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A BRIEF REVIEW ON TECHNOLOGIES FOR THE PRODUCTION OF BIOETHANOL FROM LIGNOCELLULOSIC BIOMASS

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ABSTRACT: Although the historical production of fermented beverages and alcohol in India dates back to 2000 years ago, fuel ethanol production is a recent event in India that was initiated 10 years ago by the government to offset the rapidly enlarging gap between the country's crude oil consumption, driven up by its rapid economic growth, and dwindling domestic reserves and production. Production of bioethanol from biomass is one of the ways to reduce both consumption of crude oil and environmental pollution. Bioethanol is appropriate for the mixed fuel in the gasoline engine because of its high octane number, and its low cetane number and high heat of vaporization impede self-ignition in the diesel engine. The development of second generation bioethanol from lignocellulosic biomass serves many advantages from both energy and environmental point of view. Biomass an inexpensive feedstock considered sustainable and renewable, is an option with the potential to replace a wide diversity of fossil based products within the energy sector. Lignocellulose is a major structural component of woody and non woody and consists of cellulose, hemicellulose and lignin. Biochemical conversion of lignocellulosic materials through saccharification and fermentation is a major path way for bioethanol production from biomass. To achieve an economical and environmental friendly system of bioethanol production from lignocellulosic biomass, a number of break throughs are needed, not only in individual process steps, but also in the balance and combination of these processes. This review paper gives an overview of the key technologies required and the advances achieved in recent years based on the concept of fractional conversions.

1. INTRODUCTION

Petrochemically, industrial ethanol is mainly produced through the acid-catalyzed hydration of ethylene. Ethanol for use in alcoholic beverages, and the vast majority of ethanol for use as biofuel, is produced by fermentation where certain species of yeast (e.g., Saccharomyces cerevisiae) or bacteria (e.g., Zymomonas mobilis) metabolize sugars in oxygen-lean conditions to produce ethanol and carbon dioxide. The exploitation and utilization of biomass energy have attracted much interest from the Indian government and governments around the world. Most of the remaining countries in the world collectively account for only 5% of the global bioethanol production, but India, China and Thailand are continuing to invest substantially in agricultural biotechnology and emerge as potential biofuel producers (Swart et al., 2008). It aims to replace 10% of gasoline production with biofuels by 20% by 2020 through out most of the world by the"Aggressive Biofuel Growth" scenario (Rosegrant et al., 2006). Ethanol has become an attractive alternative fuel as it can be blended with gasoline or used as neat alcohol in dedicated engines, because it has the higher octane number and higher heat of vaporization (Hahn-Hägerdal et al., 2006). Although bioethanol production has been greatly improved by new technologies, there are still challenges that need further investigations. The most controversial problem of transforming lignocellulosic raw materials into liquid fuel mainly presents in the economical feasibility attributing mainly to unilateral researches from own specialties of each researcher without regard to the characteristics of the straws themselves.

To overcome this problem, it is possible to produce bioethanol from variety of renewable agricultural sources. This paper gives an overview of the key technologies required and the advances achieved in recent years based on the concept of fractional conversion.

2. KEY TECHNOLOGIES FOR BIOETHANOL PRODUCTION FROM LIGNOCELLULOSIC BIOMASS

Lignocellulosic biomass is mainly composed of plant cell walls, with the structural carbohydrates cellulose and hemicellulose and heterogeneous phenolic polymer lignin as its primary components. However, their contents varies substantially, depending on the species, variety, climate, soil fertility and fertilization practice, but on average, for agricultural residues such as corn stover, wheat and rice straw, the cell walls contain about 40% cellulose, 30% hemicellulose and 15% ligin on a dry weight basis.

2.1. PRETREATMENT

Feed stock pretreatment is the major processing challenge in the ethanol production from lignocellulosic biomass. The lignocellulosic complex is a matrix of celluloseand lignin bound by hemicellulose chains.The objective of pretreatment is to increase the surface area and porosity of thesubstrate, reduce the crystallinity of cellulose and disrupt the heterogeneous structure ofcellulosic materials. Duringthe pretreatment, this matrix will be broken down in order to reduce the crystallinity degree of the cellulose and increase the fraction of amorphous cellulose, the most suitable form for enzymatic attack. The main part of hemicellulose is to be hydrolyzed and lignin is to be released or even degraded. The fact that the cellulose hydrolysis is affected by the porosity (accessible surface area) of lignocellulosic materials should also be considered.

2.1.1 PHYSICAL METHODS

Physical pretreatments do not use any chemicals. Size reduction by mechanical methods such as grinding or milling is one of them, through which the surface area of biomass is increased, and the degree of polymerization (DP) and crystallinity of cellulose is decreased to some extent, but the power requirement for reducing the feedstock from millimeter size to fine particles of micrometers is extremely high, which is unacceptable from the engineering point of view. Waste materials can be comminuted by a combinationof chipping, grinding and milling to reduce cellulosecrystallinity. This reduction facilitates the access of cellulases to the biomass surface increasing the conversion ofcellulose. The energy requirements of mechanical comminutionof lignocellulosic materials depend on the finalparticle size and biomass characteristics. Although, mechanical pretreatment methods increase cellulose reactivitytowards enzymatic hydrolysis, they are unattractivedue to their high energy and capital costs (Ghosh and Ghose, 2003). Pyrolysis has also been tested as a physicalmethod for pretreatment of lignocellulosic biomass since cellulose rapidly decomposes when is treated at hightemperatures.

2.1.2 PHYSICAL–CHEMICAL METHODS

Physical–chemical pretreatment methods are considerably more effective than physical. The steam explosion is the most studied method of this type. During this process, the use of saturated steam at high pressure causes auto hydrolysis reactions in which part of the hemicellulose and lignin are converted into soluble olygomers. The factors affecting steam explosion pretreatment are residence time, temperature, chip size and moisture content. To consider the combined action of both temperature and time over the performance of steam explosion pretreatment, the so-called severity index has been defined including a correction term when this process is carried out under acidic conditions (Shahbazi et al., 2005). In some cases (e.g. herbaceous waste), the use of very small particles is not desirable considering the economy of the process (Ballesteros et

al., 2002). This method is recognized as one of the most cost-effective for hardwood (poplar,oak, birch, maple) and agricultural residues, but is less efficient for soft wood (pine, cedar). Shahbazi et al. (2005) proposed a

fractionation procedure for soft wood based on steam explosion and alkaline delignification in order to produce ethanol and related co-products. Soderstromet al. (2003) propose a two-step steam pretreatment of soft wood by dilute-acid impregnation that includes a partial hydrolysis of cellulose during the second step. According to these authors, this variant of pretreatment is a promising method for increasing the overall yield during bioethanol production.

2.1.3 CHEMICAL METHODS

Chemical pretreatments employ different chemicalagents as ozone, acids, alkalis, peroxide and organic solvents. Inorganic acids as H_2SO_4 and HCl have been preferably used for biomass pretreatment. Hydrolysis with dilute sulfuric acid has been successfully developed given that high reaction rates can be achieved improving significantly the subsequent process of cellulose hydrolysis. In contrast, the costs of dilute acid pretreatment are higher than the corresponding ones of steam explosion process (Sun and Cheng, 2002). Schell et al. (2003) studied the dilute-acid pretreatment of corn stover at pilot plant level using high solid loads obtaining a xylose yield of 77% at190^0C. This pretreatment method was evaluated through a kinetic model that allowed the prediction of process conditions in order to maximize the yield. Similar kinetic studies were carried out for cane bagasse pretreated with nitric acid (Rodrı´guez-Chong et al., 2004) or without acid addition (Jacobsen and Wyman, 2002). Dilute acid pretreatment also can be accomplished in a two-stage way. For this, a first depolymerization stage of hemicellulose at 140^0C during 15 min is carried out in order to avoid the formation of furan compounds and carboxylic acids, followed by a second stage at 190^0C during 10 min to make cellulose more accessible to enzymatic hydrolysis (Saha et al., 2005). Alkaline pretreatment is based on the effects of the addition of dilute bases on the biomass: increase of internal surface by swelling, decrease of polymerization degree and crystallinity, destruction of links between lignin and other polymers, and breakdown of lignin. The effectiveness of this method depends on the lignin content of the biomass (Sun and Cheng, 2002). In general, the utilization of bases as sodium hydroxide or solvents such as ethanol or methanol (organosolv process) allows the dissolution of lignin, but their costs are so high that these methods are not competitive for large scale plants (Lynd et al., 1999).

2.1.4 LOW PRESSURE STEAM EXPLOSION TECHNOLOGY

Ghosh and Ghose (2003) reported the model process for bioethanol production proposed by Indian Institute of Technology (IIT) in Delhi (India). This process involves two pretreatment steps: steam explosion for xylose production followed by solvent pretreatment for delignification of biomass. The released pentoses are utilized for single cell protein production, where as the cellulose undergoes simultaneous saccharification and fermentation. Steam explosion is one of the most effective pretreatment technologies for breaking the crystalline structure of lignocellulose through chemical effects and mechanical forces attributed from sudden explosive decompression (Chen and Liu, 2007). During steam explosion pretreatment, hemicellulose is thought to be hydrolyzed by the acetic and other acids derived from acetyl groups at high temperatures. On the other hand, lignin is redistributed and to some extent removed from the material (Mosier et al., 2005; Pan et al., 2005). The removal of hemicelluloses is beneficial for exposing the cellulose surface and increasing enzyme accessibility to the cellulose micro fibrils (Alvira et al., 2010). Steam explosion of biomass is a pre-treatment process that opens up the fibers, and makes the biomass polymers more accessible for subsequent processes, i.e. fermentation, hydrolysis or densification processes. In general steam explosion is a process in which biomass is treated with hot steam (180 to 240 °C) under pressure (1 to 3.5 MPa) followed by an explosive decompression of the biomass that results in a rupture of the biomass fibers rigid structure. The sudden pressure release defibrillates the cellulose bundles and these results in a better accessibility of the cellulose for

enzymatic hydrolysis and fermentation. The steam explosion process offers several attractive features when compared to other pretreatment technologies including significantly lower environmental impact, less hazardous process chemicals, and greater potential for energy efficiency (Alvira et al.,2010).

2.1.5 COMBINED PRETREATMENT METHODS

Steam explosion has some limitations, such as the partial destruction of xylan, in complete disruption of the lignin-carbohydrate matrix, limited lignin removal, and lignin redistribution on the cellulose surfaces (Chen et al., 2008). With the aim of maximizing sugar recovery, some researchers have suggested a two-step steam explosion pretreatment by solubilizing hemicellulosic in the first step at low temperature. The cellulose fraction is then subjected to a second pretreatment step at temperatures higher than 210°C (Wingren et al., 2004). But, an economic evaluation is needed to determine the effectiveness of an additional steam explosion step (Alvira et al., 2010; Galbe and Zacchi, 2007). It is necessary to combine other methods with steam explosion to get the optimum pretreatment effect on lignocellulosic biomass.

It is well known that alkaline pretreatment provides an effective delignification and chemical swelling of the fibrous cellulose (Sahaand Cotta, 2006; Yang et al., 2002; Zhao et al., 2009). At the same time, the alkaline pretreatment can also cause condensation of lignin and modification of the crystal structure, which can introduce unwanted effects for lignin removal and cellulose degradation (Michael, 1985). It was reported by Yamashita et al (2010) that the maximum amountof reducing sugar extracted from bamboo by enzyme saccharification is 568 mg/(g initial dry sample) obtained in 1% (v/v) hydrogenperoxide and 1 wt.% sodium hydroxide.

2.1.6 BIOLOGICAL METHODS

Compared with physical and chemical pretreatments in which expensive equipment, chemicals and intensive energy consumption are needed, biological pretreatment by solid fermentation employs microorganisms that degrade lignocellulosic biomass at mild conditions without special requirements for equipment. Both bacteria and fungi have been explored, but rot fungi associated with wood decay are the predominant species in lignocellulose degradation for the purpose of biofuel production, particularly white-rot fungi due to their abundant ligninolytic enzymes, including lignin peroxidase, manganese peroxidase, laccases and other enzymes, and better selectivity in lignin degradation. Biological pretreatment has low energy requirements and mild environmental conditions. However, most of these processes are too slow limiting its application at industrial level. Many white-rot fungi degrade the ligninand, for this reason, they have been utilized for ligninases production and lignocellulose degradation. Lee (1997) reports the main microorganisms producing lignin degrading enzymes and indicates the fermentation processes for producing them by both submerged culture and solid-state fermentation.

2.2. CELLULASE PRODUCTION: SOLID-STATE FERMENTATION

Agricultural and industrial wastes are among the main causes of environmental pollution.Their conversion into useful products may reduce the intensity of the problems caused by them. These wastes include green gram husk, black gram husk, rice bran, wheat bran etc. are under utilized in India especially in Andhra Pradesh and Telangana. In most parts of A.P and T.S these materials are mainly used as animal feeds. A large quantity is left in farm lands to be decomposed by microorganisms such as bacteria and fungi (Okafor *et al*., 1987). Economically, the most important industrial material other than food stuffs affected by microorganisms are cellulose and wood products (Debing Jing *et al*., 2007). Proper utilization of these wastes in the environment will eliminate pollution and convert them into useful by products (Milala*et al*., 2005). Cellulose is commonly degraded by an enzyme called Cellulase. This enzyme is produced by several microorganisms, commonly by bacteria and fungi (Shin *et al*., 2000; Immanuel *et al*., 2006). Filamentous fungi are preferred for

commercially important enzymes production, because the level of the enzymes produced by these cultures is higher than those obtained from bacteria (Bakri *et al*., 2003).
Traditional SSF was operated under static conditions, resulting in poor heat and mass transfer effects: steep gaseous concentration gradients, and heat gradients (Raghavarao et al., 2003), which may adversely affect

solid-state fermentor. Forced aeration in SSF, such as agitation and rotation, were often carried out to improve mass and heat transfers, but the shear force from agitation and rotation has adverse effects on medium porosity and disruptsfungal mycelia (Chen et al., 2002; Marsh et al., 2000; Stuart et al.,1999). The gas double dynamic solid-state fermentation (GDD-SSF) reactor devised by our group, consisting of internal air circulation and periodic pulsation of air pressure, could not only provide sufficient O_2, but also provided more room for fungal propagation and improved heat transfer within the substrate (Chen et al., 2002).

2.3. ENZYMATIC HYDROLYSIS

Following pretreatment, enzymatic hydrolysis is needed to further depolymerize the cellulose component to glucose, which can be used for ethanol fermentation together with sugars released from the hydrolysis of hemicelluloses during the pretreatment. Despite intensive R & D worldwide for decades, two barriers still remains to be overcome for developing viable processes to make bioethanol economically competitive. Another basic method of hydrolysis is enzymatic hydrolysis. Enzymes are naturally occurring plant proteinsthat cause certain chemical reactions to occur. There aretwo technological developments: enzymatic and direct microbial conversion methods (Demirbas, 2005). Slow hydrolysis rates, exacerbated by the product inhibition, havebeen recognized as the major obstacle in achieving economical feasibility and commercial operation of the enzymatic hydrolysis ofcellulose. Enzymatic hydrolysis of natural lignocellulosic materialsis a very slow process because cellulose hydrolysis is hindered by structural parameters of the substrate, such as lignin and hemicellulose content, surface area, and cellulose crystallinity (Pan et al.,2006). Since enzymatic hydrolysis of native lignocellulose usually results in solubilization of V20% of the originally present glucan, some form of pretreatment to increase amenability to enzymatic hydrolysisis included in most process concepts for biological conversion of lignocellulose. Pre-treatment, under appropriate conditions, retains nearly all of the cellulose presentin the original material and allows close to theoretical yields upon enzymatic hydrolysis (Zhang et al., 2004). Utility cost of enzymatic hydrolysis is low compared toacid or alkaline hydrolysis because enzyme hydrolysis isusually conducted at mild conditions (pH 4.8 and temperature 318–323 K) and does not have a corrosion problem (Sun, 2002). Enzymatic hydrolysis is attractive because itproduces better yields than acid-catalyzed hydrolysis and enzyme manufacturers have recently reduced costs substantially using modern biotechnology (Pan et al., 2005). During the enzymatic hydrolysis of cellulosic substrates, several factors restrict the sustained catalytic activity of the cellulase mixture. It has been suggested that these limitations are owing to both substrate- and enzyme-related factors (Lu et al.,2002). The rate of enzymatic hydrolysis of the cellulosic materials always decreases rather quickly. Since Ghose and Kostic first coupled the fermentation reactor with a stirred tank type membrane module to retain the cellulase, several types of membrane bioreactor have been reported (Alfani et al., 1983; Ghoseand Kostick, 1970; Lucyna et al., 1998; Rios et al., 2004). However, a limitation of the membrane bioreactor system presently is the low concentration of reducing sugar, which has to be concentrated to reachthe optimal conditions for the subsequent fermentation, condensation,and distillation of ethanol (Yang, et al., 2006).

2.4. FERMENTATION AND PRODUCT RECOVERY

As biomass hydrolysis and fermentation technologies approach commercial viability, advancements in product recovery technologies will be required. For cases in which fermentation products are more volatile than water, recovery by distillation is often the technology of choice. Distillation technologies that will allow the economic recovery of dilute volatile products from streams containing a variety of impurities have been

developed and commercially demonstrated (Madson et al.,2000). Lignocellulose is often hydrolyzed by acid treatment; the hydrolysate obtained is then used for bioethanol fermentationby microorganisms such as yeast. Because suchlignocellulose hydrolysate contains not only glucose, butalso various monosaccharides, such as xylose, mannose, galactose, arabinose, and oligosaccharides, microorganisms should be required to efficiently ferment these sugars for the successful industrial production of bioethanol (Katahira et al.,2006).Carbon

dioxide gas stripping coupling with activated carbon adsorption Product inhibition is one of the inherent problems associated with liquid biofuel (such as ethanol or butanol) fermentation, since the toxicity of ethanol limits the final product concentration. This results in high energy requirement for solvent distillation and high wastewater pollution. Therefore, various alternative techniques to remove ethanol or butanol from the broth online have been investigated to increase the sugar solution concentration and reduce butanol inhibition. These methods include liquid–liquid extraction (Ishizakiet al., 1999), pervaporation (Liu et al., 2005), membrane distillation (Banat and Al-Shannag, 2000), and gas stripping (Ezeji et al., 2003, 2004; Qureshi and Blaschek, 2001).Gas stripping is a simple technique which does not require expensive apparatus, harm the culture, remove nutrients, or affect reaction intermediates, but reduces butanol toxicity (inhibition) (Qureshi and Blaschek, 2001). Inert gases used for ethanol or butanol gas stripping include nitrogen, carbon dioxide, hydrogen, and others (Ezeji et al., 2004; Qureshi and Blaschek, 2001).

4. CONCLUSIONS

Conversion from lignocelluloses to bioethanol holds great potentialdue to the wide spread availability, abundance, and relatively low costof cellulosic materials. There are still several hindrances on the way ofdeveloping an economically feasible technology, due to the complicated structure and in homogeneous nature of the raw material. The economical and environmentally-friendly development of bioethanol from lignocellulose requires highly efficient process integration.Considerable successes on pretreatment, enzymatic hydrolysis, fermentation, and separation of ethanol have been achieved over the past few decades. Many new ideas, such as biorefinery and the concept of oriented conversion of classified composition, have been proposed and practiced in many ethanol plants using lignocellulose as raw material. By an intelligent combination of pretreatment, hydrolysis, fermentation and product separation, the maximum efficacy and benefit of process can be achieved due to the simultaneous production of manyhigh-value co-products with ethanol from agricultural residues

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