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Bioethanol from Fresh and Dried Banana Plant Pseudostem

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Lignocellulosic wastes have stood out in the study and development of processes aimed at producing biofuels. Brazil is one of the world's largest food producers and ranks fifth in world production of bananas with an average production of seven million tons per year. For each ton of bananas harvested, around four tons of lignocellulosic wastes are generated, among which 75% consists of banana plant pseudostem. This work investigated the production of bioethanol by Saccharomyces cerevisae using this residue (pseudosteam) as a fermentation substrate previously hydrolyzed under the following conditions: (a) 250 g/L of fresh biomass (equivalent to 11.75 g/L of dry matter) cut into about 1 mm long pieces and (b) 70 g/L of dry and milled biomass (drying in 60 ºC forced air draft tray dryer and milling in Solab knife mill until particles of size smaller than 30 mesh). The biomass pre-treatments with H_2SO_4 2% m/m and NaOH 3% m/m, both conducted at 120 ºC, 15 min were evaluated. For saccharification of the pre-treated biomass, enzymatic hydrolysis (24 h, pH 5.5, 45 °C) using Novozymes® enzymatic complex composed of cellulase, beta-glucosidase and xylanase was used. The experiments were conducted in Erlenmeyer flasks containing 100 mL of work volume. The greatest percent yield in glucose (Y_{RS} = 79.5±4.4 %), calculated based on the theoretical yield of cellulose hydrolysis to glucose (1.1 g/g), was obtained with fresh biomass pre-treated with NaOH. This value was 84% higher than the percent yield resulting from the pretreatment of 70 g/L of dry biomass with the same type of hydrolysis catalyst (Y_{RS} = 43.2±1.2 %) and 31% higher than the value reached in the pretreatment of this same biomass with H_2SO_4 (Y_{RS} = 60.7±6.7%). The maximum RS value in hydrolyzed liquor (without prior concentration by evaporation) was obtained from dry biomass saccharification with H_2SO_4 (26.6±1.1 g/L). The fermentation of this liquor, after concentrating to RS ≤ 62.1 g/L, resulted in an ethanol production of 22.1±0.8 g/L with respective values of $Y_{P/RS}$ = 0.47±0.03 g/g, ethanol productivity (Q_P) 1.83±0.12 g/L.h and effectiveness of alcoholic fermentation (E_P) 80.4±0.12 %.

1. Introduction

The use of lignocellulosic biomasses has been considered an attractive alternative for the substitution of non renewable energy sources, both for the generation of thermal or electrical energy as well as the production of fuels for vehicles (Dias et al., 2013; Fernandes et al., 2013; Sellin et al, 2013). Due to their availability and low acquisition cost, the agro industrial wastes stand out within these biomasses.

Brazil is one of the world's biggest food producers and currently occupies 5th place in the production of bananas with an annual production of around seven million tons (CEPA, 2012). According to Souza et al (2010), for each ton of fruit harvested around 4 tons of pseudostem remain at the harvest location undergoing natural degradation. Making use of this biomass for the production of alcohol for fuel could be a very attractive alternative by not only contributing to the preservation of the environment through removing this waste from the land but also by adding value to the fruit production matrix, transforming the residue into a commodity.

Regarding ethanol production from this type of biomass, the search for processes able to efficiently perform the dissociation of the lignin-cellulose-hemicellulose complex and enable the attainment of high yields in fermentable sugars in hydrolyzed liquor and, most importantly, without generating elements that inhibit the fermentation process, is one of the great challenges for producing 2nd generation ethanol that is

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competitive with 1st generation ethanol from sugarcane, corn or sugar beet (Limayen and Ricke, 2012, Menon and Rao, 2012, Viikari et al., 2012, Mood et al., 2013).
Routes most studied till now have been the use of chemical pretreatment with acids or bases, followed by enzymatic hydrolysis, of which the acid pretreatment is considered as one of the most important techniques and aims for high yields of sugars from lignocellulosics (Sarkar *et al*., 2012).
The pretreatment is done to break the matrix in order to reduce the degree of crystallinity of the cellulose and increase the fraction of amorphous cellulose, the most suitable form for enzymatic attack (Sanchez and Cardona, 2008).
According to Souza et al. (2012) *Musa cavendischii* banana tree pseudostems under natural conditions has 95.3% of moisture content, 44.0% of cellulose and 8.1% of lignin on a dry basis. This cellulose levels is higher than wheat straw (30%), grasses (25%-40%) and olive ter Wood (31.4%) and similar levels to pine Wood (46.4%). However, the high percentage of moisture content present in this biomass leads to the need to assess the influence of this variable on the bioethanol production.
The aim of this study was to produce 2nd generation ethanol from pseudostem as received (without drying) and dry biomass (dry powder). For each condition, two different methods of chemical pretreatment (NaOH and H2SO4) were evaluated, followed by enzymatic hydrolysis.

2. Material and Methods

The assays with chemical pretreatments, enzymatic treatment and fermentation were conducted in 500 mL Erlenmeyer flasks, with 100 mL work volume. The results obtained were analyzed using the ANOVA method with Tukey test for $p<0.05$ using the Origin 7.5 software.

2.1 Biomass and treatments

The pseudostem from the *Musa cavendischii* banana plant was evaluated in the form of fresh particles and dry bran. Initially the biomass was cut into four slices, 30 cm length, and crushed to remove its natural liquor and then sent for pretreatment.
For the fresh biomass assays, the pressed pieces were crushed in an aqueous medium in a concentration of 250 g/L wet weight (11.75 g/L dry matter), until obtaining particles smaller than 2 mm; for the dry biomass, the crushed pieces were dried in a tray dryer equipped with forced air draft at 60 °C and milled in a Solab knife mill until attaining particles smaller than 30 mesh. The biomass concentration in powder form used in pretreatment was 77.8 g/L dry matter (dm). Both biomass concentrations (fresh and powder) were defined as the maximum possible for obtaining a satisfactory flow of the sample to be removed from the Erlenmeyer flasks. Two types of pretreatments were evaluated, both at 120 ºC/15 min: (1) Acid hydrolysis (H_2SO_4 2% m/m) and (2) alkaline hydrolysis (NaOH 3% m/dm).
The pretreated biomass, without filtering to remove the hydrolyzed extract, was depolymerized by a pool of enzymes Novozymes® using the operating conditions given by the manufacturer: (NS50013 Celulase-complex, 6% m/dm, NS50010 β-glucosidase, 0.6 % m/dm, NS50012, Enzymatic complex, 0.4 % m/dm, NS50030 Xylanase, 0.5 m/m, NS22002 Hemicelulase, 2 % m/dm) in sodium acetate/acetic acid 0.1 M buffer (pH 5,5, 45 ºC, 24 h). The mixture was then filtered and the saccharified liquor (fermentation broth) was fermented by wild Saccharomyces cerevisae.

2.2 Fermentation

The fermentation assays were carried out in 250 mL Erlenmeyer flasks with a working volume of 100 mL (80 mL fermentation broth and 20 mL inoculum), agitation frequency 120 min^{-1}, 30 ºC and initial pH 4.5. The inoculum was cultivated for 24 h in synthetic medium consisting of (g/L): glucose, 20, yeast extract, 3, $(NH_4)_2SO_4$, 0.5, K_2HPO_4, 0.5, $MgSO_4.7H_2O$, 0.1 and $CaCl_2$, 0.1, according to that proposed by Souza et al (2012). The filtered liquor was fermented under two conditions: (a) without prior concentration of reducing sugars (RS) and (b) with RS concentrated by atmospheric evaporation at 70-80 ºC.

2.3 Analytical methods and calculations

The samples were centrifuged at 3500 rpm (2000 g) for 10 min and the supernatant was removed using a glass pipette and frozen until analyzed. For the depolymerized liquors, the reducing sugars, RS (fructose + glucose+ xylose) were determined using high-performance liquid chromatography (HPLC) employing a Merck Hitachi D- 7000 IF chromatograph equipped with a Merck RI-71 refractive index detector and a Knauer Eurokat Pb column, series SE212. Phenol-sulfuric acid method (Dubois et al., 1956) was used to determine the Total Sugar concentrations (TS). The ethanol concentrations (P) were determined by gas chromatography in Agilent-6890 equipment with HP-1 column with helium as the carrier gas (2.2 mL/min).

The RS yield percentage values (Y_{RS}) after enzymatic hydrolysis of the biomass were calculated according to Eq. 1 based on the reducing sugars concentration. The yield efficiency values of the conversion of *RS* in ethanol in the fermentation assays were determinated by Eq. 2.

$$Y_{RS} = \frac{RS_f - RS_0}{1.11\,C} \quad 100 \tag{1}$$

$$R_P = \frac{P_f - P_0}{0.511(RS_0 - RS_f)} 100 \tag{2}$$

C – mass fraction of cellulose in dry biomass = 0.44 (Gonçalves Filho et al., 2012)
RS_0 – reducing sugars at the beginning of fermentation (g/L)
RS_f – reducing sugars in t_f (g/L)
0.511 – theoretical yield of glucose to ethanol by fermentation (g/g)
P_f – ethanol concentration in t_f (g/L)
P_0 – ethanol concentration at the beginning of fermentation (g/L)

3. Results and Discussion

The solubilized RS values before chemical pretreatment (RS_0) and after enzymatic treatment (RS_f) are shown in Table 1.

Table 1: Concentration of reducing sugars in banana plant pseudostem liquor before pretreatment (RS_0) with NaOH 3% m/m (experiment AlcH) or H_2SO_4 2% m/m (experiment AcidH) and after enzymatic hydrolysis (RS_f) and respective yield percentage values (Y_{RS})

Parameters	Experiments			
	Fresh biomass (11.75 g/L dry matter)		Dried biomass (77.8 g/L dry matter)	
	AlcH-F	AcidH-F	AlcH-D	AcidH-D
RS_0 (g/L)	0.6±0.4	0.6±0.4	4.5±0.6	4.5±0.6
RS_f (g/L)	5.1±0.3	5.3±0.1	19.4±0.4	25.3±2.3
Y_{RS} (%)	79.5±3.5	82.0±6.5	43.9±1.2	60.7±6.7

There was no significant difference ($p<0.05$) between the RS_f and Y_{RS} values obtained in the pretreatments of the fresh biomass with NaOH 3% (AlcH-F) or H_2SO_4 2 %(AcidH-F). Pretreatment of the dried biomass by acid (AcidH-D) presented RS_f (30.4 %) and Y_{RS} (38.3%) higher than that in alkaline pretreatment (AlcH-D); thus being indicated under the operating conditions evaluated as ideal for the saccharification process of the pseudostem. Comparing the yields between fresh and dried biomass, for both alkaline and acid pretreatment, can be observed that the use of fresh biomass led to greater Y_{RS}. This behavior can be explained by the lower concentration of insoluble particles in the reaction mixture in experiments with fresh biomass. The influence of this variable on the saccharification of lignocellulosic residues has been confirmed by Hodge et a. (2008), Viamajala et al (2009) and Dunaway et al. (2010)..

It is important to note that the highest concentration of RS in the the saccharified liquor (25.3±2.3 g/L) remains low as compared with normally used in the industrial production of ethanol from sugar cane (100-130 g / L), in accordance with Gonçalves Filho et al. (2013).

This fact requires a search for new alternatives able to increase this variable in the fermentation broth. More appropriate reaction conditions such as time and mixture homogeneity during hydrolysis can provide an increase in the yield of the saccharification process and consequentially greater RS. The evaporation of excess water in the liquor is also a possibility. The higher the initial concentration of sugar in the fermentation broth, higher will be the concentration of ethanol in the fermented broth, provided that the fermentation process is not inhibited to the point of significantly reducing the alcoholic fermentation yield.

Chu et al. (2012) investigated the fermentation of high glucose concentration (about 200 g/L) from hydrolyzate corn stover by Saccharomyces cerevisae and obtained 91.3 g/L ethanol. Sugarcane bagasse pretreatment with formic acid solution was hydrolyzed and converted to ethanol by a subsequent fermentation using S.cerevisae with ethanol titer of 60-70 g/L (Zhao et al., 2013).

According to Koppram et al. (2014) with high gravity fermentation (HG) is possible to produce 10-15% (v/v) ethanol, resulting in improved overall productivity, reduced capital coast, and reduced energy input

compared to processing at the normal gravity. However, the authors emphasize that ethanol, similarly to other inhibitors, affects the composition, structure, and function of cell membranes, and cell morphology and also the cell viability. Thus, synergy between ethanol and degradation products could be expected, especially in HG process where the concentrations of sugar and ethanol are elevated.
For the purpose of verifying the influence of the increase in substrate concentration (RS) on the production of ethanol (P) from pseudostem, the broth from dried biomass pretreated with H_2SO_4 2% (AcidH-D, Table 1) was fermented by wild S. cerevisae in two different conditions: not concentrated liquor (Figure 1a) and pre-concentrated liquor through atmospheric evaporation (Figure 1b).

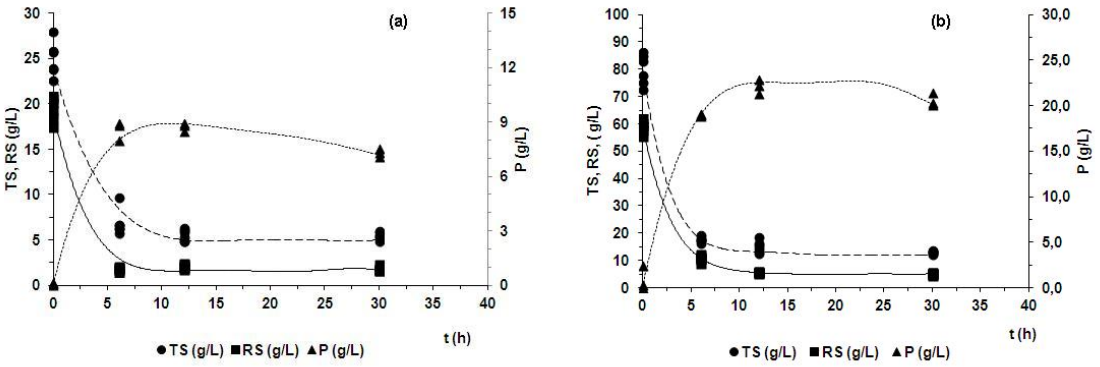

Figure 1: Fermentation of saccharified banana plant pseudostem (dried biomass pretreated with H_2SO_4 2%): (a) not concentrated liquor and (b) concentrated liquor. The multiples points of TS, RS and P for each t represent the fermentation assay and its duplicate with multiple analyzes of samples. The kinetic curve was plotted using the polynomial fit using the average values for each variable.

From the analysis of reducing sugars in HPLC (data not shown) it was possible to observe the existence and permanence of residual RS from beginning to end of all runs (1,5 g / L xylose in the not concentrated liquor, Fig. 1a, and 6 g/L in the pre-concentrated, Fig. 1b). Glucose and fructose were completely consumed by the yeast showing that no limitation of substrate consumption due to lack of nutrients. It should be remembered that in all races nutrient concentration was equal to those used in the preparation of the inoculum, except for carbon source. The residual TS content (5 and15 g / L) suggests the presence of other compounds besides xylose (not identified compounds) which were not metabolized by S. cerevisiae.
From the fitted curves (Figures 1a and 1b), the values of t_f and RS and P variables were obtained and the yield and efficiency of alcoholic fermentation were calculated. The yield ethanol from RS ($Y_{P/RS}$) and the production efficiency of ethanol (E_P) with the hydrolyzed liquor within concentration (L-nc) were higher than the respective values obtained with the concentrated liquor (L-c); However, its productivity (Q_P = 1.42 g/L.h) was lower than L-c, according to that observed in Table 2.

Table 2: Final fermentation time (t_f) and respective mean values for the concentration of reducing sugars (RS) and ethanol (P), yield ethanol from RS ($Y_{P/S}$), alcoholic efficiency (E_P) and productivity (Q_P) obtained in the fermentation of hydrolyzed liquor from previously dried banana plant pseudostem (L-nc: non concentrated liquor; L-c: concentrated liquor) assays.

Experiment	Fermentation Parameters					
	t_f (h)	RS (g/L)	P (g/L)	$Y_{P/S}$ (g/g)	E_P (%)	Q_P (g/L.h)
L-nc	6	2,12±0,22	8,57±0,49	0,49	97,0	1,42±0,08
L-c	12	5,67±0,28	22,13±0,80	0,41	80,4	1,83±0,05

The L-nc assay $Y_{P/S}$ and E_P presented values of around 20% higher than the L-c assay. This behavior may be due to the fact that on seeking to increase the initial concentration of sugars in the hydrolyzed liquor through evaporation, the concentration of potential inhibitors in the hydrolyzed medium was also increased. According to Palmqvist and Hahn-Hägerdal (2000), on being hydrolyzed, even partially, the lignin forms some phenolic compounds which are toxic to the majority of alcoholic fermentation micro-organisms. Further according to the authors, inhibitors such as acetic acid, furfural and

hydroxymethylfurfural can be produced from the decomposition of hemicellulose by sulfuric acid. These compounds were not quantified in this study.

In spite of the concentrated liquor requiring double the fermentation time of the non concentrated liquor for the consumption of sugars, this process presented a higher concentration of ethanol and higher ethanol productivity. The Q_P value for L-c (1.83 g/L.h) was 29% higher than that reached with L-nc (Q_P = 1.42 g/L.h).

Steckelberg *et al.* (2009), on using 21 different *S. cerevisae* strains in alcoholic fermentation in synthetic culture medium containing 150 g/L glucose for 24 h, obtained maximum Q_P, $Y_{P/S}$ and E_P values of around 2.8 g/L.h, 0.47 g/g and 92%, respectively. The yield and efficiency factor values were very similar to those presented in this study using the L-nc liquor; however, productivity was 50% higher. The use of synthetic medium without the presence of inhibitors resulting from substrate hydrolysis enhances the kinetics of microbial growth, thus enabling faster product formation. The use of hydrolyzed liquor detoxification before fermentation can contribute to reducing this difference in Q_P.

Gonçalves Filho *et al.* (2013), on fermenting pseudostem liquor in a concentration of 11.75 g m_s/L, which had previously undergone delignification with NaOH 1% m/m (120 °C, 30 min) and hydrolyzed with H_2SO_4 1% m/m (120 °C, 30 min), but without previous drying, obtained values of Q_P = 0.90±0.09 g/L.h, $Y_{P/S}$ = 0.31±0.01 g/g and E_P of 61.0±2.1%, lower than those presented in Table 3. Although the author, in addition to using fresh substrate in the hydrolysis instead of dry substrate as used in this study, and having washed the biomass before the enzymatic hydrolysis phase, this study achieved higher Q_P and $Y_{P/S}$ values. New studies need to be conducted to better understand this behavior.

4. Conclusions

The *Musa cavendischii* banana plant pseudostem, previously dried and milled according to the operating conditions used in this study, showed potential for being used as an alcoholic fermentation substrate. Use of the dried and milled banana plant pseudostem in a concentration of 70 g/L pretreated with H_2SO_4 2% with subsequent enzymatic hydrolysis led to a greater conversion of cellulose in glucose than pretreatment with NaOH 3%, thus being indicated for the continuation of studies.

The concentration of liquor previously hydrolyzed with acid enabled the attainment of up to 62 g/L of reducing sugars in fermentation medium, resulting in a higher concentration of ethanol in fermented liquor; however, without harming process productivity. It is known that the higher the concentration of a product in an aqueous medium, the lower the operating expenses required for its extraction and purification.

Considering the value of the RS yield in the saccharification process and the efficiency value in the fermentation process, it would be possible to obtain 147 kg de ethanol (187 L) from each ton of dry biomass treated. This yield could be increased by optimizing the saccharification and fermentation processes through the use of other pretreatment techniques such as steam-explosion, ammonia fiber explosion and autohydrolysis and the use of pseudostem liquor detoxification.

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References

CEPA (Center for Socioeconomic and Agricultural Planning of the State of Santa Catarina, Brazil), 2012. Banana p. 21-29 <cepa.epagri.sc.gov.br/Publicacoes/Sintese_2012/sintese%202012.pdf> accessed 25.11.2013 (in Portuguese).

Chu, Q., Li, X., Ma, B., Xu, Y., Ouyang, J., Zhu, J., Yu, S., Yong, Q., 2012. Bioethanol production: An integrated processo of low substrate loading hydrolysis-highe sugar liquid fermentation and solid state fermentation of enzymatic hydrolysis residue. Bioresource Technology, 123, 699-702

Dias, A., Toullec, J.L., Blandino, A., Ory, I., Caro, I. Pretreatment of Rice Hulls with Alkaline Peroxide to Enhance Enzyme Hydrolysis for Ethanol Production. Chemical Engineering Transactions, 32, 949-954,

Dubois, M., Gilles, K.A., Hamilton, J.K., 1956. Colorimetric method for determination sugars and related substances. Analytical Chemistry, 28, 350-356.

Dunaway, K.W., Dasari, R.K., Bennett, N.G., Berson, R.E., 2010. Characterization of changes in viscosity and insoluble solids content during enzymatic saccharification of pretreated corn stover slurries. Bioresource Technology, 101, 3575-3582.

Fernandes, E.R.K., Marangoni, C., Souza, O., Sellin, N., 2013. Thermochemical characterization of banana leaves as a potential energy source. Energy Conversion and Management, 75, 603-608, DOI: 10.1016/j.enconman.2013.08.008

Gonçalves Filho, L.C., Fischer, G.A.A., Sellin, N., Marangoni, C., Souza, O., 2013. Hydrolysis of banana tree pseudostem and second-generation ethanol production by Saccharomyces cerevisae. Journal of Environmental Science and Engineering A 2, 65-69.

Hodge, D.B., Karim, M.N., Shcell, D.J., McMillan, J.D., 2008. Soluble and insoluble solids contributions to high-solids enzymatic hydrolysis of lignocellulose. Bioresource Technology, 99, 8940-8948.

Limayen, A., Ricke, S.C., 2012. Lignocellulosic biomass for bioehtanol production: current perspectives, potential issues and future prospects. Progress in Energy and Combustion Science, 38, 449-467.

Meno, V., Rao, M., 2012. Trends in bioconversion of lignocelluloses: biofuels, platform chemicals & biorefinery concept. Progress in Energy and Combustion Science, 38, 522-550.

Mood, S.H., Golfeshan, A.H., Tabatabaei, M., Jouzani, G.S., Najafi, G.H., Gholami, M., Ardjmand, M., 2013. Lignocellulosic biomass to bioethanol: a comprehensive review with a focus on pretreatment. Renewable and Sustainable Energy Reviews, 27, 77-93.

Palmqvist, E., Hahn-Hägerdal, B., 2000. Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. Bioresource Technology, 74, 25-33.

Sanchez,Ó.,J, Cardona, C.A., 2008. Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresource Technology, 99, 5270-5295.

Sarkar, N., Ghosh, S.K., Bannerjee, S., Aikat, K., 2012. Bioethanol production from agricultural wastes: An overview. Renewable Energy, 37, 19-27, DOI: 10.1016/j.renene.2011.06.045

Sellin, N., Oliveira, B.G., Marangoni, C., Souza, O., Oliveira, P.N., Oliveira, T.M.N., 2013. Use of Banana Culture Waste to Produce Briquettes. Chemical Engineering Transactions, 32, 349-354, Somogyi, M.,1952. Notes on sugar determination. Journal of Biologycal Chemistry, 195, 19-23.

Souza, O., Federizzi, M., Coelho, B., Wagner, T.M., Wisbeck, E., 2010. Biodegradation of lignocellulosics residues generated in banana cultivation and its valorization for the production of biogas. Revista Brasileira de Engenharia Agricola e Ambiental, 14, 4, 438-443 (in Portuguese

Souza, O., Schulz, M.A., Fischer, G.A.A., Wagner, T.M., Sellin, N., 2012. Alternative energy from biomass: bioethanol from banana pulp and peels. Revista Brasileira de Engenharia Agricola e Ambiental, 16, 8, 915-921 (in Portuguese).

Steckelberg, C. Andrietta, M.G.S., Andrietta, S.R., 2009, Yeasts isolated from industrial processes of producing bioethanol - Numerical taxonomy and kinetic characteristics. XVII National Symposium Bioprocess, Sao Paulo, Brazil (in Portuguese)

Viamajala, S., McMillan, J.D., Schell, D.J., Elander, R.T., 2009. Rheology of corn stover slurries at high solids concentrations – Efflects of saccharification and particle size. Bioresource Technology, 100, 925-934.

Viikari, L., Vehmaanpera, J., Koivula, A., 2012. Lignocellulosic ethanol: from science to industry. Biomass and Bionergy, 46, 13-24.

Zhao, X., Dong, L., Chen, L., Liu, D., 2013. Batch and multi-step fed-batch enzymatic saccharification of Formiline-pretreated sugarcane bagasse at high solid loadings for high sugar and ethanol titers. Bioresource Technology, 135, 350-356.