use of live microorganisms and there is no need for numerous biosafety approvals before practical applications. Some results of an application of dead but urease-active bacterial cells are shown below.

## 7.15 Bioclogging of the Sand Using Dead but Urease-Active Cells of Yaniella sp. VS8

Bioclogging of sand, measured by the decrease of hydraulic conductivity, using dead but urease-active cells was about two times lower than that with live cells of *Yaniella* sp. VS8. Probably, it is due to weaker adhesion of destructed bacterial cells than that of the intact cells. The hydraulic conductivity dropped from $5.2 \times 10^{-4}$ m/s to $7.5 \times 10^{-7}$ m/s in experiment with dead but urease-active cells after seven treatments. After 9 treatments, it was dropped in experiment to $2.9 \times 10^{-8}$ m/s at the content of precipitated $CaCO_3$ in sand of 15 % (w/w). This value of hydraulic conductivity of sand could be sufficient for the majority of the applications on sand clogging.

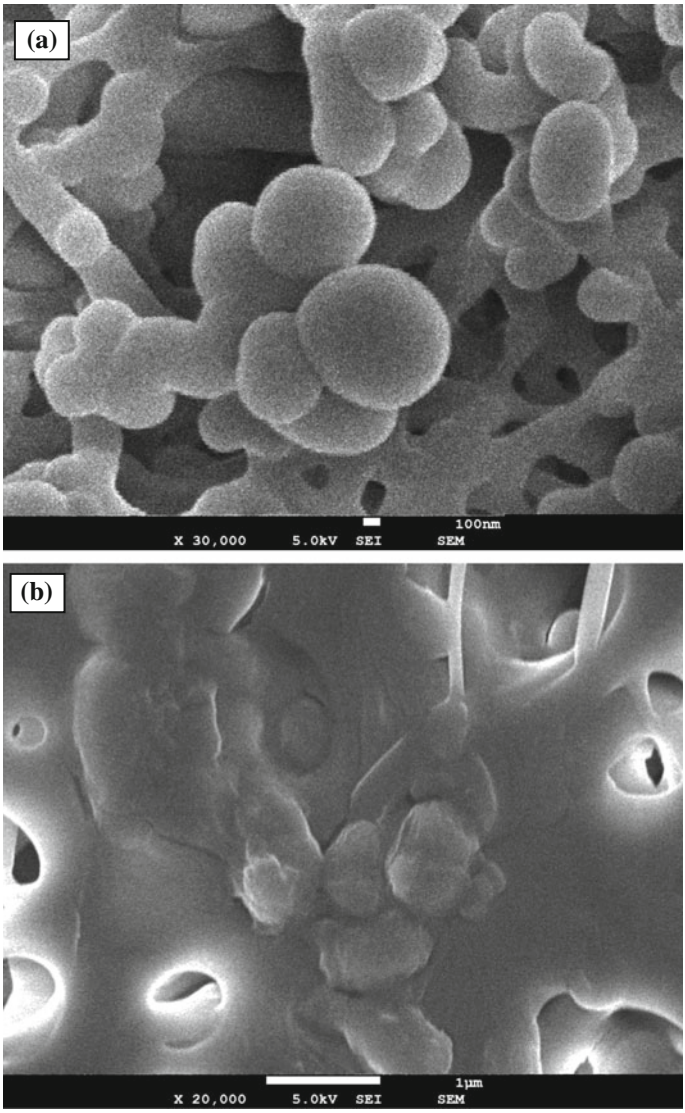



**Fig. 7.3** SEM images of *Yaniella* sp. VS8 cells on membrane filter: nontreated cells (**a**) and disrupted cells after treatment with sodium dodecyl sulfate solution (**b**)

Use of dead bacterial cells is a priori safe because dead cells do not cause infections. So, application of safe pure bacterial culture and additional killing of bacterial cells, retaining their urease activity intact, resolves the biosafety issue for the biocementation/bioclogging of porous materials.

## 7.16 Biocementation by Injection, Percolation, and Spraying

Liquid biocement is a bacterial suspension supplied into soil altogether or separately with the solution of calcium salt and urea. Biocementation can be performed as (1) surface percolation of liquid biocement (Cheng and Cord-Ruwisch 2012; Stabnikov et al. 2011), (2) spraying of liquid biocement onto surface (Stabnikov et al. 2011, 2013a; Chu et al. 2012a), or (3) bulk biocementation through the injection of liquid biocement. These treatments produce different results: (1) the crust on surface of soil due to percolation (Fig. 7.4a), biocemented layer of defined thickness due to spraying (Fig. 7.4b) or biocemented monolith due to injection (Fig. 7.4c).


**Fig. 7.4** Different spatial types of biocementation: formation of the crust on surface of sand (**a**), formation of the biocemented layer of the defined thickness (**b**), bulk biocementation (**c**)

## 7.17 Types of Crystals Produced in MICP

Quality of biocementation after MICP depends on the type of produced mineral. The shares of calcite, vaterite, or aragonite crystals (Fig. 7.5) in the biocemented material are controlled by the rate of enzymatic reaction (Cuthbert et al. 2012) and concentration of magnesium.

The formation of the crystals could be in the contact zones between sand grains (Fig. 7.6a), or on the whole surface of sand grains (Fig. 7.6b). In last case, the biocementation was done at the molar ratio Ca:Mg = 1:1. Biocementation by the crystallization on the whole surface of sand grains gave the highest unconfined compressive strength up to 12.4 Mpa at the content of precipitated Ca and Mg about 6 % (w/w).

There were appeared mainly needle-shaped aragonite crystals when biocementation was done at molar ratio Ca:Mg 1:1 (Fig. 7.4c). In the case when precipitation of calcium carbonate was going from 9 to 24 mM solution of calcium bicarbonate,

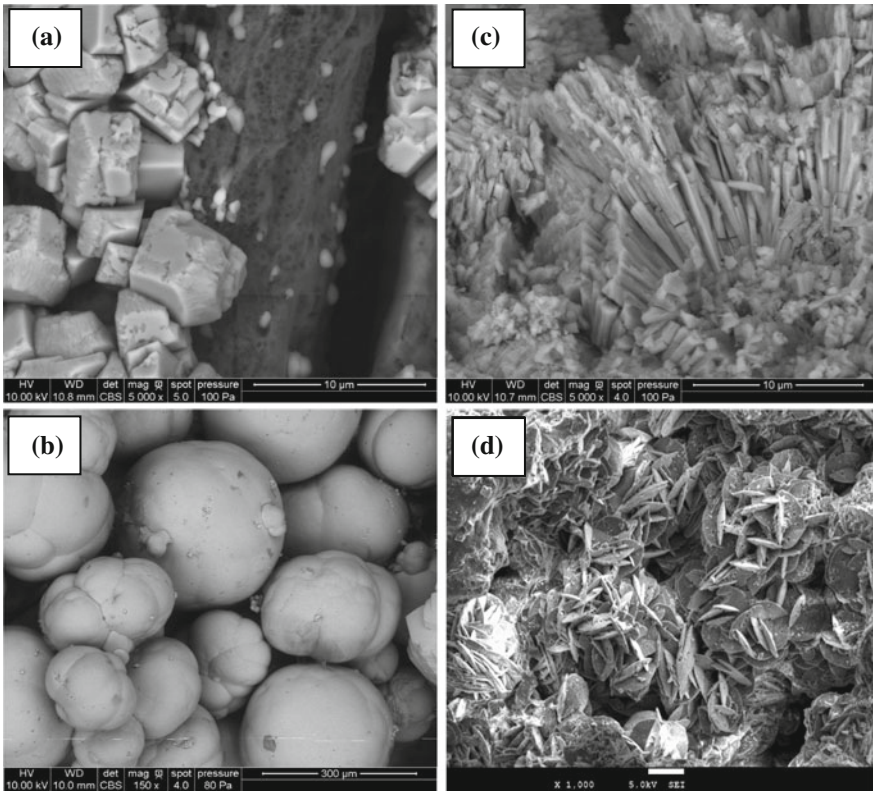



**Fig. 7.5** Calcite (**a**), vaterite (**b**), aragonite (**c**) and plain (**d**) crystals of $CaCO_3$ produced during different types of MICP

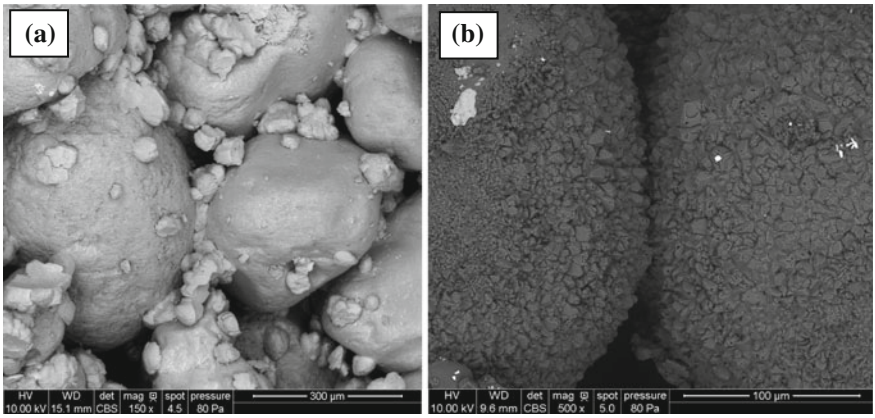



**Fig. 7.6** Formation of calcium carbonate crystals in the contact zone between sand grains (**a**) and on whole surface of the sand grains (**b**)

produced by dissolution of calcium carbonate in reactor with 100 % of $CO_2$, there appeared flat crystals (Fig. 7.4d). Type of the crystals was confirmed not just by their SEM shape but also using XRD analysis, for exemption of crystals shown in Fig. 7.4d that were produced and adhered to the surface from calcium bicarbonate solution.

Viscosity of solution also affects the type of the crystals during MICP. For example, in our experiments formation of vaterite was enhanced in viscous 1 % solution of microbial polysaccharide xanthan (Fig. 7.4b). It was shown also that formation of spherical crystals of calcium carbonate enhanced not only xanthan but also aminoacids, mainly aspartic and glutamic acids (Braissant et al. 2003). However, crystals looking as the short needles were produced in xanthan-free medium with glutamine (Braissant et al. 2003).

## 7.18 Effect of Chemical Factors on MICP

Sand biocemented at high concentration of bacteria had a much higher strength than sand treated at a lower concentration of cells (Kim et al. 2012, 2014). It was also determined that slower reaction rates may in some circumstances be desirable for maximum MICP efficiency because ureolysis is limited eventually by the encapsulation of the bacterial cells by calcite (Cuthbert et al. 2012). The characteristics of cementation of soil depend on the soil content because bioprecipitation of $CaCO_3$ was twice bigger in sand than in silt (Kim et al. 2014).

Any factors inhibiting production of urease in bacterial cells or its activity affect the MICP rate. For example, inhibiting effect of heavy metals on ureolytic culture increased in the following order: Cd(II) > Cu(II) > Pb(II) > Cr(VI) > Ni(II) > Zn (II) (Kurmac 2009).

## 7.19 Problems of MICP Applications

There are several drawbacks in the conventional MICP process:

(1) by-product of urea hydrolysis is ammonium and ammonia that are toxic substances for workers, harmful for aquatic environment and atmosphere, and increases the risk corrosion because of high pH (Pacheco-Torgal and Labrincha 2013a);
(2) the brittleness of calcite crystals bonding the soil particles; and
(3) the cost of calcium reagent and urea are higher than the cost of conventional cement. Therefore, the improvements of MICP as well as new types of biocementation have to be developed to overcome these disadvantages of conventional MICP.

## 7.20 Media for Production of Bacterial Biomass for Biocement

As biomass of urease-producing bacteria is one of the major components for biocement production, the important task for the development of biotechnology for biocementation is the method to obtain UPB biomass for large-scale applications using cheap raw material.

The medium ATCC 1376 NH4-YE containing yeast extract (YE), 20 g; $(NH_4)_2SO_4$, 10 g; 0.13 M Tris buffer (pH 9.0), 1L, is recommended for cultivation of urease-producing bacteria *S. pasteurii* (former *B. pasteurii*) and is most often used for laboratory studies of biocementation. This medium was used for cultivation of *S. pasteurii* ATCC 11859 (Chou et al. 2011; Li et al. 2011) and *B. pasteurii* ATCC 6453 (Stocks-Fischer et al. 1999). Sometimes, Tris buffer is replaced with distilled water, but the pH of the medium is adjusted to 9.0 (Whiffin et al. 2007).

Very often ammonium sulfate is replaced with urea, 20 g/L, and addition of urea to medium for cultivation of UPB is usually recommended. Liquid medium is sterilized by autoclaving for 15–20 min at 121 °C, meanwhile solution of urea has to be sterilized by filtration through 0.2 μm Millipore filter to avoid thermal decay. The strain of *S. pasteurii* DSMZ 33 was grown in Nutrient Broth supplemented with 2 % (w/v) urea (333 mM) (Gat et al. 2014). Tryptic Soy Broth with urea, 20 g/L, was recommended for cultivation of *S. pasteurii* DZM 33 by Collection of Microorganisms and Cell Cultures, Braunschweig, Germany (Stabnikov et al. 2011, 2013a, b). YE with urea was used for cultivation of *B. sphaericus* LMG 225 57 (De Muynck et al. 2010a, b; Wang et al. 2010). Sometimes, medium contains both ammonium sulfate and urea (Bang et al. 2011; Cheng and Cord-Ruwisch 2013a, b).

In some researches, extra source of carbon was added to the medium to enhance cultivation conditions for UPB. For example, 100 mM sodium acetate was added to

the medium containing YE, urea, and ammonium sulfate for cultivation of enrichment culture of UPB (Al-Thawadi 2013), and calcium acetate, 26 g/L, was added to the medium containing Nutrient Broth, 3 g/L, ammonium chloride, 10 g/L, sodium bicarbonate, 2.1 g/L, and urea, 10 g/L, for cultivation of urease-producing bacteria *S. pasteurii* and *B. sphaericus* (Kim et al. 2013).

Urease is a nickel-containing enzyme; therefore some authors used media with an addition of nickel chloride. Addition of 10 μm $Ni^{2+}$ ions to the YE medium increased urease activity, however, it is decreased under higher concentrations of nickel (Al-Thawadi 2008). The similar results were obtained for recombinant strain of *Esherichia coli*, containing a plasmid that encodes *B. pasteurii* urease (Bachmeier et al. 2002). Addition of nickel in concentration of 5–100 μM enhanced microbial calcite precipitation. Optimal concentration of $Ni^{2+}$ was 5 μM, however, concentration of $Ni^{2+}$ above 1000 μM totally suppressed calcium precipitation. Addition of 0.1 mM $NiCl_2$ to the medium with YE, 20 g/L, and 0.17 M ammonium sulfate, was used for cultivation of *Bacillus shaericus* MCP-11 (Cheng and Cord-Ruwisch 2012). Another medium for cultivation of UPB contained 10 μM of nickel ions (Verma et al. 2015).

The pH of medium for cultivation of UPB is usually adjusted to 9.0–9.5 (Cheng and Cord-Ruwisch 2012, 2013a, b; Verma et al. 2015; Whiffin et al. 2007), but sometimes medium has lower pH, for example 8.6 (Bang et al. 2011). However, all media described above are too expensive for the large-scale construction application of biocementation.

One of the ways to reduce the cost of biocementation is the use of low cost medium for cultivation of UPB. It was shown that replacement of YE with cheap soybean meal in the medium that was used for cultivation of *S. pasteurii* increased urease activity in comparison with ATCC 1376 NH4-YE medium (Li et al. 2011). The industrial effluent of the dairy industry, lactose mother liquor (LML), was proposed as a growth medium for cultivation of UPB *S. pasteurii* (Achal et al. 2009a, b). Urease activity of culture liquid and concentration of UPB biomass grown in LML—urea media (10 % LML, 5 g NaCl, 2 % urea, and 25 mM $CaCl_2$) were almost the same that were obtained on YE medium (1 g YE, 5 g NaCl, 2 % urea, and 25 mM $CaCl_2$).

## 7.21 Constitutive and Inducible Urease

Culture liquid of *Bacillus* sp. VS1 after 48 h of cultivation had the urease activity 20.3 mM hydrolyzed urea/min in the YE medium with ammonium chloride, 19.2 mM hydrolyzed urea/min in the YE medium without any addition of nitrogen source, and 10.7 mM hydrolyzed urea/min in the YE medium with urea. So, urease of *Bacillus* sp. VS1 is a constitutive enzyme. However, *Yaniella* sp. VS8 grown in the medium without urea had no urease activity. However, it demonstrated fast

increase of urease activity being transformed into the medium containing urea. For example, urease activity on the level of 4.5 mM hydrolyzed urea/min was observed when urea, 20 g/L, was added to the YE medium after 48 h of cultivation. Thus, the cells of *Yaniella* sp. VS8 have an inducible urease enzyme. What is important, there is no need to add aseptically filtrated solution of urea into the fermenter for cultivation of biocement agent because the level of urease activity can be increased by addition of urea to produce bacterial biomass very quickly after cultivation.

## 7.22 Activated Sludge of Municipal Wastewater Treatment Plants as Raw Material

It is known that hydrolyzed activated sludge (HAS) can be used for cultivation of different microorganisms (Belyaev et al. 1978; Nkhalambayausi-Chirwa and Lebitso 2012). There was attempt to use activated sludge, produced on wastewater treatment plant, for growth of urease-producing bacteria *S. pasteurii* ATCC 11859 (Whiffin 2004). Sludge was treated with 0.5 M NaOH for 20 min with following neutralization to pH 8.0 with $H_2SO_4$. However, this hydrolysate did not sustain growth of UPB. So, until now, the majority of researchers use expensive microbiological media with addition of urea after its cold sterilization by filtration. This technique is not suitable for large-scale cultivation of UPB in case of their practical, large-scale use for biogrouting and biocementation.

Activated sludge from MWWTP was tested as possible source of nutrients for UPB. Sludge was allowed to settle down, then supernatant was removed, and the concentrated sludge was used for the medium preparation. Content of total suspended solids (TSS) in this concentrated sludge was 5.7 g TSS/L. Acid hydrolysis of sludge using 98 % $H_2SO_4$ was conducted. Concentrated sulfuric acid, 3 mL, was added per 100 mL of sludge. Acid hydrolysis of activated sludge was conducted at 80 °C for 1 h. The hydrolysate of activated sludge (HAS) was neutralized to the pH value of 8.0 with 5 M NaOH, and was supplemented with NaCl, 20 g/L. The medium was not transparent and had a lot of suspended flocks of biomass. This medium, 100 mL, was placed in each of three 300 mL conic flasks. Nothing was added to the flask 1. Solution of $NiCl_2$, 24 mg/L, was added to the flasks 2 and 3. Glucose, 3 g/L, was added to the flask 3. No urea was added into the medium for cultivation of *Bacillus* sp. VS1. Sterile solution of urea, 20 g/L, was added into the medium for cultivation of *Yaniella* sp. VS8. The flasks were placed on the shaker, and bacteria cultivation was conducted at 200 rpm and the temperature of 25 °C.

The most often used source of carbon and energy for cultivation of urease-producing bacteria are YE (Chou et al. 2011; Li et al. 2012; De Muynck et al. 2010a, b; Stocks-Fischer et al. 1999; Wang et al. 2010; Whiffin et al. 2007) and tryptic soya broth (Chu et al. 2012a, b, c; Stabnikov et al. 2011, 2013a, b). According to the results of batch cultivation of *Yaniella* sp. VS8 in media with YE

**Table 7.1** Use of different media for cultivation of *Yaniella* sp. VS8 and *Bacillus* sp. VS1

| Medium | *Yaniella* sp. VS8 | | *Bacillus* sp. VS1 | |
|---|---|---|---|---|
| | UA*, mM of hydrolyzed urea/min | Biomass, g TSS/L | UA*, mM of hydrolyzed urea/min | Biomass, g TSS/L |
| TSB, 30 g/L | 6.6 | 2.9 | 14.1 | 4.5 |
| TSB, 30 g/L + YE, 10 g/L | 8.7 | 3.2 | n.d. | n.d. |
| YE, 20 g/L | 13.7 | 5.6 | 11.2 | 5.0 |
| YE, 40 g/L | 11.3 | 4.9 | 11.2 | 7.1 |
| YE, 40 g/L + acetate, 10 g/L | 5.7 | 5.3 | 12.4 | 6.9 |

*Note* *accuracy of UA measurement (percentage of standard deviation of triplicates to average) was about 10 %

or TSB added with urea, the best medium for growth and urease synthesis was YE with concentration 20 g/L (Table 7.1).

Higher concentration of YE diminished concentration of accumulated bacterial biomass and urease activity of culture liquid. An addition of acetate to medium with YE slightly increased biomass concentration, but significantly reduced urease activity.

*Cells of Bacillus* sp. VS1, grown in the medium with TSB, showed the highest urease activity. Addition of TSB, 30 g per 1 L of distilled water, gives the medium containing in (g/L): casein peptone (pancreatic), 17.0; soya peptone (papain digest), 3.0; glucose, 2.5; sodium chloride, 5.0; dipotassium hydrogen phosphate, 2.5.

YE and TSB both rich in organic sources of nitrogen-containing proteins, peptides, amino acids, B vitamins, and trace elements. However, these media are expensive for the large-scale applications.

Waste activated sludge from wastewater treatment plants (WWTPs) that treat domestic wastewater contains organic matter, N, P, K, and other nutrients (Nkhalambayausi-Chirwa and Lebitso 2012). HAS was proposed for cultivation of different microorganisms: yeasts (*Candida*, *Hansenula*, etc.), mycelium fungi (*Spicaria*, *Endomyces*, *Trichosporon*, *Penicillium*, etc.), Actinomycetes (*Streptomyces,* etc.) and rod-shaped bacteria (*Bacillus*, *Bacterium*, etc.) (Belyaev et al. 1978). There was attempt to use sludge produced on WWTP for cultivation of urease-producing bacteria *S. pasteurii* ATCC 11859 (Whiffin 2004). Sludge was treated with 0.5 M NaOH for 20 min with following neutralization to pH 8.0 with $H_2SO_4$. However, this hydrolysate did not sustain growth of urease-producing bacteria.

In our study stronger hydrolysis conditions were applied to activated sludge. HAS was nonhomogeneous liquid with content of TSS 5.7 g/L. Urease activity of culture liquid and biomass concentration were detected in the media every day.

**Table 7.2** Use of hydrolyzed activated sludge (HAS) for cultivation of *Yaniella* sp. VS8 and *Bacillus* sp. VS1

| Medium | *Yaniella* sp. VS8 | | *Bacillus* sp. VS1 | |
|---|---|---|---|---|
| | UA*, mM of hydrolyzed urea/min | Biomass, CFU/mL | UA*, mM of hydrolyzed urea/min | Biomass, CFU/mL |
| YE, 40 g/L | 11.3 | $1.1 \times 10^8$ | 11.2 | $1.8 \times 10^9$ |
| HAS | 7.5 | $6.2 \times 10^8$ | 3.6 | $1.2 \times 10^8$ |
| HAS + $NiCl_2$ | 7.5 | $5.7 \times 10^8$ | 3.7 | $1.4 \times 10^8$ |
| HAS + $NiCl_2$ + glucose | 8.2 | $8.1 \times 10^8$ | 4.5 | $7.2 \times 10^8$ |

*Note* *accuracy of UA measurement (percentage of standard deviation of triplicates to average) was about 10 %

During cultivation of *Yaniella* sp. VS8 and *Bacillus* sp. VS1 on HAS, duration of lag-phase increased in comparison with YE medium, probably due to adaptation of bacteria to initial high TSS concentration. So, results for 4 days of bacterial cultivation in the HAS were compared with ones obtained for 3 days of bacterial cultivation in the YE medium (Table 7.2).

The cell concentration of urease-producing bacteria *Yaniella* sp. VS8 grown in the medium from HAS was almost the same and even a little bit higher than the cell concentration in the medium with 4 % of YE. Meanwhile, urease activity in the HAS medium was lower than in the YE medium. The reason of this phenomenon may be the inactivation of urease by proteases, produced during bacterial growth in the protein-containing medium (Chu et al. 2014a, b). Urease activity of culture liquid of *Bacillus* sp. VS1 grown in HAS medium diminished more significantly in comparison with YE medium. The possible explanation is that this strain synthesized both endocellular and extracellular urease, and inactivation of urease by proteases of culture liquid is going faster. Addition of glucose, 0.3 %, or salt of Ni did not change significantly either urease activity of culture liquid or bacterial growth yield. However, cells of the strain *Bacillus* sp. VS1, growing in HAS medium or HAS medium with addition of glucose, hydrolyzed for 24 h in the solution of 1 M urea 45 and 72 % of initial urea, respectively.

So, cultivation of urease-producing bacteria *Yaniella* sp. VS8 and *Bacillus* sp. VS1 for large-scale biocementation/bioclogging applications could be conducted using the cheap medium from HAS of MWWTP. The strain *Bacillus* sp. VS1, which synthesized constitutive urease, grew well in HAS medium without urea addition and showed urease activity that is sufficient for biocementation/bioclogging. Cultivation of the strain *Yaniella* sp. VS8 in HAS medium can be conducted without addition of urea but urea has to be added after cultivation to induce urease synthesis. Urease can be induced during biocementation/bioclogging process in the solution of calcium salt and urea.

## 7.23 Dry Calcium-Based Biocement

Flowchart of the conversion of waste-activated sludge into bioagent for biocementation/bioclogging is shown in Fig. 7.7.

After that, dry bacterial biomass can be used separately as component A of biocement or has to be mixed with the component B, which is calcium salt and urea. Molar ratio of urea and calcium salt in the component B should be between 1.25 and 2, depending on application.

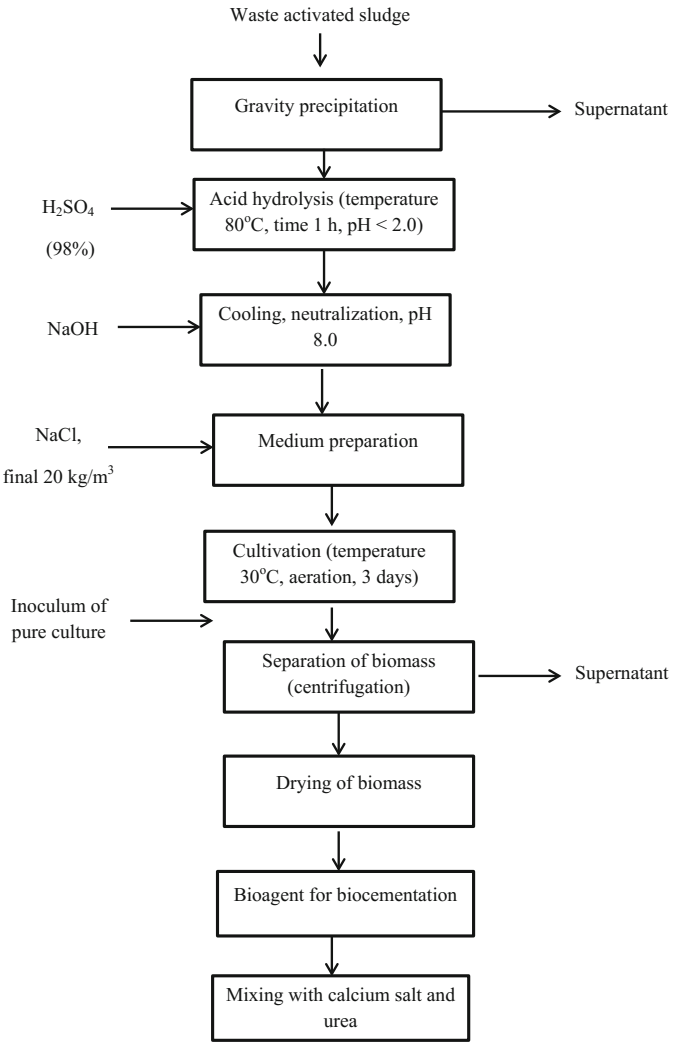



**Fig. 7.7** Flow chart of dry biocement production

If calcium chloride is used as calcium salt, it must be calcium chloride dihydrate to avoid significant heat release during dissolution of biocement in water before application. If it dehydrated, calcium chloride will be used in dry biocement temperature of solution after dissolution of biocement can be so high that urease will be denatured. Dry biocement must be standardized by urease activity that is produced after 10–20 min after dissolution of biocement in water. It could be different for different applications but approximately should be on the levels between 0.1 mM/min to 1 mM/min.

Application of biocement in two steps: treatment with the component A (bacteria) for 0.5 2 h for adsorption of cells on the surfaces, and after that treatment for 2–24 h with the component B with concentrations of calcium salt and urea on the levels 0.5–1.5 M and 0.75–2 M, respectively, is needed in cases when (1) bacterial agent form aggregates in solution of calcium salt; (2) if urease activity of bacteria is too high so significant quantity of calcium will be precipitated not on the biocemented surface but in the solution.

## 7.24 Unconfined Compressive (UC) Strength of Sand After MICP

Calcium carbonate crystals produced from calcium salt and attached to surface of sand or rock as the result of biocementation are environmentally neutral substance that can significantly increase fractured rocks strength of soil. It depends on the content of produced calcium minerals (Fig. 7.8).

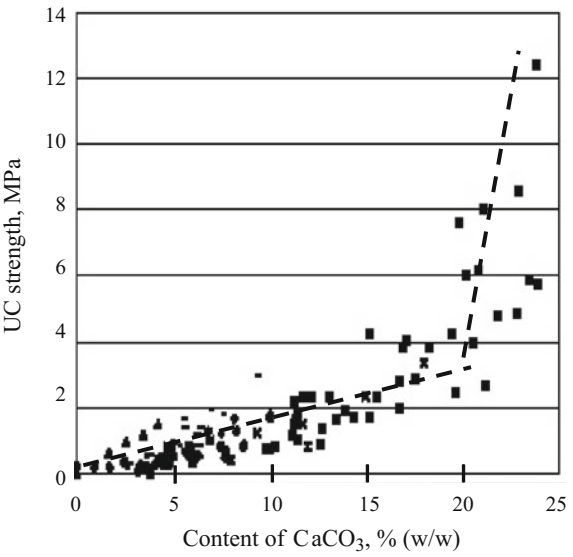



**Fig. 7.8** Unconfined compressive (UC) strength of sand after MICP (after Li and Qu 2015; Al Qabany and Soga 2013; Stabnikov et al. 2013a, b; Whiffin et al. 2007). The *dashed line* shows average values for different types and sizes of sand grains

These data can be conventionally interpolated by two lines. Hypothetically, the different slopes of these two lines are explained that bigger quantity of $CaCO_3$ is needed at the beginning of biocementation to fill in big pores and much smaller quantity of $CaCO_3$ is needed at the end of biocementation to fill in small channels.

Minimum of unconfined compressive strength ($Y_{min}$, MPa) of biocemented sand depends on the content (%, w/w) of precipitated $CaCO_3$ ($X$) by the following equations:

$$Y_{min} = 0.1X \quad \text{if } X < 20\,\%, \tag{7.11}$$

$$Y_{min} = 2 + 1.3(X - 20) \quad \text{if } X > 20\,\%. \tag{7.12}$$

Average of unconfined compressive strength ($Y_{min}$, MPa) of biocemented sand depends on the content (%, w/w) of precipitated $CaCO_3$ ($X$) by the following equations:

$$Y_{min} = 0.15X \quad \text{if } X < 20\,\%, \tag{7.13}$$

$$Y_{min} = 3 + 4(X - 20) \quad \text{if } X > 20\,\%. \tag{7.14}$$

## 7.25 Engineering Applications of MICP

Due to the formation of strength and decrease of permeability of porous materials, biocementation of porous soil can be used for numerous geotechnical applications (Seagren and Aydilek 2010; DeJong et al. 2010, 2013; Sarayu et al. 2014):

- to enhance stability of the slopes and dams (van Paassen et al. 2010; Harkes et al. 2010);
- for road construction and prevention of soil erosion (Mitchell and Santamarina 2005; Whiffin et al. 2007; Ivanov and Chu 2008; Ivanov 2010);
- for the construction of the channels, aquaculture ponds, or reservoirs in sandy soil (Chu et al. 2013a, b; Stabnikov et al. 2011);
- for sand immobilization and suppression of dust (Bang et al. 2011; Stabnikov et al. 2013a);
- to reinforce sand in near-shore areas (van der Ruyt and van der Zon 2009).

The applications of MICP in civil engineering can be also:

- the production of bricks (Sarda et al. 2009; Dhami et al. 2012; Raut et al. 2014);
- the remediation of cracks in concrete and rocks and increase of durability of concrete structures (De Muynck et al. 2008a, b, 2010a, b; Achal et al. 2010a, b; Van Tittelboom et al. 2010; Ghosh et al. 2005; Li and Qu 2012);
- the concrete improvement (Pacheco-Torgal and Labrincha 2013a);

- the self-remediation of concrete (Jonkers 2007; Jonkers et al. 2010; De Muynck et al. 2008a, b; Wiktor and Jonkers 2011; Ghosh et al. 2006; Siddique and Chahal 2011; Wang et al. 2012);
- the modification of mortar (Ghosh et al. 2009; Vempada et al. 2011);
- consolidation of porous stone (Jimenez-Lopez et al. 2008);
- the bioremediation of weathered-building stone surfaces (Fernandes 2006; Webster and May 2006; Achal et al. 2011);
- the fractured rock permeability reduction (Cuthbert et al. 2013);
- dust suppression (Bang et al. 2011; Stabnikov et al. 2013a);
- the construction of ponds and channels (Chu et al. 2012b, 2013a; Stabnikov et al. 2011);
- the mitigation of earth quake-caused soil liquefaction (DeJong et al. 2006, 2013; Chu et al. 2009a; Weil et al. 2012a, b; Montoya et al. 2012);
- the encapsulation of soft clay (Ivanov et al. 2014a, b, c);
- the coating of surfaces with calcite for enhanced marine epibiota colonization (Stabnikov and Ivanov 2016a).

Biocement based on MICP cannot be considered as alternative to cement because its applications are limited for the cases when: (1) low viscosity of solution for injection into porous material or spraying onto the surface of porous material is essential factor; (2) pure neutral calcium carbonate is an essential binder of the particle; (3) when treated surface can be placed perpendicular to gravity vector; (4) when porosity of the treated material and size of its particles are lower than at least porosity and size of the sand grains.

So, such applications of biocement as the binding of the recycled concrete aggregate (Grabiec et al. 2012), repair of the cracks in concrete (De Muynck et al. 2008a, b, 2010a, b; Achal et al. 2010a, b; van Tittelboom et al. 2010; Ghosh et al. 2005; Li and Qu 2012); the bioremediation of weathered-building stone surfaces (Fernandes 2006; Webster and May 2006; Achal et al. 2011a, b), self-remediation of concrete (Jonkers 2007; Jonkers et al. 2010; De Muynck et al. 2008a, b; Wiktor and Jonkers 2011; Ghosh et al. 2006; Siddique and Chahal 2011; Wang et al. 2012), biocementation of gravel, and many other applications can be probably more effectively performed with cement or other chemical binders rather than with biocement.

## 7.26 Biocementation Based on Production of Carbonates by Aerobic Heterotrophic Bacteria

Nadson (1903) discovered that microbes play a role in calcium carbonate precipitation, however, practical applications of this process were started for study only in 1990s. Precipitation of calcium carbonate can be due to increase in pH and production of carbonate by heterotrophic bacteria during aerobic oxidation of organics

(Ehrlich 1999; Wright and Oren 2005), for example, during oxidation of calcium acetate:

$$(CH_3COO)_2Ca + 4O_2 \rightarrow CaCO_3 \downarrow + 3CO_2 \uparrow + 3H_2O. \quad (7.15)$$

Calcium carbonate precipitation due to oxidation of organics was used for biocementation of the porous stones (Rodriguez-Navarro et al. 2003; Jimenez-Lopez et al. 2008).

Dissolved salts or chelates of Fe(II) produced by iron-reducing bacteria under strong anaerobic conditions can be also transformed to ferrous carbonate by anaerobic heterotrophic bacteria:

$$(CH_3COO)_2Fe + 4O_2 \rightarrow FeCO_3 \downarrow + 3CO_2 \uparrow + 3H_2O. \quad (7.16)$$

## 7.27 Biocementation Based on Production of Carbonates by Anaerobic Heterotrophic Bacteria

Precipitation of calcium carbonate due to increase in pH and production of carbonate by heterotrophic bacteria during anoxic oxidation of organics, for example due to bioreduction of nitrate:

$$(CH_3COO)_2Ca + 8/5NO_3^- \rightarrow CaCO_{3\downarrow} + 4/5\,N_2 \uparrow + 3CO_2 \uparrow + 3H_2O + 8/5OH^-. \quad (7.17)$$

Calcium carbonate precipitation due to nitrate bioreduction of organics (van Paassen al. 2009c) could be useful for the combination of biocementation with nitrogen gas production in situ during partial desaturation of sandy soil, which is an effective method for the mitigation of earthquake-caused soil liquefaction (Chu et al. 2009a; Rebata-Landa and Santamarina 2012; He et al. 2013). Bioreduction of nitrate (bacterial denitrification process) can also increase pH and initiate precipitation of $CaCO_3$ without pH buffering (Hamdan et al. 2011). For example, precipitation of $CaCO_3$ can be performed using bioreduction of calcium nitrate by ethanol:

$$12NO_3^- + 5C_2H_5OH \rightarrow 6N_2 \uparrow + 10CO_2 \uparrow + 9H_2O + 12OH^-, \quad (7.18)$$

$$6Ca^{2+} + 12OH^- + 6CO_2 \rightarrow 6CaCO_3 \downarrow + 6H_2O, \quad (7.19)$$

$$12NO_3^- + 5C_2H_5OH + 6Ca^{2+} \rightarrow 6CaCO_3 \downarrow + 6N_2 \uparrow + 4CO_2 \uparrow + 15H_2O. \quad (7.20)$$

**Fig. 7.9** Signs of sulfate-reducing bacteria growth (*black color* of CaS and rotten egg smell) appeared after denitrification in the tube containing denitrification medium and 0.05 M of calcium acetate. The tubes contain sand with denitrification medium and following concentration of calcium acetate (from *left* to *right*): 1.0, 0.75 0.5, 0.25, 0.1, and 0.05 M

However, our experiments showed that denitrification is inhibited at concentration of $Ca^{2+}$ above 0.1 M. Moreover, after finishing of denitrification sulfate reduction bioprocess is developing producing highly toxic and bad smelling $H_2S$ (Fig. 7.9).

The biogeochemical reactions in case of denitrification and iron-based biocementation/bioclogging are similar but also inhibition of denitrification by $Fe^{2+}$ ions could be expected as follows:

$$12NO_3^- + 5C_2H_5OH \rightarrow 6N_2 \uparrow + 10CO_2 \uparrow + 9H_2O + 12OH^-, \tag{7.21}$$

$$6Fe^{2+} + 12OH^- \rightarrow 6Fe(OH)_2 \downarrow, \tag{7.22}$$

$$12NO_3^- + 5C_2H_5OH + 6Fe^{2+} \rightarrow 6Fe(OH)_2 \downarrow + 6N_2 \uparrow + 10CO_2 \uparrow + 9H_2O. \tag{7.23}$$

## 7.28 Effect of Magnesium Ions on MICP

Dolomite, $CaMg(CO_3)_2$, is one of the most common carbonate minerals, which was deposited mostly in the Phanerozoic, produced probably at high temperature and alkaline pH which was created by anaerobic microorganisms (Rodriguez-Blanco et al. 2015).

The biocement can be produced through dissolution of mineral dolomite, which is a common raw material for the production of cement, in hydrochloric acid or organic acids. The salts of calcium and magnesium chlorides can be mixed with urease-producing bacteria and urea following with precipitation of calcium and magnesium carbonates:

$$Ca^{2+} + Mg^{2+} + CO(NH_2) + UPB \rightarrow CaMg(CO_3) \downarrow + 2NH_4{}^{+}. \quad (7.24)$$

Effect of magnesium ions on biogenic precipitation of calcium carbonate is significant. Our experiments on MICP with the mixture of 1 M solution of magnesium chloride and 1 M solution of calcium chloride giving molar ratio of Ca:Mg = 11 showed that this biocementation process produced mainly $CaCO_3$, aragonite crystals that coat whole surface of sand grains. The unconfined compressive strength of the biocemented sand was 12.4 MPa at the content of precipitated Ca 6 % (w/w). However, the hydraulic permeability of the biocemented sand was high, $7 \times 10^{-4}$ $ms^{-1}$. For calcium-and urea-based biocementation the hydraulic conductivity and strength are usually positively correlated. So, high strength is accompanied with low hydraulic conductivity of the biocemented sand. Probably, high strength but at the same time high permeability of sand after calcium + magnesium-based biocementation was due to the coating of whole surface of sand grains with crystals, while calcium- and urea-based biocementation produced crystal mainly in the contact areas of the sand grains (Fig. 7.6).

Significant effect of $Mg^{2+}$ ions was also shown in experiments on enzyme-induced biocementation of sand: precipitation rate decreased and replacement of calcium ions for even 10 % of magnesium ions initiated formation mainly aragonite crystals instead of calcite by Putra et al. (2016). At ratio of Ca:Mg 1:1 the average size of the crystals was 50 % of initial one.

Hypothetically, it could be possible that the combined calcite and struvite ($NH_4MgPO_4$) precipitation using triple superphosphate and magnesium salt to avoid the production of free ammonium and release of ammonia to atmosphere during biocementation:

$$Ca(H_2PO_4)_2 + 2Mg^{2+} + CO(NH_2)_2 + H_2O + \text{acid urease} \rightarrow 2NH_4MgPO_4 \downarrow + CaCO_3 \downarrow . \quad (7.25)$$

However, such reaction and process was not demonstrated yet, probably, because of contradictory conditions to maintain pH above 9 to produce struvite and low solubility of calcium and magnesium phosphates at this pH.

## 7.29 Calcium Phosphate Biocementation

Calcium phosphate precipitation from calcium phytate (*myo*-inositol hexakisphosphate, calcium salt) solution, (the main storage form of phosphorus in the plant seeds) using phytase activity of microorganisms (Roeselers and van Loosdrecht 2010) producing a mixture of the crystal forms such as monetite ($CaHPO_4$), whitlockite [$Ca_9(Mg,Fe^{2+})(PO_4)6HPO_4$], and hydroxyapatite [$Ca_5(PO_4)_3OH$] with the Ca-to-P molar ratio 1.55. The problem of this type of biocementation is a low solubility of calcium phytate (in the described study the concentration was 5.6 mM), so big volumes of solution must be pumped through soil.

Akiyama and Kawasaki (2012) used for calcium phosphate precipitation microbial suspension from slightly acidic soil (to select acidotolerant urease-producing species) and urea to increase pH. Unconfined compressive strength (UCS) of the biocemented sand samples was over 50 kPa. An addition of tricalcium phosphate powder increased UCS to 261 kPa after 28 days of curing (Kawasaki et al. 2013).

The microbially induced precipitation of barium hydrogen phosphate-bonded loose quartz sand particles but chemical precipitation did not bind loose sand particles into sandstone (Yu et al. 2015a). The maximum compressive strength of bio-sandstone was 2.1 MPa when the content of the slurry reaches 50 % with 24 h standing time (Yu et al. 2015b).

Hypothetically, triple superphosphate (monocalcium phosphate), which is a relatively cheap commodity, could be used for calcium phosphate precipitation using acidotolerant urease-producing microorganisms as shown below. There may be monetatite precipitation from monocalcium phosphate:

$$\begin{aligned} &Ca(H_2PO_4)_2 + CO(NH_2)_2 + H_2O + \text{acid urease} \rightarrow CaHPO_4 \\ &\quad \downarrow + CO_2 + (NH_4)_2HPO_4, \end{aligned} \tag{7.26}$$

and hydroxyapatatite precipitation:

$$\begin{aligned} &5Ca(H_2PO_4)_2 + 8CO(NH_2)_2 + 8H_2O + \text{acid urease} \rightarrow Ca_5(PO_4)_3(OH) \downarrow \\ &\quad + 2NH_4HCO_3 + 6CO_2 + 7(NH_4)_2HPO_4. \end{aligned} \tag{7.27}$$

However, monocalcium phosphate has low solubility about 0.08 M, so big volume of grouting solution must be supplied for calcium phosphate bioclogging and biocementation.

All these reactions, mediated by phytase, phosphatase, or acidotolerant urease could be very important for construction industry but the related biotechnologies for practical applications are not developed yet and could be too expensive for large-scale geotechnical applications.

## 7.30 Self-healing of Concrete Using MICP

It is considered that one prospective application of biocementation is repair of the cracks in concrete and self-healing concrete. The repair of the cracks in the surface layer of concrete is a major portion of multi-billion maintenance and repair cost of the concrete structures (Neville 1996; FHWA 2001). Self-healing concrete is based on the embedding into concrete the glass or biodegraded plastic capsules with material, which could be released after simultaneous cracking of concrete and capsules and penetration of outside water into the broken capsules. One type of material for repair and self-repair of the concrete proposed to be based on MICP (Ramachandran et al. 2001; Jonkers 2007; Ghosh et al. 2006; van Tittelboom et al. 2010; Siddique and Chahal 2011; Talaiekhozan et al. 2014; Jonkers et al. 2010, 2016; Seifan et al. 2016). The pH and temperature of fresh concrete could be up to 13 and 70 °C (Talaiekhozan et al. 2014), so microorganisms, or better their thermostable spores, must tolerate high pH and temperature and their biomass in concrete must be protected from high pH by the capsule or embedded material.

However, following the calculations below volume of produced $CaCO_3$ ($V_{calcite}$) will be always significantly smaller of the volume of the crack ($V_{crack}$) to be filled. The volume of the capsulated material ($V_{capsule}$) cannot be bigger 2–5 % (v/v) of the volume of concrete and, respectively, not more than 5 % (v/v) of the cracks volume in concrete ($V_{cracks}$). Otherwise, the decrease of the concrete strength will be not acceptable. Material of the capsules, a mixture of bacterial biomass, dihydrate of calcium chloride and urea (molar ratio = 1:1.5), will contain about 169 kg Ca/1000 kg of mixture (=40 g Ca/(146 g of calcium chloride dihydrate + 90 g of urea + 1 g of bacterial biomass) and even being densely packed 2000 kg $m^{-3}$ the content of Ca in the capsules will be 338 kg Ca $m^{-3}$ or 845 kg $CaCO_3$ $m^{-3}$. Under density of calcite 2900 kg $m^{-3}$ the maximum volume of calcite produced from the capsules is 0.291 $m^3$ of calcite/$m^3$ of the cracks volume. Therefore, the whole concept of bacterial self-healing is under question because only 30 % of the concrete cracks volume could be filled by calcite produced from 5 % (v/v) of the capsules introduced into concrete. Probably, this technology can work if the calcite crystals are deposited not in the whole volume of the crack but mainly in the mouth of the crack being moving there by the flow of $CO_2$ gas, which is produced during urea hydrolysis.

Actually, notwithstanding numerous reviews on the bright future of microbial self-healing concrete there are no experimental data yet on the commercially and technically acceptable biotechnologies. From our calculations above, absence of real data on the calcite formation inside the cracks of concrete, brittleness of calcite, and absence of data on sustainability of the calcite sealing of the cracks are the reasons of the doubts on the microbial self-healing concrete biotechnologies. Chemical reactions of the self-healing with an increase of the volume of the filling material to 10–20 times after formation of the crack in concrete could be more suitable than highly sensitive microbial processes.

# Chapter 8
# Bioclogging and Biogrouts

## 8.1 Microbial Processes of Bioclogging

Chemical grouting is a process to fill the soil voids with fluid grouts. It is often used to control water flow (Karol 2003). Common grouts are solution or suspension of sodium silicate, acrylates, acrylamides, and polyurethanes. Bioclogging is a grouting using microbial biopolymers or microbially mediated precipitation of inorganic compounds in situ for water flow control. Different possible microbial processes that can lead potentially to bioclogging are summarized in Table 8.1. These include formation of impermeable layer of algal and cyanobacterial biomass; production of slime in soil by aerobic and facultative anaerobic heterotrophic bacteria, oligotrophic microaerophilic bacteria and nitrifying bacteria; production of undissolved sulfides of metals by sulfate-reducing bacteria; formation of undissolved carbonates of metals by ammonifying bacteria; and production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil by iron-reducing bacteria. Not all of these processes have been tested in the laboratory and in the field.

## 8.2 Parameters to Measure Bioclogging

Major parameter to measure efficiency of bioclogging is hydraulic conductivity in m/s or permeability ($k$) measured in Darcy/milliDarcy (mD) defined by Darcy's law

$$v = k\Delta P/(\mu \Delta X), \tag{8.1}$$

where $v$ is the superficial fluid flow velocity through medium; $\Delta P$ is the applied pressure difference; $\mu$ is the dynamic viscosity of the fluid; $\Delta X$ is the thickness of the medium. One Darcy is permeability of a medium that permits flow rate 1 $cm^3/s$



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Green Energy and Technology, DOI 10.1007/978-981-10-1445-1_8

**Table 8.1** Microbial processes that can lead potentially to bioclogging

| Physiological group of microorganisms | Mechanism of bioclogging | Essential conditions for bioclogging | Potential geotechnical applications |
|---|---|---|---|
| Algae and cyanobacteria | Formation of impermeable layer of biomass | Light penetration and presence of nutrients | Reduction of water infiltration into slopes |
| Aerobic and facultative anaerobic heterotrophic slime-producing bacteria | Production of slime in soil from organic compounds | Presence of oxygen and medium with ratio of C:N > 20 | Cover for soil erosion control and slope protection, and prevention of slope failure |
| Oligotrophic microaerophilic bacteria | Production of slime in soil from organic matter | Low concentration oxygen and medium with low concentration of carbon source | To reduce drain channel erosion |
| Nitrifying bacteria | Production of slime in soil using oxidation of ammonium and assimilation of $CO_2$ | Presence of ammonium and oxygen in soil | To reduce drain channel erosion |
| Sulfate-reducing bacteria | Production of undissolved sulfides of metals | Anaerobic conditions; presence of sulfate and carbon source in soil | To form grout curtains to reduce the migration of heavy metals and organic pollutants |
| Ammonifying and urease-producing bacteria | Formation of undissolved carbonates of metals (Ca, Mg, Fe) in soil due to increase of pH and release of $CO_2$ during ammonification or urea hydrolysis | Presence of amino acids or urea and dissolved metal salt | To prevent piping of earth dams and dikes |
| Iron-reducing bacteria | Production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil | Anaerobic conditions changed for aerobic conditions; presence of ferric minerals | To prevent piping of earth dams and dikes |

of a fluid with viscosity 1 mPa s under a pressure gradient 1 atm/cm across an area 1 $cm^2$. Sand has a permeability of approximately 1 Darcy and the rocks have permeability less than $1 \times 10^{-8}$ Darcy. One darcy is equivalent to $9.87 \times 10^{-13}$ $m^2$. In terms of flux of water through medium under hydrostatic pressure ($\sim$0.1 bar/m) at a temperature of 20 °C 1 Darcy is equivalent to 0.831 m/day = $0.96 \times 10^{-5}$ $ms^{-1}$. Microbial biocementation using one injection of MICP solution containing 0.6 M urea and 0.6 M $CaCl_2$ decreased the permeability of sand porous medium from 394 to 31 mD (Nemati and Voordouw 2003), i.e., approximately from $3.8 \times 10^{-3}$ $ms^{-1}$ to $3 \times 10^{-4}$ $ms^{-1}$.

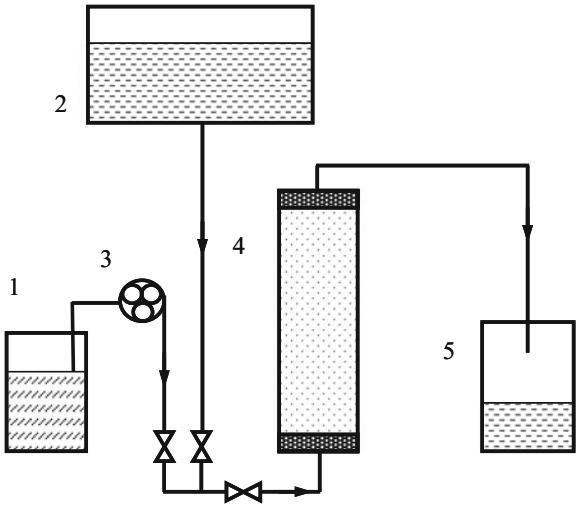



**Fig. 8.1** Schematics of the experimental facility for biocementation of sand using dead but urease-active bacterial cells: *1* the tank with either bacterial suspension or with bioclogging/ biocementing solution; *2* the tank with 2 % solution of NaCl for the measurements of hydraulic conductivity; *3* the peristaltic pump; *4* the sand column; *5* the tank for the effluent collection

In our experiments, the hydraulic conductivity of sand, $k$, was measured by falling or constant head method (Fig. 8.1) at water head falling from 0.05 to 0.02 m (for sand columns with the height less 20 cm) in or at constant water head 0.5 m (for sand columns with the height bigger 50 cm) according to the equation

**Fig. 8.2** Laboratory facility of three sand columns to study the effect of different biotreatments on hydraulic conductivity of sand. Each acrylic column had an inner diameter 60 mm and height 600 mm. One liter of Ottawa sand was placed into the column. *Outlet* and *inlet* of the sand columns were filtered by a layer of 10 mm thick sponge and a layer of 4 mm gravel

$$k = V/t \cdot S(\mathrm{m/s}), \tag{8.2}$$

where $V$ is the volume of water passed through the sand, $t$ is a time of the liquid was passed through the sand, $S$ is a cross sectional area of water flow through sand. This test was performed according to ASTM D2434-68 (2006) "Standard Test Method for Permeability of Granular Soils (Constant Head)."

Usually, we performed these laboratory tests it in the sand columns (Fig. 8.2). Each acrylic column had an inner diameter 60 mm and height 600 mm, and contains one liter of Ottawa sand. Outlet and inlet of the sand columns were filtered by a layer of 10 mm thick sponge and a layer of 4 mm gravel.

After hydraulic conductivity, the wet or dry samples were used for chemical analysis and SEM microscopy.

## 8.3 Bioclogging Using Production of Microbial Polysaccharides in Situ

Industrially produced water-insoluble gel-forming biopolymers of microbial origin such as xantan, chitosan, polyglutamic acid, sodium alginate, and polyhydroxybutyrate can also be used as grouts for soil erosion control, enclosing of bioremediation zone, and mitigating soil liquefaction (Momemi et al. 1999; Yang et al. 1993; Yen et al. 1996; Etemadi et al. 2003; Gioia and Ciriello 2006). Suitable microorganisms could be applied to soil to serve the same purpose through microbial growth and biosynthesis of extracellular biopolymers.

Production of bacterial exopolymers in situ can be used to reduce cost of soil particles binding in the soil crust formation. This has been adopted for enhancing oil recovery or soil bioremediation (Stewart and Fogler 2001).

## 8.4 Microorganisms that Can Be Used for the Formation of Polysaccharides in Situ

It is well known that almost all bacteria produce exopolysaccharides under excess of carbohydrates or other water soluble sources of carbon over source of nitrogen (Wingender et al. 1999). Therefore, such food-processing wastes or subproducts as corn glucose syrup, cassava glucose syrup, and molasses with C:N ratio >20 are used for industrial production of bacterial water-insoluble polysaccharides (Portilho et al. 2006).

The groups of microorganisms that produce insoluble extracellular polysaccharides to bind the soil particles and fill in the soil pores are oligotrophic bacteria from genus *Caulobacter* (Ravenscroft et al. 1991; Tsang et al. 2006), aerobic Gram-negative bacteria from genera *Acinetobacter*, *Agrobacterium*, *Alcaligenes*,

*Arcobacter*, *Cytophaga, Flavobacterium*, *Pseudomonas*, and *Rhizobium* (Harada 1983; Portilho et al. 2006; Ross et al. 2001). Other groups of microorganisms are cellulose-degrading bacteria from species *Cellulomonas flavigena* (Kenyon et al. 2005) and many species of Gram-positive facultative anaerobic and aerobic bacteria, such as *Leuconostoc mesenteroides* that is used for the production of a water-insoluble exopolymer dextran (Stewart and Fogler 2001). The strains of *Cellulomonas flavigena* may be suitable for large-scale soil clogging or soil grouting because these bacteria are Gram-positive (i.e., resistant to the changes of osmotic pressure) and can utilize cellulose for the production of a curdlan-type (beta-1,3-glucan) exopolysaccharide. In this case, such sources of carbon as cellulose-containing agricultural and horticultural wastes, and saw dust can be hypothetically used for the propagation of bacteria in soil and formation of the pore-clogging polysaccharide.

Probably, nitrifying bacteria that produce extracellular polysaccharides from $CO_2$ of air during oxidation of ammonium (Stehr et al. 1995) can also be used for the soil clogging. Almost every natural nitrifying biofilm includes nitrifying bacteria, which are embedded into a layer of microbial slime (Ivanov et al. 2006a). However, biofilm is usually not a plain film but rather cellular aggregates attached to surface (Ivanov et al. 2006b; Ivanov 2010). Accumulation of aggregating bacterial cells in soil pores could also contribute to soil clogging. Such bacterial strains with fast and strong cell aggregation are used in wastewater treatment in fixed biofilm reactors and for the fast formation of microbial granules (Ivanov et al. 2005; Ivanov and Tay 2006a, b).

Enrichment cultures of nitrifying and oligotrophic bacteria can be used for bioclogging. Enrichment culture of nitrifying bacteria, grown in sand with dissolved ammonium, produced microbial polysaccharides from solution of ammonium and $CO_2$ of air. The application of ammonium solution decreased the hydraulic conductivity of sand from $10^{-4}$ to $10^{-6}$ $ms^{-1}$.

## 8.5 Slow Bioclogging with Microbial Exopolysaccharides Production in Situ

Flows through the sand columns inoculated with *Pseudomonas aeruginosa* were used to investigate the effect of bioclogging (Aal et al. 2010). Bioclogging was started after 8 days of flow due to the production of extracellular substances as filling pore throats and/or as uniform covering on the sand surfaces. After 25 days a steady state was reached for porosity and conductivity. It is important to note that *P. aeruginosa* is opportunistic pathogen and on our opinion cannot be used for geotechnical experiments because of biosafety reasons.

Similar results were obtained from other experiments (Ross et al. 2001), where hydraulic conductivity decreased to $4.6 \times 10^{-6}$ m/s after 22 days of the biotreatment using activation of indigenous microorganisms. Growth of an

exopolysaccharide-producing soil by bacteria *Pseudomonas* sp. decreased saturated hydraulic conductivity of sand in sand column supplied with nutrients for microbial growth by 26-fold during 80 days of operation (Mattison et al. 2002).

## 8.6 Experimental Bioclogging of Sand with Pure Culture of *Paracoccus Denitrificans* DSMZ 413

In our experiments on bioclogging with microbial polysaccharides, we used inoculum of *Paracoccus denitrificans* DSM 413 (synonym is *Micrococcus denitrificans*; other collection numbers are ATCC 17741, ATCC 19367, CCM 982, LMD 22.21, NCIB 8944). Risk group according to German TRBA (Technical Rules for Biological Agents) is 1, meaning that the organism is unlikely to cause human or animal disease. *P. denitrificans* DSM 413 was cultivated during 48 h at 24 °C on the shaker in sterile medium of the following content, g/L: Tryptic Soy Broth DIFCO™ (TSB), 1; ethanol, 8 (10 ml); $KNO_3$, 10; $NH_4Cl$, 1; distilled water, 1 l; pH 7.0. The bottle with culture and medium was flushed with nitrogen gas for 5 min to ensure anaerobic conditions. Cultivation of inoculum was for 2 days.

The flow rate through the column was at the beginning 73 mL/h, to ensure hydraulic retention time shorter 0.5 h, i.e., shorter than bacterial generation time to ensure that only bacterial cells attached to sand grains will grow in this sand column. After 15 days the flow rate was decreased to 4.3 mL/h.

Permeability of sand treated with *P. denitrificans* DSM 413 was decreased by 58 times and consisted of 1.7 % of initial value after 19 days of biotreatment (Fig. 8.3).

There was no ammonia release in our tests on bioclogging with *P. denitrificans* DSM 413 but pH of effluent from the sand column was increased to 8.6–9.0. Therefore, it was assumed that precipitation of calcium carbonate can be observed in this process if dissolved calcium will be supplied into the sand altogether with the denitrifying medium. It could also help to decrease additionally the hydraulic conductivity because of the formation of $CaCO_3$ crystals in the microchannels between sand grains.

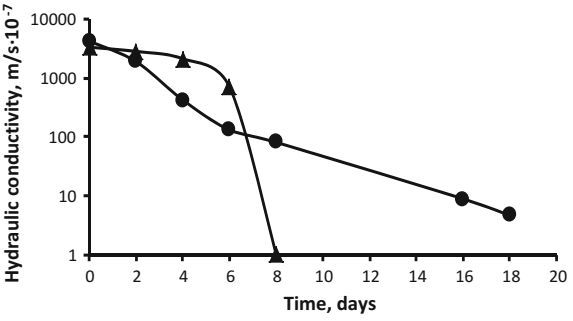



**Fig. 8.3** Hydraulic conductivity of the sand columns during continuous (*filled triangles*) or batch (*filled circles*) biotreatment with *Paracococcus denitrificans*

So, $CaCl_2$, 10 g/l, was added to medium supplied with the rate 18 mL/h during 130 h into the sand. However, there was no change in hydraulic permeability during 8 days of treatment. It was at the same level $2.1 \times 10^{-4}$–$2.4 \times 10^{-4}$ $ms^{-1}$ and pH of effluent was also varied from 6.8 to 7.4, which is a sign of the absence of denitrification. The similar results were obtained when $CaCl_2$ and $KNO_3$ in the medium were replaced with $Ca(NO_3)_2$. pH of the effluent did not increase and varied from 6.8 to 7.3. Permeability of sand was at the same level as initial value, $2.9 \times 10^{-4}$–$5.5 \times 10^{-4}$ $ms^{-1}$. It means that it was no denitrification process in the presence of dissolved calcium in medium. It is practically an important conclusion because there was proposed biocementation/bioclogging process based on the simultaneous denitrification and biocementation due to use of $Ca(NO_3)_2$. There are known several papers and PhD thesis on biocementation using denitrification. Usually, it was studied that calcium-based biocementation of sand using denitrification is performed by *Paracoccus denitrificans* DSM 413 on the medium with ethanol and nitrate, which has to increase pH to 8.5–8.7 and produce calcium carbonate due to production of $CO_2$ during denitrification and increase of pH. However, we have found that presence of calcium salt inhibited denitrification. Most probably, that it was due to the precipitation of essential for microbial growth phosphates by calcium ions.

## 8.7 Application of Enrichment Culture of Soil Microorganisms for Sand Bioclogging

Enrichment culture was selected in the laboratory sand column in The Masterflex® peroxide-cured silicone tubing EW-96400-36 (Cole-Parmer, Vernon Hills, IL, USA) with internal diameter 9.7 mm and area of cross section 0.74 $cm^2$ was filled with 50 ml of Ottawa sand with the length of sand column 68 cm. This long sand column was made to select anaerobic exopolysaccharides-producing denitrifiers. This column was inoculated with enrichment culture of soil microorganisms that was selected by anaerobic cultivation 20 g of five soil samples in 200 mL of the medium of the following content, g/L: Tryptic Soy Broth DIFCO™ (TSB), 5; ethanol, 8 (10 ml); $KNO_3$, 10; $NH_4Cl$, 1; distilled water, 1 L; pH 7.0. The bottle with culture and medium was flushed with nitrogen gas for 5 min to ensure anaerobic conditions. Cultivation of inoculum was for 7 days.

In this test, Ottawa sand was treated in long column with the small diameter to ensure gradient of redox condition. The sand column was inoculated with enrichment culture of soil microorganisms as described above. This medium was pumped constantly upwards through the sand column with the average rate at the beginning 73 mL/h, which after 15 days decreased to 4.3 mL/h. Changes of the hydraulic permeability of sand in the column treated with microorganisms under anaerobic conditions are shown in Fig. 8.4.

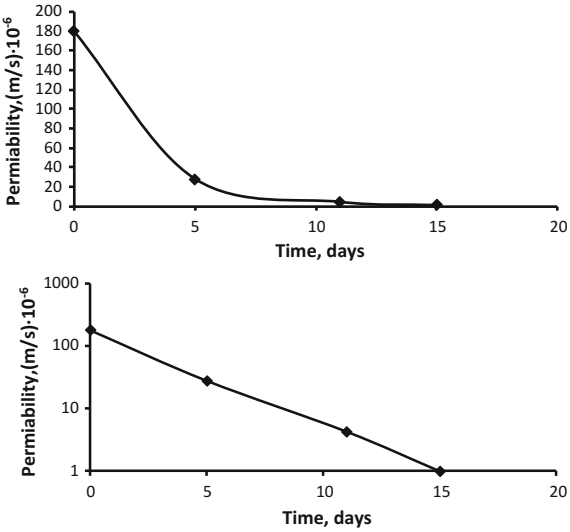



**Fig. 8.4** Changes of the hydraulic conductivity of sand (linear and logarithmic scales) in the column treated with enrichment culture of microorganisms under anaerobic conditions

**Table 8.2** Characteristics of the sections of the sand column

| Distance from the inlet, cm | Hydraulic conductivity of the sections at water head 0.5 m, $ms^{-1}$ |
|---|---|
| 0–10 | $0.9 \times 10^{-6}$ |
| 10–20 | $1.1 \times 10^{-6}$ |
| 20–30 | $1.1 \times 10^{-6}$ |
| 30–40 | $1.0 \times 10^{-6}$ |
| 40–50 | $1.5 \times 10^{-6}$ |
| 50–60 | $1.5 \times 10^{-6}$ |

Hydraulic conductivity at head 1.4 m decreased to $1 \times 10^{-6}$ $ms^{-1}$ on 15th day of the bioclogging. Totally, decrease of hydraulic conductivity was in 180 times from the initial value, $1.8 \times 10^{-4}$ $ms^{-1}$. The pH measured from day 6 to day 15 of experiment was in the range from 8.6 to 9.0.

Sand column was sectioned for the parts of 10 cm length and each part was analyzed for hydraulic conductivity (Table 8.2).

Hydraulic permeability was almost the same for all sections and increased from $0.9 \times 10^{-6}$ $ms^{-1}$ near the inlet to $1.5 \times 10^{-6}$ $ms^{-1}$ near the outlet. These values are almost same as those produced with the bioclogging by *P. denitrificans* DSM 413.

So, bioclogging with anaerobic denitrifying microorganisms is situ is not so effective, duration is at least 2–3 weeks, and finally, this clogging should be not too stable because microbial polysaccharides can be degraded in nature and after sometime the bioclogging effect should disappear.

## 8.8 Use of Waste Organic Matter for Bioclogging

Exopolysaccharide-producing bacteria may be used to modify the soil matrix in situ. After growth of these bacteria in soil, its permeability for water will be greatly reduced. This approach could be used for different geotechnical applications such as selective zonal bioremediation, harbor and dam control, erosion potential minimization, earthquake liquefaction mitigation, construction of reactive barrier, and long-term stabilization of contaminated soils (Yang et al. 1993). Organic wastes can be used as a source of organic matter for fermenting and exopolysaccharide-producing microorganisms in large-scale applications to diminish the cost of soil clogging.

For example, municipal solid wastes, sewage sludge, uncomposted, or composted poultry manure has been added to soil to diminish soil erosion. In these experiments, a positive correlation between the stability of soil aggregates and the produced microbial biomass in soil has been found (Mataix-Solera et al. 2005). It was shown in other experiments that the introduction of polysaccharide-producing algae and bacteria in irrigation channel could provide a low-cost technique for seepage control in irrigation channel. The reduction of hydraulic conductivity by 22 % of its original value within a month of inoculating soil columns with algae correlated with the amount of produced polysaccharides (Ragusa et al. 1994).

## 8.9 Use of Industrially Produced Microbial Polysaccharides for Ground Improvement

Industrially produced microbial polysaccharides, the same substances that are used as the cement admixtures (Chap. 3 of this book), can be used for ground improvement, i.e., to increase strength and diminish hydraulic permeability of soil. There are many experimental data on this topic. A comprehensive review with important data was recently published (Chang et al. 2016). Even 1 % w/w of such microbial polysaccharides as gellan or xanthan added to clay or clayey soil can increase its compressive strength in dry state up to 5 Mpa. The strength increases due to the hydrogen and ionic bonds between the biopolymers and clay particles. However, when the treated soil is hydrated, its strength decreases by one order of magnitude while the hydraulic conductivity of soil with 0.5–1 % of polysaccharide increases by 3–4 orders of magnitude (Chang et al. 2016). The stability of microbial polysaccharides and their effects in soil mechanical properties are still under question. A problem for use of industrially produced microbial polysaccharides is also high viscosity of their solutions.

## 8.10 Laboratory Bioclogging Using MICP

Important application of calcium carbonate precipitation in soil is biogrouting to reduce hydraulic conductivity of porous soil, fractured rocks, or cracked concrete (Ferris et al. 1996; Whiffin et al. 2007; Ivanov and Chu 2008; Al-Thawadi and Cord-Ruwisch 2012; Chu et al. 2012a, b; DeJong et al. 2010, 2013; De Muynck et al. 2010; Dhami et al. 2014a, b; Ivanov et al. 2015a, b; Rong et al. 2012a, b; Stabnikov et al. 2011, 2015).

In our experiments with laboratory bioclogging of sand using MICP the hydraulic conductivity after batch treatments of sand decreased to $1.3 \times 10^{-5}$ $ms^{-1}$ after 9 days, and $4.6 \times 10^{-7}$ $ms^{-1}$ on 19th day of experiment. So, hydraulic conductivity decreased by $4.3 \times 10^{3}$ times.

Continuous bioclogging using solution of 1 M $CaCl_2$ and 1.5 M urea supplied to the column with the rate 20 ml/h for the first four days, and then decreased to 4 ml/h was more efficient than batch treatments. The initial pH was in the range of 7.1–7.3 and the final pH was 8.4–8.8. Continuous bioclogging decreased hydraulic conductivity of the sand column to $1 \times 10^{-7}$ $ms^{-1}$ after 8 days of the experiment (Fig. 8.5).

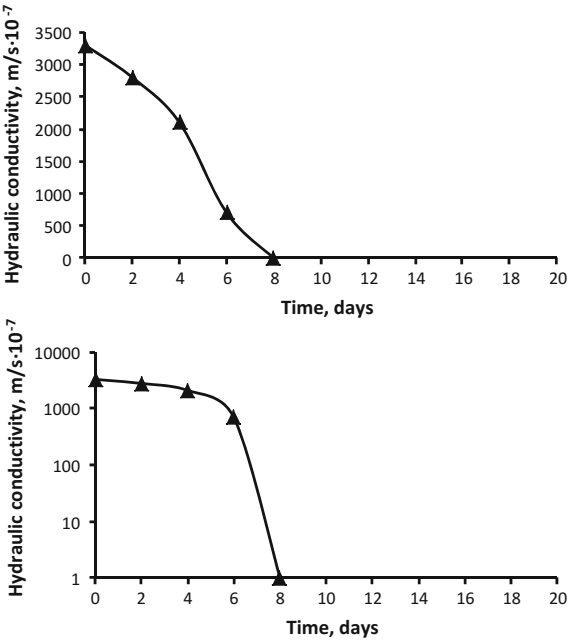



**Fig. 8.5** Hydraulic conductivity (linear and logarithmic scales) of the sand columns during continuous bioclogging using MICP

## 8.11 Effect of Precipitated Calcium Carbonate on Hydraulic Conductivity

Effect of precipitated calcium carbonate on hydraulic conductivity is shown in Fig. 8.6.

Maximum of hydraulic conductivity ($k_{max}$, $ms^{-1}$) of biocemented sand depends on the content (%, w/w) of precipitated $CaCO_3$ ($X$) by the following equations:

$$\lg k_{max} = -4 - 0.1X \quad \text{if } X < 20, \tag{8.3}$$

$$\lg k_{max} = -6 - 0.2(X - 20) \quad \text{if } X > 20. \tag{8.4}$$

Our data showed that MICP performed in the up flow of 1.5 M solution of urea and 1 M calcium chloride can decrease hydraulic permeability below $1 \times 10^{-9}$ m/s. Depending on the quantity of metal that was precipitated in the porous medium the effect can be termed as either bioclogging or biocementation. Maximum urease-dependent precipitation of Ca from the solution of 0.75 M of $CaCl_2$ and 0.75 M of urea at sand porosity 50 % (v/v) is 15 g Ca/1600 g (1 L) of sand = 0.9 % (w/w) of precipitated Ca = 2.25 % (w/w) of precipitated $CaCO_3$. To reach low hydraulic conductivity of $1 \times 10^{-7}$ $ms^{-1}$ the content of precipitated $CaCO_3$ must be about 22.5 % (w/w) (Fig. 8.6), which is based on our experimental data and published results (Stabnikov et al. 2013a, b; Al Qabany and Soga 2013; Whiffin 2007; Martinez et al. 2013). Therefore, from 2 to 10 treatments to replace 2–10 volumes of clogging solution in the pores must be used for the bioclogging. Bioclogging could be most effective in case of the filling/clogging of the channels between the particles.

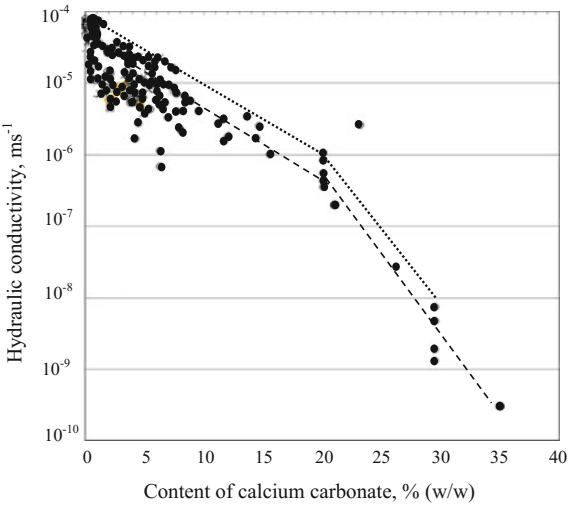



**Fig. 8.6** Correlation between hydraulic conductivity and the content of precipitated (crystallized) calcium carbonate in sand (after Stabnikov et al. 2013a, b; Al Qabany and Soga 2013; Whiffin 2007; Martinez et al. 2013). The *dotted lines* are the *trend lines* of the experimental data. The *lines* show average (*dashes*) and minimum (*dots*) values for different types and sizes of sand grains

## 8.12 Applications of MICP Clogging

Bioclogging could be a new biochemical method diminishing the hydraulic conductivity of sand or fractured rocks, which is essential for: (1) protection of the construction sites from groundwater infiltration; (2) diminishing of water losses in agricultural channels and aquaculture ponds; (3) protection of environment from the landfill or soil-polluted site leachate; (4) to stop the water seepage from the agriculture channels, aquaculture ponds, landfills, construction and polluted sites, through the dams and dykes, through the fractured rocks, flow in the tunneling space before or after excavation First experiments with sand biocementation showed that after that treatment, sand porosity diminished by 50 % and hydraulic conductivity dropped by 90 % (Kantzas et al. 1992; Gollapudi et al. 1995).

## 8.13 Bioclogging in Oil and Gas Recovery

Bioclogging of the highly permeable zones of oil field could improve oil recovery from the low permeable zones through displacement with water (Seifert 2005). MICP was used to plug fractures and reduce permeability in porous materials with the aim to diminish subsurface fluid leakage in the near wellbore environment for oil and gas development or carbon sequestration (Phillips et al. 2015, 2016). MICP was promoted in a fractured sandstone layer 340.8 m below ground surface using conventional oil field subsurface fluid delivery technologies. After 24 hours of urea and calcium solution and UPB suspension injections, the injectivity (flow rate) was decreased 1.9–0.47 L/min and the researchers suggested that MICP is a promising tool for sealing subsurface fractures in the near wellbore environment (Phillips et al. 2016).

## 8.14 Idea on Sequential Biogas Production and Biofixation of Its Bubbles in Sand

New biogeochemical processes that diminish the hydraulic conductivity of sand or fractured rocks are: (1) formation of biogas microbubbles by denitrifying bacteria and (2) fixation of these bubbles using biocementation by urease-producing bacteria.

However, the simultaneous biochemical nitrate reduction ("denitrification") and the enzymatic hydrolysis of urea in the calcium-containing medium were not applicable because denitrification activity was inhibited at concentration of $Ca^{2+}$ above 0.1 M. Meanwhile, the sequential performance of these biochemical processes in the sand columns using pure cultures of denitrifying and urease-producing bacteria was possible due to the formation of biogas microbubbles, which were

fixed in sand pores due to urease-depending bioclogging of the channels between the sand pores. The proposed sequential denitrification and biocementation decreased hydraulic conductivity of sand to $2 \times 10^{-7}$ $ms^{-1}$ at the content of precipitated $CaCO_3$ 8.0–8.4 % (w/w), while an application of biocementation alone requires content of precipitated $CaCO_3$ about 22 % (w/w). A new biochemical method could be used for a low-cost biotechnology for clogging of sand.

According to Fig. 8.6, to reach hydraulic conductivity below $1 \times 10^{-7}$ $ms^{-1}$, when hydraulic conductivity of the treated sand could be considered as low and almost equal to the hydraulic conductivity of sandstone, the content of precipitated $CaCO_3$ in sand must be at least 22 % (w/w) or 137 kg $m^{-3}$ of sand. This level of precipitation requires supply of at least 152 kg $CaCl_2$ and 123 kg of urea per 1 $m^3$ of sand, and these big quantities of reagents will restrict the application of this type of bioclogging for large-scale geotechnical or environmental works.

Therefore, another bioprocess for the clogging of water-saturated sand with potentially lower requirements for reagents was proposed and studied in this paper. The idea of the proposed biochemical process was to produce microbubbles of biogas inside the pores of sand using denitrifying bacteria and to immobilize these microbubbles in the pores using urease-dependent biocementation of the channels between the pores. So, it was expected that this process can replace an expensive filling of the sand pores with biocement making bioclogging of sand competetive with the clogging of sand by ultrafine cement.

## 8.15 Clogging Due to Biogas Production in Situ

Soil pores can be clogged by gas bubbles (Seagren and Aydilek 2010). One of the most effective ways to introduce small bubbles of nitrogen gas and carbon dioxide into water-saturated sand is denitrification, i.e., biochemical reduction of introduced nitrate to nitrogen gas in situ (van Paassen et al. 2010; Seagren and Aydilek 2010; Chu et al. 2013a, b, 2015; He et al. 2013). Different organic and inorganic substances can be used for bioreduction of nitrate but acetic acid ($CH_3COOH$) and ethanol are the most suitable electron donors because of their low cost, availability, and high solubility in water. Bioreduction of nitrate (denitrification) and production of biogas ($N_2$ and $CO_2$) are shown below

$$1.25\ CH_3COOH + 2\ NO_3^- \rightarrow N_2 \uparrow + 2.5\ CO_2 + 1.5\,H_2O + 2\,OH^-. \quad (8.5)$$

$$5\,CH_3CH_2OH + 12\,NO_3^- \rightarrow 6\ N_2 \uparrow + 10\,CO_2 + 9\,H_2O + 12\,OH^-. \quad (8.6)$$

The consumption of the reagents calculated by Eq. 8.5 is as follows: acetic acid as the electron donor, 1.34 kg/$m^3$ of biogas (or just 2.7 kg/$m^3$ of sand at sand porosity 50 % v/v), and sodium nitrate as the electron acceptor, 1.08 kg/$m^3$ of

biogas (or just 2.2 kg/m$^3$ of sand at sand porosity 50 % v/v). This quantity of reagents is about 78 times lower than quantity of reagents that must be used according to conventional MICP.

## 8.16 Instability of Biogas Bubbles in Sand

However, long-term sustainability of the gas bubbles in sand during upward or horizontal flows of groundwater is the most important for practical applications of denitrification for sand clogging. Experiments of Yegian et al. (2007) showed that under hydrostatic conditions the degree of saturation of sand (% of maximum content of water in sand) with introduced gas bubbles remained almost constant, 83–84 %, for 442 days. Similar results for hydrostatic conditions were obtained by He et al. (2013). The field data of Okamura et al. (2011) showed that gas bubbles, which were introduced in sand, remained entrapped there for 26 years.

Meanwhile, gas bubbles in sand are unstable in the case of vertical or horizontal flow of water (Chu et al. 2013a, b, 2015; He et al. 2013). The biogas bubbles disappeared from sand after 2–4 days of water flow through sand. However, the stability of biogas bubbles in sand depended on the flow rate. For example, biogas bubbles in sand were almost stable at the low flow rate of water with the hydraulic retention time 0.36 h. Probably, the stability of biogas microbubbles in sand can be increased by biocementation of the channels between sand pores using biochemical processes shown by Eqs. 8.1 and 8.2. Therefore, the aim of our study was to examine the combination of two bioprocesses: (1) production of biogas microbubbles from the solution of ethanol and nitrate in sand by denitrifying bacteria, and (2) the fixation of these microbubbles in sand using urease-dependent biocementation of the channels between sand pores.

## 8.17 Bacterial Strains Used for Biogas Production and Their Fixation in Sand

Bacterial strain of *Paracoccus denitrificans* DSMZ 413 (ATCC 17741) was used for the production of biogas in water-saturated sand. It was cultivated on the medium of the following content, g/L: Tryptic Soy Broth DIFCO™ (TSB), 5; ethanol, 8; $KNO_3$, 10; $NH_4Cl$, 1; distilled water, 1 L; pH 7.0. The bottles with bacterial suspension were flushed with nitrogen gas for 5 min to ensure anaerobic conditions during cultivation at 30 °C for 3–5 days. The concentration of bacterial biomass in suspension was measured by filtration of 10 mL of bacterial suspension through a membrane with 0.45 μm pores and drying the filter paper at 60 °C for the constant weight. The quantity of bacterial biomass introduced into the water-saturated sand for biogas production was about 100 mg of dry biomass/kg of sand.

The composition of the medium for the production of biogas in sand was similar to that was used for the cultivation of bacteria but was diluted 10 times. Bacteria were suspended in this medium and supplied to sand with the rate about 20 mL/L of sand min manually or by the peristaltic pump. For the batch treatment, the volume of the medium with suspended bacteria was slightly bigger than the pore water volume, so the level of liquid was about 1 cm above the level of sand in the column or tube. Displacement of water from the sand pores was measured as an increase of the level of liquid above this initial level due to the bioproduction of gas bubbles in the sand pores.

The strains of urease-producing bacteria (UPB) used in experiments for calcium-based biosealing of biogas bubbles was *Bacillus* sp. VS1 (Stabnikov et al. 2011, 2013). Bacteria were grown on the medium of the following composition: Tryptic Soya Broth DIFCO™, 30 g/L; urea, 20 g/L; NaCl, 20 g/L; $MnSO_4 \times H_2O$, 12 mg/L; $NiCl_2 \times 6H_20$, 24 mg/L, and phenol red, 10 mg/L. Phenol red was used as a pH indicator during cultivation and microbiological examination of culture: its color is yellow at the pH 6.8, but gradually changes to red/bright pink color at pH above pH 8.2. Cultivation of UPB was done in 300 mL of this medium in 1L flasks on the shaker at 200 rpm at 30 °C for 48 h. Urease activity of bacterial suspension was measured by the changes of electrical conductivity in 1 M solution of urea for 5 min (Stabnikov et al. 2011). It was ranged from 2 to 9 mM hydrolyzed urea/min.

The following strains with both urease and denitrifying abilities were selected and tested on the mixture 1:1 (v/v) of the above-mentioned media for growth of denitrification and urease-producing bacteria: *S. pasteurii* DSMZ 33; *Bacillus cohnii* DSMZ 6356; *Bacillus sphaericus* (*Lysinibacillus sphaericus*) DSMZ 28; *Paracoccus denitrificans* DSMZ 1405; and *Halomonas denitrificans* DSMZ 735.

## 8.18 Laboratory Examination: Biogas Production and Its Stabilization in 1 L Sand Columns

The regime of the treatment of 1L sand columns in control was as follows: (1) supply of 500 mL of *Bacillus* sp. VS1 bacterial suspension for 2 min (the flow rate was about 1000 mL/h) to the column bottom; (2) supply to the column bottom 200 mL of water for 2 min (the flow rate was about 100 mL/h) to separate bacterial suspension and entering biocementation medium and avoid chemical precipitation of $CaCO_3$; (3) supply to the column bottom the biocloggingg/biocementation solution contained 1 M (60 g/L) urea and 1.25 M (137.5 g/L) $CaCl_2$ for 7 days with the rate 20 mL/h which corresponds to the hydraulic retention time (HRT) of bacterial cells and media in the pores of sand about 25 h.

The regime of the sand treatment in experiment was as follows: (1) supply of 500 mL of *P. denitrificans* DSMZ 413 suspension from the bottom of the column for 25 min (the flow rate was about 20 mL/h); (2) continuous supply of medium for denitrifying bacteria with ethanol and nitrate from the bottom of the column for

14 days; (3) supply of 500 mL of *Bacillus* sp. VS1 bacterial suspension for 2 min (the flow rate was about 1000 mL/h) to the column top at 1st, 4th, 8th, and 12th day of biocementation; (4) supply to the column top 200 mL of water for 2 min (the flow rate was about 100 mL/h) for separation of bacterial suspension and medium for biocementation to avoid chemical precipitation of $CaCO_3$; (5) supply to the column bottom, the solution for biocementation contained 1 M (60 g/L) urea and 1.25 M (137.5 g/L) $CaCl_2$ for 5 days with the rate 20 mL/h (HRT was about 25 h).

## 8.19 Laboratory Examination of Simultaneous Denitrification and Biocementation

It was considered by some researchers (Yegian et al. 2007; van Paassen et al. 2010; Hamdan et al. 2011; Martin et al. 2013; DeJong et al. 2013; Ersan et al. 2015) that bacterial denitrification in the medium with the organic electron donor and nitrate as the electron acceptor can initiate the precipitation of calcium carbonate minerals from calcium salts due to the formation of $CO_2$ and increase of pH to 8.5–8.7 according to the Eqs. 8.2–8.4. However, this precipitation has been done from low concentration of 0.05 M of calcium salt (Ersan et al. 2015), while bioprecipitation of $CaCO_3$ using urea and urease is going usually from 0.5 to 1.0 M solution of calcium salt.

Our experiments showed that simultaneous injection of the cell suspensions of denitrifying bacteria *Paracoccus denitrificans* DSMZ413 and urease-producing bacteria *Bacillus* sp. VS1 altogether into the mixture of the media for denitrification and biocementation did not produce biogas, increase pH, and did not precipitate of $CaCO_3$ if concentration of calcium salt was bigger than 0.1 M. This was tested in two sets of experiments. In first set, the tubes with 30 mL of sand were filled with denitrifying medium containing calcium acetate, then inoculated either *P. denitrificans* DSMZ 413 or enrichment denitrifying culture produced from a mixture of the bottom sediment samples from different sites with high salinity. Concentrations of calcium acetate were 0.05, 0.1, 0.25, 0.5, 075, and 1.0 M. The tubes were flashed with nitrogen gas and sealed. After two weeks of incubation, the production of biogas was visible only in the tubes where concentration of calcium acetate was below 0.1 M. It was confirmed also by the increase of pH above 7.8, which occurred only in the tubes with concentration of calcium acetate below 0.1 M.

Another experiment was an addition of 0.1 M solution (the final concentration) either $CaCl_2$ (4 g $Ca^{2+}$/L) or $Ca(NO_3)$ to the inflow of denitrifying medium after two days of denitrification performed by bacteria *P. denitrificans* DSMZ 413 in the column with saturated sand. An addition of 0.1 M calcium ions terminated denitrification process in sand so that no biogas was produced, the pH was dropped from the values typical for denitrification 8.2–8.6 to the level of pH 6.8–7.2, and there was no clogging of sand. Termination of denitrification could be due to the precipitation of insoluble phosphate or sulfate anions by calcium cations and unavailability of these essential nutrients for growth and activity of denitrifying bacteria.

Third potential approach for simultaneous denitrification and bioclogging/biocementation was through selection of both halophilic and alkaliphilic bacterial strain with both urease and denitrifying abilities. Therefore, different strains with known urease and denitrifying abilities (Martin et al. 2013) were tested with or without presence of $Ca^{2+}$ ions at concentration 1 M: *S. pasteurii* DSMZ 33; *Bacillus cohnii* DSMZ 6356; *Bacillus sphaericus* (*Lysinibacillus sphaericus*) DSMZ 28; *Paracoccus denitrificans* DSMZ 1405; and *Halomonas denitrificans* DSMZ 735. No one strain from the above-mentioned list was able for the simultaneous biogas production and precipitation of $CaCO_3$ under anaerobic conditions in the denitrifying medium with addition of 1 M $CaCl_2$ and 2 M of urea.

Bioclogging of porous soil using denitrification in the solutions of calcium below 0.1 M will require big volume of such solution and very long time of bioclogging process. So, its practical applicability is under doubt. Therefore, all further experiments on bioclogging were based only on the sequential combination of biogas production and biogas bubbles sealing in sand.

## 8.20 Sequential Denitrification and Bioclogging in the Sand Columns

Continuous injection and extraction of the denitrification medium and bioclogging solution is more effective technology than their batch injections because of the bigger quantity of the reagents can be introduced into saturated sand. This process was studied using sequential continuous flows of denitrification medium and then bioclogging/biocementation solution through the sand columns. Bacterial strain of *P. denitrificans* DSMZ 413 with the denitrifying medium containing ethanol as electron donor and potassium nitrate as electron acceptor was used for the biogas production in situ. This selection was made because *P. denitrificans* is able for denitrification and production of non-water-soluble exopolysaccharide slime (Raguenes et al. 2004; Babenko et al. 2008), that could clog the sand pores altogether with biogas microbubbles. Bacteria-producing slime can reduce greatly the hydraulic conductivity of water-saturated sand (Vandevivere et al. 1992a, b, c; Rezic et al. 2006). Additionally, the strains of *P. denitrificans* are not pathogenic for plants, animals, or humans and it was not mentioned about its pathogenicity among 25 million citations for biomedical literature in PubMed database (www.ncbi.nlm.nih.gov/pubmed).

The hydraulic conductivity of sand decreased to the level of $10^{-7}$ $ms^{-1}$ after continuous treatment for 13 days in both cases of the treatment with *P. denitrificans* and then with *Bacillus* sp. VS1 (Fig. 8.7) or *P. denitrificans* only (Fig. 8.8).

The initial pH of the media was 6.8–7.2, the final pH was 8.2–8.8 during both denitrification and biocementation stages. Biogas bubbles were formed during denitrification stage but remained also after additional biocementation (Fig. 8.9) due to the formation of clogging between sand pores.

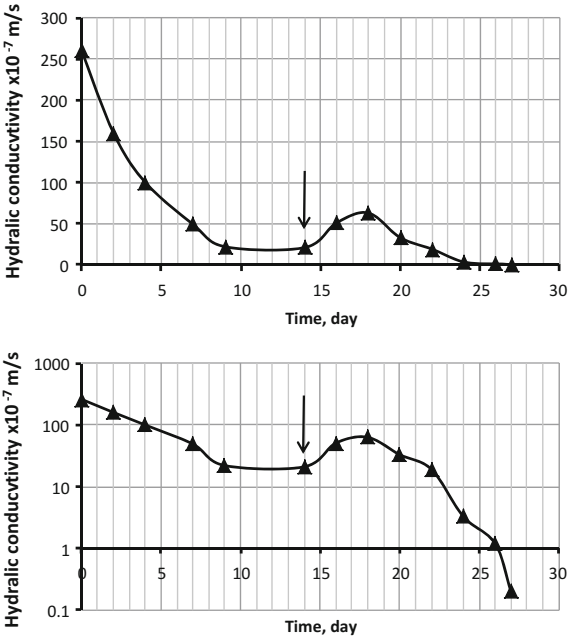



**Fig. 8.7** Changes of hydraulic conductivity in sand column during its treatment with bacteria *P. denitrificans* DSM 143 in denitrifying medium with ethanol and nitrate (from 1st to 14th day) and then with bacteria *Bacillus* sp. VS1 in biocementing solution of 1 M $CaCl_2$ and 1 M urea (from 14th to 27th day). *Arrow* shows beginning of the treatment with *Bacillus* sp. VS1 in biocementing solution

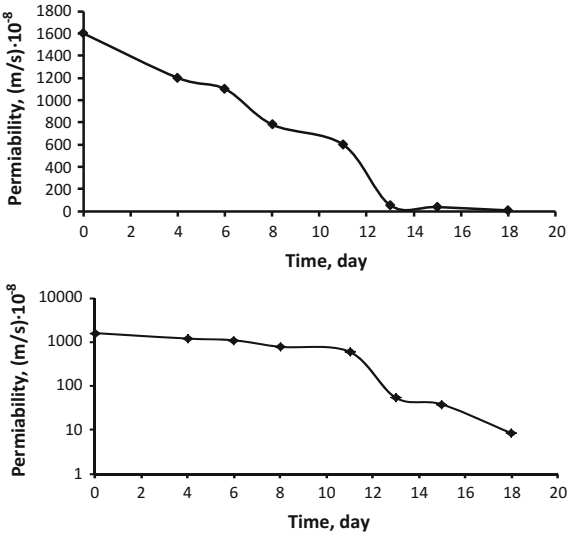



**Fig. 8.8** Decrease of hydraulic permeability (linear and logarithmic scales) of sand column during the bioclogging using treatment denitrifying medium with ethanol and bacteria *P. denitrificans* DSM 413

So, biogas bubbles produced from nitrate and ethanol in water-saturated sand can be stabilized in sand pores after biosealing using urease-induced calcium carbonate precipitation. The sequential biogas production in saturated sand and biocalcification of these gas bubbles in sand pores requires precipitation of 8 % (w/w)

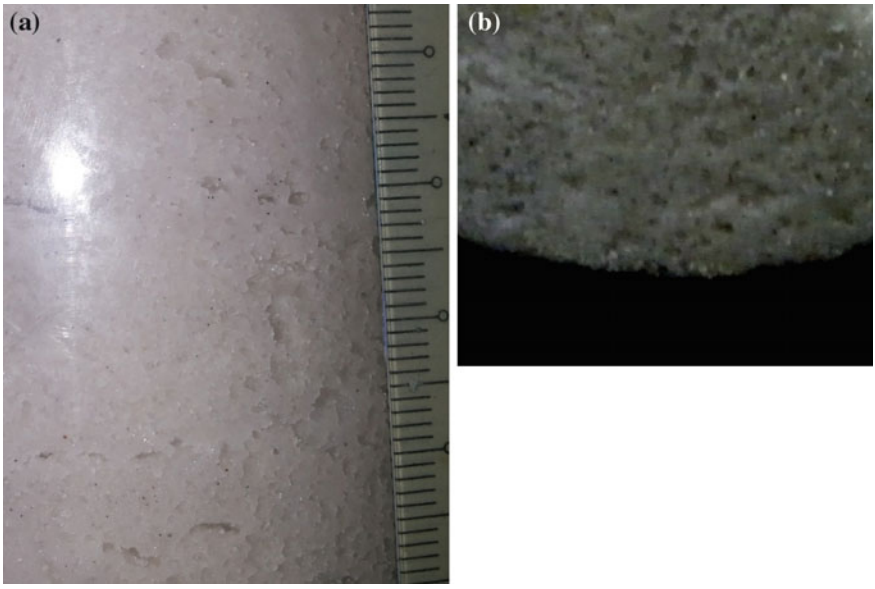



**Fig. 8.9** Bubbles of biogas in the sand column after denitrification (**a**) and remaining in the biocemented dry sand (**b**)

of calcium carbonate minerals to reduce the hydraulic conductivity of sand to $1 \times 10^{-7}$ $ms^{-1}$ meanwhile bioclogging/biocementation only without denitrification requires at least 22 % (w/w) precipitated calcium carbonate in sand. The sequential combination of two bioprocesses could find the applications for the sealing of the ponds, channels, levies, and tunneling spaces in sand, porous soil or fissured rocks.

Important engineering aspect of this combined process is that biocementation of sand was quite different whether UPB suspension for bioclogging/biocementation was supplied from the bottom or top of the column (Fig. 8.10).

After the supply from the bottom, the content of precipitated Ca was dropped to almost zero after 10 cm distance from the point of injection with geometrical mean for these 10 cm 8.0 % (w/w) and maximum 13 % (w/w) (Fig. 8.9a). Meanwhile, after supply of urease-producing bacteria from the top of the column the content of calcium in biocemented sand was between 5 and 11 % (w/w) through the whole volume of sand with geometrical mean 8.4 % (Fig. 8.10b). Hypothetically, it can be explained that bacterial cells during upward flow of the cementing solution are adsorbed mainly in the bottom part of the sand column due to retention of cells and their aggregates in the bottom part by gravity. Meanwhile, bacterial cells during downward flow of the cementing solution were evenly distributed in the bulk of the sand column because in this case it was no retention of cells and their aggregates in one part of the column by gravity.

The average content of $CaCO_3$ of 8.0–8.4 % (w/w) in both cases ensured the hydraulic conductivity of sand around $1 \times 10^{-7}$ $ms^{-1}$ and were significantly lower

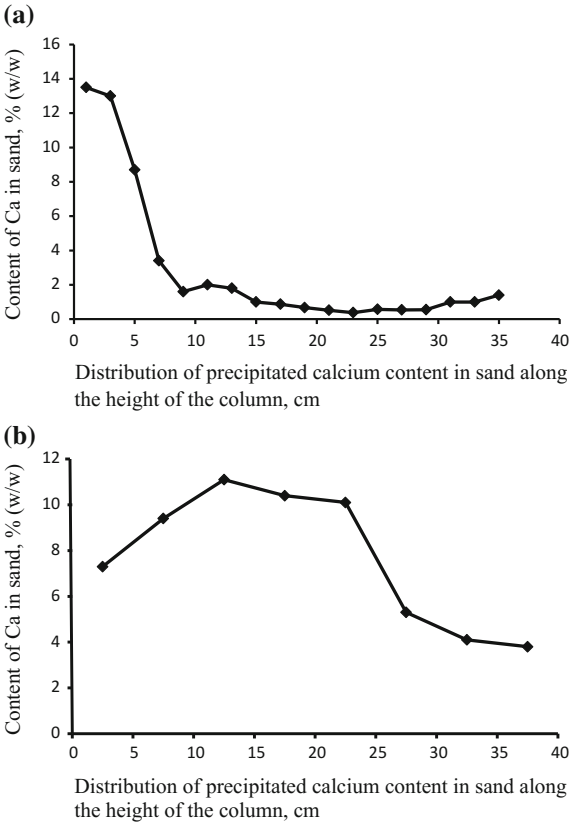



**Fig. 8.10** Content of calcium in biocemented sand after treatment with supply of UPB suspension from the *bottom* (**a**) or from the *top* (**b**)

than content of 22 % (w/w), which is required for bioclogging using only biocementation (Fig. 8.6). The dosage of precipitated Ca in case of denitrification and formation of the thin layer of biocemented sand (Fig. 8.10) was 12.8 kg Ca $m^{-2}$ of the sand surface but in case of the denitrification and biocementation of the whole volume of sand in the column (Fig. 8.10) it was about 53.8 kg Ca $m^{-2}$ of the sand surface, i.e., almost four times bigger. Therefore, denitrification and biocementation in the thin layer of sand could be the most effective way to reduce the hydraulic conductivity of water-saturated sand. This is important to stop the water seepage into the construction sites, for example into the tunneling space before excavation, and from the agriculture channels, aquaculture ponds, landfills, and polluted sites.

Hypothetically, biogas bubbles produced through denitrification in sand form a low permeable bulk of sand that could provide the basement for the formation of the low water permeability biocemented sand layer due to retaining of the biocementing solution on the boundary between the layers of water-saturated and biogas-containing sand as shown in Fig. 8.11.

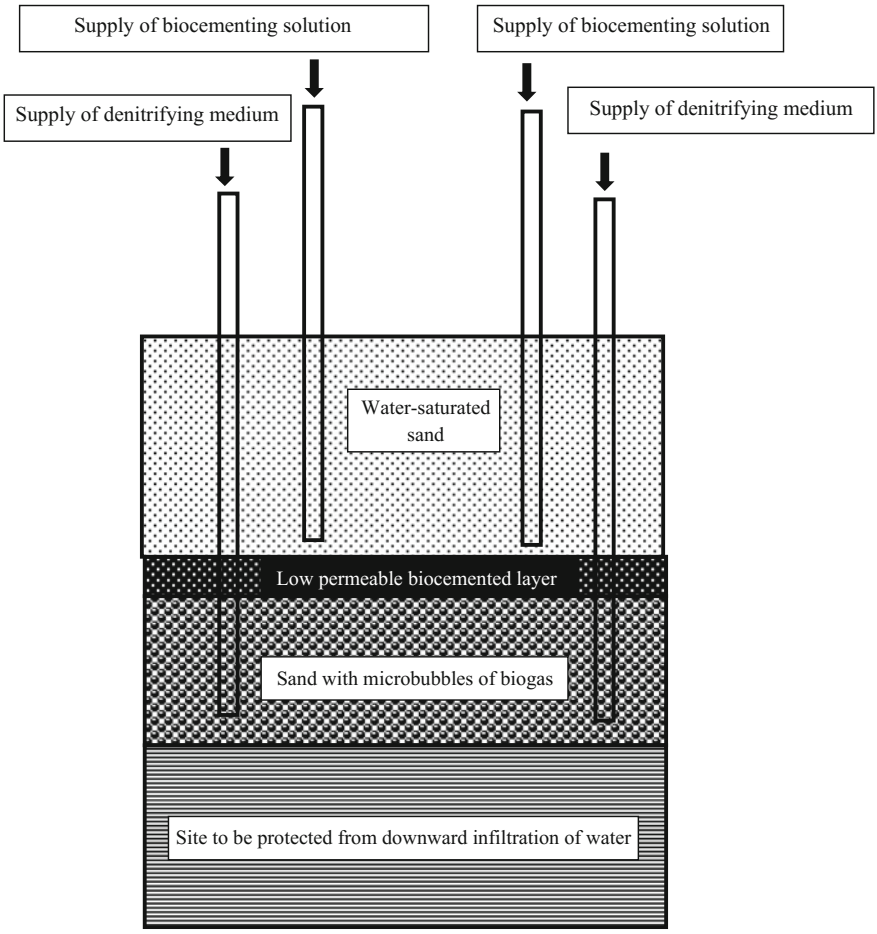



**Fig. 8.11** Hypothetical formation of the biocementated sand layer in the depth of sand due to formation of the retaining layer for biocementation solution on the boundary of water-saturated sand and sand filled with biogas microbubbles

## 8.21 Biosafe Bioclogging Using MICP

Urease-producing bacteria for bioclogging/biosealing of sand should be able to synthesize active urease under alkaline environment with high concentration of salts (Stabnikov et al. 2013a, b), which should show low aggregation ability at high concentration of calcium ions (DeJong et al. 2006; Stabnikov et al. 2011), and should be biological safe, e.g., nonpathogenic ones. Usually, the strains of *Sporosarcina pasteurii*, formerly *Bacillus pasteurii,* are commonly used for biocementation (Bang et al. 2001; Bachmeier et al. 2002; Whiffin 2004; Whiffin et al.

2007; Dupraz et al. 2009; Mortensen and DeJong 2011; Tobler et al. 2014). These bacteria belong to Risk group 1 with low individual and community risk.

However, there are a lot of studies when such opportunistic pathogens as *Bacillus cereus* (Maheswaran et al. 2014), *Bacillus mycoides* (Elmanama and Alhour 2013), *Proteus* sp., *Proteus vulgaris,* or *Proteus mirabilis* (Dosier 2014; Khanafari et al. 2011; Whiffin 2004; Varalakshmi 2014), *Staphylococcus aureus* and *Klebsiella pnemoniae* (Varalakshmi 2014), and even pathogenic bacteria *Helicobacter pylori* (Dosier 2014) were proposed to be used for MICP.

An introduction of live bacteria cells in environment is a risk, especially for the construction of ponds or reservoirs in sandy soil. In our previous research on the sand biosealing, we used live urease-producing bacteria for the construction of the model aquaculture pond using sealing of sand by microbially induced calcium carbonate precipitation (Stabnikov et al. 2011; Chu et al. 2013a, b) but the opportunity to construct the ponds using dead but urease—active bacteria was also tested (Stabnikov et al. 2016).

Treatment of bacterial cells Y*aniella* sp. VS8 was done by addition of 0.5 % (w/v) sodium dodecyl sulfate (SDS) for 960 min. Bacterial biomass was separated by centrifugation at 4 °C and 10,000 rpm for 10 min using Micro Cooling Centrifuge 5922 (Kubota, Japan), resuspended in NaCl, 2 %, and used for bioclogging of sand. Biosealing was done by spaying of the bacterial suspension and the solution of $CaCl_2$ and urea. Bacterial suspension was spread onto the surface of the model pond and left for 30 min. Then mixture of the solutions of calcium chloride and urea was spread onto the sand surface and left for 16 h. Spray of the mixture was done sometimes for each treatment thus the sand was not submerged into liquid. The treatments were repeated 11 times. After 8th, 9th, and 10th treatments the model pond was dried by placing in oven at 60 °C: after 8 and 9 treatments for night; after 10th treatment or 2 h. The study period included: (1) biotreatment—15 days, (2) drying on air—60 days, (3) additional biotreatment—4 days; (4) washing with water—3 days.

Totally, 2 L of treated bacterial suspension containing 5.8 g dry biomass, 108 g of calcium, and 225 g of urea, was used for biosealing of the model pond by spraying onto the sand surface with area of 722.5 $cm^2$. Finally, after 11 biotreatments the model pond with sizes 180 mm × 275 mm × 25 mm was constructed. Calcium in effluent was absent after every treatment. Due to this treatment, the hydraulic conductivity of sand was decreased from $5.2 \times 10^{-4}$ $ms^{-1}$ to $7.7 \times 10^{-9}$ $ms^{-1}$. Quantity of water percolated through pond bottom sand layer for the testing pond and control per day versus duration of study period is shown in Fig. 8.12. It was changed from 7000 to 96 mm $d^{-1}$.

The hydraulic conductivity of the biosealing layer in tap water did not change for at least 90 days. The content of live microbial cells in the sample taken from the pond surface was $4 \times 10^2$ CFU/g of the biocemented sand, which was almost same as initial bacterial contamination of sand, $1.1 \times 10^2$ CFU/g.

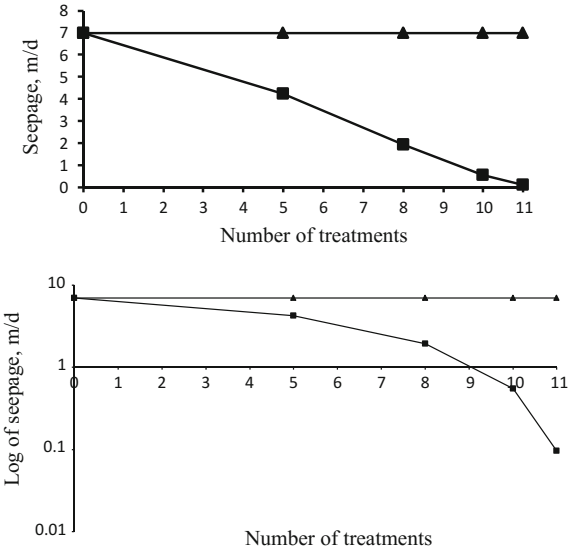



**Fig. 8.12** Dynamics of MICP bioclogging (in linear and logarithm scales) using dead but urease-active bacterial cells

Experiments with the bioclogging of the sand columns using dead but urease-active bacterial cells showed even better results (Fig. 8.13).

This sealing of sand decreased hydraulic conductivity to the level $10^{-8} \times ms^{-1}$, which is acceptable level for the aquaculture ponds. The cost of this sealing, especially when the local sources of calcium chloride brain or other calcium salts are applied, could be several times lower than any other known methods of the sand sealing, and could be used in aquaculture practice for the construction of fish, prawns, or algae ponds in sandy soil.

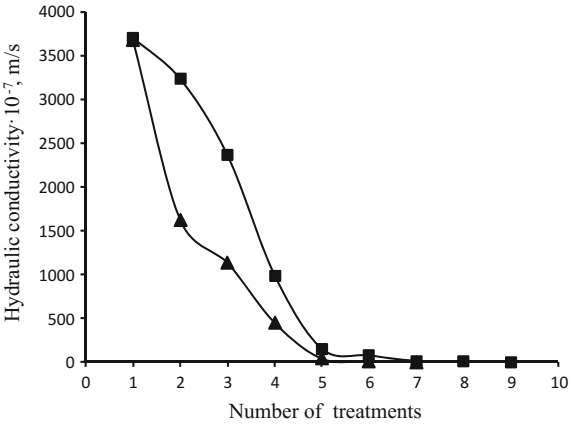



**Fig. 8.13** Hydraulic conductivity of sand columns treated with live cells (*filled triangle*) and dead, but urease-active cells (*filled square*) of *Yaniella* sp. VS8. Accuracy of the measurements was about 20 %

## 8.22 Calcium Bicarbonate Bioclogging

Important biocementation technology could be precipitation of calcium carbonate minerals using removal of $CO_2$ from solution of calcium bicarbonate (Ehrlich 1999) due to increase of pH or drop of gas phase pressure

$$\mathrm{Ca(HCO_3)_2 + OH^- \leftrightarrow CaCO_3 \downarrow + HCO_3^- + H_2O \leftrightarrow CaCO_3 \downarrow + CO_2 \uparrow + OH^-}. \quad (8.7)$$

Equation 8.7 shows that shift of equilibrium toward precipitation of calcium carbonate minerals could be because of

(1) removal of $CO_2$ from solution due to drop of pressure;
(2) removal of $CO_2$ from solution due to drying;
(3) removal of $CO_2$ from solution due to increase of temperature;
(4) initiating, not permanent increase of pH because after initiation of decay supply of hydroxide ions is performed by the reaction of decay. This initiating increase of pH can be made by urease-producing bacteria due to hydrolysis of urea

$$\mathrm{CO(NH_2)_2 + 2H_2O + urease \rightarrow 2NH_4^+ + CO_2 \uparrow + 2OH^-}. \quad (8.8)$$

The major advantage of this process over conventional MICP is that decay of calcium bicarbonate can be performed at neutral pH and 7–10 times smaller quantity of toxic ammonium ($NH_4^+$) and ammonia gas ($NH_3$) will be released. For example, we are using urea hydrolysis to initiate decay of calcium bicarbonate at molar ratio of urea: calcium ions = 0.2, while in conventional MICP this ratio is 1.5–2. This is important also because the cost of urea in conventional MICP exceeds 50 % of the total costs for bioclogging (Stabnikov et al. 2015).

Solution of calcium bicarbonate can be produced by action of $CO_2$ gas on $Ca(OH)_2$ or $CaCO_3$:

$$\mathrm{CaCO_3 + CO_2 + H_2O \leftrightarrow Ca(HCO_3)_2}, \quad (8.9)$$

which solution has slightly alkaline pH because carbonic acid is weak:

$$\mathrm{Ca(HCO_3)_2 + H_2O \leftrightarrow Ca^{2+} + 2H_2CO_3 + 2\,OH^-}. \quad (8.10)$$

After decay of calcium bicarbonate, the solution becomes slightly acidic due to hydrolysis of carbonic acid

$$Ca(HCO_3)_2 \leftrightarrow CaCO_3 \downarrow + CO_2 \uparrow + H_2O, \tag{8.11}$$

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3. \tag{8.12}$$

To shift the equilibrium to increase precipitation of $CaCO_3$ the drop of partial pressure of $CO_2$ can be used.

Due to equilibria in the Eq. 8.7 concentration of calcium bicarbonate solution produced from $CaCO_3$ and $CO_2$ is very low, it was on the levels of 22 mM at atmospheric pressure of $CO_2$ 30 atm (Fig. 8.14) and only 10 mM at atmospheric pressure.

The attempts to concentrate calcium bicarbonate solution using reverse osmosis (RO) were unsuccessful because of decay of calcium bicarbonate during membrane concentration. Only one successful way to diminish the rate of calcium bicarbonate decay was an addition of the calcium ions chelators in concentrations lower than stoichiometrical ratios for calcium ions chelating. In this case it was possible to produce calcium bicarbonate solution from solutions of $CaCl_2$, $NaHCO_3$ and calcium chelator, for example EDTA, at concentration up to 500 mM of dissolved $Ca^{2+}$. This solution decayed at atmospheric pressure for 1–24 h depending on the molar ratio between calcium ions and calcium ions chelator.

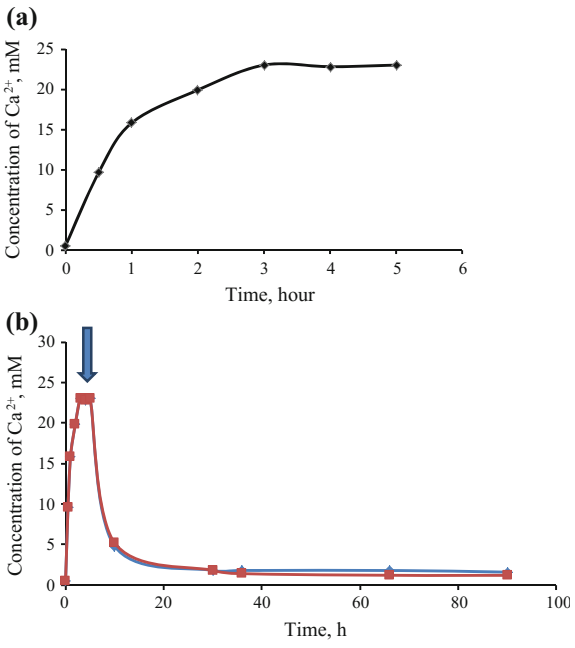



**Fig. 8.14** Production (**a**, **b**) of calcium bicarbonate solution from $CaCO_3$ to $CO_2$ at 30 atm pressure and decay of calcium bicarbonate (**b**) to $CaCO_3$ and $CO_2$ at atmospheric pressure under shaking for removal of $CO_2$ from solution. *Arrow* shows placement of solution under atmospheric pressure and beginning of shaking to remove $CO_2$ to atmosphere

## 8.23 Effect of Partial MICP on Calcium Bicarbonate Decay

To stimulate decay of calcium bicarbonate (Eq. 8.7) could be used either for removal of $CO_2$ by shanking, drying, drop of pressure, or one-time increase of pH because after initiation of decay, supply of hydroxide ions is performed by the reaction of decay.

Technologically, this one-time increase of pH can be made by enzymatic hydrolysis of urea by urease-producing bacteria. In our experiments with the repeated 24 h treatments of sand with suspension of urease-producing bacteria, solution of urea and calcium bicarbonate at molar ratio of urea:Ca = 0.1 or 0.2 the concentration of dissolved Ca in effluent decreased from 20 to 22 mM to 4 or 2 mM, respectively, but it was not decay of calcium bicarbonate without addition of urea. Initial pH of solutions was 7.7 but after treatment with urea or without urea it was 8.2 or 6.8, respectively. This type of the clogging can decrease quantity of produced ammonium/ammonia to 15–20 times because molar ratio of urea:Ca in conventional MICP is 1.5–2.0, while in decay of calcium bicarbonate it is just 0.1–0.2.

Probably, the function of urease-producing bacteria is not only one-time increase of pH, but also the formation of the centers of crystallization because even addition of the crystals of $CaCO_3$ to the solution of calcium bicarbonate also increased its rate and efficiency of decay (Fig. 8.15).

## 8.24 Bioclogging of the Fissured Rocks with Calcium Bicarbonate Solution

Bioclogging of the porous materials using calcium bicarbonate solution is not practical due to low concentration of calcium. For example, required number of the treatments of sand with porosity about 50 % (v/v) to create effect of bioclogging similar of 2 M of $Ca^{2+}$ in conventional MICP is 100–200. However, it could be

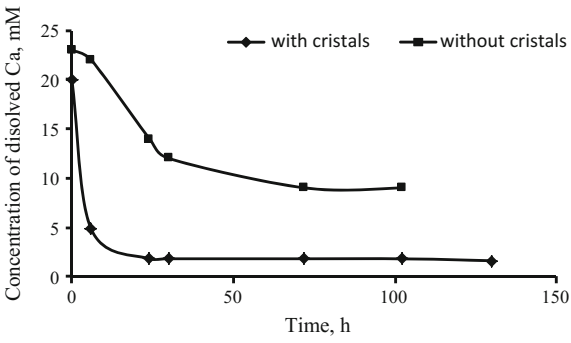



**Fig. 8.15** Decay of calcium bicarbonate under the presence or absence of the calcite crystals

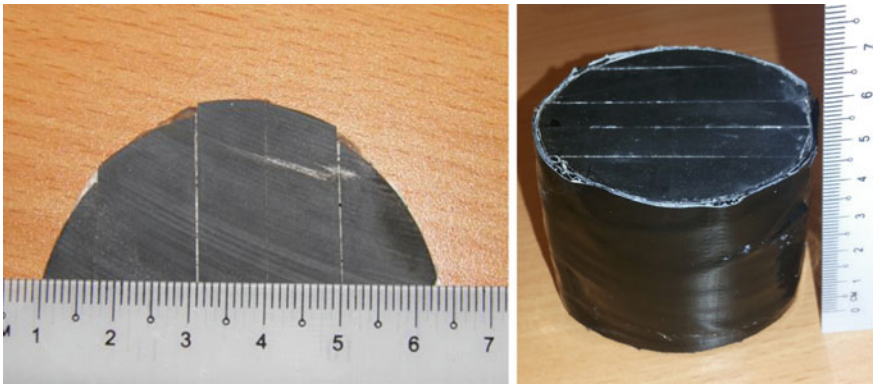


**Fig. 8.16** Sample of the clogged basalt rock with four fissures

suitable for the clogging of materials with low porosity, for example for the clogging of the fractured rocks.

In our experiments, the samples of the rocks were the basalt and granite cylinders, 5 cm diameter and 5 cm length cut along axis (Fig. 8.16) with the tape 2 mm on two alongside edges of the cuts.

Solution of calcium bicarbonate produced from $CaCO_3$ to $CO_2$ under a pressure 30 bars was used to fill in cuts in one rock specimens as shown in Fig. 8.16. Before the treatment, the water flow through the rock sample with 4 cuts was 12 L/h. After five treatments, water flow stopped (under 10 cm water head). The crystals of the clogging precipitate were tightly connected to the surface of rock fissure (Fig. 8.17).

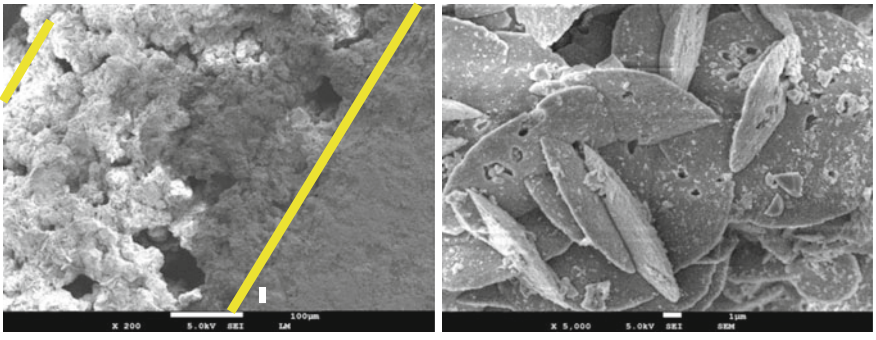


**Fig. 8.17** Connection between clogging material and rock surface (*left image*) and crystals of clogging material (*right image*). *Lines* show the surface of the rock sheets

**Fig. 8.18** Bioclogging of a cut in granite rock using solution of $Ca(HCO_3)_2$. *Left image* shows cut specimen before treatment, and *right image* shows a specimen after bioclogging

Another bioclogging test of the fissured granite rocks (Fig. 8.18) was done with five batch treatments and with use of the dead but urease-active cells of *Yaniella* sp. VS8 suspension under 10 cm water head. Before treatment, the water flow through the granite rock sample with one cut was 3–4 L/h. After five treatments, water flow stopped.

Precipitation of $CaCO_3$ from calcium bicarbonate solution in interspace 196 ± 18 μm between the granite plates 0.4 m × 0.4 m, with a portion of sand between them, using continuous supply of calcium bicarbonate solution, suspension of dead but urease-active cells of *Yaniella* sp. VS8, and urea at molar ratio of urea: Ca = 0.2–0.5 showed that at pH 7.4–8.1 concentration of $Ca^{2+}$ in effluent dropped from 20 to 22 mM to 5–7 mM, while at pH 8.4–8.7 it was dropped to 2–4 mM. Contents of $CaCO_3$ precipitate for the bottom and ceiling plates were 35 ± 14 and 13 ± 0.5 g $CaCO_3/m^{2.}$

## 8.25 Delayed Calcium Bicarbonate Decay

It is known that disodium ethylenediaminetetraacetate dihydrate, $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, ($Na_2EDTA$), forms dissociated complexes using a particular pH with calcium ions. It is widely used to dissolve limescale, which has calcium carbonate as a main component. After being bound by EDTA, metal ions remain in solution but exhibit diminished reactivity. We found that addition of calcium chelator, for example EDTA, significantly delayed decay of calcium bicarbonate (Fig. 8.19).

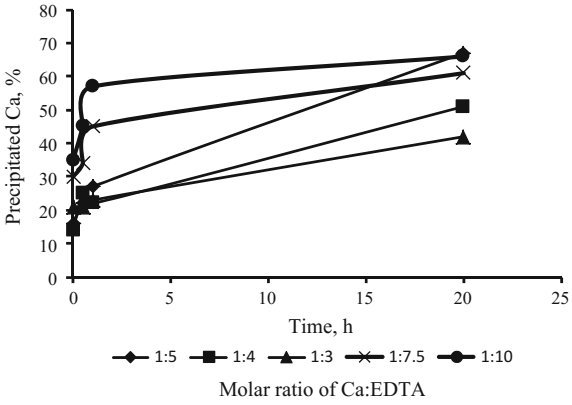
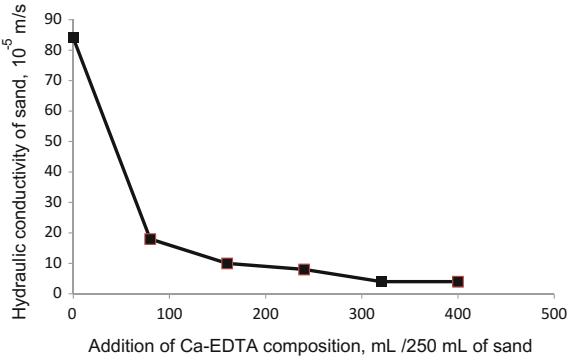



**Fig. 8.19** Quantity of precipitated calcium, % from added. Molar ratio of $CaCl_2$ and $Na_2$ EDTA was changed from 1:5 to 1:10. It was no precipitation at molar ratio 1:1 and 1:2. Solution for precipitation was produced by the mixing of 1 volume of 1 M $CaCl_2$ and 0.2 M EDTA and after pH adjustment to neutral with 1 volume of 1M $NaHCO_3$. So, initial concentration of $Ca^{2+}$ was 500 mM, which is 25 times bigger than concentration of calcium bicarbonate produced without addition of EDTA

This delayed decay could be used for the practical applications for the clogging of sand (Fig. 8.20) or fractured rocks (Fig. 8.21). For example, initial seepage through the cracked stone (Fig. 8.21) was 250 mL $min^{-1}$ but after clogging it was decreased to 0.11 mL/$min^{-1}$.

This decay, same as the calcium bicarbonate decay, is accelerated by one-time increase of pH due to addition of urease-producing bacteria and urea at low molar ratios of urea: $Ca^{2+}$ = 0.2–0.5.

**Fig. 8.20** Clogging of sand with Ca-EDTA composition



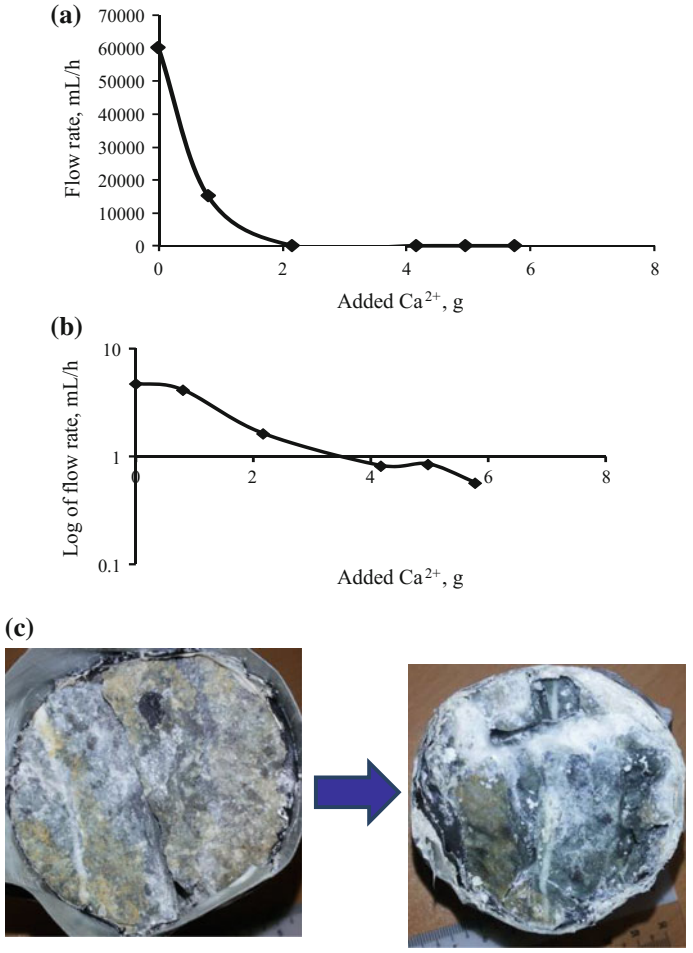



**Fig. 8.21** Influence of added calcium of the hydraulic conductivity of cracked stone. **a** linear scale, **b** logarithmic scale, **c** view of cracked rock before and after clogging

## 8.26 Microbially Mediated Precipitation of Iron Minerals

Disadvantage of MICP biogrouting is an increase of pH in soil to 8.5–9.0. To avoid this problem, another type of biogrouting, iron-based biogrout, was proposed (Ivanov and Chu 2008) and studied (Ivanov et al. 2014a, b; Stabnikov et al. 2016). Iron-based biogrout contained ferric chelate, urea, and urease-producing bacteria for the precipitation of ferric hydroxide can diminish the hydraulic conductivity of sand from $5 \times 10^{-5}$ $ms^{-1}$ to $1.4 \times 10^{-7}$ $ms^{-1}$ at the content of precipitated metals on the level about 2 % (w/w) (Ivanov et al. 2014a).

However, iron chelates are not applicable in large-scale construction, geotechnical, or environmental works because of their high cost. So, it was proposed to produce iron-based biogrout from iron ore, which is a cheap commodity, and cellulose, which is a component of cellulose-containing agricultural or solid municipal wastes. Ferric oxide, $Fe_2O_3$, is the major component of hematite iron ore and lateritic red topical soils. Fe(III) of iron oxide can be reduced by iron-reducing bacteria to dissolved ferrous ions or colloidal ferrous hydroxides using hydrogen or organic carbon under strictly anaerobic conditions (Lovley 2000; Francis et al. 2000). This dissolved ferrous, which is produced by iron-reducing bacteria, can be used in different chemical or environmental engineering applications (Ivanov et al. 2014b). The applications of microbial reduction of iron in the treatment of fat-containing wastewater (Ivanov et al. 2002), removal of phosphate during anaerobic digestion of activated sludge (Stabnikov et al. 2004) or from reject water (Guo et al. 2009; Ivanov et al. 2005, 2009), the combination of microbial reduction and microbial oxidation of iron for the treatment of food-processing wastewater (Ivanov et al. 2004; Stabnikova et al. 2010), treatment of sucralose production wastewater and many other applications (Ivanov et al. 2014b) were reported. The source of iron oxide could be hematite iron mine tailings, which are voluminous wastes that occupy land and pollute air with their dust. For example, the Godli mine (India) generates 0.6 million tons of tailings per year with an average grade of 50 % Fe. Magnetic fraction of tailing contains more than 62 % of Fe, mostly hematite. Distribution by size of hematite granules in this fraction is as follows: 64.6 % less than 12 μm; 27.4 % from 12 to 35 μm; 8 % from 35 to 45 μm (Brown et al. 2002). Totally, tailings from iron primary extraction processes only in India are estimated as 11 million tons per year (Pappua et al. 2007). As an inexpensive raw material iron ore tailings were proposed to be used as value-added product in some technologies, for example, in the production of ceramics (Wagh et al. 2002; Liu e al. 2010), in civil engineering constructions for road construction, building materials, and brick making (Yellishetty et al. 2008) or in the pigments production (Hedin 2003) but an effective way could be also production of biogrout as shown in this paper.

## 8.27 Anaerobic Bioproduction of Dissolved Fe(II)

Practical application of iron-reducing bacteria for iron ore reduction must be with cheap electron donor. Organic wastes can be such donors to produce dissolved ferrous salts of organic acids during acidogenic fermentation. Acidogenic fermentation is widely used in biotechnology to convert organic wastes into volatile fatty acids (VFA), mainly acetate (up to 58 % of total VFA), and in particular propionate, butyrate, valerate, and lactate (Arroja et al. 2012; Bengtsson et al. 2008; Traverso et al. 2000). Organic matter is degraded to VFA by hydrolytic and acidogenic bacteria in an acidogenic reactor. There are many bacterial species able to hydrolyse polysaccharides, including cellulose and hemicellulose, and then to

ferment them to organic acids, hydrogen, and carbon dioxide as the end products. Most typical material balance of this process can be shown by the following equation of acidogenic fermentation in the rumen (molar ratios of volatile fatty acids (VFA) were taken from Madigan et al. 2014):

$$\begin{aligned} C_6H_{12}O_6 + 0.82\ H_2O \rightarrow 1.13CH_3COOH\ + 0.35C_2H_5COOH \\ + 0.26\ C_3H_7COOH + 1.67CO_2 + 2.47\ H_2, \end{aligned} \tag{8.13}$$

where $C_6H_{12}O_6$ is a monomer of cellulose, $CH_3COOH$, $C_2H_5COOH$, and $C_3H_7COOH$ are acetic, propionic, and butyric acids, respectively.

In the presence calcium carbonate for neutralization of acids, iron ore, and iron-reducing bacteria these VFA can be used for bioreduction of Fe(III)-producing soluble ferric and ferrous salts and soluble calcium ions. For example, bioreduction of acetic, propionic, butyric, and lactic acids can be shown by the following equations, respectively

$$CH_3COOH + 8\,Fe^{3+} + 2\,H_2O \rightarrow 8\ Fe^{2+} + 4\,CO_2 + 8H^+, \tag{8.14}$$

$$C_2H_5COOH + 14\,Fe^{3+} + 4\,H_2O \rightarrow 14\,Fe^{2+} + 3\,CO_2 + 14\,H^+, \tag{8.15}$$

$$C_3H_7COOH + 20\,Fe^{3+} + 6\,H_2O \rightarrow 20\,Fe^{2+} + 4\,CO_2 + 20\,H^+, \tag{8.16}$$

$$C_2H_5OCOOH + 12\,Fe^{3+} + 3\,H_2O \rightarrow 12\,Fe^{2+} + 3\,CO_2 + 12\,H^+. \tag{8.17}$$

Transfer of produced solution to aerobic conditions and oxidation of $Fe^{2+}$ to $Fe^{3+}$ there can remove excessive protons

$$4\,Fe^{2+} + O_2 + 4\,H^+ \rightarrow 4\,Fe^{3+} + 2H_2O, \tag{8.18}$$

but, additional acidification could be needed due to formation and precipitation of ferrous and ferric hydroxides

$$Fe^{2+} + 2\ H_2O \rightarrow Fe(OH)_2\downarrow\ + 4H^+. \tag{8.19}$$

$$Fe^{3+} + 3\ H_2O \rightarrow Fe(OH)_3\downarrow\ + 6H^+. \tag{8.20}$$

Therefore, both reduction of $Fe^{3+}$ in anaerobic medium and formation of iron hydroxides require neutralization of pH. This could be done by two ways: (1) addition of $CaCO_3$, which produces soluble ions of $Ca^{2+}$ as shown in Eq. 8.21; (2) addition of urea and urease-producing bacteria that produce hydroxide ions according to the Eq. 8.22

$$CaCO_3 + 2H + \rightarrow Ca^{2+} + CO_2 + H_2O, \tag{8.21}$$

$$(NH_2)_2CO + 3\ H_2O \rightarrow 2NH_4^+ + CO_2 + 2\,OH^-. \tag{8.22}$$

## 8.28 Kinetics and Stoichiometry of Ferrous Bioproduction from Iron Ore

Iron-reducing bacteria are slowly growing bacteria with growth rate in the range from 0.01 to 0.09 $h^{-1}$ (Esteve-Núñez et al. 2005; Park and Kim 2001) and their enrichment and pure cultures produce Fe(II) from iron ore with the average rates from 20 to 50 g $Fe^{2+}$ $m^{-3}$ $d^{-1}$ (Stabnikov et al. 2016). Usually, concentration of Fe (II) is in the range from 100 to 500 mg (Fe(II) mg/L (Kostka et al. 2002; Park and Kim 2001; Stabnikov et al. 2016). There are known halotolerant iron-reducing bacteria (Pollock et al. 2007; Ye et al. 2004). In our experiments, an inoculum from the sediment of the Dead Sea shore on the side of Jordan, produced enrichment culture of IRB that was able to reduce iron ore at such high salinity as 20 % of sea salt. However, the rate of Fe(II) production decreased with the increase of salinity from 5 to 20 % (Stabnikov et al. 2016).

Anaerobic neutral solutions of ferrous acetate and ferrous lactate produced during anaerobic acidogenic fermentation of cellulose or lactic acid fermentations of sugars were suitable for oxidation of Fe(II) and precipitation of ferric hydroxide (s) at pH above 8.5, which can be created by the enzymatic hydrolysis of urea.

The addition of sand to the medium for fermenting and iron-reducing bacteria helped to avoid convectional transfer of oxygen into this medium and ensured formation of anaerobic conditions in the depth of sand with the medium. For example, beach sand, 350 g; iron ore, 30 g; tap water, 600 mL; glucose, 1 g; anaerobic sludge, 50 mL, were mixed in the glass bottles. The bottles were flushed with nitrogen gas, sealed and placed on the shaker at 150 rpm at 32 °C for 10 days. The changes of the oxidation reduction potential (ORP) and concentration of Fe(II) during 10 days of anaerobic cultivation was from +60 mV to −171 mV and from 0.5 to 136 mg $Fe^{2+}$ $L^{-1}$. The anaerobic conditions were established in the medium mixed with sand on the second day of cultivation.

Production of ferrous ions by iron-reducing bacteria of EC from iron ore using cellulose as a source of carbon was studied in experiments on simultaneous acidogenic fermentation and bioreduction of Fe(III) with different initial contents of cellulose and iron ore. The final Fe(II) concentrations in abiotic controls ranged from 0.07 to 0.41 mg/L. Maximum concentrations of Fe(II) in media with different concentrations of iron ore (2, 6 and 10 g/L) and cellulose (2, 6 and 10 g/L) are shown in Fig. 8.22. Concentration of Fe(II) did not depend on the concentration of cellulose in media but increased with the increasing of iron ore concentration, i.e., with an increase of the surface of the iron ore particles. Probably, it is a sign that the rate of Fe(III) bioreduction depends mainly on the surface area of iron ore particles.

The hydraulic conductivity test for sand clogged by the grout produced from 2 g/L of iron ore and 5 g/L of cellulose and final Fe(II) concentration of 712 mg/L had hydraulic conductivity of $1.6 \cdot 10^{-5}$ $ms^{-1}$ in comparison with the hydraulic conductivity in abiogenic control of $1.1 \times 10^{-4}$ $ms^{-1}$. Unconsolidated undrained triaxial test was carried out on the dry treated sand samples. The maximum deviator stress was 124 kPa for the sample treated by the grout with concentration of Fe(II)

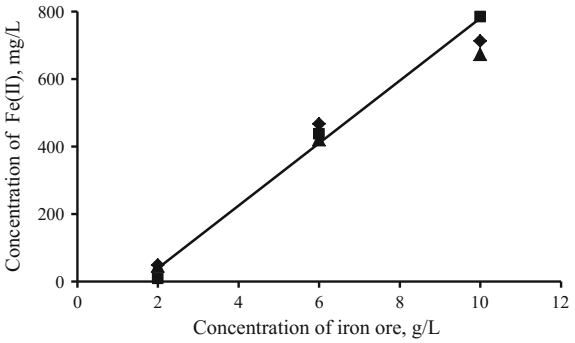



**Fig. 8.22** Production of Fe (II) in experiments with different concentrations of iron ore at following concentrations of cellulose: 2 g/L (*filled diamonds*); 6 g/L (*filled square*); 10 g/L (*filled triangle*)

of 712 mg/L. So, even one-time treatment of sand with the grout containing soluble Fe(II) decreased hydraulic conductivity of sand and increased strength of the treated sand samples. However, the content of Fe in the treated sand was low, about 0.04 % Fe (w/w). Therefore, continuous supply of the iron-containing grout was tested.

## 8.29 Combined Application of Urease-Producing Bacteria and Iron-Reducing Bacteria for the Continuous Biogrouting of Porous Soil

After 30 days of the treatment, the hydraulic conductivity of sand decreased from $7 \times 10^{-4}$ ms$^{-1}$ to $1.4 \times 10^{-6}$ ms$^{-1}$, i.e., 500 times. The average concentrations of dissolved iron in effluent after tank and after column for biogrouting were 260 and 2 mg/L, respectively, i.e., 99 % of entering to the column iron were precipitated there. The content of Fe in biocemented sand was 4.5 % (w/w). The pores of sand were filled with brown ferric hydroxide (Fig. 8.23).

SEM shows that precipitate accumulated in the channels between sand grains and partially in the pores (Fig. 8.24). XRD of the material precipitated in sand shows that this precipitate is a mixture of hematite and calcite (Stabnikov et al. 2016).

The clogging with iron hydroxide did not increase the unconfined compressive strengths of the treated sand and gravel significantly. Therefore, major geotechnical application of iron-based biogrout produced from iron ore could be bioclogging of the porous soils for the sealing of aquaculture or wastewater treatment ponds, the landfill sites, for the plugging of the piping in dams and retaining walls in sandy soil. Therefore, major geotechnical applications of iron-based biogrouts could be bioclogging of the porous soils for the sealing of aquaculture or wastewater treatment ponds, the landfill sites, and for the plugging of the piping in dams and retaining walls in sandy soil, for the reduction of hydraulic conductivity of the fractured or clastic sedimentary rocks before the dam or tunnel constructions or mine development.

**Fig. 8.23** Sand after bioclogging with iron-based biogrout

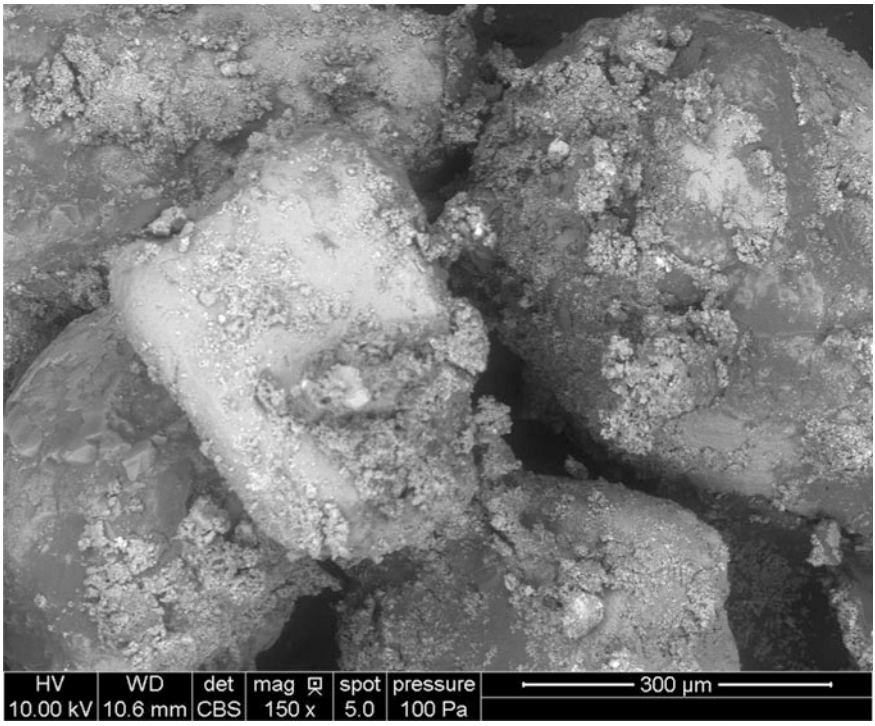



**Fig. 8.24** SEM showing precipitation in the channels between sand grains

## 8.30 Bioclogging on the Geochemical Barriers

Vertical density gradients of oxygen and other substances are developed in soil in the mm, cm, or in meter scales. Geochemical stratification and formation of microzones could be mainly due to content of water of the layer or in microzone of soil. Roughly, there are anaerobic, anoxic, aerobic, and photosynthetic layers and zones in soil systems. Strong changes of the conditions and concentrations on the boundary of these zones can cause relative biogeochemical activity and precipitation of different substances on the boundaries, which will cause clogging of the layers, the channels, or microzones of soil.

The anaerobic microorganisms in anaerobic zones produce organic acids, $CH_4$, $NH_3$, $H_2S$, $Fe^{2+}$, $N_2$. The reduced substances migrate to the anoxic or microaerophilic zones, where they are used as electron donors by anoxic and microaerophilic microorganisms, that are oxidizing these donors using products of oxidation—$NO_3^-$, $Fe^{3+}$, $SO_4^{2-}$—which are diffused from aerobic zones. The reduced substances migrate also to the aerobic and phototrophic zones, where they are used as electron donors by phototrophic and chemotrophic aerobic microorganisms. In the aerobic photosynthetic zone, $CO_2$ is reduced to organic matter using the energy of light.

Therefore, most effective way to produce a sealing layer or boundary in soil is formation of geochemical boundary, where the precipitation of the sealing substances will be facilitated by geochemical activity of microorganisms.

## 8.31 Two Different Kinetics of Bioclogging

Analysis of the bioclogging kinetics (Fig. 8.25) showed that there are existing two types of bioclogging: (1) causing slow decrease but then fast drop of hydraulic conductivity; (2) causing permanent decrease of hydraulic conductivity with almost constant rate.

First type is conventionally described as two-stages clogging and is explained by formation of the narrow channels at the first stage and clogging of these narrow channels on the second stage

$$\begin{aligned} dY/dt &= -k_1 X \quad \text{when } X < X_1 \\ dY/dt &= -k_2 X \quad \text{when } X > X_1, \end{aligned}$$

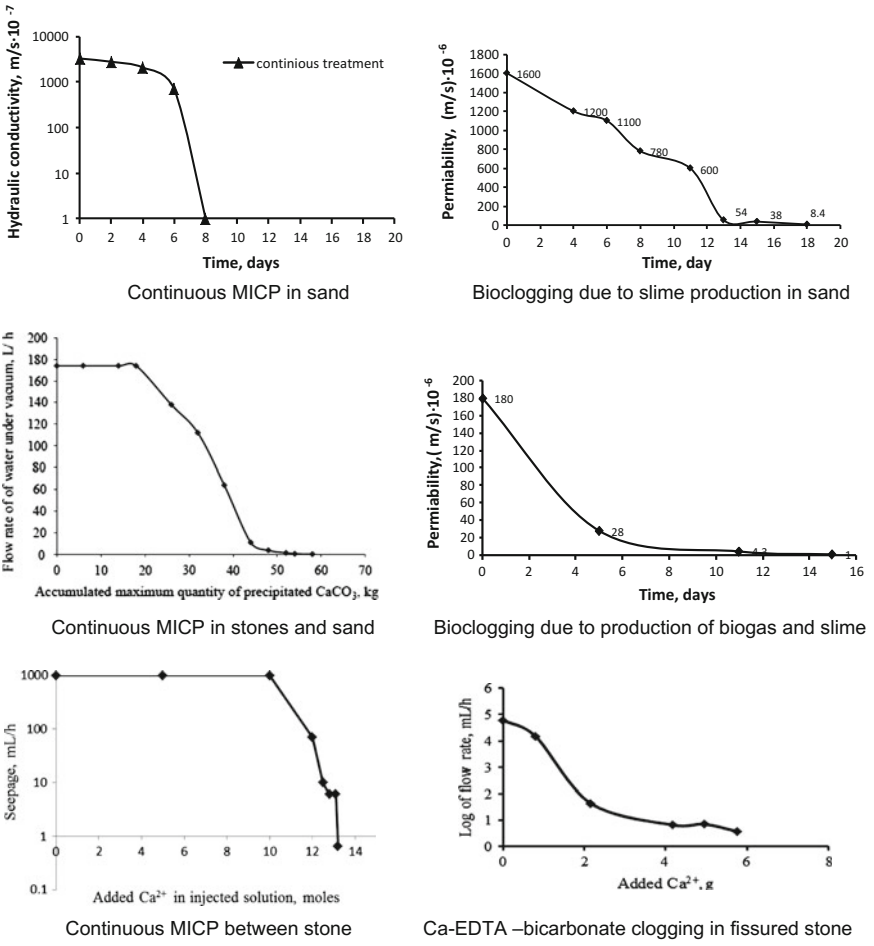



**Fig. 8.25** Two different kinetics of bioclogging

where $Y$ is hydraulic conductivity, $X$ is the content of clogging material, $k_1$ and $k_2$ are different rates.

Second type is described as one-stage clogging and explained as the filling of the voids with amorphous clogging material

$$dY/dt = -kX$$

Data on these two quite different types of bioclogging are given in Fig. 8.26.

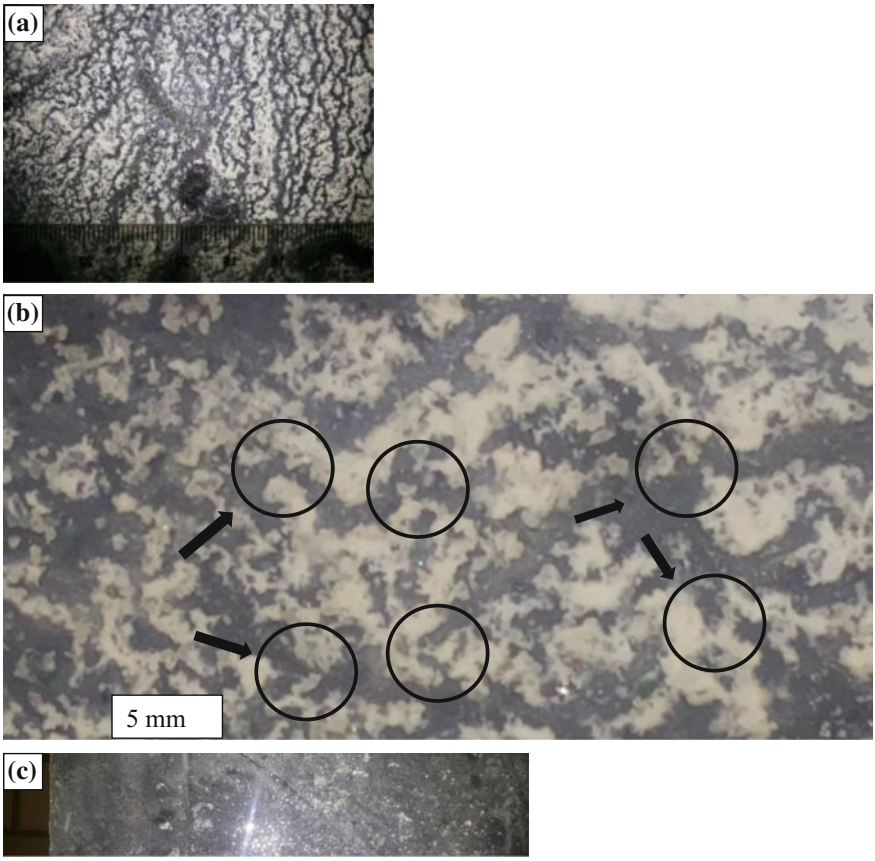



**Fig. 8.26** The mechanisms of two types of bioclogging. **a** formation of microchannels (1st stage); **b** their clogging (2nd stage); **c** precipitation of the clogging material on surface without formation of the microchannels

## 8.32 Comparison of the Biogrouting Methods

Comparison of the biogrouting methods is given in Table 8.3.

## 8.33 Development of the Biogrout

Notwithstanding the differences in geochemistry and microbiology of the biogrouts, the development of the biogrout follows through the same stages (Fig. 8.27). It is clear that for the different applications and conditions of applications there must be

**Table 8.3** Comparison of the biogrouting methods

| Biogrouting method | Advantages | Disadvantages | Practical applicability |
|---|---|---|---|
| Bioclogging using live or dead cells of urease-producing bacteria, calcium salt, and urea | (1) Strength up to 1 MPa<br>(2) Low cost | (1) $NH_3$ release<br>(2) Corrosive pH at 9.0–9.5<br>(3) Required multiple (5–10) treatments | Acceptable in the field if (1) dead cells are used; (2) pH and ammonia/ammonium concentration in air and groundwater is not a problem |
| Bioclogging using slime-producing bacteria | (1) Low cost<br>(2) No release of ammonia<br>3) No corrosive pH | (1) Slow clogging<br>(2) Expensive grout<br>(3) Hydraulic conductivity above $10^{-6}$ m/s<br>(4) Potentially instable | Applicable for the cases when carbohydrate wastes are and present and final conductivity can be higher $10^{-6}$ $ms^{-1}$ |
| Bioclogging using denitrification of calcium and nitrate salts | (1) One step of treatment<br>(2) No ammonia release<br>(3) No corrosive pH | (1) Slow clogging<br>(2) Expensive grout<br>(3) Low concentration of $Ca^{2+}$<br>(4) Required multiple treatments depending on porosity<br>(5) Production of $H_2S$ after denitrification | Not recommended for practical applications |
| Bioclogging using sequential denitrification and biocementation with dead UPB | (1) Low cost<br>(2) Low release of ammonia/ammonium | (1) Slow clogging<br>(2) Two steps of the grouting | Can be applicable for high porosity, water-saturated ground |
| Bioclogging of the fissured rocks with calcium bicarbonate and dead cells of UPB | (1) Can be produced from limestone and waste $CO_2$<br>(2) Sequestration of $CO_2$<br>(3) Low ammonia release<br>(3) No corrosive pH | (1) Needs supply of $CO_2$<br>(2) Low concentration of $Ca^{2+}$ so required multiple treatments depending on porosity | Applicable for the fractured rocks of low porosity, in case when the sources of $CO_2$ and limestone powder are present |
| Bioclogging using aerobic oxidation of calcium salt of organic acid (formate, acetate, lactate) | (1) One step of treatment | (1) Slow clogging<br>(2) Even distribution of air is required for even clogging | Can be used only for the cases where even distribution of oxygen supply is available, i.e., only for the surface treatment |
| Bioclogging using delayed decay of Ca-chelate | (1) One step of treatment<br>(2) Low ammonia release<br>(3) No corrosive pH | (1) Relatively expensive grout<br>(2) Production of waste precipitate | Applicable, if the cost of calcium chelate and sodium bicarbonate is acceptable |

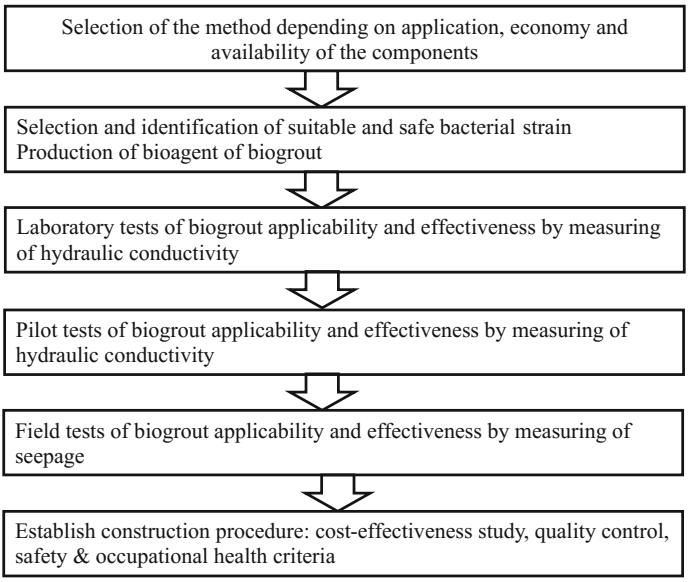



**Fig. 8.27** Development of biogrout

specific product. So, a set of the biogrouts must be developed. Additionally, very often a combination or a sequence of several processes\biogrouts must be used for better results.

# Chapter 9
# Soil Surface Biotreatment

## 9.1 Wind Erosion of Soil and Dust Emission

Wind erosion of fertile soil, the dune movement in sand deserts, the dust storms in arid and semi-arid regions, as well as the emission and dispersion of agricultural, construction, transportation, or mining dusts create a lot of problems and dangers for human life, environment, and infrastructure. Dust is also a carrying agent for soil-associated pollutants such as chemicals, viruses, and microorganisms. So, the release of the polluted dust in atmosphere can cause the deposition of these pollutants to ecosystems located downwind and negatively affect human health and environment (Boreson et al. 2004; Falkovich et al. 2004; Griffin et al. 2007; Raisi et al. 2010). Dust storms originated in desert areas effect many countries in Middle and East Asia and North Africa (Lee et al. 2006; Ci and Yang 2010).

## 9.2 Dust Control Technologies

There are known many biological, chemical, and mechanical technologies to prevent and control wind erosion of soil, wind dispersion of dust, desertification due to wind-blown sand movement as well as the release of dust from agriculture, construction, or transportation on unpaved roads. One conventional but limited by agricultural conditions way is the use of vegetation as the land surface cover to reduce wind erosion of soil (Ci and Yang 2010). Wind erosion of soil and dust formation could be prevented by the compaction of the soil surface layer using treatment with inorganic compounds such as sodium chloride, calcium chloride, magnesium chloride, lime, fly ash (Petry and Little 2002; Santoni et al. 2005; Goodrich and Jacobi 2012). Many patents have been issued on the methods to control wind erosion of soil and dust suppression. However, application of binding reagents could negatively affect plants (Goodrich and Jacobi 2012) and quality of



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Green Energy and Technology, DOI 10.1007/978-981-10-1445-1_9

surface and underground waters due to the solubility of the reagents in water and their dispersion in environment with runoff.

Another approach to suppress dust dispersion is the use of organic compounds such as an asphalt emulsion (Santoni et al. 2005), lignin and lignosuphonates (Gargulak and Lebo 1999), surfactants (Copeland et al. 2009), fly ash and polyacrylamide (Yang and Tang 2012), starch ethers, polyacrylonitrile, polyvinyl alcohol, urea–formaldehyde and their copolymers, polyacrylamide copolymers, carboxymethyl cellulose, polyvinyl acetate, epoxyresins, and many others (Nij et al. 2003; Santoni et al. 2005; US Army 2005; Orts et al. 2007; Yang et al. 2007a, b; Naemi and Ghorbanalizadeh 2010; Zandieh and Yasrobi 2010). These dust suppressors change the physical properties of the soil surface by wetting or binding the fine particles and are used in construction industry, mining, at the loading/unloading points, transportation on unpaved roads, and using of airfields. However, organic fixing reagents are usually relatively expensive for large scale application and could be environmentally harmful due to their toxicity or ability to cause eutrophication of water.

## 9.3 Biotechnological Methods for Dust and Wind Erosion Control

Alternative way for the dust suppression with chemical reagents can be an application of bio-mediated aggregation of the soil particles (Ivanov and Chu 2008; Bang et al. 2011; Chu et al. 2012a, b; DeJong et al. 2010, 2011, 2013). Bioaggregation of soil particles increases the size of soil aggregates so that soil erosion and dust emission will be reduced. The most popular technology of bioaggregation is crystallization of calcium carbonate minerals from the solution of calcium salt and urea mediated by enzyme urease or biomass of urease-producing bacteria (Ivanov 2010). Chemical precipitation of calcium carbonate in sand did not produce effect of sand grains aggregation (Chu et al. 2012b).

Even small level of sand dust bioaggregation can prevent its dispersion. There is known experimental attempts to study the effects of biotreatment on sand immobilization (Bang et al. 2011), but the published results are not applicable for sand dust suppression because of the following reasons: (1) the object of study was not fine sand or sand dust but big sand particles with the sizes from 0.075 to 4.76 mm; (2) the effect of biocementation on the sand dust formation in the wind tunnel was studied only by the mass losses of sand; (3) the content of precipitated calcium in sand was not measured; (4) the effects of some biotreatments were measured after simultaneous application of urease-producing bacteria, calcium chloride, and urea to sand, which can cause chemical precipitation of calcium carbonate due to the high pH of medium after cultivation of urease-producing bacteria.

## 9.4 Biotechnological Control of Air-Born Movement of Sand Dust and Dust-Associated Chemical and Bacteriological Pollutants

One study examined the effects of small dosage, calcium-based bio-mediated aggregation of fine sand on the air-born movement of sand dust, and dust-associated chemical and bacteriological pollutants (Stabnikov et al. 2013a, b). The bioaggregation reagent was a solution of calcium chloride and urea sprayed over the sand surface, which was preliminary treated with the suspension of urease-producing bacteria. Quantity of calcium used for the sand dust suppression was 15.6 g Ca/$m^2$. After biotreatment of the fine sand, the release of the sand dust and its artificial pollutants to atmosphere decreased in comparison with control by 99.8 % for dust, 92.7 % for phenantherene, 94.4 % for led nitrate, and 99.8 % for bacterial cells of *Bacillus megaterium.* This immobilization of dust and dust pollutants was due to bioaggregation of the fine sand particles. The sizes of 90 % of the sand dust particles increased from 29 μm in control to 181 μm after bioaggregation. Bioaggregation treatment of the soil surface could be useful method to prevent the dispersion of dust and the dust-associated chemical and bacteriological pollutants in water, air, and soil (Stabnikov et al. 2013a, b).

## 9.5 Formation of Soil Crust by Filamentous and Photosynthetic Microorganisms

The soil surface crust is created due to physical or biological (biotic) processes. Abiotic formation of soil crust is due to raindrops or run off breaking soil aggregates, then physical redeposition of fine soil particles, clogging soil pores with these particles, and drying these particles on soil surface forming sealing crust where salts, lime, and silica are also deposited at surface as water evaporates (Jeffery et al. 2010; Belnap et al. 2001). Physical crust of soil is often impenetrable for germinating seeds of plants. Soils especially susceptible to physical crusting are those with low content of organic matter and high content of silt (Belnap et al. 2001).

Biotic crusting is due to filamentous and slime-producing microorganisms binding the soil particles together. Microbial crust at soil surface is formed mainly by such phototrophic microorganisms as cyanobacteria and algae alone or in symbiosis with mycelial fungi, which are chemotrophic organisms consuming organic compounds (Fig. 9.1).

In last case, the phototrophic and chemotrophic microorganisms form symbiotic organisms called lichens, where the major function of the fungal component is to extract inorganic nutrients from soil, while the photosynthetic cyanobacteria or algae are producing photosynthetic organic compounds, which are used also by

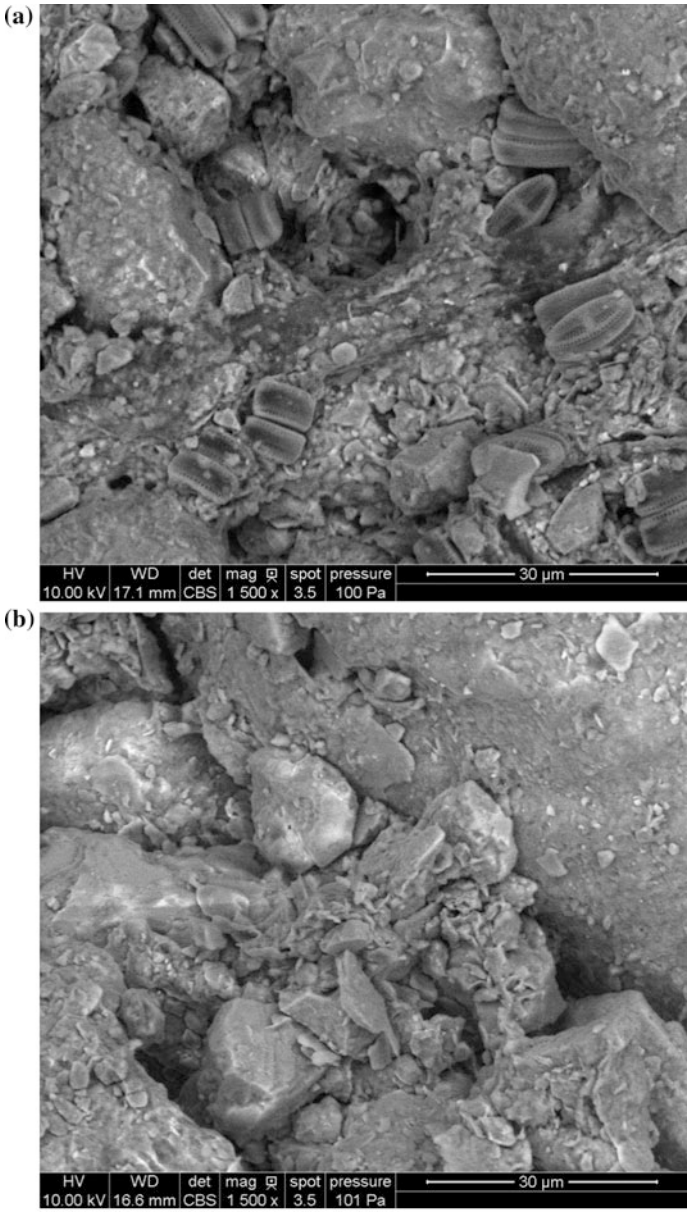



**Fig. 9.1** Surface of soil crust with algae (**a**) and profile of soil crust produced by deposition of fine particles between bigger sand grains (**b**)

fungi. Filamentous fungi and cyanobacteria weave through soil particles gluing them together (Belnap et al. 2001, 2003). Microbially formed crust creates and maintain fertility of desert soils fixing carbon and nitrogen from air and capture

nutrient-rich dust, but they are vulnerable to climate change and grazing livestock and their recovery is very long (Belnap 2003). So, artificial inoculation of soil surface by cyanobacteria could be useful for the formation or recovery of desert soil crust.

## 9.6 Functions of the Soil Crust

One of the most important roles of the soil crust is stabilization of the soil surface and its protection from erosion. The microbiotic crust at soil surface could be of a few mm thickness, but this small structure in hyper-arid regions diminishes water infiltration rate, the rates of water and wind erosion of soil, increases runoff and enhanced the slopes stability (Li et al. 2002; Auzet et al. 2004; Belnap et al. 2001; Belnap 2003, 2006; Jeffery et al. 2010). However, biological soil crusts in semi-arid cool and cold drylands can increase infiltration and reduce runoff (Belnap 2006).

The microbial crust can create the favorable conditions for the growth of plants contributing to soil fertility due to water retention, capturing nutrient-rich dust, biological nitrogen fixation from air, extraction of phosphates, and other nutrients from soil particles (Belnap et al. 2001; Belnap 2003, 2006; Jeffery et al. 2010) or decrease the soil fertility due to reduction of infiltration rate. Annual cyanobacterial fixation of atmospheric nitrogen in biological soil crusts can be up to 365 kg N/ha (cited from Belnap et al. 2001), while optimum dose of fertilizer for wheat or rice is between 40 and 125 kg N/ha.

Microbial crust affects not only hydrology and soil properties but also ecology of terrestrial systems. For example, development of microbial crust changed desert vegetation from shrubs to herbs because of decreased soil moisture in deeper soil layers (Li et al. 2002).

## 9.7 Functions of Microorganisms in Soil Crust

Microorganisms of the desert soil crust are able to tolerate extreme temperatures, drought, and solar radiation; however, the strength is low and the soil crust is vulnerable to off-road vehicles, grazing livestock, and even foot trampling (Kuske et al. 2012). For example, the impact of 10 years of annual foot trampling on soil biocrusts was examined in replicated field experiments at three cold desert sites of the Colorado Plateau, USA. Trampling detrimentally impacted lichens and mosses as well as major cyanobacterium *Microcoleus vaginatus* resulting almost in average almost twice reduction of carbon and nitrogen contents; the contents of extractable DNA and chlorophyll a and increased soil erosion (Kuske et al. 2012). Geotechnical treatment of soil such as supply of soil particles binder, nutrients,

water-retention agents, and microbial bioagents can probably reduce time of soil crust recovery, which is usually measured in decades or centuries (Belnap 2003, 2006). The surface of the quarz and other rock particles can be also covered with the microbial biofilm of so-called "hypolithics" (Chan et al. 2012), which are aggregating the solid particles due to formation of microbial biofilm. Nonphotosynthetic microorganisms, such as actinomycetes and fungi as well as oligotrophic bacteria, can also form soil crust due to "glue" (binding of the particles by biopolymers) and "rope" (binding of the particles by the microbial filaments) effects (Wu et al. 2010). Microbiotic crust is important for sand fixation in deserts, protecting from wind erosion. Wind erosion rates for sandy soil without crust cover was about 46, 21, 17 times that in the soil with crust cover at wind velocities of 18, 22, 25 m $s^{-1}$, respectively (Zhang et al. 2006). The strength of the crust formed by microbial polysaccharides and cyanobacterial filaments is low, about 13–33 Pa, but microorganisms and their polymers enhance soil cohesion and resistance to erosion (Zhang 2005).

Biocrusting can increase not only resistance of soil to water and wind erosion but also stability of soil on the slopes. In the experiments, the added biomass of some fungal strains binds the sand grains and increases slope stability (Meadows et al. 1994). The fungal colonies of *Penicillium chrysogenum* in quartz sand maintained slope stability after avalanching more effectively than the colonies of bacteria *Pseudomonas atlantica,* because the hyphal filaments bound the sand particles stronger than bacterial polysaccharides (Meadows 1994).

## 9.8 The Role of Microbial Exopolysaccharides in Biocrusting

The role of microbial polysaccharides in soil particles aggregation is well known (Chenu 1993). A lot of gel-forming water-insoluble microbial polysaccharides are produced in industry (Sutherland 1990) and some of them, for example xanthan as well as its or co-polymers or composites can be used for soil particles binding and crust formation to reduce wind and water erosion of soil (Yang et al. 2007a, b). However, big scale geotechnical sand fixation using addition of microbial products could be not feasible because of the high cost involved.

Production of bacterial exopolymers in situ can be used to reduce cost of soil particles binding in the soil crust formation. However, the production of bacterial polysaccharides by heterotrophic microorganisms requires addition of organic matter up to 60 tons/ha (see calculations above). Therefore, production of exopolysaccharides by phototrophic microorganisms remains most economical way for the bioenhanced formation of soil crust. It was shown in experiments that the introduction of polysaccharide—producing algae in irrigation channel could provide a low cost technique for seepage control in irrigation channel. The reduction of

hydraulic conductivity by 22 % of its original value within a month of inoculating soil columns with algae correlated with the amount of produced polysaccharides (Ragusa et al. 1994). Microbial biopolymeric adhesive for soil particles could be three times stronger than superglue. It is produced by oligotrophic bacteria Caulobacter crescentus (Hopkin 2006).

## 9.9 Artificial Formation of Biocrust

Enhanced engineering formation of soil crust using addition of water-retention polymers, nutrients, and specific microbial biomass could be used for the stabilization of sand surface in deserts. To enhance formation of the biotic crust on soil surface, the specific microorganisms and materials must be spread onto the soil surface. Selection of microorganisms depends on region, season, precipitation, type of soil, and materials that can be added altogether with microorganisms. For example, inoculation of soil surface with oligotrophic bacteria can facilitate formation of soil crust (Wu et al. 2010). Application of three indigenous cyanobacterial isolates to an organically poor semi-arid clay loam soil showed after 240 days water holding capacity, hydraulic conductivity, and structural stability of soil were increased (Nisha et al. 2007). Cyanobacteria with urease activity can participate in the artificial formation of calcium carbonate minerals from calcium salts (Ariyanti et al. 2011, 2012). These minerals can bind particles forming solid in the soil crust.

There are different successional stages in the development of biological soil crust: (1) cyanobacterial crust where lichen and moss coverages <20 %, 2) lichen crust where lichen coverage >20 % but moss coverage <20 %, semi-moss crust where moss coverage >20 % but <75 %), and moss crust (moss coverage >75 %). Cyanobacterial and microalgal biomass content decreased in moss crust but the content of nitrogen-fixing cyanobacteria and heterotrophic microbes increased in the semi-moss and moss crusts (Lan et al. 2013). There must be different hydraulic conductivities, water retention capacity, and strength of soil crust at these successional stages.

In the field experiments in Hobq Desert (Inner Mongolia, China), the formation of biologically induced soil crust was studied 3 to 8 years after a massive inoculation in sandy soil with average dunes of 5 m height by mixed cultures of filamentous cyanobacteria *M. vaginatus* and *Scytonema javanicum* (Colica et al. 2014). Cyanobacteria *Microcoleus* exist as a cluster of filaments, surrounded by a gelatinous sheath. The living filaments can migrate through the soil, leaving abandoned sheath material, and a stabilized soil matrix behind (Belnap et al. 2001). This technology also was tested in five different types of desert soils, i.e., Qubqi, Ulan Buh, Mu Us Deserts, Horqin Sand Land, and the Hulun Buir Sand Land, covering an area of 40 $km^2$ (http://english.ihb.cas.cn/rh/as/201303/t20130326_100145.html).

The strains of cyanobacteria that are typical organisms of the soil crust in arid lands were isolated, and the biomass of cyanobacteria was grown in raceway ponds. The spray inoculation of mixed cultures onto the sand dune topsoil of the four experimental areas was at the dosage of $\sim$0.35 g chlorophyll *a* (Chl *a*) per $m^2$ of soil. After inoculation, the sand was intermittently irrigated for 15–18 days with 20 mm of groundwater per day by automatic micro-irrigation sprinkler facilities, until the initial growth of cyanobacterial crusts was visible. The soil in the experimental areas consisted of unconsolidated sand and almost devoid of any plant coverage at the time of inoculation (Colica et al. 2014). Hydraulic conductivity of soil without crust was at the level of $3 \times 10^{-8}$ m/s, meanwhile it was decreased to of 0. $5 \times 10^{-8}$ m/s when the content of high molecular weight polysaccharides (>100 kDa) in soil, produced by microorganisms, increased to 0.2–0.6 mg/g of soil. Produced soil crust absorbed 1.78 $\pm$ 0.69 mg of water per gram, while underlying soil absorbed only 0.30 $\pm$ 0.20 mg of water per gram of sample. The study showed the crucial role played by the exopolysaccharides of biologically induced soil crust in trapping and retaining humidity in sandy soils, thus increasing the water availability in the first layers of sandy soils and reducing water infiltration protecting soil from erosion (Colica et al. 2014).

## 9.10 Formation of Thick Crust Using Calcium-Based Biocementation

Calcium-based biocementation, often called as microbially induced calcium carbonate precipitation (MICP), is a technology based on application of urease-producing microorganisms together with urea and calcium ions in a permeable soil (Ivanov and Chu 2008; Mitchell and Santamarina 2005; DeJong et al. 2010; De Muynck et al. 2010a, b). It has been shown that urease-producing bacteria or enzyme urease can be used to bind sand particles through calcite formation, a process known as biocementation (Bang et al. 2001a, b; Ramachandran et al. 2001; Hammes et al. 2003).

MICP can be performed either on the surface or in the bulk of sand (Chu et al. 2012). If the level of calcium salt solution was below the sand surface, MICP took place in the bulk of sand. On the other hand, if the level of calcium salt solution was above the sand surface, MICP was performed on the sand surface and formed a thin layer of crust of calcium carbonate. After six sequential batch treatments with suspension of urease-producing bacteria and solutions of urea and calcium salt, the permeability of sand was reduced to 14 mm/day (or $1.6 \times 10^{-7}$ m/s) in both cases of bulk and surface MICP. Quantities of precipitated calcium after six treatments were 0.15 and 0.60 g of Ca per $cm^2$ of treated sand surface for the cases of bulk or surface MICP, respectively.

The flexural strength of biocemented layer was determined by beam test in which a simply supported beam specimen was loaded by a point load in the middle of the beam, which was simply supported at the two ends (Fig. 9.2).

The test specimen was 1 cm thick, 2 cm wide, and 4 cm long. The modulus of rupture (R) was calculated using the following equation:

$$R = 3PL/2bh^2 \tag{9.1}$$

where: $P$ is load; $L$ is span length between the two supports; $b$ is width of specimen at point of fracture; and $h$ is height of specimen at point of fracture. The module of rupture of the upper crust layer was measured as 35.9 MPa for the surface MICP. This value is comparable to that of limestone. Therefore, the crust layer has not only low permeability to provide an impervious layer but also adequate strength for the prevision of bank erosion or slope instability. The soil treated using bulk MICP had a much lower mechanical strength and is thus only suitable for bioclogging.

About 95–98 % of supplied calcium was precipitated in the sand in both MICP processes. For the MICP with the level of liquid above the sand surface, calcium was precipitated mainly in the upper layer and formed a calcite crust (Fig. 9.3).

The calcium contents in the crust and in the sand below the crust were 20–21 and 2–4 % (w/w), respectively (Fig. 9.3). For this type of MICP, the mass ratio of the quantities of calcium in the bulk of sand and in the crust was 1.25. Quantity of precipitated calcium after six treatments was 0.60 g of Ca per $cm^2$ of treated sand surface for this case of surface MICP.

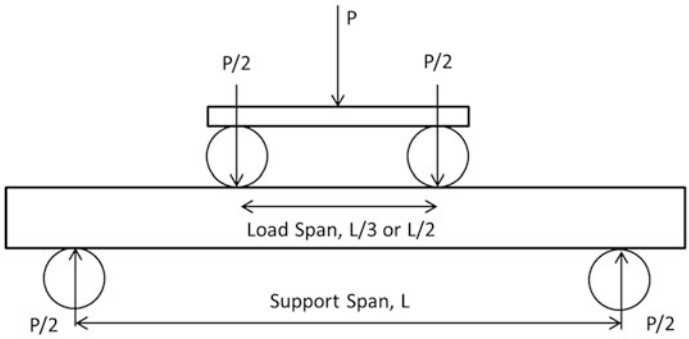



**Fig. 9.2** Setup for four points bending test

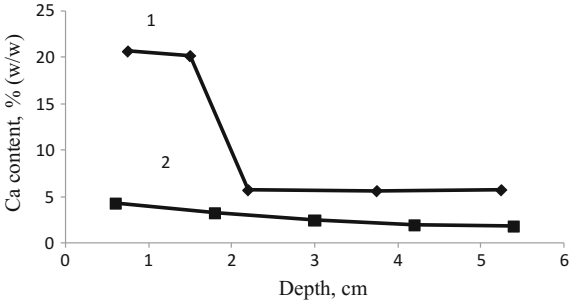



**Fig. 9.3** Content of precipitated calcium in sand after MICP at the different depth from the sand surface: *A* (*filled diamond*), treatment with the level of solution above the sand surface; *B* (*filed square*), treatment with the level of solution below the sand surface

For the MICP with the level of liquid below sand surface, calcium was precipitated almost evenly along the depth of sand after MICP (see Fig. 9.3), although the calcium content reduced gradually with depth from 4.3 % (w/w) to 1.8 % as shown in Fig. 9.3.

The calcium precipitated on the top layer of sand formed crystallized layer above the sand surface as shown in Fig. 9.4. Quantity of precipitated calcium after six treatments was 0.15 g of Ca per $cm^2$ of sand surface for this case of bulk MICP.

The formation of crust can be explained by a simple model of MICP describing the quantity of deposed calcium ($Q$) as multiplication of calcium concentration ($C$) and volume of calcium salt solution ($V$). In the case when volume of sand pores ($V_p$) is filled with calcium salt solution and there is no layer of calcium salt solution above the sand surface, the maximum deposition of calcium will be estimated as follows:

$$Q = CV_p \tag{9.2}$$

In the case when volume of pores ($V_p$) is filled with calcium salt solution and there is a layer of calcium salt solution of volume $V_s$ above the sand surface, the maximum deposition of calcium ($Q$) may be calculated as:

$$Q = CV_p + CV_s \tag{9.3}$$

or

$$Q = CA\left(H_pP + H_s\right) \tag{9.4}$$

where $A$ is treated area, $H_p$ is height of the treated soil; $P$ is soil porosity; $H_s$ is height of the calcium salt solution above soil surface. Considering that the rate of MICP in upper layer of soil is significantly higher than the diffusion rate of calcium ions in soil liquid, the calculated ratio of the quantities of calcium in the bulk of the soil and in the soil crust for our experiment is 1.20, which is almost equal to experimental value 1.25.

Under natural conditions, the depth distribution of MICP in soil could depend on the depth distribution of natural urease-producing bacteria. In the experiments, the major factor of the calcite crust formation on the surface of sand is a layer of calcium salt and urea solution above this surface.

## 9.11 Formation of Crust to Diminish the Hydraulic Conductivity

Conventionally, bentonite, and geosynthetic liners have been used to seal ponds, channels, and reservoirs in sand. However, these methods are costly. For example, about 60 kg of bentonite is required to seal 1 $m^2$ of pond area according to Pillay and Kutty (2005). The technology with geosynthetic liner is more expensive depending on the design situations (Pillay and Kutty 2005).

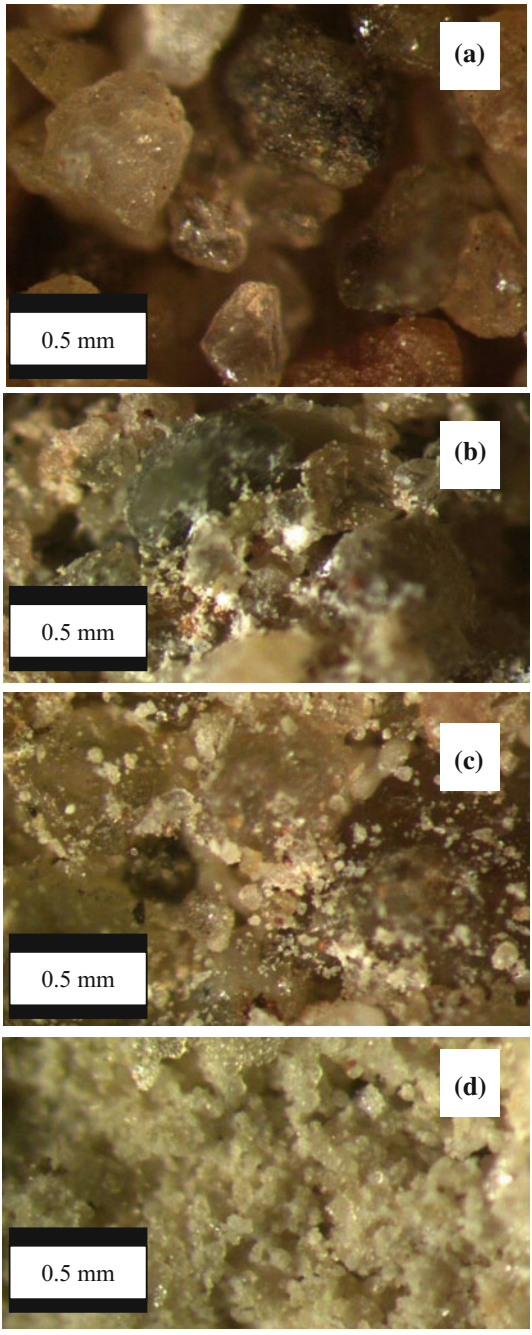



**Fig. 9.4** The stages (from the *top* to the *bottom*) of the crust formation of sand surface

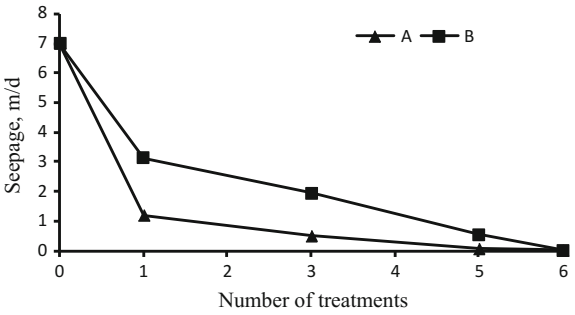



**Fig. 9.5** Effect of the MICP treatment numbers on the seepage through the sand: *A* (*filled triangle*), MICP with level of calcium salt above the sand surface; *B*(*filled square*), MICP with level of calcium salt below the sand surface

MICP process can be used to increase the shear strength and decrease the permeability of sand due to the filling of the sand pores (Gollapudi et al. 1995; Mitchell and Santamarina 2005; Whiffin et al. 2007; Chu et al. 2009; DeJong et al. 2010; Tobler et al. 2011). In our experiments on formation of the crust or top layer of biocemented sand, the hydraulic conductivity of the treated sand can be diminished to $1.6 \times 10^{-7}$ $ms^{-1}$ (or 14 mm/d) after six treatments in both types of MICP, as shown in Fig. 9.5.

This value is accepted for the construction of aquaculture ponds, because the seepage rates in soil of such permeability will be comparable with the leakage values reported for tropical fish ponds in the range of 19–58 mm/d (Teichert-Coddington et al. 1988, 1989) or for shrimp mariculture ponds in the range of 136–182 mm/d (Weisburd and Laws 1990).

Several MICP treatments were required for the reduction of permeability of sand because of two reasons: (1) the volume of sand voids that has been filled with calcium solution is not sufficient to supply quantity of calcium for the clogging of the pores after one MICP treatment; (2) repeated cycles of MICP ensure an even distribution of precipitated calcium diminishing channeling in the treated sand.

Formation of an impermeable crust layer of several millimeters or centimetres thick on top of sand surface through the MICP process has been developed (Fig. 9.6) (Stabnikov et al. 2011; Chu et al. 2012). Potential applications of the

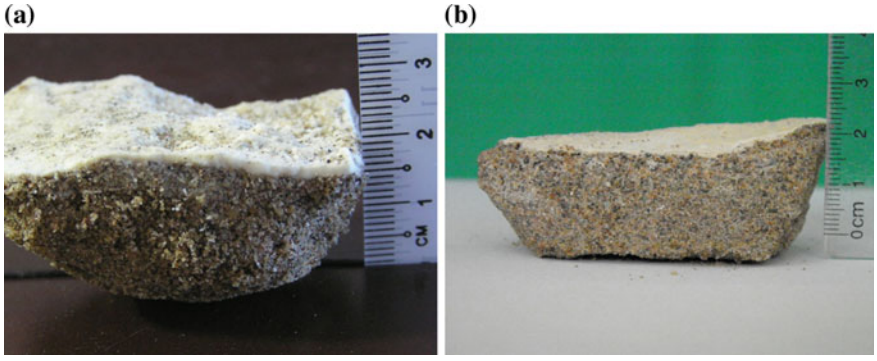



**Fig. 9.6** Formation of the calcite crust (**a**) by precipitation from solution above surface and a layer of biocemented sand (**b**) by spraying of cementing solution

method presented may include the construction of runoff collection ponds in deserts sand (Boers 1994), aquaculture ponds (Lucas and Southgate 2012; Kitto and Reginald 2011), or algal biofuel production ponds (Campbell 2008).

## 9.12 Formation of Crust to Diminish Soil Erosion and Dispersion of Soil Pollutants

In our experiments, two regimes have been studied: erosion of very fine sand with liner velocity of water flow 0.17 cm/s and erosion of coarse sand with liner velocity of water flow 17 cm/s. The dosage of precipitated calcium 6.4 g/m$^2$ of sand or 9 g Ca/kg of sand for biocementation/bioaggregation of fine sand diminished erosion of sand as well as release of chemical pollutants by 70 % and the release of the model bacteriological pollutant *B. megaterium* by 90 %. The dosage of precipitated calcium of 0.8 g/kg of coarse sand decreased the content of lead in the removed coarse sand by 50 % of initial content. So, MICP can be used to control water erosion of sand and the dispersion of sand-associated pollutants to environment.

## 9.13 Biosafe Formation of Crust or Layer of Calcite

Urease-producing bacteria for biocementation/biosealing of sand should be able to synthesize active urease under alkaline environment with high concentration of salts (Stabnikov et al. 2013a, b), show low aggregation ability at high concentration of calcium ions (DeJong et al. 2006; Stabnikov et al. 2011), and should be biological safe, e.g., nonpathogenic ones. Usually, the strains of *Sporosarcina pasteurii*, formerly *Bacillus pasteurii*, are commonly used for biocementation (Bang et al. 2001a, b; Bachmeier et al. 2002; Whiffin 2004; Whiffin et al. 2007; Dupraz et al. 2006; Mortensen and DeJong 2011; Tobler et al. 2014).

These bacteria belong to Risk group 1 with low individual and community risk. However, there are a lot of studies when such opportunistic pathogens as *Bacillus cereus* (Maheswaran et al. 2014), *Bacillus mycoides* (Elmanama and Alhour 2013), *Proteus* sp., *Proteus vulgaris* or *Proteus mirabilis* (Dosier 2014; Khanafari et al. 2011; Whiffin 2004; Varalakshmi 2014), *Staphylococcus aureus* and *Klebsiella pnemoniae* (Varalakshmi 2014), and even pathogenic bacteria *Helicobacter pylori* (Dosier 2014) were proposed to be used for biocementation. So, the critical point of biocementation is an introduction of live bacteria cells in environment, which is a risk.

To improve biosafety of the treatment the dead but urease active bacterial cells have been used (Stabnikov and Ivanov 2016a, b). Biosealing with $CaCO_3$ was done

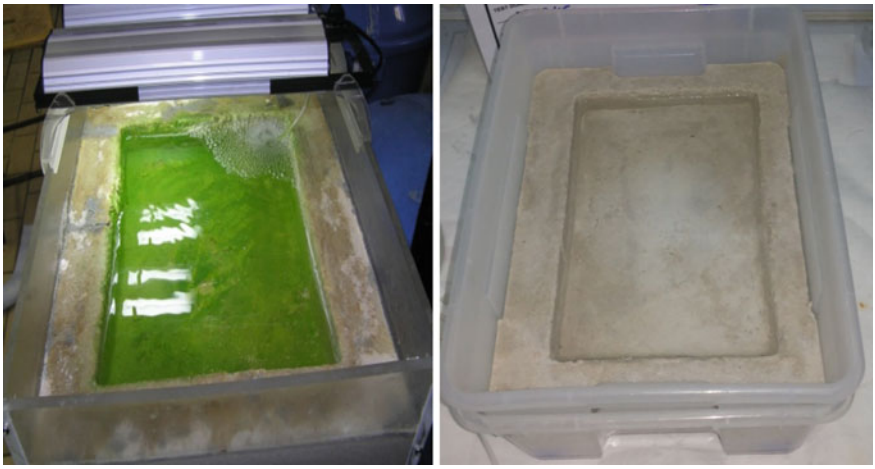


**Fig. 9.7** Images of model ponds constructed in sand using live cells of urease-producing bacteria *Bacillus* sp. VS1 and then inoculated with cyanobacteria (*left image*) and biocemented using dead but urease active cells of *Yaniella* sp. VS8 (*right image*)

by spaying of bacterial suspension and solution of $CaCl_2$ and urea. Bacterial suspension was spread onto the surface of artificial pond and left for 30 min. Then mixture of the solutions of calcium chloride and urea was spread onto the sand surface and left for 16 h. Spray of the mixture was done some times for each treatment thus the sand was not submerged into liquid. The treatments were repeated 11 times. After 8th, 9th, and 10th treatments the model pond was dried by placing in oven at 60 °C: after 8 and 9 treatments for night; after 10th treatment or 2 h. The study period included (1) biotreatment—15 days, (2) drying on air—60 days, (3) additional biotreatment—4 days, (4) washing with water—3 days. The water ponds formed are shown in Fig. 9.7.

## 9.14 Design of the Biocemented Layer of Sand

The formation of biocemented layer of sand in construction of the pond can be calculated using the following model. The maximum deposition of calcium ($Q$, kg) in the biocemented layer of sand is as follows:

$$Q = C \cdot V_p + C \cdot V_s \tag{9.5}$$

or

$$Q = C \cdot A\left(H_{\mathrm{p}} \cdot P + H_{\mathrm{s}}\right), \tag{9.6}$$

where C is calcium concentration (kg/m$^3$), $V_p$ is volume of pores in the soil, m$^3$; $V_s$ is volume of calcium salt solution, m$^3$; $A$ is treated area, m; $H_p$ is height of the treated sand, m; $P$ is sand porosity; $H_s$ is height of the calcium salt solution above soil surface, m. The calculated ratio of the quantities of precipitated calcium in the biocemented crust and in the bulk of sand for the model pond was 0.83, which was almost the same as the experimental value of 0.80. This ratio could be diminished by the formation of low permeable layer on the top of sand by sequential spaying of biocementing solution performed before major precipitation of calcium from solution above surface of sand.

The thickness of the biocemented layer, $H_p$, of sand can be calculated as follows:

$$H_{\mathrm{p}} = V_{\mathrm{p}}/A \cdot P, \tag{9.7}$$

where $V_p$ is volume of bacterial suspension and solution of calcium chloride and urea, which was used for spraying of the sand surface. The thickness of the biocemented layer was from 20 to 30 mm for bottom and 15–20 mm for the walls.

## 9.15 Cost Comparison for Biosealing

The calculated consumption of calcium during construction of model pond was 0.07 g Ca/cm$^2$ (or 0.7 kg Ca/m$^2$) for biocementation by spraying, 0.12 g Ca/cm$^2$ (1.2 kg Ca/m$^2$) for biocementation through precipitation of calcite from covering solution, and 0.02 g Ca/cm$^2$ (0.2 kg Ca/m$^2$) was used for the biocementation of the small cracks in the dried model pond. Total consumption of calcium for the construction of the model pond was 2.1 kg Ca/m$^2$ of the sand surface. Total consumption of microbial biomass was about 0.16 kg of dry biomass/m$^2$. So, evaluated quantities of the biosealing materials are 58 ton $CaCl_2$/ha, 63 tons of urea/ha, and 1.6 ton of dry bacterial biomass/ha.

Considering that the costs of dry calcium chloride, urea, and bacterial suspension are about US$250, US$360, and US$500 per ton, respectively, the evaluated total cost of the biosealing materials is about US$38,200/ha. It is lower than the cost of the sealing with bentonite, US$48,000/ha, and is significantly lower than sealing cost with geosynthetic liners, which is about US$100,000/ha.

When the bulk of sand is required to be treated, a much bigger quantity of calcium salt is required; for example, 7.6 kg of Ca/m$^2$ of surface in the study of

Harkes et al. (2010) or 113 kg of Ca/$m^2$ of surface in the study of Whiffin et al. (2007). The total calcium consumption was 2.1 kg Ca/$m^2$ of the sand surface for the model test. Therefore, the proposed method is much more cost effective. The weight of the biocemented sand layer is also significantly lower than the weight of clay introduced if about 60 kg of bentonite per 1 $m^2$ of pond area is used in the conventional method.

## 9.16 Scale-up Factors

One of the most important scale-up factors is the thickness of the biosealing layer which has to be sufficiently mechanically strong to prevent cracking because of the bending from hydraulic head and/or thermal expansion. This thickness must be determined in the field tests at the defined area at different water head and solar radiation. In every case, it will be not bigger several cm.

The hydraulic conductivity and strength after biotreatment is probably the major scale-up factor, which is determined by the content of precipitated $CaCO_3$. In our experiments the contents of $CaCO_3$ in sand on the bottom were 45, 6, 8, 8 % (w/w) on the distance 1, 2, 3, and 4 cm from surface, while there were 13 and 19 % of $CaCO_3$ on the distance of 1–2 cm from the top and side surfaces of wall.

The seepage rate from the model pond was 96 mm/d at the content of calcium in 1 cm layer on the bottom 45 % and 18 % (w/w) in 1 cm layer of the side surface of wall. This seepage was almost the same as the seepage rate from the model pond constructed in sand using un-inactivated cells of UPB *Bacillus* sp. VS1, when an average 2.1 kg of calcium per $m^2$ of sand surface was precipitated (Stabnikov et al. 2011; Chu et al. 2013). These seepage rates are comparable with the seepage rates for the aquaculture ponds (Teichert-Coddington et al. 1989; Weisburd and Laws 1998).

The technology with sprayed calcium-based biogrout simplifies considerably the procedure because there is no use of live microorganisms and thus there is no need for the numerous and complicated biosafety approvals needed for the real construction projects. The technology with percolated iron-based biogrout simplifies considerably the production of the sealing materials because raw materials, low grade iron ore and cellulose-containing waste, need for construction are abundant everywhere. However, essential part of the sand biogrouting is drying of the treated sand under sun light. Our data showed that this drying removes ammonia and ammonium from the treated sand, which is essential for fish and prawn aquaculture, but not favorable for algae cultivation. Sand sealed with iron-based biogrout is more mechanically stable for drying under sun light than sand treated with calcium-based biogrout. In last case, the treated sand is more brittle and there is possible the

formation of fine cracks due to thermal expansion of the treated sand. Repair of these cracks require additional treatment of sand after sun light drying.

Major limitation of the proposed biosealing method of the sand pond is the release of ammonia to atmosphere and ammonium to water, which can produce toxic effects for human being, animals, fishes, and prawns. So, the best solution is the use of the constructed pond for the cultivation of oil-producing algae in the coastal desert area. Then, when ammonium will be washed out from the cemented sand and consumed by the oil-producing algae during one or several cycles of their cultivation, the pond could be used for the fish aquaculture. There must be a source of carbon dioxide for algae growth, so additional layer of calcium carbonate on the bottom of the pond can be a source of carbon dioxide as well as a source of calcium ions for further strengthen and biosealing of the pond bottom.

The cost of biosealing of sand using calcium salt biogrout could be several times lower than any other known methods of the sand sealing; the biosealing has to be test in aquaculture practice for the construction of fish, prawns, or algae ponds in sand of the arid deserts.

## 9.17 Aerobic Bioaggregation and Biocementation of Soil Surface

Any aerobic oxidation of organic matter is accompanied with production of $CO_2$ and production of calcium carbonate if calcium ions are present in medium. For example, it was described precipitation of calcium carbonate from oxidation of calcium formate by methyloptrophic bacteria *Methylocystis parvus* (Ganendra et al. 2014).

Major problem for application of this process is to ensure sufficient mass transfer rate of dissolved oxygen into soil and even distribution of dissolved oxygen concentration in soil. It is very difficult to make it by an injection of air into saturated soil because of the channeling of air flow.

So, aerobic oxidation of calcium salts of organic acids can be most useful for aggregation and biocementation on the soil surface because of sufficient air access and even distribution of dissolved oxygen concentration nearby surface. For example, in our experiment the surface of sand of 450 $cm^2$ area was spread over four times with 500 mL, two times with 150 mL, and finally three times with 100 mL of the medium containing calcium acetate, 0.5 M; bacterial suspension of enrichment culture of acetate-oxidizing bacteria, 200 mL/L. Due to these treatments the seepage decreased to $3 \times 10^{-6}$ $ms^{-1}$. Major portion of calcium bicarbonate is concentrated in upper 2 cm depth layer (Figs. 9.8 and 9.9).

**Fig. 9.8** Cross section of sand treated by aerobic oxidation of calcium acetate. A layer of calcium carbonate is visible on sand surface

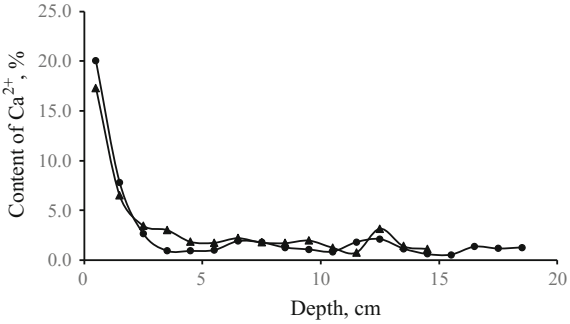



**Fig. 9.9** Profile of precipitated calcium for two independent sites in sand

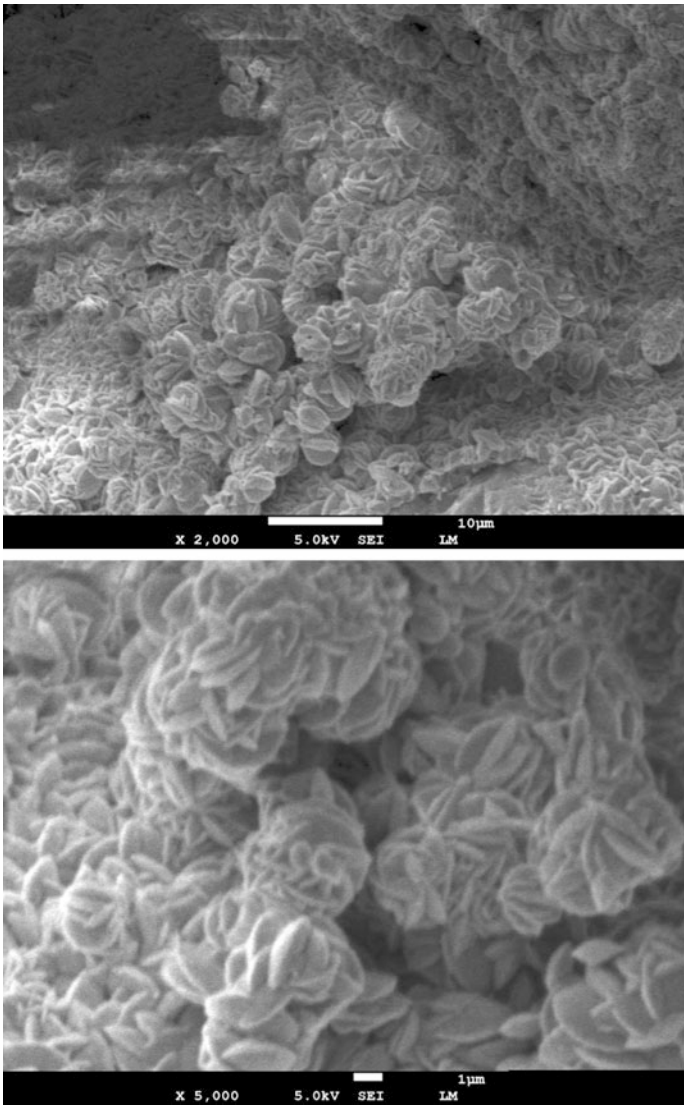



**Fig. 9.10** Crystals of calcium carbonate producing during bacterial oxidation of calcium acetate

Crystals of calcium carbonate produced after oxidation of calcium acetate have rose-like shape (Fig. 9.10). There were two major cultures in enrichment culture. One strain was identified by 16S rRNA gene sequencing as *Bacillus ginsengi* strain VSA1, another one was *Brachybacterium paraconglomeratum* strain VSA2.

# Chapter 10
# Biocoating of Surfaces

## 10.1 Coating of Concrete Surface

Cement-based materials are major construction materials but the concrete surface of the coastal constructions can be corroded under action of (1) acidic or salty water, (2) sulfate, sulfides, chloride, and nitrate ions, (3) carbon dioxide dissolved in water, (4) magnesium ions of seawater, (5) damage by freezing-thawing, (6) alternate drying and wetting, (7) acids produced by bacteria (Allahverdi and Skvara 2000; Basheer et al. 2001; Batchelor et al. 2011; Chavez-Ulloa et al. 2013; Jensen et al. 2009; Takatokuand and Mizobuchi 2014; Visalakshi et al. 2014; Wang et al. 2006). Concrete corrosion appears as the peeling, deterioration, increase of porosity due to disintegration of concrete components and infiltration of aggressive agents in its thickness through the cracks and pores of concrete, which deteriorate mechanical properties of concrete (Finozzi and Saetta 2014; Mehta and Monteiro 1993).

The organic polymer or resin mixed with calcium carbonate particulates can be used for the coating protecting concrete from corrosion (Schwotzer et al. 2010; Thiery et al. 2007; Xu et al. 2014; Yao et al. 2011). However, it is usually expensive and incompatible with concrete because of the differences in their thermal expansion coefficient (Duarte et al. 2014; Jumaat et al. 2006).

Thin, 5–10 μm, protective layer of calcium carbonate on the concrete surface is formed naturally in the case when the concrete is exposed for a long time to open air. This layer does not dissolve in water and does not interact with sulfate. The produced precipitate can block the water flow through the cracks or prevent further contact between the bicarbonate solution and the concrete (Brodersen 2003; Thiery et al. 2007). Carbonation of concrete increases mechanical strength of concrete and reduces the permeability of concrete (Owens et al. 2007; De Schutter and Audenaert 2004).

The formation of $CaCO_3$ covering layer on the surface of cement-based materials is used as a method for surface protection from aggressive aqueous environment (Schwotzer et al. 2010; Thiery et al. 2007; Xu et al. 2014). U.S. Patent



V. Ivanov and V. Stabnikov, *Construction Biotechnology*,
Green Energy and Technology, DOI 10.1007/978-981-10-1445-1_10

No. 8,182,604 "Composition suitable for aquatic habitat repair, replacement and/or enhancement," issued to Fernandez on August 16, 2011 discloses the combination of calcium carbonate (preferably aragonite), a resin, and a catalyst results in a composition that is strong, durable, and easy to use as a coating for concrete in structures such as artificial reefs. However, organic polymers and resins are expensive and not compatible to concrete because of difference in the thermal expansion coefficients of the concrete and coating material, which may lead to mechanical instability of the coating during changing temperature of environment.

## 10.2 Biocoating of Concrete Surface

Calcium carbonate layer on the surface of concrete can be formed using so-called "microbially induced calcium carbonate precipitation" (MICP) (Qian et al. 2009; Ivanov and Chu 2008; Ivanov et al. 2015a, b, 2016), which is the formation of calcium carbonate from calcium salt and urea due to hydrolysis of urea to $CO_2$ and ammonium ions. This process was described above in this chapter .

MICP produces crystals of calcium carbonate adhered to the surface but chemical precipitation of $CaCO_3$ did not produce adhered crystals (Stabnikov et al. 2011; Ivanov et al. 2016). The applications of MICP on formation of the layer of calcium carbonate on the surface of cement-based materials to repair cracks in concrete, to protect concrete from corrosion, or to change the surface properties of recycled concrete aggregates were described in numerous papers (Ramachandran et al. 2001; De Muynck et al. 2008a, b; 2010; Qian et al. 2009; van Tittelboom et al. 2010; Achal et al. 2011a, b; Kim et al. 2013; Kim and Lee 2015; Li and Qu 2012, 2015; Pan et al. 2015; Shirakawa et al. 2011, 2015). We also demonstrated previously that it is possible to produce the solid crust of calcium carbonate crystals on the surface using MICP (Stabnikov et al. 2011; Chu et al. 2012a, b; Ivanov et al. 2015a, b; Stabnikov et al. 2015).

## 10.3 The Biocoating Procedure

The following procedure has been used in our experiments on biocoating: (1) spraying of the suspension of UPB biomass in 0.2 % solution of xanthan in dosage about 0.1 mL/cm$^2$ of surface, (2) dried on air for 1 h, (3) immersed into the solution of calcium chloride (final concentration 0.75 M) and urea (final concentration 1.5 M) for 18 h, (4) dried on air at the room temperature for at least 6 h. This treatment was repeated until the formation of the layer of calcium carbonate on the frontal surface of the concrete. Typically, five rounds of biocoating were done for the formation of the coating layer with thickness from 0.3 to 2.3 mm.

## 10.4 Calcium Carbonate Layer on the Concrete Surface

The surface of the concrete specimens after coating procedure was covered with a layer of calcium carbonate that is strongly adhered to concrete. The thickness of this layer was from 0.3 to 2.3 mm (Fig. 10.1) with the coating density within a range from 0.1 to 0.7 g $CaCO_3/cm^2$.

SEM images showed that the coating layer was tightly adhered to concrete surface (Fig. 10.2), and was made from prismatic calcite crystals (Fig. 10.3), which was confirmed by XRD analysis of the coating material using Bruker D8 Advance X-ray diffractometer with $CuK_\alpha$ radiation of wavelength $\lambda = 1.54056$ Å, measurements in the locked coupled mode in the $2\theta$ range of 5 to $80^o$.

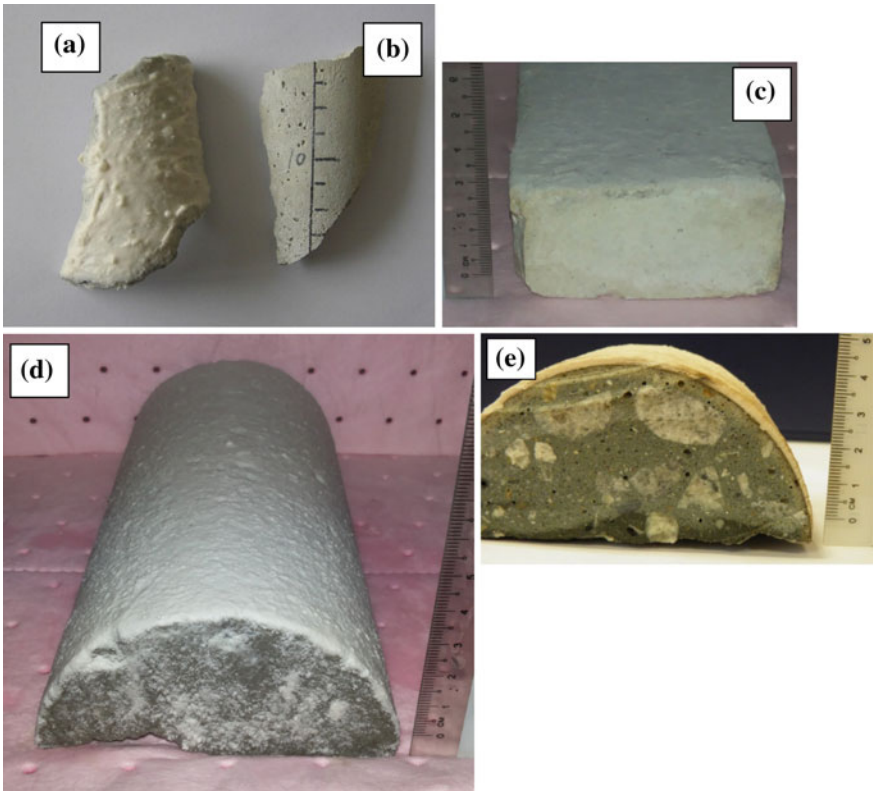



**Fig. 10.1** The concrete surfaces after biocoating (**a**, **c**, **d**, **e**) and before the biocoating (**b**)

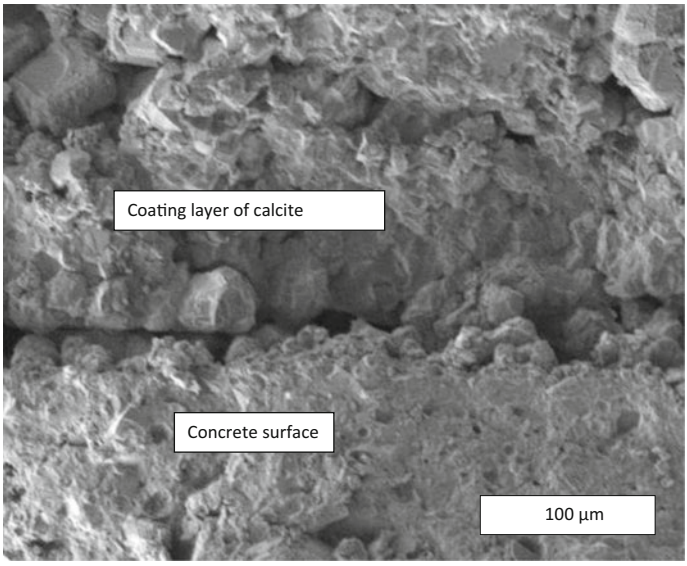



**Fig. 10.2** SEM image of calcite-coating layer (*upper layer*) on concrete surface (*bottom part*)

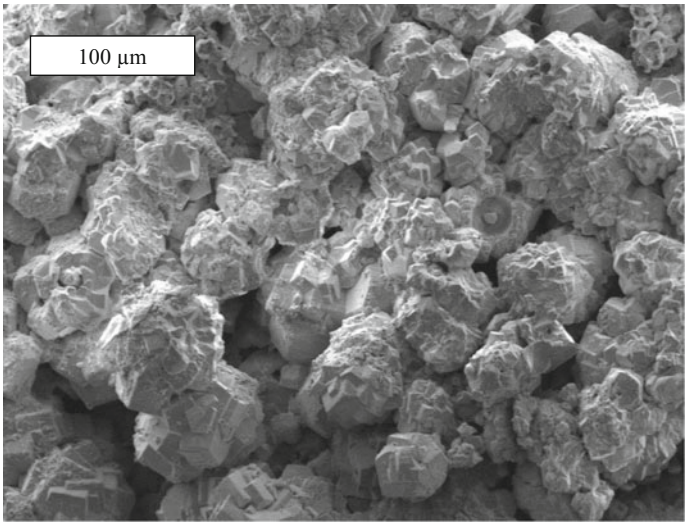



**Fig. 10.3** Calcite crystals produced during biocoating

## 10.5 The Mechanism of Biocoating Using MICP

The mechanism of MICP biocoating could be either crystallization of calcium carbonate directly on surface or formation of the crystals and their precipitation to surface by gravity and then adhesion of the precipitated crystals to the surface. In first case, coating of surface with calcium carbonate will not depend on the angle between plain of surface and gravity vector but in case of the second mechanism the coating rate will depend significantly on this angle and the concrete specimen must be rotated in the coating solution to even the thickness of the coating layer.

It was shown in our experiments that the biocoating rate depended significantly on the angle between plain of surface and gravity vector. Therefore, crystallization of calcium carbonate under action of bacterial urease is going not on the surface but in solution and after that calcium carbonate crystals precipitated onto the surface by gravity forming after some contact time an adhesion bonding between the precipitated crystals and the concrete surface. So, most probably that coating of the surface of concrete object with even layer of calcium carbonate crystals must be done with slow rotation in the treatment tank during spraying by bacterial suspension and during the precipitation of calcium carbonate crystals. It is important for the practice of concrete biocoating. During the immersion biocoating without movement of the object, the plain of the biocoated surface must be horizontal to ensure maximum of bacterial cells adhesion and calcium carbonate coating of the surface.

Precipitation of $CaCO_3$ on the vertical plane of the concrete surface was only 3 % of those on horizontal plane. For the curved surface quantity of precipitated $CaCO_3$ depended on the angle between the plane of concrete surface and vector of gravity (Figs. 10.4 and 10.5).

This difference shows significant effect of gravity on the coating due to the following mechanism of the coating: (1) formation of calcite crystals due to microbial hydrolysis of urea and formation of carbonate and increase of pH; (2) precipitation of calcite crystal to concrete surface by gravity; (3) then adhesion of the precipitated crystals to the concrete surface. These steps are shown conventionally on Fig. 10.6.

Therefore, widely used term “microbially induced calcite precipitation” (MICP) is not correct because they are involved not only calcite and not just precipitation process but also involved adhesion of bacterial cells and $CaCO_3$ crystals, which are affected by gravity. Based on these data, the more correct term for MICP could be something like “calcium carbonate minerals formation and gravity-dependent adhesion to surface induced by surface-adhered microorganisms.” However, it is not convenient to use correct but very long term in practice. So, MICP is practically convenient abbreviation but its content should be quite different from initial understanding of MICP process.

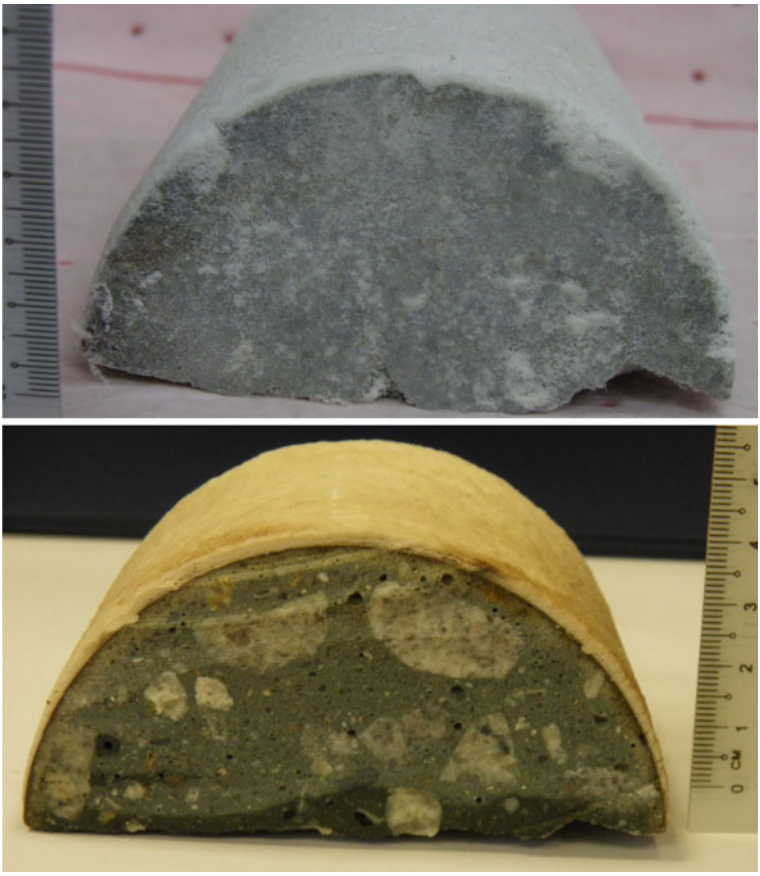
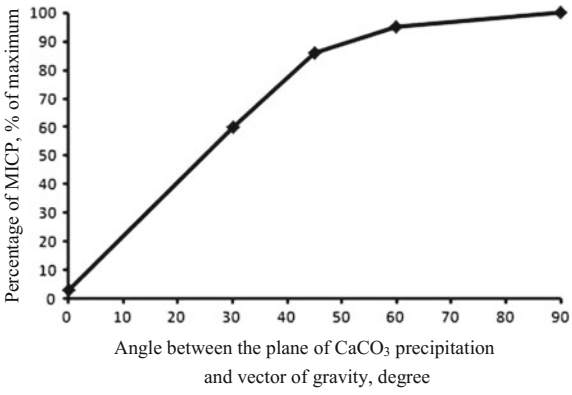


**Fig. 10.4** Effect of gravity on MICP on concrete semicylinder before (*upper image*) and after cross section (*bottom image*)

**Fig. 10.5** Effect of the angle between the plane of concrete surface and vector of gravity on the thickness of the coating layer (on the quantity of precipitated $CaCO_3$)



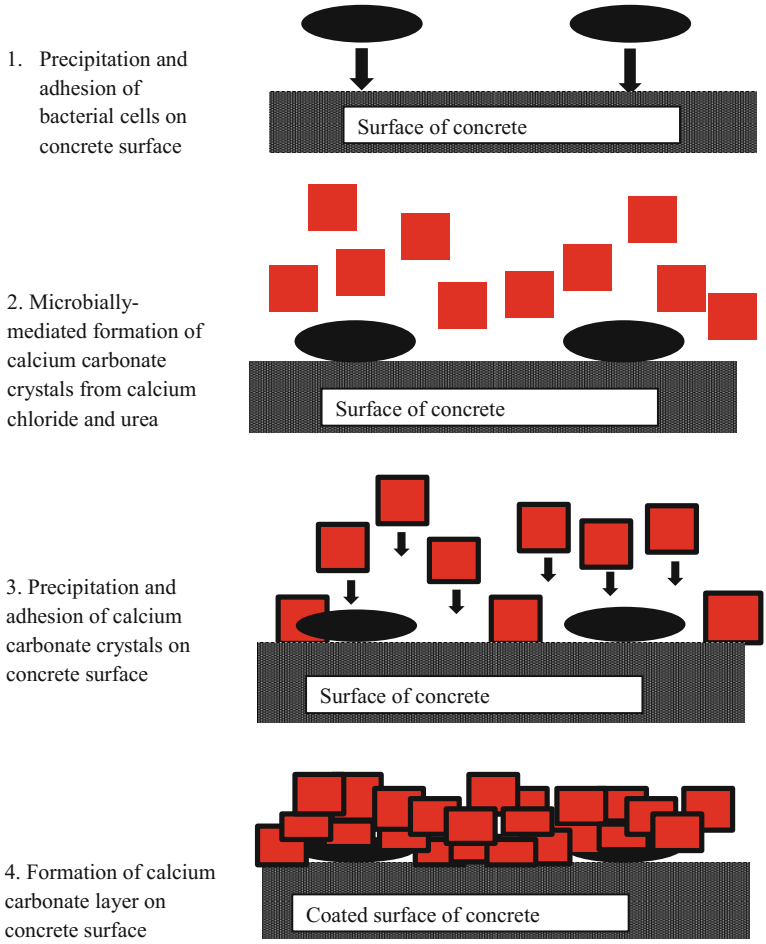



**Fig. 10.6** Steps of concrete surface biocoating with a layer of calcium carbonate explaining effect of gravity on the efficiency of coating of concrete surface with calcite crystals

## 10.6 Effect of Gravity on Adhesion of UPB Cells and Calcite Crystals

Gravity affects adhesion of both bacterial cells of urease-producing bacteria (UPB) and calcite crystals (Table 10.1).

The effect of gravity on adhesion of bacterial cells was evaluated by the ratio of $CaCO_3$ attachment in experiments 1 and 3 (=2.3) as well as in experiments 2 and 4 (=1.8). Much stronger positive effect of gravity on adhesion of $CaCO_3$ crystals was evaluated by the ratio of $CaCO_3$ attached to surface in experiments 1 and 2 (=5.9)\ as well as in experiments 3 and 4 (=4.8). Finally, due to effect of gravity on

**Table 10.1** Effect of gravity on biocoating

| No of the sample | Specimen was incubated in | Vertical or horizontal surface of the specimen | Quantity of precipitated and adhered to surface $CaCO_3$, $mg/cm^2$ |
|---|---|---|---|
| 1 | UPB suspension | Horizontal | 3.36 ± 0.26 |
| | Solution of calcium chloride and urea | Horizontal | |
| 2 | UPB suspension | Horizontal | 0.57 ± 0.1 |
| | Solution of calcium chloride and urea | Vertical | |
| 3 | UPB suspension | Vertical | 1.47 ± 0.2 |
| | Solution of calcium chloride and urea | Horizontal | |
| 4 | UPB suspension | Vertical | 0.31 ± 0.01 |
| | Solution of calcium chloride and urea | Vertical | |

biocoating, an attachment of $CaCO_3$ on vertical surface was only 9 % of adhesion of $CaCO_3$ crystals on horizontal surface. Therefore, the best biocoating (100 %) was at the horizontal position of the coated surface and the worst coating (9 % of maximum) was at the vertical position of the coated surface.

## 10.7 Effect of Biocoating on Water Adsorption

The performance and durability of concrete in aggressive environment depend on its penetrability for water, which can be evaluated by absorption of water due to capillary rise in unsaturated concrete. Therefore, a protective effect of the surface coating was investigated using determination of the water absorption by capillarity according to DIN EN 1925:1999 "Determination of water absorption coefficient by capillarity" and ASTM C1585-13

"Standard Test Method for Measurement of Rate of Absorption of Water by Hydraulic-Cement Concretes." Prior to the absorptivity test, the stones were dried in an oven at 80 °C, until a constant weight was obtained, then the sample was immersed 3 mm of water on one of its sides and the increase in mass is measured. The absorptivity (water uptake rate) coefficient was calculated from the slope of the linear curve presenting the amount of water absorbed per unit of surface and the square root of time. To determine the permeation resistance of the treated concrete, a capillary water absorption test was conducted by other authors to evaluate the effects of calcium carbonate coating on water diffusion and absorption, which may affect the durability of the concrete (Demirci and Sahin 2014; Qian et al. 2009; Zhang and Zong 2014).

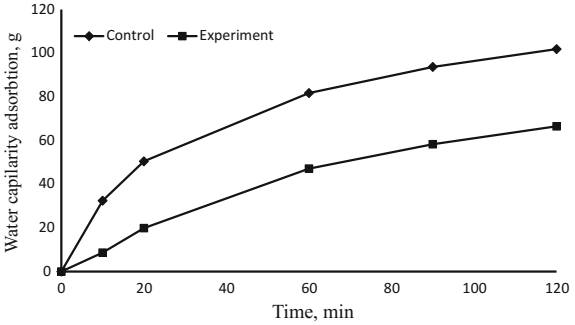



**Fig. 10.7** Water capillary adsorption by the initially dry concrete blocks that were uncoated (control) or coated by calcite layer (experiment)

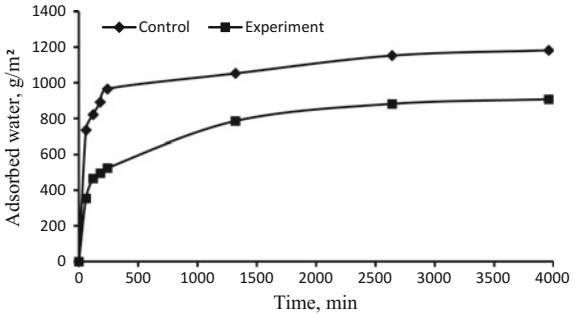



**Fig. 10.8** Water capillary adsorption by concrete semicylinder covered with a layer of microbially induced calcium carbonate (experiment) and uncovered concrete semicylinder (control)

The coating of concrete rectangle blocks with a layer of calcium carbonate crystals decreased water absorption for 35 % (Fig. 10.7).

Concrete semicylinder covered with a layer of microbially precipitated calcium carbonate (experiment) showed lower water absorption capacity in comparison with uncovered semicylinder (control) (Fig. 10.8).

Water absorption for 66 h was 1183 g/m$^2$ in control and 908 g/m$^2$ in experiment. Thus, biocoating of concrete surface with a layer of microbially induced calcium carbonate diminished water absorption by 30 %.

## 10.8 Freezing—Heating and Wetting-Drying Tests of the Coated Surfaces

To check the stability of the coating layer under changed temperature, the concrete semicylinder and the blocks were placed in the fridge at temperature −20 °C for 24 h, and then were heated for 24 h at 25 °C.

To check the stability of the coating layer under wetting-drying conditions, the concrete semicylinder and the blocks were placed in water for 48 h, and then were dried for 48 h at 60 °C.

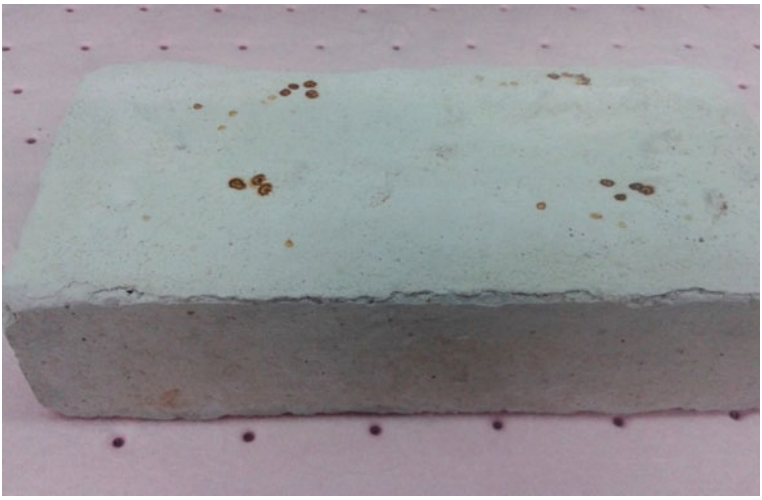


**Fig. 10.9** Photo of the coated concrete block after eighth cycles of freezing—heating

Photo of the samples showed that there were no cracks and no stripping of the coating after 10 cycles of freezing—heating (Fig. 10.9). Same was for the samples after several cycles of 2 days wetting and 2 days of drying at 60 °C. Mechanical stability of the coating layer during changes of temperature and humidity is a sign of similar coefficients of thermal expansion of the concrete and coating layer.

## 10.9 Corrosion-Protecting Carbonate Layer

The research showed that the concrete surface can be coated by a strongly attached calcite layer using microbially induced calcite precipitation. This layer could protect the concrete surface from magnesium- and carbonic acid corrosions as shown in Fig. 10.10. It can probably reduce the rate of penetration of other corrosive agents.

The calcite-coating layer on concrete surface is sustainable because of same thermal expansion coefficient as concrete. This coating decreased capillary absorption of water by concrete, which may positively affect the durability of the concrete and reduce its corrosion in seawater.

Usually, the concrete surface is colonized slowly because its pH is strictly alkaline and natural biocalcification process is slow. It is known that artificial calcium carbonate structures increased the rate of the coral reefs restoration (Sabater and Yap 2004; Stromberg et al. 2010). Therefore, the calcite-coated concrete surface could be faster and stronger colonized by aquatic algae, aquaculture shellfishes, and coral larvae because the pH of calcium carbonate is neutral and it is a major component of the shells and coral exoskeleton (Precht 2006). So, important application of the calcite biocoating of concrete could be the construction of

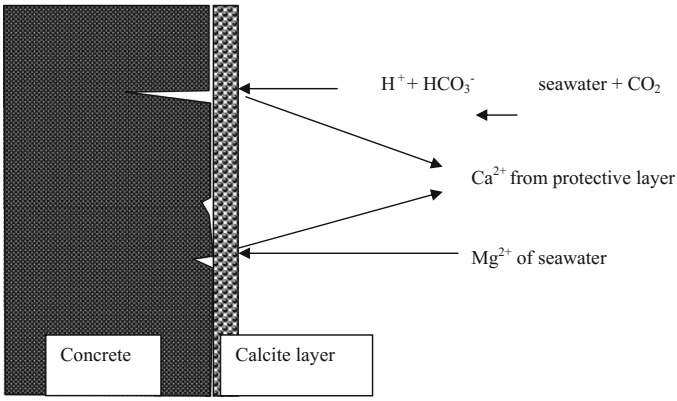



**Fig. 10.10** Schematics of the concrete surface protection from magnesium- and carbonic acid corrosions

artificial concrete reefs to replace the damaged coral reefs maintaining marine biodiversity and productivity as well as the recreational fishing and diving (Westmacott et al. 2000; Goreau and Hilbertz 2005). These artificial concrete reefs coated with calcium carbonate could be effectively colonized by marine epibiota capturing light energy and $CO_2$ reducing the rate of global climate changes. Similar constructions could be used also for shellfish aquaculture.

## 10.10 MICP on Granite Surface

Gravity had approximately the same effect on biocoating of the horizontal granite plates 40 × 40 × 2 cm with the horizontal flow of biocoating MICP solution between them. Content of calcium carbonate on the floor (bottom) plate after treatment was 113 mg of $CaCO_3/cm^2$ of the surface, but the content on the ceiling (upper) plate was only 17 mg of $CaCO_3/cm^2$ of surface, i.e., adhesion of calcite crystals was only 15 % of maximum at the angle 180° between gravity vector and plane of crystals adhesion (Fig. 10.11). Similar results were produced for the UPB-enhanced precipitation of calcium carbonate from calcium bicarbonate solution with concentration of $Ca^{2+}$ 20 mM and urea 2 mM. Precipitation of calcium carbonate on the floor (bottom) plate after treatment was 3.5 mg of $CaCO_3/cm^2$ of the surface, but the content on the ceiling (upper) plate was only 1.3 mg of $CaCO_3/cm^2$ of surface, i.e., adhesion of crystals during urease-induced decay of calcium bicarbonate was 37 % of maximum at the angle 180° between gravity vector and plane of adhesion.

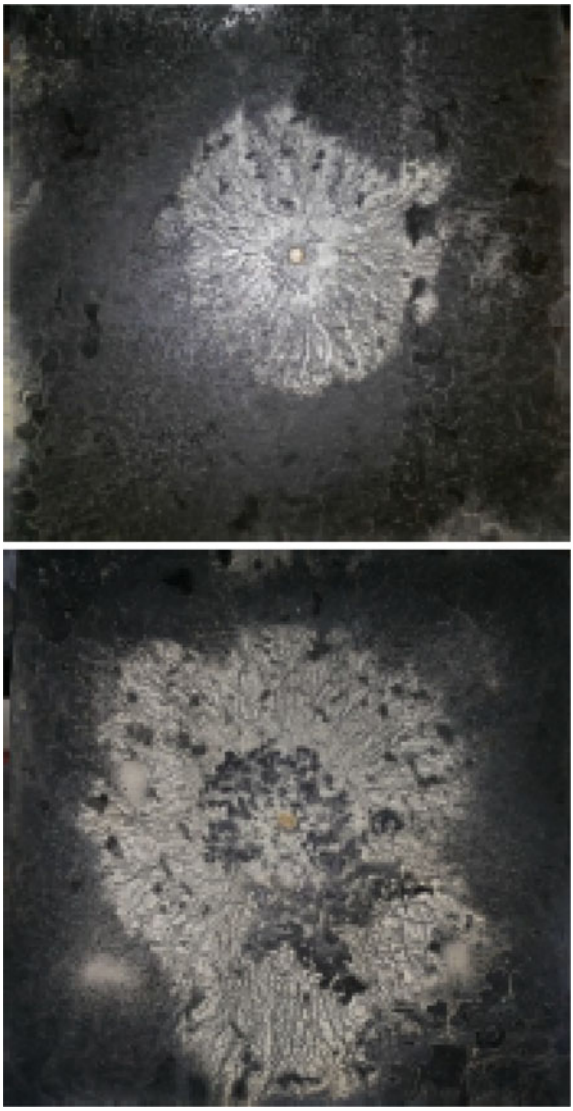


**Fig. 10.11** Precipitation of calcium carbonate on the ceiling (*upper*) plate (*upper image*) and on the floor (*bottom*) plate (*lower image*). Inlet for supply of the coating solution was in the center of the upper plate

## 10.11 MICP Coating of the Surface of Different Materials

We showed that it is possible to coat the surface not only with concrete but also with bricks, rocks, wood, plastic, glass, rubber with 1–3 mm layer of calcium carbonate minerals using MICP. The surfaces of granite and basalt (Fig. 10.12) were coated after one treatment with strongly adhered calcite layer on their surface. Surface of glass (Fig. 10.13), polyethylene fiber (Fig. 10.14), silicone rubber (Fig. 10.15), and many other materials can be coated with the layer of calcite,

**Fig. 10.12** Biocoating of granite and basalt with calcite crystals

vaterite, or aragonite crystals after one or several treatments. Biocoating of the bricks (Fig. 10.16) can be used for their "marble-like" decoration and to diminish their water adsorption capacity. We showed that it is possible to make polished "marble-like shining" surface of the coating layer if to use highly hydrophobic (greasy) polished surface as a bottom for the coating layer. In this case, the coated surface will be ceiling (upper) surface for the coating layer, so the biocoating of this upper surface will be several times slower than biocoating of the bottom surface.

**Fig. 10.13** Biocoating of glass

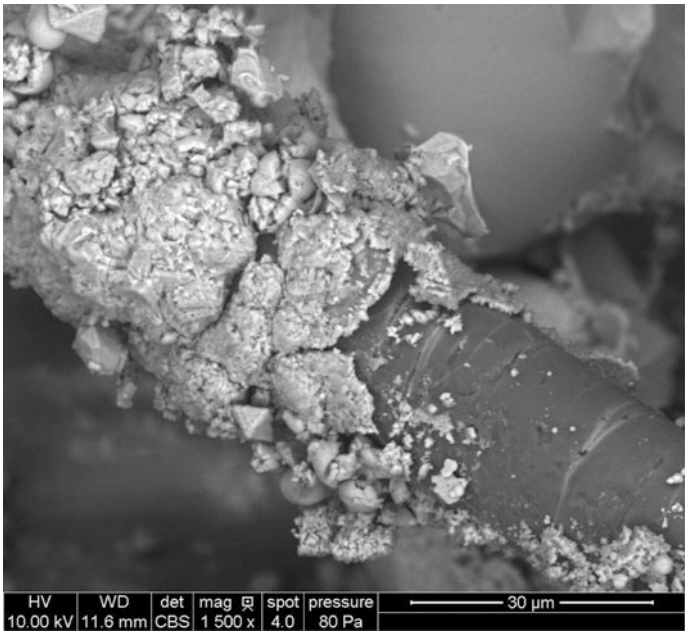



**Fig. 10.14** Biocoated polyethylene fiber

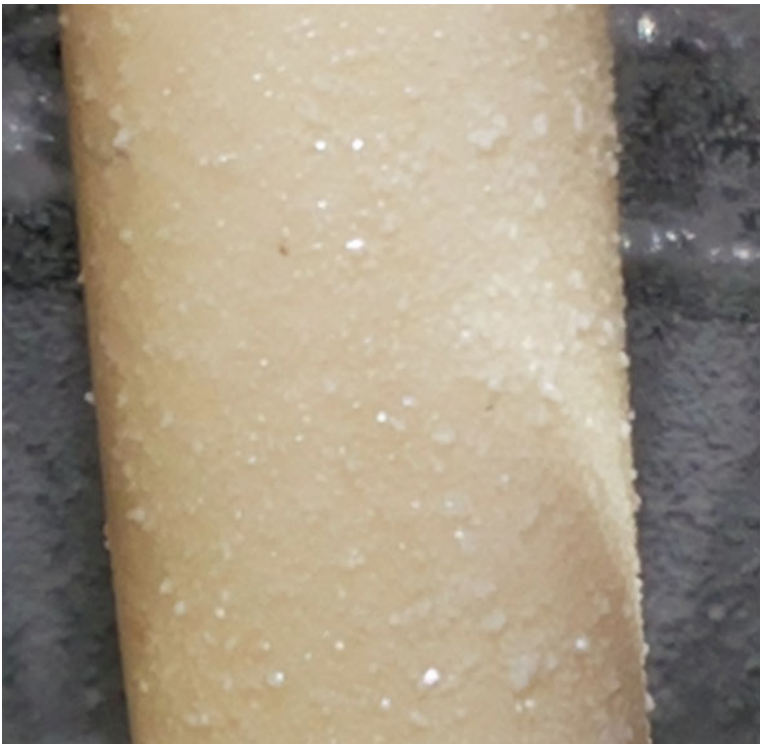


**Fig. 10.15** Biocoated surface of silicon rubber

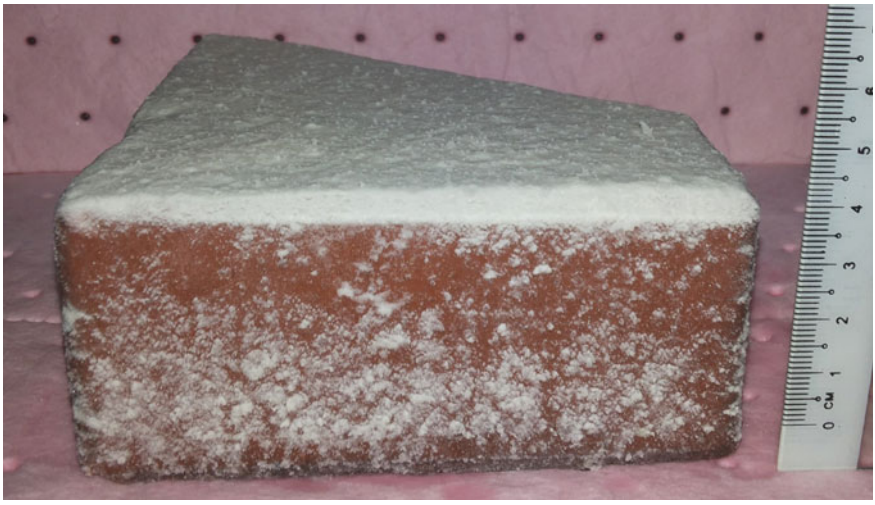



**Fig. 10.16** Coated surface of the brick

Sporogenic cells of UPB have high hydrophobicity and due to this property they are concentrated in air–water interphase. Therefore, in our experiments a film of calcium carbonate minerals is produced on water surface after addition of calcium salt and urea. This could be used to immobilize hydrophobic pollutants in water or to control mosquitoes breeding because their larvae are developing in air–water interphase.

## 10.12 Biotechnological Enhancement of Low-Crested Coastal Defense Structures

Coastal defense concrete structures that protect sedimentary coastlines from erosion are poor colonized by marine epibiota because of a high pH of the concrete surface. However, they can be designed to perform not only engineering function but also ecological service enhancing coastal ecosystems (Moschella et al. 2006; Firth et al. 2013). In the THESEUS research project 2009–2014), which optimized the design of coastal defense structures with the aim to conserve or restore native species diversity, the following solutions have been proposed: (1) creation of artificial rock pools, pits, and crevices on breakwaters; (2) precast habitat enhancement units; (3) the use of a mixture of stone sizes in gabion baskets; (4) gardening native habitat-forming species, such as canopy-forming algae, on coastal defense structures (Firth et al. 2013).

In recent years, interest in coastal defense structures with a low-crested structures (LCS) has been growing together with awareness of the sensitivity to environmental impacts produced by these structures (Burcharth et al. 2007). Here we proposed to use biocoating of the surface of low-crested structures with calcite to improve colonization and water quality in coastal area with low-crested coastal defense structures. Calcite coating has a neutral pH and is known as a favorable material for colonization by freshwater and marine epibiota such as coral and shellfish larvae, cells of microalgae, and photosynthetic cyanobacteria.

## 10.13 Artificial Coral Reefs

Another important application of the calcite biocoating could be the construction of artificial reefs. Any a human-made underwater construction made to support marine life can be considered as artificial reefs. The European Artificial Reef Research Network (EARRN) defined artificial reef as “any structure that has been deliberately submerged on the substrate (sea bed) to imitate some of the characteristics of natural reefs” (Ammar 2009). Coral reefs are declining and degrading around the

world due to anthropogenic pollution of water and acidification of seawater due to global climate change. Creation of the artificial reefs with a structure suitable for coral larvae and symbiotic microscopic algae to grow on is considered an effective way for the restoration of the damaged coral reefs to maintain marine biodiversity and productivity as well as for the recreational fishing and diving (Westmacott et al. 2000; Goreau and Hilbertz 2005). The artificial reefs, placed at certain depth and effectively capturing light energy, could be useful for the optimization and maintenance of the large-scale aquatic photosynthetic ecosystems capturing $CO_2$ reducing the rate of global climate changes.

## 10.14 Biotechnological Construction of Artificial Coral Reefs

The composition of the artificial reef surface is the most important factor for its colonization with aquatic microorganisms and sustainability of their colonies, and calcium carbonate is the most suitable substance for the construction of artificial coral reefs because it is a major component of the coral exoskeleton (Precht 2006). However, the natural biocalcification process is slow. Therefore, construction of the artificial calcium carbonate structures can be used to increase the rate of the coral reefs restoration. One way for the construction of artificial calcium carbonate coral reef is an electrochemical deposition of calcium carbonate from seawater (Sabater and Yap 2004; Stromberg et al. 2010). Our idea of the artificial reefs from calcium carbonate is to use calcium carbonate biocoating of concrete, plastic, or wooden frames. Calcium carbonate is the backbone of coral reefs, and it takes a long time for reef building organisms to build up their skeletons. Calcium carbonate coating of the surfaces of such coastal engineering and aquaculture objects as artificial reefs, concrete dams, low-crested coastal defense structures, or shellfish frames could enhance colonization of these surfaces with larvae of corals and shells as well as with microscopic algae and photosynthetic bacteria. The biocolonized calcite-coated surfaces of the artificial can also improve removal of the pollutants from water and photosynthetic capturing of $CO_2$ to reduce global warming. In 2015, the authors of this book proposed to make underwater recreational park of artificial coral reefs coated by calcite and aragonite in Singapore (Fig. 10.17).

Also, interesting is an idea of underwater to create “underwater gardens” using biocoating of terrestrial plants as the frames for fast colonization by the corals (Figs. 10.18 and 10.19). After four treatments, the thickness of calcite layer on the surface of plants was about 1 mm. The weights of the plants after MICP coating increased in average three times of the initial weights. The surface of the terrestrial plants has been evolutionary optimized to maximize their capture of light energy.

**Fig. 10.17** Artificial coral frame coated with calcium carbonate layer for enhanced colonization of corals

Therefore, their coating with calcite followed by colonization with photosynthetic microorganisms could be a way to enhance photosynthesis in coastal areas.

The plants coated with calcite layer were placed for colonization in seawater aquarium with hard corals (Fig. 5). The biofilm on the light-exposed side of calcite-coated surfaces was formed after 6 weeks of colonization and developed up to the thickness of about 250 μm.

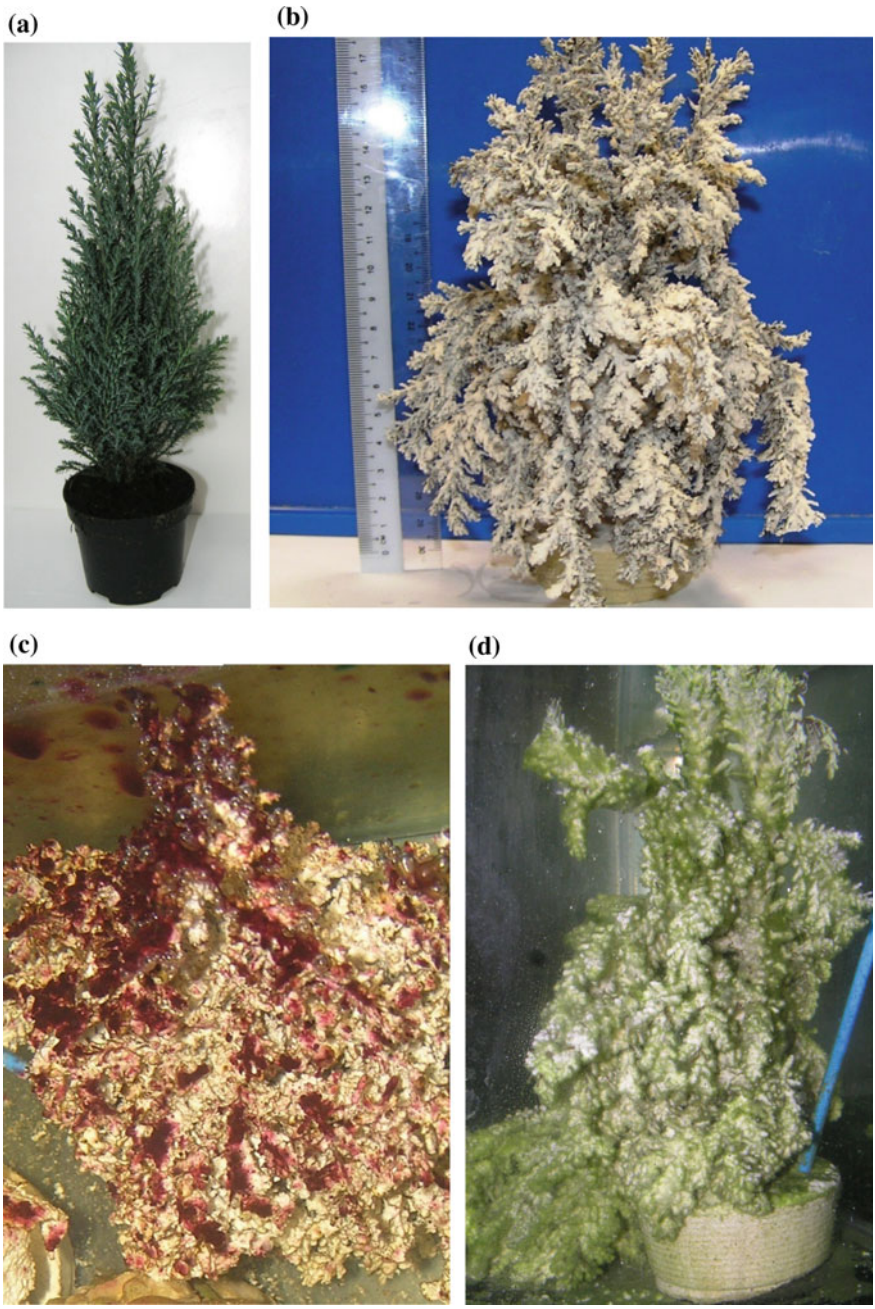



**Fig. 10.18** *Chamacyparis ellwoodi* plant before (**a**) and after biocoating with calcite (**b**), and after 6 weeks of surface colonization with photosynthetic microorganisms in seawater (**c**) and freshwater (**d**)

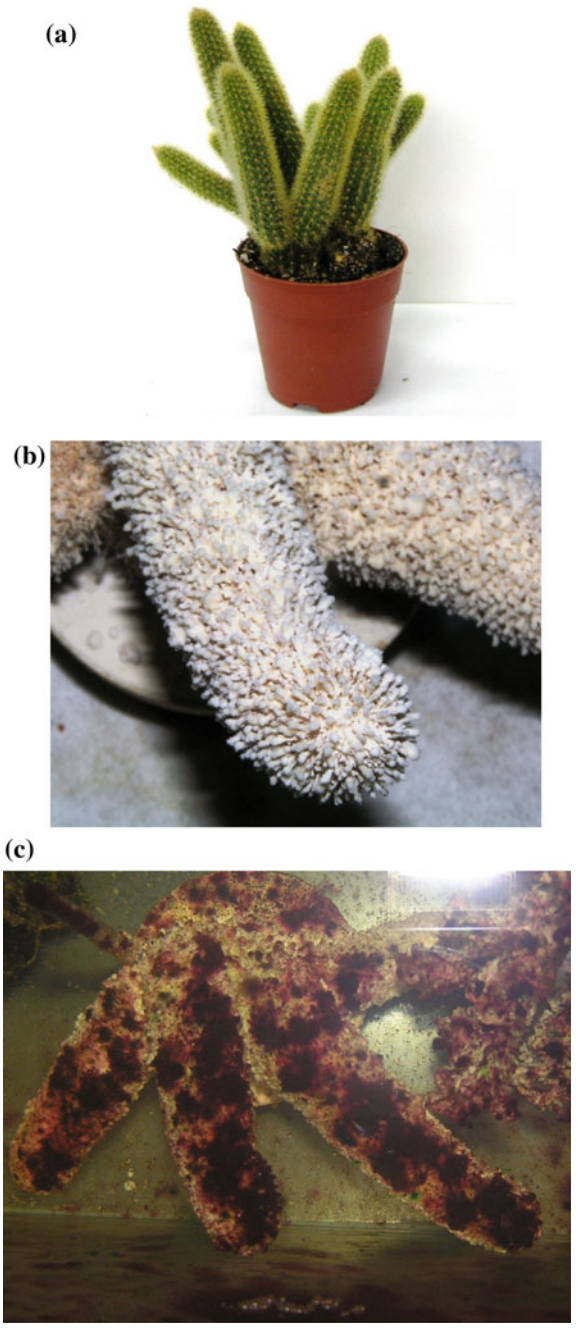



**Fig. 10.19** Cactus *Arthrocereus spinosissimus* plant before (**a**) and after biocoating with calcite (**b**), and after 6 weeks of surface colonization with photosynthetic microorganisms (**c**)

## 10.15 Biocoating of Aquaculture Frames

Biocoating of other types of frames, including plastic ropes and wooden or metal frames, could be also be useful for the coastal oysters, pearls, and other shellfish aquaculture, because calcium carbonate surfaces connected to the rope or frame are used for the centuries for colonization and cultivation of shellfish.

## 10.16 Biocoating (Biocapsulation) of Soft Clay Aggregates

There are many sediments deposited at the bottom of seas, rivers, lagoons, and lakes. It is important to use these sediments as a substitute material to natural resources (Sharma and Singh 2015). Marine clay is one of the predominant geological materials found in coastal regions around the world. After excavation or dredging works in coastal area it is remained as a waste, which is usually landfilled (Chan et al. 2011). At the same time, there is a big demand for solid fill materials for land reclamation projects and different wastes have been studied as a fill material for land reclamation (Lim and Chu 2006). Therefore, soft marine clay transformation from construction waste to the solid fill material for land reclamation is important from environmental and economic points of view. Transformation of dredged or excavated soft marine clay to solid construction material is the engineering task of great economic and environmental importance. This construction waste could be transformed to solid construction material using thermal treatment or mixing with cement but these treatments are too expensive.

One way for utilization of soft clays and clay suspension is their mixing with lime, sand, or metal works wastes, following with drying or thermal treatment to form solid material. However, drying or thermal treatment of big volume of soft marine clay are too expensive methods to be used in large-scale reclamation works.

Soft marine clay could be strengthened also by the mixing with cement (Rafalko et al. 2007; Liu et al. 2008; Chan et al. 2011). When marine clay is blended with Portland cement in the presence of water, hydration reactions will take place and calcium silicate hydrates are formed. The gel of calcium silicate hydrates fill up the void space and binds the clay particles together increasing strength of material. Usually, the best results were obtained at cement to clay ratio from 30 to 50 % (w $w^{-1}$). Therefore, conventional cementation of soft marine clay is expensive method for its transformation to a fill material. Therefore, a new opportunity to strengthen waste marine clay has to be found. An alternative way for conventional cement usage could be strenthening of marine clay using encapsulation by calcite.

An effective way for the conservation or utilization of soft solid or semisolid waste like sludges and slurries could be their encapsulation. Encapsulation of soft marine clay also could be a way to reduce the cost of clay strengthening and production of value-added solid fill material. We examined biotechnological transformation of soft marine clay to solid construction material through


**Fig. 10.20** Soft clay aggregates coated with calcite

encapsulation of clay aggregates with biogenic calcite (Ivanov et al. 2015a, b). This encapsulation was performed by the mixing of soft marine clay with dry biomass of urease-producing bacteria, following with the treatment of clay aggregates in the solution of calcium chloride and urea. The encapsulated clay aggregates (Fig. 10.20) contained up to 15 % (w $w^{-1}$) of precipitated calcium due to formation of the calcite shell. Optimal size of aggregates was about 5 mm (Fig. 10.21). Encapsulation increased the unconfined compressive strength of marine clay

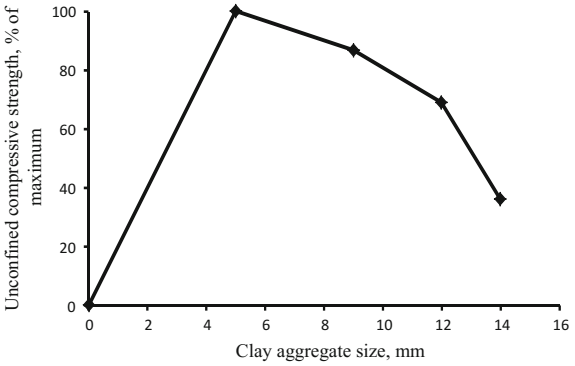



**Fig. 10.21** Effect of size on strength of clay aggregate

aggregates of 5 mm size from almost 0 to more than 2000 kPa, so that encapsulated marine clay can be used as fills. Biotechnological encapsulation could be considered as an alternative technology for the conventional strengthening of soft marine clay by cement.

## 10.17 Other Biotechnologies of Biocoating

Only the cases with the coating of the surface by MICP were considered in this chapter. However, it is clear that other biotechnologies that were considered in the chapters on biocementation and bioclogging can be also used, example are the following:

(1) biocoating using aerobic biooxidation of organic salts of calcium onto surface. For example, oxidation of calcium salt by *Bacillus pumilus* coated marble with a fine layer of vaterite which could be used for on-site stone conservation (Daskalakis et al. 2015);
(2) biocoating using calcium phosphates precipitate onto the surface;
(3) biocoating using calcium bicarbonate decay during its drying;
(4) biocoating using delayed decay of high concentration calcium bicarbonate solution using calcium chelates;
(5) biocoating using self-decay of dialkyl carbonates, for example dimethylcarbonate, in solution of calcium salt (Faatz et al. 2004). This could be an effective process if the cheap dimethylcarbonate can be produced.

# Chapter 11
# Bioremediation and Biodesaturation of Soil

## 11.1 Toxic Pollutants

Soil, surface water, groundwater, and atmosphere on the construction sites are often contaminated by pollutants, which are toxic substances for human, animals, and plants. After disposal or discharge of toxic chemicals to soil their transport and fate are almost not controlled. Pollutant can be transported by different mechanisms to air, water, and food (Fig. 11.1). Serious soil and groundwater contamination problems result from spills during production, transport, and storage of chemicals.

## 11.2 Bioremediation of Soil

There are known physical and chemical methods of the remediation of polluted soil but biological methods are often most cost effective but usually much slower than physical and chemical methods. Bioremediation of soil is an application of microbial biodegradation capability to clean up contaminated sites. In the majority cases, petroleum products are involved in the pollution of sites.

Bioremediation can be done through following types of the biogeochemical reactions:

(1) oxidation of toxic substance $S_1$ with formation of non-toxic substance $S_2$

$$S_1 - e^- \rightarrow S_2, \tag{11.1}$$

(2) Reduction of toxic substance $S_1$ with formation of nontoxic substance $S_2$

$$S_1 + e^- \rightarrow S_2, \tag{11.2}$$



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Green Energy and Technology, DOI 10.1007/978-981-10-1445-1_11

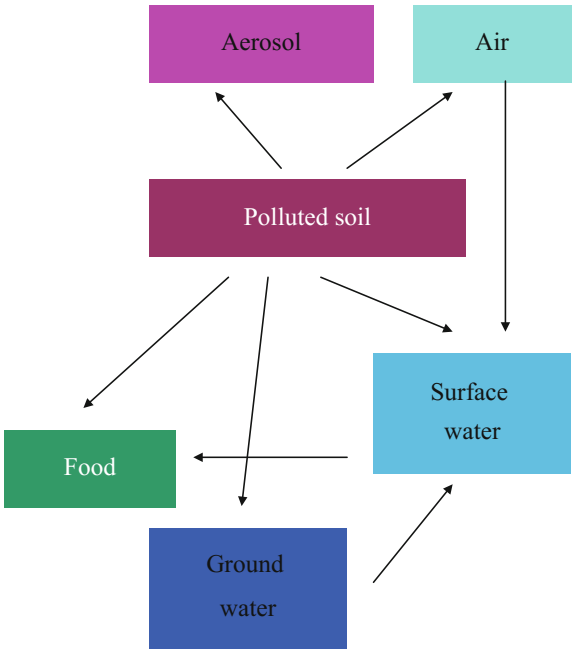


**Fig. 11.1** Distribution of pollutant from soil

(3) Mineralization of toxic organic substance $S_1$, i.e., its oxidation to $CO_2$

$$S_1 \rightarrow CO_2, \tag{11.3}$$

(4) Chemical transformation of toxic substance $S_1$ by addition/removal of radical $R$ with formation of nontoxic substance $S_2$

$$S_1 \pm R \rightarrow S_2, \tag{11.4}$$

(5) Physical or chemical immobilization of toxic dissolved substance $S_1$ by addition/removal of substance $S_2$ with formation of undissolved complex of $S_1$ and $S_2$

$$S_1 \pm S_2 \rightarrow S_1 - S_2. \tag{11.5}$$

## 11.3 Bioremediation Options

Bioremediation of the polluted construction site can be performed in the following options:

(1) in situ ("in its original place") treatment through the injection of microorganisms and nutrients;

(2) "on site" treatment through the recycle of microorganisms and medium through the bulk of polluted soil;
(3) "off site" biotreatment through excavation and slurry-phase biotreatment or composting. The clean solids are redeposited in the original site, while the liquid may be used in the next soil treatment as an inoculum.

## 11.4 Advantages and Disadvantages of Biogeotechnologies for Remediation

Bioremediation offers several advantages over physical and chemical treatment processes used to treat contaminated water and soil

(1) cleanup costs using bioremediation typically several times lower than conventional technologies such as incineration or secure landfilling;
(2) final goal of bioremediation is biodegradation or at least detoxication of the hazardous contaminant, whereas other physical, physico-chemical, or chemical technologies transfer the contaminant to a location or to another form. This is acceptable for such conventional technologies of remediation as adsorption onto activated carbon, solidification with cement, soil washing and venting, disposal to landfill. It is always a risk of the pollutant secondary leaching after these treatments;
(3) bioremediation is a relatively simple technology with minimal secondary pollution of soil, water, and air, with minimal health risk to residents or occupants if the process is performed properly. However, if not proper fulfilled and not finished biodegradation can produce more toxic chemicals than initial ones and can be a reason of secondary pollution of environment with microbial spores and toxic metabolites.

## 11.5 Problems of Bioremediation

1. Absence of indigenous soil microorganisms capable to degrade soil pollutant so pure or enrichment culture of microorganisms must be introduced, which requires a lot of the tests and approvals;
2. Aerobic microorganisms are most effective on biodegradation but transfer of oxygen into polluted soil or water for microbial growth and oxidation is difficult technical problem;
3. There must be supply of soluble nutrients for microbial growth and biodegradation but growth of microbial biomass can plug the soil pores.
4. The difficulty of predicting performance and the scaling up from laboratory to practice. Success of a bioremediation project depends on too many physical, chemical, and biological factors.

5. Microorganisms are sensitive to temperatures, pH, contaminant toxicity, contaminant concentration, moisture content, nutrient concentration, and oxygen concentration. A decrease in microbial activity will slow down the biodegradation and extend the treatment period. If microbial activity stops (e.g., due to accumulation of toxic metabolites), restarting the process may be difficult.
6. Clean up goals may not be achievable during bioremediation because some contaminants are non-biodegradable or only partially biodegradable, or because the levels of pollution is too high. As the contaminant levels diminish, the biological degradation slows down, and the microorganisms may switch to other energy sources or stop growing.
7. Bioremediation process is time-consuming. The time required generally depends on the rate at which the contaminants are degraded but the expected duration of soil bioremediation is typically about several months.

## 11.6 In Situ, on-Site, and off-Site Bioremediation

During in situ treatment, the activity of the degrading microorganisms in the polluted soil is stimulated by the introduction of oxygen, nutrients, and if needed, inoculum of microorganisms through the wells.

Most common approach of on-site treatment is that polluted groundwater with diffused from soil contaminant(s) is pumped to the surface, mixed with nutrients and microbial suspension, treated by stirring and aeration for a time sufficient for biodegradation of the pollutant, usually several hours or days, and then returned to the polluted site by percolation through the bulk of soil or by injection into polluted soil. Surface biotreatment may also be physical, and involve adsorption onto activated carbon or air stripping.

Excavation and off-site treatment can be performed by landfarming/composting in windrows, in aerated piles, or in case of heavy pollution in the rotating drum or in the slurry-phase reactors. Oxygen of air can be replaced by other oxidants such as ozone, hydrogen peroxide, or nitrate. Exhausted gas after reactors must be also treated to retain microbial cells or their spores, volatile pollutants or products of their partial biodegradation. The solids after treatment are redeposited in the site of excavation, while a portion of the slurry is retained in the slurry-phase reactor to be used as microbial inoculum to the next cycle of the treatment.

## 11.7 Microbiological Preparations for Bioremediation

Several microbial strains can perform different steps of biodegradation so not in all cases one pure culture can be used for bioremediation of polluted soil. Mixed culture of several strains can also be more stable than pure culture in terms of

**Table 11.1** Steps in microbial culture development for bioremediation

| Step | Objective | Comment |
|---|---|---|
| 1. Selection of microbial sources | To find microbial groups or species capable of degrading the target contaminants | More than one source should be used. Soil and activated sludge should be included |
| 2. Growth of cultures in the laboratory | To select the best sources of microorganisms for future use | Mixing sources may be a good strategy |
| 3. Determination of kinetic and stoichiometric parameters | To develop design and operating parameters | Design of oxygen transfer and nutrient addition requirements will depend on this step |
| 4. Determination of toxicity limitations | To determine concentration limits on operation | Toxicity to microorganisms is a common problem |
| 5. Growth of dense cultures | To provide microorganisms for treatment processes | In general, the higher the population density, the better |

process performance. The actual strains and species present may change with time, but there may be not significant effect of these changes on process performance if a number of species can carry out the biodegradation reactions.

Steps in microbial culture development for bioremediation are shown in Table 11.1.

## 11.8 Biotechnological Control of Dispersion of Pollutants

Water erosion of soil is one of the most important environmental problems causing land degradation as well as pollution of surface water (Pimentel et al. 1995). It is estimated that soil erosion on Earth is about 0.38 mm/year (Yang et al. 2003a, b) and the rate of the world's arable land loss is more than 10 million hectares per year (Pimentel et al. 1995). Water erosion is considered the main type of soil erosion in Europe, and the area of land degraded by water erosion in Europe is about 114 million hectares (Gobin et al. 2004).

Water erosion of soil on the fields fertilized with the cattle or chicken manure can be also a source of bacterial contamination of surface and ground waters (Jamieson et al. 2002; Ramos et al. 2006). Runoff from the surface of soil containing fertilizers, pesticides, heavy metals, and other pollutants causes pollution of natural water bodies and has strong economic and ecological impacts (Gilley et al. 2012; Stoate et al. 2001).

A number of techniques can be used to control water erosion of soil. These include tillage techniques, for example, leaving straw on the soil surface (Chambers et al. 2009) or application of non-toxic anionic polyacrylamides to stabilize soil structure through aggregation of soil particles (Mamedov et al. 2010; Lee et al. 2011; Sojka et al. 2006). However, treatments with chemical reagents have not been used in large scale because of their high cost.

Microbial aggregation of soil particles could be more cost-effective approach because it is a common soil bioprocess in nature. Microorganisms can produce different effects on soil texture and structure:

- bioaggregation of soil or particles is an increase of the fine particles size due to microbially mediated formation of minerals, biopolymers or microbial biofilms. Bioaggregation significantly diminish wind erosion of soil and dust emission (Bang et al. 2011; Stabnikov et al. 2013a), so similar effect of bioaggregation could be expected for water erosion of soil;
- biocrusting of soil surface is formation of mineral or biofilm crust onto soil surface by microorganisms, usually photosynthetic ones, so that the erosion, dust emission, and water infiltration will be reduced. The thickness of the mineral biocemented crust can be up to several mm (Stabnikov et al. 2011; Chu et al. 2012a);
- bioclogging of soil or porous matrix is the filling of the pores and channels in soil by microbially produced minerals or biopolymers so that hydraulic conductivity of soil will be significantly reduced (Ivanov et al. 2012);
- biocementation of soil is the binding of the particles to increase significantly strength of soil (Ghosh et al. 2005; Mitchell and Santamarina 2005; Whiffin et al. 2007; Ivanov and Chu 2008; De Muynck et al. 2008a, b, 2010a, b; Sarda et al. 2009; van der Ruyt and van der Zon 2009; Achal et al. 2010a, b; Ivanov 2010; Van Tittelboom et al. 2010; Dosier 2014; Chu et al. 2012a; DeJong et al. 2010, 2013; van Paassen et al. 2010; Harkes et al. 2010; Dhami et al. 2012; Li and Qu 2012; Pacheco-Torgal and Labrincha 2013a, b; Raut et al. 2014);
- biodesaturation of soil is decreasing water saturation of soil and liquefaction potential of saturated soil through biogas production in situ (Chu et al. 2009a, 2013b; He et al. 2013; Rebata-Landa and Santamarina 2012);
- bioencapsulation of soft clayey soil is an increase of the strength of soft soil through formation of strong shell around a piece of soft material (Ivanov et al. 2014a, b, c);
- bioremediation of polluted soil is removal of the pollutants from soil or immobilization of the pollutants in soil to diminish the release of the pollutants from soil to air, water, human, plants and animals (Warren et al. 2001; Fujita et al. 2004; Mitchell and Ferris 2005).

Therefore, microbial control of water erosion of soil can include bioaggregation of fine soil particles, biocrusting of soil surface, biocementation of soil layer, and bioremediation of polluted soil. There are possible different technologies of biocementation (Ivanov and Chu 2008) but most popular method is microbially induced calcium carbonate precipitation (MICP) performed by urease-producing bacteria in the presence of urea and calcium ions (Mitchell and Santamarina 2005; Ivanov and Chu 2008; DeJong et al. 2010, 2013; Ivanov 2010). MICP includes formation of calcium carbonate minerals such as calcite, vaterite, or aragonite due to: (1) adhesion of cells of urease-producing bacteria (UPB) on the surface of

particle; (2) creating a microgradient of concentration of carbonate and pH in the site of cell attachment due to hydrolysis of urea by urease of UPB.

## 11.9 Leaching of the Pollutants from Sand

In our experiments, 50 g of the fine sand was artificial polluted by 20 mL stock solution of phenantrene and was dried at 30 °C for one day, then this sample of the fine sand was incubated with 20 mL of stock solution of $Pb(NO_3)_2$ and was dried at 30 °C for one day. After this, sample of the fine sand was treated by 20 mL of cultural liquid of *Bacillus megaterium* DSMZ 90 with concentration of dry biomass 3.5 g/L and was dried at 30 °C for one day. For pollution of coarse sand with led, an aliquote of 300 mL of stock solution containing ions of $Pb^{2+}$ was added to 1 kg (about 0.6 L) of coarse sand. Polluted sand was placed in the channels and dried at 60 °C.

The laboratory facility to study the fine sand bioaggregation consisted of three plastic channels with length 10 cm, width 0.7 cm and heights 1.5 cm each placed on the plastic tray with slope 11°. Inlet and outlet of the channel were closed with the plastic shields 0.4 cm height that corresponds to the height of fine sand layer in the channel. Polluted fine sand, 500 mg, was placed in each channel. Nothing except the polluted fine sand was placed in control 1. Sodium chloride solution with concentration 0.85 %, 150 μL, was added to the fine sand in control 2. Bioaggregation of the fine sand was performed in experiment where the culture liquid of UPB, 150 μL, and calcium chloride, 0.75 M (82.5 g/L), and urea, 1.5 M (90 g/L) solution, 150 μL, were added for 2 days incubation of fine sand at room temperature. The volume of the added solutions (0.3 mL) was about twice bigger than the volume of the fine sand pores. The dosage of Ca was 6.4 $g/m^2$ or 9 g Ca/kg of sand. To model water erosion of fine sand, 40 mL of distilled water was supplied to the top of each plastic channel with the rate 3 mL/min, so that linear velocity of water in the channel was 0.17 cm/s.

Almost all wet fine sand, 87 % of initial weight, was removed with the flow of water in control. However, after bioaggregation only 27 % of fine sand was removed with the flow of water (Table 11.2). The calculated erosion rates before

**Table 11.2** Bioagregation control of the leaching of the sand-associated pollutants

| Control and experiment | Fine sand removed with water, % of initial quantity | Quantity of modeling pollutant removed altogether with the fine sand, % of initial content | | |
|---|---|---|---|---|
| | | Phenanthrene | Pb | Bacterial cells |
| Control (erosion of wet fine sand) | 87 | 99 | 82 | 45 |
| Experiment (erosion of bioaggregated fine sand) | 27 | 39 | 26 | 5 |

and after bioaggregation of fine sand were 65.8 and 20.2 kg/m$^2$d, respectively. So, removal of fine sand in experiment was about 30 % of that in control.

The particle size distributions showed that the maximum size of 90 % of the particles ($d_{90}$) was 40 and 80 μm before and after bioaggregation, respectively. So, bigger bioaggregated particles were removed by water with lower rate from the fine sand. These results were similar to ones obtained in the study of the wind erosion of aggregated fine sand, where the sizes of 90 % of the fine sand particles increased from 29 to 181 μm after bioaggregation (Stabnikov et al. 2013b).

Formation of biological or mineral soil crust is a major factor controlling soil erosion in nature (Pietrasiak et al. 2013). Control of sand erosion in our experiments was due to the formation of the crust produced by the binding of the sand particles due to microbially induced calcium carbonate precipitation. Usually, such crust is formed by percolation (Stabnikov et al. 2011; Chu et al. 2012b, c; Cheng and Cord-Ruwisch 2012) but we did it using spraying because it is most practical way to form the aggregated sand.

MICP was studied for solid-phase capture of inorganic contaminants of common groundwater contaminants (Sr, $UO_2$, Cu) in laboratory batch experiments (Warren et al. 2001) but the immobilization of lead, polycyclic aromatic hydrocarbons, and bacterial cells was shown at first time.

## 11.10 Biomediated Immobilization of Sand-Associated Lead

Lead was removed due to water erosion from the content of 1.5 mg/g of sand to 0.4 mg/g of sand; however, even low dosage of precipitated calcium of 0.8 g/kg of sand decreased the content of lead in the removed sand by 50 % of initial content. Immobilization of lead in the coarse sand could be due to formation of insoluble lead hydroxides at pH 9.5, which is typical value of pH in the sites of urease-dependent calcium carbonate precipitation. It could be also as precipitation of lead carbonate in the sites of biocementation with high concentration of carbonates there. There are known many other low-cost methods of lead immobilization in soil (Cao et al. 2011; Lim et al. 2013) however, the described method ensures both immobilization of the pollutants in soil and its high resistance to erosion.

## 11.11 Potential Application of MICP Against Accidental Pollution

Usually, the aim of MICP in environmental engineering is to prevent the dispersion of hazardous substances from the accident site into the environment. It is performed through biocementation of either soil surface or the bulk of soil and using artificial

formation of geochemical barrier. MICP has the ability to co-precipitate toxic radionuclides $^{90}Sr$, $^{60}Co$, and metal contaminants such as Cd and this can be used to prevent their dispersion in environment (Fujita et al. 2004; Mitchell and Ferris 2005). MICP capturing 95 % of the 1 mM Sr added to soil (Warren et al. 2001). After MICP treatment of sand surface with the quantity just 15.6 g Ca/$m^2$ the release of the sand dust and its artificial pollutants to atmosphere decreased in comparison with control by 99.8 % for dust, 92.7 % for phenantherene, 94.4 % for led nitrate, and 99.8 % for bacterial cells of *Bacillus megaterium.* This immobilization of dust and dust pollutants was due to bioaggregation of the fine sand particles. The sizes of 90 % of the sand dust particles increased from 29 μm in control to 181 μm after bioaggregation. Bioaggregation treatment of the soil surface could be useful method to prevent the dispersion of dust and the dust-associated chemical and bacteriological pollutants in water, air and soil (Stabnikov et al. 2013b), so it could be useful in construction and probably to protect atmosphere in fixation response to a radiation dispersal devise attack (Cordesman 2002; Parra et al. 2009).

Another way of environmental protection from the polluted site can be construction of permeable, reactive, biogeochemical barriers, for example $Fe^0$- or $Fe^{3+}$-reducing barriers, which will precipitate heavy metals and degrade toxic chemicals (Kavamura and Esposito 2010; Gibert et al. 2013). There may be sulfate-reducing biogeochemical barrier for precipitation of heavy metals and disinfection of groundwater or aerobic biogeochemical barrier for biodegradation of organic pollutants.

## 11.12 Biomitigation of Soil Liquefaction Through Biogas Production in Situ

Specific case of ground improvement is partial soil biodesaturation that diminishes the risk of soil liquefaction and accompanying damages of infrastructure during the earthquake.

Biocementation of loose sand using a MICP process to increase the liquefaction resistance of sand has also been reported by DeJong et al. (2006) and Montoya et al. (2012). It was shown (Montoya et al. 2012) that the resistance of sand to liquefaction, as measured by a decrease in the excess pore water pressure ratio, was significantly increased after MICP. However, this process may be more expensive as the costs of calcium salt and urea are relatively high. The generation of ammonium can also cause environmental concerns.

Energy-effective approach for compaction grouting of loose sands is induced partial saturation that can be performed by introduction of gas in saturated sand and entrapment of gas bubbles there (Yegian et al. 2007). This induced partial desaturation in loose saturated sands can decrease an excess of pore water pressure and increase the bearing capacity and shear strength of the soil, which is beneficial in

foundation design and roadway construction (Seagren and Aydilek 2010). It has been demonstrated that the liquefaction resistance of saturated sand can be significantly increased when the sand is slightly desaturated with some voids displaced by gas (Yegian et al. 2007; He et al. 2013; He and Chu 2014). Even small decrease in the degree of sand saturation to 99–97 % increased resistance of water-saturated sand to liquefaction by 30–40 % (Xia and Hu 1991; Yang et al. 2003a, b), while reduction of the sand saturation to 90 % can increase resistance of water-saturated sand to liquefaction twice (Chaney 1978; Yoshimi et al. 1989). An injection of air into ground to desaturate the sand and increase its liquefaction resistance was done in the real scale (Yoshimi et al. 1989; Okamura et al. 2006, 2011; Okamura 2006). However, gas injected in this way may be not evenly distributed. Eseller-Bayat et al. (2012) has proposed a method to generate oxygen bubbles in situ using a chemical compound sodium percarbonate ($Na_2CO_3{\cdot}3H_2O_2$). However, oxygen tends to react with minerals in soil. Thus, the amount of gas may reduce with time.

## 11.13 Denitrification as a Source of Biogas Production in Situ

Microbiological production of gas in soil was proposed to introduce smaller and more stable gas bubbles in saturated soil (Rebata-Landa 2007; He et al. 2013). This method is an introduction of nitrogen gas bubbles into soil using biochemical reduction of nitrate (denitrification) in situ. It is most preferable approach because nitrogen gas is chemically inert substance (Rebata-Landa and Santamarina 2012; Seagren and Aydilek 2010).

Different organic and inorganic substances can be biooxidized by nitrate but ethanol ($C_2H_5OH$), acetic acid ($CH_3COOH$), or glucose ($C_6H_{12}O_6$) that can be as used as 75 % (w/v) syrup from corn, are most suitable electron donors because of their low cost, availability, and high solubility in water. Their biooxidation by nitrate (denitrification) is shown below

$$1.67C_2H_5OH + 2NO_3^- \rightarrow N_2 \uparrow + 3.33CO_2 + H_2O + 2OH^-, \tag{11.6}$$

$$1.25CH_3COOH + 2NO_3^- \rightarrow N_2 \uparrow + 2.5CO_2 + 1.5H_2O + 2OH^-, \tag{11.7}$$

$$0.42C_6H_{12}O_6 + 2NO_3^- \rightarrow N_2 \uparrow + 2.5CO_2 + 1.5H_2O + 2OH^-. \tag{11.8}$$

The stoichiometrical parameters of these reactions are almost same: consumption of electron donor is 3.4 kg/m$^3$ of $N_2$ and consumption of electron acceptor (sodium nitrate) is 7.6 kg/m$^3$ of $N_2$. The consumption of electron donor and acceptor for 10 % (volume of gas/volume of water) desaturation of soil with porosity 50 % is 0.55 kg/m$^3$ of saturated soil. Production of carbon dioxide in reactions 11.6–11.8, which is from 120 to 159 g/m$^3$ of $N_2$ or from 12 to 16 g/m$^3$ of water in saturated

soil with 50 % porosity, is not accounted for desaturation of soil because solubility of $CO_2$ in water at 10 °C is 2500 g/m$^3$.

There is almost no cost difference between these electron donors: the cost of electron donor is from \$0.5 to \$0.7/kg, the cost of electron acceptor (sodium nitrate) is from \$0.4 to \$0.5/kg, so the estimated cost of electron donor and acceptor for partial desaturation is from \$5.1 to \$6.2/m$^3$ of $N_2$. The estimated cost of electron donor and acceptor for 10 % (volume of gas/volume of water) desaturation of soil with porosity 50 % is from \$0.25 to \$0.31/m$^3$ of saturated soil.

## 11.14 Stability of Biogas Bubbles in Soil

A problem of a long-term sustainability of gas bubbles in sand during upward or horizontal flows of groundwater is remained most important for practical application of ICP. Stability of nitrogen gas bubbles can be ensured by low hydraulic conductivity of soil due to the decrease in the size of the water-conducting pores because of soil pores clogging by the gas bubbles (Baveye et al. 1998; Seagren and Aydilek 2010). Experiments of Yegian et al. (2007) showed that under hydrostatic conditions the degree of saturation of sand with introduced gas bubbles slightly increased from about 83 to 84 % after 442 days. Similar results for hydrostatic conditions were obtained by He (2013). Field data of Okamura et al. (2006) showed that gas bubbles introduced in sand during sand compaction piling remained entrapped there for 26 years.

Meanwhile, there are controversial data on the stability of gas bubbles in partially saturated sand in case of vertical or horizontal flow of water. It is known that from the work of He (2013) on the production of biogas in situ using denitrifying bacteria that nitrogen gas bubbles are not stable in 1 m length sand column during upward or downward flows of water with hydraulic gradient 0.1. These bubbles disappeared from sand after 2–4 days increasing degree of sand saturation up to 100 %. For one day of experiment, saturation of sand increased only from 89 to 92 %. Other data (Eseller-Bayat et al. 2012) showed that after 18 h of a vertical upward flow with hydraulic gradient from 0.05 to 0.5 with average hydraulic retention time 0.36 h the degree of saturation increased only from 82.6 to 83.6 %. So, long-term sustainability of the gas bubbles in sand under hydrostatic conditions has no doubts but under conditions of vertical or horizontal flows of groundwater in

**Table 11.3** Parameters of sand after sequential biotreatment

| Parameter | Denitrification | Denitrification + surface biocementation | Denitrification + bulk biocementation |
|---|---|---|---|
| Gas bubbles instability, % | 26 ± 6 | 0 | 0 |
| Compressibility of loose sand, % | 1.6 ± 0.5 | 0.6 ± 0.5 | 0 |

partially saturated sand the gas bubbles will be probably not stable. Therefore, for practical implementation of induced partial desaturation in loose sands using biogas production in situ some additional technological solutions must be developed to ensure long-term sustainability of sand desaturation under groundwater flows. In our experiments, the combination of biogas production in situ with the biosealing of the biogas bubbles in sand using small quantity of biocement for long-term sustainability of partial saturation of loose sand was the solution to stabilize gas bubbles in soil. Denitrifying bacteria reduced sand saturation due to production of biogas but up to half of this biogas volume was removed from sand pores under centrifuge acceleration $600 \times g$. However, if the wet sand with gas bubbles was biosealed with calcium carbonate crystals produced by urease-producing bacteria the instability of biogas bubbles decreased to zero (Table 11.3). Approximately same results of biogas production and stability have been produced in other experiment denitrifying bacteria *Paracoccus denitrificans* DSM 143 in the sand columns and then activity of UPB *Bacillus* sp. VS1. Their application described in the chapter on bioclogging. So, the biotechnology for mitigation of soil liquefaction has to combine two steps: (1) production of gas microbubbles in sand by denitrifying bacteria on the medium with ethanol (acetate) and nitrate, and (2) fixation of these microbubbles in sand using MICP with calcium salt (0.75 M solution) and urea (1.25–1.5 M solution).

## 11.15 Biogas Production in Situ Decreased Primary Consolidation Settlement in Clayey Soils

One experimental and theoretical study was done on production of biogas in clayey soil by anaerobic fermenting bacteria *Clostridium acetobutylicum* to partially force water out of the pore space to make the pore fluid compressible, so that during loading a portion of total stress is immediately transferred to effective stress (Puzrin et al. 2010). The materials used for land reclamation must be consolidated prior to loading, using expensive preloading techniques. This treatment shifted up to 50 % of total displacement from primary consolidation settlement to immediate settlement without changing total displacements or the coefficient of consolidation. The potential benefit of this in situ biogas production could be the shorter time required for preloading (Puzrin et al. 2010).

# Chapter 12
# Optimization and Design of Construction Biotechnology Processes

## 12.1 Urease Activity of MICP Agent

Many factors could affect the efficiency of UPB production and their urease activity—pH value, urea concentration, calcium concentration, and temperature (Ferris et al. 2003; Fujita et al. 2000; Stocks-Fischer et al. 1999; van Paassen 2009a, b; Whiffin 2004; Cheng and Cord-Ruwisch 2012; Chu et al. 2013a, b; DeJong et al. 2006, 2010; Whiffin et al. 2007). The major factor is usually the strain of UPB that is used as a bioagent.

Pure culture of halophilic, alkaliphilic, urease-producing bacteria *Sporosarcina pasteurii* DSMZ 33 (ATCC 11859, CCM 2056, NCIB 8841, and NCTC 4822) was used in majority of MICP experiments and tests. *S. pasteurii* is widely used in all related studies, because of its halotolerant and alkaliphilic characters (Bachmeier et al. 2002; Bang et al. 2001a; DeJong et al. 2006; Ferris et al. 1996; Mitchell and Ferris 2005; Mortensen and DeJong 2011; Whiffin et al. 2007). Furthermore, the strains have to produce high urease activity, not repressed by $NH_4^+$, while being nonpathogenic (Whiffin 2004). A lot of phylogenetically diverse bacterial strains able to produce urease constitutively in the presence of high levels of ammonia were isolated (Burbank et al. 2012).

Urease activity of different strains of *S. pasteurii* varies from 5 to 20 mM urea/min (Harkes et al. 2010); 2.2 to 13.3 mM urea/min (Whiffin 2004); average 11.3 mM urea/min (Cheng and Cord-Ruwisch 2012). A strain MCP11 with high urease activity of 11–28 mM urea/min was selected (Al-Thawadi 2008).

Some other isolated Bacillus strains of UPB have urease activity varied from more than 3.3 mM urea/min (Al-Thawadi and Cord-Ruwisch 2012b); $8.3 \pm 0.1$ mM urea/min for strain VS1, and $9.0 \pm 0.0$ mM urea/min for stain VUK5 (Stabnikov et al. 2013a, b).



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## 12.2 Kinetics of Urease

Kinetics of urease for the suspended cells of *S. pasteurii* can be described by a Michaelis–Menten equation ($R^2 = 0{\cdot}95$) with $K_m = 0.3$ M of urea and $V_{max} = 0.2$ M $h^{-1}$ or 3.3 mM $min^{-1}$. A first-order model with $k = 0{\cdot}35$ $h^{-1}$ fits the data even better ($R^2 = 0{\cdot}99$) for urea concentrations up to 0.33 M. Maximum of urease activity for one cell was $6.4 \times 10^{-9}$ mmol $CFU^{-1}$ $h^{-1}$. Rate of ureolysis linearly correlated with cell concentrations in the range from $1 \times 10^7$ to $2 \times 10^8$ CFU $ml^{-1}$. pH in the range from 6 to 9 or ammonium concentrations up to 0.19 M did not affect the ureolysis rate (Lauchnor et al. 2015). These parameters of ureolysis can be used in design of MICP.

## 12.3 Genetically Engineered Strains of UPB

Genetically engineered strains with high urease activity can be constructed and used for bioclogging/biocementaton (Bergdale et al. 2012). In one experiment, the entire *S. pasteurii* urease gene sequences including ureA, ureB, ureC, ureD, ureE, ureF, and ureG from plasmid pBU11 were subcloned into the shuttle vector, pUCP18. The constructed plasmid, pUBU1, was transformed into two *Pseudomonas aeruginosa* strains, 8821 and PAO1, to develop recombinants capable of inducing calcite precipitation in addition to their own ability to produce EPS. Nickel-dependent urease activities were expressed from the recombinant *P. aeruginosa* 8821 (pUBU1) and *P. aeruginosa* PAO1 (pUBU1), at 99.4 and 60.9 % of the *S. pasteurii* urease activity (Bergdale et al. 2012). It was just the proof of principle of the recombinant engineering creation of a new bioclogging/biocementing bacterial strain. Practically, it could be not too suitable to produce, cultivate, and use the genetically engineered strains of UPB because the critical point of MICP is to create maximum of the crystallization and adhesion points on the surface rather than highest level of urease activity of these points.

## 12.4 Media for Cultivation of Bioagent

Most popular for biocementation/bioclogging species of *S. pasteurii* requires for cultivation medium with high concentration of nutrients, especially amino acids, so different kind of broths after enzymatic digestion of soya flour, casein, or meet are used for their cultivation. Production of bacterial biomass for MICP affects economy of biocement and technology of the biocementation (Achal 2015). Selection of strain, its cultivation, raw material for growth, methods to increase urease activity, post-cultivation treatment of biomass, its storage are essential points in the technology of MICP.

Cheap lactose mother liquor (liquid after recovering lactose from cheese or casein whey) can be used as an alternative source for standard media because there were no significant difference in the growth, urease production, and compressive strength of mortar among this and conventionally used media (Achal et al. 2009a, b).

Most often, the media containing yeast extract and soy broth are used, for example, g/L: Tryptic Soy Broth DIFCO™, 30; Yeast Extract, 10 g; NaCl, 20; $MnCl_2 \cdot 4H_2O$, 14 mg/L; $NiCl_2 \cdot 6H_20$, 24 mg. Urea, 10–20 g/L is added to the sterile medium using the Millipore system for membrane sterilization.

An example of batch cultivation on this medium at 30 °C, aeration rate 1.5 L of air/L of liquid/min with addition of silicone antifoam, and pH control at 7.3 using 1 M HCl is shown in Fig. 12.1. Maximum of biomass concentration was at 24 h, while urease activity increased even after 72 h of cultivation. However, in majority experiments optimal time for UPB *Bacillus* sp. VS1 batch cultivation was around 48 h because no increases of biomass concentration and total urease activity were found after that time (Stabnikov et al. 2013a, b).

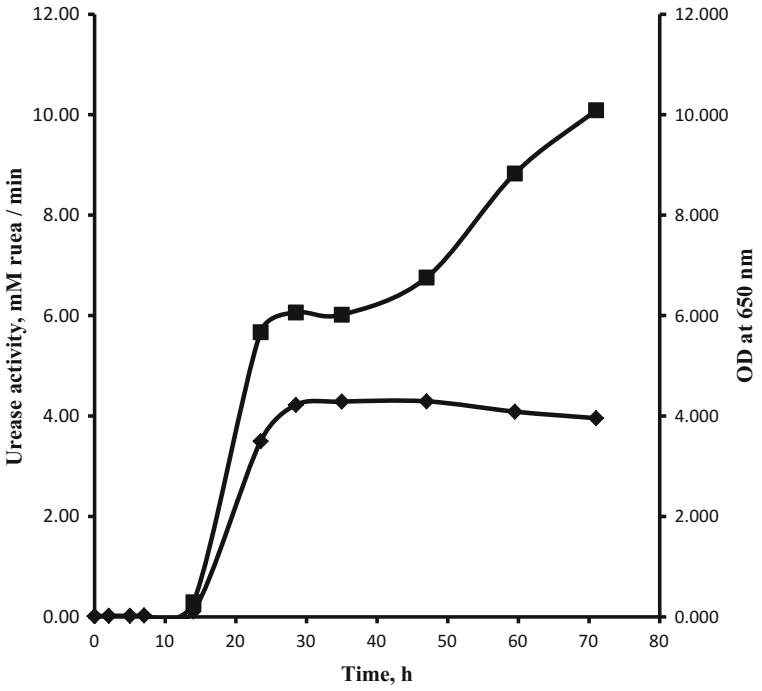


**Fig. 12.1** Growth of biomass measured by OD at 650 nm and urease activity of culture. Measurement of urease activity was done for a period of 30 min (measured value depends on the period of measurement)

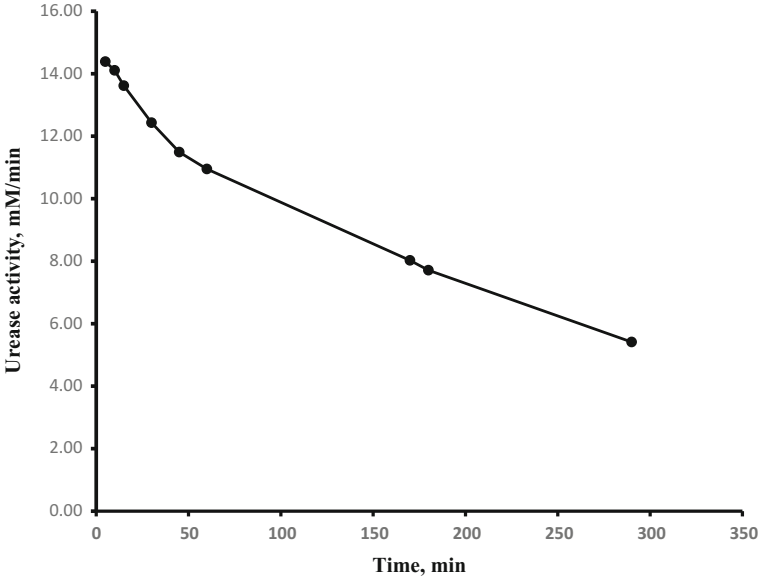


**Fig. 12.2** Urease activity of biomass before freeze-drying. Concentration of dry biomass in 1 % NaCl solution was about 10 g/L

pH of biomass suspension after centrifugation was 6.1 and initial urease activity was 14.4 mM/ min but decreased twice for about 220 min (Fig. 12.2).

In our experiments on production of dry biomass of *S. pasteurii* as a component of biocement the Medium 220 + urea, 20 g/L was used. Content of the Medium 220 (CASO AGAR, Merck 105458) is as follows: peptone from casein, 15.0 g; peptone from soymeal, 5.0 g; NaCl, 5.0 g; distilled water, 1L, pH 7.3. Cultivation of the strain *S. pasteurii* DSMZ 33 has been done in the 50 L sterilizable-in-place fermenter (ABEC, Inc., Bethlehem, PA, USA) at 30 °C. Centrifugation of biomass was done in CEPA Z-41 high-speed centrifuge (Eppendorf AG, Hamburg, Germany), then biomass was suspended in 1 % of NaCl and freeze-dried in VirTis Ultra 35L Pilot Lyophilizer (VirTis SP Scientific, Stone Ridge, NY, USA). Biomass can be used as a component of dry biocement or as suspension in 1 % of NaCl.

Important point is post-cultivation treatment of biomass. Drying or addition of calcium salt to bacterial suspension form bacterial aggregates, which cannot penetrate deeply into porous soil or fissured rocks (Stabnikov et al. 2011). Some strains of bacteria with urease activity have flocculating, self-aggregating cells (Al-Thawadi 2008). This genetic property can limit the depth of penetration of bacterial cells in soil.

## 12.5 Optimum of Urease Activity for MICP

Although urease activity is a most important factor of MICP, the increase of urease activity does not mean better MICP. Cheng and Cord-Ruwisch (2014) proposed a simple mathematical model to predict the amount of calcite precipitation along the depth of a saturated sand column at different urea hydrolysis rates. For a fixed infiltration rate, the predicted homogeneity of the precipitated calcite distribution along the depth of a sample improves as the urease activity decreases. Therefore, using UPB with low activity could lead to a more uniform cementation within sand. Such observation, i.e., stronger MICP at lower urea hydrolysis rate, was also made by Qian et al. (2009). The results of other researchers (Fidaleo and Lavecchia 2003; Martinez et al. 2013) showed that the experimental sets with lower urea hydrolysis rate than in control resulted in a more uniform MICP. So, optimum of urease activity must be determined for each case.

## 12.6 Extra- and Intracellular Urease

The majority of urease activity, about 90 %, is connected with cell and only about 10 % is extracellular urease. Therefore, it is easy to increase urease activity of bioagent just by centrifugation. Extracellular polymeric substances (EPS) of bacterial suspension inhibited MICP crystallization probably due to chelation of $Ca^{2+}$ ions (Qian et al. 2010b). Another reason could be presence of protease degrading urease in bacterial suspension (Chu et al. 2013a, b).

Bacterial ureolysis is going at either aerobic or anaerobic conditions (Parks 2009). So, even majority of UPB are aerobic bacteria, it could be possible to consider them in MICP process models as the small containers with enzyme urease. Adhered on surface bacterial cell with urease activity can produce much stronger center of crystallization of calcite on the surface than adsorbed molecules of enzyme urease.

## 12.7 Influence of Calcium Concentration on MICP

The sensitivity of urease can be influenced by reactants (urea and calcium salt in MICP) and products (ammonium) of the biocementation process. Maximum of urea and calcium salt concentrations of 1.5 M was found to produce the optimum amount of MICP by UPB strain *S. pasteurii*. The concentration of calcium cation itself could influence both the bacterial growth and the urease activity (Qian et al. 2009). In our experiment, growth of UPB *Bacillus* sp. VS 1 was inhibited in the presence of 2.5 M calcium cations.

Biotreatment of sand under 0.1 M $CaCl_2$ and 0.1 M urea solution produced high unconfined compressive strength (UCS), 2 MPa at 7 % of calcium carbonate in sand, while solutions with concentration of calcium and urea 0.5–1 M produced in average twice lower UCS of the samples (Al Qabany and Soga 2013). It was assumed by these authors from SEM images that a solution with lower concentrations of calcium salt and urea produces smaller crystals and ensures more uniform precipitation pattern. By data of (Al-Thawadi 2008), the maximum in situ urease activity was achieved by supplementing the cementation solution with growth media, and the use of 0.5 M urea and $Ca^{2+}$ as cementation solution. It should be noted that the operation cost would increase as bigger volume of low concentration solution should be injected into the soil. Similar conclusion was made by Muynck et al. (2010a, b) that it was improvement of limestone consolidation at higher concentration calcium. Our data showed that concentration of $Ca^{2+}$ above 1 M decreased percentage of calcium precipitation. It was no precipitation in 3 M $Ca^{2+}$ solution even by specially selected halophilic strains with urease activity. A lot of urease-producing extreme halophiles are known in the genus *Haloarcula: H. aidinensis, H. hispanica, H. japonica,* and *H. marismortui* (Mizuki et al. 2004), however, they were not tested for MICP under high concentration of $Ca^{2+}$ and urea.

## 12.8 Calcium: Urea Molar Ratio for MICP

Martinez et al. (2013) showed that higher urea to calcium ratio is favorable for MICP. When molar ratio of calcium to urea was 1:3, which is three times bigger stoichiometrical value for urea hydrolysis and calcium ions precipitation, pH reached 9.0, urea hydrolyzed and concentration of ammonium increased significantly, while concentration of dissolved calcium dropped to almost zero. Meanwhile, when molar ratio of calcium to urea was 1:1, which is stoichiometric value for urea hydrolysis and calcium ions precipitation, pH was on the level of 7, small portion of urea was hydrolyzed and concentration of ammonium was low, while concentration of dissolved calcium remains relatively stable. Our data showed that optimal calcium: urea molar ratio for MICP is on the level 1.0: 1.35–1.5. Lower ratio cannot produce high pH essential for MICP but higher ratio will produce more toxic ammonium and ammonia as well as will cost more.

## 12.9 Source of Calcium for MICP

Theoretically, all highly soluble and strongly dissociated in water inorganic salts of calcium are suitable for MICP. Salts of calcium with organic acids can be even effective if the conditions are favorable for biodegradation of organic acids and formation of additional quantity of $CO_2$. However, some tests showed that calcium chloride was better source of calcium for MICP in terms of precipitated calcium and

the strength of consolidated sand than calcium nitrate or acetate (Abo-El-Enein et al. 2012). From economy point of view, the brine of calcium chloride, which is widely used as the road de-icing solution containing 30–35 % of $CaCl_2$, could be the best source for MICP.

## 12.10 UPB Distribution and Immobilization

The crucial factor of biocementation is a uniform MICP formation due to even distribution of bacterial cells on the surfaces of the soil particles (Martinez et al. 2013). Transportation of bacterial cells inside soil depends on surface texture, grain size distribution, mineralogy of soil, cell wall charge, chemical composition, and viscosity of solution (Foppen and Schijven 2005; Torkzaban et al. 2008). It is now well known that the use of fixation solution with high salinity after applying UPB can increase the absorption of bacteria by reducing electrostatic repulsion of bacteria and immobilizing them on the particles surface (Whiffin et al. 2007; Harkes et al. 2010). High salinity and bacterial concentration can increase adsorption of bacterial cells resulting in higher effectiveness of UPB activity in sand.

Retention time (contact time) of bacterial cells is also important for bacterial cells adhesion. Recirculation of bacterial cells through soil increases their retention time. Quantity of bacterial suspension also can be a factor of MICP (Rowshanbakht et al. 2016).

Bacterial suspension and cementation solution should be supplied differently. Martinez et al. (2013) suggested a lower flow rate or stopped flow for bacterial suspension. Opposite flow directions of bacterial suspension and cementation solution are preferred to achieve a more uniform cementation, as plugging effect near the injection source might be alleviated (Martinez et al. 2013).

Positive relationship between calcite content and UCS was observed in all tests. It was argued by Cheng and Cord-Ruwisch (2012) that for the top of the percolated column, i.e., the unsaturated zone, MICP forms exactly between sand grains and performed as bridge, at where the menisci were saturated. On the contrary, at fully saturated bottom part, most calcite crystal precipitated on surface of the sand grain instead of acting as binder thus reduced the effectiveness of MICP.

In case of injection of MICP solution, plugging near injection point caused by low permeability could result in local cementation and preventing the further transfer of bacterial cells and cementation solution. However, the biocementation process is also self-adjustable and when the voids are filled by calcite, flow rate is higher and force the solution to travel further (Martinez et al. 2013).

To ensure MICP test at even distribution of bacterial cells, air-dried soil was mixed with UPB and compacted into the mold. Cementation solutions containing different concentration of cementation chemicals $CaCl_2$ and urea were then introduced into soil sample by air compressor. Calcite crystals bonding soil particles were observed (Soon et al. 2014).

## 12.11 Rate of MICP Per Cell During Bioclogging

Hypothetically, the rate of calcium carbonate precipitation in ICP is limited by concentration of adsorbed cells, which depends on specific surface. Experiments showed that bioclogging of the columns with glass beads between 0.01 and 3 mm in diameter using MICP by *S. pasteurii* decreased hydraulic conductivity from $3.70 \times 10^{-3}$–$8.4 \times 10^{-3}$ up to $3.3 \times 10^{-6}$ and $3 \times 10^{-7}$ $ms^{-1}$, respectively (Eryuruk et al. 2014). The amount of precipitated calcium carbonate was proportional with the bacterial cells number in the column. The specific $CaCO_3$ precipitation rate by the urease-producing bacterial cells was estimated as $4.0 \pm 0.1 \times 10^{-3}$ μg $CaCO_3$ $cell^{-1}$. Larger amounts of $CaCO_3$ precipitate were in the columns packed with smaller glass beads (Eryuruk et al. 2014).

## 12.12 Effect of Temperature on MICP

Usually, mesophilic strains of *S. pasteurii* with optimum temperature for growth and urease activity about 30 °C are used in MICP experiments. It was shown that relatively bigger proportion of existing $Ca^{2+}$ was precipitated at the lower temperature, however, long lag duration about 13 h was observed at 20 °C (Tirkolaei and Bilsel 2015). However, temperature in soil could be significantly lower than 20 °C. Therefore, ureolytic microorganisms were screened for their growth and ureolytic activity at temperatures 10, 20, 28, and 37 °C (De Muynck et al. 2013). Psychrophilic ("cold-loving") strain of *Sporosarcina psychrophila* did not produce significant amounts of calcium carbonate at 10 °C but the production of calcium carbonate at this temperature by mesophilic strain of *Bacillus sphaericus* was fastest and most effective. So, there are existing microorganisms performing MICP at temperatures at 10–15 °C. However, microbiological screening and selection could reveal psychrophilic UPB with urease active at temperatures between 5 and 10 °C.

## 12.13 Formation of Nanocomposites

A problem of MICP is the brittleness of calcite or aragonite crystals binding the soil particles. In nature it is solved by the formation of 3D micro- and nanocomposites. For example, molecules of specific proteins are present in the composites of $CaCO_3$-containing biominerals thus reducing their brittleness (Mayer and Sarikaya 2002). These proteins can increase or inhibit $CaCO_3$ precipitation rate at very low concentrations, for example 0.02 mg/L for stimulation and 1 mg/L for inhibition (Heinemann et al. 2011).

By analogy with other binders, involvement of micro- and nanofibers in MICP binding of the particles hypothetically can improve mechanical properties of

biocemented soil. Some experiments showed that MICP with *B. sphaericus* was strengthened by 37 % by addition of 20 % of carbon nanotubes to the total sealant (Annamalai and Arunachalam 2013).

## 12.14 Effect of Surfactants

Surfactants can change the surface properties of both soil particle and cell and affect also the rate of biochemical reactions. In experiments with *S. pasteurii* and *Bacillus megaterium*, precipitation of $CaCO_3$ and the type of minerals depended on the type of surfactant (Cho et al. 2015).

## 12.15 Design of Biocementation and Bioclogging Using MICP

Modeling of bioclogging and biocementation in a porous medium is very important or engineering practice because it can give better understanding of the process, and the opportunity to design and control it (Murphy and Ginn 2000; Laloui and Fauriel 2011; Fauriel and Laloui 2012; van Wijngaarden et al. 2012; Afanasyev et al. 2013; Shigorina and Strokova 2014). Bio-related parameters usually include: (1) enzymatic rate of ureolysis described by either a Michaelis–Menten equation ($R^2 = 0.95$) or first-order equation ($R^2 = 0.99$); (2) parameters of a linear correlation between ureolysis rate and bacterial cells concentration (Lauchnor al. 2015). In the above cited paper, half-saturation coefficient was 0.35 M for a Michaelis–Menten equation and $k_1$ for a first-order equation was 0.35 $h^{-1}$. Ammonium concentration up to 0.19 M had no significant effect on the ureolysis rate. A 3D model of the clogging due to the formation of biofilm in porous media showed a significant influence at large biofilm concentrations even when the permeability of the biomass is about 1 % of that of the free pore space and considering that biomass of biofilm accumulation increased by a factor of $\sim 3$ over 40 h (Pintelon et al. 2012). Our 2D model of bioclogging (see a chapter on bioclogging) includes the description of two steps: in case of the deposition of large calcite crystals, there are slow formations of the channels following with fast clogging of these channels. In the case when small non-calcite crystals or amorphous particles of $CaCO_3$, $Fe(OH)_3$, or microbial slime are produced during biotreatment, the kinetics of precipitation can be described as one-stage process

For the proper description of the process, the models have to include description of adhesion and activity of microorganisms, gas, and liquid flows between solid soil particles, interaction of biocementing/bioclogging precipitate with soil particles,

kinetics of pH and concentrations of soluble organic and inorganic matter. Finally, the models have to be validated in the laboratory and field tests.

## 12.16 Parameters of Design

Major parameters of calcium-based bioclogging and biocementation using MICP are as follows:

- $X_o$, concentration of bacterial cells or biomass in suspension used for the biocementation particles, cells/$m^3$ or g of dry biomass/$m^3$;
- $X_s$, concentration of bacterial cells or biomass adhered to surface of solid particles, cells/$m^2$ or g of dry biomass/$m^2$;
- $U_0$, initial urease activity of bacterial cell or biomass used for the soil treatment, mmol/cell/s or mmol/g dry biomass/s
- U, average urease activity of bacterial cell or biomass for the period of soil treatment, mmol/cell/s or mmol/g dry biomass/s
- $k_u$, rate of urease activity decay in situ, 1/s;
- HRT, hydraulic retention time for calcium salt and urea solution in the treated zone;
- pH of effluent;
- $A_g$, release of ammonia to gas, kg;
- $A_w$, release of ammonium to water, kg;
- C, content of precipitated/crystallized calcium carbonate in biocemented soil, % (w/w);
- UCS, unconfined compressive strength of biocemented soil, Pa;
- HC, hydraulic conductivity of biocemented soil, m/s,
- S, share of different crystals, and many others related to the process.

## 12.17 Stoichiometry of Bioclogging and Biocementation

Consider for this sample of calculations, that the aim of biocementation is an increase of UCS of sandy soil to 2 MPa, which requires precipitation/ crystallization of $CaCO_3$ to the level about 7.5 % (w/w), see related figure. The aim of biogrouting is a decrease of the coefficient of permeability of sandy soil to $1 \times 10^{-7}$ m/s, which requires precipitation/ crystallization of $CaCO_3$ to the level about 25 % (w/w), see related figure. This content corresponds to the ratio of pore volume (for the porosity of sandy soil about 50 %) to the volume of precipitated $CaCO_3$, which is equal to 0.17 $m^3$ of $CaCO_3/m^3$ of pores.

So, for bioclogging of the fissured rocks with the porosity P, $m^3$ of pores/$m^3$ of rock, the quantity of $CaCO_3$ to be supplied for precipitation is $0.17 \times P \times 2900$ kg $CaCO_3/m^3$ of $CaCO_3 = 493 \times P$ kg $CaCO_3/m^3$ of rock. Considering that porosity

of the sedimentary rocks is from 1 to 10 % (v/v) the quantity of $CaCO_3$ precipitated for bioclogging is from 5 to 50 kg of $CaCO_3/m^3$ of sedimentary rock. For the fractured rocks, the porosity could be 10–100 times lower, so depending on porosity, 0.5–5 kg of $CaCO_3/m^3$ of the fractured rock, i.e., about 0.02–0.2 % (w/w) could be sufficient for bioclogging.

## 12.18 Technological Calculations

There are two major processes in MICP bioaggregation/ bioclogging/ biocementation: (1) supply of suspension of urease-producing bacteria to the treated zone of porous material resulting in gravity precipitation and then adsorption of bacterial cells onto solid surface, and (2) crystallization and gravity precipitation of $CaCO_3$ crystals from the solution of calcium salt and urea due to activity of bacterial urease. The technological calculations are performing in backward order.

(1) Quantity of $CaCl_2$ supplied to sand to increase content of $CaCO_3$ to 7.5 % (w/w) following the reaction:

$$CaCl_2 + (NH_2)_2CO + 2H_2O \rightarrow CaCO_3 + 2NH_4^+ + 2Cl^- \qquad (12.1)$$

(110 kg of $CaCl_2$/ 100 kg $CaCO_3$) × (0.075 kg of $CaCO_3$/kg of sand) × 1600 kg of sand/$m^3$ of sand = 132 kg of $CaCl_2$/ $m^3$ of sand = 1200 mol $CaCl_2/m^3$ of sand.

(2) Quantity of urea at molar ratio of urea/Ca of 1.5 = 1200 × 1.5 = 1800 mol of urea/$m^3$ of sand = 108 kg of urea/ $m^3$ of sand.
(3) Quantity of solution 1 M $CaCl_2$/ and 1.5 M of urea to be supplied for biocementation = 1.2 $m^3/m^3$ of sand.
(4) Consider that designed time of biocementation is 1 day = 24 h = 1440 min, average urease activity of biomass in bacterial suspension is 0.1 mM/min, efficiency of cells adsorption is 100 %. Quantity of urea that will be hydrolyzed for 1 day = $1 \times 10^{-4}$ mol of urea/L x min) × 1440 min = 0.144 mol/L = 144 mol/$m^3$ of bacterial suspension.
(5) Quantity of bacterial suspension to be supplied for biocementation is:

1800 mol of urea/$m^3$ of sand/ 144 mol/$m^3$ of biocementing solution = 12.5 $m^3$ of bacterial suspension/ $m^3$ of sand.

So, the technological process is as follows:

(1) Add 12.5 $m^3$ of bacterial suspension/$m^3$ of sand and incubate or recirculate suspension for sufficient time, not less 2 h for adhesion of bacterial cells to surface of solid particles.
(2) Add in four batch treatments, or recycle, or supply and remove continuoulsy with HRT not less 24 h 1.2 $m^3$ of solution of 1 M $CaCl_2$ and 1.5 M of urea.

(3) Monitor the permeability of sand, as well as pH, concentration of $Ca^{2+}$ and $NH_4^+$ in effluent and control the process of biocementation. Equilibrium between gaseous $NH_3$ (ammonia, ammiak) and water-soluble $NH_4^+$ (ammonium) depends at pH. At pH 7.0 all nitrogen will be as soluble $NH_4^+$, at pH 8.2 about 80 % of N will be in the form of ammonium, and at pH 9.0 about 50 % of nitrogen will be in the form of ammonia (ammiak), which can be released to atmosphere as a toxic gas, and 50 % will be in the form of water-soluble ammonium. Ratio of urea/calcium should be diminished to decrease pH to at least 8.5 but an efficiency of calcium carbonate precipitation/crystallization will be also diminished.

## 12.19 Optimization and Design of Biodesaturation

Microbial bioreduction of nitrate by organics in water-saturated sandy soil produces a big quantity of nitrogen gas partially desaturating this soil and thus mitigating earthquake soil liquefaction (Hamdan et al. 2011; He et al. 2013; Eseller-Bayat et al. 2012; Montoya et al. 2012; Rebata-Landa and Santamarina 2012; Weil et al. 2012; Yegian et al. 2007). The major advantages of the biogas production in situ are as follows: (1) the distribution of the gas bubbles in soil is uniform because a biogrout is a liquid with the same viscosity as water and can be distributed evenly in porous soil; (2) nitrogen gas is inert and has low solubility in water (Chu et al. 2013a, b, 2014b; Landa and Santamarina 2012).

There are many species of denitrifying bacteria, for example, *Pseudomonas denitrificans* or *Paracoccus denitrificans* that can transform nitrate to nitrogen gas. Almost all these bacteria can be used for partial desaturation of water-saturated sand under anoxic conditions. However, only selected, identified, and tested for biosafety strain/species of denitrifying bacteria can be used for soil desaturation because almost all facultative aerobic bacteria can produce energy by nitrate respiration (denitrification) and many species are enterobacteria that are living in the guts of human and animals. Therefore, there are many pathogens among denitrifying bacteria, especially from the genera *Salmonella*, *Shigella*, and *Vibrio.*

Ethanol is a good donor of electron but there may be restriction for its use for soil treatment in some countries. So, acetic acid ($CH_3COOH$) is one of the most suitable electron donors because of its low cost, availability, and high solubility in water. Its biooxidation by nitrate (denitrification), is shown below

$$1.25\,CH_3COOH + 2NO_3^- \rightarrow N_2 \uparrow + 2.5\,CO_2 + 1.5\,H_2O + 2OH^-. \qquad (12.2)$$

The stoichiometrical parameters of this reaction is as follows: consumption of electron donor is 3.4 kg/m$^3$ of $N_2$ and consumption of electron acceptor (sodium nitrate) is 7.6 kg/m$^3$ of $N_2$.

The consumption of electron donor and acceptor for 10 % (volume of gas/volume of water) desaturation of soil with porosity 50 % is 0.55 kg/m$^3$ of saturated soil. Production of carbon dioxide in this reaction, which is from 120 to 159 g/m$^3$ of $N_2$ or from 12 to 16 g/m$^3$ of water in saturated soil with 50 % porosity, is not accounted for desaturation of soil because solubility of $CO_2$ in water at 10 °C is 2500 g/m$^3$.

The cost of electron donor is about $0.5–$0.7/kg, the cost of electron acceptor (sodium nitrate) is about $0.4–$0.5/kg, so the estimated cost of electron donor and acceptor is from $5.1 to $6.2/m$^3$ of $N_2$. So, the estimated cost of electron donor and acceptor for 10 % (volume of gas/volume of water) desaturation of soil with porosity 50 % is from $0.25 to $0.31/m$^3$ of saturated soil.

## 12.20 Field and Pilot Tests of the Biotreatment of Sand and Porous Soil

The way of the treatment of the porous soil is important for the process. One method of producing high strength cemented samples includes addition of bacterial suspension with a medium into the sand column by growing them 48 h in the presence of calcium ions as little as 6 mM. Then, continuous supply of cementation solution with equimolar concentrations of calcium and urea was for 20 volume replacements which produced finally a mechanical strength of up to 30 MPa, which is equivalent to construction cement (Al-Thawadi 2008). One of the advantages of that method is the absence of the clogging the injection-end due to sequential flushing of the bacterial cells and cementation solution. Uniform cementation was along 1 m packed sand column (Al-Thawadi 2008).

MICP in half-meter column experiments showed that the stop-flow injection of a urea- and calcium-rich solution produces a more uniform calcite distribution as compared to a continuous injection method, even when both the methods involve flow in opposite direction to that used for bacterial cell emplacement (Barkouki et al. 2011).

Field scale trials were conducted in the port area of Rotterdam, The Netherlands, and a practical scale test was performed along a newly constructed aqueduct beneath the Haarlemmermeer ring canal in the Netherlands. Both the tests were successful. Biosealing is a promising technology to reduce leakage in civil engineering applications. The technique is simple, makes use of naturally present microorganisms in the soil and is self-regulating for leak detection and reduction (van Beek et al. 2007). Probably, long bioclogging period in laboratory columns, several weeks after the injection of nutrients, was caused by low concentration of urease-producing bacteria in sand.

Biogrouting was compared to jet grouting in a large road project in Stockholm, Sweden (Suer et al. 2009). Both technologies were used to seal the contact between sheet piling and bedrock. Biogrouting was cheaper than jet grouting due to transport

and use of heavier equipment for jet grouting. Biogrouting also used less water and produced less landfilled waste. However, the production of urea and $CaCl_2$ for biogrouting required much energy (Suer et al. 2009). So, cheaper and less energy consuming salt of calcium is needed for the practical applications.

The regime of injections is very important for the MICP process (Inagaki et al. 2011). Tobler et al. (2012) studied differing injection strategies for the bioclogging. When the ureolytic bacteria *S. pasteurii* and the cementation fluid were injected at the same time (parallel injection), a heterogeneous calcite fill along the column occurred, where most calcite was precipitated closely to the inlet area. In contrast, when bacterial suspension was injected first, followed by the cementation fluid (staged injection), a more homogeneous distribution of cells was developed. Bacterial immobilization was enhanced in the presence of freshly precipitated calcite surfaces, which induced higher rates of ureolysis and calcite precipitation (Tobler et al. 2012).

Field tests of MICP have been done by the team of Delft Technical University, The Netherlands: within 12 days 40 $m^3$ of sand was biologically cemented stretching over a length of 5 m between three injection and three extraction points. It was correlation between $CaCO_3$ content and strength of the samples. From the strength tests the parameters describing the failure criterion, cohesion and friction angle, could be derived, which enable engineering design (van Paassen et al. 2009a, b, 2010).

## 12.21 Tests of the Biotreatment of the Fractured Rocks

Long-term experiments on bioclogging of the fractured rocks were conducted on six fractured chalk cores (20 cm diameter, 23–44 cm long) containing a single natural fracture embedded in a porous matrix with a flow of solution containing 2,4,6-tribromophenol (TBP) as a model contaminant of groundwater (Arnon et al. 2005). Transmissivity reduction was clearly related to TBP removal rate, following an initial slow decline and a further sharp decrease with time. The fracture's transmissivity was reduced by as much as 97 % relative to the initial value, with no leveling off of the clogging process. The reductions in fracture transmissivity occurred primarily because of clogging by bacterial cells and extracellular polymeric substances (EPS) produced by the bacteria.

MICP can be used as an alternative of grouting technologies to create barrier to contaminated groundwater flow (Cuthbert et al. 2013). The first field experiments on MICP application to reduce fractured rock permeability in the subsurface using *S. pasteurii* cells fixation in the fractured rock following with subsequent injection of cementing fluid with calcium chloride and urea resulted in a significant reduction in the transmissivity of a single fracture over an area of several $m^2$ for 17 h of treatment. The field tests and the modeling results show that MICP can be manipulated under field conditions to reduce the permeability of fractured rock. The numerical modeling of the fractured rocks bioclogging using MICP showed that

permeability on the distances 3 m and 5 m from the injection point can be decreased between 10 and 3 times, respectively (Shigorina and Strokova 2014).

The technology of bioclogging, called BioSealing, for underground constructions like sheet piles, clay layers, and contamination retaining barriers, being developed at GeoDelft in cooperation with Delft University of Technology and Visser & Smit Bouw BV (Veenbergen et al. 2005). BioSealing can decrease the flow through geotechnical constructions as well as to prevent migration of contaminants out of contaminant-retaining constructions as it was shown by results from the laboratory and the field experiments (Veenbergen et al. 2005).

The results of the tests on bioclogging and biocementation can be monitored in real time using seismic velocity and resistivity measurements (Weil et al. 2012; Martinez 2013; Montoya and DeJong 2015). Shear wave velocity (S-wave) test results were used to develop a correlation to the precipitated calcite mass and this enables prediction of changes in porosity, density, and shear modulus during treatment (Qabany et al. 2011; Weil et al. 2012). Shear wave velocity is used to nondestructively monitor the change in small-strain stiffness during shearing, which provides an indication of cementation degradation as a function of strain level. Because shear wave velocity is influenced by both the level of cementation and the change in effective mean stress during shearing, the normalized shear modulus is used to evaluate the degradation of cementation during shearing (Montoya and DeJong 2015). Biocementation in a half-meter sand column resulted in a change from a shear wave velocity of 140 m/s to an average of 600 m/s (Martinez 2013).

Compression wave velocity (P-wave) measurements can be determined under different saturation conditions and used in combination with S-wave measurements to observe how the Poisson's ratio evolved during treatment (Weil et al. 2012). MICP in a 61 cm long sand-filled column clogged a hydraulically fractured, 74 cm diameter Boyles sandstone core (Phillips et al. 2013). Permeability of the fractured rock after biotreatment decreased between 2 and 4 orders of magnitude and the sandstone core withstood three times higher well bore pressure than during the initial fracturing event showing that MICP is applicable for the bioclogging (biosealing) of the fractured rocks (Phillips et al. 2013, 2015).

A field trial was conducted to determine the effect of nutrient addition on bioclogging in a fractured rock using stimulation of indigenous bacteria in a single fracture through the additions of bioavailable carbon, nitrogen, and phosphorus sources (Knight 2008). Molar ratios of bioavailable carbon, nitrogen, and phosphorus were 100:9:4 and a carbon source concentration was 8.9 g/L. The field trial was conducted in an uncontaminated area adjacent to an abandoned quarry of flat-lying dolostone pervaded by bedding plane fractures in southern Ontario, Canada. An arrangement of three boreholes isolated a single fracture at a depth of 17 m using straddle packer systems. A groundwater recirculation system was created with groundwater withdrawal at BH7 and reinjection of amended water at BH9. Throughout the three-week biostimulation experiment, microbial numbers and diversity were evaluated before and after the experiment.

Bacterial ureolysis can be used for improved gelation of colloidal silica of low viscosity in rock grouting (Maclachlan et al. 2013). The bacterial ureolysis using

*S. pasteurii* accelerated the destabilization of colloidal silica in situ thus producing a more uniform gel structure than destabilization by the direct addition of chemical accelerators such as sodium chloride, calcium chloride, or ammonium chloride.

## 12.22 MICP Bioclogging of the Mixture of the Rocks and Sand

A pilot test on MICP bioclogging was carried out for 0.8 $m^3$ of rock and sand mass in a 1 $m^3$ plastic container. The top cover plate of the container was cut away and granite stones with weight from 300 g to 5 kg, totally about 1400 kg, were placed into the container layer by layer with the filling on space between the stones with sand (Fig. 12.3).

**Fig. 12.3** Assembling of the test facility

Totally, the filling was a mixture of 1400 kg (480 L) of stones and 640 kg (400 L) of sand. The procedure was as follows: river sand was placed into the tank to form a bottom layer with thickness 5 cm, then a layer of limestones was placed over the sand layer. The tubes with inner diameter 20 mm for supply and removal of liquids and for collection of the samples were fixed in facility (Fig. 12.3). The space between the limestones was filled with sand. In such way the second and the third layers of stones were settled altogether with sand. Then, the cover plate was placed from above and fixed with the self-bonding tape. The tubes were installed for the sampling. Sampling was at the height of 0.25, 0.50, and 0.75 m from sand surface.

The tubings were connected to the pumps for supply/removal of culture liquid and bioclogging solution. The inlets and outlets were arranged to ensure diversity of the flows pattern and sampling ports (Fig. 12.4).

Water, 100 L, was pumped into the tank and recirculated 72 h. Supply of water was through the tubes 1 and 3; removal of water was through the tubes 2 and 4. This treatment was done to ensure the uniform distribution of sand and to avoid the presence of air bubbles in sand. The facility included tank with stones and sand, pumps, absorber of ammonia, and intermediary tanks for solutions.

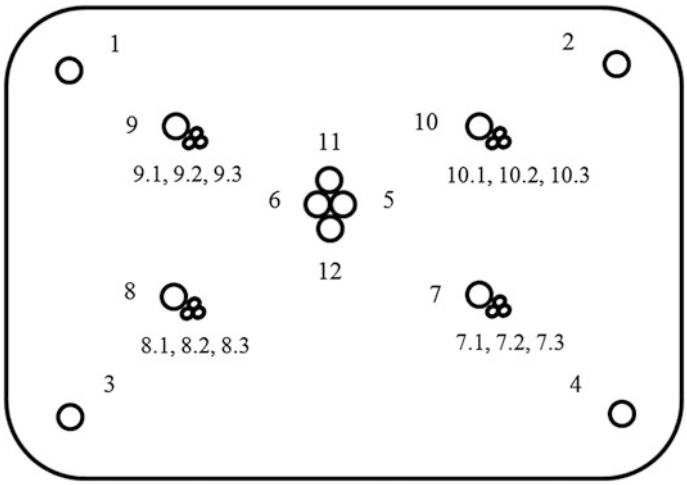


**Fig. 12.4** Inlets and outlets arranged in the plastic tank filled with stones and sand. 1, 2, 3, 4 are the tubes with the sealed bottom and with the orifices holes (diameter 3 mm, distance between orifices 50 mm); 5, the tube with the sealed bottom and with 4 lines of orifices (diameter 3 mm, vertical distance between orifices was 50 mm); 6, flexible tubing under surface of sand; 7, 8, 9, 10 are the tubes with open outlets; 11—gas inlet from absorber of ammonia; 12—gas outlet to ammonia absorber; 7.1, 8.1, 9.1, 10.1 are the tubes for sampling on the distance from the bottom of the tank 50 mm; 7.2, 8.2, 9.2, 10.2 are the tubes for sampling on the distance from the bottom of the tank 300 mm; 7.3, 8.3, 9.3, 10.3 are the tubes for sampling on the distance from the bottom of the tank 600 mm

The flow directions of 20 L of bacterial suspension of *Bacillus* sp.VS1 and bioclogging solution 1 M $CaCl_2$ and 1.37 M urea were changed as shown below to ensure the combinations of downward, upward, horizontal parallel, and diagonal flows, as well as the flows to the center and out of the center.

Treatments 1 and 2: Culture liquid and biocementation solution were supplied through the tubes 1–4; extraction of liquid for recirculation was through the tubes 5.

Treatments 3 and 4: Culture liquid and biocementation solution were supplied through the tubes 5; extraction of liquid for recirculation was through the tubes 1–4.

Treatment 5: Culture liquid and biocementation solution were supplied through the tubes 1 and 3; extraction of liquid for recirculation was through the tubes 2 and 4.

Treatment 6: Culture liquid and biocementation solution were supplied through the tubes 2 and 4; extraction of liquid for recirculation was through the tubes 1 and 3.

Treatment 7: Culture liquid and biocementation solution were supplied through the tubes 5; extraction of liquid for recirculation was through the tubes 7–10.

Treatment 8: Culture liquid and biocementation solution were supplied through the tube 6; extraction of liquid for recirculation was through the tubes 7–10.

The regime of the treatments is shown in the Table 12.1.

pH of effluent after second treatment was in the range 8.6–8.8 showing that bioclogging process (accompanying by hydrolysis of urea and increase of pH) was successful. The flow rate of water from the top to the bottom under vacuum was decreased during the treatment from 174 L $h^{-1}$ to 0.145 L $h^{-1}$ (Fig. 12.5).

So, initial seepage of water through the volume was $4.8 \times 10^{-5}$ $ms^{-1}$ and final seepage of water through volume was $4.2 \times 10^{-8}$ $ms^{-1}$ at maximum content of precipitated $CaCO_3$ 9 % (w/w). These data demonstrated efficiency of bioclogging in the pilot scale system. The sample after treatment is shown in Fig. 12.6. Overall the sample was solid and dense. However, there were voids along the boundary, which were mainly due to the nonuniformity of the treatment.

Content of calcium in biocemented sand in different sections varied from 2 to 19 % (average 8.0 %) for crust layer; from 2 to 5 % (average 3.1 %) for next from the top layer a; from 1 to 3 % (average 1.9 %) for the next two layers including the bottom layer. Calcium precipitation was concentrated in the upper layer of the mixture and in the points of injection, probably due recycling of the clogging solution. So, for practical applications better avoid recycling of the clogging solution to prevent formation of the crust and clogging in the points of injection.

Contents of live bacterial cells were determined by spreading of 10-fold dilution of biocemented sand onto Petri dishes filled with solidified TSB medium with urea. Numbers of colony formed units (CFU) were enumerated after 2 days of incubation on Petri dishes at temperature 25 °C for vegetative cells and after 5 days for enumeration of spores. Average contents of live cells of urease-producing bacteria, CFU/g of biocemented sand, were $17.6 \times 10^6$; $24.3 \times 10^6$; and $27.4 \times 10^6$ for

**Table 12.1** The regime of the treatments of the pilot test system

| No of treatment | Description |
|---|---|
| 1 | Supply of 20 L of cultural liquid (CL) + 17 L of water + 3 L (1 M $CaCl_2$ + 1.375 M urea). Recirculation for 5 h with the flow rate 1000 mL/min. Removal of used solution for 1 h. Supply of 60 L of 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 24 h. There was no Ca in effluent. Removal of used solution (approximately 1 h) |
| 2 | Supply of 20 L CL + 20 L 2 % NaCl. Recirculation for 5 h with the flow rate 1000 mL/min. Removal of used CL for 1 h. Supply of 40 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 24 h. There was no Ca in effluent. Removal of used solution for 1 h. Supply of 40 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 24 h. There was no Ca in effluent. Removal of used solution for 1 h |
| 3 | Supply of 20 L CL + 20 L2 % NaCl. Recirculation for 16 h with rate 1000 mL/min. Removal of used CL for 1.5 h. Supply of 40 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 24 h. High concentration of Ca was detected in effluent. Recirculation was continued for next 24 h. After this operation there was no Ca in effluent. Removal of used solution for 1.5 h |
| 4 | Supply of 20 L CL + 20 L 2 % NaCl. Recirculation for 4 h with rate 1000 mL/min. Removal of used CL for 2 h. Supply of 40 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 68 h. There was no Ca in effluent. Removal of used solution for 2 h. Supply of 40 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 24 h. There was no Ca in effluent. Removal of used solution for 2 h |
| 5 | Supply of 20 L CL + 10 L2 % NaCl. Recirculation for 10 h with flow rate 1000 mL/min. Removal of used CL for 3 h. Supply of 30 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 24 h. There was no Ca in effluent. Removal of used solution for 3 h. Supply of 30 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 68 h. There was no Ca in effluent. Removal of used solution for 3 h |
| 6 | Supply of 20 L CL + 10 L 2 % NaCl. Recirculation for 10 h with flow rate 1000 mL/min. Removal of used CL for 4 h. Supply of 30 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 24 h. There was no Ca in effluent. Removal of used solution for 4 h. Supply of 30 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 68 hrs. There was no Ca in effluent. Removal of used solution for 5 h |
| 7 | Supply of 20 L CL + 10 L 2 % NaCl. Recirculation for 10 h with flow rate 1000 mL/min. Removal of used CL for 5 h. Supply of 30 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 24 h. There was no Ca in effluent. Removal of used solution for 5 h. Supply of 30 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 460 h. There was no Ca in effluent. Removal of used solution for 5 h |
| 8 | Supply of 20 L CL. Recirculation for 5 h with flow rate 1000 mL/min. Removal of used CL for 5 h. Supply of 20 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 24 h. There was no Ca in effluent. Removal of used solution for 12 h. Supply of 20 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 68 h. There was no Ca in effluent. Removal of used solution for 5 h |
| 9 | Supply of 20 L CL. Recirculation for 5 h with flow rate 1000 mL/min. Removal of used CL for 6 h. Supply of 20 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 68 h. There was no Ca in effluent. Removal of used solution for 12 h. Supply of 20 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 24 h. There is no Ca in effluent. Removal of used solution for 16 h |

(continued)

**Table 12.1** (continued)

| No of treatment | Description |
|---|---|
| 10 | Supply of 20 L CL. Recirculation for 5 h with flow rate 1000 mL/min. Removal of used CL for 10 h. Supply of 20 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 68 h. There was no Ca in effluent. Removal of used solution for 24 h |
| 11 | Supply of 20 L CL. Recirculation for 3 h with flow rate 1000 mL/min. Removal of used CL for 24 h. Supply of 40 L 1 M $CaCl_2$ + 1.375 M urea. Recirculation for 68 h. There was no Ca in effluent. Removal of used solution for 67 h |
| | Total: supply of 580 L containing 64 kg of $CaCl_2$ |

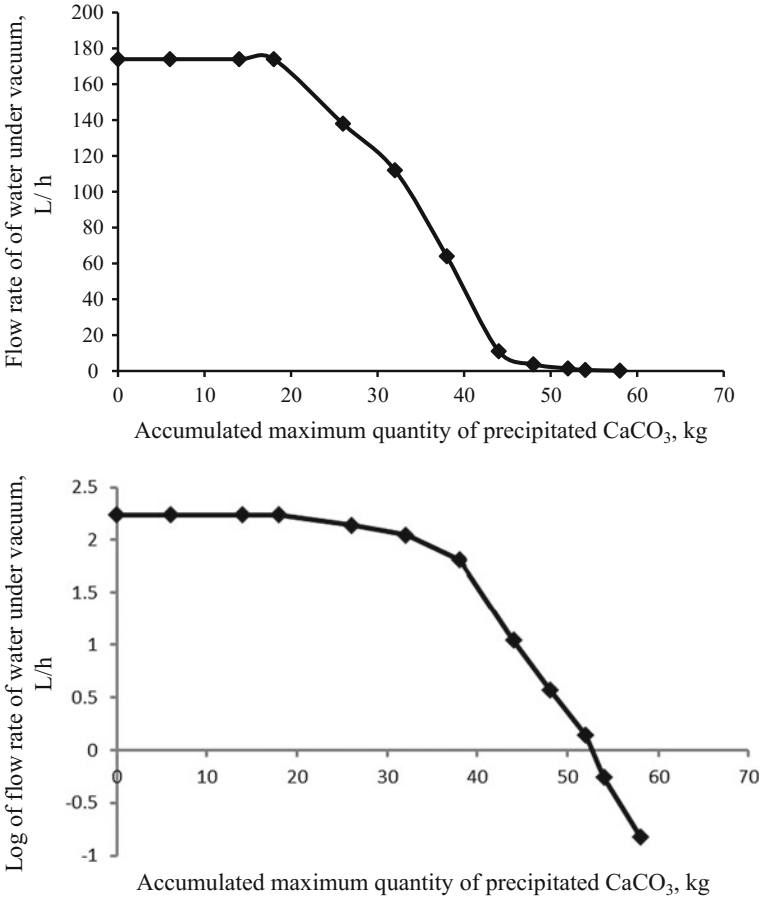


**Fig. 12.5** Flow rate of water from the *top* to the *bottom* under vacuum (in linear and logarithmic scales)

**Fig. 12.6** Treated mixture of rocks and sand

three layers of the samples. Average content of spores in live cells of UPB, %, were 77.2; 79.4, and 94.6 for these layers. Contents of UPB cells increased slightly from top to bottom, as well as percent of spores. So, bacterial cells were almost evenly distributed in sand and it was no correlation between the contents of bacterial cells and precipitated calcium in sand.

## 12.23 MICP Pilot Bioclogging of the Space Between Granite Sheets Using Dead but Urease-Active Bacterial Cells

The pilot test of the biosafe bioclogging with dead but urease-active bacterial cells was carried out with 18 granite plates of $0.4 \times 0.4 \times 0.02$ m as shown in Fig. 12.7. The orifice with a diameter 8 mm was drilled in the center of 16 sheets to supply bioclogging solution into the space between the sheets. The width of the gap between the sheets was about 200–400 μm with the inclusion of sand grains with particle sizes of 200–400 μm in the gaps. The granite plates were placed on the special iron frame so all the plates were fixed in a block with the gaps 20 mm

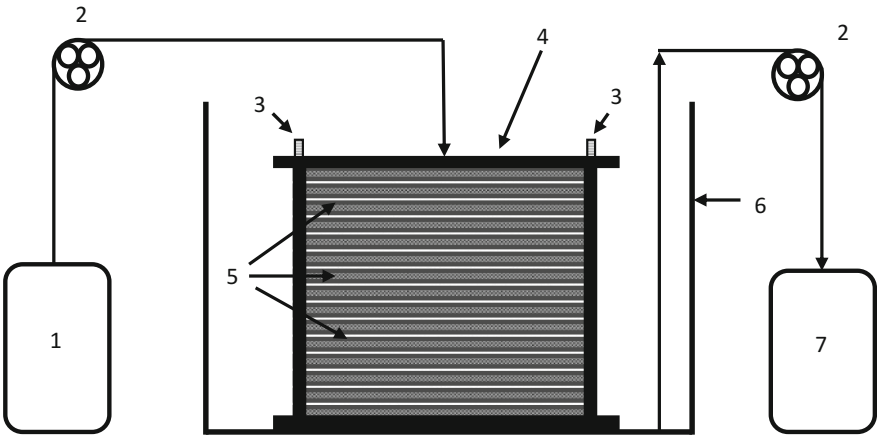


**Fig. 12.7** Test facility for bioclogging of the space between the stone plates. *1*, tank for influent (culture liquid or biocementation solution) with volume 5 L; *2*, peristaltic pumps; *3*, bolts for frame fixation; *4*, iron frame; *5*, 18 granite plates with the orifice in the center; *6*, tank, *7*, tank for effluent

between the plates (Fig. 12.7). The biogrout was injected through the orifice using a peristaltic pump as shown in the setup is schematically shown in Fig. 12.7.

Seven treatments of the packed granite plates in the testing facility have been done. Initial seepage through the space between horizontal sheets was about 1000 mL/h. Removal of liquid from tank 6 was by the peristaltic pump for treatments 4–7 with the rate 250 mL/h. The regime of the treatments of the pilot system of the horizontal granite plates is shown in Table 12.2.

**Table 12.2** The regime of the treatments of the pilot system with horizontal granite plates

| No of treatment | Description |
|---|---|
| 1 | 500 mL of culture liquid (CL) with dead but urease-active cells of *Yaniella* sp. VS8 were injected into the space between the plates by peristaltic pump and was recirculated for 4 h with the rate 500 mL/min. After this operation, 5000 mL of solution of 1 M $CaCl_2$ + 1.5 M urea were injected into the space between the plates and the recirculation was for 20 h with flow rate 1000 mL/h |
| 2 | 500 mL of CL with dead but urease-active cells of *Yaniella* sp. VS8 were injected into the space between the plates by peristaltic pump and was recirculated for 4 h with flow rate 500 mL/min. After this operation, 5000 mL of solution of 1 M $CaCl_2$ + 1.5 M urea were injected in the space between the plates and the recirculation was done for 20 h with the rate 1000 mL/h |
| 3 | 300 mL of CL of dead but urease-active cells of *Yaniella* sp. VS8 were injected in the space between the sheets by peristaltic pump and was recirculated for 4 h with flow rate 100 mL/min. After this operation, 2000 mL of solution of 1 M $CaCl_2$ + 1.5 M urea were injected in the space between the plates and the recirculation was done for 20 h with flow rate 100 mL/h |
| 4 | 150 mL of CL of dead but urease-active cells of *Yaniella* sp. VS8 were injected into the space between the plates with the rate 10 mL/h. After this operation 500 mL solution of 1 M $CaCl_2$ + 1.5 M urea were inject in the space between the sheets with rate 10 mL/h |
| 5 | 150 mL of CL of dead cells VS8 were injected into the space between the plates with the rate 6 mL/h. After this operation, 300 mL of solution of 1 M $CaCl_2$ + 1.5 M urea were injected in the space between the sheets with the rate 6 mL/h |
| 6 | 150 mL of CL of dead but urease-active cells of *Yaniella* sp. cells VS8 were injected into the space between the plates with the rate 6 mL/h. After this operation, 300 mL of solution of 1 M $CaCl_2$ + 1.5 M urea were injected into the space between the plates with the rate 6 mL/h |
| 7 | 150 mL of CL of dead but urease-active cells of *Yaniella* sp. cells VS8 were mixed with 250 mL of solution of 1 M $CaCl_2$ + 1.5 M urea and were injected into the space between the plates with the rate 6 mL/h. Just 150 mL of this mixture were injected during 240 h. The final seepage was 0.65 mL/h (125 mL of water per 192 h) |

This pilot test was a model of the sealing of seepage flow through horizontally fissured rocks. The flow rate recorded as a function of volume of biogrout, shown in Fig. 12.8, decreased from initial rate of $1.4 \times 10^{-8}$ $m^3s^{-1}$ to $1.8 \times 10^{-12}$ $m^3s^{-1}$ at pressure about 100 kPa (1 atm).

Considering that distance between the sheets is $200 \times 10^{-6}$ m and the length of four sides is 1.6 m, the area of one cross section of flow was $9.9 \times 10^{-4}$ $m^2$. There were 16 spaces between the rock sheets so the hydraulic conductivity of the clogged rock sheets at injection pressure 100 kPa was about $8.1 \times 10^{-8}$ $ms^{-1}$.

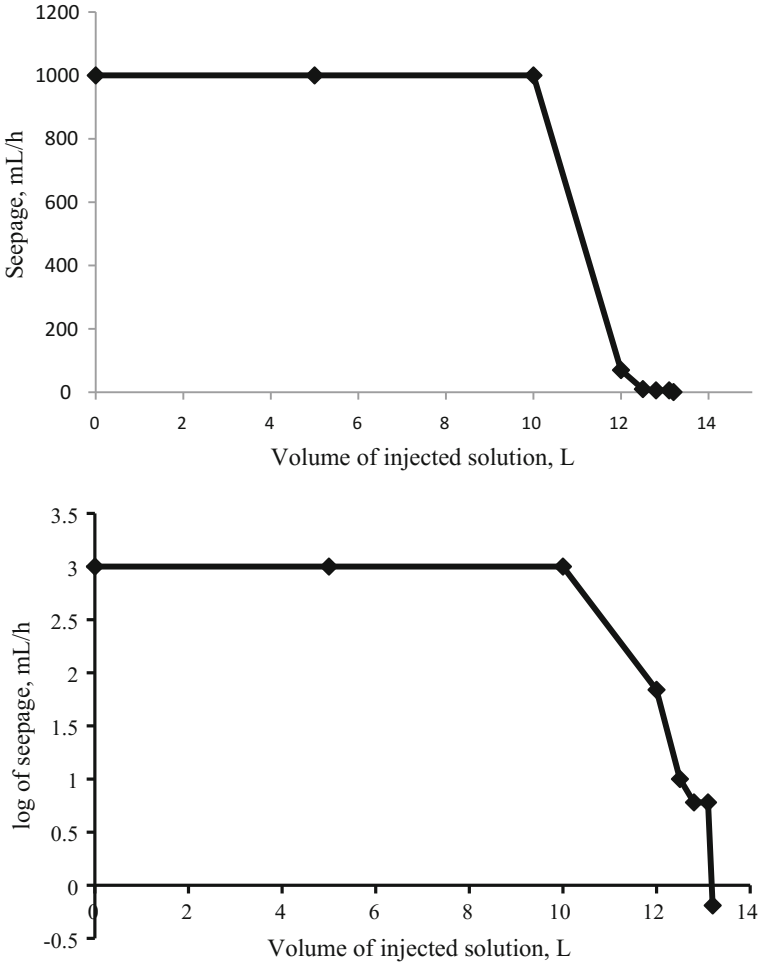


**Fig. 12.8** Seepage through the pilot facility of 18 rock plates modeling bioclogging of horizontally fissured rocks (in linear and logarithmic scales)

Images of the plate surfaces are shown in Fig. 12.9. As expected from the data on effect of gravity on bioclogging, the thick layer of calcium carbonate was formed on the surface of the lower (bottom) side of the plates (Fig. 12.9), while just the thin layer of calcium carbonate was observed on the surface of the ceiling side of the rock plates (Fig. 12.9).

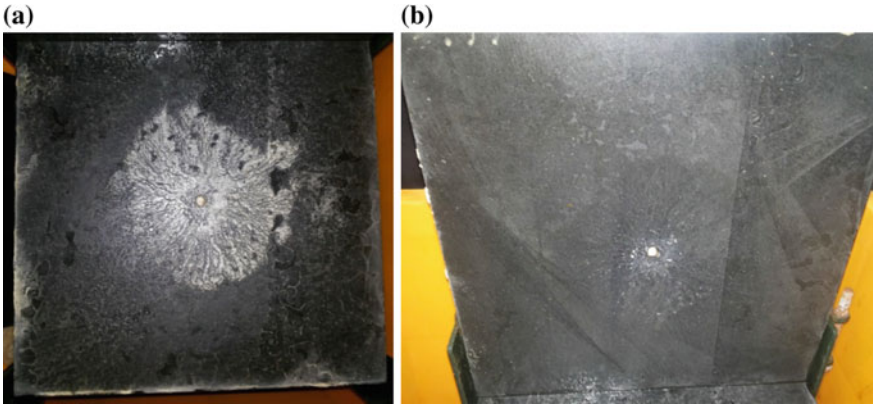


**Fig. 12.9** Images of the bottom side (**a**) and ceilingside (**b**) sides of the rock plates

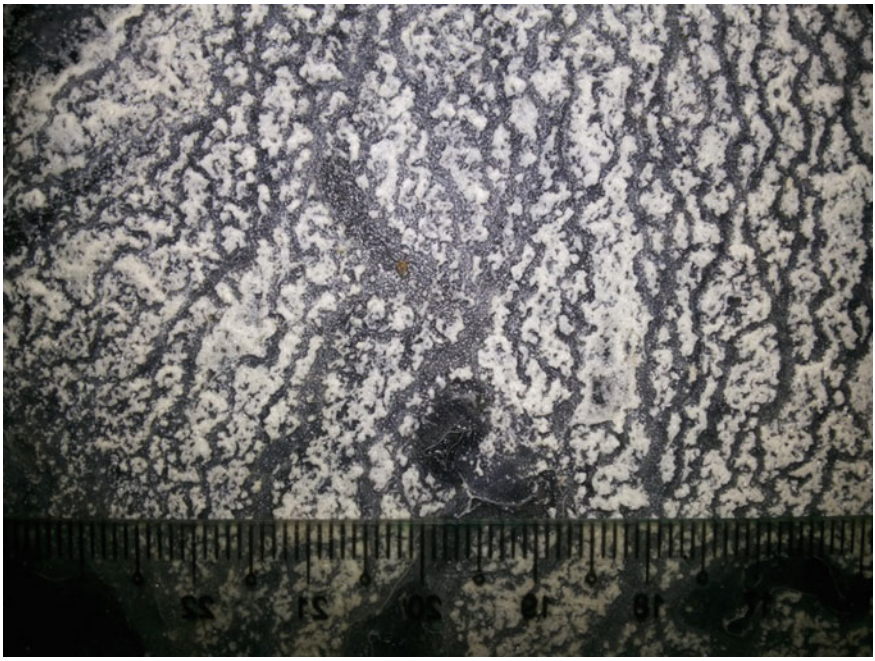

**Fig. 12.10** Formation of the radial channels and clogging of these channels by crystals (some examples shown in the *circles)*

Images of the plate surface (Fig. 12.10) and analysis of the calcium carbonate density on surface (Fig. 12.11) showed that precipitation is accompanied with the formation of narrow channels (Fig. 12.11), which are clogged by crystals.

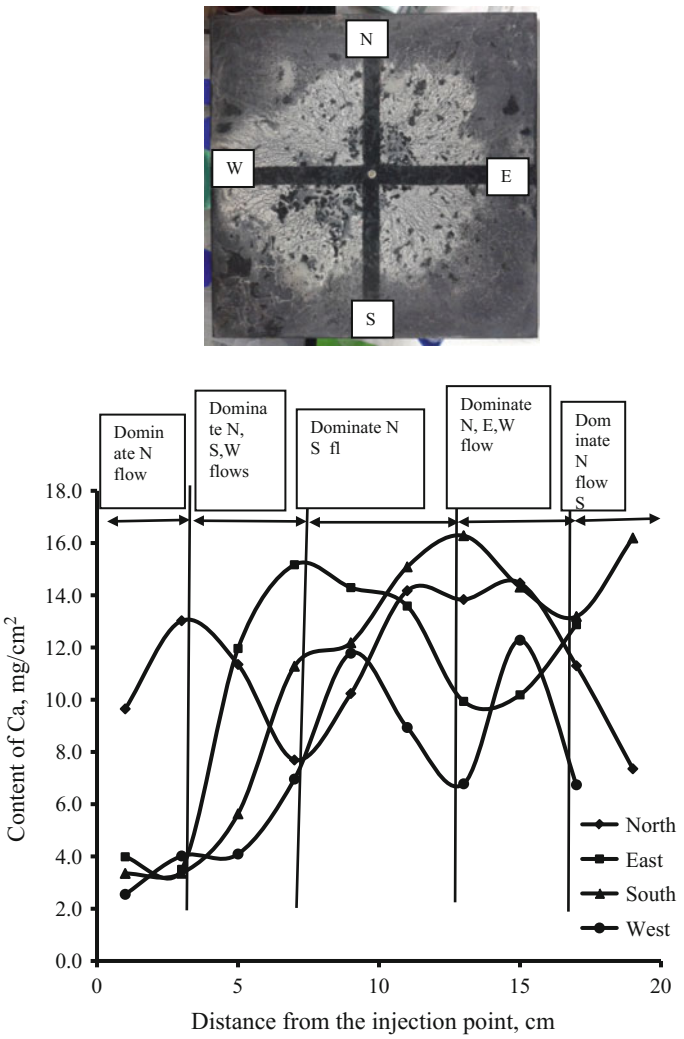


**Fig. 12.11** Radial sampling of the plate (*upper image*) and waves of the calcium carbonate precipitation (*lower graph*) showed that flow in one or two directions replaced after sometime by flow in another direction

Therefore, flow in one or two directions are replaced after sometime by flow in another direction (Fig. 12.11).

# Chapter 13
# Biocorrosion, Biodeterioration, and Biofouling in Civil Engineering

## 13.1 Microbial Biodeterioration of Construction Materials

Almost all construction materials can deteriorate due to microbial oxidation/reduction, hydrolysis, production of acids, alkali, and oxygen radicals. Timber in buildings and other wooden structures decay due to growth on their surface fungi and bacteria that hydrolyze cellulose, hemicelluloses, and degrade lignin. Dry rot, brown rot, and white rot of the timber are due to the different species of fungi specializing on biodegradation of cellulose or lignin.

Mineral acids or alkali excreted by the microorganisms cause a microbial deterioration of construction materials. For example, corrosion of steel and cement, and deterioration of marble constructions can be caused by the formation of sulfuric acid

$$S + 1.5O_2 + H_2O \rightarrow 2H^+ + SO_4^{2-} \tag{13.1}$$

Performed, for example by sulfur-oxidizing bacteria from the genus *Acidithiobacillus*, or nitric acids

$$NH_4^+ + 1.5O_2 \rightarrow 2H^+ + NO_2^- + H_2O \tag{13.2}$$

Performed, for example by ammonium-oxidizing bacteria from the genus *Nitrosomonas*.



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## 13.2 Deterioration of Cultural Heritage

Wood and paper can be easily biodegraded by the microorganisms under humid conditions. Objects of art, sculptures, and historical buildings are also deteriorated by microorganisms in humid and polluted atmosphere. For example, the marble statue shown in Fig. 13.1 has black plums due to the formation of black CaS, which can be described by the following reactions:

$$SO_2\ (\text{released to air by the vehicles}) + H_2O + 0.5O_2 \rightarrow H_2SO_4 \tag{13.3}$$

$$H_2SO_4 + 2CH_2O \rightarrow H_2S + 2CO_2 + 2H_2O\ (\text{bacterial sulfate reduction}) \tag{13.4}$$

$$CaCO_3\ (\text{white marble}) + H_2S \rightarrow CaS\ (\text{black matter}) + H_2O + CO_2 \tag{13.5}$$

Another example is biodeterioration of the column in the ancient Roman Temple of Minerva in Assisi caused probably by ammonifying and nitrifying bacteria (Fig. 13.2), in the upper layer of soil where the bottom part of the columns was embedded at some period of time:

$$CO(NH_2)_2 + 3H_2O \rightarrow 2NH_4^+ + CO_2 + 2OH^- \tag{13.6}$$

$$R(COO)NH_2 + \text{chemical energy} + [2H = \text{bioreductant}] \rightarrow RCOOH + NH_3 \tag{13.7}$$

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \tag{13.8}$$

$$CaCO_3 + 2H^+ \rightarrow Ca^{2+} + CO_2 \uparrow + H_2O \tag{13.9}$$

## 13.3 Microbial Biofouling

Microbial biofouling is the undesirable accumulation of microorganisms or products of their biogeochemical activity on submerged structures or facilities contacting with water such as groundwater wells, the pipes, coastal structures, equipment of municipal and industrial water systems, heat exchanger surfaces, air conditioners. Fouling could be due accumulation of microbial biomass, slime of microbial exopolysaccharide, scale of amorphous or crystal precipitate of calcium carbonate, ferric hydroxide, struvite $Mg(NH_4)PO_4$ induced by increase of pH and carbonate concentration by microorganisms oxidizing or hydrolyzing organic pollutants of water.


**Fig. 13.1** Biodeterioration caused by sulfate-reducing bacteria on marble statue (black plums of CaS)

## 13.4 Microbially Influenced Corrosion

Microbially influenced corrosion (MIC) is corrosion that is caused by the presence and activities of microbes. In all biochemical reactions, corrosion reactions occur with an exchange of electrons. The electrons are released in one area, the anode, travel through a metallic path and are consumed through a different biochemical

**Fig. 13.2** Biodeterioration of the columns of ancient Roman temple caused by nitrifying bacteria

reaction in another area, the cathode. The major reasons of microbially induced biocorrosion are formations of nitric, sulfuric, organic acids, $H_2S$, and $H_2$.

Microorganisms can induce corrosion:

- By stimulation of the anodic reaction by formation of acids;
- By stimulation of the cathodic reaction by microbial production of $H_2S$;
- The biodegradation of protective films;
- The increase in conductivity of the liquid environment;

## 13.5 Microbial Formation of Acids

Such organic acids as formic, acetic, propionic, butyric, lactic, gluconic, and other acids produced by bacteria during fermentation and fungi during oxidation, as well as sulfuric and nitric acids produced by bacterial oxidation (see Eqs. 13.1 and 13.2) are active corrosive agents. Biocorrosion results in pitting, crevice corrosion, selective de-alloying, stress corrosion cracking, and under-deposit corrosion. The formation of a microbial biofilm at the surface is an essential stage of the corrosion process. A biofilm provides a localized decrease of pH, concentration of oxygen, ORP, and an increased concentration of microbial metabolites, initiating corrosion.

Very common bioagents of biocorrosion are anaerobic sulfate-reducing bacteria, anaerobic fermenting bacteria, aerobic nitrifying bacteria, aerobic sulfur-oxidizing bacteria, and acid-producing fungi. Stress corrosion cracks in structures can be initiated by the action of nitrifying bacteria at the concrete surface or in the cracks where ammonium is produced from organic wastes. A bridge collapse in 2007 in Minnesota, which killed 13 people, has been attributed to a buildup of pigeons' droppings. Some experts considered that the birds' droppings deposited over the bridge's framework helped the steel beams to rust faster because of the microbial formation of nitric acid from nitrogen compounds of pigeons' droppings.

Microbially induced corrosion (MIC) can be a serious problem in stagnant water systems, such as the fire-protection system because of fermentation and production of organic acids.

## 13.6 Prevention of Microbially Influenced Corrosion, Biofouling and Biodeterioration

All microbially caused problems can be controlled by: (1) biocides; (2) maintenance of the conditions unfavorable for microbial growth and activity, for example, low humidity and water content, and absence of electron donors and acceptors; (3) conventional corrosion control methods.

Biocides are widely used in all industries. There is big diversity of chemical substances, for example, the classification of biocides in the Biocidal Products Regulation of EU 528/2012 includes 22 product types.

In some cases, microbial biofilm itself protects material from corrosion. For example, iron-reducing bacteria can protect steel from corrosion due to the formation of a protective biofilm.

## 13.7 Wood Preservatives

Timber is destroyed mainly by fungi hydrolyzing cellulose, hemicellulose, and oxidizing lignin of the timber, so the wood loses its integrity and strength. Depending on the types of fungi and mechanism of decay wood decay differentiated as wet rot, dry rot, white rot, and brown rot. To prevent microbial decay of wood the maintenance of the conditions unfavorable for microbial growth and activity, firs of all low humidity of air and water content in wood, and absence of electron donors and acceptors. To ensure long-term service of the wood construction the timber is treated with wide range of the preservatives includes copper compounds (micronized copper, alkaline copper quaternary, copper azole, copper naphthenate, chromated copper arsenate), boric compounds (boric acid, oxides and borates, PTI —Propiconazole-Tebuconazole-Imidacloprid), sodium or potassium silicates, pentachlorophenol, creosote, tung oil (China wood oil), furanic resins. All of these preservatives, for exemption of silicates and furanic resins, are highly toxic and biocumulative. Preservatives must be not leachable from wood, nontoxic for mammals, enabling the wood to be recycled at the end of its life cycle, ecologically safe and biodegradable, readily available, and cheap. Majority of existing wood preservatives do not correspond to these criteria, therefore, ecologically safe and nontoxic biotechnological wood preservatives are developing (Patachia and Croitoru 2016). Natural preservatives are waxes and resins of vegetal or animal origin category (carnauba, beeswax, rosin, dammar, copal, and shellac resins), vegetable oils with oleic, linoleic, linolenic acids, oils with alpha-linoleic acid (tall oil, tung oil, or linseed oil). Biopolymers able to diffuse into the wood, impregnating it and protecting from microbial attack. Derivates of chitin, polymer of N-acetyl-D-glucosamine, deacetylated and partially depolymerized with the different enzymes (chitinases) or by alkaline treatment to chitosan is a new biotechnological wood preservatives (Patachia and Croitoru 2016). Chitosan is inhibitor of wood-decaying fungi alone or in combination with zinc or copper salts, and fixation chemical in wood using tall or linseed oil. Proteins of different origin and tannins, which are polyphenols synthesized by plants, can be used for the fixation of the copper- and boron-based preservatives to the wood structure.

## 13.8 Bioaerosols

Negative role of microorganisms in constructions is also formation of bioaerosol, which is a collection of airborne biological particles such as viruses, cells of bacteria, spores of fungi, cells of algae, with the size ranged from 0.1 to 30 μm. Microorganisms are often attached to the dust particles. Human exposure to bioaerosols may result in infections and allergic reactions, depending on the size of bioaerosol particles and the depth of penetration into the lungs, types of microorganisms and their pathogenicity.

Typical concentrations of live bacteria in bioaerosols are 100–1000 cfu/$m^3$ for outdoor air and same or less for indoor air of ventilated space. However, it could be less 10 cfu/$m^3$ in mountain area, less than 100 cfu/$m^3$ in clean rooms, or more than 10,000 cfu/$m^3$ in air of farms or near open aeration tanks of municipal wastewater treatment plant.

## 13.9 Sources of Bioaerosols in the Buildings

Bioaerosols inside the buildings come from such major sources:

1) natural sources, including coughing, sneezing, shedding of infected human skin, pets, plants;
2) dispersion of soil dust;
3) dried droplets from aquatic environment dispersed by wind;
4) droplets from fountains, showers, air-conditioners; bacteria grow in areas where water is stagnant or condenses within air conditioning or ventilation systems;
5) microorganisms growing indoor on the surfaces of construction and decorative materials, on furniture, on organic wastes;
6) flows of indoor ventilation and outdoor air source.

## 13.10 Virus Aerosols

Viruses are intracellular parasites that can reproduce only inside a host cell. They consist usually of RNA or DNA covered with protein. Although naked viruses range from 0.02 to 0.3 μm, most airborne viruses are found as part of droplet nuclei or attached to other airborne particles. Aerolization can occur by coughing, sneezing, or talking. Virus bioaerosols spread infectious airborne diseases, such as colds, flu, chicken pox.

## 13.11 Bacterial Aerosols

Bacteria are single-celled organisms with sizes from 0.3 to 10 μm. Some bacteria form dormant spores with high survivability in dry aerosol particles, which are easily carried by air flow. There are many bacterial pathogens spread by air and causing such airborne infectious diseases such as tuberculosis and legionellosis. Aerolization can occur from the surface of soil containing up to $10^9$ bacterial cells/g or water containing up to $10^6$ bacterial cells/mL. In indoor environments, bacteria can colonize wet sites in ventilation system and become aerosolized by air flow.

Control strategies are intended to prevent formation of aerosols and exposure of susceptible individuals to aerosols containing infectious agent. For example, to prevent the outbreak of legionellosis, cooling towers should be located so that cooling tower exhaust would not be carried directly into the ventilation systems of buildings.

## 13.12 Fungal Aerosols

The majority of the fungi are saprophytes, which are organisms feeding on dead organic matter. They live in soil and in sites having decaying vegetation. Many species of fungi release spores, which are dispersed in air. These fungal spores are particles of 0.5–30 μm in size, are resistant to environmental stresses, and are adapted to airborne transport. Inhalation of fungi or their spores can cause an infection or allergic disease. Fungi and their spores can be a major problem in buildings where moisture control is poor.

Some fungi produce mycotoxins, for example, aflatoxins that are produced by the representatives of genus *Aspergillus* and the trichothecenes that are produced by species of genera *Trichoderma, Fusarium, Stachybotrys,* and some others. They are accumulating in crops during storage or on the walls at high humidity of air, polluting finally air, feed, food, cosmetics, and can cause intoxication and different diseases of human.

## 13.13 Fate of Bioaerosols

A significant number of released microorganisms is quickly die after aerosolization due to drying in air. The persistence of vegetative cells of microorganisms is increased at high humidity, low temperature, and low or absence of solar radiation. The endospores of bacteria and spores of actinomycetes and fungi have a high survival rate in the environment. The death of microorganisms in an aerosol can be described conventionally as exponential decay:

$$N = N_o e^{-kt}, \tag{13.10}$$

where $N$ is the viable microorganisms concentration in air at time $t$, $N_o$ is the viable microorganisms concentration in air at time zero, $k$ is the decay (dying) rate constant. Decay (dying) rate of viruses and microorganisms depends on such primary conditions as air temperature and humidity. Generally, but not obviously, higher temperature and lower humidity increase decay (dying) rate of bioaerosols.

To prevent production of aerosols:

1) the selection of proper materials, humidity, ventilation, and air conditioning conditions must be made;
2) in the cases when infectious agent is present in air, disinfection of this air by UV light or purification of air by adsorption filtration through activated carbon of fine fibers must be made, and a source of pathogens has to be removed or eliminated.

## 13.14 Treatment of Odorous and Toxic Gases

A lot of odorous and even toxic gases containing for example ammonia or dihydrogen sulfide can be produce during biotreatment of construction materials and soil. These gases can be treated in a wet scrubber using chemical oxidation by chlorine, hydrogen peroxide, or ozone. To remove ammonia from gas, strong sulphuric acid has to be used. The gases can be combusted at temperatures ranging from 500 to 800 °C in flame or oxidized by catalysts (palladium or platinum) at temperatures between 300 and 500 °C. The gases can be adsorbed on granulated activated carbon or other adsorbents, for example, iron oxide. Activated carbon is particularly effective at removing sulfur-containing odorous compounds. However, all these methods consume reagents, adsorbents, and energy. Not consuming reagents biofitration of these gases can be made using microbial oxidation of their components in either column biofilters with the recycle of liquid or in the compost bed. The most common way to remove gaseous pollutants from gas- or air-streams is to pass contaminated gases through bioscrubber containing suspensions of biodegrading microorganisms or through a biofilter packed with porous carriers covered by biofilms of degrading microorganisms. Depending on the nature and volume of polluted gas, the biofilm carriers in a biofilter may be cheap porous substrates, such as peat, wood chips, compost, or regular artificial carriers, such as plastic or metal rings, porous cylinders and spheres, fibers and fiber nets.

The bacterial suspension, interacting with the polluted gas, is collected at the bottom of the biofilter and recycled to the top part of the biofilter to ensure an adequate contact of polluted gas and suspension in counter or in parallel flows of liquid and gas. Addition of nutrients and fresh water to the bioscrubber or the biofilter must be made regularly or continuously. Fresh water can be used also to replace water that has evaporated in the bioscrubber or in the biofilter.

# Chapter 14
# Advances and Future Developments of Construction Biotechnology

## 14.1 Advances of Biotechnological Construction Materials

Advances in area of biotechnological construction materials are as follows:

1. admixtures for cement are produced commercially, used widely in practice, and new biotechnological admixtures are developing for industrial use;
2. bioplastic PLA is produced industrially but the construction applications are still in development because of the relatively high cost of this bioplastic;
3. bioplastic PHAs for construction applications is developing in the laboratory scale;
4. biotechnological nanomaterials for construction are studied in laboratory scale;
5. biotechnological preservatives for timber are studied and tested in pilot scale;
6. MICP biocement and biogrout is tested in pilot and field scales, but it is not environmentally friendly material due to production of ammonia/ammonium during biotreatment;
7. different types of biocements and biogrouts different from MICP are tested in pilot and field scales, but low cost and environmentally friendly materials have been tested only in laboratory;
8. biogrouts for soil desaturation and mitigation of soil liquefaction are still in the stages of pilot and field tests;
9. biomimetic 3D construction composites are still the scientific idea; there are no even successful laboratory prototypes.

So, the majority of the construction biotechnological materials for exemption of cement admixtures are not the commercial products yet and are developing and tested on the level of laboratory studies. Therefore, scientific and engineering discipline of construction biotechnology is on the stage of initial development and exponential growth with few commercial applications.



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## 14.2 Known Applications of Biocements and Biogrouts

There are known the following applications of biocements and biogrouts:

1. to enhance stability of the slopes and dams (DeJong et al. 2013; van Paassen et al. 2010);
2. road construction and prevention of soil erosion (Ivanov and Chu 2008; Ivanov 2010; Mitchell and Santamarina 2005; Whiffin et al. 2007);
3. construction of the channels, aquaculture ponds, or reservoirs in sandy soil (Chu et al. 2013; Stabnikov et al. 2013a);
4. sand immobilization and suppression of dust (Bang et al. 2011);
5. suppression of the dust-associated chemical and bacteriological pollutants (Stabnikov et al. 2013b);
6. production of the MICP treated bricks (Bernardi et al. 2014; Dhami et al. 2012; Dosier 2011; Raut et al. 2014; Sarda et al. 2009);
7. remediation of cracks in concrete and rocks and increase of durability of concrete structures (Achal et al. 2010a, b; Ghosh et al. 2005; Li and Qu 2012; Ramachandran et al. 2001; Van Tittelboom et al. 2010);
8. the reduction of hydraulic conductivity of landfill clay liners (Eryuruk et al. 2014);
9. concrete durability improvement (Pacheco-Torgal and Labrincha 2013a, b);
10. self-remediation of concrete (De Muynck et al. 2008a, b; Ghosh et al. 2006; Jonkers 2007; Jonkers et al. 2010; Siddique and Chahal 2011; Wang et al. 2012; Wiktor and Jonkers 2011; Wu et al. 2012); The repair of the cracks in the surface layer of concrete is a major portion of multibillion maintenance and repair cost of the concrete structures (Neville 1996; FHWA 2001). Self-healing concrete is based on the embedding into concrete the glass/plastic/bioplastic capsules with material, which could be released after simultaneous cracking of concrete and capsules. One type of material for self-repair of the concrete proposed to be based on MICP (Ramachandran et al. 2001; Jonkers 2007; Jonkers et al. 2010). However, volume of produced $CaCO_3$ will be always significantly smaller of the volume of capsule and the crack to be filled.
11. modification of a mortar (Ghosh et al. 2009; Vempada et al. 2011);
12. consolidation of a porous stone (Jimenez-Lopez et al. 2008);
13. bioremediation of the weathered-building stone surfaces (Achal et al. 2011a, b; Fernandes 2006; Webster and May 2006);
14. reduction of the fractured rock permeability (Cuthbert et al. 2013);
15. sealing of porous soil with biopolymers (Bergdale et al. 2012);
16. partial desaturation of sand due to nitrate bioreduction ("denitrification") using of organic compounds as electron donors. This oxidation/reduction bioprocess is following with nitrogen gas production in situ, which is causing partial desaturation of soil mitigating earthquake-induced soil liquefaction (Chu et al. 2011, 2013; Eseller-Bayat et al. 2012; Hamdan et al. 2011; He et al. 2013; Montoya et al. 2012; Rebata-Landa and Santamarina 2012; Seagren and Aydilek 2010; Weil et al. 2012; Yegian et al. 2007). This induced partial saturation as wells as

MICP biocementation of water-saturated soil were proposed as new methods of liquefaction mitigation to replace colloidal silica grouting, bentonite suspension grouting, and air injection (Huang and Wen 2015);
17. encapsulation of marine clay to produce solid filler (Ivanov 2015);
18. manufacturing of artificial coral reefs (Ivanov and Stabnikov 2016, this book) using MICP or other biocoating of concrete, plastic, wooden artificial reefs with a layer of calcite or aragonite;
19. immobilization of pollutants in soil (Fujita et al. 2004; Mitchell and Ferris 2005; Stabnikov et al. 2013a; Warren et al. 2001).

## 14.3 The Existing Problems of Biotechnological Ground Improvement and the Ways of Their Potential Solution

Future developments in biotechnological ground improvement will solve the existing problems and create new applications. The existing problems and the ways of their potential solution are shown in Table 14.1.

## 14.4 New Potential Applications of Biotechnological Ground Improvement

New potential applications of biotechnological ground improvement are shown in Table 14.2.

## 14.5 Future Products of Construction Biotechnology and Their Applications

New products and applications of construction biotechnology could be developed and used in practice, examples are the following:

1. environmentally safe and low cost biogrouts for bioclogging of the tunneling space before or after excavation;
2. environmentally safe and low cost biogrouts for bioclogging of aquaculture ponds, water reservoirs, channels, landfills, soil-polluted sites;
3. commercially available bioimmobilizers of arid desert sand surface, for dust and soil surface pollutants control, and soil erosion control;
4. commercially available biocoating materials and acceptable technologies of their application;

**Table 14.1** The existing problems of biotechnological ground improvement and the ways of their potential solution

| Existing problem | The potential solutions |
|---|---|
| Environmental safety of the MICP applications | To diminish production of ammonia and high pH during urea hydrolysis in MICP, the ratio of urea/Ca must be as low as possible<br>Develop other than MICP technologies with low or zero production of ammonia at neutral pH<br>Aerobic conditions must be created in situ to avoid the release of toxic and bad smelling dihydrogen sulfide, products of acidogenic fermentation, or deamination of aminoacids in anaerobic conditions |
| Brittleness of biocemented material | Use for bioclogging and biocementation not brittle compounds, ferric hydroxide, for example<br>Use for biocementation the bioorganic 3D composites, biomimetic analogs of shells and bones<br>Use for biocementation the composites of the brittle minerals with chemical polymers, elastic fibers, and nanomaterials |
| Sensitivity to freeze-thaw and acid rains (Cheng et al. 2013) | Use of composites<br>Sealing/biosealing of calcium carbonate depositions from penetration of acidic solution |
| Low cohesion between surface of soil particle or rock fissure and cells or produced crystals | Modification of zeta potential or hydrophobicity of surface with inorganic or organic substances to increase cohesion<br>Modification of zeta potential or hydrophobicity of surface with chemical or biopolymers to increase cohesion<br>Addition of fine dispersed material (sand, nanomaterials) to increase cohesion |
| Low predictability of the results | Modeling of the processes<br>Field tests for verification of the models |
| Stability of bioclogging, biocementation, and biodesaturation | Use formation of more stable minerals than carbonates, for example, phosphates and silicates, or ferric hydroxide<br>Immobilization of biogas bubbles using bioclogging<br>Using protection of carbonate minerals by polymers |
| Biosafety of the field application of live bacteria | Use of selected and tested for biosafety pure culture<br>Aseptic production of bacterial biomass for applications to avoid growth of potentially pathogenic strains<br>Replacement of live bacterial cells by dead but enzymatically active cells or by technical grade enzymes |
| High cost of raw materials for soil bioaggregation, bioclogging, and biocementation | Use of cheap raw materials, for example fine powder of limestone, iron ore, and organic wastes like activated sludge of municipal wastewater treatment plants, or agricultural cellulosic waste to produce soluble sources of calcium and iron |

**Table 14.2** New potential applications of biotechnological ground improvement

| Area of applications | New potential applications |
|---|---|
| Increase stability of the slopes | Lake and river shore stabilization |
| Increase stability of the dams | Strengthening of the levies |
| Road construction | Construction of country roads<br>Roadside slope stabilization |
| Prevention of soil erosion | Formation of the soil crust |
| Soil sealing | Construction of the channels, aquaculture ponds, and reservoirs in sandy soil<br>Sealing of porous soil with microbial biopolymers in situ<br>The reduction of hydraulic conductivity of landfill clay liners |
| Sand immobilization and suppression of dust | Control of dust-related chemical, radioactive, and bacteriological pollutants<br>Control of desertification and dune movement<br>Suppression of dust on country roads |
| Reduction of the fractured rock permeability | Bioclogging of the fractured rocks prior or after tunneling works |
| Mitigation of soil liquefaction | Sequential partial desaturation and biocementation of water saturated sand |
| Production of solid filler for land reclamation from marine clay | Bioencapsulation of soft marine clay |
| Bioimmobilization of pollutants in clay suspension | Bioprecipitation of pollutants from clay suspension |

5. commercially available biogrouts and technologies of their application for partial soil desaturation and mitigation of soil liquefaction;
6. low cost biocements producing low brittleness cemented materials;
7. low cost admixture to cement from sewage sludge or activated sludge of municipal wastewater treatment plants;
8. environmentally safe and low cost production of biocements and biogrouts from limestone, dolomite, cement dust, and other cement-related materials on the cement plants;
9. development of biomimetic, 3D composite biotechnological construction materials. Template-directed nucleation and template-directed fabrication can be a way for the formation of 3D composites (Dujardin and Mann 2002);
10. manufacturing of artificial reefs for recreation, aquaculture, and as the tourism attraction;
11. development of biomimetic,
12. development of self-growing construction; it was found in 2016 in Zakynthos Island (Greece) authigenic cementation of marine sediments that has formed pipe-like, disk, and doughnut-shaped concretions of ferroan dolomite forming from anaerobic oxidation of thermogenic methane in the sulfate-methane transition zone. Exposure on the modern seabed in the shallow subtidal zone

has caused confusion, as concretion morphology resembles archeological stonework of the Hellenic period (Andrews et al. 2016).
13. development of self-repairing constructions;
14. ceramic processing with biogenic additives—cells, nanofibers, and nanospheres of biopolymers as the matrices for micro- and nanostructuring of ceramics;
15. biotechnological self-healing of construction materials (Harbottle et al. 2014);
16. biotechnological paints, coatings, and adhesives for construction industry (Karak 2016; Mathias et al. 2016).

## 14.6 Eco-Efficient Biocement

By the analogy with eco-efficient concrete (Pacheco-Torgal and Jalali 2011; Pacheco-Torgal et al. 2012), eco-efficient biocement can be produced using mining tails/residuals of limestone, dolomite, iron ore and organic agricultural, food-processing, or municipal wastes using acidogenic fermentation and bioreduction of iron with production of dissolved salts of calcium, magnesium, and iron. The problem with the brittleness of biocementation could be solved using biomimetic approach (Sarikaya 1994; Mayer and Sarikaya 2002) using composite strengthening through combination of mineral and organic nano- and microparticles. By the analogy with nanomaterials in cement (Pacheco-Torgal and Jalali 2011), applications of composite micro- and nanomaterials can also be useful to increase strength and ductility of biocement. Theoretically, ductile biocement could be made as a bioinspired material (Pacheco-Torgal and Labrincha 2013b), with the 3D composite structure of hierarchically arranged nano- and micrometric units (Imai and Oaki 2010), or just simply with the layers or inclusions, where inorganic crystals of calcium carbonate (calcite, aragonite, vaterate), calcium phosphate (hydroxyapatite), oxides of Si and Fe and others create the hardness and the organic components such as proteins and polysaccharides ensure flexibility of the biocemented structure. This property is well known from the structure of the natural biominerals such as bones, shells, and corals as well as artificial engineering composite materials (Yao et al. 2011; Mayer and Sarikaya 2002). However, the cost of micro- and nanocomposites could be too high to be suitable for construction practice.

## 14.7 Calcium Carbonate Precipitation and $CO_2$ Sequestration

The microbially induced calcium carbonate precipitation process is considered some time as one of the methods for $CO_2$ sequestration (Okyay and Rodrigues 2015; Anbu et al. 2016). However, to produce major reagent, dissolved calcium

salt, $CaCO_3$ of limestone has to be dissolved in acid with the release of $CO_2$. The same quantity of $CO_2$ will be released from the system that could be precipitated (sequestrated) during precipitation in the form of $CaCO_3$. So, the balance of $CO_2$ release and precipitation is zero one. When urea is added to enhance precipitation, the balance will be positive, i.e., more $CO_2$ will be released then sequestrated. Only in case when calcium chloride brine is a reagent, $CO_2$ sequestration could be a real process.

The same is related to the production of $Ca(HCO_3)_2$ from $CaCO_3$ and $CO_2$ and utilization of this solution for bioclogging as it was described in the chapter on bioclogging in this book. There could be some but minor sequestration effect because a portion of $CO_2$ produced during self-decay of calcium bicarbonate will be retained in the bioclogged voids.

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