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Review paper

Hydrolysis of lignocellulosic materials for ethanol production: a review ☆

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Abstract

Lignocellulosic biomass can be utilized to produce ethanol, a promising alternative energy source for the limited crude oil. There are mainly two processes involved in the conversion: hydrolysis of cellulose in the lignocellulosic biomass to produce reducing sugars, and fermentation of the sugars to ethanol. The cost of ethanol production from lignocellulosic materials is relatively high based on current technologies, and the main challenges are the low yield and high cost of the hydrolysis process. Considerable research efforts have been made to improve the hydrolysis of lignocellulosic materials. Pretreatment of lignocellulosic materials to remove lignin and hemicellulose can significantly enhance the hydrolysis of cellulose. Optimization of the cellulase enzymes and the enzyme loading can also improve the hydrolysis. Simultaneous saccharification and fermentation effectively removes glucose, which is an inhibitor to cellulase activity, thus increasing the yield and rate of cellulose hydrolysis. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Cellulase; Cellulose; Ethanol; Fermentation; Hydrolysis; Lignocellulosic biomass; Pretreatment

1. Introduction

Energy consumption has increased steadily over the last century as the world population has grown and more countries have become industrialized. Crude oil has been the major resource to meet the increased energy demand. Campbell and Laherrere (1998) used several different techniques to estimate the current known crude oil reserves and the reserves as yet undiscovered and concluded that the decline in worldwide crude oil production will begin before 2010. They also predicted that annual global oil production would decline from the current 25 billion barrels to approximately 5 billion barrels in 2050. Because the economy in the US and many other nations depends on oil, the consequences of inadequate oil availability could be severe. Therefore, there is a great interest in exploring alternative energy sources.

Unlike fossil fuels, ethanol is a renewable energy source produced through fermentation of sugars. Ethanol is widely used as a partial gasoline replacement in the US. Fuel ethanol that is produced from corn has been used in gasohol or oxygenated fuels since the 1980s. These gasoline fuels contain up to 10% ethanol by volume. As a result, the US transportation sector now consumes about 4540 million liters of ethanol annually, about 1% of the total consumption of gasoline (Wang et al., 1999). Recently, US automobile manufacturers have announced plans to produce significant numbers of flexible-fueled vehicles that can use an ethanol blend – E85 (85% ethanol and 15% gasoline by volume) – alone or in combination with gasoline. Using ethanol-blended fuel for automobiles can significantly reduce petroleum use and exhaust greenhouse gas emission (Wang et al., 1999). Ethanol is also a safer alternative to methyl tertiary butyl ether (MTBE), the most common additive to gasoline used to provide cleaner combustion (McCarthy and Tiemann, 1998). MTBE is a toxic chemical compound and has been found to contaminate groundwater. The US Environmental Protection Agency recently announced the beginning of regulatory action to eliminate MTBE in gasoline (Browner, 2000). However, the cost of ethanol as an energy source is relatively high compared to fossil fuels. A dramatic increase in ethanol

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Table 1
The contents of cellulose, hemicellulose, and lignin in common agricultural residues and wastes[a]

Lignocellulosic materials	Cellullose (%)	Hemicellulose (%)	Lignin (%)
Hardwoods stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	25–35
Nut shells	25–30	25–30	30–40
Corn cobs	45	35	15
Grasses	25–40	35–50	10–30
Paper	85–99	0	0–15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15–20	80–85	0
Cotton seed hairs	80–95	5–20	0
Newspaper	40–55	25–40	18–30
Waste papers from chemical pulps	60–70	10–20	5–10
Primary wastewater solids	8–15	NA[b]	24–29
Swine waste	6.0	28	NA[b]
Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12.0

[a] Source: Reshamwala et al. (1995), Cheung and Anderson (1997), Boopathy (1998) and Dewes and Hünsche (1998).
[b] NA – not available.

production using the current cornstarch-based technology may not be practical because corn production for ethanol will compete for the limited agricultural land needed for food and feed production. A potential source for low-cost ethanol production is to utilize lignocellulosic materials such as crop residues, grasses, sawdust, wood chips, and solid animal waste.

Extensive research has been completed on conversion of lignocellulosic materials to ethanol in the last two decades (Dale et al., 1984; Wright, 1998; Azzam, 1989; Cadoche and López, 1989; Reshamwala et al., 1995; Bjerre et al., 1996; Duff and Murray, 1996). The conversion includes two processes: hydrolysis of cellulose in the lignocellulosic materials to fermentable reducing sugars, and fermentation of the sugars to ethanol. The hydrolysis is usually catalyzed by cellulase enzymes, and the fermentation is carried out by yeasts or bacteria. The factors that have been identified to affect the hydrolysis of cellulose include porosity (accessible surface area) of the waste materials, cellulose fiber crystallinity, and lignin and hemicellulose content (McMillan, 1994). The presence of lignin and hemicellulose makes the access of cellulase enzymes to cellulose difficult, thus reducing the efficiency of the hydrolysis. The contents of cellulose, hemicellulose, and lignin in common agricultural residues are listed in Table 1. Removal of lignin and hemicellulose, reduction of cellulose crystallinity, and increase of porosity in pretreatment processes can significantly improve the hydrolysis (McMillan, 1994).

2. Pretreatment of lignocellulosic materials

The effect of pretreatment of lignocellulosic materials has been recognized for a long time (McMillan, 1994). The purpose of the pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials. Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis; (2) avoid the degradation or loss of carbohydrate; (3) avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes; and (4) be cost-effective. Physical, physico-chemical, chemical, and biological processes have been used for pretreatment of lignocellulosic materials.

2.1. Physical pretreatment

2.1.1. Mechanical comminution

Waste materials can be comminuted by a combination of chipping, grinding and milling to reduce cellulose crystallinity. The size of the materials is usually 10–30 mm after chipping and 0.2–2 mm after milling or grinding. Vibratory ball milling has been found to be more effective in breaking down the cellulose crystallinity of spruce and aspen chips and improving the digestibility of the biomass than ordinary ball milling (Millet et al., 1976). The power requirement of mechanical comminution of agricultural materials depends on the final particle size and the waste biomass characteristics (Cadoche and López, 1989). A comparison is shown in Table 2.

2.1.2. Pyrolysis

Pyrolysis has also been used for pretreatment of lignocellulosic materials. When the materials are treated at temperatures greater than 300 °C, cellulose rapidly decomposes to produce gaseous products and residual char (Kilzer and Broido, 1965; Shafizadeh and Brad-

Table 2
Energy requirement of mechanical comminution of agricultural lignocellulosic materials with different size reduction (Cadoche and López, 1989)

Lignocellulosic materials	Final size (mm)	Energy consumption (kWh/ton)	
		Knife mill	Hammer mill
Hardwood	1.60	130	130
	2.54	80	120
	3.2	50	115
	6.35	25	95
Straw	1.60	7.5	42
	2.54	6.4	29
Corn stover	1.60	NA[a]	14
	3.20	20	9.6
	6.35	15	NA[a]
	9.5	3.2	NA[a]

[a] NA – not available.

bury, 1979). The decomposition is much slower and less volatile products are formed at lower temperatures. Mild acid hydrolysis (1 N H_2SO_4, 97 °C, 2.5 h) of the residues from pyrolysis pretreatment has resulted in 80–85% conversion of cellulose to reducing sugars with more than 50% glucose (Fan et al., 1987). The process can be enhanced with the presence of oxygen (Shafizadeh and Bradbury, 1979). When zinc chloride or sodium carbonate is added as a catalyst, the decomposition of pure cellulose can occur at a lower temperature (Shafizadeh and Lai, 1975).

2.2. *Physico-chemical pretreatment*

2.2.1. *Steam explosion (autohydrolysis):*

Steam explosion is the most commonly used method for pretreatment of lignocellulosic materials (McMillan, 1994). In this method, chipped biomass is treated with high-pressure saturated steam and then the pressure is swiftly reduced, which makes the materials undergo an explosive decompression. Steam explosion 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. The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis. Ninety percent efficiency of enzymatic hydrolysis has been achieved in 24 h for poplar chips pretreated by steam explosion, compared to only 15% hydrolysis of untreated chips (Grous et al., 1986). The factors that affect steam explosion pretreatment are residence time, temperature, chip size and moisture content (Duff and Murray, 1996). Optimal hemicellulose solubilization and hydrolysis can be achieved by either high temperature and short residence time (270 °C, 1 min) or lower temperature and longer residence time (190 °C, 10 min) (Duff and Murray, 1996). Recent studies indicate that lower temperature and longer residence time are more favorable (Wright, 1998).

Addition of H_2SO_4 (or SO_2) or CO_2 in steam explosion can effectively improve enzymatic hydrolysis, decrease the production of inhibitory compounds, and lead to more complete removal of hemicellulose (Morjanoff and Gray, 1987). The optimal conditions of steam explosion pretreatment of sugarcane bagasse have been found to be as following: 220 °C; 30 s residence time; water-to-solids ratio, 2; and 1% H_2SO_4 (Morjanoff and Gray, 1987). Sugar production was 65.1 g sugar/100 g starting bagasse after steam explosion pretreatment.

The advantages of steam explosion pretreatment include the low energy requirement compared to mechanical comminution and no recycling or environmental costs. The conventional mechanical methods require 70% more energy than steam explosion to achieve the same size reduction (Holtzapple et al., 1989). Steam explosion is recognized as one of the most cost-effective pretreatment processes for hardwoods and agricultural residues, but it is less effective for softwoods (Clark and Mackie, 1987). Limitations of steam explosion include destruction of a portion of the xylan fraction, incomplete disruption of the lignin–carbohydrate matrix, and generation of compounds that may be inhibitory to microorganisms used in downstream processes (Mackie et al., 1985). Because of the formation of degradation products that are inhibitory to microbial growth, enzymatic hydrolysis, and fermentation, pretreated biomass needs to be washed by water to remove the inhibitory materials along with water-soluble hemicellulose (McMillan, 1994). The water wash decreases the overall saccharification yields due to the removal of soluble sugars, such as those generated by hydrolysis of hemicellulose. Typically, 20–25% of the initial dry matter is removed by water wash (Mes-Hartree et al., 1988).

2.2.2. *Ammonia fiber explosion (AFEX)*

AFEX is another type of physico-chemical pretreatment in which lignocellulosic materials are exposed to liquid ammonia at high temperature and pressure for a