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Comparison of Pretreatment Methods for Production of Ethanol from Sugarcane Bagasse

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Sugarcane bagasse (SCB) was modified by steam explosion pretreatment at 190 °C for 10 min and 210 °C for 5 min using green liquor (GL) combined with hydrogen peroxide (GL-H_2O_2) and ethanol (GL-Ethanol) for simultaneous saccharification and fermentation (SSF)-based ethanol production. The results showed that 85.02% and 100% of hemicelluloses were solubilized by steam explosion pretreatment at 190 °C for 10 min and 210 °C for 5 min, respectively. Moreover, 20.08% and 73.77% of the lignin was removed through GL-H_2O_2 and GL-Ethanol pretreatments, respectively. The steam explosion pretreatments greatly improved the specific surface area of the SCB and led to the highest ethanol yield of 92.20% at 190 °C for 10 min and 93.19% of 210 °C for 5 min, respectively. In addition, the ethanol yield reached 72.58% for the GL-Ethanol pretreatment, and about 70% of active lignin could be recovered from the pretreatment liquid. When the GL-H_2O_2 pretreatment was used, the maximum ethanol yield of 20.92% was achieved.

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INTRODUCTION

Lignocellulosic residues, such as sugarcane bagasse (SCB), have been considered one of the most promising feedstocks for bioethanol productions in the future because of their abundance, low cost, and wide distribution. Brazil has a large supply of this feedstock, which is an abundant product of the sugar and alcohol industries (Souza *et al.* 2012). In China, most SCB is used for producing paper pulp (Alam *et al.* 2013). However, because of the low strength of paper produced from SCB, this material has not been widely used in pulp and paper mills (Yu *et al.* 2015a). Considering the high carbohydrate content of bagasse and the amount of bagasse that would be used for 3 billion liters per year, it is an attractive raw material for cellulosic ethanol production, and ethanol production could approach 3 billion liters per year (Gomes *et al.* 2014).

The task of hydrolyzing lignocelluloses to fermentable monosaccharides is still a technical problem because the digestibility of cellulose is hindered by many physico-chemical, structural, and compositional factors (Wanderley *et al.* 2013). The natural cellulose is protected by the surrounding matrix of lignin, hemicelluloses, and pectin against enzymatic attacks, which makes it a challenging material for ethanol production. Hence, a pretreatment must be performed on the raw material to improve the accessibility of cellulose (Chang and Holtzapple 2000).

Different pretreatments lead to materials with different characteristics, which influence the enzymatic hydrolysis and fermentation steps (Costa *et al*. 2014). Several methods have been investigated for improving the enzymatic hydrolysis of bagasse, including steam explosion (Biswas *et al*. 2014), liquid hot water extraction (Alvira *et al*. 2010), alkaline peroxide (Arantes and Saddler 2010), dilute acid (Rocha *et al*. 2011), lime, and organosolv pretreatments (Mesa *et al*. 2011). Currently, pretreatment methods such as fiber explosion (steam, ammonia, and CO_2), hot water extraction, and acid hydrolysis can remove hemicellulose efficiently to increase the accessibility of cellulose (Yu *et al*. 2015a). On the other hand, alkaline pretreatments solubilize lignin and a small percentage of the hemicelluloses (Wandery *et al*. 2013).

Steam explosion is the most widely employed physico-chemical pretreatment for lignocellulosic biomass (Wanderley *et al*. 2013). However, this pretreatment strategy used in saccharification has additional impacts on the efficiency of the enzymes and can also inhibit the growth of the fermenting microorganisms (Alvira *et al*. 2010). Chemical methods utilizing alkali (sodium hydroxide and hydrogen peroxide) or organic solvents (*e.g.*, ethanol and methanol) have been developed to remove lignin. Generally, the alkaline pretreatment methods are effective on certain types of material by increasing the available surface area, dissolving the lignin, and breaking the bonds between lignin and the carbohydrate polymers. However, alkaline pretreatment causes environmental pollution, especially through the release of waste water (Yu *et al*. 2014). Organosolv pretreatment is milder than organosolv pulping. Organosolv pretreatment has some typical advantages compared with other pretreatments: (1) the lignin degradation products can be applied in the fields of adhesives, films, and biodegradable polymers and other co-products; and (2) the organic solvents could be easily circulated by distillation (Mesa *et al*. 2011; Yu *et al*. 2014). Among these organosolv pretreatments, ethanol pretreatment is considered the preferable method because of its low toxicity and low boiling point, making it easily recovered by distillation.

Green liquor (GL) produced from pulping processes, a mixture of sodium carbonate and sodium hydroxide, is a kind of alkaline liquid. Recently, pretreatment with GL has been developed as a better method to increase the enzymatic digestibility of biomass, retaining maximum levels of polysaccharides in the substrate for enzymatic hydrolysis (Yu *et al*. 2013) compared with the toxic, corrosive, hazardous, and inhibitory characteristics of acid catalysts. In the present study, SCB was pretreated by steam explosion at 190 °C for 10 min and 210 °C for 5 min, using GL combined with hydrogen peroxide (GL-H_2O_2) and GL combined with ethanol (GL-Ethanol). The effects of these pretreatment conditions on the chemical composition and ethanol concentration or the yield of SCB were investigated. The aim of this study was to compare the pretreatment methods on ethanol yield from SCB.

EXPERIMENTAL

Materials

Sugarcane bagasse, which was kindly provided by Guitang Corporation (Guangxi, China), was water-rinsed and then dried at 60 °C for 12 h. The chemical composition of the raw material was 50.74% cellulose, 23.70% hemicelluloses, 21.12% lignin, and 2.38% ash (Table 1). The dry SCB was divided into two parts. One was ground and screened with 40-mesh sieves. The powders obtained were collected as the experimental

samples for GL pretreatment. The green liquor (GL) was supplied by Chenming Group (Shandong, China). The supernatant was obtained for utilization after precipitating overnight. The main components of GL were sodium carbonate (75.2 ± 0.25 g/L) and sodium hydroxide (23.04 ± 0.25 g/L). There were also other metal elements in GL, such as iron (1.14 ± 0.08 g/L) and calcium (0.39 ± 0.03 g/L). The other SCB portion was steam-pretreated at National Power Grid (Beijing). Anthraquinone (AQ, 0.01 g, Sigma Co., St. Louis, MO) was used in GL-Ethanol pretreatment. All the chemicals used in this study were of analytical grade.

Methods

Pretreatment with steam explosion (SE)

The SCB was impregnated with 4% (v/v) concentrated sulfuric acid at a ratio of solid to liquid of 1:10 for 1 h. Then, steam explosion pretreatment was performed at 190 °C for 10 min and 210 °C for 5 min (Rocha *et al*. 2012; Wanderley *et al*. 2013) using the SCB with a final moisture content of 55 wt% in a high-pressure batch reactor (20 L) located at National Power Grid (Beijing). The reactor was heated with saturated steam. In order to quench the reaction at the end of the steaming, the pressure was instantaneously released and the sample was blown in the receiver (Rocha *et al*. 2012; Wanderley *et al*. 2013). A portion of the lignocellulose after steam explosion was stored at 4 °C for subsequent chemical analyses and SSF processes, and the liquid was reserved and pretreated with and without acid hydrolysis for the determination of sugars (mono-meric and oligomeric fraction) by HPLC.

Pretreatment of sugarcane bagasse by green liquor combined with hydrogen peroxide (GL-H$_2$O$_2$)

GL-H$_2$O$_2$ pretreatment was carried out in a polytetrafluoroethylene (PTFE) reactor at 80 °C and rotated at 100 rpm for 3 h according to previous optimization studies (Yu *et al*. 2013, 2015a,b). The SCB was slurried in water (5%, w/v) containing the desired amount of H$_2$O$_2$ (0.6 g/g-dry substrate (DS)), GL (2 mL/g-dry matter), and 1% (w/w) ethylenediaminetetraacetic acid (EDTA, 98%, Sinopharm Chemical Reagent Beijing Co., Ltd). The effect of EDTA on enhancing delignification was to lessen the consumption of peroxide by reducing the inhibition of metal ions (Yu *et al*. 2013). After pretreatment, the pretreated SCB was collected by filtration and washed with distilled water until the pH was neutral.

Pretreatment of sugarcane bagasse by green liquor combined with ethanol (GL-Ethanol)

GL-Ethanol pretreatment was carried out in a PTFE reactor with a total volume of 200 mL according to a previous optimization study (Yu *et al*. 2014). Ten grams of dry sugarcane bagasse was pretreated by 1.5 mL /g-DS at 140 °C for 3 h with a solid/liquid ratio (w/v) of 1:10. The liquid was a 50:50% (v/v) ethanol: water mixture. The system (PTFE reactor + stainless steel tank) was placed in a chamber equipped with a shaft where the PTFE reactor was fixed well with a large stainless steel tank. The system was heated at an average rate of 5 °C/min and rotated at 100 rpm until the desired temperature of 140 °C was reached. The system was rapidly cooled with tap-water after the pretreatment. The pretreated SCB was obtained by filtration prior to washing with 200 mL of an ethanol-water mixture (50:50%, v/v). Then, the solid fraction thus obtaned was washed with distilled water until neutral pH was reached and dried in an oven at 103 ± 2 °C.

The solid yield was calculated using the following equation:

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$$Solid\ yield\ (\%) = \frac{mass\ of\ pretreated\ dry\ solid\ (g)}{mass\ of\ untreated\ dry\ solid\ (g)} \times 100$$

(1)

The percentage of the pretreated SCB based on the initial amount of SCB was obtained by multiplying the yield by the percentage of each component of the pretreated SCB.

Microorganisms and enzyme preparation

The microorganism *Saccharomyces cerevisiae* in the form of dry yeast was purchased from Angel Yeast Company (YiChang, China). Dry yeast was activated in a 2% glucose solution at 36 °C for 15 min and then at 34 °C for 1 h before SSF (Tang *et al*. 2011).

Cellulolytic enzymes used included Celluclast 1.5L, with a cellulase activity of 75 FPU/mL, and Novozym 188, with a β-glucosidase activity of 43.9 IU/mL; both were kindly donated by Novozymes A/S (Bagsvaerd, Denmark). The activity of these enzymes was measured with UV spectrophotometric method (Feng *et al*. 2011).

Simultaneous saccharification and fermentation

The SSF experiments were performed under nonsterile conditions in a 100-mL conical flask with 60 mL with a special sealing means for the discharge of carbon dioxide, which could reduce the loss of ethanol. The amounts of the enzymes Celluclast 1.5 L and Novozym 188 were 30 FPU/g-cellulose and 37.5 IU/g-cellulose, respectively. The concentration of insoluble fibrous material was 5% (w/v) on the basis of dry material in all the experiments.

The initial inoculum concentration of yeast was approximately 5 g/L. Fermentation with full medium (yeast powder, 40 g/L; $(NH_4)_2HPO_4$, 20 g/L; $MgSO_4 \cdot 7H_2O$, 1 g/L) was carried out, as well as fermentation with the raw material as a control case. In each experiment, SCB in the conical flask and nutrients were separately sterilized (121 °C, 20 min). The fermentation was conducted in an air bath shaker at a speed of 120 rpm at 38 °C for 120 h.

Analytical methods

The cellulose and hemicellulose contents of samples were analyzed according to the National Renewable Energy Laboratory (NREL) methods (Sluiter *et al.* 2004). Acid-insoluble lignin of SCB was determined by the TAPPI method (TAPPI T222 om-06 2006) (Ji *et al*. 2015). The yeast cell concentration and cell death ratio were determined by blood-count method, as previously described (Tang *et al*. 2011).

Ethanol and other by-products 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 present in the liquid theoretically gave 0.568 g of ethanol and is expressed as the percentage of the theoretical yield based on the amounts of SCB and cellulose (Ji *et al*. 2015). Assays were performed for two repeated experiments, and mean values are presented.

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RESULTS AND DISCUSSION

Effect of Pretreatment Method on Chemical Composition

In this work, the SE, GL-H_2O_2, and GL-Ethanol processes were used for SCB pretreatment. Specific pretreatment conditions were employed to achieve the solubilization of hemicellulose and of lignin. The carbohydrates (glucan and xylan) of the raw material accounted for 74.44% of the dry substrate (Table 1). These values are important in terms of the biorefinery concept. Lignocellulosic materials with high glucan and xylan contents are preferable candidates for bio-ethanol production (Chandel *et al*. 2011).

As previously noted by Bura *et al*. (2009), steam explosion is usually a compromise method, where conditions of temperature, residence time, and acid catalyst loading must be adjusted to apply a sufficiently severe pretreatment to facilitate enzymatic hydrolysis and solubilize hemicelluloses, but it must not be so severe as to result in sugar degradation. Table 1 shows that nearly 21% and 35% of the raw material was solubilized by steam explosion pretreatments at 190 °C for 10 min and 210 °C for 5 min, respectively. The degree of solubilization of the whole material increased moderately as the reaction temperature rose. The hemicelluloses were the component displaying the most remarkable solubilization, followed by cellulose. However, lignin was solubilized to a lesser extent (Oliveira *et al*. 2013). Compared with the raw material, both lignin and cellulose contents increased in water-insoluble steam-treated bagasse, 9% to 11% (w/w) for lignin and 9% to 11% (w/w) for glucan, respectively, as a result of hemicelluloses removal. However, there was a noticeable decrease (19% to 23%, w/w) in the amount of hemicelluloses during the steam pretreatment, especially at 210 °C for 5 min, at which condition hemicellulose was almost completely solubilized, and ash was also not detected in the solids. These results revealed that steam explosion pretreatment had an obvious effective on the SCB. Remarkable solubilization of hemicelluloses and partial removal of lignin by the steam explosion were beneficial for the accessibility of the enzyme during fermentation.

It is well known that the aim of alkaline pretreatment involving active alkaline with sodium hydroxide is to break down the chemical bonds of lignin and cellulose of sugarcane bagasse and to remove lignin. As can be seen in Table 1, approximately 12% and 26% of the raw material was removed during the GL-H_2O_2 and GL-Ethanol pretreatment processes, respectively. The content of lignin decreased sharply from 21.12% to 5.54%, and a preferable amount of glucan increased from 50.74% to 62.59% when 1.5 mL of GL/g-DS was applied during GL-Ethanol pretreatment with the use of anthraquinone (AQ) (1%, w/w), better than the two steam explosion pretreatment methods. Previous studies have shown that AQ can be used in soda pulping processes to improve delignification and reduce carbohydrate decomposition (Jiménez *et al*. 2009; Kanungo *et al*. 2011). Approximately 74% of active lignin could be recovered from the GL-Ethanol pretreatment. Conversely, only 20.08% lignin removal was observed with a H_2O_2 loading of 0.6 g/g-DS during GL-H_2O_2 pretreatment; meanwhile, the amounts of glucan and xylan decreased, indicating that glucan and xylan were solubilized under this dosage of H_2O_2. Therefore, it is necessary to reduce the dosage of H_2O_2 during the alkaline pretreatment to minimize the glucan degradation for the untreated SCB. These results indicated that the SE pretreatment was appropriate for solubilizing hemicelluloses and GL-Ethanol pretreatment had an effective on removing lignin; meanwhile, a considerable amount of cellulose for the following bioconversion was retained.

Table 1. Chemical Composition Before and After Pretreatment with Steam Explosion (SE), Green Liquor with Hydrogen Peroxide (GL-H_2O_2), and Green Liquor with Ethanol (GL-Ethanol)

Pretreatment conditions	Composition (%)				
	Glucan	Xylan	Klason lignin	Ash	Solid yield
Raw material	50.74±0.2	23.70±0.6	21.12±0.2	2.38±0.01	--
190 °C/10 min steam explosion	61.43±0.1	3.99±0.3	30.18±0.3	2.71±0.04	78.77
210 °C/ 5 min steam explosion	59.35±0.2	ND	30.68±0.2	ND	65.47
GL-H_2O_2 pretreatment (0.6 g H_2O_2/g-DS)	49.55±0.2	16.19±0.3	16.88±0.2	1.97±0.03	88.20±0.8
GL-Ethanol pretreatment (1.5 mL GL/g-DS)	62.59±0.1	21.38±0.2	5.54±0.3	1.58±0.02	73.90±0.6
ND, not detected; GL, green liquor; DS, dry substrate.					

All values are based on the oven-dried weight of samples.

Effect of Pretreatment Method on Ethanol Production

For comparison purposes, SSF with three different pretreatment methods was carried out, and the results are shown in Fig. 1. In the early stage, the overall kinetics were limited by the fermentation step because of the high concentration of sugars (Tang *et al.* 2011), which were found to be parallel to the concentration of the raw material at the beginning of SSF. Compared with the raw material, similar high concentration profiles were observed for GL-ethanol and steam explosion pretreatments at 190 °C for 10 min and 210 °C for 5 min; the GL-H_2O_2 pretreatment led to a different profile. It also could be seen that the theoretical ethanol yields were 92.20% and 93.19% for steam explosion pretreatment at 190 °C for 10 min and 210 °C for 5 min, respectively. It was worth noting that the rate of ethanol production with the GL-Ethanol pretreatment was higher than that with steam explosion pretreatment at the beginning of 20 h of SSF. The sugar concentrations of the fermentation liquids at 120 h are very low (data are not shown). Moreover, the ethanol yield increased to almost 60% in the initial 20 h. Others have reported that enzymatic hydrolysis was improved as a result of increased surface area (Koo *et al.* 2012). However, in the case of GL-Ethanol pretreatment, small adherent reactive lignin may generate some inhibitors, and could influence the activity of the yeast.

More interestingly, compared with the raw material, it was obvious that the GL-H_2O_2 pretreatment was not effective for ethanol production. The amount of lignin and hemicellulose was similar with the raw material (Table.1). That might be why the ethanol yield from the GL-H_2O_2-pretreated SCB was lower than that of the raw material. Moreover, the ethanol yield after GL-H_2O_2 pretreatment was much less than that after GL-Ethanol. A previous study showed that the glucose yield after 72 h of enzymatic hydrolysis with 1.5 mL of GL/g-DS during GL-Ethanol pretreatment was much greater than that with 0.6 g of H_2O_2/g-DS during GL-H_2O_2 pretreatment (Yu *et al.* 2015a). These results indicated that SCB pretreated with GL-Ethanol had a lower contact angle, implying that the substrate was more hydrophilic compared with GL-H_2O_2-pretreated SCB (Yu *et al.* 2015b). This was probably due to the removal of the most of the lignin in the GL-Ethanol pretreatment. Research has shown that lignin was the main factor that

contributes to the recalcitrance of biomass. Another explanation was that lignin and hemicellulose could only be partially removed while some cellulose was solubilized during the GL-H$_2$O$_2$ pretreatment process.
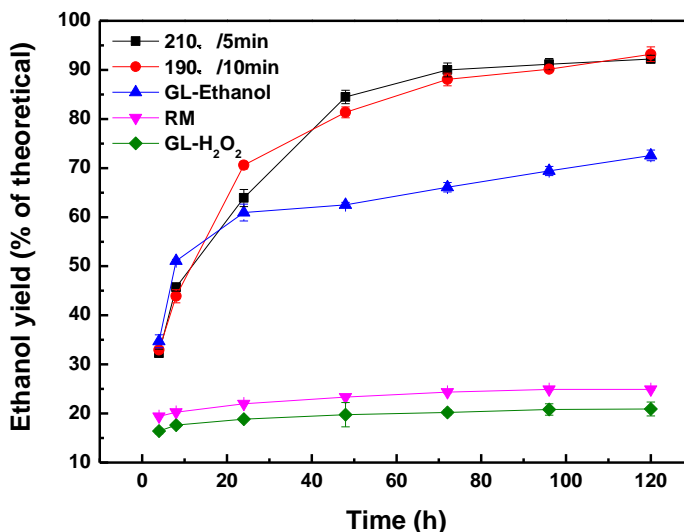

Fig. 1. Effect of pretreatment method on ethanol production from 5% (w/v) pretreated and raw material (RM) during the SSF processes with the microorganism *Saccharomyces cerevisiae* at 38 °C and initial pH 5.5

It is clear that even under mild conditions the steam explosion pretreatment and alkaline delignification processes caused a substantial cellulose loss, which for the ethanol production from this component required a final product cost analysis (Rocha *et al*. 2012). Therefore, steam explosion pretreatment at 190 °C for 10 min was judged to be an appropriate pretreatment method for improving the ethanol yield of theoretical from SCB and saving energy.

Effect of Pretreatment Method on the Yeast Cells during SSF

Figure 2 shows that the number of live yeast cells increased in the first 18 h, and decreased after 18 h, then remained relatively stable up to 120 h. The yeast-cell death ratios differed in pretreatment methods during the SSF processes (Fig. 3). Compared with the raw material, steam explosion at 190 °C for 10 min and 210 °C for 5 min and GL-Ethanol pretreatment showed similar curves of live yeast cells during SSF, and the highest contents of live yeast cells were 20.5×10^7 mL^{-1}, 18.23×10^7 mL^{-1}, and 15.1×10^7 mL^{-1} for these pretreatments, respectively. Meanwhile, steam explosion at 190 °C for 10 min and 210 °C for 5 min had relatively low yeast cell death ratio during the whole fermentation processes, illustrating that there was more yeast proliferation with the pretreated substrate in the SSF. It was likely that little oxygen from the later period of sampling promoted the reproduction of the yeast at 210 °C for 5 min pretreated SCB at 96 h. However, no marked change occurred in the SSF process of GL-H$_2$O$_2$ pretreated substrate, which had the highest yeast mortalities of 5.19% and 25.25% during 18 h and 48 to 120 h, respectively. The large amount of yeast-cell death in the SSF process of GL-H$_2$O$_2$ was also due to low hydrolysis yield in SSF, which contributed to low glucose concentration in the medium required for growth of the yeast.
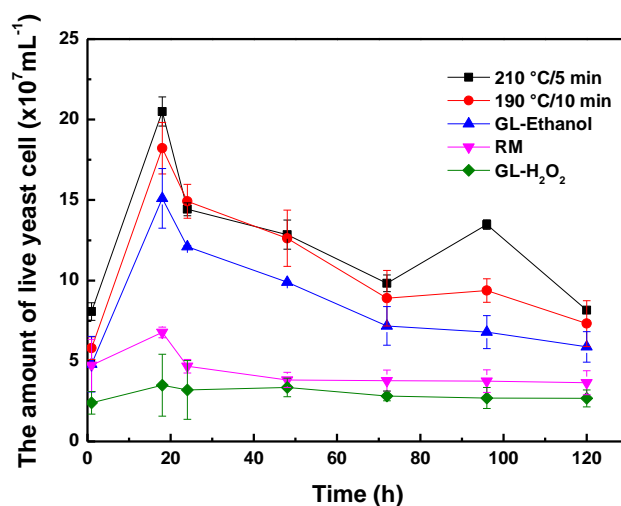
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Fig. 2. The number of live yeast cells of 5% (w/v) pretreated and raw material (RM) during the SSF process at 38 °C with initial pH 5.5
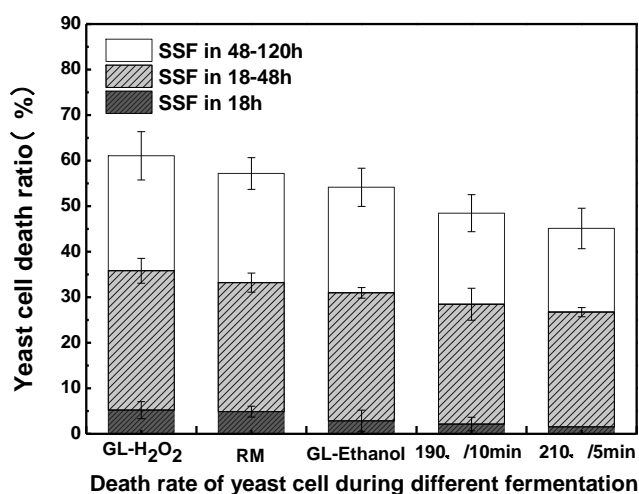

Fig. 3. The yeast-cell death ratio of 5% (w/v) pretreated and raw material (RM) during the SSF process at 38 °C with initial pH 5.5

In this study, in view of the amount of live yeast and yeast cell death ratio, the steam-exploded pretreatment was remarkably effective for ethanol production. However, milder steam pretreatment would improve the economic benefits and had a better retention of cellulose. Therefore, steam explosion at 190 °C for 10 min was judged to be more appropriate for ethanol production during the SSF processes compared with the other pretreatments.

The experimental conditions (low solid/liquid loading, high chemical use) were outside the range for a viable industrial process. The solid/liquid ratios were same in different pretreatment methods in this experiment. The conditions of high solid/liquid loading and low chemical use should be considered in the following scaled up experiments.

CONCLUSIONS

1. The findings showed that 81.27% and 100% of hemicelluloses were solubilized by steam explosion pretreatments at 190 °C for 10 min and 210 °C for 5 min, respectively. On the other hand, 20.08% and 73.77% of lignin were removed through GL-H$_2$O$_2$ and GL-Ethanol pretreatments, respectively. Steam explosion pretreatment removed the steric hindrance of hemicelluloses of the SCB. Steric hindrance of the lignin was cleaved and maximum retention of hemicelluloses with GL-Ethanol pretreatment was obtained.

2. SSF with the three different pretreatment conditions was carried out with an initial solid loading of 5% (w/v) for 120 h and gave the highest yields of 92.20% at 190 °C for 10 min pretreated material and 93.19% at 210 °C for 5 min. The highest yield of ethanol with GL-Ethanol pretreatment reached 72.58%. Compared with the raw material, no significant effect on the ethanol yield of GL-H$_2$O$_2$ pretreatment was determined.

3. The maximum theoretical ethanol yield only was 20.92% for a 120-h fermentation, which could be due to the similar amount of lignin and hemicelullose from GL-H$_2$O$_2$ pretreatment compared with the raw material

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