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Kinetics of Enzymatic Hydrolysis of Lignocellulosic Materials Based on Surface Area of Cellulose Accessible to Enzyme and Enzyme Adsorption on Lignin and Cellulose

Scientific Note

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Index Entries: Enzymatic hydrolysis; pretreatment; adsorption; lignocellulosics; kinetics.

INTRODUCTION

We adopt the following view: pretreatment, by either partial acid hydrolysis followed by explosive decompression in a flow reactor, or steam explosion in a batch reactor, reduces particle size, opens up pores by hydrolysis of the xylan, and, at high enough temperature, melts the lignin, causing aglomeration. Thus, the exposed surface area of the cellulose is increased owing to size reduction and pore formation, and yet, the area of the lignin is reduced owing to agglomeration.

The subsequent enzymatic hydrolysis involves adsorption of the cellulase enzymes on both the cellulose and lignin surfaces (*1*). In this manner, lignin acts as an inhibitor since enzyme adsorbed on lignin cannot hydrolyze cellulose. As the cellulose is hydrolyzed, and thus solubilized, pores expand and collapse, causing the exposed surface area to vary in a complex manner as a function of the extent of reaction (*2*). This variation

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in surface area, in turn, affects the cellulase adsorption and, in this manner, the rate of hydrolysis.

The objective of this paper is to present a kinetics model that is consistent with this view. Previous work does not account directly for the change in surface area during the reaction nor the effect of pretreatment conditions on adsorption characteristics, as is done herein.

THEORY

The rate of hydrolysis is given by

$$dS / dt = - k_1 (E_{a,c})^{k2} (E_T)^{k2} / (S_o f_c) \quad (1)$$

where: S = dimensionless substrate concentration, as fraction of glucose potential in original substrate; S_o = concentration of original substrate, mg/mL; $E_{a,c}$ = dimensionless concentration of enzyme adsorbed on cellulose, fraction of total enzyme; E_T = concentration of total enzyme, mg/mL; f_c = fraction of cellulose in original substrate; and k_1, k_2 = parameters.

The enzyme adsorption on cellulose is represented by

$$dE_{a,c} / dt = k_3\{E_2[k_4 f_c S_o SA(S) - E_{a,c}E_T] - k_5 E_{a,c}\} \quad (2)$$

where: E_s = dimensionless enzyme concentration in solution as a fraction of E_T; $A(S)$ = fraction of original surface area, a function of S; and k_3, k_4, k_5 = parameters.

Although the representation of the adsorption kinetics involves three parameters, the equilibrium conditions are set by k_4 and k_5 only.

Enzyme adsorption on the lignin is represented by

$$dE_{a,L} / dt = k_6 \{E_s[k_7 S_o f_L - E_{a,L}E_T] - k_8 E_{a,L}\} \quad (3)$$

where: $E_{a,L}$ = dimensionless concentration of enzyme adsorbed on lignin; f_L = fraction of lignin in original substrate; and k_6, k_7, k_8 = parameters, equilibrium being determined by k_7 and k_8. Enzyme is conserved; hence

$$E_s = 1 - E_{a,c} - E_{a,L} \quad (4)$$

In terms of k_7 and k_8, the Langmuir adsorption isotherm is given by

$$E_{a,L} = (k_7 / k_8 S_o f_L) E_s / (1 + E_s / k_8) \quad (5)$$

where: $E_{a,L}$ = concentration of enzyme adsorbed on lignin, mg/mL; E_s = concentration of enzyme in solution mg/mL; f_L = fraction lignin in the substrate; $k_7 = W_{max,L} S_o f_L$; k_8 = adsorption constant, mg/mL; and $W_{max,L}$ = adsorption capacity, mg enzyme/mg lignin.

Similarly

$$E_{a,c} = (k_4 / k_5 S_o f_c) E_s / (1 + E_s / k_5) \quad (6)$$

and

$$k_4 = W_{max,c} S_o f_c \quad (7)$$

Table 1
Langmuir Parameters for the Adsorption of Cellulase on Lignin at 40°C and pH 4.8

Lignin	Maximum amount of adsorbed cellulase, $W_{max,l}$ (mg/g)	Langmuir constant, K_l (mL/mg)
LIG180	100	0.408
LIG200	66.6	0.655
LIG220	12.3	0.807

Table 2
Langmuir Parameters for the Adsorption of Cellulase on the Cellulose Contained in the Pretreated Wood at 40°C and pH 4.8

Lignin	Maximum amount of adsorbed cellulase, $W_{max,c}$ (mg/g)	Langmuir constant, K_c (mL/mg)
PTW180	14.1	12.5
PTW200	30.5	4.25
PTW220	80.6	1.82

where: f_c = fraction of cellulose in the substrate; S_o = original substrate concentration; and $W_{max,c}$ = adsorption capacity of cellulose, mg enzyme/mg cellulose.

PARAMETER EVALUATION

Adsorption

Values for $W_{max,c}$, K_c ($=1/k_5$), $W_{max,L}$, K_L ($=1/k_8$), taken from Ooshima et. al. (*1*), are summarized in Tables 1 and 2 as a function of pretreatment conditions. The lignin used in the adsorption study was prepared from pretreated hardwood by extensive enzymatic hydrolysis, followed by treatment with urea and washing to remove the adsorbed enzyme. This material was then exposed to fresh enzyme and the equilibrium behavior determined. Langmuir behavior was observed; however desorption owing to dilution was not studied. Adsorption on the cellulose fraction was then determined by the difference between total adsorption on the cellulose-containing pretreated substrate and the adsorption on the lignin fraction, as determined by the method indicated above. Again, Langmuir behavior was observed. In doing this, it was assumed that, for the wood pretreated at 180°C, only 33% of the lignin surface is exposed initially when the cellu-

lose is still present. For wood pretreated at 200 and 220°C, it was assumed that 100% of the lignin surface is exposed. This is consistent with the observation that at the higher pretreatment temperatures, lignin agglomerates into small particles, largely separate from the cellulose (*3*). These values were chosen so that the correlation presented would be independent of pretreatment temperature. As discussed in greater detail in (*1*), this is the first attempt to partition the enzyme adsorption during hydrolysis. Such accounting is necessary in a kinetics model that considers enzyme adsorption since the proportions of lignin and cellulose change during hydrolysis. The model presented in this paper is the first to do so.

It should be noted that enzyme adsorption on lignin is undesirable both because the enzyme is thereby ineffective during the hydrolysis, and because it is lost along with the lignin left at the end of the hydrolysis. The observation, presented in Table 1, that the cellulase adsorption capacity of lignin decreases as the pretreatment temperature is increased, thus, is of considerable practical importance.

The parameters, k_3 and k_6, are set during simulation so that equilibrium is achieved quickly. They do not affect the equilibrium values. Their use avoids the quadratic expressions, or iterative procedures, required when equilibrium is assumed to be instantaneous. Furthermore, they allow one to study the effect of adsorption dynamics.

Surface Area

Surface area, as a function of conversion, presented in Fig. 1, is taken from Ooshima et al. (*4*). The data in Fig. 1 indicates that the function is independent of substrate concentration. Figure 2 demonstrates that the area function does, however, depend on pretreatment conditions. We postulate that this change may be owing to increased external surface area.

Figure 1 is based on pore volume measurements made by the solute exclusion technique. Two limitations should be kept in mind. In order to estimate area from the volume measurements, the pore configuration must be assumed. Following Stone and Scallen (*5*), a planar configuration was assumed. Secondly, pores larger than 56 nm and external surface area are not detected.

Reaction Rate

The substrate, 60 mesh Wilner mixed hardwood, was pretreated by partial acid hydrolysis with explosive decompression in a flow reactor developed by McParland et al. (*6*) at 180, 200, and 220°C. Residence time for the slurry containing 1 wt.% H_2SO_4 was 8.2 to 8.7 s. Experimental methods are described in detail by Ooshima et al. (*1,2*). Initial rate studies were conducted at different substrate concentrations of 5, 10, and 15 wt.%. The enzyme concentration in solution, as well as the sugar formed, was

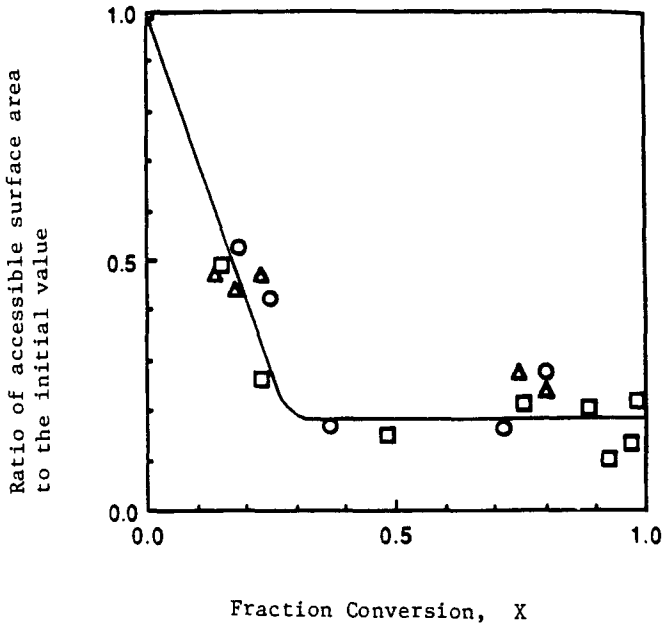

Fig. 1. Changes in accessible surface area as a function of the extent of hydrolysis. Enzyme concentration = 2.52 mg protein/mL. Initial substrate concentration (wt%) = 5 (□), 10 (○), and 15 (△).

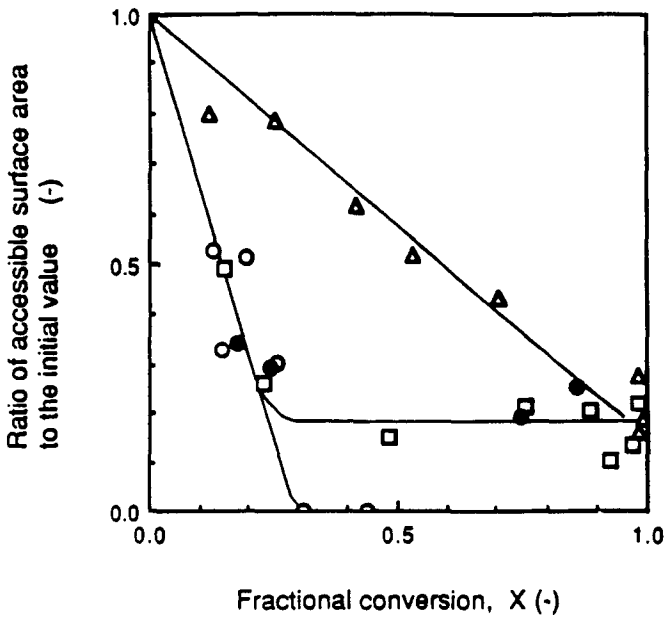

Fig. 2. Changes in accessible surface area as a function of the extent of hydrolysis. Initial substrate concentration = 5 wt%. Enzyme concentration = 2.52 mg protein/mL. Untreated wood (○), PTW180 (●), PTW 200 (□), and PTW220 (△).

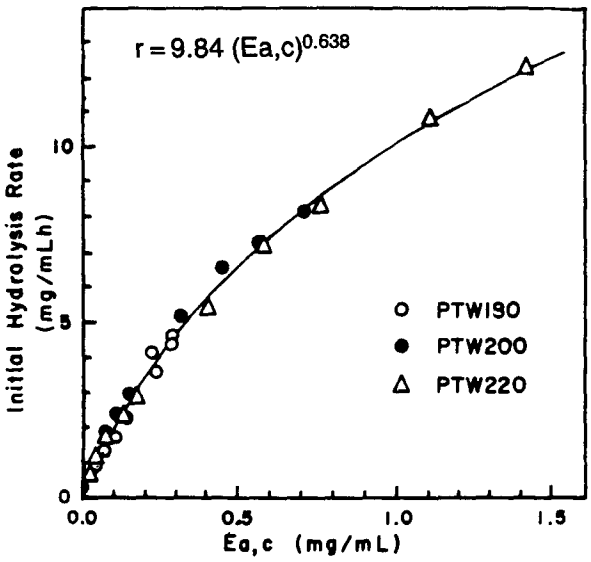

Fig. 3. Initial rate of enzymatic hydrolysis as a function of the concentration of enzyme adsorbed on the cellulose fraction of pretreated hardwood. Initial substrate concentration = 10 wt%. Enzyme concentration = 2.52 mg/mL. Pretreatment temperatures 180, 200, and 220°C.

measured. From these data and the adsorption behavior described above, the concentration of enzyme adsorbed on the cellulose fraction, $E_{a,c}$, was calculated. Figure 3 demonstrates good correlation between the reaction rate and $E_{a,c}$ for hardwood substrates pretreated at three different temperatures. The relationship is nonlinear, yielding a value for k_1 of 9.84 (when the rate is mg/mL/h and the concentration of adsorbed enzyme is in mg/mL) and for k_2, 0.64.

CONCLUSION

Comparison of the model with experimental data is currently in progress. It appears that more detailed studies of the adsorption dynamics, not just adsorption equilibrium, are needed.

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