

RESEARCH ARTICLE

Ethanol production from sugarcane bagasse by fed-batch simultaneous saccharification and fermentation at high solids loading

Yueshu Gao¹  | Jingliang Xu² | Zhenhong Yuan² | Jianchun Jiang³ |
Zhenjia Zhang¹ | Chunjie Li¹

¹School of Environmental Science & Engineering, Shanghai Jiao Tong University, Shanghai, China

²Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, Guangzhou, China

³Institute of Chemical Industry of Forest Products, Chinese Academy of Forest, Nanjing, China

Correspondence

Jianchun Jiang, Institute of Chemical Industry of Forest Products, Chinese Academy of Forest, Nanjing, China.
Email: bio-energyi@163.com

and
Chunjie Li, School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai, China.
Email: cjli@sjtu.edu.cn

Funding information

National Natural Science Foundation of China, Grant/Award Number: 21808141; Natural Science Foundation of Jiangsu Province for Youth Science Funds, Grant/Award Number: BK20160150; Natural Science Foundation of Shanghai City, Grant/Award Number: 16ZR1417400; Fundamental Research Funds for the Central Universities, Grant/Award Number: 17X100040069

Abstract

To make the bioethanol conversion process economically viable, improving solids loading (>15% [w/v]) in different unit operations is crucial. In this study, alkali pretreatment of sugarcane bagasse was carried out at high solids loading. Temperature and pH were optimised via batch simultaneous saccharification and fermentation (SSF) experiments using *Saccharomyces cerevisiae* Y-2034 or *Kluyveromyces marxianus* NCYC-587. The effects of enzyme addition mode and solids loading on ethanol production were studied in fed-batch SSF experiments. It was demonstrated that the optimised pH and temperature for both *S. cerevisiae* Y-2034 and *K. marxianus* NCYC-587 were 5.2 and 37°C. The mode of enzyme addition “one-time dose” improved ethanol productivity better than the “batch addition” mode. With fresh substrates added, solids loading strongly increased from 19% to 33% (w/v), corresponded to a small decline in the ethanol yield. At the final solids loading of 33% (w/v), the highest ethanol concentration of 75.57 ± 2.14 g/L was obtained, with ethanol productivity of 0.63 ± 0.02 g/(L h) and a yield of $66.17 \pm 1.87\%$.

KEYWORDS

ethanol production, fed-batch process, high solids loading, simultaneous saccharification and fermentation, sugarcane bagasse

1 | INTRODUCTION

Sugarcane is a common crop widely planted for sugar extraction and ethanol production. According to the data of the Food and Agriculture Organization of the UN, the global production quantity was approximately 2000 Mt in 2017. China,

as the third biggest producer, generates >25 Mt of sugarcane bagasse (SCB) accounting for 6% of global sugarcane quantity. SCB is chiefly constituted with cellulose (32%-48%), hemicellulose (19%-24%), and lignin (23%-32%) and is an abundant and renewable source of lignocellulosic biomass.¹ To improve its added value, a promising utilisation route is production of

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2018 The Authors. *Energy Science & Engineering* published by the Society of Chemical Industry and John Wiley & Sons Ltd.

bioethanol, which is considered a suitable substitute of fossil fuels with benefits for the economy and environment.²

In the past decades, bioethanol manufacture from lignocellulosic materials received much research attention.³ Many researchers have investigated the influences of high solids content (>15% solids, w/w) on different unit operations in order to make the process of converting lignocellulosic biomass to ethanol economically viable.⁴ It is expected that performing this process at high solids loading—particularly with inclusion of high-solids pretreatment following up high-solids hydrolysis and fermentation—is a great means to improve the process efficiency.⁵ Less water is required for the pretreatment and enzymatic hydrolysis. Capital costs are decreased due to the shrinking sizes of reactors and equipment for even saccharides and ethanol concentrations. Energy consumption during heating, cooling, agitating, and ethanol distillation is decreased.⁶

Technical challenges are also caused by high-solids loading, for example, enhanced difficulties with stirring and mixing, limited heat and mass transfer and thus a prolonged process.⁷ To decrease these negative impacts and simultaneously reduce the inhibitory effect of high glucose concentration on cellulase, a simultaneous saccharification and fermentation (SSF) process combined with a fed-batch measure have been regarded as a feasible approach.⁸ During saccharification, the substrate is degraded and converted to glucose, which will be quickly transformed into ethanol by a fermenting microorganism.⁹ More free water will be liberated. The system viscosity in the slurry is decreased, and diffusion and mixing limitations are minimised. The liquefaction could thereby be beneficial to add a fresh substrate and mitigate the issue of high viscosity.

Strategies which contribute to the fermenting efficiency of SSF mainly include increasing substrate loading, choosing a favorable yeast strain, and optimizing temperature and pH value.¹⁰ It was proposed that the pH value between 4.5 and 5.0 was favorable for saccharification of lignocellulosic materials, while the most suitable pH range for yeast fermentation was 5.0–6.0.¹¹ The conflict of optimal pH value during enzymatic hydrolysis and fermentation processes decreased the ethanol concentration produced by SSF process. The optimal enzyme activity of cellulases is shown in the range of 45–55°C, while most microorganisms have good ethanol productivity and yield in the range of 28–35°C.¹² To improve the effectiveness of SSF process, variations between the optimal temperatures for the cellulase and microorganisms may as well be alleviated. Many researchers have focused on carry out SSF of cellulosic substrates via thermotolerant strains such as *Kluyveromyces marxianus*¹³ and *Kluyveromyces fragilis*.¹⁴ The comparison of fermenting capacities was necessary between normal temperature strain—*Saccharomyces cerevisiae* and thermotolerant strain—*K. marxianus* under optimized conditions for SSF.

There had been many reports on combining fed-batch additions of substrate with SSF, which showed a good potential

for increasing ethanol concentrations. A research group¹⁰ reported that a high ethanol concentration of 84.7 g/L can be achieved using corncob pretreated by combinatorial methods at a solids loading of 25% (w/v). However, both dilute sulfuric acid and sodium hydroxide pre-treatments were involved, which obviously increased the energy consumption and equipment investment. Another group¹⁵ employed diluted NaOH-pre-treated SCB as a feedstock and obtained an ethanol concentration of 68.05 g/L and an ethanol productivity of 0.57 g/(L h) at 30% (w/v) solids loading. The pretreatment process was conducted at low solids loading of 5% (w/v), which led to huge water consumption. Some researchers¹⁶ yielded only 40 g/L ethanol using spruce pretreated by SO₂ and steam explosion at a solids loading of 20% (w/v). To make the process of converting lignocellulosic biomass to ethanol economically viable on an industrial scale, selecting a substrate pretreated by a low-energy-consuming way at high-solids loading and optimisation of the fed-batch SSF process for high bioethanol production might be necessary but lack of systematic studies.

Alkali pretreatments may increase the specific surface area and carbohydrate accessibility to cellulase. They are effective at dissolving lignin while preserving most of cellulose and hemicellulose.¹⁷ As one of the most ordinary means applying to the industrial production for pretreating a cellulosic biomass, alkali pretreatment is considered promising for high-solids pretreatments.^{18,19} In the present research, crude SCB was pretreated with diluted NaOH at a solid to liquid ratio of 1:6. Temperature and pH were optimised in batch SSF processes with *S. cerevisiae* Y-2034 or *K. marxianus* NCYC-587, which represent conventional yeast and thermotolerant yeast. To increase the final ethanol concentration and productivity, different enzyme addition modes and solids loading levels were evaluated in the fed-batch SSF processes. The findings of this investigation will help in understanding bioethanol production at high-solids loading during all unit operations as an important utilization means of SCBs.

2 | MATERIALS AND METHODS

2.1 | Materials

Sugarcane bagasse was supplied by Guangxi Feng Hao Group Company Limited. (Pingxiang, China). After milling, the segments between mesh sizes 20 and 80 were screened and employed for experiments. Cellic CTec2 was used as cellulase provided by Novozymes A/S (Bagsævrød, Denmark); the activity of 310 FPU/mL was tested on the basis of the method of the IUPAC.²⁰

Two fermenting microorganisms, *K. marxianus* NCYC-587 and *S. cerevisiae* Y-2034, were used in the batch SSF processes, which were supplied by the National Collection of Yeast Cultures (Norwich, UK) and National Center for

Agricultural Utilization Research (Peoria, IL), respectively. Both inoculums were prepared in 150 mL Erlenmeyer flasks with 50 mL growth medium consisted of 20 g/L glucose, 20 g/L peptone, and 10 g/L yeast extract. *K. marxianus* NCYC-587 was cultivated at 42°C for 12 hours at 150 rpm on a shaker, whereas *S. cerevisiae* Y-2034 was grown at 30°C for 12 hours. Each inoculum was employed in all SSF experiments at 0.1 g/L (dry weight/volume).

2.2 | Methods

2.2.1 | Alkali pretreatment

For removing lignin from the complex cellulosic material and to improve the subsequent processes, SCB was pretreated with a 1.8% (w/v) NaOH solution for 60 minutes at 110°C according to the method published elsewhere.^{18,19} The solid residues were washed with tap water to neutral and then dried in a forced-air oven at 50°C. It was kept in a desiccator for the subsequent SSF experiments.

2.2.2 | Batch SSF experiments

For batch SSF experiments with *K. marxianus* NCYC-587 or *S. cerevisiae* Y-2034, optimal pH and temperature were investigated. A fermentation medium that consisted of 2 g/L yeast extract, 5 g/L KH_2PO_4 , 2 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.4 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 12% (w/v) alkali-pre-treated SCB was loaded in 250 mL Erlenmeyer flasks with 100 mL of 0.2 mol/L sodium acetate buffer. Cellulase loading was 10 FPU/(g substrate). Firstly, a series of batch SSF experiments was conducted at the initial pH of 4.8, 5.2, 5.6, or 6.0. Our 12% (w/v) SCB was prehydrolysed for 12 hours at 50°C and 150 rpm. As for *S. cerevisiae* Y-2034 and *K. marxianus* NCYC-587, the temperatures were turned down to 30–42°C; and the inocula were added into the flasks for the SSF experiments. After ethanol yield detection, the optimal pH for SSF was determined. As for *S. cerevisiae* Y-2034, after prehydrolysis, the temperatures were set to 30, 34, 37, or 40°C to conduct the SSF process. For *K. marxianus* NCYC-587, the temperatures were set to 37, 40, 42, or 45°C to conduct the SSF experiments. The overall SSF duration including prehydrolysis and fermentation was 96 hours. Rubber plugs were used to seal the Erlenmeyer flasks. Time points for collecting samples were set at 0, 12, 24, 48, 72, and 96 hours.

2.2.3 | Fed-batch SSF experiments

The optimal pH and temperature determined in the batch SSF were taken for the initial condition for the fed-batch SSF experiments. The fed-batch SSF experiments were initiated with 12% (w/v) solids loading. To confirm two modes of enzyme, adding of enzyme—“one-time dose” and “batch

addition”, fed-batch processes “A” and “B”—were executed. The fresh substrates (7% [w/v]) were fed after 6 hours to get the final solids loading of 19% (w/v), and the enzyme loading of 10 FPU/(g substrate) was used for both experiments. For process A, cellulosic enzyme was added into the flask at the beginning of hydrolysis in accordance with 19% (w/v) dry solids loading. For process B, enzyme was fed accompanying with the corresponding amount of the substrate. To further increase the ethanol concentration and survey the effect of solids loading on ethanol production, more fresh solids were fed. After prehydrolysis for 12 hours, 4% (v/v) *S. cerevisiae* Y-2034 was added into flasks, and simultaneously, the temperature was set to the optimal one. After that, 7% (w/v) of fresh solids was fed consecutively at 6 and 12 hours to attain the final solid loading of 26% (w/v) and one more at 24 hours to achieve the final solids loading of 33% (w/v). Time points for collecting samples were set at 0, 12, 24, 48, 72, 96, and 120 hours to detecting the ethanol and sugar concentrations. All the experiments for each operation condition were carried out in duplicate.

2.2.4 | Analytical methods

The proportions of cellulose, hemicellulose, and lignin were estimated using the standardised protocols established by the National Renewable Energy Laboratory (NREL, Golden, CO).²¹ Concentrations of glucose, xylose, and cellobiose were analysed on a high-performance liquid chromatography system (Waters 2695, Milford, MA, USA). Ethanol concentrations were measured by means of a gas chromatograph system (Agilent HP 6820, Santa Clara, CA, USA). The detailed detecting conditions were described in previous literature.¹²

2.2.5 | Calculations

The following formula was used to calculate the ethanol yield

$$Y_{\text{Ethanol}} (\%) = \frac{\text{Ethanol (g/L)} \times 100}{\text{Solid (g/L)} \times \text{Percentage}_{(\text{Glucan})} \times 0.568}$$

Ethanol (g/L) is the concentration of ethanol in fermentation broth, Solid (g/L) is the solid loadings, $\text{Percentage}_{(\text{Glucan})}$ is the percentage of glucan in the pretreated SCB, and the factor of 0.568 is the theoretical factor for the conversion of cellulose to ethanol.²²

3 | RESULTS AND DISCUSSION

3.1 | Alkali pretreatment of SCB

Pretreatment is one important step for production of ethanol from lignocellulosic materials and has a significant effect on the process cost by concluding the extent of sugar recovery, fermentation

toxicity, enzymatic hydrolysis rates, enzyme loading, and other process variables.²³ In the present work, dry SCB was pretreated with 1.8% (w/w) NaOH at the S/L ratio of 1:6, and both untreated SCB and pretreated SCB were assayed. The concentrations of glucan, xylan, and lignin in the raw material were $41.95 \pm 0.51\%$, $21.7 \pm 0.12\%$, and $24.11 \pm 1.21\%$. After alkali pre-treatment, the glucan and xylan concentrations increased to $61.01 \pm 1.31\%$ and $26.94 \pm 0.68\%$, respectively. Simultaneously, the lignin concentrations decreased obviously from $24.11 \pm 1.21\%$ to $8.95 \pm 0.33\%$ after structural disruption caused by NaOH. After the swelling of NaOH and the removal of lignin, the compact structure in raw SCB was broken severely, and some small fragments with porous morphology were formed. All these properties

should positively affect the interaction between the carbohydrates and enzyme and increase fermentation efficiency.²⁴ Many studies involving alkali pretreatment of SCB have been reported. Some researchers²⁵ pretreated SCB at 80°C for 120 minutes at an S/L ratio of 1:10 and produced substrates with glucan, hemicellulose, and lignin concentrations of $63.80 \pm 2.19\%$, $24.30 \pm 0.82\%$, and $7.80 \pm 0.79\%$. This approach may increase the consumption of water and energy and also the cost of equipment dealing with equivalent biomass materials. A pretreatment performed at high solids loading might be more efficient (than that at low solids loading), since a greater amount of biomass available in the reaction would produce higher sugar concentrations and thus ethanol concentrations.²⁶

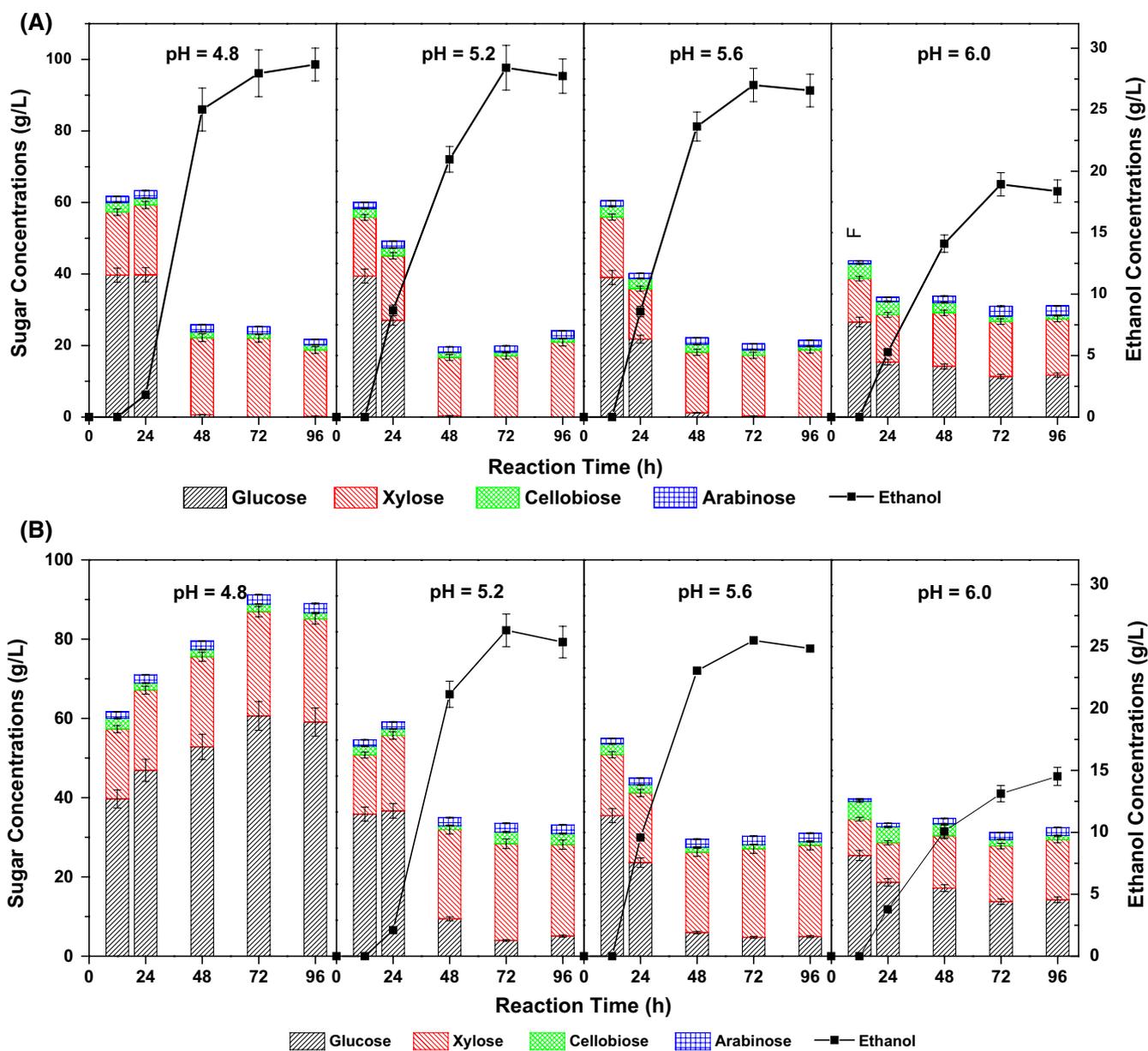


FIGURE 1 Effects of pH variation on the ethanol and sugar concentrations during the simultaneous saccharification and fermentation (SSF) process with *Saccharomyces cerevisiae* Y-2034; A, the SSF process with *S. cerevisiae* Y-2034, B, the SSF process with *Kluyveromyces marxianus* NCYC-587

3.2 | Batch experiments with SSF

3.2.1 | The effect of pH on the SSF process

Four initial pH values were chosen to determine the optimal pH of the SSF process with *S. cerevisiae* Y-2034 or *K. marxianus* NCYC-587. As presented in Figure 1, the ethanol concentration varied depending on the initial pH value. When *K. marxianus* NCYC-587 was added into the system at initial pH 4.8, no ethanol was detected. It may be due to that a lower pH value was harmful to the yeast and blocked the metabolic activity.^{18,19} Then, with the initial pH increasing, the ethanol

concentration decreased, and the maximum ethanol concentration of 26.29 ± 0.31 g/L was obtained in the system at pH 5.2 at the time point of 72 hours with productivity of 0.37 g/(L h). No obvious difference in terms of ethanol production was observed between the systems at pH 5.2 and 5.6. The enzymatic hydrolysis process was severely hindered with the increase in pH, and thus ethanol concentration produced was the lowest at pH 6.0.

Saccharomyces cerevisiae Y-2034 was added into the system at initial pH 4.8, it had a short lag phase. Less than 10 g/L ethanol was produced and sugar concentration had a small increase at 24 hours. When pH was 5.2, the highest

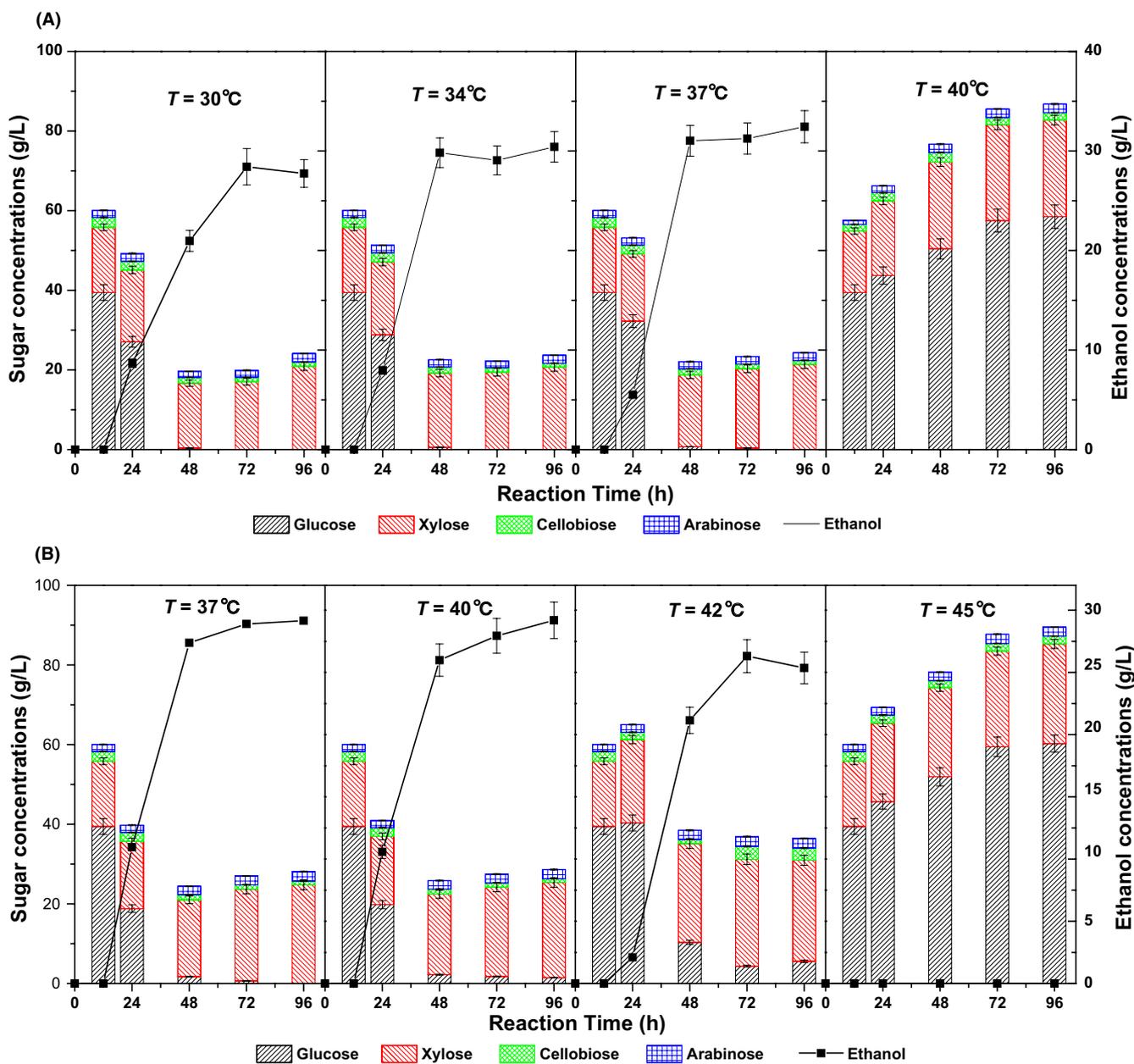


FIGURE 2 Effects of temperature variation on the ethanol and sugar concentrations during the simultaneous saccharification and fermentation (SSF) process; A, the SSF process with *Saccharomyces cerevisiae* Y-2034, B, the SSF process with *Kluyveromyces marxianus* NCYC-587

ethanol concentration of 28.41 ± 1.82 g/L corresponding to a productivity of 0.39 ± 0.02 g/(L h) was detected for *S. cerevisiae* Y-2034 after 72 hours of SSF. When the initial pH increased to 6.0, the glucose concentration of enzymatic hydrolysis produced after 12 hours was the lowest, which meant that the enzymatic hydrolysis process was restrained because the enzymatic activity was affected by high pH. The concentration of glucose was detected to be >10 g/L in the slurry after 72 hours, indicating that the fermentation process was blocked. At initial pH of 6.0, the maximum ethanol concentration for *K. marxianus* NCYC-587 was only 13.12 ± 0.66 g/L corresponding to a productivity of 0.18 ± 0.01 g/(L h) and for *S. cerevisiae* Y-2034, the maximum ethanol concentration was 18.93 ± 0.95 g/L corresponding to a productivity of 0.26 ± 0.01 g/(L h). Therefore, pH 5.2 was chosen as optimal for the SSF process with either *S. cerevisiae* Y-2034 or *K. marxianus* NCYC-587.

3.2.2 | The effect of temperature on the SSF process

Effects of temperature variation on the ethanol and sugar concentrations during the SSF process with *S. cerevisiae* Y-2034 were depicted in Figure 2A. After prehydrolysis for 12 hours at 50°C, the rate of hydrolysis was high, and nearly 40 g/L glucose was released. *S. cerevisiae* Y-2034 inocula were added to the hydrolysates, and system temperatures were adjusted to 30, 34, 37, or 40°C. As for the former three temperature conditions, the fermentation rates could be faster than the rate of enzymatic hydrolysis. The glucose concentration sharply decreased as glucose was converted to ethanol. The glucose build-up was small and vanished after 36 hours. With the process temperature rising from 30 to 37°C, the highest ethanol concentration increased from 28.41 ± 1.82 to 31.25 ± 1.56 g/L after 72 hours of the SSF process. At 37°C, due to the higher temperature stress on yeast, it needed more time to adapt to the environment and yield the lowest ethanol concentration between hours 12 and 24. In contrast, the increase in temperature enhanced the enzymatic activity, which may have promoted enzymatic hydrolysis and eventually yielded the highest ethanol concentration. Therefore, 37°C has been chosen as the optimal temperature of the SSF process with *S. cerevisiae* Y-2034. Temperature at 40°C has been tested during the fermentation of *S. cerevisiae* Y-2034 and no ethanol was yielded owing to the limited thermotolerance of this strain.

Effects of temperature variation on the ethanol and glucose concentrations during the SSF process with *K. marxianus* NCYC-587 are presented in Figure 2B. After prehydrolysis for 12 hours at 50°C, *K. marxianus* NCYC-587 inocula were added to hydrolysates, and system temperatures were

adjusted to 37, 40, 42, or 45°C, respectively. It was found that ethanol concentrations of the SSF processes showed no big difference between 37 and 40°C, which yielded the highest concentration of approximately 29.20 ± 1.46 g/L at 96 hours. According to our previous experiment, *K. marxianus* shows an excellent ethanol yield when utilising pure glucose at 50 g/L as the sole carbon source at 42°C. In contrast, with the process temperature rising from 37 to 42°C, the remaining glucose in three fermenting liquors increased from 0 to 5.58 ± 0.28 g/L, which meant that the fermentation was the rate-limiting step in this SSF process. This result might be related to the death of yeast under high temperature.²⁶ In addition, 45°C was tested as fermentation temperature of *K. marxianus* and yielded no ethanol owing to the limited thermotolerance of this strain.

Moreover, high-concentration ethanol as the end-product severely blocked the glucose transport and metabolic system and thus suppressed the downstream metabolic pathway.²⁸ The ethanol tolerance of strains should be taken into consideration to reach high-solids loading and ethanol concentration in fed-batch SSF experiments. The influence of the initial ethanol concentration on the growth of *S. cerevisiae* Y-2034 and *K. marxianus* NCYC-587 was studied next. The cell growth was remarkably decreased with the exogenous ethanol concentration increasing, especially for *K. marxianus* NCYC-587, whose tolerable ethanol concentration was below 31.57 g/L, while the cell growth of *S. cerevisiae* Y-2034 could still proceed at the ethanol concentration of over 63.15 g/L. According to all the above experiments, the performance of *S. cerevisiae* Y-2034 was superior to that of *K. marxianus* NCYC-587, and thus *S. cerevisiae* Y-2034 was chosen as the fermenting strain for the subsequent fed-batch SSF experiments.

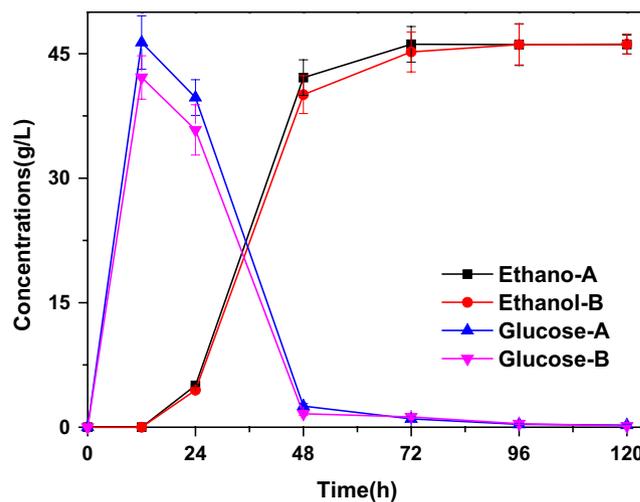


FIGURE 3 Profiles of ethanol and glucose concentrations during fed-batch simultaneous saccharification and fermentation in different enzyme addition modes

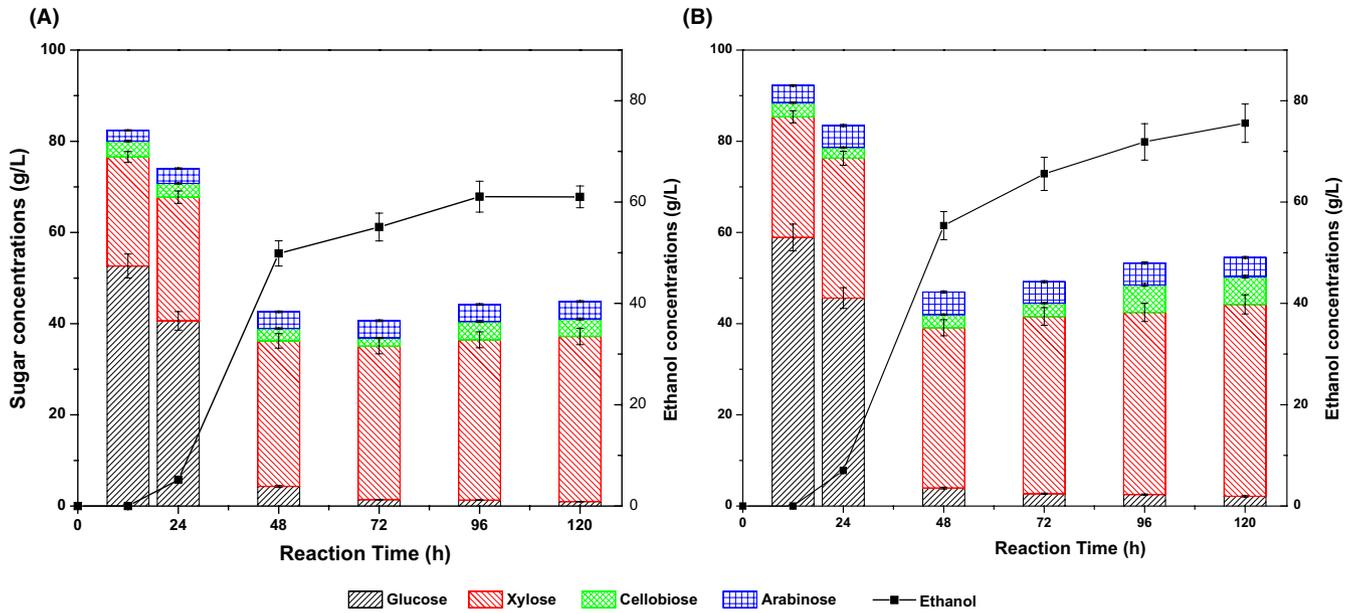


FIGURE 4 The profiles of ethanol and sugar concentrations during fed-batch simultaneous saccharification and fermentation processes; A, the solids loading of 26% (w/v), B, the solids loading of 33% (w/v)

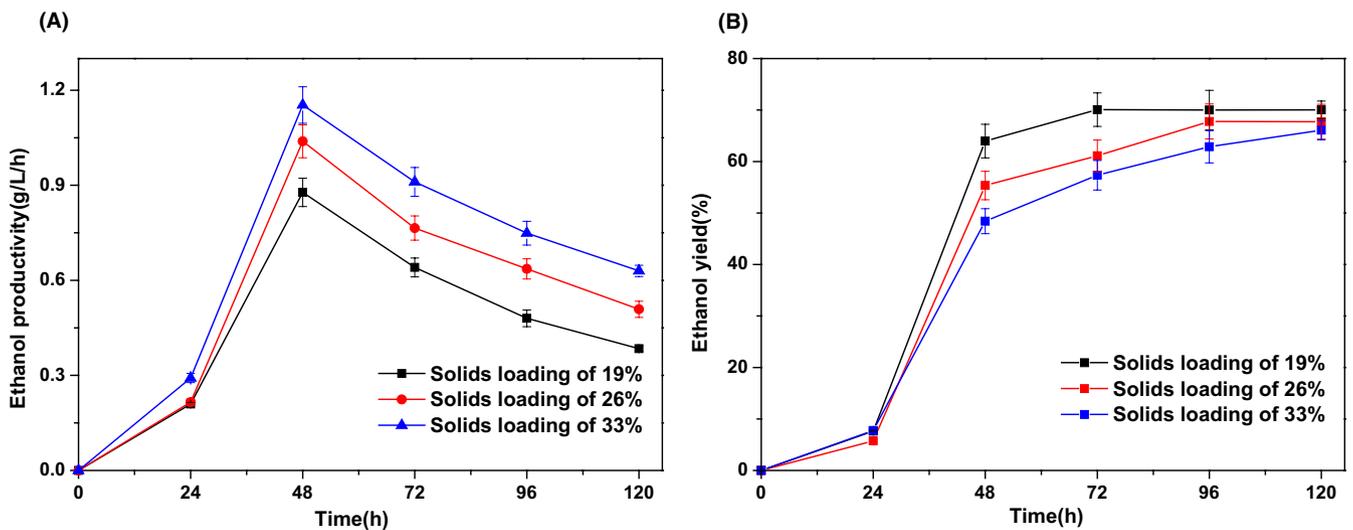


FIGURE 5 Effects of solids loading levels on the ethanol productivity and yield during the fed-batch simultaneous saccharification and fermentation process

3.3 | Fed-batch SSF experiments

3.3.1 | The effect of enzyme addition mode on ethanol production

The effect of enzyme addition mode on the ethanol production was presented in Figure 3. For mode “A”, all the necessary enzymes were dosed at the beginning. This approach accelerated the enzymatic hydrolysis of SCB owing to the existence of more cellulase. The amount of insoluble solids and the viscosity of slurry decreased.

Mixing and mass transfer were facilitated. Much more sugars were released in process A than in process B during the first 12 hours. After *S. cerevisiae* Y-2034 was introduced, more ethanol was produced in process A than process B until the final stage of 72 hours. It was demonstrated that the mode of enzyme addition “one-time dose” improved ethanol productivity better than the “batch addition” mode. Considering the result above and the simplified procedures of process A, all the cellulase was completely fed at the beginning in the subsequent experiments.

3.3.2 | Ethanol production at high solids loading

In the previous study,^{e29} an optimised fed-batch mode was determined in an enzymatic hydrolysis system. All the feeding steps can be completed within only 24 hours at the final solids loading of 33% (w/v). The highest concentrations of cellobiose, glucose, and xylose obtained by that enzymatic hydrolysis system were 9.376, 129.50, and 56.03 g/L. These preferable conditions of substrate addition, including the addition interval and added amount during fed-batch saccharification and the aforementioned optimal conditions of SSF (including pH, temperature, and the yeast strain) were referred to in order to accomplish high solids loading during SSF.

The variations of ethanol and sugar concentrations during fed-batch SSF processes at solids loading 26% and 33% (w/v) are depicted in Figure 4. After prehydrolysis for 12 hours, *S. cerevisiae* Y-2034 was added to system “a” and to system “b”. During the initial fermentation phase of 12 hours, ethanol concentration was below 10 g/L, together with low consumption of glucose. After that, significant increases of approximately 50 g/L emerged during the next 24 hours in the two groups. Because glucose, which was accumulated in the prehydrolysis stage, was running out and owing to a limited release of fresh monosaccharide, the increases in ethanol concentration became slow after 48 hours. As for group with the 26% loading (w/v), the maximum ethanol concentration of 61.07 ± 3.05 g/L was obtained at 96 hours, and nearly no further increase was observed after extension to 120 hours. As for the solids loading of 33% (w/v), the maximum ethanol concentration of 75.57 ± 2.14 g/L was obtained when the residue time was prolonged to 120 hours. With the prolongation, the ethanol concentration continued to increase, and a small quantity of glucose remained in the final liquor. This finding means that extra 24 or 48 hours may be beneficial for a further increase in the ethanol concentration. Park et al³⁰ utilised alkali-pretreated palm empty fruit bunches as a feedstock and obtained 62.5 g/L ethanol concentration at 30% solids loading. Li et al³¹ took phosphoric acid-acetone-pretreated reed as feedstock and attained 69.3 g/L ethanol concentration at 36% solids loading. Zhang and Zhu³² used SCB pretreated by Fenton reaction combined with NaOH as substrate, ethanol concentrations of 70.45 g/L were obtained in fed-batch mode at a solid loading of 30% (w/v). Compared with these reports at high solids loading, a higher ethanol concentration (75.57 ± 2.14 g/L) was implemented in this study. From the standpoint of economically viable ethanol production from lignocellulosic biomass on an industrial scale, these values meet the requirements, and the fed-batch SSF process performed at high-solids loading showed satisfactory performance.

To identify the effect of solids loading on ethanol production, fermentation differences among three levels of solids loading in fields of the ethanol productivity and yield were presented in Figure 5. Throughout the fed-batch SSF process, the solids loading increased, and much more time was needed to achieve

maximum ethanol concentration. The biggest ethanol productivity for loadings 19% (w/v), 26% (w/v), and 33% (w/v) was achieved at 48 hours corresponding to 0.88 ± 0.04 , 1.04 ± 0.05 , and 1.15 ± 0.06 g/(L h). The ethanol productivity began to decrease with time. At the solids loading of 19% (w/v), the maximum ethanol concentration of 46.13 ± 2.14 g/L was obtained in 72 hours, which corresponded to ethanol productivity of 0.64 ± 0.03 g/(L h). At the solids loading of 26% (w/v), the maximum ethanol concentration of 61.07 ± 3.05 g/L was obtained in 96 hours, which corresponded to ethanol productivity of 0.64 ± 0.03 g/(L h). At the solids loading of 33% (w/v), the maximum ethanol concentration of 75.57 ± 2.14 g/L was achieved in 120 hours, which corresponded to ethanol productivity of 0.63 ± 0.03 g/(L h). During the SSF process, ethanol yields for the three levels of solids loading all gradually decreased. At the final stage of 120 hours, ethanol yields reached $70.06 \pm 3.25\%$, $67.78 \pm 3.39\%$, and $66.08 \pm 1.87\%$. No obvious differences were observed among the three levels of solids loading in fields of the final ethanol productivity and yield. It was demonstrated that this fed-batch SSF condition was effective at increasing ethanol concentration with a small decrease in productivity (from 0.64 ± 0.03 g/[L h] to 0.63 ± 0.03 g/[L h]) and yield (from $70.06 \pm 3.25\%$ to $66.08 \pm 1.87\%$).

4 | CONCLUSIONS

Compared to *K. marxianus* NCYC-587, *S. cerevisiae* Y-2034 had higher fermenting ability and ethanol tolerance, which was beneficial to be applied in high concentration ethanol production with SSF process. Alkali pretreatment was identified as an efficient method to increase SCB digestibility at high-solids loading. Throughout the fed-batch process, solids loading could be efficiently increased, and the maximum ethanol concentration increased remarkably to 75.57 g/L. It can be concluded that a combination of high-solids NaOH pretreatment and a fed-batch SSF process was effective at achieving a high ethanol concentration as well as high ethanol productivity. This study could provide useful information for ethanol production at high solids loading from lignocellulosic materials of the theoretical yield.

ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (21808141), Natural Science Foundation of Jiangsu Province for Youth Science Funds (BK20160150), the Natural Science Foundation of Shanghai City (16ZR1417400), and the Fundamental Research Funds for the Central Universities (17X100040069).

ORCID

Yueshu Gao  <http://orcid.org/0000-0001-5885-3311>

REFERENCES

- Saini JK, Saini R, Tewari L. Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. *3 Biotech*. 2015;5(4):337-353.
- Menegol D, Fontana RC, Pinheiro Dillon AJ, Camassola M. Second-generation ethanol production from elephant grass at high total solids. *Biores Technol*. 2016;211:280-290.
- Bhutto AW, Harijan K, Qureshi K, Bazmi AA, Bahadori A. Perspectives for the production of ethanol from lignocellulosic feedstock – a case study. *J Clean Prod*. 2015;95(6):184-193.
- Gao Y, Xu J, Zhang Y, et al. Effects of different pretreatment methods on chemical composition of sugarcane bagasse and enzymatic hydrolysis[J]. *Bioresource Technology*. 2013;144(144C):396-400.
- Modenbach AA, Nokes SE. Enzymatic hydrolysis of biomass at high-solids loadings – a review. *Biomass Bioenerg*. 2013;56:526-544.
- Qureshi AS, Zhang J, Bao J. High ethanol fermentation performance of the dry dilute acid pretreated corn stover by an evolutionarily adapted *Saccharomyces cerevisiae* strain. *Biores Technol*. 2015;189:399-404.
- Ye G, Zeng D, Zhang S, Fan M, Zhang H, Xie J. Ethanol production from mixtures of sugarcane bagasse and dioscorea composita, extracted residue with high solid loading. *Biores Technol*. 2018;257:23-29.
- Modenbach AA, Nokes SE. The use of high-solids loadings in biomass pretreatment – a review. *Biotechnol Bioeng*. 2012;109(6):1430-1442.
- Sewsynker-Sukai Y, Kana EBG. Simultaneous saccharification and bioethanol production from corn cobs: process optimization and kinetic studies. *Biores Technol*. 2018;262:32-41.
- Zhang M, Wang F, Su R, Qi W, He Z. Ethanol production from high dry matter corncob using fed-batch simultaneous saccharification and fermentation after combined pretreatment. *Biores Technol*. 2010;101(13):4959-4964.
- Limayem A, Ricke SC. Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects. *Prog Energy Combust Sci*. 2012;38:449-467.
- Gao YS, Xu JL, Yuan ZH, Yu Z, Liang CY, Liu YY. Ethanol production from high solids loading of alkali-pretreated sugarcane bagasse with an SSF process. *BioResources*. 2014;9(2):3466-3479.
- Suryawati L, Wilkins MR, Bellmer DD, Huhnke RL, Maness NO, Banat IM. Simultaneous saccharification and fermentation of Kanlow switchgrass pretreated by hydrothermolysis using *Kluyveromyces marxianus* IMB4. *Biotechnol Bioeng* 2008;101(5):894-902.
- Ballesteros I, Ballesteros M, Cabanas A, et al. Selection of thermotolerant yeasts for simultaneous saccharification and fermentation (SSF) of cellulose to ethanol. *Appl Biochem Biotechnol*. 1991;28-29(1):307-315.
- Liu Y, Xu J, Zhang Y, et al. Sequential bioethanol and biogas production from sugarcane bagasse based on high solids fed-batch SSF. *Energy* 2015;1:1199-1205.
- Koppram R, Olsson L. Combined substrate, enzyme and yeast feed in simultaneous saccharification and fermentation allow bioethanol production from pretreated spruce biomass at high solids loadings. *Biotechnol Biofuels*. 2014;7(1):1-9.
- Rastogi M, Shrivastava S. Recent advances in second generation bioethanol production: an insight to pretreatment, saccharification and fermentation processes. *Renew Sustain Energy Rev*. 2017;80:330-340.
- Zhang Y, Xu J-L, Yuan Z-H, Liu Y-Y, He M-C. Comparison of different pretreatment methods on sugarcane bagasse and fractal-like kinetics of enzymatic hydrolysis. *Adv New Renew Eng*. 2013;1(2):166-169.
- Zhang W, Lin Y, Zhang Q, Wang X, Wu D, Kong H. Optimisation of simultaneous saccharification and fermentation of wheat straw for ethanol production. *Fuel*. 2013;112:331-337.
- Ghost TK. Measurement of cellulase activities. *Pure Appl Chem*. 1987;59(2):257-268.
- Sluiter A, Hames B, Ruiz R, et al. *Laboratory Analytical Procedure (LAP): determination of Structural Carbohydrates and Lignin in Biomass*. Golden, CO: National Renewable Energy Laboratory; 2008. Report No.: NREL/TP-510-42618.
- Varga E, Klinke HB, Reczey K, Thomsen AB. High solid simultaneous saccharification and fermentation of wet oxidized corn stover to ethanol. *Biotechnol Bioeng*. 2004;88(5):567-574.
- Ding J-C, Xu G-C, Han R-Z, Ni Y. Biobutanol production from corn stover hydrolysate pretreated with recycled ionic liquid by *Clostridium saccharobutylicum* DSM 13864. *Biores Technol*. 2016;199:228-234.
- Alvira P, Tomas-Pejo E, Ballesteros M, Negro MJ. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Biores Technol*. 2010;101(13):4851-4861.
- Wang W, Wang Q, Tan X, et al. High conversion of sugarcane bagasse into monosaccharides based on sodium hydroxide pretreatment at low water consumption and wastewater generation. *Biores Technol*. 2016;218:1230-1236.
- Roche CM, Dibble CJ, Knutsen JS, Stickel JJ, Liberatore MW. Particle concentration and yield stress of biomass slurries during enzymatic hydrolysis at high-solids loadings. *Biotechnol Bioeng*. 2009;104(2):290-300.
- Lin YS, Lee WC, Duan KJ, Lin YH. Ethanol production by simultaneous saccharification and fermentation in rotary drum reactor using thermotolerant *Kluyveromyces marxianus*. *Appl Energy*. 2013;105(105):389-394.
- Mohapatra S, Mishra C, Behera SS, Thatoi H. Application of pretreatment, fermentation and molecular techniques for enhancing bioethanol production from grass biomass – a review. *Renew Sustain Energy Rev*. 2017;78:1007-1032.
- Gao Y, Xu J, Yuan Z, Zhang Y, Liu Y, Liang C. Optimization of fed-batch enzymatic hydrolysis from alkali-pretreated sugarcane bagasse for high-concentration sugar production. *Biores Technol*. 2014;167:41-45.
- Park I, Kim I, Kang K, et al. Cellulose ethanol production from waste newsprint by simultaneous saccharification and fermentation using *Saccharomyces cerevisiae* KNU5377. *Process Biochem*. 2010;45(4):487-492.
- Li H, Kim N-J, Jiang M, Kang JW, Chang HN. Simultaneous saccharification and fermentation of lignocellulosic residues pretreated with phosphoric acid–acetone for bioethanol production. *Biores Technol*. 2009;100(13):3245-3251.
- Zhang T, Zhu MJ. Enhanced bioethanol production by fed-batch simultaneous saccharification and co-fermentation at high solid loading of Fenton reaction and sodium hydroxide sequentially pretreated sugarcane bagasse. *Biores Technol* 2017;229:204-210.

How to cite this article: Gao Y, Xu J, Yuan Z, Jiang J, Zhang Z, Li C. Ethanol production from sugarcane bagasse by fed-batch simultaneous saccharification and fermentation at high solids loading. *Energy Sci Eng*. 2018;6:810–818. <https://doi.org/10.1002/ese3.257>