

Review

Lignocellulosics to ethanol: The future of the chemical and energy industry

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Accepted 17 September, 2010

Energy and environmental issues are among the major concerns facing the global community today. Biofuel technology is now globally embraced as the promising technology to replace fossil fuels. Lignocellulosic waste biomass from forestry, agriculture and municipal sources are abundant, inexpensive and potential feedstock for bioenergy production. To initiate the cellulosic bioenergy production, saccharification of cellulosic biomass is essential; however; recalcitrant nature of the waste materials, crystallinity of cellulose fiber, lignin and hemicellulose content presents a major obstacle in the conversion processes. Several pretreatment methodologies were discussed in details by which the crystalline structure of lignocellulosic biomass becomes more susceptible for cellulase enzymes. This review also addresses the different strategies for the enzymatic hydrolysis and fermentation. This article reviews the developments in the technology for ethanol production from lignocellulosic materials/biomass. Furthermore, the detailed biochemical basis of lignocellulosic biomass to ethanol is also reviewed.

Key words: Ethanol, lignocellulosic biomass, cellulase, pretreatment technologies, biofuels, fermentation.

INTRODUCTION

At the start of the 21st century, significant energy and environment challenges were faced. Our economy and lifestyle mostly rely on the use of fossil fuels because major energy source (about 80%) comes from fossil fuels (Demirbas, 2007). Fossil fuels are the main global energy resources for the industrialization and economic growth of countries during the past century. Depending on the production and consumption rates, the presently known reserves of fossil fuels will not appreciably run out for at least 100 years or more, but the demand for oil is expected to exceed production from known and anticipated oil reserves ten or twenty years from now (Goldemberg, 2007). In addition, the unfettered use of fossil fuels shows negative impacts on the environment because of emission

of greenhouse gases (CO₂, CH₄ and CO) resulting in global warming and pollution (Saratale et al., 2008). Hence, the overwhelming scientific evidence was that the unfettered use of fossil fuels has caused the world's climate to change, with potential disastrous effect. Thus an energy paradigm is based on the fossil fuel dependency, leading to economic and environmental challenges (Lo et al., 2009). For these reasons, in this century, large efforts are being conducted worldwide in order to develop technologies that generate clean, sustainable energy sources which could substitute fossil fuels (Ragauskas et al., 2006; Levin et al., 2006). Biofuels are the only alternate energy source for the foreseeable future and can still form the basis of sustainable development in terms of socioeconomic and environmental concerns (Demirbas, 2007). As biofuels can be produced from common biomass, represent CO₂ cycle, ecofriendly, cost competitive with fossil fuels and biodegradable, it contributes to sustainability that becomes important and promising alternative energy source for fossil fuels to protect the biosphere and prevent more localized forms of pollution (Puppan, 2002). Although the worldwide

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Abbreviations: GHG, Greenhouse gases; DP, degree of polymerization; AFEX, ammonia fiber expansion; PEG, polyethylene glycol; BSA, bovine serum albumin.

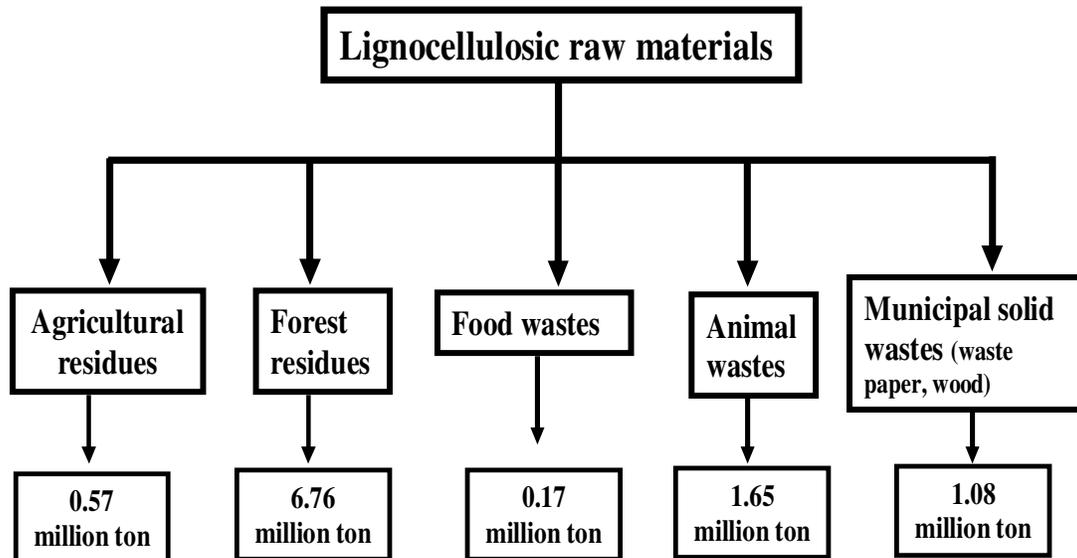


Figure 1. Annual production of total lignocellulosic biomass in South Korea (NAEK, 2008).

annual production of biofuels increased from 4.4 - 50.1 billion liters, the political and public support for biofuels has been countermined. Some recent reports argued that use of food crops or croplands for biofuels production resulted in food shortages and the increased prices of staple food crops such as maize and rice (James et al., 2008; Keeney and Hertel, 2008). Nevertheless, biofuels from renewable carbon sources (such as lignocellulosic biomass) are particularly attractive based on bioresource sustainability, ecofriendly, inexpensive and these resources do not compete directly with food production, or with land that may be needed for food production (Ragauskas et al., 2006; Schubert, 2006; Slade et al., 2009).

Lignocellulosic biomass in the form of wood and agricultural residues is virtually inexhaustible, since their production is based on the photosynthetic process which is about 60% of the total biomass produced (Kuhad et al., 1997). It was estimated that terrestrial plants produce about 1.3×10^{10} metric tons per annum which is energetically equivalent to about two-thirds of the world's energy requirement (Kim and Yun, 2006). Moreover, agricultural residuals or byproducts are annually renewable, abundantly available and account for more than 180 million tons per year (Kapdan and Kargi, 2006). The most abundant lignocellulose agricultural residues are corncobs, corn stover, wheat, rice, barley straw, sorghum stalks, coconut husks, sugarcane bagasse, switchgrass, pineapple and banana leaves can be produce every year (Demain et al., 2005). Apart from the aforementioned lignocellulosic waste, cereal crops, pulse crops and harvestable palm oil biomass are being produced in large amount worldwide annually (Rajaram and Verma, 1990). Wood and paper industries also produces huge amount of lignocellulosic biomass. In addition, lignocellulosic

wastes are also derived from commercial and industrial activities including municipal solid wastes (paper, cloth, garden debris) and commercial and industrial wastes (paper, packing materials, textiles, bagasse, demolition wood) which also create the waste disposal problem. Recycled paper waste was found to be an efficient resource for biofuels production (Duff and Murray, 1996). In South Korea, the annual production of total lignocellulosic biomass is about 10.231 million tons per year (Figure 1). In addition, the utilization of aquatic biomasses including marine macroalgae has been evaluated as the most feasible under Korean environmental conditions and acts as promising feedstock for cellulosic ethanol. Moreover, several Korean companies are now actively promoting the use of wastes from palm or cassava plantations in Southeast Asia. Thus, a huge amount of plantation residues including palm wastes and cassava residues among others might be available for bioethanol production (NAEK, 2008). There is enormous worldwide interest in the development of new and cost-efficient processes for converting plant-derived biomass to bioenergy in view of fast depletion of oil reserves and food shortages (Gong et al., 1999). Thus, biomass utilization for energy production (mainly ethanol), could solve waste disposal problems and also help to displace growing dependence on fossil fuels by providing a convenient and renewable source of energy (Kumakura, 1997; DOEUS, 2006; Schubert, 2006).

Ethanol is regarded as a sustainable and cost-effective source of energy. Ethanol has attracted special attention due to its potential use as an automotive fuel and its environmental, energy and socioeconomic advantages relative to fossil fuel consumption and greenhouse gases (GHG) emissions reduction (Tampier et al., 2009; Kumar et al., 2009). For instance ethanol, being an excellent

transportation fuel, can be blended with gasoline, 10 and 22% blends are being used in the US and Brazil, respectively (Wyman, 1994). It is an oxygenated fuel that contains 35% oxygen, which reduces particulate and NO_x emission from combustion. It may be used directly (95% ethanol and 5% water) as fuel, such nearly pure ethanol fuel provides a number of environmental benefits, due to their low pressure and reduced emission of ethanol in to the atmosphere along with their clean burning characteristics (Lynd et al., 1991). Ethanol-blended gasoline oxygenates also reduces the formation of carbon monoxide and ozone, which is desirable for the implementation of Clean Air Act Amendments (Prasad et al., 2007; Wyman, 1994; González-García et al., 2010). Thus with the increasing shortage of petroleum, urban air pollution and accumulation of carbon dioxide in the atmosphere, ethanol is expected to play a more significant role in the future.

Lignocellulosic biomass composed of cellulose (insoluble fibres of β -1,4-glucan), hemicellulose (non-cellulosic polysaccharides, including xylans, mannans and glucans) and lignin (a complex polyphenolic structure). Generally, lignocellulosic biomass contains 35 - 50% cellulose, 25 - 30% hemicelluloses and 20 - 25% lignin (Mabee et al., 2006). Thus lignocellulosic biomass is being considered as the largest renewable energy resource all over the world and being promising and economically feasible carbohydrate source for the production of ethanol (Kim and Yun, 2006). However, cellulosic materials are usually not readily fermentable by microorganisms because the factors affecting the hydrolysis of cellulose include porosity (accessible surface area) of the waste materials, crystallinity of cellulose fiber and low fiber porosity (Zhang et al., 2006; Saratale et al., 2010). In addition, the molecular organization of the different components of the plant fiber cell wall, that is, cellulose, hemicellulose, and lignin which are jointly termed as the lignocellulose complex, also limits the accessibility of microorganisms and their enzymes to wood and its fiber components. For this reason, pretreatment is required to get rid of lignin and hemicellulose, to reduce the crystallinity of cellulose and increase the surface area of materials which can improve the formation of fermentable sugars for the production of bioenergy products (Zhang et al., 2009; Kumar et al., 2008). The article first describes the chemical components of lignocellulosic biomass and the methods for pretreatment and hydrolysis of lignocellulosic feedstock. It also discusses the existing technologies leading to fermentative ethanol production by using lignocellulosic feedstock and their biochemical basis.

MAJOR COMPONENTS OF LIGNOCELLULOSICS

On this planet, lignocellulosic biomass are the most abundant, renewable and consisting of complex polymer

of sugars. However, it's extremely complex and well designed nanoscale composite makes it resistant to microbial and enzymatic attack. A detailed understanding of these chemical constituents of the cell wall components will be helpful to develop and optimize the mechanistic model for its conversion (Kotchoni et al., 2003). Cellulose (molecular formula $(C_6H_{10}O_5)_n$) is a linear condensation polymer of glucose joined together by β (1-4) glycosidic bonds with a degree of polymerization (DP) from 100 - 20,000 which is water insoluble and recalcitrant to hydrolysis into its individual glucose subunit because of tightly packed, highly crystalline structure with straight, stable supra-molecular fibers of great tensile strength and low accessibility in its polymer form (Demain et al., 2005). About 33% of all plant matter is composed of cellulose (Crawford, 1981). The multiple hydroxyl groups on the glucose residues from one chain of hydrogen bonds with the oxygen molecules on the same or on a neighbor chain holds the chains firmly together side-by-side and forming microfibrils that makes recalcitrant a compact structure. The microfibrils are group of (about 30) individual cellulose chains, and approximately 100 microfibrils are packed to form fibrils and these fibrils are further packed to form the cellulose fiber (Brown and Saxena, 2000). It was observed that the degree of crystallinity of cellulose depends on its origin for example cotton cellulose is about 70%, while other commercial celluloses are in between 30 - 70% degree of crystallinity. Moreover, amorphous cellulose is degraded at a much faster rate whereas crystalline cellulose is highly resistant to microbial attack and enzymatic hydrolysis (Zhang et al., 2006; Kumar et al., 2008).

Hemicelluloses are the most abundant renewable biomass consisting of short chains of branched heteropolysaccharides containing both hexoses and pentoses (Saha, 2000). The contribution of hemicellulose is approximately 25-35% of lignocellulosic biomass. Hemicellulose contains many different sugar monomers such as pentoses (D-xylose, L-arabinose), hexoses (D-mannose, D-glucose, D-galactose) and sugar acids, whereas, cellulose contains anhydrous glucose. The major hemicellulose components in softwood are glucomannans and galactomannans while in hardwood mainly the xylan is present. It was estimated that hemicelluloses account averagely for about 22% of softwood, 26% of hardwood and 30% of various agricultural residues (Zhang et al., 2007). The chemical composition and hemicellulose content usually depends on the plant materials, growth stage and growth conditions (Niehaus et al., 1999). Hemicellulose consists of shorter chains about 500 - 3000 sugar units, whereas, about 7,000 - 15,000 glucose molecules per polymer were seen in cellulose. Moreover, hemicellulose is a branched polymer, while cellulose is unbranched polymer (Kumar et al., 2008). In addition, xylan is a major ingredient of hemicelluloses which comprises of about 15 - 30% of annual plants, 20 - 25% of hardwoods and 7 - 12% of softwoods. Generally, xylan

consists of 4-O-methyl-D-glucuronic acid, L-arabinose, or acetyl groups as substituents on the D-xylose backbone. In grasses and cereals, the xylan contains L-arabinose linked as a single unit substituent to a D-xylose backbone (Romanowska et al., 2006). Xylan appears to be a major interface between lignin and other carbohydrate components since they are probably covalently linked to phenolic residues of lignin via the arabinosyl and/or glucuronosyl residues. Xylan plays a major role in cell wall cohesion and makes them resistant. It was also observed that xylan and mannan protects cellulose from enzymatic attack (Rabinovich et al., 2002).

Lignin fills the spaces in the cell wall between cellulose, hemicellulose, and pectin components. Afterwards, it covalently linked to hemicellulose and thereby crosslinks different plant polysaccharides, gives mechanical strength to the cell wall and extend the whole plant. The highest concentration of lignin is found in the middle lamella, but is most abundant in the secondary walls of some vascular plants (Kapdan and Kargi, 2006). Lignin is a collection of various phenylpropanoid components having similar chemical properties with molecular weight higher than 100 KD. Lignin contains mainly three cinnamyl alcohol precursors such as p-coumaryl, coniferyl and sinapyl alcohol. Its composition differs with the plant species, plant tissues and its location within the plant cell wall. Lignins are highly branched polymeric molecules containing phenylpropane-based monomeric units linked together by different types of bonds, including alkyl-aryl, alkyl-alkyl, and aryl-aryl ether bonds (Kumar et al., 2008). Due to its insolubility in water and optically inactive nature, it is quite difficult for microorganisms to penetrate and start degrading it. They are generally acid stable but can be solubilized under alkaline conditions. In addition, lignin acts as a bonding agent between cells making a wood composite material which is highly resistant to microbial attack. Due to its water permeation-reducing property, lignin plays an important role in the internal transport of water, nutrients, and metabolites in plant. The ecological and environmental factors such as location, climate, sunlight, age of the wood and plant sustenance influences the chemical structure of lignins (Crawford, 1981). In addition, several studies demonstrated that chemical substituents of the backbone of the hemicelluloses, such as arabinose, galactose and 4-O-methylglucuronic acid are covalently linked with lignin (Fromm et al., 2003). Due to these sequences, lignin makes the plant wall more resistant to microbial attack and degradation. In addition to cellulose, hemicellulose and lignin, some structural proteins called extensins and structural polysaccharide pectin, is abundantly found in sugar beet and in some fruits cell wall (Brummell, 2006; Lagaert et al., 2009).

PHYSICAL AND CHEMICAL PRETREATMENT METHODS OF LIGNOCELLULOSIC MATERIALS

The purpose of the pretreatment is to remove lignin and

hemicellulose, reduce cellulose crystallinity and increase the porosity of the materials (McMillan, 1994). The features of an effective pre-treatment strategy include: Breaking the lignocellulosic complex; decreasing the cellulose crystallinity; preserving the hemicellulose sugars; limiting the formation of degradation products that are inhibitory to hydrolysis and fermentation; minimizing energy inputs and use of extraneous chemicals; requiring a simple set up; generating high value lignin coproduct; minimizing the production of toxic and hazardous wastes; generating minimum amount of waste water and cost effective treatment (Kumar et al., 2008; Saratale et al., 2008; Zhang et al., 2009). Literature reports a number of pre-treatment options that have been tried for various biomass types. Table 1 lists some of the most promising pre-treatment strategies that can be considered when commercializing the lignocellulose to bioethanol process. Up to now, several methods have been used to treat cellulosic feedstock (polysaccharides to corresponding monomers) and each generates a different pretreatment product stream (Table 1) (Chandrakant and Bisaria, 1998). Physical pretreatment (mechanical comminution and pyrolysis) was found to be effective in breaking down the cellulose crystallinity but requires more cost for power and gives all the three major compounds in one product stream (Cadoche and López, 1989). Chemical methods such as (ozonolysis; acid hydrolysis; alkaline hydrolysis; oxidative delignification; solvent extraction) are also effective pretreatment procedure, but requires more energy and chemicals than biological processes and may cause secondary pollution problems (Sivers and Zacchi, 1995). Dilute acid hydrolysis has been successfully developed for the pretreatment of lignocellulosic materials. Dilute sulphuric acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis (Esteghalalian et al., 1997). At moderate temperature, direct saccharification suffered from low yields because of sugar decomposition, whereas, at higher temperature in dilute acid, treatment is favorable for cellulose hydrolysis (McMillan, 1994). Although dilute acid pretreatment can significantly improve the cellulose hydrolysis, its cost is usually higher than some physicochemical pretreatment processes such as steam explosion or ammonia fiber expansion (AFEX). Some bases can also be used for the pretreatment of lignocellulosic materials and the effect of alkaline pretreatment depends upon the lignin content of the materials (Fan et al., 1987; McMillan, 1994). The mechanism of alkaline hydrolysis is believed to be saponification of intermolecular ester bonds crosslinking xylan hemicellulosic and other components, for example, lignin and other hemicellulose. The porosity of the lignocellulosic materials increases with the removal of the crosslinks (Kumar et al., 2008). Dilute alkaline treatment of lignocellulosic materials caused swelling, leading to an increase in internal surface area, decrease in the degree of polymerization and crystallinity, separation of structural linkages between lignin and carbohydrates and disruption of the lignin structure (Fan et al.,

Table 1. Various physical, chemical and biological methods for the pretreatment of lignocellulosic feedstock (Kumar et al., 2008; Saratale et al., 2008; Zhang et al., 2009).

Pretreatment process	Type	Source	Advantages	Limitations and disadvantages
Physical pretreatment	Mechanical	Milling, grinding, extrusion, pressing	Reduces cellulose crystallinity	Power consumption usually higher than inherent biomass energy
	Autohydrolysis	Steam pressure, steam explosion, hydrothermolysis, steam and mechanical sheer, pyrolysis, dry heat expansion, moist heat expansion	Causes hemicellulose degradation and lignin transformation; cost-effective	Destruction of a portion of the xylan fraction; incomplete disruption of the lignin-carbohydrate matrix; generation of compounds inhibitory to microorganisms
	Irradiation	Gamma, electron beam, photooxidation	Increase accessible surface area, removes lignin and hemicellulose to an extent; does not produce inhibitors for downstream processes	Not efficient for biomass with high lignin content
Chemical pretreatment	Alkali	Sodium hydroxide, ammonium hydroxide	Hydrolyzed hemicellulose to xylose and other sugars; alters lignin structure	Long residence times required; irrecoverable salts formed and incorporated into biomass
	Acids	Dilute or concentrated sulfuric acid, dilute or concentrated hydrochloric acid, nitric, phosphoric, acetic	Remove hemicelluloses and lignin; increase accessible surface area	High cost; equipment corrosion; formation of toxic substances
	Oxidizing agents	Peracetic acid, sodium hypochlorite, sodium chlorite, hydrogen peroxide	Increase accessible surface area, removes lignin and hemicellulose to an extent	Expensive and not effective for biomass.
	Solvents	(Organosolv) Methanol, ethanol, butanol, phenol, ethylamine, hexamethylenediamine, ethylene glycol	Hydrolyzes lignin and hemicelluloses	Solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost
	Gases	Ammonia, chlorine, nitrous oxide, ozone, sulfur dioxide.	Reduces lignin content; does not produce toxic residues Increase accessible surface area; cost-effective; does not cause formation of inhibitory compounds	Does not modify lignin or hemicelluloses Large amount of ozone required; expensive
Biological pretreatment	Cellulolytic microorganisms	Bacteria, Fungi and Actinomycetes	Degrades lignin and hemicelluloses; low energy requirements	Rate of hydrolysis is very low; Utilization of reducing sugar by microorganisms for their growth limits the application

Table 1. Contd.

Cellulolytic enzymes	<p>Endoglucanases (endo-1,4-[3- D-glucan-4-glucanohydrolase, EC 3.2.1.4)</p> <p>Exoglucanases (exo-1,4-[3-D-glucan-4-cellobiohydrolase, EC 3.2.1.91)</p> <p>β-glucosidases (β-d-glucoside glucohydrolase; EC 3.2.1.21)</p> <p>Cellobiose phosphorylase (EC 2.4.1.20)</p>	<p>Increase accessible surface area; does not cause formation of inhibitory compounds</p> <p>Hydrolysis of cellulose into fermentable sugars for the production of biofuels.</p> <p>Little energy requirement and mild reaction conditions, high substrate specificity, high yield of sugars, and high hydrolysis efficiency</p>	Due to enzyme cost process becomes expensive
	Hemicellulose degrading enzymes		
	Endoxylanases (1,4-[3-D-xylan xylanohydrolase, EC 3.2.1.8)	Depolymerization of hemicellulose to monomeric sugars for biofuels and other valuable chemicals production. Increase accessible surface area; does not cause formation of inhibitory compounds; Little energy requirement and mild reaction conditions, high substrate specificity, high yield of sugars, and high hydrolysis efficiency	Due to enzyme cost process becomes expensive
	Exoxylanase (1,4-[3-D-xylan xylohydrolase, EC 3.2.1.37)		
	Xylosidase (1,4-[β -D-xylan xylohydrolase, EC 3.2.1.37)		
α-L-arabinofuranosidase (EC 3.2.1.55)			
	1,4- β -D-Mannanase (1,4- β -D-mannan mannanohydrolase, EC 3.2.1.78)		
	1,4- β –mannosidases (β -D-1,4-mannoside mannohydrolase, EC 3.2.1.25)		
	α-galactosidase (α -galactoside galactohydrolase, EC 3.2.1.22)		
	Lignin degrading enzymes		
	Lignin peroxidase (ligninase, EC 1.11.1.14)	Useful biological tool for the degradation of lignin. For the delignification of wood and agricultural residues to increase the digestibility.	Due to enzyme cost process becomes expensive
	Manganese peroxidase (EC1.11.1.13)	Increase accessible surface area; does not cause formation of inhibitory compounds	
	Laccases (benzenediol: O ₂ oxidoreductase, EC 1.10.3.2)		

1987; Saratale et al., 2008).

Among physicochemical pretreatment procedures, steam explosion is recognized as one of the most cost-effective pretreatment processes for hardwoods and agricultural residues, but having limitation due to incomplete disruption of the lignincarbohydrate matrix and generates compounds that may be inhibitory to microorganisms used in downstream processes (Mackie et al., 1985). To remove the inhibitory products, pretreated biomass needs to be washed by water but water wash decreases the overall saccharification yields. AFEX pretreatment for various lignocellulosic materials shows better performance (Holtzapfle et al., 1991) but ammonia makes the process expensive and also causes secondary pollution problems. Although all these methods, in general, have potential for cellulose hydrolysis, they usually involve complicated procedures or are economically unfeasible (Mes-Hartree et al., 1988). The advantages and disadvantages of the different pretreatment processes are summarized in Table 1.

BIOLOGICAL METHODS FOR LIGNOCELLULOSIC PROCESSING

As pretreatment of lignocellulose waste is the rate-limiting step and has a major influence on the costs of both prior operation (e.g., lignocellulose particle size reduction) and subsequent operations (e.g., enzymatic hydrolysis and fermentation) (National Research Council, 1999). Conventional physicochemical methods for cellulose hydrolysis not only require large inputs of energy but also make secondary pollution. Biorefineries on the other hand, utilize the activities of whole microbial cells and their enzymes to convert cellulose into fermentable sugars. Biorefinery is the overwhelming choice for lignocellulosic waste treatment due to their ease of use, high efficiency and low energy requirement, as compared to conventional physicochemical methods. Biological pretreatment of cellulosic materials could be achieved by using cellulolytic microorganisms for a combined microbial and enzymatic hydrolysis or using cellulolytic enzymes directly.

Microbial hydrolysis of cellulosic materials

Effective degradation of lignocellulosic biomass could be achieved by the use of efficient cellulolytic micro-organisms which can produce effective cellulolytic enzymes during hydrolysis. Filamentous fungi are found ubiquitous in the environment, inhabiting ecological niches such as soil, living plants and lignocellulosic waste material. The ability of fungi to rapidly adapt their metabolism to varying carbon and nitrogen sources is achieved through the production of a large set of intra- and extracellular enzymes that are able to degrade various complex organic pollutants (Saratale et al., 2007a,b). Several studies have shown that white-rot fungi are the most effective microorganisms for the pretreatment of lingo-

celluloses, such as wood chips, wheat straw, bermuda grass and softwood (Hatakka, 1983; Akin et al., 1995). Some reports suggested that application of mixed bacterial culture is also useful for efficient hydrolysis of lignocellulosic waste (Datar et al., 2007). Processes of using fungal and bacterial systems for cellulose hydrolysis are inexpensive and easy to operate. However, they suffer the drawback of the consumption of the hydrolyzed products (such as reducing sugars) due to cell growth. To overcome these problems, some studies suggest a two-stage process using mixed or pure microbial culture for hydrolysis and the subsequent fermentative bioenergy production (Lo et al., 2009; Saratale et al., 2010).

Direct application of cellulolytic enzymes

Enzymatic hydrolysis of cellulose is carried out by the cellulose-hydrolyzing enzyme cellulases, a mixture of several enzymes that hydrolyze crystalline/amorphous cellulose to fermentable sugars (Duff and Murray, 1996). The conversion of lignocellulosic biomass to fermentable sugars through biocatalyst cellulase derived from cellulolytic organisms has been suggested as an economically feasible process and offers potential to reduce the use of fossil fuels and reduce environmental pollution relative to physicochemical processes (Dale, 1999). Formation of soluble sugars from cellulose in agricultural residues relies on the sequential/coordinated action of individual components such as β -endoglucanase (EC 3.2.1.4), β -exoglucanase (EC 3.2.1.91) and β -D-glucosidase (EC3.2.1.21) in cellulase enzymes (Bhat and Bhat, 1997; Lynd et al., 2002). Endoglucanases cleave intramolecular β -1,4-glucosidic linkages randomly and releases reducing sugars in the reaction mixture; having more applications in textile and detergent industries. Generally, exoglucanases acts on the accessible ends of cellulose molecules to liberate glucose and cellobiose but cellobiohydrolase (CBH I and II) by *Trichoderma reesei* act on the reducing and non-reducing cellulose chain ends reported earlier (Zhang et al., 2006). β -D-glucosidases hydrolyze soluble cellobiose and other cellodextrins to produce glucose in the aqueous phase (Zhang et al., 2006). In addition to the three major groups of cellulase enzymes, there are also a number of ancillary enzymes that attack hemicellulose, such as glucuronidase, acetylsterase, xylanase, β -xylosidase, galactomannanase and glucomannanase (Table 1) (Duff and Murray, 1996).

The interaction between hydrolytic enzymes and cellulosic substrates is complex, in part due to the significant number of possible interactions in the system involving a multi-enzyme complex (including cellulose, hemicellulose and lignin degrading enzymes) that adheres to a multi-component insoluble biomass substrate and acts catalytically upon it (Rabinovich et al., 2002; Zhang et al., 2006).

Enzymatic hydrolysis of cellulose consists of three steps: Adsorption of cellulase enzymes onto the surface of the cellulose, biodegradation of cellulose to ferment-

able sugars, and desorption of cellulase (Saratale et al., 2010a). Retardation of cellulase activity during hydrolysis may be because of the irreversible adsorption of cellulase on cellulose (Converse et al., 1988; Zhang et al., 2006). There are several advantages of enzymatic hydrolysis, including little energy requirement and mild reaction conditions, high substrate specificity, high yield of sugars, and high hydrolysis efficiency (Table 1). High sugar release resulted from enzymatic hydrolysis is dependent not only on pretreatment of cellulosic biomass but also on cellulolytic enzymes concentration (Sun and Cheng, 2002). The high dosage of expensive cellulases for high hydrolysis yields remains another obstacle to cellulosic ethanol commercialization (Wyman, 2007). Thus enzymatic hydrolysis is another most important step in the bioconversion of lignocelluloses into ethanol. To make the process economically feasible, it is expected to produce higher sugar yields at much lower cellulase enzyme loading.

From the economic point of view, there is need to increase the cellulase enzyme mass productivity with higher stability and specificity (substrates) for the specific processes. Conventional chemical pretreatment using acid or alkali could disrupt the crystalline structure of lignocelluloses and make cellulose more accessible to the enzymes for the conversion of the polysaccharides into fermentable sugars (Mosier et al., 2005). Conducting such treatment at high temperature with thermostable enzymes offer advantages in the lignocellulose bioconversion processes which are operated at high temperature and requiring viable enzyme recovery (Turner et al., 2007). Stability of the enzymes at higher operation temperature also have a significant influence on the bioavailability and solubility of organic compounds and thereby provides efficient hydrolysis of cellulosic biomass and increased flexibility with respect to process configuration, all contributing towards the overall improvement of the economy of the process (Viikari et al., 2007). Supplementation of surfactants (e.g. Tween 20 and Tween 80) during hydrolysis is capable of modifying the cellulose surface property and minimizing the irreversible binding of cellulase on cellulose (Eriksson et al., 2002). The addition of polymers such as polyethylene glycol (PEG) can also effectively increase enzymatic hydrolysis of lignocelluloses due to a higher availability of enzymes for cellulose degradation (Borjesson et al., 2007). In lignin-containing substrates, addition of bovine serum albumin (BSA) reduced adsorption of cellulase on lignin resulting in an increase in the activity (Ferreira et al., 2009). Recently, Saratale et al. (2010) reported that addition of certain metal additives, such as Mn^{2+} , could effectively enhance the multicomponent cellulase enzyme system of *Cellulomonas biazotea* NCIM-2550. Additional research efforts have been taken to improve the cellulase enzyme system by studying the cellulase structure and mechanism of action, the reconstitution of cellulase mixtures (cocktails), enzyme immobilization, random mutagenesis

as well as genetic engineering approaches for cost effective cellulase enzyme production (Cherry and Fidantsef, 2003; Zhang et al., 2006).

MICROBIAL FERMENTATION OF LIGNOCELLULOSICS

Bioethanol can be produced by using feedstocks containing sucrose (e.g. sugar cane, sugar beet, sweet sorghum and fruits), starch (e.g. corn, wheat, rice, potatoes, cassava, sweet potatoes and barley) and lignocellulose (e.g. wood, straw, and grasses) (Goh et al., 2009). Most industrial ethanol production uses sugarcane molasses or enzymatically hydrolyzed starch (from corn or other grains), and yeast (*Saccharomyces cerevisiae*) (Balat and Balat, 2009). Byproducts of this process are carbon dioxide, low amounts of methanol, glycerol, etc. Yeast fermentation of glucose syrups to ethanol has been well progressed in recent years but found economically infeasible. Thus, abundant and renewable lignocellulosic biomass feedstock has been considered as the low-cost feedstock for bioethanol production (Gray et al., 2006). Lignocellulosic biomass such as crop residues and sugar cane bagasse are included in feedstock for producing bioethanol (Lee and Dale, 2004). There are about 73.9 Tg dry wasted crop in the world that could potentially produce 49.1 Gt year⁻¹ of bioethanol. For several decades, microbial utilization of sugars obtained from the hydrolysis of lignocellulosics for the production of fuel ethanol has been an active area of research (Dien et al., 1997; Ho et al., 1999; Ingram, 2000; Sreenath and Jeffries, 2000; Jeffries et al., 1994; Lawford and Rousseau, 2002). This has been largely due to the absence of suitable ethanologens that can utilize the mixture of the various pentose, hexose and higher sugars present in hydrolysates (Singh and Mishra, 1995).

Research has confirmed considerable differences in the uptake and utilization of the various sugars by bacteria, yeast and molds (Ho et al., 2000; Ingram, 2000; Jeffries et al., 1994; Green et al., 2001; Zhang, 2002; Picataggio et al., 1994). Some studies reported that alcohol fermentation using lignocellulosic hydrolysates has some technological problems such as enzymatic hydrolysis reaction of cellulose which is about two orders of magnitude slower than the average ethanol fermentation rate with yeast (Antoni et al., 2007). Bacterial ethanol fermentation can use all sugars derived from cellulosic biomass; however, it suffers from catabolite repression. The widely studied *Zymomonas mobilis* is considered the work horse of bacterial ethanol fermentation (Alterthum and Ingram, 1989). Recently a new recombinant *Escherichia coli* B strain LY165 has been developed for bioethanol plant located in the Bay of Osaka, Japan (Ohta et al., 1991). *Streptococcus fragilis* and *Kluyveromyces fragilis* are used widely for commercial ethanol production (Pesta et al., 2006). The thermophilic bacterium *Clostridium thermocellum* could readily hydrolyze

cellulosic biomass; degrade hemicellulose and cellulose for ethanol production (Lynd et al., 2002; Wu et al., 2008). Cellulolytic microorganisms give significant cellulose hydrolysis but after hydrolysis diversion towards different metabolic shifts gives mixed gaseous acidogenic fermentation products (Lynd et al., 2002; Demain et al., 2005).

Some studies reported that after hydrolysis of lignocellulosic biomass, the produced pentose sugars (mainly D-xylose and L-arabinose) create problem in yeast alcohol fermentation because yeast strains lack the xylose utilization enzymes (mainly xylose reductase and xylitol dehydrogenase) (Hahn-Hägerdahl et al., 2007). Thus, the efficient utilization of the xylose component of hemicellulose in addition to hexoses offers opportunity to significantly reduce the cost of bioethanol production (Goldemberg, 2007). In agricultural, raw material and hardwoods, pentose sugars are present in larger proportion, which cannot be neglected if we want to increase the yield of ethanol and complete substrate utilization. Several strategies have been employed to remedy these limitations ranging from host selection, host modification by classical strain development approaches and genetic engineering of new strains (Ho et al., 2000; Jeffries et al., 1995; Ingram, 2000; Picataggio et al., 1994). In bacteria, D-xylose utilization involves the action of D-xylose isomerase followed by phosphorylation of D-xylulose by D-xylulose kinase (Amore et al., 1989; Gulati et al., 1996; Ho et al., 2000; Singh and Mishra, 1995). By comparison, the utilization of D-xylose in fungi proceeds with the action of D-xylose reductase with the formation of xylitol as the product (Bruinenberg et al., 1984; Jeffries et al., 1994; Jeffries et al., 1995; Singh and Mishra, 1995; Jeppsson et al., 1999). This is followed by dehydrogenation of xylitol by the action of xylitol dehydrogenase to D-xylulose, which in turn is acted on by D-xylulose kinase (Singh and Mishra, 1995; Amore et al., 1989). The product of the phosphorylation, D-xylulose-5-phosphate, is assimilated into the pentose phosphate pathway, which feeds into the glycolytic pathway leading to the production of ethanol. A significant difference in cofactor use exists between the naturally pentose-fermenting yeast such as *Pichia stipitis* and the brewing yeast, *S. cerevisiae* was observed. In *P. stipitis* and in other genera of pentose fermenting yeast, the reductases that reduce D-xylose to xylitol can utilize either NADH or NADPH. In contrast, in *S. cerevisiae* the host reductase that acts on D-xylose is limited to NADPH. This cofactor use in *S. cerevisiae* is responsible for the cofactor regeneration imbalance and is one of the reasons why this yeast is unable to produce significant levels of ethanol from D-xylose under anaerobic conditions (Amore et al., 1989; Bruinenberg et al., 1984; Jeffries et al., 1995). Cloning of the *P. stipitis* D-xylose reductase that utilizes NADH into *S. cerevisiae* improved the ability of this yeast to utilize D-xylose and resulted in improved ethanol production by the genetically engineered *Saccharomyces*

strain. Further genetic engineering of *S. cerevisiae* to increase D-xylulose kinase activity yielded new recombinant yeast strains with significant ethanol production from D-xylose (Ho et al., 2000; Eliasson et al., 2000). Most recently, employment of metabolic flux analysis to recombinant *S. cerevisiae* grown on a mixture of D-glucose and D-xylose has been used to further delineate additional genes that can be targeted to improve ethanol production from D-xylose. This latest approach highlights the great potential offered by metabolic engineering to achieve further improvements in ethanol yield and productivity in recombinant *S. cerevisiae* (Nielsen, 2001; Ostergaard et al., 2000).

Simultaneous saccharification and fermentation

The list provided in Table 2 illustrates some of the available options that can be used in the fermentation of sugars in lignocellulosic hydrolysates by combining an appropriate hydrolysate and process. Among which simultaneous saccharification and fermentation (SSF) is more advantageous process because of higher ethanol yields, requires lower amounts of enzyme, and no end-product inhibition from cellobiose and glucose formed during enzymatic hydrolysis (Banat et al. 1998). However, due to their low rates of cellulose hydrolysis, it results in lower alcohol production. In addition, there is a theoretical gap in simultaneous saccharification of cellulosic biomass and ethanol fermentation as well as proportion of pentose and hexose sugar concentration (Hahn-Hägerdahl et al., 2007; Torney et al., 2007). Moreover, most microorganisms used in this process lack the ability to utilize xylose, a hemicellulose hydrolysis product, which also limits the application. Recently some ethanologens such as *E. coli*, *Klebsiella oxytoca*, and *Z. mobilis* are found promising strains for industrial exploitation (Matthew et al., 2005). In addition, some strains of genus *Clostridium*, *Cellulomonas*, *Trichoderma*, *Penicillium*, *Neurospora*, *Fusarium*, *Aspergillus* which has ability to produce high cellulolytic and hemicellulolytic activity, are capable of simultaneous fermentation of monosaccharides to ethanol. Moreover, to make the SSF process more effective, it has also been found necessary to search for thermostable strains capable of producing substantial amounts of ethyl alcohol at temperatures optimal for saccharification and suitably resistant to ethanol as well as the necessity to develop strain capable of hydrolyzing cellulose and xylan along with fermentation of glucose and xylose to ethanol by applying recombinant DNA technology (Lan and Tanaka, 2005).

Conclusions

Lignocellulosic biomass has several advantages over

Table 2. Summary of the effective ethanol fermentation methods.

Method	Organisms/enzymes
Single or pure microbial culture	Pentose utilizing ethanolgenic yeast or recombinant bacteria or yeast
By developing microbial co-culture	Combination of brewing yeast with ethanolgenic pentose fermenting yeast
Isomerization followed by fermentation	Carry out isomerization at neutral to slightly alkaline pH further lowering the pH and temperature and sequential fermentation using brewing yeast.
Simultaneous saccharification and fermentation	Combination of cellulolytic enzymes with an ethanolgenic organism or applying genetically engineered ethanolgen having ability to utilize lignocellulosics.

conventional sugar- and starch-based raw materials and has been projected to be one of the main sources of bioethanol in the near future. The major factor affecting the efficiency of the conversion of lignocellulosic materials into energy products is the hydrolysis/saccharification of lignocellulose. The key to a successful cellulosic ethanol production is to develop effective pretreatment technology leading to rapid and high yield hydrolysis of lignocellulose; converting it to fermentable sugars for subsequent fermentative production of ethanol. The conversion of cellulose continues to be economically and technically challenging due to the current high cost of commercially available enzymes as well as the energy cost for the pretreatment and hydrolysis and cost of subsequent detoxification. For the commercial production of lignocellulosic ethanol, attention should be geared towards cellulosic feedstock and their handling, the biomass processing and pre-treatment strategy, cost of cellulase enzyme, optimum enzyme loadings, robust and hyper-producing (recombinant) microbial strains having the ability to produce cellulases, hexose and pentose utilization and high ethanol tolerance. In addition, there is need to develop efficient fermentation configuration and process strategy as well as integration of the ethanol plant with a power generation and/or biomethane/biohydrogen plant.

ACKNOWLEDGEMENT

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0013038). This review is dedicated to the memory of Advocate Mahesh D. Saratale who passed away on November 3, 2009.

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