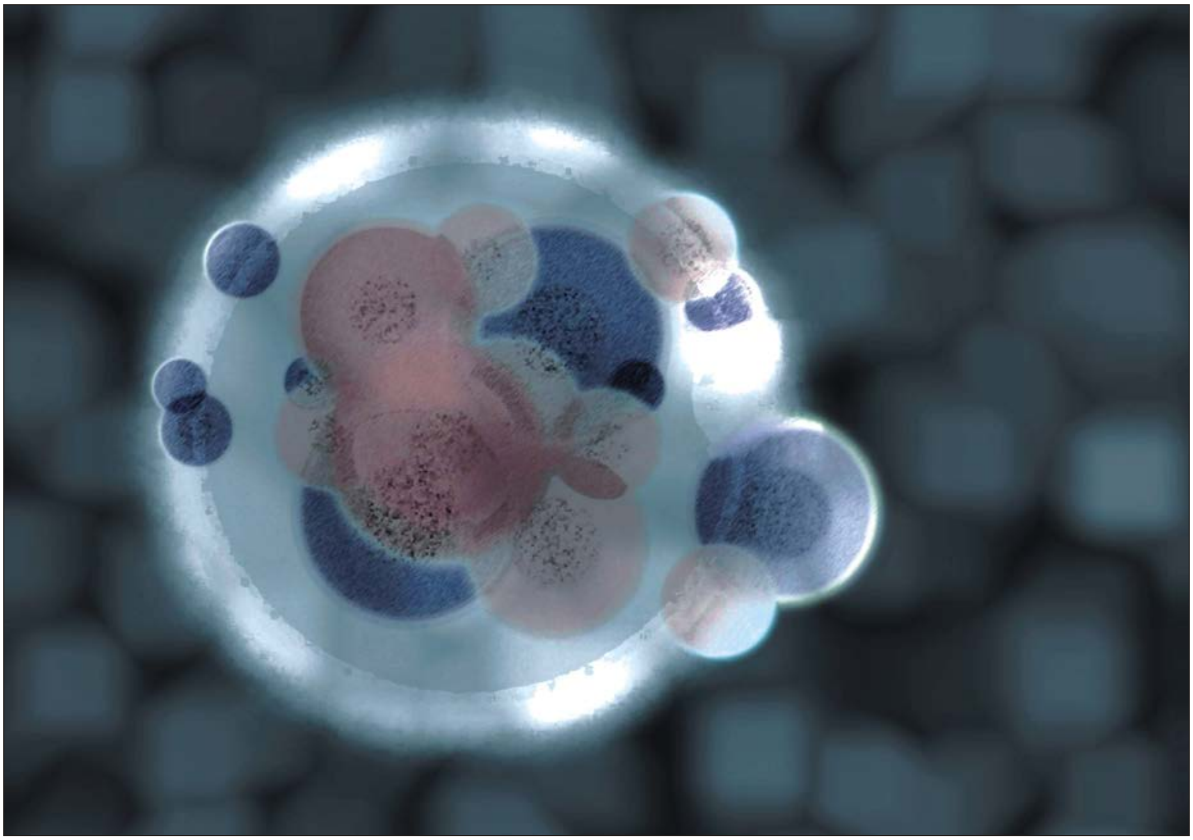

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Biofuel Production: A Promising Alternative Energy for Environmental Cleanup and Fuelling Through Renewable Resources

[1]Simeon O. Kotchoni and [2]Emma W. Gachomo
[1]Department of Agronomy, Purdue University,
Lilly Hall, 915 West State Street, West Lafayette, IN 47907, USA
[2]Department of Biology, Rutgers University, Science Building, 315 Penn Street, Camden, NJ 08102, USA

Abstract: The increasing demand of energy and concern of green house gas emission has resulted in searching for a renewable form of energy that is less pollutant and economically efficient. To achieve this goal, biofuel technology is now globally embraced as the promising technology to replace petroleum fuels with alternative fuels produced from renewable sources such as cellulosic biomass. In this review, we highlight the advantages and disadvantages of the petroleum fuels, been in use this far. In addition, we focus on the processes of alternative fuel production and their impacts, both positive and negative, at environmental, economical and ecological levels. The review also addresses how the available cellulosic biomass resources at our disposal can be used to positively affect the future generations.

Key words: Biofuels, bioconversion, ethanol, lignocellulose, biorefinery, renewable energy

INTRODUCTION

Acceptance of a new technology generally depends on the way it is presented to the public. This was well demonstrated in the context of genetically modified crops when they were commercially introduced in the market (Somerville, 2000). There was a worldwide uproar concerning the release of Genetically Modified Organisms (GMOs), which still persist up to date especially in Europe, despite series of sustainable informative programs. Learning from such observations, it is imperative to follow-up introduction of any new technology with non-biased sustainable informative programs showing the advantages and disadvantages of the technology over what has been in use so far.

Access to an adequate energy supply at reasonable cost is crucial for economic growth. Currently the world is heavily dependent on petroleum fuels for most of its energy needs. Biofuels technology aims at replacing petroleum fuels with an alternative energy source from renewable resources, thereby improving the global economic growth and environmental conditions (Sheehan *et al*., 2003; Greene *et al*., 2004). It is important to bring to the attention of the public and decision makers all aspects of this technology. It is of long-term benefit to determine the different biological materials needed for the production of biofuels and whether biofuels are viable alternatives. Without a doubt, the development of energy from biological materials seems to be a promising alternative, especially with the possibility of recycling processes. The intriguing question is why up to date this alternative energy source has not been implemented worldwide. The next question is whether biofuel production is sustainable and what advantages biofuel production has over petroleum fuels.

This review discusses the pros and cons of biofuel production from a neutral stand point by highlighting the importance of energy production from renewable resources and the environmental and ecological factors of large-scale production of cellulosic biomass in years to come.

PRODUCTION OF CELLULOSIC BIOMASS FOR BIOFUELS

Biotechnology offers the promise of dramatically increasing ethanol production using celluloses and hemicelluloses, the most abundant biological materials on earth (Himmel *et al*., 1999; Farrell *et al*., 2006). Plant residues including postharvest corn plants (stover) and timber residues are the primary resources for biofuel production (Sheehan *et al*., 2003; Council For Agricultural Science and Technology, 2007). Other specialized high-biomass energy crops such as domesticated poplar trees, switchgrass, crop residues and forestry biomass are also considered as readily available cellulosic materials for bioconversion (Council For Agricultural Science and Technology, 2007; Schmer *et al*., 2008). The biochemical

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Corresponding Author: Dr. Simeon O. Kotchoni, Department of Agronomy, Purdue University, Lilly Hall, 915 West State Street, West Lafayette, IN 47907, USA Tel: +1-765-496-6914 Fax: +1-765-496-7255

conversion of cellulosic biomass to ethanol involves three basic steps; (i) pretreatments to increase the accessibility of cellulose to enzymes and the solubilization of hemicellulose sugars, (ii) hydrolysis with special enzyme preparations to break down cellulose to sugars, (iii) the fermentation to ethanol (Lynd *et al*., 2005). To make ethanol production from cellulosic biomass conversion more economical and practical will require a science-based molecular redesigning of numerous enzymes, biochemical pathways and entire cellular systems.

To access the cellulosic material of the plants or plant residues, the outer protective material, the lignin, must first be degraded. Unfortunately, lignocellulose is an extremely complex and well-designed nanoscale composite that is resistant to enzymatic attack (Durot *et al*., 2003). Although considerable research has focused on converting biomass cellulose to fermentable glucose, less has been done on bioconversion of hemicellulose and lignocellulose into ethanol. A better understanding of the complex structures and composition of these polysaccharide groups will help identify and optimize the mechanistic models being developed for hemicellulose bioconversion (Kotchoni *et al*., 2006). Lignin degradation is the key to making polysaccharide components of cell walls available for breakdown. Lignin, a major component of plant cell walls, gives strength to wood. It is the second most abundant natural polymer on earth. This amorphous and insoluble aromatic material lacks stereoregularity and unlike hemicellulose and cellulose it is not susceptible to hydrolytic attack. In the cell wall structure, lignin protects cellulose, making it inaccessible for cellulosic enzymes. Therefore, pretreatment of the biomass with heat, specific enzymes and acids is required to first remove the lignin from the cellulose core before hydrolysis. This pretreatment is one of the most expensive processing steps of biofuel production. Therefore, improving the production of enzymes such as ligninases and hemicellulases will significantly help in lowering the cost of biomass pretreatment and in speeding the production of biofuels/ethanol through biological degrading processes.

KEY ENZYMES FOR BIOFUEL PRODUCTION

Ligninases and hemicellulases are the key enzymes in biofuel production. They are inadequately understood and few examples are known. Optimizing these enzymes will help the ultimate goal of consolidating pretreatment and saccharification. Consequently, research needs to identify, characterize, improve and economically produce the most effective enzyme systems for biomass preconditioning. These enzymes would be applied before or after traditional pretreatment to minimize and eventually, replace thermochemical processes, thus simplifying processing and reducing the effects of overall pretreatment severity at the macromolecular level. Attention must therefore be focused on identifying more enzymes in this class and characterizing their principal actions. The ultimate goal is to produce a recombinant ligninase-hemicellulase microbial system with enhanced stability and catalytic activity.

Despite its vital importance, the enzymatic basis for *in vivo* lignin depolymerization has remained elusive. Research on putative ligninases, which can both degrade and polymerize lignin preparations, has not yielded reliable insights into the mechanisms of lignin cleavage. In plants, lignins are biopolymers characterized by a (C6-C3)n skeleton (Foerster *et al*., 2000; Vogt and Jones, 2000; Lim *et al*., 2001). Lignin is the polymeric product derived from the dehydrogenative polymerization of three monolignol precursors: coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol (Lim *et al*., 2001, 2005). Lignin is ranked second after cellulose as the most abundant organic material on earth and provides strength and protection against pathogenic attacks to plants. The way forward for biofuel production from plant residues requires a good understanding of regulatory expression network of genes involved in lignin biosynthesis in order to design efficient genetic strategies for its degradation. In this contest, Rice and *Arabidopsis thaliana* are among the most promising models to successfully study lignin degradation in plants. The Arabidopsis and rice genomes are completely sequenced plant genomes (Meinke *et al*., 1998) and this allows us to examine the phylogenetic-molecular relationship and regulatory expression of all genes involved in lignin biosynthesis. Based on the whole gene expression analysis, regulatory pathway(s) and functional features of the corresponding gene products of lignin biosynthesis, we can easily propose more efficient molecular approaches to digest the lignocellulosic plant residues during biofuel production. The genus *Arabidopsis* belongs to the Brassicaceae (mustard or crucifer) family in the tribe Sisymbriae that contains several species including the most well known *Arabidopsis thaliana*. Although *A. thaliana* is not a promising cellulosic plant for biofuel production, this particular species has emerged as model plant for studies in classical and molecular genetics, developmental biology, physiology, biochemistry and functional genomics for several reasons. A summary of reasons to adopt *Arabidopsis thaliana* as a model system is well documented (Meyerowitz and Pruitt, 1985; Meinke *et al*., 1998; Meyerowitz, 2001). Many biological functions are shared between *Arabidopsis thaliana* and other plants with more complex genomes and prolonged life cycles (Meyerowitz, 2001). Therefore, biological properties found in *Arabidopsis thaliana* are likely to be found in all other flowering plants (Meyerowitz, 2001). Analyses of the structure and functions of genes in *Arabidopsis thaliana* can lay the groundwork for studying the molecular basis of lignin degradation necessary for biofuel production from plant residues. Databases, web-browser data mining interface for Affymetrix GeneChips and analysis toolbox have been made available for various genomic, metabolic and proteomic analyses in *Arabidopsis thaliana* that have not been developed for many other plant species (Zimmermann *et al*., 2004).

Potential candidates that can efficiently degrade lignin polymers were recently identified in white rot fungi (Martinez *et al*., 2004), but the genes encoding a range of ligninases need to be identified. The genomic sequence of a white rot fungus, *Phanerochoaete chrysosporium*, is up to date the most promising candidate for bioconversion of lignin. Since heterologous ligninase expression and manipulation have had limited success, a suitable host organism will still be needed to produce heterologous ligninases in sufficient quantities after the most promising lignocellulose degrading candidates have been identified. In this regard, possible hosts to consider include *Escherichia coli*, *Saccharomyces cerevisiae*, *Pichia pastoris* and *Aspergillus oryzae* to mention but few.

WHITE ROT FUNGI, THE LIGNIN- AND HEMICELLULOSE-HYDROLYZING ORGANISMS: LEARNING FROM THE MASTER

Although lignin is one of the most abundant polymers known, it is unfortunately not susceptible to hydrolytic attack (Adler, 1977). Only a small group of fungi are able to completely degrade lignin to carbon dioxide and to thereby gain access to the carbohydrate polymers of plant cell walls, which they use as carbon and energy sources (Blanchette, 1991; Martinez *et al*., 2004). Selective degradation of lignin by these fungi leaves behind crystalline cellulose with a bleached appearance that is often referred to as white rot (Eriksson *et al*., 1990; Blanchette, 1991) hence the name white rot fungi. These filamentous wood decay fungi are common inhabitants of forest litter and fallen trees and have potential in a wide range of biotechnological applications including hazardous waste remediation and the industrial processing of paper and textiles (Blanchette, 1991). White rot fungi are the primary degraders of lignin (Martinez *et al*., 2004). These organisms also degrade the cellulose and hemicellulose components of plant cell walls. The most intensively studied white rot basidiomycete, *P. chrysosporium* (Burdsall and Eslyn, 1974), is phylogenetically distant from other sequenced fungi, all of which are members of the Ascomycotina, e.g., *Saccharomyces cerevisiae* (Goffeau *et al*., 2002), *Schizosaccharomyces pombe* (Wood *et al*., 2002) and *Neurospora crassa* (Schulte *et al*., 2002). White rot fungi catalyze the initial depolymerization of lignin by secreting an array of oxidases and peroxidases generating highly reactive and nonspecific free radicals, which in turn undergo a complex series of spontaneous cleavage reactions (Kirk and Farrell, 1987; Martinez *et al*., 2004). Major components of the *P. chrysosporium* lignin depolymerization system include multiple isozymes of lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP). LiP and MnP require extracellular H_2O_2 for their *in vivo* catalytic activity and one likely source is the copper radical oxidase, glyoxal oxidase (GLOX) (Martinez *et al*., 2004). To understand how white rot fungi degrade lignin cellulose and hemicellulose components, the genome of a white rot fungus, *Phanerochoaete chrysosporium*, was sequenced (Martinez *et al*., 2004). This is a great contribution toward understanding the biologically degrading process of lignin.

This fungus degrades brown lignin, the protective matrix surrounding cellulose microfibrils of plant cell walls, leaving behind crystalline white cellulose (Martinez *et al*., 2004). Elucidating the regulation of genes, proteins and metabolites from this organism and other lignocellulosic organisms will enhance our understanding of how the individual and collective mechanisms of hydrolyzing enzymes work to promote lignin and hemicellulose degradation as well as their interactions with other organisms in ecosystems. Such advances are necessary for generating the framework to engineer large-scale processes for biomass utilization.

IMPROVING ACCESSIBILITY TO CELLULOSIC BIOMASS THROUGH GENETIC AND MUTAGENIC APPROACHES: CURRENT PROGRESS AND PERSPECTIVES

As mentioned earlier, understanding the physical, chemical and molecular properties of cell wall, especially how lignin is synthesized, will be a stepping stone toward generating transgenic plants with modified lignin content. Such transgenic plants will provide a unique advantage of bypassing the need for acid pretreatment and facilitating enzymatic digestion destined for fermentation to ethanol/biofuel. Several genetic resources are available to modify and thereby improve accessibility of crop based lignocellulosic biomass. These include; the use of existing mutants, forward and reverse genetics to obtain novel mutants and transgenic approaches in which the expression of genes of interest is modified. The gene of interest can be up regulated via introduction of a transgene under control of a strong promoter. Alternatively, the gene can be down regulated or knocked out through the use of co-suppression mediated by the introduction of an antisense construct, or an RNA interference construct. In the latter case, two short fragments of the target gene are cloned in opposite orientation from each other separated by a spacer sequence. Transcription of such a transgene results in the formation of a dsRNA molecule that is targeted for destruction. As a consequence of this, the native mRNA from the target gene is also degraded reviewed by Baulcombe (2004).

Progress has been made in mining genetic resources to improve lignocellulosic biomass accessibility to enzymatic degradation. Draude ***et al.*** (2001) demonstrated that a removal of 67% of the lignin from soft wood pulp resulted in a nearly threefold increase in the yield of reducing sugars, an 88% increase in the ultimate yield of glucose and a twofold increase in the initial hydrolysis rate (over the first hour of hydrolysis). Similar results were reproduced in soft wood pulps (Charles *et al*., 2003) as well as in corn stover (Yang and Wyman, 2004). Recently, antisense-mediated gene down regulation approach has been used to demonstrate that reducing the level of lignin content in plant can increase ethanol yields and decreasing thereby processing inputs (Chapple *et al*., 2007; Chen and Dixon, 2007). In stems of transgenic alfalfa lines, independently down regulated six different lignin biosynthetic enzymes, recalcitrance to both acid pretreatment and enzymatic degradation is directly proportional to lignin content. These transgenic lines yielded nearly twice as much sugar from cell walls as wild-type plants (Chen and Dixon, 2007). Their work proves that Lignin modification could bypass the need for acid pretreatment and thereby facilitate bioprocess consolidation. In addition, plant breeding can be implemented to improve biomass yield, biomass quality and biomass conversion efficiency, either through selection among progeny obtained by crossing parents with desirable traits, or as a way to enhance the agronomic performance of promising mutants and transgenic plants (Vermerris *et al*., 2007). Considerable effort and progress has been made in generating promising mutant lines that can be explored to improve biofuel production. The most promising classes of mutants are the *brown midrib* maize and sorghum mutants in which cell wall composition is altered (Vermerris *et al*., 2007). These mutants are easily recognized by the reddish-brown coloration of the vascular tissue in the leaf blade and sheath. The genetic mutations are designated as *bm* in maize (Chabbert *et al*., 1994a, b; Marita *et al*., 2003; Barrière *et al*., 2004) and *bmr* in sorghum (Bout and Vermerris, 2003).

In summary, the application of genetic and genomic strategies is very likely to pay great dividends in biofuel production technology. If for example the approach used by Chen and Dixon (2007) can be translated from the lab to the field and pretreatment can be eliminated, the savings on acid alone would be about 5.5 pounds of acid per 20 cents per gallon of ethanol, with elimination of other costs associated with pretreatment doubling or tripling these savings (Chapple *et al*., 2007). This proves what can be done by genetic manipulation to improve the quality of crops for biofuel production.

BIOFUEL: A RENEWABLE ENERGY TO EMPOWER THE WORLD

The development of biorefinery is here shown as a very promising technology to produce renewable energy from plants and plant residues. The main objective of the technology is to convert waste into energy. A biorefinery could be loaded with various kinds of wastes, which can be converted into biofuel/ethanol via parallel refinery processes (Lynd *et al*., 2002). This project has been implemented by Purdue University, USA (Purdue Agricultures, 2007). The trends toward adopting the use of alternative fuels by using readily available crop residues to supply electricity, steam and chilled water are of great value to the environment. Surely, the technology will promote less waste production into the environment. Universities are generally known for their high-energy demands and such projects can easily be set up in universities as a backup energy system in case of electric failure. In this case, switchgrass is being used, but any other plant residue can be used to generate biofuels. Purdue researchers are already heading toward the next step, which is developing a new delivery system to improve the energy productivity of the bioenergy.

Late in 2007 the USA signed an energy bill that will have a great and long-term impact on US. agriculture (Purdue Agriculture, 2007; www.epa.gov/otaq/renewablefuels/). By increasing the Renewable Fuels Standard (RFS) to 36 billion gallons by the year 2022, the bill provides a road map for the production of renewable fuels (biofuels) from farms and forests (www.epa.gov/otaq/renewablefuels/). This new energy bill will have huge impacts on agriculture and forestry in the very near future. Of the 36 billion gallons of RFS to be produced, (i) cornstarch ethanol will contribute 15 billion gallons per year; (ii) about 13.5 billion gallons per year will be in place by the end of 2008; (iii) cellulosic ethanol will be the dominant portion of the industry to grow after 2010. Total cellulosic ethanol is expected to grow from zero to 21 billion gallons by 2022; (iv) the existing cornstarch based ethanol plants will focus on adding cellulosic ethanol production to their capacity at existing sites. The cellulosic portion of the plant will use cornstalks or other crop residues. More than likely corn producers will not meet the demands of the rapidly expanding corn ethanol plants in the near future. Under such conditions, grazing lands will be targeted to shift to fuel crops such as prairie grasses and may develop into fuel-crop production areas (Purdue Agriculture, 2007; www.epa.gov/otaq/renewablefuels/). Other lignocellulosic feedstocks that have been proposed include switchgrass, woody plants and mixtures of prairie grasses and forbs (Schmer *et al*., 2008).

Conservation Reserve Program (CRP) acres may largely shift toward cellulosic energy crops in various areas. Forests and woodlands may be shifted to energy crops as well. A large search for new energy crops will be the next step in optimizing biofuel production. Some of these may be nontraditional crops such as sweet sorghums, tropical maize, or sugar cane. A large amount of research, development and experimentation will be conducted to discover the most economic ways to produce, store and transport these new cellulosic energy crops. Most of the crops will be land and natural resource based which means agriculture will be called to meet these enormous challenges and to also reap the potential rewards. In summary various reserved forest areas might very soon be lost to cellulosic energy crop production.

Another subsequent effect of this new technology is the hidden problem of deforestation linked to biofuel production due to higher demand for land to grow biofuel-producing crops (Fargione *et al*., 2008). In addition, increased diversion of cropland to biofuels has resulted in greatly increased prices of food crops because of high demand and low supply (Fargione *et al*., 2008). The high demand for biofuels has lead to the replacement of natural wilderness with plants for biofuels to the extent that they are now considered among the world's top carbon emitters as well as source of deforestation (Fargione *et al*., 2008). However, with caution and adequate ecosystem management programs, this disadvantage can be appropriately tackled to avoid a negative impact on the ecosystem.

THE ENERGY SYSTEM USED SO FAR: ADVANTAGES AND DISADVANTAGES

The vast majority of energy sources used so far is powered by petroleum products, i.e., gasoline or diesel fuel. However, looking into the future, there are increasing concerns about the hidden costs of petroleum dependency. There is therefore an increasing need to find alternative energy sources to reduce our petroleum (gasoline or diesel fuel) dependency. So far petroleum fuel deserves the merit of empowering the world in many if not all aspects of life. But it is associated with several disadvantages that lead to the search for alternative energy sources.

Beside the increasing price of petroleum products, the complete combustion of petroleum products (hydrocarbon fuels) leads to the production of carbon dioxide (CO_2), which contributes to the global warming (Farrell *et al*., 2006). In case of an incomplete combustion of the hydrocarbon fuels, there is formation of carbon monoxide (CO), a highly poisonous gas, released in the atmosphere. Even in case of complete combustion of hydrocarbon fuels into water and carbon dioxide, there is now growing concern about carbon dioxide as a greenhouse gas. In addition, some of the unburned carbon atoms can react with nitrogen oxides (another pollutant from combustion) in the presence of sunlight to form ozone, which is a lung irritant (although ozone layer in the stratosphere is a shield against the sun's ultraviolet light, but at ground level ozone is the main component of photochemical smog) (Farrell *et al*., 2006). These unburned carbon atoms can also remain stuck to one another with few or without hydrogen atoms, especially during incomplete combustion of diesel fuel, producing soot. In contrast, alternative fuels produced from biological materials are less polluting than gasoline and diesel. Biofuels, are simpler molecules, therefore easier to burn more completely and producing less carbon monoxide, soot and unburned hydrocarbons. For example, an alternative fuel such as methane is almost incapable of forming smog.

Some alternative fuels are not hydrocarbons; in this group belong the alcohols and biodiesel, which contain oxygen atoms as well as carbon and hydrogen. The subgroup of alcohol fuels includes methanol (CH_3OH), ethanol (C_2H_5OH) and the biodiesel (monoalkyl esters). The presence of oxygen in these alternative fuels promotes more complete combustion, so that less carbon monoxide, soot and unburned hydrocarbons are produced. The alternative fuels inherently produce less carbon dioxide than gasoline or diesel fuel when burned completely as shown by the following example: 100 oxygen atoms are needed for four molecules of isooctane (gasoline) to produce 32 carbon dioxide molecules and 36 water molecules, while the same number of oxygen atoms will combine with 25 methane(natural gas) molecules to produce 25 carbon dioxide molecules and 50 water molecules. That is, a given amount of air oxygen will produce about 25% less carbon dioxide if used to burn natural gas (CH_4, methane) than if used to burn gasoline.

AVOIDING CARBON DIOXIDE EMISSIONS IN THE ENVIRONMENT

If the carbon atoms of the fuel came from the carbon dioxide in the air (atmosphere), for instance an alcohol fuel produced by fermentation of biomass, then returning it to the air later would add nothing to the net flow of carbon dioxide into the atmosphere. Alcohol fuels or biodiesel produced from plants, when burned, just return to the air the carbon dioxide that the plants had taken out from the air while growing. The alternative fuels such as liquefied petroleum gas (LPG, commonly known as propane), Compressed Natural Gas (CNG), Liquefied Natural Gas (LNG), Methanol (M85), Ethanol (E85) and biodiesel (B20) are potential bioenergic entities that have to various degrees some advantages over the petroleum fuels because they emit less pollutants than petroleum fuels. They are however associated to various disadvantages, or else the world system would not still be almost 100% dependent on petroleum products. The disadvantages of alternative fuels will be discussed in detail in sections below. Because of the restricted number of pages, only a few alternative fuels such as Methanol (M85), Ethanol (E85) and biodiesel (B20) will be emphasized in this review.

METHANOL (M85)

Methanol is often produced from natural gas. It can be produced by fermentation of biomass and that is why it is also called wood alcohol. Methanol is often blended at 85% methanol with 15% unleaded gasoline, hence the name M85. Methanol is not carcinogenic. It is less toxic, but more corrosive than gasoline (NESCAUM, 2001). Therefore special chemical additives are necessary before it can be used. A given volume of M85 will produce approximately 70% less energy compared to the same volume of gasoline. Methanol is most commonly mixed with 15% gasoline to correct two disadvantages of pure methanol: (i) a pure methanol flame is colorless, therefore invisible. Gasoline (15% V/V) is added to give the flame some color in order to allow rescuers to know whether a fire is present when a M85-engine is involved in an accident; (ii) pure methanol has a single boiling point. This often causes cold-start problems in cold weather, or vapor lock in hot weather. Gasoline, being a mixture of compounds with different boiling points, overcomes this problem.

ETHANOL (E85)

Ethanol is produced by fermentation of biomass, generally corn, though other, lower-value feedstocks like brewery waste and cheese-factory effluent can be used in an effort to reduce costs. Ethanol is a renewable resource that contributes nothing to greenhouse-gas loading of the atmosphere and therefore causes no concerns of global warming. It is usually blended in a mixture of 85% ethanol, 15% unleaded gasoline, hence the name E85 (NESCAUM, 2001). Like methanol, it can be blended with any amount of gasoline. The main disadvantage of E85 is the high price, even with the available subsidies. However, research is under way to ferment lower-grade feedstocks such as corn cob to make alcohol more cost efficient. In addition, Ethanol is also corrosive, though less than methanol and there are concerns about flame visibility in case of accidental burning of ethanol.

BIODIESEL (B20)

Biodiesel is a substitute of petroleum diesel made from biomass. It is inherently renewable and therefore contributes nothing to carbon-dioxide loading of the atmosphere. Biodiesel is commonly produced from soybean or canola oil as material source, but animal fat or recycled cooking oil can also be used. To reduce its cost over petroleum diesel, biodiesel is a blend of 20% biodiesel and 80% petroleum diesel fuel, hence the name B20. Biodiesel can cut greenhouse-gas emissions as well as ordinary pollutants (particularly soot) by displacing petroleum diesel. The main disadvantage of B20, like that of E85, is its high cost.

BIOFUEL PRODUCTION: THE HIDDEN THREAT ON DEFORESTATION AND GREEN HOUSE GAS EMISSION

Although biofuels are been viewed as the promising alternative fuels to solve problems associated with increasing energy demand, climate change and environmental pollution, their production is still accompanied with far extended list of hidden threats that need to be dealt with to avoid what otherwise might be more devastating and far out weigh the advantages of biofuels over the fossil fuels. Studies that have proposed that substituting biofuels for gasoline will reduce greenhouse gases because biofuels sequester carbon through the growth of the feedstock, have however failed to count the carbon emissions that occur as farmers worldwide respond to higher prices and convert forest and grassland to new cropland to replace the grain (or cropland) diverted to biofuels (Searchinger *et al.*, 2008).

Converting native habitats to cropland releases CO_2 as a result of burning or microbial decomposition of organic carbon stored in plant biomass and soils (Fargione *et al.*, 2008).

Converting the world's lowland tropical rainforests into cellulosic crop lands will not only promote desertification, but also increase directly or indirectly the green house gas emission. According to recent study conducted by Fargione *et al.* (2008), converting rainforests, peatlands, savannas, or grasslands to produce food crop-based biofuels in Brazil, Southeast Asia and the United States creates a "biofuel carbon debt" by releasing 17 to 420 times more CO_2 than the annual greenhouse gas (GHG) reductions that these biofuels would provide by displacing fossil fuels. Their analyses suggested that biofuels, if produced on converted land, could, for long periods of time, be much greater net emitters of Green-House Gases (GHG) than the fossil fuels that they typically displace (Righelato and Spracklen, 2007; Fargione *et al.*, 2008). Greater biofuel production might decrease overall energy prices, which could increase energy consumption and GHG release (Perlack *et al.*, 2005; Tilman *et al.*, 2006). In the same study, Fargione *et al.* (2008) shows that biofuels made from waste biomass or from biomass grown on degraded and abandoned agricultural lands planted with perennials incur little or no carbon debt and can offer immediate and sustained GHG advantages. Using a worldwide agricultural model to estimate emissions from land-use change, Searchinger *et al.* (2008) have found that corn-based ethanol, instead of producing a 20% savings, nearly doubles greenhouse emissions over 30 years and increases greenhouse gases for 167 years. Their work showed that biofuels from switchgrass, if grown on U.S. corn lands, increase emissions by 50%. These studies raise concern about large biofuel mandates and highlight the value of using waste products for biofuel production.

CONCLUSION AND FUTURE PERSPECTIVES

In an effort to decrease greenhouse gas emissions, expand domestic energy production and maintain economic growth, scientists aim at producing biofuel from feedstock using current biotechnology (Chappel *et al.*, 2008; Chen and Dixon, 2007; Searchinger *et al.*, 2008). For reasons mention above, decreasing GHG emission by switching from fossil fuels to biofuel production will greatly depend on direct and indirect effect of land clearing for biofuel production (Fargione *et al.,* 2008; Searchinger *et al.*, 2008). Unlike food crops grown for grain-based ethanol (e.g., corn), which require high inputs of fertilizers and pesticides, proposed lignocellulose-based energy crops (e.g., switchgrass) typically require relatively few economic or environmental inputs and can be cultivated on marginal, lower-productivity land (Hill *et al.*, 2006). Thus, in a rapidly growing industry related to cellulosic plant species selection, cultivar improvement and conversion technologies it is imperative to promote cost effectiveness. A variety of plant species, including grasses, herbs and trees, are being considered for use as biofuel crops (McLaughlin *et al.*, 2002). The leading candidates for lignocellulose-based energy, however, are primarily rhizomatous (i.e. having below ground vegetative reproductive structures) perennial grasses. Generally, most of these grasses are not native to many of the regions where production is proposed (Lewandowski *et al.*, 2003). From an agronomic point of view, their life history characteristics, rapid growth rates and tonnage of biomass produced by these non-native grasses make them ideal feedstock crops, but they may easily become invasive species and pose a trait to the ecosystem. Though the technology is promising, care must be taken through adequate planning and management to avoid transforming farmlands into cellulosic-biomass areas for biofuels.

The hurried trends with which biofuel crops currently are being sought to replace petroleum fuels will likely end in a new dimension of biologically based industrial energy. To sustain the industrial need, non-native perennial species will soon be globally selected mainly for their rapid growth rates, annual production and low economic and environmental inputs. This may pose a serious threat to the ecosystem. To preserve the ecosystem, biofuel feedstocks should be propagated easily in highly managed agricultural systems and should not be able to survive outside of such cultivation (Paine *et al.*, 1996). This practice is also applied for nearly all major crops currently grown world wide, including rice, wheat, corn, soybean, cotton, tomato and alfalfa.

Therefore, similar expectations should apply to biofuel crops, without which, the benefits of dedicated biofuel feedstock production may be counterbalanced by the ecological damage caused by their invasion into sensitive natural ecosystems and agricultural production systems (Paine *et al.*, 1996). Although introducing some plant species, as biofuel sources may be safe, the environmental and ecological risks associated with each species must be evaluated along with the agronomic and economic benefits.

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