

# BIOMASS CROPS

PRODUCTION,  
ENERGY AND THE  
ENVIRONMENT

*Alfred P. Haggerty*  
*Editor*



*Environmental Science, Engineering and Technology*

NOVA

# **BIOMASS CROPS: PRODUCTION, ENERGY AND THE ENVIRONMENT**

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# **BIOMASS CROPS: PRODUCTION, ENERGY AND THE ENVIRONMENT**

**ALFRED P. HAGGERTY**  
**EDITOR**



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## PREFACE

In the energy industry, biomass refers to biological material which can be used as fuel or for industrial production. Biomass includes plant matter grown for use as biofuel, as well as plant or animal matter used for production of fibres, chemicals or heat. This new book presents current research in the study of biomass crops, including the conversion of wood into liquid fuels; alfalfa biomass production; gasification of biomass in aqueous media and biofuel production potential.

Chapter 1 - Statistics show that energy consumption has increased exponentially since the Industrial Revolution. Around 495 EJ of primary energy were consumed in the world in 2008, of which over 81% were met by combustion of fossil fuels (IEA, 2009). Considering a global population of 6.8 billion, this represents on average the equivalent of approximately 2 tons of petroleum per person and year. Projections for the coming decades reveal an average annual growth above 2.0%, owing primarily to an increase in world population and the rapid development of emerging economies like China, India and Brazil (IEA, 2009). At this rate, global energy demand is expected to double and global electricity demand will triple between 2008 and 2050 (EIA, 2010).

Chapter 2 - The looming energy challenges around the world will have to be tackled with a portfolio of different raw materials and technologies. Biomass is a widely available renewable carbon source and includes organic wastes and energy crops, which can be used for the production of biofuels to contribute to the reinvigoration of the biomass industry, among others. Energy crops have to be produced in a cheap and environmentally benign way in order to be utilized for the sustainable production of biofuels. Energy crops include mainly three categories, namely oil-rich crops, sugar crops, which contain sugars directly available for biofuel production, and lignocellulosic crops, which contain tightly bound cellulose, hemicellulose and lignin.

In this Chapter focus is given on fermentative biofuel production from sugar-rich and lignocellulosic biomass. Ethanol production from energy crops has been studied in the literature since the 1980s, but in the last decade significant research efforts have been addressed towards biological hydrogen production. Therefore, ethanol and hydrogen are considered two representative options for short- and long-term biofuel production, respectively. In particular, biofuel production from lignocellulosic crops or agricultural residues is more intensively discussed, given the higher degree of complexity of the utilization of these raw materials for biofuel production. Various pretreatment methods can be

applied to enhance the accessibility of lignocellulosic carbohydrates for enzymatic hydrolysis and the production of fermentable substrates. The efficiency of ethanol/hydrogen production from these substrates is dependent on their quality, which largely depends, in turn, on the amount of degradation products which act as inhibitors in the fermentations. Therefore, significant discussion is dedicated to the key aspects of the role of pretreatment of biomass on the efficiency of biofuel production.

The development of dedicated pretreatment techniques which are tuned to special characteristics of different energy crops is discussed. Sweet sorghum and sugar beet are regarded as two energy crops that can be instrumental in the promising field of biofuels. Sweet sorghum is interesting because it constitutes a highly productive sugar crop which, after sucrose extraction, provides a yet not well studied lignocellulosic residue, sweet sorghum bagasse. Sweet sorghum bagasse is currently unexploited, poses a disposal problem and its usage as fodder for animals is not a sufficiently viable solution. Similarly, sugar beet constitutes a traditional sugar crop, which can provide the biofuel industry with innovative raw materials with no competition with food production. From the viewpoint of sustainability, it is necessary to simultaneously assess the impact of the aforementioned biomass and biofuels on the environment and the economic growth. In principle, the use of organic wastes can be a win-win solution; however, biomass crops can present peculiar advantages for their potential in protecting/reclaiming vulnerable and marginal soils, sequestering CO<sub>2</sub>, bio-depurating wastewater and enhancing biodiversity and wildlife.

Chapter 3 - Increasing atmospheric CO<sub>2</sub> results in enhanced photosynthesis in C<sub>3</sub> plants like alfalfa. However, after long-term exposure, the photosynthetic rate decreases. This phenomenon, often described as down-regulation, is explained by most authors as the consequence of the disappearance of strong plant sinks leading to leaf carbohydrate accumulation and thus resulting in a photosynthetic decrease. The initial photosynthesis response to elevated CO<sub>2</sub> induces plant growth and enhanced yield production. After long term CO<sub>2</sub> exposure, when photosynthesis is acclimated, increased plant biomass is also shown due to the initial enhancement of plant dry matter. Management of alfalfa as a forage crop entails periodic cutting of shoots. In this situation, photosynthetic down-regulation is avoided and the alfalfa taproot is the main source organ that provides C and N compounds to new growing shoots. This source and sink organ role inversion allows us to study alfalfa biomass production before and one month after cutting (regrowth). Alfalfa is used as a forage crop for animal feeding as a source of protein and amino acids; therefore not only is the quantity of crop production important but also the biochemical composition of shoots is a key factor. During the present study, elevated CO<sub>2</sub> reduced leaf protein concentration probably due to the dilution effect derived from starch accumulation in these conditions. Forage plants may also be the primary source of antioxidants. Elevated CO<sub>2</sub> altered reactive oxygen species (ROS) production in leaves, reducing their production, and resulted in the relaxation of the antioxidant system, which may induce changes in the antioxidant value of forage biomass.

Chapter 4 - Phytochemical induction of monoterpenes following herbivory by insects and mechanical damage, was studied in *Minthostachys mollis* (Lamiaceae), a plant native to Central Argentina with medicinal and aromatic uses in the region. The monoterpenes pulegone and menthone were analyzed in *M. mollis* 24 and 48 h after leaves were mechanically damaged or exposed to insects with different feeding habits (chewing, scraping, sap-sucking, and puncturing). Essential oil composition and emission of volatiles were assessed. Mechanical damage resulted in an increase of pulegone and menthone concentration

in *M. mollis* essential oil during the first 24h. Menthone content generally decreased whereas pulegone concentration increased in all treatments where insects were involved. The changes observed after insect feeding occurred also in the adjacent undamaged leaves, but induced changes after mechanical wounding were restricted to the damaged site, suggesting that an elicitor related to the insects may be required for a systemic response to be induced.

Changes in the volatiles released from *M. mollis* damaged leaves were also detected, most noticeably showing an increase in the emission of pulegone. Inducible chemical changes in aromatic plants might be common and widespread, affecting the specific compounds on which commercial exploitation is based.

Chapter 5 - The lignocellulosic biomass materials are abundant, cheap and renewable feedstocks suitable for biofuel and biochemical production. They can be derived from forestry wastes such as residues of the trees and shrubs, energy crops like maize, sorghum, miscanthus, kenaf, switchgrass, jatropha, corn, sugarcane and any agricultural residues such as corn stovers, wheat straw etc. The use of biofuels derived from lignocellulosic biomass does not cause additional increase in the carbon dioxide level in the earth's atmosphere. The release of carbon dioxide during biofuel utilization is balanced by the carbon dioxide consumed in biomass growth.

In many cases, because of large water content and high drying cost, biomass is not a suitable feedstock for conventional thermochemical gasification technologies. Thermochemical gasification techniques such as biomass gasification and pyrolysis are energy intensive processes and produce relatively high amounts of char and tar with low conversion of biomass into gas. Among other various conversion methods, hydrothermal gasification, using super- or sub-critical water as the reaction medium, is seen as a promising way to produce hydrogen from biomass with high efficiency. These processes can be applied to the conversion of biomass with high moisture content without drying. While processes applied in sub- and super-critical water around 250-400°C, methane and carbondioxide are the major products in addition to the target gas hydrogen but the formation of these major side products can be minimized by using appropriate catalysts and adjusting processing temperature and pressure conditions.

Aqueous phase reforming (APR) process is a rather new evolving technology involving decomposition of the oxygenated hydrocarbons to produce hydrogen-rich gas. The main advantage of APR is its relatively low gasification temperature where CO concentration within the hydrogen stream is rather low. The process produces high yield of hydrogen gas with low CO byproduct due to the water-gas shift reaction ( $\text{CO} + \text{H}_2\text{O} \leftrightarrow \text{H}_2 + \text{CO}_2$ ) which is effective at the processing temperature. APR of carbohydrates take place at considerably lower temperatures compared to conventional alkane steam reforming process. A lower temperature reduces unwanted decomposition reactions that normally observed when carbohydrates are heated to elevated temperatures. Carbohydrates such as sugars (e.g., glucose) and polyols (e.g., ethylene glycol, glycerol) can be efficiently converted in the aqueous phase over appropriate heterogeneous catalysts at relatively mild processing conditions to produce hydrogen rich gas mixture. Lignocellulosic materials containing high level of polysaccharides are potential biomass sources for the APR gasification provided that, by using ecological pre-treatment techniques, the water-insoluble polysaccharides are hydrolyzed into relatively smaller carbohydrates which are soluble in water. This chapter will focus on APR and summarize the relevant research and development activities including the authors' work on conversion of lignocellulosics.

Chapter 6 - The biofuels are ecologically acceptable energy sources resulting in reduction in release of large quantities of CO<sub>2</sub> and other harmful greenhouse gases into the atmosphere causing global warming and climate change. Biofuels like biodiesel and bioethanol are the most important transportation fuels either used alone or as an additive to fossil fuels. The production technologies such as transesterification and fermentation are used for biodiesel and bioethanol respectively. The biofuel strategy should focus on use of biomass for blending ethanol with gasoline and non-edible oilseeds for production of biodiesel for blending with petro-diesel. Besides providing relief against local air pollution and global environmental change problems such diversification could also be useful in managing interruptions of fossil fuel supply, volatility of its prices and thus ensuring sustainable economic development.

Chapter 7 - There are many farmlands that have been contaminated with heavy metal (HM) in central Taiwan resulted from the irrigation using river water contaminated with HMs. According to the Soil and Groundwater Pollution Remediation Act (SGWPR Act) of Taiwan, these lands cannot plant edible crops until suitable techniques are conducted to decrease the total concentration of HM in soils to conform to the Soil Control Standard (SCS). However, some of the foliar crops still accumulated a high concentration of HM in the edible parts; even the concentration of HM of the remediated sites is below the SCS. Planting suitable crop species is especially important in this situation and these contaminated sites after remediation can be reused. Soybean, a biomass crop further used to produce biodiesel, seems feasible to plant in the farmlands in this situation. This manuscript reports previous results that used pot experiments to investigate the accumulation of HM by various parts of soybean planted in the artificially cadmium- (Cd-), copper- (Cu-), or zinc (Zn-) contaminated soils with different concentrations. The aim is to assess the feasibility of planting soybean in the HM-contaminated soils to produce biodiesel.

Chapter 8 - Declining soil fertility is a critical agricultural challenge facing smallholders in central Kenya. A study to improve soil fertility and farm productivity in the area was carried out during the period 2003 to 2007. Problem-solving tools were used to build the broad conceptual and methodological approaches needed to address farming constraints. The study identified farming systems constraints and disseminated “best-bet” integrated soil fertility management (ISFM) interventions using participatory methods and mutual collaborative action. This paper describes processes in the participatory approaches, project milestones and joint experiences that were gained. The participatory approaches included Participatory Rural Appraisal (PRA), Mother-baby approach (M-B approach), Farmer training groups (FTGs), Annual stakeholder planning meetings, Village training workshops, Cross-site visits and Participatory Monitoring and Evaluation (PM & E). Food shortage was the main problem identified by farmers resulting from low crop yields. The causes of poor yields were biophysical factors, but several socio-economic factors influenced farmer ability to manipulate farm productivity. Village training workshops attracted a 20% higher farmer turnout than mother trial field days. Farmer and experimental evaluations showed that the most favoured technologies were tithonia, manure, manure-fertilizer combinations, and tree legumes while the most effective dissemination pathways included demonstrations, farmer training grounds, field days and farmers’ groups. Using PM& E procedures, farmers developed indicators that they used to monitor progress, and annual ISFM milestones were achieved, leading to the achievement of overall project objectives. Innovative adjustments to ISFM technology dissemination were proposed by both farmers and scientists.

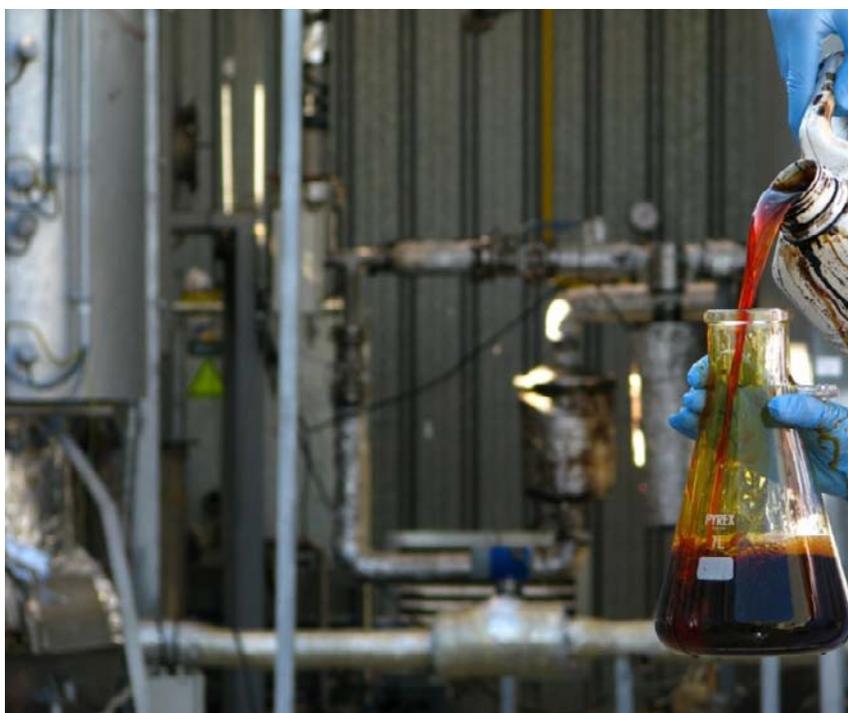
*Chapter 1*

**CONVERSION OF WOOD INTO LIQUID FUELS:  
A REVIEW OF THE SCIENCE AND TECHNOLOGY  
BEHIND THE FAST PYROLYSIS OF BIOMASS**

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# 1. INTRODUCTION TO BIOMASS ENERGY

## 1.1. The Current Energy System

Statistics show that energy consumption has increased exponentially since the Industrial Revolution. Around 495 EJ of primary energy were consumed in the world in 2008, of which over 81% were met by combustion of fossil fuels (IEA, 2009). Considering a global population of 6.8 billion, this represents on average the equivalent of approximately 2 tons of petroleum per person and year. Projections for the coming decades reveal an average annual growth above 2.0%, owing primarily to an increase in world population and the rapid development of emerging economies like China, India and Brazil (IEA, 2009). At this rate, global energy demand is expected to double and global electricity demand will triple between 2008 and 2050 (EIA, 2010).

It becomes evident that this trend is unsustainable. From an environmental perspective, the massive combustion of fossil fuels has been held responsible for irreversible changes to the global climate, with profound consequences to the planet and our way of life (IPCC, 2007). With regard to the economy, the imbalance between increasing demand and the ongoing depletion of finite natural resources will push the price of fossil fuels up in the medium term. Unless the use of alternative energy sources is sufficiently widespread, this scenario will lead to international conflicts and severe contraction of the global economy with detrimental consequences to the standard of living of most people. The solution to this situation rests on two pillars: on the one hand, a reduction in the overall consumption of primary energy that should be achieved through implementation of energy efficiency measures and technologies. On the other, the promotion of locally available and less carbon intensive alternative energy sources that should progressively replace fossil fuels (Stern, 2006; IPCC, 2007; Demirbas et al., 2009).

At present, renewable sources collectively supply 14.0% of all the primary energy consumed in the world. The largest contribution comes from biomass, accounting for almost 10% of the global energy supply. Most of this bioenergy (87%) is obtained by direct combustion of wood for heat generation in domestic, agricultural, farming or small scale production activities. Biomass energy prevails, primarily in less industrialized economies owing to its use in domestic activities and also in environmentally conscious countries with abundant natural resources like Scandinavian countries and Brazil (IEA Bioenergy, 2002 and 2010).

Owing to the environmental and strategic benefits associated with the use of renewable energy sources, most governments have drafted programs with ambitious objectives intended to facilitate a smooth transition into this new energy model. A clear example is the "Energy-Climate Legislative Package" approved in 2009 by the Council of the European Union. This document requires Member States to meet the following targets by 2020: reduce greenhouse gas emissions by 20% of 1990 levels; increase the contribution of renewable energy sources to 20% of the total; and reduce primary energy consumption by 20% through implementation of improved efficiency technologies and strategies. The European Directive 2009/28/EC on the promotion of renewable energy has recently established additional objectives, including a 10% share of renewable energy in the transport sector by 2020.

Biomass will certainly have a significant role to play in the development of this sustainable energy model. Biomass contains chemical energy that has been captured from the sun radiation through photosynthesis. As illustrated in Figure 1, this energy can be made available by the application of different technologies, which are usually classified in two categories: biological such as anaerobic digestion and fermentation; and thermochemical like combustion, gasification and pyrolysis. This paper deals with advanced thermochemical routes and their potential in the efficient transformation of biomass feedstocks into valuable energy and chemical products.

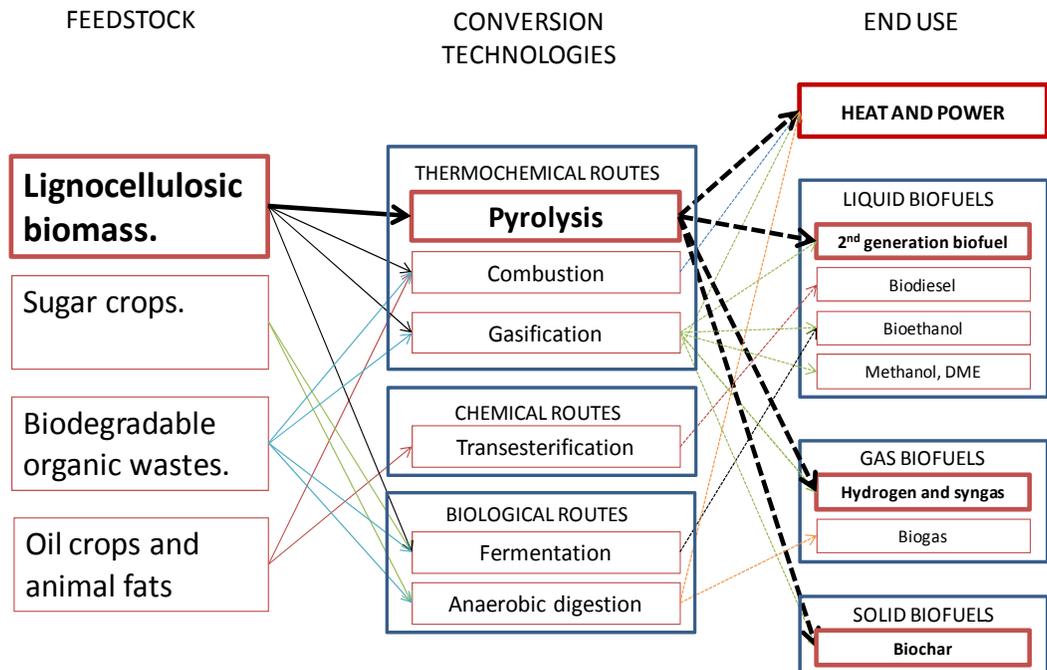


Figure 1. Alternative biological and thermochemical routes for the energy valorization of biomass.

## 1.2. Conventional Thermochemical Technologies: Biomass Combustion

Combustion of wood for heat generation is a well established technology, commonly associated with agricultural and industrial activities that produce biomass as a by-product (such as wood processing, paper making, forest management and food production and processing like olive oil, cereals and nuts). Around 83% of the solid biomass consumed in Europe is dedicated to heat production and only 17% to power generation. However, the latter is expanding rapidly, primarily in Northern Europe, North America and Brazil owing to favorable policies and abundant resources (IEA Bioenergy, 2002; Van Loo and Koppejan, 2008; Euroobserver, 2010).

Due to the low energy density and high transportation costs of biomass fuel, dedicated biomass power plants are usually smaller (5-25 MWe) than those using conventional fossil fuels, resulting in lower energy conversion efficiencies (typically between 20-25% depending on plant size and technology) and higher investment costs (2000-4000€/kW). Biomass

combustion plants also require expensive maintenance programs due to corrosion, fouling and slagging caused by inorganic elements present in the biomass fuel (Cl, K, Na, Ca, Mg) (Jenkins et al., 1998; Demirbas, 2005). Economic risks are also higher than in conventional power plants owing to seasonal and yearly variability in the production and quality of the biomass feedstocks, their scattered geographical distribution and high transportation costs (Caputo et al., 2005).

As a result, the production of electricity from biomass remains expensive in most cases and highly dependent on public subsidies (Badcock and Lenzen, 2010; San Miguel et al., 2010). Electricity costs have been calculated to range between US\$20/MWh for the co-firing of a locally available charge-free biomass feedstock to a more realistic \$30-\$50/MWh for medium size (10-25 MWe) dedicated power plants using conventional biomass at a standard cost of US\$3-US\$3.5/GJ (Caputo et al., 2005). This compares unfavorably with electricity generation costs calculated for more conventional energy sources like hydroelectric power (15 US\$/MWh), natural gas in a combined cycle (25 US\$/MWh), coal (26 US\$/MWh) and nuclear (16 US\$/MWh) (CNE, 2008). At present, biomass only accounts for 1.3% of the electricity generated in the world, 3.7% in the European Union and 3.4% in the USA (IEA Bioenergy, 2010; EIA, 2009).

### 1.3. Advanced Thermochemical Technologies: Gasification and Pyrolysis

The increase of scale in the consumption of biomass fuels expected in the following years will definitely require the development of innovative technologies capable of providing greater energy efficiency, improved cost effectiveness and a higher overall environmental performance. Gasification and pyrolysis are two thermochemical technologies that have the potential to contribute in this direction and play a key role in the expansion of bioenergy. As shown in Table 1, the former involves the transformation of a carbonaceous feedstock into a gas, usually called syngas or producer gas, by exposure to high temperatures (850-1000°C) under mildly oxidizing conditions (usually substoichiometric oxygen or/and steam). Gasification was developed in the early 19<sup>th</sup> century for the transformation of mineral coal into town gas that was used for lighting and domestic energy applications. It was subsequently adapted for the treatment of organic wastes, petroleum fractions and biomass. Syngas can be used with higher energy efficiency than the original feedstock in burners, engines and turbines for the generation of heat and electricity. After necessary processing and upgrading, syngas may also be used as a chemical feedstock for the synthesis of ammonia, methanol, synthetic liquid fuels or purified hydrogen.

Biomass gasification has already passed the demonstration stage and can be regarded as a young commercial technology, with plants of varying scale operating around the world. However, it is also true that the penetration of this technology in the energy market is still limited due to technical problems associated with the formation of tars and the fuel quality of the resulting gases (Knoef, 2005; Badeau et al., 2009).

Pyrolysis is another thermochemical technology capable of transforming lignocellulosic biomass into high value products. The term *pyrolysis* is very self explanatory in its root, deriving from the Ancient Greek words *pyro* (πρ) meaning heat and *lysis* (λύσις) meaning rupture. Pyrolysis is mainly associated with thermal decomposition of organic compounds, as they are heated in the absence of oxygen or any other reactive element. The pyrolysis of

lignocellulosic biomass gives rise to its transformation into a non-condensable gas, condensable oil and a solid char, which can be used for their energy content or as a chemical feedstock. As will be explained throughout this paper, this technology is highly versatile, with product yields and characteristics highly dependent not only on biomass feedstock but also processing conditions. As shown in Table 1, pyrolysis processes are usually classified in two categories depending on the target product: solid charcoal in slow pyrolysis and liquid oils in fast pyrolysis.

**Table 1. Typical product yields, reaction conditions and enthalpy in the pyrolysis of biomass, compared against combustion and gasification technologies**

Process		Product yields (wt%)			Conditions	Enthalpy
		Liquid	Char	Gas		
Pyrolysis	Slow	15-25	30-40	30-40	Low temperature (300-600°C). Long vapor residence time (> 1 min) Inert atmosphere	Endothermic/ Exothermic
	Fast	60-75	10-15	15-30	Moderate temperature (450-500°C). Short vapor residence time (< 2 sec) Inert atmosphere	Slightly endothermic
Gasification		0-5	5-10	85-95	Very high temperature (850-1000°C). Mildly oxidizing conditions	Endothermic
Combustion		0	0-5	95-100	High temperatures (700-850°C). Highly oxidizing conditions.	Highly exothermic

Slow pyrolysis or carbonization is a traditional technology that has been used for centuries in rural societies for the production of charcoal (Lehmann and Joseph, 2009). Charcoal is more stable and has a higher energy density than the original biomass, which allows for storage for longer periods of time and produces higher temperatures upon combustion. Slow pyrolysis involves heating the wood slowly to temperatures around 300-600°C in the absence of oxygen over long periods of time. The process results in the thermal decomposition of the feedstock into a volatile fraction and a solid char. The resulting vapors are allowed very long residence times inside the reaction chamber in order to maximize recombination and polymerization reactions, leading to the formation of the solid char (30-40 wt%). The anoxic conditions were traditionally achieved by covering the piles of wood with turf or moistened clay. At present, the process is conducted in kilns and retorts which achieve higher carbon efficiencies and reduce processing times. The vapors generated in slow pyrolysis processes are usually condensed in order to reduce emissions into the atmosphere. This condensate, called pyrolytic oil, amounts to 15-25 wt% of the original biomass feedstock and consists of a mixture of water, methanol, acetic acid, phenols and other insoluble organic tars (Strezov et al., 2007; Lehmann and Joseph, 2009).

Fast pyrolysis is based on the same principles as carbonization, but relies on the careful control of the process conditions in order to maximize the formation of condensable compounds at the expense of other gas or solid fractions. In short, the production of high oil yields requires very rapid thermal degradation of the biomass feedstock followed by very rapid removal of the volatile products in order to minimize secondary reactions. This objective is achieved using reactors that allow efficient heat transfer into the biomass particle,

small particle size of the feedstock, fine control of the reaction temperature (usually between 450°C and 500°C) and reduced residence time of the pyrolysis vapors in the reaction chamber (usually below 2 seconds). Oil yields up to 85 wt% have been reported in the literature, although typical values in optimized plants usually range between 60-75 wt%.

Bio-oils produced through fast pyrolysis have significantly higher energy density (around 19-22 GJ/m<sup>3</sup>, compared to 4-5 GJ/m<sup>3</sup> in the original biomass and 38-40 GJ/m<sup>3</sup> of petroleum derived fuels) and more homogeneous characteristics than the original material. Therefore, they can be handled, stored, transported and processed more effectively than the original biomass feedstock. Pyrolysis oil is intended to serve as a secondary energy carrier for subsequent conversion into heat, electricity, chemicals or transport fuels.

The vision expressed in numerous communications describes an energy system in which bio-oil is produced in distributed pyrolysis units close to the sources of generation and then transported to centralized plants for energy generation or to bio-refineries for subsequent processing into advanced fuels and high-value chemicals (Bridgwater et al., 1999; Czernik and Bridgwater, 2004; Mohan et al., 2006; Demirbas and Balat, 2006; Babu, 2008; Badger and Fransham, 2006; Venderbosch and Prins, 2010). This model benefits from the improved economy of scale of large energy generation and processing plants. Unlike first generation bio-fuels (bio-ethanol and biodiesel), which only make use of a small fraction of the biomass (sugars and fats, respectively), fast pyrolysis processes make use of all the organic matter present in the biomass feedstock. This results in higher overall energy efficiencies and greater greenhouse gas abatement potential.

The basis of modern, fast pyrolysis was established in the early 1980's with pioneering work conducted at the University of Waterloo (Canada). This was followed by numerous research institutions and companies that contributed in different areas like fast pyrolysis process design, characterization of pyrolysis oils and development of energy applications. The most renowned of these research groups include Aston University (United Kingdom), VTT Technical Research Centre (Finland), the University of Western Ontario (Canada), the National Renewable Energy Laboratory (USA) and the University of Twente (Netherlands), while the most active private companies in this field include Biomass Technology Group (BTG, The Netherlands), Envergent Technologies (USA), Dynamotive Energy Systems (Canada) and Ensyn Technologies (Canada). Interest in this technology is evidenced by the large number of technical documents published in the last two decades including numerous scientific reviews (Graham et al., 1984; Elliot et al., 1991; Maschio et al., 1992; Freel et al., 1993; Scott et al., 1999; Bridgwater, 1999; Bridgwater et al., 1999; Meier and Faix, 1999; Oasmaa and Czernik, 1999; Bridgwater and Peacocke, 2000; Bridgwater, 2003; Czernik and Bridgwater, 2004; Yaman, 2004; Mohan et al., 2006; Demirbas and Balat, 2006; Zhang et al., 2007; Babu, 2008; Goyal et al., 2008; Qiang et al., 2009; Zhang et al., 2010; Venderbosch and Prins, 2010), manuals (Bridgwater et al., 1999; Bridgwater 2002 and 2005) and compilations (Bridgwater, 2001, 2003, 2008 and 2009; Bridgwater and Boocock, 2006). The intense activity in this field of international scientific networks and organizations like ThermalNet ([www.thermalnet.co.uk](http://www.thermalnet.co.uk)), PyNe (<http://www.pyne.co.uk>) and IEA Bioenergy ([www.ieabioenergy.com](http://www.ieabioenergy.com)) is also noteworthy.

Despite the considerable scientific and technical progress achieved in the last few decades, fast pyrolysis of biomass is not a complete reality yet. The production of bio-oil is not particularly expensive but there is limited acceptance of this novel fuel in the energy and chemical markets. Existing plants operate intermittently, usually in association with research

programs designed to improve the efficiency of the pyrolysis process, upgrade the fuel properties of the resulting oils and develop new energy technologies capable of using them efficiently. Despite these uncertainties, private companies have been making substantial investments in the last few years in preparation for new technical developments that allow a wider application of these oils. This includes the construction of increasingly larger biomass pyrolysis plants, such as the 50 T/day BTG plant constructed in Malaysia in 2005, and based on rotating cone technology; the 130 T/day and the 200 T/day Dynamotive plants in West Lorne and Guelph (Canada) constructed in 2005 and 2006 based on the fluidized bed technology; the 100 T/day; the 100 T/day RTP<sup>TM</sup>-1 plant constructed by Ensyn in 2007 in Renfrew (Canada) based on circulating fluidized bed technology; and the 36 T/day Bio Oil Holding-1 plant in Tessenderlo (Belgium) based on the Auger technology. In addition, a few more commercial plants are being planned for the next few years or are under construction, like the Pytec Mallis plant at Brahlstorf (Germany), the Bio Oil Holding plants at Tessenderlo (Belgium) and Delfzijl (Netherlands), and the BTG Empyro BV at Hengelo (Netherlands).

The situation at present has been compared to the early days of the petrochemical industry, owing to the enormous potential of this new material (bio-oil) but the lack of experience and technical means that allow its use and transformation in a cost-effective manner (Mohan et al., 2006). The path will be progressively cleared with the advent of green chemistry, the development of biorefinery concepts (Demirbas, 2009; Cherubini, 2010) and the progressive depletion of fossil fuel resources.

The aim of this paper is to provide a review of existing knowledge regarding the fast pyrolysis of biomass and provide a critical evaluation of its potential in the mass production of renewable energy. The paper has been divided into six sections, including this introduction, to biomass energy. Section two provides a review of scientific and technical aspects associated with the pyrolysis of biomass, including a description of the biomass feedstock, the chemistry behind the thermal degradation of the biomass components and the conceptual description of the essential features in a typical fast pyrolysis plant. Owing to the importance of reactor design in this technology, section three provides a review of the existing alternatives, with information about technical and economic aspects associated with each option. This section is illustrated with a description of the 25 kg/hour pilot scale biomass fast pyrolysis pilot plant designed and developed at Ikerlan-IK4 (Miñano, Spain). Section four contains information about the chemical, physical and fuel characteristics of the bio-oils and evaluates potential applications primarily in the energy sector. Section five provides a summary of alternative technologies aimed at improving the fuel properties of pyrolysis oils. This upgrading is essential in order to counteract the adverse properties of the oils in terms of instability and corrosive behavior, and to facilitate its use in conventional or adapted energy technologies like burners, engines and turbines. Finally, section six provides a historic review of the commercial development of fast pyrolysis technology, with a description of the most representative plants and future developments.

## 2. SCIENTIFIC PRINCIPLES OF BIOMASS PYROLYSIS

### 2.1. Biomass as an Energy Feedstock

#### 2.1.1. Introduction to Lignocellulosic Biomass

The term biomass in its broadest sense is used to describe organic matter produced by living organisms. Electromagnetic radiation from the sun is captured by green plants during photosynthesis and stored as chemical energy in the form of sugar molecules. These molecules are subsequently transformed into other biomass constituents (fats, proteins and carbohydrates) through different metabolic routes and passed around to other organisms through the food chain (Stassen et al., 1999). Biomass is currently the largest source of renewable energy in the world and a continuous expansion is expected in the following years in order to comply with existing programs aimed at reducing the consumption of fossil fuels (EIA, 2010; IEA, 2009; Euroobserver, 2010). The combustion of biomass and its derivatives is regarded as low carbon activity because the CO<sub>2</sub> emitted into the atmosphere has been recently captured by the plants through photosynthesis. Substitution of fossil fuels with locally generated biomass provides additional benefits in the form of energy security, improved trade balances, opportunities for economic and social development in rural communities and the possibility of reducing waste disposal problems (Stern, 2006; IEA Bioenergy, 2010).

Although any organic material can be subjected to fast pyrolysis, this technology was developed primarily for the conversion of lignocellulosic biomass. Wood parts, shavings, sawdust and tree bark produced as by-products of forest management, wood processing and agricultural activities are the most common feedstocks for fast pyrolysis processes. Other abundant types of residual lignocellulosic materials with the potential to be subjected to fast pyrolysis include sugarcane and sorghum bagasse, corn and cotton stalks, rice hulls, cereal straws, peanut and oat hulls, organic fibers from the pulp and paper industry, nut shells and fruit stones (Mohan et al., 2006). Residual lignocellulosic biomass is being increasingly demanded for various energy and non-energy applications, resulting in reduced availability and higher market prices. In addition, due to their residual nature, they tend to be subjected to changes in quality and supply. Hence, dedicated energy crops like short-rotation coppice (eucalyptus, poplar, willow) and grasses (miscanthus, switchgrass) are increasingly being considered a means of producing large amounts of lignocellulosic biomass in a more controlled environment. Intensive energy crops need to be adequately managed in order to minimize other adverse effects on local biodiversity and land use (Berndes et al., 2003; Bassam, 2010).

However, not all lignocellulosic materials are the same. Hard woods, soft woods, herbaceous plants and other biomass feedstocks differ largely in their chemical composition and physical characteristics, which have a notable effect on their thermal behavior. The main differences relate to the proportion and exact characteristics of the polymeric components (lignin, cellulose and hemicellulose), water content and the presence of additional organic compounds (resins, fats, sugars and other extractives), and inorganic elements (ash) (Salisbury et al., 1992). These features have a notable effect on the pyrolysis behavior of the feedstock, affecting the yield and characteristics of the final products (Oasmaa et al., 2003;

Yang et al., 2007; Livingston, 2007; Fahmi et al., 2008; Guo et al., 2010). Hence, adequate selection and conditioning of the biomass feedstock has to be regarded as an essential part of the fast pyrolysis process.

### 2.1.2. Polymeric components of lignocellulosic biomass

Lignocellulosic biomass is a composite material consisting primarily of three organic polymeric constituents: cellulose, hemicellulose and lignin (Salisbury and Ross, 1992; Sjostrom, 1993; Walker, 2009). As shown in Figure 2, cellulose is a linear homopolysaccharide typically consisting of between 10,000-15,000 units of D-glucose, linked by  $\beta(1\rightarrow4)$  glycosidic (ether) bonds. Cellulose is a structural component of the primary cell wall in all green plants and typically represents 40-60 wt% of the wood. It has a crystalline structure stabilized by  $(O_6 \rightarrow H-O_2)$  and  $(O_3-H \rightarrow O_5)$  intermolecular hydrogen bonds, which make it highly stable and resistant to chemical and thermal degradation. The chemical formula of cellulose is  $(C_6H_{10}O_5)_n$ , with oxygen representing 49% of its mass (O'Sullivan, 1997).

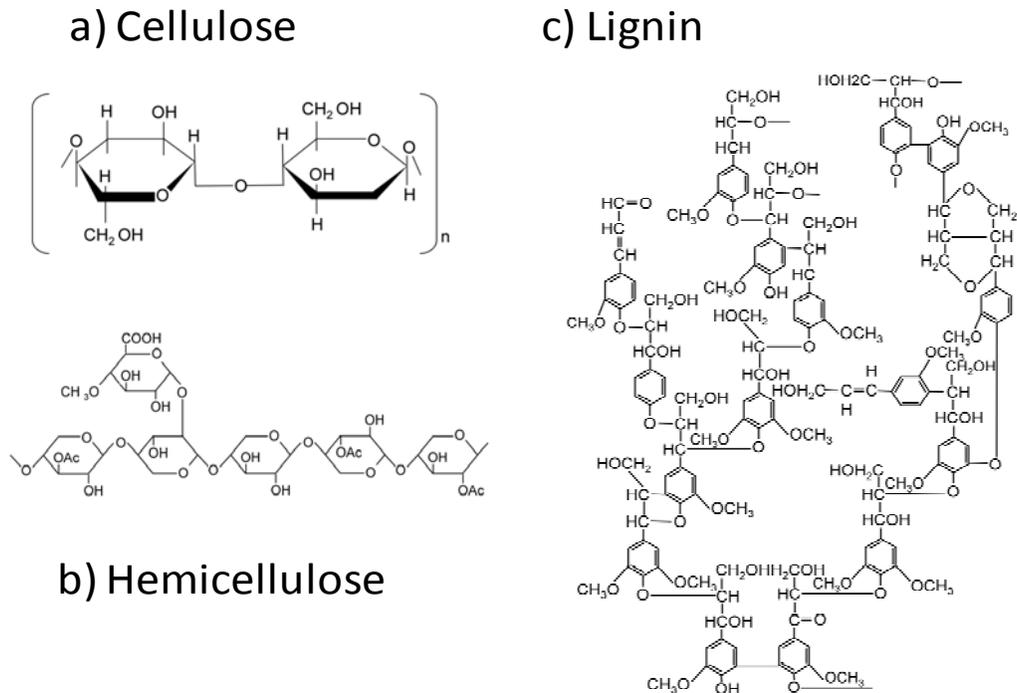


Figure 2. Chemical structures of main biomass components (Salisbury and Ross, 1992).

Hemicellulose is a heteropolysaccharide that is found together with cellulose in the primary cell wall of plants. It is made of pentoses (mainly D-Xylose but also L-arabinose), hexoses (mainly D-glucose, D-mannose and D-galactose) and glucuronic acid linked by glycosidic bonds, with a degree of polymerization of around 200-1000 units. Unlike cellulose, hemicellulose has a highly branched, random and amorphous structure that can be easily hydrolyzed into basic sugars by the application of heat or chemical reagents. The composition and specific structure of hemicellulose differs depending on plant species (soft and

hardwoods, herbaceous plants) and the part of the anatomy (stem, branches, roots, bark, leaves). It represents between 20-35 wt% of the wood, having a chemical formula similar to cellulose (Sjostrom, 1993; Walker, 2009). The combined carbohydrate fraction of a biomass (cellulose and hemicelluloses) is also known as hollocellulose.

**Lignin** is a highly aromatic and amorphous biopolymer present in the secondary cell wall of plants. It forms covalent bonds with the fibrous cellulose and hemicellulose polymers, increasing mechanical strength, reducing water permeability and protecting the plant structure from chemical and biological attack (Salisbury and Ross, 1992; Sjostrom, 1993). Lignin has a three-dimensional highly branched structure consisting of phenyl propane units linked to each other by ether and C-C bonds. As illustrated in Figure 3, the most common of these units can be associated with the following structures: 4-propenyl phenol (p-coumaryl alcohol), 4-propenyl-2-methoxy phenol (coniferyl alcohol) and 4-propenyl-2,5-dimethoxy phenol (sinapyl alcohol). Hardwood lignin (Dicotyledonous angiosperm) contains higher proportions of coniferyl and sinapyl units, while softwood lignin (Gymnosperm) mostly consists of coniferyl monomers. The molecular weight of lignin structures ranges widely between 10000 and 50000 units, which represents around 60-200 phenyl propane monomers. The oxygen content is around 20-25 wt%, much lower than in cellulose. Lignin is therefore highly resistant to chemical and thermal degradation (Sjostrom, 1993; Walker, 2009; Faravelli et al., 2010).

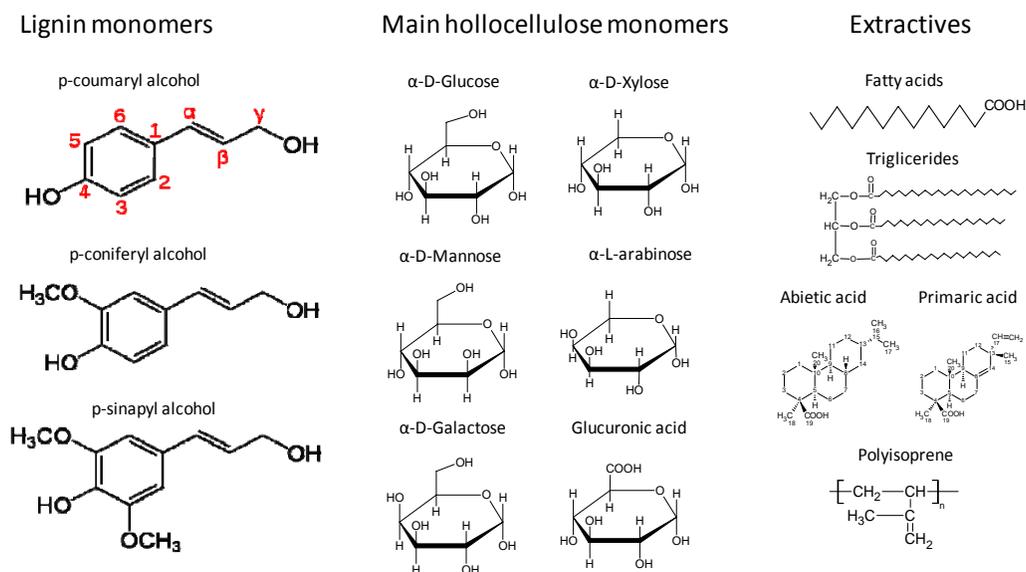


Figure 3. Monomeric building blocks of lignin, cellulose and hemicellulose.

### 2.1.3. Other Components in Lignocellulosic Biomass

#### 2.1.3.2. Extractives

Lignocellulosic biomass also contains variable amounts (1-15 wt% in total) of extractives that confer additional properties (odor, color, protection against mechanical and biological attack) to different elements in the anatomy of the plant (Sjostrom, 1993; Walker, 2009; Guo et al., 2010). Owing to their defensive and structural needs, wood bark and leaves usually

contain much higher proportions of extractive elements than internal wood tissues. Extractives are outside the primary cell walls and can be readily separated from the biomass using non-polar solvents such as toluene, acetone or n-hexane (Thurbide and Hughes, 2000). Extractive elements have been classified into various categories including fatty acids, triglycerides, terpenoids, tannins, rubbers, waxes, alkaloids, proteins, carbohydrates and other organic species. Depending on their concentration and characteristics, these elements may interfere with the mechanical and chemical aspects of the pyrolysis process (Oasmaa et al., 2003; Guo et al., 2010).

#### 2.1.3.2. *Inorganic elements*

Ash content and composition has been reported to have a dominant effect on the thermochemical conversion of biomass (Raveendran et al., 1995; Livingston, 2007; Fahmi et al., 2008). This is due to the fact that alkaline (mainly K and Na), alkaline-earth (mainly Ca and Mg) and other inorganic species have a catalytic effect on the oxidative (combustion, gasification) and thermal cracking reactions that take place on the organic molecules when exposed to high temperatures. The presence of these inorganic elements affects the mechanism of thermal decomposition in biomass, promoting ring scission reactions instead of the predominant depolymerization mechanism that takes place in their absence. The pyrolysis of lignocellulosic biomass containing high proportions of mineral elements, as happens with cereal straw and rice husks, is associated with the production of reduced oil yields and the formation of higher proportions of gas products (Fahmi et al., 2008; Livingston, 2007; Richards and Zheng, 1991). In contrast, fast pyrolysis of a biomass previously demineralized by acid washing resulted in the production of high oil yields rich in levoglucosan and other anhydrosugars (Piskorz et al. 1989; Raveendran et al., 1995).

Most of the inorganic matter present in the biomass feedstock concentrates is in the solid char fraction upon pyrolysis, although some of it also ends up in the pyrolysis oil. The presence of these inorganic elements in the liquid fuel is highly detrimental due to deposition, corrosion and erosion in the equipment. In addition, the presence of alkaline inorganic elements in the pyrolysis oil has also been associated with accelerated aging as they catalyze recombination and polymerization reactions (Livingston, 2007). The mineral content and composition of a plant is affected by a number of parameters including plant species, part of the anatomy, type of soil and weather conditions. High ash contents are typically found in cereal and in herbaceous plants (5-15 wt%), with high concentrations of Si, K, Ca, Na and Cl. Lower ash contents (< 0.5 wt%) are characteristic of softwood from conifers (Livingston, 2007). Although demineralization of biomass feedstock by acid washing is technically viable, this activity is unlikely to be economically and environmentally acceptable in large scale commercial processes.

#### 2.1.3.3. *Water*

Moisture content in biomass feedstock represents a very important parameter in the design of a fast pyrolysis process. The lignocellulosic biomass delivered to energy plants usually contains between 30 and 60 wt% water, depending on plant species, location, weather and storage conditions (Fagernas et al., 2010). Some of this is in the form of free water, filling the lumens, open pores and intercellular spaces of the biomass tissues. This free water can be easily removed by air drying at temperatures slightly above 100°C. Biomass also contains bound water physisorbed within molecular size cavities and inside plant cell walls.

Elimination of this water requires more severe conditions in order to counteract the attractive interactions (hydrogen bonds and Van der Waals forces) that exist between the water molecules and the solid (Amos, 1998; Brammer and Bridgwater, 1999; Walker, 2009).

This water has a negative impact on the energy balance of the pyrolysis process. All the moisture introduced into the reactor is evaporated and then heated to the pyrolysis temperature (usually around 500°C). Additional energy is also consumed in order to cool and condense the water present in the volatile fraction. This water is recovered together with the pyrolysis oil, reducing its energy value and affecting its fuel properties.

However, excessive elimination of moisture from the biomass feedstock may be detrimental to the fast pyrolysis process. Flash evaporation of internal water has been reported to favor the mechanical rupture of the biomass structures, thus facilitating the evolution of volatile products from the interior of biomass particles. Steam has also been reported to favor heat transfer and act as an absorbent inside the pyrolysis reactor, capturing of water soluble elements and favoring their transfer outside the reaction chamber (Diebold and Bridgwater, 1999). In addition, biomass pyrolysis oils with very low water content exhibit very high viscosities and are more chemically unstable, affecting their potential use as fuels.

## 2.1. The Chemistry of Biomass Pyrolysis

### 2.1.4. Thermal Degradation of Biomass Components

The term pyrolysis is used to describe the degradation of a substance by exposure to high temperatures. By definition, pyrolytic processes take place in the absence of oxygen or any other external element that may interact with the substance under consideration. The addition of *heat causes the atoms in the molecule to increase their energy of vibration*. When the kinetic energy associated with this vibration is greater than the potential energy of the chemical bond, the interaction is broken resulting in the transformation of the original compound and the formation of new species.

Thermal degradation processes are usually highly complex, owing to the numerous reactions taking place at one time, the unstable nature of intermediate products generated and the high temperatures involved. Primary decomposition reactions in a simple molecule like ethanol or acetic acid may involve several transformations (elimination, chemical rearrangement, substitutions, additions) occurring simultaneously, or sequentially, as the molecule is progressively heated to increasingly high temperatures (Moldoveanu, 1998). This complexity is far greater when the subject of the pyrolysis reaction is a solid composite material like lignocellulosic biomass, and when this material is heated very rapidly as happens in the fast pyrolysis processes. This section provides an introduction to the chemical basis of biomass pyrolysis including information about primary degradation reactions taking place on the biomass components and secondary homogeneous and heterogeneous reactions taking place on the resulting products.

The thermal degradation of biomass can be investigated by monitoring the sample mass as it is progressively heated under inert atmospheric conditions. Figure 4 shows the thermogravimetric analysis (TGA) of lignocellulosic biomass and its main components (lignin, cellulose and hemicellulose). It has been described that each one of the components

behaves rather independently during the thermal degradation of lignocellulosic biomass (Yang et al., 2007).

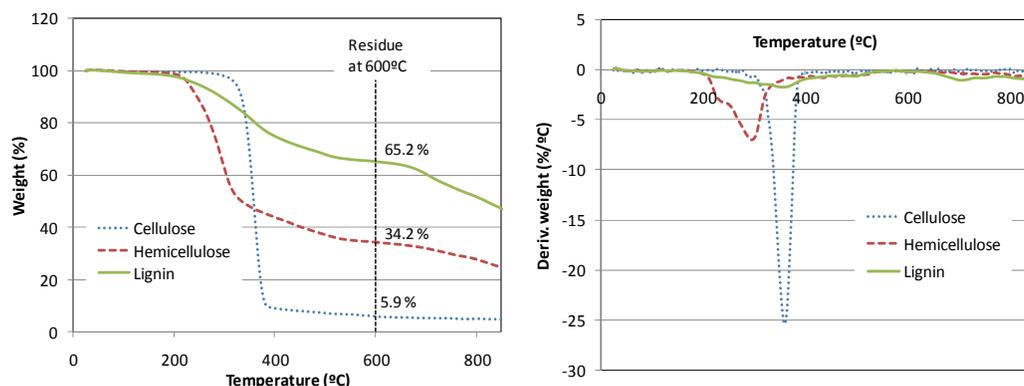


Figure 4. TGA-DTG analysis of biomass components (cellulose, hemicellulose and lignin).

The TGA analysis shows three areas of weight loss that may be associated with different stages in the pyrolysis process. The first one takes place at temperatures between 120–200°C and involves the volatilization of free water and light organic products. The second stage takes place at temperatures between 200–450°C and involves the thermal degradation of the main biomass components. The third stage takes place at temperatures above 450–500°C, and involves the progressive cracking of resilient elements present in the original biomass (lignin) and carbonized products (Yang et al., 2007; Wu et al., 2009).

The TG analysis in Figure 4 also shows that hemicellulose decomposes at lower temperatures (225–300°C) than crystalline cellulose (350–400°C), leaving a residue of solid char that represents around 32 wt% of the original mass at 500°C. Despite the higher degradation temperature required, pyrolysis of cellulose generates virtually no solid residue, which may be attributed to low cross linking reactions during the degradation process. In contrast, the thermal degradation of lignin pyrolysis takes place over a wider temperature range (200–600°C) and results in the formation of a highly resilient solid char residue (50 wt% of original mass at 800°C).

#### *Thermal degradation of cellulose*

In the case of cellulose, controlled exposure to low temperatures (between 230°C and 250°C) leads to intra-molecular elimination of water in position C<sub>2</sub>-C<sub>3</sub> and the formation of anhydro-cellulose (Scheirs et al., 2001). As shown in Figure 4, char formation is very limited in the thermal degradation of cellulose. This may be increased by exposing the cellulose to temperatures around 250°C over long periods of time, which promotes exothermic cross-linking reactions in the dehydrated polymer (Pastorova et al., 1994; Hosoya et al., 2006). Higher temperatures (above 350°C) are required to affect the fracture of the dehydrated polymer chain. This takes place by rupture of C-O glycosidic bonds between adjacent glucose units, far more unstable than the C-C bonds that form the cellulose rings. This rupture appears to take place through random scission mechanisms that lead to the formation of anhydro-oligomer fragments and levoglucosan (Piskorz et al., 2000). Owing to its reduced thermal stability, these new fragments are readily degraded into smaller oxygenated species like acetic

and formic acids, hydroxyacetaldehyde, 2-butanone, 1-hydroxy-2-propanone, acetone and furfural (Wu et al., 2007).

The degradation mechanism described for the thermal degradation of cellulose is significantly altered by the presence of inorganic elements (mainly alkali and alkali-earth cations) that catalyze the cleavage of C-C bonds in glucose rings (Richards and Zheng, 1991; Piskorz et al., and 2000; Patwardhan et al., 2010). The presence of alkali salts has been reported as having greater influence on the reaction mechanism than temperature. The presence of small concentrations of these elements (below 0.1 wt%) has been associated with reduced yields of levoglucosan and the formation of higher proportions of smaller molecular weight aldehydes and ketones.

#### *Thermal degradation of hemicellulose*

Hemicellulose has a more diverse chemical composition than cellulose and has a lower thermal stability due to its non-crystalline structure. Hence, dehydration reactions on the polymeric components take place at comparatively lower temperatures (around 200°C). Cross-linking reactions in anhydro-hemicellulose polymers are more favored than in the anhydro-cellulose molecule, as evidenced by the formation of large amounts of char residue. At temperatures between 250-300°C, the anhydropolymer undergoes depolymerization reactions following a mechanism similar to the one described for cellulose. These intermediate products are highly unstable and rapidly undergo C-C scission reactions to give rise to the formation of acetone, furfural, acetic acid and other small molecular weight oxygenated compounds (Wu et al., 2009; Shen et al., 2010).

#### *Thermal degradation of lignin*

Owing to its highly complex and heterogeneous structure, lignin contains many different types of chemical bonds that may respond differently to thermal stress. As a result, thermal degradation of lignin occurs over a very wide temperature range (200-900°C). It has been described that the pyrolytic process is initiated by the rupture of the weaker ether bonds in the  $\beta$ -O-4 position of the phenyl propane units (see Figure 3). This leads to the formation of radical structures that stabilize themselves through hydrogen abstraction, resulting in the fracture of the original lignin macromolecule. This depolymerization process leads to the formation of phenolic monomers and oligomers containing the characteristic p-coumaryl, p-coniferyl and p-sinapyl structures (Faravelli et al., 2010).

**Table 2. Main condensable products derived from the fast pyrolysis of biomass components (Bridgwater et al., 1999; Wu et al., 2009).**

Biomass component	Main condensable products
Hemicellulose	Acetic acid, furfural, furan, 1-hydroxy-propanone, 1-hydroxy-2-butanone.
Cellulose	Levoglucosan, hydroxyacetaldehyde, 5-hidroxymethylfurfural, acetol, formaldehyde, furfural.
Lignin	Monomeric phenolic structures (including phenols, p-coumaryl alcohols, coniferyl alcohols, sinapyl alcohols) and oligomeric phenols of molecular mass between 200-3000 u.

At temperatures above 500°C, this depolymerization mechanism is increasingly overlapped by intermolecular and intramolecular condensation reactions, leading to the formation of polyaromatic hydrocarbons. This process is accompanied by the release of volatile components and a reduction in O and H content, characteristic of char formation (Faravelli et al., 2010; Nakamura et al., 2008). As in the case of hollocellulose, the presence of mineral elements (mainly K, Na and Ca) promotes ring fractionation in the lignin molecule (Dobele et al., 2005; Richards and Zheng, 1991). Table 2 describes the main condensable products derived from the fast pyrolysis of biomass components.

### ***2.2.2. Chemical Reactions in the Fast Pyrolysis Process***

The reaction mechanisms described in Section 0 are substantially complicated in fast pyrolysis processes because the biomass feedstock is heated almost instantly. As a result, a large number of degradation routes are activated simultaneously on the biomass components leading to the formation of a very complex mixture of primary products. In addition, the high temperatures inside the reactor favor further cracking of these primary products, and also promote secondary homogeneous (gas-gas) and/or heterogeneous (gas-solid) reactions between the reactive pyrolysis vapors and the solid char (Garcia-Perez, 2008).

The severity of the pyrolysis process can be controlled by adjusting the temperature inside the pyrolysis reactor and the residence times of the evolving products at such high temperatures. High pyrolysis temperatures promote the cracking of primary and secondary products, leading to the formation of higher concentrations of small molecular weight non-condensable gases. Application of high reaction temperatures and allowing longer residence times of the vapors inside the reactor chamber also promote recombination and polymerization reactions leading to the formation of solid char (Elliott et al., 1991; Meier and Faix, 1999; Bridgwater, 1999).

Particle size of the biomass feedstock also has a notable effect on the secondary reactions. Pyrolysis vapors generated in the interior of biomass particles need to diffuse into the bulk of the gas phase before they can be removed from the reaction chamber. This transit may be hampered in the case of large biomass particles, increasing the residence time of the pyrolysis products inside the reactor and favoring recombination reactions with the external surface of the solid biomass and char. In contrast, this mass transfer may be favored when the pyrolysis process is conducted under vacuum conditions, reducing the extent of heterogeneous and homogeneous secondary reactions taking place inside the particles (Brackmann et al., 2003).

## **2.3. Introduction to Fast Pyrolysis Processes**

A fast pyrolysis plant consists of an integrated series of operating units where the received biomass feedstock is conditioned to reduce moisture content and particle size, pyrolyzed under controlled conditions and the resulting products separated into non-volatile solids (charcoal), condensable volatile products (pyrolysis oil) and non-condensable volatile products (pyrolysis gas). The yield and composition of each of these fractions depends on many factors, the most important involving the nature and conditioning of the biomass feedstock (mainly drying and reduction of particle size); primary and secondary thermal reactions (influenced by reactor configuration, temperature profile of the system, heat transfer

and vapor residence time) and product separation technologies and conditions (cyclones and filters for solids and quenching for condensable products) (Elliot et al., 1991; Meier and Faix, 1999; Bridgwater and Peacock, 2000; Mohan et al., 2006; Venderbosch and Prins, 2010).

### 2.3.1. Fast Pyrolysis Plant Layout

Figure 5 illustrates the main features in a typical fast pyrolysis system. The process starts with the initial collection, transportation and storage of the selected biomass feedstock. Hence, selection of the biomass and location of the pyrolysis plant has a marked effect on the economics of the process and needs to be decided taking into consideration geographical, seasonal and yearly production of the biomass feedstock and transportation costs (Caputo et al., 2005). As discussed above, biomass characteristics also have a decisive effect on product yields and characteristics. A review describing the fast pyrolysis of different biomass feedstocks (bark, different types of wood, agricultural wastes, nuts and seeds, algae, grasses, forestry residues, cellulose, lignin) is included in Mohan et al. (2006).

The biomass feedstock needs to be conditioned in order to reduce moisture content and particle size. Drying is essential as it affects the energy requirements of the pyrolysis process and the fuel properties of the resulting bio-oil. The drying system is usually integrated with existing energy sources within the pyrolysis plant in order to optimize the energy efficiency (Amost, 1998). Selection of the drying technology requires analysis of energy sources, plant size and throughput, emission requirements and fire risks associated with the specific characteristics of the biomass feedstock. Owing to its reduced investment and maintenance costs, most biomass to energy plants use direct rotary dryers, although faster and more energy efficient steam drying systems are increasingly being considered (Fagernas et al., 2010; Brammer and Bridgwater, 1999).

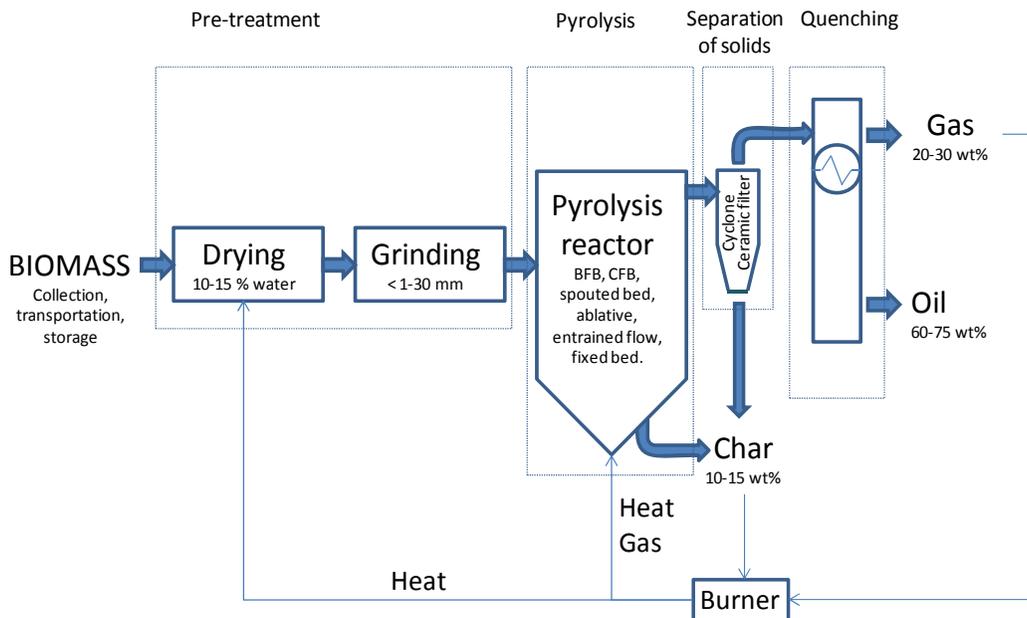


Figure 5. Simplified process diagram of a biomass fast pyrolysis plant.

In most cases, drying units use process heat obtained directly from the hot flue gases as they exit the pyrolysis reactor. Additional energy can be obtained by combustion of the pyrolysis char and gases (Brammer and Bridgwater, 1999). The use of direct and indirect solar dryers is also being considered particularly in warm countries owing to the lower energy requirements (Sharma et al., 2009). Optimum moisture content in the biomass feedstock needs to be reduced to 10-15 wt%.

Feedstock particle size is a key parameter in biomass pyrolysis as it affects heat transfer and determines the extent of intra-particle secondary reactions. Small particles are heated more rapidly and homogeneously than larger particles. In addition, secondary reactions (mainly recombination but also cracking) are promoted in larger particles as a result of impeded transference of volatile pyrolysis products from the interior of the biomass particle to the bulk of the gas phase. This results in reduced oil yields and a marked variation in product composition (Shen et al., 2009). Feed specifications depend on reactor design and usually range between less than 0.5 mm in rotating cone reactors, 1-5 mm for bubbling fluidized beds and 1-10 mm for circulating fluidized beds. Ablative reactors may use much larger biomass particles (5-10 cm) owing to the different heat transfer mechanisms involved (Bridgwater and Beacock, 2000). Size reduction of biomass is usually conducted in commercial knife and hammer mills. This processing involves high economic and energy costs which increase exponentially with the reduced particle size (Esteban and Carrasco, 2006; Zhu and Pan, 2010).

Dry and particle size reduced biomass may then be fed into the pyrolysis reactor, where the thermal decomposition takes place. The pyrolysis process usually takes place at atmospheric pressure and temperatures between 450°C and 500°C. This temperature represents an equilibrium aimed at maximizing the thermal decomposition of the original biomass feedstock while minimizing secondary reactions of the pyrolysis vapors. Secondary reactions are influenced not only by the thermal profile of the pyrolysis process but also by exposure time of the pyrolysis vapors. It has been described that, although secondary reactions are slowed at temperatures below 400°C, some secondary reactions continue to occur even at room temperature, which contributes to modifying the composition and fuel properties of the final oils (Bridgwater and Peacock, 2000). Gas residence times are largely affected by the scale of the pyrolysis process. Thus, very low residence times (below 0.1 s) can be achieved in small laboratory reactors. Gas residence times around 2-10 s are usually achieved in larger scale commercial fluidized bed reactors. Vapor residence times are minimized in ablative reactors owing to their particular design (Peacock and Bridgwater, 1994; Mohan et al., 2006).

The reactor is the most important part of the pyrolysis process, as it has a crucial effect both on energy efficiency and also on product yields and composition. Pyrolysis reactors need to comply with the following requirements: high heating and heat transfer rates; careful control and homogeneity in the temperature profile; and rapid cooling of the pyrolysis vapors after they have been produced (Bridgwater and Peacock, 2000). Some of the reactor designs have been adapted from conventional industrial processes, like bubbling and circulating fluidized beds. Others have been developed specifically for fast pyrolysis, taking into consideration the specific requirements of this technology. These include ablative reactors (vortex, rotating blades); spouted bed reactors, rotating cones and vacuum reactors. Although the latter category may produce better results in laboratory experiments, it also entails higher technical and economic risks due to the limited experience in the development of large scale

units (Meier and Faix, 1999; Bridgwater and Peacock, 2000; Venderbosch and Prins, 2010). The characteristics of each one of these reactor designs are described in more detail in Section 0 of this paper.

The low density char fines generated during the pyrolysis process are usually entrained in the gas stream as it exits the reactor. The presence of solids in the pyrolysis oil is highly detrimental to its fuel properties as described in Section 0 of this paper. Fuel solids need to be below 0.01 wt% in turbines and engines, although higher levels may be acceptable in burners and other less restrictive applications (Oasmaa et al., 2009). Separation of these solids from the condensed oil is highly problematic owing to its high viscosity and large molecular weight.

Cyclone separators are usually employed to remove solid char particles from pyrolysis gas streams because of their low cost and reliable design. However, the efficiency of these devices is very limited for very small particles. Reactors employed in the fast pyrolysis of biomass subject the char to some degree of attrition. This effect is particularly severe in fluidized bed reactors owing to physical interaction of the char particles with circulating sand and the high gas velocities employed. Hence, single gas cyclones are usually not sufficient to ensure efficient removal of solids in this type of systems. Multi-cyclone systems (operating either in series or in parallel) and ceramic filters achieve higher solid separation efficiencies, particularly in the case of particles smaller than 10  $\mu\text{m}$ . However, ceramic filters involve higher maintenance and operating costs, and cause greater pressure drops in the system. The installation of hot gas filtration technologies has been reported to reduce bio-oil yields and modify oil properties owing to enhanced thermal and catalytic cracking reactions in the cyclones and filters (Scahill et al. 1996).

Rapid quenching of the pyrolysis vapors is required to separate the condensable pyrolysis oils from the non-condensable gas fraction. The collection of liquids also represents a significant technology challenge, as a high proportion of the condensable products present in the gas stream are in the form of aerosols. Capture of these aerosols simply by cooling to temperatures below dew point in indirect heat exchanging devices involves very low efficiencies (Meier and Faix, 1999). Large scale pyrolysis plants typically employ direct contact quenching towers using organic absorbents like fuel oil, bio-diesel and bio-oil. Careful design and temperature control are essential in order to avoid clogging by fast condensation of heavier lignin derived products. Gas exit temperature needs to be sufficiently low to ensure effective recovery of light products. Additional collection of aerosols may be achieved using electrostatic separators.

### **3. TECHNOLOGY PRINCIPLES OF BIOMASS FAST PYROLYSIS**

#### **3.1. The Fast Pyrolysis Reactor**

##### ***3.1.1. Fundamentals in the Design of the Pyrolysis Reactor***

Numerical modeling is a fundamental tool in the design of large scale fast pyrolysis reactors. Pyrolysis kinetics coupled with transport phenomena must be solved in order to predict the reactor performance and optimize the operating conditions.

### Fast Pyrolysis Kinetics

For engineering purposes, primary decomposition of wood is usually simplified as taking place according to a single degradation mechanism described in Figure 6 (Shafizadeh, 1975; Shafizadeh and Chin, 1977; Chan et al. 1985; Samolada and Vasalos 1991; Di Blasi, 2008). This reaction pathway is extended to include secondary reactions leading to the formation of gases and char (Antal, 1983; Di Blasi, 1993).

Kinetic models use a first order Arrhenius equation to relate reaction rate and temperature. Activation energy values determined for the three parallel primary reactions are comparable to each other (between 56 and 106 kJ/mol for pyrolysis temperatures between 425-525°C). Therefore, it is not possible to displace the selectivity toward the production of specific compounds (Di Blasi, 2008). For most of the biomass species, the overall reaction rate constant (addition of  $k_1+k_2+k_3$ ) at 500°C is greater than  $0.5 \text{ s}^{-1}$ , which substantiates the fast nature of the pyrolytic reaction (Van de Velden, 2006).

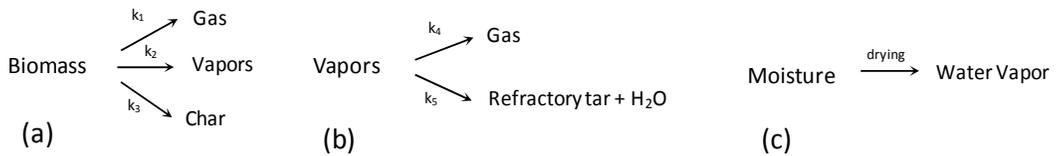


Figure 6. Simplified mechanism for the thermal degradation of biomass (Shafizadeh, 1975).

Thermogravimetric analysis (TGA) has been used to determine the kinetics of biomass pyrolysis. It has been described that these values may not be directly applicable to fast pyrolysis due to the low heating rates employed (usually 5-20°C/min compared to above 100°C/s in fast pyrolysis) and the thermal lag characteristic of this technology (Narayan and Antal, 1996; Van de Velden et al 2006). Kinetic parameters have also been determined for different biomass feedstocks and temperature ranges using tube furnaces (Thurner and Mann, 1981, Di Blasi and Branca, 1999), fluidized bed (Samolada and Vasalos, 1991) and spouted beds (Olazar et al., 2001).

### Heat Requirements

The amount of heat that needs to be delivered to the pyrolysis reactor is reported to be in the range between 0.82 - 3.47 MJ/kg (Piskorz et al., 1999). The energy demand is the addition of the sensible heat required to heat the biomass to the pyrolysis temperature (around 500°C), the energy required to evaporate the moisture introduced into the reactor and the energy absorbed by the pyrolysis reaction. The average steady state heat requirement ( $Q_{pyr}$ ) of the process may be expressed using the following formula:

$$Q_{pyr} = m_w C_{p,w} (T_r - T_0) + m_w \Delta H_{pyr} + m_{H_2O} C_{p,H_2O} (T_r - T_0) + m_{H_2O} \Delta H_{evap}$$

Considering the following values for the pyrolysis of 1 kg of biomass containing 10 wt% moisture (Van de Velden et al., 2006): temperature of the pyrolysis process ( $T_r = 500^\circ\text{C}$ ), specific heat capacity of the biomass ( $C_{p,w} = 1.0 \text{ kJ/kg K}$ ), enthalpy of the pyrolysis process ( $\Delta H_{pyr} = 434 \text{ kJ/kg}$ ), and enthalpy of water vaporization ( $\Delta H_{evap} = 2440 \text{ kJ/kg}$ ). The heat input required to pyrolyze 1 kg of biomass can be calculated as follows:

$$Q_{pyr} = 1 \text{ kg}_w * 1 \frac{\text{kJ}}{\text{kg K}} (500 - 25)K + 0,1 \text{ kg}_{H_2O} * 4,18 \frac{\text{kJ}}{\text{kg K}} (100 - 25)K + 0,1 \text{ kg}_{H_2O} * 2440 \frac{\text{kJ}}{\text{kg}} + 0,9 \text{ kg}_w * 434 \frac{\text{kJ}}{\text{kg}} = 1140 \text{ kJ}$$

This represents around 7% of the heating value contained in the original biomass. In laboratory scale prototypes, this energy is usually supplied by electric resistances that heat the external walls of the pyrolysis reactor. However, this approach is not economically viable and may not be considered for larger scale commercial processes.

One way of providing reaction heat is by controlled combustion of the pyrolysis gases (CO, CH<sub>4</sub>, H<sub>2</sub> and light hydrocarbons and organics) inside the reactor, in a procedure usually referred to as oxidative pyrolysis (Senneca et al., 2004; Scott et al., 1995). This is achieved by controlled injection of air into the system. A key drawback of this method relates to reduced oil yields resulting from localized high temperatures inside the reactor which results in excessive cracking of the volatile products. In addition, bio-oils produced in oxidative pyrolysis processes usually contain excessive water generated during the combustion process and lack lighter hydrocarbons consumed during the combustion reaction. These two aspects negatively affect the fuel properties of the oils.

A more widely used alternative to heat supply involves the reheating of process gases or the fluidizing sand. These are usually extracted from the pyrolysis reactor and re-heated in an external burner, which usually employs pyrolysis gases and chars as fuels. The reheated gases also provide the inert atmospheric conditions required in pyrolysis processes, although their heat transfer capacity is limited. An optimum value needs to be reached between gas temperature (which promotes unwanted secondary reactions on the pyrolysis products) and gas flow rate (which negatively affects condensation process). In bubbling and circulating fluidized bed reactors, most of the heat transfer is achieved by reheating and circulating the fluidizing sand (Mohan et al., 2006; Bridgwater and Peacocke, 2000; Meier and Faix, 1999). In ablative reactors (vortex, cyclone or rotating disk), heat is transferred indirectly through the reactor wall by conduction. Heat transfer rate is increased by centrifugal or mechanical forces that facilitate the contact of biomass particles with the hot reactor walls (Peacocke and Bridgwater, 1994). A more detailed description of the functioning of these reactors can be found in the next paragraph.

### 3.2. Reactor Designs for Biomass Fast Pyrolysis

The cost of the pyrolysis reactor amounts to approximately 10-15% of the total plant cost (Huber et al. 2006). However, the reactor is the central part of the process, having a key effect not only on the energy efficiency of the process but also the yields and characteristics of the resulting products. Reactors intended for the fast pyrolysis of biomass need to fulfill three basic requirements: first, very fast heat transfer in order to ensure rapid heating of the biomass particles at relatively low temperatures; second, very low residence time of the vapors inside the reaction chamber in order to minimize secondary reactions; and third, reduced purge gas flow in order to reduce dilution of volatile products and condensation requirements.

Other aspects that need to be taken into consideration include attrition of the chars in order to avoid contamination of volatile products, and biomass particle size requirements in

order to avoid excessive energy and economic costs in feedstock preparation. Various reviews have been dedicated to describing alternative reactor designs for fast pyrolysis (Meier and Foix, 1999; Bridgwater and Peacocke, 2000; Mohan et al., 2006). These reactor designs can be classified in two main categories, fluidized bed and non-fluidized beds.

In fluidized bed reactors, a fluidizing gas (recycled pyrolysis gas) is passed through a granular material (usually inert silica sand) at velocities sufficiently high to suspend the solid and cause it to behave like a fluid. Heat is transferred primarily by conduction between the hot sand and the biomass, allowing for very fast heating rates of the feedstock. Fluidized bed reactors are widely used in many industrial applications owing to its rapid heat transfer characteristics and uniform temperature profiles. Hence, the use of these conventional technologies involves lower technical and economic risks. A key drawback with fluidized bed reactors is the small particle size of the biomass feedstock required to ensure adequate fluidizing behavior of the bed and fast heat transfer conditions. Size reduction may involve significant economic costs. Figure 7 show a schematic representation of conventional (bubbling and circulating bed) and non conventional (conical spouted bed and spout fluid bed) fluidized bed reactors.

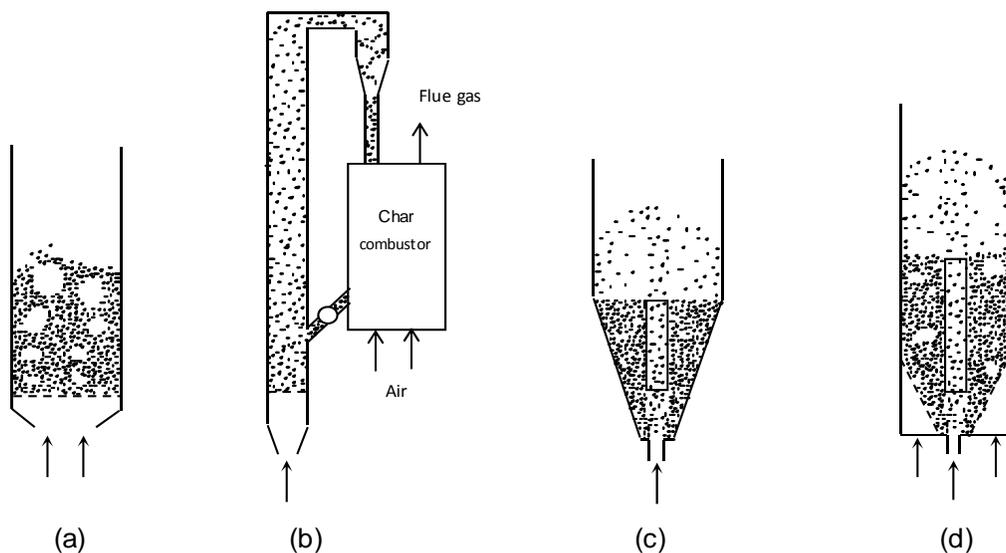


Figure 7 a). Bubbling fluid bed, b) circulating fluid bed, c) conical spouted bed, d) spout fluid bed.

The non-fluidized reactor category includes a wide range of designs developed or adapted to the specific requirements of fast pyrolysis. This includes ablative reactors, rotating cones, horizontal stirred moving bed and the Lurghi twin screw reactor. These designs do not require recirculation of inert gas, which results in improved thermal efficiency and a higher concentration of the volatile products. They also exhibit high heating rates owing to direct contact between the hot surface and the biomass, allowing the possibility of using larger particle size feedstocks, and minimizing secondary reactions due to localized heat supply surfaces. However, these non-fluidized designs suffer from the need to have moving parts operating at high temperatures. In addition, they assume higher technology and economic

risks due to limited previous experience in large scale applications. The following sections provide further information about the design and operating characteristics of these reactors.

### **3.2.1. Conventional Fluidized Beds**

#### *Bubbling Fluid Beds (BFB)*

The bubbling fluid bed is a reliable and well developed technology widely used in commercial processes like combustion, gasification and oil cracking. This type of reactor is simple to construct and operate, providing very effective heat and mass transfer characteristics and thermal control. Sand is used as the solid phase of the bed and the residence time of the vapors is controlled by the fluidizing gas flow rate. Owing to their cost effectiveness and high proportion of oil yields, bubbling fluidized beds are the most widely used reactor design for the fast pyrolysis of biomass (Scott and Piskorz, 1984; Samolada et al., 1992; Gerdes et al., 2002; Wang et al., 2005).

Fluidized bed reactors were first adapted for the fast pyrolysis of biomass in the Department of Chemical Engineering at University of Waterloo (Scott and Piskorz, 1982). The process, usually referred to as the Waterloo Flash Pyrolysis Process (WFPP), operates at temperatures between 450-500°C, involves vapor residence times around 2 seconds and has been claimed to produce liquid yields between 70-74 wt%. Large scale WFPP reactors usually employ lower reaction temperatures (430°C) and longer residence times (10 s) in order to reduce gas recycling flow rates (Scott and Piskorz, 1982; Scott et al., 1999).

Biomass particle size is a critical issue in bubbling fluidized beds. Small particle size is necessary to ensure good bed mixing and avoid segregation. Typical biomass particle size are between 0.1-2.0 mm. Owing to the poor fluidization properties of the biomass, high sand to biomass ratios (around 10:1) are usually required (Ramakers et al. 2004).

#### *Circulating Fluid Beds (CFB)*

Circulating fluidizing bed reactors are also common in industrial chemical processes, providing higher throughputs than conventional BFB. CFB reactors require higher gas velocities (3-7 m/s) in order to carry bed particles out of the main reactor. As a result, bed porosity is much higher than in conventional bubbling beds and the residence time of biomass particles and volatile products is similar (Van de Velden and Baeyens 2006). The entrained mixture of sand and carbonized biomass is usually driven to a secondary reactor where the char is combusted and the sand is heated for process energy. At this point, ash content needs to be eliminated in order to reduce ash recirculation, which affects secondary reactions during the pyrolysis process. Standard sand to biomass ratios in circulating fluidized beds are between 10:1 and 20:1, and residence times for both gas and solids are lower than 2 seconds (Freel and Graham 1991, Boukis et al. 2007). CFBs can handle particles up to 10 mm but due to the low residence times of the solids biomass particles usually have to be smaller than 1.5 mm (Boukis et al. 2007). Owing to the higher gas velocities, attrition of the char is more severe, resulting in higher char contents in the volatile fraction.

### **3.2.2. Non Conventional Fluidized Beds**

Non-conventional fluidized beds include spouted beds and spout fluid beds. They provide some advantages to fast pyrolysis processes associated with the lower fluidizing gas velocities

required. However, since they are scarcely used in commercial high temperature applications, they involve higher technological risks.

### *Spouted Beds*

Spouted bed reactors are gas-particle contactors in which the gas is introduced through a single nozzle at the center of a conical base. Spouted beds are divided into three different regions, each one of them having its own specific flow behavior. In the central region, the particles move upwards, entrained by the incoming gas as in a transported bed. In the area surrounding this central region, particles move downwards as in a moving bed. In the fountain region the particles are uplifted into the main reactor, closing the cycle. Spouted beds achieve higher thermal efficiencies than conventional fluidizing beds because they need less fluidizing gas and also because only part of the bed is fluidized, resulting in reduced pressure drops.

Although large scale spouted beds are not common in industrial processes, the upper limit seems to be around 3 m in diameter (Lim and Grace, 1987). This is one order of magnitude smaller than fluidized beds that have been scaled up to a 30 m diameter. The inclusion of an element in the spout-annulus interface holding the annulus region, called draft tube, stabilizes the functioning of the reactor, reducing its limitations for scaling up. Thus, the draft tube is indispensable for large size spouted beds (Konduri et al., 1999).

Spouted beds provide good mixing and very efficient bed material contact. Conical geometry spouted beds are able to handle biomass particles such as chips and shavings with no inert material being required (Olazar et al., 1994). They are especially useful for applications where irregular, sticky or coarse particles, such as biomass, need to be dealt with vigorously. The research center Ikerlan-IK4 (Ikerlan website, 2010) has developed a biomass fast pyrolysis pilot plant based on a draft tube Conical Spouted Bed reactor in an attempt to demonstrate the performance of this technology and obtain experimental know-how for scaling up.

### *Spout Fluid Beds*

Spout fluid beds represent a combination of spouted and bubbling fluidized bed reactors (Chatterjee, 1970). This gas particle interaction can be achieved by feeding supplementary gas into the annulus region of a spouted bed. As a result, both spouting and fluidizing phenomena take place in the reactor bed. Higher circulation velocities than in spouted beds and better solid recycling and mixing than in the bubbling fluid beds is achieved (Berruti et al., 1988). The most important work on fast pyrolysis with a spout fluid bed is being conducted by the Anhui University of Science and Technology (China) with a 6 kg/h pilot plant.

### **3.2.3. Non Fluidized Reactors**

#### *Rotating Cone Reactor*

As illustrated in Figure 8, the Rotating Cone Reactor relies on the application of centrifugal forces on a mixture of hot sand and biomass particles. As a result, the mixture is transported in spiral movements from the bottom to the top of the reactor and across the cone wall. Typical rotating speeds of the cone may vary between 180 and 600 rpm. Using sand as a heat carrier, the reactor operates like a mechanical fluidized bed. Heat transfer occurs by the

combination of gas and solid phase convection, and also by conduction against the hot wall, allowing high heat transfer even in large scale reactors (Janse et al., 1999; Wagenaar et al., 2001). Char and sand are continuously transported through a hole to a fluid bed combustor and reheated sand is returned to the reactor through a standpipe, a riser and a cyclone.

Pyrolysis vapors are ejected at the top of the reactor and there is negligible vapor transfer to the char combustor. Solid residence time is very short, between 0.05 and 3 seconds and vapor residence time is around 0.3-0.5 seconds (Wagenaar et al., 1994). Hence, this technology requires very small particle size and very high reaction temperatures to ensure complete pyrolysis of the feedstock (Janse et al. 1999). Conical reactors do not require the recirculation of inert gas, thus minimizing dilution of the volatile products derived from the biomass pyrolysis process.

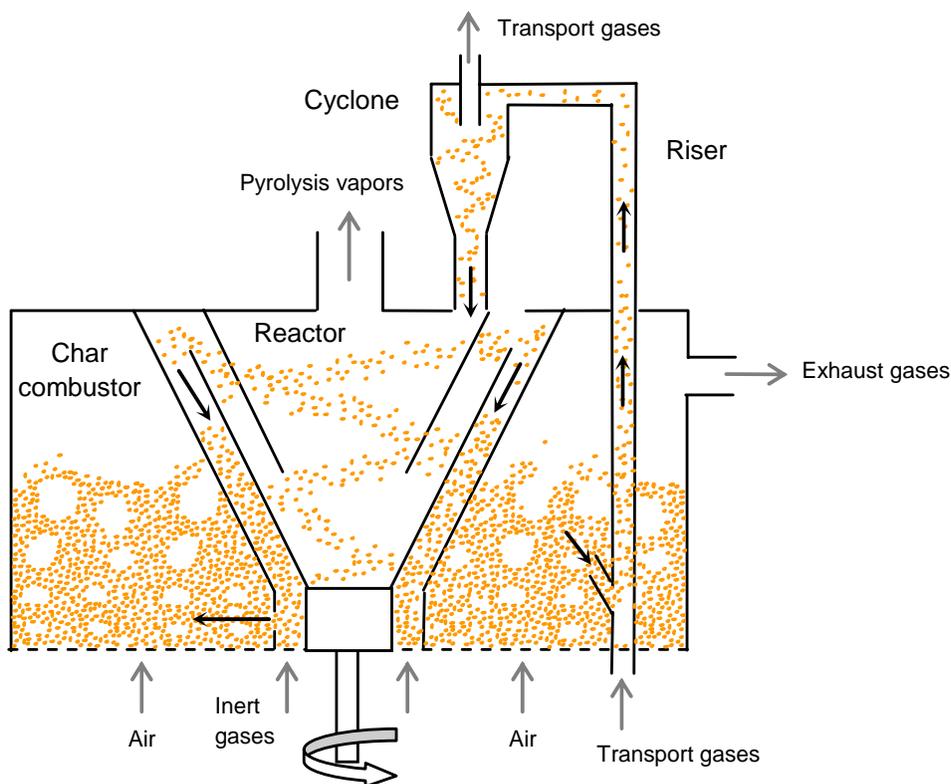


Figure 8. Rotating cone reactor developed by Twente University and BTG-BTL. (Figure published in Janse et al. 2000).

#### *Twin Screw Reactor and the Auger Reactor*

In the Auger Reactor, a single rotating screw is used to transport a mixture of pre-heated sand and ground biomass along the reaction chamber. An improvement to this initial design is the Twin Screw Reactor; where two parallel intermeshing and self cleaning screws are forced to rotate in the same direction.

Hot sand, used as a heat carrier, and coarse biomass particles are fed on one end of the twin screw system at a ratio of 20:1. The rotating movement transports the mixture along the reactor, allowing a good radial and low axial mixing between these two elements. Coarse char

particles are separated from the heat carrier particles and these are reheated and recycled to the reactor. The temperature of the incoming sand is around 550°C and this is reduced by around 100°C in each cycle. Vapor residence time is less than 1 s (Henrich, 2007). The experience obtained by Lurgi GmbH gasifying coal has been used to adapt this reactor design for the pyrolysis of biomass. No inert gas is required in this type of reactor. However, this reactor design entails mechanical challenges due to the moving parts at high temperatures. In addition, the high friction between pipe walls, screws and particles causes erosion. The control of the biomass residence time is another advantage of this reactor. The average solid residence time depends on screw rotation and can vary from 10 to 55 seconds (60-300 rpm respectively) for 15 kg/h (Raffelt et al., 2006).

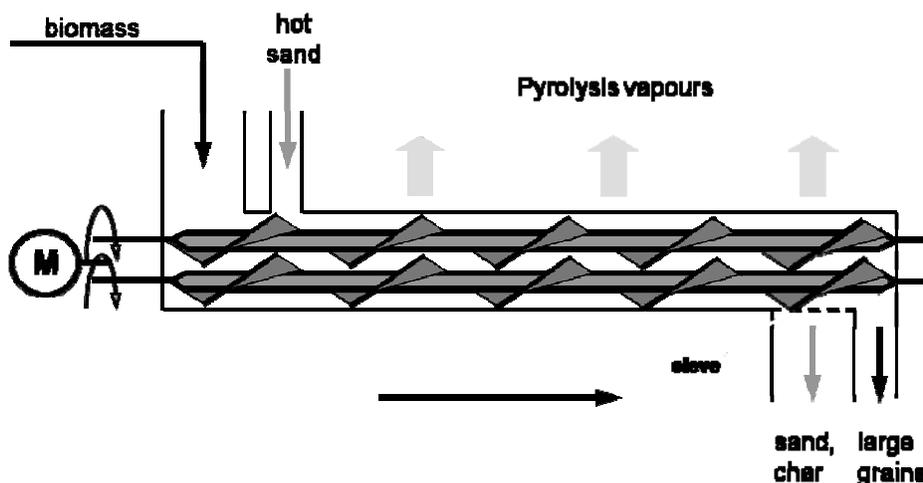


Figure 9. Twin Screw reactor developed by FZK.

#### *Ablative Reactors*

Ablation pyrolysis is a novel technology which fits well with the requirements of fast pyrolysis processes. The reaction process has been resembled to buttering bread (Ringer et al. 2006). Melting velocity of the biomass particles increases with pressure against the hot wall. This is typically achieved by exerting pressure directly on biomass particles against a rotating disk (Schöll et al. 2004, Peacocke and Bridgwater, 1994) or by applying centrifugal forces against a hot wall, as in cyclonic and vortex reactors (Diebold 1980, Lede et al. 2006). Contact pyrolysis involves high heat transfer due to the high thermal conductivity of metals. Wall temperature should be higher than 600°C, and in the case of cyclonic reactors the relative velocity between the particle and wall must be high (Bridgwater et al., 1999). Owing to the low thermal conductivity of the biomass, only the outer layer of the particle is subjected to pyrolysis. The volatile products generated as a result are rapidly removed from the hot surface, ensuring very low secondary reactions. The carbonized outer layer of the biomass particle is rapidly removed owing to attrition in the ablative reactor, leaving a second layer of fresh biomass exposed, which is subjected to further pyrolysis by contact with the hot surface.

The most important feature of ablative reactors, besides avoiding inert gases, in direct contact configuration, is that internal heat transfer of biomass does not limit the reaction rate.

This means that large biomass particles can be used. Nevertheless the process is limited by the hot wall surface area and the mechanical system makes it scale up still harder.

In direct contact against a rotating disk reactors, pressure generating devices are designed as hydraulic pistons (Figure 10), in order to apply the required pressure (5-200 bar) accurately and then maintain it (Meier et al., 2008). In the case of PYTEC's disk pyrolyser, the hot disk rotates at around 60 rpm (relative motion of 2 m/s), at contact pressure of 10-12 bar, and the ablation rate is around 3 mm/s (Schöll et al., 2006). The housing of the reactor could have a one lower aperture to collect solid products and at least one second aperture at the upper end connected to a cyclone and a condenser (Meier et al. 2008).

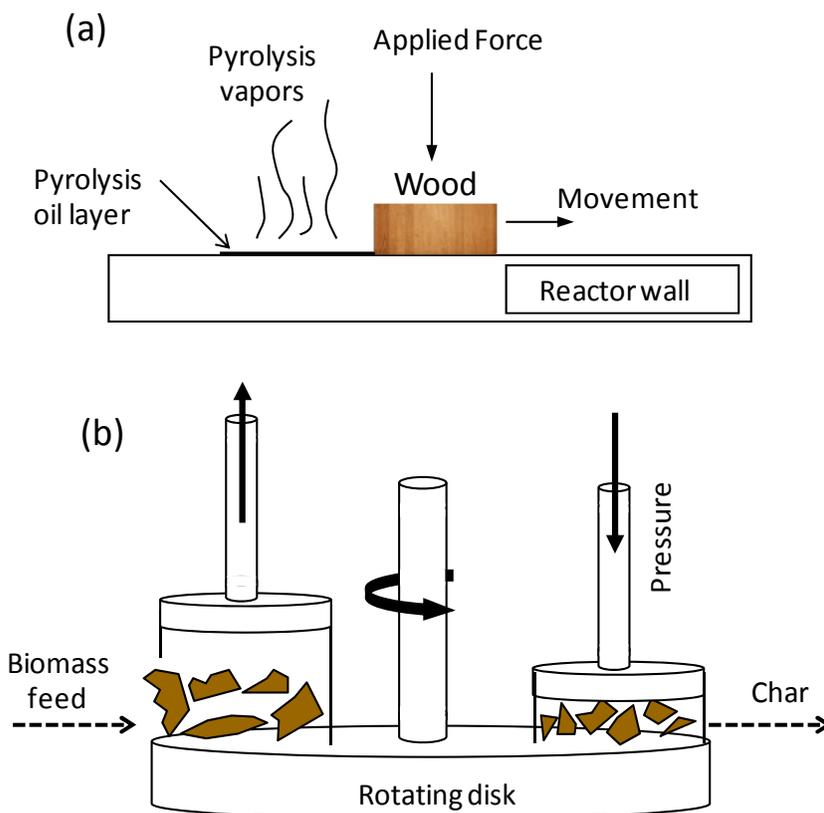


Figure 10. a) Biomass ablation scheme b) Rotating disk ablative reactor developed by Pytec.

#### *Vacuum Pyrolysis Reactor*

Vacuum pyrolysis takes place under low pressures of typically 0.15-0.20 atmospheres and moderate temperatures (400-500°C). The use of a vacuum serves to decrease the resistance experienced by molecules diffusing out of the biomass particles. This effect is greater with bigger particles where diffusion is the limiting step (Knoetze and Rabe 2005). Hence, pyrolysis vapors are rapidly volatilized and removed from the reactor, minimizing secondary reactions. This low pressure is achieved by a vacuum pump connected to the main reactor. Vacuum pyrolysis processes do not employ carrier gases, hence reducing the dilution of evolving gases.

New reactor designs, the horizontal moving and stirred bed reactors, were developed specifically for vacuum pyrolysis by Roy et al. (2000) with the aim of improving heat transfer in the reactor compared to previous reactors, such as the multiple hearth furnace where the heat source is the external wall (Lemieux et al. 1987). The pyrolysis reactor consists of a cylinder with two heating plates, one above the other. Plates are made up of tubes through which molten salt is circulated as a heating medium. There are some mechanical agitators that periodically stir the biomass on the belt. Feedstock is heated slowly by conduction and radiation. Non condensable gases are fired together with natural gas in the molten salt heater. Oil yields are quite low and mechanical difficulties, such as solid feeding and discharging devices to maintain a good seal and high temperature (500°C) mechanical transport, makes this technology complex.

Table 3 summarizes the main characteristics and working parameters of different types of reactors.

**Table 3. Reactor types and its main features**

Reactor	Optimum product distribution (tar/char/gas)	Gas to biomass wt% ratio	Typical Feedstock size	Heat transfer coefficient (W/m <sup>2</sup> K)	Maximum plant size	Reference
Deep Bubbling Fluid Bed	74,3/12,5/10,1 (430°C hardwood sawdust)	<2	<2mm	150-500	100-200 ton/day	(Scott et al. 1999 ; Papadakis et al. 2009 ; Dynamotive)
Shallow Bubbling Fluid Bed	78/7,7/10,8 (500°C hardwood sawdust)	6-8	<2mm	150-500	160 kg/h (out of work)	(Scott et al 1994 ; Papadakis et al. 2009)
Circulating Fluid Bed	78/10,9/11,1 (520°C hardwood sawdust)	1,3-1,9	<2mm	200-650	100 ton/day	(Freel and Graham 1991; Boukis et al. 2007; Ensyn)
Spouted Beds	72/12/16 (450°C softwood sawdust)	<1	Sawdust, flakes	180-260	25 kg/h	(Aguado et al. 2000; Zabrodsky et al. 1967)
Spout Fluid Beds	73,2/14/12,8 (480°C, sawdust)	~<2	<3 mm	-	6 kg/h	(Chen et al. 2003)
Rotating Cone	71/15/14 (500°C, mixed wood sawdust)	0	<0,3 mm	500-1500	2 ton/h	(Wagenaar et al. 2001 ; Janse et al. 1999, BTG)
Rotating disk Ablative reactor	68/10/22 (disk at 650°C, wood chips)	0	Chips	502-1675	6-48 ton/day	(Meier et al. 2007 ; Di Blasi 1996)
Twin Screw	66/17/17 / (~500°C, beech flour)	0	Sawdust, Chops...	100-200	12 ton/day	(Henrich 2007, Bioliq)
Vacuum reactor	56/26/18 (475°C, 0,4mm particles)	0	Bark pellets	86-155	3,5 ton/h	(Garcia-Perez, 2007; Yang et al. 2000)

### 3.3. Fast Pyrolysis Plant: Ikerlan Ik4 Pilot Plant

In addition to the pyrolysis reactor, a fast pyrolysis plant also includes a number of elements that are common to most of them. This includes a biomass pretreatment system; a feeding system; a recycled gas pre-heater; a char recovery system; and a liquid recovery system. These systems are illustrated in this section using the fast pyrolysis pilot plant developed at Ikerlan IK4 (Miñano, Spain) as a reference. The typical fluid bed plant layout is illustrated in Figure 11. The following sections describe the basic aspects and equipment of the Ikerlan-IK4 fast pyrolysis plant.

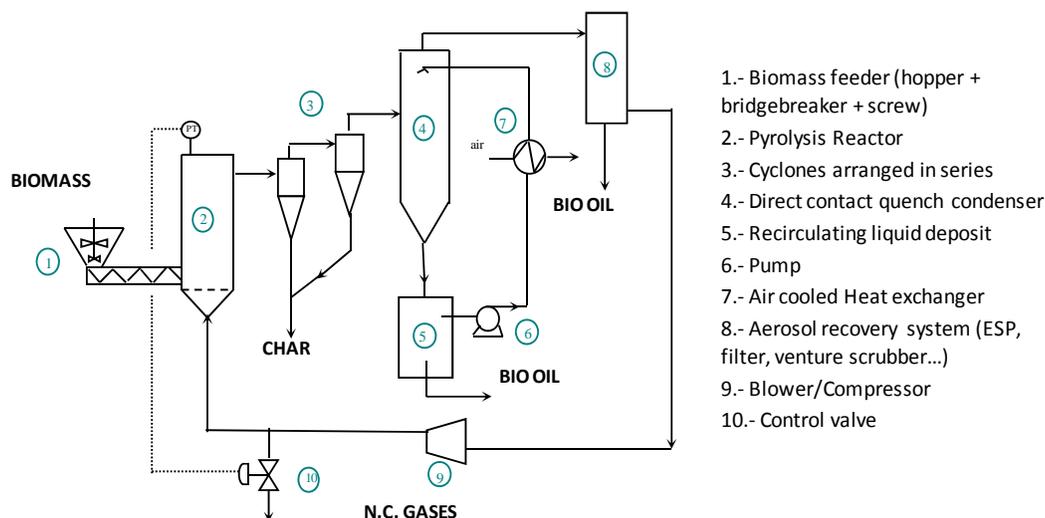


Figure 11. Conventional scheme for a biomass fast pyrolysis plant based on fluidized bed technology.

#### 3.3.1. Ikerlan-IK4 Pilot Plant

The Ikerlan IK4 pilot plant (Pyne Newsletter 26, 2009; Diaz, 2008; Fernández-Akarregi, 2010; Makibar, 2010) with a capacity of up to 25 kg/h, is based on a conical spouted bed reactor. This option was chosen because of its simplicity, good heat transfer characteristics, very low flow gas requirements and the ability of treating coarse and heterogeneous particles of biomass. The plant has been working with the reactor heated by electrical resistances (Fernández, 2009), but the next phase is the thermal integration of the plant, using the heat contained in the non condensable gases produced in the process.

After exiting the reactor, solid char, gas, vapors and aerosols pass through a dual in-line cyclone system. These cyclones remove most of the entrained solid char particles. Liquid recovery, after the cyclone system, is performed using a light hydrocarbon spray. The condensable vapor portion of the gases is cooled and collected in a product tank. Quench liquid goes through an air cooled heat exchanger and is then recycled to the spray tower. The remaining aerosols are separated from the non-condensable gases with coalescence filters. Aerosols coalesce into tiny droplets which gather and drip down to an oil pot. The clean, inert gas is then recycled back to the spouted bed reactor. The fluidizing gas is adjusted by means of a rotary piston blower, a vortex flow meter and an inverter. Pressure at the reactor is maintained at ambient pressure, burning the excess of non-condensable gas in a flare with a pilot burner.

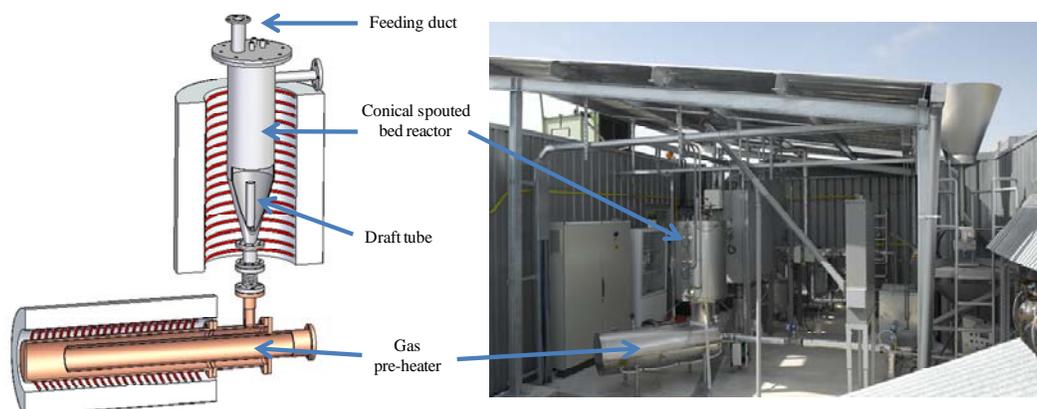


Figure 12. Ikerlan IK4 pilot plant reactor and gas preheater.

### 3.3.2. Biomass Pretreatment

As discussed above, the particle size of the biomass feedstock needs to be small enough to ensure effective heating rates. The specific particle size distribution depends on reactor design. Biomass size reduction is usually conducted using knife mills and hammer mills. The cost of processing is high and grows exponentially with the reduced particle size. In a study conducted by NREL (Diebold and Schahill, 1997) the energy consumption to obtain a 0.72 mm sawdust from ½" wood chips using a hammer mill was 105 kWh/tonne (5% of the energy contained in the feedstock).

Freshly harvested biomass contains around 40-60 wt% moisture and agricultural crop residues exposed to open-air solar drying may contain around 15 wt% (Klass, 1998). Reducing moisture content to 10-15% is also necessary to achieve high heating rates and to obtain pyrolysis oils with relatively low water content (15-25 wt%). Each kg of water fed, generates approximately 1.25 kg of water content in the product oil, however, drying to values below 10% is considered expensive (Bridgwater et al. 2009). Direct heat drying using hot air, flue gas or superheated steam such as in rotary driers, silo driers or conveyor belt driers are lower in cost compared with indirect-heat drying, where heat is transferred by hot surfaces (Klass, 1998). As an example, the energy consumption in an improved rotary drier for wood chips varies between 2.7 MJ/kg of biomass to 2.8 MJ/kg of water (Meza et al. 2008). In most cases, heat for biomass drying is obtained by combustion of the pyrolysis gas and char by-products, although alternatives have also been studied (solar energy, biomass combustion, fossil fuels). It has been described that drying of the biomass feedstock at temperatures above 200°C may cause condensation and cross linking reactions resulting in reduced oil yields (Dobele et al. 2007).

### 3.3.3. Feeding System

Most fast pyrolysis reactors operate at near atmospheric pressure, which means that feeding systems are not very complex. Pneumatic systems (Scott and Piskorz 1982, Berruti et al. 2009) and screw conveyors are widely used in reactors of different sizes and designs. In conventional bubbling fluidized beds, biomass is fed just above the distribution plate to increase solid residence time in the dense phase (Rapagna and Mazziotti, 2008). Top feeding would be possible depending on bed superficial velocity and biomass particle size. In

contrast, feeding in spouted bed reactors is best at the top in order to ensure at least a complete bed cycle (Figure 13). Feeding lines must be maintained at low temperatures to avoid deposition of condensed tars and clogging. Hence, bottom feeding seems are usually avoided.



Figure 13. Biomass top feeding at Ikerlan IK4 pilot plant.

Typically upstream, an airlock system such as the lock hopper or a rotary valve is necessary to avoid air inflow or vapor backflow. A screw feeder with tapered and extended sections can also help to plug seal formations (Dai and Grace, 2008). Biomass particles may attract each other by electrostatic charges or molecular forces. These problems become significant with small particle sizes, and careful designs are necessary to avoid bridging or blocking feeding lines due to the cohesive nature of biomass.

### **3.3.4. Recycle Gas Pre-Heater**

Recycled gas is usually preheated in a heat exchanger prior to being introduced into the main fast pyrolysis process. The temperature of the recycled gas needs to be, at least, comparable to the pyrolysis temperature. In some cases, process heat is introduced via recycled gas, in which case this gas needs to be preheated to higher temperatures. It has been described that gas temperatures above 600°C should be avoided because of the promotion of secondary cracking and coking reactions (Scott et al., 1997 and 1999).

### **3.3.5. Char Recovery System**

Pyrolysis vapors usually contain high proportions of entrained solids. Depending on process characteristics, these may consist of char fines, inorganic ashes and bed particles. Separation of these elements from the volatile fraction is essential to ensure high pyrolysis oil quality. Cyclones are the cheapest and simplest equipment to recover the char from the product stream. This technology is effective for particles greater than 10 $\mu$ m. However, char fines smaller than 1  $\mu$ m are usually produced in fluidized bed reactors, owing to attrition.

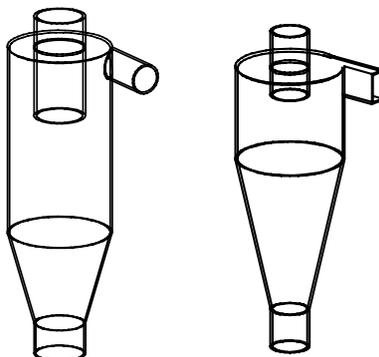


Figure 14. First and second stage cyclones at Ikerlan-IK4 pilot plant.

Solid collection efficiency can usually be enhanced by arranging cyclones in series. For example, in the Ikerlan-IK4 pilot plant a second stage cyclone was installed in 2010 to remove fine char particles (Figure 14). The second stage cyclone has an inlet gas velocity higher than that of the first stage cyclone to increase the centrifugal force on the solids and to increase solid recovery. Energy loss is mainly determined by the pressure drop over the cyclone, typical values are around 0.2-0.4 kWh/1000m<sup>3</sup>. Hot gas filtration using ceramic filters can follow conventional cyclones in order to reduce the presence of solids in the volatile fraction even further.

### 3.3.6. Liquid Recovery System

Pyrolysis vapors need to be quenched and condensed very rapidly in order to minimize secondary reactions and maximize oil yields and reduce the formation of heavy tar deposits (Bridgwater et al. 2009). The volatile fraction contains a high proportion of aerosols (Daugaard and Brown, 2006) that are very difficult to recover with conventional equipment. These aerosols are generated by direct ejection of oligomeric compounds in the reactor (Bayerbach and Meier 2009). Direct contact condensers (spray towers) are best suited to achieve rapid cooling of the volatile fraction. Vapors are cooled from approximately 350°C to almost ambient temperature (further cooling is not justified for energy applications) using generated pyrolysis oil or other organic liquids as a coalescing medium. The use of mineral oils may also be considered, as they are able to withstand higher temperatures without cracking or evaporating. Mineral oil viscosity could be lower than the pyrolysis oil in order to ensure the generation of small droplets in nozzles.

Dilution of the pyrolysis vapors in the carrier gas reduces the recovery efficiency owing to reduced vapor pressures. Relatively low exit gas temperature is essential to ensure effective condensation of volatile products. Pyrolysis oils generated in this way exhibit lower viscosities owing to the presence of high concentrations of lighter hydrocarbons and water. Figure 15 shows the fast pyrolysis vapors condenser employed at Ikerlan IK4, which consists of two 90° full cone nozzles and a woven wire mesh demister. This demister is efficient for the recovery of entrained mineral oil droplets.

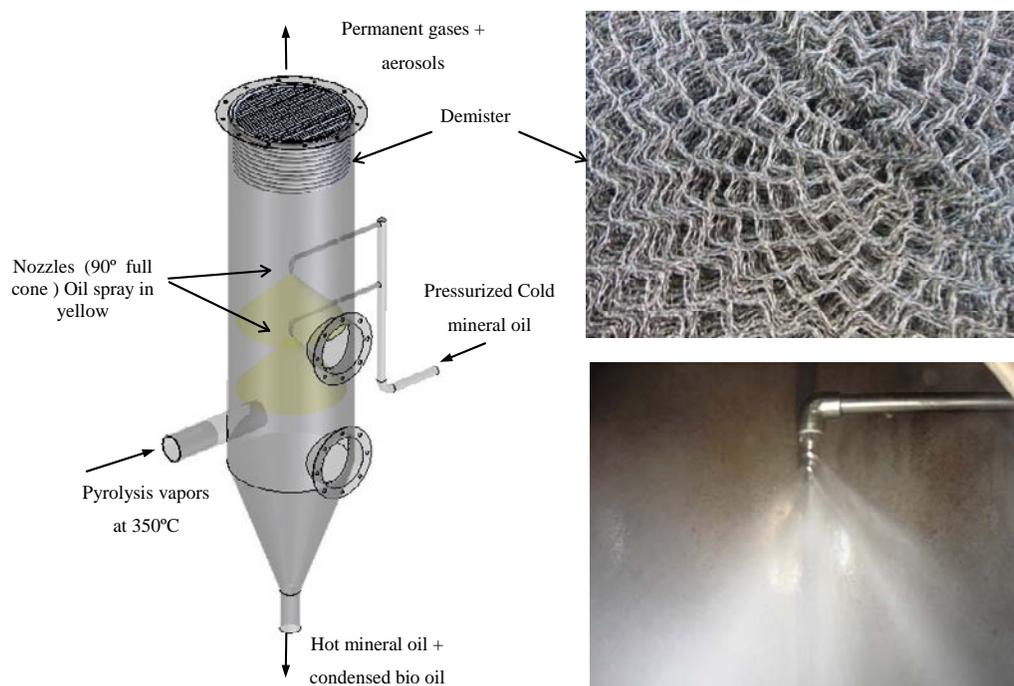


Figure 15. Pyrolysis vapors condenser at Ikerlan IK4 pilot plant.

Recovery of aerosols is not effective in the scrubbing system, and requires the use of additional equipment downstream such as coalescing filters, venturi scrubbers (Barynin and Kawaguchi 2009), electrostatic precipitator (ESP), (Bologa et al. 2008), or rotary demisters (Xu et al. 2010, Brouwers, 1991). Electrostatic precipitators are very efficient and have low energy consumption of 0.2-0.4 kWh/1000m<sup>3</sup> compared, for instance, with venturi scrubbers which consume between 1.5-6 kWh/1000m<sup>3</sup>. Nevertheless ESPs require high initial investment costs and simpler equipment would be more attractive for commercial plants.

Fractional condensation at different stages/temperatures could be interesting to produce low water and low molecular weight compounds of bio oil (Westerhof et al. 2007) or to recover a desired product easily, such as phenolic rich oil (Effendi et al. 2008). High condenser temperature oils exhibit higher viscosity values and heating values.

## 4. PYROLYSIS BIO-OILS AND OTHER PYROLYSIS PRODUCTS

### 4.1. Pyrolysis Products

#### 4.1.1. Product Yields and Overall Characteristics

As discussed throughout this paper, the thermal degradation of lignocellulosic biomass results in the production of a solid fraction of carbonized char and a vapor/aerosol fraction that can be subsequently separated into non-condensable gases and a complex mixture of condensable oils. The yield and composition of these products depends on the nature of the original biomass and the precise conditions employed in the pyrolysis process. This section

provides a summary of the characteristics and potential applications of these products, paying special attention to the condensable oils.

### *Pyrolysis Gases*

Gas yields in fast pyrolysis processes typically range between 15-30 wt%, although this value may be reduced in small laboratory scale reactors where secondary reactions are minimized. Gas yields may rise significantly (above 30 wt%) by increasing the severity of the pyrolysis process (higher reaction temperatures, longer residence times of pyrolysis vapors in the reactor) (Bridgwater et al., 1999; Chen et al., 2003). Certain inorganic elements often present in the biomass feedstock (K, Na, Ca) or added to the process by impregnation of the biomass by incorporation into the fluidizing medium (olivine, dolomite, limestone, acid zeolites) may catalyze the cracking of larger molecular weight species into non-condensable gas products, hence favoring gas yields at the expense of condensable liquid products (Bridgwater, 1994 and 1996). Fast pyrolysis of biomass feedstocks that contain high proportions of these inorganic elements, like cereal straw, have been reported to produce higher gas yields, and consequently lower oil yields (Livingston, 2007).

Owing to differences in process characteristics, reactor design, feedstock composition and operating conditions, there is a wide variability in the composition of pyrolysis gases reported in the literature. A typical gas composition may be as follows: CO (30-40%), nitrogen (0-60%), CO<sub>2</sub> (20-25%), hydrogen (5-10%), methane (5-15%) and C<sub>2</sub>-C<sub>5</sub> oxygenated and non-oxygenated organics (5-10%). Heating values usually range between 10-15 MJ/Nm<sup>3</sup>, although this may be reduced in oxidative pyrolysis due to partial combustion inside the reactor for process heat, or may rise above 15 MJ/Nm<sup>3</sup> by promotion of thermal and catalytic cracking reactions (Chen et al., 2003). Commercial pyrolysis plants usually recycle pyrolysis gases for process energy and also for drying of biomass feedstock. The use of pyrolysis gas for other applications (syngas) has also been suggested, although this would involve significant technical and economic challenges (Bridgwater, 1996).

### *Pyrolysis Chars*

Most of the solid char produced in fast pyrolysis results from the dehydration and subsequent carbonization of the original biomass feedstock components. Additional carbonized matter is formed as a result of recombination and polymerization reactions taking place between solid and vapor pyrolysis products (Demirbas, 2001).

Char yields in optimized fast pyrolysis processes typically range between 10-15 wt%, compared to 30-40 wt% in traditional biomass carbonization processes designed to promote recombination and polymerization reactions on the pyrolytic products (Bridgwater and Peacock, 1999; Demirba, 2001). Owing to its amorphous structure, pyrolysis char does not conduct electricity. The carbon usually makes up most of the inorganic components and therefore exhibits higher ash content than the original biomass feedstock. Owing to its calorific value (30-35 MJ/kg), pyrolytic char can be used as fuel in boilers. Its use as an adsorbent and gasification feedstock has also been suggested (Sharma et al., 2004; Goyal et al., 2008). Recent studies also propose the incorporation of biochar into agricultural soils. This activity contributes to reducing the emission of greenhouse gases into the atmosphere and increases agricultural productivity, as it favors nutrient fixation and microbial activity (Lehman and Joseph, 2009; Laird et al. 2009).

### *Pyrolysis Oils*

As much as 60-75 wt% of the original biomass can be transformed into condensable bio-oils in an optimized fast pyrolysis process. The use of this oil as an energy carrier involves economic and technical advantages over the original biomass, owing to its higher energy density, homogeneous structure and liquid state (Bridgwater, 1999; Mohan et al., 2006). However, it also involves some challenges associated with production costs and particularly with the exceptional chemical and fuel properties of the oil, that have little in common with conventional hydrocarbon products (Oasmaa and Peacocke, 2001). The following section provides a more detailed description of the characteristics of these bio-oils and their potential applications.

#### **4.1.2. Characteristics of Pyrolysis Oil**

Characterization of the chemical, physical and fuel properties of the pyrolysis bio-oils is essential in order to determine their potential use in different energy and chemistry applications. Various publications have been dedicated specifically to investigate and review the characterization of pyrolysis oils (Sipila et al., 1998; Oasmaa and Czernik, 1999; Oasmaa and Peacocke, 2001; Oasma and Meier, 2005; Scholze and Meier, 2001; Zhang et al., 2007; Qiang et al., 2009; Bahng et al., 2009; Oasmaa et al., 2009).

In order to compare the characteristics of bio-oils produced in different plants and make them more accessible to the market, the need to develop and validate standard methods of analysis soon became apparent. This work was led by the Finish research center VTT which produced “A guide to physical property characterization of biomass derived fast pyrolysis liquids” (Oasmaa and Peacocke, 2001). The methods described in this document were validated in a series of “round robin” tests coordinated by the international organizations Pyrolysis Network (PyNE) and IEA Bioenergy (Oasmaa and Meier, 2005). These analytical methods have become standard practice for the characterization of fuel and physical properties in fast pyrolysis bio-oils. However, the methods proposed to determine chemical composition and chemical stability did not produce consistent results in “round robin” tests and no standard method has been accepted for chemical composition so far (Oasmaa et al., 2009).

When it comes to analyzing pyrolysis oils, there are two issues that need to be considered. The first one is that the fuel properties and chemical composition differ significantly from conventional liquid fossil fuels. Owing to the high water content and the presence of oxygenated compounds, pyrolysis oils are highly acidic, polar and thermally unstable. These characteristics need to be taken into consideration when handling, characterizing and using the oils. The second is that the pyrolysis oil is not a unique material, and notable differences may be observed depending on the biomass feedstock and production conditions. This variability may cause difficulties in the commercialization of the bio-oil, owing to the lack of consistency in the quality of pyrolysis oils generated by different producers. However, this versatility may also be regarded as a positive aspect, allowing the pyrolysis process to be designed and operated in order to optimize the yields and characteristics of target products.



Figure 16. Image of a typical pyrolysis oil.

As shown in Figure 16, biomass pyrolysis oil is a red-brownish liquid with a strong smoky odor. Bio-oils containing high proportions of fine chars may have a darker or even completely black color. The oil is described as a micro-emulsion that consists of an aqueous phase containing lighter oxygenated organic compounds and a non-aqueous phase containing heavier water insoluble aromatic compounds. The elements in the aqueous phase derive primarily from the thermal decomposition of the polysaccharide components of the biomass (cellulose and hemicellulose) while the non-aqueous phase contains hydrophobic elements generated from the decomposition of lignin. This emulsion is stabilized by polar constituents (sugars) that behave as bridging agents between the aqueous and the hydrophobic elements (Mohan et al., 2006; Bayerbach et al., 2006 and 2009).

Freshly made pyrolysis oil is a rather homogeneous liquid, with both aqueous and organic components forming a single phase. However, this homogeneity is lost spontaneously with time due to so called “aging” reactions (Diebold, 2000; Hilten and Das, 2010). The homogeneity of a bio-oil may be monitored by analyzing water content at different depths, although the reproducibility of this method has not been validated (Oasmaa and Peacocke, 2001). Table 4 shows the main characteristics of a typical pyrolysis oil compared with various conventional petroleum derived and biomass derived liquid fuels.

#### 4.1.2.1. Water Content and Oil Miscibility

The presence of small amounts of water in conventional fossil fuels has been associated with corrosion and damage to the engine and fuel filters. Hence, standard fuel specifications required very low water contents ( $< 0.05$  wt% in transport fuels and  $< 0.1$  wt% in heavy fuel oil) (ASTM, 2009, 2009A and 2010). In contrast, water is an integral part of the pyrolysis oil with concentrations typically ranging between 15-30 wt%. Some of this water comes from free moisture present in the biomass feedstock and some is generated as a result of dehydration and thermal degradation of the biomass components. Water content in pyrolysis

oils cannot be determined by evaporation methods owing to its thermal instability. The accepted method is Karl-Fischer titration according to ASTM E203 (Oasmaa and Peacocke, 2001).

**Table 4. Typical characteristics of freshly produced biomass pyrolysis oil compared against more conventional liquid fuels.**

	Pyrolysis oil	Heavy fuel oil (No. 5)	Diesel fuel (No. 2)	Biodiesel	Ethanol (100%)	
Moisture content (wt%)	15-30	< 0.1	< 0.05	< 0.05	< 0.05	
pH	2.5-3.0	-	-	-	-	
Density (kg/m <sup>3</sup> )	1100-1250	950	820-860	860-900	794	
Elemental analysis (wt%)	C	40-50	85	84-87	75-80	52.2
	H	6-8	11	15-32	12-15	13.1
	N	0.1-0.5	0.3	< 0.1	< 0.1	-
	O	45-55	1.0	< 0.1	10-11	34.7
	S	< 0.05	1.5- 2.5	< 0.001	< 0.001	-
Chemical formula	Aqueous emulsion of oxygenated compounds.	Aliphatic, olefinic and aromatic hydrocarbons C <sub>18</sub> -C <sub>60</sub>	Aliphatic, olefinic and aromatic hydrocarbons C <sub>9</sub> -C <sub>25</sub>	Methyl esters of C <sub>12</sub> -C <sub>22</sub> fatty acids	C <sub>2</sub> H <sub>5</sub> OH	
Average molecular weight (u)	500-1000	Aprox. 500	Aprox. 200	Aprox. 250	46.07	
High heating value (MJ/kg)	16-19	40	45	36	30	
Energy density (GJ/m <sup>3</sup> )	19-22	38	38	31	24	
Viscosity at 40°C (cP)	20-500	180	2-5	3.5-5.0	1.2	
Solids (char and ash) (wt%)	0.05-0.5	1	< 0.01	< 0.01	< 0.01	
Flash point (°C)	45-100	90	60	150	12.8	
Autoignition temp (°C)	> 500	290	260	180	365	
Water solubility	Aprox. 50%	Negligible	Negligible	Negligible	100	

Representative values from liquid fuels characterized at Laboratorio de Productos Petroliferos, Fundación Gómez Pardo, Universidad Politécnica de Madrid, (Spain).

This water is detrimental to the heating value and flame temperature of the pyrolysis oil, hence affecting its ignition properties. However, water has to be in sufficient amounts to reduce viscosity (Diebold, 2000). In addition, very low (below 10 wt%) water content has been reported to reduce stability of the aqueous emulsion, favoring phase separation. Since water cannot be removed by distillation, the easiest way to adjust its content in the bio-oil is by controlling the moisture levels in the original biomass feedstock. Drying of the biomass to reach moisture levels around 10-15 wt% is usually recommended (Bridwater et al., 2000, Mohan et al., 2006). Fractionated condensation can also be used to reduce the presence of water in certain pyrolysis oil fractions. Heavy lignin derived components condense at higher temperatures than water and lighter water soluble elements (Bayerbach and Meier, 2009).

Owing to the high water content and the presence of oxygenated organic compounds, pyrolysis oils are highly polar, and therefore immiscible with conventional hydrocarbon fuels. In contrast, pyrolysis oil tolerates the addition of water up to a limit. Phase separation usually occurs at water contents above 40 wt% (Bayerbach and Meier, 2009). Blending of pyrolysis

oils with alcohols (methanol and ethanol) is also possible and has been proven to improve chemical stability and fuel properties (Diebold, 2000; Boucher et al., 2000; Nguyen and Honnery, 2008).

#### 4.1.2.2. *Elemental Composition*

Elemental analysis in pyrolysis oils can be performed using a standard microelemental apparatus (ASTM D5291). The elemental composition of the pyrolysis oil is similar to that of the original biomass. The main elemental components of the oil (on a dry basis) are carbon (45-50 wt%) and oxygen (40-50 wt%). Oxygen is found in the form of reactive functional groups (including carboxyl, carbonyl, hydroxyl, ether, ester and aldehyde) that provide the oil its unstable characteristics. Nitrogen content is highly variable, depending on the feedstock, and very undesirable if the oil is to be combusted due to the formation of nitrogen oxides. The concentration of sulfur in the oil is in most cases negligible (Oasmaa and Peacocke, 2001).

#### 4.1.2.3. *Mass and Energy Density*

The density of pyrolysis oil is very high (1100-1250 kg/m<sup>3</sup>) compared to conventional biomass feedstocks (300-500 kg/m<sup>3</sup>) and also to conventional liquid fuels (800-950 kg/m<sup>3</sup>). In terms of energy content, pyrolysis oils exhibit high heating values (HHV) in the range 16-19 MJ/kg. On a weight basis, this is around 60% lower than petroleum derived fuels (40-45 MJ/kg) and about 40% less than conventional biofuels (biodiesel and bio-ethanol have 36 and 30 MJ/kg, respectively). However, owing to the high mass density of the pyrolysis oil, this difference is significantly reduced when represented on a volume basis. This low heating value is related to the high water and oxygen content (on a water-free basis) of the oil. Owing to its comparable elemental composition, the pyrolysis oil has a similar energy density to the original biomass. However, its energy density per unit of volume is 3-5 times higher than the original feedstock (19-22 GJ/m<sup>3</sup> compared to 4-10 GJ/m<sup>3</sup> in the biomass).

#### 4.1.2.4. *Viscosity*

Viscosity in conventional fuels has to be within a certain range in order to ensure adequate performance of the engine. Viscosity values need to be sufficiently high to ensure that the fuel provides sufficient lubrication to the injection pumps and the pistons. Excessively high viscosity values may affect atomization of the fuel, hence reducing combustion efficiency and increasing the emission of particles, CO and volatile organic compounds.

Viscosity values (determined at 40°C) for freshly made pyrolysis oil have been reported to range between 20-500 cP, as determined according to standard method ASTM D445. This is significantly higher than in conventional transportation fuel (< 5.0 cP) and comparable to heavy fuel oil. However, viscosity in the pyrolysis oil varies depending on many parameters including water content and average molecular weight. Viscosity also increases with time as a result of aging reactions, where organic molecules recombine and polymerize into larger molecular weight oligomers. Hence, values in excess of 1000 cP have been reported for oils with low water content and exposed to ambient conditions or high temperatures for long periods of time. Conventional injectors may not work for liquids with such high viscosity values. Moderate preheating of the pyrolysis oil has been suggested as a means of reducing viscosities and facilitating pumping and atomization (Zheng and Kong, 2010). However, exposure to high temperature accelerates aging reactions, thus altering the fuel properties of

the oil rapidly (Hiltén and Das, 2010). Viscosity may also be reduced by the addition of alcohol or by forming emulsions with conventional fossil fuels (Czernik and Bridgwater, 2004).

#### 4.1.2.5. Solids and Ashes

Pyrolysis oils contain variable amounts of solids in suspension, usually consisting of char fines and in some cases bed solids entrained from the reaction zone. The analytical method for solids in suspension was developed specifically for pyrolysis oils, and involves dissolving the oil in methanol-dichloromethane and determining the insoluble fraction (Oasmaa and Peacocke, 2001). Solid contents typically range between 0.05 and 0.5 wt%, depending on the pyrolysis technology employed and the efficiency of solid separation devices (ceramic filters, cyclones) (Oasmaa and Peacocke, 2001; Oasmaa and Meier, 2005). The presence of these solids in the oil is highly undesirable due to mechanical erosion and blocking of burners, injectors, pumps, valves and turbine blades. In addition, these chars usually make up the inorganic fraction of the original biomass (ash), primarily consisting of alkali and alkaline-earth oxides. Alkali metals (potassium, sodium) are damaging due to their corrosive properties during combustion, and alkaline-earth elements (calcium, magnesium) are detrimental due to deposition (Czernik and Bridgwater, 2004).

#### 4.1.2.6. Chemical Stability

As discussed above, biomass pyrolysis oils are chemically unstable, which poses a significant challenge to their use in commercial applications (Diebold, 2000; Oasmaa and Kuoppala; 2003; Hiltén and Das, 2010). The oil contains reactive species that tend to recombine and polymerize according to the following reactions:

- Polymerization of unsaturated hydrocarbon chains.
- Esterification between carboxyl and hydroxyl groups  $\rightarrow$  polymerization + water.
- Etherification between carbonyl and hydroxyl groups  $\rightarrow$  polymerization + water.

This causes an increase in the viscosity of the bio-oil, a reduction in the concentration of volatiles and eventual phase separation. Chemical instability in pyrolysis oils can be evaluated using accelerated aging tests. Exposure of the oils to 80°C over 24 hours has been reported to be equivalent to one year storage at room temperature in terms of aging (Hiltén and Das, 2010; Oasmaa and Kuoppala, 2003). It has also been observed that alkaline metals present in the solid chars effectively catalyze aging reactions. The addition of polar solvents like ethanol and methanol has been reported to increase the stability of the oil, which can be stored for longer periods of time without deterioration of fuel properties (Diebold, 2000).

#### 4.1.2.7. Corrosion and Acidity

Owing to the formation of carboxylic acids during the thermal degradation of biomass components, primarily acetic and formic acid, pyrolysis oils have very low pH values (usually 2-3) and are highly corrosive to conventional construction materials (carbon steel, aluminum, plastics and elastomers). This corrosive nature is intensified even further at high temperatures in boilers, engines and turbines (Czernik and Bridgwater, 2004; Chiaramonti et al., 2007). Hence, the oil has to be stored and transported in acid-proof materials like stainless steel or

polyolefins. Any other elements that are in contact with the oil during processing and energy generation (fuel lines, pipework, vessels, gaskets, burners, nozzles, pistons, combustion chambers, blades, etc) also need to be resistant to acid corrosion (Diebold, 2000; Pootakham and Kumar, 2010).

#### 4.1.2.8. Flash Point and Ignition Characteristics

Flash point indicates the minimum temperature at which a fuel will ignite on application of an ignition source and may be determined by ASTM D93. Low flash point values are required to ensure safe handling, storage and transportation of the fuel. Experimental tests show flash point values in pyrolysis oils comparable to conventional diesel fuel, and significantly higher than ethanol. However, significant differences have been observed depending on the proportion of light organic products, which are promoted under severe pyrolysis conditions (Oasmaa and Peacocke, 2001; Oasma and Meier, 2005). Unlike hydrocarbon fuels, pyrolysis oils do not show a correlation between flash point temperature and ignition properties. This is so because the combustion of the oil at low temperatures is rapidly extinguished by the evolution of evaporated water. Hence, pyrolysis oils exhibit very high autoignition temperatures (> 500°C) as shown in Table 4.

#### 4.1.2.9. Chemical Composition

Biomass pyrolysis oils are extremely complex in their chemical composition owing to emulsinated state, the lack of predominant compounds (apart from water), the wide range of molecular weights, polarities and functional groups with high reactivity even at low temperatures. Hence, characterization of the chemical composition of pyrolysis oil is a highly challenging activity that needs to be approached cautiously.

The precise composition of the oils is highly affected by the nature of the biomass feedstock and processing conditions, and it changes with time owing to aging reactions. In terms of molecular size, it has been described that vapors exiting the pyrolysis reactor have molecular weights below 200 u, as determined by molecular beam mass spectrometry (Evans and Milne, 1987; Brown et al., 2001). However, freshly made pyrolysis oils have an average molecular weights around 500-1000 u (as determined by Gel Permeation Chromatography). This molecular weight increases with time due to the formation of oligomeric structures by recombination and polymerization reactions characteristic of the aging process (Bayerbach et al., 2006).

Approximations of the chemical composition of pyrolysis oils can only be achieved by preliminary fractionation by solvent extraction (Mohan et al., 2006). The chemical components of pyrolysis oils can be separated into the following fractions: water soluble species (acids, alcohols, ethers); ether soluble species (aldehydes, ketones, phenols); ether insoluble species (sugars, hydroxyl acids), dichloromethane soluble (small lignin fragments) and dichloromethane insoluble species (larger lignin fragments and solids) (Oasmaa and Kuoppala, 2003; Oasmaa et al., 2003).

These fractions may be subjected to different analytical techniques in order to elucidate some components. Gas Chromatography (GC) has been widely used to characterize pyrolysis oils (see Figure 17). Although it provides useful information, it needs to be acknowledged that this technique can only detect volatile components, which represents only a small proportion of the compounds in the pyrolysis oils (around 30-50%). Furthermore, exposure to

high temperatures in the GC injection system and column are likely to affect the original composition of the oils (Oasmaa et al., 2009).

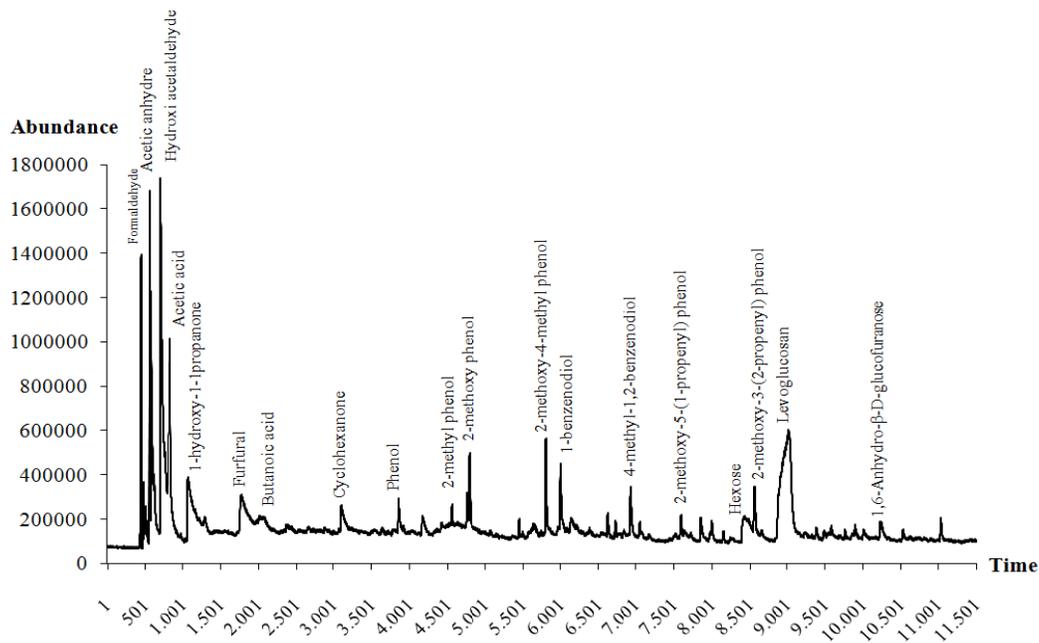


Figure 17. Characteristic GC of the aqueous fraction of a biomass pyrolysis oil.

**Table 5. Chemical composition of a fractioned pyrolysis oil as determined by GC/MS (Oasmaa and Kuoppala, 2003).**

Fraction	Compound	wt (%)	Fraction total (wt%) <sup>a</sup>
n-hexane solubles (extractives, fatty acids, resins)		5.7	5.7
Water solubles (acids, alcohols, ethers)	Formic acid	1.5	5.6
	Acetic acid	3.4	
	Propionic acid	0.2	
	Glycolic acid	0.6	
Ether solubles (aldehydes, ketones, lignin monomers, etc)	Ethylene glycol	0.3	23.2
	Isopropanol	2.6	
	Nonaromatic Aldehydes	9.7	
	Aromatic Aldehydes	0.009	
	Nonaromatic Ketones	5.36	
	Furans	3.4	
Ether insolubles (sugars, hydroxyl acids)	Pyrans	1.1	45.3
	Anhydro-β-D-arabino-furanose	0,27	
	Anhydro-β-D-glucopyranose <sup>b</sup>	4,0	
DCM solubles low molecular lignin fragment	Dianhydro-α-D-glucopyranose	0,17	17.1
	Catechols	0,06	
	Lignin derived phenols	0,09	
	Guaiacols (Methoxy phenols)	3,8	
DCM insolubles (high molecular lignin fragments, solids)		2.6	2.6

a = including other non identified compounds b= levoglucosan DCM = Dichloromethane

Table 5 shows the chemical characterization of the different fractions separated by solvent extraction as determined by GC/MS (Oasmaa and Kuoppala, 2003). The results are evidence that only a small amount of the sugars (ether insoluble) and lignin derived (DCM soluble) products can be identified by this technique. For this particular bio-oil, the highest concentrations of any individual species were observed for levoglucosan (4.0 wt%); acetic acid (3.4 wt%); isopropanol (2.6 wt%); and formic acid (1.5 wt%).

Analysis of non-volatile species can also be achieved using liquid phase chromatography like HPLC and GPC, although the results are not always adequate due to the multiphase character of the sample and the formation of aggregates (Mohan et al., 2006). Other techniques that have been used to obtain information about the chemical composition of pyrolysis oils include Fourier Transformed Infra-red (FTIR) spectroscopy to characterize the functional groups in the pyrolysis oils; Gel Permeation Spectroscopy (GPS) to determine molecular size distributions; and Nuclear magnetic resonance (NMR) to investigate the presence of hydrogen and carbon atoms in certain structural groups (Mohan et al., 2006; Bahng et al., 2009;). Hyphenated techniques such as gas chromatography coupled with mass spectrometry (GC/MS) and multidimensional spectrometry (GC-MS) provide additional information about the presence of specific compounds (Fullana et al., 2005).

#### 4.2.2.10. Health And Safety and Environmental Issues with Pyrolysis Bio-Oils

Health and safety issues and environmental considerations need to be adequately managed in the production, transportation and utilization of biomass pyrolysis oils. The importance of these issues grows with the number and scale of the pyrolysis projects undertaken and the wider acceptability of this technology. Regarding environmental issues, work conducted in the proximity of traditional biomass carbonization kilns demonstrated that exposure to pyrolysis vapors increased the acidic character of the soils, its biological activity and the concentration of biologically active polyaromatic hydrocarbons (PAH) (Uvarov, 2000; Fischer and Bienkowski, 1999). However, this issue would not be applicable to fast pyrolysis processes since these plants are designed to contain all the pyrolytic vapors within the plant, with minimum release of volatile components into the atmosphere.

Biodegradation is the main elimination route for pyrolysis oils released into the environment, as may occur in the event of accidental discharge during transport, storage or processing of the bio-oils. Experimental tests have shown comparable biodegradation curves in bio-oils produced using different conversion technologies and feedstocks. The biological response was very fast and spontaneous, with values ranging between 41-50 wt% degradation after 28 days. This is substantially higher than conventional fossil fuels, but slightly lower than vegetable oils (Piskorz and Radlein, 1999; Blin et al., 2007).

Experimental work described pyrolysis oils as exhibiting slight but discernible mutagenic and teratogenic activity. This has been associated with the presence of PAHs and other biologically active species such as formaldehyde, acetaldehyde and furfural (Diebold, 1999, Putnam et al., 1999; Lewis et al., 1988). The degree of toxicity is highly dependent on processing conditions and is reported to be lower than that observed in conventional fossil fuel oils. A more in depth study conducted on 19 different bio-oil samples confirmed these results (Blin et al., 2005). This same document described bio-oils as benign in their effect on algae and small water animals, even showing a slight fertilizing effect.

The concentration of polyaromatic hydrocarbons (PAH) in pyrolysis oils appears to correlate with toxicity and carcinogenic activity. The concentration of PAHs in bio-oils is

primarily dependent on processing conditions. High reaction temperatures and long vapor residence time promote aromatization and condensation reactions leading to the formation of these undesirable compounds. PAH concentrations are usually below 10 ppm in fast pyrolysis oils produced at temperatures below 500°C. Far higher contents (above 0.5 wt%) may be found in oils produced using higher temperatures and in the case of slow pyrolysis processes (Pakdel and Roy, 1991; Blin et al., 2005; García-Pérez, 2008). The formation of other potentially toxic compounds like dioxins and furans during the pyrolysis of biomass is expected to be negligible, owing to the low temperatures involved (García-Pérez, 2008). Owing to the high concentration of corrosive species, like formic and acetic acid, pyrolysis bio-oils produce acute irritation to the eyes, skin and respiratory system (Blin et al., 2005).

A very complete “Material and Safety Data Sheet” (CIRAD, 2006) and a guide on “Transport, storage and handling of biomass derived fast pyrolysis liquids” (CIRAD, 2006B) have been recently published. These documents contain complete information about chemical and physical properties, stability and reactivity, handling and storage conditions, specific measures in the case of fire or accidental release measures, exposure controls and personal protection, disposal consideration and transport information. For transportation purposes, pyrolysis oil is classified as flammable and corrosive, and has to be packaged and handled accordingly.

## 4.2. Potential Applications of Pyrolysis Oils

As illustrated in Figure 18, pyrolysis bio-oils have the potential to be used in a wide range of energy and chemical applications. The higher energy density and liquid state of the bio-oil facilitates storage and reduces transportation costs from the biomass producing areas to energy generation plants. The liquid state of the bio-oil also allows for transformation in higher efficiency devices (engines and turbines) for power generation. The most evident option involves direct combustion for the generation of heat and electricity. Higher revenues and a wider distribution would be achieved if the oil could be used as a substitute to transportation fuels (Czernik and Bridgwater, 2004; Chiaramonti et al., 2007).

Experimental tests have shown that conventional equipment and processes, designed for the use of fossil fuels, are in most cases inappropriate for pyrolysis oils. The extent of these changes depends on the robustness of the technology and the fuel properties of the bio-oil, which may change depending of processing technology and biomass feedstock. For instance, large boilers accept alternative fuels more readily than engines and turbines. Upgrading the oil may be used to reduce the need for extensive modifications in the conventional equipment and to optimize fuel properties. A review of upgrading alternatives (blending with alcohols, emulsions with hydrocarbon fuels, catalytic hydrotreatment, catalytic cracking, solvent fractionation and fractional condensation) is included in Section 5 of this paper. Another alternative involves the gasification and/or steam reforming of the pyrolysis oils in centralized plants, with the aim of producing a gas fuel with applications in the energy and chemical industry.

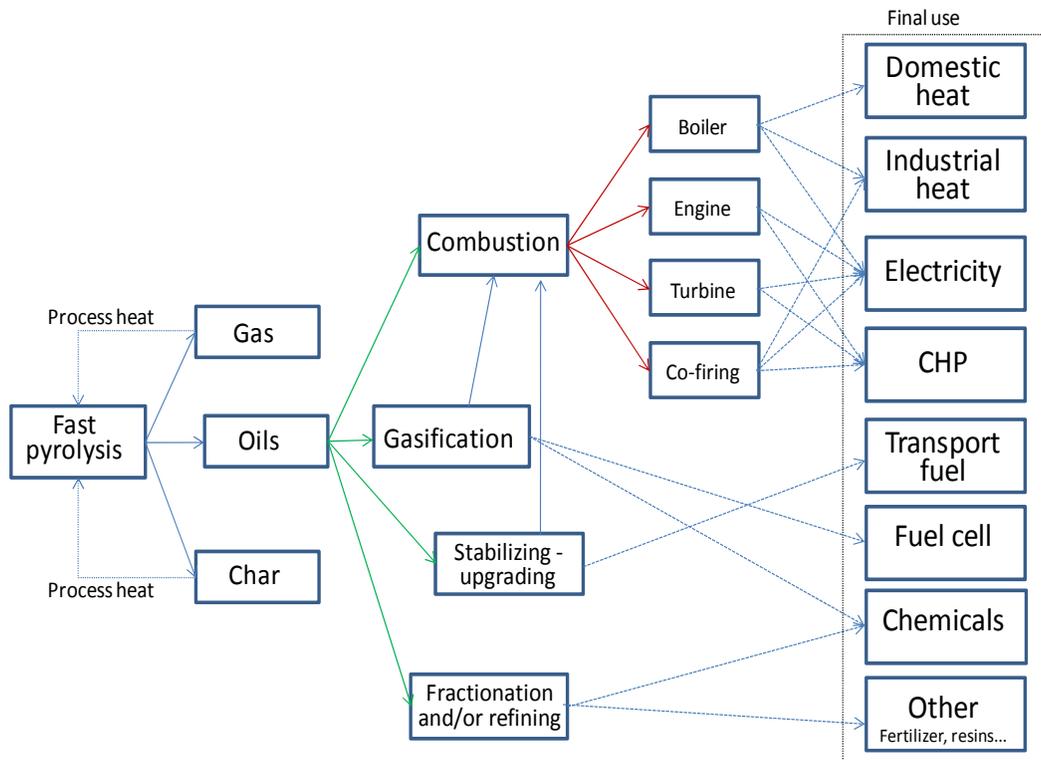


Figure 18. Alternative applications for pyrolysis oils.

Pyrolysis oils can be processed and refined to extract certain fractions or chemicals with commercial applications such as flavoring agents in the food industry, fertilizers and adhesive resins. The use of pyrolysis oil as feedstock in conventional refineries has also been investigated, although the notable differences in the composition and properties compared with crude oil generates problems. The development of bio-refineries, specially designed for the processing of pyrolysis oil, is an area of great commercial and scientific interest. The special characteristics of the oil in terms of corrosive behavior, viscosity and instability need to be taken into consideration in the transportation, handling and storage of biomass derived fast pyrolysis oils.

#### 4.2.1. Pyrolysis Oils for Energy Applications

Pyrolysis oils can be burnt efficiently in boilers, engines and gas turbines. However, these trials have also identified numerous challenges resulting from the unusual chemical and fuel properties of the oils (Czernik and Bridgwater, 2004; Chiaramont et al., 2007). Despite the high expectations created around this novel fuel, the long term use of this fuel in commercial systems has not been demonstrated. Work is ongoing in order to adapt existing technology to the particular properties of the pyrolysis oil in terms of ignition properties, heating value, viscosity, energy content, thermal and chemical stability, and corrosion capacity. In addition, work needs to be directed towards the development of upgrading methods capable of improving the fuel properties of the oils. In addition, improved pyrolysis processes (fractionated condensation, hot gas filtration) and upgrading of the oil (by blending with alcohols, emulsification with fossil fuels and catalytic processing) create new opportunities

that may bring this fuel closer to commercial applications. Various research groups and organizations are also working in the production of fuel specifications for specific applications which will facilitate the development of technology and market penetration (Brammer et al., 2006).

#### *4.2.1.1. Flame Combustion of Pyrolysis Oils for Heat Generation*

Commercial furnaces and boilers for heat generation are designed to accept different types of gas, liquid and solid fuels such as natural gas, petroleum distillates, wood, biomass residues and coal. The main requirements are adequate combustion properties, acceptable emission levels and economic viability. Various papers investigated the flame combustion of pyrolysis bio-oils for the production of heat and power (Oasmaa et al., 2001; Kyto et al., 2002; Czernik and Bridgwater, 2004; Stamatov et al., 2006; Calabria et al., 2007; Chiaramonti et al., 2007; Khodier et al., 2009; Zheng and Kong, 2010; Tzanetakis et al., 2010).

Regarding heat generation, the chemical company Red Arrow (Wisconsin, USA) operates a 5 MWt swirl burner stoked with mixtures of gas, liquid and solid by-products derived from the operation of a fast pyrolysis plant. This company employs fast pyrolysis technology for the production of food additives and generates process energy by combustion of the pyrolysis by-products (Czernik and Bridgwater, 2004). Additional work has been conducted at two Finish companies, Neste Oy (Gust, 1997) and Oilon Oy (Oasmaa et al., 2001), who tested two industrial boilers (2.5 MWt and 8 MWt) on bio-oils supplied by the Dutch company BTG.

Experimental results prove that furnaces and boilers intended to operate on pyrolysis bio-oils need to be adapted to the special characteristics of the fuel. Owing to the low pH of the pyrolysis oil, standard carbon steel and polymers cannot be used in nozzles, pumps, fuel lines, tanks or any other part of the equipment that is in contact with the fuel. This needs to be replaced with stainless steel, Teflon, halogenated polymers and other advanced materials. Fuel/air mass ratios need to be reduced in order to account for the reduced heating value of the bio-oils (Calabria et al., 2007). Owing to its high ignition temperature ( $> 500^{\circ}\text{C}$ ), furnaces and boilers using bio-oil usually need to be started using an auxiliary fuel (natural gas, fuel oil). The bio-oil needs to be pre-heated and the atomization pressure increased in order to counteract the higher viscosity of the fuel. However, this pre-heating needs to be conducted at moderate temperatures and over short periods of time to avoid polymerization and carbonization reactions. A better option may be pre-heating of the atomization air, facilitating bio-oil combustion without affecting its thermal stability. It is recommended that the furnace and boiler is shut down on the auxiliary fuel, in order to prevent deposition and clogging of the thermally unstable oil. The presence of solids in the pyrolysis oil has been reported to be highly detrimental to the combustion equipment, causing blocking and erosion in the nozzles and pumps (Gust, 1997; Oasmaa et al., 2001; Czernik and Bridgwater, 2004).

There is no agreement regarding the emissions in furnaces and boilers using bio-oils due to differences in the characteristics (water content and composition) and conditioning (pre-heating) of the oil, and also to the design and operating conditions of the burner. It appears that  $\text{NO}_x$  levels are primarily determined by the nitrogen content of the fuel. Particle and CO emissions are higher due to the lower fuel properties of the bio-oil (Gust, 1997; Oasmaa et al., 2001; Khodier et al., 2009; Zheng and Kong, 2010; Tzanetakis et al., 2010).

In order to promote the acceptance of pyrolysis oil in the energy market, it is necessary to standardize its fuel quality. This requires development of standard methods of analysis and

determination of quality values for specific applications, as happens with conventional fuels. ASTM is preparing a first initiative on the standard characteristics of pyrolysis oils for burner applications. Table 6 describes the specifications and test methods for these oils. Additional standards for other energy applications (engines and turbines) should follow in the next years (Oasmaa et al., 2005 and 2009).

**Table 6. Expected Parameters For Biomass Pyrolysis Fuels In ASTM Burner Fuel Standard (Oasmaa Et Al., 2009).**

Property	Test method	Specification
Gross heat of combustion (MJ/kg)	D240	> 15
Water content (wt%)	E203	< 30
Solids content (wt%)	Annex A1	< 2.5
Kinematic viscosity at 40°C (mm <sup>2</sup> /s)	D445	< 125
Density at 20°C (kg/dm <sup>3</sup> )	D4052	1.1-1.3
Sulfur content (wt%)	D4294	< 0.05
Ash content (wt%)	D482	< 0.25
pH	E70-07	Report
Flash point (°C)	D93-B	> 45
Pour point (°C)	D97	< -9

#### 4.2.1.2. Pyrolysis Oil for Power Generation

Electricity is a more valuable energy product than heat, owing to its higher commercial value and ease of distribution. Pyrolysis bio-oils can be transformed into electricity using different technologies which differ in their energy efficiency and fuel requirements. These include stationary diesel engines, gas turbines and co-firing in large-scale power stations.

#### Co-firing

Co-firing of pyrolysis bio-oil in large power plants represents the least troublesome alternative for the energy valorization of pyrolysis oils. Large power plants only require minor changes to accept this fuel, and benefit from greater energy efficiencies (owing to the higher overall capacity of the process), reduced investments (compared to dedicated biomass power plants) and reduced administrative troubles due to existing industrial activity (Czernik and Bridgwater, 2004).

The co-firing of pyrolysis oil with coal was demonstrated at the Manitowac power station (Wisconsin, USA) (Czernik and Bridgwater, 2004; Chiaramonti et al., 2007). The pyrolysis oils employed were produced by Red Arrow and contributed to 5% of the thermal input of a 20MWe power block. It was reported that the plant was operated without significant problems after a few modifications in the boiler.

In the year 2002, fifteen tones of pyrolysis oils produced by the Dutch company BTG were fired at a 350 MWe combined cycle natural gas power plant in Harculo (The Netherlands). Tests lasted four day and no adverse effects were observed in terms of emission levels or damage to the equipment. At present, the main obstacle to the use of pyrolysis oils in co-firing involves the availability of the fuel and economics (Wagenaar, et al., 2002; Chiaramonti et al., 2007).

### *Stationary Diesel Engines*

Diesel engines and turbines offer higher efficiency (up to 45%) than Rankine cycles in the generation of electricity and can be adapted for the production of combined heat and power (CHP). Medium and slow speed Diesel engines are particularly robust and can operate on low-grade fuels. Hence, various companies dedicated to the manufacture of this type of equipment, most notably the Finish Wärtsila and the British Ormrod, have investigated their performance on pyrolysis bio-oils (Solantausta et al., 1993 and 1994; Ormrod, and Webster, 2000; Shihadeh and Hochgreb, 2000 and 2002; Czernik and Bridgwater, 2004; Chiaramonti et al., 2007; Murugan et al., 2009). The use of bio-oils in smaller conventional engines is far more troublesome.

Although these tests demonstrate that slow Diesel engines may run on pyrolysis bio-oils, it is also true that this fuel generates important problems which can be associated with its corrosive nature, thermal instability (coke formation), high viscosity, abrasiveness (due to high concentration of solids in suspension), fuel variability (formation of lumps, phase separation and stratification), low heating value and high water content. Regarding the operation of the engine, adjustments need to be made on the injection system to adapt to the lower heating value and higher density of the pyrolysis oils (reduced air intake). Owing to their high ignition temperature, the engine also needs to be started using conventional fuel. Dual operation of the engine using conventional Diesel improves the performance of the engine (Ormrod and Webster, 2000; Chiaramonti et al., 2007). Engine shut off also needs to be conducted on conventional fuel to minimize coke deposition. Owing to the high water content and combustion behavior, the bio-oils exhibit ignition delays which can be partly overcome by pre-heating the fuel (55°C) and the combustion air (50-70°C) (Shihadeh and Hochgreb, 2000 and 2002).

Diesel engines and equipment in contact with the pyrolysis oil suffer from severe corrosion problems. This detrimental effect can be avoided by using advanced construction materials like 300 series stainless steel, silicon carbide coatings and fluorinated polyethylene. Erosion in fuel injectors, exhaust valves and pumps was caused by the abrasive effect of suspended particles. Carbon deposits were formed in the combustion chamber and feeding system due to thermal instability of the bio-oil fuel (Solantausta et al., 1993 and 1994; Shihadeh and Hochgreb, 2000 and 2002; Murugan et al., 2009).

Engine performance is highly dependent on the bio-oil fuel properties. This can be modified in different ways including: hot filtration of pyrolytic vapors to reduce solids in suspension; thermal and catalytic cracking to increase the concentration of volatile products; selection of biomass feedstock; blending with alcohols; and forming emulsions with conventional hydrocarbon fuels and bio-diesel (Czernik and Bridgwater, 2004; Chiaramonti et al., 2007).

### *Turbines*

Experimental work has shown that pyrolysis oil can also be used on gas turbines (Chiaromonti et al., 2007; Bridgwater, 2003; López-Juste and Salvá-Monfort, 2000; Boucher et al., 2000 and 2000B). The Canadian company Orenda Aerospace carried out an extensive program to evaluate the suitability of using bio-oils on conventional turbines and adapt existing designs to the characteristics of this fuel.

As in the case of engines, standard gas turbines were able to operate on this fuel, although notable deficiencies in operation and damage to the equipment were detected. Owing to the high viscosity of the bio-oil, fuel preheating to temperatures around 70-90°C was required in order to improve atomization and overall combustion behavior. This pre-heating was also required to counteract the high ignition temperature and low volatility of the bio-oil. As with engines, this pre-heating had to take into consideration the low thermal stability of the oil. Gas turbines need to be started with hydrocarbon fuels to facilitate the ignition of the pyrolysis oil. Conventional fuel is also required for shutdown in order to remove deposits and avoid clogging. Blending of the bio-oil with alcohols (methanol and ethanol) improved combustion properties and reduced soot formation and emission of unwanted species in the exhaust gases (particulates, NO<sub>x</sub>, CO and hydrocarbons) (López-Juste and Salvá-Monfort, 2000; Boucher et al., 2000).

The combustion chamber and nozzles of the gas turbine need to be adapted to the lower heating value and higher viscosity. Combustion instability due to stratification and phase separation in the bio-oil fuel was also reported to be a problem. Erosion of the engine and other equipment (valves, pumps) caused by solid particles was a major problem which can be readily addressed by using hot filtered pyrolysis oils. Elimination of these solids also favored corrosion and deposition problems derived from the presence of inorganic constituents (mainly K, Na and Ca). Owing to the low pH and corrosive nature of the pyrolysis bio-oils, another key issue relates to the compatibility of materials used in the construction of the engine and other elements that are in contact with the oil. Austenitic 300 series stainless steel and fluorinated high density polyethylene are appropriate for metal and polymeric elements (López-Juste and Salvá-Monfort, 2000; Boucher et al., 2000 and 2000B; Chiaromonti et al., 2007).

#### ***4.2.2. Gasification/Steam Reforming for Syngas Generation***

Pyrolysis oils can be used as feedstock in the production of syngas (usually called producer gas when diluted with nitrogen) via gasification/steam reforming. Reforming usually refers to one of the stages in the gasification process involving the oxidation of the volatile products using steam. Gasification of bio-oils implies economic benefits over conventional gasification of the original biomass owing to its higher energy density and reduced transportation costs (Zwart et al., 2006). The liquid state of the bio-oil also favors the efficiency of the process towards the production of target species (mainly H<sub>2</sub> and CO). The resulting producer gas consists of a mixture of CO, H<sub>2</sub>, CO<sub>2</sub>, CH<sub>4</sub> and other light hydrocarbons, usually diluted with variable amounts of nitrogen. Owing to its calorific value (12-18 MJ/Nm<sup>3</sup>) it may be used directly in turbines or engines. Gasification conducted on pure oxygen or steam results in the formation of an undiluted syngas, which may be used more efficiently in the production of synthetic transportation fuels (via Fischer-Tropsch), chemical synthesis (methanol, ammonia) or separation of purified hydrogen (Knoef, 2005).

Bio-oil gasification/steam reforming is an area of great economic and scientific interest, as demonstrated by the large number of papers published in this field (Wang et al., 1997 and 1998; Czernik et al., 2002; Takanahe et al., 2004; Bimbela et al., 2009; Bona et al., 2008; García et al., 2000). Commercial nickel based catalysts have been found to be highly effective in the steam reforming of oil surrogates like toluene, acetic acid, acetol or butanol. Even higher activities were observed in catalysts based on cobalt-molybdenum and also based on noble metal (Pd, Pt, Ru, Rh) supported on metal oxides (Al, Ce, Zr), although the cost of these materials would be significantly higher (Domine et al., 2008; Wu et al., 2008; Rioche et al., 2005; Basagiannis and Verykios, 2007). The use of multifunctional catalysts (Ni, K, Ca, Mg supported on Al<sub>2</sub>O<sub>3</sub>) that catalyze steam reforming, gasification and water-gas shift reactions, is described in Czernik et al. (2007)

The main problems associated with this technology relate to the thermal instability of the bio-oil and the formation of carbon deposits in the gasification reactor, which affect the transit of gases and vapors, and also the activity of the catalysts. This negative effect may be reduced by separating the aqueous and lignin fractions of the pyrolysis oils (Kechagiopoulos et al., 2006 and 2009; Panigrahi et al., 2003; Gayubo et al. 2009). Fluidized beds (bubbling and spouted bed) are better suited for this type of processes than fixed beds owing to the reduced formation of carbon deposits.

#### **4.2.3. Chemical Feedstock**

The production of refined chemicals from pyrolysis oil has also been investigated in numerous papers. Chemicals have a much higher added value than fuels. As discussed in Section 2 of this paper, pyrolysis oils contain hundreds of chemicals in the form of carboxylic acids, aldehydes, ketones, ethers, esters, sugars and dehydrosugars, and phenolic compounds. These come in a very wide range of molecular sizes, some of which combine different functional groups.

If pyrolysis oils are to be a source of chemicals, effective separation methods must be developed. Numerous papers have been published describing and testing alternative solvent fractionation methods for pyrolysis oils (Garcia-Perez et al., 2007; Oasmaa and Kuoppala, 2003; Sipila et al., 1998; Mohan et al., 2006). The bio-oils can be separated into a lighter aqueous fraction (65-70%) and a higher density viscous organic fraction (25-30%) simply by the addition of water. The former contains most of the products derived from the fractionation of the carbohydrate elements of the biomass (cellulose and hemicelluloses) and the latter concentrates lignin derivatives.

At present, the recovery of pure compounds is not economically attractive owing to the relatively low proportions of individual species and the high diversity of species present in the oil. Adapting pyrolysis conditions of pyrolysis vapors may be used to improve selectivity of compounds of interest. Chemical compounds that could be targeted for extraction and purification from pyrolysis oils include phenolic compounds, sugars (levoglucosan) (Bennett et al., 2009) and certain carboxylic acids (formic and acetic). Catalytic pyrolysis may be used to enhance the yield of certain target compounds like levoglucosan (Dobele et al., 2005).

#### **4.2.4. Other Applications**

Pyrolysis bio-oils are used in the production of commercial flavoring agents. The American company Red Arrow Products uses Ensyn RTP technology to produce smoke flavorings and food additives (Bridgwater, 2003). In addition, the lignin fraction of the bio-

oils has been investigated for its use as a replacement for petroleum-based phenolic resins, which are employed in the production of wood panels. The adhesive capacity of the pyrolysis oil is related to the presence of phenolic compounds that react with carbonyl and carboxyl groups when heated to temperatures around 250-300°C. A review on this technology has been published by Effendi et al. (2008).

The use of pyrolysis oils for the production of fertilizers has also been evaluated. Carboxyl groups present in pyrolysis oils combine with ammonia and urea present in natural fertilizers resulting in the formation of amides. These compounds are less water soluble than the original compounds, allowing for the slow release of nutrients into the soil (Radlein et al. 1997).

## 5. PROCESSING AND UPGRADING OF PYROLYSIS OILS

As discussed above, fuel properties of pyrolysis bio-oils differ significantly from conventional petroleum derived hydrocarbons. This affects their potential use in standard energy conversion devices (furnaces, boilers, engines and turbines). These fuel properties can be improved by selecting adequate biomass feedstocks, by optimizing pyrolysis process design and operating conditions, and also by subjecting the oils to additional processing or upgrading. Regarding upgrading strategies, pyrolysis bio-oils may be blended with alcohols or may be emulsified with conventional fossil fuels in order to improve fuel properties. This is a straightforward option which does not involve high technology or economic costs. Alternatively, condensed oils or volatile pyrolysis vapors may be subjected to catalytic cracking or hydrotreatment in order to modify average molecular weight and reduce oxygen content. This second option is more technologically challenging and requires further investigation.

### 5.1. Mixtures of Pyrolysis Bio-Oils with Conventional Fuels

#### 5.1.1. Formation of Bio-Oil Emulsions with Diesel

Although pyrolysis oils are immiscible with non-polar hydrocarbon fuels, emulsions of conventional Diesel with bio-oils can be stabilized with the addition of commercial surfactants (Chiaramonti et al., 2003; Ikura et al., 2003). The stability of these emulsions is affected by the characteristics of the bio-oil (in terms of biomass feedstock, pyrolysis technology and conditions and aging), type and concentration of surfactant and Diesel proportion. Emulsions containing up to 75% bio-oil have been produced and have been reported to be more stable than the pure bio-oil (Chiaramonti et al., 2003). The viscosity of the bio-oil emulsions was reduced with the addition of increasing proportions of Diesel. High surfactant concentrations favored the stability of the emulsions but also increased viscosity values. Optimum surfactant concentrations were determined to range between 0.5 - 2.0%.

Engine tests of these emulsions were promising, showing improved behavior over the original bio-oil. However, the equipment was still affected by the low pH and presence of suspended solids in the emulsions. This damage was reduced in emulsions containing low bio-oil contents. Diesel fuel containing 5% bio-oil exhibited viscosity and ignition properties

acceptable to be used in conventional engines and turbines (Shihadeh and Hochgreb, 2002; Chiaramonti et al., 2003B). The main weakness of this upgrading strategy relates to the high cost of the commercial surfactants and the high energy consumed in the emulsification process.

Another strategy investigated involves extraction of soluble components of the bio-oil using conventional bio-diesel fuel. The lignin fraction of the bio-oil is more soluble in bio-diesel than the carbohydrate fraction. Up to 3.5 wt% of the bio-oil is transferred into the bio-diesel by simple extraction. Bio-diesel enriched with bio-oil showed improved chemical stability but exhibited lower fuel properties due to higher solid content and acid number (Garcia-Perez et al., 2010).

### **5.1.2. Blending with Alcohols**

Pyrolysis oils are miscible with polar alcohols and experimental work has been conducted to evaluate the fuel properties of bio-oils blended with methanol, ethanol and diethylene glycol dimethyl ether (dyglyme) (Diebold, 2000; Chiaramonti et al., 2007; Boucher et al., 2000). The addition of alcohol improved the fuel properties of the oils in terms of reduced acidity and corrosiveness, improved volatility and ignition properties, higher heating values and lower mass densities. The addition of alcohols also improved the thermal and chemical stability of the bio-oil. Methanol was described as being more beneficial than ethanol in the stability of the bio-oils (Nguyen and Honnery, 2008; Diebold, 2000; Chiaramonti et al., 2007; Boucher et al., 2000).

Alcohols can also react with carbonyl and carboxyl groups present in the bio-oil to produce acetals and ester groups. This reaction could be used to reduce the acidity of the oils and modify its fuel properties. However, the equilibrium of these reactions is unfavorable due to the competition with water and complete neutralization cannot be achieved (Diebold, 2000; Moens et al., 2009).

## **5.2. Chemical Upgrading**

Two types of routes are being explored for the chemical upgrading of pyrolysis bio-oils: catalytic cracking and catalytic hydrotreatment. These two processes are widely used for the transformation of petroleum fractions in conventional refineries. The challenge involves adapting these technologies to the particular characteristics of the pyrolysis bio-oils. Despite the enormous potential, these technologies are in the development stage and still suffer from notable drawbacks in terms of reduced product yields and catalyst deactivation.

### **5.2.1. Catalytic Cracking**

Catalytic cracking is performed in conventional petroleum refineries to convert heavy hydrocarbon fractions into lighter products, primarily liquid C<sub>5</sub>-C<sub>12</sub> gasolines and olefinic gases. The process takes place in fluidized bed reactors at temperatures around 500°C and low pressure (1.5-2.0 bar) in the presence of acid catalysts (most commonly synthetic zeolites). Numerous authors have investigated the catalytic cracking of pyrolysis oils with the aim of improving fuel properties (Adjaye and Bakhshi, 1995 and 1995 B; Olazar et al., 2000; Gayubo et al., 2004; Adam et al., 2005 and 2006; Aho et al., 2008, 2008 B and 2009;

Antonakou et al., 2006; Williams and Nugranad, 2000; Vitilo et al., 1999; Sharma and Bakhshi, 1991).

Catalytic cracking can be performed either directly on the hot volatile products or by re-vaporization of already condensed bio-oil. The former, which benefits from improved energy efficiencies, may be achieved by incorporating acid (zeolites) or alkaline (limestone, olivine, dolomite) catalysts into the bed of the fluidizing reactor. The organic vapors formed as a result of the pyrolysis process are in contact with the catalysts, which promote cracking reactions and the formation of smaller molecular weight species (Aho et al., 2008 and 2008; Corella et al., 2004; Zhang et al., 2009). A second alternative, which allows for more flexibility in the design and operation of the catalytic process, involves upgrading the hot pyrolysis vapors in a secondary catalytic reactor separated from the main pyrolysis process (Olazar et al., 2000; Gayubo et al., 2004). Catalytic cracking is also affected in the case of biomass feedstocks containing high ash contents. The presence of alkaline or alkaline-hearth minerals (primarily K, Na and Ca) has been proven to promote catalytic cracking of the pyrolysis products (Livingston, 2007; Fahmi et al., 2008). Impregnation of the biomass feedstock with these inorganic elements has also been proposed and tested as a way of promoting catalytic cracking (Bru et al., 2007).

Another option involves the catalytic upgrading of previously condensed pyrolysis oils. The oils could be produced in distributed pyrolysis units and transported into a centralized catalytic plant for upgrading. This process is energetically less efficient and has to overcome the problems associated with the thermal volatilization of the pyrolysis oils.

The aim of catalytic cracking is to reduce the average molecular weight of the volatile products, hence improving the volatility of the pyrolysis oils and increasing gas yields at the expense of heavier condensable liquids. Cracking processes also promote the formation of char as a by-product. The extent of these transformations depends on the severity of the cracking process, which is dictated primarily by the temperature (usually 400-600°C) of the cracking process, residence time of the vapors and catalytic activity of the solids. Synthetic zeolites like the ones employed in conventional FCC cracking of petroleum fractions (HZSM-5 zeolite, HY-zeolite, Beta zeolite) exhibit strong catalytic activity in the cracking of pyrolysis bio-oils (Vitilo et al., 1999; Gayubo et al., 2004; Aho et al., 2008, 2008 B and 2009). Despite exhibiting comparatively lower acid properties, mesostructured aluminosilicates (Al-MCM-41, Al-SBA-15) have also been effective in the cracking of biomass pyrolysis oils (Iliopoulou et al., 2007; Twaiq et al., 2003; Adam et al., 2005). Alkaline minerals (dolomite, olivine, hydrotalcite, limestone, zinc oxide) also exhibit catalytic activity in the cracking of biomass tars and oils (Nokkosmaki et al., 2000; Hernandez et al., 2010; Corella et al., 2004). Certain cracking catalysts also promote the formation of aromatic products and deoxygenation of the pyrolysis products. This oxygen is released in the form of water, CO<sub>2</sub> and CO.

A key drawback to this technology is related to the formation of coke deposits (8–25 wt%) in the cracking reactors, which affects the overall functioning of the process and the performance of the catalysts. The use of centralized catalytic cracking facilities would benefit from an economy of scale, but would face problems associated with the re-volatilization of the oil.

### **5.2.2. Catalytic Hydrotreating**

Hydrotreating is performed in conventional refineries to remove impurities (mainly sulfur and oxygen) from petroleum products. The process involves the reaction of the hydrocarbon feedstock with hydrogen or a hydrogen donating solvent at high temperatures (300-400°C) and pressures (150-300 bar) in the presence of catalysts (Co/Mo or Ni/Mo supported on alumina). A similar strategy has been proposed for upgrading pyrolysis oil (Elliott et al., 1991; Elliott, 2007; Elliott and Neuenschwander, 1996; Pindoria et al., 1998; Zhang et al., 2005; Fisk et al., 2010; Medrano et al., 2010). Hydrotreatment under severe reaction conditions (300-350°C and 200 bars) has been reported to be successful in the removal of oxygen (up to 90 wt%) from pyrolysis oil, which is eliminated in the form of CO<sub>2</sub> and water. The upgraded oil exhibited reduced acidity, was soluble in hydrocarbon fuels and had a caloric value comparable to petroleum derived fuels (up to 40 MJ/kg). However, efficiency of the process was low, with oil yields typically below 50-55 wt% and gas yields in excess of 15 wt%. The process also generated large amounts of char (above 25 wt% yield), which caused operational problems (Wildschut et al., 2009 and 2009 B).

Hydrotreatment may also be conducted under milder reaction conditions (< 280°C and 2-3 bars) in order to reduce the formation of char (1-10 wt%) and gases (3-6 wt%), and increase oil yields. In this case, only less stable oxygen groups (carbonyl and carboxyl) are eliminated. Owing to their lower oxygen content, the lignin fraction of pyrolysis bio-oils may be a better candidate for hydrotreatment than the whole oil (Holmgren et al., 2006). As in catalytic cracking, the inactivation of catalysts and the clogging of the system due to excessive formation of char need to be resolved.

### **5.2.3. Co-Processing in Conventional Refineries**

The use of bio oil in conventional refineries is a promising alternative, although this alternative has several drawbacks due to the high acidity of the feedstock, high moisture content, and the presence of oxygenated species and metals. In Europe, the Biocoup research program (Biocoup, 2010) is studying the feasibility of co-processing 2-10 wt% upgraded bio oils in FCC and hydrotreating or hydrocracking units.

Partially upgraded bio-oil should have a smoother entry in existing refineries. Co-processing low severity upgraded hydrodeoxygenated bio oils (HDO) and Vacuum Gas Oil in FCC units with standard catalyst results in a similar gasoline yield, with higher coke and dry gas yield, compared to the FCC feed alone (Biocoup executive summary, 2010). On the other hand, co-processing with a straight run gas oil in a HDS (hydrodesulphurization) unit decreases the desulphurization rate (Biocoup executive summary, 2010). All studies have been developed in lab scale laboratories and sugar polymerization problems have been published related to the HDO process (Solantausta, 2006).

### **5.2.4. Biorefineries and Biomass Pyrolysis Oils**

Biorefineries are facilities that integrate a range of conversion processes (both biological and thermochemical) to produce refined fuels, chemicals, electricity and heat from biomass (Briens et al., 2008; Demirbas, 2009; Cherubini, 2010). They will change the current biomass use models and will make it possible to arrive at new integration methods of fuel and chemical production, waste management and consumer demands. According to most visions, biomass which is typically in a low density form will be transported to big central processing

plants. Thus, pretreatments for energy densification at the local level, such as fast pyrolysis, torrefaction, or pelletization can be useful strategies. Bio-oil is a high density liquid that has two key advantages, it is pumpable and storable. The “bio-crude” would then be refined to biofuels and some of its fractions could be converted to chemicals, analogous to today’s petroleum refinery.

Recently fast pyrolysis, in addition to being considered a pretreatment technology, has gained interest within the biorefinery itself. This concept involves biomass fractionation into its constituent components (cellulose, hemicellulose and lignin) followed by a (catalytic) fast pyrolysis. The biochemical process always leaves 15 to 30 percent of the input biomass mass as unconverted lignin, therefore, its valorization to phenolics, resins and/or adhesives by fast pyrolysis could be integrated in the biorefinery (Nowakowski et al., 2010).

## **6. COMMERCIAL FAST PYROLYSIS OF BIOMASS**

### **6.1. Historical Evolution of Biomass Fast Pyrolysis**

Biomass is a renewable energy source available all around the world, but transport cost to the conversion plant makes its use complicated. To enhance the implementation of biomass to energy, the fast pyrolysis process is one of the most promising technologies. It can overcome the biomass disadvantages with its conversion from solid to liquid, increasing the energy density and facilitating the transport and storage.

The first steps of the fast pyrolysis processes were carried in the 1980s, with slow pyrolysis for liquid production. Some companies such as Alten, Bio-alternative, Cres and BBC work in these fields, but most of the plants are being abandoned. On the other hand, in Brazil, Biocarbo (Biocarbo web site) is a good example of slow pyrolysis bio oil exploitation. The company is the pioneer in the use of water diluted wood pyrolytic acids such as agricultural bio stimulant, a product called Biopirol, and heavy tar as a boiler fuel. The first fast pyrolysis commercially successful plant in the world was built in 1989 in the USA with RTP™ technology (Graham, 1991). The plant was used for the production of boiler fuel and high value chemicals, such as flavorings and browning agents, with a capacity of around 100 kg/h hardwood feedstocks. Operating conditions were around 450-600°C reaction temperature for 0.6-1.1 s residence time. The oil product yield reaches a 73% (Graham, 1995).

Since 1989, commercial plants with a capacity up to 70 Tn/day were built basically in North America, while only demonstration plants were constructed in Europe (Cueva, 1995; Prins, 1997; Klass, 1998; Boukis, 2001; Wagenaar, 2001; Bridgwater, 2002; Bridgwater, 2003; Venderbosch, 2010). Nevertheless in recent years, more commercial plants are under design and construction. Although most of the larger pyrolysis commercial plants are currently located in North America, several large commercial plants are also under study, design or construction in Europe. The fast pyrolysis technology has been growing in recent years, increasing its importance in bio-oil generation. Table 7 contains a review of the characteristics of some of the most representative fast pyrolysis plants constructed and operated since the 1980’s, with information such as year of construction, capacity, location, type of technology, operating conditions, operating company and current status.

**Table 7. Review of main characteristics of pilot scale, demonstration and commercial scale biomass fast pyrolysis plants since 1983**

Plant	Year built	Capacity	Location	Owned by	Technology	Designed	Operating conditions	End-use	Design modifications	Status
GTRI	1983	57 kg/h	USA	GTRI	Rotating tube	GTRI	T <sup>a</sup> reactor 500°C, 1.5 mm particle size up to 60% bio-oil optimisation (HHV 24.3 MJ/kg, 1.23 kg/m <sup>3</sup> , 0.2% char content) and 21% char	Bio-oil	1985, replacement reactor tube and addition of quench vessel and second demister	In operation until 1989
Alten	1985	500 kg/h (designed for Italy 1000kg/h)	Avezzano,	Alten	Stirred /Fluid bed	Alten	T <sup>a</sup> reactor 500°C, 1 atm, slow pyrolysis, up to 20% bio-oil (HHV1 29.7 MJ/kg, high charcoal content 15% in oil) and 20% char,	Bio-oil to fire a 27MWe power station. Convert wood and agricultural wastes into marketable fuel products.		Dismantled, 1985-1990
Bio-Alternative	1900's	50kg/h. 2Tn/h	Switzerland	Bio-Alternative	Fixed Bed	Bio-Alternative	T <sup>a</sup> reactor 475°C, up to 72% bio-oil (20% water content)	Charcoal production, Oil as by product		Abandoned 1993
NREL	1990	50 kg/h (achieved 36 kg/h)	USA	NREL	Ablative Vortex	NREL	T <sup>a</sup> wall 650°C, 5mm particle size, 400 m/s tangentially speed, up to 67% bio-oil (HHV1 18-23MJ/kg, 25% water content, 2-3 pH)	R&D plant, to produce liquid fuels.	1995 Reactor vertically installed and hot filter added	Abandoned 1997
CRES	1990	10 kg (maf)/h	Greece	CRES	Circulating Fluidised bed	CRES	T <sup>a</sup> reactor 550°C, vapours residence time 450 s, Heat carrier sand, up to 70% bio-oil (LHV 16.7 MJ/kg)	Demostration plant	1998	New commissioning needed due to move to a new lab Shut down
BBC	1990's	10-25 kg/h	Canada	BBC	Ablative reactor	BBC	T <sup>a</sup> reactor 470-540°C, 6mm particle size (1-3 mm), vapours residence time 0.88 s, tyres disposal up to 54% biooil (HHV 26.3 MJ/kg, 14% water content, 9.2% char content)	Demonstration plant, method for		
Castle Capital	1990's	50 Tn/d	Halifax, Canada	Castle Capital	Ablative reactor	BBC	T <sup>a</sup> reactor 500°C, 34.5-55.15 kPa. If delivered to a gas turbine 500-700°C at 2000kPa	Solid waste treatment (Steam boilers). Convert into high value fuels and chemicals		Dismantled

**Table 7. (Continued)**

Plant	Year built	Capacity	Location	Owned by	Technology	Designed	Operating conditions	End-use	Design modifications	Status
Interchem	1990	32.7 Tn/d (only 180 kg/h achieved)	Springfield, USA	Interchem	Ablative Vortex	NREL	Reactor heated by pyrolysis non-condensable gases.	Production fuel-oil and charcoal.	Tested until 1992. Redesigned and relocated to Kansas (New reactor), but never completed	Project abandoned in 1993
RTP <sup>TM</sup>	1991	100 kg/h	USA		Transported bed	Ensyn	400-600°C; 0.6-1.1s; 73 wt% oil	Demonstration plant: boiler fuel and high value chemicals		In operation*
Egemin	1991	200 kg/h	Belgium	Egemin	DownFlow	Egemin	T <sup>a</sup> reactor 490°C, 0.6 s residence time, 1-5 mm particle size, up to 40% biooil (high char content in oil)	Demonstration plant. Bio-oil production from wood.		Abandoned in 1993, Research EEC Joule Programme finished. Problems to get sufficiently high rate of heat transfer.
Manitowoc RTP <sup>TM</sup> -1	1993	30 Tn/d	Manitowoc, WI, USA	Red Arrow	Transported bed	Ensyn	3-6 mm particle size, up to 70% biooil (HHV 20 MJ/kg) 15% Char	Commercial plant		In operation*
Union Fenosa	1993	200 kg/h (achieved 160 kg/h)	Meirama, Spain	Union Fenosa	Fluid bed	Waterloo WFPP (1989)	Up to 70% biooil (15-25% water content) and 20% char.	Demonstration, samples for testing in combustion and engines	1996-1998 Improvements on Feeding system and liquid collection	Dismantled
TWT	1993	50 kg/h	Canada	TWT	Stirred/Fluid Bed	TWT	T <sup>a</sup> reactor 350-400°C, self contained in a trailer	Telegraph poles, chemicals recovery. Chicken Farm	1986 200kg/h for wood/peat pyrolysis	Closed down in 1996
ABRI_1	1990's	200 kg/h	Alabama, USA	ABRI		ABRI	T <sup>a</sup> reactor 400°C 3-6 mm particle size, up to 60% biooil (HHV 20 MJ/kg) 15% Char			In operation* 15Tn/d and 50Tn/ plants planned (information website)
Rhineland RTP <sup>TM</sup> -1	1995	35 Tn/d	Rhineland, WI, USA	Red Arrow	Transported bed	Ensyn	3-6 mm particle size, up to 70% biooil (HHV 20 MJ/kg) 15% Char	Commercial plant		In operation*

**Table 7. (Continued)**

Plant	Year built	Capacity	Location	Owned by	Technology	Designed	Operating conditions	End-use	Design modifications	Status
Rhineland Chemical #2	1995	2 Tn/d	Rhineland, WI, USA	Red Arrow	Transported bed	Ensyn	3-6 mm particle size, up to 70% biooil (HHV 20 MJ/kg)	Commercial plant		In operation*
BFH/IWC	1997	5 kg/h	Hamburg, Germany	BFH/IWC	Fluid Bed	Univ. Waterloo Univ. Hamburg	T <sup>a</sup> reactor 400-525°C, 0.3-0.5 m particle size, fluidization 5-10 m <sup>3</sup> /h, up to 75% biooil (HHV 19 MJ/kg, 18% water content)	Demonstration plant for contaminated wood wastes	Prior in 1993 100-200 g/h unit	In operation*
Biotherm <sup>TM</sup>	1997	80 kg/h	Canada	Dynamotive	Fluid bed	RTI assistance	T <sup>a</sup> reactor 500-600°C, 0.37-0.45 s residence time, 2 mm particle size, fluidization non-condensable gases, up to 61% biooil	Demonstration plant', Biomass Refinery Products. High value chemicals and Bio-Lime <sup>TM</sup>	Prior 1991 BC Research Vancouver	In operation*
BTG	1998	50 kg/h	Netherlands	BTG	Rotation cone	Twente University	biooil (HHV 20.9-22.2 MJ/kg, 14% water content, 2.7 pH, 1.216 kg/m <sup>3</sup> )	Demonstration plant. Further scale to 250kg/h in 2001	In 1989 10 kg/h prototype in Univ. Twente (1994 fully heat integrated)	In operation*
ENEL	1998	15Tn/d	Bastardo, Italy	ENEL, VTT and Fortum	Transported bed	Ensyn, RTP <sup>TM</sup>	3-6 mm particle size, up to 70% biooil (HHV 20 MJ/kg)	Demonstration plant	Improvements second cyclone and bio-oil recovery	In operation*
PYROVAC-1	1999	3.5 Tn/h	Saguenay, Quebec, Canada	Pyrode Inc.	Vacuum pyrolysis. Moving Stirred reactor	Pyrovac	475°C, 20 kPa absolute; biomass 10% moisture, 0.5-40 US mesh sieve. Yields (% w/w): 29% Oils 26% Biochar 27% Aqueous phase 18% Gas	1. Commercial production of pyrolytic lignins to partially replace phenols in PF resins. 2. Solid and liquid biofuel industrial demonstration plant.	Reactor proved to be operational after design modification performed in 2001	Plant put in moth-balls in 2003 due to change in asset ownership. It is planned to restart operation during 2011.

**Table 7. (Continued)**

Plant	Year built	Capacity	Location	Owned by	Technology	Designed	Operating conditions	End-use	Design modifications	Status
Wellman	2000's	250kg/h	Oldbury, England	WELLMAN	Fluid bed	Consortium Aston Univ, Inst. Chemistry Hamburg, Biomass Tech. Group, Kara Energy Systems, Omrod Diesel, Wellman	1-2 mm particle size, up to 75% biooil (HHV MJ/kg, water content) 15% Char, Heat provided with cahar and gases combustion	Demonstration plant, CHP (Modified Diesel engine)		Abandoned*
Rhinelander RTP™-2	2001-2002	45 Tn/d	Rhinelander, WI, USA	Red Arrow	Transported bed	Ensyn	3-6 mm particle size, up to 70% biooil (HHV 20 MJ/kg) 15% Char	Commercial plant		In operation*
Dinamotive-Vancouver	2001	15Tn/d	Vancouver, Canada		Fluid bed	Dynamotive	T <sup>n</sup> reactor 450-500°C, 1-2 mm particle size, up to 72% biooil (HHV 16-19 MJ/kg, 20-25% water content, 2-3pH, 1.2 kg/m <sup>3</sup> )	Commercial plant		In operation*
Pytec, Hamburg	2002	10 kg/h	Hamburg, Germany	PYTEC	Ablative pyrolyzer	PYTEC-TEC	60 wt% oil (16-18 MJ/kg, 20-35% water content, 2-3 pH) No heat carrier	Research work R&D		In operation
Rhinelander Chemical #3	2003	1 Tn/d	Rhinelander, WI, USA	Red Arrow	Transported bed	Ensyn	3-6 mm particle size, up to 70% biooil (HHV 20 MJ/kg) 15% Char	Commercial plant		In operation*
Lurgi- KIT (FZK)	2004	10 kg/h	Karlsruhe, Germany	KIT	Twin screw	KIT-Lurgi		Process Demonstration Unit		
Bio Oil Holding-1	2004	1.5 Tn/h (36 Tn/d)	Tessenderlo, Belgium	Bio Oil Exploitation	Auger	Bio Oil Holding	400 – 650°C 1 – 5 s residence time	Pilot plant	First 100kg/h pilot plant tested	Operational

**Table 7. (Continued)**

Plant	Year built	Capacity	Location	Owned by	Technology	Designed	Operating conditions	End-use	Design modifications	Status
Bioware-unicamp	2004	300kg/h	Piracicaba, Brazil	unicamp	Bubbling Fluidized Bed	unicamp	T <sup>a</sup> reactor 480-500°C, 10.4 fluidizing gas/feedstock mass ratio, up to 40% biooil (HHV 31.4MJ/kg, % water content, 2.2pH, 1.14 kg/m <sup>3</sup> ), % char	Demonstration plant		In operation* 500kg/h and 1000kg/h projected in bibliography. No data available about them
Petroleum Demo#1	2005	300 BDP	Bakersfield, CA, USA	Ivanhoe Energy	Transported bed	Ensyn	3-6 mm particle size, up to 70% biooil (HHV 20 MJ/kg) 15% Char	Commercial plant		In operation*
BTG Malaysia	2005	50Tn/d	Malaysia		Rotating cone	BTG	70% Bio-oil (16-18 MJ/kg, 25% water content, 2.5-3.8 pH, 1.15-1.25 kg/m <sup>3</sup> )	Plant.		In operation*
Dynamotive-West Lorne	2005	130 Tn/d	West Lorne, Canada		Fluid bed	Dynamotive	T <sup>a</sup> reactor 450-500°C, 1-2 mm particle size, up to 72% biooil (HHV 16-19 MJ/kg, 20-25% water content, 2-3pH, 1.2 kg/m <sup>3</sup> )	Commercial plant. Main purpose oil for combustion in Orenda's GT 2500 gas turbine to produce electricity.		In operation* (Did not reach designed production capacity)
PYTEC Bulkau	2005	6 Tn/d	Bulkau, Germany	PYTEC	Ablative pyrolyzer	PYTEC-TEC BtO-Process	60 wt% oil (16-18 MJ/kg, 20-35% water content, 2-3 pH) No heat carrier	CHP 300kWh Diesel	Pilot plant	In operation
Dynamotive-Guelph	2006	200 Tn/d	Guelph, Canada		Fluid bed	Dynamotive	T <sup>a</sup> reactor 450-500°C, 1-2 mm particle size, up to 72% biooil (HHV 16-19 MJ/kg, 20-25% water content, 2-3pH, 1.2 kg/m <sup>3</sup> )	Commercial plant		In operation*
Agritherm mobile plant	2006	200 kg/h	Western Ontario, Canada	Agritherm	Bubbling fluid bed	Agritherm, Western Ontario Univ.		Demonstration plant		In operation*
Renfrew RTP <sup>TM</sup> -1	2007	100 Tn/d	Renfrew, Canada	Ensyn	Transported bed	Ensyn	3-6 mm particle size, up to 70% biooil (HHV 20 MJ/kg) 15% Char	Commercial plant		In operation*
Ikerlan IK4 Miñano	2008	25 kg/h	Miñano, Spain	Ikerlan IK4	Spouted bed	Ikerlan IK4	450-600°C, <1s; Sand heat carrier	Research work R&D	2009, 2010	In operation, R&D

**Table 7. (Continued)**

Plant	Year built	Capacity	Location	Owned by	Technology	Designed	Operating conditions	End-use	Design modifications	Status
RTI	2009	7 kg/h	Waterloo, Canada.	RTI	Fluid bed	RTI	T <sup>a</sup> reactor 425-575°C, 1 s residence time, 2 mm particle size, up to 75% bio-oil (HHV 16-19 MJ/kg, 18-23% water content, 2-3 pH, 1.2 kg/m <sup>3</sup> )	Commercial plant for chemicals production		Operational on daily basis
Tampere	2009		Tampere, Finland	Consortium Metso-UPM-Fortum-VTT				2 MW fuel fast pyrolysis unit integrated with 4 MWth circulating fluidized bed boiler		In operation
Pytec Oberhausen	2010	10 kg/h	Oberhausen, Germany	Fraunhofer Umsicht	Ablative pyrolyzer	PYTEC-TEC	60 wt% oil, 17 MJ/kg carrier	No heat	Research work R&D	Under construction
Pytec Mallis	2010	48 Tn/d	Brahlstorf, Germany	PYTEC	Ablative pyrolyzer	PYTEC-TEC	60 wt% oil, 17 MJ/kg carrier	No heat	CHP 8x300kWh	Commercial plant Under construction
Bio Oil Holding-2010 Tessenderlo		2 x 5 Tn/h (120 Tn/d)	Tessenderlo, Belgium	Bio Oil Exploitation	Auger	Bio Oil Holding	400 – 650°C		Commercial plant	Under Construction
Bio Oil Holding-2010 Delfzijl		5 Tn/h (120 Tn/d)	Delfzijl, Netherlands	Bio Oil Exploitation	Auger	Bio Oil Holding	400 – 650°C		Commercial plant	Planning
Empyro BV	2010	120 Tn/d	Hengelo, Netherlands	BTG	Rotating cone	BTG			Commercial plant, CHP	Planning

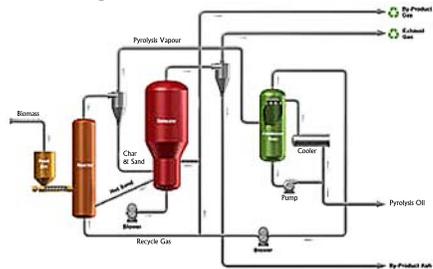
## 6.2. Commercial Key Players in Biomass Fast Pyrolysis

In order to have an understanding of the current situation with fast pyrolysis of biomass, this section provides brief information about the most representative technologies and demonstration plants currently operating in the world.

### 6.2.1. *Envergent Technologies*

Envergent Technologies (Envergent web site, 2010) is a joint venture between UOP and Ensyn created in 2008. The objective of this company was to design and built fast pyrolysis plants, providing pyrolysis oil technology for fuel oil substitution and electricity generation, and to develop technology for upgrading pyrolysis oil to transportation fuels in a near future. ENSYN has more than 20 years experience in the construction and operation of commercial fast pyrolysis plants. This company patented in the 1990's the RTP™ pyrolysis process, which has been commercially proven in 7 plants.

Envergent Technologies	RTP™	Source: Envergent Non-Confidential Presentation, 2009
N° commercial plants	7 (6 in USA, 1 in Canada)	Process flowsheet of RTP™, Envergent Technologies
Reactor technology	Circulating transported fluidized bed reactor	
Reactor temperature	510°C	
Reaction time	< 2s	
Heat Carrier	Sand	
Gasifying agent	Pyrolysis gases	
Biomass feeding rate	Up to 100 Tn/day (commercial) Up to 1.000 Tn/day (availability)	
Biomass size	3-6 mm	
Biomass max. humidity	5-6 wt%	
Yields: Oil - Char - Gases	>70% - 15% - 15%	
HHV: Oil - Char	19.9 MJ/l	



Envergent Technologies  
25 East Algonquin Road  
Des Plaines, IL 60017-5017, U.S.A.

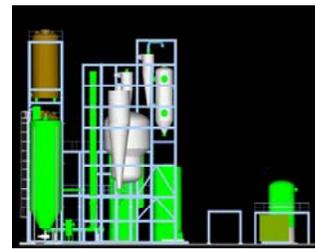


Figure 19. Technical characteristics of the Envergent Technologies biomass fast pyrolysis plant.

This RTP technology is a fast thermal process in which biomass is rapidly heated to approximately 510°C in the absence of oxygen in less than 2 seconds. A circulating transported fluidized bed reactor system similar to the one used in the UOP Fluid Catalytic Cracking (FCC) technology, is at the heart of the process. Inside the reactor hot sand vaporizes the biomass, and the gases are then rapidly quenched, typically yielding 65wt% to

75wt% pyrolysis oil. The char produced is recovered with the sand and burnt to heat the sand again, prior to being introduced to the inside of the reactor. This pourable liquid can then be used as fuel for industrial heat or electrical generation, or it can eventually be further upgraded to produce transportation fuels. The main characteristics of this technology are shown in Figure 19

Envergent offers standard sized modular units, from 100, 200, 400 and 1.000 BDMTPD (Bone Dry Metric Tons Per Day) and considers pyrolysis oil for energy applications in at least 3 different ways (Envergent, 2009):

- Heat generation, replacing fossil fuels, with a reduction in 70-90% of GHG and with a 40% lower cost compared to # 2 Fuel-Oil.
- Green electricity production, with an estimation of 0.10 \$USA/kWh cost in production including RTP operating.
- Transportation fuels, expected to be commercially available in 2012.

### **6.2.2. BTG**

BTG company (BTG world website, 2010) was established in 1970 at Twente University (The Netherlands) with the aim of commercializing the rotating cone reactor technology for fast pyrolysis of biomass. Up to now they have designed and constructed several plants including a 5 kg/h laboratory unit in 1995, a 200 kg/h pilot plant in 1998 and the most recent 48 Tn/day plant in Malaysia in 2005. They are currently constructing a larger 120 Tn/day installation in Netherland.

BTG-BTL is the daughter company commercializing pyrolysis technology (BTG-BTL web site, 2010). BTG-BTL's fast pyrolysis technology was originally based on the rotating cone reactor (RCR). Biomass particles at room temperature and hot sand particles are introduced near the bottom of the cone, where the solids are mixed and transported upwards by the rotating action of the cone. The reactor temperature is around 450 to 600°C and the sand is heated in a combustor with the char produced in the process. It is characterised by an intense mixing without the need of an inert carrier gas, so a minimum down stream equipment size is required. The main characteristics of this technology are shown in Figure 20.

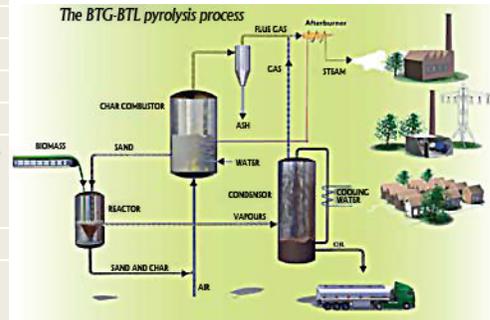
BTG is in close collaboration with other companies, such as Opra Turbines (Opra Turbines web site, 2010), for the development of turbines and engines suited for pyrolysis bio-oil. An economical overview of a commercial plant estimates a bio-oil cost production around 75 to 300 €/Tn (4 to 18 €/GJ), considering a biomass cost from 0 to 100 €/Tn. For example, for its 48 Tn/day installation, BTG assumes a production cost about 85 €/Tn (approx 5 €/GJ), with a biomass cost of 25 €/T (Venderbosch and Prins, 2010).

### **6.2.3. Dynamotive**

The Dynamotive company (Pyne Newsletter 4, 1997; Dynamotive web site, 2010), was founded in 1990 to develop and commercialize alternative energy technologies. In 1996, the Company licensed the Fast Pyrolysis process from Resource Transforms International Ltd. (RTI), a partner company located in Waterloo, Ontario. Up to now they have designed and constructed several plants at Canada: a 15 Tn/d at BC Research Facility in Vancouver in 2001, a 130Tn/d pilot plant in West Lorne in 2005 to generate electricity and a 200 Tn/day plant in Guelph in 2007.

BTG	
N° commercial plants	1 (Malaysia 2005; Netherlands expected in 2011)
Reactor technology	Rotating cone reactor
Reactor temperature	450-600°C
Heat Carrier	Sand
Gasifying agent	No need
Biomass feeding rate	Up to 48 Tn/day (commercial) Up to 120 Tn/day (expected 2011)
Biomass size	<6 mm
Biomass max. humidity	< 10 wt%
Yields: Oil - Char - Gases	>75% - % - %
HHV: Oil	16-20 MJ/kg
Water content: Oil	15-30 wt%
pH Oil	2.8-4.0
Density (kg/l)	1.1-1.25
Oil: %C-%H-%N-%S-%O	55 to 64 - 5 to 8 - 0.05 to 1 - 0.05 - balance

Source: BTG-BTL  
BTG-BTL pyrolysis process



BTG Bioliquids in HQ of BTG Biomass Technology Group  
Josink Esweg 34,  
7545 PN, Enschede  
The Netherlands



1-5 kg/h BTG-BTL process (laboratory)



250 kg/h (pilot plant)



Malaysia, de 2Tn/h (commercial plant)



Hengelo plant scheme for BTG-BTL process

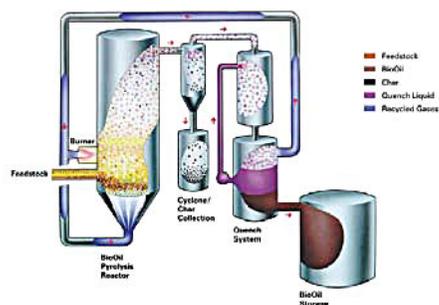
Figure 20. Technical characteristics of the BTG biomass fast pyrolysis plant.

Prepared biomass feedstock (<10% moisture and 1-2 mm particle size) is fed into the bubbling fluid-bed reactor, which is heated to 450–500°C in the absence of oxygen. The biomass is vaporized and the resulting gases pass into a cyclone where solid particles and char are extracted. The gases enter a quench tower where they are quickly cooled using BioOil already made in the process. The BioOil condenses and falls into the product tank,

while non-condensable gases are returned to the reactor to maintain process heating. The entire reaction from injection to quenching takes only two seconds. The main characteristics of this technology are shown in Figure 21.

DYNAMOTIVE	
N° commercial plants	3 (Vancouver, West Lorne, Guelph, Canada)
Reactor technology	Fluid bed
Reactor temperature	450-500°C
Reaction time	<2 s
Gasifying agent	Non-condensable gases
Biomass feeding rate	Up to 15 Tn/day , 130 Tn/day y 200 Tn/day
Biomass size	1-2 mm
Biomass max. humidity	<10%
Yields: Oil - Char - Gases	60-75% - 10-20% - 10-20%
HHV: Oil	16-19MJ/kg
Water content:Oil	20 wt%
pH Oil	2.2
Density (kg/l)	1.207
Kinematic viscosity	47.18 mm <sup>2</sup> /s at 20°C (ASTM D445)
Solids content	0.06 wt%
Ash content	0.0034 wt%
Oil:%C-%H-%N-%S-%O	to 47 - 6 to 8 - <0.1 - <0.02 - balance

Source: Dynamotive website



Canadian Offices  
Suite 140, 13091 Vanier Place,  
Richmond BC, V6V 2J1  
Canada

Figure 21. Technical characteristics of the Dynamotive biomass fast pyrolysis plant.

#### 6.2.4. Bio-Oil Holding NV

Bio Oil Holding, a privately owned company, was founded in 1999, where it commenced with its own R&D on the thermochemical conversion technology, flash pyrolysis. Bio Oil Exploitation, located in Tessenderlo, Belgium, is the main subsidiary of Bio Oil Holding. Bio Oil Exploitation started with a 100 kg/h test installation by which it converted different (organic) waste streams such as pig manure, paper sludge, sunflower husk, etc. Based on these test runs a 1.5 ton/h pilot installation, which is still operated today, has been constructed. In 2009 Bio Oil Exploitation started with the construction of two industrial 5 ton/h pyrolysis installations. Their completion is expected at the end of 2010.

Bio Olie Nederland, another subsidiary of Bio Oil Holding, is planning to install four 5 Tn/h pyrolysis installations at the Energy Valley in Delfzijl – Groningen – The Netherlands. Bio Olie Nederland is in the final stage of obtaining its environmental permit, whereafter it will immediately start its construction. A new subsidiary, Bio Oil R&D, is ready to be launched as well. Here, all research activities (process optimisation, bio-oil upgrading, predictive pyrolysis, chemical extraction, etc.) will be centralized.

The technology is based on the combination of an Auger reactor and a fluidized bed, where sand is applied as the heat transfer medium. Operating temperatures range between 400°C-650°C depending on the characteristics of the feedstock. Feeding rates and the

residence times are adjusted depending on feedstock characteristics. The biomass feedstock is ground to a particle size between 8-10 mm. No need for drying is reported. A schematic three-dimensional plant lay-out and the injection system of the 1.5 ton/h installation is shown in the figures below.

The aim of the company is to recycle different waste streams, mainly containing biomass and plastic material, and upgrade the obtained pyrolysis oil, initially, into a marine fuel (slow running diesel engine). The pyrolysis process can be specifically operated so as to obtain a pyrolysis oil with a relatively neutral pH (around 6 to 7), which is one of the few requirements in order to guarantee its applicability as a (marine) fuel. At this moment, waste streams such as Solid Recovered Fuel (SRF), Automobile Shredder Waste (ASR) and even roofing material, are being investigated successfully. In the future, subsequent developments in application will be executed to conform to the type of input material. The extraction of value-added chemicals is also put forward as a future milestone. The main characteristics of this technology are shown in Figure 22.

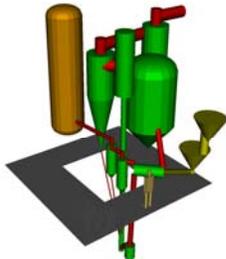
<b>Bio Oil Holding N.V.</b>	Process flowsheet of Bio-Oil Holding	
N° commercial plants 1 Tessenderlo (+ 2 under construction)	Holding	
Reactor technology Auger		
Reactor temperature 400 - 650°C		
Reaction time 1 - 5 s		
Heat Carrier sand		
Gasifying agent -		
Biomass feeding rate 1.5 Tn/hour or 36 Tn/day + Multiple 5 Tn/hour or multiple 120 Tn/day		
Biomass size 50 mm x 50 mm x 8 mm, max. thickness: 8 - 10 mm		
Biomass max. humidity 10 wt%		
Yields: Oil - Char - Gases 40 - 70%, 20 - 50%, 10 - 20% (depends on input)		
pH 3 - 7		
Density (kg/l) 1.2		
		BIO-Oil Holding NV Industrieterrein Ravenshout Zone 3 - nr 3154 B-3980 Tessenderlo Belgium



Figure 22. Technical characteristics of the BioOil Holding NV biomass fast pyrolysis plant.

### 6.2.5. *Metso-UPM-VTT-FORTUM*

Metso (Metso web site, 2010) , UPM (UPM web site, 2010) and FORTUM (Fortum website, 2010) are working in close collaboration with VTT (Pyne Newsletter 4, 1997; VTT web site, 2010) to develop a new bio-oil production concept. A 2 MW fuel fast pyrolysis unit has been integrated with Metso's 4 MWth circulating fluidized bed boiler located at Metso's R&D Centre in Tampere (Finland).

Since June 2009, the Metso R&D Center in Tampere, Finland, has been producing high-quality bio-oil from sawdust and forest residues. The consortium has developed a bio-oil production process in which a reactor, linked to a conventional fluidized bed boiler can first gasify solid biomass and then compress it into liquid form. Through their five months of pilot testing and utilization of the R&D Center's 2 MW plant, the partners have improved the bio-oil production methods and the efficiency of the process.

METSO-UPM-VTT		VTT pilot plant. photo
N° commercial plants	1 (Tampere, Finland)	
Reactor technology	2 MW fuel fast pyrolysis unit integrated with 4 MWth circulating fluidized bed boiler	
Boiler	4 MWth circulating fluidized bed boiler	
Heat Carrier	Sand	
Heat for the process	Char and non-condensable gases	

Figure 23. Technical characteristics of the METSO-UPM-VTT-FORTUM biomass fast pyrolysis plant.

The Finnish technology developers are committed to ongoing improvements in the production of bio-oil from renewable resources. It is the companies' vision that combining bio-oil production with bioenergy-based power plants will also provide them with new business potential, as well as remarkable cost and efficiency benefits. The companies' agreement for bio-oil test production extends through 2010. The construction of a commercial-scale demonstration plant will be planned based on the results and experiences garnered from the test runs in 2009 and 2010.

Metso is in charge of the technological development of the pyrolysis reactor integrated into the fluidized bed boiler. UPM adds to the project expertise in the use of biomass as raw material. The bio-oil production process is based on VTT's earlier R&D and patents. In the test production phase, VTT focused on the control and quality of the bio-oil. In the longer term, bio-oil refining will also be developed, for example, for feeding a conventional oil refinery. The main characteristics of this technology are shown in Figure 23. UPM's focus is on using bio-oil as a substitute for light and heavy fuel oil in heating and combined heat in power plants. Oilon developed a new burner for pyrolysis oils.

### 6.2.6. PYTEC

PYTEC (Pytec web site, 2010) was founded in 2002, with the purpose of developing the ablative pyrolysis technology for the liquefaction of renewable raw materials, organic residues and waste materials. PYTEC's objective in cooperation with its sister company TEC, is the development of the the BtO-process, an innovative pyrolysis process for the thermal conversion of wood to bio-oil. Furthermore, they are also focused on the economical upscale and realization of the BtO-Technology, implying the planning, construction, distribution, operation, and servicing of plants. From the very beginning, there has been a close cooperation between PYTEC, TEC and the Institute of Wood Technology and Wood Biology (vTI website, 2010). This ensures development and engineering on a highly scientific and technical level.

Since December 2005 the first pilot scale plant has been in operation to produce bio-oils from 250 kg/h wood chips by using the process of Fast Ablative Pyrolysis. Aimed at the economical generation of heat and power from biomass, the produced bio-oil will be combusted in a CHP-plant (Combined Heat and Power plant). The plant is of modular designed, every plant component is fitted in a container, therefore the capacity can be enhanced easily. This allows the implementation of the obtained experience for the construction of a new 48Tn/d pyrolysis plant in Malliss (Germany). Beside this, PYTEC also works with a labscale R&D unit in Hamburg (Germany) and a duplicate of this unit is being built for research work at Fraunhofer Umsicht in Oberhausen (Germany).

PYTEC	
N° commercial plants	2 (Bulkau, Malliss. Germany)
Reactor technology	Ablative reactor
Reactor temperature	475°C
Reaction time	1 s
Heat Carrier	No needed
Gasifying agent	CO <sub>2</sub> exhaust gases from CHP engine
Biomass feeding rate	Up to 6 Tn/day Up to 48 Tn/day
Biomass size	chips
Biomass max. humidity	15%
Yields: Oil - Char - Gases	60% - 20% - 20%
HHV: Oil	17 MJ/kg
Water content:Oil	wt%
pH Oil	2.3
Density (kg/l)	1.2
Oil: %C-%H-%N-%S-%O	48.9-6.2-0.5-0-44.4

Source: PYTEC



Office Hamburg:  
Durchdeich 10  
D-21037 Hamburg  
Germany

Figure 24. Technical characteristics of the PYTEC biomass fast pyrolysis plant.

PYTEC has developed a unique and patented Fast Pyrolysis system based on a reactor in which the ablative process takes place continuously. Chipped biomass or other bulk material is evaporated on a hot plate. A thin liquid film of Bio-Oil is continuously generated and evaporated so that the movement of the wood on the hot plate provokes no significant friction. At PYTEC's laboratory plant ablation rates of 4 mm/s have been achieved. The condensation of the bio-oil is realized by the combination of a spray column and an

electrostatic filter. The non-condensable part of the product gases are combusted and the resulting heat is utilized in the drying of the feedstock. The charcoal is eliminated by a cyclone and subsequently combusted, its energy is utilized to heat the reactor. The main characteristics of this technology are shown in Figure 24

### 6.2.7. RTI

In the years of 1979 – 2000, University of Waterloo, under D.S. Scott carried out a well known and recognized pioneering work in biomass conversion, focused mostly on fluid bed fast pyrolysis processing (~100 papers, reports, patents). Some intellectual aspects arriving from that work were later granted to Encon, Union Fenosa, Ensyn and Red Arrow (Underwood G., 1992).

In 1992, D.S. Scott with his collaborators started a small R&D company called Resource Transforms International. In 1996, the company was approached by Dynamotive Co. of Vancouver to improve on its original pyrolysis technology. After intensive due diligence (Stone&Webster), a new improved fast pyrolysis processing was proven, demonstrated, patented and later scaled up by Dynamotive Co. Since 2000s, Dynamotive Co. is the sole owner of this technology.

In 2006, RTI was granted US Patent # 7094932, “Production of glycol aldehyde by hydrous thermolysis of sugars”. The company utilizes this technology on a daily basis to manufacture solutions of hydroxyacetaldehyde for the food industry. Recent R&D activities of the Company include application of fast pyrolysis condensates, targeting a variety of agricultural products like slow release fertilizers, bio-cides, seed germination agents (US Patent # 5676727 and US patent application 20090126433).

<b>RTI</b>	
N° commercial plants	1 (Waterloo, Ontario, Canada)
Reactor technology	Fluid bed
Reactor temperature	425-575°C
Reaction time	Approx. 1 s
Heat Carrier	Sand
Gasifying agent	Steam and Non-condensable gases
Biomass feeding rate	Up to 5 kg/h
Biomass size	< 2 mm
Biomass max. humidity	< 10%
Yields: Oil - Char - Gases	60-75% - 10-20% - 10-20%
HHV: Oil	16-19MJ/kg
Water content:Oil	18-23 wt%
pH Oil	2 - 3
Density (kg/l)	1.2

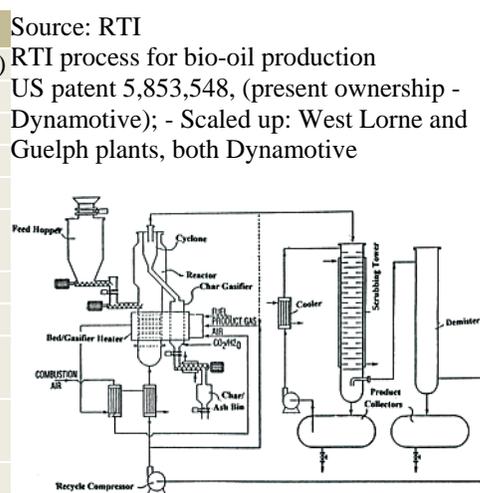


Figure 25. Technical characteristics of the RTI biomass fast pyrolysis plant.

At RTI's 5 kg/h laboratory plant the fluid bed reactor operating temperatures range between 425°C-575°C depending on the characteristics of the feedstock (< 2 mm particle size). The condensation of the bio-oil is realized by the combination of a scrubber column and

a demister. The non-condensable part of the product gases are combusted and the resulting heat is utilized to heat the reactor, with sand as the heat carrier. The charcoal is eliminated by a cyclone and collected. The main characteristics of this technology are shown in Figure 25.

### 6.2.8. CHOREN

The Carbo-V Process of CHOREN (Choren web site, 2010) in Germany is a three-stage gasification process involving low temperature gasification, high temperature gasification and endothermic entrained bed gasification. During the first stage of the process, the dry biomass is continually carbonized through partial oxidation (low temperature pyrolysis) with air or oxygen at temperatures between 400 and 500°C, i.e. it is broken down into a gas containing tar and char. During the second stage of the process, the gas is post-oxidized hypostoichiometrically using air and/or oxygen in a combustion chamber operating above the melting point of the fuel's ash to turn it into a hot gasification medium.

During the third stage of the process, the char is added to the hot gasification medium. They react endothermically generating a synthesis gas. Once this has been treated in the appropriate manner, it can be used as a combustible gas for generating electricity, steam and heat, or as a synthesis gas for producing fuel from biomass, e. g. wood chips. The main characteristics of this technology are shown in Figure 26.

CHOREN		Source: CHOREN
N° commercial plants	1	
Year built	mechanical completion 2009	
State (operational, R&D, other)	commissioning phase	
Reactor technology	Carbo-V-technology	
Reactor temperature	400-500°C and 1400-1600°C	
First stage	Low temperature pyrolysis	
Second stage	Post-oxidized (hot gasification medium)	
Third stage	Char added to the hot gasification medium, generating synthesis gas	
Gasifying agent	Air or oxygen	
Biomass max. humidity	15%	
		CHOREN Industries GmbH Frauensteiner Str. 59 D-09599 Freiberg Germany

Figure 26. Technical characteristics of the Choren biomass pyrolysis plant.

## 6.3. Economic Aspects of Biomass Pyrolysis

The economics of the fast pyrolysis installations is one of the key factors for further application in the future. Some external parameters could affect the economical viability of the process, such as crude oil price evolution, waste biomass availability, consumer energy requirements, etc. It can be assumed that the price of crude will rise in the future mainly due

to an increase in world demand and the reduction in the crude extractions. Consumer energy requirements will probably be at least the same or higher than in recent years, although energy efficiency will be improved pushed by the international politics, due to an increase in world population. The use of waste biomass, such as waste forest biomass, solid urban waste, and plastics, as biomass feeding of fast pyrolysis installations, will allow its economic viability for bio-oil and char production, and at the same time rubbish storage in dumps will disappear.

Taking into consideration the published economics data from the key companies about fast pyrolysis plants, the range of capital costs for the plant alone is in the range of 200 to 500 €/kW<sub>th</sub> (Venderbosch and Prins, 2010). Therefore, the capital investment cost is expected to be at least 20 million € for a 0.2 Gt/year biomass plant (Henrich and Weirich, 2009). An important feature of this technology is that the idea of several fast pyrolysis plants supplying bio oil to a central gathering plant includes a “cost reduction by learning” due to accumulated experience (Henrich and Weirich, 2009). Furthermore, replication saves costs because it takes advantage of repeated equipment and engineering costs (Henrich and Weirich, 2009).

Other specific costs for transport, storage, pretreatment, infrastructure, etc should be considered and could add up to another 50 to 100%, depending on the case. Studies indicate that pyrolysis oil can be produced at a range of 4 to 15 €/GJ, corresponding with 65 to 225 €/Tn, with a feedstock between 0 and 100 €/Tn (Peacocke and Bridgwater, 2006; Ringer et al., 2006).

There are many studies, such as UOP (UOP web site, 2010) Final Technical Report for USA DOE, that show the opportunities for biorenewables in oil refineries (UOP, 2005; Sarkar et al., 2010). For example UOP reported that producing gasoline from pyrolytic lignin hydrocracking becomes economically viable for 18 \$/bbl of pyrolysis oil at a crude price of 50 \$/bbl, considering financial incentives and CO<sub>2</sub> credits. As pyrolysis oil price decreases and crude price increases, producing gasoline from pyrolytic lignin becomes more attractive. Meanwhile, others (Sarkar et al., 2010) reported that large scale bio-hydrogen production from bio-oil delivered a cost in the range of 2.40-4.55 \$/kg H<sub>2</sub>.

In regards to Fisher Tropsch liquids production, as have been mentioned in the previous section, it is very dependent of the economy of scale. In an analysis conducted by Wright (Wright et al., 2008), they concluded that cheaper biofuel could be produced in distributed fast pyrolysis plants followed by centralized gasification and synthesis compared with centralized biomass processing facilities with capacities exceeding 2.4 Mton of biofuel per year. In this study, costs as low as 0.33 \$ per gasoline liter could be achieved assigning a 50 \$/ton credit to biochar production, which assumes a carbon sequestration equivalent to 16 \$/ton carbon dioxide. Another interesting techno-economic study is based on the Bioliq process, where char is mixed with bio oil to form a high energy density slurry (Henrich and Weirich, 2009), for a plant producing 1 Mton per year of Fischer Tropsch biofuel at a cost of 0,8 € per liter (around 1 €/kg). Both studies reflect the important influence of biomass feedstock price and transport costs in the final biofuel cost.

## 6.4. Analysis of Current Status and Perspectives

The status of fast pyrolysis is very optimistic. Further improvements in the pilot plants have promoted the construction of large commercial plants. Joint ventures and new

companies are emerging to commercialize its technologies for the production and upgrading of pyrolysis bio-oil from biomass waste. Furthermore, turbines and engines are being modified and adapted to work with pyrolysis bio-oil or its upgraded products, so much research and development work is carried out on this field. Some projects, evaluate the opportunities for bio-oil in the heat and power markets of Europe (14 countries) compared with conventional (fossil) alternatives for the same heat and power. At this time, only heat applications were found to be the most economically competitive, followed by CHP applications, and finally, rarely competitive electricity applications (Brammer et al., 2006).

As idea combination of the perspectives for the close future, some companies are planning their expansion with new commercial plants and upgrading technologies for the use of bio-oil for combined heat and power purposes as well as for transport fuel and high value chemical uses. The flexibility of pyrolysis plants and the viability to be decentralized if needed enhanced the interest of this technology (Pootakham et al., 2010). New plants are under design and viability is highly dependent on fossil fuel price evolution.

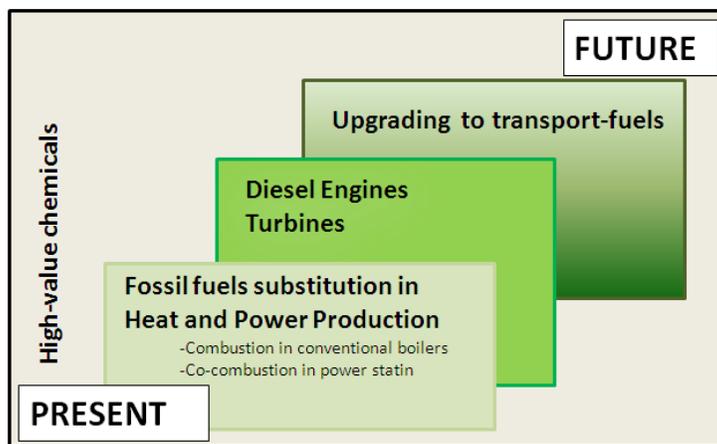


Figure 27. Fast Pyrolysis market opportunities from the present to a near future.

## CONCLUSIONS

The need to change the current energy model requires the development of novel technologies capable of making use of renewable energy sources like wind, solar radiation and biomass. Most developed countries have set ambitious objectives regarding the use of bio-energy for the production of heat, electricity and transport fuels. However, existing technologies (mainly combustion of solid biomass for heat and power, and the production of first generation transport bio-fuels) are facing significant problems owing to their reduced energy efficiencies, poor greenhouse gas emission balance, limited economic viability and complicated supply logistic.

Fast pyrolysis of biomass is a novel technology developed in the 1980's capable of transforming lignocellulosic biomass (wood) into liquid oils, with yields typically above 65-70 wt%. The pyrolysis process can be designed to be energy self sustained by combusting gas and solid by-products. This transformation entails notable economic and logistic advantages

regarding storage, transportation, processing and utilization of this natural resource in different energy and chemical applications.

Research and development in the field of biomass pyrolysis has been absorbing substantial amounts of human and economic resources in the last two decades. This effort has focused on various areas including: development of robust, economic and energy efficient fast pyrolysis technologies; characterization and upgrading of pyrolysis bio-oils; development of technologies capable of making use of the energy and chemical potential of the bio-oils.

However, pyrolysis bio-oils differ substantially from petroleum derived hydrocarbons which prevent their use in conventional energy applications. Pyrolysis bio-oils contain high water levels (15-40 wt%) and exhibit a high concentration of oxygenated compounds (oxygen content around 40-50 wt%). They are also highly corrosive (pH around 2-3) and viscous, present low volatility, and are thermally and chemically instable. Hence, most of the technology developed in the past 150 year for the use of petroleum derived fuels is not directly applicable to pyrolysis bio-oils. Hence, the market penetration of this technology is still very limited, due primarily to the unconventional fuel properties of the oils.

R&D work is currently ongoing in different areas including the testing and development of alternative reactor designs that allow high oil yields and energy efficiencies; the chemical, physical and catalytic upgrading of pyrolysis bio-oils in order to improve fuel and chemical properties; the development of novel energy technologies (furnaces, boilers, engines, turbines) capable of operating on this unconventional fuel; the preparation of standard specifications describing the fuel properties of the oils for different commercial applications; and determination of toxicological and environmental aspects associated with the production and use of the liquids. A number of private companies (BTG, ENEL, Ensyn, Dynamotive, Fortum, Wellman) are investing substantial amounts of economic resources in the development and operation of demonstration and small commercial plants.

There is widespread agreement that the energy and chemical potential of liquid biomass is enormous. There are high hopes that the technical problems encountered in the commercial development of this technology will soon be overcome and fast pyrolysis will become a reality. Bio-oil prices are being reduced with the optimization of new pyrolysis technologies (ablative reactors, fluidized beds). The fuel quality of these oils is also being improved with the development of catalytic, chemical and physical upgrading technologies. Blending of the pyrolysis oils with conventional fuels (diesel and alcohols) may also provide a solution to the unconventional fuel properties of these fuels. Advances are being made in the development of energy technologies adapted to the properties of this fuel, and the chemical potential of the bio-oil is being realized for the production of higher added value of organic products.

It has been described that the current situation with pyrolysis oils resembles that of the early days of the petrochemical industry. The path to using these oils will be progressively cleared with the advent of green chemistry, the development of biorefinery concepts and the progressive depletion of fossil fuels resources.

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*Chapter 2*

## **BIOMASS CROPS FOR FERMENTATIVE BIOFUEL PRODUCTION**

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### **ABSTRACT**

The looming energy challenges around the world will have to be tackled with a portfolio of different raw materials and technologies. Biomass is a widely available renewable carbon source and includes organic wastes and energy crops, which can be used for the production of biofuels to contribute to the reinvigoration of the biomass industry, among others. Energy crops have to be produced in a cheap and environmentally benign way in order to be utilized for the sustainable production of biofuels. Energy crops include mainly three categories, namely oil-rich crops, sugar crops, which contain sugars directly available for biofuel production, and lignocellulosic crops, which contain tightly bound cellulose, hemicellulose and lignin.

In this Chapter focus is given on fermentative biofuel production from sugar-rich and lignocellulosic biomass. Ethanol production from energy crops has been studied in the literature since the 1980s, but in the last decade significant research efforts have been addressed towards biological hydrogen production. Therefore, ethanol and hydrogen are considered two representative options for short- and long-term biofuel production, respectively. In particular, biofuel production from lignocellulosic crops or agricultural residues is more intensively discussed, given the higher degree of complexity of the

utilization of these raw materials for biofuel production. Various pretreatment methods can be applied to enhance the accessibility of lignocellulosic carbohydrates for enzymatic hydrolysis and the production of fermentable substrates. The efficiency of ethanol/hydrogen production from these substrates is dependent on their quality, which largely depends, in turn, on the amount of degradation products which act as inhibitors in the fermentations. Therefore, significant discussion is dedicated to the key aspects of the role of pretreatment of biomass on the efficiency of biofuel production.

The development of dedicated pretreatment techniques which are tuned to special characteristics of different energy crops is discussed. Sweet sorghum and sugar beet are regarded as two energy crops that can be instrumental in the promising field of biofuels. Sweet sorghum is interesting because it constitutes a highly productive sugar crop which, after sucrose extraction, provides a yet not well studied lignocellulosic residue, sweet sorghum bagasse. Sweet sorghum bagasse is currently unexploited, poses a disposal problem and its usage as fodder for animals is not a sufficiently viable solution. Similarly, sugar beet constitutes a traditional sugar crop, which can provide the biofuel industry with innovative raw materials with no competition with food production. From the viewpoint of sustainability, it is necessary to simultaneously assess the impact of the aforementioned biomass and biofuels on the environment and the economic growth. In principle, the use of organic wastes can be a win-win solution; however, biomass crops can present peculiar advantages for their potential in protecting/reclaiming vulnerable and marginal soils, sequestering CO<sub>2</sub>, bio-depurating wastewater and enhancing biodiversity and wildlife.

## 1. SUSTAINABLE BIOMASS PRODUCTION

### Introduction

A discussion focusing on biomass production sustainability is difficult because the definition itself of sustainability is still being debated. Its dynamic character has been acknowledged: “*Sustainability is a moving target*” wrote Hoag and Skold (1996) and later the Project Group ‘Sustainable production of biomass’ confirmed: “*Sustainability is a continuous process of improvement and adjustment*” (Project Group ‘Sustainable production of biomass’, 2006).

The process of evaluating sustainability is made more complex because besides “negative externalities” impairing environmental quality, agriculture can give origin to “positive externalities” such as carbon sequestration, soil protection, wastewater depuration, flood reduction (Pretty, 2003; DFID, 2004).

In all cases, when undertaking a sustainability evaluation process, it is necessary that rigorously selected key input assumptions and standardized procedures be adopted to avoid the frequent risk of biased approaches leading to misinformation. Such a need has been luminously demonstrated by the long dispute opposing Pimentel and associates, maintaining that the energy input to obtain ethanol from corn is higher than the output (Pimentel, 2001, 2003; Pimentel and Patzek, 2005; Pimentel et al., 2007) to the advocates of bioethanol, including the USDA (Graboski, 2002; Shapouri et al., 2002), in a seemingly endless debate (e.g. USDA, 2009; Aljama, 2010).

The debate does not seem to be soothed by the recent re-orientation towards the so-called second generation raw material, aimed at cancelling the dilemma “food or fuel” by focusing on lignocellulosic biomass: at the root of this long dispute is in fact the absence of an unambiguous evaluation protocol, which gives room to strong biases due to contrasting ideological positions and/or economic interests.

The dispute has been further fuelled by the addition of “indirect impacts” (such as indirect land use change and competition with food) to “direct impacts” (such as land use change effects when starting a biofuel crop plantation, plus fertilizer, water and fuel use during feedstock production, just to limit the analysis to the biomass production segment, i.e. limiting it to the farm gate).

Presently there is a wide divergence of opinions between those who are sceptical on the possibility of achieving sustainability in large scale production of bioenergy in the short term (e.g. Bindraban et al., 2009) and those who are optimistic (e.g. Hettenhaus, 2006). The European Council has shown concern for the sustainability of bioenergy production, issuing some basic rules (OJEU, 2009), and a number of official and unofficial organizations are now forming, with the target of setting clear procedures for achieving the sustainable production of biomass and biofuels (e.g. Project Group ‘Sustainable production of biomass’, 2006; CSBP, 2009; Lattimore et al., 2009; Roundtable on Sustainable Biofuels, 2010; Ismail and Rossi, 2010).

The issue has grown so complex, however, that simulation through the adoption of mathematical models has been suggested (Robertson et al., 2010; Dale et al., 2010), but of course simulation models are only useful as long as sufficient good quality inputs are available. The present section aims at contributing by illustrating the results of experiments conducted during the last eighteen years in two environments, Sicily (Italy) and Beijing (China), widely different in environmental, economic and social factors.

## Potential Biomass Production

As observed by Parton and Pouyat (2008):

“There is a need to determine which crops are best suited in which ecoregion. No one plant species will be the best biomass producer in all climatic regions. However, at the present time, there is not a sufficient understanding from an energy efficiency perspective of which crops are appropriate for which ecoregion or production system. More comparative multispecies field trials and monitoring are needed at a range of climatic and soil conditions to fully understand the energy efficiency of various feedstock systems. Specifically, what are the geographical ranges, overall yields, and stabilities of feedstock species, preferably perennial species, as biofuel crops?”

In 2003 Lewandoski et al. produced a review of yields obtained from perennial grasses in the US and Europe, and the species reportedly able to yield in excess of 30 Mg ha<sup>-1</sup> year<sup>-1</sup> dry matter were only five, three of them in the US (*Panicum virgatum*, *Pennisetum purpureum* and *Saccharum spp*) and two in Europe (*Arundo donax* and *Miscanthus spp*). Later, in a paper of Boehmel et al. (2008) it was reported that in Hohenheim, Germany, the highest yields were obtained from miscanthus, switchgrass and energy maize, never exceeding 20 Mg

ha<sup>-1</sup> year<sup>-1</sup> dry matter. Scholz et al. (2010) confirmed that in Germany no lignocellulosic crop is able to reach that level of yield.

Here we refer the results of experiments conducted in Sicily and in Beijing area; in Figure 1 the main climatic characteristics of the two experimental sites are shown (Lieth et al., 1999). The opposite pattern of precipitations, with dry summers in Sicily and wet summers in Beijing, as well as the smoother seasonal excursions in Mediterranean temperatures can be appreciated; the dotted areas evidence the larger seasonal droughts in Sicily.

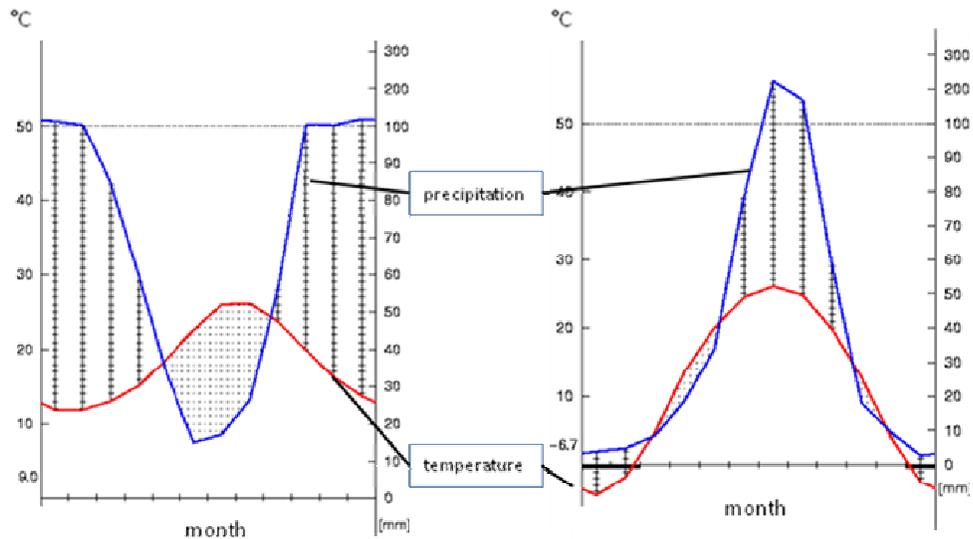


Figure 1. Yearly distribution of temperature and precipitation in Messina, Sicily (left) and Beijing, China (right).

**Table 1. The main species tested in the course of experiments in China and Sicily.**

Species	Site (C=China S=Sicily)	Common name	Yield (Mg dm ha <sup>-1</sup> year <sup>-1</sup> )	Irrigated	Cycle
<i>Arundo donax</i>	S	Giant reed	40 (38-51) <sup>a</sup>	Yes	Perennial
<i>Arundo donax</i>	C	Giant reed	34 <sup>b</sup>	No	Perennial
<i>Cynara cardunculus</i>	S	Wild artichoke	18 (8-31) <sup>c</sup>	No	Perennial
<i>Eucalyptus globulus</i>	S	Eucalyptus	7	No	Perennial
<i>Miscanthus sacchariflorus</i>	C	Miscanthus, Silvergrass	29 <sup>b</sup>	No	Perennial
<i>Panicum virgatum</i>	C	Switchgrass	28 <sup>b</sup>	No	Perennial
<i>Pennisetum purpureum</i>	S	Napiergrass	30 <sup>d</sup>	Yes	Perennial
<i>Pennisetum purpureum</i>	C	Hybrid pennisetum	43	Yes	Annual
<i>Ricinus communis</i>	S	Castorbeans	10 (plus seeds)	Yes	Perennial
<i>Saccharum officinarum</i>	S	Energy cane	33 <sup>e</sup>	Yes	Perennial
<i>Sorghum bicolor</i>	S	Fiber sorghum	30	Yes	Annual

<sup>a</sup> Average of 3 years x 2 sites; <sup>b</sup> Average of 2 years after establishment; <sup>c</sup> Average of 3 years x 3 sites; <sup>d</sup> Average of 3 years x 2 cultivars; <sup>e</sup> Average of 3 years

Although in total some twenty species have been scrutinized, only the main data are reported here with the focus on perennial crops (Table 1), due to their outstanding sustainability level. Only data referring to two annuals are reported, one (hybrid pennisetum) because it gave the highest yields, and another (sorghum) because in some way it can be considered a standard in biomass production.

Figures 2 and 3 show the harvesting of perennial grasses during the winter period, mild and humid in Sicily, cold and dry in Beijing.



Figure 2. Harvesting biomass in Sicily.



Figure 3. Harvesting biomass in Beijing.

## The Holistic Approach

Gradually, the simplistic initial approach, aimed at assessing exclusively the productive potential, was modified into a holistic approach, encompassing ever more aspects impacting sustainability, while some insight was also gained into the physiology of the plants.

Figure 4 gives an example of the results achieved when investigating the influence of PAR (photosynthetically active radiation) on plant photosynthetic activity in Sicily: it is interesting to note how reeds, the only C3 plant in the group of the five under comparison, although having the lowest net photosynthetic activity particularly at high PAR values, gave the highest biomass yield. We speculated that it can depend on their ability to start vegetating earlier than the other plants, with a soil temperature of 14°C at 10 cm depth, thus obtaining a development level that the other plants cannot reach, since they begin growing at about 25°C (Merlo et al., 1998). Also in China, where the soil is frozen about 100 days per year, reed outyielded miscanthus and switchgrass, both C4 plants (Zuo et al., 2010).

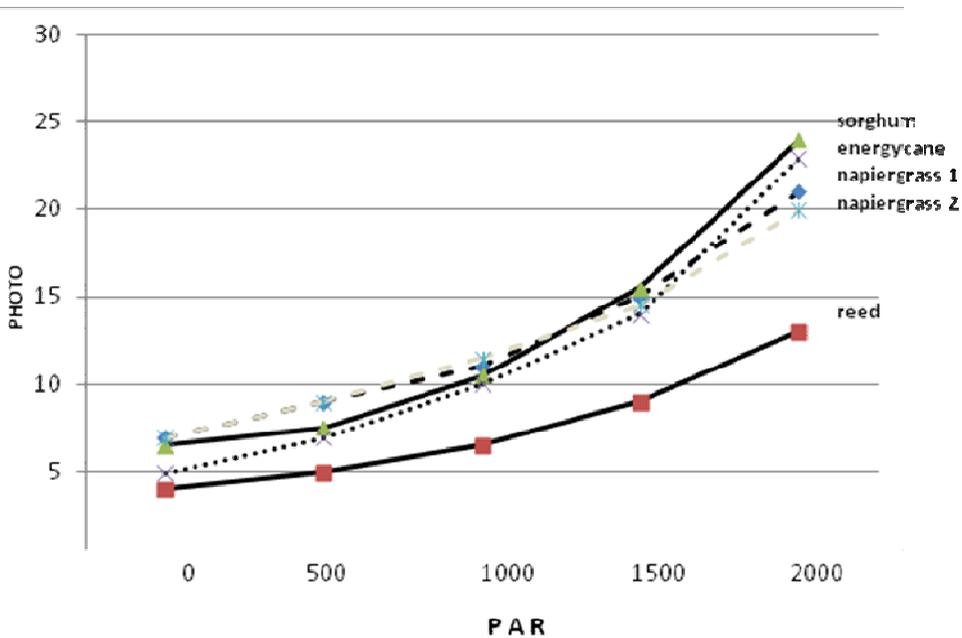


Figure 4. Influence of photo-active radiation, PAR on net photosynthetic activity, PHOTO - PAR in  $\text{mE m}^{-2}\text{sec}^{-1}$ , PHOTO in  $\text{mmol CO}_2 \text{m}^{-2} \text{sec}^{-1}$ .

Budgets for energy were worked out separately for the crops in Sicily and those in Beijing, and results are synthetically reported in Table 2. The differences between results in Beijing and Sicily depend mainly a) on the higher energy inputs in Beijing, owing to the extensive use of their cheap human labour (paid at euro 0.51/hour) and b) on the rainy summer season in Beijing, permitting to avoid irrigations (Zuo et al., 2010). From the investigations in Sicily it was found that for grass irrigation in the Mediterranean environment a mean crop coefficient  $E_c = 0.5$  is sufficient (Merlo et al., 1998), implying approximately the application of a net volume of  $5,000 \text{ m}^3 \text{ha}^{-1}$  per year.

One distinct advantage of most perennials, including wild artichoke, is the practical absence of any pest significantly attacking the crops, and consequently averting any risk of pollution due to pesticides; this did not apply to the two napiergrass cultivars tested in Sicily, however, severely attacked by the European corn borer (*Prausta nubilalis*). Furthermore, when properly managed, perennial grasses as well as wild artichoke do not require any fertilization after the establishment period, as demonstrated by the non-significant response to fertilizers in spite of the considerable nutrient exportation with biomass (Lewandowski et al., 2003; Robertson et al., 2010; Glover, 2010; Glover et al., 2010). This had been observed before in one of the giant reed fields in Sicily (Merlo et al., 1998), in production for over 50 years without any fertilizer addition.

**Table 2. Energy budget of the tested biomass species.**

Species	Common name	Site (C=China S=Sicily)	Energy input (GJ/ha)	Gross energy yield (GJ/ha)	Net energy yield (GJ/ha)	Energy efficiency (GJ/GJ)
<i>Arundo donax</i>	Giant reed	S	14	720	706	50.4
<i>Arundo donax</i>	Giant reed	C	18	619	601	33.4
<i>Cynara cardunculus</i>	Wild artichoke	S	11	324	313	28.5
<i>Eucalyptus globulus</i>	Eucalyptus	S	7	126	119	17.0
<i>Miscanthus sacchariflorus</i>	Miscanthus, Silvergrass	C	19	522	503	26.5
<i>Panicum virgatum</i>	Switchgrass	C	17	506	489	28.8
<i>Pennisetum purpureum</i> (perennial)	Napiergrass	S	18	540	522	29.0
<i>Pennisetum purpureum</i> (annual)	Hybrid pennisetum	C	40	792	752	18.8
<i>Ricinus communis</i>	Castorbeans	S	6	128	122	20.3
<i>Saccharum officinarum</i>	Energy cane	S	18	594	576	32.0
<i>Sorghum bicolor</i>	Fiber sorghum	S	17	540	523	30.8

One further important aspect to be considered in the assessment of sustainability is the crop impact on soil conservation: perennial crops offer a superior protection compared to annuals thanks to the absence of cultivation after the establishment and to the thorough cover they offer to soil surface, encouraging rainwater infiltration, thus reducing overland flow and erosion while in turn enriching the aquifer (Figure 5).



Figure 5. The superb protection offered by the powerful root system of giant reed.

One environment-related aspect of biomass crops sustainability is their ability to sequester atmospheric CO<sub>2</sub>: typically the fresh weight of root system in 3 years old giant reed to the depth of 1.5 m resulted in Sicily of about 44 kg/m<sup>2</sup>, as opposed to 18 kg/m<sup>2</sup> for napiergrass (average between the two cultivars) and only 6.5 kg/m<sup>2</sup> for energy cane (Merlo et al., 1998). Such a considerable amount of below-ground organic matter in giant reed is expected to have also a potentially appreciable impact on farm economic budgets if carbon tax is activated. Experiments conducted in Beijing area gave similar results; the dry matter to fresh matter ratio in roots ranged from a minimum of 17.3% in hybrid pennisetum to a maximum of 35.7% in miscanthus (unpublished results).

From the viewpoint of biodiversity, the impact of perennial grasses can be considered on the same level of planted forests (Lysen and van Egmond, 2008). Notoriously, eucalyptus is a plant that does not admit competition, due to the presence of eucalyptol (birds do not nest in eucalyptus trees) and the typical allelopathies determined by its leaves (almost no undergrowth can be found in eucalyptus plantations besides ferns and *Ampelodesma* spp.). Opposite to it, miscanthus encourages wildlife:

“miscanthus stands also contain more large animals (mammals, birds) than other herbaceous crops (corn or reeds), possibly because of the greater diversity of canopy structure leading to a higher number and greater range of ecological niches” (Jodl et al., 1998).

Glover et al. (2010) give an interesting, thorough account of the environmental impact of perennials, including their influence on insect communities concluding that:

“Perennial fields supported overall greater insect species richness; in particular, they supported greater richness of insect herbivores, pollinators and detritivores” and “Perennial fields likely provided a greater number and diversity of pollen sources which would account for the consistent presence of wild pollinators in the perennial fields.”

Based on the data collected in the course of our experiments enriched with specific literature reports, the matrix of Table 3 was elaborated.

**Table 3. Matrix of sustainability evaluation of the tested biomass species.**

Species	Common name	Factors				
		soil protection	aquifer protection	gas emissions	biodiversity	net energy production
Arundo donax	Giant reed	++	++	++	+	++
Cynara cardunculus	Wild artichoke	++	++	+	+	-
Eucalyptus globulus	Eucalyptus	++	+	+	--	--
Miscanthus sacchariflorus	Miscanthus, Silvergrass	+	+	+	++	+
Panicum virgatum	Switchgrass	+	+	+	+	+
Pennisetum purpureum (perennial)	Napiergrass	+	+	+	+	+
Pennisetum purpureum (annual)	Hybrid pennisetum	--	-	-	--	+
Ricinus communis	Castorbeans	+	+	+	+	--
Saccharum officinarum	Energy cane	+	+	+	+	+
Sorghum bicolor	Fiber sorghum	--*	-	-	--	+**

\* if no-tillage is applied the -- mark becomes +

\*\* the + mark becomes ++ in the case of sweet sorghum

## Discussion

“At the moment the possibilities for testing biomass for its sustainability are inadequate” honestly admit the Project Group ‘Sustainable production of biomass’, 2006; but they also warn: “Sustainability in the long term can only be achieved, if a start is made with it now”.

We are well aware that sustainability is such a complex issue that factors and marks listed in Table 3 can be considered at best a preliminary qualitative approach to its assessment. However, there is certainly sufficient matter for a first screening; for instance it is evident that giant reed is by far the best choice, in spite of problems potentially arising from its high silica content; the relatively poor performance of annual crops compared to perennials can also be appreciated. A protocol should be enacted, supported by appropriate indicators, permitting to achieve a quantitative evaluation of sustainability at local, meso- and macro-level, giving the

due weight to all concurring factors and considering their interactions as well as all the negative and positive externalities. Data should be collected and processed in standardized modes permitting their easy input in simulation models.

More aspects need to be assessed, including the biomass production under marginal conditions: we know for instance that giant reed is able to cope with some level of salinity but is not tolerant to drought, while the opposite is true for wild artichoke. Eucalyptus, switchgrass and castorbeans are known to be tolerant to a number of abiotic stresses, albeit at the cost of some reduction in yield. Halophytes, partially explored in our former experiences, are one untapped source of low-cost biomass in saline environments.

Assessing the potential biomass production under marginal pedo-climatic conditions is of interest in order to reduce the risk that good quality arable land be subtracted to food production: this should be one of the major objectives for research in next years.

## **2. PRETREATMENT OF LIGNOCELLULOSIC BIOMASS FOR BIOFUEL PRODUCTION**

Most advanced biofuels production technologies are focused towards converting lignocellulosic biomass into transportation fuels. Lignocellulosic biomass, or lignocellulose, consists of plant biomass that is comprised of cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are natural polymers of carbohydrates and are thereby potential sources of sugars for fermentation. Lignin however is not fermentable under anaerobic conditions. Lignocellulose comes in many different types, such as wood residues such as sawmill residues, agricultural crop residues such as straw and stove, industrial residues from agro-food processing as well as dedicated energy crops, such as the energy grasses *Miscanthus* and switchgrass, and several fast-growing wood species. Lignocellulose is widely available, relatively inexpensive, sustainable in terms of CO<sub>2</sub> emissions and non-competitive to food applications.

There are many different routes for producing biofuels from lignocellulose. Given that the primary biofuel in the world today, bioethanol, is made through fermentation, this section of the current Chapter focuses on using lignocellulose for fermentative biofuel production. Primary examples are cellulosic ethanol, ABE/butanol, and bio-hydrogen. A general schematic of the conversion of lignocellulose to ethanol, butanol or bio-hydrogen is provided in Figure 6. The process generally consists of a pretreatment step, a hydrolysis step, and a fermentation step. Lignin is discharged as a co-product and can be used to generate electricity to supply the process with energy. Pretreatment is necessary to break open the lignocellulosic structures and to facilitate the separation of the main carbohydrate fractions hemicellulose and cellulose from lignin, in order to make these better accessible for hydrolysis, the next step in the process. Hydrolysis is the process to convert the carbohydrate polymers cellulose and hemicellulose into fermentable sugars. While hydrolysis can be achieved by using concentrated acids, most biofuel pathways developed today are based on enzymatic hydrolysis, by employing cellulases and hemicellulases. Fermentation is the main process used to convert fermentable sugars, produced from the previous hydrolysis step, into ethanol, higher alcohols (e.g. butanol), solvents or hydrogen.

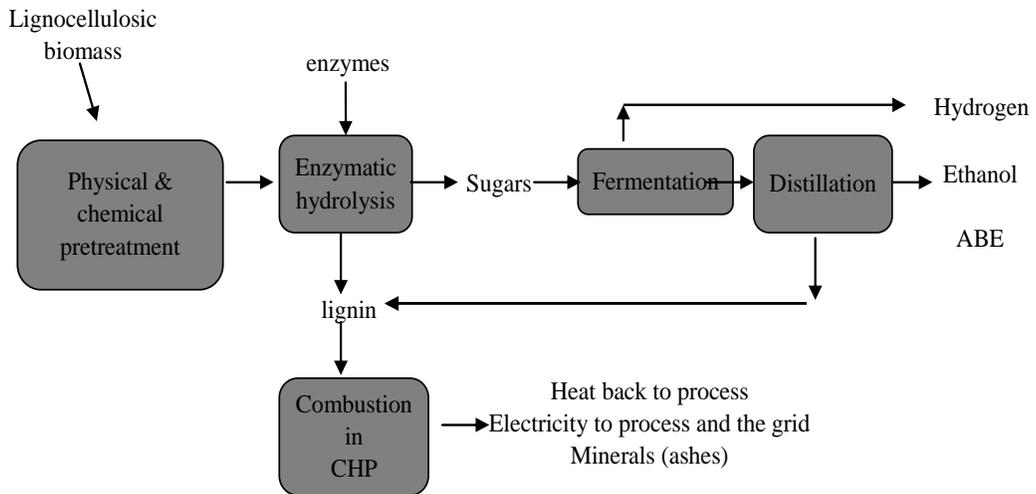


Figure 6. Generic scheme for biochemical conversion of lignocellulosic biomass to biofuels.

A large number of pretreatment methods have been studied and some have been implemented at pilot scale (Talebna et al., 2010). Current pretreatment methods include steam explosion, liquid hot water, dilute acid-, lime-, ammonia pretreatments, and pretreatment by using organic solvents. Pretreatments that combine mechanical treatment with chemical pretreatment are also common. The pretreatment process is often optimized towards reaching the objective of the biomass pretreatment, such as the yield of fermentable sugars. Other factors in the optimization are the pretreatment cost, the energy requirement of the pretreatment process, or its environmental impact. This section highlights a particular challenge in the development of biochemical biofuel production, which is the fermentability of hydrolysates produced through pretreatment of lignocellulose. As pretreatment may produce substances that have an inhibitory effect on the fermentation, the reduction or elimination of fermentation inhibitors is a crucial factor in the development of lignocellulose pretreatment.

Fermentation inhibitors have toxic effects on the fermenting organisms, thus reducing the biofuel yield and productivity. The level of toxicity depends in part on fermentation variables including cell physiological conditions and pH of the medium. In addition, the fermenting organisms may, to some extent, be resistant to inhibitors or may become gradually adapted to their presence.

Compounds inhibiting microbial activity can be either originally present in the biomass, or produced due to too severe pretreatment conditions (Maas et al., 2008). Based on common inhibitory compounds present in lignocellulosic hydrolysates from spruce, willow, wheat straw, sugar cane bagasse and corn stover, Almeida et al. (2007) distinguished three major groups of fermentation inhibitors furan derivatives, aliphatic acids, and phenolic compounds.

The inhibitors that are present in lignocellulose are liberated relatively easy during the pretreatment step. For instance, under relatively mild conditions, acetylated hemicellulose is hydrolyzed and beside monosaccharides, the organic acid acetic acid is liberated. Inhibitors that are produced under too extreme pretreatment conditions generally consist of degradation compounds of sugars. For example, acidic conditions combined with high temperatures

during lignocellulose pretreatment often lead to formation of furfural or 5- hydroxymethyl furfural (HMF; Mussatto and Roberto, 2004). Both furfural and HMF are further degraded to formic acid and levulinic acid. Degradation products of lignin, formed during pretreatment, can also lead to inhibition. Examples are phenolic compounds such as vanillin, syringaldehyde and catechol which are all formed by partial breakdown of lignin (Palmqvist et al., 1996).

Furfural and HMF affect cell growth and respiration. While HMF is considered less toxic than furfural and its concentration in (hemi)cellulose hydrolysates is usually low, it is generally considered that extensive degradation of (hemi)cellulose is responsible for the formation of the latter inhibitor compounds. Kinetic studies have shown that the production of furfural strongly increases with temperature and reaction time (McKillip and Collin, 2002). A variety of compounds (e.g. aromatic, polyaromatic, phenolic and aldehydic) may be released from the lignin fraction. Phenolic compounds have a considerable inhibitory effect on fermentation and are generally more toxic than furfural and HMF, even at low concentrations. Phenolic compounds cause partition and loss of integrity of cell membranes of the fermenting organisms reducing cell growth and sugar assimilation. At very low concentrations, some compounds may improve fermentation kinetics, whereas at higher concentrations they lead to inhibition.

Acetic acid is derived from the acetyl groups in hemicellulose. At low pH in the fermentation medium the acetic acid is in the undissociated form, and may diffuse into the cells. Once in the cell, the acid dissociates causing a lowering of cell pH that inhibits cell activity. The toxicity varies according to the fermentation conditions. Since the formation of acetic acid is inherent to hemicellulose hydrolysis, its formation cannot be prevented. However, a higher fermentation pH can reduce this effect or the acid can be neutralized before fermentation.

Another group of potential fermentation inhibitors are the so-called extractives. Extractives are soluble compounds derived from lignocellulose and include acidic, tannic and terpene acids. Although much is unknown about the effects of these compounds on fermentation, these extractives are generally considered to be less toxic compared to the lignin degradation products or acetic acid.

The inhibitory effects of the compounds listed above have been extensively studied for the conversion of lignocellulosic hydrolysates to ethanol by yeast and bacteria (Taherzadeh et al., 1997; Klinke et al., 2002; Klinke et al., 2004; Maas, 2008), as well as other biofuels, including biohydrogen by extreme thermophilic bacteria (de Vrije et al., 2009; Panagiotopoulos et al., 2009). The research shows the often complex role of fermentation inhibitors in lignocellulosic biomass conversion, and suggests several strategies for improvement of fermentation.

Taherzadeh et al. (1997) investigated the role of acetic acid in fermentation to ethanol. They reported that the concentration of the undissociated form of acetic acid should not exceed 5 g/l in the fermentation broth for growth to occur. Interestingly, acetic acid was also shown to have a beneficial effect on fermentation. At a concentration of 3.3 g/l of undissociated acetic acid, the ethanol yield was 20% higher than without added acetic acid. In a related paper (Taherzadeh, 1999). fermentation techniques for conversion of dilute acid hydrolysates made from spruce and birch woods were examined, using *Saccharomyces cerevisiae*. Hydrolysates containing high initial concentrations of furfural (2.2 to 5.7 g/l) and HMF (2.4 to 7.3 g/l), were found to be strongly inhibiting the yeast. However, using a fed-

batch technique with an optimized feed rate, results show to completely ferment the glucose and mannose sugars in both hydrolysates. Most of the furfural (90%), and part of the HMF (40–70%), present in the hydrolysates was converted during the fed-batch operation. To what extent furfural was volatilized during the fermentation, was not clear. The results suggested that detoxification, normally considered of ethanol from lignocellulose, is not necessary.

Maas (2008) investigated fermentation of lime-pretreated wheat straw with *Saccharomyces cerevisiae*. Fermentation tests of unwashed hydrolysates showed good fermentability to ethanol under standard pH conditions (5.0). At lower fermentation pH however, acetic acid originating from the lignocellulose affected the ethanol productivity moderately (pH 4.5) to severely (pH 4.0; Figure 7). Inhibitory effects of acetic acid at lower fermentation pH could be overcome by washing the lime-pretreated wheat straw prior to enzymatic hydrolysis. They concluded that, depending on raw material type and composition, either acetic acid removal prior to enzymatic hydrolysis or selection of more acid-tolerant yeasts should be considered when employing lime pretreatment at the industrial scale.

De Vrije et al. (2009) investigated the fermentability of lignocellulose-derived hydrolysates for thermophilic hydrogen fermentation, based on alkaline chemical pretreatment of *Miscanthus*, followed by enzymatic hydrolysis. They reported poor fermentability of lime-pretreated *Miscanthus* whereas fermentation of sodium hydroxide pretreatment hydrolysate yielded 2.4 to 2.9 mole hydrogen per mole C6 sugar. The results suggest that the choice of chemicals in lignocellulose pretreatment can have a large effect on fermentability of hydrolysates, and therefore the selection of chemicals should be done with fermentation in mind. Panagiotopoulos et al. (2009) investigated fermentability of pretreated lignocellulose for thermophilic hydrogen production as well, but used dilute-acid pretreated corn stover and barley straw, two common agricultural field residues. They showed that pretreated barley straw in particular indicated good hydrogen production by *C. Saccharolyticus*, but that hydrolysates derived under similar conditions from corn stover indicated poor fermentability. Differences in the two raw materials were believed to be an indication of the role of sugar degradation products furfural and HMF in fermentability. Most likely, the hemicellulose fraction of barley straw was more recalcitrant in comparison with corn stover, which led to lower furfural concentrations in the barley straw hydrolysate, and therefore better fermentability.

As the results of previous research reports suggest, there are several strategies to overcome inhibition of fermentation. A variety of detoxification methods (e.g. precipitation, active charcoal, vacuum evaporation) can be applied to lignocellulose hydrolysates in order to reduce the concentration of inhibitors prior to enzymatic hydrolysis and fermentation (Mussatto and Roberto, 2004). Overliming with calcium hydroxide (raising the pH to 10 and separating the precipitate from the liquid prior to reducing the pH; Galbe and Zacchi, 2002) is probably the most common detoxification step employed for lignocellulose hydrolysates. The cost of detoxification, which are essentially additional pretreatment costs, should however be taken into account in the development of pretreatment processes. A different approach to avoid fermentation inhibition is to adapt pretreatment process for example by employing a two-stage pretreatment, where hemicellulose-derived sugars are released under mild conditions, followed by more severe cellulose pretreatment. By removing the hemicellulose-derived sugars prior to cellulose pretreatment, the formation of degradation products from pentose sugars can be avoided (Galbe and Zacchi, 2002). A different approach was taken in alkaline pretreatment, as suggested by Maas et al. (2008), where prior to enzymatic hydrolysis

and fermentation, pretreated lignocellulose is washed to remove inhibitors (e.g. acetic acid; lignin degradation products) generated during the alkaline pretreatment. In this case, only a small amount of pentose sugars are released, as the relatively mild conditions did not lead to extensive hemicellulose hydrolysis.

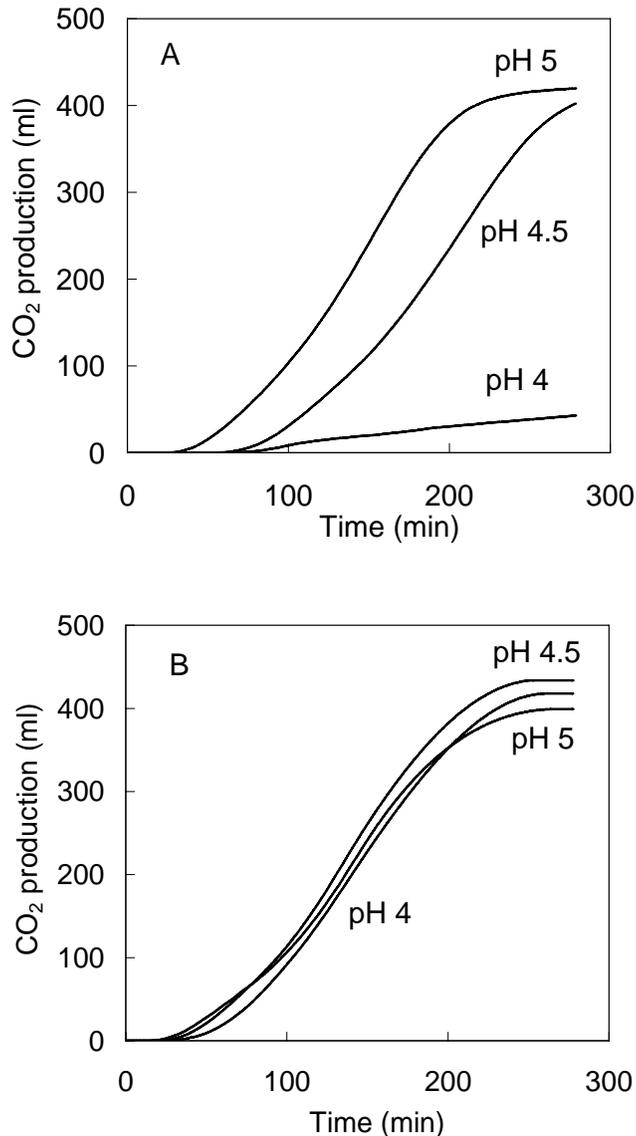


Figure 7. Production of CO<sub>2</sub> (as equimolar of ethanol) during fermentation of unwashed (A) and washed (B) lime-pretreated wheat straw by *S. cerevisiae* at 32°C and pH 4, 4.5 and 5. (adapted from Maas, 2008).

Strategies to overcome inhibition that do not involve removal of inhibitors involve changing fermentation conditions in order to reduce the inhibiting effects of certain compounds. Examples of these strategies are fed-batch feeding of lignocellulosic hydrolysates or changing pH during fermentation, as was noted above. It should be noted that

changing fermentation conditions may also lead to less-than-ideal conditions for the microorganism, longer residence times and therefore higher process costs. Therefore, employment of these strategies to overcome inhibition should be balanced against possible disadvantages. Finally, the development of more robust microorganisms can be an efficient strategy to overcome inhibition. For instance, several yeast strains have been identified that can tolerate much higher levels of furfural and HMF compared to standard baker's yeast. (Liu et al., 2005; Almeida et al. 2007)

The prevention of fermentation inhibitors should form an integral part in the development of lignocellulose pretreatment. In many cases, formation of inhibitors (e.g. furfural) can be avoided by adjusting pretreatment conditions. In some cases, the formation of inhibitors (e.g. acetic acid) cannot be prevented as they originate directly from the raw material. For many fermentation inhibitors, strategies other than pretreatment adjustment can be developed to overcome fermentation inhibition. Maximum allowable concentrations for fermentation inhibitors can only be given in certain circumstances. The concentrations of common inhibitors are often only an indication as toxicity depends on various factors including the sugar concentration, the type of organism and fermentation variables, and the number and concentration of inhibitors and their synergistic effects.

### **3. SUGAR BEET TO BIOETHANOL**

This section of the current chapter is devoted to the utilization of sugar beet for ethanol production. Sugar beet has been commercially grown as a source of table sugar (sucrose) since beginning of the 19<sup>th</sup> century, initially in Europe. It was introduced as a European replacement for sugar cane which is grown in tropical climate. Today sugar beet is an important crop mainly in the industrialized countries of the northern hemisphere – also in Asia and North America – where the climate is temperate. It is usually planted in the spring and harvested in the autumn but in warmer climates it is a winter crop, planted in the autumn and harvested in the spring. Farmers can choose between numerous sugar beet varieties suitable for a range of climatic conditions, soil types and local risks including pest and disease. The farming methods are very well developed and all field operations are mechanized.

In 2008, with the global annual crop of about 172,500,000 t, sugar beet accounted for 20% of the world sucrose output (Sugar Economy, 2010). However, its importance is not restricted to its use as a raw material for sucrose production. It plays a key role in sustainable crop rotation systems and the byproducts of its processing, and mainly beet pulp and molasses, are widely used as fodder components; molasses also has many other applications. With the progress of industrial biotechnology, it becomes more and more clear that sugar beet is an excellent raw material for biorefinery processing (Villadsen, 2010). As the interest in bioenergy is increasing, it is also recognized as a highly competitive energy crop. Owing to its average yield of 62 t/ha which is equivalent to attainable ethanol yield about 6000 l/ha, sugar beet is the most productive energy crop under European conditions where the attainable ethanol yield for corn is 3300 l/ha and for wheat 2200 l/ha (authors' own estimates based on 2009 statistics).

Sugar beet is a biennial plant which during its first growing season produces a large (1–2 kg) storage root (Figure 8A,B). If the plant is not harvested at this time, then during its second growing season, nutrients in the root will be used to produce flowers and seeds and the root mass will decrease. In commercial beet production, the root is harvested after the first growing season. A typical composition of the fresh beet root is about 25% dry substance (DS) including up to 19% sucrose, 2.3% cellulose and hemicellulose, 2.4% pectins, and small amounts of proteins, saponins and mineral substance (Bohn et al., 1998). The root tissue should preferably be processed directly after harvesting because its sucrose content is reduced during prolonged storage.



Figure 8. Sugar beet. A, complete plant; B, roots ready for processing; C, cossettes transported to extraction equipment.

Beet root tissue can be converted into fermentable raw material using juice production processes well established in the sugar industry (van der Poel et al., 1998; Hinkova and Bubnik, 2001). After reception at the processing plant, the beet roots are washed, mechanically sliced into thin strips called cossettes (Figure 8C), and passed to juice extraction equipment. Counter-current water extraction carried out at elevated temperature (up to 80 °C)

yields the so-called raw juice typically containing about 15% DS in which sucrose fraction is about 0.90; it also contains small amounts of glucose and fructose. The exhausted cossettes are discharged from the extractor and subsequently pressed to remove excess moisture thus yielding a byproduct known as beet pulp. While this process is economic in large-scale sugar factories, alternative methods of raw juice separation are considered for smaller-scale biotechnological processing. A possible approach is to apply low-temperature, counter-current washing of beet brei. In order to destroy cell membranes effectively so that sucrose can be washed out at low temperature, disintegration of beet tissue in a homogenizer or beater mill is required. The obtained raw juice is similar to that produced by extraction while exhausted and pressed beet brei is a byproduct to replace beet pulp.

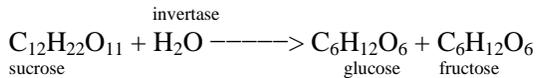
Raw juice is an excellent raw material for biotechnological processing but it is not suitable for storage as some of its components are not chemically stable. When used for sugar production, it undergoes a purification procedure to remove impurities and improve chemical stability. First, the raw juice is mixed with milk of lime and heated up to 80–90 °C causing multivalent anions such as sulfate, phosphate etc. to precipitate as calcium salts while the simple sugars, glucose and fructose, along with the amino acid glutamine, are converted to chemically stable carboxylic acids. Next, carbon dioxide is bubbled through the alkaline solution, precipitating calcium carbonate particles; small particles aggregate, entrap or adsorb impurities, settle out in clarifiers and are discharged as lime mud. Clear solution, which after second treatment with carbon dioxide and subsequent filtration contains less than 15% DS in which the sucrose fraction is about 0.93, is called thin juice. It can be used as fermentation raw material but in sugar factories, it is concentrated by evaporation to about 70% DS. The resulting thick juice can be fed directly into a crystallization subsystem, or alternatively can be stored for a period of up to several months, for later processing. If not used for sugar crystallization, it can be diluted with water to give a fermentable raw material. Thick juice storage – now applied in many sugar factories – makes it possible to process this raw material year around.

Sucrose crystallization from thick juice is done in several (typically three) stages, each stage including production of crystals and their separation from sucrose-containing run-off syrup. Usually, crystals from the first stage are discharged as marketable sugar but those from the remaining stages are dissolved and recycled. With the exception of the last stage, the stream of run-off syrup or its part is also recycled to increase the overall crystal yield; alternatively, at the cost of reduced sugar output, it can be discharged as a byproduct which can be diluted with water and fermented. The solution obtained from the last crystallization stage is a byproduct called molasses. It contains about 75% DS in which sucrose fraction typically is about 0.62, and has traditionally been in use as a raw material for the fermentation industries including distilleries.

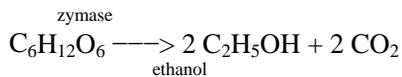
The basic steps for large scale production of ethanol from any sugar-containing raw material are:

- fermentation of sugars employing certain species of yeast (e.g., *Saccharomyces cerevisiae*),
- distillation,
- dehydration (required for typical fuel applications).

Fermentation is a series of chemical reactions wherein the simple sugars (glucose, fructose and others) are converted into ethanol and carbon dioxide. From the chemical point of view, sucrose is an organic compound made up of one molecule of fructose and one molecule of glucose joined in a chemical bond. In a process known as hydrolysis, that is, reaction with water catalyzed by the yeast-produced enzyme invertase, this bond breaks, resulting in individual molecules of fructose and glucose:



This is followed by a process in which another enzyme, zymase, catalyzes the fermentation reaction, that is, the breakdown of molecules of simple sugars:



This process is carried out continuously or in batches at 30–40°C (Rankovic et al., 2009). A simplified scheme of industrial fermentation of molasses is shown in Figure 9 (after Sapporo Engineering Ltd, 2010).

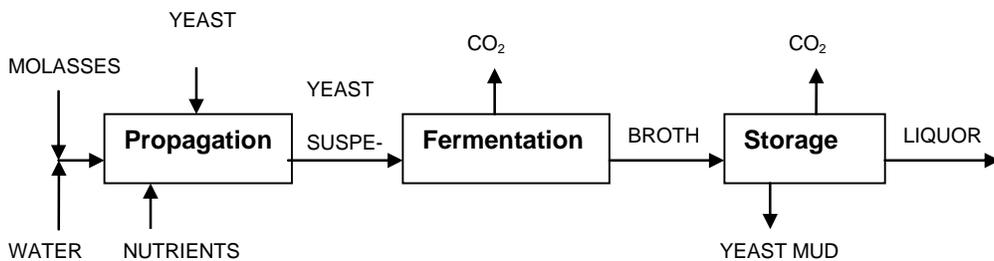


Figure 9. Principle scheme of industrial fermentation of molasses.

The initial sucrose concentration in the feed is below 20% and the ethanol concentration obtainable by fermentation is limited by ethanol's toxicity to yeast. The most ethanol-tolerant strains of yeast can survive up to approximately 15 vol% ethanol in the broth. When the fermentation is over, yeast biomass is removed leaving fermentation liquor from which ethanol must be separated. The established separation process employs distillation to obtain water-ethanol mixture in which ethanol content is limited to 95–96% due to the formation of a water-ethanol azeotrope. This may be used as fuel alone but unlike anhydrous ethanol it is immiscible in petrol. In order to obtain a product which can be used in combination with petrol as an engine fuel, the water fraction is typically removed in further treatment (dehydration).

The byproduct of distillation, called vinasse, is a liquid with about 7% DS, rich in organic compounds and potassium and calcium salts with exact composition dependent on the kind of raw material fermented (raw juice, diluted thick juice, mother syrup from crystallization or molasses). For the vinasse derived from molasses, the established practice includes

concentration by evaporation and subsequent marketing for use as fodder additive, fertilizer or raw material for biogas production (Cortez and Brossard Perez, 1997; Fernandez et al., 2009). Vinasse concentrated to 60–70% DS can also be used for co-firing with natural gas or fuel oil for steam generation; alternatively, it can be burned in a specially designed firebox leaving a residue from which potash can be recovered.

In order to reduce the energy demand of separation and simultaneously intensify fermentation, research is conducted on other separation methods that could partly replace energy intensive distillation. Among promising options, one can name gas stripping or membrane separation in gas phase (pervaporation) coupled with fermentation, alternatively extractive fermentation, that is, liquid extraction coupled with fermentation (Cardona and Sanchez, 2007).

Regarding dehydration, it is typically carried out as a physical adsorption process using a molecular sieve. Molecular sieves specially developed for ethanol dehydration are commercially available. In this process, the mixture of ethanol and water vapor under pressure passes through a bed of molecular sieve particles. The particles have the property of allowing adsorption of water while excluding ethanol. After a period of time, the bed is regenerated under vacuum or in the flow of inert atmosphere (e.g. nitrogen) to remove the adsorbed water. Ethanol content of 99.95% in the dehydrated product can be attained.

Ways to reduce the overall energy demand of fuel ethanol production by modifying the dehydration step are under investigation. An interesting option is to apply pervaporation which can be coupled with the preceding distillation step (Cardona and Sanchez, 2007).

As was indicated above in a review of beet processing procedures, a beet-sugar factory produces a number of material streams which can be processed to ethanol (Figure 10). This constitutes a basis for various concepts of coupling between sugar production and ethanol production (Hinkova and Bubnik, 2001; Hempelmann, 2007). The idea of small-scale molasses-based distilleries connected with sugar factories is as old as is the industrial sugar production but on a really large scale, ethanol production coupled with cane sugar production has been implemented in Brazil since 1970's (Sandalow, 2006). Stimulated by the Brazilian example and supported by research on the most economic options of process integration (Schweitzer, 2006; Henke et al., 2006; Krajnc and Glavic, 2009), the coupling of ethanol and beet sugar production is practiced in Europe where a number of large sugar beet-based ethanol plants were recently put into operation. In the British factory Wisington, residual streams from sugar crystallization are processed to ethanol, betaine and feed additives (British Sugar, 2010). In the factory Klein Wanzleben, Germany, ethanol is produced from raw juice during the beet campaign and from stored thick juice during the inter-campaign period (Seemann and Boelcke, 2008; Harten, 2009). Another German sugar factory in Anklam uses fermentation raw material prepared from thick juice taken either directly from beet processing, or from storage (Praj Industries, 2008).

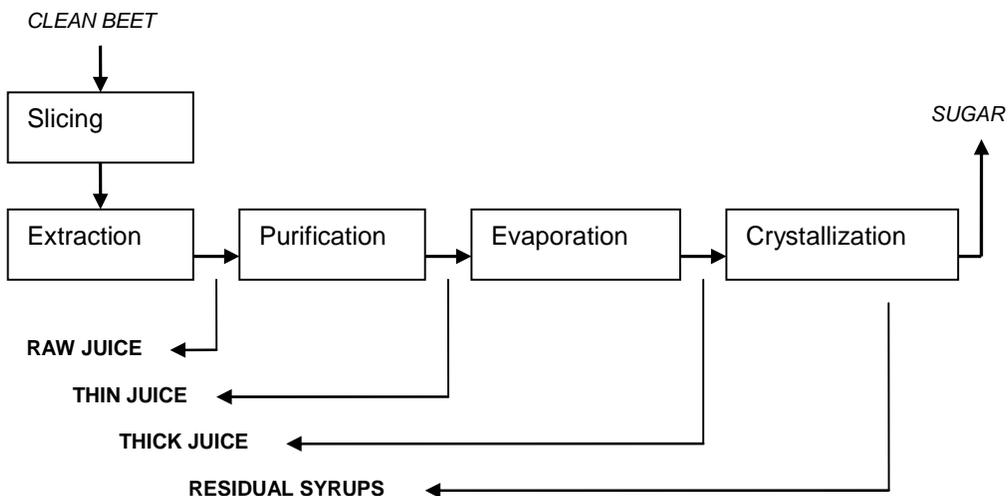


Figure 10. Material streams generated in sugar production from sugar beet and potentially available for fermentation. Residual syrups include run-off syrups and molasses.

In 2009, the global bioethanol output was 73.7 billion l of which about 60% produced from cereals, about 37% from sugar cane and less than 3%, or about 2.0 billion l, from sugar beet ((S&T)<sup>2</sup> Consultants Inc, 2009). Further expansion of the production of ethanol from sugar beet is expected in Europe (Keil et al., 2009).

#### 4. SWEET SORGHUM TO BIOHYDROGEN

In this section of the current Chapter the utilization of sweet sorghum for biological hydrogen production is discussed. Sweet sorghum (*Sorghum bicolor*) has a great potential as an energy crop and becomes even more interesting when its production can be oriented towards the sustainable production of clean fuels, such as hydrogen (Stolten, 2010). It belongs to the C<sub>4</sub> family, it has a high photosynthetic efficiency, and is a heat- and drought-tolerant crop. One of the most important inherent characteristics of sweet sorghum is its high yields of 20–30 ton dry weight per ha (Figure 11A). Currently about 10% of sweet sorghum production in the USA is used for ethanol production (Corredor et al., 2006). In the EU there is currently no large commercial production of biofuels from sweet sorghum. However, it is regarded as an energy crop that has the potential to be instrumental in the medium-term future in the fulfilment of a robust biobased economy (EUBIA, 2010). The investigation of its adaptation in Southern Europe is in an advanced level (Dalianis, 1996; Curt et al., 1998; Dolciotti et al., 1998; Habyarimana et al., 2004; Karaoglanoglou et al., 2007), whereas trials in cooler northern regions are being recently conducted (Martínez-Pérez et al., 2007).



Figure 11. Sweet sorghum (A) on the field, (B) after removal of grains and leaves, (C) bagasse. Sweet sorghum bagasse before (D) and after (E) pretreatment with sodium hydroxide. The pretreatment with 10% NaOH (w/w dry matter) resulted in a delignification of 46%. (photo A by A. Glynos, Peloponnesos, Greece).

Sweet sorghum (Figure 11A,B) can be fractionated via sugar extraction of the stalks to a liquid fraction, rich in sucrose, the so-called juice, and a remaining solid fraction, sweet sorghum bagasse (Figure 11C). Traditionally, sweet sorghum juice has served as syrup. Utilization of sweet sorghum juice for ethanol production (Coble et al., 1984; Mamma et al., 1996) has been studied in the literature since the 1980s, and more recently significant research efforts have been addressed towards biological hydrogen production (Claassen et al., 2004; Ntaikou et al., 2008). Sweet sorghum bagasse is a lignocellulosic raw material, with approximately 60% of the total dry matter fermentable polysaccharides, 18% lignin and a low ash content of 4% (Panagiotopoulos et al., 2010). Sweet sorghum bagasse was previously used as animal feed or soil fertilizer (Negro et al., 1999). At present, it is mostly unexploited and its usages as fodder for animals or for energy production by combustion (Monti & Venturi, 2003) are not sufficiently viable solutions. Moreover, sweet sorghum bagasse contains 8% sucrose (Figure 12), which constitutes a considerable quantity of sugar available for ethanol/hydrogen production that is wasted if the bagasse is not used for biofuel production. Currently, the processing of sweet sorghum bagasse for hydrogen production has attracted interest. In this Chapter, critical features of hydrogen production from sweet sorghum are discussed, and examples from current research are used to show progress in the

field. The presented trends are drawn from a collaborative work (Claassen et al., 2010) by our research groups and others, and, in particular, focus is given on the processing of sweet sorghum bagasse for biological hydrogen production.

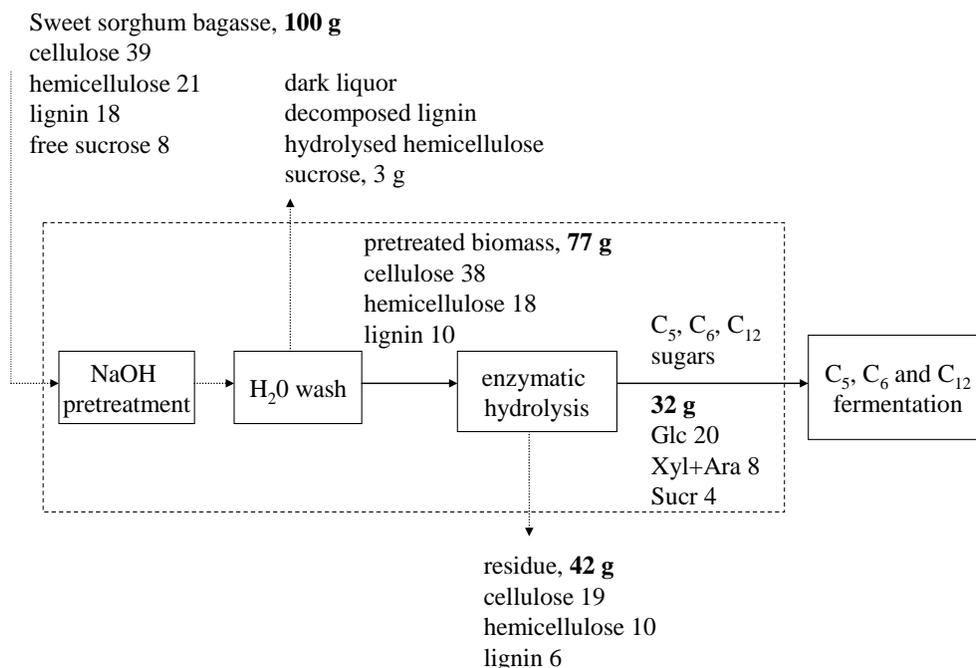


Figure 12. Flowsheet of chemical (NaOH) pretreatment and enzymatic hydrolysis of sweet sorghum bagasse for the production of fermentable substrates and (non)fermentable coproducts. (from: Panagiotopoulos et al., 2010).

Sweet sorghum juice consists mainly of soluble sugars (sucrose, glucose, fructose) that are readily fermentable. Relative percentages of each sugar are approximately 80%, 15%, and 5% for sucrose, glucose, and fructose, respectively. However, from our own work with processing of sorghum, it has become clear that the sugar composition of the juice is dependent on the origin of the material and/or the conditions of the processing. To give an example, the pressing of milled sweet sorghum results in 10–15% higher sugar yields when a pretreatment of the material at 70°C has been originally conducted. Under the influence of this thermal pretreatment, higher glucose and fructose production has been observed. This observation can be important in conjunction with the finding of a previous work of our groups (Claassen et al., 2004), according to which the extreme thermophilic bacterium *Caldicellulosiruptor saccharolyticus* has a preference on the consumption of sucrose over glucose when used in the fermentation of sweet sorghum juice for hydrogen production. A hydrogen yield on glucose of 2.3 mol/mol was obtained, which is equal to 58% of the maximal theoretical value. Another bacterium which seems suitable for efficient hydrogen production from sweet sorghum juice is the rumen bacterium *Ruminococcus albus*, which resulted in a hydrogen yield of 2.6 mol/mol glucose (Ntaikou et al., 2008). However, the latter fermentations took place at an initial sugar concentration of 3 g/l, compared to 25 g/l in the fermentations with *C. saccharolyticus*.

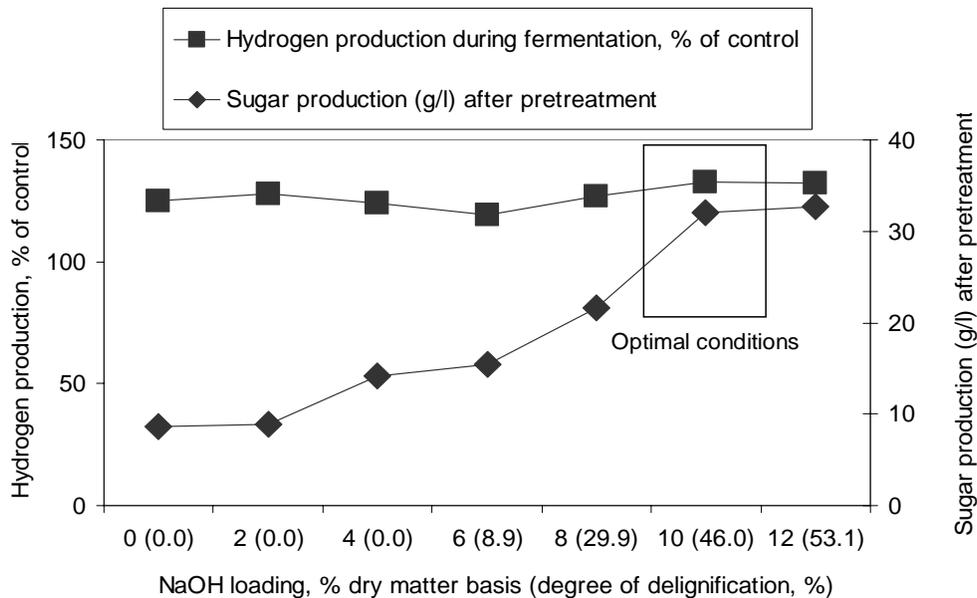


Figure 13. Optimization of the pretreatment of sweet sorghum bagasse based on the chemical (NaOH) loading (and concomitant degree of delignification), the production of fermentable sugars and the fermentability for hydrogen production. In control fermentations pure sugars were used.

Previous work of our groups has indicated that that the energy balance of the cultivation of sweet sorghum for hydrogen production can significantly improve if the remaining bagasse is fermented at a high hydrogen yield and productivity. Before fermentation takes place, chemical pretreatment of sweet sorghum bagasse is needed (Figure 11D,E), to enhance the accessibility of the carbohydrates of the bagasse for enzymatic hydrolysis and the production of fermentable substrates. The efficiency of hydrogen production from these substrates is dependent on their quality, which partly depends on the amount of degradation products which act as inhibitors in fermentations. In general, a successful pretreatment of the bagasse toward hydrogen production is characterized by simultaneous satisfaction of three criteria: mobilization of sugars, limitation of the release of fermentation inhibitors and preservation and/or easy recovery of nonfermentable components for valorization in the biorefinery (Figure 12). All of the aforementioned criteria are linked to the lignin of sweet sorghum bagasse. Lignin is generally not desirable in anaerobic fermentations because a) it is not degraded anaerobically and b) lignin that is chemically degraded, and possibly solubilized during pretreatment, is inhibitory to the growth of hydrogen-producing bacteria. On the other hand, the reduction in lignin content, achieved by mutations by some researchers, can be combined with undesirable decrease in sorghum yield (Pedersen et al., 2005). Therefore, optimization of the delignification can be performed towards high sorghum conversion efficiency (Figure 13). Through this methodology, the use of chemicals, such as NaOH (de Vrije et al., 2002) or  $\text{Ca}(\text{OH})_2$  (Kim and Holtzapple, 2005), to achieve delignification can be conducted in a rational way, and, simultaneously, natural characteristics of the plant are not disturbed. Recently, results on the delignification of *Miscanthus*, which has similar composition with sweet sorghum bagasse, were published (de Vrije et al., 2009). The delignification achieved with NaOH was substantial and comparable with the reports of Maas

(2008) and Panagiotopoulos et al. (2010), whereas the delignification with  $\text{Ca}(\text{OH})_2$  was found insignificant.

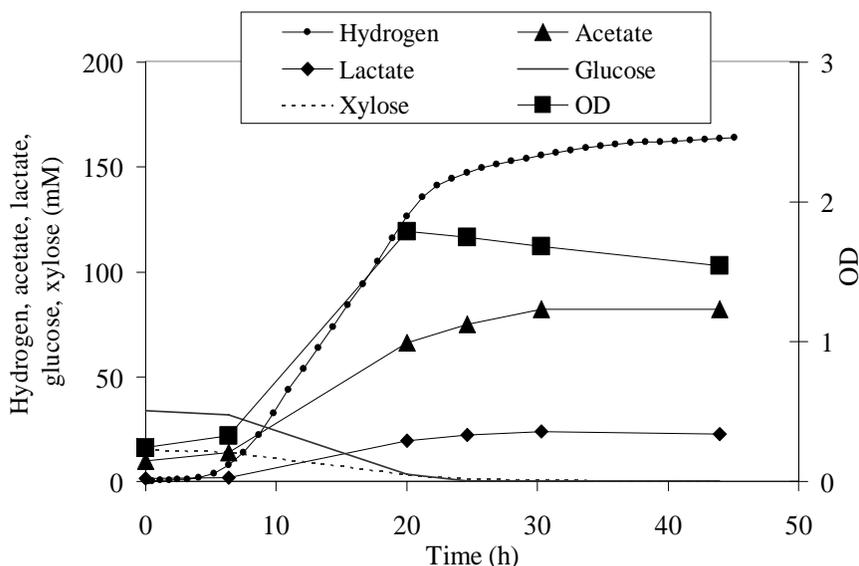


Figure 14. Growth (squares), production of hydrogen (circles), acetate (triangles) and lactate (diamonds), and consumption of glucose (solid line) and xylose (dashed line) in cultures of *C. saccharolyticus* grown on the hydrolysate of sweet sorghum bagasse.

Fermentation of sweet sorghum bagasse can be investigated under uncontrolled or controlled conditions. Our methodology is to originally perform fermentations without control of pH and hydrogen pressure. These experiments, known as fermentability tests (Panagiotopoulos et al., 2009), are rapid tests that evaluate the fermentability of the bagasse for hydrogen production and are often used to optimize the pretreatment of lignocellulosic materials, as described in previous paragraph. This paragraph discusses the fermentative production of hydrogen from sweet sorghum bagasse in batch fermentations under pH and hydrogen pressure controlled conditions. To evaluate hydrogen production efficiency the fermentations took place at neutral pH and relatively low partial hydrogen pressures. It has been reported (van Niel et al., 2003) that in fermentations with *C. saccharolyticus* high partial hydrogen pressures (>10 kPa) may result in a metabolic shift from hydrogen and acetate towards lactate, which is undesirable. Therefore, nitrogen is usually used as stripping gas, whereas other gases, such as  $\text{CO}_2$  (Willquist et al., 2009) may be interesting. Apart from hydrogen, acetate and lactate (Figure 14),  $\text{CO}_2$  was also produced in the fermentations. A key parameter used to characterize hydrogen production efficiency is hydrogen yield, which is expressed as mol hydrogen per mol C6 sugar. The results showed that sweet sorghum bagasse is fermentable and the extent of the fermentability depends on the initial sugar concentration of the culture medium. Specifically, in fermentations on 10 g sugar/l hydrogen yield was 2.6 mol per mol C6 sugar and was comparable to that of paper sludge hydrolysate (Kádár et al., 2004) with similar sugar concentration. At higher substrate concentrations (20 g sugar/l) the production of lactate increased at the expense of hydrogen production. Another critical parameter is hydrogen productivity, which is usually used in its volumetric version and

expressed as mmol hydrogen per l culture medium and h of fermentation. The maximum volumetric hydrogen productivity is typically calculated from the time interval with the highest percentage of hydrogen in the off-gas of the fermentation. For sugar concentrations of 10 and 20 g/l the maximum volumetric hydrogen productivities were comparable to a similar experiment with *Miscanthus* (de Vrije et al., 2009) on 14 g sugar/l. Table 4 provides a summary of the most recent studies dedicated to produce hydrogen with *C. saccharolyticus*, which is regarded as one of the most interesting bacteria due to its superiority with respect to hydrogen yield (de Vrije & Claassen, 2003; Hallenbeck, 2005; Jones, 2008; Panagiotopoulos et al., 2010) and range of utilizable substrates (Blumer-Schuetz et al., 2008; Panagiotopoulos et al., 2009). To complete the discussion, the use of *Ruminococcus albus* in fermentations of sweet sorghum bagasse at an initial sugar concentration of 3 g/l has also been reported (Ntaikou et al., 2008). Notable is that, to our knowledge, the last time that hydrogen production from the aforementioned bacterium was studied, was in 1973 by Iannotti et al. and Miller and Wolin, before it attracts again attention nowadays with sweet sorghum.

**Table 4. Hydrogen yields and production rates from sweet sorghum and similar raw materials, with the extreme thermophilic bacterium *Caldicellulosiruptor saccharolyticus*.**

Biomass	Substrate	g sugar/l	$Y_{H_2}$ mol/mol C6	max. $Q_{H_2}$ mmol/(l · h)	Reference
Sweet sorghum bagasse	Glucose, xylose, sucrose	10	2.6	10.6	Panagiotopoulos et al., 2010
Sweet sorghum bagasse	Glucose, xylose, sucrose	20	1.3	10.2	Panagiotopoulos et al., 2010
Sweet sorghum juice	Sucrose, glucose, fructose	25	2.3		Claassen et al., 2004
Molasses	Sucrose	15	2.1	7.1	Özgür et al., 2010
Miscanthus	Glucose, xylose	14	3.3	10.4	de Vrije et al., 2009
Paper sludge	Glucose, xylose	8	2.0-2.1	5.3-6.0	Kádár et al., 2004
Artificial medium	Glucose, xylose	10	3	17	Zeidan and van Niel, 2009

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*Chapter 3*

## **ALFALFA BIOMASS PRODUCTION AND QUALITY DURING CUTTING REGROWTH CYCLE UNDER ELEVATED CO<sub>2</sub>**

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### **ABSTRACT**

Increasing atmospheric CO<sub>2</sub> results in enhanced photosynthesis in C<sub>3</sub> plants like alfalfa. However, after long-term exposure, the photosynthetic rate decreases. This phenomenon, often described as down-regulation, is explained by most authors as the consequence of the disappearance of strong plant sinks leading to leaf carbohydrate accumulation and thus resulting in a photosynthetic decrease. The initial photosynthesis response to elevated CO<sub>2</sub> induces plant growth and enhanced yield production. After long term CO<sub>2</sub> exposure, when photosynthesis is acclimated, increased plant biomass is also shown due to the initial enhancement of plant dry matter. Management of alfalfa as a forage crop entails periodic cutting of shoots. In this situation, photosynthetic down-regulation is avoided and the alfalfa taproot is the main source organ that provides C and N compounds to new growing shoots. This source and sink organ role inversion allows us to study alfalfa biomass production before and one month after cutting (regrowth). Alfalfa is used as a forage crop for animal feeding as a source of protein and amino acids; therefore not only is the quantity of crop production important but also the biochemical composition of shoots is a key factor. During the present study, elevated CO<sub>2</sub> reduced leaf protein concentration probably due to the dilution effect derived from starch accumulation in these conditions. Forage plants may also be the primary source of antioxidants. Elevated CO<sub>2</sub> altered reactive oxygen species (ROS) production in leaves, reducing their production, and resulted in the relaxation of the antioxidant system, which may induce changes in the antioxidant value of forage biomass.

## INTRODUCTION

Rising global population will entail an increased demand for food and energy. This, together with the uncertainty of fossil fuels reserves, makes it urgent for plant biology research to improve yield and quality. In the context of global climate change, which may limit production due to stress factors such as rising temperatures, drought episodes, etc., it is specially outstanding the higher vegetable requirement. In this sense it is essential that research concerning forage production and quality in crops like alfalfa is carried out, as this is the key for livestock feeding.

Economic development and progressive industrialization has increased the atmospheric CO<sub>2</sub> concentration from 280  $\mu\text{mol mol}^{-1}$  in 1750, the preindustrial period, to 387  $\mu\text{mol mol}^{-1}$  currently (Keeling et al., 2009). These values have been recorded since 1958 in the Mauna Loa observatory in Hawaii. Nevertheless, this observatory, as at other global monitoring sites, samples air at high elevations away from anthropogenic sources, and actual ground-level CO<sub>2</sub> concentration can be significantly higher as a result of urbanization (Idso et al., 1998).

In 1988 the United Nations Environment Programme (UNEP) and the World Meteorological Organization (WMO) established the Intergovernmental Panel on Climate Change (IPCC) to provide the world with a clear scientific view on the current state of climate change and its potential environmental and socio-economic consequences. Predictions of future atmospheric CO<sub>2</sub> concentration are calculated with integration of both natural and anthropogenic sources of the gas. Calculations include variables of economic growth and demography. The IPCC predicts that the CO<sub>2</sub> concentration may be between 660 and 790  $\mu\text{mol mol}^{-1}$  from 2060 to 2090 (IPCC, 2007). With the increase in greenhouse gases the models project a global surface temperature rise from 1.4 to 5.8 °C over the period 1990 to 2100.

Plants are sensitive to factors such as CO<sub>2</sub> and temperature, which are implicated in global change. The effect of doubled CO<sub>2</sub> atmospheric concentration in plants has been described extensively. Plant growth stimulation is the most common response to elevated CO<sub>2</sub> in the literature. At the current CO<sub>2</sub> concentration, ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) is not saturated (Drake et al., 1997). This enzyme catalyzes both CO<sub>2</sub> and O<sub>2</sub> fixation and despite its low affinity for CO<sub>2</sub> (Bowes, 1993), the increase in CO<sub>2</sub> concentration will favour carboxylation relative to oxygenation.

Due to Rubisco's response to high CO<sub>2</sub>, doubling the gas concentration may enhance tiller (Ziska et al., 2004) and leaf formation, leading to promoted growth and size in C<sub>3</sub> (Bazzaz, 1996) and some C<sub>4</sub> species (Ziska and Bunce, 1997). However, elevated CO<sub>2</sub> not only affects shoots but increases root growth (Ziska et al., 1996) and morphology, enhancing root diameter and cortex width (Rogers et al., 1992). Under elevated CO<sub>2</sub> the limitation on other essential nutrients may take place so the new fixed C is also transported to roots in order to improve mineral uptake and satisfy the demand from shoots. In this sense, a review of the root/shoot (R/S) ratio in crops showed a significant increase in this parameter (about 60%) in studied species (Rogers et al., 1994).

However, the photosynthetic response to elevated CO<sub>2</sub> usually decreases under long-term exposure, and plants acclimate to elevated CO<sub>2</sub> concentrations (Sage et al., 1989). This acclimation process is often referred to as "down-regulation" (Saralabai et al., 1997). This reduction in photosynthetic efficiency may be caused by stomatal closure phenomena

(Sánchez-Díaz et al., 2004) or by a reduced amount/activity of rubisco (Long et al., 2004). Under elevated CO<sub>2</sub> conditions, non-structural carbohydrates accumulate and the expression of genes encoding for the photosynthetic apparatus is suppressed through the possible increased hexose cycling within the leaf, resulting in decreased photosynthetic capacity and a notable decrease in the amount of rubisco (Drake et al., 1997; Moore et al., 1999).

As mentioned above, increasing CO<sub>2</sub> reduces the stomatal conductance at the cellular and biochemical level (Assman, 1999). Elevated CO<sub>2</sub> generally decreases stomatal conductance but without limitation of photosynthesis (Drake et al., 1997). The available literature about CO<sub>2</sub> agrees that this effect on conductance and transpiration results in increased water use efficiency (WUE) of plants (Jones, 1998). The WUE is a quantitative measure of the gas exchange of the leaf and is defined in physiological terms as the ratio between the rate of C fixed and the rate of water transpired. In agronomic terms it has been defined as the ratio of dry matter production to water use (Boyer, 1996), and is usually referred to as water use efficiency of productivity (WUEp). This parameter may take into account the dry matter production and water consumption over the entire growing season which is more informative than temporary gas exchange ratios and suitable for ecological, agricultural and forestry applications (Larcher, 2003).

Alfalfa (*Medicago sativa* L.) is one of the most widely distributed forage crops in the world. Its cultivation began 9000 years ago on the Caucasian plateau, Iran and Turkey (Bolton, 1962) and these days is adapted to grow from the equator to almost Arctic Circle regions (Michaud et al., 1988). Alfalfa is recognized as an important source of protein and amino acids, especially those considered essential for animal feeding (Mauriès, 1994).

The field management of alfalfa includes defoliation by periodic cutting and removal of shoots. The subsequent regrowth phase, like other periods of intensive growth such as spring growth or seed development, requires the mobilization of C and N reserves accumulated previously in specific storage organs. In alfalfa, N reserves in the roots have been shown to be very important after partial or total removal of the photosynthetic area (Bewley, 2002). Root C reserves are mainly used for respiration in the remaining organs (Avice et al., 2003; Ta et al., 1990), and contrasts with the view that shoot regrowth is carbohydrate driven (Volenc et al., 1996). The N reserves, however, are used to support shoot regrowth (Avice et al., 2003; Barber et al., 1996). Since defoliation causes great reduction of mineral N uptake and symbiotic N<sub>2</sub> fixation (Ourry et al., 1994), the mobilization of stored N is the key to providing N to the new regrowing shoots during the first days of cutting (Avice et al., 2003).

Atmospheric CO<sub>2</sub> concentration increases alter photosynthesis, respiration, and therefore affect plant growth. During the plant growth cycle, reactive oxygen species (ROS), such as the superoxide anion (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and the hydroxyl radical (OH<sup>•</sup>) are continuously produced as a consequence of aerobic metabolism (Foyer and Harbinson, 1994). Under physiological steady-state conditions, these molecules are scavenged by different antioxidant defence components (Alscher et al., 1997). However, the equilibrium between production and scavenging of ROS may be perturbed by adverse environmental factors, and as a consequence intracellular levels of ROS may rise rapidly (Polle, 2001). Plant growth under elevated CO<sub>2</sub> may be enhanced but simultaneously coupled to higher metabolic ROS production. Contrariwise, other authors argue that increasing CO<sub>2</sub> may lead to a reduction in the basal rate of oxygen activation, and consequently, a decrease in ROS formation (Halliwell and Guteridge, 1989). This situation results in a relaxation of the antioxidant defence system of the plant cell (Badiani et al., 1993). ROS detoxification includes the major cellular redox

buffers ascorbate and glutathione (Apel and Hirt, 2004), which are known antioxidant substrates that are usually supplemented in forage diets.

As elevated CO<sub>2</sub> and temperature may enhance plant growth, higher forage production may be available under climate change conditions. Nevertheless the biochemical characteristics of plant tissues may be altered. In this chapter, parameters related to plant biomass production and leaf biochemistry are presented as a first approach to the effects of elevated CO<sub>2</sub>, temperature and water availability during the cutting regrowth cycle of alfalfa.

## ALFALFA BIOMASS PRODUCTION UNDER CLIMATE CHANGE CONDITIONS

Alfalfa plants were grown in two temperature gradient greenhouses (Aranjuelo et al., 2005) and divided into 8 treatments combining two CO<sub>2</sub> levels (ambient, around 350  $\mu\text{mol mol}^{-1}$  and 700  $\mu\text{mol mol}^{-1}$ ), two temperature regimes (ambient and ambient + 4°C), and two water availability conditions (well-irrigated, control and partially irrigated, drought). Drought treatments were kept at 50% of the maximum soil volumetric water content. Three harvests were carried out during the experiment. The first harvest was performed after one month in the greenhouses (60-day-old plants), and plants were cut to a 5 cm plant height. The second harvest was performed 15 days after cutting (15-day regrowth), and the third 30 days after cutting (30-day regrowth).

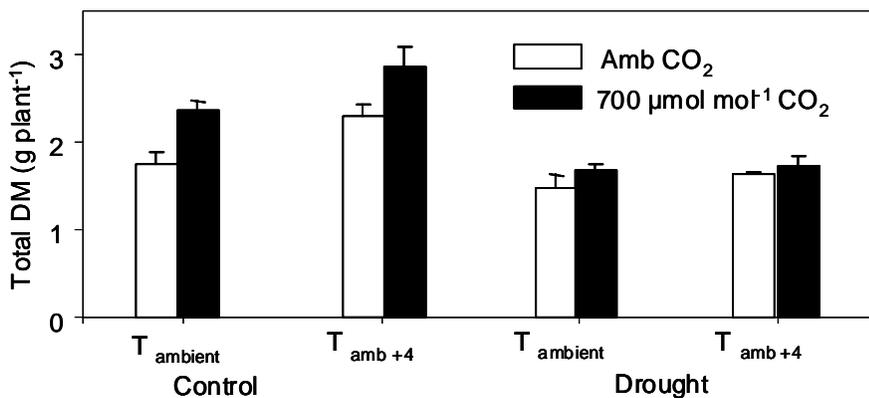


Figure 1. Effect of CO<sub>2</sub>, temperature and water availability on Total DM in nodulated alfalfa after one month of growth in the temperature gradient greenhouses at the end of the pre-cutting period.

At the end of one month of growth in the temperature gradient greenhouses, elevated CO<sub>2</sub> enhanced plant growth in optimum water conditions with a positive interaction with elevated temperature (Fig. 1). It is well known that the effect of elevated CO<sub>2</sub> in stimulating plant growth depends on temperature (Idso and Kimball, 1989). The CO<sub>2</sub> effect is usually greater at elevated temperature (Aranjuelo et al., 2006). Nevertheless, under drought conditions, no effect of CO<sub>2</sub> was observed (Fig. 1). As expected, low water availability reduced alfalfa total

dry matter (DM) production by 30% (Fig. 1). Elevated atmospheric carbon supply and water stress may affect dry matter partitioning between the aboveground and belowground organs, resulting in decreased root/shoot ratio.

Differences in the response of aboveground and belowground organs to environmental factors were observed (Erice et al., 2006a). Thus, at the end of one month of growth in the temperature gradient greenhouses, elevated CO<sub>2</sub> increased aboveground organ DM (leaf and stem) (Fig. 2), but not the DM of below ground components (root and nodule) (Fig. 2). The response of DM partitioning to elevated CO<sub>2</sub> shows no clear behaviour in the available literature but Harmens et al. (2000) observed a transient increase in DM allocation into the shoot during the early stages of *Dactylis glomerata* growth under low nitrogen supply at elevated CO<sub>2</sub> concentrations. Nodule DM showed no positive response to rising CO<sub>2</sub> (Fig. 2), and is consistent with the responsiveness of root mass to CO<sub>2</sub> enrichment being smaller in nodulated soybean plants than in non-nodulated soybean plants (Ainsworth et al., 2004). However, under elevated CO<sub>2</sub> conditions it is well known that additional carbohydrate is available (Erice et al., 2006b) and it may be used to increase specific nodule N<sub>2</sub>-fixing activity.

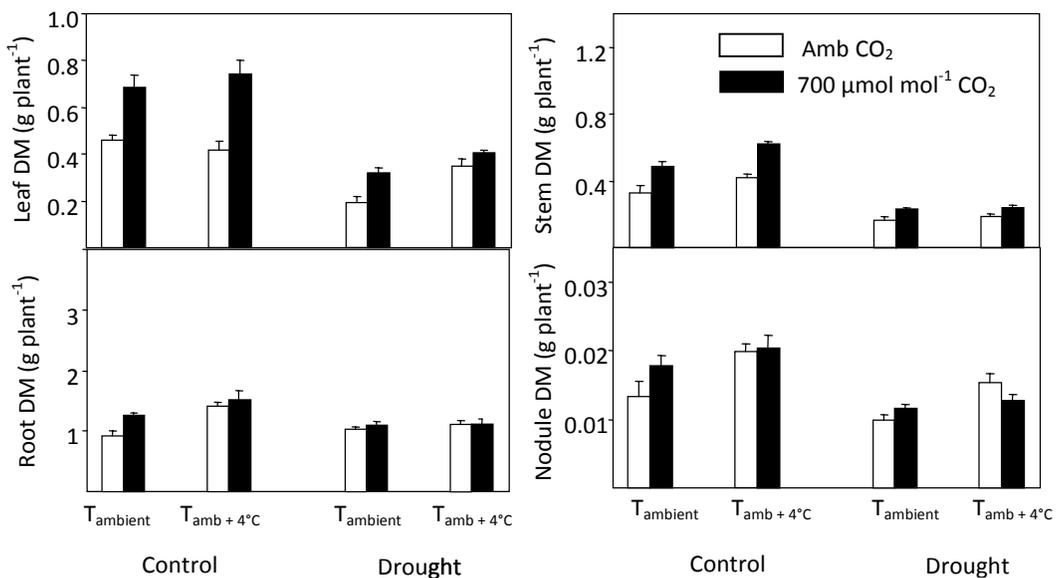


Figure 2. Effect of CO<sub>2</sub>, temperature and water availability on leaf, stem, root and nodule DM in nodulated alfalfa after one month of growth in the temperature gradient greenhouses at the end of the pre-cutting period.

After one month in the temperature gradient greenhouses the aboveground parts of the plants were cut at 5 cm stem height. The remaining organs (shoots, taproots and nodules) were maintained in the greenhouses for a further month under the same treatments in order to develop new shoots (regrowth). As mentioned above, in pre-cutting results, when plants were cut, it seemed that elevated CO<sub>2</sub> interacted positively with temperature at the end of regrowth period (Fig. 3).

As in the preceding growth period, 30 days after cutting, elevated atmospheric CO<sub>2</sub> concentration enhanced DM accumulation in aboveground organs (Fig. 4). However, contrary

to the observation in the pre-cutting period, root DM also increased, especially under elevated temperature conditions (Aranjuelo et al., 2006; Erice et al., 2006a), showing the positive interaction between these two factors. It is well known that temperature modifies the CO<sub>2</sub> effect (Idso and Kimball, 1989), and it is greater at higher temperatures or even negative at low temperatures (Rawson et al., 1995).

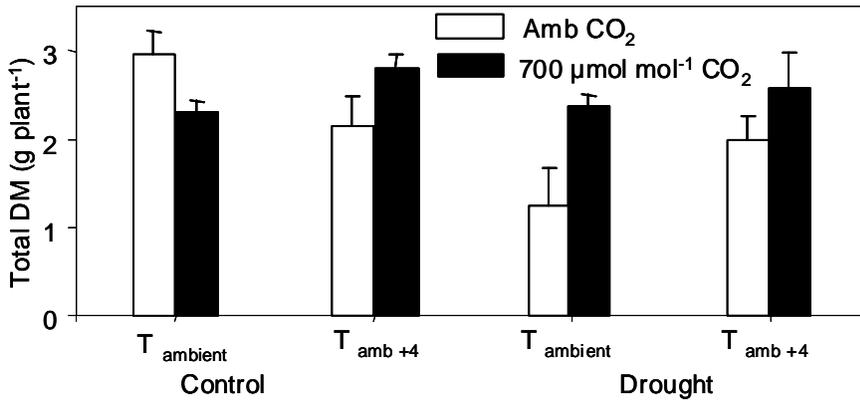


Figure 3. Effect of CO<sub>2</sub>, temperature and water availability on Total DM in nodulated alfalfa after two months of growth in the temperature gradient greenhouses at the end of the regrowth period.

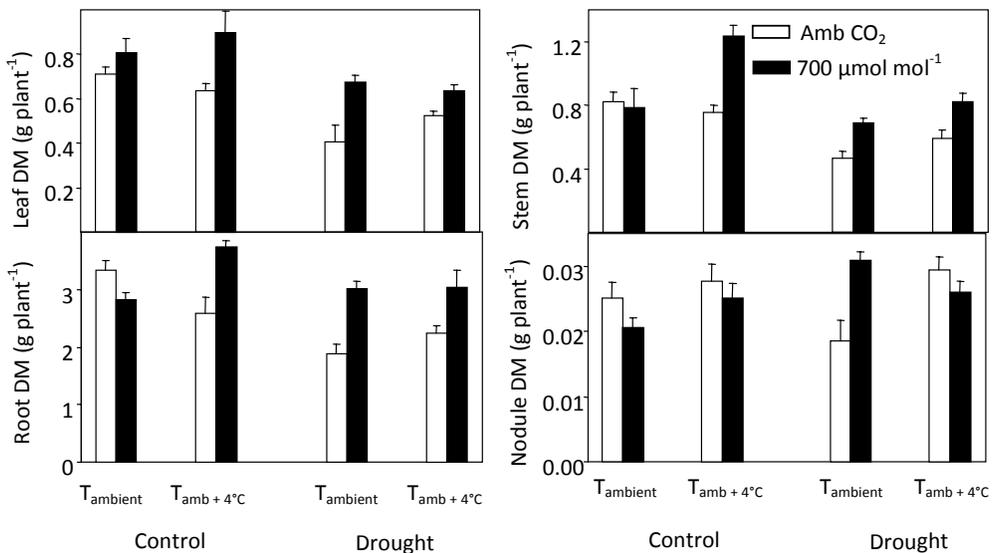


Figure 4. Effect of CO<sub>2</sub>, temperature and water availability on leaf, stem, root and nodule DM in nodulated alfalfa after two months of growth in the temperature gradient greenhouses at the end of the regrowth period.

At the end of the pre-cutting harvest as well as 30 days after cutting, drought reduced both aboveground and belowground DM, with a greater decrease shown by aboveground components (Fig. 4). Shoot and root biomass is affected differently by water shortage, and shoots are generally more sensitive. Some authors argue that transpiring parts of the plant

usually develop higher water deficits (Kramer and Boyer, 1995; Nielsen and Halvorson, 1991).

One of the most widely reported effects of elevated CO<sub>2</sub> is the reduction in stomatal conductance (Wall et al., 2000). This is an indirect effect of CO<sub>2</sub> and may be especially important in drier ecosystems, when soil water limits plant growth (Morgan et al., 2004). This stomatal closure often results in increased water use efficiency (WUE). WUE is a parameter which may be altered by elevated CO<sub>2</sub> due to decreased transpiration and/or increase in DM accumulation (Rogers et al., 1996). In the present study, in order to compare WUE at the leaf and whole plant levels, two parameters were analysed: water use efficiency of productivity (WUE<sub>p</sub>) and water use efficiency of photosynthesis (WUE<sub>ph</sub>) (Larcher, 2003). WUE<sub>p</sub> was calculated as the ratio of produced DM to the water consumption in a period of time and WUE<sub>ph</sub> was analysed as the relationship between photosynthesis and transpiration from measurements of leaf gas exchange. WUE<sub>p</sub> was calculated for the pre-cutting and regrowth periods while WUE<sub>ph</sub> was measured at the end of pre-cutting and at the regrowth periods.

During the pre-cutting growth period, WUE<sub>p</sub> was increased by high CO<sub>2</sub> and low water availability (Fig. 5), as reported previously (Aranjuelo et al., 2006). This increase was due to higher DM production (Fig. 5) and lower water consumption, as was also shown by a lower specific transpiration in the case of elevated CO<sub>2</sub> (Erice et al., 2006a). At the end of this period, the increase in WUE<sub>ph</sub> was related to decreases in leaf conductance (Erice et al., 2006a; 2006b). During the regrowth period, WUE<sub>p</sub> was increased by CO<sub>2</sub> due to DM accumulation and not by reduction of transpiration in control plants (Erice et al., 2006a) whereas, enhanced WUE<sub>p</sub> in droughted plants could be explained by the reduced leaf area with no changes in specific transpiration as reported for the pre-cutting period (Erice et al., 2006a). At the end of the regrowth period, the increased WUE<sub>ph</sub> under elevated CO<sub>2</sub> and elevated temperature (Fig. 5) was not due to increased net photosynthesis but was due to decreased leaf conductance (Erice et al., 2006a).

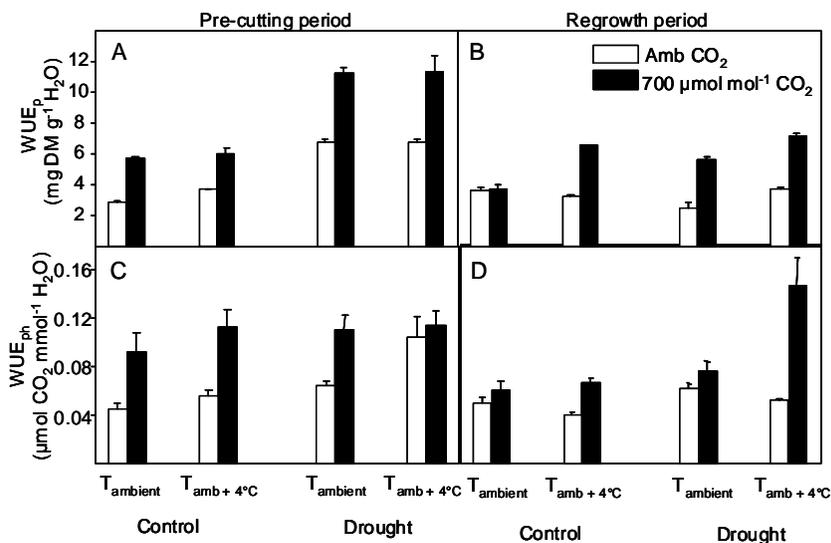


Figure 5. Effect of CO<sub>2</sub>, temperature and water availability on water use efficiency of productivity (WUE<sub>p</sub>) and water use efficiency of photosynthesis (WUE<sub>ph</sub>) at the end of the pre-cutting period (A and C) and after 30 days of regrowth (B and D).

## ALFALFA LEAF BIOCHEMISTRY UNDER CLIMATE CHANGE CONDITIONS

Alfalfa is one of the most important sources of proteins, with more protein content per hectare than soybean, another plant commonly used for animal protein feeding (Mauriès, 1994). Alfalfa produces large quantities of amino acids and all amino acids are present in the nitrogen mass of alfalfa, especially those considered essential for animal feeding (Mauriès, 1994).

As shown before, atmospheric CO<sub>2</sub> enrichment typically enhances growth and DM accumulation (Drake, 1992; Poorter et al., 1996) but as Goldman et al. (1999) have noted “with the burgeoning consumer interest in foods that optimize health, attention is shifting from concerns over the constituents of foods that may promote health”. One of the most reported consequences of rising atmospheric CO<sub>2</sub> related to the quality of animal forage is decreased foliar nitrogen (Luo et al., 1994; Biswas et al., 1996). Cotrufo et al. (1998) found that most of the experiments (82%) related to this subject reported a reduction in plant N concentration under enriched CO<sub>2</sub> conditions.

This N depletion (Bowes, 1993; Schenk et al., 1995) is probably due to a dilution phenomenon derived from carbohydrate accumulation (Campbell et al., 1988; Luo et al., 1994; Erice et al., 2006b). Another factor that may influence this effect is nitrogen translocation to other parts of the plant in order to increase the sink size and/or activity and avoid C accumulation (Hocking and Meyer, 1991; Conroy and Hocking, 1993). In our case, leaf total soluble protein (TSP) content was decreased by elevated CO<sub>2</sub> after one month of growth in the temperature gradient greenhouses (pre-cutting period) (Table 1). This reduction was concomitant with starch enhancement. Contrary to this, the other non structural carbohydrates measured, total soluble sugars, showed no differences under elevated CO<sub>2</sub>.

The high leaf starch concentration is a widely reported characteristic of plants grown under elevated CO<sub>2</sub> (Erice et al., 2006b). Some authors argue that this starch enrichment in leaf tissue is the main cause of CO<sub>2</sub>-induced nitrogen concentration reduction (Huber et al., 1984; Vu et al., 1989; Rowland-Bamford et al., 1990; Kuehny et al., 1991; Baxter et al., 1994; 1995). But there are other factors that may be responsible for the reduced nitrogen observations, in addition to the biomass dilution effect (Norby et al., 1986; O’Neill et al., 1987; Conroy et al., 1990) such as limitations in Calvin cycle enzymes (Besford, 1990; Besford et al., 1990; Stitt, 1991) due to the reduced carboxylation enzyme requirement in CO<sub>2</sub> enriched air (Roumet et al., 1996).

**Table 1. Effect of CO<sub>2</sub>, temperature and water availability on leaf starch, total soluble sugars (TSS) and total soluble protein (TSP) concentration in nodulated alfalfa at the end of the pre-cutting period and after 30 days of regrowth.**

Treatments H <sub>2</sub> O - CO <sub>2</sub> - T			Pre-cutting			30 days of Regrowth		
			Starch (mg g <sup>-1</sup> DM)	TSS (mg g <sup>-1</sup> DM)	TSP (mg g <sup>-1</sup> DM)	Starch (mg g <sup>-1</sup> DM)	TSS (mg g <sup>-1</sup> DM)	TSP (mg g <sup>-1</sup> DM)
Control	Amb	Amb	35.1±0.6	57.3±1.9	171.6±7.9	51.7±5.4	39.4±1.3	167.8±6.5
“	700	Amb	268.2±9.3	42.6±1.7	138.6±2.2	161.9±4.4	39.5±1.2	190.0±1.4
“	Amb	Amb+4°C	158.5±15.8	63.7±8.1	123.8±2.5	79.2±5.4	46.7±3.2	165.5±3.7
“	700	Amb+4°C	239.5±7.9	54.8±5.0	92.8±3.9	166.7±2.9	35.6±2.2	159.7±9.8
Drought	Amb	Amb	46.7±2.4	21.5±8.8	161.7±4.6	59.6±6.6	32.7±1.6	200.4±3.8
“	700	Amb	269.6±3.4	29.9±2.6	159.3±8.4	116.6±17.7	34.9±2.7	158.8±13.9
“	Amb	Amb+4°C	108.2±6.3	42.8±2.9	131.7±5.9	82.1±17.4	39.7±4.4	220.8±3.3
“	700	Amb+4°C	317.4±17.1	33.5±5.0	103.0±6.2	105.8±15.2	35.0±2.2	177.3±11.4

As a consequence of reduced leaf N concentration under elevated CO<sub>2</sub> it may be possible to observe the depletion in leaf protein concentration (Idso and Idso, 2001). This situation is particularly interesting because alfalfa is commonly used as a protein source for animal feeding. For that reason some authors consider that the consumption of this forage produced under elevated CO<sub>2</sub> may have deleterious effects on both wild and domestic ruminants (Owensby et al., 1996) and insect herbivores (Lindroth, 1996). Nevertheless, the reduced soluble protein concentration is not universally observed and some theories to this respect have been developed. A first observation is that protein depletion due to high CO<sub>2</sub> is observed much less in experiments conducted in the field (Idso et al., 1996; Zanetti et al., 1996). The basis for this result apparently is related to unrestricted root limitation in these conditions allowing to more fully explore the soil profile in search of needed nutrients (Bowler and Press, 1993; Stulen and den Hertog, 1993). The plants in our experiments were grown in 13 L pots to avoid becoming pot-bound. This is particularly important because the limitation to root development may be one of the causes for photosynthetic acclimation to elevated CO<sub>2</sub> (Erice et al., 2006a).

The drop in leaf protein due to a CO<sub>2</sub> enriched atmosphere has also been related to plants without actively growing organs (Vivin et al., 1996). In our study, plants were 60-days-old, and after one month in the temperature gradient greenhouses they were acclimated to CO<sub>2</sub> (Erice et al., 2006a; 2006b) and reaching the first stages of flowering.

However, after cutting, photosynthetic acclimation disappeared in alfalfa plants (detected as no changes in photosynthetic rates,  $V_{cmax}$  or rubisco total activity) (Erice et al., 2006b) and significant decreases in TSP and increased starch concentrations were observed (Table 1). These plants also showed increased relative growth rates during all regrowth periods (Erice et al., 2007) under elevated CO<sub>2</sub>. After cutting, and in spite of a high growth rate with carbohydrate consumption, the leaf protein concentration decreased in alfalfa because the elevated photosynthetic rate led to a dilution phenomenon due to starch accumulation.

Another characteristic observed under elevated CO<sub>2</sub> that is not well understood is the plant antioxidant status. Knowledge about the effect of increasing CO<sub>2</sub> on plant antioxidants is scarce. Plant biomass is the primary source of antioxidants for certain animals (Wheeler et al., 1998) and it is important to know what effects an increase in air CO<sub>2</sub> concentration might have on the concentrations of these health-promoting compounds in edible plants tissues.

In studies conducted with alfalfa it has been shown that in the pre-cutting harvest, after one month of exposure to elevated CO<sub>2</sub>, the activity of plant antioxidant enzymes was reduced (Aranjuelo et al., 2008). This relaxation in antioxidant defences was also revealed in reduced antioxidant substrates such as glutathione and ascorbate. These results are in accordance with others that have shown a reduction in plant antioxidant compounds under high CO<sub>2</sub> (Schwanz and Polle, 1998; Niewiandomska et al., 1999). This generally accepted response has been interpreted as meaning that plants growing in elevated CO<sub>2</sub> usually experience less oxidative stress, thus needing less antioxidant protection (Idso and Idso, 2001).

Nevertheless, based on our previous results, the antioxidant response may be altered by plant growth during regrowth where different phases of growth were characterized (Erice et al., 2007). During the first 15 days of regrowth, plants develop new photosynthetic regions with the mobilization of C and N reserves from roots, mainly in the form of starch, amino acids and proteins (Avicé et al., 2003). During the second 15 days of regrowth, a less intense

growth phase results in the recovery of photosynthate fluxes from shoots to roots (Avice et al., 2003).

Results obtained after 2 weeks of regrowth relate the higher growth to an increased catalase and ascorbate peroxidase activity, despite no observed differences in superoxide dismutase or glutathione reductase (Fig. 6). These results suggest a higher ROS production associated with the growth enhancement induced by CO<sub>2</sub> enrichment. Nevertheless, in the last 2 weeks of regrowth, CO<sub>2</sub> did not affect the plant growth rate but significantly reduced catalase, superoxide dismutase and glutathione reductase activity (Fig. 6).

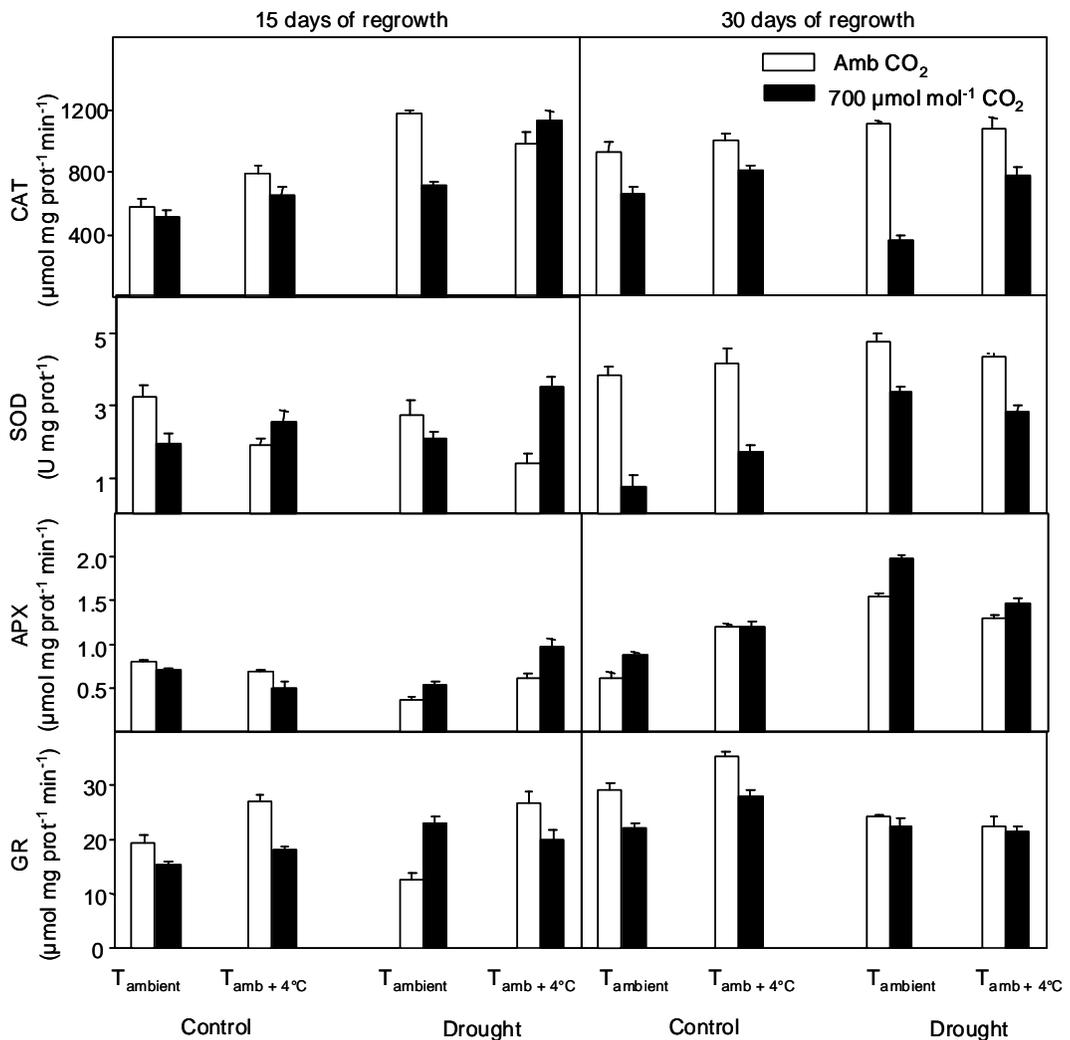


Figure 6. Effect of CO<sub>2</sub>, temperature and water availability on leaf catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) activities in nodulated alfalfa after 15 days and 30 days of regrowth.

## CONCLUSION

Future climate conditions include the rising of atmospheric CO<sub>2</sub> as well as global temperature. Moreover, in Mediterranean areas, reduction in precipitation regimes is also expected. Elevated CO<sub>2</sub> through enhancement of photosynthesis may stimulate alfalfa plant growth, especially that in the aboveground organs (leaves and stems) which are cut for animal feeding. After practising cutting of shoots and during regrowth, shoot dry matter was promoted by CO<sub>2</sub> but contrary to that observed in the precutting period, root matter also increased. Throughout the experiment, water shortage reduced DM accumulation. Nevertheless, shoots were always more affected than roots by water shortage. Drought and elevated CO<sub>2</sub> enhanced WUE, measured as WUE<sub>p</sub> and WUE<sub>ph</sub> by decreasing leaf conductance comparatively more than dry matter production.

As a source of forage crop protein for animal feeding, the biochemical characteristics of alfalfa leaves are important parameters with agronomic interest. In the present study, leaf protein concentration was always reduced by CO<sub>2</sub> and this phenomenon was related to a dilution effect derived from leaf starch accumulation. It is remarkable that this protein depletion may be related to growth conditions and more experiments in field conditions are needed to confirm the results. Elevated CO<sub>2</sub> also alters ROS production in plant tissues reducing their production and leading to a generalized relaxation of the antioxidant system. In this respect, the antioxidant value of forage may also be altered under elevated CO<sub>2</sub> and further studies should be carried out to demonstrate the implications.

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*Chapter 4*

## **DAMAGE-INDUCED VARIATIONS IN ESSENTIAL OIL COMPOSITION AND VOLATILE EMISSION IN AROMATIC PLANTS**

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### **ABSTRACT**

Phytochemical induction of monoterpenes following herbivory by insects and mechanical damage, was studied in *Minthostachys mollis* (Lamiaceae), a plant native to Central Argentina with medicinal and aromatic uses in the region. The monoterpenes pulegone and menthone were analyzed in *M. mollis* 24 and 48 h after leaves were mechanically damaged or exposed to insects with different feeding habits (chewing, scraping, sap-sucking, and puncturing). Essential oil composition and emission of volatiles were assessed. Mechanical damage resulted in an increase of pulegone and menthone concentration in *M. mollis* essential oil during the first 24h. Menthone content generally decreased whereas pulegone concentration increased in all treatments where insects were involved. The changes observed after insect feeding occurred also in the adjacent undamaged leaves, but induced changes after mechanical wounding were restricted to the damaged site, suggesting that an elicitor related to the insects may be required for a systemic response to be induced.

Changes in the volatiles released from *M. mollis* damaged leaves were also detected, most noticeably showing an increase in the emission of pulegone. Inducible chemical

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changes in aromatic plants might be common and widespread, affecting the specific compounds on which commercial exploitation is based.

**Keywords:** Phytochemical induction, aromatic plants, Lamiaceae, mechanical wounding.

## INTRODUCTION

As a consequence of the selection pressure imposed by herbivores, of which insects represent the dominant group [1], plants have developed a sophisticated array of chemical defensive mechanisms [2]. Such defenses may directly affect the herbivores (Direct Defenses) as in the case of chemical compounds with toxic, repellent, or digestibility-reducing activity. Alternatively, defensive effects may be mediated by an increased efficiency of the herbivore's natural enemies (Indirect Defenses), as when the emission of plant volatiles aids pathogens, parasitoids and predators in locating their hosts or prey [3], thus facilitating top-down control of herbivore populations [4]. The distinction between both types of defenses is not clear-cut, since some direct defenses, particularly those that slow herbivore growth by reducing their digestive efficiency, may need the third trophic level to be effective [5,6].

Defensive compounds are assumed to be costly for the plant because resource allocation to their biosynthesis should reduce the resources available for growth and reproduction, whereas self-toxicity for the plant or ecological consequences of their accumulation could also negatively affect plant fitness [7-9]. Therefore, investment in defense could be expected to decrease when enemies are absent and increase when they are present. This reasoning is supported by the frequent existence of a lower baseline of constitutive defenses (always expressed in the plant and preventing herbivore attack) which can be reinforced through the expression of inducible defenses when the plant is actually damaged [10].

Chemical changes following damage by herbivore insects [7, 11-13] have been repeatedly reported and could thus represent a way to reduce the cost of defensive compounds, since the latter are synthesized only when necessary. Plants or plant parts under high herbivory risk should invest in constitutive defenses, whereas those receiving only sporadic insect damage are expected to rely predominantly on inducible defenses [9]. However, both kinds of defensive mechanisms may co-occur on the same plant, and they may provide different benefits [14]. The molecular and genetic basis of plant defense and insect counter-defense mechanisms, are currently studied not only from a basic science perspective, but also for biotechnology-based pest control practice [15].

Insect-induced changes in plant chemistry depend on quantity of area consumed, phenology of damaged leaves [16], distribution and type of damage [11, 17]. Inducible compounds seem to represent a specific plant response to herbivore damage, insect egg deposition [18-19] or even mechanical damage [10, 20]. Herbivore insects deploy a variety of feeding mechanisms, which allow them to exploit plant resources in different ways. This is the basis of the "feeding guild" concept, i.e. groups of species utilizing in a similar manner a particular plant structure [21]. There is evidence that the same plant can show differential responses to damage from different feeding guilds and even from different species within a guild [22, 23].

Induced defenses may extend beyond the site of damage to other parts of the plant, resulting in a systemic response, as a consequence of intraplant communication mediated by molecules that act as signals among the different plant tissues [24,25].

At the biochemical and physiological level, induction mechanisms involve a number of plant hormones, such as jasmonic acid, salicylic acid, ethylene and abscisic acid [26, 27]. Phytohormones involved in the transduction of induced signals alter both primary and secondary metabolites in damaged plants [10].

Phytochemical induction can affect the activity of the insect species eliciting the response and of other species feeding on the plant [28-31], with repellent or toxic effects for some insects [32-37] and attractive or stimulant for others [4, 38-40].

Defensive induction may involve quantitative or qualitative changes in plant chemistry, and the induced compounds can either remain within plant tissues or be emitted as volatiles [20,26]. Volatile emission represents a permanent loss of energy, in contrast with the compounds that remain locked within plant tissues, which can be recycled if necessary [32].

Various terpenes, aromatic phenols, alcohols, aldehydes and other compounds are easily volatilized when air exposed, and can be emitted by damaged plants [41]. Newly developed methods of "head space" collection allowing measurement of volatiles in the air surrounding plants, in combination with gas chromatography techniques and mass spectrometry, have provided abundant information on chemical composition of natural emissions [42, 43]. Hundreds of volatiles have been detected in the plant surrounding air, but the fragrance is usually dominated by a few major compounds, mainly monoterpenes, sesquiterpenes and homoterpenes [41]. The profile of emitted volatiles changes dramatically when plants are damaged, with differential responses to mechanical or herbivore damage [26, 31, 40].

Plant chemical responses include a great variety of compounds with convenient properties for specific functions in defence [44, 45]. Such compounds are not homogeneously distributed on all parts of the plant, but rather concentrated on the plant surface, thus favouring a protective function against herbivory by occupying a location where they could be easily perceived by insects and other herbivores [44].

Lamiaceae, a plant family that includes various species with aromatic and/or medicinal uses, presents terpenes, and particularly monoterpenes (the C<sub>10</sub> members of the terpenoid family of natural products), as major chemical compounds. Terpenes, which are responsible for the characteristic fragrances of aromatic plants [32], can be emitted as soon as plant structures are damaged [9, 44]. Lamiaceae accumulate essential oils in specific places, frequently in glandular hairs [41], probably as an adaptation to prevent autotoxicity since many terpenes are potentially toxic for the plant itself [46].

Monoterpenes occupy a prominent place among the best studied examples of plant secondary metabolites with defensive functions. These colourless, lipophilic, volatile substances represent the major constituents in plant essential oils, showing defensive activity (toxic, repellent, feeding and ovipositing deterrent, etc) against a variety of insects and pathogens [32-37]. Furthermore they seem to be involved in plant intraspecific communication [44]. However their role as inducible defences has been largely overlooked.

Inducible chemical changes would be particularly important in medicinal and aromatic plants, not only as defensive mechanisms against possible insect pests, but also because the altered substances could be the specific compounds with aromatic or therapeutic attributes that give economic value to the plant [47]. A better knowledge of factors affecting essential oil quantity and quality in aromatic plants is needed in order to improve the production of

these natural products and for pest management programs [48]. However, studies on induced responses in aromatic or medicinal plants are extremely scarce [47, 49].

In the present contribution we have integrated our results on experimental analyses of induced responses in *Minthostachys mollis* (Kunth.) Griseb., a Lamiaceae native to Central Argentina with medicinal and aromatic uses in the region [50-52]. We have considered changes induced by different types of damage, in the essential oil composition and emission of volatiles. Damage-induced changes in essential oil and emitted volatiles have rarely been considered simultaneously [33]. Our study focuses on monoterpenes, since the most important compounds of *M. mollis* belong to this group [47], and because increased production of such compounds has been found after herbivory in various plant species [10, 40, 47]. We have also studied temporal and spatial (translocation) variations in the plant responses.

## METHODS AND MATERIALS

Bioassays for essential oil induction. Healthy, pest free, 6-month-old plants (average 18 leaves) of *M. mollis*, grown in a glasshouse without supplementary lighting, were used in all experiments.

For mechanical damage experiments, five leaves on each plant, all approximately the same age and size but from different nodes, were punched in the middle of the lamina leaving a 5-cm-diam hole and thus removing 30 to 40% leaf area.

For insect damage experiments, plants were exposed to insects representing the following feeding habits:

- a) leaf chewing: adults of *Systema sp.* (Coleoptera: Chrysomelidae), surface feeders with chewing mouthparts, ingest whole pieces of the leaf lamina, leaving holes or notches;
- b) leaf scraping: nymphs and adults of “thrips”, *Tubulifera sp.* (Thysanoptera), remove the contents of leaf epidermal or parenchymal cells, producing a characteristic mottling of the affected surfaces [1];
- c) sap-sucking: adults of *Halticus sp.* (Hemiptera: Miridae) insert their feeding stylets a short distance into the leaf tissue, leaving small, discolored spots where the spongy mesophyll cells are broken and emptied, causing local necrosis [1];
- d) leaf puncturing: female adults of *Liriomyza huidobrensis* (Diptera: Agromyzidae) pierce the leaf tissues with their ovipositor and suck up the exuded cellular contents.

The insects were collected from field *M. mollis* plants, either cultivated or growing spontaneously near Córdoba city, in central Argentina. Adults of *L. huidobrensis* were reared from leafmining larvae collected on *Vicia faba* crops in the same location.

In each test, a plant was exposed for 4-6 h in a cage (wood, glass, and gauze, 30 cm on each side) to at least five insects from one of the feeding types above-mentioned. All experiments were made under similar conditions of light, temperature ( $22 \pm 2^\circ\text{C}$ ), and relative humidity (70%). At least five leaves (of approximately the same age and size) from different

nodes were offered to the insects; the leaf opposite to each of the former was covered with a thin plastic film to avoid insects feeding on them. Film-covered leaves from undamaged plants were used as controls for the damage-adjacent leaves. The essential oil composition of control film covered leaves did not significantly differ from that of the uncovered leaves on the same plant (Wilcoxon signed ranks test,  $p > 0.05$ ), thus possible effects of the film cover were dismissed. Care was taken that the whole amount of physical damage to the plant was not larger than 30-40% of the leaf area. All insects were then removed, and the plants were placed in an insect-free environment.

In both mechanical and insect damage experiments, all damaged leaves and their adjacent (undamaged) ones were removed from the plant 24 h or 48 h after damage (two treatments for response time), and kept separately in order to assess chemical induction and translocation effects. Five leaves, similar in size and age to the damaged ones, from an undamaged plant were used as controls. Different plants were used for each treatment (damage type and response time); the youngest and oldest pairs of leaves were never included in the analyses. All leaves were frozen until the chemical analyses were carried out. Ten different plants (replications) were used for each treatment.

**Essential Oil Extraction.** After weighing, the plant material was submitted to hydrodistillation in a micro Clevenger-like apparatus for 40 min and the volatile fraction was collected in dichloromethane. Internal standard was added (12  $\mu\text{g}$  of tymol in 2  $\mu\text{l}$  dichloromethane). Essential oils of *M. mollis* contain 50 different compounds, with two monoterpenes accounting for about 80% of their volume: pulegone and menthone [47]. These two compounds were used for the present study.

**Collection of Plant Volatiles.** The volatile collection system consisted of a vacuum pump that created a constant air flow (300 ml/min) through a polyethylene terephthalate (PET) chamber (1500 ml in volume) containing a plant; the chamber was closed at one end with a cap pre-drilled to fit exactly the collection trap. At the other end a cap, with a hole through which the plant stem passed, separated the bottom of the chamber from the plant pot ground. Air exited the chamber throughout a reusable glass collection trap packet with 30 mg Super Q absorbent (80-100 mesh)(Alltech), which was rinsed prior to each volatile collection with 5-10 ml dichloromethane to remove impurities. Volatiles were collected for 2 hr; immediately after, the compounds were eluted from the absorbent traps with 200  $\mu\text{l}$  dichloromethane, and internal standard was added (12  $\mu\text{g}$  of tymol in 2  $\mu\text{l}$  dichloromethane). Collected volatiles were analyzed with GC as described below. Once the volatiles were collected, the plant was cut and weighed.

For each plant, volatiles were collected 24 and 48 hr after receiving mechanical damage and puncturing by adult female *L. huidobrensis* as indicated in the bioassays above described. Volatiles were also collected from undamaged plants, to be used as controls. All plants were similar in age and size, and collections were taken under similar conditions of light, temperature and time of day. Collections made without plants, from an empty chamber, established that the background of monoterpenes present was negligible. Each treatment was repeated at least five times.

**Chemical Analyses.** Analyses were accomplished with the use of a Perkin-Elmer Q-700 gas-chromatograph equipped with a CBP-1 capillary column (30 m x 0.25 mm) and a mass selective detector. Analytical conditions: injector and detector temperatures 250°C and 270°C, respectively; oven temperature programmed from 60°C (3 min) to 240°C at 4°/min; carrier gas helium at a constant flow of 0.9 ml/min; source 70 eV. The oil components were

identified by a combination of mass spectral and retention time data, which were compared both with those of authentic compounds and with those published in Zygadlo et al. [54]. GC analyses were performed with a Shimadzu GC-RIA gas-chromatograph, fitted with a 30 m x 0.25 mm fused silica capillary column coated with a Supelcowax 10. The GC operating conditions were: oven temperature programmed from 60°C (3 min) to 240°C at 4°/min, injector and detector temperatures 250°C; detector FID; carrier gas nitrogen at a constant flow of 0.9 ml/min. Identification of the components was performed by comparison of their retention times with those of pure authentic samples.

**Volatile Emission Rate.** An estimation of the amount of monoterpenes emitted in relation to their content in the plant tissues was obtained by calculating, for each treatment, the quotient between the concentration values of menthone or pulegone in the headspace and their respective concentration in the essential oil (headspace/essential oil).

**Statistics.** Differences in menthone and pulegone content between treatments were tested for statistical significance using Mann-Whitney and Kruskal-Wallis tests, since data were not normally distributed.

## RESULTS

**Essential Oil Composition.** Both herbivore insect feeding as well as mechanical damage resulted in significant changes in *M. mollis* essential oil chemistry.

The concentration of menthone (Figure 1) significantly decreased 24 h after leaf exposure to most types of insect feeding damage ( $p < 0.05$ , Kruskal-Wallis,  $H = 28.99$ ), whereas mechanical damage elicited the opposite reaction, increasing up to six times in comparison with undamaged control plants. The same trend was maintained for insect damage after 48 h, but levels of menthone in mechanically damaged leaves returned to normal levels ( $p < 0.05$ , Kruskal-Wallis,  $H = 18.30$ ). The only exception to this pattern was a lack of response in scraped leaves.

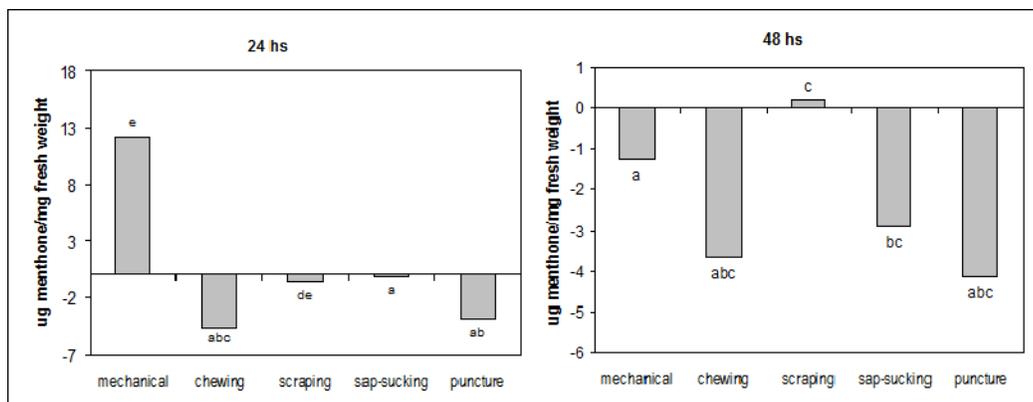


Figure 1. Changes in menthone concentration (means  $\pm$  SE) in the essential oil of *Minthostachys mollis* leaves, as a result of different types of damage, at different post-wounding time. Different letters indicate statistically significant differences among damage treatments ( $p < 0.05$ , Kruskal-Wallis).

Pulegone concentration (Figure 2) increased in all damage treatments ( $p < 0.05$ , Kruskal-Wallis,  $H=31.31$ ) 24 h after damage, with the highest response being elicited by mechanical damage and followed by scraping. These two damage types differed from the others by inducing higher pulegone levels 24 h post-wounding, whereas for all other insect damage types this compound was still increasing 48 h after damage ( $p < 0.05$ , Kruskal-Wallis,  $H=31.71$ ).

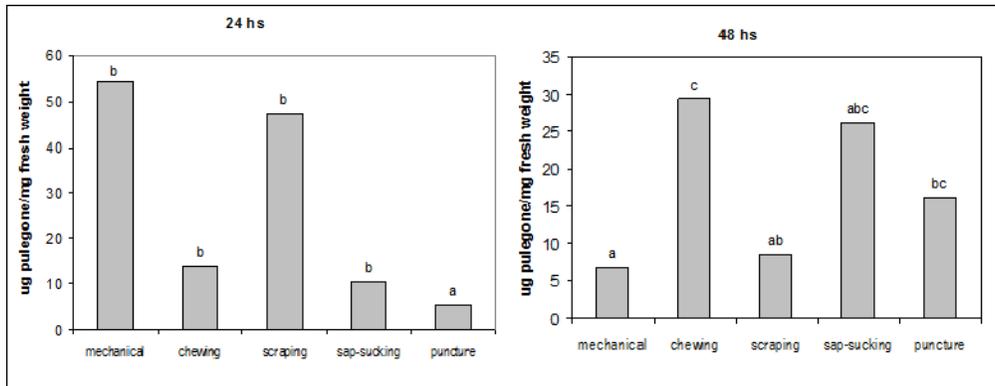


Figure 2. Changes in pulegone concentration (means  $\pm$  SE) in the essential oil of *Minthostachys mollis* leaves, as a result of different types of damage, at different post-wounding times. Different letters indicate statistically significant differences among damage treatments ( $p < 0.05$ , Kruskal-Wallis).

Systemic response. Menthone concentration in undamaged leaves adjacent to leaves wounded by insects did not differ from the latter (Figure 3) after either 24 or 48 h ( $p > 0.05$ , Wilcoxon signed ranks test), indicating a systemic induction. However, leaves adjacent to those mechanically damaged did not reflect the dramatic increase observed in the latter after the first 24 hr, their menthone content being significantly lower (Wilcoxon test,  $p > 0.05$ ).

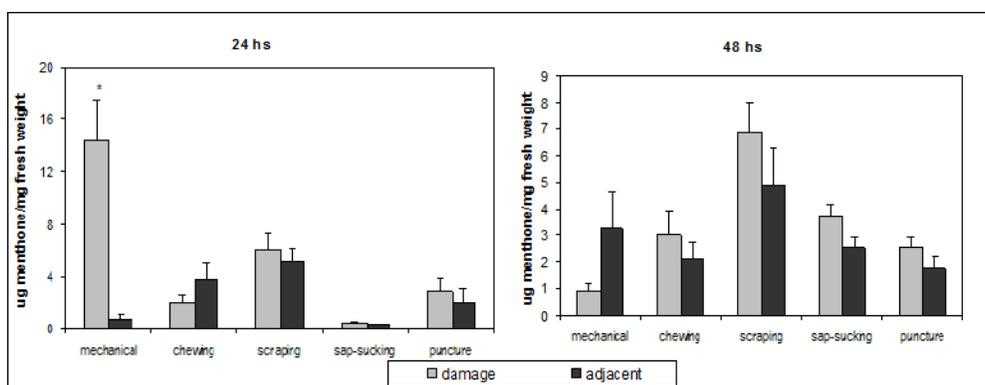


Figure 3. Menthone concentration in the essential oil of *Minthostachys mollis* leaves subjected to different types of damage and in their adjacent leaves, at different post-wounding time. Asterisks (\*) indicate significant differences between damaged and adjacent leaves within each damage type ( $p < 0.05$ , Wilcoxon signed ranks test).

Furthermore, pulegone content in leaves growing next to mechanically damaged ones (Figure 4), did not reflect the increase observed in the latter after 24 h (Wilcoxon test,  $p < 0.05$ ), whereas leaves adjacent to those damaged by insects presented increased levels of pulegone, similar to those found in the actual damaged leaves (Wilcoxon test,  $p > 0.05$ ). Leaves adjacent to those damaged by scraping and sap-sucking insects showed a significant increase at 24 and 48 h after wounding, respectively ( $p < 0.05$ , Wilcoxon signed ranks test), thus magnifying the changes observed in the damaged leaves (Figure 4).

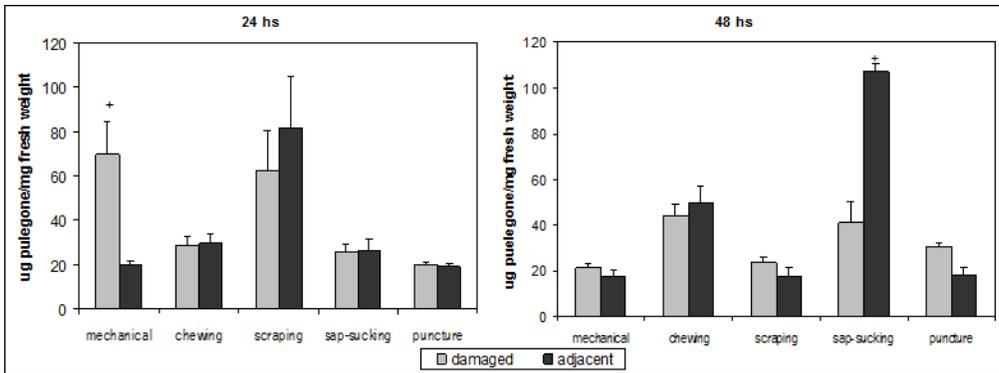
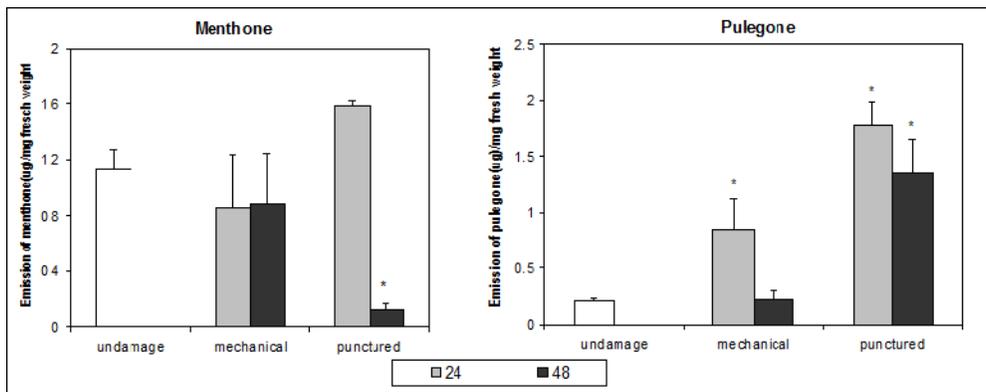


Figure 4. Pulegone concentration in the essential oil of *Minthostachys mollis* leaves subjected to different types of damage and in their adjacent leaves, at different post-wounding times. Asterisks (\*) indicates significant differences between damaged and adjacent leaves within each damage type ( $p < 0.05$ , Wilcoxon signed ranks test).



(\*) values significantly different from controls ( $p < 0.05$ , Mann-Whitney test).

Figure 5. Variations in the main volatiles released from *Minthostachys mollis* plants as a result of mechanical damage and leaf puncturing by the leafminer *Liriomyza huidobrensis*. The values are expressed in ng/mg fresh weight (means  $\pm$ SE).

**Plant Volatiles.** Results from headspace experiments showed different changes in volatile emission after mechanical damage and leaf puncturing by the leafminer *L. huidobrensis*. Mechanical damage resulted in unaltered emission of menthone ( $p > 0.05$ ,  $U$  24hr=46,  $U$  48hr= 73.5) whereas the emission of pulegone increased dramatically 24 h after wounding ( $p < 0.05$ ,  $U$  24hr=105) and then decreased back to pre-wounding levels ( $p > 0.05$ ,  $U$  48hr= 73.5) (figure 5). Instead, emission of menthone from plants damaged by leafminer punctures,

was similar to that from undamaged plants when measured 24 h after exposure to the insects ( $P=0.02$ , Kruskal-Wallis  $H=7.95$ ), but decreased by 90% of the baseline level after 48 h (fig. 5). In contrast, emission of pulegone increased up to 8.4-fold in damaged plants ( $P=0.01$ , Kruskal-Wallis  $H=13.04$ ), with highest levels observed after 24 h.

**Emission Rate of Volatiles.** The emission rate of volatile pulegone with regard to its concentration in the essential oil did not show important changes following either mechanical or puncture damage, whereas the emission rate of menthone was noticeably lower in mechanically damaged plants at 24 h and higher at 48 h than that of control plants (Figure 6). In contrast, the emission rate of menthone after 48 h was dramatically lower in punctured plants compared to undamaged plants.

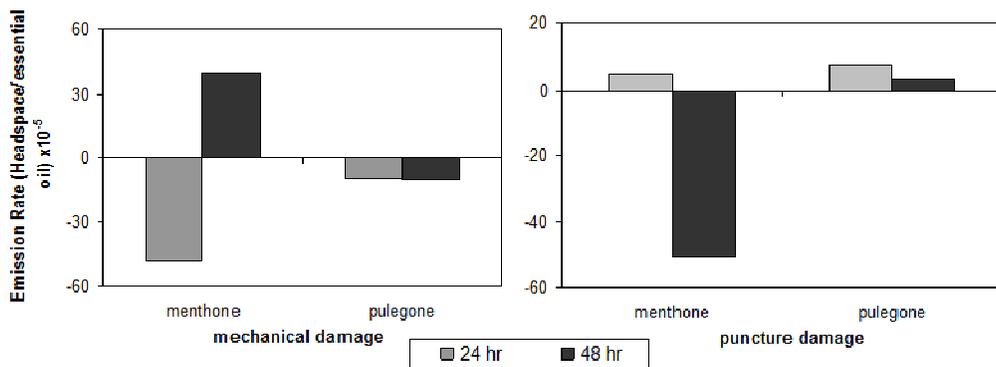


Figure 6. Volatile emission rates of menthone and pulegone from *Minthostachys mollis* plants, 24 and 48 h after being mechanically damaged or punctured by female adults of *L. huidobrensis*. (Values represent differences from corresponding rates in undamaged plants.)

## DISCUSSION

**Oil Concentration.** The essential oil of *Minthostachys mollis* plants showed phytochemical changes as a response to leaf damage. Mechanical damage induced a dramatic increase, after 24 h, in the concentration of the two most abundant monoterpenes, menthone and pulegone. Zabarás and Wyllie [53] found a comparable response in another aromatic plant, *Ocimum minimum*, whose main compound linalool also increased 24 h after suffering mechanical wounding. However, and in sharp contrast with the 6.5-fold menthone increase observed after mechanical damage, different types of insect feeding resulted in decreased contents of menthone in *M. mollis* plants. The only exception was the lack of inducible effects on menthone concentration following scraping damage by thrips.

On the other hand, pulegone content significantly increased after 24 h in all damage treatments, whether mechanical or by insect feeding. Similar results were observed in *Melaleuca sp.* (Myrtaceae) where herbivory induced a 1.75 fold increase of  $\alpha$ -pinene [55]. Levels of pulegone returned to normal after 48 h when leaves were mechanically wounded, but the response to insect damage was even more pronounced at that time. Leaves facing scraping damage by thrips showed again a different response, being most intense 24 h upon wounding and declining at 48 h, in coincidence with the response to mechanical damage. The observation of changes in essential oil concentration during the first 24 h after damage

suggests that biosynthetic processes are elevated during the postwounding period [40]. From an ecological perspective, such rapid chemical changes may be interpreted as an attempt to minimize subsequent predation [56]. A longer lasting induction, as observed following insect feeding in comparison with mechanical damage, can reinforce the defensive effect and seem to be related to the magnitude of damage [57].

For an induced response to represent a defensive mechanism, it should protect the damaged plant from further attack. In the present case, such defensive role can be expected from the observed increase in pulegone, since this compound has shown toxic [37, 58-61], growth inhibiting [62], repellent and oviposition deterrent [63] properties on various insect species. Pulegone is probably the most potent inhibitor of acetylcholinesterase (AChE) present in the neuromuscular junction in invertebrates [37, 64]. Additionally, it has been proved to destroy symbionts of herbivores, thus creating a biochemical barrier for host plant utilization [37].

Although in a lesser degree, menthone has also showed insecticidal [65] and genotoxic activity [59]. Moreover, the observed decrease in menthone after insect damage could magnify the toxic effect of pulegone, since the two compounds seem to be antagonistic [59].

Phytochemical induction after insect damage can differ qualitatively and/or quantitatively depending on the insect species and feeding habits [22, 28, 66, 67]. Presumably, variations in induced responses to different damage types could be due to changes generated at the wound site. These signals may originate from the plant (e.g., cell wall fragments) or the insect (e.g., salivary factors) [68] or may even involve both plant and insect [69]. Factors affecting the strength of an induced response include: the number of wounded cells in contact with healthy cells; the duration, persistence, and magnitude of a single damage event; the types of cell / tissue affected, and the kinds of forces acting on the leaf [4, 26, 70]. Although the herbivores here studied differed greatly in their feeding habits, remarkably similar changes in monoterpene concentration were induced in *M. mollis*. Such changes occurred whether insects removed pieces of leaf, punctured epidermal cells with the ovipositor, or penetrated the plant cuticle to feed from the mesophyll cells, injecting relatively large quantities of saliva [1]. Only feeding by thrips, which scrape the leaf epidermis to feed into the parenchyma cells, caused a slightly different reaction which was closer to that elicited by mechanical damage.

The relatively uniform response observed in *M. mollis* to a variety of insect wounds could be attributable to the possession of the extremely active compound pulegone, which in high concentrations might be an insurmountable obstacle for most insect herbivores. Whether plants containing one strong active compound tend to show more homogeneous responses to damage than plants with a more diversified defensive structure is a question that has not yet been addressed.

Active plant defences frequently involve systemic induction, i.e. occurring not only at or near the site of damage, but extended throughout the plant, as a result of signalling molecules enabling communication between different plant tissues [3]. Our results suggest such a systemic induction in *M. mollis* leaves, as a response to insect damage: menthone levels decreased and pulegone increased in undamaged leaves adjacent to insect-wounded leaves, mirroring or even magnifying the changes observed at the site of damage. Such systemic induction after insect feeding is consistent with several reports where the defensive reaction was recorded throughout the plant [3, 4, 24, 67, 71]. In this regard, the response elicited by mechanical damage was clearly different, since no translocation was observed, suggesting

that an elicitor related with the insects may be required for a systemic response to be induced [40].

**Plant Volatiles.** Mechanical damage as well as leaf punctures from adult leafminers affected the release of volatile terpenoids in *M. mollis*. With leafminer damage, the emission of volatile pulegone increased while emission of menthone decreased, reflecting the general response pattern observed in the essential oil. However, with mechanical damage the changes in volatile emission differed from those observed in the essential oil: although both major monoterpenes increased in the essential oil, volatile emission only reflected the pulegone increase. In agreement with other studies (e.g. [31, 42]), our results show that damage can induce the release of volatile terpenoids. Zabarás et al. [49] also found a dramatic increase of volatile emission in *Melaleuca alternifolia* leaves after mechanical damage, although in that case the compounds involved remained unchanged in the essential oil.

The increase in pulegone emission rate 48 hr after insect damage may be interpreted as an attempt to minimize subsequent predation [37] or as a potential cue for natural enemies of herbivores [30].

In aromatic herbs, significant amounts of volatile compounds accumulate in leaf glandular trichomes, whereas emitted volatiles usually represent only a small fraction (usually less than 1%) of the total pool produced [46, 72]. The proportion of emitted monoterpenes may differ from those in the plant oil [41, 46, 73]. In undamaged *M. mollis* plants, volatile emissions were dominated by menthone, whereas pulegone was the major component of the essential oil within the plant [50]. Leaf punctures made by *L. huidobrensis* females with their ovipositors, induced a decreased emission of volatile menthone at 48 h. so that at that point, pulegone became the dominant component of both the essential oil and the emitted volatiles.

**Emission Rate of Volatiles.** Considering the small fraction of total monoterpenes that are emitted as volatiles, extrapolation over a 6-month plant growing period suggests that < 1% of total monoterpenes would be released to the atmosphere. A low rate of volatilization is consistent with observations in other Lamiaceae (e.g. [46, 72]). Induced volatiles could provide a direct defence for the plant by affecting herbivore behaviour or performance [73-76] whereas they could also provide indirect defence by improving prey or host location for predators and parasitoids [4, 22, 31, 71].

Even in undamaged plants, the relative concentration of the various monoterpenes emitted from *M. mollis* was quite different from that found in the essential oil within the plant. Emitted monoterpenes contained higher proportions of menthone (60.2% in the headspace versus 10% in the oil) and lower amounts of pulegone (11.1% versus 85%). Studies on other plant species have also shown that composition of emitted monoterpenes may differ from their presence in the plant oil [41, 46, 72]. Membranes of storage compartments might be selectively more permeable to particular monoterpenes, or the emitted monoterpenes may be associated with entirely different secretory compartments than the stored compounds [46].

It is likely that the chemical changes here observed in both oil concentration and emissions of volatiles serve important ecological roles, particularly for plant defense. This is important considering the tremendous negative impact of insect pests on cultivated plants, leading to the annual loss of almost a quarter of world crop production [1]. In addition, from a commercial point of view the effects of wounding on leaf oil concentration may be significant. Given the economic importance of monoterpenes for the fragrance, flavor, and pharmaceutical industries, knowledge of the processes that affect monoterpene production

and emission in plants can be of value in increasing the yields of these commercially valuable natural products.

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*Chapter 5*

## **GASIFICATION OF BIOMASS IN AQUEOUS MEDIA**

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### **ABSTRACT**

The lignocellulosic biomass materials are abundant, cheap and renewable feedstocks suitable for biofuel and biochemical production. They can be derived from forestry wastes such as residues of the trees and shrubs, energy crops like maize, sorghum, miscanthus, kenaf, switchgrass, jatropha, corn, sugarcane and any agricultural residues such as corn stovers, wheat straw etc. The use of biofuels derived from lignocellulosic biomass does not cause additional increase in the carbon dioxide level in the earth's atmosphere. The release of carbon dioxide during biofuel utilization is balanced by the carbon dioxide consumed in biomass growth.

In many cases, because of large water content and high drying cost, biomass is not a suitable feedstock for conventional thermochemical gasification technologies. Thermochemical gasification techniques such as biomass gasification and pyrolysis are energy intensive processes and produce relatively high amounts of char and tar with low conversion of biomass into gas. Among other various conversion methods, hydrothermal gasification, using super- or sub-critical water as the reaction medium, is seen as a promising way to produce hydrogen from biomass with high efficiency. These processes can be applied to the conversion of biomass with high moisture content without drying. While processes applied in sub- and super-critical water around 250-400°C, methane and carbon dioxide are the major products in addition to the target gas hydrogen but the formation of these major side products can be minimized by using appropriate catalysts and adjusting processing temperature and pressure conditions.

Aqueous phase reforming (APR) process is a rather new evolving technology involving decomposition of the oxygenated hydrocarbons to produce hydrogen-rich gas. The main advantage of APR is its relatively low gasification temperature where CO concentration within the hydrogen stream is rather low. The process produces high yield of hydrogen gas with low CO byproduct due to the water-gas shift reaction ( $\text{CO} + \text{H}_2\text{O} \leftrightarrow \text{H}_2 + \text{CO}_2$ ) which is effective at the processing temperature. APR of carbohydrates take place at considerably lower temperatures compared to conventional alkane steam reforming process. A lower temperature reduces unwanted decomposition reactions that normally observed when carbohydrates are heated to elevated temperatures.

Carbohydrates such as sugars (e.g., glucose) and polyols (e.g., ethylene glycol, glycerol) can be efficiently converted in the aqueous phase over appropriate heterogeneous catalysts at relatively mild processing conditions to produce hydrogen rich gas mixture. Lignocellulosic materials containing high level of polysaccharides are potential biomass sources for the APR gasification provided that, by using ecological pre-treatment techniques, the water-insoluble polysaccharides are hydrolyzed into relatively smaller carbohydrates which are soluble in water. This chapter will focus on APR and summarize the relevant research and development activities including the authors' work on conversion of lignocellulosics.

## 1. INTRODUCTION

Environmental problems arising due to energy production by utilizing fossil fuels are the major challenges facing the world. In addition to the environmental problems caused by fossil fuels, rapid depletion of the corresponding resources forced the world communities to develop alternative technologies for making use of clean and renewable energy sources. Currently, much research and development activities are being focused on the production of energy from renewable sources where greenhouse gas emissions during the production or utilization stages are either null or neutral. In this respect, biomass resources are carbon dioxide neutral and therefore the fuels to be obtained from these sources are good candidates which will at least partially replace fossil-derived alternatives at this transition stage. Gasification or liquefaction of biomass are those sustainable and environmental friendly approaches. In gasification technologies, hydrogen-rich gas or synthesis gases are the target secondary fuels whereas in liquefaction processes oils and oxygenated fuels are obtained. At this point, it is important to mention that those biomass materials which are not edible should be the main alternatives for converting into fuel products. In this respect, lignocellulosic biomass such as grass, shrubs, woods, by-products of agricultural activities, and energy plants can be converted into various fuel types. Energy crop is a plant grown as a low cost and low maintenance harvest used to make biofuels, or directly exploited for its energy content. They are fast-growing plants, trees or other herbaceous biomass harvested specifically for energy production. Energy crops can be food crops such as corn, sugarcane, sugar beet, sweet sorghum or non-food crops such as poplar trees, switchgrass, kenaf, miscanthus, and other similar plants.

Lignocellulosic biomass can be converted into liquid and gaseous fuels by using a variety of thermal and hydrothermal gasification processes [1-4]. Microbiological processes are also other alternatives for converting lignocellulosics into gaseous or liquid fuels [5]. Hydrothermal gasification processes that are being developed for conversion of lignocellulosics are applied in either gaseous phase (steam reforming), in supercritical water, or in liquid phase (aqueous phase reforming: APR). Steam reforming needs the lignocellulosic material to be dried prior to gasification. The temperatures applied in this conversion are also high, i.e. 800°C or above and considerable tar and char are formed. The thermodynamic critical points for water are 374.3 °C and 221.2 bar for temperature and pressure, respectively. Effective gasification of lignocellulosics in supercritical water needs to go quite higher values than the critical points, e.g. above 600°C for complete gasification [6]. Despite high initial investment costs and moderately high operational costs, SCW gasification

of lignocellulosics seems to be still advantageous compared to steam gasification because no tar or char is produced.

## 2. GASIFICATION OF BIOMASS BY AQUEOUS-PHASE REFORMING

A rather new method involving the decomposition of oxygenated hydrocarbons to produce hydrogen-rich gas in aqueous medium at moderately low temperature and pressure conditions was developed by Dumesic and co-workers [2]. Biomass-derived oxygenated hydrocarbons such as ethylene glycol, glycerol, glucose and sorbitol were successfully converted into  $H_2$ , CO,  $CO_2$  and gaseous alkanes by the catalytic aqueous-phase reforming (APR) using supported metal catalysts [2, 7-9].

Hydrogen production from biomass hydrolysates, e.g. carbohydrates by APR has several advantages over existing methods. The conversion takes place at moderate temperatures, around 225-265 °C, and at pressures of 27-54 bar, conditions that prevent steam formation and ensure that the reaction sequence takes place in the aqueous phase [10-11]. A lower temperature reduces unwanted decomposition reactions that are normally observed when carbohydrates are heated to elevated temperatures. The water-gas shift reaction ( $CO + H_2O \leftrightarrow H_2 + CO_2$ ) is thermodynamically favored at the temperatures applied in APR, thus lead to low levels of CO in a single step catalytic process [2].

### 2.1. Types of Biomass Feedstocks used in APR

Hydrolysis products of biomass materials or biomass-derived oxygenated hydrocarbons such as sugars and sugar alcohols (ethylene glycol, glycerol, glucose, fructose, sorbitol, sucrose etc.) can be used in APR [2, 7-10]. However, efficiency of the process highly depends on the biomass feedstock and the reforming catalyst used. Selectivity for hydrogen production from biomass-derived oxygenated hydrocarbons was observed to be in the order of glucose < sorbitol < glycerol < ethylene glycol < methanol [10].

The lower operating temperatures of APR resulted in higher hydrogen selectivity but lower conversions were achieved at low temperatures. The hydrogen yield for reforming of glucose decreased further as the feed concentration increased to about 10 wt.%. However, hydrogen yields for reforming of the polyols were not sensitive to the concentration of the aqueous feed (e.g., from 1 to 10 wt.%) [12-13].

Majority of the previous studies on hydrogen production by aqueous phase reforming have been focused on single polyalcohol or sugar which were soluble in water [7, 9, 14-16].

Glycerol is expected to be one of the largest feedstocks for gasification via APR [17]. One source of glycerol is the low-value waste stream from the trans esterification of plant oils [18-19] and animal fat [18] in the production of biodiesel. The concentration of glycerol in this waste may be quite high; e.g. 80% [20]. Glycerol can also be produced by fermentation of sugars such as glucose, either directly [21] or as a by-product of the industrial conversion of lignocellulose into ethanol [22]. It is expected that this growing industry may be a major biofuel provider in the future [17].

## 2.2. Catalysts in APR

The desired catalytic pathway for H<sub>2</sub> production from oxygenated hydrocarbons via APR involves cleavage of C–C, C–H and O–H bonds throughout effective interaction with the catalyst surface. An effective catalyst in APR while facilitating cleavage of these bonds, should also promote removal of adsorbed CO species from catalyst surface by the water-gas shift reaction, but must not facilitate C–O bond cleavage and hydrogenation of CO or CO<sub>2</sub> [2, 23-24]. Wawrzetz et al. (2010) investigated the aqueous phase reforming of glycerol over Pt/Al<sub>2</sub>O<sub>3</sub> catalyst and concluded that the overall process needed a bifunctional catalyst; i.e. an acid-base catalyst and a metal catalyst [25]. According to them, the initial reactions were dehydrogenation and dehydration which were followed by decarbonylation and subsequent water-gas shift reaction. They declared that, under the reaction conditions they have used, hydrogenolysis of the C–O and C–C bonds in the alcohols did not take place. They have also observed that larger Pt particles favor hydrodeoxygenation over complete deconstruction to hydrogen and CO<sub>2</sub>.

Group VIII metals generally show higher activities in breaking C–C bonds, thus they can be used as effective catalysts for reformation of oxygenated hydrocarbons [26-27]. A very important point for the economic use of precious metal catalysts is their recovery, refining and recycling of the catalysts. This procedure is simplified by the use of supports on which the metals are doped. Typically, these support materials provide physical surfaces for dispersion of small metal particles, which are necessary in achieving high surface area [28]. Precious metal catalysts are widely used in the supported form on porous materials such as activated carbon,  $\gamma$ -alumina, silica, silica-alumina, organic polymers, and so forth, with a variety of properties (e.g., particle size, surface area, pore volume and size, mechanical strength and resistance to attrition, thermal stability, etc.). The final catalytic properties depend on the combination of the type of metal [29-31] and supportive material [15-16, 30].

Shabaker et al. (2003) reported that Pt-black and Pt supported on carbon, and alumina exhibited high activity for the production of H<sub>2</sub> by aqueous-phase reforming of ethylene glycol solutions [8]. Feng and co-workers (2008) showed that catalyst support can significantly affect the catalytic performance of ruthenium based catalysts in the glycerol hydrogenolysis reactions [32]. Shabaker et al. (2003) and Huber et al. (2006) reported that Pt is the best monometallic catalyst in terms of activity and selectivity for the production of hydrogen by APR and these functions can be improved further by supporting the metal on carbon, alumina, or titanium oxide [8, 33].

Bimetallic catalysts were also used in APR studies. Huber et al. (2006) suggested that activity of Pt catalysts could be improved further by adding Ni, Co or Fe to a Pt/Al<sub>2</sub>O<sub>3</sub> catalyst [33]. They have attributed this improving due to the lowering of the d-band center [34-36].

Raney Nickel is another alternative catalyst to be used in APR. Shabaker, Huber, and Dumesic (2004) carried out an intensive study by using Raney-Ni in decomposing glycerol via APR [37]. They have observed that Raney-Ni and Raney Ni-Sn displayed good stability with time on stream during APR. Addition of Sn to Ni improved the selectivity for production of hydrogen. A Ni:Sn ratio of 14 improved the selectivity upto 90%. It was observed that the turnover frequencies for hydrogen production at 498 K over Raney-Ni catalysts were several times lower than that over 3 wt% Pt/Al<sub>2</sub>O<sub>3</sub> based on CO chemisorption. However, the high

CO uptakes and high densities of Raney-Ni based catalysts lead to comparable rates of hydrogen production per unit reactor volume as 3 wt% Pt/Al<sub>2</sub>O<sub>3</sub> at 498 K.

### 2.3. Decomposition of Polyalcohols and Sugars via Aqueous Phase Reforming

Most of the APR studies involved decomposition of small oxygenated hydrocarbons which can be derived from various biomass sources. The target compounds to be decomposed were either polyalcohols like ethylene glycol and glycerol or sugars and sugar-derived compounds like glucose, sorbitol, and others.

#### 2.3.1. Glycerol

Glycerol was one of the polyalcohols whose gasification via APR was investigated thoroughly due to its abundance as a feedstock and expectations on further growth of this feedstock in the future [17]. Shabaker et al. (2004) investigated glycerol gasification via APR by using Raney-Ni and Pt/Al<sub>2</sub>O<sub>3</sub> [37]. They have shown that when Raney-Ni was modified with Sn, hydrogen production efficiency and selectivity were almost same as those achieved when Pt/Al<sub>2</sub>O<sub>3</sub> was used as the catalyst (pls. refer the previous section: Catalyst in APR).

Wen et al. (2008) used various catalysts to decompose 10% glycerol solution at 503 K and 3.2 MPa temperature and pressure, respectively [15]. The catalysts used were Pt/Al<sub>2</sub>O<sub>3</sub>, Pt/MgO, Pt/SiO<sub>2</sub> and Pt/AC (active carbon). It was observed that Pt/Al<sub>2</sub>O<sub>3</sub> was the most stable and effective in hydrogen production. It was also observed that the basic catalyst Pt/MgO was effective in hydrogen production but its stability was too low.

Luo et al. (2008) have investigated the effect of the amount of Pt loaded on Al<sub>2</sub>O<sub>3</sub> and concluded that the catalyst 0.9 wt% Pt /Al<sub>2</sub>O<sub>3</sub> amongst the others used was the most effective for hydrogen production from glycerol at the processing conditions of 220°C, 2.5 MPa where the initial glycerol concentration was 5 wt% [38].

A study on the effect of the type of Pt precursor compound and the type of the alumina as the support material was carried out by Lehnert and Claus [39]. The concentrations of the precursor solutions were so adjusted that the final metal loadings were 3 wt%. The method for loading the Pt precursors was incipient wetness and it was observed that the difference in the type of precursors did not result a significant difference in catalyst activity during APR of glycerol at the processing conditions of 250°C and 20 bar with an initial glycerol concentration of 10 wt%. Three different aluminas were used as the support; namely puralox® (Sasol),  $\gamma$ -alumina (Alfa Aesar), and Catapal B® (Sasol). Puralox® contained a mixture of  $\gamma$ -,  $\delta$ - and  $\theta$ - phases and Pt/Puralox® was the most effective catalyst amongst others used for the production of hydrogen.

The most recent study on decomposition of glycerol via APR belongs to Wawrzetz et al. [25] (pls. refer the previous section: Catalyst in APR). They have investigated the effect of particle size of Pt on Al<sub>2</sub>O<sub>3</sub> as the catalyst. The conclusions they have reached are summarized in the previous section.

### 2.3.2. Ethylene Glycol

Ethylene glycol was the other polyalcohol whose decomposition via APR was investigated thoroughly mainly because of its simple structure having all the characteristic bonds (C-C; C-H; C-O; O-H) which are being cleaved during aqueous phase reforming.

Shabaker et al. and Davda and Dumesic studied the decomposition of ethylene glycol and methanol via APR by using alumina-supported Pt catalysts at two different processing temperatures of 483 K and 498 K for which the corresponding processing pressures were 22.4 bar and 29.3 bar, respectively [8, 23]. Comparing the hydrogen production efficiency from methanol and ethylene glycol, they have concluded that C-C bond cleavage reactions were not the rate limiting steps for ethylene glycol reforming. They have also declared that since hydrogen production selectivities were almost 100%, methanation and Fischer-Tropsch reactions involving CO/CO<sub>2</sub> and H<sub>2</sub> were negligible under the processing conditions of the APR. The same group [Shabaker, Huber et al, 2003] also studied the decomposition of ethylene glycol via APR by using various different catalysts; namely Pt-black and Pt supported on TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, carbon, SiO<sub>2</sub>, SiO<sub>2</sub>-Al<sub>2</sub>O<sub>3</sub>, ZrO<sub>2</sub>, CeO<sub>2</sub>, and ZnO. High activities for the catalysts Pt-black, and Pt/ TiO<sub>2</sub>, Pt/ Al<sub>2</sub>O<sub>3</sub>, and Pt/carbon were observed where the turn over frequencies for hydrogen production were around 8-15 min<sup>-1</sup> at 498 K.

Valiente et al. (2010) also carried out an APR study on decomposition of ethylene glycol at 500 K/27-36 bar by using a Pt/Al<sub>2</sub>O<sub>3</sub> catalyst and investigated the effect of catalyst weight/ethylene glycol flow rate ratio [40]. They have also tested the effect of a Ni/Al coprecipitated catalyst which has exhibited a significant influence on hydrogen and alkane selectivities.

In another study, Liu et al. (2010) subjected ethylene glycol to APR in the presence of KOH so that due to carbonation, CO<sub>x</sub> free hydrogen-rich gas with the composition of 97% H<sub>2</sub> and 3% CH<sub>4</sub> was obtained [41].

## 2.4. Hydrogen Production from Glucose, Sorbitol and Lignocellulosic Biomass via APR

Lignocellulosic biomass of various origins when hydrolyzed effectively produces glucose as the main carbohydrate. Further, sorbitol is obtained when glucose is reduced. Therefore, it may be logical to discuss the gasification of glucose, sorbitol and lignocellulose-derived hydrolysates together in this section.

### 2.4.1. Glucose and Sorbitol

Davda and Dumesic (2004) investigated the gasification of glucose and sorbitol via APR separately and observed that while sorbitol gave a high yield and selectivity for hydrogen production, the corresponding values for glucose were rather low, especially when the concentration of the substrates are high [9]. They have observed that selectivity for hydrogen production is insensitive to the sorbitol concentration in the range 1 wt% to 10 wt% whereas this was not the case with glucose. Therefore, they have proposed a two-step process for the production of hydrogen from glucose in which glucose would be converted into sorbitol in a hydrogenation reactor in the first step that would be followed by reforming of sorbitol to convert it into H<sub>2</sub> and CO<sub>2</sub> in the second step.

Swami et al. (2008) converted glucose into ethanol via fermentation by using *Saccharomyces cerevisiae* and then used this broth for hydrogen production via aqueous phase reforming in the presence of a Pt-based catalyst [14]. The hydrogen obtained from APR of the fermentation broth was compared against the yield from a feed containing 5% ethanol in water. While the catalyst was stable for an extended time on stream during APR of 5% ethanol solution, very rapid deactivation was observed during APR of fermentation broth.

Wen et al. (2009) prepared Ni/Al<sub>2</sub>O<sub>3</sub> catalysts in one- and two-step impregnation of the metal on the support and tested the performances of these catalysts in APR of glucose [42]. They have observed that those catalysts prepared in the two-step method showed higher performances in the yield of hydrogen gas and selectivity for hydrogen increased as the metal particle sizes in the catalysts were increased. Carbon conversion into gas phase was only one third of the initial carbon value while another one third of the carbons were carbonized and stucked on the catalyst surface.

Another investigation on APR of sorbitol was carried out by Kirilin et al. (2010) over Pt/Al<sub>2</sub>O<sub>3</sub> catalyst [43]. The study was concentrated on the formation of intermediates during APR of sorbitol. Over 260 intermediates were recognized but only 50 of the major products were identified with high reliability. This study showed that even decomposition of a rather simple molecule like sorbitol throughout APR created a large number of intermediates which were formed in various competing reactions. Therefore, adjusting the processing parameters in APR of oxygenated hydrocarbons is rather a complicated issue when targeting certain final products; e.g. H<sub>2</sub>.

#### **2.4.2. Lignocellulosic Biomass**

Since lignocellulosic biomass is the most abundant non-edible source, attempts to gasify these materials directly via APR were noted. Valenzuela et al. (2006) subjected pine sawdust to APR directly in one-pot, two-step process in which sulphuric acid (1% to 5%) aqueous solution was used as the reaction medium [44]. The heterogeneous catalyst used was Pt/Al<sub>2</sub>O<sub>3</sub> and it was suggested that with the effect of both sulphuric acid solution and the heterogeneous catalyst, lignocellulosic material should have first hydrolyzed into glucose and others from cellulose and hemicellulose which were decomposed over Pt/Al<sub>2</sub>O<sub>3</sub> to give the final gaseous and water soluble products. They have declared that the amount of hydrogen produced from biomass was in comparable magnitude per gram of feed versus the hydrogen obtained in other APR runs with glucose at the reaction temperature of 225°C.

Wen et al. (2010) subjected solid cellulose directly to APR over Pt/C catalyst at 533 K [45]. They have declared that the hydrogen selectivity and the yield were significantly higher than those for the glucose reforming and even comparable with those for the sorbitol reforming under similar conditions. The relatively high efficiency for H<sub>2</sub> production was ascribed to the slow hydrolysis of cellulose which was catalyzed by the H<sup>+</sup> reversibly formed in water during the reaction.

Irmak, Erbatur and Hesenov [46] and Irmak and Öztürk [47] have carried out an APR investigation on the hydrolysates of kenaf and wheat straw. They have applied a two-step process in which the biomass first subjected to a catalytic thermal treatment in water under carbondioxide pressure at 250°C. This treatment resulted in a solubilization of more than 75% of biomass material [48]. GPC analysis showed that the solubilized carbohydrates were gathered into two main groups where the most abundant ones had average molecular weights in between 15000-25000 Da whereas the other group had average molecular weights in

between 50000-70000 Da. It was not certain whether these measured values correspond to free carbohydrates or aggregates. Further investigation is in progress. The filtered hydrolysate solutions obtained from kenaf and wheat straw were subjected to APR at 250°C by using various commercial and home-made catalysts under 30-50 bar pressures. Solid kenaf, solid wheat straw and glucose were also subjected to APR so as to compare the corresponding gasification results with the one obtained from biomass hydrolysates. A few selected results are given in Table 1 (unpublished data; to be published elsewhere).

Table 1. Comparison of APR of biomass hydrolysates, solid biomass and glucose

Feed <sup>1</sup> /Catalyst	Gas volume (ml)	Gas Composition (mol%)					H <sub>2</sub> mol/g catalyst
		H <sub>2</sub>	CO	CO <sub>2</sub>	CH <sub>4</sub>	C <sub>2</sub> H <sub>6</sub>	
Solid Kenaf-Pt/AC	134.4±22.7	32.0±1.3	16.6±2.0	46.1±5.3	4.6±0.2	0.70±0.1	0.29
Solid wheat straw-Pt/AC	131.0±7.0	16.4±0.8	3.2±0.2	72.0±3.4	7.0±0.3	1.4±0.1	0.15
Kenaf hydrolysate-Pt/AC	104.2±1.7	60.0±1.9	n.d. <sup>2</sup>	32.4±0.1	6.6±0.1	1.0±0.1	0.43
Wheat straw hydrolysate / Pt-SWCNT	57.4 ±1.1	66.7 ± 2.0	n.d.	27.3 ±1.9	5.5 ± 0.7	0.5 ± 0.5	0.26
Wheat straw hydrolysate/ Pt-MWCNT	29.3 ±1.3	42.7 ± 2.9	n.d.	45.7 ± 4.7	11.6 ±1.8	n.d.	0.085
Glucose-Pt/AC	312.1± 2.1	57.4±1.4	n.d.	34.0±3.2	5.4±0.7	3.2±0.8	1.21
Glucose/ Pt-SWCNT	193 ± 4.2	19.5 ± 2.1	n.d.	63.0 ± 3.2	14.0 ± 1.1	3.5 ± 1.1	0.26
Glucose/ Pt-MWCNT	239 ±15.6	25.0±1.3	n.d.	65.0 ± 1.6	6.0 ± 0.1	4.0 ± 0.1	0.41

<sup>1</sup>Kenaf hydrolysate obtained at 250°C contains 4400 mg/l of total organic carbon with two polysaccharide fractions of 53892 and 15262 Da average MWs; glucose 4400 mg/l; Pt/AC: 5% Pt on activated carbon. Wheat straw hydrolysate obtained at 250°C contains 4500 mg/l total organic carbon with two polysaccharide fractions of 69866 and 25429 Da MWs; glucose 4000 mg/l; SWCNT: Single-walled carbon nanotube; MWCNT: Multi-walled carbon nanotube.

<sup>2</sup> n.d.: not detected

Table 1 shows that the total gas volumes obtained from APR of solid biomass samples exceed the corresponding volumes obtained from hydrolysates but the selectivities for hydrogen production are rather low compared to the values obtained from hydrolysates. On the other hand, selectivities for hydrogen from hydrolysates are quite high, though the extent of gasification needs to be improved.

## CONCLUSION

Aqueous Phase Reforming (APR), a clean and sustainable technology, which has been developed mainly for gasification of oxygenated hydrocarbons promises to be technically and economically feasible to produce hydrogen and/or synthesis gas in the near future. Glycerol will be one of the abundant feeds for this process since it comes as by-product of biodiesel production and possibly will come from fermentation of sugars such as glucose, either directly or as a by-product of the industrial conversion of lignocellulose into ethanol.

The results obtained on APR by the authors of this chapter are not in close agreement with the results of Valenzuela et al. [44] and also with Wen et al. [45] such that gasification of solid biomass via APR does not seem to be promising for direct hydrogen production. Selectivities for hydrogen production are rather low and separation of catalysts for further usage will be problematic. Additionally, a continuous process does not seem to be applicable. On the other hand, gasification of the hydrolysates to be obtained from lignocellulosics via APR seems to be promising provided that environmentally benign hydrolysis methods should be developed further for effective hydrolysis of the polysaccharides and possibly the hydrolysis step should be combined with hydrogenation so that the final products of the pretreatment should be sorbitol and/or sorbitol type compounds which can be effectively gasified via APR into hydrogen-rich gaseous products.

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*Chapter 6*

## **BIOFUEL PRODUCTION POTENTIAL, PROCESSING AND UTILIZATION**

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### **ABSTRACT**

The biofuels are ecologically acceptable energy sources resulting in reduction in release of large quantities of CO<sub>2</sub> and other harmful greenhouse gases into the atmosphere causing global warming and climate change. Biofuels like biodiesel and bioethanol are the most important transportation fuels either used alone or as an additive to fossil fuels. The production technologies such as transesterification and fermentation are used for biodiesel and bioethanol respectively. The biofuel strategy should focus on use of biomass for blending ethanol with gasoline and non-edible oilseeds for production of biodiesel for blending with petro-diesel. Besides providing relief against local air pollution and global environmental change problems such diversification could also be useful in managing interruptions of fossil fuel supply, volatility of its prices and thus ensuring sustainable economic development.

**Keywords:** biofuel, transesterification, fermentation, biodiesel, bioethanol, biomass, non edible oilseeds.

The primary source of energy all over the world is fossil fuels such as coal, oil, petroleum products, natural gas etc. About 75% of the world energy use is of fossil fuel, followed by 19% of renewable energy resources (mainly biomass and hydropower) and 6% of nuclear energy (FAO, 2000). There has been rapid growth in energy consumption in most developing countries during last two decades. Energy, in India, is derived from several sources such as fossil fuels, biogas, wood, coal, oil and natural gas. Petroleum products constitute almost 50% of the total national energy demand. The government has to spend huge amount of foreign

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exchange on its imports. At present the consumption of petroleum products in India has been increasing at annual growth rate of 5–6% and in the year 2005–2006, it was about 112 million tonnes (Mt)/year. According to annual report (2006–2007) of Ministry of Petroleum and Natural Gas (MoPNG), India has imported about 99 Mt of crude oil during the year 2005–2006, causing a heavy burden of Rs. 171,702 crores on foreign exchange. For the 2007–2008, the crude oil import bill was Rs. 272,699 crores (\$68 billion), having more than 75% of oil import dependency (GOI, 2007) (Fig. 1). These figures are likely to go up further in view of the development targets of the future.

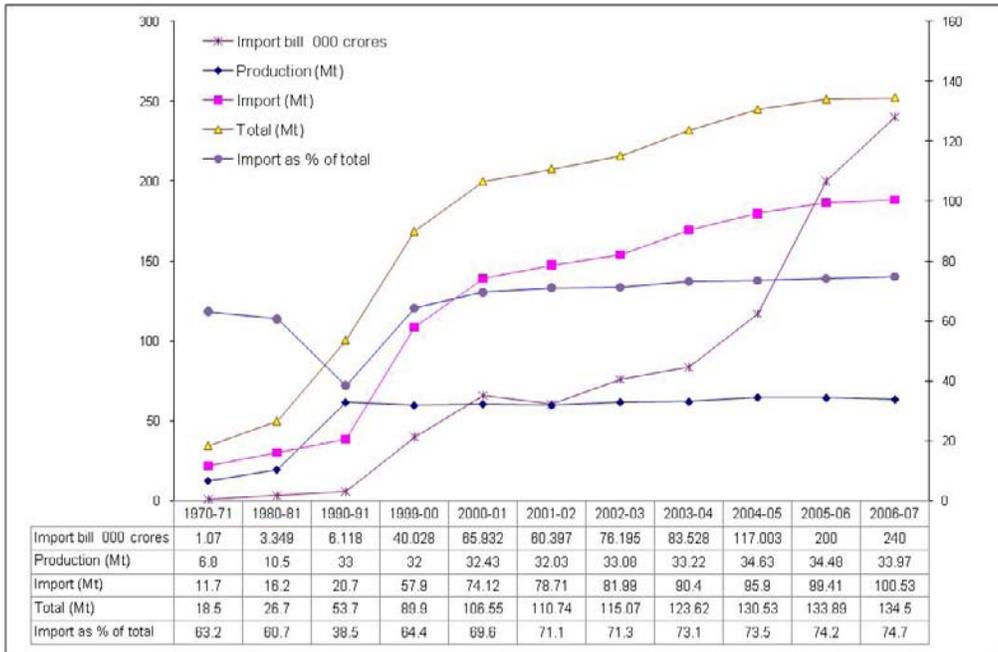


Figure 1. Scenario of crude oil import and production in India.

The combustion of fossil fuels is now being held responsible for several local as well as global environmental problems. The local air pollution caused by smoke and other exhausts of the automobiles such as benzene, PAH and NO<sub>x</sub> has led to widespread problems of human health. Considering these, in several metropolitan cities including Delhi, a gradual shift from diesel to CNG and other safer modes of fuels is being advocated (MoPNG, 2006). The carbon exhausts of fossil fuel combustion have also led to global environmental change. It is now projected that accumulation of CO<sub>2</sub> and other greenhouse gases resulted in a 0.7°C increase in temperature during last 100 years. In next 100 years it is projected that such GHG induced global warming would be to the extent of 1.5 to 5.8°C in different parts of the world (IPCC, 2001). Measures that would reduce accumulation of greenhouse gases and mitigate global warming are intensively being studied and discussed. Under Kyoto protocol and other similar instruments, mechanisms are being sought/evolved that would have considerable impact on energy use and policy of many countries. There appears to be a trade-off between energy use and development. Since overall economic development remains the primary goal of developing countries leading to increasing use of energy, it is important that alternate sources of energy are evaluated urgently.

## BIOFUEL AS RENEWABLE ENERGY RESOURCES

Renewable energy resources are an important means of providing this diversity that can mitigate considerable extent of local and global environment problems associated with fossil fuel usage (Nigam and Singh, 2010). Biofuels are referred to liquid, gas and solid fuels predominantly produced from biomass. Biofuel, including biogas, biohydrogen, biodiesel and ethanol from agricultural sources, crop residues and energy crops have been considered as a possible source, which could be used as a substitute to the conventional fuels (Prasad *et al.*, 2007b). The biggest difference between biofuels and petroleum feedstocks is oxygen content. Biofuels have oxygen levels of 10–45% while petroleum has essentially none, making the chemical properties of biofuels very different from those of petroleum (Kralova and Sjoblom, 2010). To cope with the global fuel crisis, many countries of America, Asia and Europe have started to produce biofuel from plants and food crops. Biofuels provided 1.8% of the world's transport fuel in 2008. The US has fixed a target of reduction in petroleum consumption by 20 per cent in the next 10 years, largely by turning to ethanol and other alternative fuels. India is a country with a positive outlook towards renewable energy technologies and committed to the use of renewable sources to supplement its energy requirements. The country is one among the few nations to have a separate ministry for renewable energy which address the development of biofuels along with other renewable energy sources. In the year 2003, the Planning Commission of the Government of India brought out an extensive report on the development of biofuels (Planning Commission, 2003) and bio-ethanol and biodiesel were identified as the principal biofuels to be developed for the nation. Elaborate policies for promoting both bio-ethanol and biodiesel were formulated and the time frames for enacting the development of biofuels and implementation of policies were defined. The blending targets for ethanol and biodiesel in gasoline and petroleum diesel respectively were proposed as 10% and 20% by 2011–2012 (Planning Commission, 2003) and a 5% ethanol blend in gasoline was made mandatory in 11 states and three union territories of the nation. According to a document published by Government of India, Ministry of Petroleum and Natural Gas for 5% ethanol blend in gasoline required 500 M l ethanol per annum (MoPNG, 2006). The National Biofuels Policy (NBP) has set a target level of biofuel blending of 20%, to be achieved by 2017.

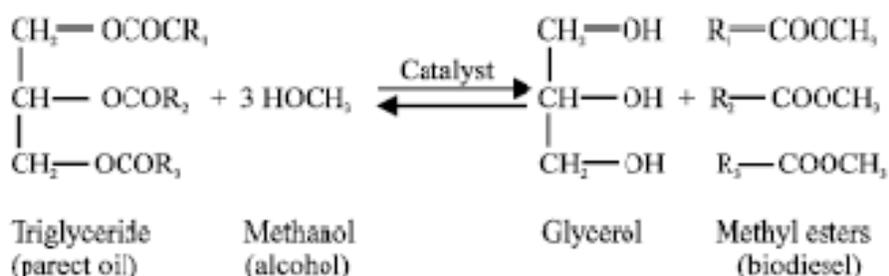
## CLASSIFICATION OF BIOFUELS

Biofuels are broadly classified as primary and secondary biofuels. The primary biofuels are used in an unprocessed form, primarily for heating, cooking or electricity production such as fuel wood, wood chips and pellets, etc. The secondary biofuels are produced by processing of biomass e.g. ethanol, biodiesel, DME, etc. that can be used in vehicles and various industrial processes. The secondary biofuels are further divided in to first, second, third and fourth generation biofuels on the basis of raw material and technology used for their production (Larson, 2008). The first generation biofuels refer to the fuels that have been derived from sources like starch, sugar, animal fats and vegetable oil. The second generation biofuels use biomass to liquid technology including cellulosic biofuels, biohydrogen, biomethanol, bioethanol, etc. Algae are considered to belong to the third generation of

biodiesel feedstock. Fourth generation biofuels are derived from the bioconversion of living organisms (microorganisms and plants) using biotechnological tools (Rutz and Janseen 2007; FAO 2008). Biofuels are also classified according to their source and type. They may be derived from forest, agricultural or fishery products or municipal wastes, also including by-products and wastes originated from agro-industry, food industry and food services. Biofuels can be solid such as fuelwood, charcoal, and wood pellets; or liquid such as ethanol, biodiesel and pyrolysis oils; or gaseous such as biogas (Nigam and Singh, 2010).

## Biodiesel

Biodiesel is made from vegetable oils, animal fats or recycled greases using transesterification. It refers to a non-petroleum-based diesel fuel consisting of mono alkyl esters of long chain fatty acids derived from these renewable lipid sources (Meher et al. 2006). The vegetable oil is composed of triglycerides of long chain fatty acids attached to glycerol. The *transesterification* is a process of transforming one type of ester into another. This process involves combining any natural oil (vegetable or animal) with virtually any alcohol and a catalyst. According to a comparative study of different alkaline catalysts used in transesterification of vegetable oil with methanol at the following conditions: 238 K, 6:1 molar ratio of methanol to oil and 1% wt sodium methylate catalyst gives the highest yield (98.6%) i.e 12% higher than sodium hydroxide. The reaction is shown below.



Petroleum diesel fuel is made up of hundreds of different hydrocarbon chains (roughly in the range of 14–18 carbons in length) and contains aromatic hydrocarbons (benzene, toluene, xylenes, etc.), sulphur and crude oil residue contaminants. However, the chemical composition of biodiesel is different from that of petroleum based diesel fuel. Biodiesel hydrocarbon chains are generally 16–20 carbons in length and contain oxygen at one end. Biodiesel contains 11% oxygen by weight and no sulfur. The higher oxygen content encourages more complete combustion (Kralova and Sjoblom, 2010). These properties improve combustion efficiency and emission profile. Biodiesel fuel blends reduce particulate matter (PM), hydrocarbon (HC), carbon monoxide (CO) and sulfur oxide (SOx) emissions (Tyson, 2004; Sukumar *et al.* 2010). Hence it is a clean, renewable and domestically produced diesel fuel, which has many characteristics of a promising alternative energy resource. It can be used as a fuel for vehicles in its pure form or blended with diesel; these can be used in existing diesel engines without many modifications. The worldwide annual production of bio diesel is estimated to be over 0.5Mt in 1998 (IEA, 1998) and 14.6Mt in 2008 respectively (Licht, 2009).

The crop used for producing a fuel is referred to as the feedstock. All over the world edible oils are used for manufacture of biodiesel. Oil crops such as rapeseed or mustard, sun flower, olive, soybean, canola, cotton seed, palm, coconut, peanut and jojoba can be used for biodiesel production (FAO, 2008). Currently, soy bean in United States, rape seed in Europe, and palm in South East Asia are the primary feedstock for biodiesel production. The main commodity sources for Biodiesel in India can be non-edible oils obtained from plant species such as *Jatropha curcas* (Ratanjyot), *Pongamia pinnata* (Karanj), *Calophyllum inophyllum* (Nagchampa), *Hevea brasiliensis* (Rubber) and other oil-based crops (Sukumar *et al.*, 2010). Another focusing feedstock group is high oil algae, which are particularly appealing due to their extremely high growth rates (due to high photosynthetic efficiency and low amount of energy expended on things other than growing), and the potential for high oil content (Christi, 2007). The algae that are used in biodiesel production are usually aquatic unicellular green algae. This type of algae is a photosynthetic eukaryote characterized by high growth rates and high population densities. Under good conditions, green algae can double its biomass in less than 24 hours (Christi, 2007; Schneider, 2006). Additionally, green algae can have huge lipid contents, frequently over 50% (Christi, 2007; Schneider, 2006). This high yield, high density biomass is ideal for intensive agriculture and may be an excellent source for biodiesel production.

In India, the Indian Railways has been experimenting with the new eco-friendly bio-diesel fuel to run passenger trains and was successful in its trial run by using 5% bio-diesel as fuel in the Delhi–Amritsar Shatabdi Express on 31 December 2002. It has plans to use the vast tracts of land all along the railway tracks for growing biofuel trees. RSDO (Railways' Research Design and Standards Organization) located at Varanasi (Uttar Pradesh, India) are conducting initial trials on test beds. The railways are cultivating *Jatropha* in 500 hectares of its land in West Bengal. The GOI's Planning Commission set an ambitious target of 11.2-13.4 million hectares of wasteland to be planted with *Jatropha* by 2012, in order to produce sufficient biodiesel to blend at 20 per cent with petro-diesel (GOI, 2007).

## Bioethanol

The liquid biofuel, usually in the form of alcohol or ethanol, can be produced from the plant carbohydrates after enzymatic hydrolysis and fermentation mostly from sugar and starch crops. With advanced technology being developed, cellulosic biomass, such as trees and grasses, are also used as feedstocks for ethanol production. Methanol can also be produced from crop residues and other biomass feed stocks. Ethanol from biological systems is considered of particular importance because it can be readily used as a fuel for spark ignition engines without necessitating any modifications. It is usually used as a gasoline additive to increase octane and improve vehicle emissions. Ethanol, being the low-carbon fuel, appears to have large potential benefits to minimise the risk of greenhouse gas (GHG) emissions (IEP, 1998). It also reduces carbon monoxide emissions and smog due to sulfur deposits in gasoline. The dependency on imported oil is also reduced. World ethanol production for transport fuel tripled between 2000 and 2007 from 17 billion to more than 52 billion litres. From 2007 to 2008, the share of ethanol in global gasoline type fuel use increased from 3.7% to 5.4%. In 2009 worldwide ethanol fuel production reached 19.5 billion gallons (73.9 billion liters).

The use of ethanol as an automobile fuel in the United States dates as far back as 1908. However, its use in engines remained at a negligible level. Interest in ethanol was renewed in the 1970s, when oil supply disruptions in the Middle East started and the efforts to remove lead in the gasoline were concentrated. Ethanol was blended directly into gasoline in a mix of 80-95 percent gasoline and 5-20 percent ethanol called gasohol. Brazil, today has a successful industrial-scale ethanol project which produces ethanol from sugar cane for blending with petrol. Competition between land for food, export crops and energy crops is not significant in Brazil. The use of ethanol has helped to create rural jobs in Brazil, improve air quality and provided a carbon substitution route. In USA, about 1% of the fuel utilized by U.S. automobiles is gasohol (Pimentel, 1998). Since, the sugars are easier to convert to glucose than the cellulosic or lignocellulosic substrates, the majority of ethanol currently produced in the United States and Brazil is made from sugarcane and corn which contain large quantities of simple sugars.

Since the primary aim of agriculture, however, would remain meeting food needs of the ever-increasing population (Kumar, 1998), we need to explore options for simultaneously meeting the ethanol needs of the transport sector. Indian land use is primarily demonstrated by agriculture and hence it generates a large quantity of agro residues (Kumar, 2009). Most of agricultural residues are at present used for meeting rural energy needs in the form of direct burning, biogas production, and manure or as a feed for livestock. Utilization of agricultural land for producing biomass for alcohol production has been advocated as an alternative policy option. It is also imperative to explore the available bio-resources for alcohol production within economic and eco-friendly perspectives (Prasad *et al.*, 2007a; Kaparaju *et al.*, 2009).

## LIGNOCELLULOSIC BIOMASS CONVERSION TO ETHANOL

Plant cell walls consist of cellulose microfibrils embedded in lignin, hemicellulose and pectin with an altered amount of each compound among plant species and plant parts. Lignocellulosic biomass could support the sustainable production of liquid transportation fuels. About 73.9 Tg dry wasted crop in the world could potentially produce 49.1 Gt year<sup>-1</sup> of bioethanol. Lignocellulosic biomass could produce up to 442 Gt year<sup>-1</sup> of bioethanol. Thus, the total potentiality of bioethanol production from crop residues and wasted crops is 491 Gt year<sup>-1</sup>, about 16 times higher than the current world ethanol production. The potential bioethanol production could replace 353 Gt of gasoline (32% of the global gasoline consumption) (Prasad *et al.*, 2007).

The use of energy crop systems along with agricultural, forest, orchard residues and municipal solid wastes using second- generation biofuel technology can greatly decrease GHG emissions (Farrell, 2006) while decoupling food and biofuel production (Kaparaju *et al.*, 2009). In India, biomass fuels dominate the rural energy consumption patterns accounting for over 80% of total energy consumed. Fuelwood, crop residues (including plantation crops) and livestock dung are the biomass fuels used in rural areas.

The residues from arable land can be categorized as residues of agricultural crops from the production per se (e.g. straw, maize cob-stover and cotton stalks) and agricultural by-products (e.g. sugarcane bagasse, barley hull, wheat barn, rice husks and rice washing drainage) originating from the processing of the agricultural commodity. Oilseed crops (e.g.

soybean, sunflower and canola) tend to produce fewer residues than grain crops and they are not ideal feedstock for soil sustainability reasons (Nelson, 2007). Sugarcane bagasse among other resources dominates in terms of tonnage in tropical and subtropical regions (Kumar *et al.*, 2008). Forestry products, particularly sawdust and forest thinnings are considered as potential feedstock for bioethanol production. The maximum theoretical yield of ethanol can be estimated as  $0.32 \text{ g g}^{-1}$  wood, assuming full conversion of cellulose and hemicellulose to sugars and subsequent conversion of sugars to ethanol at the theoretical yield of  $0.51 \text{ g g}^{-1}$  of sugar (Tahezadeh, 1999). Dedicated energy or solid crops such as Short Rotation Coppice *Miscanthus giganteus* (miscanthus) and *Panicum virgatum* (switchgrass) amongst others (e.g. *Hibiscus cannabinus* or kenaf, *Phalaris arundinacea* or reed canary grass, *Arundo donax* or giant reed, *Eucalyptus globulus* or eucalyptus) are considered as short term crops for the production of bioethanol (Monti *et al.*, 2009).

## **CROP RESIDUE PRODUCTION AND THEIR AVAILABILITY FOR ETHANOL PRODUCTION IN INDIA**

The major crop residues produced in India are straws of paddy, wheat, millet, sorghum, pulses, oilseed crops; maize stalks and cobs; cotton stalks; jute sticks; sugar cane trash; mustard stalks; etc (Kumar, 2009). Some past and future projected data for various agro-residues at the national level are given in Table 1. The total crop residue production during 1996–1997 is estimated to be 626 Mt. The dominant residues are those of rice, wheat, sugarcane and cotton accounting for 66% of the total residue production. Sugarcane and cotton residue production is 110.8 and 50.0 Mt respectively. As 390Mt of crop residues, which are used as fodder, will not be available as feedstock for energy in the year 2010. The total potential of non-fodder crop residues available for energy is estimated to be 325 and 450 Mt for 1996–1997 and 2010, respectively (Table 1). India has the potential to produce around 713 million gallons of bioethanol annually in comparison to its present production of 4% to the world total production (Prasad *et al.*, 2007a).

Besides this, a portion of the agri-residues (rice straw, wheat straw, corn stover, etc) that are presently burnt or left in the field due to mechanized farming may also be harvested for bioethanol production. The crop residues are available through out the year and dependency on a single feedstock is hence not required. The amount available depends upon the harvesting time, storage-related characteristics, storage facility, etc. Rice straw in Northwest India is currently not utilized as a livestock feed or for other uses. The farmers in order to vacate fields for timely wheat planting generally burn much of it. This straw could also be an ideal and cheap substrate for ethanol production provided such technologies are effective. A rough estimate indicates that such rice straw is more than 5 million tons in Punjab and Haryana alone. The ethanol potential of this straw alone is 1250 million liters, which is sufficient to meet the 10% blending requirement for gasohol of the whole country at present. Producing biomass for ethanol may not be expected to result into any serious aggravation of environmental problems since the land and resources required for producing biomass for ethanol is rather small (Prasad *et al.*, 2007a).

**Table 1. Total crop residue production and their availability for ethanol production in India.**

Crop	Total residue production (Mt air dry weight)		Non fodder crop residues (Mt air dry weight)	
	1996-97	2010	1996-97	2010
Rice	146.5	213.9	26.7	41.0
Wheat	110.6	157.6	8.8	21.4
Jowar	22.3	12.2	-	-
Bajra	15.8	13.6	0.0	1.4
Maize	26.3	32.5	5.3	-
Other cereals	9.4	2.8	-	-
Red gram	13.5	11.2	13.5	11.2
Gram	9.3	13.5	9.3	13.5
Other pulses	17.1	17.1	17.1	16.5
Groundnut	20.7	28.1	20.7	28.1
Rapeseed & Mustard	13.8	24.1	13.8	24.1
Other oilseeds	18.2	27.1	18.2	27.1
Cotton	50.0	55.7	50.0	55.7
Jute	15.7	10.5	15.7	5.9
Sugarcane	110.8	185.4	99.7	163.5
Coconut+ Arecanut	20.0	28.2	20.0	28.2
Mulberry	3.0	3.3	3.0	3.3
Coffee	3.42	3.9	3.4	3.9
Total	626.5	840.6	325.3	450.7

Source: Prasad *et al.* (2007 b).

## TECHNOLOGIES FOR BIOETHANOL PRODUCTION

Extensive research has been completed on the conversion of lignocellulosic materials to ethanol production in the last two decades. This conversion includes two processes: (i) hydrolysis of cellulose in the lignocellulosic materials to fermentable reducing sugars and (ii) fermentation of the sugars to ethanol. The hydrolysis is usually catalyzed by cellulase enzymes and the fermentation is carried out by yeast or bacteria. The factors that have been identified to affect the hydrolysis of cellulose include porosity, i.e., accessible surface area of the waste materials, cellulose fiber crystallinity lignin and hemicellulose content. The presence of lignin and hemicellulose makes the access of cellulase enzymes to cellulose difficult, thus reducing the efficiency of the hydrolysis. The contents of cellulose, hemicellulose and lignin in common agricultural residues and wastes are presented in Table 2. Removal of lignin and hemicellulose, reduction of cellulose crystallinity and increase of porosity in pretreatment processes can significantly improve the hydrolysis (Jørgensen *et al.*, 2007; Alvira *et al.*, 2010).

Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by enzyme hydrolysis; (2) avoid the degradation or loss of carbohydrate; (3) avoid the formation of byproducts inhibitory to subsequent hydrolysis and fermentation processes; (4) be cost effective. Novel bioreactors consisting of

more than one bioreactor along with genetic recombination techniques are being developed at laboratory and pilot scale to improve the yield and productivity of bioethanol.

**Table 2. Cellulose, hemicellulose and lignin content in residues and wastes.**

Agricultural residue	Cellulose	Hemicellulose	Lignin
Hardwood Stem	40-50	24-40	18-25
Softwood stem	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Wheat straw	33-40	20-25	15-20
Rice straw	40	18	5.5
Leaves	15-20	80-85	0
Cotton seed hairs	80-90	5-20	0
Coastal Bermuda grass	25	35.7	6.4
Switch grass	30-50	10-40	5-20
Primary wastewater solids	8-15	NA	24-29

Source: Prasad *et al.* (2007 b).

Thermophilic fermentation seems a promising technique. Additionally, the use of supercritical CO<sub>2</sub> as a pre-treatment option has increased the ethanol yield by 70% (Zheng *et al.*, 1998). The conversion of by-products into value added products under a biorefinery concept may further reduce the associated process costs with additional energy in the form of fuels, heat and electricity such as formation of xylitol from xylose, methyl furate from furfural and plastic from hydroxymethyl furfural (Kumar *et al.*, 2009). The world wide bioethanol production potential is 49.1 GL year<sup>-1</sup> from 73.9 Tg dry wasted crop material which is about 16 times higher than the current world ethanol production. Overview of an integrated biorefinery producing fuel, chemicals and energy from a variety of lignocellulosic materials (Jørgensen *et al.*, 2007) is shown by Figure 2.

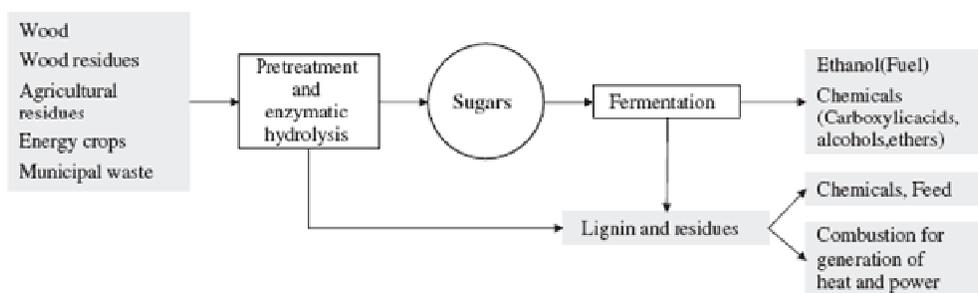
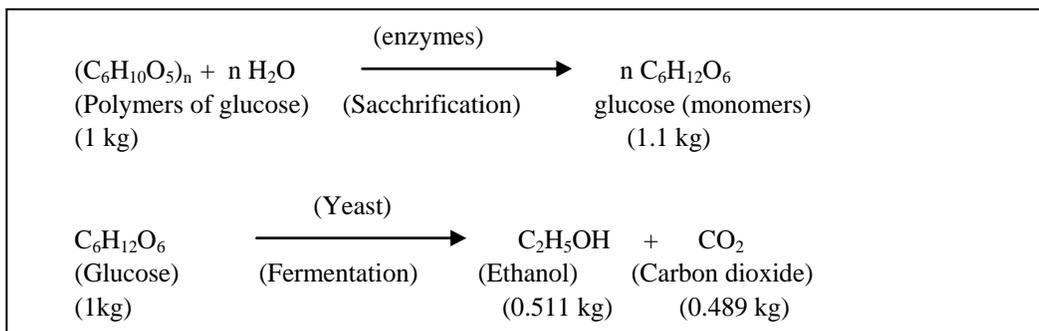


Figure 2. Overview of an integrated biorefinery producing fuel, chemicals and energy from a variety of lignocellulosic materials. Source: Jørgensen *et al.* (2007).

Nevertheless, estimation of GHG emissions of these products as they are shaped into marketable products is required. The main technological issues have been summarized recently by Prasad *et al.* (2007b) described the pros and cons of various pre-treatment options for ethanol production from lignocellulosic biomass. Moreover, lignocellulosic biomass can be exploited as a potential feedstock for bioethanol.

## ETHANOL PRODUCTIVITY PER UNIT MASS

The agri-resources constitute a vast resource of biomass consisting of sugars, starch, cellulose and ligno-cellulosic residues. The enormous quantity of available biomass has a considerable potential of ethanol production using appropriate enzymes. By comparison, the straw of all crops had 470-502 kg carbohydrate equivalent per ton of biomass. The carbohydrates (polymers of glucose) on hydrolysis and saccharification would yield glucose (monomers) which on fermentation give ethanol as per the following reaction (Garcha *et al.* 1987).



In the process of conversion, 1 kg of glucose polymer yields 1.1 x 0.511 kg ethanol. The substrate energy from the biomass is used by the enzymes catalysing the conversion for their sustenance. It was assumed on the basis of the earlier reports as 10% of energy is also lost during the process of conversion due to inefficiency of the industrial process. These losses were assumed as 5% for sugarcane (sugars based conversion), 10% for starchy cereals and tubers and 20% for cellulose/hemicellulose based straw products. Considering the density of ethanol as 0.785 g / cc at 25<sup>0</sup> C and concentration 95% (by weight), the ethanol potential could be calculated for each product as follows:

- Ethanol (95%) litres/ton fresh biomass = 1000 kg fresh biomass x dry weight fraction x carbohydrate fraction x 1.1 x 0.511 x (0.9\*conversion efficiency)/0.785/0.95

The ethanol equivalent of major crops and their crop residues could be calculated assuming the appropriate dry weight fraction of different products. The results showed that the ethanol potential was highest for wheat being 403 liters per ton of biomass followed by rice and corn (394 liter/ton), jowar (382 liter/ton) and bajra (366 liter/ton). By comparison, cassava with relatively less water content and high carbohydrates had a high ethanol potential of 157 liter/ton of biomass, As against these, crop residues ( straw of different crops) had similar ethanol potential of 250-265 liters/ton of biomass.

## NEEDS FOR FOOD VERSUS FUEL POLICY

A proper auditing of environmental costs of producing biomass for gasohol and biodiesel versus use of gasoline and diesel in terms of carbon sequestration and improved local air quality need to be considered in any policy decision. It needs to be resolved if subsidized ethanol production industry (including subsidies in biomass production) is more competitive than the cost of gasoline. Brazil had been a large producer of ethanol, but has abandoned subsidizing ethanol resulting in decreased use of ethanol in automobiles. Once agri-biomass is utilised for ethanol industry, it is also likely that the cost: benefit ratio of farm production may undergo considerable changes. There is also a policy of reducing subsidies currently provided to agriculture. Agricultural planning has to ensure sufficient food production, employment generation and rural income without increasing the risk to environment. Alternate land use systems can meet the demand of food for ever increasing population together with fuel requirement of domestic rural system as well as industrial processes are needed. Such options should be economically viable, technically feasible, socially acceptable, and environmentally safe (World Energy Outlook, 2007).

## CONCLUSIONS

Bio-fuels are considered to be a viable option for achieving energy security and environmental protection from green house gas emissions. Biomass energy has the potential to be a significant source of liquid and gaseous fuel energy in selected regions and of economic and environmental benefit to the global infrastructure. Ethanol and biodiesel are the two biofuels primarily used in transport either in pure or blended form along with petrol and diesel. The agricultural residues which currently left unused or of less utilization can be useful and cheap resource to meet the entire demand of biomass for biofuel production provided the technology for its conversion is available and cost effective. There is need to pay attention on research and development of new technology for efficient biomass utilization for liquid and gaseous fuel energy. A proper auditing of environmental costs of producing biomass for biofuel versus use of fossil fuels in terms of carbon sequestration and improved environmental quality is needed for implementing any policy decision.

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*Chapter 7*

**PLANTING SOYBEAN IN Cd-, Cu-, OR Zn-  
CONTAMINATED SOILS TO ASSESS ITS FEASIBILITY  
IN FURTHER PRODUCING BIODIESEL**

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**ABSTRACT**

There are many farmlands that have been contaminated with heavy metal (HM) in central Taiwan resulted from the irrigation using river water contaminated with HMs. According to the Soil and Groundwater Pollution Remediation Act (SGWPR Act) of Taiwan, these lands cannot plant edible crops until suitable techniques are conducted to decrease the total concentration of HM in soils to conform to the Soil Control Standard (SCS). However, some of the foliar crops still accumulated a high concentration of HM in the edible parts; even the concentration of HM of the remediated sites is below the SCS. Planting suitable crop species is especially important in this situation and these contaminated sites after remediation can be reused. Soybean, a biomass crop further used to produce biodiesel, seems feasible to plant in the farmlands in this situation. This manuscript reports previous results that used pot experiments to investigate the accumulation of HM by various parts of soybean planted in the artificially cadmium-(Cd-), copper- (Cu-), or zinc (Zn-) contaminated soils with different concentrations. The aim is to assess the feasibility of planting soybean in the HM-contaminated soils to produce biodiesel.

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**Keywords:** biodiesel, heavy metal, Soil Control Standard (SCS), soybean

## 1. INTRODUCTION

### 1.1 Heavy Metal Contamination in Formerly Taiwan (Chen, 1991)

The most famous heavy metal (HM) pollution event first happened on the paddy farmlands in northern Taiwan irrigated with illegal wastewater discharges from two chemical plants, which produced the stabilizing materials for plastics. The total contaminated area was approximately 100 ha. Soil samples in the two plants were collected and analyzed for cadmium (Cd) and lead (Pb) in different fractions and 0.1 N HCl extractable concentrations. The root, stem and leaf, hull, and grain (or brown rice) parts of the rice plants and the irrigation water were sampled near the polluted area. The concentration of Cd and Pb in soils closest to the chemical plants was higher relative to other regions and was in the levels of 180 to 378 mg kg<sup>-1</sup> and 352 to 3,145 mg kg<sup>-1</sup>, respectively. The Cd predominantly existed in the exchangeable fraction (27-47% of total) and organic matter bounded fraction (28-40% of total). Soil texture affected the distribution of Pb in the contaminated sites and it existed in the order of residual fraction (28-48% of total), free Fe and free Mn fraction (28-31% of total), and exchangeable fraction (16-28% of total).

Analytic results of rice samples show that about 88% of total Cd uptake and 70% of total Pb uptake occurred in the roots. Although the content of Cd in the brown rice is low (ranged from 0.50 to 7.94 mg kg<sup>-1</sup>), it still exceeded the forward threshold (0.5 mg kg<sup>-1</sup>) and current threshold (0.4 mg kg<sup>-1</sup>) adopted by the Department of Health of Taiwan. The concentration of Pb in the brown rice grown in polluted regions is not detectable, which indicates that Pb is difficult to transport from the root to the brown rice.

### 1.2 Survey of HMs-Contamination Farmlands in Taiwan

The total area of farmlands in Taiwan is approximately 850,000 ha. Since 1982, four stages of survey have been conducted by the Environmental Protection Administration of Taiwan (Taiwan EPA). The objective is to understand the HM contamination of farmlands in Taiwan (Taiwan EPA, 2010).

- Stage one (1983-1987): Each representative survey unit is 1,600 ha. According to the survey result, more than 300,000 ha of farmlands were contaminated with HMs.
- Stage two (1987-1991): Each representative survey unit is 100 or 25 ha. The results showed that about 50,000 ha of farmlands were contaminated with HMs potentially.
- Stage three (1992-2000): The survey lands were selected according to the results of stage two which have a relatively high concentration of HMs in soil. Each representative survey unit was 1.0 ha. There were approximately 950 ha of

farmlands that had been contaminated with HMs. Most of them were located in central and northern Taiwan and mainly contaminated with chromium (Cr), copper (Cu), and zinc (Zn).

- Stage four (2000 to 2008): This stage was conducted following the Soil and Groundwater Pollution Remediation Act (SGWPR Act) announced in 2000. The control sites or remedial sites were announced based on the criteria Soil Control Standard (SCS) in the SGWPR Act.

Taiwan EPA reported that the areas of contaminated rural soil, which were higher than the SCS, based on the results of 319 ha rural soil survey in 2002, were 159 ha of nickel- (Ni-) contaminated soils, 148 ha of Cu-contaminated soils, 127 ha of Cr-contaminated soils, 113 ha of Zn-contaminated soils, 17 ha of Cd-contaminated soils, 4 ha of Pb-contaminated soils, and 0.3 ha of mercury- (Hg-) contaminated soils. Moreover, the total areas of contaminated rural soil were about 251 ha (Taiwan EPA, 2010).

### 1.3 Remediation of HMs-Contaminated Soils in Taiwan

If the concentrations of contaminants exceed the SCSs the contaminated site is announced as a "control site" according to the SGWPR Act (Taiwan EPA, 2010) (Fig. 1). The polluter must then provide a control plan and take necessary actions to avoid the expansion of contaminants. A "remediation site" is announced if the control site poses a threat to public health and environment after going through set assessment procedures. At such time, the polluter will be required to provide a remediation plan to decrease the concentrations of pollutants in the contaminated site to below the SCSs. A flexible remediation target according to risk assessments will be set if there is no way to treat a site to below SCSs due to the limitations of geographic nature, unique character of the pollutant, or remedial technologies. In addition, if the source of contamination is unclear, but pollutant concentrations exceed SCSs, the implementation of control or remedial procedures will be implemented by the proper government authority as the same as described above.

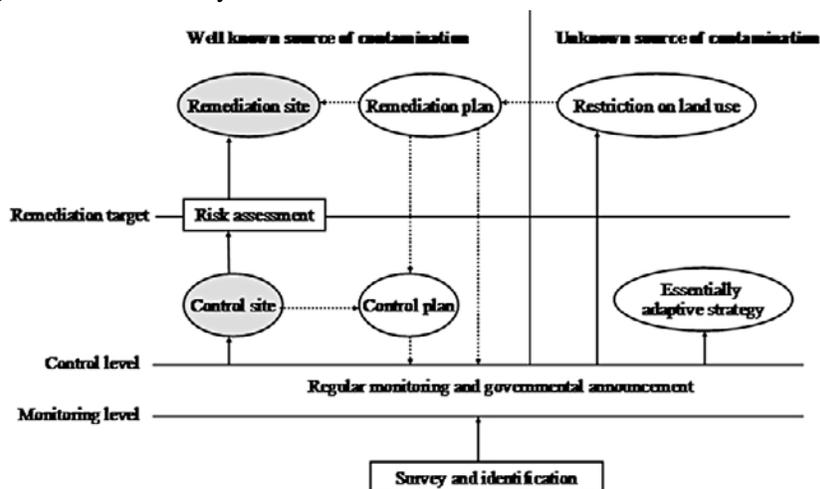


Figure 1. Management and remediation approach for HM-contaminated sites in Taiwan according to SGWPR Act.

According to the SGWPR Act, eight HMs were used as the target contaminants. Total concentrations of arsenic (As), Cr, Cd, Cu, Hg, Ni, Pb, and Zn in soils digested by aqua regia were used as the thresholds in determining contamination. Soil turnover and acid washing were the two most used techniques to decrease the total concentration of HMs in soils in Taiwan. Because the soils in upper layers always have lower concentrations of HM compared with that in the deeper layers, vertical mixture of them can efficiently reduce the total concentration of HM in soils. Soil turnover, with the advantage of lower cost and easy operation, was used to treat approximately 250 ha of the HM-contaminated farmlands in Taiwan. However, the farmlands after being treated with the two techniques may be deficient in organic matters (OMs) and essential nutrients. The HM-contaminated soils after acid washing have lower pH values which are not suitable for the growth of most crops. Suitable practices should be further conducted to adjust the soil pH and to increase the contents of OMs and nutrients

## 2. ACCUMULATION OF HM BY SOYBEAN GROWN IN HM-CONTAMINATED SOILS

Leafy vegetables are high accumulators compared to non-leafy vegetables (Podlesáková et al., 2001). The bioconcentration factor (BCF is the ratio of shoot HM concentration to soil HM concentration) of Cd of pak choi, the most consumed leafy vegetable in Taiwan, was in the levels of 2.1 to 5.5 (Chen et al., 2010a; Chen et al., 2010b). For lettuce grown in Cd-spiked soils, the BCF values were between 1.9 and 2.2 (Podlesáková et al., 2001). In previous cases, even the total concentration of Cd in farmland was below the SCS ( $5.0 \text{ mg kg}^{-1}$ ) of SGWPR Act; some varieties of rice still accumulated more than  $0.5 \text{ mg Cd kg}^{-1}$  in their brown rice (Römkens et al., 2009). In able to reuse the HM-contaminated farmlands and also to produce non-harmful vegetables, non-leafy vegetable is a better choice. Beans of soybean can be further used to produce biodiesel and its agricultural management is mature. Seeds of soybean were sowed in artificially and potted Cd-, Cu, or Zn-contaminated soils to assess their accumulation in different parts, especially for beans.

### 2.1 Pot Experiment I

Soil samples were collected in an HMs-contaminated site in central Taiwan. After air drying, soil samples were ground and screened with stainless steel sieves (10 or 80 mesh). The basic characteristics analyzed namely soil water content (Gardner, 1986), texture (Gee and Bauder, 1986), pH value (Thomas, 1996), organic carbon content (OC) (Nelson and Sommers, 1982), and the total concentration of HMs. Texture of the used soil is silty clay (sand 8.80%, silt 50.9%, clay 40.3%) with slight acidity (pH 6.20) and moderate content of OC (2.52%). Appropriate volume of solutions of  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , or  $\text{CuSO}_4$  were added to the soil samples according to their initial concentrations to make their final concentrations ( $\text{mg kg}^{-1}$ ) as Cd-5, Cd-20, Zn-1000, Zn-2000, Cu-200, and Cu-400 (coded as HM-concentration).

Approximately 2.5 kg of artificially HM-contaminated soil samples were added in each pot (16 cm diameter and 20 cm height) and sowed five seeds of soybean (*Glycine max* L.). Pot experiment was conducted in the phytotron (day/night = 30 °C/25 °C) located in MingDao University with three replicates. Water content of soil was controlled at 50-70% of field capacity by weighing and adding DI water every 2-3 days. Soybeans were sampled after growing for 75 days, separated into different parts (root, shoot, pod, and bean) and rinsed with DI water. After oven drying at 65 °C for 72 hours, plant tissues were digested by a mixture solution of concentrated HNO<sub>3</sub>/HClO<sub>4</sub> (v/v = 87/13).

Soil samples after pot experiment were collected, air dried, ground, and then passed through stainless steel sieves (10 or 100 mesh). Soil samples passed through a 100 mesh sieve were digested by aqua regia to determine their total concentrations of HMs. The available concentrations of HMs in soils passed through 10 mesh stainless sieves were extracted by 0.1 M HCl or DI water. The concentrations of Cd, Cu, or Zn in the digestant or extracting solutions were determined with a flame atomic absorption spectrometer (FAAS; Perkin Elmer AAnalyst 200).

## 2.2. Accumulation of HM by Soybean

The target concentrations of Cd, Cu, and Zn in artificially spiked soils were set according to the SCSs of SGWPR Act. After the pot experiment, the total and extractable concentrations of Cd, Cu, and Zn in soils are shown in Table 1. Except for Zn, the final concentrations of HMs in soils were in the levels of 85-125% of the target concentration. Approximately 70-100% of the spiked HMs can be extracted by 0.1 M HCl.

**Table 1. Concentration of Cd, Cu, or Zn in soils digested by aqua regia or extract by 0.1 M HCl and deionized water (DI water).**

	Concentration in soil (mg kg <sup>-1</sup> )		
	Aqua regia	0.1 M HCl	DI water
	Cd		
CK	0.80±0.26	0.50±0.09	ND*
Cd-5	6.27±0.36	4.87±0.07	ND
Cd-20	19.4±0.5	18.9±0.2	0.02±0.03
	Cu		
CK	50.0±0.8	47.0±2.5	ND
Cu-200	210±13	151±5	ND
Cu-400	340±10	258±9	0.05±0.05
	Zn		
CK	202±2	94.8±1.4	0.07±0.05
Zn-1000	1271±41	884±19	19.9±2.0
Zn-2000	3,135±166	2,583±81	480±79

\*ND: not detectable

Similar to that reported by Jung and Thornton (1996), the accumulation of Cd in the different parts of soybean increased when the Cd concentrations in soils increased. Different

parts of soybean accumulated less than  $3.0 \text{ mg kg}^{-1}$  in Cd-CK because of the low Cd concentration in soil ( $0.80 \pm 0.26 \text{ mg kg}^{-1}$ ). However, the Cd concentrations were in the levels of 9.0 to  $220 \text{ mg kg}^{-1}$  in the treatment of Cd-20 (Fig. 1). The Cd concentration accumulated in the different parts of soybean was in the decreasing order of root, shoot, pod, and bean. Roots of soybean accumulated more than 4-fold of Cd concentration compared with shoots, pods, and beans. Experimental result shows that the soybean did not accumulate Cd in its bean when growing in Cd-5 ( $6.27 \pm 0.36 \text{ mg Cd kg}^{-1}$ ). Planting soybean in Cd-5 will not produce Cd-contained beans and the other parts can accumulate more than  $5.0 \text{ mg kg}^{-1}$ . The shoots of soybean can remove Cd from contaminated soils and the produced beans can be used to produce biodiesel when it is planted in soils containing less than  $6.0 \text{ mg Cd kg}^{-1}$ .

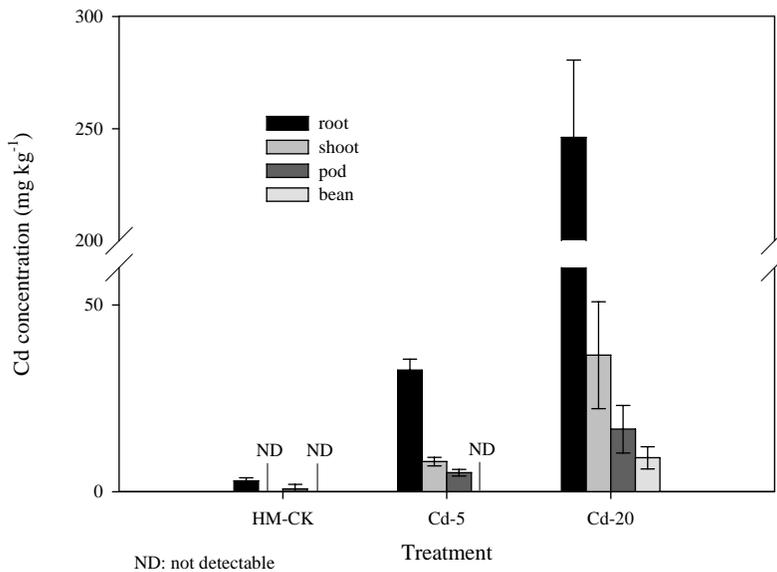


Figure 2. The accumulation of Cd by various parts of soybean grown in artificially Cd-contaminated soils with different concentrations (Replicated (n) = 3).

Similar to the experimental result of Cd, roots of soybean accumulated higher concentrations of Cu compared with other parts (Fig. 2). Copper is an essential element for health of the plants and for the nutrient supply to animals. Its content in plant shoots did not often exceed  $20 \text{ mg kg}^{-1}$ , which is often considered to indicate the threshold of exceeded contents (Kabata-Pendias and Pendias, 2001). Less than  $25 \text{ mg Cu kg}^{-1}$  was accumulated in the bean of soybean, but were not detectable in the shoot and pod for most of the treatments. The experimental result is in agreement with the foregoing reference.

Because the total Zn concentration in Zn-2000 was 1.6-fold higher than the target concentration, seeds of soybean after sowing did not germinate at all. In the treatment of Zn-1000, roots of soybean accumulated the highest concentration of Zn compared with other parts (Fig. 3). The concentrations of Zn in the roots, shoots, and pod were in the levels of 900-2,200  $\text{mg kg}^{-1}$ , which is more than three times of that in the beans. The toxic critical concentration of Zn in the plants is reported to be 150-200  $\text{mg kg}^{-1}$  for sensitive plants and 300-400  $\text{mg kg}^{-1}$  for young barley and oats. However, the critical concentration of Zn in root tissues is much higher because Zn is immobilized in cell walls (Kabata-Pendias and Pendias,

2001). Experimental result of this study is in agreement with the foregoing reference and the Zn concentration in roots is 1.3-fold than that in the shoots. The toxic critical concentration of Zn in the shoot of soybean may be higher than  $1,500 \text{ mg kg}^{-1}$  because no toxic symptom was observed during the pot experiment.

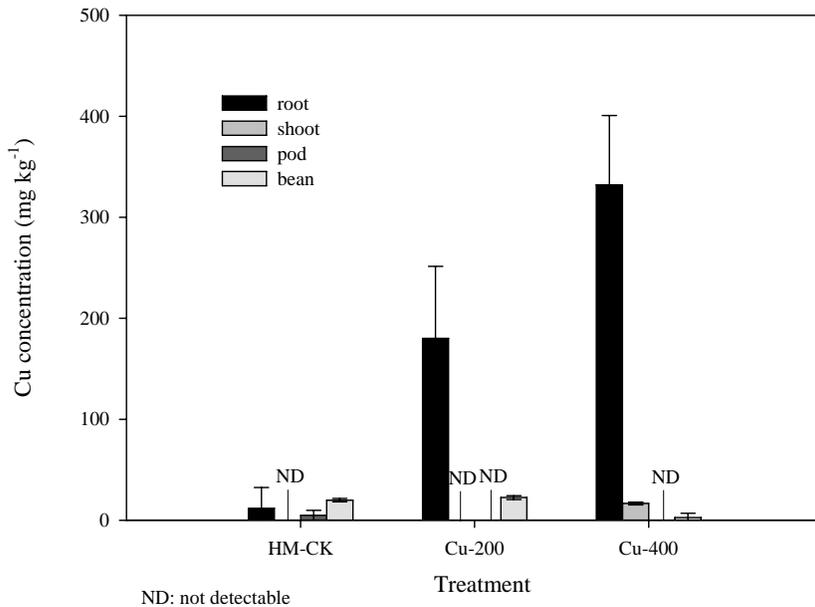


Figure 3. The accumulation of Cu by various parts of soybean grown in artificially Cu-contaminated soils with different concentrations (Replicated (n) = 3).

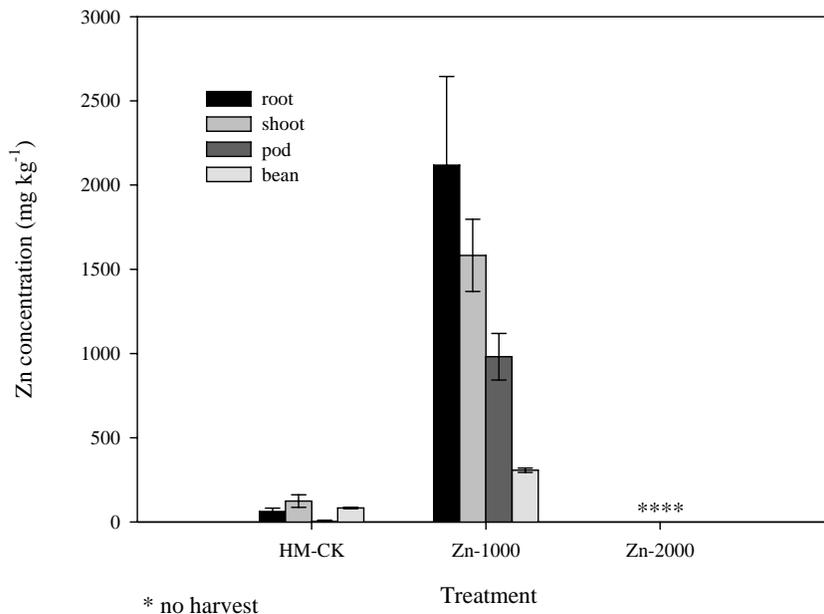


Figure 4. The accumulation of Zn by various parts of soybean grown in artificially Zn-contaminated soils with different concentrations (Replicated (n) = 3).

### 2.3. Bioconcentration Factor of HM of Soybean

Bioconcentration factor (BCF), as described in equation [1], was used as an indicator to assess the accumulation capacity of the different parts of soybean grown in contaminated soils. The soil's treatments used to calculate the BCF values include Cd-5, Cd-20, Cu-200, Cu-400, and Zn-1000. HM-CK and Zn-2000 were not used in the calculation because of the lower concentration of HMs in CK and plants died, respectively.

$$\text{BCF} = \frac{\text{Concentration of HM in plants}}{\text{Concentration of HM in soils}} \quad [1]$$

Except for the root, similar BCF values were found for Cd and Zn (Fig. 4). The translocation of Cd and Zn within the plant was faster than Cu, in which its BCF was less than 0.06 and can be neglected except for the root. Many researchers reported that Cd was transferred more easily from soil to the tissues of vegetables than other HMs (Wang et al., 2003; Zheng et al., 2007), with translocation decreasing in the order Cd > Zn > Pb > Cu > Cr > Ni (Li et al., 2010). The experimental result of this study is in agreement with the foregoing literatures. For the same HM, the roots of soybean have higher BCF compared with other parts and their values were mostly all more than unity, especially in Cd-5 (BCF = 5.18) and Cd-20 (BCF = 11.1). In relative to other parts, the beans of soybean had the lowest BCF, which were all less than 0.24 excluding the highest value (0.47) in Cd-20. Experimental result is in agreement to the experimental result of Murakami et al. (2007) who planted two cultivars of soybean in potted Cd-contaminated soils with total concentration ranging from 0.83 to 4.29 mg kg<sup>-1</sup>. Lavado (2006) reported that the Cd concentration in beans and leaves of soybean when growing in the soil with 1.25 mg Cd kg<sup>-1</sup> was 0.11 and 0.20 mg kg<sup>-1</sup>, respectively. The BCF of bean and leaf was only 0.09 and 0.16, respectively. Roots of two cultivars of soybean accumulated a higher concentration of Cd compared with shoots; their BCF values for roots and shoots were in the levels of 2.40-4.14 and 0.56-3.66, respectively.

A hyperaccumulator is a plant that can accumulate higher than 100 mg Cd kg<sup>-1</sup>, or 1,000 mg kg<sup>-1</sup> for Cu, or 10,000 mg kg<sup>-1</sup> for Zn (Brooks et al., 1977; Reeves, 1992). The translocation factor (TF; the ratio of HM concentration in the shoot to that in the root) and BCF should be greater than unity (Sun et al., 2009). The soybean used in this study accumulated more than 100 mg Cd kg<sup>-1</sup> in the roots and its BCF of shoots were more than unity. Even so, it still can not be regarded as a Cd hyperaccumulator because of its lower TF values.

### 3. EFFECT OF APPLICATION OF AMENDMENTS ON THE ACCUMULATION OF HM BY SOYBEAN

The application of OM can efficiently improve the soil conditions and thus the growth of plants. Biosolid (BS), which is enriched in OM, was applied to Cd-contaminated soils as an amendment to test its effect on the accumulation of Cd. The following experiment result is excerpted partially from our previous research (Chen et al., 2010a) and reorganized.

### 3.1. Pot Experiment

The surface soils (0-20 cm) of a contaminated site in central Taiwan were sampled and used for the pot experiment. Biosolid was sampled from a wasted sludge tank of a domestic wastewater treatment plant. Soils and BS were air dried, ground, and passed through a 10 mesh sieve before analysis. Solution of  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  was artificially added to the soils to make their final total concentration ( $\text{mg kg}^{-1}$ ) as Cd-CK, Cd-3, and Cd-5 (coded as HM-concentration).

BS was homogeneously mixed with artificially Cd-contaminated soils and then 3.0 kg of mixture (5% of BS) was added in each pot (16 cm diameter and 20 cm height). Ten seeds of soybean (*Leichardt* species) were sowed in each pot and all pots were then placed in a greenhouse (day/night = 30 °C/25 °C) with four replicates. Soil water content during the experiment was controlled in the levels of 50-70% of field capacity by adding DI water every 3-4 days. Soybeans were harvested after growing for 90 days and separated into beans, pods, and shoots. The plant tissues were rinsed and oven dried at 65°C for 72 hours, weighted, ground, and digested using  $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$  method (Harmon and Lajtha, 1999). Soil samples after pot experiment were collected, air dried, ground, and digested by aqua regia. The Cd concentrations in the digestant of plants and soils were determined with a FAAS (Hitachi 180-30).

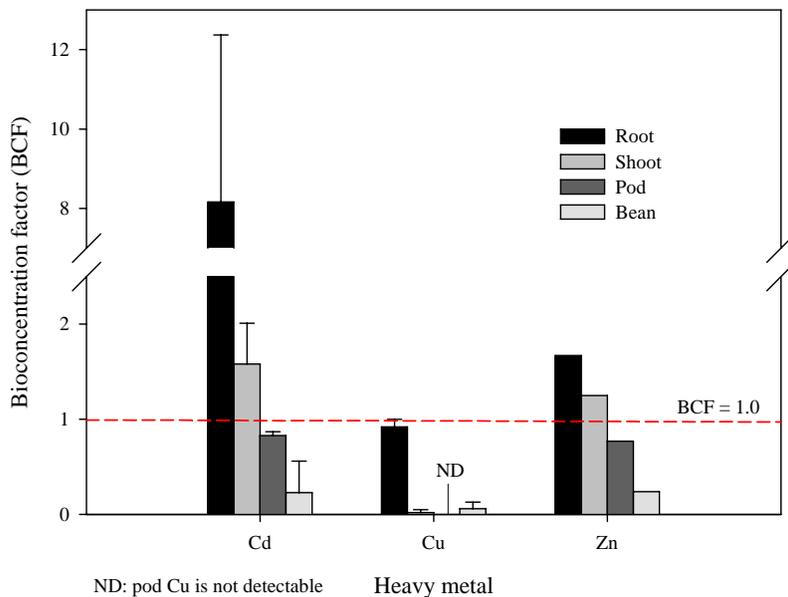


Figure 4. The bioconcentration factor (BCF) of Cd, Cu, and Zn by various parts of soybean grown in artificially contaminated soils (Replicated (n) = 3).

### 3.2. Effect of BS on the Accumulation of Cd

The texture of the tested soil was silty clay (sand 8.80%, silt 50.9%, clay 40.3%) with moderate CEC ( $13.2 \pm 0.4 \text{ cmol}_{(+) } \text{ kg}^{-1}$ ) and water content ( $2.98 \pm 0.48\%$ ). The soil pH values

were in the levels of 5.9 to 6.7. The total concentration of Cd of Cd-CK, Cd-3, and Cd-5 was  $1.02\pm 0.32$ ,  $2.94\pm 0.54$ , and  $5.28\pm 0.92$  mg kg<sup>-1</sup>, respectively.

Figure 5 shows the Cd concentration in the shoots, pods, and beans of soybeans grown in Cd-contaminated soils amended with or without BS. In agreement with that reported by Jung and Thornton (1996), the accumulation of Cd in the shoots and pods of soybean increased with the increasing of spiked Cd concentrations in soils. The Cd concentrations in the various parts of soybean grown in Cd-CK were not detectable because of the lower Cd concentration in soils. The accumulation of Cd by different parts of soybean grown in Cd-3 and Cd-5 is in the decreasing order of shoot, pod, and bean. Soybeans accumulated higher concentrations of Cd in its shoot, but were all less than 10 mg kg<sup>-1</sup> and have large variation possible resulting from the soil pH (ranged from 5.9 to 6.7). Beans only accumulated a low concentration of Cd ( $0.25\pm 0.35$  mg kg<sup>-1</sup>) in their beans in the treatment of Cd-3. For other treatments, beans did not accumulate Cd even amended with BS.

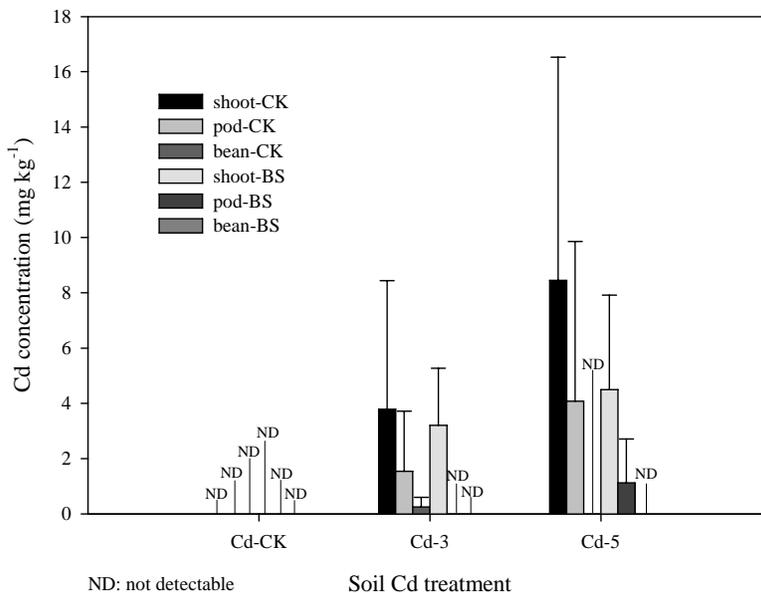


Figure 5. Effect of applying biosolid (BS) on the Cd concentration in the shoots, pod, and beans of soybean grown in artificially Cd-contaminated soils (Replicates (n) = 4).

### 3.3. Bioconcentration Factor of Cd of Soybean

After growing in Cd-contaminated soils for 90 days, the BCF values of Cd of various parts of soybean is shown in Fig. 6. The shoots of soybean had higher BCF in relation to other parts and some of them were more than unity (dotted line in Fig. 6). There was no significant effect of applying BS on the BCF. The BCF of *Brassica rapa* grown in the artificially Cd-contaminated soils was in the levels of 1.9 to 2.2 (Chen et al., 2010b). For lettuce grown in Cd-spiked soils, the BCF values were between 1.9 and 2.2 (Podlesáková et al., 2001). Various parts of soybean and especially the bean had lower BCF compared with

leafy vegetables which revealed that soybean is a safer crop when planting in the Cd-contaminated soils.

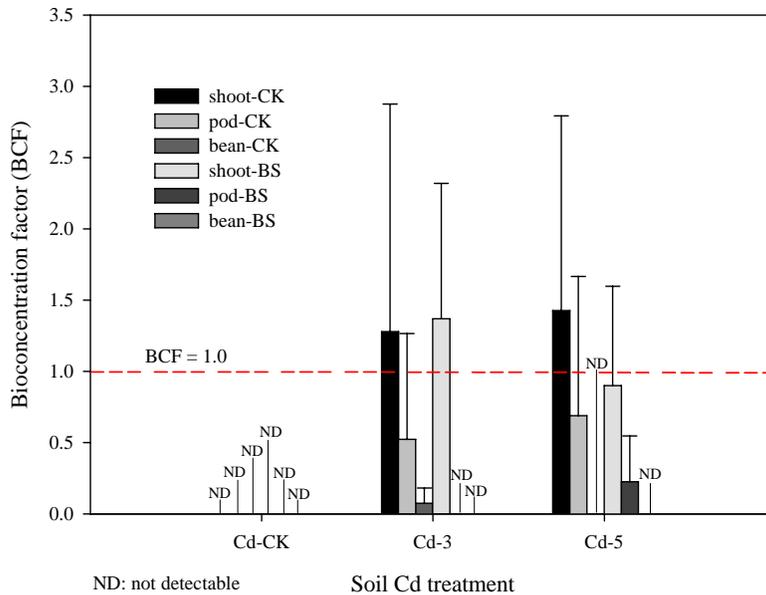


Figure 6. Effect of applying biosolid (BS) on the bioconcentration factor (BCF) of Cd in the shoots, pod, and beans of soybean grown in artificially Cd-contaminated soils (Replicates (n) = 4).

## CONCLUSION

The accumulation of Cd, Cu, and Zn by different parts of soybean grown in artificially spiked soils was investigated using pot experiments. The roots and shoots of soybean accumulated higher concentration of HMs compared with other parts. Even so, the Cd concentration accumulated in the beans of soybean was not detectable for most of the treatments. Planting soybeans in Cd-contaminated soils with a total concentration of less than  $5 \text{ mg kg}^{-1}$  seems to be feasible because their bean did not accumulate considerable concentration of Cd. These HMs-contaminated soils can be reused and the following procreative beans can be further used to produce biodiesel. More researches should be conducted from yield and quality of soybean oil and biodiesel to assess its feasibility.

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*Chapter 8*

**DISSEMINATION OF INTEGRATED SOIL FERTILITY  
MANAGEMENT PRACTICES USING PARTICIPATORY  
APPROACHES IN THE CENTRAL  
HIGHLANDS OF KENYA**

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**ABSTRACT**

Declining soil fertility is a critical agricultural challenge facing smallholders in central Kenya. A study to improve soil fertility and farm productivity in the area was carried out during the period 2003 to 2007. Problem-solving tools were used to build the broad conceptual and methodological approaches needed to address farming constraints. The study identified farming systems constraints and disseminated “best-bet” integrated soil fertility management (ISFM) interventions using participatory methods and mutual collaborative action. This paper describes processes in the participatory approaches, project milestones and joint experiences that were gained. The participatory approaches included Participatory Rural Appraisal (PRA), Mother-baby approach (M-B approach),

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Farmer training groups (FTGs), Annual stakeholder planning meetings, Village training workshops, Cross-site visits and Participatory Monitoring and Evaluation (PM & E). Food shortage was the main problem identified by farmers resulting from low crop yields. The causes of poor yields were biophysical factors, but several socio-economic factors influenced farmer ability to manipulate farm productivity. Village training workshops attracted a 20% higher farmer turnout than mother trial field days. Farmer and experimental evaluations showed that the most favoured technologies were tithonia, manure, manure-fertilizer combinations, and tree legumes while the most effective dissemination pathways included demonstrations, farmer training grounds, field days and farmers' groups. Using PM& E procedures, farmers developed indicators that they used to monitor progress, and annual ISFM milestones were achieved, leading to the achievement of overall project objectives. Innovative adjustments to ISFM technology dissemination were proposed by both farmers and scientists.

**Keywords:** Farmer groups; Mother-baby approach; Participatory Rural Appraisal; Participatory Monitoring and Evaluation; Tithonia

## INTRODUCTION

### Background

Soil nutrient depletion in sub-Saharan Africa, a phenomenon that has ultimately led to soil degradation and reduced per capita food production, has become a serious issue of global concern (Bationo et al., 2004). High population growth rates in sub-Saharan Africa (SSA), resulting in reduced fallow periods and subsequent intensified continuous cropping without proper soil management is the primary cause of nutrient depletion (Smaling et al., 1997; Nandwa and Bekunda, 1998). The humid highlands of central Kenya are characterized by high population pressure and intensively cultivated farmlands. Farmers in this region use insufficient amounts of inorganic fertilizers and other sub-optimal inputs (Woomer and Muchena, 1996) due to high cost, low returns to soil input investments, lack of know-how and poor incentives to utilize adequate inputs (Okalebo, 2000; Makokha et al., 2001).

Management of soil fertility at the smallholder farmers' level in Africa is complex due to socio-economic and biophysical heterogeneity of farming systems. Several researchers and global development experts have raised concerns over declining agricultural performance and rising rural poverty in the SSA, especially in the period after the 1990s SAP (Structural Adjustment Program) economic market reforms (Camara and Heinemann, 2006), leading to a paradigm shift in soil fertility management. Researchers consequently recognized the need for integrated soil fertility management (ISFM) as a guiding principle and a basic requirement for increasing agricultural productivity in SSA (Heerink, 2005; Vanlauwe et al., 2003). Studies in the central highlands of Kenya and other areas in sub-Saharan Africa have identified promising ISFM interventions with proven potential to improve soil productivity. Long term experiments have shown that combined organic and inorganic soil treatments lead to net soil bio-physical (Bekunda et al., 1997; Mugendi et al., 1999) and economic gains (Mugwe and Mugendi, 1999) than sole applications. These include practices such as, integrated organic and in-organic fertilizers, intercropping systems, improved manure management, integration of multipurpose legumes, biomass transfer and improved fallows (Mugendi et al., 1999;

Gitare et al., 2000; CGIAR 2002). ISFM improves the agronomic efficiency of external inputs used, reduces the risks of acidification that results from sole fertilizer application (Bekunda et al., 1999; Mugwe et al., 2010), provides a more balanced supply of nutrients, besides offering several unquantifiable ecosystem services. These practices result in more yield and nutrient use efficiency than that expected from mere additive effects of sole applications (Bekunda et al., 1997; Mugendi et al., 1999; Gachengo et al., 1999). Despite these positive results, up-scaling has been slow and adoption of ISFM technologies in Kenya has been minimal (Ali et al., 2007).

Apart from socio-economic and several farm-level factors, poor research, extension, and dissemination methodologies are some of the causes of low ISFM adoption (MoA, 2002). Availability of sufficient quantities of organic materials and germplasm is also a major challenge to full utilization of ISFM potential (Vanlauwe et al., 2003; Nwalozie and Mbaye, 2003). Before the 1970s, the research and development paradigm was centralized and relied on top down transfer of technology models that did not consider the needs of multiple clients (Hall and Clark, 1995) or diverse and dynamic contexts. While it is recognized that farmers have valuable knowledge that scientists could integrate to improve agricultural research and development, past models were unsatisfactory in terms of enhancing widespread technology adoption (Biggs, 1978; Rhoades and Booth, 1982; Richards, 1995). This led to emergence of participatory methods (techniques that include multiple stakeholder involvement in problem solving) in agricultural research and extension. Chambers and Ghildyal (1985) proposed the “farmer first and last” model. As a result, the use of participatory approaches in the agricultural context proliferated since the 1980s. There is empirical evidence supporting the effectiveness of participatory approaches such as participatory rural appraisal (PRA), in enhancing the relevance and adoption of technologies by smallholders (Witcombe et al., 1999; Onduru et al., 2002), especially when multiple stakeholders are involved in mutual collaboration (Haverkort, 1991).

A major challenge in the dissemination of soil fertility management technologies includes their highly variable performance due to heterogeneity in smallholder farms (Tittonell et al., 2005). Local farmer adaptation is a key means of optimizing agricultural performance in heterogeneous environments that exist in smallholder farming systems. Involvement of farmers is important because they understand their complex biophysical, social-cultural and economic environment better and therefore better in adapting new technologies which are crucial in accelerating adoption. However, knowledge gaps still remain on how this can be achieved as there are relatively few studies with farmer-participatory approaches dealing with diverse ISFM practices (Place et al., 2003). Moreover, involvement of farmers in soil fertility technologies has proved problematic, with few successful proven models (Kanyama-Phiri et al., 1998). The purpose of this study was therefore to use participatory methodologies to diagnose farming systems constraints and to involve farmers in the development and dissemination of ISFM practices in Meru south District, Kenya. The ISFM technologies disseminated included integrated organic and inorganic nutrient sources, improved manure management, legume intercrops, and biomass transfer using high quality organics. This paper documents the processes in the participatory methods used and outlines achievements, milestones, lessons and some empirical results emanating from the participatory soil management approaches.

## Conceptual Model

In the evolution of agricultural extension approaches, there has been major paradigm shifts in an effort to transfer information from research to farmers more efficiently. Traditionally, in the 1950's and 1960's, transfer of information to farmers was the responsibility of government extension services where farmers were regarded as users of research findings. This linear extension mode usually referred to as "the top-down approach" had drawbacks and there was limited adoption of agricultural technologies and limited impact on agricultural productivity (Farrington and Martin, 1988). The major reason for low adoption was poor communication mechanisms for linking researchers to extension agencies and farmers. This led to rethinking in late 1980s and 1990s with a focus to generate technologies that fit farmers' conditions (Chambers and Thrup, 1989). A family of approaches labeled "Farmer first", "farmer-back-farmer" (Rhoades and Booth, 1982), "Farmer First and last" (Chambers and Gildyal, 1985) and "Farmer participatory research" (Farrington and Martin, 1988) were coined. These approaches emphasized participation in generation, evaluation and promotion of sustainable agricultural production. In the 2000s, issues of farmer empowerment, change in institutions and policies become pertinent with Integrated Agricultural Research for Development (IAR4D) coming into the limelight. Critical analysis of these evolving approaches and methodologies reveal that the major focus is effective exchange of information between research, extension and farmers to enhance farmer empowerment and adoption of improved agricultural technologies.

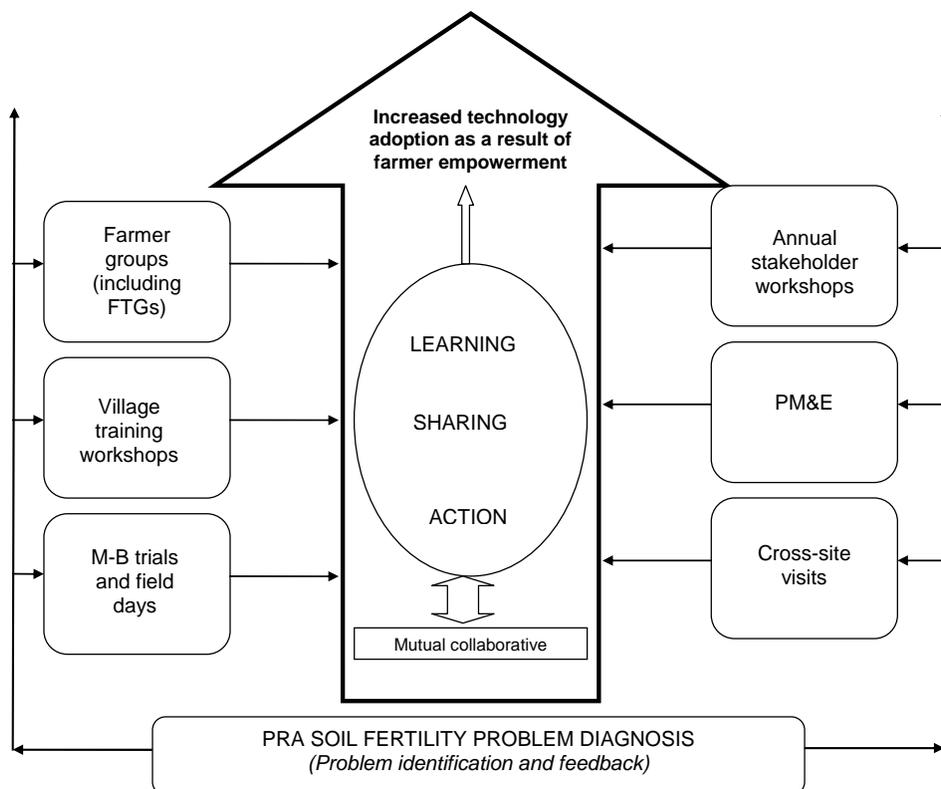


Figure 1. Conceptual model for disseminating ISFM practices in central Kenya between 2003 and 2007.

Therefore to enhance effective exchange of information among partners and promote adoption of ISFM practices, this study adopted a methodological design incorporating farmers' participation, and the innovation diffusion model that considers access to information about an innovation to be the key factor in determining adoption decisions (Rogers, 1995; Feder et al., 1985). According to the innovation diffusion model, adoption is a mental process that involves successive events beginning with initial exposure (first hearing about an innovation) to full utilization of an idea. The model emphasizes extension contact and other methods that create widespread knowledge and awareness among farmers. Its (model) assessment has revealed improvement in agricultural technology adoption due to increased in farmer knowledge (Adesina and Zinnah, 1993; Hussain et al., 1994). Similarly, increased knowledge as a result of farmers' attendance in training seminars has also been shown to positively influence technology adoption (Adesina and Baidu-Forson, 1995). In the current study, information to farmers was disseminated using participatory-based approaches that emphasized mutual collaborative learning, sharing and joint action (Figure 1).

## MATERIALS AND METHODS

### Description of the Study Area

Meru South District of Kenya lies between latitudes and longitudes 00°03'47" N and 00°27'28" S 37°18'24" E and 28°19'12" E. The district covers an area of about 1035 km<sup>2</sup>. According to agro-ecological conditions, the area lies in the Upper Midland Zone (UM2-UM4) (Jaetzold et al., 2006) on the eastern slopes of Mt. Kenya with an annual mean temperature of 20° C and a bimodal rainfall pattern totaling 1200–1400 mm. The rainfall comes in two seasons; the long rains (LR) lasts from March through June, while short rains (SR) commences in October through December.

The area is dominated by farming systems with a complex integration of crops and livestock, and smallholder farms that are intensively managed. Land sizes are small ranging from 0.1 to 1.5 ha (mean=1 ha), and slope cultivation is widespread. The main cash crops are coffee and tea while the main staple food crop is maize, which is cultivated from season to season mostly intercropped with beans. Other food crops include potatoes, bananas and vegetables that are mainly grown for subsistence consumption. Improved dairy farming is a major enterprise. Other livestock in the area include sheep, goats and poultry.

The study was carried out in (four) 4 sites. These were Muthambi Division (Murugi), Chuka Division (Mucwa and Kirege) and Magumoni Division (Mukuuni). These four project sites were chosen using GPS to extensively cover the district as possible. Kirege [1473 meters above sea level (masl)] and Mucwa (1373 masl) are located in the Upper midlands (UM3) agro-ecozone while Mukuuni and Murugi (both 1287 masl) are located at the transition of the UM3 zone to the UM4 zone.

The soils in Kirege, Mukuuni and Mucwa are Rhodic Nitisols while in Murugi they are Humic Nitisols. These are deep, well weathered soils with moderate to high inherent fertility but over time soil fertility has declined due to continuous cropping, slope cultivation (up to 60% slope) (Lekasi et al., 2001) and minimal use of fertilizers. The soils have generally low

levels of organic carbon (< 2.0%), nitrogen (<0.2%), phosphorus (< 10 ppm) and are moderately acidic (pH ranges from 4.8 – 5.4) (Jaetzold et al., 2006).

## **Approaches**

The disseminated approaches included in the study were as follows; Participatory Rural Appraisal (PRA), Mother- Baby trials (M-B trials), Village training workshops, farmers' groups, annual workshops, and Participatory Monitoring and Evaluation (PM&E), which are described in the following section.

### ***Participatory Rural Appraisal (PRA)***

The goal of PRA is to implement projects that are socially acceptable, ecologically sustainable and economically viable, through wide participation of community stakeholders. PRA helps communities to mobilize human and natural resources, define prevailing problems, prioritise opportunities, evaluate local institutional capacities, and develop systematic site plans of action (Chambers, 1994). This project used the PRA to evaluate the status quo regarding soil management in the target area. The PRAs were conducted in all the 4 project sites during the month of February in 2004 and were implemented in two phases. Phase one involved diagnosis of problems (diagnosis phase) while phase two involved the research team providing feedback of the diagnosis phase to the farmers and identification of potential solutions to commonly identified agricultural constraints.

### **Mother-Baby Approach**

The Mother-Baby trial model (M-B trial) is an upstream participatory research methodology designed to improve the flow of information between farmers and researchers about technology performance and appropriateness under farmer conditions (Snapp, 1999). The trial design consists of two types of trials; mother and baby. The mother trial is researcher-designed and conforms to scientific requirements for experimentation, data collection and analysis. The baby trial consists of a single replicate of one or more technologies or components from the mother trial that is managed by farmers. According to Johnson et al. (2003), the M-B trial methodology has three goals. First, to generate data on which to assess the technology performance under realistic farmer conditions. Secondly, to complement the agronomic trial data with farmer assessment of the adoption potential of technologies. The third is to encourage farmers to actively participate in the trials, and this is expected to stimulate farmer experimentation with, and adoption of new or introduced technologies and practices. All farmers within the vicinity of the "mother" sites were given equal opportunities to participate in the study. The methods used to disseminate and scale up the practices included field days, and individual farmer visits to the experimentation and demonstration sites. The field days were held every season in the demonstration site to get feedback concerning the performance of various experimental treatments.

## **Village Training Workshops**

The project highly borrowed on the methodology described by Bunch (1982) that advocates a combination of 80% practical training and 20% theoretical component. Trainings were conducted for both farmers and extension staff. The objectives of training were to equip farmers and extension staff with the principles of ISFM strategies and to practically demonstrate technologies in the mother trials. Farmers were trained on the best usage and application methods of different soil resources, research benefits, record keeping and group dynamics, so that they train other farmers. Each training workshop was organized into five workstations (i) Organic materials (*tithonia*, *calliandra* and *leucaena*): sole or in combination, (ii) Manure management, (iii) Mineral fertilizers, (iv) Herbaceous and grain legumes (v) Community mobilization and technology transfer and (vi) Soya bean utilization. In the manure and fertilizer training, appropriate agronomic procedures (timing, rates and application procedures) were emphasized.

## **Farmer Groups**

During field days, farmers were requested to form groups in each village, because it would not have been conceivable to reach individual farmers in large and dispersed areas. Farmer groups are found on the principle that they develop effective working relationships and synergy. Farmer groups and community based organizations have been used successfully in western Kenya to promote improved fallows (Place et al., 2004) and in central Kenya to scale up fodder production using *calliandra* (Wambugu et al., 2001).

## **Stakeholders' Annual Planning Workshops**

A strong partnership with stakeholders that includes institutional support has been advocated as an important factor for successful scaling-up strategies especially with soil fertility technologies (Snapp et al., 2002; Sanchez, 1999). Stakeholders in this work included researchers from Kenyatta University, Kenya Forestry Research Institute (KEFRI), Kenya Agricultural Research Institute (KARI), and Tropical Soil Biology and Fertility Institute of CIAT (TSBF-CIAT), extension staff from the Ministry of Agriculture, field technicians and farmers.

## **Participatory Monitoring and Evaluation (PM&E)**

There is often a danger with all new approaches to rural development that “promising ideas” will fall out of favour before potential practitioners have had sufficient time to evaluate results and improve on methods (Belshaw, 1997). This points to the need for greater attention to monitoring and evaluation of participatory research projects (McAllister and Vernooy, 1999). Participatory monitoring and evaluation (PM&E) involves multiple stakeholders including local people in joint decision making regarding how progress should be measured,

in defining criteria for success and in deciding how results should be acted upon. The purposes of PM&E includes assessing program inputs, processes, outputs and outcomes; generating information for both community and development partners (Aubel, 2004), capacity building and assessing program impact (Estrella et al., 2000; Jackson, 1995). Interest in PM&E emerged first in agriculture and in rural development in the 1970s (Estrella and Gaventa, 1998). The development of PM&E draws primarily on various participatory traditions including: farming systems research; farmer participatory research (Farrington and Martin, 1988); participatory action research; participatory learning and action, including Rapid Rural Appraisal (RRA) and Participatory Rural Appraisal (PRA)(Chambers, 1997). The PM&E used in this project framework was produced by different stakeholders, where different indicators for monitoring and evaluating the project were developed (Mugendi and Kung'u, 2006). PM&E was designed to increase program impact assessment and planning skills among farmers. The cropping indicators that were selected included crop foliage color, height, vigor, inflorescence size and general crop condition.

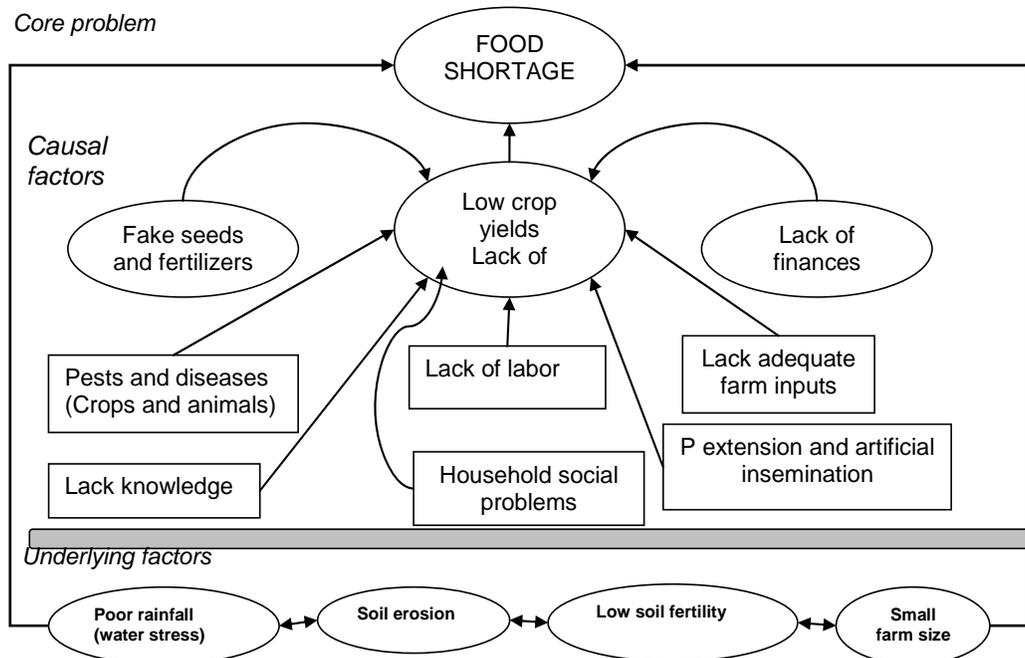


Figure 2. Diagrammatic presentation of the primary problem status as outlined by farmers during PRA in 2003, Meru south district, Kenya.

## RESULTS

### Participatory Rural Appraisal (Pra)

A total of 1,428 farmers (831 males and 597 females) attended the problem diagnosis meeting while 2,118 farmers (1186 males and 932 females) attended the feedback meetings to rank problems and suggest solutions. The results indicated that the immediate problem was

food shortage in all the sites in Meru South District (Figure 2). The farmers attributed the food shortage to low yields. The causal factors for low yields of both crop and fodder were outlined as lack of finances to purchase farm inputs. Farmers also argued that sometimes the seeds were adulterated. Low yields resulted into low finances, leading to household poverty. There was also a problem of inability to connect with extension workers and this led to lack of knowledge on what inputs to apply on the farms for efficient productivity. The farmers also outlined the underlying factors as lack of adequate water and rainfall, presence of poor soils, soil erosion and small farm sizes (Figure 2). The cause for this was identified as poor tillage methods, lack of proper knowledge on how to take care of the soils and lack of finances. Consequently it emerged that all these problems have a cyclical nature and are inter-related (Figure 2).

**Table 1. Problems diagnosed, ranked and suggested solutions by farmers in all the sites during the PRA in Meru south district, Kenya**

Problem	Rank	Solutions
Poor soil fertility and soil erosion <sup>#</sup>	1	<ul style="list-style-type: none"> <li>Practice soil conservation (bench terraces, contour hedges, crop rotation, grass strips &amp; planting trees)</li> <li>Government to help in the provision of demonstration and training on soil fertility improvement technologies</li> <li>Practice early planting and mixed cropping</li> <li>Provision of water for irrigation</li> <li>Be assisted in soil testing</li> </ul>
Animal and crops pests and diseases <sup>#</sup>	2	<ul style="list-style-type: none"> <li>Increase extension staff to teach farmers</li> <li>Use indigenous knowledge to control pests and diseases</li> </ul>
Lack of knowledge and extension services <sup>S</sup>	3	<ul style="list-style-type: none"> <li>Increase extension staff</li> <li>Farmers be consulted so as to participate in decision making with extension staff</li> </ul>
Inadequate finances to purchase fertilizer <sup>S</sup>	4	<ul style="list-style-type: none"> <li>Use of organic materials plus mineral fertilizers</li> <li>Be provided with credit facilities</li> </ul>
Erratic rainfall <sup>#</sup>	5	<ul style="list-style-type: none"> <li>Be provided with demonstration and training on soil fertility improvement</li> <li>Practice soil and water conservation (bench terraces, contour hedges, crop rotation grass strips &amp; planting trees)</li> <li>Be provided with water for irrigation and practice mixed cropping</li> </ul>
Poor markets <sup>S</sup>	6	<ul style="list-style-type: none"> <li>Farmers to form marketing groups in order to eliminate middlemen in the marketing of farm produce</li> <li>Government to ensure that farmers get quality seeds verified through KARI and other organizations</li> <li>Government to ensure that farmers get markets and good market linkages through formation of marketing groups</li> </ul>
Inadequate fodder <sup>#</sup>	7	<ul style="list-style-type: none"> <li>Plant more fodder (Napier grass and improved fodder)</li> </ul>
Lack of fertilizers and chemicals. Fake seeds <sup>S</sup>	8	<ul style="list-style-type: none"> <li>Provide technical know-how through seminars, farmer field days and educational tours etc</li> <li>Government to lower prices of inputs such as fertilizers and seeds</li> <li>Government to ensure that farmers get quality seeds verified through KARI and other organizations</li> </ul>
Small farms <sup>#S</sup>	9	<ul style="list-style-type: none"> <li>Improve production within small farms</li> <li>Hire land for cultivation</li> <li>Offer labour for sale to enable farmers buy food</li> </ul>
Food insecurity <sup>#S</sup>	10	<ul style="list-style-type: none"> <li>Improve production within small farms by improving soil fertility</li> <li>Use indigenous knowledge to control pests and diseases</li> <li>Offer labor for sale to enable farmers buy food</li> </ul>

Physical and socio-economic farm constraints have been identified. <sup>#</sup> Refers to physical factors; while <sup>S</sup> Refers to socio-economic and market factors.

During phase two of the PRA in March 2003 (feedback meetings), farmers ranked the agricultural problems (10) they had identified during the diagnosis phase and suggested solutions. The priority problem ranked first was low soil fertility and erosion and the solution for these were proper agronomic practices and training in soil management (Table 1). The other top three problems were animal and crop diseases, lack of extension services and inadequate finances in that order. Since it was not practical to address all the 10 problems identified by the farmers, the project agreed together with the farmers to address soil fertility and food security related problems through demonstrations and training. The farmers identified locations where the demonstration sites (mother trials) would be situated and also agreed that the land should be; i) in a public area, ii) secure, iii) accessible to all participants, and iv) at least 2 – 3 acres in size.

## Mother-Baby Trials

Four demonstration trials (mothers) were established in Kirege, Mucwa, Mukuuni and Murugi. They were all situated in public schools except in Mukuuni, where there wasn't enough public land and as such one farmer agreed to avail land for the demonstration. The mother trials addressed different soil fertility themes; in Kirege and Mucwa, the theme was biomass transfer using *tithonia*, *calliandra*, *leucaena*, manure and *mucuna* while Mukuuni had maize/grain legume intercrops (groundnuts, beans and cowpea) Murugi had maize and grain legume (soya beans and green grams) rotations.

Farmers were invited to attend joint field days in the mother trials during the grain filling stage every season. The field days were attended by a wide range of other stakeholders who included, teachers and students from local secondary and primary schools, local administration, extension personnel from the Ministry of Agriculture, researchers and lecturers from Kenyatta University. The field days involved visits to the demonstration plots and discussions where farmers interacted with researchers and extension personnel. Due to the different themes in each of the sites, cross-site visits were also organized during the field days. This enabled farmers to visit other sites and share experiences. During the field days, farmers gained knowledge on various technologies by learning and seeing what was being done in the demonstration (mother) plots and then started testing technologies of their choice on their farms (babies).

Four (4) field days were held in all sites with a total of 1,644 farmers attending. The data shows that more female farmers attended the field days during the 2<sup>nd</sup> (55%) and 4<sup>th</sup> (52%) field days compared to the 1<sup>st</sup> and 2<sup>nd</sup> field day where there were 35% and 50% females, respectively. Murugi recorded a consistent increase in the female farmers' attendance as time progressed (Table 2). The high number of farmers who participated in the field days could be due to the fact that they perceived soil fertility to be a problem since they had identified it as a priority during the PRA. Versteeg and Koudokpon (1993) also reported an enhanced farmer participation in their study when farmers perceived soil fertility to be a serious agricultural production constraint.

**Table 2. Field day and village training workshop attendance by gender in the different sites in Meru South, Kenya**

Field days								
Site	Event 1		Event 2		Event 3		Event 4	
	Male	Female	Male	Female	Male	Female	Male	Female
Kirege	64	38	-	-	-	-	-	-
Mucwa	45	37	20	31	46	40	-	-
Mukuuni	145	55	101	114	75	63	80	56
Murugi	81	53	91	109	46	62	77	115
Total	335	183	212	264	167	165	157	171
Village training workshops								
Kirege	337	235	62	50				
Mucwa	100	33	68	78				
Mukuuni	220	185	107	151				
Murugi	96	103	72	94				
Total	753	556	309	373				

### Village Training Workshops

Village training workshops were held at every site in September 2004 and July 2005 with a total of 1,971 farmers participating (Table 2). The 1<sup>st</sup> village training workshop was well attended and most of the farmers were males (58%). During the 2<sup>nd</sup> village training, the number of female farmers attending increased to 55%. The village workshops provided a forum for learning, practical participation and sharing of experiences among farmers, extension personnel and scientists.

Before the start of the project, farmers were using varied vegetative materials such as maize stover, grevillea, banana leaves, etc, as livestock bedding material and to increase quantity of manure. Manure made from such material was, however, of poor quality and farmers lacked adequate knowledge on its management and application rates. During the field days and village training workshops, farmers were trained on proper manure management and appropriate use of mineral fertilizers. *Calliandra*, and *tithonia* + *calliandra* were new organic materials introduced to the farmers. After the field days and training, farmers were encouraged to test inputs of their choice on their farms (babies). To find out what farmers were testing, a farm survey involving 563 farmers who had registered as having tried new technologies was conducted during 2005 LR season. Results showed that farmers were testing a wide range of inputs (Table 3). Most farmers tested mineral fertilizer (47%) followed by manure plus mineral fertilizer, manure alone and *tithonia* with 43%, 24% and 12%, respectively. No farmers tested the legumes possibly because the soil fertility improvement by the legumes was not visible within the short term to attract interest among farmers. Soil improvement by legumes is variable depending on the amount of N availed and in some cases gains are realized over the long-term period (Amede 2003).

The modifications mainly involved combining different organic materials, an aspect not learnt at the demonstration site. At the demonstration site, single type organic materials were solely applied or combined with mineral fertilizer. Farmers reported that they mixed the organic resources because the amount of single biomass types was inadequate.

**Table 3. Soil fertility replenishment technologies tested by farmers during 2005 LR in Meru South, Kenya**

Technology	Kirege N= 73	Mukuuni N= 195	Mucwa N= 62	Murugi N= 233	Total N= 563
<i>Tithonia</i>	3	22	11	32	68 (12.1%)
<i>Tithonia</i> + fertilizer	0	10	0	14	24 (4.3%)
* <i>Tithonia</i> + manure	2	3	8	5	18(3.2%)
* <i>Tithonia</i> + manure + fertilizer	0	1	1	2	4 (0.7%)
Manure	15	63	22	37	137 (24.3%)
<i>Calliandra</i>	1	0	1	2	4 (0.7%)
Fertilizer	27	135	26	78	266 (47%)
Manure + fertilizer	38	69	31	108	246 (43%)
* <i>Mucuna</i> + manure	2	0	0	0	2 (0.4%)
<i>Leucaena</i>	0	0	0	1	1 (0.2%)
<i>Leucaena</i> + fertilizer	0	1	0	0	1 (0.2%)
* <i>Leucaena</i> + calliandra + manure	0	0	1	0	1 (0.2%)
* <i>Calliandra</i> + manure + <i>tithonia</i>	0	0	1	0	1 (0.2%)

\*Farmers innovated technologies, Percentages add to more than 100% because farmers tried more than one technology.

**Table 4. Farmers' experiences and constraints in testing soil fertility technologies in Meru south District, Kenya**

Technology	Experiences	Constraints	Mean score	Rank
<i>Tithonia</i>	<ul style="list-style-type: none"> <li>Crops not affected by nematodes</li> <li>Improved soil fertility</li> <li>High yields</li> </ul>	<ul style="list-style-type: none"> <li>Biomass not readily available</li> <li>Labor intensive</li> </ul>	8.5	1
Manure + fertilizer	<ul style="list-style-type: none"> <li>High maize vigour</li> <li>High yields</li> </ul>	<ul style="list-style-type: none"> <li>Fertilizer is expensive</li> <li>No livestock, no manure</li> <li>Labor intensive</li> <li>Limited know-how on manure management</li> </ul>	7	2
<i>Leucaena/calliandra</i>	<ul style="list-style-type: none"> <li>Good fodder for livestock</li> <li>Increase milk production</li> <li>Increase yields</li> </ul>	<ul style="list-style-type: none"> <li>Land is limited</li> <li>Inadequate biomass</li> </ul>	7	3
Manure	<ul style="list-style-type: none"> <li>Requires fertilizer</li> <li>Must be fully decomposed</li> <li>Gives high yields</li> <li>Encourages soil micro organisms</li> </ul>	<ul style="list-style-type: none"> <li>Labor intensive</li> <li>Cannot be used directly from the cowshed</li> </ul>	5.5	4
Fertilizer	<ul style="list-style-type: none"> <li>High yields</li> <li>Required all seasons</li> <li>May acidify soils</li> </ul>	<ul style="list-style-type: none"> <li>Costly</li> <li>Not readily available</li> </ul>	5	5

During the fourth year (2006), a forum was held for farmers to share their experiences in testing technologies on their farms. In this forum, farmers used yields, soil improvement seen through crop responses, soil color change and labour as their criteria to rank the soil fertility

technologies. *Tithonia* was ranked the best technology followed closely by manure + fertilizer and *calliandra* and *leucaena* (Table 4). The favourable characteristics of *tithonia* as a green manure shown by Jama et al. (2000) included high leaf biomass and nutrient content, and favourable decomposition and nutrient release characteristics.

The least ranked technology was fertilizer, mainly because of its high costs. The high ranking of *tithonia* is associated with high yield obtained (data not shown), while the leguminous trees were favoured because of their multiple roles of improving soil fertility and providing fodder. Other studies in the region have reported preference of these inputs by the farmers (Mugwe et al., 2010; Kiptot et al., 2007).

## Farmer Groups

A total of 40 farmer groups were formed and trained on tree nursery establishment and management, group dynamics and book keeping. The group membership consisted of 60.2% females and 39.8% males. A survey carried out in June 2005 indicated that group membership encouraged technology testing. For example, in Kirege, 43% of the female headed households who were testing the technologies were group members. Meanwhile in Mukuuni, Murugi and Mucwa, 42%, 73% and 78% of the female farmers trying technologies were group farmers. Male headed households that were group members were 70% in Murugi, 56% in Mukuuni, 55% in Mucwa and 27% in Kirege. This shows that both male and female headed households found group membership an important avenue to learn and try new technologies.

One of the major achievements of these groups was the ability to propagate nursery seedlings and promulgate tree planting, especially *calliandra* and *leucaena* on their farms. During the three seasons that they propagated seedlings (2004 SR, 2005 LR and 2005 SR), farmer groups from Murugi produced the largest quantity with 89,631 seedlings followed by Mukuuni farmer groups with 45,196 seedlings. Kirege and Mucwa farmer groups produced the least number of seedlings with 32,343 and 24,130 seedlings, respectively. The difference in quantities produced was mainly attributed to the number of groups in each site and membership per group. The use of group nurseries as a means of availing propagation materials to farmers was effective and considerable success was registered as noted in the number of seedlings farmers received within the short time period. At the end of the three seasons, about a third of all members in group nurseries received close to 500 seedlings. This amount is enough to supplement animal protein for one dairy cow for one year (Roothaert et al., 1998).

Though the major objective of forming the group nurseries was to enable propagation of *calliandra* and *leucaena* seedlings and consequently increase on-farm planting, farmers got an opportunity to interact and enhance mutual relations. For example, other tree and vegetable species were also propagated and the groups started other social activities as well. For instance, the groups started activities revolving around generating income for the group. Merry-go-round was the most common with more than 50% of the groups having merry-go-round activities as a way of raising funds for the group and also as a group welfare activity. This was reflected by some of the groups transforming themselves to more formal outfits through registration in the ministry of culture and social services. Registration means that the group is formal and is recognized by the government and would be able to benefit from

government funding. These groups are therefore likely to be sustainable in the long term even after the end of project activities.

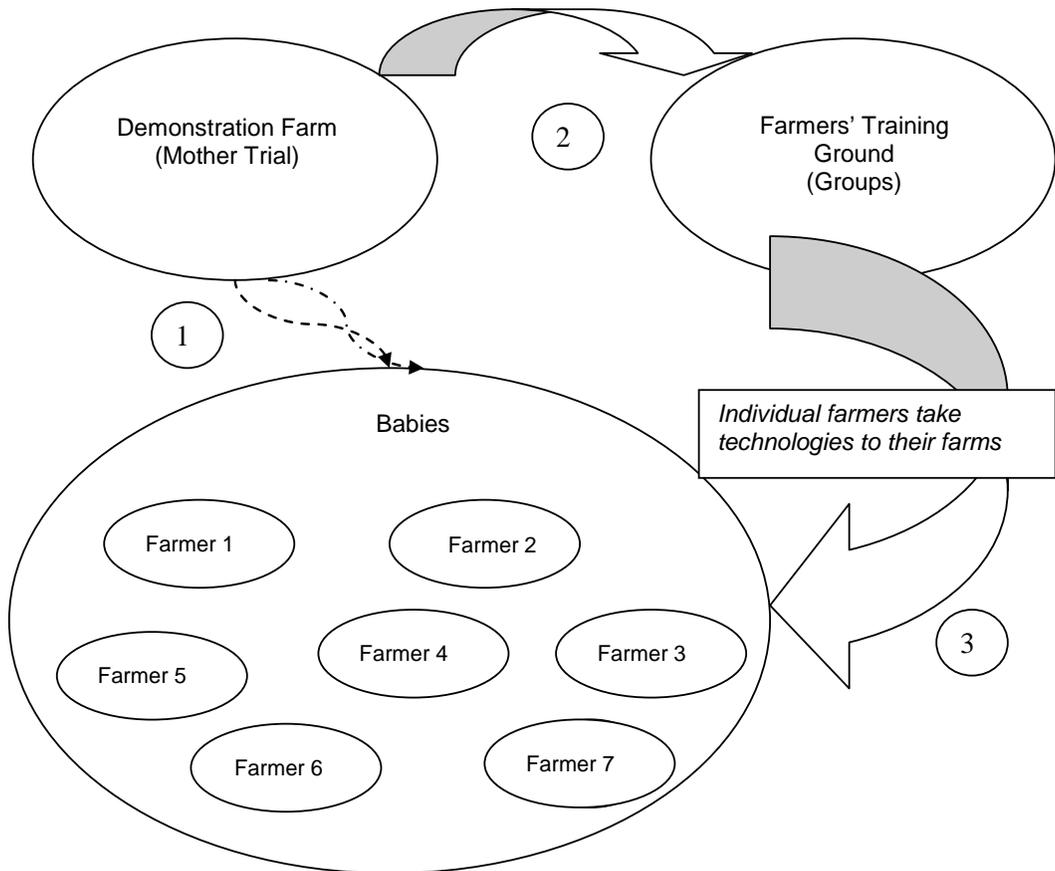


Figure 3. The adopted new mode of participatory technology transfer by farmers in Meru South district, Kenya.

### Annual Stakeholders' Planning Workshops

The partnerships between different stakeholders were formalized through the development of memorandum of understanding (MOUs) between research organizations, farmers and extension personnel. Stakeholders' workshops were held once every year to review and evaluate project progress and plan for the following year. In these workshops, two representative farmers from each of the groups participated. Different representative farmers were selected each year to enhance full participation by members. A total of 280 representatives attended stakeholders' workshops during the four years. The stakeholders highlighted progress they had made during each year and the challenges they had experienced. They also planned together on activities for the coming year and shared responsibilities. During the second annual stakeholders' workshop (January 2005), farmers suggested a new approach, "farmers training ground" (FTG) (Figure 3) to complement the M-

B approach. Farmers indicated that they would be more confident to transfer technologies to their farms if they first practiced together in small groups (arrow 3, Figure 3). They indicated that they would be able to confirm whether the good performance they observed at the demonstration sites would be replicable on their farms. However, they also indicated that some farmers who were willing to test demonstration site technologies were free to do so (Arrow 1, Figure 3). In the FTG approach, the farmers form groups, and one of the farmers' avails a piece of land where farmers practice some of the technologies demonstrated in the mother trial site. The farmers agreed on few technologies to test at the training grounds.

The farmers noted that the new approach (FTG) was more practical and realistic as it led to a "hands on" opportunity to implement what is done in the demonstration sites. Farmers planned together and selected a few technologies from the demonstration trial which they tested within their groups and then eventually started testing them on-farm. Development of this approach by farmers is an indication that the conventional M-B approach may be less effective compared to FTGs in ISFM dissemination.

### Participatory Monitoring and Evaluation (PM&E)

Farmers identified four major outcomes and indicators to measure project achievements (Table 5). The first outcome was higher quality yields and the indicators to measure this were i) presence of technologies on farms, ii) increased food supply at household level, and iii) increased food for sale. The second outcome was increased knowledge on better and improved farming methods and the indicators to measure this were i) farm presence of technologies, ii) technology knowledge increase, iii) confidence in teaching other farmers, and iv) confidence in inviting diverse experts to assess and train farmers. The third outcome was improved soil conservation and the indicators to measure this were i) reduced soil erosion, and ii) formation of bench terraces. The fourth outcome was improved livestock health and the indicators to measure this were i) presence of more healthy animals, and ii) more milk production.

**Table 5. Expected outcomes, indicators and farmer assigned percentages of achievements after about 5 years in Meru South, Kenya**

Expected Outcome	Indicators	(%)
High yields	Presence of technologies on farms	68.1 ab
	Increased food supply at household level	68.7 ab
	Increased food for sale	33.7 c
Knowledge on better and improved farming methods	Presence of technologies on farms	68.1 ab
	Increased knowledge about the technologies	80.0 a
	Confidence in teaching others	80.0 a
	Confidence in inviting other experts & asking questions	74.5 a
Improved soil conservation	Reduced soil erosion	63.8 ab
	Formation of bench terraces	61.3 b
Improved animals	More healthy animals	80.0 a
	More milk production	77.7 a
LSD		16.3
P		<.001

Stakeholder workshops were also an important means to find out farmers' views on technologies, potential impacts and general project performance in terms of achieving set out objectives. Therefore, during the last stakeholder meeting held in March 2007, farmers used indicators developed during the PM&E to evaluate achievements (Table 5). Generally, increased ISFM knowledge, confidence in teaching others and more healthy animals scored highest (80%). This could be attributed to intensive training that was conducted throughout the project period in terms of field days and village training workshops. FTGs also strengthened the training process. Using the knowledge gained, farmers were able to use technologies (68.1%) and consequently increase food supply at the household level to about 68.1%. Increase in milk production (77.7%) as a result of improved animal health (80%) could be explained by feeding fodder trees to animals. At the start of the project, farmers had on average less than 10 fodder trees per farmer but after 3 years, the number of trees per farmer had increased to about 300 trees (Mugwe, 2007).

Farmers also evaluated the participatory approaches that the project had been using in disseminating technologies by scoring, using a scale of 10 (for most effective) to 1 (for least effective). FTGs were ranked the best overall followed by demonstration farms (77.5%), nurseries (76.2%) and field days (67.4%) (Table 6). During discussions, farmers reported that these approaches were the most effective in learning and enhanced technology uptake. This finding agrees with results from Western Kenya which reported that farmer participation in field days and demonstrations significantly increased adoption (Makokha et al., 1999). This is because field demonstrations exposed benefits associated with technologies, therefore influencing farmers to select the best technology option sets.

**Table 6. Farmers' evaluation of the different approaches used in the project in Meru South District, Kenya**

Approach	Mean (%)	Rank
Field days	68.7 abc	4
Training workshops	55.0 bc	6
Farmer groups	58.7 abc	5
Nurseries	71.2 abc	3
Demonstration farms	76.2 ab	2
Farmer training grounds	77.5 a	1
Cross-site visits	50.0 c	7
LSD	22.2	

## Project Milestones

Overall, the project achieved reasonable progressive milestones that were realized annually, leading to cumulative impact at the end of 4 years. During the end of first year, several important milestones were achieved. Soil fertility problems, their inter-linkages and solutions were identified with multiple-stakeholder involvement. ISFM training had been disseminated and demonstration sites had also been established. Apart from suggesting new approaches (FTG), farmers were able to test and modify technologies by year 2005. By the third year, there was a large increase in seed propagation and fodder production. At the end of

4 years, farmers were able to evaluate project progress and dissemination methods. Impact was characterized by increased food and fodder production, improved livestock health and soil fertility. The project also generated spill-over effects. Apart from agro-ecosystem impacts, social linkages and enhanced mutual collaboration in on-farm and off-farm activities was also realized, for instance formal welfare groups which enhanced social capital.

## DISCUSSIONS

The outcomes of this study provided broad insights, lessons and physical evidence relating to how project objectives were achieved by farmers and scientists using the consultative PRA-based dissemination framework. Causal factors related to agricultural constraints and their solutions were identified and ranked by farmers. Problem diagnosis depicted that farmers had a structured and functional understanding of the main farming system constraints in central Kenya. Farmers identified underlying factors which mainly encapsulated bio-physical variables of which most farmers have minimal control and likely to impose relative production-side limitations (FAO, 1995). The second category of causal factors mostly included farm-level socio-economic elements and market based constraints, which directly or indirectly influences household potential to manipulate its' physical production limits. This understanding of farming constraints is cogent and acceptable with scientific views (FAO, 1995; Murage et al., 2000).

The village workshops were important to farmers who are said to learn faster by doing and therefore able to apply key lessons learnt (PLA, 1998). In addition, the village training strategy was more attractive to women who are usually not able to attend training in venues far from their villages due to their gender roles or decision-making roles in the household (Chale, 1990). Technology modifications are an implication of farmer interest to improve fertility of their soils. Farmer modification of introduced practices has been reported by several other authors in participatory studies (Adesina et al., 1999; Pisanelli et al., 2000; Obonyo and Franzel, 2005). Farmers make technology modifications because they are rarely able to manage any single enterprise in the 'optimal' manner prescribed by researchers and therefore adapt technologies to their agronomic circumstances (Hu et al., 2007; Franzel and Scherr, 2002). This view of technology development is particularly relevant to the diverse smallholder farming systems in developing countries (Hu et al., 2007), where no single technology is appropriate without considerable adaptation by individual farmers, which leads to a final package that is technically feasible, profitable and acceptable (Adesina et al., 1999).

The annually held stakeholder workshops offered an opportunity for participants to plan project activities and build broad consensus. According to Franzel et al. (2002), farmers' workshops can be an important means to discuss issues related to planning, new practices, exchanging of opinions and lessons, and to clarify differences. The annual meetings encouraged mutual collaborative sharing and learning and gave partners a feeling of project ownership. This is important in building confidence, strong partnerships between researchers and farmers hence facilitating ISFM adoption (Kanyama-Phiri et al., 2000). During annual meetings, farmers suggested 'farmers training grounds' (FTGs) as a necessary modification to the M-B trial. The findings of Johnson et al (2001) showed that the rigidity of M-B trials in soil fertility work in Malawi limited real impact on human and social capital. FTGs are

superior because joint sharing of experiences, scarce land, labour, time and knowledge among low-resource endowed farmers may be more efficient, interactive and cost effective during ISFM development and up-scaling processes. The training grounds developed for testing candidate technologies could be equated to “model farms” in a study by Tonello et al. (2001). These authors reported that farmers preferred to evaluate technologies they had chosen, in model farms located close to their residences. FTGs are solely owned by farmers and therefore they would have full confidence to practice and therefore likely to adopt. The results from farmer group-led nursery tree propagation in this study suggest that FTGs, when theoretically operational under the aegis of farmer groups, could enhance ISFM development and dissemination because germplasm development is key to scaling-up ISFM.

The PM&E exercise achieved its core theoretical objectives as outlined by Aubel et al. (2004), Estrella and Gaventá (1988), Chambers (1997) and Estrella et al. (2000). During this key activity, farmers developed biophysical and qualitative milestones to evaluate project progress. Tree, crop and livestock yield increments, soil fertility improvement, increased farm sales and enhanced ISFM learning and knowledge-building were designated as the long-term motifs and their parts served as milestones to measure incremental project progress. The dissemination methods best ranked highest had a key aspect of involving majority stakeholders in a social learning interaction mode. Social learning has increasingly gained recognition as a participatory approach because it is accompanied by individual and group reflection on what is being learnt as well as iterative attempts to apply what is learnt (Chambers and Thrup, 1989). The FTGs were significantly more effective in ISFM dissemination than cross-site visits and village training workshops (Table 6). Explanation for the effectiveness of the training grounds in disseminating technologies is that farmers had joint experiential learning during technology development. In training, participant ability to learn and apply lessons to their work situations is quicker if practical elements are initially tried (PLA, 1998). Possible explanation for the lower effect by cross-site visits is that only a few farmers participated in cross-site visits due to transport costs or other social responsibilities.

## Lessons Learnt

A number of lessons were learnt during the project and this agrees with Franzel et al. (2002) who noted that participatory research offers researchers, extensionists, policy makers and farmers an opportunity to learn important lessons about achieving effective dissemination, as well as feedback on further research priorities.

The broad lesson is that farmers have a structured and functional understanding of farming systems, and appreciated the broad synergy and linkages in agro-ecosystem constraints, technological solutions, and project participation. Some of the lessons learnt include:

- The use of M-B approach to scale up ISFM technologies may not be very effective for all technologies compared to FTG methods, which were more effective in confidence building and in facilitating technology transfer.

- Working in joint partnership is very constructive and enhances sustainability as well as impact. The project witnessed a renewal of synergy, recognition and appreciation of the knowledge of agricultural officers. For instance, the project activities have awakened some retired agricultural officers who are now being invited by the groups to discuss agricultural matters with farmer groups. In other areas, farmers made an effort to forge links with the Forestry Department, and training on seedling management has been on-going. We experienced a positive change of attitude by the farmers who at the start of the project who were accusing agricultural extension staff of neglect and overall reticence.
- The framework of participatory interaction enabled farmers to view the “outsiders” as partners in development and over time, the farmers gained confidence in engaging the partners in development discourse. Future projects that use participatory approaches are therefore likely to receive good interaction from these farmers.
- The use of PM&E has been a major eye opener for all stakeholders on how the farmers would like to direct the course of a project for their optimal benefit.
- Farmer ranking of technologies recognized the importance of organic inputs and integrated systems in enhancing soil fertility.
- Keeping a vibrant and an effective working partnership is very challenging and more expensive than had been anticipated. To maintain harmony and good will among multiple partners, constant meetings and regular field team visits must be put in place.

## CONCLUSIONS AND RECOMMENDATIONS

Conceptual and methodological pathways for scaling up of ISFM technologies involving problem-solving participatory tools were explored and evaluated in the study. The results indicated that integrated soil management and stakeholder involvement approaches offered the best solutions to agricultural challenges facing farmers in central Kenya by both farmers and scientists.

The core problem identified during the PRA was food shortage caused by low crop productivity. Multiple production and market-based challenges were identified to be limiting agricultural productivity. Farmers’ solutions to the problems of low soil fertility and soil erosion were use of proper agronomic practices and training on soil fertility management. Low erratic rainfall (leading to water stress in the farming system) is critical and there is need to address this through exploration of strategies to enhance water use efficiency. Due to annual decline in per capita farm size, options to intensify agricultural production efficiently need to be urgently sought by all stakeholders.

The inputs most favoured by farmers and rated highly were *tithonia*, manure plus fertilizer, manure, and leguminous trees. These inputs are likely to be adopted as shown by the high number of testing farmers in 2005 LR. This is consistent with results from several long-term experiments in SSA which have proven the long-term biophysical and economic benefits of ISFM systems. Fertilizer was rated least mainly due to high costs. To attain high and sustainable production, integrated use of the organics and fertilizer is key and as a result there is need for government intervention to enhance fertilizer availability cost-effectively

and to support the organic components of high potential ISFM systems through suitable policy frameworks. SSA governments can enhance ISFM development by facilitating key germplasm production and distribution systems. The participatory approaches in this study emphasized training (capacity building), mutual sharing, learning and action with an aim to enhance farmer knowledge on ISFM practices. Farmer rating of outcomes expected after 4.5 years of dissemination activities showed that increased knowledge and confidence in teaching others was rated highest with about 80%. This was attributed to farmer involvement in interactive learning activities that encouraged learning and decision making at individual and community levels.

FTGs, demonstration farms, field days and farmer groups emerged as important approaches to address agricultural constraints. These dissemination methodologies received high ratings by farmers in terms of effectiveness, knowledge and information transfer to farmers. Meanwhile, technologies that included organic components emerged as the most effective solutions to farm production constraints. Strong partnerships within and between research, extension and farmers are important in enhancing knowledge and overall participation by stakeholders. Inclusive and complementary approaches in soil fertility management and stakeholder involvement regimes resulted in the most effective ISFM interventions in terms of dissemination tools, technology effectiveness and acceptability. A policy framework should therefore be developed so as to encourage appropriate capacity development and utilization within the national agricultural research and extension system. Germplasm availability is usually a major constraint to ISFM development and dissemination in most SSA regions. Farmer groups were effective in activities that involved bulking of planting materials as revealed by the success in propagating tree seedlings and bulking of new germplasm. When coupled with farmer groups that are propagating germplasm, FTGs could enhance scaling-up of ISFM. Farmers modified the conventional M-B approach that assumed that they would choose technologies from mother trials and transfer them directly to their farms. FTGs and other joint systems could enhance efficiency in ISFM dissemination, through savings in labour, land, resource inputs, as well through improved social interaction. Most farmers chose to test selected technologies on their own in groups, and preferred dissemination systems that engendered this process.

The possibility that some farmers could benefit from both M-B and FTG dissemination methods should not be overlooked. For successful dissemination and adoption of ISFM technologies, this study further recommends that a mid phase, “farmer training and testing phase” be incorporated within the overall M-B approach for successful ISFM technology dissemination. In summary, project milestones in this work progressed reasonably through annual phases that first involved problem-solution definition, ISFM technology development and dissemination and final realization of outputs and outcomes through participatory stakeholder involvement. With a longer time-frame and more input, the project demonstrates that further successes and spill-over effects could potentially be realized.

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