Enhanced Ethanol Production with Mixed Lignocellulosic Substrates from Commercial Furfural and Cassava Residues

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Simultaneous saccharification and fermentation (SSF) is an attractive process configuration for bio-ethanol production. Further reductions in process cost of SSF are expected with the use of waste agricultural or industrial materials as feedstock. In the current study, two industrial lignocellulosic wastes, cassava residues (CR) and furfural residues (FR), were combined during SSF for ethanol production due to their value-added applications and positive environmental impacts. After CR were liquefied and saccharified, saccharification liquid was added to SSF of FR. The effect of substrate fractions was investigated in terms of ethanol yield, byproduct concentration and the number of yeast cells. Besides, a natural surfactant, *Gleditsia* saponin, was added to investigate the effect of FR lignin on SSF with 20% substrate concentration. The results showed that increasing the ratio of CR/FR improved the ethanol yield and that the ethanol yield was also increased gradually by increasing the substrate concentration from 6% to 12%. A high ethanol concentration of 36.0 g/L was obtained under the condition of CR:FR = 2:1 with 12% substrate concentration, reaching 71.1% of the theoretical yield. However, *Gleditsia* saponin did not affect the ethanol yield, indicating the insignificant effect of lignin in SSF with low lignin content in the reaction system.

Keywords: Ethanol; Cassava residue; Furfural residue; Glycerol; Mixed lignocellulosic substrates.

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INTRODUCTION

The fossil energy crisis and its related global environmental impacts have resulted in an urgent search for renewable energy sources (Kerr 2007), such as solar, wind, and biomass. Of the biomass that has been harnessed as biofuel, bioethanol is currently the most widely used, as it can replace liquid petroleum and thus help reduce greenhouse-gas pollution. However, because of serious food security concerns and other issues, recent research has focused on replacing bioethanol's most common feedstock (*i.e.*, starch and sugars from existing food crops) with lignocellulosic biomass (Farrell *et al.* 2006; Hahn-Hägerdal *et al.* 2007; Mabee 2007; Okano *et al.* 2010). Unfortunately, to date, researchers have struggled to demonstrate the commercial viability of converting lignocelluloses to ethanol.

Generally, two process configurations, simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF), have been compared as methods to produce bioethanol from lignocellulosic materials. Simultaneous

saccharification and fermentation is generally regarded as having many advantages over SHF, such as reducing equipment capital cost and contamination risk (Kang *et al.* 2012), shorter residence time, and overall high ethanol yield. However, as the structural material in plants, lignocellulose has evolved to withstand fast and effective microbial/enzymatic degradation, a characteristic known as biomass recalcitrance (Himmel *et al.* 2007). Biomass recalcitrance makes pretreatment necessary for efficient saccharification in the SSF of lignocelluloses, which obviously increases the overall process cost. Moreover, lignin accumulation reduces the capacity during SSF to obtain high final ethanol concentration.

One way to reduce the process cost of SSF is through the use of waste agricultural or industrial materials as feedstock. Furfural residue (FR) is a main waste of the furfural industry and is rich in cellulose that can be easily utilized without pretreatment. However, lignin remains a major bottleneck in increasing the final ethanol concentration in SSF of FR (Alkasrawi *et al.* 2003). Cassava residues (CR), as a by-product of the cassava starch industry, contain about 30 to 50 wt.% of starch and certain cellulose. Because of its abundant carbohydrates and low content of acid insoluble matter, CR can serve as an ideal substrate for bioethanol production by SSF. To fully convert starch and cellulose in CR, this complex cellulose-starch waste could be liquefied and saccharified by double enzyme methods, followed by cellulose hydrolysis using cellulases.

In an earlier phase of the current research, SSF of FR and corn was conducted to improve the final ethanol concentration, reduce the negative effect of FR lignin, and decrease the consumption of nutrients. Cassava residues (CR) from the cassava industry also offer potential opportunities for better economic utilization of agro-industrial residues (Chen *et al.* 2014). Compared with corn, the high-energy efficiency of cassava fuel, combined with the large area of cassava plantation in southwest China, may better facilitate more sustainable ethanol production from cellulosic and starchy materials.

As a result, and given the scarcity of research in this area, the purpose of this study was to assess the feasibility of bioconverting these two industrial wastes (*i.e.*, cellulose and starch) to ethanol and yield-determining the factors in the bioconversion process.

EXPERIMENTAL

Materials

Cassava residues with a moisture content of 10.7% were kindly provided by GuangXi Key Laboratory of Chemistry and Engineering of Forest Products (Nanning, China). Furfural residues were kindly provided by Chunlei Company (Xingtai, China), and water-rinsed before being used. The chemical composition of CR and FR is presented in Table 1. *Gleditsia* saponin extracted from the pod wall of *Gleditsia sinensis* Lam. with a purity of 81.5% was kindly supplied by Shexian Forestry Bureau (Hebei, China).

Table 1. Composition of Cassava Residue and Furfural Residue

	Cellulose	Acid insoluble lignin	Hemicellulose	Starch	Ash	Protein
CR	25.0%	6.8%	nd	47.2%	nd	3.7%
FR	42.2%	38.7%	1.9%	nd	0.8%	nd
Nd, not detected						

Methods

The experimental procedure is illustrated in Fig. 1. The two materials were mixed in different proportions and concentrations to investigate the effects on the ethanol yield and concentration.

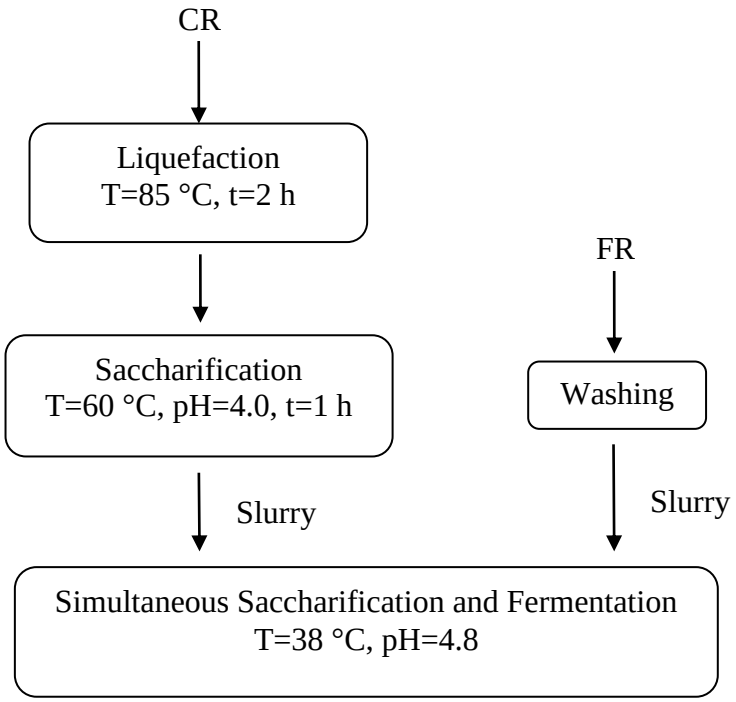

Fig. 1. Experimental procedure used to assess the effects of mixtures of CR and FR on simultaneous saccharification and fermentation (SSF)

Microorganisms and enzyme preparation

The microorganism *Saccharomyces cerevisiae* in the form of dry yeast was purchased from Angel Yeast Company (YiChang, China). Before SSF, dry yeast was activated in a 2% glucose solution at 36 °C for 15 min, then at 34 °C for 1 h. Cellulolytic enzymes were Celluclast 1.5L with a cellulase activity of 75 FPU/mL and Novozym 188 with a β-glucosidase activity of 43.9 IU/mL, which were both kindly donated by Novozymes A/S (Bagsvaerd, Denmark). The enzyme loading used in SSF was 8 FPU/g-cellulose for Celluclast 1.5 L and 10 IU/g-cellulose for Novozym 188. α-amylase and glucoamylase were the commercial enzymes chosen and were obtained from the Beijing Aoboxin Co. (China). Their enzyme activities were 4 and 100 KU/g, respectively.

Gleditsia saponin preparation

Gleditsia saponin is a natural surfactant separated from *Gleditsia* fruits. The pods were manually separated from the seeds (13 mm × 8 mm × 6 mm) and dried in air. The air-dried pods were then milled to nominal sizes of 40 to 60-mesh. *Gleditsia* saponin was extracted with water from the pods of *Gleditsia* fruits. The critical micelle concentration (CMC) of *Gleditsia* saponin was 0.16 g/L, and the addition of *Gleditsia* saponin was 0.4 g/L.

CR starch saccharification

The CR starch saccharification liquid was prepared by double enzymes methods, and the concentration ranged from 5% to 20%. Briefly, CR was firstly liquefied at 85 °C

for 2 h using commercial amylase enzyme at an amount of 150 u/g CR, followed by adjusting pH to 4.0 with 10% of sulfuric acid. Addition of the amylase can decrease the viscosity of CR, which is conducive to the role of glucoamylase (Liu *et al.* 2005). Finally, saccharification was conducted at 60 °C for 1 h by glucoamylase at an amount of 20 u/g CR. The use of incomplete saccharification aims to reduce high concentrations of sugar osmotic pressure of yeast (Erdei *et al.* 2010). The pH of CR saccharification liquid was adjusted to 5.5 with 10% sodium hydroxide before SSF.

Simultaneous saccharification and fermentation

In the SSF test, a 100 mL Erlenmeyer flask was used at a working volume of 60 mL with a special sealing means for the discharge of carbon dioxide, which could reduce the loss of ethanol. The amount of the enzymes Celluclast 1.5L and Novozym 188 was 15 FPU/g-cellulose and 17 IU/g-cellulose, respectively. The FR concentration ranged from 2% to 10%, while the CR saccharification concentration ranged from 2% to 13.3%. The CR/FR weight ratio was from 2/1 to 1/5, and the concentration was from 6% to 12%. The initial inoculum concentration of yeast was about 3.3 g/L. The FR was sterilized prior to being added to flasks (121 °C, 20 min). The enzyme and cool saccharification liquid of CR were added to fermentation directly. The fermentation was conducted in an air bath shaker at a speed of 120 rpm and 38 °C.

Analysis of substrate composition

The cellulose and hemicellulose contents of samples were analyzed according to the National Renewable Energy Laboratory (NREL) methods (Sluiter *et al*. 2004a). Acid-insoluble lignin of FR and CR was determined by the TAPPI method (TAPPI T222 om-06 2006). The total starch was determined by complete saccharification of CR starch according to McCleary's method (McCleary *et al*. 1994). The yeast cell concentration and cell death ratio were determined by blood-count method, as previously described (Tang *et al.* 2011a). A muffle furnace was used at 500 °C to calculate the percentage of total ash according to the residue weight. The content of nitrogen was determined by the Kjeldahl method (Bremner 1960), and then the protein content was obtained by multiplying the nitrogen content by a factor of 6.25. The fermentation samples were filtered (pore size, 0.22-μm) to detect sugars and ethanol. The total amounts of reducing sugars were measured using the Somo-gyi-Nelson colorimetric method employing glucose as a standard (Nelson 1944; Wang *et al.* 1987). Ethanol and glycerol were analyzed by high performance liquid chromatography (HPLC) (Waters 2695e, USA) using an Aminex HPX-87H column (300 × 7.8 mm; Bio-Rad Laboratories, USA) at 65 °C and refractive index detection detector at 30 °C. The injection volume of the sample was 10 μL, and 5 mM sulfuric acid was used as the eluent at a flow rate of 0.6 mL/min. The ethanol yield was calculated assuming that 1 g of cellulose or starch present in the liquid theoretically gave 0.568 g of ethanol, and is expressed as the percentage of the theoretical yield based on FR and cellulose and CR starch and cellulose. Assays were performed in two repeated experiments, and mean values are presented.

RESULTS AND DISCUSSION

Glucose Release from Cassava Residue by Different Enzymes

Cellulolytic enzymes have been shown to be efficient in the release of glucose from CR. It is also confirmed that CR loading affects the release degree of glucose in

enzyme hydrolysis (SriZnorakutara *et al.* 2004). In this study, glucose release from CR decreased from 477 g to 356 g per kg CR when the substrate loading increased from 5% to 20%. This differs from prior research, in which 704.8 g glucose was released per kilogram CR (Divya *et al.* 2011). The difference is likely due to the difference in chemical composition and the cellulolytic enzymes used, as the extraction technique affects the chemical composition of CR (Ziska *et al.* 2009; Divya *et al.* 2011; Shanavas *et al.* 2011; Nguyen *et al.* 2014). When the substrate consistency was higher than 15%, a high viscosity was initially observed in CR enzyme hydrolysis. However, after 4 h of hydrolysis, the materials became well liquefied, indicating that celluloses play an important role in the viscosity (Gong *et al.* 2009). For general lignocellulosic materials, lignin is regarded as a major bottleneck for the enzymatic hydrolysis at high substrate consistency (Mooney *et al.* 1998; Berlin *et al.* 2005). Because CR contains only 6.8% of the acid insoluble lignin (Table 1), it is an attractive option for bioconversion.

Double enzyme method is always used for the release of glucose from starch materials (Zhang *et al.* 2011). Normally, complete saccharification of corn after liquefaction is not necessary for efficient ethanol fermentation. The use of incomplete saccharification aims to reduce high concentrations of sugar osmotic pressure of yeast (Erdei *et al.* 2010). According to our experimental results, 3 h of saccharification was found to be sufficient for bioconversion of CR to ethanol by a double enzyme method (data not shown). The glucose concentration reached 93.9 g/L and 116.7 g/L respectively, when using 15% and 20% of substrate consistency. Since CR contains 73.2% glucan, neither cellulolytic enzymes nor amylase could fully release the glucose in CR. Therefore, both of them were coupled for better use of CR carbohydrate.

The Effect of CR/FR Ratios on Ethanol Production

Figure 2 illustrates the concentration profiles of reducing sugars and ethanol in SSF of different CR/FR ratios ranging from 1:5 to 2:1. In the early stage, the overall kinetics was limited by the fermentation step because of the high concentrations of sugars, which were found to be parallel to the concentration of CR at the beginning of SSF (Tang *et al.* 2011a).

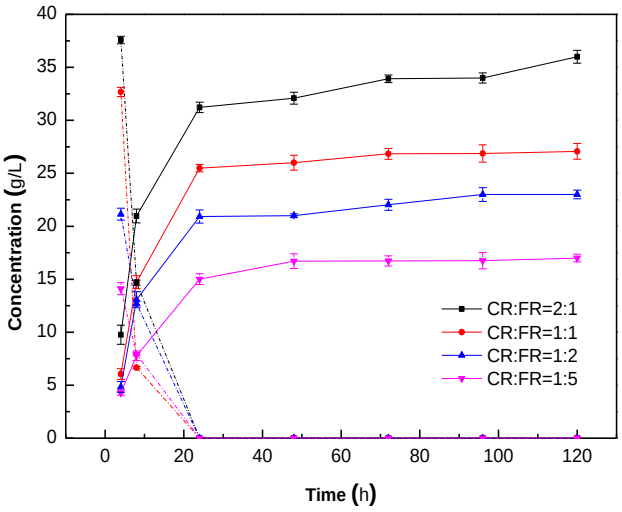

Fig. 2. The effect of CR/FR ratios on concentration of ethanol (solid line) and concentration of reducing sugars (dashed line) during SSF at 38 °C with initial pH 6.0 under 12% substrate concentration of cassava residue (CR) and furfural residue (FR)

In the four cases reflected in Fig. 2, the sugar level was totally exhausted at 24 h after inoculation with the yeast. Obvious increases in ethanol concentration and yield were achieved as a result of CR addition. The ethanol concentration increased from 17.1 g/L to 36.2 g/L, when decreasing the CR/FR ratio from 1:5 to 2:1. It has been reported that FR contains more than 40% lignin (Yu *et al.* 2014), and lignin is known to inhibit ethanol fermentation because of its invalid adsorption to cellulose (Sun *et al.* 2011). The ethanol-yield profiles differed with different ratios of CR/FR; the highest ethanol yield reached 71.1% of the theoretical as CR:FR=2:1, whereas the yield went down to 45.2% of CR:FR = 1:5.

The Effect of Substrate Concentration on Ethanol Production

CR were liquefied and saccharified, and the saccharification liquid was then added to SSF of FR. The CR contained 3.72% protein, which was lower than that of corn. When CR was loaded at a consistency of 20%, the nutrients were sufficient for fermentation (data not shown).

Ethanol production of SSF at different substrate concentrations with a 2:1 CR/FR ratio is shown in Fig. 3. The final ethanol concentration increased with an increasing substrate concentration, which is beneficial in reducing the cost of ethanol separation (Wingren *et al.* 2003). The rate-limiting step in the overall SSF was cellulose conversion, and the total reducing sugars stayed below 1.0 g/L after 8 h. In the current study conditions of 20% CR with FR addition at a CR/FR ratio of 2/1, much higher ethanol concentration than that from 6% was obtained: 42.7 g/L *vs*. 12.6 g/L. Öhgren *et al.* (2006) obtained ethanol concentrations of about 25 g/L ethanol at 10% WIS over the SSF step from steam-pretreated corn stover.

By using a high concentration of substrates, a high final ethanol concentration was also obtained from cellulosic ethanol (Zhang *et al.* 2010). However, a high concentration of lignocellulosic substrate (>10%) is known to reduce the final ethanol yield by reason of higher viscosity and higher levels of inhibitor in the fermentation system (Hoyer *et al.* 2009). Interestingly, in this study, the average yield of ethanol was observed to increase mildly but consistently, from 63.2%±3.1% to 71.2%±1.6% of the theoretical, when the substrate concentration increased from 6% to 12%. The relationship between yield and substrate concentration was not linear but parabolic. The ethanol yield of theoretical was increased due to the increasing protein content when the substrate concentration was increased from 6% to 12%, while higher level of cellulose results in the viscosity increment of the system made the yield decreased under the substrate concentration of 12% to 20%. When using FR alone, the ethanol yield was only 28% of theoretical of 10% substrate concentration (data not given). Enhancing the substrate concentration appropriately can play a positive role in ethanol production, but a high concentration has an inhibitory effect on ethanol yield of the theoretical (Huang and Penner 1991; Penner *et al.* 1994). Zhang *et al*. (2011) managed to obtain ethanol in the final fermentation from 24.7, 31.0, and 39.3 g/L to 40.6 g/L with increasing pretreated corn stover loading from 15.0%, 20.0%, 25.0% to 30.0% (w/w), while the ethanol yield to the theoretical value decreased from 76.5%, 68.0%, 64.8% to 52.1% with increasing corn stover loading.

Addition of surfactant is a potential method for performing SSF with lignocellulose (Ballesteros *et al.* 1998). In this study, the ethanol yield of SSF was 62.8% with 20% substrate concentration, and increased to 63.0% with the addition of 0.4 g/L *Gleditsia* saponin. Only a slight increase in both concentration and yield was observed when *Gleditsia* saponin was added after 8 h. *Gleditsia* saponin addition has a positive

effect on SSF when there is a high amount of lignin. Tang *et al*. (2011b) demonstrated that the addition of Gleditsia saponin could reduce the enzyme loading, which has a significant economical effect on the ethanol production. This indicates that lignin inhibition is no longer a major factor in the fermentation of a high substrate concentration with a CR/FR ratio at 2, in which the amount of lignin is relatively low.

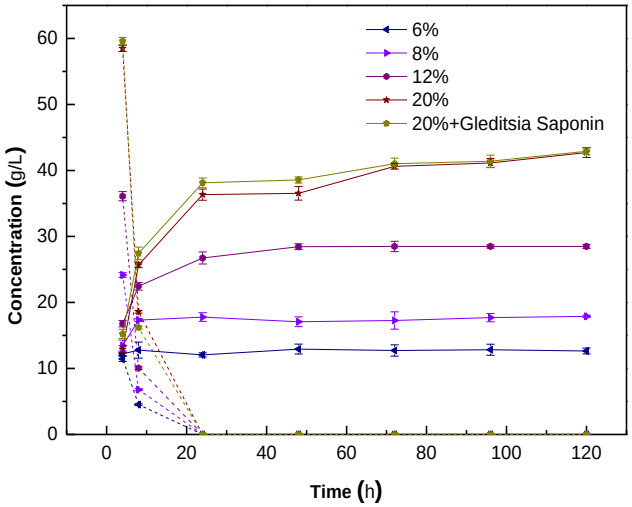

Fig. 3. The effect of substrate concentration on ethanol (solid line) and reducing sugars (dashed line) concentrations during SSF of CR/FR ratio at 2 at 38 °C with initial pH 6.0

The Effect of CR/FR Ratios on Glycerol By-Product

The effect of glycerol by-product on ethanol production during fermentation of *Saccharomyces cerevisiae* is important to study, because the generation of glycerol consumes at least 4% of the carbon source for the fermentation (Zi *et al.* 2000). When osmotic pressure changes and low oxidation-reduction potential occurs, glycerol is obtained (Zi *et al.* 2000). In the process of ethanol fermentation, the main function of glycerol is to maintain the balance of NAD^+/NADH in yeast cells, and it plays an important role in starting ethanol fermentation (Ribereau-Gayon *et al.* 2000). As can be seen in Fig. 4, the reaction had more glucose at the beginning, and the yeast cells lacked acetaldehyde as a hydrogen acceptor, resulting in increased NADH as concentration increased (Brown *et al.* 1978). Thus, there was a high concentration of glycerol, ranging from 5.61 to 11.09 g/L, with the change of substrate ratio of CR/FR from 2/1 to 1/5 (Fig. 3). Specifically, 3-phosphoric acid glycerol dehydrogenase catalyzed the dihydroxy acetone phosphate reduction reaction, generated 3-phosphoric acid glycerol, and resulted in NADH reduction of NAD^+. Then under the action of 3-glyceride, hydrolysis of glycerol occurred. In this process, enough acetaldehyde was accumulated in yeast cells as hydrogen, and then ethanol fermentation started. Glycerol decreased at 8 h because of the low amount of glucose, and glycerol was converted to dihydroxyacetone (Shams *et al*. 2008).

In addition, glycerol can be involved in energy metabolism or synthesis of glycogen and fat. Glycerol not only plays an important role in starting-up ethanol fermentation, but it can also be used as a metabolic regulation substance for osmotic pressure produced by the high concentration of sugar and ethanol in the fermentation process (Rehm *et al.* 1988; Agarwal 1990; Tang *et al.* 2011b). In this study, the glucose ran out at 24 h, and a quantity of glycerol was produced in the system to maintain

intracellular osmotic pressure. Comparing the four groups, the least amount of glycerol byproduct appeared at the CR/FR ratio of 2, because the lowest FR includes the lowest lignin amount in this group (Fig. 4). After 48 h, glycerol concentration was relatively stable because of the stabilization of ethanol production.

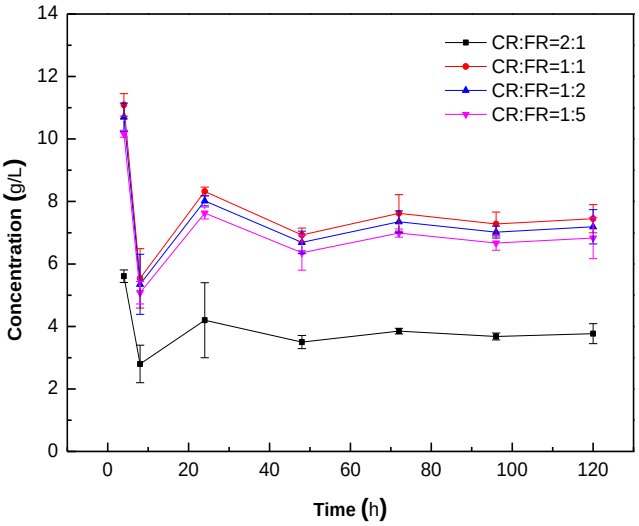

Fig. 4. Concentration of glycerol during SSF of cassava residue (CR) and furfural residue (FR) at 38 °C with initial pH 6.0

The Number of Live Yeast Cells and the Yeast-Cell Death Ratio during SSF

The number of live yeast cells increased during the first 17 h, and then decreased from 17 to 120 h (Fig. 5). The yeast-cell death ratio decreased with increasing concentration during SSF (Fig. 5), illustrating that there was more yeast proliferation with high substrate concentration in SSF.

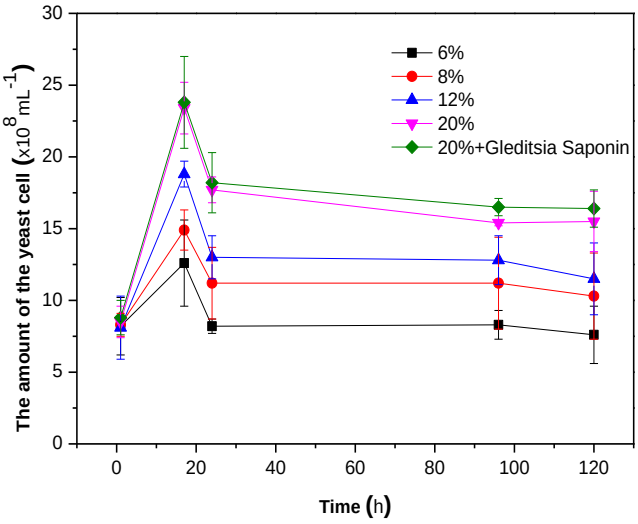

Fig. 5. The number of live yeast cells during SSF of CR/FR ratio at 2 at 38 °C with initial pH 6.0

The addition of *Gleditsia* saponin did not affect the number of live yeast cells during SSF of 20% substrate concentration. However, SSF of 20% concentration had a

slightly higher yeast-cell death ratio than that with *Gleditsia* saponin (Fig. 6). The number of live yeast cells in SSF of 20% concentration with *Gleditsia* saponin was nearly twice that of SSF of 6% substrate concentration, whereas different yeast-cell death ratios were obtained for both groups. The number of live yeast cells increased in SSF with increasing substrate concentration. Moreover, the promoting effect that occurred between substrate concentration and *Gleditsia* saponin seemed to be strengthened with increasing substrate concentration.

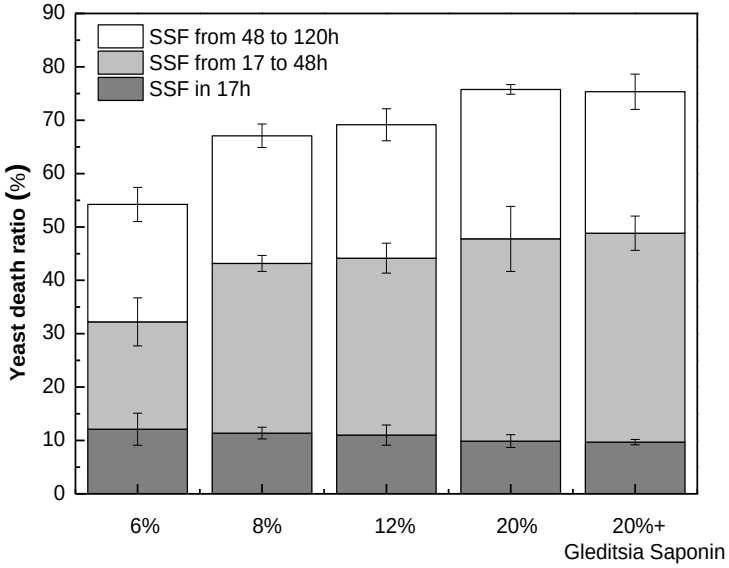

Fig. 6. The number of dead yeast cells during SSF at 38 °C with initial pH 6.0. Data show the mean ± standard deviation

CONCLUSIONS

1. The results of this investigation indicate that a mixture of cassava and furfural residues can provide a reliable feedstock for ethanol production. The final ethanol yield was raised obviously when increasing the CR/FR ratio from 0.2 to 2. The maximal yield reached 71.1% with a CR/FR ratio of 2 and a substrate loading of 12%. The CR/FR ratio also affected the glycerol formation during SSF.
2. The ethanol yield increased when the substrate consistency increased from 6% to 12%. However, the ethanol yield decreased when the substrate concentration was further raised to 20%, which was partly due to high viscosity and the inhibitory effect of glucose to yeast and enzymes.
3. Addition of *Gleditsia* saponin did not improve the ethanol yield of SSF, but it reduced the yeast-cell death ratio at a high substrate consistency of 20%. These interesting results indicated that lignin was not a major factor in SSF of the mixtures at a high substrate consistency with a CR/FR ratio of 2.
4. The mixture of CR and FR could dilute the lignin amount of FR to some extent, which benefited cellulose hydrolyzation. Meanwhile, the addition of CR provided nutrition to ethanol fermentation and reduced the demand for medium as a consequence, which would have some economic benefits.

ACKNOWLEDGMENTS

The authors are grateful for the financial support of this research from the China Ministry of Science and Technology (2014DFG32550) and the Guangxi Key Laboratory of Chemistry and Engineering of Forest Products (GXFC12-07).

REFERENCES CITED

Agarwal, G. P. (1990). "Glycerol," in: *Microbial Bioproducts*, L. de Boer, H. Brandl, L. Dijkhuizen, and R.C. Fuller (eds.), Springer Berlin Heidelberg, pp. 95-128. DOI: 10.1007/BFb0010233

Alkasrawi, M., Eriksson, T., Börjesson, J., Wingren, A., Galbe, M., Tjerneld, F., and Zacchi, G. (2003). "The effect of Tween-20 on simultaneous saccharification and fermentation of softwood to ethanol," *Enzyme Microb. Technol.* 33(1), 71-78. DOI: 10.1016/S0141-0229(03)00087-5

Ballesteros, I., Oliva, J. M., Carrasco J., Cabanas, A., Navarro, A. A., and Ballesteros, M. (1998). "Effect of surfactants and zeo-lites on simultaneous saccharification and fermentation of steam-exploded poplar biomass to ethanol," in: *Biotechnology for Fuels and Chemicals*, M. Finkelstein and B. H. Davison (eds.), Proceedings of the Nineteenth Symposium on Biotechnology for Fuels and Chemicals, pp. 369-381. DOI: 10.1007/978-1-4612-1814-2_35

Berlin, A., Gilkes, N., Kurabi, A., Bura, R., Tu, M., Kilburn, D., and Saddler, J. (2005). "Weak lignin-binding enzymes-a novel approach to improve activity of cellulases for hydrolysis of lignocellulosics," *Twenty-Sixth Symposium on Biotechnology for Fuels and Chemicals*, B. H. Davison, B. R. Evans, M. Finkelstein, and J. D. McMillan (eds.), pp. 163-170. DOI: 10.1007/978-1-59259-991-2_14

Bremner, J. M. (1960). "Determination of nitrogen in soil by the Kjeldahl method," *The J. of Agric. Sci.* 55(1), 11-33. DOI: 10.1017/S0021859600021572

Chen, C., Ding, S., Wang, D., Li, Z., and Ye, Q. (2014). "Simultaneous saccharification and fermentation of cassava to succinic acid by *Escherichia coli* NZN111," *Bioresour. Technol.* 163, 100-105. DOI: 10.1016/j.biortech.2014.04.020

Divya Nair, M. P., Padmaja, G., and Moorthy, S. N. (2011). "Biodegradation of cassava starch factory residue using a combination of cellulases, xylanases and hemicellulases," *Biomass Bioenerg.* 35(3), 1211-1218. DOI: 10.1016/j.biombioe.2010.12.009

Erdei, B., Barta, Z., Sipos, B., Réczey, K., Galbe, M., and Zacchi, G. (2010). "Ethanol production from mixtures of wheat straw and wheat meal," *Biotechnol. Biofuels* 3, 16. DOI: 10.1186/1754-6834-3-16

Farrell, A. E., Plevin, R. J., Turner, B. T., Jones, A. D., O'hare, M., and Kammen, D. M. (2006). "Ethanol can contribute to energy and environmental goals," *Science* 311(5760), 506-508. DOI: 10.1126/science.1121416

Gong, X. F., Li, P., Liang, L., and Zhu, M. (2009). "Investigation on the fed-batch hydrolysis of cassava pulp by multi-enzyme and ethanol fermentation by saccharomyces cerevisiae," *Food and Fermentation Industries* 37(4), 112-116.

Hahn-Hägerdal, B., Karhumaa, K., Fonseca, C., Spencer-Martins, I., and Gorwa-Grauslund, M. F. (2007). "Towards industrial pentose-fermenting yeast strains," *Microb. Biotechnol*. 74(5), 937-953. DOI: 10.1007/s00253-006-0827-2

Himmel, M. E., Ding, S. Y., Johnson, D. K., Adney, W. S., Nimlos, M. R., Brady, J. W.,

and Foust, T. D. (2007). "Biomass recalcitrance: Engineering plants and enzymes for biofuels production," *Science* 315(5813), 804-807. DOI: 10.1126/science.1137016

Hoyer, K., Galbe, M., and Zacchi, G. (2009). "Production of fuel ethanol from softwood by simultaneous saccharification and fermentation at high dry matter content," *J. Chem. Technol. Biot.* 84(4), 570-577. DOI: 10.1002/jctb.2082

Huang, X., and Penner, M. H. (1991). "Apparent substrate inhibition of the *Trichoderma reesei* cellulase system," *J. Agric. Food. Chem.* 39(11), 2096-2100. DOI: 10.1021/jf00011a042

Kang, H. W., Kim, Y., Kim, S. W., and Choi, G. W. (2012). "Cellulosic ethanol production on temperature-shift simultaneous saccharification and fermentation using the thermostable yeast *Kluyveromyces marxianus* CHY1612," *Bioproc. Biosyst. Eng.* 35(1-2), 115-122. DOI: 10.1007/s00449-011-0621-0

Kerr, R. A. (2007). "The looming oil crisis could arrive uncomfortably soon," *Science* 316(5823), 351-351. DOI: 10.1126/science.316.5823.351

Liu, Z., Wang, J. P., Zhang, L. F., Zeng, A. W., and Yuan, X. G. (2005). "Production of ethanol by simultaneous saccharification and fermentation from cassava," *Chin. J. Proc. Eng.* 5(3), 353. DOI: 1009−606X(2005)03−0353−04

Mabee, W. E. (2007). "Policy options to support biofuel production," in: *Advances in Biochemical Engineering/Biotechnology*, O. Lisbeth (ed.), Springer Berlin Heidelberg, pp. 329-357. DOI: 10.1007/10_2007_059

Mooney, C. A., Mansfield, S. D., Touhy, M. G., and Saddler, J. N. (1998). "The effect of initial pore volume and lignin content on the enzymatic hydrolysis of softwood," *Bioresour. Technol.* 64(2), 113-119. DOI: 10.1016/S0960-8524(97)00181-8

Nelson, N. A. (1944). "A photometric adoption of Samogyi method for the determination of glucose," *J. Biol. Chem.* 153, 375-380. DOI: 10003538069

Nguyen, C. N., Le, T. M., and Chu-Ky, S. (2014). "Pilot scale simultaneous saccharification and fermentation at very high gravity of cassava flour for ethanol production," *Ind. Crop. Prod.* 56, 160-165. DOI: 10.1016/j.indcrop.2014.02.004

Okano, K., Tanaka, T., Ogino, C., Fukuda, H., and Kondo, A. (2010). "Biotechnological production of enantiomeric pure lactic acid from renewable resources: Recent achievements, perspectives, and limits," *Microbiol. Biotechnol.* 85(3), 413-423. DOI: 10.1007/s00253-009-2280-5

Öhgren, K., Rudolf A., Galbe M., and Zacchi G. (2006). "Fuel ethanol production from corn stover: Optimization of steam pretreatment and improvement of simultaneous saccharification and fermentation," *Biomass Bioenergy* 30, 863-869. DOI: 0.1016/j.biombioe.2006.02.002

Penner, M. H., and Liaw, E. T. (1994). "Kinetic consequences of high ratios of substrate to enzyme saccharification systems based on *Trichoderma* cellulose," in: *Enzymatic Conversion of Biomass for Fuels Production*, M. E. Himmel, J. O. Baker, and R. P. Overend (Eds.), American Chemical Society, Washington, DC, pp. 363-371.

Rehm, H. J. (1988). "Microbial production of glycerol and other polyols," in: *Biotechnology Set, Second Edition*, H.-J. Rehm and G. Reed (eds.), Wiley-VCH Verlag GmbH, Weinheim, Germany. pp. 51-69. DOI: 10.1002/9783527620999.ch5f

Ribéreau-Gayon, P., Glories, Y., Maujean, A., and Dubourdieu, D. (2000). *Handbook of Enology. The Chemistry of Wine and Stabilisation and Treatments*, Volume 1, 2nd Edition, John Wiley & Sons. DOI: 10.1002/0470010398.ch6

Shams, Yazdani, S., and Gonzalez, R. (2008). "Engineering *Escherichia coli* for the efficient conversion of glycerol to ethanol and co-products," *Metab. Eng.* 10(6),

340-351. DOI: 10.1016/j.ymben.2008.08.005

Shanavas, S., Padmaja, G., Moorthy, S. N., Sajeev, M. S., and Sheriff, J. T. (2011). "Process optimization for bioethanol production from cassava starch using novel eco-friendly enzymes," *Biomass Bioenergy* 35(2), 901-909. DOI: 10.1016/j.biombioe.2010.11.004

Srinorakutara, T., Suesat, C., Pitiyont, B. Kitpreechavanit, W., and Cattithammanit, S. (2004). "Utilization of waste from cassava starch plant for ethanol production," *The Joint International Conference on Sustainable Energy and Environment (SEE)*, Hua Hin, Thailand, pp. 1-3.

Sun, R., Song, X., Sun, R., and Jiang, J. (2011). "Effect of lignin content on enzymatic hydrolysis of furfural residues," *BioRes.* 6(1), 317-328. DOI: 10.15376/biores.6.1.317-328

Tang, Y., Zhao, D., Cristhian, C., and Jiang, J. (2011a). "Simultaneous saccharification and cofermentation of lignocellulosic residues from commercial furfural production and corn kernels using different nutrient media," *Biotechnol. Biofuels* 4(1), 22. DOI: 10.1186/1754-6834-4-22

Tang, Y., Su, Z. Q., Zhao, D. Q., Qi, X., and Jiang, J. X. (2011b). "The effect of *Gleditsia* saponin on simultaneous saccharification and fermentation of furfural residue for ethanol production," *Adv. Mater. Res.* 236, 108-111. DOI: 10.4028/www.scientific.net/AMR.236-238.108

TAPPI T222 om-06. (2006). "Acid-insoluble lignin in wood and pulp," *TAPPI Press*, Atlanta, GA.

Wang, Y. W., Tang, R., Tao, J., Gao, G., Wang, X., Mu, Y., and Feng, Y. (1987). "Quantitative analysis program fermentation of lignocellulosic materials in the solid matrix of hemicellulose, cellulose and lignin," *Microbiol. Biotechnol*. 14(2), 81-84.

Wingren, A., Galbe, M., and Zacchi, G. (2003). "Techno-economic evaluation of producing ethanol from softwood: Comparison of SSF and SHF and identification of bottlenecks," *Biotechnol*. 19(4), 1109-1117. DOI: 10.1021/bp0340180

Yu, H., Xing, Y., Lei, F., Liu, Z., and Jiang, J. (2014). "Improvement of the enzymatic hydrolysis of furfural residues by pretreatment with combined green liquor and ethanol organosolv," *Bioresour. Technol.* 167, 46-52. DOI: 10.1016/j.biortech.2014.05.111

Zhang, J., Chu, D., Huang, J., Yu, Z., Dai, G., and Bao, J. (2010). "Simultaneous saccharification and ethanol fermentation at high corn stover solids loading in a helical stirring bioreactor," *Biotechnol. Bioeng.* 105(4), 718-728. DIO: 10.1002/bit.22593

Zhang, S. H., Li, H. M., Pan, S. M., and Lin, Y. X. (2011) "Saccharification of cassava starch with enzymatic catabolism," *Fujian J. Agric. Sci.* 26(5), 818-821. DOI: 1008-0384(2011)05-818-04

Zi, L. H., Liu, C. G., Wang, N., Yuan, W. J., and Bai, F. W. (2013). "Very high gravity ethanol production under different aeration schemes," *China Biotechnol*. 33(6), 86-92.

Ziska, L. H., Runion, G. B., Tomecek, M., Prior, S. A., Torbet, H. A., and Sicher, R. (2009). "An evaluation of cassava, sweet potato and field corn as potential carbohydrate sources for bioethanol production in Alabama and Maryland," *Biomass Bioenergy* 33(11), 1503-1508. DOI: 10.1016/j.biombioe.2009.07.014

Article submitted: Oct. 2, 2014; Peer review completed: Nov. 29, 2014; Revised version received and accepted: Dec. 10, 2014; Published: January 5, 2015.