BIOMASS HYDROLYSIS*

by

Wilfredo I. Jose**

INTRODUCTION

Much research has been done on the conversion of cellulose to fermentable sugars. Since biomass typically contain 30 to 40 percent cellulose, other uses must be found for the remaining components. Hemicellulose and lignin have some value as feedstocks for fuels and other chemicals. The utilization of a wide variety of biomass depends on several schemes being developed for the conversion of cellulosic and lignocellulosic materials. The basic problem is the disruption of lignin and the breaking down to smaller or simple sugars before fermentation or any suitable utilization. Biomass hydrolysis offers solution to the above problem. The method of hydrolysis could be by acid hydrolysis or by enzymatic processes. Normally, pretreatment of the biomass is necessary before the hydrolysis proper. This presentation deals with the fundamentals and does not attempt to give a comprehensive review, which could be found in the literature. The acid hydrolysis, the enzymatic process, and the pretreatment methods are discussed.

---

* Paper presented at the Regional Training Workshop on Bioenergy, sponsored by UNESCO and ESCAP, Asian Institute of Technology, Bangkok, Thailand, Feb. 22-28, 1988.

** Chairman, Department of Chemical Engineering, University of the Philippines.

THE STRUCTURE OF BIOMASS

Cellulose, hemicellulose, and lignin in roughly 4:3:3 ratios comprise most lignocellulosic materials. Some biomass contain protein that could be recovered and most are structurally strong. Therefore biomass can be used as sources of chemicals, energy, food and feeds, and building materials.

Cellulose is a polymer of glucose linked at the 1,4 positions, similar to starch in percentage composition and in yielding glucose only on complete hydrolysis. The cellulose molecule consists of long chains of beta glucosidic residues. Starch molecule on the other hand consists of alpha glucosidic residues linked on the same positions. The linkages are similar but the difference in configuration causes a higher crystallinity in cellulose. Thus cellulose is less accessible to enzymes or acid catalysts. In contrast, starch is readily hydrolyzable. Cellulose provides the structural rigidity to plants which is maintained until death.

Hemicellulose consists of straight chain xylose units joined at 1,4 linkages with side chains consisting of arabinose, mannose, glucuronic acid, and other pentoses and hexoses. In the plant structure, hemicellulose is intimately associated with the cellulose. The molecular weight of hemicellulose is much lower than cellulose (100 to 200 monomers per molecule) and the crystallinity is much lower than that of cellulose. Cellulose is in the order of a hundred times more difficult to hydrolyze than starch. The rate of hydrolysis of hemicellulose is in between that of starch and cellulose. Hemicellulose is insoluble in water, soluble in dilute alkali, and easily hydrolyzed by dilute acid.

Lignin is a three dimensional polymer consisting of several phenolic compounds, interspersed and acts as a cementing material in the fibrous constituents. The ether linkages between the monomers are possible at several positions, thus the three dimensionality of the structure. The ether bond can be broken by catalytic hydrogenation to give aromatic products. Microbial process to produce is unlikely as the phenolic products are toxic. A limited number of microorganisms are able to degrade lignin.

CONSTRAINTS TO CELLULOSE HYDROLYSIS

The effectivity of hydrolysis depends on the susceptibility of cellulose to the cellulolytic agents. There must be direct physical contact between these agents and the cellulose molecules for hydrolysis to proceed. [1] However, several structural complexities can hinder this process. Some of these are: water content, capillary structure of the cellulose fiber, crystallinity of the cellulose, and the presence of other substances associated with the cellulose in the fiber such as lignin and hemicelluloses. [2] The primary problem is the combination of the lignin and cellulose.

1. Moisture plays a major role in the hydrolysis of cellulose. It swells the fiber and opens up the fine structure making the cellulose more accessible to the hydrolytic agent. Water is added to the cellulose during the hydrolytic cleavage of the glycosidic links.

2. The capillary structure of the cellulose fiber defines the accessible surface area of the cellulose. The dimensions of the capillary are in the same order of dimension as the enzymes. The access to these regions will determine the success of the reaction. [3]

3. The more crystalline the cellulose molecule is, the less accessible it is to the hydrolytic agents. One reason is that the molecular arrangement and steric rigidity of the anhydrous units within the crystalline regions can also be a constraint to cellulose hydrolysis. [1, 3].

4. The presence of other compounds such lignin, hemicellulose, minerals, and ash in the cellulose structure can inhibit cellulose hydrolysis to some extent. The combination of lignin with cellulose has been proven to be a primary problem in cellulose degradation.

PRETREATMENT METHODS

From the above discussion, it is clear that cellulose hydrolysis should be preceeded by a pretreatment process to render the cellulose more accessible to the hydrolytic agents. These methods may be physical, chemical or microbial treatments. They involve some manipulation and transformation of the cellulose structure and its existence with other associated materials. The objective of the pretreatment step is to disrupt the lignin structure and overcome the crystalline structure of the cellulose. For commercial viability, the pretreatment steps should 1) require cheap chemicals and simple equipment, 2) solubilize the lignin and the hemicellulose, 3) reduce the crystallinity of the cellulose, and 4) be applicable to a wide variety of ligno-cellulosics.

1. Physical methods

Some of the physical methods are particle size reduction, irradiation, variation in temperature and pressure, and steam processes. The major objective is to enhance susceptibility of cellulose to hydrolysis by increasing the surface area & decreasing crystallinity. [4]

1.1 Particle Size Reduction.

Comminution is accomplished by using vibratory ball milling, pot milling, attrition grinding, or shredding. The bacterial or enzymatic hydrolysis of wood was found to be 50 to 75% complete if it was in the form of sander dust [5].

1.2 Irradiation.

Gamma radiation disrupts lignocellulose complex to make the cellulose available to hydrolytic attack [6]. The use has been tested for rice hull and straw and sawdust and other agricultural by-products. Results show that dilute acid hydrolysis is enhanced and the digestibility is improved. [7, 8, 5]

1.3 Variation in Temperature and Pressure.

Subjecting of cellulosic materials to extremes of temperatures and pressures such as heating in kerosene or dry air, or freezing and thawing enhance chemical reactivity of the cellulosic materials. [5]

1.4 Steam Processes.

Several methods employ steam or water plus heat. Some are: Autohydrolysis, wet oxidation, steam explosion, rapid steam hydrolysis/continuous extraction.

1.4.1 Autohydrolysis.

The process of heating the material with water at temperature up to 185°C can be termed as autohydrolysis or prehydrolysis or steaming. The organic acids are formed as the reactive ester groups in the hemicellulose component of the substrate cleave. These organic acids act as catalysts for the subsequent hydrolysis of the hemicellulose to soluble oligosaccharides. Small amounts of inorganic acids like sulfuric can be added to improve the rate and effectiveness of the reaction. However, at higher temperatures or longer periods, furfural and 5-hydroxymethyl furfurals are produced which later leads to self-condensation to form a copolymer. Considerable degradation and modification are observed to occur in lignin.

1.4.2 Wet Oxidation.

It is autohydrolysis in the presence of high pressure oxygen or air. [9] The initial reaction produces acetic and formic acids as major organic acids, methanol and small amount of neutral organic compounds brought about by the solubilization of the acidic hemicellulose components, deesterification of acetate groups, and by oxidation. [10] Consequently, a greater number of the hemicelluloses are broken down into lower molecular weight fragments that are water soluble and the hydrolytic reactions become more favorable. [11] This has been tested on some carbohydrate compounds like xylose, glucose, glucitol, cellulose and dextran at high temperature.

1.4.3 Steam Explosion.

The commercial process consists of a vertical cylinder filled with wood chips and sealed and pressurized with saturated steam at pressures up to 1000 psig. High internal pressures arise as the chips are permeated by the saturated steam. When the bottom of the cylinder is opened, the wood chips are defibrated by the sudden decompression. Several changes have been effected by the process. For one, the lignin is broken down into products with a molecular weight range from 150 to 7000 and which retains the basic lignin structure and are moderately reactive. Hemicelluloses are partially broken down and are soluble in hot water. The lignin content is also increased with the degraded products condensing with lignin. Furthermore, the cellulose is more accessible to enzymatic hydrolysis which may have been caused by the disruption of the lignin polymer. Eighty percent (80%) of the theoretical amount of glucose can be converted. [13, 14]

1.4.4 Rapid Steam Hydrolysis/Continuous Extraction.

The process is more commonly called RASH process and is similar to steam explosion. The difference lies on the continuous removal of the soluble or gaseous products from the reaction zone and cooled to minimize further degradation.

The RASH process starts with the addition of saturated steam to a biomass filled reactor resulting in the hydrolytic depolymerization of the hemicelluloses and lignin. As part of the steam cook, it condenses to the reactor bottom. The depolymerized carbohydrate and lignin fragments are carried down at the bottom where a transfer line removes and cooks the products. No catalyst is needed provided it can yield better results. The RASH method fractionated out the greatest number of hemicelluloses and lignin with minimal degradation leaving a solid residue with greatly enhanced cellulose residue as compared to the other processes discussed. [14, 15]

2. Chemical Pretreatment.

Pretreatment methods using chemicals are fastly gaining recognition in modifying the rigid crystalline cellulose structure prior to hydrolysis.

2.1 Use of Swelling Agents.

There are two basic modes of swelling for cellulose namely intercrystalline and intracrystalline. The former involves the entry of the swelling agent between crystalline units with a volume change approximately equal to the volume of the adsorbed agent and a maximum adsorption of about 30%. Removal of the swelling agent leads to the original network dimensions and structure [16] Intracrystalline, on the other hand, results in new crystalline modifications or unlimited swelling of the cellulose with the swelling agent penetrating the crystalline as well as the amorphous regions. Some examples are sodium hydroxide, amines and anhydrous ammonia which causes definite alterations in the original structure. Thus, intracrystalline swelling agents provide one pathway towards alteration of cellulose crystalline structure with enhanced hydrolytic activity.

2.1.1 NaOH Swelling.

Extensive swelling and separation of structural elements to produce fibers of different crystallinity are observed upon using NaOH solution of 17% or higher. The alkali-treated cellulose is hydrolyzed by the acid 40% faster than the untreated cellulose. This is also done to remove or alter silica and other components to improved digestability. Digestability coefficient of dry matter hulls increased. NaOH treatment of straw is known as Beckman process and involves soaking the straw in a 1.5% NaOH solution at atmospheric temperature and pressure. Digestability of the straw can be doubled at higher temperature and pressure. [17]

2.1.2 Ammonia Treatment.

Anhydrous ammonia can effect a phase change in the crystalline structure of cellulose. Ammonia is believed to hydrolyze the glucoronic acid ester cross-links, providing ready access to structural carbohydrate by rumen microorganisms.

2.1.3 Other Swelling Agents.

Other swelling agents proposed are calcium oxide, calcium hydroxide, cuproammonium hydroxide, and cadoxen. However these chemicals are expensive and some are toxic.

2.2 Extraction and Degradation of Lignin.

Delignification procedure is used in the sulfate and sulfite pulps and Kraft pulp. This is based on the removal of lignin through the selective action of chemical pulping and bleaching agents. Other chemicals that can disrupt the lignin are alkaline peroxides and sodium hypochlorite. [18]

3. Microbial or Enzymatic Pretreatment Method

In nature, lignin is degraded continuously by microbial enzymes. Wood-rot fungi attacks lignin and some of the enzymes responsible for lignin degradation are laccase and polyphenoloxidase. Further developments have to be awaited to utilize this processes with whole microorganisms rather than isolated enzymes. [19, 20]

ACID HYDROLYSIS OF CELLULOSE

The oldest method to produce glucose or sugars from cellulose is acid hydrolysis. Acid hydrolysis starts a sequence of reactions. The rate of reaction for hemicellulose is about 1,000 times that for cellulose. As the sugar products are formed, some are degraded to resins, polymers, and furfural derivatives. The conditions of the reaction should be set so that there is a favorable balance of unreacted materials, sugar product, and undesired products. Considerable degradation products leads to major losses. The maximum yield of sugars is 55% of the initial cellulose. Hemicellulose can be removed by treatment with dilute acid without any effect on cellulose. Dilute acid hydrolysis at high temperature can be equivalent to strong acid hydrolysis at low temperature. Any savings on vessel requirement is offset by the cost of neutralization when using strong acids at low temperature. Sulfur dioxide can be used and results in less water requirement.

1. Kinetics of Dilute Acid Hydrolysis [21]

Cellulose hydrolysis closely proceeds according to the following:

$$\underset{(A)}{\text{Cellulose}} \xrightarrow{k1} \underset{(B)}{\text{Glucose}} \xrightarrow{k2} \underset{(C)}{\text{Degradation}} \text{ products}$$

The differential equation are :

$$dC_A/dt = k_1 C_A$$

$$dC_B/dt = -k_1 C_A - k_2 C_B$$

$$dC_C/dt = k_2 C_B$$

where

C_A = cellulose concentration

C_B = glucose concentration

C_C = degraded glucose concentration

k_1, k_2 = rate constants

t = time

By the Arrhenius equation, the temperature dependency of the rate constant is obtained. Also, additional coefficients on substrate dependency have been determined. The plot of the expected yield of sugars from cellulose as a function of time and temperature shows that the best yields are at high temperatures and very short times. [22] The predicted yield does not increase above 55%. By increasing the value of k_1, the yield can be increased to 85% by pretreating the biomass and reducing the time for the degradation reaction.

2. Some Dilute Acid Saccharification Processes

2.1 Single Stage Saccharification.

The earliest plants involved the cooking of pine wood with 1% sulfuric acid for thirty minutes at 170°C. The maximum yield based on the kinetic analysis above is 20%.[23, 24]

2.2 Continuous Single Stage Hydrolysis.

An extension of the single stage process was using a plug flow reactor and employing higher temperatures. Higher yields were achieved. [25]

2.3 Two Stage Hydrolysis. [26]

This two stage batch hydrolysis separated the acetyl and hemicellulose in the first stage and the glucose in the second stage. The yield was 48-49%. The first stage was at 190°C and required 3 minutes while the second stage 215°C and 3 minutes.

2.4 The Percolation Process. [27]

This process had dilute acid washing sugar as it was formed from wood chips. The sugar yield was doubled but the sugar concentration was less than half of the original single stage operation.

2.5 Other Acid Processes.

Several technologies that have been developed are the Hokkaido process, Bergius-Rhenau process, and several hydrogen chloride gas processes, among others.

ENZYMATIC HYDROLYSIS OF CELLULOSE

To offset the high cost of chemicals and the disadvantages associated with acid hydrolysis, enzymatic hydrolysis of cellulose is an alternative solution. Cellulases are enzymes capable of degrading cellulose because of their specific affinity for the beta-1,4-glucosidic bonds. Some microorganisms with cellulase activity are Trichoderma viride, Cellumonas sp. and Chetomium globosum. The enzymes from these microorganism are produced by growing the microorganism on the same cellulosic material to be used later as the substrate for sugar production, and separating the mycelium and the residual cellulose by centrifugation or filtration, with the filtrate being the enzyme isolate. The cellulase enzymes consists of endo- and exo-splitting hydrolases, together with enzymes that transform cellobiose to sugar product. The cellulase complex of Trichoderma which is the representative of fungal cellulases contains the following components: 1) endoglucanase, 2) cellobiohydrolase 3) beta-glucosidase. [28, 29, 30] Synergistically, the three enzymes act on the cellulose substrate. The endoglucanase starts the attack at the internal sites of the crystalline cellulose, followed by the action of the cellobiohydrolase. The beta-gludosidase transforms the cellobiose to glucose, preventing the inhibitory effect of the cellobiose on the endo-glucanase, and cellobiohydrolase. [31, 32]

The cellulase system may be complex, but current technologies have resulted in greater productivity and it is expected that enzymatic hydrolysis will be able to compete favorably with chemical processes.

CONCLUSION

It is difficult to compress such a wide subject of biomass hydrolysis into a short presentation such as this. The point is that there are several technologies available which could be suitable to particular types of biomass. Some biomass may require combinations of treatment schemes. the acid hydrolysis is the simplest method of hydrolyzing biomass but it also offer several disadvantages. On the other end is enzymatic hydrolysis which may be efficient but is expensive. By using pretreatment processes, more efficient acid or enzyme processes can be attained.

REFERENCES

COWLING, E., Biotech, Bioeng, Symp. 5, 163-82 1975.

COWLING E., and W. BROWN, Adv. in Chem. Ser., 95, 157-87 1969

FAN, L., Y. LEE, and D. BEARDMORE, Biotech. Bioeng,, 22, 177-99, 1980.

NYSTROM, J. Biotech Bioeng, Symp. 5, 221-24, 1975.

MILLET, M. A. BAKER, and L. SATTER, Biotech. Bioeng, Symposium No. 6, 125-53, 1976.

PIGDEN, W., and D. HEANEY, Adv. in Chem Ser. 95, R.F. Gould, Ed., 1969.

MACMANUS, W., J. Agric, Sci, Camb. 79, 55-66, 1972.

KITTS, W., C. KRISHNAMURTI, J. SHELFORD, and G. HUFFMAN, Adv. in Chem. Series 95, 279-97, 1969.

MCGINNIS, G, W. WILSON, and C. BIERMANN, unpublished paper, Forest Products Lab and Chem. Dept., Mississippi State University, 1984.

MCGINNIS G., W. WILSON, and C. MULLEN, , Ind. Eng. Chem Prod. R. & D., 22, 352-57, 1983.

SCHULTZ, T., and C. BIERMANN, Energy for Biomass and Wastes VIII, Institute of Gas Technology, Mississippi, 1984.

SCHULTZ T., C. BIERMANN, and G. MCGINNIS, Ind. Eng. Chem., Prod. R. & D., 22, 34448, 1983.

FOODY P., Optimization of Steam Explosion Pretreatment. Iotech Corp., Ottawa, Ontario, Canada.

SCHULTZ, T. and MCGINNIS, unpublished paper, Forest Products Lab and Chem. Dept., Mississippi State University, 1984.

BIERMANN, C., T. SCHULTZ, and G. MCGINNIS, J of Wood Chem. & Tech., 4:1, 111-28, 1984.

MILLET, M.A., A. BAKER, and L. SATTER, Biotech. & Bioeng. Symp. 6, 125-53, 1976.

MILLET, M.A., A. BAKER, and L. SATTER, Biotech. & Bioeng. Symp. 5, 193-219, 1975.

BAKER, A., M. MILLET, and L. SATTER, Cellulase Technology Research, ACS Simp. Ser. No. 10, 1975.

HAN, Y., Adv. Appl. Micro. 26, 781-87, 1978.

ISHIHARA, T., in Lignin Biodegradation: Microbiology, Chemistry, and Potential Applications. Vol. II Kirk, Haguchi, and Chang, Eds., Florida, CRC Press, 1980.

SAEMAN, J.F., Ind., Eng. Chem. 37, 43, 1945.

KNAPPERT, D., H. GRETHLEIN, and A. CONVERSE, Proceedings of Fourth Annual Joint US/USSR Conference on Microbial Enzyme Reactions, NTIS PB80-132913, 403-19, 1979.

SHERRARD, E.C. and KRESSMAN, F., Ind. Eng. Chem., 37, 5, 1945.

WENZL, H., Chemical Technology of Wood. Academic Press, N.Y., 1970.

GRETHLEIN, H., Proc. of the Second Annual Fuels from Biomass. Symp. Paper no. 26, Rensselaer Polytechnic Institute, Troy, N.Y., June 1978.

CEDERQUIST, K., Production and Use of Power Alcohol in Asia and the Far East Report of a Seminar, Lucknow, India, 23 Oct. - 2 Nov., 1952.

LUERS, H.Z., Angew. Chem, 45, 369, 1932.

GADEN, E, M. MANDEL, E. REESE, and L. SPANO, eds., Enzymatic Conversion of Cellulosic Materials: Technology and Applications, Biotech. Bioeng. Symposium No 6. John Wiley and Sons, N.Y. 1976.

HALLIWELL, G., Prog. Indust. Microbial., 15, 1-58, 1978.

RYU, D. and M. MANDELS, Enzyme Microbial Technology, 2, 185-93, 1980.

WOOD T, Proc. of the International Symposium on Wood and Pulping Chemistry. The Ekman Days, Stockholm, Sweden, 3, 31-38, 1980.

ERIKSSON, K., Cellulases of Fungi, in Trends in the Biology of Fermentations, A. Hollaender, ed., Plenum Press, N.Y., 1981.