This article was published in the October 2010 issue of the journal Biomass and Bioenergy, volume 34, pages 1427-1439. (http://www.elsevier.com/locate/biombioe). The article was received for publication 15 April 2009; reviewer's comments were sent to the authors 13 November 2009; the article was received in revised form 26 March 2010; and accepted for publication 16 April 2010.

Bioenergy and the potential contribution of agricultural biotechnologies in developing countries

John Ruane[a, *], Andrea Sonnino[b] and Astrid Agostini[c]

[a]FAO Working Group on Biotechnology
[b]FAO Office of Knowledge Exchange, Research and Extension
[c]FAO Investment Centre
UN Food and Agriculture Organization (FAO)
Viale delle Terme di Caracalla
00153 Rome
Italy

[*]Corresponding author. Tel. +39 06 57052997; Fax +39 06 57056361; e-mail address john.ruane(at)fao.org

ABSTRACT

We provide an overview of the current status of bioenergy development, focusing on first- and second-generation liquid biofuels, considering drivers of growth and risks that have raised concerns over recent years. We also describe the main areas where biotechnologies are being, or can be, applied for production of first- and second-generation biofuels as well as microalgal biodiesel and biogas. Greatest attention is paid to second-generation biofuels in the review because of the large expectations they have created and because of the significant role that biotechnology applications are likely to play in their development. We close with some specific considerations regarding applying biotechnologies for bioenergy development in developing countries.

1. Introduction

In a relatively short time, large-scale cultivation of feedstocks for production of liquid biofuels has become a reality, a phenomenon that is predicted to expand, driven by concerns about climate change, petrol prices and national energy security, among others. The potential social, economic, environmental and human rights impacts have been much debated and have been the subject of considerable controversy with e.g. the UN Special Rapporteur on the Right to Food highlighting grave concerns that "biofuels will bring hunger in their wake", arguing that "the sudden, ill-conceived, rush to convert food - such as maize, wheat, sugar and palm oil - into fuels is a recipe for disaster" [1].

In this context, the subject of bioenergy has very actively engaged governments and their policy makers worldwide. For example, in June 2008 representatives from 181 countries, including 42 Heads of State or Government, gathered at FAO Headquarters in Rome for the High-Level Conference on World Food Security: the Challenges of Climate Change and Bioenergy. The Summit concluded with the adoption by acclamation of a Declaration and, regarding biofuels, the Declaration stated: "It is essential to address the challenges and opportunities posed by biofuels, in view of the world's food security, energy and sustainable development needs. We are convinced that in-depth studies are necessary to ensure that production and use of biofuels is sustainable in accordance with the three pillars of sustainable development and takes into account the need to achieve and maintain global food security. We are further convinced of the desirability of exchanging experiences on biofuels technologies, norms and regulations. We call upon relevant intergovernmental organizations, including FAO, within their mandates and areas of expertise, with the involvement of national governments, partnerships, the private sector, and civil society, to foster a coherent, effective and results-oriented international dialogue on biofuels in the context of food security and sustainable development needs" [2].

Because of concerns about the current first-generation of liquid biofuels, there is major interest in moving to alternative systems of biofuel production, such as second-generation liquid biofuels based on lignocellulosic biomass, and applications of biotechnologies will be important if they are to become widely available in the future. As the topic is of current global relevance and interest, this paper reviews the role that application of agricultural biotechnologies may play for production of bioenergy in developing countries, with a major focus on liquid biofuels.

Biotechnology represents a broad collection of tools that can be used for a variety of purposes, such as the genetic improvement of plant varieties and animal populations to increase their yields or the genetic characterization and conservation of genetic resources. Some of the technologies may be applied to all the food and agriculture sectors, such as the use of genomics, molecular DNA markers or genetic modification, while others are more sector-specific, such as tissue culture (in crops and forest trees) or embryo transfer (livestock).

This review focuses on the use of biotechnologies both to produce biomass for bioenergy purposes and to convert biomass to biofuel. It covers biotechnology applications for first- and second-generation biofuels and, to a lesser degree, for biogas production and for biodiesel production from microalgae. It therefore covers applications of biotechnologies to bioenergy production systems that are currently a reality (first-generation biofuels and biogas) as well as to those that are still at the experimental stage (second-generation biofuels and microalgal biodiesel).

In the paper, we start with an overview of the current status of bioenergy development, focusing on first- and second-generation liquid biofuels (Section 2), including the major risks and opportunities related to their rapid expansion. Some of the potential ways in which biotechnology could contribute to this area are then considered, covering production of biomass as well as conversion of the biomass to first- or second generation liquid biofuels, in addition to production of biodiesel from microalgae and production of biogas (Section 3). Finally, a small number of issues of specific relevance to developing countries are briefly considered in Section 4.

2. Bioenergy and first and second-generation liquid biofuels

2.1 Bioenergy

The term bioenergy refers to energy obtained from biomass, which is the biodegradable fraction of products, waste and residues from agriculture (of vegetable and animal origin), forestry and related industries, as well as the biodegradable fraction of industrial and municipal waste [3]. A wide range of biomass sources can be used to produce bioenergy in a variety of forms. For example, food, fibre and wood process residues from the industrial sector; energy and short-rotation crops and agricultural wastes; and forest and agroforest residues from the forestry sector can all be used to generate electricity, heat, combined heat and power, and other forms of bioenergy [4].

Traditional biomass materials, including fuelwood, charcoal and animal dung, continue to be important sources of bioenergy in many parts of the world and, to date, woodfuels represent by far the most common sources of bioenergy. Modern bioenergy relies on efficient conversion technologies for applications at the household, small business and industrial scale. Solid or liquid biomass inputs can be processed to be more convenient energy carriers. These include solid biofuels (e.g. firewood, wood chips, pellets, charcoal and briquettes), gaseous biofuels (biogas, synthesis gas, hydrogen) and liquid biofuels (e.g. bioethanol, biodiesel) [4]. Among the different segments of the bioenergy sector, the largest and most rapid growth has been seen in liquid biofuels [3]. For this reason, and because of their predicted further expansion in the future, they are the main focus of this paper.

The major use of liquid biofuels is for transport, where the biofuel is either blended with traditional transport fuels (biodiesel with diesel or bioethanol with petrol) for conventional engines or used on its own in vehicles with specialised engines. There is also much interest in liquid biofuels as a cooking or heating fuel, although significant barriers, such as the need for more affordable stoves, still remain [5].

2.2 First-generation liquid biofuels

In the current debate, biofuels are generally divided into "first-generation" and "second generation" biofuels. The division is not strict and is based on different parameters, such as the level of establishment in the market of a particular technology, the type of processing technology or the type of feedstock [6]. Following [7], in this paper we distinguish "first-generation" and "second-generation" biofuels by the biomass (feedstock) used. First-generation fuels are generally made from sugars, grains or seeds, i.e. using only a specific (often edible) portion of the above-ground biomass produced by a plant, and relatively simple processing of the biomass is required to produce a finished fuel [7].

The two main first-generation liquid biofuels are currently biodiesel and bioethanol, representing about 15 and 85% of current global production respectively [8]. A brief overview of the way they are produced is provided here.

2.2.1 Biodiesel

For biodiesel production, the feedstocks involved include vegetable oils (e.g. derived from oilseed crops such as soybean, sunflower, jatropha, oil palm or rapeseed), used frying oil (e.g. from restaurants) or animal fat (e.g. pork lard) [9]. The major components of vegetable oils and animal fats are triacylglycerols (TAGs, also called triglycerides), which consist of three long-chain fatty acids linked to a glycerol backbone. Natural oils are too viscous to be used in modern diesel engines. However, in the 1980s a chemical modification of natural oils was introduced that helped to bring the viscosity of the oils within the range of current petroleum diesel. Thus, by reacting these TAGs with simple alcohols such as methanol (a chemical reaction known as "transesterification", already commonplace in the oleochemicals industry), alkyl esters (methyl esters), generically known as biodiesel, are formed whose properties are very close to those of petroleum diesel [10].

2.2.2 Bioethanol

Ethanol, also known as ethyl alcohol, can be produced from any biomass that contains appreciable amounts of sugar or materials that can be converted into sugar, such as starch or cellulose. Sugar cane, sweet sorghum and sugar beet are examples of feedstocks that contain sugar. Maize, wheat and other cereals contain starch (in their kernels) that can relatively easily be converted into sugar.

In producing bioethanol from sugar crops, they are first processed to extract the sugar (e.g. through crushing). The sugar is then fermented to yield ethanol. Ethanol fermentation is the biochemical process by which sugars, such as glucose, fructose and sucrose, are converted into ethanol and carbon dioxide using yeast or other micro-organisms. Glucose and fructose are monosaccharides with six carbon atoms, and are thus termed 6-carbon sugars. Sucrose is a disaccharide made of glucose and fructose joined together. A final step distils (purifies) the ethanol to the desired concentration and usually removes all water to produce "anhydrous ethanol" that can be blended with petrol. With sugar cane, the "bagasse" (i.e. the crushed stalk of the plant) can be used as a solid fuel and burned for heat and electricity.

In producing bioethanol from starchy materials, the process is more difficult compared to sugar crops because an additional step, hydrolysis of the feedstock, is required. Starch is a polysaccharide consisting of long chains of glucose molecules. Through hydrolysis, where the starch reacts with water, the starch is broken down to fermentable glucose molecules. Hydrolysis, also known as saccharification, can either be enzymatic (using a mixture of enzymes known as amylases) or acid-based [11]. Once the starch is broken down to glucose syrup, the process is similar to that for sugar crops (i.e. the sugars are fermented to ethanol, typically using the yeast called *Saccharomyces cerevisiae*, followed by distillation of the ethanol to the desired concentration and removal of water). The process also yields several by-products, such as protein-rich animal feed (e.g. dried distillers' grains with solubles, DDGS).

2.3 Second-generation liquid biofuels

Second-generation fuels are generally those made from non-edible lignocellulosic (LC) biomass, either residues of forest management or food crop production (e.g. corn stalks or rice husks) or whole plant biomass (e.g. grasses or trees grown specifically for biofuel purposes) [7]. LC biomass, also called cellulosic biomass, is a complex composite material consisting primarily of cellulose, hemicellulose and lignin bonded to each other in the plant cell wall [12].

There is major interest in moving from the current first-generation of liquid biofuels to the second-generation biofuels. As an illustration, [7] summarises: "By comparison to feedstocks for first-generation biofuels, lignocellulosic biomass is generally (a) not edible and therefore does not compete directly with food production; (b) can be bred specifically for energy purposes, thereby enabling higher production per unit land area; and (c) represents more of the above-ground plant material, thereby further increasing land-use efficiency. These basic characteristics of lignocellulosic materials translate into substantial energy and environmental benefits for second-generation biofuels compared to most first-generation biofuels". Similarly, at the Roundtable dedicated to 'Bioenergy and Food Security' during the FAO Summit in June 2008, several countries "noted the sustainability challenges related to the production of first generation biofuels and highlighted the promise of second generation technologies to reduce competition for natural resources" [2]. However, it is important to highlight that while LC feedstocks processed into bioenergy would not otherwise be used for food, they compete with agricultural crops for agricultural inputs, land and water and therefore do not completely eliminate food competition. LC feedstocks can be grown on land of lower potential than that typically needed for crop production, but higher yields will be obtained on land of good potential and profitability will be higher in areas that are well connected to markets.

The potential importance of second-generation biofuels is clear from the observation that most plant material is not sugar or starch but is LC biomass. In fact, cellulose is the most abundant biological material on earth. It is a polysaccharide that makes up about 40-50% of the weight of dry wood. In higher plants it is organized into microfibrils, each containing up to 36 glucan chains having thousands of glucose residues, which are largely responsible for the plant cell wall's mechanical strength [12]. Hemicellulose is also a polysaccharide, accounting for 25-35% of dry wood [11]. It is a mixture of various polymerised monosaccharides, such as xylose and arabinose (both 5-carbon sugars, or pentoses) and glucose, mannose and galactose (all 6-carbon sugars, or hexoses). Lignin, instead, is not a polysaccharide and this highly branched polyphenolic macromolecule is strongly resistant to chemical and biological degradation. It is not fermented to produce liquid biofuels, but instead can be recovered and used as a fuel for heat and electricity at an ethanol production facility [7]. The relative proportions of these three materials in LC feedstocks vary, depending on the species involved. For example, the biochemical composition of biofuel feedstock from the pine tree is about 45% cellulose, 22% hemicellulose (mainly mannose followed by xylose sugars), 28% lignin and 6% others, while for switchgrass these proportions are 32% cellulose, 25% hemicellulose (almost all xylose sugars), 18% lignin and 25% others [11].

LC biomass can be converted to biofuels by thermo-chemical or biochemical processing and many efforts are being made worldwide to commercialise second-generation biofuels through both routes (e.g. [7]). The thermo-chemical processes generally use much higher temperatures and pressures, begin with gasification (where the biomass is converted into synthesis gas, also called syngas, that is a mixture of hydrogen and carbon monoxide) or pyrolyis (heating of organic material in the absence of oxygen), and can produce a wider variety of fuels than biochemical conversion processes (see e.g. [7, 13]). Many of the second-generation thermo-chemical fuels, such as demethyl ether, refined Fischer-

Tropsch liquid (FTL) and methanol, are fuels that are already made commercially from fossil fuels. For example, FTL is a mixture of hydrocarbon compounds, resembling a semi-refined crude oil, that can be refined to produce different hydrocarbon fuels, the primary one being a diesel-like fuel for compression ignition engines. In addition to LC biomass, coal and natural gas can also be used as feedstocks for FTL production [7]. Thermo-chemical processing of LC biomass is not described in any detail here as, with few exceptions (see Section 3.2.4), it does not depend on applications of biotechnology.

In biochemical processing of LC biomass to produce bioethanol, the process is more complicated than converting starch to bioethanol. There are two key parts. First, the cellulose and hemicellulose portions of the biomass must be broken down into sugars. This is a major challenge, and a variety of thermal, chemical and biochemical methods are being developed to carry out this saccharification step in an efficient and low-cost manner [9]. Second, these sugars must be fermented to make bioethanol. The yielded sugars, however, are a complex mixture of 5-carbon and 6-carbon sugars and this provides a greater challenge for complete fermentation into bioethanol.

2.4 Global production of liquid biofuels

Although some pilot plants currently exist, second-generation biofuels still remain a product for the future. Larson [7] predicts that substantial commercial production using biochemical processing will only begin in 10-20 years (versus 5-10 years for thermo-chemical processing). Such estimates vary, depending on factors such as expected private sector investments and oil prices, but it seems that it will take a minimum of five years [14]. First-generation biofuels, on the other hand, are already being produced in significant commercial quantities in a number of countries. World production has increased steadily in recent years, with production currently dominated by two countries, the United States and Brazil, and one type of fuel, bioethanol. See [7] for further details.

What about the future? OECD-FAO [15] provides an assessment of future prospects in the major agricultural commodity markets over the period 2008 to 2017 and includes an analysis of and projections for global biofuel markets for bioethanol and biodiesel. While noting that a number of uncertainties (such as oil prices and government policies) affect their projections, they predict that global ethanol production will continue to increase so that the quantity produced in 2017 will double that of 2007 [15]. It predicts that the United States and Brazil will continue to be the largest ethanol producers through to 2017 but also that production in several other countries, including China, India and Thailand, will grow rapidly. Regarding global biodiesel production, the report suggests that it will grow at slightly higher rates than for bioethanol to reach 24 billion litres by 2017 and that production in 2017 will continue to be dominated by the EU (over 50%), followed by Indonesia, Brazil, the United States and Malaysia respectively.

Policy interventions, especially in the form of subsidies and mandated blending of biofuels with fossil fuels, are driving the rush to liquid biofuels [8]. For example, the EU decided in March 2007 to set mandatory targets of a 20% share of renewable energies in overall EU energy consumption by the year 2020, and a mandatory 10% minimum target for the share of renewable fuels in overall EU transport petrol and diesel consumption by 2020, most of which is expected to be met by biofuels. Also, the United States Congress in December 2007 passed the Energy Independence and Security Act which, *inter alia*, sets required minimum annual levels of renewable fuel (biofuel) in United States transportation fuel, beginning at about 34 billion litres in 2008 and rising to about 137 billion litres in 2022 [16]. Indeed, a recent study of bioenergy development in the G8+5 countries (i.e. the G8 countries - Canada, France, Germany, Italy, Japan, Russia, United Kingdom and United States - plus Brazil, China, India, Mexico and South Africa), shows that all except one (Russia) have set either mandatory or voluntary biofuel transport targets [4].

Biofuel development in OECD countries has therefore been promoted and supported by government policies and a growing number of developing countries are also beginning to introduce policies to promote biofuels [8]. Analysis indicates that, with the exception of bioethanol from sugar cane in

Brazil, biofuels are generally not economically competitive with fossil fuels without subsidies [8]. In most countries, biofuel production is therefore dependent on public support and the ongoing discussion about the potential and actual benefits of supporting biofuel production/use will have a major influence on biofuel production in the future. As summarised by [15], "changes in biofuel policies, either to raise or to lower domestic targets or to review current policy incentives downwards, could be of major importance for agricultural markets given that biofuel production is one of the important factors lending strength to these markets over the medium term".

2.5 Reasons for major current focus on liquid biofuels

Government policies play a key role in influencing investment in bioenergy. The rapid growth in biofuels has been the result of substantial public policy support. OECD [17] highlights three major categories of support – budgetary support measures, including tax concessions and direct support to production, blending or use mandates and trade restrictions. Support amounted to about 11 G$ in nominal terms in 2006 and is projected to rise to 25 G$ in the medium term (2013-2017 average). There are four main factors behind this policy support and that are driving the current interest in liquid biofuels: the recent high oil prices; the increased emphasis on achieving national energy security; potential climate change mitigation; and rural development. See [4] and [8] for more details.

2.6 Current concerns about production of liquid biofuels

Public concern has centred on a number of risks related to rapid bioenergy expansion, such as:

2.6.1 Increasing food prices

Agricultural commodity prices rose sharply towards the end of 2006 and in 2007 and continued to rise even more sharply in early 2008 before stabilising and then declining rapidly. The FAO Food Price Index rose on average 7% in 2006 compared with the previous year, in 2007 it increased by 26% compared to 2006 and in 2008 it increased by 24% compared to 2007. While average prices for the first quarter of 2009 (the latest data available) are lower than the average prices for 2008 or 2007, they are nevertheless 16 and 24% higher than the average prices for 2006 and 2005 respectively [18].

This recent surge in food prices was seen in almost all major food and feed commodities and its driving forces were many and complex, where both supply-side and demand-side factors played a part. One of the demand-side factors underlying the state of the markets was the demand from the biofuel industry for agricultural commodities such as sugar, maize, cassava, oilseeds and palm oil [19]. It is estimated that about 100 million tonnes of cereals (nearly 5% of global cereal production) were used for biofuel production in 2007-08 [19]. The proportion of oil and sugar crops is even higher, with 9% of the world’s oilseeds production and 10% of sugar cane production being converted to biofuels. Over half of the increase in the total use of cereals and oilseeds during 2005-2007 was accounted for by biofuels [20]. Increased demand for these commodities was one of the leading reasons for the increase in their prices in world markets, which in turn led to higher food prices.

For the future, OECD-FAO [15] projects that food commodity prices will continue to be higher than in the past. Compared to the period 1998-2007, it predicts that average agricultural commodity prices will be substantially higher for the period 2008-2017 (e.g. 40-60% higher for wheat, maize and skim milk powder, over 60% higher for butter and oilseeds and over 80% higher for vegetable oils). The demand for biofuels is one of the main factors underlying their projections as "biofuel demand is the largest source of new demand in decades and a strong factor underpinning the upward shift in agricultural commodity prices" [15].

2.6.2 Land use changes

The Earth’s land surface covers about 134 Mm^2 . Of these, roughly 15 are used for crop production, 35 as grassland, 39 for forests, 2 for urban settlements and the remaining 42 Mm^2consist of desert, mountains and otherwise land that is unsuitable for productive use [21, 22]. The large demand for liquid biofuels has led to increasing proportions of certain crops being used for biofuel instead of for food/feed. For example, in the United States the estimated proportion of maize cultivated that is used for biofuels has steadily increased from less than 5% in 1997 to about 30% in 2008 [7, 23]. It has also resulted in farmers switching from non-biofuel crops to biofuel crops. Furthermore, it has led to forests, peatlands, savannas and grasslands being converted to agricultural lands for biofuel production (or for non-biofuel crop production, to replace agricultural land that has already been diverted to biofuel production) [24].

The conversion of natural lands, such as wetlands and natural forests, for biofuel production represents an important threat to biodiversity through the loss of habitats, their biodiversity components and the loss of essential ecosystem services. In addition, the large-scale ploughing of non-agricultural land and pasture land as well as peatland degradation could result in substantive release of carbon emissions into the atmosphere [25]. For example, [24] looked at six different cases of habitat conversion situations currently taking place as a result of biofuel production: conversion of 1) Brazilian Amazon rainforest to soybean 2) Brazilian Cerrado (i.e. tropical savannah) to soybean 3) Brazilian Cerrado to sugar cane 4) Indonesian or Malaysian lowland tropical rainforest to oil palm 5) Indonesian or Malaysian peatland tropical rainforest to oil palm and 6) United States central grassland to maize. Their results suggested that if produced on converted land, then biofuels could, for long periods of time, be much greater net emitters of GHGs than the fossil fuels they typically displace.

2.6.3 Increased pressure on scarce water resources

Scarcity of water is one of the major global problems facing humankind at the moment and it is likely to be an ever increasing problem in the future [26]. Furthermore, there will be more intense competition from the industrial and municipal sectors for the water resources available for agriculture in the future, despite the fact that there will also be an ever-increasing demand for water in agriculture to meet the needs of the growing world population [26]. In water-short countries where agriculture relies essentially on irrigation, increasing production of biofuels will simply add to the strain on stressed water resources because of the large quantities of water required for production of the feedstock and its conversion to biofuel. Sugar cane and oil palm have high water requirements (1500-2500 mm per year), while cassava, castor bean, cotton, maize and soybean, all crops considered suitable for biofuels, require medium levels of water (500-1000 mm per year) [3]. However, it is the share of irrigation water used to meet these requirements which will influence pressure on water resources. For example, a recent report from the International Water Management Institute points out that a litre of ethanol made from irrigated sugarcane in India can require more than 25 times as much irrigation water as a litre of ethanol made from mostly rainfed sugarcane in Brazil and concludes "unless planned properly, biofuel crops are likely to escalate competition for water, especially in areas where it is already scarce” [27].

3. Biotechnologies and bioenergy production

As described in the Introduction, a wide range of biotechnologies are available and many of them can be applied for bioenergy production in developing countries. They include, among others, fermentation, genomics and genetic modification and cover applications to micro-organisms, crops and forest trees. In the context of bioenergy production, they can be used to increase the efficiency of both parts of the production cycle i.e. the production of biomass for bioenergy purposes and the conversion of the biomass to biofuels.

Here, we will briefly consider some of the kinds of areas where biotechnologies are being, or can be, applied for production of first-generation biofuels (Section 3.1), second-generation biofuels (3.2) as well as microalgal biodiesel and biogas (3.3). Greatest attention is paid to second-generation biofuels because of the large expectations they have created and because of the significant role that biotechnology applications are likely to play in their development.

3.1 Application of biotechnologies for first-generation biofuels

Apart from a range of factors including the amount of rainfall etc., yields of liquid biofuel also depend on the crop that is cultivated and the part of the world where it is grown. Estimated bioethanol yields per hectare have been calculated to be about 5500 and 4500 litres (L) from sugar cane in Brazil and India respectively, 3800 and 2000 L from maize in the United States and China respectively and about 1900 and 1500 L from cassava in Brazil and Nigeria respectively, while for biodiesel, estimated yields per hectare are 4700 and 4100 L from oil palm in Malaysia and Indonesia respectively and 550 and 500 L from soybean in the United States and Brazil respectively [8].

3.1.1 Production of biomass

One way in which biotechnologies (or, indeed, conventional plant breeding) could contribute is by improving biomass production. The plant varieties currently being used for first-generation biofuels worldwide have been genetically selected for agronomic characteristics relevant to food and/or feed production and they have not been developed considering their characteristics as potential feedstocks for biofuel production. Varieties could be selected with increased biomass per hectare, increased yields of oils (biodiesel crops) or fermentable sugars (bioethanol crops) or with improvements in characteristics relevant for their conversion to biofuels. As little genetic selection has been carried out in the past for biofuel characteristics in most of these species, considerable genetic improvement should be possible.

The field of genomics is likely to play an important role here. Genomics is the study of an organism's genome i.e. the entire complement of its genetic material (genes plus non-coding sequences). The goal of modern plant genomics is to understand how plants do what they do i.e. to discover the function of each gene; the cells in which each gene functions (and when); the relationship each gene has with all other genes; and the consequences of altered gene function [28]. Draft genomes of several first-generation feedstocks, such as maize, sorghum and soybean, are in the pipeline or have already been published. For example, the project to sequence the genetic code of soybean (*Glycine max*) began in 2006 and the first chromosome-scale assembly of its genome was made available in December 2008 [29]. Using the information this will provide on the genetic make-up of soybean, research can aim to produce better varieties for biofuel production by changing the type, quantity and/or location of the oil produced by the plant [30]. Apart from genomics, a range of other biotechnologies can also be used, such as marker-assisted selection and genetic modification. For example, [31] describes how the task of oil palm breeders can be facilitated by biotechnologies such as marker-assisted selection (where DNA markers can be used to identify genetically-superior individuals when they are just weeks old rather than when the trees are 5-7 years old, after they produce the fruits that are the source of the oil) or tissue culture (applied to multiply up genetically superior trees).

3.1.2 Conversion of biomass to liquid biofuels

Another area where biotechnology can be applied is in improving the conversion of biomass to liquid biofuels. For example, as the yeast *S. cerevisiae* cannot directly ferment starchy materials (e.g. corn starch), the feedstock must first be hydrolysed using acids or enzymes, in particular a family of enzymes called amylases, normally alpha-amylase and glucoamylase. In the past, enzymes were isolated primarily from plant and animal sources, and thus a relatively limited number of enzymes were available. Today, bacteria and fungi are exploited and used for the commercial production of a diversity of enzymes. Several strains of micro-organisms have been selected or genetically modified to increase the efficiency with which they produce enzymes. In most cases, the modified genes are of microbial origin, although they may also come from different kingdoms [32]. Many of the current commercially available enzymes, including amylases, are produced using genetically modified (GM) micro-organisms where the enzymes are produced in closed fermentation tank installations (e.g. [33]). The final enzyme product does not contain GM micro-organisms. Royal Society [13] suggests that as the current usage of GM micro-organisms within fermentation systems involves keeping them in contained environments such as fermentation vats, then genetic modification is a far less contentious issue here than with GM crops.

To reduce costs and increase the efficiency of bioconversion, research is also ongoing to develop GM yeast strains which produce the amylases themselves so that the saccharification and fermentation steps can be combined, as well as to develop GM maize plants which can produce the amylases [13]. After fermentation, the ethanol produced needs to be separated from the dilute solution using distillation. The step requires a lot of energy and could be made more efficient by genetically improving the micro-organisms used in the fermentation process so that the ethanol concentration is increased prior to distillation [13].

3.2 Application of biotechnologies for second-generation biofuels

In view of the risks related to first generation biofuels production mentioned in Section 2.6, there is great interest in moving from first-generation biofuels towards use of LC biomass for second-generation biofuels. This has brought with it major R&D investments in this area, where e.g. in 2007 venture entities invested in nominal terms an estimated 2.9 G$ in the biofuel industry sector in the United States alone and where investments worldwide are expected to increase significantly in coming years [34]. If second-generation biofuels are to become a reality in the future some technological breakthroughs are needed, and applications of biotechnology in this context are discussed here.

However, it should be noted that these biotechnology breakthroughs alone will not be enough. Second-generation biofuels will also have to be economically viable and environmentally sustainable, which will depend on a series of factors, including the logistical challenge of collecting and transporting large amounts (in quantity and volume) of LC biomass to the biofuel production facilities [21]. This may require that the LC biomass is produced close to the processing site, which can be a disadvantage for developing countries which at the moment have the option of producing feedstock that can be shipped, processed or semi-processed, for further conversion in the country of use. Also, competition for land and other inputs will remain a challenge and it is not certain that all the concerns related to use of first-generation biofuels will be alleviated by second-generation biofuels. For example, [24] suggests that, like first-generation biofuels, second-generation biofuels may also result in land clearing and land use changes. FAO [8] also notes that excessive withdrawal of agricultural residues for bioenergy purposes could negatively impact soil fertility and quality by removing decomposing biomass.

The LC biomass needed for second-generation biofuels can come from two main sources. The first source is from by-products, such as agricultural residues like sugar cane bagasse, corn stover, straws from barley, oats, rice, wheat and sorghum; residues from the pulp and paper industry; and municipal cellulosic solid wastes. For example, [35] predicts that in Brazil there will eventually be significant production of bioethanol from sugar cane bagasse and straw, materials that are available on a large-scale. The second source is from dedicated biomass feedstocks, grown specifically for the purpose of biofuel production, such as perennial grasses and short-rotation forest trees [28]. As with first-generation biofuels, applications of biotechnologies can be considered separately for production of biomass and for conversion of the biomass to biofuels.

3.2.1 Production of LC biomass from by-products

Concerning the by-products of crop production, relatively little R&D has yet been carried out with biofuels in mind. For example, cereal production has been optimised for grain yield but the crops have not been bred for straw quality in relation to its use as biomass for biofuel purposes [13]. In fact, breeding has aimed to reduce straw and straw quantity [36]. Substantial room for genetic improvement therefore exists. Thus, information from genomic projects of first-generation biofuel crops, such as those mentioned in Section 3.1.1, can also be used in genetic improvement programmes to breed varieties with LC biomass characteristics that are more suitable for biofuel purposes [30]. Some examples of ongoing research projects in this area include attempts to: identify and isolate genes in sweet sorghum that control the high stalk sugar trait and a decreased stalk lignin trait, in order to combine both traits within the same plant; identify genes that regulate cell wall synthesis in rice, in order to genetically manipulate them to change the cell wall composition for cost efficient ethanol fermentation; and optimise the use of DNA markers to simultaneously breed for high grain yield (for energy or non-energy purposes) and high stover quality (for ethanol production) in maize [37].

3.2.2 Production of LC biomass from dedicated feedstocks

Concerning dedicated biomass feedstocks, a range of potential candidates are of interest. They include perennial grasses (i.e. which flower for several years) such as switchgrass, miscanthus, reed canary grass and giant reed [13]. They also include tree species such as the poplar and eucalyptus. As for some of the first-generation biofuel species, the genomes of a number of second-generation species are also being sequenced. For example, the recent announcement that the eucalyptus tree genome is to be sequenced is important because eucalyptus species are the most widely planted hardwood trees in the world (occupying more than 18 million hectares), supplying woody biomass for several industrial applications. The challenges and potential of applying new molecular techniques and approaches to eucalyptus breeding for traits such as those relevant to biofuel purposes have recently been reviewed [38].

The eucalyptus genome will be the second tree genome to be sequenced following that of the poplar already published in 2006. Tuskan [28] describes how the genomics information of the poplar can be used in combination with the extensive knowledge already available about important identified genes of other species, such as rice or the model species *Arabidopsis,* to identify equivalent (homologous) genes in the poplar so that trees with desirable properties for biomass production can be developed. Among other things, the trees would ideally: accumulate greater carbon allocation in the stem, through the development of a less extensive root system and through reduced height and minimal perennial branch formation and growth; be relatively short but with a large stem diameter, generating lower amounts of low quality wood, more harvested biomass and improved harvesting/handling efficiencies; display higher productivity per unit area and drought/stress tolerance; not produce flowers; and be modified to produce optimal feedstocks for energy conversion (e.g. by increasing the polysaccharide component in the wood at the expense of lignin, for biochemical conversion of the biomass to liquid biofuels) [28]. Apart from genomics, other biotechnologies can also be applied. For example, [39] reviews some of the ways in which genetic modification can be applied to improve the biomass characteristics of plants for biofuels, including development of crop varieties that produce less lignin; that self-produce cellulase enzymes for cellulose degradation and ligninase enzymes for lignin degradation; or that have increased cellulose or overall biomass yields.

3.2.3 Conversion of LC biomass to liquid biofuels

LC biomass can be converted to biofuels in two main ways, by thermo-chemical or biochemical processing [7] and here we will discuss biochemical processing, because of the extensive applications of biotechnology involved. Depending on factors such as the kind of feedstock available, biochemical conversion of LC biomass to liquid biofuels can follow a number of different pathways, in which four major steps can generally be identified [11].

First is pre-treatment of the biomass, which promotes the physical disruption of the LC matrix. This is necessary because the LC materials are structured for strength and resistance to biological, physical and chemical attack [35]. Pre-treatment can be carried out in a number of ways e.g. using dilute acids (such as sulphuric or hydrochloric acid), alkalines (such as calcium hydroxide), liquid ammonia (the ammonia fibre explosion pre-treatment) or steam explosion [11].

Second is hydrolysis i.e. breakdown of the polysaccharides to their simple sugars, which is carried out using either acid (dilute or concentrated) or enzymes. According to [13], the current trend is towards enzymatic hydrolysis to avoid costly recovery and wastewater treatment requirements resulting from the use of acid. Balat et al. [11] also indicate that enzymatic hydrolysis is attractive because it produces better yields than acid-catalysed hydrolysis and that enzyme manufacturers have recently reduced costs substantially using biotechnology.

The importance and interest in enzymatic hydrolysis has renewed and increased the focus on several aspects of cellulases (i.e. enzymes, such as endoglucanases, cellobiohydrolases and beta-glucosidases, which break down cellulose) and hemicellulases (i.e. enzymes, such as xylanases, mannanases, xylosidases, glucosidases or arabinosidases, that break down hemicelluloses). These include the search for high cellulase-producing organisms; the production of hypercellulolytic mutants (i.e. which are highly efficient at degrading cellulose) of organisms suitable for cellulase production; genetic modification to develop high cellulase-producing organisms with high specific activity; and theoretical studies on the mechanism of action of a multi-enzyme system on a complex polymer [35]. Engineering of enzymes using advanced biotechnologies is ongoing to develop enzymes with improved characteristics such as higher efficiencies, increased stability at elevated temperatures and at certain pH levels and higher tolerance to end-product inhibition [35].

Regarding the search for efficient biomass-degrading organisms, a wide range of micro-organisms can produce cellulases and hemicellulases in nature and are at the centre of major R&D initiatives. Among others, these include strains of fungi (of *Hypocreaceae Trichoderma*, *Penicillium* or *Chrysosporium* species) and bacteria (of *Bacillus, Clostridium* or *Cellulomonas* species). For example, [28] describes some genome sequencing projects that are aiming ultimately to find genes to produce new enzymes for plant cell wall breakdown. These include projects focusing on specific micro-organisms known to have desirable biomass-degrading characteristics, such as the bacterium *Clostridium thermocellum* (which degrades cellulosic materials using a large extracellular cellulase system called the cellulosome) or the white rot fungus *Phanerochaete chrysosporium* (which produces enzymes that degrade lignin) or the bacterial community resident in the hindgut of a wood-feeding termite.

The third step is fermentation of the sugars, resulting from the breakdown of cellulose and hemicellulose, to bioethanol. Unlike production of bioethanol from first-generation sugar crops or starchy materials, fermentation is more complicated here as it is a mixed-sugar fermentation (involving pentose and hexose sugars) and it takes place in the presence of inhibiting compounds released and formed during the first two steps of the process, i.e. pre-treatment and hydrolysis. Because of their larger sizes, thicker cell walls, better growth at low pH, less stringent nutritional requirements and greater resistance to contamination, yeasts are preferred to bacteria for commercial fermentations [40]. However, LC biomass, in particular hardwood and agricultural raw materials, can contain 5–20% (or more) of the pentose sugars xylose and arabinose which are not fermented to ethanol by the yeast *S. cerevisiae*, the most commonly used industrial fermentation micro-organism [41].

To overcome these problems, several different approaches are being explored. One is to develop efficient xylose-fermenting strains of *S. cerevisiae* using a range of biotechnologies, including genetic modification (where genes to enable xylose fermentation are transferred from the yeast *Pichia stipitis*, the bacteria *Thermus thermophilus* or the fungus *Piromyces* species) and global gene expression analysis combined with targeted deletion or altered expression of key genes [40]. Another approach is to focus on yeast species that naturally ferment xylose. For example, *P. stipitis* is a well-studied natural xylose-fermenting yeast. The recent reporting of its genome sequence, predicting over 5800 genes, is important in this context as the genetic information can be employed to improve usefulness of this yeast for commercial fermentation operations [42]. The optimism regarding these approaches is summarised by [40]: "Genomic and expression analysis of *Pichia stipitis* along with new strains from nature should continue to drive this field forward. The eventual goal is a yeast that is capable of efficiently fermenting glucose, xylose and other minor sugars to ethanol, and progress is being made on multiple fronts".

Another approach is to focus on bacteria instead of yeast. Three bacterial species that have received much attention are *Escherichia coli, Klebsiella oxytoca* and *Zymomonas mobilis* and GM strains have been produced for each of them for bioethanol purposes. For example, *Z. mobilis* has been shown to have higher ethanol yields and productivity than traditional yeast fermentations. However, like *S. cerevisiae* it cannot naturally ferment pentose sugars. To overcome this, GM strains using genes from *E. coli* have been developed which can also ferment xyloses and/or arabinoses [11].

The fourth step is removal of the bioethanol. The step involves distillation which separates the bioethanol from water in the liquid mixture. See [11] for more details.

For simplicity, the process above has been described in four sequential steps. In practice, enzymatic hydrolysis (2nd step) and fermentation (3rd step) can also be carried out together, called simultaneous saccharification and fermentation (SSF). This has a number of advantages, as they take place in the same reactor, thus reducing costs, and increase the hydrolysis rate, since the sugars resulting from hydrolysis are fermented and thus do not inhibit cellulase activity. On the negative side, the ideal pH or temperature conditions for the saccharification step may differ from those for the fermentation step [11]. For the future, it would also be desirable to combine cellulase production, enzymatic hydrolysis and fermentation - called consolidated bioprocessing (CBP) [43].

Through the steps described above, two of the three main components of LC biomass, i.e. cellulose and hemicellulose, are converted to bioethanol. The third component, lignin, as well as its by-products, need to be removed before fermentation takes place as they are often toxic to micro-organisms and the enzymes used for hydrolysis, which can reduce the conversion efficiency. According to [13], this could be partly addressed by using low lignin feedstocks or developing new strains of lignin tolerant and lignin degrading micro-organisms. Lignin can be burnt to provide a source of heat and power for the conversion process. Alternatively new developments may make it valuable as a chemical feedstock.

The importance of processes for converting LC biomass to liquid biofuels was recently underlined by [43] in an analysis of the economics of second-generation bioethanol production in the United States, concluding that "the immediate factor impeding the emergence of an industry converting cellulosic biomass into liquid fuels on a large scale is the high cost of processing rather than the cost or availability of feedstock". In their analysis, they also looked at the different steps involved in converting the biomass to bioethanol and estimated that the cost savings of improving the conversion of LC biomass to sugars (e.g. by eliminating pre-treatment, reducing the amount of cellulase needed, using CBP) were in general much larger than from improving the conversion of sugars to bioethanol (e.g. by increasing fermentation yield).

3.2.4 Biotechnology in thermo-chemical conversion of LC biomass

As mentioned in Section 2.3, in addition to biochemical conversion, LC feedstocks can also be converted to biofuels using a number of thermo-chemical conversion processes. In one of these processes, i.e. the production of alcohol fuels (ethanol or butanol) from syngas, biotechnology may also play an important role as one option is to use micro-organisms to ferment the syngas [7]. Production of ethanol from fermentation of syngas has already been demonstrated but considerable improvements could be made (e.g. isolation of micro-organisms that act well in hot temperatures, as gasification results in syngas with a high temperature) and approaches such as metabolic engineering will be important here [44]. If this process could be made commercially viable, it would be particularly advantageous as, unlike biochemical conversion, the lignin in LC biomass, as well as cellulose and hemicellulose, would be converted to a liquid biofuel [7].

3.3 Applications of biotechnologies for some other biofuels

Apart from using sugars, grains, or seeds (first-generation biofuels) or LC biomass (second-generation biofuels), a number of other biomass sources can be used to produce biofuels. Two of them will be briefly mentioned here, as well as the role that biotechnologies may play for them. The first one, involving microalgae, is an option for the future while the second one, involving biogas production, is currently available in both developed and developing countries.

3.3.1 Biodiesel from microalgae

The potential importance that microalgae might have for production of biodiesel has long been recognised (see e.g. [10] for some historical background). Microalgae are unicellular photosynthetic micro-organisms, living in saline or freshwater environments, that convert sunlight, water and carbon dioxide to algal biomass. They are categorised into four main classes: diatoms, green algae, blue-green algae and golden algae. Like higher plants, they produce storage lipids in the form of TAGs. Many species exhibit rapid growth and high productivity, and many microalgal species can be induced to accumulate substantial quantities of lipids, often greater than 60% of their dry biomass ([10].

The advantages of using microalgae instead of crops for biodiesel production include that they represent much higher potential biodiesel yields per hectare (so that they could theoretically, unlike biodiesel crops, meet the global demand for transport fuels); can be harvested throughout most of the year, thus giving a regular supply of biomass; and use less freshwater [45, 46]. As microalgal production can also take place in ponds or bio-reactors on non-arable land or in a marine environment, it need not compete with food production for land or water. Apart from high efficiency production of TAGs for biodiesel, [46] argue that microalgae are also well suited for the production of feedstocks for other biofuels, including biohydrogen, bioethanol and biogas. Algae can also be efficiently grown when coupled with CO_2-emitting flue gases from power plants and can contribute to atmospheric CO_2 reductions when the biomass remaining after extracting the oil for biodiesel is fed into carbon sequestration processes [46].

There are however, some serious hurdles to be overcome before the process becomes a realistic alternative. For example, [45] estimates that the price of biomass production needs to fall about 9-fold for it to become feasible, underlining the importance of improving the production technology through e.g. developing efficient methods for recovering algal biomass from the dilute broths produced in the bioreactors. Chisti [45] also argues that genetic modification and metabolic engineering are likely to have the greatest impact on improving the economics of production of microalgal biodiesel and that some specific applications of biotechnologies that might be considered include increasing the biomass yield; increasing the biomass growth rate as well as the oil content in the biomass; and improving temperature tolerance of the microalgae so that there is a reduced need for cooling, which is expensive. In a similar vein, [46] argues that the biggest challenge over the next few years will be to reduce costs for cultivation and to further improve the biology of oil production from the microalgae. They also emphasise the role that advanced biotechnologies will play in this context, where "future algal strain improvement will utilize methodologies such as lipidomics, genomics, proteomics, and metabolomics to screen for and develop new strains that exhibit high growth and lipid biosynthesis rates, broad environmental tolerances, and that produce high value-add by-products". Currently, there are a number of companies setting up pilot plants with anticipation to scale up to commercial biodiesel production from algae within the next few years.

3.3.2 Biogas

In the absence of oxygen, certain bacteria will ferment biomass into methane and carbon dioxide, a mixture called biogas. In this anaerobic digestion process, the feedstocks used to obtain biogas may include sewage sludge, agricultural by-products and wastes (especially animal manure), industrial wastes (e.g. organic solid wastes) or municipal solid wastes. They may also include dedicated feedstocks grown for the purpose of biogas production. The resulting fuel can be used for heat, electricity and as a vehicle fuel (after the gas has been compressed and using the same engine and vehicle configuration as natural gas). As methane is a GHG, its capture and use as a biofuel prevents the release of methane into the atmosphere.

Biogas can be produced at landfill sites, centralised co-digestion units (co-operative units, using different biomass sources) or in farm-scale units. These farm-scale digestion plants, mainly using animal wastes, are widespread throughout the developing and developed world [47]. For example, in

China 17 million biogas users are reported in 2005, in India there are an estimated 3.8 million household-scale biogas plants while in Nepal over 170 000 plants, using cattle and buffalo manure, are in operation [48]. These plants (also called digesters) are generally used to provide gas for cooking and lighting for a single household.

At the biochemical level, anaerobic digestion is complex, consisting of a series of reactions catalysed by a mixture of different bacterial species. Four stages of anaerobic digestion are generally distinguished: hydrolysis, acidogenesis, acetogenesis and methanogenesis. In the first stage, bacteria secrete enzymes which hydrolyse polymers, such as lipids, proteins and carbohydrates, to smaller molecules such as fatty acids, amino acids and glucose. For example, proteins are generally hydrolysed to amino acids by protease enzymes secreted by *Bacteroides*, *Butyrivibrio*, *Clostridium*, *Fusobacterium*, *Selenomonas* and *Streptococcus* species. Second, in acidogenesis, these products are metabolised by groups of bacteria and fermented to produce organic acids, such as butyric acid, propionic acid and acetic acid, as well as hydrogen and carbon dioxide. Third, acetogenic bacteria (i.e. that make acetic acid as their sole or primary metabolic end-product) convert organic acids to acetic acid plus hydrogen and carbon dioxide. Fourth, methanogenic bacteria produce methane from acetic acid or from hydrogen and carbon dioxide [49].

Anaerobic digestion happens slowly in nature and could be accelerated in several ways, such as using more efficient micro-organisms in these processes, although knowledge of these microbial communities is generally still quite basic. However, to improve the understanding and efficiency of biogas production, some studies on the roles of the different populations of micro-organisms have been carried out, on specific types of micro-organisms such as cellulolytic bacteria and methanogenic bacteria in specific environments like landfill sites or solid waste or sewage sludge digesters [50].

As an example of one such study, [50] looked at the microbial community involved in the first stage (hydrolysis) of anaerobic digestion of two different kinds of organic substrates (sugar beets and grass/clover) using a technique called fluorescence in situ hybridisation (FISH), where fluorescently labelled DNA sequences are added to bacterial cells, making it possible to identify, quantify and localise different bacterial species in complex microbial communities without having to actually cultivate the microbes. From the study they were able to identify the general bacterial groups involved, concluding that their results "could be considered as a first step towards the development of strategies to stimulate hydrolysis further and ultimately increasing the methane production rates and yields from reactor-based digestion of these substrates". A range of other biotechnologies are also being applied in this context, such as the use of metagenomics (i.e. isolating, sequencing and characterising DNA extracted directly from environmental samples) to study the micro-organisms involved in a biogas producing unit in order to improve its operation (e.g. [51]).

4. Some issues related to the applicability in developing countries

A small number of issues of specific relevance to application of biotechnologies for bioenergy purposes in developing countries are briefly described below.

4.1 Technology relevance

Considering second-generation biofuel technologies, [7] argues that since they are primarily being developed in industrialised countries, there are important issues that must be considered about their relevance for developing countries. Thus, he writes: "Technologies developed for industrialized country applications will typically be capital-intensive, labour-minimizing, and designed for large-scale installations to achieve best economics. In addition, the biomass feedstocks for which technologies are designed may be quite different from feedstocks that are suitable for production in developing countries. To capitalize on their comparative advantages of better growing climates and lower labour costs, developing countries will need to be able to adapt such technologies. Tailoring feedstocks to local biogeophysical conditions will be important for maximizing biomass productivity per hectare and minimizing costs. In addition, adapting conversion technologies to reduce capital intensities and increase labour intensities will be important for providing greater employment opportunities and reducing the sensitivity of product cost to scale".

Putting these considerations into the specific context of the subject of this paper, biotechnologies (for production of biofuel feedstocks and/or for conversion of feedstocks to biofuels) developed in industrialised countries may need to be adapted for appropriate use in developing countries. This may involve the need to adapt biotechnologies or biotechnology products developed elsewhere to different crop/tree species and agro-ecological zones or to different kinds of biomass production and/or bioconversion systems in developing countries. The adaptation of complex and sophisticated technologies to local needs and capacities in developing countries is, however, a challenge [16]. Further, the capacity of developing countries to develop tailor-made technologies for their specific contexts is very limited.

4.2 Intellectual property rights

Related to the previous issue, major investments are being made today in R&D in biofuels, primarily by the private sector and in developed countries. This is clearly reflected in the major increase in biofuel-related patents. An analysis by [34] indicates that the number of biofuel-related patents (defined as U.S. patents applied for or granted, plus Patent Cooperation Treaty (PCT) international applications) has risen from about 150 in 2002, to about 400 in 2005 and to over 1,000 in 2007, when the number of patents was higher than the combined total of patents related to solar and wind power. For 2006-2007, the major focus of patents was on biodiesel rather than bioethanol and most patents were owned by the private sector, with 57% owned by corporate entities, 11% by universities or other academic institutions and 32% undesignated. The authors predict that for the future, the number of biofuel patents will continue to increase steadily; the number of agricultural biotechnology biofuel patents will significantly increase as transgenic plant technology is directed to biofuel applications; and that LC biofuel patents will increase in number.

Zarrilli [16] notes that forthcoming biofuel technology will be proprietary and points out that strong intellectual property rights regimes may mean that access to technology is problematic, especially for developing countries. Intellectual property rights are generally considered an important issue for biotechnology applications, impacting both GMOs and non-GMO biotechnologies, and their consequences are often perceived as negative, with concerns expressed that they might for example, act as a constraint to biotechnology research in developing countries [32].

4.3 Non-transport biofuels

While the major focus today is on production of liquid biofuels for transport purposes, it is also important to keep in mind, when considering the potential contribution of agricultural biotechnologies in this context, that the production of biofuels for non-transport needs (lighting, heating, cooking) could have tremendous advantages for developing countries. For example, for regions such as sub-Saharan Africa, breakthroughs in the area of liquid biofuels for cooking would be very important as energy for cooking is a priority since 95% of all staple foods must be cooked and traditional cookstoves, powered by fuelwood and dung, have negative health and social impacts. According to [5]: "transition to improved cookstoves using biobased feedstocks could free women and children from the collection and transport of wood and dung which can account for up to one-third of their productive time, and reduce the effects of indoor air pollution which is responsible for more deaths of women and children than malaria and tuberculosis combined".

Acknowledgements

We gratefully acknowledge the comments of Denis Murphy on an earlier draft of the manuscript as well as those from our FAO colleagues (Jean-Marc Faurès, Hannes Johnson, Preetmoninder Lidder, Tony Piccolo and Wim Polman) and non-FAO colleagues (Elba P.S. Bon, Maria Antonieta Ferrara and Peer Schenk) on specific parts of the document. An anonymous reviewer is also gratefully acknowledged. Finally, we thank Shivaji Pandey and Jeff Tschirley, Chair of the FAO Working Group on Biotechnology and former Chair of the FAO Working Group on Bioenergy respectively, for their support.

REFERENCES

[1] UN. Note by the Secretary-General transmitting the interim report of the Special Rapporteur on the Right to Food. 62nd General Assembly of the United Nations. 2007. http://www.un.org/ga/third/62/docslist.shtml (accessed on 26.3.10)

[2] FAO. Report of the High-Level Conference on World Food Security: the Challenges of Climate Change and Bioenergy, 3-5 June 2008, FAO Headquarters, Rome. http://www.fao.org/foodclimate/conference/doclist/en/?no_cache=1 (accessed on 26.3.10)

[3] FAO. Opportunities and challenges of biofuel production for food security and the environment in Latin America and the Caribbean. Document prepared for the 30th Session of the FAO Regional Conference for Latin America and the Caribbean, held in Brasilia, Brazil, 14-18 April 2008. http://www.fao.org/Unfao/Bodies/RegConferences/Larc30/Index_en.htm (accessed on 26.3.10)

[4] GBEP. A review of the current state of bioenergy development in G8 + 5 countries. Global Bioenergy Partnership. 2007. http://www.fao.org/docrep/010/a1348e/a1348e00.htm (accessed on 26.3.10)

[5] UNCSD. Small-scale production and use of liquid biofuels in Sub-Saharan Africa: Perspectives for sustainable development. Background document to 15th session of the UN Commission on Sustainable Development, 30 April to 11 May 2007, New York, United States. http://www.energyandsecurity.com/images/csd15_bp2.pdf (accessed on 26.3.10)

[6] WBGU. World in transition – Future bioenergy and sustainable land use. German Advisory Council on Global Change. Earthscan, London. 2010.

[7] Larson ED. Biofuel production technologies: status, prospects and implications for trade and development. United Nations Conference on Trade and Development (UNCTAD). 2008. http://www.unctad.org/en/docs/ditcted200710_en.pdf (accessed on 26.3.10)

[8] FAO. The State of Food and Agriculture 2008: Biofuels: prospects, risks and opportunities. http://www.fao.org/publications/sofa-2008/en/ (accessed on 26.3.10)

[9] IEA. Biofuels for transport. International Energy Agency. 2004. http://www.iea.org/textbase/nppdf/free/2004/biofuels2004.pdf (accessed on 26.3.10)

[10] Sheehan J, Dunahay T, Benemann J, Roessler P. A look back at the U.S. Department of Energy's Aquatic Species Program—Biodiesel from algae. National Renewable Energy Laboratory. 1998. http://www.nrel.gov/docs/legosti/fy98/24190.pdf (accessed on 26.3.10)

[11] Balat M, Balat H, Oz C. Progress in bioethanol processing. Progress in Energy and Combustion Science 2008; 34: 551-573.

[12] USDOE. Breaking the biological barriers to cellulosic ethanol: A joint research agenda. Summary of the Biomass to Biofuels Workshop, held 7-9 December 2005, Rockville, United States, sponsored by the U.S. Department of Energy. http://genomicsgtl.energy.gov/biofuels/b2bworkshop.shtml (accessed on 26.3.10)

[13] Royal Society. Sustainable biofuels: Prospects and challenges. 2008. http://royalsociety.org/Sustainable-biofuels-prospects-and-challenges/ (accessed on 26.3.10)

[14] Rotman D. The price of biofuels. Technology Review, January/February 2008.

[15] OECD-FAO. OECD-FAO Agricultural Outlook 2008-2017. 2008.
[16] Zarrilli S. Global perspective on production of biotechnology-based bioenergy and major trends. Paper prepared for the FAO seminar on "The role of agricultural biotechnologies for production of bioenergy in developing countries", held in Rome, Italy, 12 October 2007 http://www.fao.org/biotech/docs/zarrilli.pdf. (accessed on 26.3.10)

[17] OECD. Biofuel support policies: An economic assessment. 2008.

[18] FAO Food Price Indices website. http://www.fao.org/worldfoodsituation/FoodPricesIndex/en/ (accessed on 26.3.10)

[19] FAO. Assessment of the world food security and nutrition situation. Document prepared for the 34th Session of the FAO Committee on World Food Security, Rome, 14-17 October 2008. http://www.fao.org/UNFAO/Bodies/cfs/cfs34/index_en.htm (accessed on 26.3.10)

[20] FAO. The market and food security implications of the development of biofuel production. Document prepared for the 67th Session of the FAO Committee on Commodity Problems, Rome, 20-22 April 2009. ftp://ftp.fao.org/docrep/fao/meeting/016/k4477e.pdf (accessed on 26.3.10)

[21] Doornbosch R, Steenblik, R. Biofuels: Is the cure worse than the disease? Presented at the 20th meeting of the OECD Round Table on Sustainable Development, 11-12 September 2007, Paris, France. http://www.oecd.org/dataoecd/15/46/39348696.pdf (accessed on 26.3.10)

[22] FAO. World Agriculture: Towards 2015/2030. An FAO perspective. Edited by J. Bruinsma.2003. http://www.fao.org/docrep/005/y4252e/y4252e00.htm (accessed on 26.3.10)

[23] FAO. Soaring food prices: Facts, perspectives, impacts and actions required. Information document prepared for the High-Level Conference on World Food Security: the Challenges of Climate Change and Bioenergy, Rome, 3-5 June 2008. http://www.fao.org/foodclimate/conference/doclist/en/?no_cache=1 (accessed on 26.3.10)

[24] Fargione J, Hill J, Tilman D, Polasky S, Hawthorne, P. Land clearing and the biofuel carbon debt. Science 2008; 319: 1235-1238.

[25] SBSTTA. New and emerging issues relating to the conservation and sustainable use of biodiversity - Biodiversity and liquid biofuel production. Document UNEP/CBD/SBSTTA/12/9 prepared for the 12th meeting of the Subsidiary Body on Scientific, Technical and Technological Advice, 2-6 July 2007, Paris, France. http://www.cbd.int/meetings/sbstta-12/documents.shtml?t=0&mtg=sbstta-12 (accessed on 26.3.10)

[26] FAO. Coping with water scarcity: What role for biotechnologies? By J. Ruane, A. Sonnino, P. Steduto and C. Deane. FAO Land and Water Discussion Paper No. 7. Rome. 2008. http://www.fao.org/docrep/011/i0487e/i0487e00.htm (accessed on 26.3.10)

[27] IWMI. Water implications of biofuel crops: Understanding tradeoffs and identifying options. Water Policy Brief 30. International Water Management Institute, Colombo, Sri Lanka. 2008. http://www.iwmi.cgiar.org/Publications/Water_Policy_Briefs/PDF/WPB30.pdf (accessed on 26.3.10)

[28] Tuskan GA. Bioenergy, genomics, and accelerated domestication: A U.S. example. Paper prepared for the FAO seminar on "The role of agricultural biotechnologies for production of bioenergy

in developing countries", held in Rome, Italy, 12 October 2007 http://www.fao.org/biotech/docs/tuskan.pdf (accessed on 26.3.10)

[29] Phytozome website. United States Department of Energy Joint Genome Institute and the Center for Integrative Genomics. http://www.phytozome.net/soybean.php (accessed on 26.3.10)

[30] JGI. DOE JGI releases soybean genome assembly to enable worldwide bioenergy research efforts. Press release of the United States Department of Energy Joint Genome Institute, 17 January 2008. http://www.jgi.doe.gov/News/news_1_17_08.html (accessed on 26.3.10)

[31] Murphy DJ. Future prospects for oil palm in the 21st century: Biological and related challenges. European Journal of Lipid Science and Technology 2007; 109: 296-306.

[32] FAO. Results from the FAO Biotechnology Forum: Background and dialogue on selected issues. By J. Ruane and A. Sonnino. FAO Research and Technology Paper No. 11. Rome. 2006. http://www.fao.org/docrep/009/a0744e/a0744e00.htm (accessed on 26.3.10)

[33] Novozymes. Enzymes produced by genetically modified microorganisms. 2008. http://www.novozymes.com/en/MainStructure/AboutUs/Positions/Enzymes+produced+by+GMMs.htm (accessed on 26.3.10)

[34] Kamis R, Joshi, M. Biofuel patents are booming. Baker & Daniels LLP report. 2008. http://www.bakerdstreamingvid.com/publications/Biofuel%20Report.pdf (accessed on 26.3.10)

[35] Bon EPS, Ferrara, MA. Bioethanol production via enzymatic hydrolysis of cellulosic biomass. Paper prepared for the FAO seminar on "The role of agricultural biotechnologies for production of bioenergy in developing countries", held in Rome, Italy, 12 October 2007. http://www.fao.org/biotech/docs/bon.pdf (accessed on 26.3.10)

[36] Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, et al. `Green revolution' genes encode mutant gibberellin response modulators. Nature 1999; 400: 256-261

[37] USDOE. Plant feedstock genomics for bioenergy. USDA and DOE award 11 grants for biomass genomics research. Announcement by the U.S. Department of Energy and the U.S. Department of Agriculture. 2007. http://genomicsgtl.energy.gov/research/DOEUSDA/awards.shtml (accessed on 26.3.10)

[38] Grattapaglia D. Marker-assisted selection in Eucalyptus. Chapter 14 in "Marker-assisted selection: Current status and future perspectives in crops, livestock, forestry and fish", Guimaraes, E., Ruane, J., Sonnino, A., Scherf, B.D. and J. Dargie (eds.). FAO. 2007. http://www.fao.org/docrep/010/a1120e/a1120e00.htm (accessed on 26.3.10)

[39] Sticklen M. Plant genetic engineering to improve biomass characteristics for biofuels. Current Opinion in Biotechnology 2006;.17: 315-319.

[40] Jeffries TW. Engineering yeasts for xylose metabolism. Current Opinion in Biotechnology 2006; 17: 320–326.

[41] Hahn-Hagerdal B, Galbe M, Gorwa-Grauslund MF, Liden G, Zacchi, G. Bio-ethanol – the fuel of tomorrow from the residues of today. Trends in Biotechnology 2006; 24: 549-556

[42] Jeffries TW, Grigoriev IV, Grimwood J, Laplaza JM, Aerts A, Salamov A, et al. Genome sequence of the lignocellulose-bioconverting and xylose-fermenting yeast Pichia stipitis. Nature Biotechnology 2007; 25: 319-326.

[43] Lynd LR, Laser MS, Bransby D, Dale BE, Davison B, Hamilton R, et al. . How biotech can transform biofuels. Nature Biotechnology 2008; 26: 169-172.

[44] Henstra AM, Sipma J, Rinzema A, Stams AJM. Microbiology of synthesis gas fermentation for biofuel production. Current Opinion in Biotechnology 2007; 18: 200-206.

[45] ChistiY. Biodiesel from microalgae beats bioethanol. Trends in Biotechnology 2008; 26: 126–131.

[46] Schenk MP, Thomas-Hall SR, Stephens E, Marx UE, Mussgnug JH, Posten C, et al. Second generation biofuels: High-efficiency microalgae for biodiesel production. BioEnergy Research 2008; 1: 20-43.

[47] IEA. Biogas production and utilisation. International Energy Agency. 2005. http://www.ieabioenergy.com/LibItem.aspx?id=182 (accessed on 26.3.10)

[48] Nepal G. Policies for promoting investment in energy sustainability: A case of biogas sector of Nepal. A paper prepared for the OECD Global Forum on International Investment, held 27-28 March 2008, Paris, France. http://www.oecd.org/dataoecd/45/31/40311609.pdf (accessed on 26.3.10)

[49] FAO. Renewable biological systems for alternative sustainable energy production. Edited by K. Miyamoto. FAO Agricultural Services Bulletin 128. 1997. http://www.fao.org/docrep/w7241e/w7241e00.htm (accessed on 26.3.10)

[50] Cirne DG, Lehtomaki A, Bjornsson L, Blackall, LL. Hydrolysis and microbial community analyses in two-stage anaerobic digestion of energy crops. Journal of Applied Microbiology 2007; 103: 516-527.

[51] JGI. Why sequence a biogas-producing microbial community? United States Department of Energy Joint Genome Institute. 2007. http://www.jgi.doe.gov/sequencing/why/99203.html (accessed on 26.3.10)

ABBREVIATIONS: CBP = Consolidated bioprocessing; EU = European Union; FTL = Fischer-Tropsch liquid; GHGs = Greenhouse gases; GM = Genetically modified; GMOs = Genetically modified organisms; IPR = Intellectual property rights; LC = lignocellulosic; OECD = Organisation for Economic Co-operation and Development; R&D= Research and development; TAGs = Triacylglycerols.

KEYWORDS: Bioenergy; Biotechnology; Developing Countries