period of time, and then the pressure is swiftly reduced. The concept of AFEX is similar to steam explosion. In a typical AFEX process, the dosage of liquid ammonia is 1–2 kg ammonia/kg dry biomass, temperature 90 °C, and residence time 30 min. AFEX pretreatment can significantly improve the saccharification rates of various herbaceous crops and grasses. It can be used for the pretreatment of many lignocellulosic materials including alfalfa, wheat straw, wheat chaff (Mes-Hartree et al., 1988), barley straw, corn stover, rice straw (Vlasenko et al., 1997), municipal solid waste, softwood newspaper, kenaf newspaper (Holtzapple et al., 1992a), coastal Bermuda grass, switchgrass (Reshamwala et al., 1995), aspen chips (Tengerdy and Nagy, 1988), and bagasse (Holtzapple et al., 1991). The AFEX pretreatment does not significantly solubilize hemicellulose compared to acid pretreatment (to be discussed in the following section) and acid-catalyzed steam explosion (Mes-Hartree et al., 1988; Vlasenko et al., 1997). Mes-Hartree et al. (1988) compared the steam and ammonia pretreatment for enzymatic hydrolysis of aspenwood, wheat straw, wheat chaff, and alfalfa stems, and found that steam explosion solubilized the hemicellulose, while AFEX did not. The composition of the materials after AFEX pretreatment was essentially the same as the original materials. Over 90% hydrolysis of cellulose and hemicellulose has been obtained after AFEX pretreatment of Bermuda grass (approximately 5% lignin) and bagasse (15% lignin) (Holtzapple et al., 1991). However, the AFEX process was not very effective for the biomass with high lignin content such as newspaper (18–30% lignin) and aspen chips (25% lignin). Hydrolysis yield of AFEX-pretreated newspaper and aspen chips was reported as only 40% and below 50%, respectively (McMillan, 1994).

To reduce the cost and protect the environment, ammonia must be recycled after the pretreatment. In an ammonia recovery process, a superheated ammonia vapor with a temperature up to 200 °C was used to vaporize and strip the residual ammonia in the pretreated biomass and the evaporated ammonia was then withdrawn from the system by a pressure controller for recovery (Holtzapple et al., 1992b). The ammonia pretreatment does not produce inhibitors for the downstream biological processes, so water wash is not necessary (Dale et al., 1984; Mes-Hartree et al., 1988). AFEX pretreatment does not require small particle size for efficacy (Holtzapple et al., 1990).

2.2.3. CO_2 explosion

Similar to steam and ammonia explosion pretreatment, CO_2 explosion is also used for pretreatment of lignocellulosic materials. It was hypothesized that CO_2 would form carbonic acid and increase the hydrolysis rate. Dale and Moreira (1982) used this method to pretreat alfalfa (4 kg CO_2/kg fiber at the pressure of 5.62 MPa) and obtained 75% of the theoretical glucose released during 24 h of the enzymatic hydrolysis. The yields were relatively low compared to steam or ammonia explosion pretreatment, but high compared to the enzymatic hydrolysis without pretreatment. Zheng et al. (1998) compared CO_2 explosion with steam and ammonia explosion for pretreatment of recycled paper mix, sugarcane bagasse, and repulping waste of recycled paper, and found that CO_2 explosion was more cost-effective than ammonia explosion and did not cause the formation of inhibitory compounds that could occur in steam explosion.

2.3. Chemical pretreatment

2.3.1. Ozonolysis:

Ozone can be used to degrade lignin and hemicellulose in many lignocellulosic materials such as wheat straw (Ben-Ghedalia and Miron, 1981), bagasse, green hay, peanut, pine (Neely, 1984), cotton straw (Ben-Ghedalia and Shefet, 1983), and poplar sawdust (Vidal and Molinier, 1988). The degradation was essentially limited to lignin and hemicellulose was slightly attacked, but cellulose was hardly affected. The rate of enzymatic hydrolysis increased by a factor of 5 following 60% removal of the lignin from wheat straw in ozone pretreatment (Vidal and Molinier, 1988). Enzymatic hydrolysis yield increased from 0% to 57% as the percentage of lignin decreased from 29% to 8% after ozonolysis pretreatment of poplar sawdust (Vidal and Molinier, 1988). Ozonolysis pretreatment has the following advantages: (1) it effectively removes lignin; (2) it does not produce toxic residues for the downstream processes; and (3) the reactions are carried out at room temperature and pressure (Vidal and Molinier, 1988). However, a large amount of ozone is required, making the process expensive.

2.3.2. Acid hydrolysis

Concentrated acids such as H_2SO_4 and HCl have been used to treat lignocellulosic materials. Although they are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive and hazardous and require reactors that are resistant to corrosion. In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible (Sivers and Zacchi, 1995).

Dilute acid hydrolysis has been successfully developed for pretreatment of lignocellulosic materials. The dilute sulfuric acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis (Esteghlalian et al., 1997). At moderate temperature, direct saccharification suffered from low yields because of sugar decomposition. High temperature in dilute acid treatment is favorable for cellulose hydrolysis (McMillan, 1994). Recently developed dilute acid hydrolysis

processes use less severe conditions and achieve high xylan to xylose conversion yields. Achieving high xylan to xylose conversion yields is necessary to achieve favorable overall process economics because xylan accounts for up to a third of the total carbohydrate in many lignocellulosic materials (Hinman et al., 1992). There are primarily two types of dilute acid pretreatment processes: high temperature (T greater than 160 °C), continuous-flow process for low solids loading (5–10% [weight of substrate/weight of reaction mixture]) (Brennan et al., 1986; Converse et al., 1989), and low temperature (T less than 160 °C), batch process for high solids loading (10–40%) (Cahela et al., 1983; Esteghlalian et al., 1997). Although dilute acid pretreatment can significantly improve the cellulose hydrolysis, its cost is usually higher than some physico-chemical pretreatment processes such as steam explosion or AFEX. A neutralization of pH is necessary for the downstream enzymatic hydrolysis or fermentation processes.

2.3.3. Alkaline hydrolysis

Some bases can also be used for pretreatment of lignocellulosic materials and the effect of alkaline pretreatment depends on the lignin content of the materials (Fan et al., 1987; McMillan, 1994). The mechanism of alkaline hydrolysis is believed to be saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components, for example, lignin and other hemicellulose. The porosity of the lignocellulosic materials increases with the removal of the crosslinks (Tarkow and Feist, 1969). Dilute NaOH treatment of lignocellulosic materials caused swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure (Fan et al., 1987). The digestibility of NaOH-treated hardwood increased from 14% to 55% with the decrease of lignin content from 24–55% to 20%. However, no effect of dilute NaOH pretreatment was observed for softwoods with lignin content greater than 26% (Millet et al., 1976). Dilute NaOH pretreatment was also effective for the hydrolysis of straws with relatively low lignin content of 10–18% (Bjerre et al., 1996). Chosdu et al. (1993) used the combination of irradiation and 2% NaOH for pretreatment of corn stalk, cassava bark and peanut husk. The glucose yield of corn stalk was 20% in untreated samples compared to 43% after treatment with electron beam irradiation at the dose of 500 kGy and 2% NaOH, but the glucose yields of cassava bark and peanut husk were only 3.5% and 2.5%, respectively.

Ammonia was also used for the pretreatment to remove lignin. Iyer et al. (1996) described an ammonia recycled percolation process (temperature, 170 °C; ammonia concentration, 2.5–20%; reaction time, 1 h) for the pretreatment of corn cobs/stover mixture and switchgrass. The efficiency of delignification was 60–80% for corn cobs and 65–85% for switchgrass.

2.3.4. Oxidative delignification

Lignin biodegradation could be catalyzed by the peroxidase enzyme with the presence of H_2O_2 (Azzam, 1989). The pretreatment of cane bagasse with hydrogen peroxide greatly enhanced its susceptibility to enzymatic hydrolysis. About 50% lignin and most hemicellulose were solubilized by 2% H_2O_2 at 30 °C within 8 h, and 95% efficiency of glucose production from cellulose was achieved in the subsequent saccharification by cellulase at 45 °C for 24 h (Azzam, 1989). Bjerre et al. (1996) used wet oxidation and alkaline hydrolysis of wheat straw (20 g straw/l, 170 °C, 5–10 min), and achieved 85% conversion yield of cellulose to glucose.

2.3.5. Organosolv process

In the organosolv process, an organic or aqueous organic solvent mixture with inorganic acid catalysts (HCl or H_2SO_4) is used to break the internal lignin and hemicellulose bonds. The organic solvents used in the process include methanol, ethanol, acetone, ethylene glycol, triethylene glycol and tetrahydrofurfuryl alcohol (Chum et al., 1988; Thring et al., 1990). Organic acids such as oxalic, acetylsalicylic and salicylic acid can also be used as catalysts in the organosolv process (Sarkanen, 1980). At high temperatures (above 185 °C), the addition of catalyst was unnecessary for satisfactory delignification (Sarkanen, 1980; Aziz and Sarkanen, 1989). Usually, a high yield of xylose can be obtained with the addition of acid. Solvents used in the process need to be drained from the reactor, evaporated, condensed and recycled to reduce the cost. Removal of solvents from the system is necessary because the solvents may be inhibitory to the growth of organisms, enzymatic hydrolysis, and fermentation.

2.4. Biological pretreatment

In biological pretreatment processes, microorganisms such as brown-, white- and soft-rot fungi are used to degrade lignin and hemicellulose in waste materials (Schurz, 1978). Brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. White-rot fungi are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials (Fan et al., 1987). Hatakka (1983) studied the pretreatment of wheat straw by 19 white-rot fungi and found that 35% of the straw was converted to reducing sugars by *Pleurotus ostreatus* in five weeks. Similar conversion was obtained in the pretreatment by *Phanerochaete sordida* 37 and *Pycnoporus cinnabarinus* 115 in four weeks. In order to prevent the loss of cellulose, a cellulase-less mutant of *Sporotrichum pulverulentum* was developed for the degradation of lignin in wood chips

(Ander and Eriksson, 1977). Akin et al. (1995) also reported the delignification of Bermuda grass by white-rot fungi. The biodegradation of Bermuda grass stems was improved by 29–32% using *Ceriporiopsis subvermispora* and 63–77% using *Cyathus stercoreus* after 6 weeks.

The white-rot fungus *P. chrysosporium* produces lignin-degrading enzymes, lignin peroxidases and manganese-dependent peroxidases, during secondary metabolism in response to carbon or nitrogen limitation (Boominathan and Reddy, 1992). Both enzymes have been found in the extracellular filtrates of many white-rot fungi for the degradation of wood cell walls (Kirk and Farrell, 1987; Waldner et al., 1988). Other enzymes including polyphenol oxidases, laccases, H_2O_2 producing enzymes and quinone-reducing enzymes can also degrade lignin (Blanchette, 1991). The advantages of biological pretreatment include low energy requirement and mild environmental conditions. However, the rate of hydrolysis in most biological pretreatment processes is very low.

3. Enzymatic hydrolysis of cellulose

Enzymatic hydrolysis of cellulose is carried out by cellulase enzymes which are highly specific (Béguin and Aubert, 1994). The products of the hydrolysis are usually reducing sugars including glucose. Utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 45–50 °C) and does not have a corrosion problem (Duff and Murray, 1996). Both bacteria and fungi can produce cellulases for the hydrolysis of lignocellulosic materials. These microorganisms can be aerobic or anaerobic, mesophilic or thermophilic. Bacteria belonging to *Clostridium*, *Cellulomonas*, *Bacillus*, *Thermomonospora*, *Ruminococcus*, *Bacteriodes*, *Erwinia*, *Acetovibrio*, *Microbispora*, and *Streptomyces* can produce cellulases (Bisaria, 1991). *Cellulomonas fimi* and *Thermomonospora fusca* have been extensively studied for cellulase production. Although many cellulolytic bacteria, particularly the cellulolytic anaerobes such as *Clostridium thermocellum* and *Bacteroides cellulosolvens* produce cellulases with high specific activity, they do not produce high enzyme titres (Duff and Murray, 1996). Because the anaerobes have a very low growth rate and require anaerobic growth conditions, most research for commercial cellulase production has focused on fungi (Duff and Murray, 1996).

Fungi that have been reported to produce cellulases include *Sclerotium rolfsii*, *P. chrysosporium* and species of *Trichoderma*, *Aspergillus*, *Schizophyllum* and *Penicillium* (Sternberg, 1976; Fan et al., 1987; Duff and Murray, 1996). Of all these fungal genera, *Trichoderma* has been most extensively studied for cellulase production (Sternberg, 1976).

Cellulases are usually a mixture of several enzymes. At least three major groups of cellulases are involved in the hydrolysis process: (1) endoglucanase (EG, endo-1,4-D-glucanohydrolase, or EC 3.2.1.4.) which attacks regions of low crystallinity in the cellulose fiber, creating free chain-ends; (2) exoglucanase or cellobiohydrolase (CBH, 1,4-β-D-glucan cellobiohydrolase, or EC 3.2.1.91.) which degrades the molecule further by removing cellobiose units from the free chain-ends; (3) β-glucosidase (EC 3.2.1.21) which hydrolyzes cellobiose to produce glucose (Coughlan and Ljungdahl, 1988). In addition to the three major groups of cellulase enzymes, there are also a number of ancillary enzymes that attack hemicellulose, such as glucuronidase, acetylesterase, xylanase, β-xylosidase, galactomannanase and glucomannanase (Duff and Murray, 1996). During the enzymatic hydrolysis, cellulose is degraded by the cellulases to reducing sugars that can be fermented by yeasts or bacteria to ethanol.

4. Improving enzymatic hydrolysis

The factors that affect the enzymatic hydrolysis of cellulose include substrates, cellulase activity, and reaction conditions (temperature, pH, as well as other parameters). To improve the yield and rate of the enzymatic hydrolysis, research has focused on optimizing the hydrolysis process and enhancing cellulase activity (Cantwell et al., 1988; Durand et al., 1988; Orpin, 1988).

4.1. Substrates

Substrate concentration is one of the main factors that affects the yield and initial rate of enzymatic hydrolysis of cellulose. At low substrate levels, an increase of substrate concentration normally results in an increase of the yield and reaction rate of the hydrolysis (Cheung and Anderson, 1997). However, high substrate concentration can cause substrate inhibition, which substantially lowers the rate of the hydrolysis, and the extent of substrate inhibition depends on the ratio of total substrate to total enzyme (Huang and Penner, 1991; Penner and Liaw, 1994). Huang and Penner (1991) found that the substrate inhibition occurred when the ratio of the microcrystalline substrate Avicel pH 101 to the cellulase from *Trichoderma reesei* (grams of cellulose/FPU [filter paper unit, defined as a micromole of reducing sugar as glucose produced by 1 ml of enzyme per minute] of enzyme) was greater than 5. Penner and Liaw (1994) reported that the optimum substrate to enzyme ratio was 1.25 g of the microcrystalline substrate Avicel pH 105 per FPU of the cellulase from *T. reesei*. The susceptibility of cellulosic substrates to cellulases depends on the structural features of the substrate in-