Developing a Fundamental Understanding of Biomass Structural Features Responsible for Enzymatic Digestibility

Jonathan O'Dwyer, Li Zhu, and Mark T. Holtzapple. Department of Chemical Engineering, Texas A&M University, College Station, TX 77843

Although many people advocate reducing energy usage, consumption continues to grow with increasing population. Because petroleum is a nonrenewable resource, humans must seek alternative energy sources that are inexhaustible. Lignocellulosic biomass is one of the most valuable alternative energy sources because it is renewable, widely available, and environmentally friendly. Biomass can be converted to liquid fuels (such as ethanol) or chemicals (such as carboxylic acids). This technology has many potential benefits such as reduced dependence on oil, reduced greenhouse gas emissions, and generating further employment opportunities through the creation of a new industry.

Overcoming limitations to thoroughly hydrolyze biomass with enzymes has been the main focus of a massive amount of research over the last three decades. Unfortunately, no study to date can predict with certainty the digestibility of pretreated biomass. A concerted effort with Auburn University and Michigan State University has been undertaken to study hydrolysis mechanisms on a fundamental level. Predicting enzymatic hydrolysis based solely on structural features (lignin, acetyl, and crystallinity index) would be a major breakthrough in understanding enzymatic digestibility thereby lowering pretreatment and hydrolysis costs and accelerating commercialization of biomass technologies.

Throughout this research, a simplified form of a theoretical model of cellulose hydrolysis, the HCH-1 Model, developed by Holtzapple *et al.* (1984) will be used to model enzymatic hydrolysis

$$x = B \ln(E_0) + A \qquad (1)$$

where *x* and E_o are sugar conversion (%) and enzyme loading (FPU/g dry biomass), respectively.

Several features deemed important in effecting enzymatic digestibility include lignin content, the presence of acetyl groups, cellulose crystallinity, degree of polymerization, surface area/pore volume of cellulose fiber, and particle size. However, elucidating the relative importance of these factors is complicated because of the complex nature of lignocellulosic biomass, and modifying one barrier can disguise the importance of others. For the purpose of this research, crystallinity index (CrI), lignin content, and acetyl content will be investigated as the major factors influencing enzymatic hydrolysis.

Poplar wood model samples were prepared with a variety of lignin contents, acetyl contents, and crystallinities. The structural features of the samples were directly manipulated via selective delignification with peracetic acid, selective deacetylation with KOH, and selective decrystallization with ball milling. The treatment techniques employed sought to minimize cross effects. The 147 poplar wood model samples will be utilized as substrates throughout this research. Theoretically, it is possible to predict the enzymatic digestibility of lignocellulose if the function of chemical and physical features that determine digestibility can be modeled.

The fundamental understanding of enzymatic digestibility as a function of structural features (lignin, acetyl, crystallinity) gained from this work has the potential to aid in the design of more effective and economically feasible conditions of the two major contributors to the high cost of current biomass technologies: pretreatment techniques and enzyme loading.

Erlenmeyer flasks (50-mL), which contained 0.2 g dry weight of pretreated poplar wood, 18 mL of distilled water, 1.0 mL of 1-M citrate buffer and 0.6 mL of 1% sodium azide solution, were placed inside a 100-rpm air-bath shaker at 50°C. When the solution reached 50°C (~1 h), the reaction was initiated by adding 0.2 mL of appropriately diluted cellulase and 0.05 mL of cellobiase. Experiments were performed at three incubation periods (1, 6, and 72 h) and strategically selected enzyme loadings to ensure the linearity of Equation 1 was obeyed. After the desired incubation time (1, 6, or 72 h), the Erlenmeyer flasks were removed from the air-bath shaker, boiled for 15 minutes to denature the enzymes, cooled, centrifuged, and the filtrate was frozen until sugars were analyzed with HPLC.

The slopes (B) and intercepts (A) from Equation 1 were determined for each of the 147 samples at all three times (1, 6, and 72 h). A total of 1323 experiments (147 samples x 3 time periods x 3 enzyme loadings) were conducted. Average correlation coefficients (R^2) from plots using Equation 1 for glucan conversion at 1, 6, and 72 h were 0.96 ± 0.019, 0.98 ± 0.018, and 0.98 ± 0.04, respectively. Average correlation coefficients (R^2) from plots using Equation 1 for xylan conversion at 1, 6, and 72 h were 0.94 ± 0.03, 0.98 ± 0.032, and 0.98 ± 0.032, respectively. It was shown that intercepts for glucan, xylan, and total sugar increased with increasing time (e.g. 72 h > 6 h > 1 h). The 1 h slopes were consistently smaller than those for 6 and 72 h, and 6 and 72 h slopes spanned a broader range. This is likely due to the extended incubation times for 6 and 72 h resulting in the biomass being in contact with the enzymes for longer periods in conjunction with the biomass' inherent reactivity.

The neural network toolbox in MATLAB® and SAS® were used to develop nonparametric and parametric empirical models, respectively, to predict enzymatic digestibility. Preliminary models show promise in predicting the parameters A and B from Equation 1. Model performance parameters are summarized in Table 1 (neural network) and Table 2 (SAS®). Correlation coefficients (R^2) were determined from plots of actual A and B values calculated from Equation 1 versus A and B values as predicted by the models. A total of 18 models were developed with both MATLAB® and SAS®: 6 for glucan, 6 for xylan, and 6 for total sugar (not shown).

The models will be tested to determine if they can predict A and B and ultimately enzymatic reactivity of biomass samples pretreated via aqueous ammonia, FIBEX, lime, neutral water, and dilute acid. The analyses will be compared with outputs from the mathematical models to determine if they can predict enzymatic digestibility based solely on acetyl content, lignin content, and crystallinity or determine if there are other structural features that play a major role in the enzymatic hydrolysis of biomass.

Table 1. Modeling of enzymatic hydrolysis with neural networks.

	glucan						xylan					
	1 h		6 h		72 h		1 h		6 h		72 h	
	B	A	B	A	B	A	B	A	B	A	B	A
R^2	0.95	0.92	0.94	0.95	0.92	0.94	0.83	0.83	0.88	0.94	0.81	0.97
MSE	3.2	0.5	4.7	9.7	5.2	48.3	0.6	0.6	2.8	9.0	2.5	18.8

Table 2. Modeling of enzymatic hydrolysis with SAS[®].

	glucan						xylan					
	1 h		6 h		72 h		1 h		6 h		72 h	
	B	A	B	A	B	A	B	A	B	A	B	A
R^2	0.96	0.89	0.93	0.95	0.90	0.96	0.77	0.71	0.86	0.89	0.64	0.95
MSE	2.5	0.7	4.5	8.4	7.4	34.4	0.7	0.9	2.9	13.7	4.5	28.0