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Process Simulation of Dilute Acid Pretreatment of Coastal Bermudagrass for Bioethanol Production

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Abstract. *Coastal bermudagrass is a promising lignocellulosic feedstock for bioethanol production. It is well suited for the Southeastern United States where it is currently grown for hay production and nutrient management in animal farming operations. Prior experiments have generated sugar and sugar degradation data from the dilute acid pretreatment and enzymatic hydrolysis of bermudagrass over a range of pretreatment conditions. Experimentally, the yield of total glucose and xylose was maximized at 93 % of the theoretical value for the pretreatment conditions 140 ^{o}C and 1.2 % sulfuric acid (w/w) for a residence time of 30 minutes. To explore further potential optimum pretreatment conditions, an artifical neural network (ANN) was created to model both the pretreatment and enzymatic hydrolysis steps using the prior experimental data to train it. The ANN took the only the three pretreatment conditions as inputs and output glucose from the enzymatic hydrolysis step, with an R^2 of 0.97, xylose from the pre-hydrolyzate, with an R^2 of 0.95, total glucose and xylose from both steps, with an R^2 of 0.97, and furfural from the pre-hydrolyzate, with an R^2 of 0.93. From the ANN, several optimal sets of pretreatment conditions were found with total glucose and xylose levels greater than 93% of the theoretical yield with the maximum being just under 100% for the conditions 150 ^{o}C and 0.9 % sulfuric acid (w/w) for a residence time of 30 minutes. A simple fermentation simulation reinforced the need for co-fermenting xylose and glucose.*

Keywords. Dilute acid pretreatment, coastal bermudagrass, neural network, process simulation, ethanol production

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Introduction

Domestic concerns over increasing oil prices, the limited supply of oil, and global warming are providing an impetus to find a domestically producible and environmentally friendly liquid fuel to replace gasoline. Ethanol produced from cellulosic biomass has the potential to meet this need. Cellulosic biomass is ubiquitous and a variety of feedstocks are possible depending on locale. Additionally, cellulosic feedstocks provide non-food crop options which can be grown on non-arable land keeping crop input costs low and avoiding the current competitions between food and energy that ethanol from corn encounters.

Much of the research being done on the conversion of cellulosic biomass to ethanol concerns three parts of the overall process: pretreatment, enzymatic hydrolysis and fermentation. Pretreatment is of particular interest because it is upstream of both enzymatic hydrolysis and fermentation and can affect both processes. An effective pretreatment renders the biomass susceptible to enzymatic hydrolysis for maximum fermentable sugars without providing inhibition of either enzymatic hydrolysis or fermentation. Many of the most popular pretreatment methods being investigated involve a combination of heating and either an acid or alkali chemical (Mosier et al. 2005). Of the many pretreatment options, dilute sulfuric acid pretreatment, is a near term pretreatment technology which appears to be inexpensive at scale when compared to other technologies and has been investigated for a large number of feedstocks (Eggeman and Elander 2005; Mosier et al. 2005). Examples in the United States (U.S.) cover a range of potential cellulosic biomass feedstocks including converting woody biomass from Colorado and Tennessee, corn stover in the midwestern U.S., and herbaceous biomass in the southeastern U.S. (Torget et al. 1991; Lloyd and Wyman 2005; Sun and Cheng 2005). In each case, the researchers are looking for optimum pretreatment conditions that maximize sugar yield for a particular biomass requiring significant time and resources to come to a conclusion. Regression and kinetic models have been used in the past to model the dilute acid pretreatment of biomass, but artificial neural networks (ANN) have been less frequently employed. An ANN offers the benefit of modeling a complex system without the underlying mathematical descriptions as well as the option to model nonlinear systems easily and could save time and resources when searching for optimum pretreatment conditions (Dwyer et al. 2008).

Artificial Neural Network

An ANN is mathematical way of simulating the way biological neurons in a brain learn the relationship between input and output data after training with example input and output data sets. ANNs have been used already in biofuel research to help optimize pretreatment conditions for biodiesel production and to associate cellulosic biomass structural features with digestibility (Rajendra et al. 2008; Dwyer et al. 2008). Figure 1 below shows a basic schematic for an ANN. Generally an ANN has an input layer, a specified number of hidden layer and an output layer. The hidden layers and the output layer both contain the neurons, input weights, biases and transfer functions associated with processing the data. A simple type of ANN is the feedforward neural network which is set up to feed inputs forward through processing layers without feedback. An ANN must be trained and the most common technique for training is by a backpropagation algorithm. Backpropagation refers to how the algorithm adjusts the neuron weights in order to minimize output error. This means that training the network well greatly affects the performance of the network. Optimal training depends on the number neurons chosen for a hidden layer, the number of hidden layers, the transfer functions in the output layer, and the learning method chosen. Generally for fewer than five inputs the optimal network performance occurs when the number of neurons in the hidden layer are double the number of inputs (Priddy et al. 2005). Too many neurons can result in over-fitting and too few can result in

under-fitting. Additionally, the simpler the ANN, the better it will learn so minimizing the number of hidden layers is ideal. One of the most commonly used transfer functions is the sigmoid and when paired with a linear transfer function, the ANN can be used to generalize almost any problem (Priddy et al. 2005; Demuth et al. 2008). Once trained optimally, the ANN can be used as a generalized model to accurately give output data for a given input.

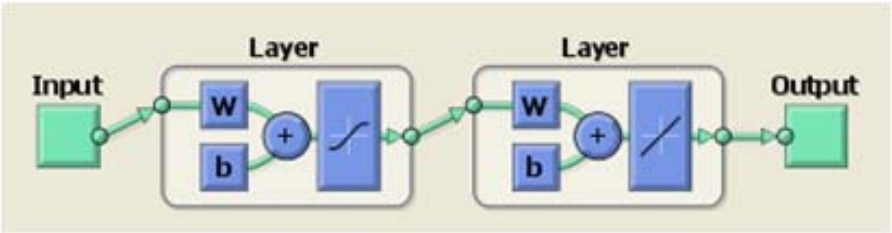

Figure 1. Sample ANN diagram taken from MATLAB where w is the matrix of weights corresponding to number of neurons and b is the bias term.

Project Outline

This project concentrates on the pretreatment step of the conversion process. Dilute sulfuric acid pretreatment was examined on coastal bermudagrass, a perennial grass used in southeastern U.S. for both swine waste nutrient management as well as a source of animal feed, to assess the potential of this feedstock for use in ethanol production. Experiments completed prior to this paper have yielded multiple pretreatment conditions that resulted in maximum xylose monomer production from the dilute acid pretreatment pre-hydrolyzate liquor (PreH), maximum glucose monomer production from enzymatic hydrolyzate (EH), and maximum total sugar production (combined total glucose and total xylose from both pretreatment and enzymatic hydrolysis) while keeping furfural production less than 1 g/L in each case. This paper offers a further analysis of the prior experimental data through a simple process model using a feedforward neural network trained with a backpropagation algorithm to simulate the pretreatment and enzymatic hydrolysis steps and yields from literature to simulate the fermentation step. This offers a way to examine pretreatment results not obtained experimentally and to draw further conclusions on optimum pretreatment conditions. Additionally, the utility of using a neural network as opposed to other modeling techniques for optimizing pretreatment options and developing relationships between inputs and outputs will be assessed.

Materials and Methods

Pretreatment and Hydrolysis

A factorial design was developed to examine the effect of the pretreatment conditions (reaction temperature, acid concentration and residence time) on the sugar yields in the both the PreH and the EH as well as the generation of sugar degradation products in the prehydolyzate. The values of the design are based on literature reviewed and prior work on the dilute acid pretreatment of bermudagrass done by Sun and Cheng (2005). Sulfuric acid concentrations of 0.3, 0.6, 0.9, and 1.2% (w/w) were examined at temperatures 120, 140, 160, and 180 ^{o}C and residence times of 5, 15, 30, and 60 minutes. Each pretreatment combination was preformed in triplicate.

Coastal bermudagrass was obtained in 2007 from Central Crops Research Station located in Clayton, NC courtesy of Dr. Joseph Burns of the Crop Science Department at North Carolina State University. The bermudagrass was ground to particle sizes no greater than 2mm and stored in sealed bags at ambient room temperature in the lab until used. Stainless steel vessels were loaded with 3 g of ground bermudagrass and 30 ml of dilute sulfuric acid before being mixed and sealed. The vessels were heated indirectly in an oil bath with approximately a 12 minute heating period to get to temperature before a planned residence time at that temperature. After pretreatment, the vessels were removed and placed in cold water for immediate cooling prior to being vacuum filtered. The solids were rinsed with 60 ml of water to capture all the hydrolyzed sugars from the pretreatment step. This filtrate, the PreH, was stored at -20 ^{o}C for analysis later. The solids were then rinsed with another 140ml of water and stored in a sealed plastic bags at 4 ^{o}C for hydrolysis.

Enzymatic hydrolysis was preformed in 50 ml centrifuge tubes for 72 hours at 55 ^{o}C, 165 rpm agitation by an automated shaking water bath, and in 0.05 M sodium citrate buffer to maintain a pH of 4.8. The enzymes were loaded in excess at 40 filter paper units (FPU) of cellulase per gram of dry biomass and 70 cellobiose units (CBU) of cellobiase per gram of dry biomass to avoid any limitation in monomeric sugar production caused by enzyme deficiency. Sodium azide at a concentration of 0.3% (w/v) was added to each tube to inhibit of microbial growth.

Sugar Analysis

The EH and PreH were analyzed using a high performance liquid chromatography system (HPLC) to quantify the sugar monomers. The HPLC was also used to quantify prehydolyzate sugar degradation products. An Aminex HPX-87P column was used to distinguish amounts of glucose, xylose, galactose, arabinose in the hydrolyzate samples. For the prehydrolyzate samples, an Aminex HPX-87H column was used to quantify the levels of glucose, xylose, furfural, 5-hydroxyfuranmethal (HMF), forminc acid, and levulinic acid. Total sugars, monomeric sugars, and degradation products were calculated on a per gram of raw biomass basis.

Statistical Analysis

The data was run through the GLM procedure in SAS 9.1.3 (SAS Institute Inc., Cary, NC) to identify statistically significant and insignificant differences. The data was adjusted using Tukey's adjustment and was evaluated as significant differences where $p < 0.01$.

MODEL

General Process Model

A simple process model was developed to assist in further differentiating statistically similar sugar outputs in order to identify optimal pretreatment conditions. The process that was examined included a pretreatment step, an enzymatic hydrolysis step, and a fermentation step. The model was used to make inferences on pretreatment resources required based on a relationship between the severity of pretreatment and the resulting sugar and degradation product levels.

Neural Network Model of Pretreatment and Enzymatic Hydrolysis

MATLAB version 7.6.0.324 R2008a was used to generate a feed-forward back-propagation neural network which modeled the pretreatment and enzymatic hydrolysis steps together. Three inputs, the pretreatment conditions temperature (^{o}C), acid concentration (% w/w), and time (minutes), were mapped to four outputs, glucose from the EH, xylose from the PreH, total sugars, and furfural. Experimental data was randomly divided so that 80% of the data was used for training the network, 20% of the data was used for testing the network, and 20% of the data was used for validation. All of the data input into the neural network was subject to normalization of means and standard deviations.

The neural network was composed of one hidden layer with six neurons and a sigmoid transfer function and one output layer with one neuron and a linear transfer function. To ensure that six hidden layer neurons was the best choice, the network was run 100 times over a range of four to ten neurons to see the effect on the coefficient of determination (R^2) and root mean square error (RMSE). In data not shown, six neurons minimized the R^2 and RMSE values, supporting this choice. The backpropagation training algorithm *trainlm* was used to train the neural networks in MATLAB.

Fermentation Simulation

The fermentation step quantified ethanol yields based on the separate fermentation of monomeric glucose from the EH and the co-fermentation of the total monomeric glucose and xylose from both EH and PreH. All conversion rates were taken from the study done by Krishan et al. which examines the xylose conversion and co-fermentation characteristics of *Saccharomyces* 1400(pLNH33) for ethanol production (1999). For glucose fermentation alone a conversion rate of 0.48 grams of ethanol per gram of sugar was used. A conversion rate of 0.46g grams of ethanol per gram of sugar was used for glucose and xylose co-fermentation.

RESULTS AND DISCUSSION

ANN Assessment

The accuracy of the ANN after training was assessed using the R^2 value and RMSE for each of the outputs. Since the ANN divides the data up randomly for training, validation and testing, the resulting network after each training session will simulate the actual data slightly differently. To examine this change, the ANN was run 100 times to find the average R^2 values and RMSE values for the outputs over all of the runs and to show the standard deviation of these statistical assessments to ensure they did not vary largely. The results, shown below in Table 1, show that the standard deviations of the R^2 statistic are reasonable compared to the average values suggesting that the ANN is consistently learning similar relationships between inputs and outputs independent of how the data is chosen. However, the standard deviation of the RMSE is large in comparison to the average value suggesting alternatively that some values predicted by the ANN can be affected by how the training data is chosen. In these cases, there is more chance of a few large outliers despite a seemingly good fit otherwise.

Table 1**.** Average R^2 and RMSE and corresponding standard deviations for ANN outputs

Output	Average R^2	Std. Dev. R^2	Average RMSE	Std. Dev. RMSE
Glucose from EH	0.87	0.09	16.84	4.93
Xylose from PreH	0.93	0.05	13.45	3.35
Total Sugars	0.93	0.06	24.63	7.95
Furfural	0.88	0.09	6.08	2.01

A single ANN with low RMSE and high R^2 for each output was chosen to find the optimum pretreatment conditions and corresponding high sugar yields for use in the process model. Additionally, manual visual inspection of predicted versus actual values was made to choose an ANN with few large outliers. Figure 2 show plots of predicted versus actual values for glucose from EH, xylose from PreH, total monomeric sugars, and furfural respectively from the ANN which was used. As seen in the plots, the ANN predicts the majority of values very closely to the experimental data with few outlying values when predicting glucose from EH, xylose from PreH, and total sugars. Furfural values are predicted less accurately with many values being either higher or lower than the actual value by a relatively large amount. Negative furfural predictions are considered as zeros in reference to the process simulation. Table 2 contains the R^2 and RMSE values for the ANN plots shown in figure 2.

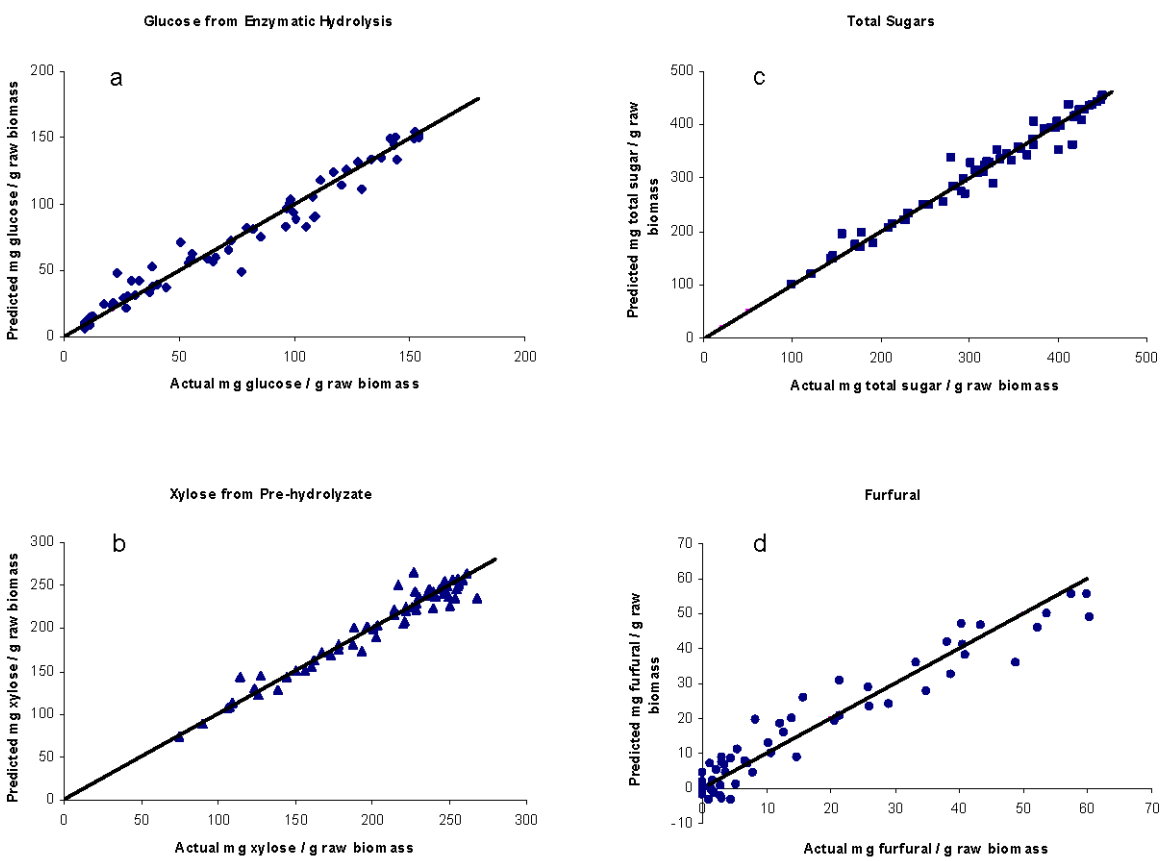

Figure 2. ANN predicted values versus actual experimental values for (a) glucose from enzymatic hydrolysis, (b) xylose from pre-hydrolyzate, (c) total monomeric sugars, and (d) furfural.

Table 2**.** Average ANN R^2 and RMSE

Output	R^2	RMSE
Glucose from EH	0.97	8.96
Xylose from PreH	0.95	11.52
Total Sugars	0.97	16.81
Furfural	0.93	4.73

Experimental Optimum Pretreatment Conditions

Theoretically, the maximum total glucose available is 284 mg/g raw biomass and the maximum total xylose available is 180 mg/g raw biomass. Ignoring minor sugars, the maximum theoretical total sugar yield is therefore 464 mg/g raw biomass. From reviewed literature, it was found that furfural levels beyond 1 g/L, HMF levels beyond 1 g/L, and total weak acid concentrations beyond 2 g/L can begin to inhibit fermentation (Almeida et al., 2009; Navarro, 1994; Palmqvist and Hahn-Hagerdah, 2000). If the degradation product amounts represented in this paper were constant per gram of biomass at a higher solid loading, the concentrations would increase by the same multiple as the solid loading. For this reason, the degradation product cut-off values are kept pessimistically low since ideally the solid loading in a production environment would be higher that 10%. Additionally, it was found that furfural levels were much higher than the other degradation product levels in the experiments reported, so to simplify the process of picking optimum pretreatment conditions with minimal expected inhibition downstream, only furfural levels were assessed.

Table 3 contains the set of optimum pretreatment conditions corresponding to statistically similar total sugar levels each with less than 1 g/L furfural. Biomass neutralization effects on pH and heat effects on pH were ignored. The combined severity factor (CS) (Chum et al. 1990):

$$\log CS = \log(t \bullet \exp[(T_H - T_R) / 14.75]) - pH, \quad (1)$$

where t is residence time in minutes, T_H is reaction temperature, and T_R is the reference temperature 100 ^oC is also provided in table 3 for use in comparing between the severity of the other experimental pretreatments as well as the ANN generated pretreatments in the next section.

Table 3**.** Total Sugar Optimum Pretreatment Conditions

Temperature, ^oC	Concentration, % w/w	Time, min	Log CS	Xylose in Pre-hydrozylate Yield, %	Glucose in Hydrolyzate, Yield, %	Total Sugars Yield, %
120	1.2	60	1.45	76.35	78.04	86.11
140	0.9	30	1.62	80.03	80.29	88.77
140	0.9	60	1.92	75.77	84.28	90.13
140	1.2	5	0.96	74.03	80.44	87.03
140	1.2	15	1.44	78.64	84.77	91.45
140	1.2	30	1.74	81.09	84.54	92.63
160	0.9	5	1.43	80.15	83.59	90.65

ANN Optimum Pretreatment Conditions

A MATLAB program was written to search the ANN output every 5 ^{o}C from 120 ^{o}C to 180 ^{o}C picking the maximum total sugars at each temperature with less than 1 g/L of furfural and within the range of 0.3 % to 1.2 % dilute acid concentration and 5 to 60 minutes pretreatment time. Table 4 contains the entire run from this MATLAB program. The bottom three sets of pretreatment conditions are not truly maximized for total sugars because the MATLAB search program could not find any values in the ANN output at 170 ^{o}C, 175 ^{o}C, and 180 ^{o}C with furfural levels less than 1 g/L. Examining the yields in table 4, it is easy to discern that there are further optimum conditions (>93% total sugars yield) possible beyond those found experimentally. It can also be noted that although the CS values between the experimental and ANN sets vary, they are comparable. While some of the higher yields in the ANN set are associated with a higher CS value, a high CS value does not result in a high yield. In fact, the CS varies within each set even for similar yields suggesting that the severity of a pretreatment as determined by the CS does not predict yield.

Table 4**.** ANN Optimum Pretreatment Conditions

Temperature, ^{o}C	Concentration, % w/w	Time, min	Log CS	Xylose in Pre-hydrozylate Yield, %	Glucose in Hydrolyzate, Yield, %	Total Sugars Yield, %
120	1.2	60	1.45	74.66	79.17	87.37
125	1.2	55	1.56	80.38	81.23	90.63
130	1.1	55	1.67	85.19	83.66	94.02
135	1.1	50	1.78	87.79	85.30	95.81
140	1.0	35	1.73	90.29	87.91	98.33
145	1.0	30	1.81	90.85	89.32	99.15
150	0.9	30	1.91	90.14	90.98	99.73
155	1.2	5	1.41	88.44	89.32	98.80
160	0.8	20	1.98	82.74	92.29	97.10
165	0.7	15	1.94	75.47	91.35	93.30
170	0.3	5	1.25	21.54	68.06	56.74
175	0.3	5	1.39	26.09	73.58	61.63
180	0.3	5	1.54	30.99	78.89	66.57

Fermentation

The fermentation step was generated using a conversion rate of 0.48 grams of ethanol per gram of sugar for ethanol fermentation alone and 0.46g grams of ethanol per gram of sugar for glucose and xylose co-fermentation. The fermentation of glucose alone was compared to the co-fermentation of glucose and xylose in order to quantify the handicap of not using the xylose portion of the biomass. Table 5 shows the fermentation results for the experimental data and table 6 shows the fermentation results for the ANN generated data. Both tables show that there

is about a 70% more ethanol produced per kilogram of biomass if the PreH stream is utilized in the process. As expected from the sugar data, the ANN generated optimum conditions have higher ethanol yields.

Table 5**.** Fermentation Simulation Summary for Experimental Optimum Conditions

Temperature, ^{o}C	Concentration, % w/w	Time, min	Glucose-only fermentation, ml EtOH / kg biomass	Co-fermentation, ml EtOH / kg biomass	Difference, %
120	1.2	60	134.84	232.95	72.8
140	0.9	30	138.72	240.14	73.1
140	0.9	60	145.62	243.81	67.4
140	1.2	5	138.98	235.44	69.4
140	1.2	15	146.45	247.38	68.9
140	1.2	30	146.07	250.59	71.6
160	0.9	5	144.42	245.24	69.8

Table 6**.** Fermentation Simulation Summary for ANN generated Optimum Conditions

Temperature, ^{o}C	Concentration, % w/w	Time, min	Glucose-only fermentation, ml EtOH / kg biomass	Co-fermentation, ml EtOH / kg biomass	Difference, %
120	1.2	60	136.79	236.36	72.8
125	1.2	55	140.35	245.18	74.7
130	1.1	55	144.54	254.34	76.0
135	1.1	50	147.37	259.18	75.9
140	1	35	151.89	266.00	75.1
145	1	30	154.32	268.23	73.8
150	0.9	30	157.19	269.79	71.6
155	1.2	5	154.33	267.27	73.2
160	0.8	20	159.46	262.67	64.7
165	0.7	15	157.84	252.41	59.9
170	0.3	5	117.59	153.50	30.5
175	0.3	5	127.13	166.73	31.1
180	0.3	5	136.30	180.08	32.1

Conclusion

An ANN is effective for accurately modeling sugar yields after pretreatment and enzymatic hydrolysis solely based on the pretreatment conditions as inputs. It has been demonstrated that a well trained ANN can be used to search for optimum sugar yields and corresponding pretreatment conditions without performing the experiments, however this claim would be more

sound with experimental data to validate it. The final result of the simple process model reinforces the significance of fermenting the pre-hydrolyzate stream and demonstrates that a new correlation should be sought beyond the combined severity factor which can better associate pretreatment conditions with sugar yield and economic factors related to the energy and chemical requirements of the pretreatment process

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