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D. E. Brown

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Lignocellulose hydrolysis

By D. E. Brown

Department of Chemical Engineering, U.M.I.S.T., P.O. Box 88,
*Manchester M*60 1*QD, U.K.*

Sources of lignocellulose materials suitable for conversion into chemical feedstocks are reviewed and the chemical nature of the cellulosic, hemicellulosic and lignin components examined. Pulping and analytical methods of separation are discussed and a consideration is made of the potential products from lignocellulosic sources.

Recent work with dilute sulphuric acid treatment is described and particular attention is given to recent developments in the uses of anhydrous hydrofluoric acid for hydrolysis processes.

The nature of the cellulase enzyme system is explored and current reports on the mechanisms involved in this multiple-substrate–multiple-enzyme reaction system are reviewed. Various aspects of size reduction and chemical pretreatment are described and a process scheme is outlined. A brief discussion of the recent developments in the kinetic models for this complex reaction sequence is included.

Nomenclature

symbol	definition	units
A	acid concentration	kg m^{-3}
C	cellulose concentration	kg m^{-3}
C_g	glucose concentration	kg m^{-3}
C_o	decomposed glucose concentration	kg m^{-3}
E	energy of activation	J mol^{-1}
k_1	rate constant for production of glucose	s^{-1}
k_2	rate constant for decomposition of glucose	s^{-1}
K_1, K_2	constants	s^{-1}
K_i	inhibition constant	kg m^{-3}
K_m	Michaelis–Menten constant	kg m^{-3}
P	product concentration	kg m^{-3}
R	gas constant (8.314)	J K^{-1} mol^{-1}
S_0	initial substrate concentration	kg m^{-3}
t	time	s
T	temperature	K
V_m	maximum rate of reaction	kg m^{-3} s^{-1}
Y_1, Y_2	stoichiometric coefficients	kg kg^{-1}

Introduction

Lignocellulosic materials are the major components of the continuously renewable plant kingdom. Such materials represent captured energy from the Sun's radiation in combination with carbon from atmospheric carbon dioxide. The complex formulations of lignocellulosics

give structure and strength to the plants and these characteristics have been exploited by the wood-processing, building and agricultural industries. In addition, their high calorific values have resulted in their use as primitive sources of energy. Ancient deposits of modified lignocellulosics in the form of coal and oil are more sophisticated, but limited, supplies of both energy and resources. An interest in the direct use of lignocellulosics both for their contained energy but also as renewable sources of chemicals is therefore growing rapidly.

The microbial world continuously degrades the lignocellulosics, obtaining energy and raw materials for its survival. Early work on lignocellulose hydrolysis has focused its attention on this biodegradation process, with prevention as the primary goal. This work is now expanding to exploit and develop the enzymic processes associated with the microbial activities. In addition, analytical studies of lignocellulosic materials and various timber-processing procedures have generated considerable amounts of information on chemical methods of degradation and hydrolysis.

Currently, lignocellulosic materials occur in quantity as surpluses or as processing wastes in existing industries. However, it is possible that a chemical industry based on resources derived from lignocellulosics might develop, bringing new processes and products. Special programmes of plant breeding, cultivation and conservation would become associated with such ventures.

Considerable work has been undertaken in recent years on the subject of lignocellulosic hydrolysis, and several excellent reviews of the work have been produced (Ghose 1977; Linko 1977; Bisaria & Ghose 1981; Lee & Fan 1980; Lee *et al.* 1980; Ryu & Mandels 1980; Natick 1981).

Sources of lignocellulosic materials

Although many individual sources of lignocellulosic materials can be identified (Dunlap 1981), it is convenient to classify the three major groups according to Dunlap & Chiang (1980) as follows.

1. Primary cellulosics: those plants that are harvested specifically for their cellulosic content or structural or feed value. Into this category are placed such materials as cotton, timber and hay of all kinds.

2. Agricultural waste cellulosics: those lignocellulose-containing materials that accumulate or are left in the fields as by-products or wastes during agricultural harvesting operations. Such materials include timber processing residues, straw, stovers, rice hulls, sugar-cane bagasse and animal manures.

3. Municipal waste cellulosics: fibres occurring as waste paper or paper products in the solid wastes. Clearly this source has a particular advantage in that collection arrangements are well developed. However, this material will contain plastics, glass, metal, etc., unless special separation procedures are implemented. Some authorities operate elaborate separation units and produce a high-quality material with a cellulose content as high as 75% (Dunlap & Chiang 1980).

Except with municipal solid waste, there will be serious problems associated with the collection of the lignocellulosics. Waste sources are usually 'dilute' in that small amounts of material are available from a large number of suppliers or low concentrations of small particles are contained in very large volumes of liquids. Assembly of the lignocellulosic materials in suitable quantities at convenient locations will be an important item in the economic assessment of hydrolysis processes.

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Clearly, there is already a considerable conflict between the current interests of established users of lignocellulosics and the desires of others for the long-term proposals for the future. Problems may arise from aspects about which there is little information. Sloneker (1976) has warned against the removal of straw from maize-growing areas because of the deleterious effects that this may have on soil fertility. Forestry may be similarly effected (Linko 1977) by the complete removal of all the branches and roots that are currently allowed to decompose where they fall. New large-scale uses of lignocellulosic materials will clearly have to overcome various commercial, environmental and economic difficulties as well as to develop the appropriate technologies.

Nature of lignocellulosic materials

Lignocellulosic materials are composed of three major groups of polymers: cellulose, hemicelluloses and lignin. An exact formulation is not known because it can only be postulated from the breakdown products of various chemical treatments. In addition, it varies from source to source in terms of both its chemical constituents and their relative ratios. Cellulose is a major structural component of the cell wall, forming an outer support matrix for the cell membrane (Wenzl 1970). Hemicellulose forms in association with the cellulose within the spaces created by the network of cellulosic strands. As the cell dies, due primarily to the lack of flow of nutrients through the relatively rigid cellulosic wall, there are many secondary products secreted. In particular, the material of which lignin is composed appears as a form of cement between the cell walls.

(*a*) *Cellulose* has been identified as the simplest of the polymers in lignocellulosics, being composed of a continuous chain of D-glucose molecules linked in the β-1,4 configuration (figure 1). These chains or micelles may contain more than 10^4 anhydroglucopyranose units (Sihtola & Neimo 1975) giving a molecular mass of greater than 1.5 MDa. Cellulose micelles are bunched together to form thread-like microfibrils. The individual cellulosic polymer strands are hydrogen-bonded between the ring oxygen of glucose molecules and the hydroxyl groups at position 3. The cellulosic fibrils are composed of highly ordered micelles possessing crystalline structure interspersed with disorderly areas of so-called amorphous cellulose (Cowling & Kirk 1976; Dunlap & Chiang 1980). Crystalline material is sometimes referred to as α-cellulose, which is that material that will not dissolve in a solution of 17.5 % sodium hydroxide.

(*b*) *Hemicellulose* is a particularly heterogeneous polymer in that it is composed variously of the three hexoses glucose, mannose and galactose, and the two pentoses xylose and arabinose, together with their uronic acids. Three well defined groups can be identified (Wenzl 1970) as (1) xylans that have a basic backbone of poly-β-1,4-xylan with additional side links to arabinose, glucuronic acid and arabinoglucuronic acid, (2) mannans that are composed of glucomannans and galactomannans and (3) galactans appearing as arabinogalactans. The origin of the lignocellulosic material defines the nature of the hemicelluloses. Thus xylan (or pentosan) type material, although widespread, is particularly characteristic of hardwoods, whereas glucomannans are found in softwoods.

(*c*) *Lignin* (Cowling & Kirk 1976) is a three-dimensional polymer of phenylpropane units. The basic molecule is shown in figure 2 with three common variations of *p*-coumaryl alcohol in which R_1 and R_2 are hydrogen, sinapyl in which they are both methoxy (OCH_3) groups and coniferyl in which R_1 is OCH_3 and R_2 is H (Higuchi *et al.* 1981). The true formulation of lignin in the natural state can only be deduced from theoretical reconstructions of various degradation

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products. Possible structures for the repeating unit of coniferous lignin have been proposed by Forss & Fremer (1975) and Adler (1977). The various postulated linkages have been thoroughly reviewed (Higuchi *et al.* 1981) and three major groups can be identified as (1) coniferous lignin formed mainly from coniferyl alcohol unit (2) hardwood lignin containing a mixed polymer of coniferyl and sinapyl alcohols and (3) grass lignin formed from coniferyl, sinapyl and *p*-coumaryl alcohols.

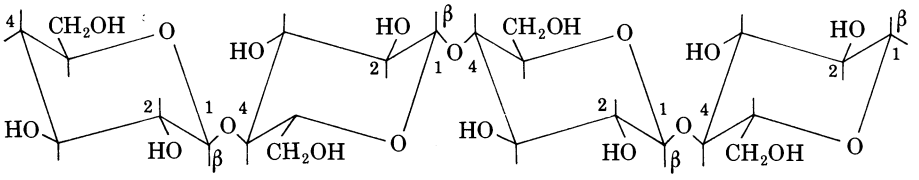

FIGURE 1. Cellulose (poly-β-1,4-D-glucosan).

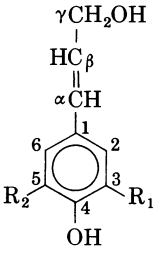

FIGURE 2. The general phenylpropane unit of lignin.

COMPOSITION OF LIGNOCELLULOSICS

The source of the lignocellulosic material has the greatest influence on the reported analysis. However, the method of analysis may also have some influence on quantities and nature of the components identified. Established pulping procedures (Wenzl 1970) are designed primarily to remove the lignin from the raw material, leaving the cellulose relatively unaffected and with varying quantities of hemicellulosic sugars remaining, depending on the final pulping requirement. Pulping with alkaline solutions, sodium carbonate and sodium hydroxide, gives the 'soda pulp', suitable for most timbers. The addition of sodium sulphide (from sodium sulphate) has produced the so-called 'kraft pulp', which first degrades the hemicellulose before leaching out the lignin. On the acid side there is the 'sulphite pulp', which is classically produced by the action of sodium or calcium bisulphite, with some free sulphur dioxide in solution, on the lignocellulosic material. The waste liquors from all of these pulping processes will contain degraded forms of hemicellulose and lignin in varying concentrations depending on the subtleties of individual processes.

Degradation of lignocellulosic materials for analytical purposes is more specific than that for pulping processes but still exhibits limitations in that it modifies the nature of the overall complex mixture. Apart from the ash content and the extractives, which are those substances that can be removed by water or various solvents, lignocellulosic materials can be degraded to preserve either the hemicellulose and cellulose or the lignin. Figure 3 represents a schematic arrangement of the overall approach to degradation for analytical purposes. Various treatments with strong sulphuric acid, concentrated hydrochloric acid and even hydrofluoric acid will effectively hydrolyse all the cellulose and hemicellulose to sugar materials. The remaining

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substance, slightly modified as the hemicellulose is removed, is termed lignin. Alternatively, lignin can be removed in a form that is considerably modified from the natural polymer by treatment with chlorine and leaching with sodium hydroxide (or by using sodium chlorite). A small amount of hemicellulose is degraded with the lignin but most remains unaffected with the cellulose as a mixture known as holocellulose. Treatment of the holocellulose with 17.5 % sodium hydroxide will remove all the hemicellulosic (i.e. pentosan) materials leaving the crystalline α-cellulose. The exact quantities of components found in lignocellulosic materials will thus depend on the method of degradation.

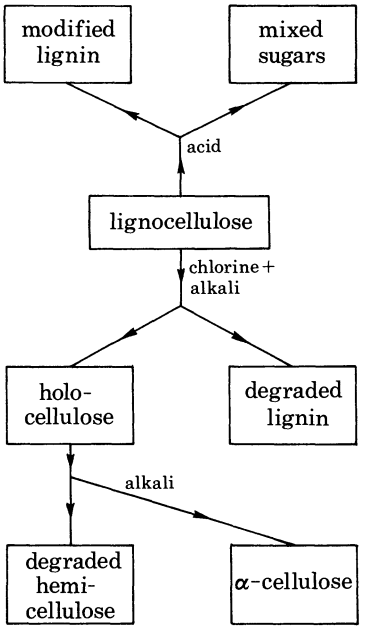

FIGURE 3. Lignocellulose degradation for analytical purposes.

Some typical values of the most significant components to be found in lignocellulosic materials are listed in table 1. Wood materials have a very low ash content of approximately 0.4 %, compared with the grass stems such as wheat straw (11 %) and rice straw (17.5 %). Lignin has an ash content of approximately 30 % for the softwoods and 20 % for the hardwoods such as birch. This is compensated for by a relatively high hemicellulose content of 39 % compared with softwoods in the range 25–30 %. Cellulose contents are reasonably constant in the range of 40–50 %. The effect of the kraft pulping process can be seen in the considerable reduction of lignin and hemicellulose, with the resulting increase in cellulose level. This level of approximately 75 % cellulose in pulp is also reflected in the cellulose content of the separated fibres in municipal waste. In the grass materials, hemicellulosic contents are similar to those of wood, but cellulosic and lignin contents are more typically lower, around 30 % and 20 % respectively. As a complete contrast is the cotton seed hair, which contains 80–95 % cellulose and no lignin (Cowling & Kirk 1976).

It is perhaps more useful to appreciate the contents of lignocellulosic materials from the point of view of their ultimate breakdown products. In table 2 are shown some recent analytical figures of the components of some grasses (Wilke *et al.* 1981) after exhaustive hydrolyses of the materials. Clearly, by their method of decomposition a portion of the lignin figures recorded

in table 1 may have been hemicellulosic or appear in the benzene–ethanol extract. Glucose contents are higher than typical reported cellulose contents because some glucose will have been contributed from the hemicellulosic material.

TABLE 1. TYPICAL COMPOSITIONS OF LIGNOCELLULOSIC MATERIALS

raw material	ash (%)	extractives (%)	lignin (%)	cellulose (%)	hemi-cellulose (%)	reference
spruce wood	0.4	1.8	28.6	43.0	27.0	Rydholm (1965)
pine wood	0.4	5.3	27.8	44.0	26.0	
birch wood	0.3	3.1	19.5	40.0	39.0	
pine kraft pulp	0.4	0.2	5.0	77.0	18.0	
municipal refuse	—	—	—	76.0	—	Dunlap (1981)
bagasse	2.4	6.0	18.9	33.4	30.0	Clark (1969)
wheat straw	11.0	3.5	18.0	30.5	28.4	
rice straw	17.5	4.6	12.5	32.1	24.0	
bamboo	3.3	1.2	20.1	—	19.6	
cotton	—	—	—	80–95	5–20	Cowling & Kirk (1976)

TABLE 2. SUGARS FROM LIGNOCELLULOSIC MATERIALS (WILKE *ET AL.* 1981)

material	ash (%)	extractives (%)	lignin (%)	hexosans (%)			pentosans (%)	
				glucan	mannan	glactan	xylan	arabinan
barley straw	10.8	9.7	13.8	37.5	1.26	1.71	15.0	3.96
corn stover	4.3	5.5	15.1	35.1	0.25	0.75	13.0	2.8
rice straw	12.4	4.4	9.9	36.9	1.6	0.4	13.0	4.0
sorghum straw	10.1	6.2	14.5	32.5	0.8	0.2	15.0	3.0
Wheat straw	9.6	7.2	14.5	32.9	0.72	2.16	16.9	2.1
average	9.4	6.6	13.6	35.0	0.93	1.04	14.6	3.2

PRODUCTS FROM LIGNOCELLULOSE HYDROLYSIS

The strategy of the degradation process of the conversion of lignocellulosic materials into useful chemicals and feedstocks will be particularly influenced by the desired end-product. Hydrolysis will compete with other routes such as direct combustion, pyrolysis and hydrogenation (Reese *et al.* 1972). Also, it is most probable that degradation procedures will be based either on the breakdown and removal of the cellulosic and hemicellulosic materials, leaving a relatively unaltered lignin, or on the removal of lignin followed by controlled hydrolysis of the cellulose and hemicellulose. If some preliminary separation is not carried out before the final hydrolysis step, a complex mixture of materials will result, such as the typical average values in table 2. Future hydrolysis schemes must be prepared to make use of both the lignin and the cellulosic and hemicellulosic materials (Humphrey 1979). Table 3 lists some of the products of lignocellulose hydrolysis that have been identified by writers.

Some possible end-products have been discussed by Goldstein (1976), but are developed on the assumption that the various components in the lignocellulosic material are quite separate. Thus cellulose can clearly be converted into glucose from which, by fermentation, there can be a variety of products such as ethanol, leading to ethylene and butadiene. Alternatively, acid treatment might be used to give hydroxymethyl furfural and ultimately laevulinic acid. Dunlap (1981) has assessed the cellulose composition of a wide range of lignocelluloses in terms of its use as a digestible carbohydrate. Xylose has a limited use in the production of solvent furfural

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and also adiponitrile. Microbial processes based on xylose and the other sugars derived from hemicellulose will be restricted to microorganisms that are able to utilize them. The potentials of products from lignin are evident (Glasser 1981), and the possibilities of its increasing availability is prompting considerable investigation into new applications. Traditional products such as phenol and benzene are discussed by Huibers & Jones (1980). Glasser (1981) also recalls the established uses of lignin sulphonates as dispersants and emulsion stabilizers for use in tertiary oil recovery, drilling muds, dyes, pesticides, etc. Perhaps of special interest for the future is the suggestion (Higuchi *et al.* 1981) of new products to be made by exploiting the microbiological reactions that are possible (Ander & Eriksson 1978; Crawford 1981). Two possible groups of materials are envisaged in which (*a*) increased reactivity and changed properties of

TABLE 3. PRODUCTS FROM THE HYDROLYSIS OF LIGNOCELLULOSE

product	application
mixed sugars liquor	fermentation processes: SCP, ethanol, butanol, organic acids, antibiotics, enzymes, etc.
	animal feed carbohydrates
glucose	fermentation processes
	fructose syrups
	ethylene, butadiene, hydroxymethyl furfural, laevulinic acid
xylose	fermentation processes with selected organisms
	furfural, adiponitrile
	xylitol sweetner
other sugars	fermentation processes with selected organisms
	animal feed carbohydrates
lignin	fuel, carbon black
	sulphonates as dispersants and emulsifiers in drilling muds, dyes, etc.
	chelating agents, humectants, resin extenders
	phenol, benzene, phenolic resins, vanillin, dimethylsulphoxide, methylmercaptan

lignin are achieved by demethylation, hydroxylation and aromatic ring cleavage, and (*b*) the production of lower molecular mass compounds is brought about by such reactions as the cleavage of α–β carbon bonds.

ACID HYDROLYSIS OF LIGNOCELLULOSES

It was mentioned above that the acidic treatment of lignocellulosic materials is used as a procedure in the degradation of the polymeric structure for analytical purposes. Acid hydrolysis is being used as a pretreatment technique (Wilke *et al.* 1981; Knappert *et al.* 1981) before enzymic hydrolysis. However, acid hydrolysis has been examined in some detail for many years (Wenzl 1970) as a method in its own right for producing sugar liquors, particularly for ultimate fermentation to ethanol, etc. Although some degradation is possible with any acidic solution, the work has tended to favour sulphuric acid as the catalyst of the hydrolysis. In addition, the use of dilute acid at elevated temperatures, derived from the old Scholler–Tornesch process, is finding most support. Unfortunately, the presence of the acid, while hydrolysing the β-1,4 links in the sugar polymer, also renders the sugars non-fermentable by further modification. Saeman (1945) proposed a scheme of cellulose hydrolysis:

$$\underset{(C)}{\text{cellulose}} \xrightarrow{k_1} \underset{(C_g)}{\text{glucose}} \xrightarrow{k_2} \underset{(C_0)}{\text{decomposed glucose.}} \qquad (1)$$

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Mass balances for the three materials can be written based on the assumption of pseudo-first-order reactions:

$$dC/dt = -k_1 C_x, \tag{2}$$

$$dC/dt = Y_1 k_1 C_g - k_2 C_1, \tag{3}$$

$$dC_o/dt = Y_2 k_2 C_g, \tag{4}$$

in which the rate constants k_1 and k_2 are a function of the acid concentration and the Arrhenius relation with temperature:

$$k_1 = K_1(A)^m \exp(E_1/RT), \tag{5}$$

$$k_2 = K_2(A)^n \exp(E_2/RT). \tag{6}$$

Some recent studies have been performed to test these kinetics by using a pure cellulosic material, solka floc (Grethlein 1975), municipal waste (Porteous 1976) and barley straw and glucose (Sinclair & Quintero-Ramirez 1981). The rate data for the decomposition of glucose seem in good agreement in the temperature range 160–200 °C and 0.5–2 % sulphuric acid, as shown in table 4.

TABLE 4. DECOMPOSITION OF GLUCOSE

workers	K_2/s^{-1}	$E_2/(J\ mol^{-1})$	n
Saeman (1945)	2.10×10^{12}	135340	1.02
Sinclair & Quintero-Ramirez (1981)	2.66×10^{12}	136588	1.05

TABLE 5. ACID HYDROLYSIS OF CELLULOSE

workers	material	K_1/s^{-1}	$E_1/(J\ mol^{-1})$	n
Saeman (1945)	Douglas fir	2.88×10^{17}	179509	1.30
Grethlein (1975)	solka floc	2.03×10^{17}	177835	1.16
Sinclair & Quintero-Ramirez (1981)	barley straw	4.62×10^{14}	152750	1.31

There is, however, some discrepancy between the results of different workers when the data for the cellulosic breakdown step are examined (table 5). It is interesting to note the similarity between the data for Douglas fir and solka floc. In the barley straw it is possible that high proportions of amorphous cellulose together with considerable ball-milling pretreatment may have been the causes of the low values for K_1 and E_1.

Because of the high activation energy constant, E_1, for cellulosic breakdown compared with that for glucose decomposition, E_2, it is advantageous to increase the temperature of the overall conversion. Nolan & Humphrey (1981) have examined the relation between the maximum conversion of cellulose to glucose and temperature, acid concentration and residence time in the continuous-flow reactor. The possibility of high temperature and short contact time is indicated. Porteous (1976) selected 0.4% (by volume) sulphuric acid at 230 °C with a mean residence time of 1.2 min. This is not dissimilar to the scheme of 1.0 % (by volume) sulphuric acid at 200 °C and a residence time of 3 min used by Sinclair & Quintero-Ramirez (1981) for their economic design study. This work suggested a feasible price for fermentable sugars at £164 t^{-1} and is an encouragement for further studies in this area.

The use of hydrofluoric acid as an agent for lignocellulose hydrolysis, although known for more than a century (Gore 1869), has been almost completely ignored, although patents for its use were taken out by Fredenhagen & Helferich (1932). Recently, renewed efforts with this reagent have been reported (Defaye *et al.* 1980; Lamport *et al.* 1980; Selke *et al.* 1982).

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Anhydrous hydrofluoric acid, although requiring some special handling precautions, is a very useful source of hydrogen ions. Defaye *et al.* (1980) reported that cellulose and pine wood dissolve very readily in anhydrous HF. Excess anhydrous HF can be removed by evaporation because it boils at 20 °C. The resulting material is predominantly glycosyl fluorides and almost unaltered lignin, when that is initially present in the raw material. The glycosyl fluorides undergo repolymerization, particularly when the fluoride ions are removed by evaporation resulting in a mixture of oligosaccharides (Selke *et al.* 1982). These polymers of glucose were found to be easily hydrolysed to fermentable monomers by treatment with 50 mM sulphuric acid solution at 140 °C for 1 h. The claims of these workers have been confirmed for pure cellulose, separated municipal refuse, softwood sawdust, hardwood chips, shredded telephone directory and barley straw (R. E. Banks, D. E. Brown & N. Dickinson, unpublished results 1982). The hydrolysis reaction is very rapid at room temperature and produces a reactive lignin as a by-product. Although repolymerization of the glucose occurs, the material is not modified irretrievably, as in high-temperature hydrolysis with sulphuric acid. Based on their findings to date, Selke *et al.* (1982) have developed various process scenarios to exploit the anhydrous HF reaction. Clearly, if the recovery and recycle of anyhdrous HF can be achieved at a high efficiency together with high yields of sugars and a reactive lignin, this route could attract considerable attention in the future.

Enzymic hydrolysis of lignocelluloses

Most of the studies of the mechanism of lignocellulose hydrolysis by enzymic methods have used lignin-free materials. At the simplest level of complexity are the straight β-1,4 linked chains of glucose (cellodextrins) and analogous materials such as carboxymethyl cellulose (CMC) and hydroxyethyl cellulose (HEC). A higher degree of complexity is achieved in high-quality filter paper and as a powder such as solka floc. Cotton fibres represent the highest proportion of naturally occurring crystalline cellulosic material. Also, Avicel is a proprietory material containing a high proportion of crystalline or α-cellulose. All of these materials and many others listed by Bisaria & Ghose (1981) have been used as substrates, particularly for analytical methods aimed at quantifying the activity of enzyme solutions. The methods of measuring the progress of the hydrolysis reaction such as the breaking strength of the cotton fibres, the formation of reducing sugars or the extent of reduction of solution viscosity did nothing to reveal the true mechanism of the reaction. The increasing use of high-performance (or pressure) liquid chromatography (h.p.l.c.) in observing the products of the reaction is contributing greatly to the increase in the knowledge of the process. However, the more indirect methods used for enzyme assays did reveal that enzymic materials from different microorganisms do not all possess the same degree of catalytic ability towards each substrate.

Although it is well known that very many bacteria and fungi are able to use lignocellulosic materials as a nutrient source, there are very few culture filtrates that show the ability to degrade such materials to simple sugars *in vitro*. Bisaria & Ghose (1981) have collated the reports of fungi that produce cellulase capable of degrading insoluble cellulose. Most of the reported research work on the nature of the enzymes and the mechanism of the hydrolysis process have been carried out with enzyme preparations from *Trichoderma reesei* (formerly *viride*), *Trichoderma koningii*, *Fusarium solani* and *Sporotrichum pulverulentum* (formerly *Chrysosporum lignorum*).

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The cellulase enzyme system

The understanding that cellulosic materials contain crystalline or α-cellulosic areas, amorphous or more reactive areas and perhaps have free ends of the cellulose chains protruding from the surface, together with the fact that only a limited number of enzyme preparations could substantially degrade natural cellulose, prompted Reese *et al.* (1950) to postulate that cellulase might be made up of a mixture of enzymes and to suggest a scheme for the reaction mechanism:

$$\text{crystalline cellulose} \xrightarrow{C_1} \text{reactive cellulose} \xrightarrow{C_x} \begin{matrix}\text{cellobiose}\\ + \\ \text{glucose}\end{matrix} \xrightarrow{\text{cellobiase}} \text{glucose.} \qquad (7)$$

Various enzyme systems have now been examined in some considerable detail and the component proteins identified (Lee & Fan 1980). A detailed discussion of the β-glucanase enzymes has been compiled by Halliwell (1979). Three general categories of β-glucanases can be specified for the highly active cellulase system.

1. Exo-β-glucanase. The most important enzyme in this category is cellobiohydrolase (EC 3.2.1.91), which removes cellobiose units from the non-reducing ends of cellulose chains. This enzyme, it is generally agreed, is synonymous with the C_1 component in (7). The possibility that the same enzyme might act as a glucohydrolase and remove glucose molecules cannot be ignored. In some cases an exo-glucohydrolase has been identified that was not a cellobiohydrolase (Lee & Fan 1980).

2. Endo-β-glucanase. This enzyme randomly attacks free cellulose chains to produce β-1,4 oligosaccharides. It is referred to as a glucanohydrolase (EC 3.2.1.4) and is believed to be comparable with the C_x component in (7).

3. β-Glucosidase. This enzyme (EC 3.2.1.21) primarily hydrolyses cellobiose into glucose. Some enzymes in this category have, however, been identified that will hydrolyse other glucose dimers and also some of the low glucose number cellodextrins (Halliwell 1979).

The three groups of enzymes are in fact able to be sub-divided into a number of separate fractions. Table 6 shows the extent to which workers have been able to fractionate the proteins present in various cellulase systems. In particular, most workers describe a number of components in the general category of endoglucanases. However, unless the separate components are all present in the system, the enzyme preparation will not function as a complete hydrolase. This synergism between the various proteins was demonstrated by Wood (1975) and Wood & McCrae (1975) in some detail with the cellulase components of *Trichoderma koningii*. More recently, Wood & McCrae (1979) reported extensive work on the recombination of exoglucanase (C_1) and endoglucanase (C_x) mixtures prepared from the separation of enzyme systems produced by different microorganisms and by growing microorganisms with different inducing reagents. They observed that only certain compatible combinations result in the maximum rate of hydrolysis of natural cellulosic materials. Their conclusion was that although the separate enzymes C_1 and C_x do show reactivity towards CMC and simple cellodextrins, they need to combine together to influence the breakdown of crystalline material. They postulate that the C_x causes the initial cleavage in the cellulose at positions where C_1 is already located. The C_1 then removes cellobiose and prevents the reforming of the β-1,4 link.

A schematic representation of the mechanism of cellulose hydrolysis can thus be drawn as shown in figure 4. Glucohydrolase (GH) can produce glucose from loose ends of cellulose

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chains in crystalline and non-crystalline materials. Cellobiohydrolase (CBH or C_1) can produce cellobiose from the same end materials and from oligosaccharides produced by the action of glucanohydrolase (GCH or C_x) on degraded and reactive cellulose. Particularly resistant areas of cellulose will be degraded into cellobiose by the combined efforts of the C_1 and C_x components. This picture is not completely accepted because there is some argument (Wood & McCrae 1979) as to whether or not the C_1 component is capable of swelling the crystalline cellulose on its own, as Reese (1977) still maintains. In addition, there is some slight evidence (Eriksson *et al.* 1975; Wood & McCrae 1979) that oxygen might be involved, together with an oxygenase enzyme in the initial attack of the crystalline cellulose for some enzyme systems.

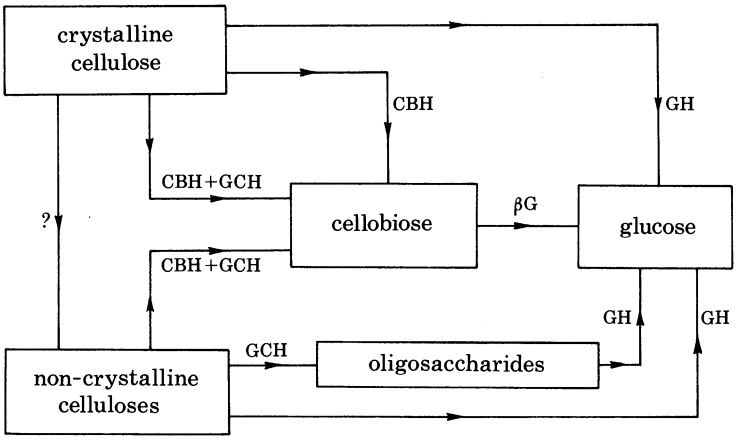

FIGURE 4. Mechanisms of cellulase hydrolysis by the cellulase enzyme system. GH, glucohydrolase (exo-β-1,4-glucanase); CBH, cellobiohydrolase (exo-β-1,4 glucanase) (C_1); GCH, glucanohydrolase (endo-β-1,4 glucanase) (C_x); βG, cellobiase (β-glucosidase).

TABLE 6. COMPONENTS OF SOME CELLULASE SYSTEMS

organism	exoglucanase	endoglucanase	β-glucosidase	references
T. reesei	Exg	Eng I, Eng II	βG	Petterson (1975), Gong *et al.* (1979)
T. reesei	Exg	Eng I, Eng II, Eng III, Eng IV	βG	Gritzali & Brown (1979)
T. koningii	Exg I, Exg II	C_{x1}, C_{x2}, C_{x3a}, C_{x3b}, C_{x4}, C_{x5}	βG I, βG II	Wood & McCrae (1979)
S. pulverulentum	Exg	T_1, T_2, T_{2b}, T_{3a}, T_{3b}	βG	Ericksson (1975)
Geotrichum candidum 3C	Exg	Eng I, Eng II, Eng III, Eng IV, Eng V	βG	Radionova *et al.* (1981)
F. solani	Exg	Eng	βG	Wood (1975)

In a typical enzyme preparation from *Trichoderma reesei* it has been found that the main protein component is the cellobiohydrolase and that only approximately 1% of cellulase protein is β-glucosidase (Natick 1981; Gong *et al.* 1979; Gritzali & Brown 1979). Thus it is common practice to improve the level of β-glucosidase by the direct addition of this enzyme separately produced by *Aspergillus* species. The basic nature of the enzyme system indicated by the relative magnitudes of enzyme assays for filter paper cellulase, CMC cellulase and cellobiase does not seem to have changed during extensive mutation programmes of the micro-organism (Andreotti *et al.* 1981). The mutations have been selected in such a way that only the quantities of cellulase enzymes have increased without changing the ratio or nature of the individual components.

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Methods of pretreatment

For the enzymic process to be able to proceed at a practical rate it is necessary to reduce the size of the lignocellulosic material to small particles. Many different shredders, hammer mills and homogenizers have been used for this purpose. A detailed study of a variety of devices was undertaken by Mandels *et al.* (1974). The overall conclusion was that the simple ball mill is the most effective device, imparting an increased reactivity at a given interfacial area. However, ball-milling is an expensive process that might have to be minimized on economic grounds, even though such techniques can be very effective (Millett *et al.* 1979). Wilke & Yang (1975) prefer the procedures of shredding and hammer-milling to give a particle size of 800 μm (Wilke *et al.* 1976). For a process scheme in which further pretreatment is envisaged, the initial particle size is not critical provided that aqueous suspensions can be adequately pumped and stirred (Wilke *et al.* 1981). Heat treatment before milling at 200 °C (Brown & Fitzpatrick 1976) greatly improves the milling effect.

Many pretreatment procedures can be considered for lignocellulose hydrolysis, derived primarily from the wood processing and pulping industry (Lipinsky 1979). An important first stage is the wetting out of the surface of the particles, particularly if the material has been through an energy-intensive milling stage. Brown & Waluizzaman (1977) showed that a period of 24 h of prewetting was required before reproducible results could be obtained for milled waste newspaper hydrolysis studies. Chemical pretreatments, although aimed primarily at increasing the accessibility of the cellulosic material to the enzyme molecules, usually result in the extraction of one or more components of the lignocellulosic material. Thus a sulphuric acid (0.9 M) treatment at 100 °C of various straw materials by Wilke *et al.* (1981), although improving the enzymic reaction slightly, removes a proportion of the hemicellulosic material. A similar improvement was obtained in the hydrolysis of cellulose in poplar (Knappert *et al.* 1981) by pretreatment with 0.5% (by volume) sulphuric acid at 200 °C.

Many of the methods currently under investigation are developments of pulping and delignification procedures. A useful technique for swelling the cellulose fibres for enzymic attack is treatment with alkaline solutions. Mandels *et al.* (1974) found that NaOH (20 g l^{-1}) at 70 °C for 90 min was adequate for this swelling process. Dunlap (1981), however, removed almost all of the lignin by hot treatment with NaOH at 300 g l^{-1}. This method also removed some of the original cellulose. An alternative approach to the removal of lignin is the break-up of the structure by oxidation with peracetic acid, sulphur dioxide (Dunlap *et al.* 1976) or nitrogen dioxide (Wilke *et al.* 1981). A very detailed study has been undertaken by Fan *et al.* (1981) of the effects of various both physical and chemical pretreatments on a range of straw materials. Most of the chemical techniques such as NaOH at 10 g l^{-1}, sodium sulphite at 137 g l^{-1}, 4–6% (by volume) sodium hypochlorite, peracetic acid, butanol with aluminium chloride and ethylene glycol with HCl caused different degrees of lignin removal. They found that the relative extent of enzymic hydrolysis increased with the degree of delignification and with the decrease in the crystallinity index. Sodium hydroxide treatment was concluded to be the most realistic and economical procedure. This finding agrees with views of Pannir Selvam & Ghose (1981) for the removal of lignin and hemicellulosic materials from rice straw and bagasse. Other methods of pretreatment have been reviewed recently by Linko (1977) and Ryu & Mandels (1980).

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The hydrolysis process

Any attempt to develop a process scheme for the hydrolysis of cellulose must take into account a variety of additional factors besides the need for size reduction and pretreatment. An optimum temperature of 50 °C is usually recommended (Natick 1981) and a pH of 4.8. However, as Linko (1977) points out, increased temperatures give increased hydrolysis rates initially. Unfortunately, the rate of loss of enzyme increases with increase in temperature. Practical schemes (Wilke & Yang 1975; Wilke *et al.* 1976) have been developed on the basis of 45 °C as the operating temperature for the hydrolyzer.

The enzyme material is a particularly expensive portion of the raw materials (Wilke *et al.* 1976) and it is thus desirable to recover and reuse it. Unfortunately the enzyme system has been found to adsorb very strongly on the cellulosic substrate and to be released only as the cellulosic molecules become degraded (Mandels *et al.* 1971; Peitersen *et al.* 1977). Wilke & Yang (1975) examined the adsorption process in some detail and incorporated a countercurrent adsorber train into their process flowsheet. However, there is insufficient practical evidence to say whether or not enzyme material might be recycled in this way. Particularly, it is not certain to what extent the β-glucosidase is recovered in this way, although the C_1 and C_x components are adsorbed equally (Natick 1981).

The main products of the hydrolysis reaction are glucose and cellobiose. In addition, other sugars such as xylose may be present in liquors from lignocelluloses. The cellobiose is known to inhibit the action of the cellulase enzymes. However, β-glucosidase acts to remove the cellobiose but that enzyme is also inhibited by its product, glucose. Considerable improvements can be achieved by including additional β-glucosidase produced by *Aspergillus phoenicis* (Bissett & Sternberg 1978) into the enzyme preparation. Alternative schemes to remove the glucose as it accumulates have been proposed, such as ultrafiltration, by growing microorganisms for single cell protein or ethanol or by conversion to fructose, which is not inhibitory (Woodward & Arnold 1981).

A schematic arrangement of the various steps that must be considered for an enzymic hydrolysis system is shown in figure 5. Such a scheme will usually be expected to form only a part of a complete plant because enzyme production and economic use of the sugar solutions would be an integral part of the operation.

Kinetics of enzymic hydrolysis

The development of kinetic equations to describe the typical results of reducing sugar assay with time has been slow owing to a lack of understanding of the mechanisms of the processes involved. In addition, with cellulose being a solid material the rate of the reaction could be limited by surface area. The early attempts to produce kinetic equations for enzymic hydrolysis have been reviewed by Brown & Waliuzzaman (1977). Owing to a lack of mechanistic information, workers assumed that the material was a single substrate (S) and that the enzyme was a single component (E). Simple equations for Michaelis–Menten reactions,

$$E + S \rightleftharpoons ES, \tag{7}$$

$$ES \longrightarrow E + P, \tag{8}$$

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were inadequate to describe data from experiments in which additions of cellobiose and glucose had been made. Ghose & Das (1971) proposed that the system was competitively inhibited by product (P):

$$E + P \rightleftharpoons EP. \tag{9}$$

The integrated form of the rate equation is then given as

$$\frac{P}{t} = \frac{V_m K_i}{K_i - K_m} - \frac{K_m(K_i + S_0)}{K_i - K_m}\left(\frac{1}{t}\right) \ln \frac{S_0}{S_0 - P}. \tag{10}$$

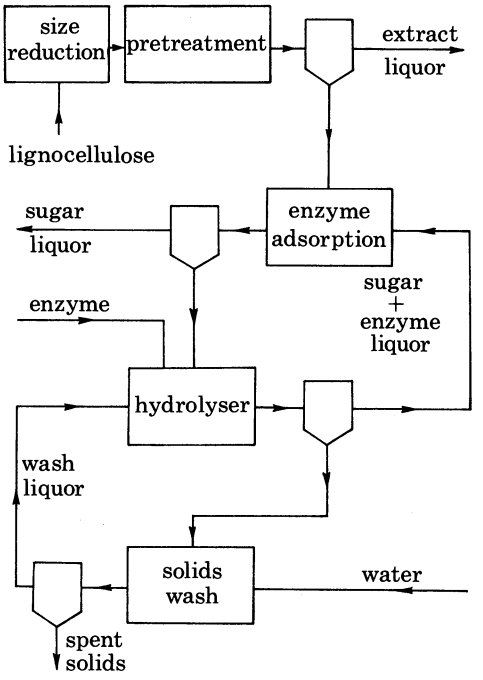

FIGURE 5. Process stages in enzymic hydrolysis of lignocellulose.

Howell & Stuck (1975) proposed that, as this approach was unable to predict the full time course of a hydrolysis process, the accumulation of the intermediate cellobiose was inhibiting in a non-competitive manner:

$$ES + P \rightleftharpoons ESP \tag{11}$$

and

$$EP + S \rightleftharpoons ESP. \tag{12}$$

This approach gave the integrated rate equation to be

$$V_m t = K_m\left(1 + \frac{S_0}{K_i}\right) \ln \frac{S_0}{S_0 - P} + \left(1 - \frac{K_m}{K_i}\right) P + \frac{P^2}{2K_i}. \tag{13}$$

Assuming that the reducing sugar assay was representative of the product cellobiose, values of the parameters have been obtained for solka floc (Ghose & Das 1971; Howell & Stuck 1975) and for newspaper (Brown & Waliuzzaman 1977) in close agreement. A further refinement was proposed by Howell & Mangat (1978) to account for a decrease in the hydrolysis rate for extended periods of hydrolysis. In this it is proposed that, in addition to inhibition by cellobiose, there is some inaccessibility of substrate and enzyme deactivation by some mechanism other than product inhibition. Good predictions were obtained for batch experiments that ran for up to 90 h.

More recent studies are taking into account some of the mechanisms that are known to exist in this complex process. Enzyme adsorption has been incorporated by Huang (1975) and Humphrey (1979) while using basic enzyme kinetics with product inhibition. Suga *et al.* (1975) tackled the problem of modelling depolymerization processes involving both exo- and endo-enzymes. More recently Okazaki & Moo-Young (1978) have applied this approach to the cellulose hydrolysis reaction. The system assumes three enzymes: cellobiohydrolase, an endo-glucanase and β-glucosidase; and considers synergism between the glucanases, the degree of polymerization of the substrate and substrate inhibition. Such models are not fully developed but with experience and the aid of computer techniques for solving equations they should be able to provide predictive expressions for the most complex situations. An extensive review of kinetic models is presented in the paper by Lee *et al.* (1980).

Conclusions

Although there are many sources of lignocellulosic materials produced within the plant kingdom, only those that can be centralized in large quantities will be feasible for processing into intermediates for chemical synthesis. These materials will probably be derived from traditional timber and agricultural processing and so will be in competition with these industries. Ecological and environmental aspects will have to be considered in any large-scale harvesting operations. Municipal refuse still remains a most attractive raw material if economical separation procedures can be established.

Lignocellulosic materials contain a variety of components in addition to cellulose, and during hydrolysis processes the hemicellulose and lignin fractions will become degraded and modified. Any process scheme for the use of lignocellulose should take account of these additional materials, the trend towards the developments of integrated scenarios for the full use of the components is evident.

Acid hydrolysis is still very possible as a technique for the conversion of lignocellulose to useful sugar liquor. It does have the disadvantage of requiring conditions of temperature that cause degradation of the sugars to unfermentable compounds. However, renewed interest in the use of hydrofluoric acid might overcome some of these difficulties.

The enzymic hydrolysis process still attracts a great deal of attention, particularly because the highly specific reaction produces sugars under reasonable conditions of temperature and pressure. However, the accessibility of the cellulosic fibres to the enzymes is so poor that size reduction and various chemical pretreatment procedures are considered essential.

Aspects of the understanding of the enzyme system and the mechanism of the reaction have greatly increased in recent years. Knowledge of synergistic factors, enzyme adsorption, product inhibition and effects of low levels of β-glucosidase has been established. Practical systems are able to be operated at a high conversion efficiency of the lignocellulosic material into usable sugar liquors.

Although the current work on lignocellulose hydrolysis has considerably increased the ability to carry out the process with understanding and permits more accurate design feasibility studies for full-scale plants, no simple procedure has yet been found to overcome the intractable nature of this material within the economic constraints of modern life.

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