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Optimization of Acid and Steam Explosion Pretreatment of Cogon Grass for Improved Cellulose Enzymatic Saccharification

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Abstract

Acid-impregnation and its combination with steam explosion were evaluated and optimized using Response Surface Methodology. At 10% solid-liquid ratio, cogon was impregnated with diluted H_2SO_4 solution (0 to 3%, w/w) at different ranges of temperature (40 to 120 °C) and varied time (0 to 130 min). Impregnated samples were then subjected to enzymatic saccharification using 60 FPU/g Accelerase 1500™. After enzymatic saccharification, the concentration of reducing sugar released was measured using colorimetry. Based on the results, Response Surface Model (RSM) showed that the optimum condition, predicting 7.18% Reducing Sugar Yield (RSY), was impregnation of cogon using 1.9% H_2SO_4 at 91.8 °C for 56 min. Experimental verification of optimum condition, done in triplicates, showed 6.35 ± 0.05% RSY. Acid-impregnated cogon was subjected to steam explosion to improve saccharifiability. Factors varied were temperature (137 to 222 °C) and exposure time (17 to 582 s). Steam-exploded samples were saccharified and RSY was determined. RSM indicated that the best steam explosion condition, predicting 7.91% RSY, was 179 °C and 500 s. Experimental verification of optimum condition showed 8.78 ± 0.02% RSY. Using RSY as basis, steam explosion improved saccharifiability of H_2SO_4-impregnated cogon by 38%, thus, increasing production of reducing sugars for potential bioethanol production.

1. Introduction

One of the major environmental issues we are facing today is climate change. It is primarily caused by the human expansion of the greenhouse emissions which according to the 5th Assessment Report of the Intergovernmental Panel on Climate Change (IPCC), is from the energy supply (47%), industry (30%), transport (11%) and buildings (3%) sectors [1]. This vast dependence on fossil fuels and its impact to the environment led to extensive researches on renewable sources of fuels in many countries. Bioethanol as biofuel is one of the most researched alternative sources of energy.

Lignocellulosic material, such as cogon, is an abundant organic resource in the Philippines. It mainly consists of three types of polymers: cellulose, hemicellulose, and lignin. The carbohydrate components (cellulose and hemicellulose) are fermentable after saccharification, which makes cogon a suitable feedstock for bioenergy production [2]. One important step towards energy conversion of lignocellulosic material is the pretreatment. It is an important process because it removes hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials. Studies have shown that appropriate type of pretreatment method varies for different sources, depending on its composition. Pretreatment methods can be divided into different categories: physical (grinding and milling), physico-chemical (steam pretreatment/autohydrolysis), chemical (alkali, dilute acid, oxidizing agents, and organosolv), and biological, or a combination of these [3]. Several studies have explored the use of dilute acid in the pretreatment of lignocellulosic materials including palm fruit bunch, rice husk, and pine tree [4], and wheat straw [5]. Meanwhile, steam explosion was used also used for various biomass such as olive tree pruning, forage sorghum,

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and rapeseed straw [6]. In this study, H_2SO_4 was used for the chemical treatment followed by a physico-chemical treatment through steam explosion.

2. Experimental

2.1. Raw material

Cogon grass samples were harvested from the marginal lands of Laguna, Philippines. Samples were sun-dried, then oven-dried (26 °C) and stored under ambient conditions (1 atm, 28 °C). Size was reduced to 2 cm using heavy duty cutter and further reduced to an average of 0.5 mm diameter particle size using the Thomas-Wiley Mill (Model ED-5, Ontario, Canada).

Untreated samples were analyzed for moisture and cellulose content. Moisture content was measured by drying the sample at 105 °C in an oven to constant weight. Cellulose content was determined using the protocol described in the Laboratory Analytical Procedures published by the National Renewable Energy Laboratory of the US Department of Energy [7].

2.2. Pretreatment

Design Expert 8.0 (DE8) software from Stat-Ease, Inc. generated the central composite experimental design (CCD) for the optimization stage of H_2SO_4-impregnation (Table 1). At 10% solid-liquid ratio, ground cogon was impregnated with diluted solutions of H_2SO_4 at varied temperatures using temperature-controlled oil bath for varied length of time. Treated samples were filtered and the solid material was washed with distilled H_2O until neutral. Duplicate experiments were carried out per pretreatment condition. H_2SO_4-impregnated samples were subjected to enzymatic saccharification and the reducing sugar content of the hydrolyzate was obtained. Results were analyzed using the DE8 software and the identified optimum acid impregnation condition was verified by an actual experimentation.

2.3. Enzymatic saccharification

Enzymatic saccharification was done to assess the effects of pretreatment on the saccharifiability of cogon. Treated samples were weighed based on the individual moisture content and calculated cellulose content such that the samples had 0.20 g cellulose on a dry basis. The samples were mixed with

Table 1
Central Composite Design
for acid-impregnation of cogon

Sample	Temperature, °C	Time, minutes	$[H_2SO_4]$, wt %	RSY, %
1	60.0	10.0	0.50	4.66 ± 0.0
2	120.0	10.0	0.50	5.99 + 0.0
3	60.0	100.0	0.50	4.04 ± 0.1
4	120.0	100.0	0.50	6.66 + 0.2
5	60.0	10.0	2.50	5.29 ± 0.0
6	120.0	10.0	2.50	5.61 ± 0.0
7	60.0	100.0	2.50	5.41 ± 0.2
8	120.0	100.0	2.50	5.35 + 0.0
9	39.5	55.0	1.50	5.12 ± 0.1
10	140.5	55.0	1.50	4.68 ± 0.3
11	90.0	0.0	1.50	5.14 ± 0.0
12	90.0	130.0	1.50	4.98 + 0.1
13	90.0	55.0	0.00	4.65 ± 0.1
14	90.0	55.0	3.18	6.69 ± 0.2
15	90.0	55.0	1.50	6.45 ± 0.1
16	90.0	55.0	1.50	7.27 + 0.2
17	90.0	55.0	1.50	8.90 ± 0.1
18	90.0	55.0	1.50	6.92 ± 0.2
19	90.0	55.0	1.50	6.81 + 0.1
20	90.0	55.0	1.50	7.24 ± 0.0

10 mL sterilized citrate buffer (0.05 M, pH 4.8) in a sterile 125-mL Erlenmeyer flask. A volume of sterile distilled water was then added so that the total reaction volume was 20 mL (volume of the enzyme added later was included in the calculation of total volume). The tubes were incubated at 50 °C. Upon obtaining a constant temperature, Accelerase 1500™ from Genencor Company, USA was added at a loading of 60 FPU mL^{-1} sample. Addition of the enzyme signified the start of the saccharification. The flasks were placed in a 50 °C-shaking incubator and the reaction was allowed to proceed for 72 h. Enzyme was deactivated by submerging samples in a boiling H_2O for 10 min. A substrate control and an enzyme control were also prepared to account for the residual sugars present in the mixture. A substrate control is composed of all mixture components except the enzyme while the enzyme control lacks the substrate. Saccharified samples were centrifuged at 1.660 × g for 10 min and stored for reducing sugar analysis via the Nelson-Somogyi Method [8]. Reducing sugar and Reducing Sugar Yield (RSY) were calculated using the following equations:

$$\%RSY = \frac{(RS_{corr})(V_{rxn})}{m_{biomass}} \times 100$$

Where: *%RSY* = reducing sugar yield per gram biomass; RS_{corr} = corrected RS; V_{rxn} = reaction volume of the concentration measurement; $m_{biomass}$ = mass of the biomass sample used

$$RS_{corr} = RS_{raw} - RS_{enzyme} - RS_{substrate}$$

Where: RS_{corr} = corrected RS; RS_{raw} = raw measurement of RS; RS_{enzyme} = RS of enzyme control; $RS_{substrate}$ = RS of substrate control.

2.4. Steam explosion

A CCD (Table 2) generated by the DE8 was used for the optimization of steam explosion pretreatment. Three hundred milliliters of optimally-impregnated cogon, tightly packed in a beaker, was fed into the reaction chamber on top of the QBS-80 Steam Explosion Technology Test Bed (Hebi Zhengdao Machine Factory, Hebi, China) through a feed hopper. The desired temperature was set and the steam was allowed to heat the material for a certain length of time. The steam pressure was released which resulted to explosive decompression of the sample. Steam exploded samples were subjected to enzymatic saccharification and the reducing sugar content of the hydrolyzates were obtained. Results were analyzed using the DE8 software and the identified best steam explosion condition was verified by an actual experimentation.

Table 2
Central Composite Design for steam explosion of acid-impregnated cogon

Sample	Temperature, °C	Time, sec	RSY, %
1	150.0	100	4.14 ± 0.2
2	210.0	100	2.98 + 0.2
3	150.0	500	4.49 ± 0.0
4	210.0	500	4.03 + 0.1
5	137.6	300	3.51 ± 0.0
6	222.4	300	3.10 ± 0.2
7	180.0	17	5.88 + 0.0
8	180.0	583	9.24 + 0.0
9	180.0	300	6.93 + 0.1
10	180.0	300	7.04 ± 0.0
11	180.0	300	7.39 ± 0.1
12	180.0	300	8.55 ± 0.0
13	180.0	300	4.14 + 0.3

Summary of the procedure

A process flow of the procedures was shown in Fig. 1.

3. Results and discussion

3.1. Cellulose and moisture content

The cellulose content of dried cogon grass was 37% (w/w, dry weight basis). It is consistent with the cellulose content of grasses (25–40%) as stated by Sun & Cheng [9]. Cellulose content estimates the amount of glucose that can be produced after enzymatic saccharification of the substrate. Moisture content of fresh cogon is 60%.

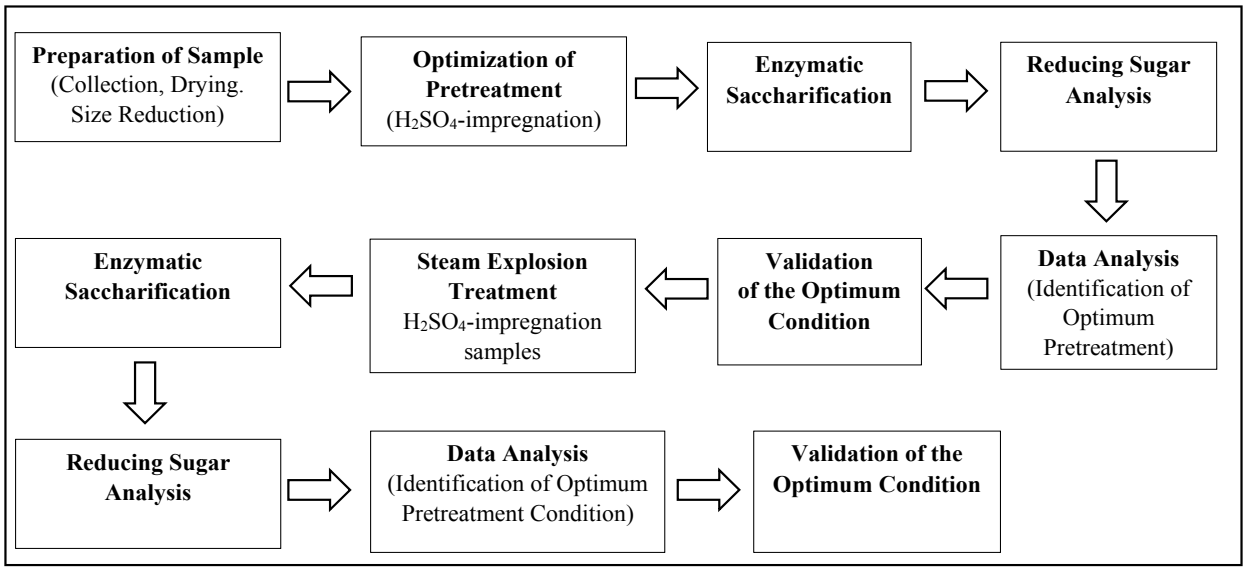

Fig. 1. Process flow of the procedures.

3.2. Effect of acid impregnation

Acid is known to be effective in removing the hemicellulose of plant materials. In this study, the firm structure of cogon grass was broken by acid hydrolysis, resulting to the removal of hemicelluloses. This is also equivalent to increased porosity and enzymatic digestibility of biomass [10, 11, 12], hence, a more exposed cellulose for enzymatic attack. The effectiveness of acid pretreatment done was measured by the RSY after enzymatic saccharification. Results showed that reducing sugars obtained after enzymatic saccharification of different treatments ranged from 4 to 9%. A response surface model generated was found to be significant and there is only a 4.59% chance that its value occurred due to noise. Moreover, the «Lack of Fit F-value» is not significant relative to the pure error, thus, the model fits with the data. The statistical model relating the RSY to the acid impregnation parameters (in terms of coded factors) is:

$$\text{SQRT}(\%RSY) = 0.38 - 0.010\,(A) - 1.094 \times 10^{-3}\,(B) - 0.013\,(C) - 5.048 \times 10^{-3}\,(AB) + 0.019\,(AC) - 9.866 \times 10^{-4}\,(BC) + 0.024\,(A^2) + 0.025\,(B^2) + 0.015\,(C^2)$$

Where: %RSY = reducing sugar yield per gram biomass; A = temperature; B = time; C = H_2SO_4 concentration

The three factors, individually, had minimal effect on the RSY. Increasing each factor, though, resulted in an increase of the RSY. The increase in temperature had almost the same effect with the increase in impregnation time, both having a more dominant positive effect on the RSY compared to the increase in acid concentration. A positive interaction involving temperature and concentration was also indicated. The graphical representation of this model (response surface) is shown in Fig. 2. The graphical view of the model predicted that the optimum condition was impregnating cogon with 1.9% w/w H_2SO_4 at 91.8 °C for 56 min. The predicted RSY using the said condition was 7.18%. Verification using actual impregnation experiment yielded 6.35 ± 0.02%.

3.3. Effect of steam explosion

In steam explosion pretreatment, the sample is exposed to high pressure and consequently high temperature. It allows the breakdown of lignocellulosic structural components by the action of heating, formation of organic acids during the process, and shearing forces resulting in the expansion of the moisture [13, 14]. A study by Lam et al. [15] suggested that steam explosion of wood resulted in a material with favorable fuel properties (high heating value, low moisture absorption). It was also an effective pretreatment in poplar as a significant increase in pores was observed [16]. However, beyond a certain temperature, sugar losses will increase. RSY after saccharification ranged from to 3.0 to 9.2%. A model generated from the response was found to be significant, having only a 0.68% chance that its value occurred due to noise. Moreover, the «Lack of Fit F-value» is not significant relative to the pure error. The statistical model relating the RSY to the acid impregnation parameters (in terms of coded factors) is:

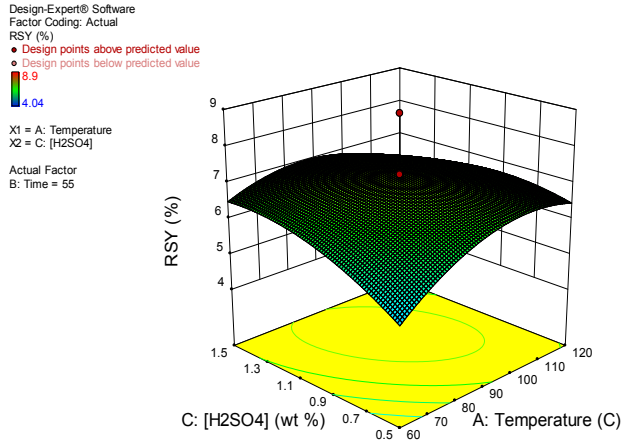

Fig. 2. Plot of the response surface model for the acid-impregnation of cogon. (Scale Units: Temperature in °C, [H_2SO_4] in %w/w, RSY in %).

$$\%RSY = 7.48 - 0.27\,(A) + 0.77\,(B) + 0.18\,(AB) - 2.47\,(A^2) - 0.34\,(B^2)$$

Where: %RSY = reducing sugar yield per gram biomass; A = temperature; B = time.

It could be seen that time, compared to temperature, had greater effect on the RSY. The increase of both the temperature and time, however, will lead to a decrease in RSY. Moreover, temperature-time interaction had a minimal effect on the RSY. The graphical representation of this model is shown in Fig. 3. The graphical view of the model suggests that the optimum temperature lies between 174 °C and 186 °C. Increasing the holding will have no significant effect on the RSY, thus, high yield can be attained at any time between 100 to 500 s. Using

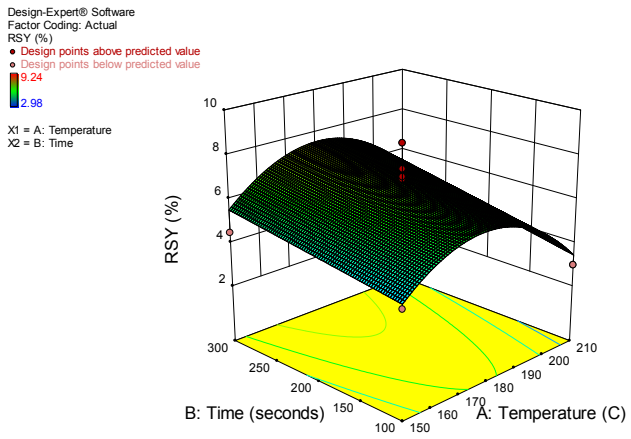

Fig. 3. Plot of the response surface model for the steam explosion of acid-impregnated cogon. (Scale Units: Temperature in °C, [H_2SO_4] in %w/w, RSY in %).

the numerical optimization feature of the DE8, the optimum condition was calculated and established to be 179 °C and 500 s. The predicted RSY using the said condition was 7.91% while the experimentally verified optimum RSY of the samples was 8.78 ± 0.05%. Subjecting acid-impregnated cogon to steam explosion pretreatment has increased RSY by 38%.

The models generated to predict the optimal conditions for both acid-impregnation and its combination with steam explosion are valid based on the results of the experimental verification. Reducing sugar yield upon using acid-impregnation as pretreatment method for cogon was 6.35% and a follow-through steam explosion resulted to an 8.78% RSY. Steam explosion improved RSY of acid-impregnated cogon by 38%, thus, increasing the potential bioethanol yield.

4. Conclusion

Pretreatment methods is a critical factor in converting lignocellulosic feedstocks into simple sugars. It loosens the structure of the material so that enzymes will be able to its work in converting cellulose into sugars through saccharification. Acid impregnation using the optimum condition was able to generate about 6.35% yield of reducing sugar while its combination with the optimum steam explosion conditions improved the yield to 8.78%.

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