ACID-BASED HYDROLYSIS PROCESSES FOR ETHANOL FROM LIGNOCELLULOSIC MATERIALS: A REVIEW

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Bioethanol is nowadays one of the main actors in the fuel market. It is currently produced from sugars and starchy materials, but lignocelluloses can be expected to be major feedstocks for ethanol production in the future. Two processes are being developed in parallel for conversion of lignocelluloses to ethanol, "acid-based" and "enzyme-based" processes. The current article is dedicated to review of progress in the "acid-based-hydrolysis" process. This process was used industrially in the 1940s, during wartime, but was not economically competitive afterward. However, intensive research and development on its technology during the last three decades, in addition to the expanding ethanol market, may revive the process in large scale once again. In this paper the ethanol market, the composition of lignocellulosic materials, concentrated- and dilute-acid pretreatment and hydrolysis, plug-flow, percolation, counter-current and shrinking-bed hydrolysis reactors, fermentation of hexoses and pentoses, effects of fermentation inhibitors, downstream processing, wastewater treatment, analytical methods used, and the current commercial status of the acid-based ethanol processes are reviewed.

Keywords: Lignocellulosic materials, Dilute-acid hydrolysis, Ethanol, Fermentation

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INTRODUCTION

Ethanol is nowadays an important product in the fuel market. Its market grew from less than a billion liters in 1975 to more than 39 billion liters in 2006 and is expected to reach 100 billion liters in 2015 (Licht 2006). Less than 4% of the ethanol is produced synthetically from oil, while the rest is produced by fermentation from bioresources. Ethanol is now produced from two major groups of bioresources: sugar substances and starchy materials. There is a competition between these two feedstocks for fuel ethanol production. While sugar substances were the feedstock for more than 60% of fuel ethanol production at the beginning of the 2000s, its share decreased to 47% by 2006, when grains accounted for 53% of the production (Licht 2006).

Ethanol has a potential market as big as the oil market. It can potentially replace the entire fuel market for gasoline. Furthermore, plastics such as polyethylene can be produced from ethanol through ethylene. However, the amounts of sugar substances and grains are limited in the world. They are relatively expensive feedstocks for ethanol production, and ethanol competes with human food for these raw materials. This competition may lead the price of grains and sugar to higher levels in the future.

Furthermore, the economy of the ethanol production process from grains depends on the market for its by-product, i.e. distillers' dried grains with solubles (DDGS) as animal food, which may not expand like the ethanol market in the future. In addition to the price, there is concern about damaging forests by increasing farming area as a result of more ethanol production from, for instance, sugar cane in Brazil.

Lignocellulosic materials are renewable, largely unused, and abundantly available sources of raw materials for the production of fuel ethanol. Lignocellulosic materials can be obtained at low cost from a variety of resources, e.g. forest residues, municipal solid waste, waste paper, and crop residue resources (Wyman 1996). These materials contain sugars polymerized in form of cellulose and hemicellulose, which can be liberated by hydrolysis and subsequently fermented to ethanol by microorganisms (Millati et al. 2002; Palmqvist and Hahn-Hägerdal 2000).

COMPOSITION OF LIGNOCELLULOSIC MATERIALS AND THEIR HYDROLYZATES

Lignocellulosic materials predominantly contain a mixture of carbohydrate polymers (cellulose and hemicellulose), lignin, extractives, and ashes. The term "holocellulose" is often used to describe the total carbohydrate contained in a plant or microbial cell. Holocellulose is therefore comprised of cellulose and hemicellulose in lignocellulosic materials.

Cellulose is an unbranched linear polymer. The length of a cellulose molecule (polymer) is determined by the number of glucan units in the polymer, referred to as the degree of polymerization. The degree of polymerization of cellulose depends on the type of plants and typically is estimated to be from 2000 to 27000 glucan units. Hemicelluloses belong to a group of heterogeneous polysaccharides. The amount of hemicellulose is usually between 11% and 37% of the lignocellulosic dry weight. Hemicelluloses are relatively easily hydrolyzed by acids to their monomer components consisting of xylose, mannose, glucose, galactose, arabinose, and small amounts of rhamnose, glucuronic acid, methyl glucuronic acid, and galacturonic acid (Morohoshi 1991; Sjöström 1993).

Lignin is a very complex molecule constructed of phenylpropane units linked in a three-dimensional structure. Generally, softwoods contain more lignin than hardwoods. Although the principal structural elements in lignin have been largely clarified, many aspects of its chemistry remain unclear. Chemical bonds have been reported between lignin and hemicellulose and even cellulose. Lignins are extremely resistant to chemical and enzymatic degradation (Palmqvist and Hahn-Hägerdal 2000; Taherzadeh 1999).

Extractives are wood compounds that are soluble in neutral organic solvents or water. The extractives usually represent a minor fraction (between 1-5%) of lignocellulosic materials. They contain a large number of both lipophilic and hydrophilic constituents. The extractives can be classified in four groups: (a) terpenoids and steroids, (b) fats and waxes, (c) phenolic constituents, and (d) inorganic components (Sjöström 1993; Taherzadeh 1999).

The amounts of the carbohydrate polymers and lignin depend on the type of material. Garrote et al. (1999) and Wyman (1996) have compiled the compositions of lignocelluloses from different hardwoods, softwoods, and agricultural residues reported in publications. The hardwoods such as white birch, aspen, red maple, *Eucalyptus*, *Populus*, and oak contain 39-54% cellulose, 14-37% hemicellulose, and 17-30% lignin. The corresponding values for softwoods, e.g. pines and firs, are 41-50% cellulose, 11-27% hemicellulose, and 20-30% lignin. The composition of different agricultural residues varies widely. For instance, rice straw consists of 32-47% cellulose, 19-27% hemicellulose, and 5-24% lignin.

The carbohydrate polymers in the lignocellulosic materials need to be converted to simple sugars before fermentation, through a process called hydrolysis. However, several products can result from hydrolysis (Fig. 1). There are several possible methods to hydrolyze lignocelluloses. The most commonly applied methods can be classified in two groups: chemical hydrolysis and enzymatic hydrolysis. Cellulose and hemicellulose can be converted to ethanol, while lignin remains as a by-product:

$$\text{Cellulose} \xrightarrow{\text{Hydrolysis}} \text{Glucose} \xrightarrow{\text{Fermentation}} \text{Ethanol}$$

$$\text{Hemicellulose} \xrightarrow{\text{Hydrolysis}} \text{Pentoses\&Hexoses} \xrightarrow{\text{Fermentation}} \text{Ethanol}$$

In addition, there are some other hydrolysis methods in which no chemicals or enzymes are applied. For instance, lignocelluloses may be hydrolyzed by gamma-ray or electron-beam irradiation, or microwave irradiation. However, these processes are far from being commercially applied (Taherzadeh 1999).

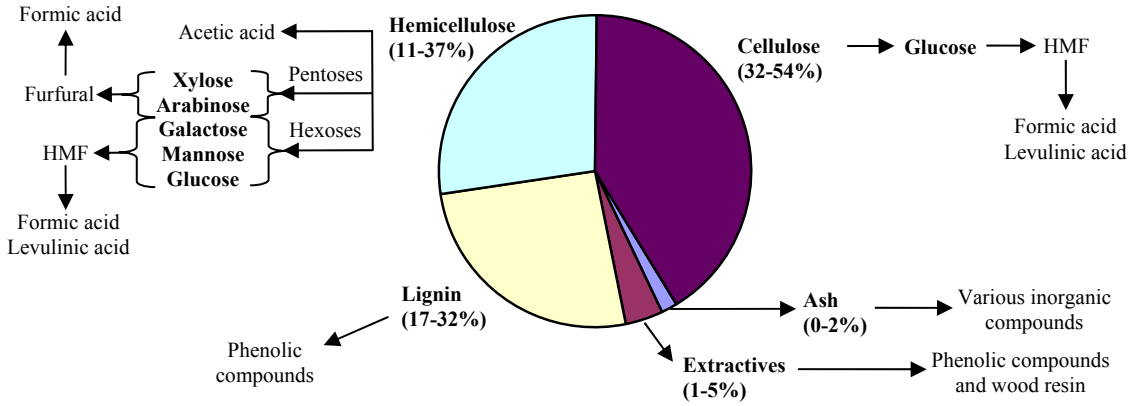

Fig. 1. Composition of lignocellulosic materials and their potential hydrolysis products

OVERALL PROCESS OF ETHANOL PRODUCTION FROM LIGNOCELLULOSIC MATERIALS BY CHEMICAL HYDROLYSIS

A generally simplified representation of the process for ethanol production from lignocellulosic materials by chemical hydrolysis is shown in Fig. 2. The lignocellulosic raw materials are milled initially to sizes of a few millimeters, and then they are hydrolyzed to obtain fermentable sugars. However, several by-products may be formed or released in this step. If highly toxic hydrolyzates are formed, a detoxification stage is necessary prior to fermentation. The hydrolyzates are then fermented to ethanol in bioreactors. The ethanol is distilled to 90-95% purity by distillation. If fuel ethanol is desired, it should be further dehydrated to >99% by e.g. molecular sieves, to enable its blending with gasoline.

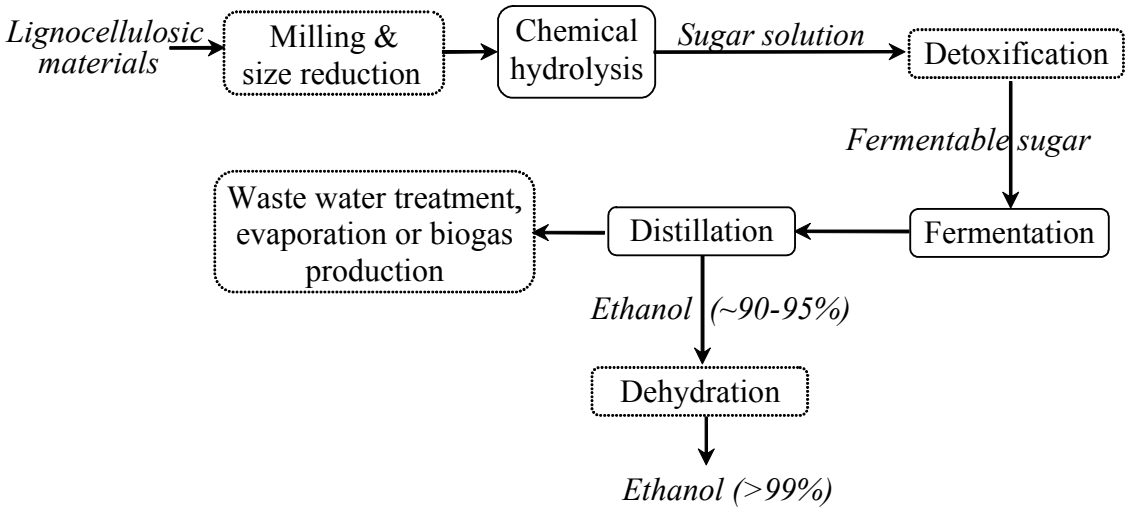

Fig. 2. Overall process scheme for ethanol production from lignocellulosic materials

CHEMICAL HYDROLYSIS

Hydrolysis involves cleaving the polymers of cellulose and hemicellulose into their monomers. Complete hydrolysis of cellulose results in glucose, whereas the hemicellulose gives rise to several pentoses and hexoses. While softwood hemicellulose is mainly composed of mannose, the dominant sugar in hemicellulose derived from hardwood and crop residues is usually xylose (Karimi et al. 2006b; Taherzadeh et al. 1997a). The hydrolysis can be carried out chemically or enzymatically.

Chemical hydrolysis involves exposure of lignocellulosic materials to a chemical for a period of time at a specific temperature, and results in sugar monomers from cellulose and hemicellulose polymers. Acids are predominantly applied in chemical hydrolyses. Sulfuric acid is the most investigated acid (Harris et al. 1945), although other acids such as HCl (Hashem and Rashad 1993) have also been used. Acid hydrolyses can be divided into two groups: (a) concentrated-acid hydrolysis and (b) dilute-acid

hydrolysis. A comparison between concentrated- and dilute-acid hydrolysis methods is presented in Table 1.

Table. 1. Comparison between Concentrated- and Dilute-Acid Hydrolysis Methods

Hydrolysis method	Advantages	Disadvantages
Concentrated-acid process	- Operated at low temperature - High sugar yield	- High acid consumption - Equipment corrosion - High energy consumption for acid recovery - longer reaction time (e.g. 2-6 h)
Dilute-acid process	- Low acid consumption - Short residence time	- Operated at high temperature - Low sugar yield - Equipment corrosion - Formation of undesirable by-products

Concentrated-Acid Hydrolysis

Hydrolysis of lignocelluloses by concentrated sulfuric or hydrochloric acids is a relatively old process. Braconnot in 1819 first discovered that cellulose can be converted to fermentable sugar by concentrated acids (Sherrard and Kressman 1945). Concentrated single-stage hydrolysis with sulfuric acid, and concentrated hydrolysis by liquid or vapor phase with hydrochloric acid were used.

Concentrated-acid processes are generally reported to give higher sugar yield (e.g. 90% of theoretical glucose yield) and consequently higher ethanol yield, compared to dilute-acid processes. Furthermore, the concentrated-acid processes can operate at low temperature (e.g. 40°C), which is a clear advantage compared to dilute-acid processes. However, the concentration of acid is very high in this method (e.g. 30-70%), and dilution and heating of the concentrated acid during the hydrolysis process make it extremely corrosive. Therefore, the process requires either expensive alloys or specialized non-metallic constructions, such as ceramic or carbon-brick lining. The acid recovery is an energy-demanding process. In addition, when sulfuric acid is used, the neutralization process produces large amounts of gypsum. Furthermore, the environmental impact strongly limits the application of hydrochloric acid. The high investment and maintenance costs have greatly reduced the potential commercial interest of this process (Jones and Semrau 1984; Katzen et al. 1995; Wyman 1996).

Despite the disadvantages, the concentrated-acid process is still of interest. It has recently been exploited by a Dutch research group in a process called "Biosulfurol." In this process, the biomass is impregnated with 70% sulfuric acid and subsequently hydrolyzed by adding water. The acid is then recovered partly by anion membranes, and partly in the form of H_2S from anaerobic wastewater treatment. The process was claimed to have a low overall cost for the ethanol produced (van Groenestijn et al. 2006). Furthermore, two full-scale cellulosics-to-ethanol projects are under development in North America by Arkenol Inc. (www.arkenol.com) and Masada Resource Group (www.masada.com) based on concentrated-acid hydrolysis.

Dilute-Acid Hydrolysis

Among the chemical hydrolysis methods, dilute-acid hydrolysis is probably the most commonly applied. It is a method that can be used either as a pretreatment preceding enzymatic hydrolysis, or as the actual method of hydrolyzing lignocellulose to the sugars (Qureshi and Manderson 1995). The first established dilute-acid hydrolysis process was probably the Scholler process (Faith 1945). This was a batch process, in which the wood material was kept in 0.5% sulfuric acid at 11-12 bar for approximately 45 minutes. Nowadays, most of dilute-acid hydrolysis processes are performed in a batch mode with a retention time of a few minutes (e.g. cf. Karimi et al. 2006b).

Batch reactors have been the most widely used reactors for kinetic study of hydrolysis and for laboratory and pilot study of ethanol production from lignocellulosic materials (e.g. Brandberg et al. 2005; Harris et al. 1984; Millati 2005; Purwadi et al. 2004; Saeman 1945; Sanchez et al. 2004; Sues et al. 2005; Taherzadeh et al. 1997a; Taherzadeh et al. 2000c). An example of a kinetic study for one-stage dilute-acid hydrolysis is presented in Fig. 3, where 0.5% sulfuric acid was used at temperatures of 188-234°C and a retention time of 7 minutes (Taherzadeh et al. 1997a). A major part of the hemicellulose (more than 80%) could be hydrolyzed by dilute-acid hydrolysis at temperatures less than 200°C, but the maximum overall glucose yield occurred at a hydrolysis temperature higher than 220°C. This is due to the larger recalcitrance of cellulose to hydrolysis.

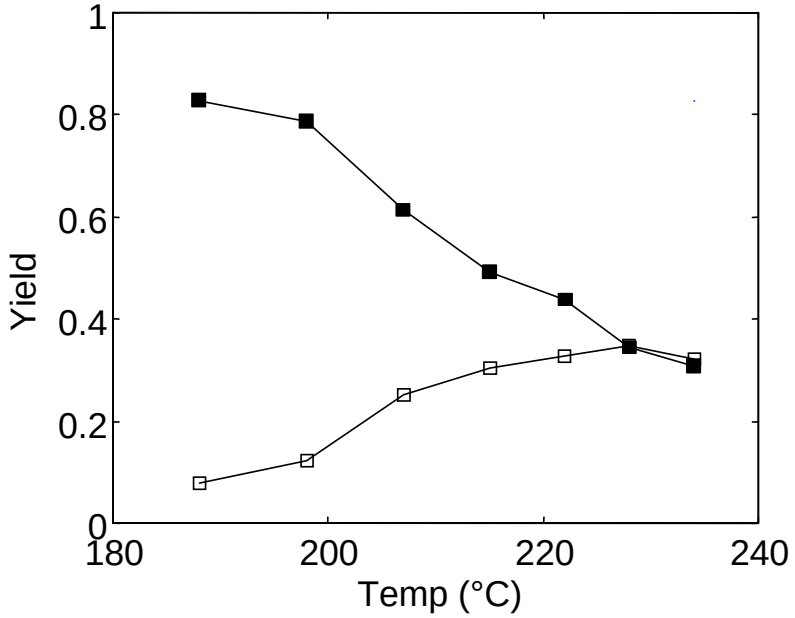

Fig. 3. Glucose yield from glucan (□) and mannose yield from mannan (■) in a one-step dilute-acid hydrolysis of (25% dry weight) spruce as a function of hydrolysis temperature (adapted from Taherzadeh 1999).

A main drawback of dilute-acid hydrolysis processes, particularly in one stage, is degradation of the sugars in hydrolysis reactions and formation of undesirable by-products. This not only lowers the yield of sugars, but also several of the by-products severely inhibit the formation of ethanol during the fermentation process. The possible

inhibitors that can be formed during the dilute-acid hydrolysis, and their original components, are illustrated in Fig. 1. Potential inhibitors are furfural, 5-hydroxymethylfurfural (HMF), levulinic acid, acetic acid, formic acid, uronic acid, 4-hydroxybenzoic acid, vanillic acid, vanillin, phenol, cinnamaldehyde, formaldehyde, etc. (Larsson et al. 2000; Taherzadeh 1999). Some inhibitors, such as terpene compounds, are initially present in the wood, but apparently most of the inhibitors are formed during the hydrolysis process.

In order to avoid degradation of monosaccharides at high temperatures and formation of the inhibitors, dilute-acid hydrolysis is carried out in two (or more) stages. In the first stage, which should be carried out under relatively mild conditions, hemicellulose is converted to sugar monomers. It is considered as equivalent to a dilute-acid pretreatment step. In the second stage, the residual solid is hydrolyzed under more severe conditions, allowing cellulose to be hydrolyzed (Harris et al. 1984). In a one-stage pretreatment, a temperature between 140 and 170°C can be used, but two treatments at about 120°C for a longer time may also be applied (Torget and Hsu 1994).

The "two-stage" dilute-acid process is usually preferred to one-stage dilute-acid hydrolysis because:

a) The separate stages for hydrolysis of the hemicellulose and cellulose should result in higher sugar yield. Furthermore, a product with high hexose sugar content can be obtained in the second-stage hydrolysis, which can easily be fermented to ethanol. Mixtures of pentose and hexose are usually problematic for fermentation, because of the difficulty in fermentation of pentoses.
b) The energy consumption should be minimized, since liquid is removed before the second-stage hydrolysis.
c) The resulting sugar solution should be more concentrated.
d) Less sugar degradation from the hydrolyzed materials in the first stage leads to a higher overall yield of sugars.
e) Fewer fermentation-inhibiting components are formed during the two-stage hydrolysis.

Generally, the maximum yield of pentoses and hexoses recovered from hemicelluloses in the first stage of the hydrolysis is high (i.e. 80-95% of the total sugars available), while the yield of cellulose hydrolysis to glucose is usually low (e.g. 40-60%). However, a low yield of glucose may not be considered as a serious problem because of the low price of lignocellulosic materials, as well as the possibility of drying and burning the residual cellulose and lignin in an energy complex to produce electricity and heat.

Dilute-Acid Hydrolysis Reactors and Processes

Batch reactors are traditionally used for hydrolysis of lignocellulosic materials in lab and pilot plants. However, research also has been carried out concerning development of other reactor types for this purpose. In this section, we review plug flow, percolation, countercurrent, and shrinking-bed reactors for hydrolysis of lignocellulosic materials by the dilute-acid processes.

Plug flow reactor

The CSTR (continuous stirred-tank reactor) and plug flow reactor are generally used in continuous processes. The CSTR has not been of significant interest for the hydrolysis of lignocellulosic materials, possibly due to problems associated with mixing of very high solid content material, the sealing of the stirrer in a high-pressure reactor, and the high energy demand for mixing. However, there have been some studies concerning continuous hydrolysis in plug flow reactors (Church and Wooldridge 1981).

Thompson and Grethlein (1979) developed an isothermal plug flow reactor to study the kinetics of acid hydrolysis of cellulosic substrates. The kinetic parameters in a model that gives glucose formation from purified cellulose (Solka-Floc) were obtained at temperatures from 180 to 240°C, sulfuric acid concentrations from 0.5 to 2.0%, and slurry concentrations from 5.0 to 13.5%. It was determined that the glucose formation from newsprint can be predicted from the kinetic model developed for Solka-Floc. It was found that at least 50% of the potential glucose can be obtained at 240°C, 1% acid, and 0.22 minutes of residence time. This was a significant improvement in yield of conversion of cellulose to glucose, compared to the batch reactor, where the maximum yield was only 40%. Moreover, the retention time is significantly shorter in plug flow reactors. Further improvement of plug flow reactors has been reported by McParland et al. (1982), who obtained 55-58% glucose yield at 240°C and only 6 seconds residence time.

The plug flow reactor, having less than 30 seconds residence time and higher yield of glucose (e.g. 50-60%) compared to batch reactors, might be a process of commercial interest. However, no more improvement may be forthcoming for this system, since it has two inherent problems: difficulty in controlling the retention time in the range of a few seconds, and heat transfer limitation within the biomass particles (Lee et al. 1999).

Percolation reactor

The percolation reactor is a packed-bed flow-through reactor. There are certain advantages in this reactor in comparison to batch and plug flow reactors for hydrolysis of lignocellulosic materials. Lower sugar decomposition can result, since the sugar product is removed immediately after formation in this reactor. A high concentration of sugar can be produced by using a packed-bed reactor, since a high solid/liquid ratio can be used. Furthermore, unlike the batch and plug flow reactors, this process does not need a solid/liquid separation (Lee et al. 1999).

In a study by the National Renewable Energy Laboratory (NREL) in the USA, a continuously flowing, two-stage percolation reactor resulted in more than 95% of the theoretical yield of solubilized xylose sugars (monomeric and oligomeric) from yellow poplar sawdust (Torget and Hsu 1994). They studied the two-temperature (140/170°C) dilute-acid prehydrolysis of hybrid poplar using a percolation process, and found soluble xylose to be 92% of the theoretical value and 2% of the xylan to be degraded to furfural. Acid-impregnated steam explosion (0.4% sulfuric acid, 200-230°C, 1-5 min) was reported to solubilize 90-95% of the hemicellulose from softwood, and 90% of the remaining cellulose can be hydrolyzed by cellulase enzymes (Nguyen et al. 1998).

Percolation reactors were used in most of the old wood sugar processes such as the Scholler process, the Madison wood sugar process, and some of the Soviet processes

(Lee et al. 1999). Two methods of modification have been applied in percolation reactors: two-stage step-change and two-stage reverse-flow. These processes involved a first treatment stage at a low temperature followed by a second stage at a high temperature. In two-stage step-change percolation reactors, the fresh biomass is first treated at a low temperature in the first reactor, followed by treatment in the second reactor at a higher temperature. In two-stage reverse-flow percolation reactors, the treated biomass at low temperature is treated at a high temperature, then the liquid is passed through the second reactor filled with fresh biomass at the low temperature (Kim et al. 1993; Lee et al. 1999; Torget and Hsu 1994).

Countercurrent reactor

A countercurrent reactor is a moving-bed reactor in which the directions of solid and liquid are reversed. The major part of the sugar is produced near the liquid outlet point of the reactor. Countercurrent flow of liquids and solids minimizes sugar degradation and product dilution by removing the sugars from the reaction zone before substantial degradation can occur, and consequently raising the yield and concentration of sugar with minimal inhibitory formation.

The progressing-batch reactor is one of the attempts that is based on a countercurrent scheme. A progressing-batch reactor uses several percolation reactors in series to simulate countercurrent flows, while retaining the simplicity of the percolation reactor. Wright et al. (1987) made the first attempt at design of such systems. They used seven percolating reactors: one reactor for filling, another for emptying, and five in operation.

Shrinking-bed reactor

Bed shrinking is a phenomenon that can improve the reactor performance. Bed shrinking occurs in a packed-bed reactor due to partial solubilization of the solid biomass during the reaction. This reactor has a fixed and a movable end; the movable end is supported by a compressed spring. Incorporation of a spring-loaded plunger into the packed-bed reactor is intended to continuously reduce the bed depth as the reaction progresses. It reduces the liquid throughput, which results in raising the sugar concentration. This method has resulted in sugar yields higher than 95% from hemicellulose and 85% from cellulose in a multiple percolation reactor system simulating countercurrent operation (Lee et al. 2000).

Kim et al. (2001) studied the kinetics of cellulose hydrolysis using batch and shrinking-bed reactors. The maximum yield of glucose obtained from batch reactor experiments was about 60% for pure cellulose, whereas the maximum glucose yields from yellow poplar feedstock were subsequently lower, falling in the range of 26-50%. In experiments using the shrinking-bed hydrolysis reactor, glucose yields of 87.5, 90.3, and 90.8% of the theoretical yield were obtained for yellow poplar feedstocks at 205, 220, and 235°C, respectively. Furthermore, the hydrolysis times for glucan were about three times lower with the shrinking-bed reactor than with the batch reactor. What causes this difference is not yet fully understood, and further research is necessary to investigate the details of the reaction mechanism of these heterogeneous catalytic reactions.

EFFECTIVE PARAMETERS IN HYDROLYSIS OF LIGNOCELLULOSIC MATERIALS

Lignocellulosic materials and hydrolysis processes are very complicated. Factors influencing the yields of the lignocellulose to the monomeric sugars and the by-products are:

(a) Properties of the Substrate

The properties of the substrate can affect the hydrolysis. These properties are: neutralizing capacity, proportion of easily hydrolyzable hemicellulose and cellulose, amount and rate of hydrolysis of the difficult-to-hydrolyze materials, the length of the macromolecules, degree of polymerization of cellulose, configuration of the cellulose chain, and association of cellulose with other protective polymeric structures within the plant cell wall such as lignin, pectin, hemicellulose, proteins, mineral elements, etc. Particle size is also one of the effective parameters (Kosaric et al. 1983; Taherzadeh et al. 1997a).

(b) The Acidity of the System

Another parameter affecting the hydrolysis is the acidity of system. The acidity is dependent on the type and concentration of acid used, amount of acid solution, amount of acid (e.g. acetic acid) released from the biomass during hydrolysis, liquid to solid ratio, the neutralizing capacity of the lignocellulose, and movement of the solution during heating. When dilute-acid hydrolysis is applied in a continuous process such as a screw-fed co-current reactor, it requires a relatively short residence time. Therefore, the penetration of acid catalyst into the biomass, as well as dispersion in the reactor, can significantly affect the overall reaction, and consequently the reactor performance. Diffusivity of sulfuric acid is dependent on the nature of the lignocellulosic materials. It has been shown that the diffusivity of sulfuric acid is significantly higher in agricultural residues than in hardwood (Kim and Lee 2002).

(c) Rate of Decomposition of Hydrolysis Products during Hydrolysis

The rate of decomposition of the products during the hydrolysis process depends on temperature, acidity, reaction time, and the concentration of sugars. Under hydrolysis conditions that produce a solution containing in excess of 10 percent glucose, reversion phenomena are suggested to be very important. The reversion phenomena result in much of the glucose being present not as free glucose but as dimers, oligomers, and anhydrosugars which are unavailable to the microorganisms used in fermentation (Harris et al. 1984). It was recently reported that metals and/or metal ions can also catalyze glucose decomposition during the acid hydrolysis of lignocellulosic materials. Thus, the material used in the construction of the hydrolysis reactor should also be carefully selected (Xiang et al. 2004).

FERMENTATION OF THE HYDROLYZATES

Fermentation of the lignocellulosic hydrolyzates is more difficult than the well-established processes of ethanol production from *e.g.* sugar-cane juice or grains. Hydrolyzates contain a broader range of inhibitory compounds, whose composition and concentration depend on the type of lignocellulosic materials and on the chemistry and nature of the pretreatment and hydrolysis processes. Secondly, the hydrolyzates of hemicelluloses contain not only hexoses but also pentoses, where xylose is the dominant sugar in the hydrolyzates from hardwood hemicelluloses (Nigam 2001; Pessoa et al. 1997; Vanzyl et al. 1988; Wyman 1996). Therefore, the fermenting microorganism should be able to produce ethanol from the hydrolyzates with a high yield and productivity, withstand potential inhibitors, and produce ethanol from pentoses, as well as being safe for humans. Baker's yeast (*Saccharomyces cerevisiae*) is the most commercially used microorganism for ethanol production, but it cannot ferment xylose (Björling and Lindman 1989; Hahn-Hägerdal et al. 1988; Ho et al. 1998; Jeffries 2006; Jeppsson et al. 2002; Sreenath and Jeffries 2000). A large number of yeasts, bacteria, and filamentous fungi are reported to produce ethanol as the main fermentation product, and have been reviewed in literature (Ingram et al. 1998; Jeffries 2006; Lin and Tanaka 2006; Olsson and Hahn-Hägerdal 1996).

The effects of the inhibitory compounds, different fermentation techniques, and the xylose-fermenting microorganisms are discussed briefly here.

(a) The Effects of Inhibitory Compounds on Fermentation

The by-products mentioned earlier inhibit fermentation by different mechanisms. As a function of conditions and method of hydrolysis, different inhibitors may dominate in terms of concentrations. However, a combination of the action of several substances is the reason for observed inhibition (Clark and Mackie 1984). Furthermore, it is not only the quantitatively dominant inhibitors that determine the fermentability of a hydrolyzate. The toxicity of a hydrolyzate is found to differ from that of a synthetic medium with the same amount of the major hydrolyzate inhibitors added, indicating the importance of other inhibitors present in trace amounts (Taherzadeh et al. 1999).

Acetic acid, formic acid, and levulinic acid are the most common carboxylic acids found in the hydrolyzates. Acetic acid is not only a by-product of hydrolysis (Schneider 1996), but is also a well-known by-product of fermentation (Oura 1977). Acetic acid is mainly formed from acetylated sugars in the hemicellulose, which are cleaved off already under mild hydrolysis conditions. Therefore, the acetic acid yield in the hydrolysis does not significantly depend on the severity of the hydrolysis process, and acetic acid can be formed at concentrations even higher than 10 g/l (Taherzadeh et al. 1997b). It is generally accepted that the effect of the undissociated part of the acid is larger than the effect of the dissociated part (Thomas et al. 2002). The undissociated carboxylic acids can diffuse through the cell membrane, dissociate there, and decrease the internal pH (Gottschalk 1987; Verduyn et al. 1992). It is reported that the baker's yeasts can tolerate up to about 5 g/l undissociated concentration of acetic acid (Taherzadeh et al. 1997b).

The rate of sugar decomposition under dilute-acid hydrolysis conditions (e.g. 0.8% acid and 180°C) is ordered as follows (Xiang et al. 2004):

Xylose> Arabinose> Mannose> Galactose> Glucose

Therefore, the xylose is more sensitive to the acidity and high temperature conditions, and is decomposed to furfural. Glucose is more resistant to harsh conditions.

Furfural and HMF are the only furans usually found in hydrolyzates in significant amounts (Taherzadeh et al. 1997a). Furfural has been found to inhibit the *in vitro* activity of several important enzymes in the primary carbon catabolism, such as hexokinase, aldolase, phosphofructokinase, triosephosphate dehydrogenase, and alcohol dehydrogenase. Among these enzymes, the latter two appear to be the most sensitive (Banerjee et al. 1981). However, the inhibition of certain non-glycolytic enzymes, such as pyruvate dehydrogenase and aldehyde dehydrogenase, is even more severe (Modig et al. 2002). Consequently, cell growth is more sensitive to the presence of furfural than is the ethanol production from glucose. Furfural can be converted by the yeast to less inhibitory compounds, furfuryl alcohol and furoic acid (Taherzadeh et al. 2000c).

HMF is not as severely toxic to *S. cerevisiae* as furfural (Taherzadeh et al. 2000b). This is in line with the observation that the *in vitro* inhibition of the enzymes pyruvate dehydrogenase and aldehyde dehydrogenase is lower by HMF than by furfural. On the other hand, the conversion rate of furfural is about 4 times faster than that of HMF. Hence, HMF remains much longer than furfural in the medium, and consequently, the effects of HMF last longer than those of furfural. It should be mentioned that early in the hydrolysis, HMF is the major degradation product of hexoses, but subsequently levulinic and formic acids predominate (Harris et al. 1984).

A large number of phenolic/aromatic compounds have been detected in dilute-acid hydrolyzates (Larsson 2000; Palmqvist and Hahn-Hägerdal 2000). These are believed to be degradation products of lignin during the hydrolysis. However, the aromatic compounds may also form as a result of sugar degradation and are present in lignocelluloses as extractives. Larsson (2000) studied the influence of lignocellulose-derived aromatic compounds on oxygen-limited growth and ethanol production by *S. cerevisiae.* The influence of hydroxymethoxybenzaldehydes, diphenols/quinones, and phenylpropane derivatives on *S. cerevisiae* cell growth and ethanol formation was assayed using a defined medium and oxygen-limited conditions. The inhibitory effect of the hydroxymethoxybenzaldehydes was highly dependent on the positions of the substituents. A major difference in inhibition by the oxidized and reduced form of a diphenol/quinone was reported, with the oxidized form being more inhibitory. Transformations of aromatic compounds occur during the fermentation, including aldehyde reduction, quinone reduction, and double bond saturation. Aromatic alcohols were detected as products of reductions of the corresponding aldehydes, namely hydroxymethoxybenzaldehydes and coniferyl aldehyde. High molecular mass compounds and the corresponding diphenol have been detected as products of quinone reduction. Together with coniferyl alcohol, dihydroconiferyl alcohol was identified as a major transformation product of coniferyl aldehyde (Larsson 2000). Among the phenolic compounds, vanillin and syringaldehyde are the important inhibitors. However, they can be assimilated by *S. cerevisiae* in the fermentation process (Delgenes et al. 1996), and

conversion of vanillin to vanillyl alcohol by *Klebsiella pneumoniae* has also been reported (Nishikawa et al. 1988).

It should be noticed that reported concentrations of the phenolic/aromatic compounds are normally a few milligrams per liter (Larsson et al. 1999). This could be due to the low water solubility of many of the phenolic compounds, or to a limited degradation of lignin during the hydrolysis process. Among the phenolic compounds, less heavily substituted phenolics are probably the most inhibitory materials in the hydrolyzates (Clark and Mackie 1984; Nishikawa et al. 1988).

(b) Fermentation Techniques

Several of the inhibitory compounds found in hydrolyzates can be biotransformed, or, in a few cases, even be fully metabolized by yeast. Conversion occurs for several of the carboxylic acids, furans, and phenolic compounds. This suggests that continuous *in situ* detoxification of the hydrolyzate during the fermentation might be possible. However, it requires a suitable mode of operation, and the bioconversion of inhibitors must be taken into account in the design of the process.

In batch processes of ethanol production, the microorganism works in a high substrate concentration initially and a high product concentration finally. Generally, batch fermentations are characterized by low productivity, and they are labor-intensive. Batch cultivation is not a suitable method for cultivation of lignocellulosic hydrolyzates, since a high concentration of the inhibitors at the beginning of fermentation deactivates the yeasts and stops the process. The "fed-batch" technique is a promising method for the fermentation of dilute-acid hydrolyzates, and its application for this purpose has recently been studied (Nilsson et al. 2001; 2002; Taherzadeh et al. 2000a; Taherzadeh et al. 1999). The basic concept behind the success of this technique is the capability of *in situ* detoxification by the cells. Since the yeast has a limited capacity for the conversion of the inhibitors, the achievement of a successful fermentation strongly depends on the feed rate of the hydrolyzate. At too high a feed rate, using an inhibiting hydrolyzate, both ethanol production and cell growth can be expected to stop, whereas at a very low feed rate the hydrolyzate may still be converted but at a very low productivity, which was experimentally confirmed (Taherzadeh et al. 1999). Consequently, there should exist an optimum feed rate (or dilution rate) specific to a particular hydrolyzate. If the hydrolyzate is only slightly inhibitory, then a high feed rate can be applied. On the other hand, for a severely inhibiting hydrolyzate, a low feed rate is necessary to prevent build-up of the concentration of inhibitors in the bioreactor to levels that completely stop the cellular metabolism. Therefore, an optimum feed rate should be provided to the bioreactor by e.g. an adaptive control of the process (Nilsson et al. 2001; 2002; Taherzadeh 1999; Taherzadeh et al. 2000c).

"Continuous cultivation" is the third mode of operation for fermentation of hydrolyzates. The major drawback of continuous fermentation is that inhibitors present in the medium will limit the specific growth rate of the cells. This will result in wash-out of the bioreactor, unless a very low dilution rate is applied, giving a very low productivity. Furthermore, at a very low dilution rate the conversion rate of the inhibitors can be expected to decrease, due to the decreased specific growth rate of the biomass. Thus, wash-out may occur even at very low dilution rate. Cell retention by "immobilization,"

"encapsulation," "filtration," and "cell recirculation" by using e.g. centrifuges or flocculating organisms are solutions to overcome the wash-out problem in continuous cultivation of dilute-acid hydrolyzates. Millati (2005) immobilized cells of *S. cerevisiae* entrapped in Ca-alginate to continuously ferment hydrolyzates. The method was then further developed by Purwadi (2006), and completed with cultivation with flocculating yeast. Cell recirculation was examined by Brandberg et al. (2005). Continuous fermentation of wheat-supplemented lignocellulose hydrolyzate with different types of cell retention was compared by Brandberg et al. (2007). Encapsulation of *S. cerevisiae* was reported to be a powerful means for continuous cultivation of toxic dilute-acid hydrolyzates (Talebnia and Taherzadeh, 2006). All of these methods indicate new possibilities of using fermentation with high-cell-density cultivation.

(c) Pentose Fermentation

An important factor in production of ethanol from lignocellulosic materials is efficient fermentation of the sugars available in hydrolyzates to ethanol with a high yield and high productivity rate. Although a large number of yeasts, bacteria, and filamentous fungi can produce ethanol as the main fermentation product, none of these microorganisms naturally meets all the requirements for the lignocellulosic ethanol production. One of the major challenges in this field is fermentation of xylose. *S. cerevisiae*, which is the most widely microorganism in ethanol production, does not have genes encoded for xylose reductase (XR) and xylitol dehydrogenase (XDH) and cannot utilize xylose (Jeffries and Jin 2004; Karhumaa et al. 2006; Katahira et al. 2004; Kötter and Ciriacy 1993; Jeppsson et al. 2002; van Maris et al. 2006). There have therefore been intensive efforts to introduce other wild type organisms that can utilize xylose and produce ethanol or genetically modify the organism for this purpose. The result of these intensive efforts is to obtain microorganisms that are able to produce ethanol anaerobically from xylose with high yield (e.g. more than 0.40 g/g sugars) and high productivity of more than 1.0 g/g.h (van Maris et al. 2006). The challenge is therefore now to introduce these strains in large-scale ethanol production from lignocellulosic feedstocks that are rich in xylose.

Organisms for producing ethanol from xylose

There are several naturally occurring ethanol-producing bacteria, yeast and fungi that utilize xylose. The yeast species *Candida, Pichia, Schizosaccharomyces, Kluveromyces* and *Pachysolen*, fungi of species *Fusarium, Mucor, Rhyzopus, Monilia*, and *Paecilomyces*, and bacteria of species *Clostridium, Bacillus, Bacteroides, Thermoanaerobacter*, and *Ervinia* are mentioned as ethanol producers from xylose (Abbi et al. 1996; Flores et al. 2000; Olsson and Hahn-Hägerdahl 1996; Jeffries and Kurtzman 1994; Millati et al. 2005; Sreenath and Jeffries 2000; Zaldivar et al. 2001). In research on ethanol production from pentoses and sugar-cane bagasse hemicellulose hydrolyzate, Ueng and Gong (1982) used *Mucor* and *Fusarium* species. Both of these are able to ferment various sugars and alditols, including glucose, pentoses and xylitol, to ethanol. *Mucor* can ferment sugar-cane bagasse hemicellulose hydrolyzate to ethanol, while *Fusarium* cannot ferment this hydrolyzate to ethanol. Zygomycetes fungi such as *Mucor indicus* and *Rhizopus oryzae* have recently been explored and showed good potential to

be used for ethanol production from xylose and other sugars in lignocellulosic hydrolyzates (Karimi et al. 2005; Karimi et al. 2006a; Millati 2005).

Genetic modification of the strains

Several attempts have been made to genetically modify *S. cerevisiae* and other microorganisms in order to produce ethanol from both hexoses and pentoses. There are some excellent reviews in this field (e.g. c.f. Jeffries 2006; van Maris et al. 2006). Several research groups tried to express either genes encoding for XR and XDH for assimilation of xylose through xylitol or xylose isomerase for direct conversion of xylose to xylulose in *S. cerevisiae*. Low ethanol yield and productivity from xylose, imbalance of NADH, and sensitivity to oxygen presence were among the big challenges in this field (van Maris et al. 2006). However, there are now engineered strains that are able to produce ethanol from xylose anaerobically with good yield of e.g. 0.41 g/g and productivity of e.g. 1.2 g/g.h (Kuyper et al. 2005).

Other strains than *S. cerevisiae* also have been engineered for ethanol production from xylose. The method principally involves addition and expression of all the genes that are not present in the genome of the microorganisms and are necessary to run the pathway from the sugars to ethanol. For example, the genes for xylose isomerase, xylulokinase, transketolase, and transladolase were inserted into bacteria *Zymomonas mobilis*, which resulted in an organism producing ethanol from xylose (Zhang et al. 1995). Ingram and his group added the genes for pyruvate decarboxylase and alcohol dehydrogenase to *Escherichia coli* (Ohta et al. 1991). The resulting recombinant was able to produce 41 g/l ethanol from 80 g/l xylose with a productivity of 0.87 g/l.h. Furthermore, Ingram et al. (1999) made a similar recombination to *Klebsiella oxytoca*, which is natively able to ferment cellobiose and cellotriose, in order to eliminate the need for β-glucosidase. The strain was further recombinated to contain endoglucanase. Recently, Japanese Research Institute of Innovative Technology for the Earth (RITE) developed a microorganism for ethanol production. RITE strain is claimed to be an engineered strain of *Corynebacterium glutamicum* that converts both pentose and hexose sugars into alcohol. They engineered the central metabolic pathway of *C. glutamicum* to produce ethanol. A recombinant strain which expressed the *Z. mobilis* gene encoding for pyruvate decarboxylase and alcohol dehydrogenase was constructed (Inui et al. 2004). Among the different fermentation parameters, xylose-utilizing cultures are known to be very sensitive to dissolved oxygen concentration. A low and well-controlled level of oxygenation is required for efficient ethanol production from xylose by most of the xylose-fermenting microorganisms. A certain level of oxygen is necessary to maintain cell viability, xylose transport, and high ethanol productivity. While many of the pentose-fermenting microorganisms rapidly lose viability without sufficient oxygen, excess oxygen completely stops ethanol production, and the cells respire the substrate to form biomass (Franzen 1997; Lee 1997; Toivari et al. 2001; Wyman 1996).

Production of ethanol from other carbon sources in lignocellulosic hydrolyzates (e.g. L-arabinose, galacturonic acid and L-rhamnose) may require extensive metabolic engineering. While xylose is the most abundant pentose sugar in the hemicellulosic fraction of biomass, L-arabinose is also present in appreciable amounts. L-Arabinose fermentation, based on the expression of a prokaryotic pathway in *S. cerevisiae*, has also

been established, but needs further optimization before it can be considered for industrial implementation. An emerging and major challenge is to achieve the rapid transition from proof-of-principle experiments under 'academic' conditions (synthetic media, single substrates or simple substrate mixtures, absence of inhibitory components) towards efficient conversion of complex industrial substrate mixtures that contain synergistically acting inhibitors (van Maris et al. 2006).

(d) Adaptation of Microorganisms

Increases in fermentation rate and ethanol yield by adaptation of microorganisms to the fermentation media have been reported in some studies (Amartey and Jeffries 1996; Oliinichuk et al. 1986). Adaptation of the microorganism to the lignocellulosic hydrolyzate was suggested as an alternative approach to detoxification.

Martin et al. (2007) reported that adaptation of a xylose-utilizing genetically engineered strain of *S. cerevisiae* to sugar-cane bagasse hydrolyzates can increase its tolerance against phenolic compounds, furaldehydes, and aliphatic acids, and lead to improved performance with respect to ethanol production. Amartey and Jeffries (1996) showed that adaptation of *P. stipitis* to corncob acid-hydrolyzed hemicellulose resulted in a significantly higher fermentation rate.

Adaptation has been shown to increase the ability of a broad range of yeast strains to grow in lignocellulosic hydrolyzates. Adaptation of *P. stipitis* CBS 5776 by repeated recycling in an acid hydrolyzate from aspen resulted in increased productivity from 0.60 to 0.85 g/g.h and increased yield from 0.32 to 0.45 g/g. In another study, *P. stipitis* CBS 5776 was adapted to a detoxified hydrolyzate (red oak acid hydrolyzate) and was then able to ferment an undetoxified hydrolyzate with a larger inoculum, giving an ethanol yield of 0.30 g/g. The unadapted *P. stipitis* could not ferment the undetoxified hydrolyzate at all (Olsson and Hahn-Hägerdal 1996). At Nanjing Forestry University, extensive work on the adaptation of *C. shehatue* to Spent Sulfite Liquor (SSL) was carried out, resulting in strains that can tolerate high temperature (38°C), high acetic acid concentration (15 g/l), and a high pentose fraction (70%) (Olsson and Hahn-Hägerdal 1996). However, the ability to adapt *S. cerevisiae* to lignocellulosic hydrolyzates was shown to be strain-dependent. For instance, Keating et al. (2006) did not observe any improvement in adaptation of *S. cerevisiae* to SSL.

The adapted strains are seldom deposited in culture collections; they have only been used in individual laboratories, and verification of the performance of these strains is difficult. In addition, the stability of the adapted strains may present a problem. Isolation of strains from natural or industrial habitats has been reported to be a useful technique for finding strains with suitable properties for cultivating lignocellulosic hydrolyzates. A strain of *S. cerevisiae* isolated from a SSL fermentation plant was shown to be able to utilize glucose and galactose simultaneously in the presence of acetic acid, in contrast to the behavior of baker's yeast. As an alternative to adaptation and isolation from harsh environments, genetic engineering might improve the microorganism to better withstand a specific inhibitor; however, this can only be attempted if the inhibiting mechanism is known (Olsson and Hahn-Hägerdal 1996).

DOWNSTREAM PROCESSING

Product Recovery

The fermentation broth (mash or beer) is a mixture of ethanol, water, cell mass, fusel oil, and other components available in the fermentation media such as residual sugars, nonfermentable sugars, and hydrolysis by-products. The concentration of ethanol produced from dilute-acid hydrolyzate is typically low (e.g. 10-35 g/l), since the total concentration of sugar is usually low (e.g. 20-80 g/l). Furthermore, it is difficult to work with highly concentrated hydrolyzates, since the concentration of inhibitors will increase by concentrating the hydrolyzates. On the other hand, if one could get rid of this problem, then the ethanol concentration in mash would be generally up to 10%. The cells can tolerate this level of ethanol concentration at 30°C, but their tolerance decreases with increasing temperature (Hamelinck et al. 2005).

Ethanol can be purified by distillation to a concentration just below its azeotropic point, i.e. 95%, which will be called "hydrated ethanol" (Hamelinck et al. 2005). However, the solid particles and fermenting microorganisms should be separated before distillation, by e.g. centrifuges or decanters. On the other hand, if flocculant yeast is used, then the natural capacity of these microorganisms for aggregation and flocculation can eliminate the need for separation of biomass and reduce the investment costs. Hydrated ethanol can be employed in high-ethanol-content fuel (e.g. E95). However, for mixing of the ethanol with gasoline, the ethanol should contain no more than 1% of water (anhydrous ethanol). Molecular-sieve technology is the common method for production of anhydrous ethanol.

Residual Solids and Wastewater Treatment

The main solid residual from the process is lignin. Its amount and quality depend on the feedstock used and the process applied. The lignin and remaining solid materials can be burned to produce steam for the process (hydrolysis, distillation, and evaporation), electricity, and perhaps central heat (Hamelinck et al. 2005). This is possible due to the high energy value of the lignin that is released during its combustion (Cardona and Sanchez 2007). However, it can also be processed through e.g. gasification and Fischer-Tropsch process to produce synthesis gas and hydrocarbon fuel additives. Lignin can replace phenol in the widely used phenol formaldehyde resins, although the production costs and market value of these products are problematic.

The stillage, or wastewater remaining after distillation, exhibits a considerable pollution potential (Callander et al. 1986). Most of the components in the stillage of ethanol production from lignocellulosic materials originate from the soils on which these materials are grown, and therefore should be returned to soil. However, application of untreated stillage to standing pasture can result in phytotoxicity (Wilkie et al. 2000). The residual water contains significant amounts of organic compounds such as acetic acid, furfural, HMF, residual sugars, and other components, and needs processing before its disposal into the environment. Wilkie et al. (2000) reviewed the characterization of ethanol stillage from several lignocellulosic materials and compared them with stillage from conventional feedstocks. Generally, the characteristics of stillage from cellulosic materials appear to be comparable to those of conventional feedstocks (e.g. sugar cane

and corn), and, therefore, methods of stillage treatment and utilization applied to conventional feedstocks might also be applicable to cellulosic feedstocks. Two possible exceptions to the similarity of cellulosic and conventional stillage characteristics which deserve attention are the potential for higher levels of heavy metals from the acid hydrolysis processes and the presence of unusual inhibitors, such as hardwood extractives, associated with phenolic compounds present in the feedstock. However, the limited number of studies on stillage from different cellulosic feedstocks and hydrolysis methods means that predictions of treatment performance are prone to error.

A solution may be to recirculate the wastewater as much as possible and then concentrate the rest by multistage evaporation and incinerate the final concentrated wastewater. However, the evaporation requires a significant amount of energy that can have a negative impact on the energy balance of ethanol production (Faust et al. 1983; Wilkie et al. 2000). Running the process at higher solids consistency or with recirculated process streams, to maintain a high concentration of ethanol and dissolved solids, can reduce the energy requirements in the distillation and evaporation units. However, the effect of this stream circulation on the fermentation and stream handling should be examined. Finally, the evaporator condensate can undergo aerobic or anaerobic biological treatment or burning. The stillage can be used for production of single-cell protein or other viable biological products such as enzymes, chitosan, astaxanthin, plant hormones, and the biopolymers alternan and pullulan.

Aerobic and anaerobic digestion can serve as an effective means for removing COD from stillage and converting it to biogas, which is another biofuel. However, the presence of inhibitors in the stillage can cause some difficulties in biological digestion of stillage.

Reducing the color of stillage, in addition to COD reduction and nutrient removal, might be required in order to discharge treated stillage into surface waters without degrading water quality (Wilkie et al. 2000). The presence of phenolic compounds that are released during the degradation of lignin, melanoidins from Maillard reaction of sugars with proteins, caramels from overheated sugars, and furfural and HMF from acid hydrolysis, can make the cellulosic stillage's color more severe than that of other stillages.

Analysis of Lignocellulosic Materials and Acid Hydrolyzates

The accurate compositional analysis of biomass is crucial in order to commercialize the technology for biomass to ethanol conversion. However, the analysis of lignocellulosic materials depends on the method of analysis and also on the sample preparation. A number of approaches involve milling a dried sample, and regrinding or discarding fines can cause major errors, since the fines can differ in composition and reactivity from the larger particles (Wyman 1996). It should be kept in mind that different parts of wood and agricultural residue have different composition.

The analysis of hydrolyzate and fermentation broths based on lignocellulosic hydrolyzates is not straightforward, since the solution contains a complex array of sugars, phenolic compounds, organic acids, furans, and other degradation products. Furthermore, the diversity of constituents in the hydrolyzates may interfere with the analysis. In addition, the ethanol produced during the process increases the solubility of hydrophobic

substances in the broth, so that the composition of the matrix continuously changes during fermentation (Olsson and Hahn-Hägerdal 1996; Taherzadeh 1999; Wyman 1996). Total reducing sugar (monosaccharide) concentration may be analyzed colorimetrically using a dinitrosalicylic acid (DNS) reagent (Miller 1959). The reducing concentrations can also be analyzed by Fehling titration (Xu et al. 2005). It is also possible to analyze total monosaccharides plus disaccharides concentration by the DNS or Fehling method, but the samples should be completely hydrolyzed by HCl before analysis. However, the presence of complex media can cause large errors in these methods. Chromatography seems to be the most reliable method for analyses of different sugars and furans in the hydrolyzate.

In research work, sugar utilization and solvent production in several fermentations of lignocellulosic hydrolyzates have been determined in two different ways: the sample components were determined using single-column liquid chromatography (CLC) analysis and with a combination of gas chromatographic analysis and calorimetric methods. The concentrations determined by the two methods were compared; the results for 15 out of 19 samples agreed within 80% similarity, but only 5 samples agreed within 95% similarity. Substantial differences in analysis results (a relative standard deviation of 12% in total sugar content) were also found when different laboratories altogether made 18 analyses of the same corncob hydrolyzates. One reason for the difference in analysis results is the lack of selectivity in the detection method employed (Olsson and Hahn-Hägerdal 1996).

During the development of analytical methods, careful evaluation of the selectivity is necessary. The use of two different detection principles may indicate impurities in the individual chromatographic peaks. In line with discussions of suitable fermentation alternatives, awareness of the difficulties and uncertainties in the analysis methods used for the determination of the fermentation characteristics is necessary. High-performance liquid chromatography (HPLC) seems to be the best and most reliable method for analysis of sugars, furfural, HMF, acetic acid, and fermentation products. However, usually the interactions between the different sugars' peaks in HPLC reduce the accuracy and reproducibility of this method for analyses of different sugars. Furthermore, separations of different peaks from the chromatograms are usually person-dependent and much care is needed for reliable and reproducible analyses of the sugars. Decomposition of some sugars such as sucrose is a typical problem in HPLC. When one analyzes the sucrose, a peak for glucose and another for fructose appears in some cases due to internal hydrolysis of the sugar. Other problems are the evaporation of ethanol during the fermentation, taking the sample, freezing and defrosting, and even during preparation of the standard samples used for calibration.

COMMERCIAL PROCESSES BASED ON THE ACID PROCESS

Pilot plant and commercial-scale facilities for converting lignocellulosic biomass to ethanol by chemical hydrolysis have existed since the early 1900s. One of the earliest commercial hydrolysis processes was a dilute-sulfuric-acid process developed by EWEN and TOMLINSO during World War I. Two plants in the United States producing sugars

from wood were operative. For economic reasons, both plants were closed at the end of World War I. Dilute sulfuric acid hydrolysis of wood was re-examined by the Forest Products Laboratory, U.S. Department of Agriculture, at the request of the War Production Board in 1943.

In total, more than twenty alcohol plants utilizing lignocelluloses were operated in Europe, Russia, China, Korea, and the US prior to or during World War II. These plants used either the Scholler dilute (0.2-1%) sulfuric acid process or the Bergius concentrated (40-45%) hydrochloric acid process for wood hydrolysis. Yields for both processes were similar, with up to 45% fermentable sugar (w/w) from coniferous sawdust or bark-free chips. The Scholler process was less capital intensive and was therefore preferred compared to the Bergius process. In this process, dilute-acid of 0.5-0.6% concentration was percolated down through the packed bed with continuous removal of 5-6% sugar solution. After hydrolysis, the lignin residue was filter pressed to 50% moisture and burned to satisfy the steam requirement of the plant (Maiorella 1983). Many of the plants were closed for economic reasons after World War II (Kosaric et al. 1983; Maiorella 1983). Today, only a few of these old plants are still operating, with virtually all of them in Russia (Badger 2002; Nguyen et al. 1996).

Tavda Hydrolysis Plant located in Russia is one of the industrial plants operating nowadays. The process is somewhat similar to the Scholler process, and it was established in 1943. In 1970, the plant was reconstructed, to give a design production capacity of 13.5 million liters per year, and it currently produces approximately 12.4 million liters per year. About 70% of the ethanol production is derived from wood-cellulose hydrolyzate, together with 20% derived from wheat-starch hydrolyzate and 10% from beet molasses. All of the three feedstocks are combined in a single fermentation process. A mixture of about 80% woodchips and 20% sawdust are processed in a dilute-acid hydrolysis process. The process is a multistage dilute-acid process under different pressures (6, 7, 9, 12, and 12.5 atmospheres) for a period of 100 minutes. The total fermentation time is 6-8 hours. The yeast used in the fermentation is a strain of the fission yeast *Schizosaccharomyces*. Further information can be found at www.distill.com.

Inventa AG (Switzerland) developed a dilute-acid hydrolysis process. The technology is based on the dilute-acid hydrolysis of wood, which produced annually 10 million liters of fuel grade ethanol, using locally available softwoods. The hydrolysis is performed on wood chips at 140-180°C with dilute acid (0.6 wt.%). Solid residue leaving the reactor is used as a fuel. The vaporized furfural is collected in a recovery section. The cooled hydrolyzate is then neutralized with limestone, and the gypsum is separated. The fermentation of hydrolyzate takes place in a series of fermenters. A yeast adapted to wood hydrolyzates is used. The mash, having an ethanol concentration of about 2%, is separated, and a concentrated yeast suspension is recycled to the fermenter. Fuel-grade ethanol produced is concentrated by distillation. The yield of alcohol is 240 liter per tone of wood dry matter. The plant operated until 1956 in Domat/Ems (Kosaric et al. 1983).

BC International Corporation (BCI) in the United States, and SEKAB in Sweden, are commercializing the new technologies based on the latest research and developments on two-stage dilute-acid hydrolysis. In the first stage, hemicellulose is hydrolyzed under milder conditions (e.g. 170-190°C), and cellulose is hydrolyzed under more severe conditions (200-230°C).

CONCLUDING REMARKS

Lignocellulosic materials are expected to be major feedstocks for ethanol production in the near future. However, the process of conversion of these materials is not as easy as conversion of sugar substances and starchy materials. Dilute-acid hydrolysis is one of the suitable methods for conversion of lignocellulosic materials to fermentable sugars. However, the process of hydrolysis is very complicated, depending on several parameters such as properties of the substrate, acidity, and rate of decomposition of the products during hydrolysis. Conversion of the hemicellulose present in lignocellulosic materials to simple sugars by dilute-acid hydrolysis can be easily carried out. However, obtaining a high yield of glucose from the cellulose part of lignocelluloses by dilute-acid hydrolysis is a challenge. A suitable reactor design can be applied in order to improve the yield of cellulose conversion by the dilute-acid process. Fermentation of a mixture of hexose and pentose and several major and minor inhibitors can introduce further problems. A suitable fermentation strategy, such as adaptive continuous or fed-batch processes, and employing a suitable natural or engineered microorganism that can convert both pentose and hexoses is necessary for the fermentation. The environmental impacts of an ethanol process should also be considered carefully. The residual solid materials and the wastewater should be further processed to produce several other products in an ethanol plant; otherwise they might create environmental problems.

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