ARTICLE

BIOTECHNOLOGY and BIOENGINEERING

Simultaneous Saccharification and Ethanol Fermentation at High Corn Stover Solids Loading in a Helical Stirring Bioreactor

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ABSTRACT: The higher ethanol titer inevitably requires higher solids loading during the simultaneous enzymatic saccharification and fermentation (SSF) using lignocellulose as the feedstock. The mixing between the solid lignocellulose and the liquid enzyme is crucially important. In this study, a bioreactor with a novel helical impeller was designed and applied to the SSF operation of the steam explosion pretreated corn stover under different solids loadings and different enzyme dosages. The performances using the helical impeller and the common Rushton impeller were compared and analyzed by measuring rheological properties and the mixing energy consumption. The results showed that the new designed stirring system had better performances in the saccharification yield, ethanol titer, and energy cost than those of the Rushton impeller stirring. The mixing energy consumption under different solids loadings and enzyme dosages during SSF operation were analyzed and compared to the thermal energy in the ethanol produced. A balance for achieving the optimal energy cost between the increased mixing energy cost and the reduced distillation energy cost at the high solids loading should be made. The potentials of the new bioreactor were tested under various SSF conditions for obtaining optimal ethanol yield and titer.

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KEYWORDS: simultaneous saccharification and ethanol fermentation; lignocellulose; high solids loading; high ethanol titer; helical impeller; mixing energy consumption

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Introduction

Increasing ethanol titer in the fermentation broth is crucially important for cost reduction of cellulose ethanol due to the great energy demand of ethanol distillation (Galbe et al., 2007; Larsen et al., 2008). The higher ethanol titer inevitably requires higher lignocellulose solids loading in the simultaneous saccharification and fermentation (SSF) process. The solid concentration above 30% (w/w) in the starch-based ethanol fermentation is a common practice for obtaining ethanol titer above 8–10% (w/w) (Bayrock and Ingledew, 2001). For lignocellulose-based ethanol fermentation, a high solids loading of the pretreated lignocellulose feedstock close to 30% (w/w) is also required to reach the ethanol concentration up to 5–10% (w/w). The high solids loading will also contribute to the reduction of water use for ethanol production (Gerbens-Leenes et al., 2009).

Many problems arise when the pretreated lignocellulose solids loading is above 15% (w/w) during SSF operation (Tolan, 2002). The solid portion of pretreated lignocellulose is gradually hydrolyzed into the liquid slurry containing monosaccharide sugars, oligomers sugars, and insoluble lignin/ashes under the catalysis of cellulase enzymes during the simultaneous enzymatic saccharification and fermentation. The mixing of the solid feedstock with the liquid cellulase enzyme deteriorates when the solid lignocellulose content in the bioreactor is increased. The mixing gets worse and the apparent viscosity of the slurry reaches maximum when all the solid feedstock is fed into the bioreactor. Furthermore, with the fermentation starts and carbon dioxide generates, a complicated gas–liquid–solid multiphase system forms and this multi-phase system leads to the low mass and heat transfer efficiency, low sugar yield, and low ethanol yield. Making the situation worse is the low cellulase enzyme dosage per unit of solid lignocellulose and

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the low inoculation seeds percentage due to the cost reduction consideration. Therefore, mixing is the central barrier for a practical SSF bioreactor with high lignocellulose solids loading to achieve high ethanol titer. The sufficient mixing capacity, the low energy consumption, and the low stress to the enzyme and microbial cells should be considered as the key factors for designing mixing processes in lignocellulose processing bioreactors.

SSF is a common practice for lignocellulose processing but few studies concerned the bioreactor design and the mixing energy consumption under high solids loading. Mohagheghi et al. (1992) reported a simple horizontally rotating fermenter for SSF at the maximum solids loading of 24.4% (w/w) dilute acid pretreated wheat straw after washed with deionized water. De Bari et al. (2002) demonstrated an experimental flowsheet for ethanol production from aspen wood at the maximum solids loading of 16% (w/w). The steam exploded aspen wood was either soaked with alkali or washed with hot water for detoxification of degradation products. Jørgensen et al. (2007) reported a gravimetric-mixing reactor for liquefaction of lignocellulose at high solid concentration with up to 40% (w/w) initial dry water insoluble solid. This unique bioreactor realized the mixing operation under high solids loading using gravimetric mixing method and scaled up to the pilot bioreactor of 11 m^3.

In this study, a bioreactor with a novel helical impeller design was applied to the SSF of steam explosion pretreated corn stover (CS) under different solids loadings and different enzyme dosages. The pretreated CS was used directly to the SSF processing without any detoxification steps such as washing or overliming. The performances using the novel helical impeller and the common Rushton impeller were compared under the same solids loading; the SSF performances were analyzed by measuring rheological properties and stirring energy cost. The results showed that the new design had better performances in saccharification yield, ethanol titer, and energy cost than those of the Rushton impeller stirring. The mixing energy consumption under different solids loadings and enzyme dosages during SSF operation were analyzed and compared to the thermal energy in the ethanol produced. The potentials of the new bioreactor were tested under various SSF conditions for the optimal ethanol yield and titer. The current bioreactor provided a practical option for future cellulose ethanol production from agriculture residues and other lignocellulosic biomass.

Materials and Methods

Raw Materials and Pretreatment

CS was grown in Northeast Province of Jilin, China, and harvested in fall, 2007. After collection, the CS was milled coarsely to a fiber length of less than 25 mm by a SF rotor speed mill, then washed with water to remove the majority of the field dirt, stones, and metals, and then air-dried. The steam explosion pretreatment was performed in StakeTech batch system (SunOpta Bioprocess, Inc., Brampton, Ontario, Canada) at the condition of 200°C, 2.0 MPa for 4 min. Only saturated steam was used without adding any inorganic and organic acids. The pretreated CS contained approximately 40.0% dry solid matter (DM), stored at 4°C, and was ground in a juice blender for a few seconds to disperse the aggregates formed during the storage at 4°C before feeding into the bioreactor.

Enzymes and Strains

The cellulase enzyme used was Accellerase 1000 from Genencor International (Rochester, NY). The cellulase and cellobiase activities were assayed separately. The cellulase activity was 65.8 FPU/mL using the protocol of NREL LAP-006 (1996). One unit of filter paper cellulase (FPU) was defined as the amount of enzyme which produces 2.0 mg of reducing sugar from 50.0 mg of filter paper within 1 h. The experiment was carried out in a reaction mixture containing 0.5 mL appropriately diluted enzyme solution, 1.0 mL of 50 mM citrate buffer (pH 4.8), and 50.0 mg of Whatman No.1 filter paper. The reaction solution was incubated at 50°C for 1 h. Then the concentration of the released reducing sugar was measured using 3,5-dinitrosalicylic acid (DNS) method.

The cellobiase activity was 152.0 IU/mL. It was assayed in a reaction mixture containing 1.0 mL of 80 mM cellobiose solution in 50 mM citrate buffer at pH 4.8 and 1.0 mL of appropriately diluted enzyme solution (Ghose, 1987). The reaction solution was incubated at 50°C for 10 min, and the reaction was terminated by boiling it in a water bath for 2 min. One unit of cellobiase activity (CBU) was defined as the amount of enzyme that forms 2.0 μmol glucose per minute from cellobiose.

A thermo- and inhibitor-tolerant baker's yeast mutant strain *Saccharomyces cerevisiae* DQ1 was obtained by our laboratory and used in all the fermentation experiments. The culture solution was aliquoted into 1.0 mL vials containing 30% (w/w) glycerol and stored at −80°C freezer. At each inoculation step, one vial of *S. cerevisiae* DQ1 was taken from the −80°C freezer and the solution in the vial was completely inoculated into the seeding culture in order to keep the same inoculation size and quality.

Cell Cultivation

A three-step adaptation procedure for SSF using pretreated CS was followed: first, a vial of *S. cerevisiae* DQ1 was inoculated into a 100 mL Erlenmeyer flask containing 20.0 mL of sterilized synthetic medium, cultured in a shaking incubator at 30°C, 150 rpm, for 18 h; then the culture was inoculated into a 100 mL flask containing 20.0 mL of sterilized medium containing 50% of CS hydrolysate and 50% of synthetic medium (pH 5.0), cultured at 30°C, 150 rpm for 15 h; finally, the culture

was inoculated into a 500 mL flask containing 200.0 mL of sterilized hydrolysate medium at pH 5.0, cultured at 30°C, 150 rpm for 15 h. The yeast cells were harvested by centrifuging at 4,000 rpm for 10 min, and the pellets were resuspended in 20.0 mL sterilized water. Then the yeast suspension was inoculated into the hydrolysate to start the SSF step. Medium and water used above were autoclaved at 115°C for 20 min.

The synthetic medium solution contained 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. The major degradation products from the steam explosion pretreatment with potential inhibition effects included acetic acid and levulinic acid. 5-Hydroxymethylfurfural and formic acid were measured and were found to be less than 0.5 g/L. Furfural, vanillin, and 5-hydroxymethylbenezate were not detected from the pretreated CS used. The concentration ranges of acetic acid and levulinic acid in the experimental were 2.0–5.3 and 1.1–4.0 g/L, respectively, due to the different pretreated CS loadings. These concentration ranges were still not high enough to inhibit the saccharification of Accellerase 1000 enzyme (Chen et al., 2009; Jing et al., 2009) as well as the fermentation by *S. cerevisiae* DQ1. Therefore, the pretreated CS was used directly for SSF without any detoxification treatment.

SSF Bioreactor and Operation

All SSF experiments of the pretreated CS were performed in the 5-L bioreactor equipped with an impeller as shown in Figure 1. The bioreactor included a drive shaft, a helix impeller, a turbine/aerofoil impeller, and a bottom impeller. The helix impeller was mounted on the drive shaft by a supported-shaft, twisting around the drive shaft from top to down, which form a frame configuration. The turbine/aerofoil impeller sets on the drive shaft within the frame configuration. The bottom impeller is located at the bottom of the combined impeller, which was close to the bottom of the bioreactor. When the Rushton impeller was used on the bioreactor, the Item 9 (helical impeller), Item 10 (turbine/aerofoil impeller), and Item 11 (bottom impeller) in Figure 1a were replaced by the Rushton impeller shown in Figure 1b. A power measuring meter was mounted in the bioreactor, and the power consumption was monitored at intervals. The power consumed on the stirring was calculated by subtracting the no-load power consumption.

The SSF process was operated at two stages, the prehydrolysis stage started at the beginning and then the real SSF stage followed until the end of the operation. In the prehydrolysis stage, the Accellerase 1000 cellulase

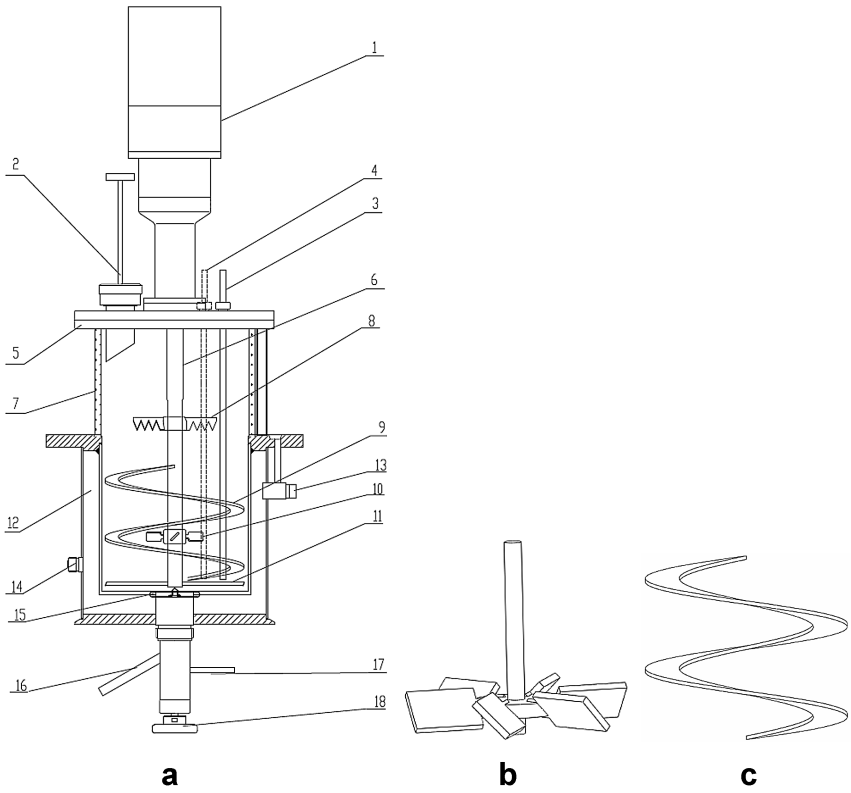

Figure 1. Bioreactor for SSF operation at high solids loading. Bioreactor description: (**a**) Diagram of the bioreactor with the helical impeller system, (**b**) Rushton impeller, and (**c**) helical impeller. The bioreactor includes (1) motor, (2) solid feeding inlet, (3) thermometer port, (4) pH-meter port, (5) tank cap, (6) drive shaft, (7) tank wall, (8) antifoaming impeller, (9) helical impeller, (10) turbine/aerofoil impeller, (11) bottom impeller, (12) water-bath jacket, (13) water-bath jacket outlet, (14) water-bath jacket inlet, (15) gas disperser, (16) discharge head, (17) gas inlet, and (18) stop valve.

enzyme was fed into the tank at the dosage of 7.0–30.0 FPU/g DM. Then the total pretreated CS including both the solid and the liquid from pretreatment was fed into the bioreactor within 12 h for prehydrolysis at 50°C. The feeding rate was adjusted so that the material inside the bioreactor maintains a liquid slurry form. In other words, the pretreated CS would not continue to feed into the bioreactor until the pretreated CS previously fed into the bioreactor was liquefied into the liquid slurry. The feeding was in a semi-continuous style. SSF started by reducing the temperature to 37°C and inoculating the *S. cerevisiae* DQ1 seeds into the hydrolysate. The SSF operation continued for 60 h and the samples were withdrawn at regular intervals and centrifuged at 10,000 rpm for 5 min. The supernatant was stored frozen until analysis on high-performance liquid chromatography (HPLC). The pH was maintained at pH 5.0 during the hydrolysis and SSF stages by addition of 5 M NaOH solution.

Analysis of Corn Stover Composition

The composition of CS was analyzed using Foss 2021 Cellulose Analyzer (Foss A/S, Hillerod, Denmark). The moisture of CS was measured at 105°C overnight until the weight was constant. The original CS contained 32.6% cellulose, 26.4% hemicellulose, and 8.1% lignin (w/w, dry weight base). The moisture of the pretreated CS was approximately 60.0% (w/w, total weight base). The detailed moisture data of each pretreated CS package were measured before use.

Analysis of Sugars, Ethanol, and Inhibitors on HPLC

Glucose, ethanol, and lignocellulose degradation compounds, such as furfural, 5-hydroxyfurfural (5-HMF), acetic acid, and levulinic acid, were analyzed using HPLC (LC-20AD, refractive index detector RID-10A, Shimadzu, Kyoto, Japan) with a Bio-Rad Aminex HPX-87H column at the column temperature 65°C. The mobile phase was 5 mM H_2SO_4 at the rate of 0.6 mL/min. All samples were centrifuged to remove the cell mass and other water insoluble substances, then filtered through a 0.22 μm filter before analysis.

Ethanol Yield Calculations

The ethanol yields were calculated according to the NREL LAP-008 (2001)

$$\text{Theoretical ethanol concentration} = 0.51f[\text{Biomass}] \times 1.111$$

$$\text{Ethanol yield} = \frac{[\text{EtOH}]_f - [\text{EtOH}]_0}{\text{theoretical ethanol concentration}} \times 100\%$$

where [Biomass] is the dry biomass weight concentration at the beginning of the fermentation (g/L); f is the cellulose fraction of dry biomass (g/g); 0.51 is the conversion factor for glucose to ethanol based on the stoichiometric biochemistry of yeast; 1.111 is the conversion factor for cellulose to equivalent glucose. $[\text{EtOH}]_f$ is the ethanol concentration at the end of the fermentation (g/L) minus any ethanol produced from the enzyme and medium; $[\text{EtOH}]_0$ is the ethanol concentration at the beginning of the fermentation (g/L), mainly from the seed inoculation. The volume in the unit of g/L only refers to the liquid fraction in the reaction system, does not refer to the volume of the whole slurry. The liquid volume was calculated based on the water mass balance of the SSF operation. The ethanol concentration in the liquid phase was analyzed using HPLC as described above. Then the accurate ethanol could be obtained using the above equation.

Analysis of Viscosities of Liquid Slurry

Apparent viscosity of the hydrolysate slurry and fermentation broth was measured by taking samples periodically during SSF using ARES Rheometer (TA Instruments, Inc., New Castle, DE) with a parallel plate geometry of 25 mm in plate diameter. The rheological experiments were conducted at 37°C and the steady rate sweeping tests were conducted within the range of 0.01–250 s^{-1}. The average viscosity of the fluid was estimated using the approach of Metzner and Otto (1957):

$$\dot{\gamma} = K_s N$$

where $\dot{\gamma}_{avg}$ is an average shear rate (s^{-1}), K_s is a constant which depends upon the vessel impeller configuration, and N is the impeller rotational speed (rev/s). For single helical impeller, K_s value of 33 was used according to Tanguy et al. (1997) whose impeller configuration was similar to the present one. From the knowledge of the speed of rotation, one can estimate the effective shear rate which in turn can be used to get the corresponding effective viscosity μ_a (Pa s), directly from the measured rheology data.

CFD Modeling

The bioreactor geometry was incorporated into the commercial CFD software CFX 11.0 (ANSYS Inc., Canonsburg, PA). The fluid was simplified as the uniform liquid phase with the same viscosity value at the SSF temperature and rotation rate. The effects of water-insoluble solid particles from the pretreated CS, the gas bubbles released during fermentation, as well as the mixing of the pretreated CS with the cellulase and inoculation on the rheology value were neglected.

Results

Mixing and Energy Consumption During SSF at High Pretreated Corn Stover Loading

Two impellers, the Rushton impeller and the helical impeller, were applied to the SSF under the high solids

loading of pretreated CS. Figure 1a shows a bioreactor with a modified helical impeller used for this study. Figure 1b shows a most commonly used Rushton impeller in the low viscous Newtonian fluid mixing. An improvement on the Rushton impeller was the slant paddle angles toward the circulation direction. On the other hand, the helical impeller and its variants shown in Figure 1c were one of the options for high viscous non-Newtonian fluid agitation. The performances of SSF under high pretreated CS loading using the two modified impellers were compared with respect to the ethanol production and energy consumption as shown in Figure 2.

Figure 2 shows that the feeding time of pretreated CS into the bioreactor using the helical impeller was at least 2 h shorter than that using Rushton impeller. The feeding of pretreated CS only proceeded when the solid CS was liquefied into slurry state. Thus, the shorter feeding time indicated that the mixing using the helical impeller was better than that using the Rushton impeller. During the SSF process, especially in the prehydrolysis stage (before inoculation), the quick liquefaction of the pretreated CS into a slurry was important and the helical impeller was clearly superior to that of Rushton impeller. Figure 2a indicates that both the glucose consumption rate and the ethanol production rate using the helical impeller were greater than that using Rushton impeller. The glucose concentration, 76.7 g/L with the helical impeller and 77.3 g/L with the Rushton impeller, was similar in the hydrolysate slurry before inoculation because of the glucose inhibition on the Accellerase 1000 enzyme. However, the ethanol concentration at the end of SSF process was significantly different, 51.0 and 43.9 g/L for the helical and the Rushton impellers, respectively. Figure 2b shows the time courses of xylose and acetic acid released from hemicellulose during the SSF operation. Xylose maintained at the low level in the hydrolysate and the fermentation broth (less than 20.0 g/L) during the SSF operation. Xylose was not consumed and even increased slightly during the SSF operation. Acetic acid was also constant with a slight increase during the operation. Although the acetic acid concentration in this experiment was up to 5.3 g/L, the enzymatic saccharification and fermentation performance was not inhibited by the existence of acetic acid significantly. Figure 2c indicates that the stirring power consumption using the helical impeller was significantly smaller than that using Rushton impeller. The results indicated that the helical impeller had better performances on ethanol yield, ethanol productivity, and energy consumption, largely because of the improved mixing by the helical impeller.

Figure 3 shows the CFD simulation results of bioreactors with the two impellers. Figure 3a indicates that velocity distribution using the helical impeller under the high viscous system was a large vertical circulation in the bioreactor, with the upward flow near the axis range and downward flow in the range between the outer edge of the impeller and the bioreactor wall. Several smaller inner circulation fields were formed around the helical spiral bladders. The velocity

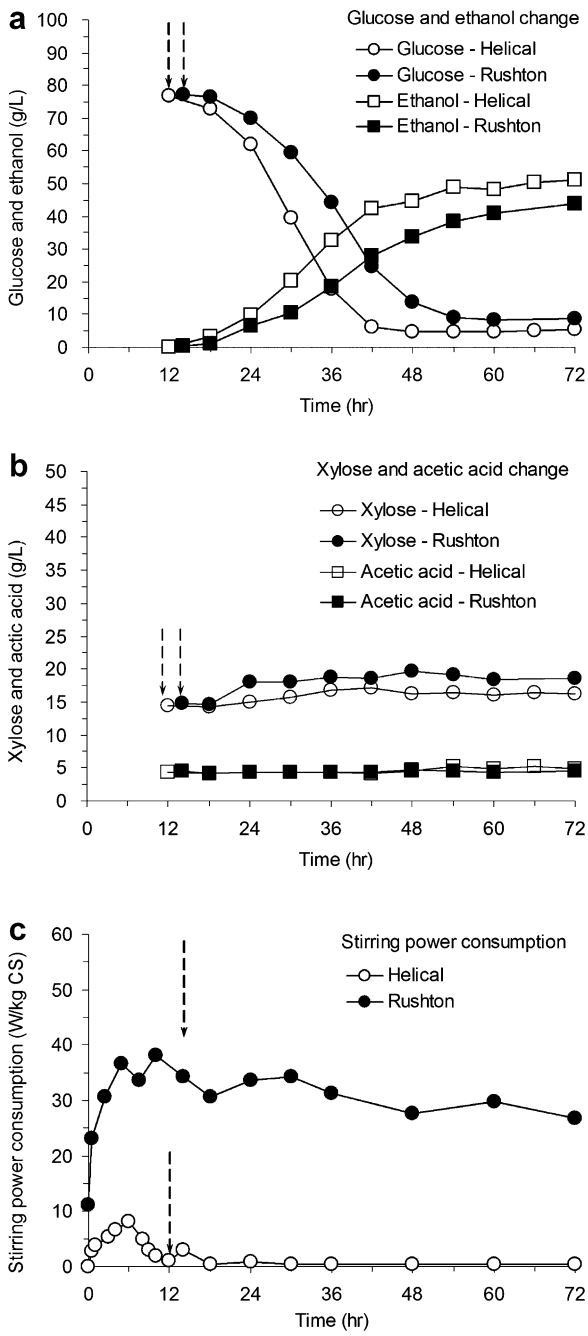

Figure 2. Simultaneous saccharification and fermentation at high pretreated CS loading using different stirring impellers. **a**: Glucose consumption and ethanol production with helical and Rushton impellers; (**b**) xylose and acetic acid formation with helical and Rushton impellers; (**c**) stirring energy consumption using helical and Rushton impellers. Dotted arrows indicate inoculation time. Conditions: Pretreated CS at 30.0% DM (w/w), Accellerase 1000 dosage 15.0 FPU/g DM, 50°C for prehydrolysis (before inoculation), and 37°C for SSF (after inoculation), 120 rpm stirring rate for helical and 200 rpm for Rushton.

values distributed uniformly both in axial and radius directions inside the whole bioreactor without showing a large difference, except the small ranges near the outer edge of the impeller bladders. The velocity distribution using

Rushton impeller as shown in Figure 3b was significantly differentiated: in the impeller stirring range the velocity value was high but low in the upper range of the viscous system. However, the mixing fields were limited to only about one-third of the whole volume of the bioreactor comparing to the whole bioreactor with helix impeller. Comparison of the results between the two fluid fields using helical and Rushton impellers indicated that the helical fluid was better in mixing efficiency than that of the Rushton fluid.

Effect of Inoculation Time and Ratio at High Pretreated CS Loading

The temperature difference between the hydrolysis (50°C) and the fermentation (30–37°C) indicates that the SSF operation was divided into two stages, the first prehydrolysis stage to achieve a higher hydrolysis yield at high temperature, and the second SSF stage both for enzymatic hydrolysis and ethanol fermentation at lower temperature. Figure 4 shows the effect of inoculation time by a time interval of 12 h and the inoculation ratio on the glucose consumption and ethanol yield under the high pretreated CS loading of 29.0% (w/w).

Figure 4a shows that the maximum glucose concentration increased with prolonged prehydrolysis time, but the accumulated glucose was consumed quickly after the inoculation in all three cases. The operations at the inoculation time of 12 and 24 h showed the almost same ethanol concentrations (51.8 and 52.1 g/L) at the fermentation ends (72 h), although the difference of glucose concentration before inoculation was great (58.5 and 81.0 g/L). The glucose concentration accumulated to a high value (92.2 g/L) when inoculation started at 36 h, but the ethanol concentration was only 48.6 g/L.

Figure 4b shows the effect of SSF operation at different inoculation ratio (10.0% and 20.0%, v/v) on the ethanol yield. The result showed that the glucose consumption was essentially the same while the ethanol yield increased a little at the end of SSF fermentation (from 51.2 to 55.1 g/L), showing that the increased inoculation ratio had minor effect on the ethanol yield at the double inoculation. Considering the ethanol production costs reduction, we choose 10.0% (v/v) as the inoculation ratio in all the other experiments.

SSF at High Solids Loading: Effect of Pretreated CS Loading on SSF Performance

The solids loading range was tested from 15.0% (w/w) to 30.0% (w/w) in the modified helical impeller bioreactor (Table I and Fig. 1) and its SSF performance was investigated. No detoxification to the pretreated CS such as water washing was used because the washing led to the high water content. In the prehydrolysis state, the power consumption for mixing was significantly greater than that

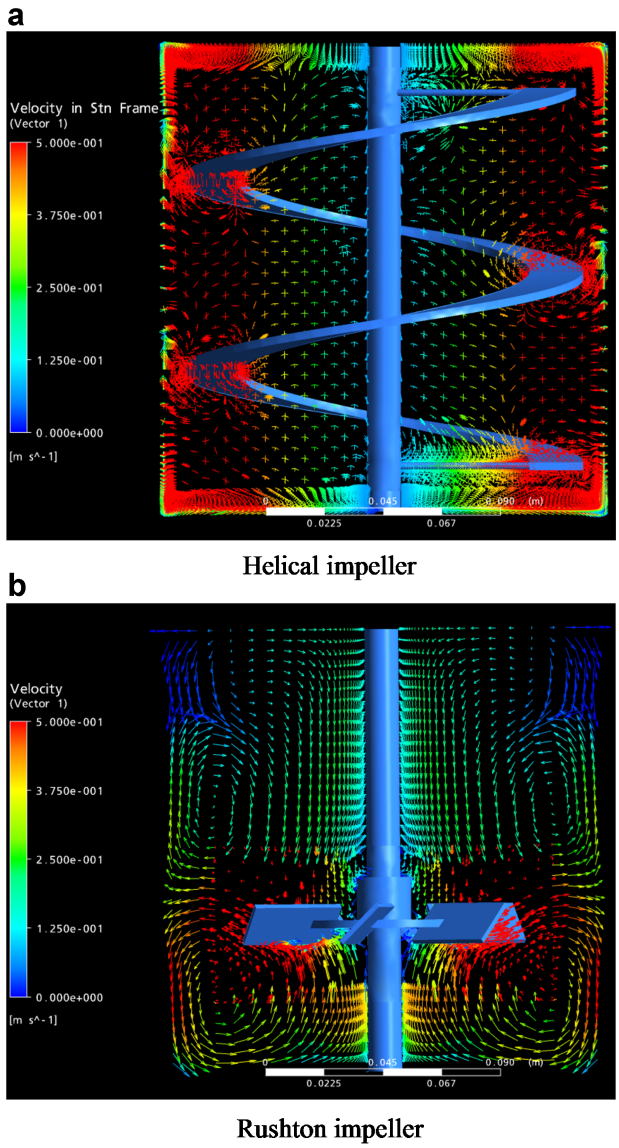

Figure 3. Fluid field distribution of bioreactors with helical impeller and Rushton impeller using CFD software. **a**: Fluid field distribution with helical impeller; and (**b**) fluid field distribution with Rushton impeller. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

in the following SSF stage after inoculation because of the existence of the unliquefied CS particles. To enhance the mixing between the cellulase liquid and the pretreated CS solids, the hydrolysis should be always carried out at a proper velocity to keep the reaction system in the liquid state or the liquid slurry state. If the hydrolysis of the pretreated CS solids was not sufficient, the large portion of the solid particles was circulated by the impeller stirring in the bioreactor. This situation would lead to the poor mixing between the cellulase liquid and the pretreated CS solids, as well as the high stirring energy consumption. The proper hydrolysis velocity required a proper feeding rate of the solid pretreated CS to maintain the reaction system at the slurry

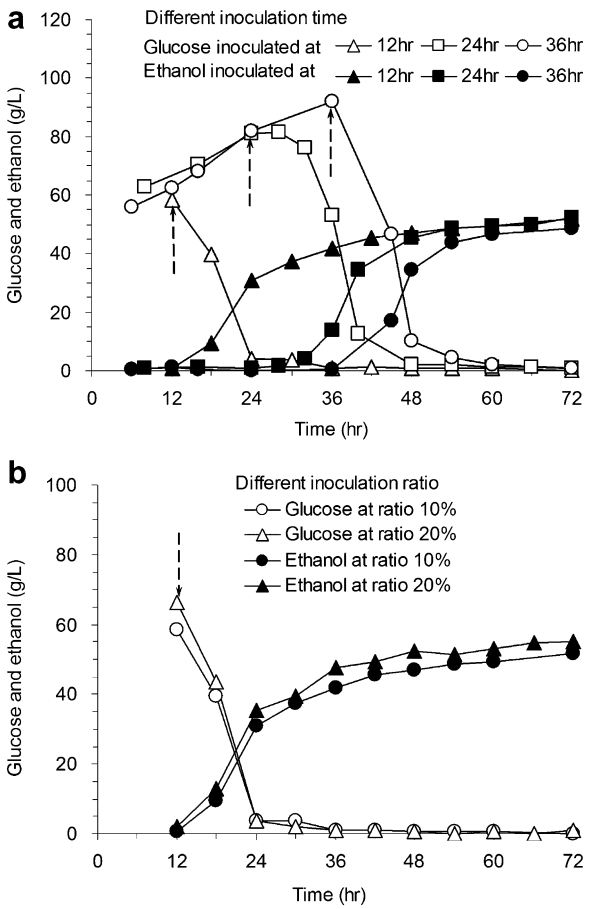

Figure 4. Effect of inoculation time and ratio on SSF performance at high pretreated CS loading at (**a**) different inoculation time and (**b**) different inoculation ratio. Dotted arrows indicate inoculation time. Conditions: Pretreated CS loading at 29.0% DM (w/w), Accellerase 1000 dosage 15.0 FPU/g DM, 50°C for prehydrolysis (before inoculation), and 37°C for SSF (after inoculation), 120 rpm stirring rate.

state under the given mixing condition. The feeding time directly reflected the mixing efficiency. Table II shows that the feeding time is increased sharply with increasing pretreated CS loading (0.5, 2.0, 5.0, and 10.0 h for the pretreated CS of 15.0%, 20.0%, 25.0%, and 30.0%, respectively) at the low cellulase dosage (7.0 FPU/g DM).

Table I. Material feeding in the SSF operations at different pretreated CS loadings.

Required solids loading (%, w/w)	15.0	20.0	25.0	30.0
Total pretreated CS (g) (*A*1)	1,000.0	1,300.0	1,800.0	2,000.0
Moisture of the pretreated CS (%, w/w) (*A*2)	64.8	63.9	65.4	65.6
Deionized water (mL) (*A*3)	1,280.0	970.0	600.0	200.0
Enzyme dosage (FPU/g DM)	7.0	7.0	7.0	7.0
Enzyme (mL) (*A*4)	38.0	50.0	67.0	74.0
Inoculum (mL) (*A*5)	20.0	20.0	20.0	20.0
True solids loading (%, w/w)	15.1	20.1	24.9	30.0

Solids loading calculation: $A1 \times (1 - A2/100)/(A1 + A3 + A4 + A5) \times 100\%$. For instance, the solids loading 30.0% was calculated by $2{,}000.0\ (1 - 65.6/100)/(2{,}000.0 + 200.0 + 74.0 + 20.0) = 30.0\%$.

Table II. Feeding time of pretreated CS at different CS loadings.

Solid CS loading (%, w/w)	15.0	20.0	25.0	30.0
Feeding time (h)	0.5	2.0	5.0	10.0

Conditions: Accellerase 1000 dosage 7.0 FPU/g DM, 50°C for prehydrolysis (before inoculation), and 37°C for SSF (after inoculation), 120 rpm stirring rate.

The result indicated that the mixing at high solids loading was getting difficult even with a small increase in pretreated CS solids, especially from 25.0% to 30.0% solids loading.

Figure 5 shows the SSF performance at different pretreated CS loadings and low dosage of cellulase enzyme. The sampling was taken from the slurry after the pretreated CS was fed completely to avoid ratio change of cellulase dosage per unit of CS dry matter. This was the reason for no sampling for solids loading over 25.0% (w/w) during the prehydrolysis. Figure 5a indicates that the glucose accumulated during prehydrolysis but quickly consumed after inoculation of *S. cerevisiae* DQ1. The glucose concentration at the inoculation time increased with the solids loading increasing, 26.6, 29.8, 39.9, and 43.1 g/L corresponding to the solids loading of 15.0%, 20.0%, 25.0%, and 30.0% (w/w), respectively. However, the glucose was consumed out within 12 h after inoculation and then remained at a low level in all solids loading cases. The result indicated that the rate-limiting step for ethanol production was the enzymatic hydrolysis of the pretreated CS into glucose, rather than the fermentation of the hydrolyzed sugar to ethanol by *S. cerevisiae* DQ1. The theoretical ethanol concentration, assuming that all the cellulose in the original CS was completely converted into glucose and then completely converted into ethanol, was calculated as shown in Figure 5b. At a low cellulase dosage of Accellerase 1000 (7.0 FPU/g DM), the ethanol concentration in the final fermentation broth increased from 24.7, 31.0, and 39.3 g/L to 40.6 g/L with increasing pretreated CS loading from 15.0%, 20.0%, 25.0% to 30.0% (w/w), while the ethanol yield to the theoretical value decreased from 76.5%, 68.0%, 64.8% to 52.1% with increasing CS loading. The difference in ethanol concentration between the theoretical value and the experimental data increased as pretreated CS loading increases. This indicated that the conversion efficiency was decreased. As the rate-limiting step, the low hydrolysis rate of the pretreated CS was the major reason for the conversion efficiency decrease.

Figure 5c shows the dynamic viscosity changed during SSF at different high pretreated CS loadings. The sampling started at the end of prehydrolysis (12 h) and the viscosity was immediately measured to avoid any change in the samples. The data were fluctuated because the non-hydrolyzed CS fiber particles and ash particles in the samples significantly affected the viscosity measurement, especially at the early stage of prehydrolysis and SSF. The viscosity data in Figure 5c were taken in the experimental shear rate of the helical impeller (120 rpm stirring rate). The viscosity of the SSF slurry increased with increasing

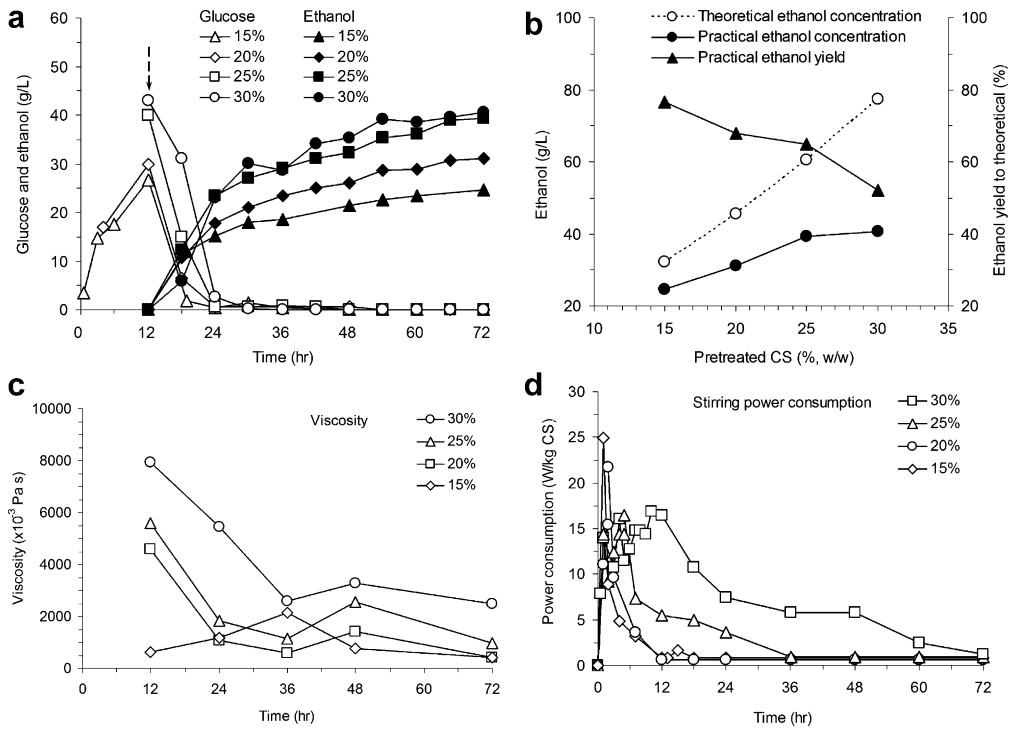

Figure 5. SSF at different pretreated CS loading. **a**: Time courses of glucose consumption and ethanol yield; (**b**) ethanol concentration and yield change with pretreated CS; (**c**) time courses of the viscosity at the real SSF shear rate; (**d**) time courses of the stirring power consumption. Dotted arrows indicate inoculation time. Conditions: Accellerase 1000 dosage 7.0 FPU/g DM, 50°C for prehydrolysis (before inoculation) and 37°C for SSF (after inoculation), 120 rpm stirring rate. The material feeding and the solids loading calculation were shown in Table III.

pretreated CS loading, decreased sharply with the SSF time in all CS loading cases. This sharp decrease corresponded to the glucose consumption and ethanol generation.

Figure 5d shows the stirring power consumption of the helical impeller at different CS loading. Very similar to viscosity and glucose consumption changes, the stirring power increased significantly with increasing pretreated CS loading and decreased sharply with prehydrolysis and SSF time. The correspondence between viscosity and the stirring power consumption indicated that the stirring resistance was from the apparent viscosity of the SSF system. The fast hydrolysis gave a quick decrease in viscosity and stirring power consumption simultaneously.

SSF at High Solids Loading: Effect of Enzyme Dosage on SSF Performance

Reducing cellulase enzyme dosage per unit of pretreated CS dry matter is important for cost reduction of cellulose ethanol production. This experiment was carried out at the high pretreated CS loading of 30.0% (w/w) with different cellulase enzyme dosage of Accellerase 1000 (Table III).

Table IV shows that the feeding time decreased with increasing enzyme dosage (10.0, 8.5, and 7.0 h for the enzyme dosage of 7.0, 15.0, and 30.0 FPU/g DM, respectively) at the high pretreated CS loading of 30.0% (w/w). The results indicated that enzymatic hydrolysis rate was greatly improved by using higher enzyme dosage.

Figure 6 shows the SSF performance with different enzyme dosages at CS loading of 30.0% (w/w). Figure 6a indicates that the initial glucose released during prehydrolysis increased from 43.1 and 59.1 g/L to 80.4 g/L with the increasing enzyme dosage from 7.0 and 15.0 FPU/g DM to 30.0 FPU/g DM. The glucose was (completely) consumed within 18 h after inoculation and then remained at a low level in the following SSF operation. Figure 6b demonstrates that the double enzyme dosage from 7.0 FPU/g DM to 15.0 FPU/g DM significantly increased the ethanol yield and concentration from 52.1% to 75.9% and 40.6 to 59.3 g/L, respectively, but the further doubling of the enzyme dosage from 15.0 to 30.0 FPU/g DM gave the limited

Table III. Material feeding during the SSF operations at different enzyme dosages.

Required enzyme dosage (FPU/g DM)	7.0	15.0	30.0
Required solids loading (%, w/w)	30.0	30.0	30.0
Total pretreated CS (g) (*A*1)	2,000.0	2,000.0	2,000.0
Moisture of the pretreated CS (%, w/w) (*A*2)	65.6	64.0	63.7
Deionized water (mL) (*A*3)	200.0	210.0	57.0
Enzyme (mL) (*A*4)	74.0	165.0	300.0
Inoculum (mL) (*A*5)	20.0	20.0	20.0
True solids loading (%, w/w)	30.0	30.0	30.6

Solids loading calculation was similar to Table III.

Table IV. Feeding time of pretreated CS at different Accellerase 1000 enzyme dosages.

Accellerase 1000 enzyme dosage (FPU/g DM)	7.0	15.0	30.0
Feeding time (h)	10.0	8.5	7.0

Conditions: Pretreated CS loading 30.0% (w/w), 50°C for prehydrolysis (before inoculation), and 37°C for SSF (after inoculation), 120 rpm stirring rate.

improvement on ethanol yield and concentration from 75.9% to 82.8% and 59.3 to 64.6 g/L, respectively. The result indicated that in the economic sense there might be an optimal enzyme dosage at a fixed operation condition to give maximum ethanol yield.

Figure 6c shows the dynamic viscosity changed at different enzyme dosages with the experimental shear rate of the helical impeller (120 rpm stirring rate). The viscosity decreased with increasing enzyme dosage, and with SSF time. Similar to the viscosity change, the stirring power consumption of the helical impeller decreased with increasing enzyme dosage and SSF time as shown in Figure 6d. The result indicated that the increased enzyme dosage significantly increased the mixing, hydrolysis, and ethanol yield, as well as decreasing stirring power consumption; the increased cellulase enzyme use would be an important help in improving the SSF performance at the high pretreated CS loading.

Discussion

Our starting point in this work was to find a practical approach to increase the ethanol titer in the SSF process under the high pretreated CS loading. The high feedstock loading efficiently improved the ethanol product concentration in the SSF system, but severe problems arose to this unusual system: high solids loading, high viscous, mixing of liquid (enzyme and seeds) with solid (pretreated CS), simultaneous enzyme reaction and fermentation, and furthermore, all these performances and properties were always changing. The mixing between the large portion of the pretreated CS solid and the small portion of the liquid enzyme and the inoculation seeds under the high solids loading during the SSF process is crucial important. A well mixed SSF system should be constantly maintained in the

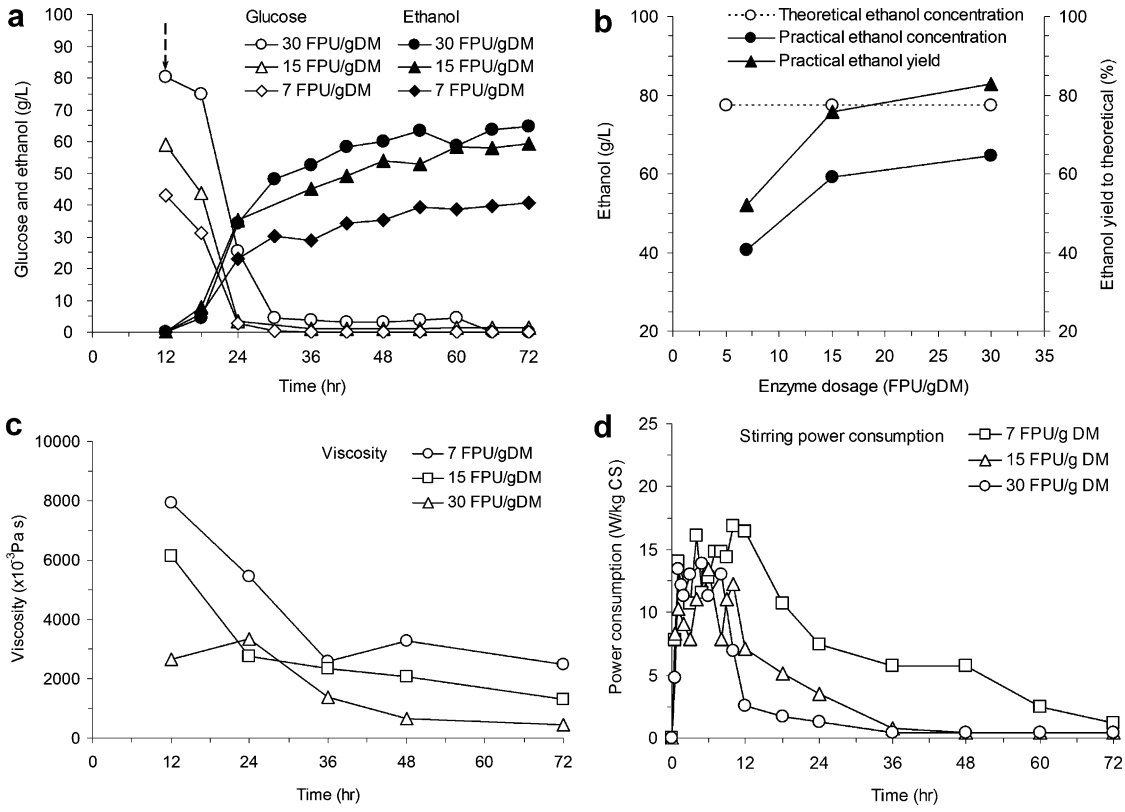

Figure 6. SSF at different Accellerase 1000 enzyme dosage. **a**: Time courses of glucose consumption and ethanol yield; (**b**) ethanol concentration and yield change with enzyme dosage; (**c**) time courses of the viscosity at the real SSF shear rate; (**d**) time courses of the stirring power consumption. Dotted arrows indicate inoculation time. Conditions: Pretreated CS loading 30.0% (w/w), 50°C for prehydrolysis (before inoculation), and 37°C for SSF (after inoculation), 120 rpm stirring rate. The material feeding and the solids loading calculation were shown in Table IV.

liquid slurry state to ensure the progressing of enzymatic hydrolysis. To realize this well mixing state, the pretreated CS should be fed slowly enough, but in the practical operation the CS solid should be fed as soon as possible to finish the fed-batch operation within a short period of time and relieve the product inhibition on cellulase enzyme by fermenting the hydrolyzed sugars into ethanol. The SSF operation is inevitably carried out under high viscous slurry containing high content of unliquefied CS particles. The mixing efficiency and the stirring energy consumption are the major concern of the practical bioreactor design.

Rushton impeller is the most commonly used paddle in the low viscous Newtonian fluid fermentation bioreactor. However, apparently the Rushton impeller is not a suitable option for the high viscous slurry system as experimentally verified in the study. The focus of this study is on a modified helical impeller bioreactor and its application for SSF under high pretreated CS loading. First the two commonly used impellers for fermentation, the Rushton and modified helical impeller, were compared in terms of stirring power consumption and SSF performances. Clearly the helical impeller showed advantages in energy conservation, ethanol yield, and the ethanol titer in the final fermentation broth. The better performances were explained by the CFD modeling, showing that the thorough and uniform velocity distribution was found in the modified helical impeller stirring system, comparing that of Rushton impeller's.

After gaining this knowledge, a series of SSF experiments were carried out and various parameters related to SSF at high solids loading were taken into account, such as prehydrolysis and inoculation conditions, different solids loading, different enzyme dosage. The effects of these parameters on the SSF performances were investigated by observing pretreated CS feeding, glucose consumption, ethanol yield and final titer, viscosity tendency, power consumption, etc. All the experiments used the pretreated CS directly without detoxification treatment. We found that the practical maximum pretreated CS loading 30.0% (w/w) could be applied for SSF operation using the modified helical impeller bioreactor, and gave a good ethanol yield at the proper condition. The fresh steam explosion pretreated CS contained approximately 50.0–60.0% (w/w) water. When the CS was used as the feedstock, the maximum solid content generally was no more than 30.0% (w/w) when the liquid cellulase enzyme, inoculation seeds, and the necessary liquid nutrition were added to the bioreactor. This was the reason for selecting 30.0% (w/w) as the ceiling value of solids loading for SSF in this study (Tables I and III).

At the highest solids loading of the pretreated CS up to 30.0%, the ethanol titer in a broth reached 40.0 and 59.3 g/L after 72 h SSF process at relative low enzyme dosage of 7.0 and 15.0 FPU/g DM, respectively. If the enzyme dosage increased to 30.0 FPU/g DM, the ethanol titer could reach 64.6 g/L. The ethanol yields were 52.1%, 75.9%, and 82.8% for the enzyme dosage of 7.0, 15.0, and 30.0 FPU/g DM, respectively. Mohagheghi et al. (1992) used the dilute acid pretreated wheat straw at a solids loading of 32.3% (w/w) and achieved the maximum ethanol concentration of 57 g/L after 144 h SSF operation. De Bari et al. (2002) used the steam exploded aspen wood at a solids loading of 16% (w/w) with hot water washing for detoxification and the optimal ethanol concentration of 47 g/L in the fermentation flask. Jørgensen et al. (2007) used the steam pretreated wheat straw at a solids loading of 35% (w/w) and the ethanol concentration reached 62 g/L (converted from the value of 48 g/kg (w/w) in the original reference for easy comparison) after 96 h SSF operation.

The mixing energy consumption is crucially important for the SSF operation under high solids loading. To evaluate the mixing energy consumption at different solids loadings and different enzyme dosages, the data in Figures 5 and 6 were translated into the mixing energy consumption ($E1$) and the thermal energy in the ethanol produced ($E2$) as shown in Tables V and VI. Table V showed that the mixing consumption increased one order of magnitude with the increased solids loading from 15.0% to 30.0% (w/w) (79.5, 113.6, 340.5, and 1,009.2 MJ/t slurry for the solids loading of 15.0%, 20.0%, 25.0%, and 30.0%, respectively). On the other hand, the thermal energy in the ethanol produced increased less than twice with the increasing solids loading (854.9, 1,150.8, 1,551.3, and 1,723.2 MJ/t slurry for the solids loading of 15.0%, 20.0%, 25.0%, and 30.0%, respectively). The ratio of the mixing energy consumption ($E1$) to the thermal energy production in the ethanol ($E2$) was increased exponentially with the increasing solids loading (9.3%, 9.9%, 21.9%, and 58.6% for the solids loading of 15.0%, 20.0%, 25.0%, and 30.0%, respectively). The analysis indicated that at the low cellulase enzyme dosage (7.0 FPU/g DM at the SSF operation in Fig. 5), the extremely high solids loading of the pretreated CS should be prohibited because of the high mixing energy consumption. For instance, the mixing energy consumption under the 30.0% (w/w) solids loading was responding to the 58.6% of the total thermal energy in the ethanol produced.

While the extremely high solids loading was prohibited at the low enzyme dosage (7.0 FPU/g DM), Table VI gives us another picture for the mixing energy consumption at the increased enzyme dosage. The mixing energy consumption

Table V. Mixing energy consumption during the SSF operations at different solids loadings.

Solids loading (%, w/w)	Mixing energy consumption ($E1$) (MJ/t slurry)	Thermal energy in the ethanol produced ($E2$) (MJ/t slurry)	$E1/E2$ (%)
15.0	79.5	854.9	9.3
20.0	113.6	1,150.8	9.9
25.0	340.5	1,551.3	21.9
30.0	1,009.2	1,723.2	58.6

For the material feeding and the SSF operation conditions refer to Table III and Figure 5. The unit MJ/t slurry here refers to the energy per ton of the slurry in the bioreactor. The higher heating value (HHV) for ethanol is 23.4 MJ/L, and the ethanol density (average) is 790 kg/L.

Table VI. Mixing energy consumption during the SSF operations at different enzyme dosages.

Enzyme dosage (FPU/g DM)	Mixing energy consumption ($E1$) (MJ/t slurry)	Thermal energy in the ethanol produced ($E2$) (MJ/t slurry)	$E1/E2$ (%)
7.0	1,009.2	1,723.2	58.6
15.0	424.7	2,511.5	17.0
30.0	347.0	2,738.2	12.7

For the material feeding and the SSF operation conditions refer to Table IV and Figure 6. The unit MJ/t slurry here refers to the energy per ton of the slurry in the bioreactor. The higher heating value (HHV) for ethanol is 23.4 MJ/L, and the ethanol density (average) is 789 kg/L.

during the SSF operation decreased quickly with the increasing enzyme dosage (58.6%, 17.0%, and 12.7% for the enzyme dosage of 7.0, 15.0, and 30.0 FPU/g DM, respectively). The result indicated that increasing the cellulase enzyme loading not only improved the ethanol yield but also significantly reduced the mixing energy consumption. The result again demonstrated the importance of low-cost cellulase enzyme from a new aspect, the mixing energy cost of SSF operation, for cellulose ethanol production. The extremely high solids loading of 30.0% (w/w) could still be considered in the application of the SSF operation if the enzyme cost was reduced significantly.

As described in the beginning of this article, the purpose using the high solids loading of the pretreated CS in the SSF operation was to increase the ethanol titer in the fermentation broth, so that the energy cost in the ethanol distillation step could be reduced. However, the results of this study also revealed the other fact, that is, the increase in solids loading could significantly increase the mixing energy consumption, especially at the extremely high solids loading and the low enzyme dosage. Therefore, there should be a balance for achieving the optimal energy cost between the increased mixing energy cost and the reduced distillation energy cost when the solids loading is increased. The improved pretreatment efficiency, the improved mixing efficiency, the improved catalytic efficiency, and the reduced cost of cellulase enzyme as well as other improvements in the lignocellulose processing would certainly contribute to the reduction of the energy cost during the SSF operation.

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