ETHANOL PRODUCTION FROM SUGARCANE BAGASSE USING SSF PROCESS AND THERMOTOLERANT YEAST

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ABSTRACT. *Sugarcane bagasse (SCB) pretreated by hydrothermolysis at 195°C for 10 min was used as a substrate for ethanol production in a simultaneous saccharification and fermentation (SSF) process with the thermotolerant yeast* Kluyveromyces marxianus *IMB3 at 45°C. SSF was carried out for 7 days using 15 and 30 filter paper units (FPU) cellulase g^{-1} treated SCB (Accellerase 1500) to determine the effect of enzyme loading on ethanol production. Different pretreated dry solids loadings of 10% and 15% (w/w) were investigated in this work during enzymatic hydrolysis. Results showed 89.7% hemicellulose removal during pretreatment and glucan recovery of 97.8%. The highest ethanol concentration of 29.2 g L^{-1} was obtained with 30 FPU cellulase g^{-1} treated SCB within 72 h, which was equivalent to 58.9% of the maximum theoretical ethanol yield.*

Keywords. *Enzymatic hydrolysis, Ethanol, Hydrothermolysis,* Kluyveromyces marxianus *IMB3, SSF, Sugarcane bagasse.*

Sugarcane bagasse (SCB) is a lignocellulosic material widely found in Brazil, particularly in the southeast of the country. SCB has been investigated by scientists in Brazil as a potential source for ethanol production (da Silva et al., 2010; Rocha et al., 2011; Rocha et al., 2012; Santos et al., 2012). In Brazil, every ton of ground sugarcane used for producing sugar and ethanol generates, on average, 250 kg of bagasse and 200 kg of sugarcane straw (UNICA, 2012). During the 2013-2014 Brazilian harvest, more than 658 million tons of sugarcane was ground, which generated about 165 million tons of bagasse and 132 million tons of sugarcane straw (CONAB, 2014). Currently, bagasse can be used in several applications, such as energy cogeneration and production of ethanol and animal feed (Rocha et al., 2012). Economical and environmentally beneficial uses of SCB are greatly desired.

SCB as well as other types of lignocellulosic biomass primarily consist of cellulose, hemicellulose, and lignin; however, the composition of each component varies with the raw material. Cellulose is a natural polymer consisting of cellobiose units linked by glycosidic linkages of the type β-(1→4) (Fengel and Wegener, 1989). Hemicelluloses are polysaccharides that are closely associated with cellulose in the plant cell wall. They consist of polymers of several pentoses (xylose and arabinose), hexoses (glucose, mannose, and galactose), and uronic acids. Lignin is a highly branched material that can be classified as a polyphenol constituting an irregular arrangement of several phenylpropane units (Silva et al., 2009). Lignin plays an important role in cell wall structure as an agent of protection against microbial damage. Cellulose and hemicellulose are not directly available for bioconversion due to their intimate association with lignin (Trajano et al., 2013; Williams and Morrison, 1982).

Ethanol production from lignocellulosic biomass consists of four steps: pretreatment, enzymatic hydrolysis, fermentation, and distillation. Pretreatment decreases the crystallinity of cellulose, removes hemicelluloses, and increases the surface area of the available biomass. Pretreatment is an important step for increasing the accessibility of cellulose to enzymes during enzymatic hydrolysis. Hydrolysis is the conversion of carbohydrate polymers into monomeric sugars (Mosier et al., 2005). These sugars are converted to ethanol by fermenting microorganisms. Finally, distillation is used to concentrate and purify the ethanol.

A great number of pretreatment methods have been studied in the bioconversion of biomass, and among them is hydrothermolysis. This pretreatment (also known as hydrothermal pretreatment, autohydrolysis, aquasolv, or liquid hot water pretreatment) can be considered an eco-friendly processing technology since it uses only water as a solvent, which reduces corrosion problems and the formation of toxic compounds while providing a high recovery of hemicellulose and making cellulose more accessible to cellulases (Perez et al., 2008; Pessani et al., 2011; Ruiz et al., 2012; Saha et al., 2013; Yu et al., 2013).

Cellulosic biomass can be converted to ethanol using dif-

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ferent processes, one of which is simultaneous saccharification and fermentation (SSF). SSF has been intensely investigated because it reduces contamination risk and lowers costs through the usage of the same reactor for hydrolysis and fermentation (Garcia-Aparicio et al., 2011; Ruiz et al., 2012). SSF combines enzymatic hydrolysis of cellulose with simultaneous fermentation of the sugars to ethanol, thereby alleviating problems caused by product inhibition (Olofsson et al., 2008; Wingren et al., 2003). Other processes have also been reported for ethanol production from lignocellulosic materials, including separate hydrolysis and fermentation (SHF), simultaneous saccharification and co-fermentation (SSCF), and presaccharification prior to simultaneous saccharification and fermentation (PSSF) (Garcia-Aparicio et al., 2011; Pessani et al., 2011). PSSF has been recently reported by Moreno et al. (2013) using the thermotolerant yeast *Kluyveromyces marxianus* CECT 10875.

K. marxianus IMB strains were isolated by Banat et al. (1992) from a wine distillery in India. These IMB yeast strains were capable of growing and producing relatively high ethanol concentrations at temperatures between 40°C and 50°C. Barron et al. (1995) reported that *K. marxianus* IMB3 was capable of ethanol production at 45°C on media containing milled paper and exogenously added commercial cellulose. Suryawati et al. (2008, 2009) reported the production of ethanol by *K. marxianus* IMB4 at 45°C using switchgrass pretreated by hydrothermolysis and SSF. One comparative study between *K. marxianus* IMB strains and *Saccharomyces cerevisiae* D_5A in SSF with switchgrass pretreated by hydrothermolysis showed similar ethanol production yields for *K. marxianus* IMB3 at 45°C and *S. cerevisiae* D_5A at 37°C (Faga et al., 2010). The IMB3 strain was also used to produce ethanol at different cellulase loadings (0.1 to 1.1 mL g^{-1} glucan), with the greatest ethanol yield (86% of maximum theoretical) achieved with 0.7 mL g^{-1} glucan using 8% switchgrass pretreated by hydrothermolysis in batch SSF (Pessani et al., 2011). Kadar et al. (2004) investigated the use of various industrial wastes (Solka Floc 200, old corrugated cardboard, waste cardboard, and paper sludge) in SSF using *K. marxianus* (Y01070) and *S. cerevisiae* (commercial strain) to produce ethanol. After 72 h, the ethanol concentrations were 17.8 and 16.6 g L^{-1} and the volumetric ethanol productivities were 0.25 and 0.23 g L^{-1} h^{-1}, respectively, for *K. marxianus* and *S. cerevisiae*. SCB was employed in experiments to produce ethanol with thermotolerant *S. cerevisiae* UFPEDA 1238 (Santos et al., 2012). The substrate was pretreated by steam explosion and then delignified with sodium hydroxide. PSSF experiments (6 h of prehydrolysis) were carried out at 37°C using 8% dry solids loading with 20 FPU g^{-1} cellulose and 10% v/v β-glucosidase. After 30 h, 25 g L^{-1} of ethanol and a 0.70 g L^{-1} h^{-1} ethanol volumetric productivity were obtained.

In this context, the present work investigated the use of hydrothermolysis-pretreated SCB as substrate for ethanol production using SSF. The effects of dry solids content (10% and 15%, w/w) and enzyme loading (15 FPU g^{-1} SCB and 30 FPU g^{-1} SCB) on ethanol yields were evaluated. The thermotolerant yeast *K. marxianus* IMB3 was employed throughout this work.

METHODS AND MATERIALS

SAMPLE PREPARATION

The SCB used in this work was supplied by Centro de Tecnologia Canavieira (Piracicaba, Sao Paulo, Brazil). The biomass was ground through a 2 mm screen using a Thomas-Wiley mill (Arthur H. Thomas Co., Philadelphia, Pa.). The ground SCB was stored in reusable bags at room temperature prior to pretreatments. The polysaccharide, lignin, ash, and extractives contents of the biomass were determined before and after pretreatment using a standard procedure developed by the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2011).

HYDROTHERMOLYSIS

SCB samples were pretreated by hydrothermolysis in a 1 L benchtop pressure reactor (Series 4250, Parr Instrument Co., Moline, Ill.) equipped with a propeller agitator, heater, and temperature controller. The reactor was first filled with 60 g of dry SCB. Moisture content of the SCB (6.7%) was determined by the method of Sluiter et al. (2008a). Deionized water was then added to achieve a 10% dry solids mixture. The completely sealed reactor was heated to 195°C and agitated at 200 rpm. The temperature was held at 195°C for 10 min. After heating was complete, the reactor was cooled in an ice bath to a temperature of 40°C. The solid and liquid fractions were separated by vacuum filtration through Whatman #5 filter paper. The solids were rinsed with 600 mL of deionized water at 60°C three times to remove any residual soluble sugars and/or fermentation inhibitors. A 5 to 6 g sample of the washed residual solids was dried in an oven for 24 h at 105°C to determine the mass of dry solids recovered after pretreatment (Sluiter et al., 2008a). The solids were stored in sealed plastic bags at 4°C until they were used for enzymatic hydrolysis.

SCB was extracted prior to pretreatment by ethanol followed by deionized water using a Dionex accelerated solvent extractor (model 300, Dionex Corp., Sunnyvale, Cal.) and a standard NREL procedure (Sluiter et al., 2008c). Removal of ethanol from extractives was done using a RapidVap N2 evaporation system (Labconco Corp., Kansas City, Kans.) at 500 mbar and 40°C for 24 h. Water-extractives samples were evaporated in an oven at 40°C for 48 h. Pretreated samples were not extracted.

The composition of degradation products in the prehydrolysate was determined using a standard NREL procedure (Sluiter et al., 2008b). Analyses of carbohydrates and organic acids were carried out using HPLC with refractive index detection (RID) (Agilent 1100 Series, Santa Clara, Cal.) on an Aminex HPX-87P column (BioRad, Sunnyvale, Cal.) at 85°C with deionized water as eluent flowing at 0.6 mL min^{-1}.

MICROORGANISM AND GROWTH CONDITIONS

Cultures of *K. marxianus* IMB3 were obtained from the University of Ulster (Coleraine, Northern Ireland) and were grown on liquid yeast peptone dextrose (YPD) medium containing (g L^{-1}): 10.0 yeast extract, 20.0 peptone, and 50.0 glucose. A loopful of *K. marxianus* IMB3 cells were aseptically transferred into 250 mL baffled culture flasks

containing 100 mL of YPD medium covered with aerobic stoppers (Bug Stopper, Whatman PLC, Florham Park, N.J.). Flasks were incubated at 45°C for 17 h at 200 rpm on an orbital shaker (MaxQ mini 4450, Thermo Scientific, Dubuque, Iowa). The cells were collected by centrifugation at 7200×*g* for 6 min (Sorvall Legend RT, Thermo Scientific, Waltham, Mass.). The supernatant was decanted, and the cells were washed twice in 0.89% (w/v) sterile sodium chloride (NaCl) solution (Ramachandriya et al., 2013). The obtained cells were resuspended in 0.89% (w/v) sterile NaCl solution to give an optical density (OD) of 5.0.

SIMULTANEOUS SACCHARIFICATION AND FERMENTATION (SSF)

Yeast fermentation medium (YFM) was prepared using DI water consisting of (g L^{-1}): 5.0 yeast extract, 20.0 KH_2PO_4, 10.0 $MgSO_4$, 20.0 $(NH_4)_2SO_4$, and 1.0 $MnSO_4 \cdot H_2O$ (Banat et al., 1992). Commercial cellulase (Accellerase 1500, kindly provided by Dupont Genencor, Palo Alto, Cal.) with activity of 99.6 FPU mL^{-1}, was used in the SSF experiments. Cellulase activity was determined using a standard filter paper assay (Ghose, 1987).

SSF was conducted in 250 mL baffled flasks sealed with rubber stoppers fitted with a one-way air valves (check valve, Fisher Scientific, Pittsburgh, Pa.) to maintain an anaerobic environment. In this study, four conditions were tested:

- 15 FPU cellulase g^{-1} pretreated dry SCB/10% (w/w) dry SCB loading.
- 30 FPU cellulase g^{-1} pretreated dry SCB/10% (w/w) dry SCB loading.
- 15 FPU cellulase g^{-1} pretreated dry SCB/15% (w/w) dry SCB loading.
- 30 FPU cellulase g^{-1} pretreated dry SCB/15% (w/w) dry SCB loading.

Each fermentation flask contained 5.0 mL of 10X YFM, 2.5 mL of 1 M sodium citrate buffer at pH 5.5, 0.5 mL of concentrated *K. marxianus* IMB3 culture (OD 5.0), 10 or 15 g dry SCB solids (w/w), and 15 or 30 FPU cellulase g^{-1} pretreated dry SCB. Deionized water was added to each flask to make the total mass in each flask 100 g.

All flasks were incubated at 45°C on an orbital shaker. The SSFs with 15% dry solids loading contained stainless steel balls (10 mm diameter) to assist in the dissolution of the SCB (Ramachandriya et al., 2013). Aliquots of 1.5 mL were collected at 0, 7, 24, 48, 72, 96, 120, 144, and 168 h. The samples were centrifuged at 16,600×*g* for 10 min (AccuSpin Micro 17, Fisher Scientific, Pittsburgh, Pa.), and the supernatant was filtered through 0.45 µm nylon syringe filters (VWR International, West Chester, Pa.) and frozen until analyzed. At the end of each fermentation cycle, the pH values of all fermentation slurries were recorded. All SSF experiments were carried out in triplicate.

ANALYSIS OF SSF SAMPLES BY HPLC

The concentrations of glucose, xylose, galacturonic acid, acetic acid, and ethanol were measured by HPLC. Ten µL of each sample were analyzed by HPLC using an Aminex HPX-87H column (BioRad, Hercules, Cal.) with a refractive index detector (RID) (Agilent 1100 Series, Santa Clara, Cal.). The mobile phase was 0.01 N H_2SO_4 flowing at 0.6 mL min^{-1} at 60°C for 30 min.

CALCULATIONS

The theoretical yield of ethanol was calculated using the following equation (Ramachandriya et al., 2014):

$$\text{Theoretical yield}\,(\%) = \frac{[\text{EtOH}_t] - [\text{EtOH}_0]}{0.51 \cdot (f \cdot [\text{biomass}] \cdot 1.11)} \cdot 100 \qquad (1)$$

where

$[EtOH_t]$ = ethanol concentration (g L^{-1}) produced at time t (h)
$[EtOH_0]$ = initial ethanol concentration (g L^{-1})
0.51 = mass conversion factor of glucose to ethanol (g g^{-1})
f = glucan fraction of dry biomass (-)
[biomass] = initial concentration of solids (g L^{-1})
1.11 =conversion factor for glucan to glucose.

RESULTS AND DISCUSSION

SCB COMPOSITION

Native SCB was subjected to extraction by ethanol-water prior to compositional analysis. The extraction of SCB resulted in the removal of 5.9% of the dry biomass as extractives, 1.7% by ethanol and 4.2% by water. The composition of SCB before and after pretreatment is shown in table 1. The total dry native SCB contained 41.7% glucan, 22.3% xylan, 1.8% galactan, 2.6% arabinan, 0.5% mannan, 20.7% lignin, 4.3% ash, and 5.9% of extractives. The mass balance of compositional analysis accounted for 99.8% of dry matter in the SCB. These results are in agreement with data reported for native SCB (Rocha et al., 2011; Wanderley et al., 2013).

The recovery of SCB solids in the water-insoluble solids (WIS) remaining after pretreatment was 62.0%. Hydrothermolysis resulted in 97.8% recovery of glucan and 10.3% recovery of xylan from the raw SCB in the WIS. Most of the lignin (90.1%) from the raw SCB remained in the WIS. The WIS contained 65.8% glucan, 3.7% xylan, and 30.1% lignin. Oliveira et al. (2014) observed similar behavior for sugarcane straw using hydrothermolysis (195°C for 10 min). Those authors reported an increase in glucan content from 38.1% to 62.6% and a decrease in xylan content from 29.2% to 3.5%. Sugars quantified in the prehydrolysate, which is the liquid collected after pretreatment before washing, were

Table 1. Compositional analysis of native and hydrothermolyzed SCB.

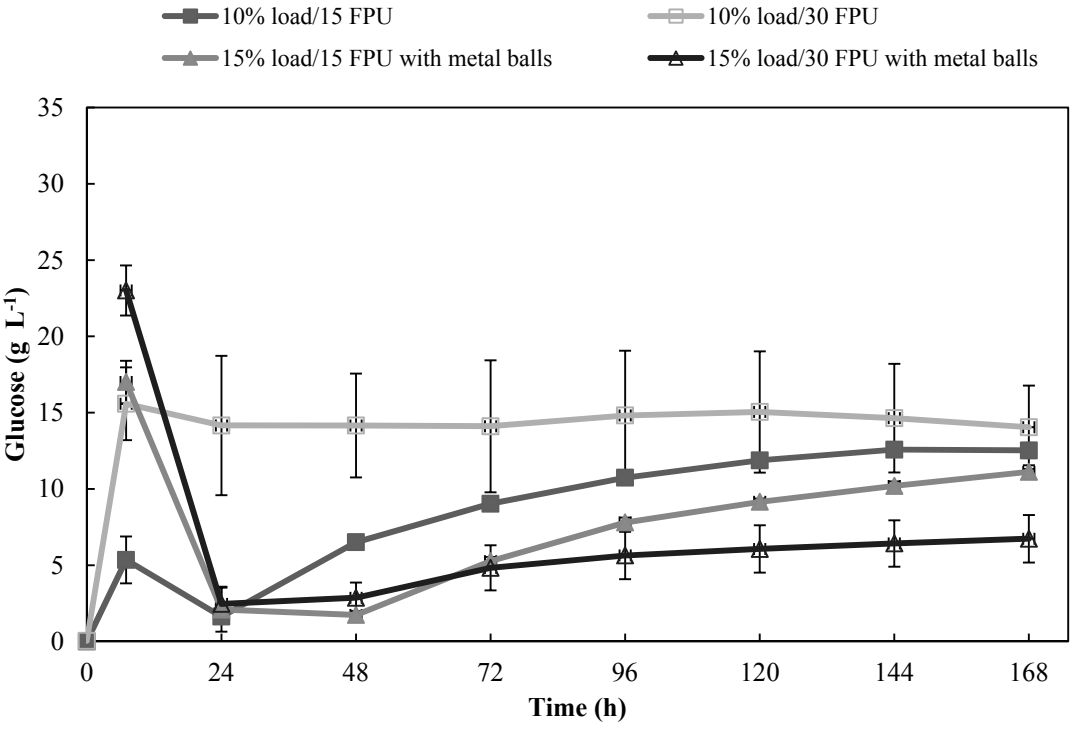
Components	Native SCB (% d.b.)[a]	Hydrothermolyzed SCB (% d.b.)[a]
Glucan	41.7 ±0.1	65.8 ±1.5
Xylan	22.3 ±0.3	3.7 ±0.6
Galactan	1.8 ±0.1	-
Arabinan	2.6 ±0.4	0.1 ±0.1
Mannan	0.5 ±0.1	0.1 ±0.2
Lignin	20.7 ±0.3	30.1 ±1.3
Ash	4.3 ±0.7	ND[b]
Extractives	5.9[c]	ND[b]

[a] d.b. = dry basis.
[b] ND = not determined.
[c] no replicates.

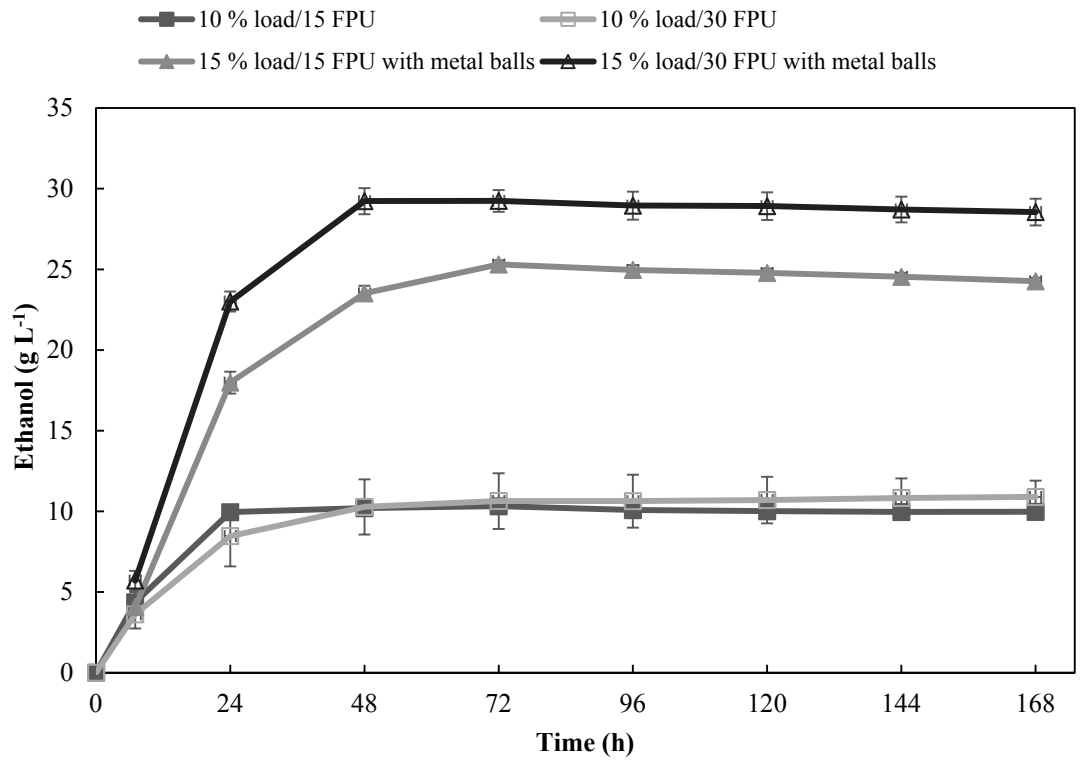
3.3 g L^{-1} glucose, 13.8 g L^{-1} xylose, 1.0 g L^{-1} galactose, 3.4 g L^{-1} arabinose, and 2.2 g L^{-1} mannose.

SIMULTANEOUS SACCHARIFICATION AND FERMENTATION

Pretreated SCB was used as substrate for ethanol production. Figure 1a shows the time course of glucose concentration in the SSFs. SSFs were performed at 10% (w/w)/15 FPU, 10% (w/w)/30 FPU, 15% (w/w)/15 FPU, and 15% (w/w)/30 FPU. Glucose concentration increased from 0 to 7 h as glucose was produced at a faster rate than the yeast could consume it. Glucose then decreased between 7 and 24 h for all treatments. Glucose was 2.1 g L^{-1} at 24 h for all flasks except the 10% (w/w)/30 FPU treat-



(a)



(b)

Figure 1. Concentrations of (a) glucose and (b) ethanol for 168 h during SSFs using *K. marxianus* IMB3 at 45°C with pretreated SCB.

ment. The 10% (w/w)/30 FPU treatment had greater glucose concentrations than all other treatments throughout the SSFs, averaging 14.2 g L^{-1}. The 15% (w/w)/30 FPU treatment had the lowest glucose concentration at the end of SSF, with 6.7 g L^{-1}, while the other treatments had glucose concentrations of 11.1 g L^{-1} or greater.

Ethanol concentrations in all SSF treatments are shown in figure 1b. All ethanol concentrations increased during the initial 7 h, which indicated that enzymatic hydrolysis of glucan to glucose and ethanol fermentation occurred early in SSF. Production of ethanol continued in all treatments until 48 h, after which ethanol concentration stabilized. The stable ethanol concentration corresponded with an increase in glucose concentration as the cellulase enzyme continued to hydrolyze cellulose to glucose. At 72 h, ethanol concentrations were 10.3, 10.6, 25.3, and 29.2 g L^{-1} for the treatments 10% (w/w)/15 FPU, 10% (w/w)/30 FPU, 15% (w/w)/15 FPU, and 15% (w/w)/30 FPU, respectively. After 72 h, ethanol concentration did not increase in any treatment, which suggested that the cells were inhibited. The causes for inhibition of fermentation are not known; however, some authors have reported the combination of high temperature and low ethanol tolerance at 45°C as a major cause for low cell viability for *K. marxianus* IMB3 at 72 h (Pessani et al., 2011; Suryawati et al., 2009). Ballesteros et al. (2004) used *K. marxianus* CECT 10875 at 42°C in SSF and reported that ethanol production ceased between 72 and 82 h, which they attributed to metabolic stress caused by low glucose concentration and the presence of ethanol in the SSF process. Suryawati et al. (2008) observed similar performance using *K. marxianus* IMB4 at 45°C and suggested that the combination of higher temperature, ethanol concentration, and decreased pH from acetic acid formation may have contributed to the cessation of fermentation.

Acetic acid production was observed during all SSFs. The profiles for acetic acid over time are shown in figure 2. The maximum acetic acid concentration obtained was 2.6 g L^{-1} in the 15% (w/w)/30 FPU SSF after 168 h. Acetic acid concentrations at 72 h were 1.0, 1.3, 2.0, and 2.2 g L^{-1} in the 10% (w/w)/15 FPU, 10% (w/w)/30 FPU, 15% (w/w)/15 FPU, and 15% (w/w)/30 FPU, respectively. Acetic acid formation during SSF can inhibit yeast growth. The lowest concentration of acetic acid (1.2 g L^{-1}) was detected in the 10% load/15 FPU SSF after 168 h. Pessani et al. (2011) reported that the increase in acetic acid concentration during SSFs was mainly due to *K. marxianus* IMB3 metabolic activity and not from the enzymatic hydrolysis of hemicelluloses. In these experiments, it was observed that higher ethanol concentration was associated with higher acetic acid concentrations, which is similar to that observed by Pessani et al. (2011). After 96 h, acetic acid slightly increased in all SSFs. Suryawati et al. (2008) observed a similar behavior in SSF experiments using *K. marxianus* IMB4 at 45°C. According those authors, acetic acid produced by IMB4 may have cause inhibition of IMB4 fermentation and ethanol production. A similar result was observed by Ballesteros et al. (2004) in SSF experiments using *K. marxianus* CECT 10875 at 42°C.

In the final SSF experiments, the measured pH values were 5.03, 5.00, 4.85, and 5.01 for 10% (w/w)/15 FPU, 10% (w/w)/30 FPU, 15% (w/w)/15 FPU, and 15% (w/w)/30 FPU, respectively. In this study, all the assays showed pH values of about 5.0. In this condition, the acetic acid was present in dissociated form. According to Oliva et al. (2003), dissociated acetic acid has little toxicity for cells and has an insignificant effect on ethanol yield. The undissociated form of acetic acid has been shown to have a greater inhibition of cellular growth than the dissociated

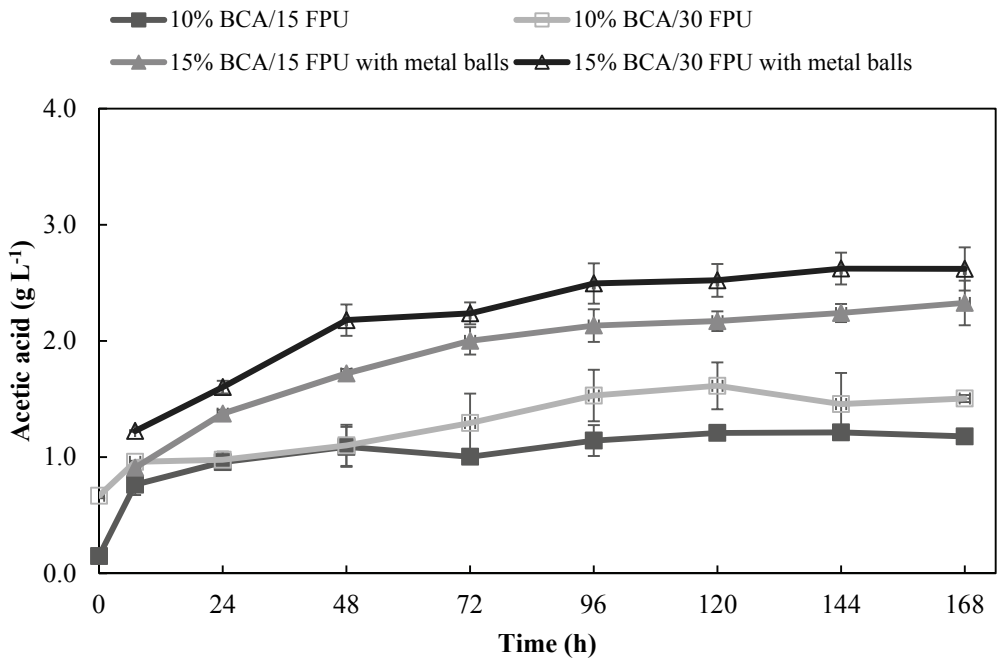


Figure 2. Acetic acid profiles in SSFs using *K. marxianus* IMB3 at 45°C.

Table 2. Summary of simultaneous saccharification and fermentation (SSF) assays with *K. marxianus* IMB3 at 48 h.

% Solids Loading (w/w)	Enzymatic Loading (FPU g^{-1} glucan)	% Glucose Conversion	Ethanol (g L^{-1})	$Y_{E/Et}$[a] (%)	% Ethanol from SSF[b]	Q_E[c] (g L^{-1} h^{-1})	P_E[d] (mg L^{-1} FPU^{-1} h^{-1})
10	15	46.1	10.3	31.2	67.9	0.20	14.3
10	30	55.3	10.6	33.8	58.2	0.20	7.4
15	15	56.9	25.3	48.1	89.9	0.46	35.1
15	30	64.3	29.2	58.9	91.8	0.58	20.3

[a] $Y_{E/Et}$ = theoretical ethanol yield assuming ethanol yields on glucose of 0.51 g g^{-1}.
[b] % Ethanol from SSF = ethanol yield based on total glucose released in the SSF (total glucose present in SSF to ethanol).
[c] Q_E = volumetric ethanol productivity.
[d] P_E = specific ethanol productivity (ethanol concentration, mg L^{-1}) related enzymatic loading (FPU).

form (Berg et al., 2007). The pH of the media was greater than the pKa of acetic acid (4.8); thus, acetic acid was dissociated and had little effect on the cells during SSF.

Data obtained in the SSFs are summarized in table 2. Glucose conversion was 64.3% of the maximum theoretical possible in the fermentation with 15% (w/w)/30 FPU. Glucose conversions were similar for the 10% (w/w)/30 FPU and 15% (w/w)/15 FPU treatments (55.3% and 56.9%, respectively). A comparison between treatments at 10% dry solids loading (w/w) showed that the 72 h ethanol concentration was about 10.0 g L^{-1} for both enzyme loadings; however, glucose conversion was 55.3% using 30 FPU and 46.1% using 15 FPU, an increase of 16.6% in glucan conversion to glucose.

Theoretical ethanol yields, calculated by equation 1, were 31.2%, 33.8%, 48.1%, and 58.9% in the 10% (w/w)/15 FPU, 10% (w/w)/30 FPU, 15% (w/w)/15 FPU, and 15% (w/w)/30 FPU treatments, respectively. These low ethanol yields may be related to lower enzyme loadings, as compared to other studies that also used Accellerase enzymes (Pessani et al., 2011; Pryor and Nahar, 2010). For 10% (w/w)/15 FPU and 10% (w/w)/30 FPU, an effect of enzymatic loading was not observed. The ethanol yields obtained from total glucose released in the SSFs were 91.8%, 89.9%, 67.9%, and 58.2% for 15% (w/w)/30 FPU, 15% (w/w)/15 FPU, 10% (w/w)/15 FPU, and 10% (w/w)/30 FPU, respectively. The results of 91.8% for 15% (w/w)/30 FPU and 89.9% for 15% (w/w)/15 FPU enhance the hypothesis that % maximum theoretical ethanol yields lower than those of other studies were due to slow enzyme hydrolysis and not due to poor yeast fermentation.

The volumetric ethanol productivity (Q_E) at 72 h increased from 0.20 to 0.58 g L^{-1} h^{-1} when solids content and enzyme loading increased. The results showed that the highest Q_E (0.58 g L^{-1} h^{-1}) resulted in the greatest ethanol concentration of 29.2 g L^{-1}. However, specific ethanol productivity was 35.1 mg L^{-1} FPU^{-1} h^{-1} for 15% (w/w)/15 FPU, which was 42.4% higher than the productivity obtained for 15% (w/w)/30 FPU (20.3 mg L^{-1} FPU^{-1} h^{-1}). These results show that, in terms of ethanol produced per enzyme activity added, the best condition was 15% (w/w)/15 FPU.

CONCLUSION

This study showed that hydrothermolysis of SCB at 195°C for 10 min had 97.8% recovery of glucan and 89.4% removal of xylan in the WIS. The greatest ethanol concentration achieved from SSF using *K. marxianus* IMB3 after 72 h at 45°C was 29.2 g L^{-1} in the treatment containing 15% (w/w)/30 FPU of cellulase. This represented a yield of 58.9% of the maximum based on the glucan present after pretreatment, but the yield was 91.8% of the maximum based on the glucose released during hydrolysis. After 72 h, an accumulation of glucose was observed as a result of a cessation of ethanol production. A possible cause of the cessation was the high temperature and ethanol stress of the yeast. When considering the ethanol productivity per FPU of cellulase activity, the 15% (w/w)/15 FPU treatment resulted in the greatest productivity of 35.1 mg ethanol L^{-1} FPU^{-1} h^{-1} at 48 h.

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