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Simultaneous saccharification and co-fermentation of dry diluted acid pretreated corn stover at high dry matter loading: Overcoming the inhibitors by non-tolerant yeast

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HIGHLIGHTS

- Efficient conversion of pretreated corn stover at high solid loading was achieved.
- Ethanol titer reached 47.2 g/L at 25% dry matter loading of pretreated corn stover.
- Inhibitors significantly reduced xylose utilization at high dry matter loading.
- The toxicity of inhibitors to yeast increased at high temperature.

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ABSTRACT

Dry dilute acid pretreatment (DDAP) is a promising method for lignocellulose bioconversion, although inhibitors generated during the pretreatment impede the fermentation severely. We developed the simultaneous saccharification and co-fermentation (SScF) of DDAP pretreated biomass at high solid loading using xylose fermenting *Saccharomyces cerevisiae*, SyBE005. Effect of temperature on SScF showed that ethanol yield at 34 °C was 10.2% higher than that at 38 °C. Ethanol concentration reached 29.5 g/L at 15% (w/w) dry matter loading, while SScF almost ceased at the beginning at 25% (w/w) dry matter loading of DDAP pretreated corn stover. According to the effect of the diluted hydrolysate on the fermentation of strain SyBE005, a fed-batch mode was developed for the SScF of DDAP pretreated corn stover with 25% dry matter loading without detoxification, and 40.0 g/L ethanol was achieved. In addition, high yeast inoculation improved xylose utilization and the final ethanol concentration reached 47.2 g/L.

1. Introduction

Bioethanol from lignocelluloses is a promising renewable energy as a substitute of fossil energy (Somerville and Youngs, 2014). The economic cellulosic ethanol production is related to several key technological issues, such as efficient pretreatment, water saving, efficient utilization of feedstock, fermentation with high ethanol concentration (Konda et al., 2014; Unrean and Srienc, 2010; Madhavan et al., 2012). Recently, a new pretreatment method, named 'dry dilute acid pretreatment (DDAP)', was developed based on the dilute-acid pretreatment (Zhang et al., 2011). In addition to the inexpensive sulfuric acid as the catalyst, the water saving was achieved due to the dry process (He et al., 2014a,b). Therefore, it will be promising for economic cellulosic ethanol production to develop an efficient approach to convert DDAP pretreated biomass.

Due to the severe conditions during the pretreatment process, various lignocellulose derived toxins, including furan derivatives, organic acids and lignin derivatives, are generated. These toxins severely inhibit the enzymatic hydrolysis and ethanol fermentation (Zhang et al., 2011). High dry matter (DM) loading fermentation is necessary to get high ethanol titers and thus decrease the cost of the distillation process (Koppram et al., 2014). However,

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high dry matter (DM) loading means higher concentration of inhibitors, which results in stronger inhibition to yeast. Therefore, a detoxification step to remove the toxins for the subsequent hydrolysis and fermentation is necessary, such as washing, chemical detoxification or biological detoxification (Qin et al., 2013). However, the detoxification prior to fermentation would increase the cost of bioethanol production. Washing increases the waste of water, while the chemical or biological detoxification increased the operation unit or process time. Another efficient approach to overcome the inhibitors is to construct tolerant strains to ferment the hydrolysates with inhibitors directly (Harner et al., 2015). However, the complex mechanisms of the tolerance to inhibitors and the variation of inhibitors according to different pretreatments impeded the strain engineering for tolerance, especially for the tolerance for inhibitor mixture. Therefore, the process design to overcome the inhibitors is very important for lignocellulose bioconversion.

Simultaneous saccharification and co-fermentation (SScF) has been proven efficient to convert the pretreated biomass with hemicellulose and cellulose (Koppram et al., 2013). Glucose and xylose is released and removed simultaneously during SScF, and the inhibition of monosaccharide to cellulases and xylanases would be reduced. However, a compromise between the optimal temperatures for the cellulolytic enzymes and yeast *Saccharomyces cerevisiae* growth is necessary in the SScF process, because the optimal temperature for yeast growth is around 30 °C while the optimal temperature for the cellulolytic enzymes is around 50 °C (Olofsson et al., 2008). Xylose utilization is another issue to be solved in the SScF process. Researchers have proposed many strategies, such as pre-fermentation (Bertilsson et al., 2009), enzyme feeding or both enzyme and substrate feeding (Olofsson et al., 2010), to improve xylose consumption. Fed-batch strategy and high yeast loading were applied to improve xylose utilization in the present study.

To take advantage of the DDAP method, we investigated the effect of temperature and DM loading on SScF of DDAP pretreated corn stover, and the effect of the inhibitors on xylose and glucose utilization was also analyzed. Finally, we performed a fed-batch SScF to convert DDAP pretreated corn stover efficiently (Fig. 1).

2. Methods

2.1. Materials

The corn stover used in this study was grown and harvested in Henan Province, China. It was washed and dried at 105 °C until the weight was constant. Then it was milled coarsely using a beater pulverizer (SF-300, Ketai Milling Equipment, Shanghai, China) and screened through a mesh with the circle diameter of 10 mm and then stored in sealed plastic bags until use.

Accellerase 1500 (Genencor, 89 mg/mL, 77 FPU/mL) and Novozyme 188 (Sigma–Aldrich, 67 mg/mL, 850 CBU/mL) were used in this study (Qin et al., 2013). The activity of enzymes is determined according to LAP method (NREL, 2008). Ampicillin with finial concentration of 50 mg/L was used to prevent bacterial contamination.

2.2. Pretreatment of corn stover

Pretreatment reactor and process were described in detail previously (He et al., 2014a,b). Corn stover in this study was pretreated for 3 min at 170 °C using 2.5% sulfuric acid at 50 rpm agitation rate. The compositions of the pretreated corn stover (dry basis) were determined following the Laboratory Analytical Procedure (LAP) of the National Renewable Energy Laboratory (NREL, 2008).

2.3. Simultaneous saccharification and co-fermentation

Microorganism used in this study was an engineered *S. cerevisiae* SyBE005 with genetically constructed xylose utilizing pathway (Zha et al., 2014). Seed culture preparation and inoculation process were described in detail by Zhu et al. (2014). Pretreated corn stover did not go through any detoxification process before the SScF process. During the SScF process, no any other extra organic nutrients and inorganic salt were added to the fermentation slurry. The flasks were sealed by a rubber stopper with a syringe needle inside in order to release the carbon dioxide produced during fermentation. All SScF experiments were carried out in duplicates under anaerobic conditions using shaking flasks in an orbital incubator at 150 rpm. The pre-hydrolysis process was conducted at 50 °C and 200 rpm. Three temperatures (30 °C, 34 °C and 38 °C) were used to investigate the effect of temperature on SScF. Then 34 °C was used at the other SScF process. Pretreated corn stover was diluted to the wanted solid loading with distilled water and 3 M KOH were used to adjust the pH to 5.5. Unless otherwise specified, the enzyme loading used in SScF process was 15 FPU/g dry matter Accellerase 1500, 30 CBU/g dry matter Novozyme 188.

Unless otherwise specified, the inoculum size for SScF experiments was 2.5 g/L. For SScF process with yeast feeding, yeast was inoculated in three parts at 0 h (1 g/L), 24 h (1 g/L) and 48 h (0.5 g/L), but the total inoculum size was 2.5 g/L.

In SScF experiments of 'Effect of high yeast inoculum size on SScF' part, the inoculum size was 7.5 g/L for batch SScF and fed-batch SScF with or without yeast feeding. In the same part, the inoculum size was 2.5 g/L at 0 h and5.0 g/L at 96 h for fed-batch SScF with yeast feeding.

For SScF process with pre-fermentation, yeast was inoculated into the fermentation broth before the addition of enzyme. Pre-fermentation was performed at 34 °C for 8 h, then the enzyme was added.

For fermentation of diluted enzymatic hydrolysate at 25% (w/w) DM loading, enzymatic hydrolysis of pretreated corn stover were performed at 50 °C and 200 rpm for 72 h. Then the hydrolysis mixtures were centrifuged at 4000 rpm for 10 min. The supernatant was diluted with sterile water to the desired times. The concentration of glucose and xylose at the beginning of fermentation was adjusted to the same as the undiluted enzymatic hydrolysate.

For fed-batch SScF process, the DDAP pretreated corn stover and enzymes were split into five equal parts to add into the fermentation broth. DDAP pretreated corn stover of 5% (w/w) DM loading and 1/5 enzyme loading were added in pre-hydrolysis for 12 h, and then yeast was inoculated to start the fermentation. Rest of the DDAP pretreated corn stover and enzymes were added into fermentation broth at 0 h, 24 h, 36 h and 72 h.

2.4. Analysis of sugars, ethanol and cell viability

The samples took from SScF process were centrifuged at 12,000 rpm for 5 min. Before analysis, the supernatant was filtered with a 0.22 μm filter to remove impurities. Ethanol, glucose and xylose in the sample were analyzed using HPLC with a Aminex HP-87H column (Bio-Rad, Hercules, CA, USA) at 65 °C, using 5 mM H_2SO_4 as eluent, at a flow rate of 0.6 mL/min (Qin et al., 2012).

In order to better understand the SScF process, the strain viability was measured. However, since it is impossible to measure OD_{600} with residual solids during SScF process, colony forming unit (CFU) was measured to determine the viability of strains. Fermentation samples were collected and then diluted to the wanted rate

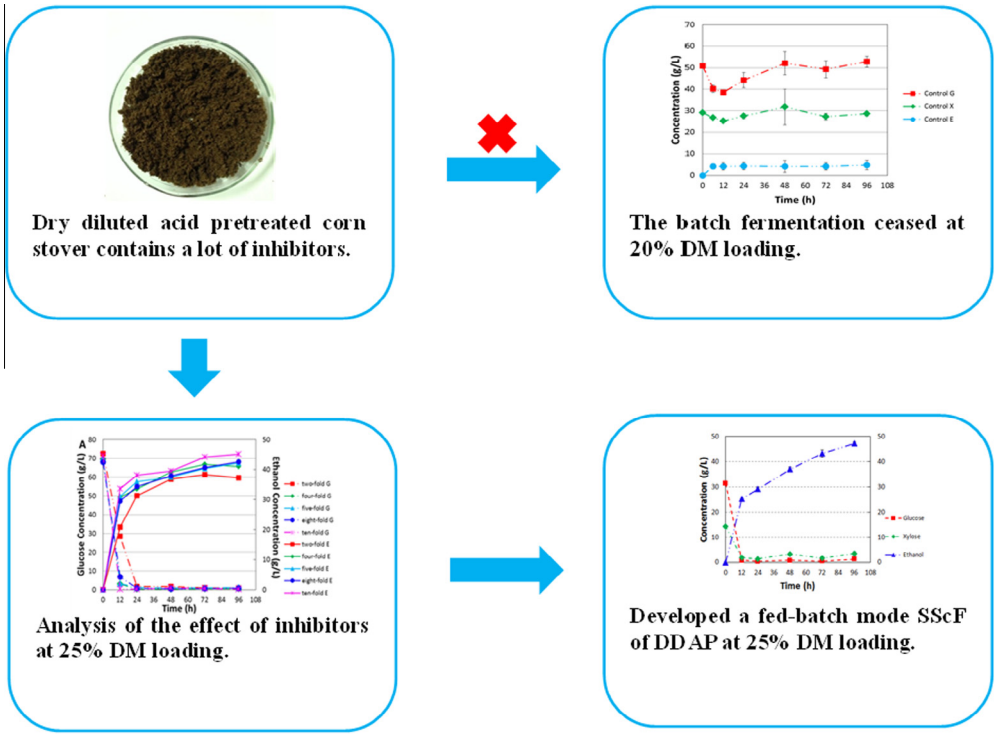

Fig. 1. A schematic diagram of the development of fed-batch SScF of DDAP pretreated corn stover.

Table 1
The compositions of dry matter fraction of DDAP pretreated corn stover (the moisture of DDAP pretreated corn stover is 53.70%).

Insoluble fraction[a]		Soluble fraction[a]			
Glucan	32.20 ± 0.20%	Glucose	1.00 ± 0.01%	Acetate[b]	2.56 ± 0.15
Xylan	3.30 ± 0.05%	Xylose	14.10 ± 0.08%	Furfural[b]	0.30 ± 0.04
Lignin	21.80 ± 0.38%	Oligo-glucose	1.00 ± 0.53%	5-HMF[b]	0.22 ± 0.03
		Oligo-xylose	3.00 ± 0.81%		

[a] Based on /g dry matter fraction of DDAP pretreated corn stover.
[b] The unit is g/100 g dry matter.

in order to obtain 20–200 colonies on a single plate using sterile water. The plates were incubated at 30 °C for 48 h. The strain viability was detected on a YPX agar medium (samples at 0 h, 24 h and 48 h) (per liter: 10 g yeast extract, 20 g peptone, 20 g xylose, and 20 g agar) and a YPD agar medium (samples at 72 h and 96 h) (per liter: 10 g yeast extract, 20 g peptone, 20 g dextrose, and 20 g agar). Then single colonies formed on the plates were counted and cellular viability was calculated accordingly.

2.5. Ethanol yield calculation

In order to facilitate the calculation, content of glucan and xylan in the pretreated corn stover and solid residue were converted into the content of glucose and xylose. The theoretical amount of glucose released during SSCF is 1.11 times the amount of glucan and 1.13 times the amount of xylan in the solid material, respectively. Ethanol yield was calculated as follows:

$$\text{Ethanol yield } (\%) = \text{ethanol in SScF}/0.51 * (\text{glucan in DDAP-CS} * 1.11 + \text{xylan in DDAP-CS} * 1.13) * 100\%$$

DDAP-CS: Dry dilute acid pretreated corn stover.

3. Results and discussion

Dilute sulfuric acid is used as the catalyst in the dry diluted acid pretreatment (DDAP), and most of xylan were degraded to xylose during pretreatment. Due to the degradation of glucose and xylose, furfural and 5-HMF were generated at high concentrations (Table 1). In addition, acetate was also in a high concentration because of the hydrolysis of the acetyl of the biomass. All the compositions are mixed in the pretreated biomass, which means that the following fermentation would be challenged by the sugar mixture and the inhibitors. Simultaneous saccharification and co-fermentation (SScF) using a xylose utilizing yeast SyBE005 was performed to convert DDAP pretreated corn stover without washing or other pre-detoxification.

3.1. Effect of temperature on SScF using SyBE005

A compromise between the optimal temperatures for the cellulolytic enzymes and yeast *S. cerevisiae* growth is applied in the SScF process, because the optimal temperature for yeast growth is around 30 °C while the optimal temperature for the cellulolytic enzymes is around 50 °C (Olofsson et al., 2008). The optimal temperature for SScF of pretreated corn stover without inhibitors using *S. cerevisiae* SyBE005, a xylose-utilizing yeast, was about 38 °C

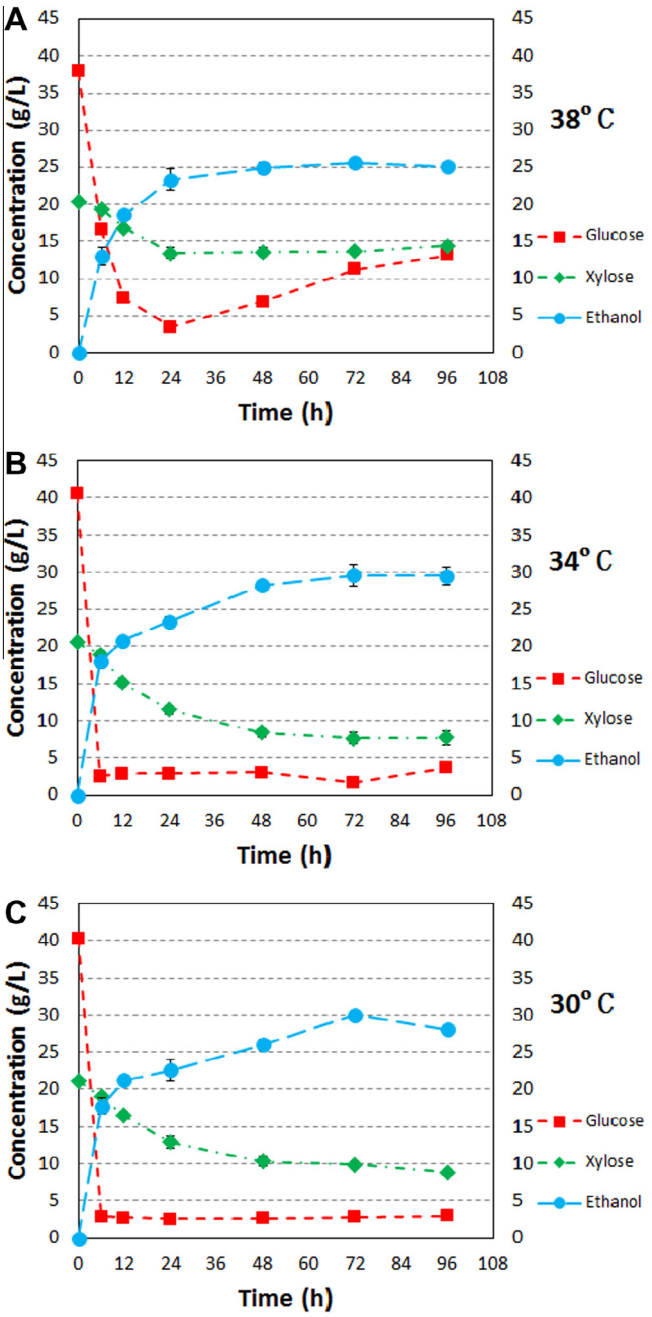

Fig. 2. The effects of temperature on the inhibitor tolerance of *S. cerevisiae* SyBE005. A: SScF at 38 °C; B: SScF at 34 °C; C: SScF at 30 °C. The fermentation conditions include: inoculation size 2.5 g/L, 12 h pre-hydrolysis, pH 5.5 and 15% (w/w) DM (dry matter) loading.

(Zhu et al., 2014). However, ethanol production is not efficient at 38 °C for SScF of DDAP pretreated corn stover. Although glucose was quickly consumed in the first 24 h, it accumulated to 13.2 g/L in the late fermentation phase (Fig. 2), indicating that the strain activity was very low and glucose release by enzymatic hydrolysis was faster than glucose consumption by strains. Xylose concentration almost maintained at 14.5 g/L from 24 h to the end of the fermentation. The final ethanol concentration at 38 °C was 25.1 g/L, and only 58.2% of ethanol yield was achieved. Such low fermentation performance should be due to the low viability of the strain, because the accumulation of xylose and glucose demonstrated the enough enzymatic activities for the system. According to the conclusion, we conducted the SScF at low temperatures (30 °C or 34 °C), and much better results confirmed the conclusion. At 30 °C and 34 °C, little glucose accumulation was observed and only 3.0 and 3.7 g/L glucose left in the broth after 96 h fermentation (Fig. 2B and C), respectively. Compared with the performance of SScF at 38 °C, the final ethanol concentration and yield increased by 2.9 g/L and 6.7% at 30 °C, and 4.4 g/L and 10.1% at 34 °C, respectively.

According to enzymatic activity, the monosaccharides are released less at 30 °C or 34 °C than that at 38 °C (Olofsson et al., 2008). However, the higher ethanol concentrations were achieved at lower temperatures. These results demonstrated the higher viability of SyBE005 at 30 °C or 34 °C than that at 38 °C during the SScF of DDAP pretreated corn stover. Our previous study showed little difference on the viability of SyBE005 at 30 °C or 34 °C or 38 °C (Zhu et al., 2014). Therefore, the low viability of SyBE005 at 38 °C was due to the synergistic effect of high temperature and the inhibitors in DDAP pretreated corn stover. Based on the findings, 34 °C was applied in the following SScF on DDAP pretreated corn stover.

3.2. Effect of dry matter loading on SScF of DDAP pretreated corn stover

In order to enhance the ethanol concentration in the final fermentation broth, high DM loading is required for the SScF. Besides 15% (w/w) DM loading applied on the above experiment, we tried the SScF of DDAP pretreated corn stover at 20% and 25% (w/w) DM loading. The fermentation ceased very early, and the final ethanol was less than 5 g/L. The high concentration of inhibitors from the high DM loading should be the main reason for the fermentation ceasing.

Pre-fermentation, the fermentation of initially available free glucose in the liquid before the addition of enzymes, was applied to reduce the competitive inhibition on xylose uptake by hexoses in fermentation broth (Bertilsson et al., 2009). Yeast feeding, which could degrade some inhibitors by the first adding yeast (Wang et al., 2013) and enhance cell viability (Koppram and Olsson, 2014), was also conducted to improve xylose consumption during SScF process. Therefore, pre-fermentation and strain feeding strategies were tried at 20% (w/w) DM loading (Fig. 3). However, neither pre-fermentation nor yeast feeding showed any improvement in SScF process at 15% (data not shown) or 20% (w/w) DM loading (Fig. 3). Pre-fermentation SScF process was conducted with no pre-hydrolysis in this study and the low glucose content

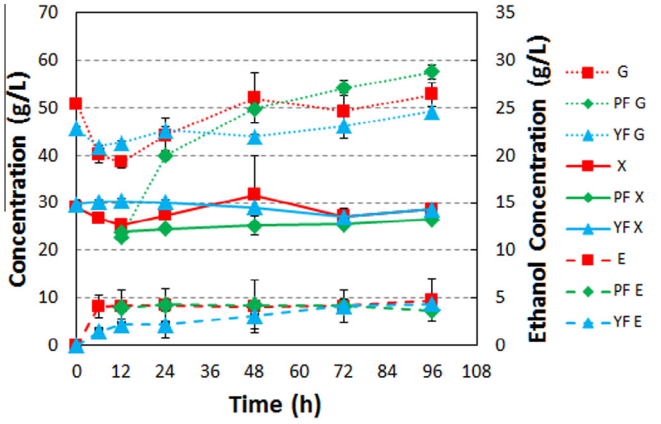

Fig. 3. SScF performance of *S. cerevisiae* SyBE005 with different strategies at 20% (w/w) DM loading. PF: SScF with pre-fermentation. YF: SScF with yeast feeding. G: glucose. X: xylose. E: ethanol. The fermentation conditions include: inoculation size 2.5 g/L, 12 h pre-hydrolysis, pH 5.5. SScF with pre-fermentation was conducted without pre-hydrolysis.

(1.5 g/L) at the initial stage of SScF was not enough for strains to maintain the growth and resist the inhibitors during the pre-fermentation. Yeast feeding strategy did not work in this study, high content inhibitors at high DM loading might be the main reason. Besides, the total inoculum size was split into three parts, and the low inoculum would also reduce the fermentation performance. The failure of yeast feeding strategy in SScF of DDAP pretreated corn stover indicated that the inoculum size played an important role in dealing with the inhibitors problem and increasing the inoculum size might improve SScF performance at high solid loading.

3.3. Effect of diluted enzymatic hydrolysates on sugar conversion

In order to investigate the effect of the inhibitors on sugar conversion of SyBE005, we designed the diluted enzymatic hydrolysates. Taking into consideration of the requirement of the high ethanol concentration (Koppram et al., 2014; Modenbach and Nokes, 2013), a high DM loading (25%, w/w) was applied in this experiment.

The glucose consumption rates in the initial 12 h of the fermentation using SyBE005 were 3.43, 5.77, 5.58, 5.44 and 5.82 g/L h when the concentration of inhibitors were diluted by twofold, fourfold, fivefold, eightfold and ten-fold from 25% DM loading, respectively. It indicated that glucose utilization was hampered even at the concentration of inhibitors diluted by twofold from 25% (w/w) DM loading, although the strain SyBE005 finished the glucose fermentation at 15% (w/w) DM loading (Fig. 2). The glucose consumption rates at different concentration of inhibitors indicated that the concentration of inhibitors at 6.25% DM loading would exhibit little inhibition.

When the concentration of inhibitors were diluted by twofold, fourfold, fivefold, eightfold and ten-fold, the final xylose concentration were 16.4, 10.0, 5.2, 5.5 and 0.6 g/L, respectively (Fig. 4). The clear tendency of the residual xylose concentration indicated that xylose utilization of SyBE005 was much more sensitive to the inhibitors, consistent with previous report on other xylose utilizing yeast (Wang et al., 2014). Therefore, the efficient utilization of xylose should be an indicator for the inhibitor concentration. In addition, compared with fermentation of twofold dilution, ethanol yield increased by 3.0%, 8.8%, 13.7% and 12.3% at fourfold, fivefold, eightfold and ten-fold dilution, respectively. The increase of ethanol yield mainly caused by the xylose utlilization. The effect of diluted hydrolysates on sugar utilization indicated that the biomass feeding at low DM loading might be a potential approach to overcome the inhibitor problem in SScF of DDAP pretreated corn stover at high DM loading.

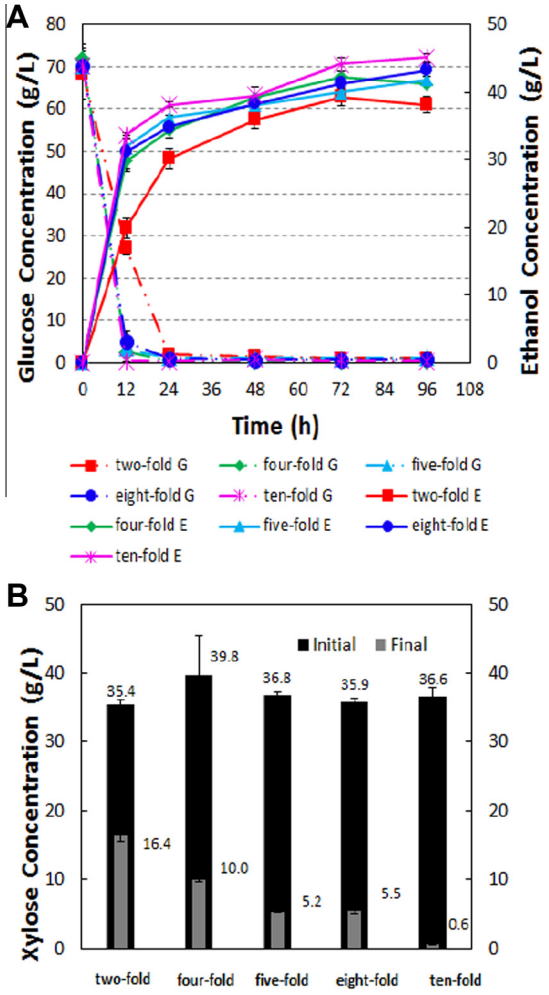

Fig. 4. The effect of diluted hydrolysates on glucose (A) and xylose (B) at 25% (w/w) DM loading. G: glucose. X: xylose. E: ethanol. Enzymatic hydrolysis of pretreated corn stover were performed at 50 °C and 200 rpm for 72 h. Then the hydrolysis mixtures were centrifuged and supernatant was diluted with sterile water to the desired times.

3.4. Fed-batch of DDAP pretreated corn stover improved SScF

Based on the effect of the diluted hydrolysate on sugar utilization, we performed the fed-batch SScF of DDAP pretreated corn stover using SyBE005, and the pretreated biomass and enzymes were split into five parts to add into the fermentation system. Glucose was utilized well at the initial stage of SScF, and xylose was partly utilized while the glucose concentration was low (Fig. 5). Along with the feeding of DDAP pretreated corn stover, the concentration of xylose started to increase at 20% (w/w) DM loading (at 36 h), and glucose started to accumulate after 48 h in the fed-batch SScF. However, although the sugar accumulation occurred after 72 h, the continuous increase of ethanol concentration indicated that the fermentation of the strain was still working at 20% (w/w) DM loading (after 36 h) or even 25% (w/w) DM loading (after 72 h). Compared with the batch SScF at 20% (w/w) DM loading in Fig. 3, these results suggested that fed-batch could effectively improve SScF process at high DM loading. Although the strain SyBE005 is not capable of detoxifying the hydrolysates from DDAP pretreated corn stover perfectly, we observed the *in situ* detoxification of furfural and HMF by SyBE005 (supplemental Figure). During the fed-batch process, part of the inhibitors were degraded at the low DM loading. When the feedstock was fed to high DM loading, the concentration of the degradable inhibitors was much lower than that of the hydrolysate at the same DM loading in batch SScF. Besides, the synergic effect of the different inhibitors was the important aspect of the toxicity of the inhibitors (Ding et al., 2011). Therefore, the decrease of one or two inhibitors would also reduce the toxicity of the mixed inhibitors.

The strain viability was monitored during the fed-batch SScF at 25% (w/w) DM loading. No increase of the strain viability was observed during the process (Fig. 5). The strain viability decreased significantly along with the feeding, i.e., the increase of DM loading. In the SSF of other pretreated corn stover, the strain viability also decreased after the maintenance for 24 h or even 48 h, and the viability decrease was mainly due to the lack of nutrients and the inhibition by the byproducts of the fermentation (Liu et al., 2014). However, the decrease of the strain viability in this study was mainly due to the high inhibitors caused by the high DM loading.

In order to decrease the sugar accumulation in the later stage of fed-batch SScF, yeast feeding was applied at three different stages,

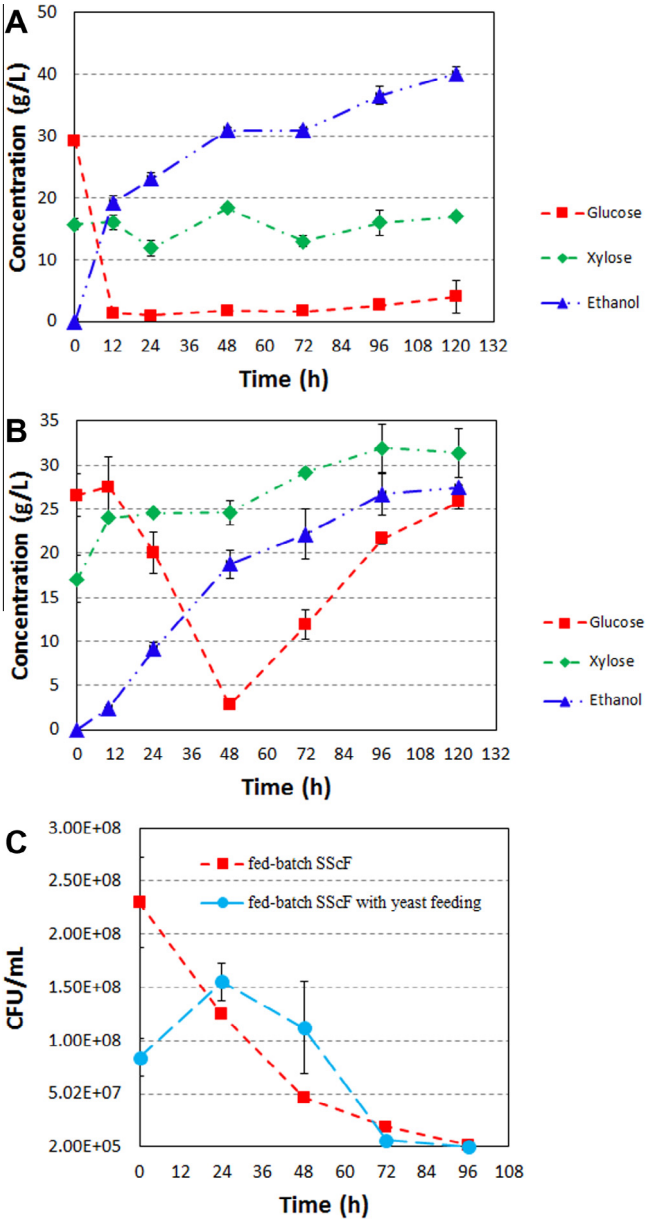

Fig. 5. The effect of fed batch of biomass and yeast feeding of SScF at 25% (w/w) DM loading. A: fermentation performance of SScF with fed batch of biomass. B: fermentation performance of fed-batch SScF with yeast feeding. C: cell viability during SScF with fed batch of biomass and yeast feeding.

i.e., the initial time, 24 h and 48 h. However, no fermentation improvement was observed for the yeast feeding process (Fig. 5). The initial glucose utilization was very slow, and due to the presence of glucose, the decrease of xylose concentration was not observed during the whole fermentation process. The finial concentration of ethanol was lower than that without yeast feeding, because less sugar was utilized during the SScF with yeast feeding. The strain viability assay showed that yeast feeding just increased the strain viability at a short period during the SScF (Fig. 5C). However, compared with SScF without yeast feeding, the higher strain viability during this period showed the worse performance in the SScF with yeast feeding. This may be caused by the fact on several aspects. The monitoring of the strain viability was conducted just after the yeast feeding, and the strain viability would be decreased significantly in a very short time due to the toxicity of the inhibitors. In addition, the later feeding strain died quickly due to the direct exposure to the higher inhibitor concentration caused by the DM loading increase, which was very similar to the ceasing SScF without fed-batch mode at high DM loading. Besides, yeast feeding strategy split the inoculum size into three parts, and the low inoculum would also reduce the fermentation performance. On another aspect, in SScF without yeast feeding, the strains adapted to the toxic hydrolysate step by step along with the fed-batch process. It was proved to be efficient to improve the fermentation performance by the adaptation (Wang et al., 2013; Yang et al., 2012; Lv et al., 2014).

To summary, in order to improve the performance of SScF at high DM loading, the key issues includes (1) bringing down the inhibitor concentration in the broth to start the fermentation, (2) reducing the toxicity of the inhibitors along with the fermentation, (3) keeping higher strain viability for a longer time.

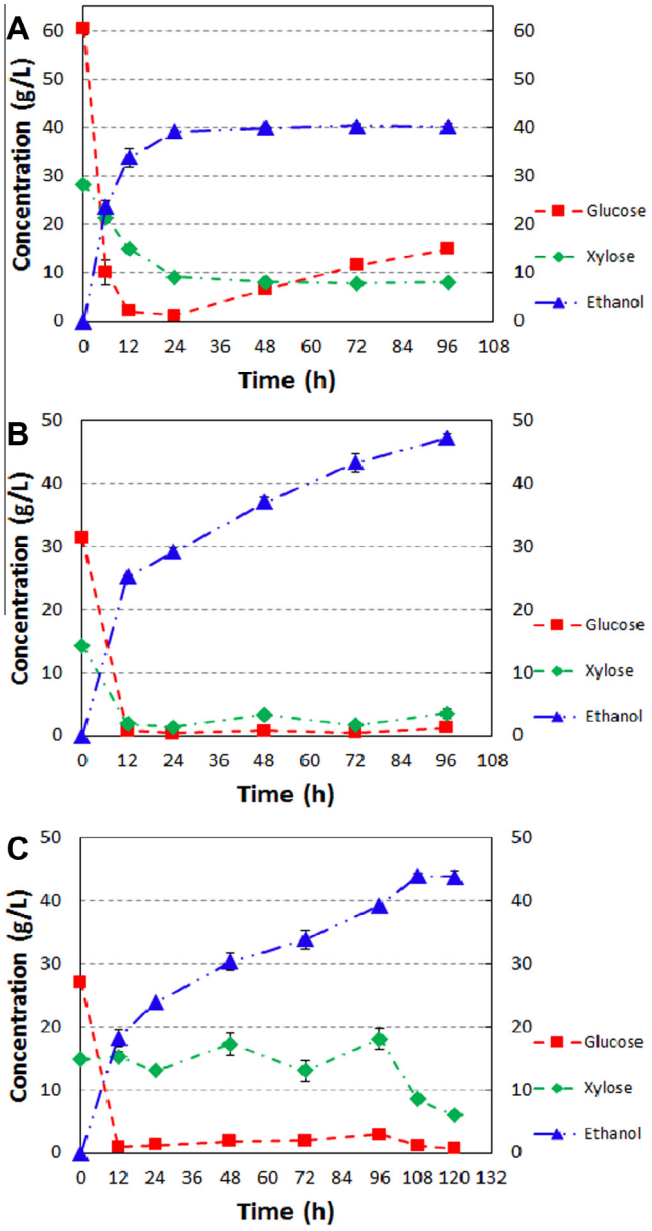

Fig. 6. The effect of high yeast inoculum size on SScF at 25% (w/w) DM loading. A: fermentation performance of batch SScF with 7.5 g/L yeast. B: fermentation performance of fed-batch SScF with 7.5 g/L yeast at 0 h. C: fermentation performance of fed-batch SScF with 2.5 g/L yeast at 0 h and 5.0 g/L yeast at 96 h.

Table 2
The performance of SScF process at 25% (w/w) DM loading.

	Inoculum size (g/L)	Glucose concentration[b] (g/L)	Xylose concentration[b] (g/L)	Ethanol titer[b] (g/L)	Ethanol yield[b] (%)
Fed-batch 1[a]	2.5	3.9 ± 2.6	17.0 ± 0.6	40.0 ± 1.1	55.5 ± 0.01
Fed-batch 2	2.5	25.8 ± 0.8	31.4 ± 2.7	27.5 ± 1.5	41.0 ± 0.12
Batch 3	7.5	14.9 ± 0.3	8.13 ± 0.1	40.3 ± 0.4	55.8 ± 0.01
Fed-batch 4	7.5	1.3 ± 0.1	3.5 ± 0.7	47.2 ± 0.8	65.5 ± 0.01
Fed-batch 5	7.5	1.0 ± 0.8	6.0 ± 0.1	43.8 ± 0.1	60.8 ± 0.01

[a] Fed-batch 1, 2, are corresponding to fed-batch SScF and fed-batch SScF with strain feeding with 2.5 g/L yeast, fed-batch 4, 5 are corresponding to fed-batch SScF and fed-batch SScF with strain feeding with 7.5 g/L yeast.
[b] Ethanol yield is calculated based on the total glucose and xylose.

Table 3
Comparison of ethanol production from pretreated corn stover at high solid loading.

Fermentation mode	Solid loading (%, w/w)	Detoxification	Ethanol titer (g/L)	Ethanol yield	Reference
SSF[a]	25%	Yes	55.68	79.46%	Qureshi et al. (2015)
SSF	30%	Yes	71.4	80.3%	Qureshi et al. (2015)
SSF	20%	No	–	5.7%	Varga et al. (2004)
SSF	20%	Yes	59.8	77.2%	Liu et al. (2014)
SHF[b]	30%	Yes	49.5	68.2%	Lu et al. (2010)
SHF	30%	No	<1	–	Lu et al. (2010)
SScF	25%	No	47.2	65.5%	This study

[a] SSF (simultaneous saccharification and fermentation) process does not include xylose fermentation.
[b] SHF (separate hydrolysis and fermentation) process does not include xylose fermentation.

3.5. Effect of high yeast inoculum size on SScF

Although glucose was utilized well in fed-batch SScF at 25% DM loading, the residual xylose was in a high concentration (17.0 g/L) after the fermentation. Increasing the yeast inoculum size could improve xylose utilization (Jin et al., 2010), and we applied a higher yeast loading (7.5 g/L) to further improve xylose utilization in the SScF of DDAP pretreated corn stover.

The final ethanol concentration of batch SScF with 7.5 g/L yeast at 25% DM loading reached 40.3 g/L (Fig. 6A), which was much higher than batch SScF with 2.5 g/L yeast at 25% DM loading (less than 5 g/L). Higher yeast inoculum size did minimize the inhibitors problem at high DM loading. However, glucose gradually accumulated to 14.9 g/L in the late fermentation phase, although it was quickly consumed in the first 24 h. These results indicated that the strain viability decreased quickly due to the high toxicity of inhibitors. Therefore, we applied the fed-batch mode to further relieve the inhibitors problem.

Compared with the performance of batch SScF with 7.5 g/L yeast inoculation, the final ethanol concentration and yield reached 47.2 g/L, 65.5% for fed-batch SScF and 43.8, 60.8% for fed-batch SScF with yeast feeding, respectively (Table 2). These results indicated that fed-batch mode was more effective in the fermentation process at high DM loading. For fed-batch SScF with 7.5 g/L yeast inoclulation, the final ethanol concentration and yield was 3.4 g/L and 4.7% higher than fed-batch SScF with yeast feeding. As shown in Fig. 6B, free glucose and xylose concentration was very low during the entire fermentation process in fed-batch SScF without yeast feeding. This low sugar concentration could relive the inhibition of sugars on cellulases and xylanases and improve the enzymatic hydrolysis of corn stover. These results were consistent with the above conclusion that yeast feeding strategy was not appropriate in dealing with inhibitors problem at high DM loading.

Although the bioconversion of the DDAP pretreated corn stover was achieved by the fed-batch SScF, the highest ethanol yield at 25% (w/w) DM loading was only 65.5%. Further improvement of ethanol yield could be on several issues, such as increasing oligomeric sugar conversion, more efficient detoxification approach, and more tolerant strain development.

A brief comparison of ethanol production from pretreated corn stover at high solid loading was shown in Table 3. Ethanol titer and yield reached 71.4 g/L and 80.3% with the fermentation of pretreated corn stover at 30% (w/w) solid loading (Qureshi et al., 2015). However, compared with fermentation of un-detoxification corn stover, the ethanol titer and yield was much higher in the fermentation of detoxification corn stover (Table 3). These results indicated that detoxification processes was very effective approaches to improve the bioethanol production at high solid loading. However, the detoxification process prior to fermentation would increase the cost of bioethanol production. Ethanol titer and yield reached 47.2 g/L and 65.5% through the fermentation of un-detoxification corn stover at 25% DM loading in this study. Besides, effective bioconversion of both glucose and xylose was also achieved here. These results suggested that the novel fed-batch mode was effective to convert the un-detoxification pretreated corn stover at high DM loading.

4. Conclusions

Fed-batch SScF using a xylose utilizing yeast was successfully developed to convert DDAP pretreated corn stover at 25% (w/w) DM loading through the analysis of the effects of the hydrolysate on sugar utilization, and efficient xylose utilization was achieved at a high yeast inoculation. The final ethanol concentration and yield reached 47.2 g/L and 65.5% in the fermentation of un-detoxification corn stover at 25% DM loading applying the fed-batch mode at a high yeast inoculation.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2015.08.140.

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