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Cellulosic Ethanol Fermentation Using *Saccharomyces cerevisiae* Seeds Cultured by Pretreated Corn Stover Material

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Abstract Utilization of lignocellulose materials to replace the pure glucose for preparation of the fermenting yeast seeds could reduce the cost of ethanol fermentation, because a large quantity of glucose is saved in the large-scale seed fermentor series. In this study, *Saccharomyces cerevisiae* DQ1 was cultured using the freshly pretreated corn stover material as the carbon source, and then the culture broth was used as the inoculation seeds after a series of seed transfer and inoculated into the ethanol production fermentor. The results show that the yeast cell growth and ethanol fermentation performance have essentially no difference when the yeast seeds were cultured by glucose, the corn stover hydrolysate liquid, and the pretreated corn stover solids as carbon sources, respectively. Approximately 22 % of the yeast cell culture cost was saved, and the process flow sheet in industrial scale plants was simplified by using the pretreated corn stover for seed culture. The results provided a practical method for materials and operational cost reduction for cellulosic ethanol production.

Keywords Seed culture · Corn stover (CS) solids · Carbon source · *Saccharomyces cerevisiae* DQ1 · Simultaneous saccharification and ethanol fermentation (SSF)

Introduction

Cost reduction is the key issue for commercialization of cellulosic ethanol production from lignocellulosic feedstock [1, 2]. Among various cost-effective items, glucose consumption used for growing fermenting microbe cells is considerably high in large-scale plants [3]. For general ethanol fermenting strains such as *Saccharomyces cerevisiae*, the seed inoculation ratio was approximately 10 % (*v*/*v*) of the fermentation volume of the next-stage fermentor. A typical design of cellulosic ethanol plant with 61 million gallon production annually in the

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NREL technical report in 2011 [3] requires two seed trains of five sequentially aligned fermentors in each train before the seeds finally enter the ethanol production fermentor with the inoculation ratio of 11 % (*v*/*v*). In all the fermentors of the seed culture, glucose is as the carbon source. Only in the final production fermentor, the pretreated corn stover is used as the carbon source for ethanol production. Apparently, if the glucose used is replaced by the corn stover materials, the overall cost of cellulosic ethanol could be reduced by a large percentage, especially in the industrial scale plants.

In the previous studies, corn stover hydrolysate, a clear supernatant liquid prepared from enzymatic hydrolysis of the pretreated corn stover, was widely used in the seed culture of fermenting strains such as *S. cerevisiae* [4–8]. However, the preparation of corn stover hydrolysate requires an independent saccharification bioreactor and a solid/liquid separation equipment to obtain the hydrolysate liquid. This step not only increases the fixed capital cost for equipment but also leads to a high risk of contamination during the solid/liquid separation [9–11].

In this study, a simple approach of seed culture using the pretreated corn stover materials as carbon source was tested. The freshly pretreated corn stover solids were directly fed into the fermentor as the carbon source without enzymatic hydrolysis step, together with the cellulase enzyme for seed cultivation. Whole culture slurry (liquid and solids phase) was sequentially transferred in the seed fermentors after a suitable culture time, and the same operation was repeated until the production fermentor. The simultaneous saccharification and ethanol fermentation (SSF) seed culture developed could be advantageous in terms of complete cellulase utilization, glucose replacement, and contamination risk-free. The practicability of this method highly depends on whether the seed quality (cell viability) and quantity (cell number) meet the requirement in the multiple seed culture and production fermentor from the relatively inexpensive carbon source (pretreated corn stover solids). The objectives include to evaluate cell growth and ethanol production of SSF seed culture, to compare ethanol production efficiency using yeast seeds cultured by different carbon sources (glucose, corn stover hydrolysate, and pretreated corn stover solids), and to estimate the economic feasibility of the pretreated corn stover solids as carbon source in industrial scale. The method offers a simple and practical approach of cost reduction for the large-scale ethanol fermentation from lignocellulosic biomass.

Materials and Methods

Raw Materials and Chemicals

Corn stover (CS) without pretreatment is defined as virgin corn stover. It was grown in Dancheng County, Henan Province, China, and harvested in fall 2012. The corn stover was milled coarsely on a beater pulverizer and screened through mesh with the circle diameter of 10 mm. The milled corn stover was washed to remove field dirts, stones, and metals and then dried until constant weight and sealed in plastic bags for use. The cellulose and hemicellulose contents of corn stover were determined according to a two-step H_2SO_4 hydrolysis method developed by the National Renewable Energy Laboratory (NREL) [12]. The virgin corn stover contained 37.2 % of glucan and 19.9 % of xylan. The pretreated corn stover contained 41.4 % of glucan and 4.0 % of xylan.

Yeast extract was purchased from Oxiod (Basingstoke, Hampshire, England). All other standard chemicals including glucose, peptone, KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4$, NaOH, and

H_2SO_4 were of reagent grade and purchased from Lingfeng Chemical Reagent Co., Shanghai, China. Agar was purchased from New Probe Bioscience Technology Co., Beijing, China.

Strains and Enzymes

The thermotolerant ethanol fermenting strain *S. cerevisiae* DQ1 was used in all experiments. This strain was stored at the Chinese General Microorganisms Collection Center, Beijing, China, with the registration number of CGMCC 2528 and was used as [4, 7].

The biodetoxification strain *Amorphotheca resinae* ZN1 was stored at the Chinese General Microorganisms Collection Center, Beijing, China, with the registration number of CGMCC 7452 for the removal of inhibitors from the pretreated corn stover [5].

The cellulase enzyme Youtell #6 was kindly provided by Hunan Youtell Biochemical Co. (Yueyang, Hunan, China). The filter paper activity of Youtell #6 was 135 FPU/g determined using the NREL protocol LAP-006 [13], and the cellobiose activity was 344 CBU/g using the method of Sharma et al. [14].

The culture media used included the following:

1. Synthetic medium: 20.0 g/L of glucose, 2.0 g/L of KH_2PO_4, 1.0 g/L of $(NH_4)_2SO_4$, 1.0 g/L of $MgSO_4 \cdot 7H_2O$, and 1.0 g/L of yeast extract
2. Adaptation medium: 2.0 g/L of KH_2PO_4, 1.0 g/L of $(NH_4)_2SO_4$, 1.0 g/L of $MgSO_4 \cdot 7H_2O$, and 1.0 g/L of yeast extract (pH 6.0)
3. Fermentation medium: 2 g/L KH_2PO_4,·1 g/L $MgSO_4$, 1 g/L $(NH_4)_2SO_4$, and 1 g/L yeast extracts
4. YPD medium: glucose 20 g/L, peptone 20 g/L, yeast extract 10 g/L, and agar 20 g/L. All media were sterilized at 115 °C for 20 min.

Pretreatment

Corn stover was pretreated using the dry dilute acid pretreatment procedure as described in Zhang et al. [6]. The corn stover feedstock was firstly presoaked with diluted sulfuric acid solution (5.0 %, *w*/*w*) with the ratio of the solid (dry corn stover) to the liquid (sulfuric acid solution) of 2:1 (*w*/*w*) and then was pretreated at 190 °C for 3 min. The pretreated corn stover was withdrawn from the reactor, and the solid content was 50 % (*w*/*w*).

The pretreated corn stover was biologically detoxified to remove the fermentation inhibitors by using *A. resinae* ZN1 strain according to the procedure in Zhang et al. [5]. The detoxified corn stover was used as the final ethanol production fermentation and was not used in the seed culture steps.

Corn Stover Hydrolysate Preparation

Freshly pretreated corn stover solid was hydrolyzed as described in Huang et al. [8]. Briefly, 15 % (*w*/*w*) freshly pretreated corn stover solids were hydrolyzed using Youtell #6 at 50 °C for 48 h with the dosage of 15 FPU/g DM (dry pretreated corn stover). The water-insoluble solids were separated from corn stover hydrolysate by centrifugation at 10,000 rpm for 10 min. The clear hydrolysate contained 61.05 g/L of glucose, 32.09 g/L of xylose, 2.23 g/L of acetic acid, 0.32 g/L of furfural, and 0.16 g/L of 5-hydroxymethylfurfural (HMF). Hydrolysate was used as carbon source for yeast seed culture.

Cell Cultivation Using Freshly Pretreated Corn Stover as Carbon Source

The schematic diagram of the ethanol fermenting strain *S. cerevisiae* DQ1 culture using the freshly pretreated corn stover (without detoxification treatment) as carbon source is shown in Fig. 1. The adaptation procedure used was to allow the yeast gradually adapting the inhibitors containing hydrolysate and assure the reproductivity of the fermenting strain. The details are described as follows:

Step 1 A vial of *S. cerevisiae* DQ1 stock culture was inoculated into a 100-mL Erlenmeyer flask containing 20 mL of the synthetic medium (20 g/L of glucose contained) and cultured for 18 h in a shaking incubator at 30 °C and 150 rpm.

Step 2 5 mL of the cell culture from step 1 was inoculated into a 250-mL flask containing 50 mL of the adaptation medium at 10 % (*v*/*v*) inoculation ratio and then cultured for 12 h at 37 °C and 150 rpm. In this step, 2.5 g (dry base) of the pretreated corn stover solids (5 % of the total medium by weight percentage) and 0.34 g of cellulase Youtell #6 at 15 FPU/g DM were added into the adaption medium before the culture started.

Step 3 5 mL of the cell culture from step 2 was inoculated into the 50 mL of the adaptation medium cultured for 12 h at 37 °C and 150 rpm. In this step, 5 g (dry base) of the same solid corn stover (10 % of the total medium by weight percentage) and 0.68 g of cellulase at 15 FPU/g DM were added into the adaption medium before the culture started.

Step 4 The process was repeated for further adaptation purpose. Five milliliters of the cell culture from step 3 was inoculated into the 50 mL of the adaptation medium cultured for 12 h at 37 °C and 150 rpm, and 5 g (dry base) of corn stover (10 %, *v*/*v*) and 0.68 g of cellulase at 15 FPU/g DM were added into the adaption medium before the culture started.

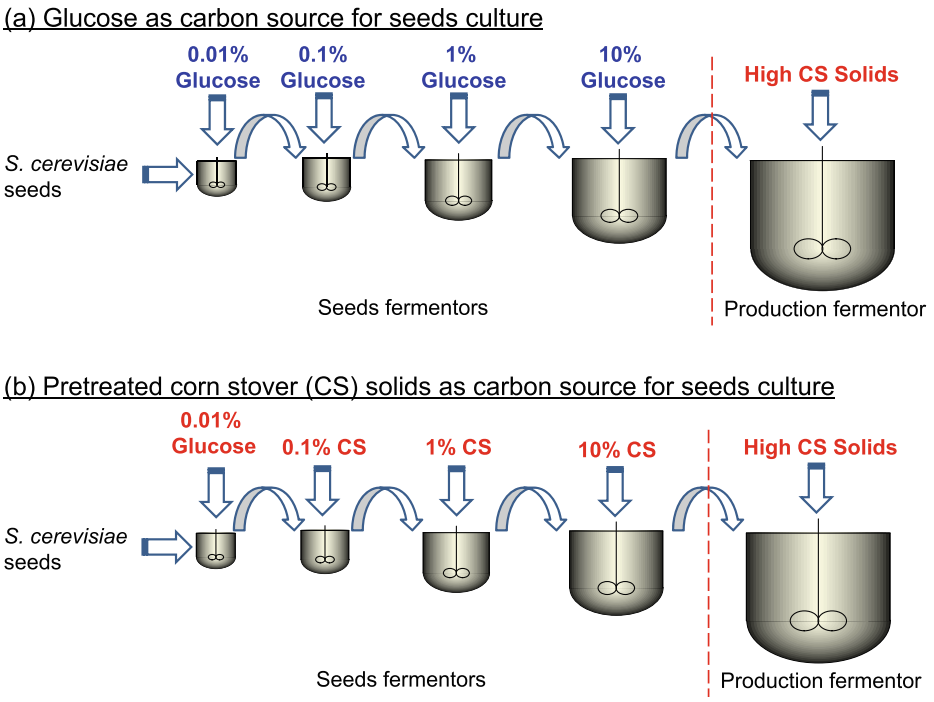

Fig. 1 Schematic diagram of yeast seed culture using freshly pretreated corn stover solids as carbon source

Step 5 The whole suspension from step 4 was used as seed culture of the simultaneous saccharification and fermentation (SSF) in the final SSF bioreactor for production of ethanol using the detoxified corn stover.

The cell cultivation using the hydrolysate liquid prepared from the pretreated corn stover followed the same procedure as reported in Zhang et al. [4].

Simultaneous Saccharification and Ethanol Fermentation (SSF) Operation

All SSF experiments were performed in a 5-L bioreactor under the solids loading of the pretreated corn stover up to 25 % (*w*/*w*). A unique helical ribbon stirrer was installed for well mixing of the solids with enzyme and nutrients as described in Zhang et al. [4]. The SSF process was operated at two stages:

Stage 1 (prehydrolysis) 6 h at 50 °C, pH 4.8 after cellulase was added at 15 FPU/g DM. The pretreated, detoxified, and disk-milled corn stover feedstock was fed into the bioreactor in semi-continuous mode during 6 h.

Stage 2 (SSF) The prehydrolyzed slurry was cooled down to 37 °C, and then the fermentation medium nutrients were added to reach the fermentation medium composition. The yeast seed culture from step 5 of "Cell Cultivation Using Freshly Pretreated Corn Stover as Carbon Source" section was inoculated to start the SSF operation. The SSF continued for 72 h, and samples were withdrawn with regular intervals. The pH was maintained at 4.8 during the overall operation by addition of 5 M NaOH or 2 M H_2SO_4 solutions. Each experiment was duplicated, and the mean value was calculated.

Analysis

The samples were centrifuged to remove the solids, and the supernatant was collected for analysis on HPLC to obtain the periodic concentration of glucose, ethanol, and acetic and lactic acid. All samples were filtered through a 0.22-μm filter before injection. The HPLC used consisted of LC-20 AD pump, RI detector RID-10A (Shimadzu, Kyoto, Japan), and a Bio-Rad Aminex HPX-87H column at 65 °C with 0.6 mL/min of 5 mM H_2SO_4 as the mobile phase.

The optical density of yeast cells in the fermentation slurry was not able to measure because of the high solids inside. An alternative method was used by counting the colony-forming unit (CFU/mL). The CFU number of the yeast cells was counted by spreading each of the samples collected at regular interval (SSF) after dilution on to the YPD agar plates and incubated at 30 °C for 48 h; the single colony numbers grown on the agar plates were counted.

Ethanol Yield Calculation

Ethanol yield was calculated according to the modified method by taking into account the ethanol formation and the water loss in the hydrolysis [15].

$$\text{Ethanol yield} = \frac{[C_1] \times W}{976.9{-}0.804 \times [C_1]0.511 \times f \times [Biomass] \times m \times 1.111} \times 100\%$$

where $[C_1]$ is the ethanol concentration in the culture broth(g/L), W is the total water input of the SSF (g), f is the cellulose fraction of corn stover feedstock, [*Biomass*] is the dry corn

stover concentration at the beginning of the SSF (g/g), *m* is the total weight of the SSF (g), 0.511 is the conversion factor for glucose to ethanol based on stoichiometric biochemistry of yeast, 1.111 is the conversion factor for cellulose equivalent to glucose, and 976.9 and 0.804 are the constants of the formula derived from the method development in the study of Zhang and Bao [15].

Results and Discussion

Seed Culture Using Freshly Pretreated Corn Stover Solids as Carbon Source

The *S. cerevisiae* DQ1 seed culture was prepared by using the freshly pretreated corn stover solids (without any detoxification treatment) as the carbon source. The cell viability and ethanol fermentation performance are shown in Fig. 2.

Figure 2a shows the *S. cerevisiae* DQ1 cell growth indicated by the CFU number. In the first seed culture, the yeast cell broth from the synthetic medium was consecutively transferred to the SSF culture containing 5 % (*w*/*w*) pretreated corn stover solids, the second seed culture was carried out by transferring the first seed culture broth into the SSF culture containing 10 % (*w*/*w*) pretreated corn stover solids, and the third seed culture again was carried out by transferring the second culture broth into the SSF culture containing 10 % (*w*/*w*) pretreated corn stover (repeating the second seed culture). The cell CFU number (2.0×10^8) reached its maximum in the period of 12–15-h culture time. In the second culture, the CFU number was relatively decreased and reached approximately 70 % of the maximum CFU number in the first culture after 15-h cultivation (1.5×10^8). The CFU number in the third culture was similar to that in the second culture, with fast growth rate and a slightly higher CFU number. The overall cell growth in the three cultures was in a satisfactory condition and met the requirement of the consequent SSF operation.

Figure 2b shows the time course of glucose consumption and ethanol formation in the seed culture. The glucose released and the ethanol produced were low because of the low pretreated corn stover solids loading (5 %, *w*/*w*); with the increased solids loading to 10 % (*w*/*w*) in the other two cultures, the glucose released and consumption, as well as the ethanol production increased, and the third culture gave the best performance, although the CFU number was slightly decreased in the second and third seed culture. The reason might come from the adaptation function of yeast cells to the inhibitors in the pretreated corn stover, similar to the adaptation function in the seed culture in the corn stover hydrolysate liquid [7]. Also, although the glucose released from corn stover and ethanol produced in the seed culture did not make a significant contribution to the final ethanol titer in the production fermentor, it still added a surplus on the ethanol titer and yield in the SSF using the pretreated corn stover by replacing glucose as the carbon source.

SSF Using Yeast Seeds Cultured by Pretreated Corn Stover Solids

The seed culture prepared by using the pretreated corn stover materials as carbon source was inoculated into the simultaneous saccharification and fermentation (SSF) of corn stover at the volume ratio of 10 %. Three SSF cases were carried out using the seeds cultured by the synthetic medium (glucose), the clear corn stover hydrolysate liquid, and the freshly pretreated corn stover, respectively. The SSF results are shown in Fig. 3.

Figure 3a shows that the CFU number in the SSF increased from the start and reached at maximum, and then declined. In the first case using the synthetic medium (glucose) for seed

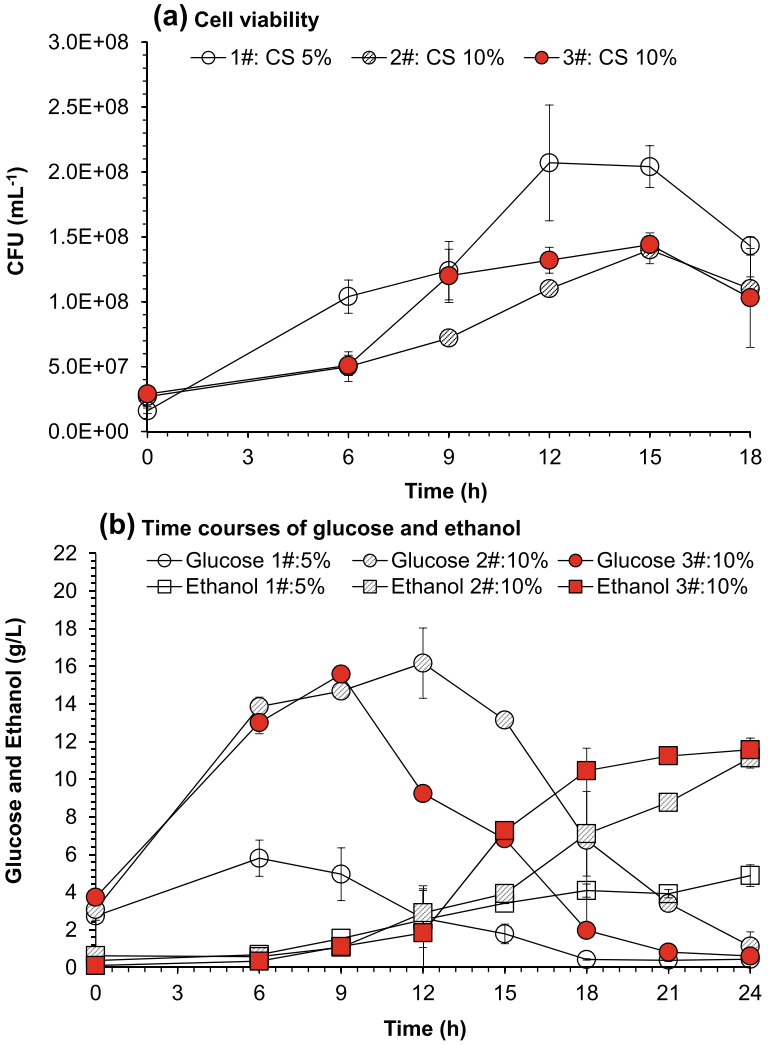

Fig. 2 Seed culture of *S. cerevisiae* DQ1 using the freshly pretreated corn stover solid as carbon source. Conditions: cellulase 15 FPU/g DM, 37 °C, pH 6.0, and shaked at 150 rpm. **a** Yeast cell viability (CFU/mL). **b** Time course of glucose utilization and ethanol production. *S. cerevisiae* DQ1 seed culture from the first step in "Cell Cultivation Using Freshly Pretreated Corn Stover as Carbon Source" section were transferred to the culture medium containing 5.0 % (*w*/*w*) of the pretreated corn stover solids for 12 h (the step 2 in "Cell Cultivation Using Freshly Pretreated Corn Stover as Carbon Source" section) (*1#*), the seed culture from the *1#* was transferred into the medium containing 10 % of the pretreated corn stover for 12 h (the step 3 in "Cell Cultivation Using Freshly Pretreated Corn Stover as Carbon Source" section) (*2#*), and the seed culture from *2#* was transferred into the culture medium containing 10 % of the pretreated corn stover for 12 h (step 4 in "Cell Cultivation Using Freshly Pretreated Corn Stover as Carbon Source" section) (*3#*)

culture, the CFU number reached 5.4×10^8 after 48 h and then declined. In the second case using the clear corn stover hydrolysate for seed culture, the CFU number reached 3.2×10^8 in the earlier culture time of 30 h, and then declined. In the third case using the pretreated corn stover solids as the carbon source, the CFU number reached 3.6×10^8 at the same culture of 30 h, similar to that using the hydrolysate liquid and as twice

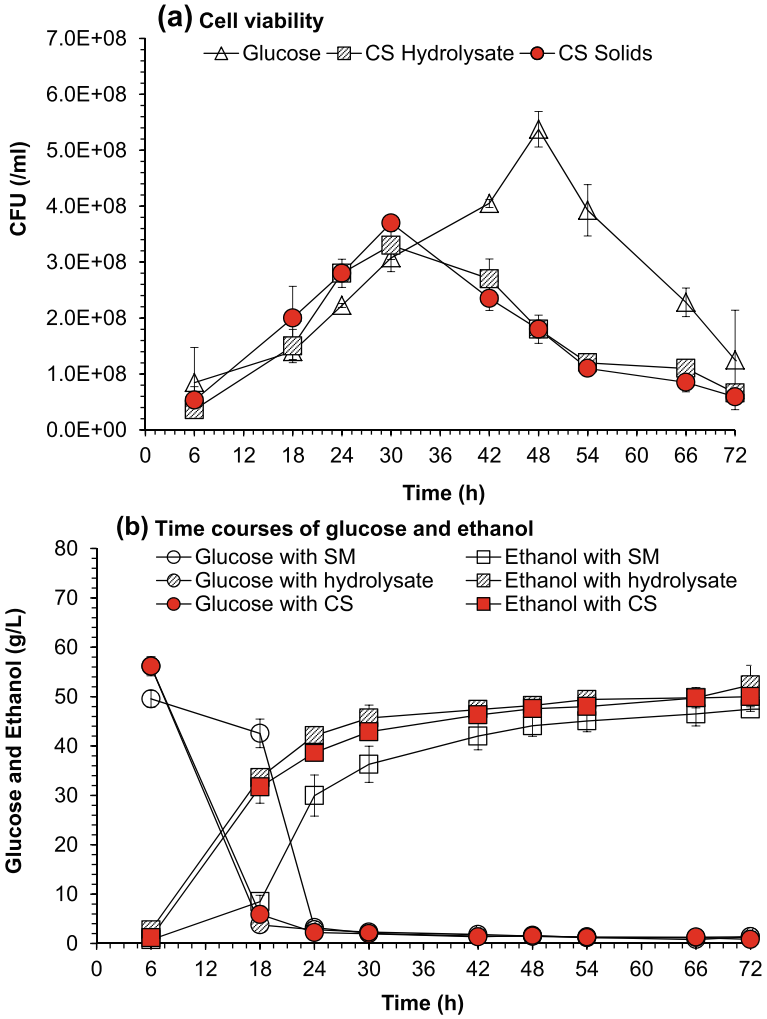

Fig. 3 SSF of corn stover using seeds cultured with different carbon sources. **a** Cell viability (CFU/mL). **b** Time course of glucose utilization and ethanol production. *S. cerevisiae* DQ1 were cultured using the synthetic medium (glucose), the clear corn stover hydrolysate, and the freshly pretreated corn stover solids as carbon sources. The SSF was carried out in a 5-L helically stirred bioreactor. Conditions: 25 % (*w*/*w*, dry base) solids loading and cellulase 15 FPU/g DM; the prehydrolysis was lasted for 6 h at 50 °C and pH 4.8, and then the SSF was operated for 72 h at 37 °C, pH 4.8

as that in the seed culture (1.5×10^8), suggesting that the cell growth in the seed culture using the corn stover solids was at least as good as that using hydrolysate for seed culture. The high CFU number of *S. cerevisiae* DQ1 indicates that the corn stover materials provided sufficient energy and nutrients and neglected the effect of inhibitors for cell growth.

Figure 3b shows the time courses of glucose consumption and ethanol production in the SSF of pretreated and detoxified corn stover at high solids loading of 25 % (*w*/*w*). In the three SSF cases, the ethanol titer and yield were 47.48 g/L and 72.07 %when the seeds were cultured using glucose, 52.34 g/L and 80.17 % by clear pretreated corn stover hydrolysate, and 49.99 g/

Table 1 Cost estimation based on 100,000 t/annum cellulosic ethanol production scale using corn stover solids as carbon source for seed culture

	Case 1	Case 2
Carbon source dosage in seed culture media (g/L)[a]	20.0 (glucose)	100.0 (CS)
Ethanol titer in the production fermentor(g/L)[a]	47.5	50.0
Fuel ethanol production annually (t/annum)	100,000	100,000
Required total ethanol fermentation broth volume (m^3)[b]	2,105,000	2,000,000
Required minimum seed fermentor stages[c]	4	4
Single-stage inoculation ratio (%, *v*/*v*)[a]	0.1000	0.1000
Overall inoculation ratio (%, *v*/*v*)[d]	0.1111	0.1111
Required total seed culture volume (m^3)[e]	234,000	222,200
Required carbon source (t, glucose or CS)[f]	4,678 (glucose)	22,200 (CS)
Carbon source cost: feedstock price ($/t)[g]	570.6	64.4
Carbon source cost: enzyme($/t)[h]	0	29.6
Overall carbon source costs on seed culture ($, million)[i]	2.69	2.09

Conditions: 25 % (*w*/*w*) solids loading in production fermentor with cellulase 15 FPU/g DM; 6-h prehydrolysis at 50 °C and pH 4.8, followed by 72-h SSF at 37 °C and pH 4.8

[a] This study, Fig. 3. The extra ethanol produced in case 2 using pretreated corn stover materials for seed culture (50 g/L) than that in case 1 using glucose for seed culture (47.5 g/L) was neglected

[b] Ethanol fermentation broth volume (m^3)

= Ethanol production annually (t/a)/[ethanol titer in the production fermentor (g/L)×1000]

=100,000/(47.5×1000)=2,105,000 (m^3) (case 1)

or

=100,000/(50.0×1000)=2,000,000 (m^3) (case 2)

[c] Humbird et al., 2011, NREL technical report [3]: pages 26–35

[d] Overall seed inoculation ratio (%, *v*/*v*)

=0.1 (stage 4)+0.01 (stage 3)+0.001 (stage 2)+0.0001 (stage 1)=0.1111

[e] Total seed culture volume (m^3)

= Total ethanol fermentation broth volume (m^3)×overall seed inoculation ratio (%, *v*/*v*)

=2,105,000×0.1111=234,000 (m^3) (case 1)

or

=2,000,000×0.1111=222,300 (m^3) (case 2)

[f] Required carbon source (t)

= Total seed culture volume (m^3) [carbon source dosage in seed culture media (g/L)/1000]

=234,000/(20.0/1000)=4678 (t) glucose (case 1)

or

=222,300/(100.0/1000)=22,220 (t) CS (case 2)

[g] Carbon source: feedstock price

Case 1: Glucose=$0.2579/lb=$570.6/t ([3]: page 63);

Case 2: Corn stover=$0.741/gal ethanol=$0.741/gal×87 gal/t=$64.4/t ([3]: page 4)

[h] Carbon source: enzyme cost

Case 1: glucose=0 assumed for glucose;

Case 2: corn stover=$0.34/gal ethanol=$0.34/gal×87 gal/t=$29.6/t ([3]: page 4)

[i] Overall carbon source costs on seed culture

Case 1: required carbon source (t)×(carbon source prices+processing cost) ($/t)

=4678 (t)×($570.6/t+0)=$2.69 million

Case 2: required carbon source (t)×(carbon source prices+processing cost) ($/t)

=22,220 (t)×($64.4/t+$29.6/t)=$2.09 million

L and 76.10 %by freshly pretreated corn stover solids, respectively. Although the CFU number in the first case was greater than that in the other two cases, the ethanol titer and yield in both the second and third cases were higher than that in the first case, suggesting that the yeast cells were went through the adaptation treatment in the corn stover hydrolysate or the corn stover solids-containing system. In the cases using the corn stover hydrolysate and solids as the carbon source, both the glucose consumption and ethanol production were close, indicating that the seed culture using the pretreated corn stover solids could be effectively applied for the consequent SSF without separate hydrolysis step.

Cost Estimation on Using Pretreated Corn Stover for Seed Culture

Preliminary cost estimation of seed culture in the two cases using glucose and corn stover solids was calculated based on an industrial scale of 100,000 t/annum ethanol production. Only the cost of seed culture materials (glucose or corn stover) in the two cases was compared as shown in Table 1. Two operational cases were selected based on the results of this study in Fig. 3 and applied on the 100,000 t/annum fuel ethanol production scale, without considering the changes in the scale-up processes. Case 1 was based on the use of synthetic medium containing 20 g/L of glucose for seed culture; case 2 was based on the use of corn stover solids containing 100 g/L of the pretreated corn stover solids (dry basis). The cost estimation process of the two seed culture cases is summarized in Table 1.

In the operational cases of Table 1, a fourth-stage seed culture system was assumed for the 100,000 t/annum ethanol production before the final production fermentor. The inoculation ratio of the single transfer between two seed fermentors or between the last seed fermentor and the final production fermentor was set to 10 %. Thus, the overall seed inoculation ratio was the summary of the four stages 11.11 %. The estimation results in Table 1 show that approximately 22 % of the seed culture material cost was saved by using corn stover solids ($2.09 million) to replace glucose ($2.69 million) in the production scale of 100,000 t/annum cellulosic ethanol plant, besides the cost reduction of equipment and operations by using the pretreated corn stover materials. Although the estimation was preliminary on a conceptual process, the cost reduction data still sufficiently demonstrated the advantages of the seed culture method using the pretreated corn stover solids as the seed culture materials.

When corn stover hydrolysate liquid was used for seed culture instead of corn stover solids, the ethanol production performance was similar as shown in Fig. 3. However, the cost for using hydrolysate liquid certainly increased due to the existence of additional processes in corn stover saccharification to obtain the hydrolysate and the solids/liquid separation to obtain clear hydrolysate liquor. Furthermore, the contamination risk was also significantly increased if the corn stover hydrolysate liquor was used because the solids/liquid separation was hard to be processed in a strictly closed circle. Another possible advantage of corn stover solids usage was that the cellulase enzyme absorbed onto the corn stover residues was not lost by feeding the overall slurry in the next-stage seed fermentor or production fermentor for recycled use on corn stover saccharification.

Conclusion

The *S. cerevisiae* DQ1 strain was sequentially transferred in the culture systems containing 5–10 % of freshly pretreated corn stover solids and then inoculated as the seed into the production fermentor. The ethanol titer and yield of the SSF using yeast seeds cultured by synthetic medium (glucose), corn stover hydrolysate liquid, and corn stover solids as carbon

sources reached to 47.48 g/L and 71.07 %, 52.34 g/L and 80.17 %, and 49.99 g/L and 76.10 %, respectively. Approximately 22 % of the cost for yeast cell culture was saved by using the pretreated corn stover solids as carbon source, and the contamination risk using hydrolysate liquid was avoided.

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