

Bioethanol Production Using Lignocellulosic Biomass - review

Part I. Pretreatments of biomass for generating ethanol

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(Received November 2, 2010; Accepted December 10, 2010)

ABSTRACT

Bio-ethanol is a promising alternative energy source for reducing both consumption of crude oil and environmental pollution from renewable resources like lignocellulosic biomass such as wood, forest residuals, agricultural leftovers and urban wastes. Based on current technologies, the cost of ethanol production from lignocellulosic materials is relatively high, and the main challenges are the low yield and high cost of the hydrolysis process. Development of more efficient pretreatment technology (physical, chemical, physico-chemical, and biological pretreatment), integration of several microbiological conversions into fewer reactors, and increasing ethanol production capacity may decrease specific investment for ethanol producing plants. The purpose of pretreatment of lignocellulosic material is to improve the accessible surface area of cellulose for hydrolytic enzymes and enhance the conversion of cellulose to glucose and finally high yield ethanol production which is economic and environmental friendly.

Keywords : *Bioethanol, pretreatment, lignocellulosic biomass.*

1. Introduction

One of the greatest challenges for society in the 21st century is not only to meet the increasing energy demand for transportation, heating and industrial processes, but also to provide raw materials for the

industry in a sustainable way 1). Demerits of fossil derived from transportation fuels i.e. greenhouse gas emissions, pollution, unsustainable supply, unequal supply-demand relations are strongly reduced or even absent with bio-transportation fuels 2,3). Environmental, long-term economic and national security concerns have motivated researches over the last 25 years into

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renewable, domestic sources of fuels and chemicals now mostly derived from petroleum 4). During the last decades, global warming from the increased amount of greenhouse gases, mainly carbon dioxide, has become a major political and scientific issue. Burning fossil fuels i.e. natural gas, coal and oil releases CO₂, which is also a major cause of global warming 5). In the past, shortages of fossil energy and environment pollutions have awaked the interest of renewable energies in many countries.

Among the clean energies, bioethanol is considered as a promising renewable fuel because of clean burning characteristics, reduction of particulate and NO_x emission from combustion 6). Diminution of world's petroleum supply and greenhouse gas (GHG) effects has resulted in a growing interest in alternative fuels 7). The increasing concerns about environmental protection, the increase of oil prices and the decrease of the world reserves of fossil energy carriers have attracted scientific interest to the use of bioethanol as a transportation fuel. Biomass residues are potential raw materials for ethanol production since they are inexpensive, abundant and rich in carbohydrates 8). Biorefinery integrates biomass conversion processes and equipment to fossil resources for production of energy carrier's fuels, power, and high value chemicals.

In most cases, commodity product bioethanol is produced from not only corn or wheat but also from cassava, rye, barley or sorghum. But these raw materials are not sufficient for supplying increasing demands for animal or even human feeding. In this case, Biological material as renewable energy source derived from living, or recently living organisms, such as forest and mill residues, agricultural crops and wastes, wood and wood wastes, animal wastes, livestock operation residues, aquatic plants, fast-growing trees and plants, municipal and industrial wastes for bioethanol production. This ethanol also can reduce net CO₂ emissions i.e. help to alleviate global warming and environmental pollution to

approximately zero 9-11). Therefore, future energy generation and fundamental sources of raw materials will come to rely more and more on renewable sources 12). Bioethanol production from renewable biomass has received considerable worldwide attention in current years. Ethanol for fuel can also decrease strong dependence on foreign oil, reduce trade deficits, create jobs in rural areas, reduce air pollution, and reduce global climate change CO₂ buildup. Currently, the National Renewable Energy Laboratory (NREL) identifies biomass pretreatment as one of the factors standing in the way of cost effective lignocellulosic bioethanol 13). Technologies that will allow cost effective conversion of biomass into fuels and chemicals consider economy of scale, low-cost pretreatment systems and highly effective and efficient biocatalysts 14,15). Different pretreatment methods have been investigated for different materials 16). A variety of techniques have been studied for the conversion of lignocellulosic materials to fermentable sugars. Pretreatment is an important tool for practical cellulose conversion processes. This is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars 17).

Different pretreatment technologies have been developed (dilute acid or alkali, flow-through, ammonia fiber explosion, ammonia recycle percolation, steam explosion, and organosolv), and the common purpose of these technologies is to break open the lignocellulosic structure.

Lignocellulosic biomass includes four main steps for bioethanol production as represented in Fig. 1:

1. Pretreatment-breaking down the structure of the lignocellulosic matrix.
2. Enzymatic hydrolysis-depolymerizing cellulose to glucose by means of cellulolytic enzymes.
3. Fermentation-metabolizing the glucose to ethanol, generally by microorganisms, bacteria or yeast strain.
4. Distillation-separating and purifying the ethanol

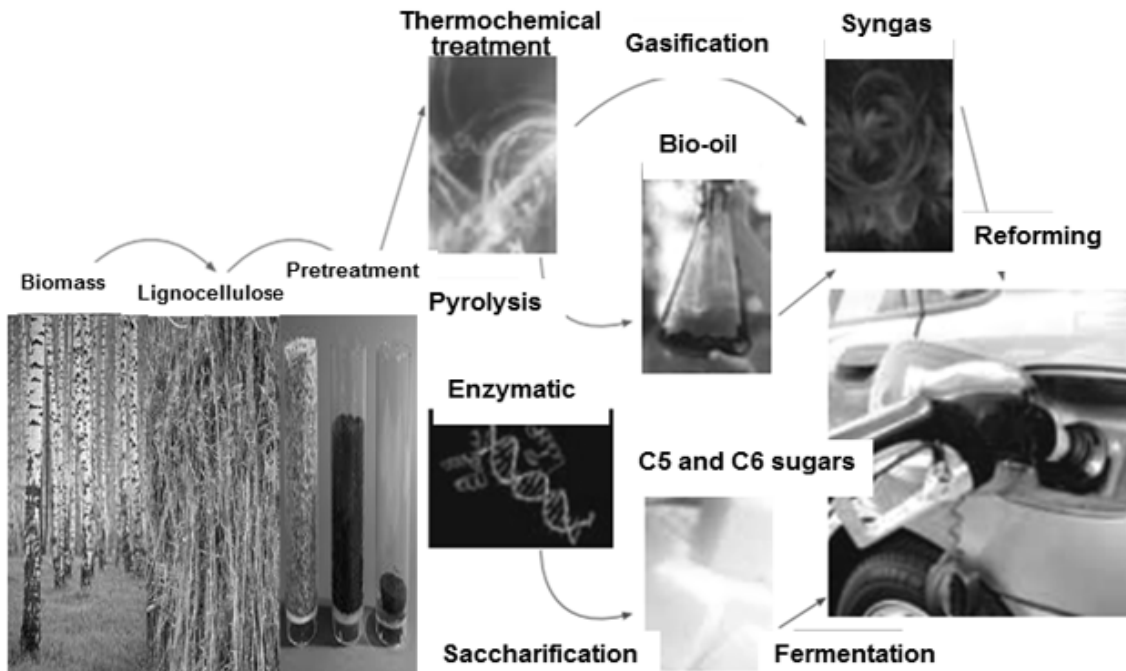


Fig. 1. A summary of the primary processing steps involved in the conversion of lignocellulosic materials to biofuels.

to meet fuel specifications.

The goal of pretreatment of lignocellulosic material is to make the cellulose accessible to hydrolysis for conversion to biofuels using microorganisms which is economic, environmental friendly and also renewable.

2. The composition of lignocellulosic materials

Lignocellulose is the primary building block of plant cell walls. Plant biomass consists of mainly three different types of polymers, namely cellulose, hemicellulose and lignin, which are associated with each other (18). Cellulose and hemicellulose comprise the carbohydrate fraction of biomass while lignin consists of poly (aromatic) moieties from phenylpropanoid building blocks and minor constituents include organic extractives (mainly terpenes) and inorganic compounds (ash) (19). Plant biomass consists of

40-55% cellulose, 25-50% hemicellulose and 10-40% lignin, depending on whether the source is hardwood, softwood, or grasses (20). Cellulose is the main part of the cell wall, with the elementary formula $(C_6H_{10}O_5)_n$, highly linear polysaccharide but hemicellulose has more branched structure and more susceptible to chemical degradation than cellulose. Lignin acts like the bounds in the cells, and gives rigidity to the cell wall. It has complex three dimension polymers of phenylpropane, namely p-coumaryl, coniferyl, and sinapyl alcohols.

3. Lignocellulosic feedstock biorefinery

It is generally known that the pulp and paper industry generates about 50% of its own energy needs from biomass residues and makes extensive use of combined heat and power. Biorefinery in pulp and

paper industry is an integrated system of interdependent processes, which converts a set of renewable feedstock effectively into the actually most profitable portfolio of value-added products with a minimum of external energy needs and practically no wastes and emissions. Such value-added products include different kinds of biomaterials and bioproducts, chemicals, biofuels and energy. Among the several definitions of biorefinery, the most exhaustive was recently formulated by the International Energy Agency Bioenergy Task 42 "Biorefinery". Utilization of biomass as raw materials in a biorefinery is a promising alternative to fossil resources for production of energy carriers and chemicals, as well as for mitigating climate change, reducing dependence on imported fossil fuels and enhancing energy security (21) as depicted in Fig. 2. In the near future, the second generation biofuel facilities in pulp and paper industry are expected to develop towards biorefinery concepts, where bioenergy, biochemicals, and biomaterials are co-produced along with transportation biofuels.

4. Pretreatment of lignocellulosic materials

An effective and economical pretreatment of lignocellulosic materials is indicated by several criteria. The useful effects of pretreatment of lignocellulosic materials have been recognized for a long time. The aim of the pretreatment is to remove lignin and hemicellulose, diminish cellulose crystallinity, and augment the porosity of the lignocellulosic substrates as represented in Fig. 3. Pretreatment should meet the following requirements: (a) production of reactive cellulosic fiber for enzymatic attack, (b) avoiding destruction of hemicelluloses and cellulose, (c) avoiding formation of possible inhibitors for hydrolytic enzymes and fermenting microorganisms, (d) minimizing the energy demand, (e) reducing the cost of size reduction for feedstocks, (f) diminishing the cost of material for construction of pretreatment reactors, (g) producing less residues, (h) consumption of little or no chemical and using a cheap chemical, (i) being simple and effective at low humidity. Several

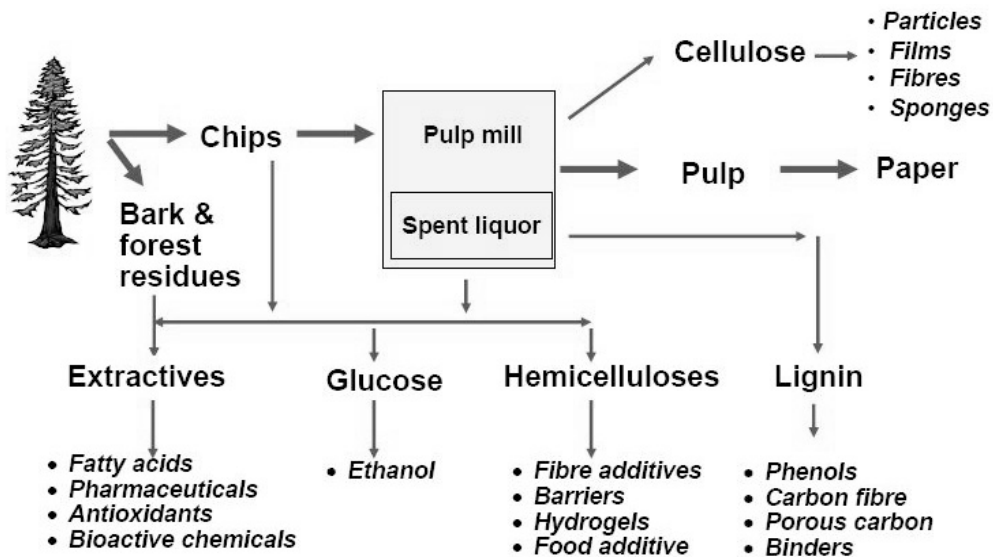


Fig. 2. Biorefinery in the pulp and paper industry. (Source: Biorefineries in pulp and paper sector, IEA Implementing Agreement, April, 2007)

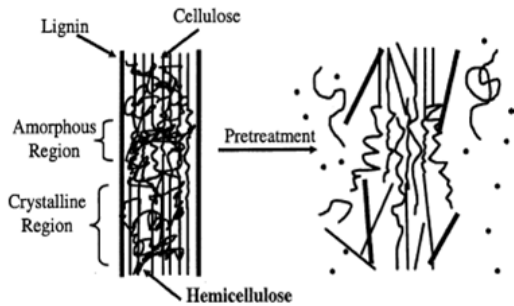


Fig. 3. Schematic of goals of pretreatment on lignocellulosic material 17).

methods have been introduced for pretreatment of lignocellulosic materials prior to enzymatic hydrolysis or digestion such as physical, chemical, physico-chemical and biological processes. 22-45).

4.1 Physical pretreatments

4.1.1 Mechanical pulverization

The objective of mechanical treatment is a reduction of particle size and crystallinity of lignocellulosic in order to increase the specific surface and reduce the degree of polymerization. This can be produced by a combination of chipping, grinding or milling depending on the final particle size of the material (10-30 mm long and 3 to 6 mm thick after chipping and 0.2-2 mm diameter holes after milling or grinding) 20). Vibratory ball milling with 80 meshes (0.177 mm) is used for more effective in breaking down the cellulose crystallinity and improving the digestibility of the biomass than ordinary ball milling 46).

4.1.2 Pyrolysis & Hydrothermal treatment

Pyrolysis is a form of incineration that chemically decomposes organic materials by heat above 300 °C and pressure in the absence of oxygen. When biomass is treated with that temperature, cellulose rapidly decomposes to gaseous products, bio-oil, or residual biochar 47,48). The decomposition is much slower and less volatile products are produced at lower

temperatures. The process can be increased with the presence of O₂ 48), called thermal treatment. During a hydrothermal process, temperature, pressure, and reaction time are the most important factors affecting the material conversion. A higher temperature and pressure lead to a faster hydrolysis rate but a faster decomposition rate as well, which consequently leads to a relatively lower yield of hexoses. Nevertheless, flow technology has the advantage of shorter reaction times, as it skips the preheating stage of the reactor 49). If zinc chloride, ZnCl₂ or sodium carbonate, Na₂CO₃ is added as a catalyst, the decomposition of pure cellulose can occur at a lower temperature 50).

4.1.3 Extrusion

Extrusion process is also a novel and promising physical pretreatment method for biomass conversion to ethanol production. In this technique, the materials are submitted to heating, mixing and shearing, resulting in physical and chemical modifications during the passage through the extruder 51). Several parameters must be taken into account to achieve the highest efficiency in the process. Screw speed and barrel temperature are considered to disrupt the lignocellulose structure causing defibrillation, fibrillation and shortening of the fibers, and finally increasing accessibility of carbohydrates to enzymatic attack.

4.2 Chemical pretreatments

4.2.1 Ozonolysis treatment

Ozonolysis is a novel pretreatment method that can enhance biomass digestibility with minimal generation of chemical waste streams and degradation of the carbohydrate components. It employs powerful oxidant O₃, which forms highly reactive free hydroxyl ions upon decomposition. This method can effectively degrade lignin and part of hemicellulose. This treatment has several advantages: (1) it effectively removes lignin; (2) it does not produce toxic residues for the

downstream processes; and (3) the reactions are carried out at room temperature and pressure 52). Furthermore, the fact that ozone can be easily decomposed by using a catalytic bed or increasing the temperature means that processes can be designed to minimize environmental pollution 53). However, a large amount of ozone and varieties of parameters i.e. oxygen cylinder, automatic gas flow control valve, ozone generator, process gas humidifier, reactor, ozone UV spectrophotometer, ozone catalytic destroyer, iodide trap to test catalyst efficiency, vent, pressure regulation valve, three-way valve, moisture content of the sample, particle size, and O_3 concentration are obligatory for this process. This is an attractive pretreatment method for industrial process since it does not leave acidic, basic, or toxic residues in the treated material 54). As for laboratory-scale, this process is expensive. It is expected that the results of this study will help in the development of a pretreatment process that provides higher specificity towards lignin removal compared than other delignifying agents/pretreatments.

4.2.2 Acid treatment

The acid pretreatment can operate either under a high temperature and low acid concentration (dilute-acid pretreatment) or under a low temperature and high acid concentration (concentrated-acid pretreatment) 16). Treatment of lignocellulosic materials with acid at a high temperature can efficiently improve the enzymatic hydrolysis. Different concentrated sulfuric acid is the most applied acid, while other acids HCl, phosphoric acid and nitric acid are also used 55). Although they are powerful agents for cellulose hydrolysis but concentrated acids are toxic, corrosive and hazardous and require reactors that are resistant to corrosion. In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible 56). Dilute-acid hydrolysis is probably the most commonly applied method among the chemical pretreatment methods. At an elevated

temperature (140-190 °C) and low concentration of acid (0.1-1% H_2SO_4), the dilute-acid treatment can achieve high reaction rates and significantly improve cellulose hydrolysis. In general, pretreatments with diluted acids involve high temperature (>160 °C) continuous processes with low solid content (5-10%) 57) and discontinuous low temperatures (<160 °C) processes with high content of solids (10-40%) 58). Its cost is usually higher than some physico-chemical pretreatment processes such as steam explosion or AFEX. The major drawback of this method, particularly at low pH, is the formation of different types of non-accessible molecules such as carboxylic acids, furans and phenolic compounds 55). These chemicals may not affect the enzymatic hydrolyses, but they usually inhibit the microbial growth and fermentation, which results in less yield and productivity of ethanol or biogas 59).

4.2.3 Alkaline treatment

Alkali pretreatment refers to the application of alkaline solutions such as NaOH, lime $Ca(OH)_2$ or NH_3 to remove lignin and a part of the hemicellulose, and efficiently increase the accessibility of enzyme to the cellulose 16). Sodium, potassium, calcium, and ammonium hydroxides are suitable agents for alkaline pretreatment. NaOH has a strong base activity which catalyzes the removal of lignin and swells celluloses 60-63). This base and sodium sulfide are used in kraft paper pulping. $Ca(OH)_2$ has the additional benefits of low reagent cost and safety 64,65) and this process is also similar to Kraft paper pulping technology. Alkali pretreatment processes use lower temperatures and pressures compared to other pre-treatment technologies. This pretreatment may be carried out at ambient conditions, but time is measured in terms of hours or days rather than minutes or seconds 66). Alkali pretreatment reduces the lignin and hemicellulose content in biomass, enhances the surface area, allowing penetration of water molecules to the inner layers, and breaks the bonds between hemicellulose

and lignin carbohydrate. Dilute NaOH is usually used for alkali pre-treatment. As for considering economic and environmental aspects, dilute NaOH treatment would be much more suitable than concentrated NaOH pretreatment.

4.2.4 Oxidative delignification

Lignin biodegradation could be catalyzed by the peroxidase enzyme with the presence of hydrogen peroxide, H_2O_2 . Almost 50% lignin and most hemicellulose were solubilized by 2% H_2O_2 at 30 °C within 8 hours, and 95% efficiency of glucose production from cellulose was achieved in the subsequent saccharification by cellulase at 45 °C for 24 hours (67). An oxidative pretreatment consists of additional oxidizing compounds, like H_2O_2 or Peracetic acid to the biomass which is suspended in water. During oxidative pretreatment, several reactions can take place such as electrophilic substitution, displacement of side chains, cleavage of alkyl aryl ether linkages or the oxidative cleavage of aromatic nuclei (68). In many cases, the used oxidant is not selective and therefore losses of hemicellulose and cellulose can occur. Oxidative lime pretreatment of poplar (69) removed 77.5% lignin from the wood chips at 150 °C for 6 hours and improved the yield of glucose by enzymatic hydrolysis from 7% (untreated) to 77% (treated) compared to the untreated and pretreated poplar wood. Peracetic acid is very lignin selective and no significant carbohydrate losses occurred.

4.2.5 Organosolv process

The organosolv process is a promising pretreatment strategy which has attracted more attention and proved the potential for utilization in lignocellulosic pretreatment (70). In the organosolv process, an organic or aqueous organic solvent mixture with inorganic acid catalysts (HCl or H_2SO_4) is used to break the internal lignin and hemicellulose bonds. Methanol, ethanol, acetone, ethylene glycol, triethylene glycol, and tetrahydrofurfuryl alcohol are the common solvents for this process (71,72). Organic acids such as

oxalic, acetylsalicylic, and salicylic acids also can be used as catalysts (73). The applied solvents should be separated by evaporation and condensation, and recycled to reduce the operational costs of the process. Scientists observed the highest ethanol yield to be 99.5% at 195 °C, 5 minutes, pH 2.0, and 1:1 ratio of acetone-water after pretreatment (74).

4.2.6 Ionic liquids (ILs) pretreatment

The use of ILs as solvents for pretreatment of lignocellulosic biomass has recently received much attention. ILs is salts, typically composed of large organic cations and small inorganic anions, which exist as liquids at relatively low temperature or room temperature. Their solvent properties can be varied by adjusting the anions and the alkyl constituents of the cations. These interesting properties include chemical and thermal stability, non-flammability, low vapour pressures and a tendency to remain liquid in a wide range of temperatures (75). In this process, no toxic or explosive gases are produced, so ILs also called “green” solvents. Carbohydrates and lignin can be simultaneously dissolved in ILs with anions activity (1-butyl-3-methylimidazolium cations) because ILs forms hydrogen bonds between the non-hydrated chloride ions of the IL and the sugar hydroxyl protons in a 1:1 stoichiometric. In this study using 1-ethyl-3-methyl imidazolium diethyl phosphate, the yield of reducing sugars from wheat straw pretreated with this ionic liquid at 130 °C for 30 min was 54.8% after being enzymatically hydrolyzed for 12 h (76). In spite of some current limitations, advanced research e.g. as potential synthesis of ILs from carbohydrates may play an important role in reducing their cost. Development of ILs pretreatment offers a great potential for future lignocellulose biorefining processes.

4.3 Physico-chemical pretreatments

4.3.1 Steam explosion (autohydrolysis)

Steam explosion is an increasingly popular

pretreatment of raw lignocellulosic biomass to separate the cellulose and lignin. This treatment is typically initiated at a temperature of 160-260 °C (corresponding pressure 0.69-4.83 MPa) for several seconds to a few minutes before the material is exposed to atmospheric pressure. 90% efficiency of enzymatic hydrolysis has been achieved in 24 hours for poplar chips pretreated by steam explosion, compared to only 15% hydrolysis of untreated chips (77). Small amounts of lignin are removed but it is melted and depolymerized and spread back over the surface of the cellulose fibre. The factors that affect steam explosion pretreatment are residence time, temperature, chip size and moisture content (78). Researchers primarily apply to improve the fermentation process for the conversion of bamboo into methane by the use of steam-explosion technology (79). Recent studies indicate that lower temperature and longer residence time are more favorable than high temperature and short residence time. Optimal hemicellulose solubilization and hydrolysis can be achieved by either high temperature and short residence time (270 °C, 1 minute) or lower temperature and longer residence time (190 °C, 10 minutes) (78). This process offers several attractive features-potential for significantly lower environmental impact, lower capital investment, more potential for energy efficiency, less hazardous process chemicals and conditions and complete sugar recovery (80).

4.3.2 Liquid hot water

The objective of this water is to solubilize mainly the hemicellulose, to make the cellulose more accessible and to avoid the formation of inhibitors at pH 4-7. This is similar to autohydrolysis, except that the larger amounts of water are employed and the pressure is elevated to ensure that the water remains liquid. This is also called hydrothermolysis (81), and aquasolv (82). Cocurrent, countercurrent or flow-through reactor configurations may be used. Cocurrent treatment involves the biomass and water

being held in slurry which is warmed and then cooled; counter-current treatment moves the biomass and water in opposite directions; and flow-through treatment involves the water passing over the stationary biomass (75). This treatment, pH is decreased for two reasons- increasing temperatures lowers the p^{Ka} of water and liberates acids from biomass. Water has an unusually high dielectric constant that enables to dissociate ionic substances and able to dissolve all of the hemicellulose. One half to two thirds of the lignin also dissolves from most biomass materials when these materials are treated at 220 oC for 2 min. Hot water cleaves hemi-acetal linkages and liberates acids during biomass hydrolysis. This facilitates the breakage of such ether linkages in biomass (83). In general, liquid hot water pretreatments are attractive from a cost-savings potential for no catalyst requirement and low-cost reactor construction due to low-corrosion potential.

4.3.3 Supercritical water

Supercritical water treatment is thought to be a promising pretreatment for bioethanol production from lignocelluloses. This treatment is more suitable for obtaining high yields of hydrolyzed products and cellulose is found to be more liable to fragment (84). The treatment (water with a temperature higher than 374 °C and a pressure higher than 22 MPa) has been considered as one of the main hydrothermal technologies because of the high dissolving and catalyzing capacity of supercritical water, which can separate lignin from cellulose and rapidly hydrolyze the cellulose through catalysis by H⁺ ions (85,86).

4.3.4 Ammonia fiber/ freeze explosion (AFEX)

Ammonia fibre explosion is very similar concept to steam explosion, with the main difference being the ammonia is used instead of superheated steam. This treatment is regarded as an attractive method for high yielding digestible cellulose (36,87). AFEX depolymerizes the lignin, dissolves the hemicellulose and decrystallizes

the cellulose 88,89). The moderate temperature and pH also minimize formation of sugar degradation products. In this process, the raw materials are exposed to liquid ammonia at high temperature and pressure for a period of time, and then the pressure is swiftly reduced. The dosage of liquid ammonia is 1-2 kg ammonia/kg dry biomass, temperature 90 °C, and residence time 30 minutes. Pressures exceeding 12 atm are required for operation at ambient temperature. For diminishing the cost and protecting the environment, ammonia NH₃ must be recycled after the pretreatment. In NH₃ recovery process, a superheated ammonia vapor with a temperature up to 200 °C is used to vaporize and strip the residual ammonia in the pretreated biomass and the evaporated ammonia is then withdrawn from the system by a pressure controller for recovery 90). The inhibitor products are not produced for the downstream biological processes, and thus water wash is not necessary 91,92).

4.3.5 Supercritical CO₂ explosion

Carbon dioxide, CO₂ explosion is similar to steam and ammonia explosion treatment methods for lignocellulosic materials. As for developing improved lignocellulose pretreatment techniques, the supercritical CO₂ explosion uses a lower temperature than steam explosion and is possibly less expensive compare to ammonia explosion. CO₂ is helpful for hydrolyzing hemicellulose and cellulose. Furthermore, low temperature prevents any appreciable decomposition of monosaccharide's by using acid 93). It is hypothesized that CO₂ will form carbonic acid and enhance the hydrolysis rate. Researchers used this method to pretreat alfalfa (4 kg CO₂/kg fiber at the pressure of 5.62 MPa) and obtained 75% glucose during 24 hours of the enzymatic hydrolysis 88). Scientists compared and found that CO₂ explosion was more cost-effective than ammonia explosion and did not cause the formation of inhibitory compounds that could occur in steam explosion 94).

5. Biological pretreatment

Biological treatment means the use of whole organisms (fungi and bacteria) or enzymes of lignocellulosic materials for safe and environmentally friendly method. These enzymes are controlled by carbon and nitrogen sources. Fungal pretreatment of agricultural residues is a new method for improvement of digestibility 95). White-rot fungi (such as *Phanerochaete chrysosporium*, *Trametes versicolor*, *Ceriporiopsis subvermispota*, and *Pleurotus ostreatus*) are the most effective for biological pretreatment of lignocellulosic materials 96,97). Biological pretreatment might be used not only for lignin removal, but also for biological removal of specific components such as antimicrobial substances. The main advantages of biological pretreatment include low energy requirement, no chemical requirement, and mild environmental conditions but this treatment is slow process.

6. Conclusions

With the possibility of depletion of the world's petroleum supply, biomass has attracted attention as an alternative energy resource. The cell-wall structural of biomass material is composed of cellulose and hemicellulose encrusted with lignin. The biodegradability of lignocellulosic biomass is limited by several factors like crystalline cellulose, available surface area, and lignin content. Such a complex structure of biomass prevents enzymatic accessibility of microorganisms to the cell-wall components and also contributes to the resistance of cellulose to hydrolysis. Pretreatment and intrinsic structures of the biomass itself are primarily responsible for its subsequent hydrolysis. The conditions employed in the opted pretreatment method affects various substrate characteristics that regulate the susceptibility of the substrate to hydrolysis and the subsequent fermentation of the released sugars. For these reasons, biomass pretreatment is an extremely important step for the synthesis of

biofuels. There is a critical need to understand the fundamentals of various processes, which can help in making a suitable option depending on the structure of the biomass substrate, operating conditions and the hydrolysis agent for low cost bioethanol production.

Acknowledgement

This study was carried out with the support of 'Forest Science & Technology Projects' (Project No.03-2009-0136) provided by Korea Forest Service.

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