H_G = gas holdup, volume of gas per unit volume of empty column
H_L = liquid holdup, volume of liquid per unit volume of empty column
k_{GS} = gas-solid mass transfer coefficient, cm/s
k_{LG} = overall gas-liquid mass transfer coefficient, cm/s
k_{LS} = liquid-solid mass transfer coefficient, cm/s
K_{GS} = gas-solid adsorption equilibrium constant, cm^3/g
K_{LG} = Henry's law solubility constant between the gas and liquid, C_G/C_L
K_{LS} = liquid-solid adsorption equilibrium constant, cm^3/g
K^* = overall mass transfer constant defined in Equation (10*a*), dimensionless
L = height or length of the packed section, cm
M_T = total amount of tracer injected in a pulse disturbance, g-mole
Q_G = volumetric flow rate of the gas or vapor stream, cm^3/s
Q_L = volumetric flow rate of the liquid stream, cm^3/s
s = Laplace transformation variable
t = time, s
u_G = superficial gas velocity, cm/s
u_L = superficial liquid velocity, cm/s
X = variable defined in Table 2
Y = variable defined in Table 2
Z = axial distance in the column, cm

Greek Letters

α_1 = dimensionless gas-liquid mass transfer coefficient, $k_{LG}a_{LG}L/u_L$
α_2 = dimensionless liquid-solid mass transfer coefficient, $k_{LS}a_{LS}L/u_L$
α_3 = dimensionless gas-solid mass transfer coefficient, $k_{GS}a_{GS}LK_{LG}/u_L$
β = dimensionless mean residence time ratio of the liquid to gas defined in Equation (12*a*) $H_Lu_G/u_LH_G = \gamma/\omega$
γ = H_L/H_GK_{LG}
δ = dimensionless liquid-solid adsorption equilibrium constant defined in Equation (12*b*) $H_L/(1 - H_L - H_G)K_{LS}$
$\delta(t)$ = delta function
λ_1 = expression defined in Table 1
λ_2 = expression defined in Table 1
λ_1' = expression defined in Table 1
λ_2' = expression defined in Table 1
ω = u_L/u_GK_{LG}, defined in Equation (10*b*)
$(\mu_n)_G$ = n^{th} moment of the gas effluent, s^n
$(\mu_n)_L$ = n^{th} moment of the liquid effluent, s^n
$(\mu_n^*)_L$ = n^{th} reduced moment of the liquid effluent
$(\mu_n^*)_G$ = n^{th} reduced moment of the gas effluent
$(\mu_0^*)_L$ = zeroth reduced moment of the liquid effluent
$(\mu_0^*)_G$ = zeroth reduced moment of the gas effluent

Subscripts

G = gas phase
L = liquid phase
S = solid phase
n = moment number

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Glucose Production by Biochemical Hydrolysis of Mesquite

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The production of glucose from cellulose by enzymatic hydrolysis has been demonstrated by many researchers (Gaden et al., 1976; Millet et al., 1975; Mandels et al., 1974). Enzymatic hydrolysis of cellulose offers a method to convert inexpensive sources of cellulose to glucose

which can be used directly as food or as a substrate for biological processes to yield other chemicals and products such as ethanol and single-cell protein or even nitrogen fixing processes to produce ammonia (Barnett et al., 1976). Most of the previous research has focused on readily available substrates with high cellulose content such as wastepaper and soft woods because of high glucose yield and reasonably high rates of reactions. Very little research has been conducted on the enzymatic hydrolysis of substrates with a high lignin to cellulose ratio.

This research was designed to determine if glucose could be produced in appreciable quantities from a substrate with a high lignin content by some suitable pretreatment. The substrate chosen was mesquite, which is a serious problem for Texas agriculture and should be an inexpensive substrate for cellulose hydrolysis (Parker, 1977). Table 1 shows the component analysis of the mesquite used in this study. Mesquite composition varies considerably throughout the year owing to the presence or lack of leaves and beans.

The structure of wood is such that the lignin tends to prevent the enzyme from coming in contact with the cellulose and thus prevents hydrolysis. Consequently, woods such as mesquite with a high lignin content are hydrolyzed very slowly. Methods to increase the rate of enzymatic hydrolysis in lignin containing woods have been demonstrated on some substrates. These methods include delignification with acids, alkali swelling, steam swelling, and ball milling (Goldstein, 1970; Goldstein and Villareal, 1971). Recently, a method was reported that utilized a differential speed two-roll mill which markedly increased the rate of hydrolysis (Tassinari and Macy, 1977).

Three of the methods were investigated in this study. These methods were alkali swelling, steam swelling, and sulfuric acid treatment. The alkali treatment produced the greatest improvement in rate of hydrolysis, but the sulfuric acid hydrolyzed much of the cellulose to glucose prior to enzyme treatment.

EXPERIMENTAL PROCEDURE

The mesquite used in this research was harvested in October, 1975, in Wilbarger County, near Lockett, Texas. The total harvested mesquite (trunk, branches, and leaves) was ground to −30 mesh particle size in a hammer mill, and this material was then used for all the tests.

The enzyme used was furnished by the U.S. Army Natick Research and Development Command, Natick, Massachusetts. It was lyophilized cellulase from the fungus *Trichoderma viride* with a filter paper activity of 0.125 units/mg and contained 0.24 mg/mg of protein and no reducing sugar. The activity of the enzyme was verified after receipt.

The mesquite was pretreated in 4.0 g quantities by placing equal amounts of mesquite and treatment fluid in 25 ml, Teflon® lined, Parr digestion bombs. This mixture was continuously tumbled in an oven at the desired temperature to insure adequate mixing. The mesquite was then washed with distilled water, dried, and weighed. The filtrate was analyzed for glucose by the o-toluidine colorimetric method.

Batch, enzymatic hydrolysis tests were conducted in stoppered, 250 ml Erlenmeyer flasks placed in a shaking water bath maintained at the desired temperature. The reaction mixture consisted of 100 ml of a potassium biphthalate-sodium hydroxide buffer, 1.0 g of substrate, and 1.0 g of lyophilized cellulase.

Table 1. Mesquite Analysis

Component	% of dry matter* by weight
Ash	2.7
Cellulose	41.8
Hemicellulose	17.7
Lignin	15.7
Cell solubles	
Crude protein	7.2
Soluble carbohydrates	14.9

* Dry matter = 94.4% of mesquite.

Table 2. Results of Mesquite Pretreatment

Pretreatment conditions	Weight loss, %	% weight loss as glucose*
5% NaOH, 150°C, 1 hr	18.0	—
5% NaOH, 150°C, 2 hr	22.9	—
10% NaOH, 150°C, 2 hr	23.3	0.56
10% NaOH, 150°C, 2 hr†	31.9	0.61
H_2O, 150°C, 1 hr	10.6	—
H_2O, 150°C, 2 hr	18.5	1.42
1% H_2SO_4, 150°C, 1 hr	17.1	—
5% H_2SO_4, 150°C, 2 hr	39.3	12.4

* Determined from glucose in filtrate after washing.
† 36.0 g mesquite sample for pH and temperature dependence studies.

Table 3. Relative Rate of Mesquite Hydrolysis as a Function of *pH* and Temperature
(Based on conversion in 1 hr)

pH	Temperature, °C 40	50	60
3.0		34.6	
3.5		58.2	
4.0		69.1	
4.5		89.1	
5.0	78.2	100	76.4
5.5		58.2	
6.0		60.0	

DISCUSSION OF RESULTS

Each of the pretreatments used in this study extracted a fraction of the mesquite which consisted of the soluble carbohydrates (pentoses, xyloses, etc.), the resins, and some of the cellulose and hemicellulose which was hydrolyzed during pretreatment. Preliminary screening experiments were conducted to evaluate the effect of pretreatment conditions on subsequent enzymatic hydrolysis rates. A summary of the pretreatment results is shown in Table 2. It can be seen that a significant fraction of the mesquite was hydrolyzed to glucose during the acid pretreatment.

The optimum conditions for enzymatic hydrolysis using crude cellulase from *T. viride* were thought to be 50°C and *pH* = 5.0 (Reese, 1977) which was verified using a sample of mesquite treated with 10% sodium hydroxide.

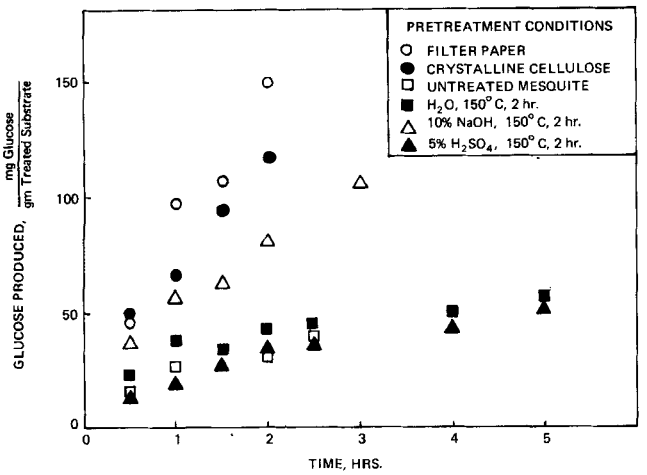

Figure 1. Conversion to glucose during initial five hours of hydrolysis tests.

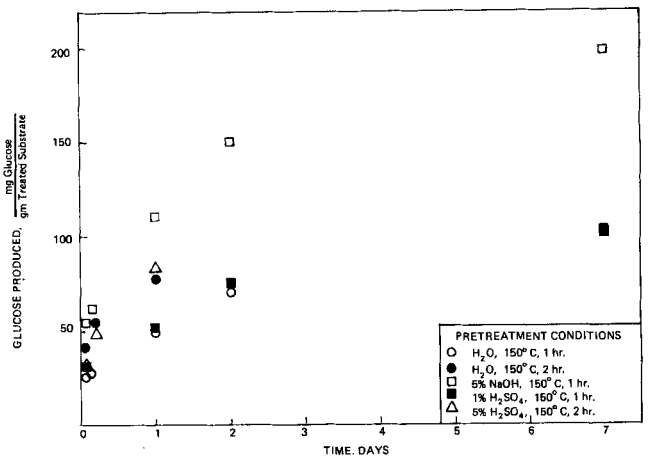

Figure 2. Conversion to glucose during entire hydrolysis tests.

This treatment was chosen to prepare a large (36.0 g) batch of mesquite for determination of temperature and *p*H dependence of the enzyme activity. The activity of cellulase as a function of *p*H and temperature is shown in Table 3.

Filter paper and crystalline cellulose (Sigmacell 50, 50μ particle size) were used as substrates to provide a basis for comparison of mesquite hydrolysis rates. It was assumed that hydrolysis of filter paper (Whatman No. 1) would result in the highest yield of glucose possible. The hydrolysis tests were conducted over a 48 hr or 7 day period. Figure 1 shows the glucose produced during the initial 5 hr, and Figure 2 shows the conversion for the total test.

The fact that the hydrolysis of crystalline cellulose proceeds at a rate comparable to noncrystalline cellulose suggests that the structure of the cellulose does not significantly inhibit the enzyme. The very low hydrolysis rate of mesquite is not a result of the cellulose structure but is due to either lignin or an inhibitor in the mesquite and is probably the result of both. It has been reported that mesquite heartwood contains a cellulase inhibitor that is not extracted during pretreatment (Goldstein and Villarreal, 1971). Because the mesquite used in this study contained some heartwood, the hydrolysis rate would be retarded. This investigation has not disproven the possibility that an inhibitor is present. The results of this study have shown that lignin inhibition can be reduced.

Caustic pretreatment produced the greatest increase in hydrolysis rate based on conversion of treated mesquite charged to the reactor. This is due partially to the fact that the caustic treatment extracted the least glucose (converted cellulose) during pretreatment. Also, the mesquite is swollen during caustic treatment, which allows the enzyme to penetrate the structure more easily.

Steam swelling of mesquite produced very little improvement in hydrolysis rate. This indicates that the mesquite is not swollen as much as with caustic treatment.

The hydrolysis rate for acid treated mesquite was essentially the same as untreated mesquite. This may be misleading in that a significant portion of the cellulose was converted to glucose by acid hydrolysis. As a result, the total glucose produced during the acid treatment followed by enzymatic hydrolysis was greater than that produced by the caustic treatment and subsequent hydrolysis.

None of these pretreatments are as effective as the two-roll milling reported by Tassinari and Macy (1977).

CONCLUSIONS

When we consider commercial processing to produce glucose from wood, the results of this study lead to the following conclusions. Acid pretreatment followed by enzymatic hydrolysis might produce the most glucose in a given amount of time. However, because the use of sulfuric acid will require a much higher capital investment, the caustic treatment may be the most economic method of producing glucose from wood. A detailed process design will be required to determine the optimum conditions. Based on the results of this study, a better pretreatment method than those considered must be developed before high lignin content woods will be suitable substrates for enzymatic hydrolysis to produce glucose.

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