Effect of pretreatment dilute acid - peroxide on napier grass *(pennisetum purpureum schumach)* to enhance reducing sugar yield by enzymatic hydrolysis

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Abstract

The napier grass (*Pennisetum purpureum Schumach.*) is a potential feedstock biomass for conversion to ethanol, bio-energy or high added value products. Variation in prehydrolysis parameters (time, acid strength, biomass %, particle size) and enzymatic saccharification conditions were examined for conversion of napier grass into fermentable sugars. A pretreatment of the water insoluble substrate ($WIS_{H_2SO_4}$) at 15.1 % of reducing sugar yield (RSY) was done with peroxide (H_20_2) at 122 °C. The relationship between RSY and the acid process parameters are described by a mathematical model derived from the experimental data.The peroxide pretreatment at 0.7 % (w/w), 4.75 mm particle size, 8% (w/v) biomass concentration, improved the RSY production from enzymatic hydrolysis in 97.6 % after 75 min. The enzymatic hydrolysis produced 287.81 mg/(g initial dry sample) of glucose and 245.81 mg/(g initial dry sample) of Xylose. Therefore, it was concluded that combination peroxide - acid pretreatment is an effective and environmentally friendly method for the enzyme hydrolysis of napier grass.

Keywords

Dilute acid, hydrolysis, reducing sugars, napier grass, *Pennisetum purpureum*, peroxide.

1. Introduction

The chance to develop a sustainable economic bioethanol project implies fast growing crop adapted to the climatic and soils conditions of a specific area and a lignocellulosic biomass efficient technology conversion to fermentable sugars at lower costs [1,2]. It means to compete with gasoline; the cost production of ethanol from biomass should be competitive [3].

Napier grass (*P. purpureum*) can be considered as a potential feedstock of biomass for conversion to ethanol. *P. purpureum* is crop for animal feed and the excess may be used as feedstocks for bioethanol production to avoid direct competition between bioethanol and food productions. The optimization of pre-treatment process parameters of the lignocellulosic biomass dilute acid pretreatment is a necessary condition to get the highest glucose and hemicellulose sugars release [4]. Pretreatment of lignocellulosic materials is done to remove lignin and enhance the hydrolysis of cellulose. Even dilute acid pretreatment of lignocellulosic biomass has been researched for several materials [5]. The pretreatment of biomass at high temperature (150 - 240 °C) and the quantity of acid used becomes an uneconomical cost production [6]. However, dilute acid pretreatment has been shown to successfully hydrolyze hemicelluloses and disrupt the ligno-cellulosic structure for a wide range of feedstocks [7,12].

The pretreatment of potential biomass to increase RSY gives the opportunity to develop new pro-

cedures with the environmental requirements, low concentration of inhibitory substances for saccharification and fermentation [13,14]. Peroxide (H_2O_2) an oxidative agent under appropriate conditions reacted readily with lignin and related phenolics [15,18]. Besides, alkaline peroxide pretreatment combination with steam explosion and hydrothermal process of lignocellulose biomass showed to improve enzymatic saccharification [19-21].

Different pretreatment variations of alkali peroxide procedure especially with acids have been investigated for lignin removal to enhance hydrolysis [22-29]. A variation peroxide-sulphuric acid pretreatment has not received enough attention. However, a pretreatment peroxide 1% -acid 1 % on sawdust produced between 47 – 56 % of fermentable sugars [23]. The high cost of peroxide do not use feasible for pretreatment. This paper describes the effect of peroxide pretreatment on washed at 15.1 % RSY. A factorial surface response methodology was employed to describe the relationship between RSY and the major process variables, namely acid concentration, pre-treatment temperature, size particle and time. The procedure improves the RSY by enzymatic hydrolysis about 81.1 %. This research can give new clues to improve lignocellulosic pretreatment procedure on the commercial use of *P. purpureum* for large-scale ethanol production.

2. Material and methods

Samples of napier grass (P. purpureum) were provided by Pharmavicola. Four random samples of 16-wk-old material were gathered and weighed immediately after cutting. The grass samples were a mixture of leaf and stem. The material was dried at 55 ºC by 12 hours. Then it was milled to a particle size smaller than 0.5 mm using a laboratory mill, homogenized and stored in a desiccator at room temperature until used. A sieve shaker was used. The chemical composition of raw material and water insoluble fraction were performed using standards [30].

Ash was determined as the percentage of dry residue remaining after the oxidation between 550 ºC - 600 ºC. The liquor was analyzed for total RSY using a DNS assay [31,21].

The concentrations of main components were as follows: Cellulose 35.5±0.801 %, lignin 11.3±0.638, xylose 14.3±0.302, extractives 16.0± 0.456, ash 5.84± 0.076.These values are in the range found for this kind of materials [33-38].

To reducing sugars (in prehydrolyzate), pretreatments samples were autoclaved in a 10-L stainless steel reactor at 105 °C and 20 psi. Biomass samples were loaded into the reactor at the different solid (2, 4, 6, and 8 g of biomass)/liquid ratios containing 0.5 to 1 g H_2SO_4/100 g liquor on dry basis. Samples were collected from the reaction media at several reaction times in the range 30–90 min. A factorial design based on the variation of the pretreatment conditions acid concentration, size particle, biomass concentration and residence time was used to identify the best pretreatment conditions for RSY in the aqueous fraction are summarized in Figure 1.

The hemicellulose from washed water insoluble substrate ($WIS_{H_2SO_4}$) at 15.1 % RSY was solubilized with peroxide (0.4 – 0.7) % (w/w) at 30 °C with agitation at 130 rpm for 72 h. The parameters used for acid pretreatment are shown on Table 1.

Table 1. Parameters used for acid pretreatment of napier grass.

Code	Parameter	Low level	High level	Relation	F	R^2
K_1	Acid, % (w/w)	0,5	1	k1-k2	0.76	0.86
K_2	Biomass, % (w/w)	2	8	k1-k3	0.67	0.69
K_3	Size (mm)	2.36	4.75	k1-k4	1	0.89
K_4	Residence time (min)	30	90	k1-k4	0.99	0.76

The reduction sugar yield production (arabinose, glucose and xylose) was calculated by Eq. 1 and 2.

RSY Production (%) = 100 (RSY /Total theoretical RSY) (1)

Total theoretical RSY = (Biomass concentration) (dry biomass) (hemicellulose) (2)

For enzyme hydrolysis filter paper procedures were used to determine the activities of the cellulase and cellobiase. Comercial cellulose 1500 and multifect CXB enzymes were used for the enzymatic hydrolysis step.

Accellerase1500 (Genencor, USA), endoglucanase (2200−2800 CMCase units/g), and β-glucosidase (525−775 pNPG units/g) enzyme were used in this study. Enzymes were kindly provided by Genencor- Merquiant LTDA. Multifect CX B, β-Gluconase (2250 BGL U/mL) obtained from Trichoderma reesei. A dosis of 0.05 g enzyme/g subtracts was used, specific gravity 1.3 g/mL. The washed insoluble subtracts after peroxide pretreatment were used for enzymatic hydrolysis. Enzymatic hydrolysis was performed in 100mL Erlenmeyer flasks, each containing 25 mL buffer of 0.029 M sodium citrate buffer (pH 4.8) at 8 % (w/v) dry pretreated substrate loading at 50 °C at 150 pmr for 100 min. Samples were taken at timed intervals, boiled for 2 min to denature the enzymes, and analyzed for RSY concentrations [39]. All hydrolytic experiments were performed in triplicate and the average results are shown in Figure 3. Data of enzymatic hydrolysis yield (EHY) was calculated in mg per 100 g of pretreated substrate by Eq 3:

$$EHY = \frac{\text{Released reducing sugars x 0.9}}{\text{Glucose from cellulose (dry)}} \quad (3)$$

The water-insoluble phenolic fraction was analyzed by UV– vis spectroscopy at 280 nm. Glucose and xylose yield from peroxide-enzymatic hydrolysis were analized by HPLC-Trangenomic ION-300 column (oven temperature at 45 °C) with isocratic elution (flow rate 0.4 ml/min; mobile phase: H_2SO_4 0.005 N). Standard samples were prepared for determination.

The effect of the parameters acid concentration, size particle, biomass concentration on the RSY by acid pretreatment was found to be not linear. A factorial surface response methodology was employed to setup the experiment. Data were used to construct a polynomial mathematic model with the following form presented by Eq 4:

$$RSY\ \% = \sum_{i=0}^{N} B_i X^i \quad (4)$$

Where is a polynomial that depends of biomass (w/w) by eq 5:

$$B_i = \sum_{j=0}^{M} B_{ij} C_i \quad (5)$$

The lowest RSY by acid pretreatment were determinated. The model was analyzed using a 95 % confidence F test, and the coefficient, r2, was checked.

3. Results and discussion

The use of bioethanol supposes a reduction our dependence upon foreign expensive oil and reduces greenhouse gas emissions [13]. Napier grass (*Pennisetum purpureum Schumach.*) biomass can be an adequate substrate for ethanol production due to the high carbohydrate content of 60.0 % of the dry weight. The hemicellulose fraction comprises 24.7 % of raw material, xylose being the main sugar (80%). Cellulose and lignin content is around 35.3 % and 8.0 %, respectively. From literature review, particle size has not considered as an important parameter in pretreatment of lignocellulose biomass [33, 38, 39].

In our study, a dilute acid pretreatment of napier grass (*P. purpureum)* biomass was done under different process parameters: temperature (122 °C), solid concentration in the reactor (2-6%, w/v), sulfuric acid concentration (0.5–1.0 %, w/w) and size particle (2.0 – 5.0 mm).

On Figure 1, shows the estimated response surface (R^2 = 0.79) for acid pretreatment of napier grass where RSY increases with decrease biomass feed. A low RSY production of 15.1 % for a biomass feed of 8.0 %.

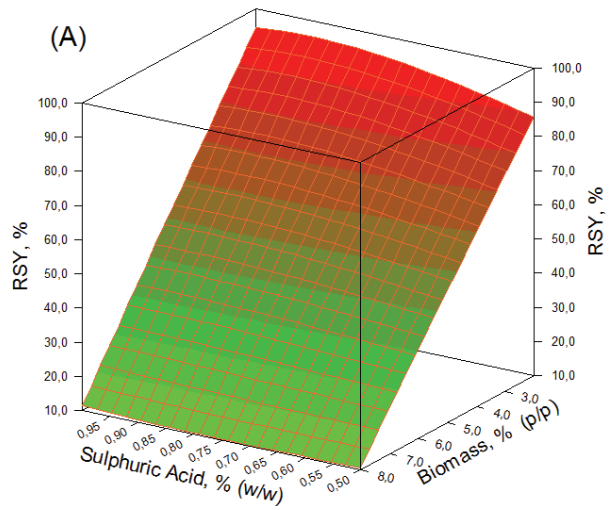

Figure 1. Estimated response surface for acid pretreatment showing the influence of acid and biomass on RSY.

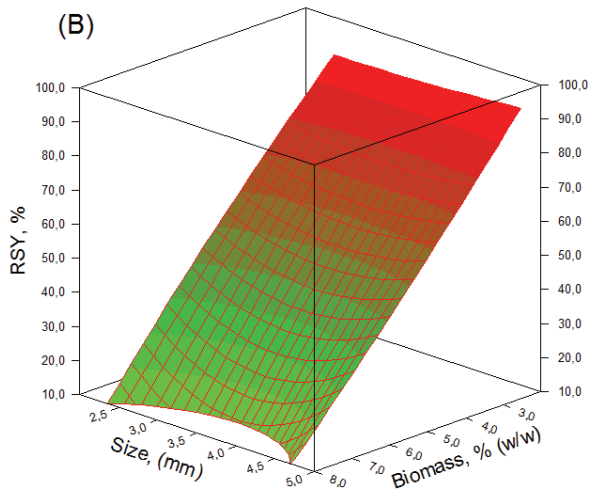

Figure 2. Estimated response surface for acid pretreatment showing the influence of size particle and biomasa on RSY.

The maximum of RSY of 98 % was found at 2.0% (w/v) biomass concentration, and acid 0.8 %. The results suggest that RSY is negative affected at high biomass concentration. From the economical and environment perspectives low biomass feed charge implies an important limitation to the industrial process [40,41].

As shown in Figure 2, two important groups of data with high RSY production at 2.5 mm and 4.5 mm particle size (r^2 =0.81) are found. From the results on Figure 3, the best RSY was found at 60 min and around 3 % (w/w). But for biomass concentration above 6 % (w/w) the RSY is around 10 %. RSY % varies from 77 - 98% from 30 - 60 min residence time. Pretreated $WIS_{H_2SO_4}$ were washed 3 volumes of water prior to enzymatic hydrolysis to remove inhibitors [39]. The $WIS_{H_2SO_4}$ obtained from the peroxide pretreatment was carefully washed and used to enzymatic hydrolysis for 120 min.

From the above results it was decided to use washed $WIS_{H_2SO_4}$ at 15.1% RSY (2.455±0.02 g/L) dilute acid (0.5 %, w/w), biomass feed (8 %, w/v), size particle (4.75 mm) and residence time (30 min) for the next pretreatment with a peroxide solution (0. 4 - 0.7 %,w/w). The experimental conditions at pH 11,4 for second pretreatment were biomass feed (8 %, w/v), residence time (72 h), particle (4.5 mm), 130 rpm at 30 °C was done on $WIS_{H_2SO_4}$.

From the results on Figure 3, the best RSY was found at 60 min and around 3 % (w/w). But for biomass concentration above 6 % (w/w) the RSY is around 10 %.

Even peroxide pretreatment has showed to be an efficient agent procedure. However, the cost of peroxide, and the high concentration of reagent used, deeply affects the bioethanol production. For this reason, new studies to decrease peroxide concentration without affecting RSY are useful [15].

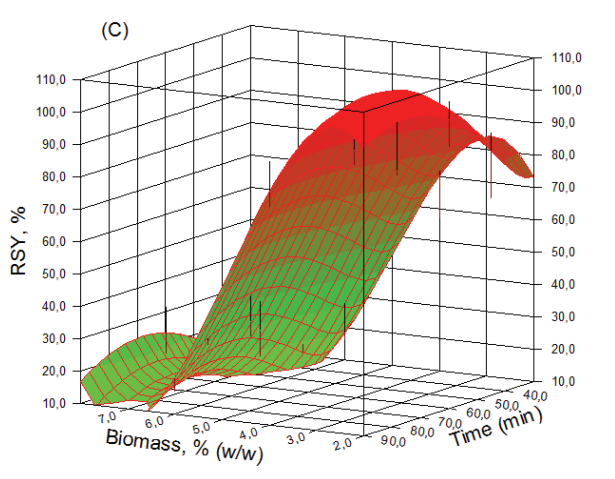

Figure 3. Estimated response surface for acid pretreatment showing the effect of biomass and time residence on RSY.

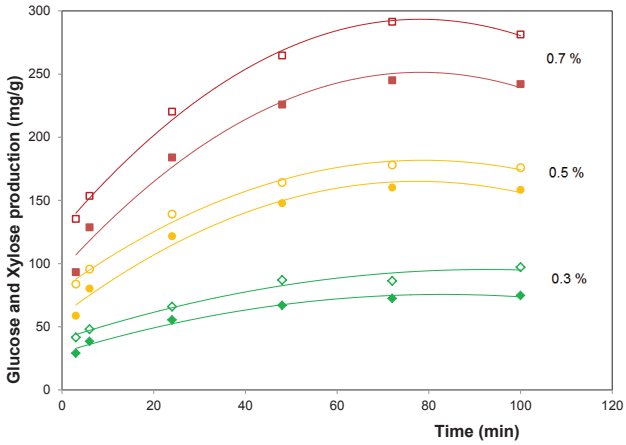

Figure 4. Experimental dependence of Glucose and Xylose production on time at several peroxide concentrations by enzymatic hydrolysis. Peroxide: 0.3 %(w/w), Glucose (□), Xylose (■); 0.5 % (w/w), Glucose (○), Xylose (●); 0.5 % (w/w), Glucose (♦), Xylose (◊).

Figure 4 shows 97.6 % of RSY obtained from washed $WIS_{H_2SO_4}$ treated with peroxide 0.7 % (w/w) by enzymatic hydrolysis at 75 min. The compositional analysis shows significant differences in glucose and xylose production between samples treated with peroxide at 0.3 %, 0.5 % and 0.7 %. The peroxide treatment produced 287.81 mg/g (initial dry sample) of glucose and 245.81 mg/(g initial dry sample) of Xylose, respectively, by enzymatic hydrolysis after 80 min. In the dilute acid pretreatment, on rye straw it was found 146 mg xylose/g (acid 1.5%, 90 min, 121 °C) and, on Bermuda grass 137 mg xylose/g, acid 1.5% (w/w), 60 min and 121 °C. 37 But also, alkaline peroxide 10 % (w/w) pretreatment combined with steam explosion (20 atm) on bamboo produced 456 mg/(g initial dry sample) of glucose and 460 mg/(g initial dry sample) of reducing sugar by enzymatic saccharification at 20 h. [19]. The peroxide treatments of sucarcane bagasse at 70 °C for 48 h produced xylose and glucose as 250 mg/100 g and 230 mg/100 g respectively [22]. However, by alkaline hydrogen peroxide treatment the higher glucose yield: 691 mg/ g of glucose were found for pretreated bagasse after hydrolysis of bagasse pretreated for 1 h at 25 °C with 7.35% (v/v) of peroxide [15]. Also, a combined steam-pretreated material (184 °C for 4min) and 2% peroxide at 60 °C for 24 h, obtain a glucose yield of 503.5mg/g raw material with a substrate concentration of 3.3% [42]. These pretreatment are very expensive because the peroxide high concentration and the low substrate concentration used.

The peroxide pretreatment of $WIS_{H_2SO_4}$, released glucose should proceed from hemicelluloses mainly. From literature, it was found that pretreatment of sawdust with acid 1%, sawdust-to-acid (w/v) ratio of 1:10, 120°C at 15 psi for 40 min., then the acid hydrolyzate of sawdust treated with peroxide 1% produced 47 - 56 % RSY [43,44].

The grass *P. purpureum* with cellulose and hemicellulose fractions of 40% and 30%, and low lignin content (23.89 %) respectively showed to be a promising biomass for high RSY production and low inhibitors substances [33].The analysis of the liquor after peroxide pretreatment showed low concentrations of 0.33 g furfural/L and 0.73 g hidroximetilfurfural/L. Furfural is considered affect hydrolysis above 5 g/L only after 48 hours [45].

The data of the RSY resulted from the enzymatic hydrolysis of the napier grass pretreated found to depend on the peroxide concentration.

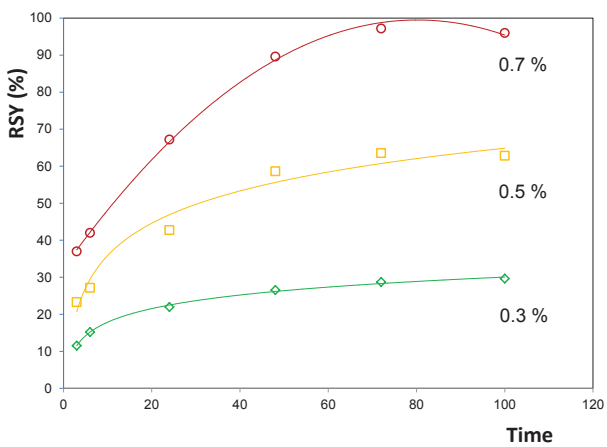

Figura 5. Experimental dependence of RSY (%) on time at several peroxide concentrations by enzymatic hydrolysis. Peroxide % (w/w): 0.3 %, (○), 0.5 % (□); 0.7 % (◊).

Conclusions

Our objective was to combine the effect of peroxide acid pretreatment to enhance RSY by enzymatic hydrolysis. A factorial design based on the variation of the pretreatment conditions of the dilute sulfuric acid pretreatment at loading (0.5–1.0 %, w/w), residence time (30 – 90 min), particle size (2.36 - 4.76 mm) and solid/liquid ratio (2–8%, w/v) on RSY production in a batch reactor at 122 °C was done. The best RSY production of 99.71 % (7.001 ± 0.255 g/L) was found at dilute acid (1 %, w/w), biomass feed (2 % w/v), size particle (2.36 mm) and residence time (90 min).

In this study, peroxide - acid pretreatment of napier grass (*P. purpureum*) were investigated to enhance RSY enzymatic hydrolysis as a potential substrate for bioethanol production. The combination of peroxide acid pretreatment dramatically improved the hydrolyzability of the substrate ($WIS_{H_2SO_4}$). This study aims to combine peroxide –acid dilute pretreatment to low cost production. A pretreatment of the water insoluble substrate ($WIS_{H_2SO_4}$) at 15.1 % of reducing sugar yield (RSY) was done with peroxide. The relationship between RSY and the acid process parameters could be well described by a mathematical model derived from the experimental data. On Figure 5, peroxide acid pretreatment at 0.7 % (w/w), 4.75 mm particle size, 8% (w/v) biomass concentration, improved the RSY production from enzymatic hydrolysis in 97.6 %. After enzymatic hydrolysis, this produced 287.81 mg/(g initial dry sample) of glucose and 245.81 mg/(g initial dry sample) of Xylose. Therefore, it was concluded that peroxide acid pretreatment is an effective and environmentally friendly method for the enzyme hydrolysis of napier grass.

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