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Optimization of liquid ammonia pretreatment variables for maximum enzymatic hydrolysis yield of energy cane bagasse

Saeed Oladi, Giovanna M. Aita*

Audubon Sugar Institute, Louisiana State University Agricultural Center, St. Gabriel, LA 70776, United States

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ABSTRACT

This study aimed at optimizing a liquid ammonium hydroxide pretreatment for energy cane bagasse for maximum sugar yields (glucose and xylose) via response surface methodology. Optimized pretreatment parameters included temperature (160–220 °C), ammonium hydroxide to biomass ratio (0–0.5:1) and residence time (30–60 min). Temperature was found to be the dominant pretreatment parameter followed by ammonium hydroxide to biomass ratio. High temperatures and long residence times had a negative effect on sugar yields. Sugar yields had the highest correlation with lignin removal. Based on our quadratic models fitted on the experimental results, optimum pretreatment conditions for maximum glucose yield were 208 °C, for 36 min and at an ammonium hydroxide to biomass ratio of 0.4:1. A glucose yield of 30.77 g glucose/100 g (dry weight) untreated biomass and a xylose yield of 3.99 g xylose/100 g (dry weight) untreated biomass were predicted by the model. Optimum pretreatment conditions for maximum xylose yield were 160 °C, for 60 min and at an ammonium hydroxide to biomass ratio of 0.31:1. These conditions resulted in a predicted xylose yield of 9.10 g xylose/100 g (dry weight) untreated biomass and a glucose yield of 23.34 g glucose/100 g (dry weight) untreated biomass. The low xylose yields observed were attributed to the high amounts of xylan lost due to solubilization. The quadratic models were found to be reliable for the prediction of sugar yields within the design space. All predicted values were experimentally confirmed with 95% confidence of the predicted values.

1. Introduction

Cellulose and hemicellulose from lignocellulosic material can be utilized in the sustainable production of bio-based fuels and chemicals (Kim et al., 2003). However, the crystalline structure of cellulose and the presence of lignin tightly linked to the hemicellulose create a rigid structure that resists bioconversion (Aita et al., 2011). Therefore, a pretreatment technology is needed in order to disrupt the recalcitrant nature of lignocellulosic biomass (Haghighi et al., 2013). Unless some type of pretreatment is performed prior to enzymatic hydrolysis, no more than 20% cellulose digestibility can be achieved (Mosier et al., 2005).

There are still inherent shortcomings with each type of pretreatment available to date (Pérez et al., 2008). Among all the available technologies, ammonia-based pretreatments have some advantages in that they are non-corrosive, non-pollutant and non-toxic, remove lignin, and preserve most carbohydrates with minimal generation of by-products. These by-products (i.e., furans, carboxylic acids, phenolic compounds) can inhibit enzymes and microorganisms during enzymatic hydrolysis and downstream fermentation processes (Behera et al., 2014). Ammonia-based pretreatment technologies are versatile in terms of applied temperature, residence time and ammonia to biomass ratio. In addition, the volatility of ammonia facilitates its recovery post pretreatment. Residual amounts of ammonia can serve as a nitrogen source for microorganisms during fermentation (Kim et al., 2008; Salvi et al., 2010; Tae Hvun et al., 2006; Wyman et al., 2005). Ammonia-based pretreatments can increase the porosity and surface area of the biomass due to swelling. Furthermore, ammoniation of the methoxyl groups present in lignin prevents the lignin from non-productively binding to cellulases (Yoo et al., 2011). Choosing the right crop for a bioconversion process is crucial since 60–75% of the total cost of biofuel production is allotted to the purchase price of the feedstock (Salassi et al., 2014). Energy cane has great potential as an energy crop and it is a cross breed between sugarcane and its wild ancestors. It is more resistant to cold, drought and disease than sugarcane (Kim and Day, 2011). Variety Ho 02-113 (used in this study) is a non-commercial energy cane variety with a replanting period of seven years compared to three years for sugarcane (Salassi et al., 2014). This variety yields 9% Brix in pressed juice, a fiber content

* Corresponding author.
E-mail address: gaita@agcenter.lsu.edu (G.M. Aita).

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Table 1
Coded level of independent variables in the central composite design (CCD).

		Coded level					
Variables	Unit	Coding	$-\alpha$[a]	−1	0	+1	$+\alpha$[a]
Temperature	°C	A	160	172	190	208	220
Residence Time	min	B	30	36	45	54	60
Ammonium Hydroxide[b] to Biomass Ratio	v/w	C	0	0.10	0.25	0.40	0.50

[a] Axial distance $= \sqrt[4]{N}$, where N is the number of experiments of the factorial design.
[b] H_4OH, 28% v/v solution.

of 264 g/kg (dry basis) and a cane yield of 88.9 t/ha (dry basis) as compared to sugarcane commercial variety LCP 85-384 which has 13.5% Brix in pressed juice, a fiber content of 135 g/kg (dry basis) and a cane yield of 69.2 t/ha (dry basis) (Knoll et al., 2013; Kim and Day, 2011).

Response surface methodology (RSM) is a statistical modeling technique that determines a multivariate equation by utilizing quantitative data from an appropriately designed experiment (Bas and Boyacı, 2007). This modeling technique solves the equation by finding an optimal response. RSM has become a practical method for optimizing different chemical and biochemical processes (Bas and Boyacı, 2007). The main advantage of RSM is minimizing the number of experimental trials required to evaluate the effect of multiple variables as well as their interactions (Kim and Han, 2012).

This study is the first one to evaluate the interactive effect of liquid ammonia pretreatment variables including temperature, residence time, and ammonium hydroxide to biomass ratio on the glucose and xylose yields from energy cane bagasse. Subsequently, these variables were optimized for maximum sugar yield (glucose, xylose) using RSM modeling technique.

2. Material and methods

2.1. Biomass

Energy cane non-commercial variety Ho 02-113 was bred in Houma, LA through collaboration between the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) and the Sugar Research Station at Louisiana State University Agricultural Center in St. Gabriel, LA. Energy cane was harvested at the Sugar Research Station and the entire plant was milled three times using a roller press (Farrel Company, Ansonia, CT) to extract the juice. The remaining solids or fibrous material (bagasse) was oven dried at 45 °C overnight to a final moisture content of 10%. Partially dried energy cane bagasse was milled (Wiley Mill, Arthur Thomas Co, PA), sieved (2 mm mesh sieve) and stored at −20 °C until further use.

2.2. Experimental design and statistical analysis

Central Composite Design (CCD) was employed utilizing the software Design-Expert 9.0.3 (State Ease Inc., Minneapolis, MN) to assess the effect of temperature, residence time and ammonium hydroxide to biomass ratio on the glucose yield of pretreated energy cane bagasse. CCD consists of 2^k factorial points, $2k$ axial points ($\pm\alpha$), and six center points for replications, where k is the number of variables. The range and levels of independent variables are shown in Table 1. Range of independent variables (temperature, residence time and ammonium hydroxide to biomass ratio) were selected based on published literature and preliminary studies. A total of 20 experiments were performed in duplicate and shown in Table 2. Center points of the design were replicated six times to estimate the pure error sum of squares. A quadratic polynomial equation (Eq. (1)) was assumed to approximate the true function.

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii}X_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ii}X_iX_j + \in_i \quad (1)$$

Where Y, response variable; X_i and X_j, the explanatory variables; β_0, constant coefficient; β_{ij}, two factor interaction coefficient; $\in$, random error. Significance of each coefficient was evaluated with analysis of variance (ANOVA).

Xylose yields collected from experimental data were used to statistically optimized the pretreatment conditions for maximum xylose yield (independently from glucose yield) using the software Design-Expert 9.0.3.

2.3. Liquid ammonia pretreatment

Liquid ammonia pretreatment of energy cane bagasse was carried out in a 4 L stirrer reactor (Autoclave Engineers, Erie, PA). Experiments were carried out at ammonium hydroxide (NH_4OH, 28% v/v solution, Fisher Scientific, Pittsburgh, PA) to dried biomass to water ratio of 0–0.5:1:20. Pretreatment temperatures ranged from 160 to 220 °C and residence times ranged from 30 to 60 min. Residence time was monitored once the desired temperature had been reached. At the end of each pretreatment, the bioreactor was cooled down to room temperature using cold water spray to accelerate the process. The pretreated material was passed through a stainless steel sieve (0.2 mm mesh) to separate the liquid and solid fractions. The solid fraction was used for chemical composition analysis and enzymatic hydrolysis assessments. All pretreatments were carried out in duplicate and the mean values calculated.

2.4. Chemical composition analysis

Untreated energy cane bagasse and liquid ammonia pretreated energy cane bagasse samples (solid fraction) were analyzed for glucan, xylan, lignin, arabinan, mannan, and also ash content following NREL's Laboratory Analytical Procedures (LAP TP-510-42618, 42619, 42622). NREL reference material 8491 (for sugarcane bagasse) was analyzed as an internal sample to ensure the accuracy of the procedures.

2.5. Enzymatic hydrolysis

Enzyme concentrations used were kept constant for all the treatments in order to evaluate the effect of pretreatment on biomass digestibility. Spezyme CP (Genencor, Danisco US Inc., Rochester, NY, USA), and Novozyme 188 (Sigma-Aldrich, Inc., St. Louis, MO, USA) were used in combination during enzymatic hydrolysis. Activity of the enzymes were measured following Ghose (1987) method for cellulase activity and Bailey et al. (1992) method for xylanase activity. Subsequently, Spezyme CP at 30 FPU/g glucan and Novozyme 188 at 30 CBU/g glucan were used in enzymatic hydrolysis of samples following NREL LAP TP-51043629. A biomass loading of 5% (w/v) was mixed with 0.1 M sodium citrate buffer (pH 4.8) and