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Simultaneous saccharification and microbial lipid fermentation of corn stover by oleaginous yeast *Trichosporon cutaneum*

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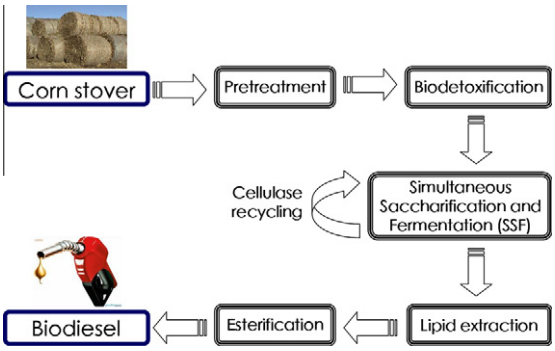
HIGHLIGHTS

- The SSF of corn stover was tested on microbial lipid fermentation for the first time.
- The SSF of corn stover was effective in both the small scale (5 L) and the enlarged scale (50 L) bioreactors.
- Cellulase enzyme could be partially recycled in the SSF of corn stover.

GRAPHICAL ABSTRACT



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ABSTRACT

Simultaneous saccharification and fermentation (SSF) is the most commonly practiced operation in lignocellulose bioconversion to avoid the sugar product inhibition to cellulase enzymes. In this study, for the first time SSF was tested on microbial lipid fermentation using the diluted acid pretreated and biodetoxified corn stover. The results show that SSF was effective than the separate hydrolysis and fermentation (SHF) on lipid accumulation of *Trichosporon cutaneum* CX1 cells in both the small scale (5 L) and the enlarged scale (50 L) bioreactors. The solutions for the oxygen transfer and the lipid extraction in SSF practically worked well. The process parameters were optimized and the lipid yield obtained were 3.03 g/L in the 5 L, and 3.23 g/L in the 50 L, respectively. The result also shows that the cellulase enzyme could be partially recycled in the SSF. The study provided a practical and efficient way for microbial lipid production from lignocellulose material.

1. Introduction

Biodiesel industry has been heavily restricted by the shortage of lipid feedstock supply and the searching for new lipid resources is the key issue for the survival of biodiesel industry. Among various options, microbial lipid provided a relatively practical option comparing to other methods (Koblitz et al., 2006). Oleaginous microorganisms quickly accumulate vegetable oils like intracellular lipids with high lipid titer in fermentation broth (Ratledge and Wynn, 2002). If lignocellulose biomass such as corn stover, wheat straw, switchgrass could be used as the feedstock of microbial lipid fermentation, the microbial lipid would be much more cost competitive than that using starch or sucrose because of its abundance and low value property.

Several groups had investigated the microbial lipid production from lignocellulose using the separate hydrolysis and fermentation (SHF) process: lignocellulose feedstock was first pretreated and hydrolyzed into the liquid hydrolysate containing the fermentable sugars such as glucose and xylose, then the hydrolysate was

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fermented into lipid by oleaginous microorganisms. Huang et al. (2009) prepared the hydrolysate from the sulfuric acid treated rice straw and then for lipid fermentation by *Trichosporon fermentans*. Yu et al. (2011) prepared the dilute sulfuric acid pretreated wheat straw hydrolysate and used for lipid fermentation by several yeast strains including *Cryptococcus curvatus*, *Rhodotorula glutinis*, *Rhodosporidium toruloides*, *Lipomyces starkeyi*, and *Yarrowia lipolytica*. In our previous study, a mutant *Trichosporon cutaneum* strain was obtained and used to convert the dilute sulfuric acid pretreated and biologically detoxified corn stover into lipid of 3.1 g/L under 15% solid loadings by SHF process (Chen et al., 2009; Huang et al., 2011).

The most commonly practiced operation in lignocellulose bioconversion is simultaneous saccharification and fermentation (SSF) to avoid the product inhibition of the accumulated sugars to cellulase enzymes (Kim et al., 2008; Lynd et al., 2008). The SSF operation was typically used for cellulosic ethanol production, but the application of SSF to microbial lipid production from lignocellulose has not been reported. The major consideration of the absence of SSF in lipid fermentation may come from the intracellular existence of lipid in the oleaginous microorganisms. The intracellular lipid has to be recovered in solid phase and will go to the solids residues composed of lignin components if the SSF is used for lipid fermentation. The recovery of lipid may be difficult by the common solvent extraction method. Another possible consideration may be the difficulty of oxygen input to the aerobic oleaginous microorganism fermentation, because of the viscous property of the SSF operation, especially at the high solids loading of lignocellulose (Ageitos et al., 2011; Beopoulos et al., 2011; Zhang et al., 2010a).

In the present study, the SSF operation using the diluted acid pretreated and biologically detoxified corn stover was tested on the microbial lipid production using a mutant strain *T. cutaneum* CX1 developed in our previous studies (Chen et al., 2009; Huang et al., 2011). The results show that the SSF operation well fits the microbial lipid production and the lipid yield was better than that of the SHF using hydrolysate in both 5 L and the enlarged 50 L fermentors. The two major considerations for SSF, lipid recovery and oxygen input, were proved to be not the essential barriers for the SSF of lipid production. This study may provide an interesting and perhaps a practical method for lipid production from lignocellulose material.

2. Methods

2.1. Strains and mediums

T. cutaneum CX1 was a mutant strain of *T. cutaneum* CGMCC 2.1374 from China General Microbiological Culture Collection Center (CGMCC), Beijing, China. The strain was cultured in yeast peptone dextrose (YPD) medium (glucose 20 g/L, peptone 10 g/L, yeast extract 10 g/L, pH 6.0) for 24 h, then the culture was aliquoted into 1.0 mL microtubes containing 30% (w/w) glycerol and stored at −80 °C freezer. The strain was maintained at 4 °C on YPD agar slants and sub-cultured from the −80 °C freezer once a month. Each YPD agar slant containing the colonies of *T. cutaneum* CX1 was picked by sterilized stick, and then this stick was incubated into 20 mL inoculum medium and cultured at 30 °C for 12 h as inoculum. The inoculum medium contains: 20 g/L glucose; 0.5 g/L yeast extract; 5 g/L $(NH_4)_2SO_4$; 1 g/L KH_2PO_4; 0.5 g/L $MgSO_4{\cdot}7H_2O$ and pH adjusted to 5.8–6.0.

The biodetoxification strain *Amorphotheca resinae* ZN1 was isolated and stored in our laboratory (Zhang et al., 2010a). A. resinae ZN1 was stored at 4 °C on potato dextrose agar (PDA) slants (potato 200 g/L, glucose 20 g/L, agar 20 g/L).

2.2. Reagents and raw materials

Corn Stover (CS) was grown in Shandong, China, and harvested in fall 2009. After collection, the materials were milled coarsely using a beater pulverizer and screened through a mesh with the circle diameter of 5 mm. The milled CS were washed to remove the field dirts, stones and metals, and then dried at 105 °C until the weight was constant and stored in sealed plastic bags for use.

All chemicals including KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4{\cdot}7\ H_2O$, yeast extract, and petroleum ether were purchased from the local supplier Lingfeng Chemical Reagent Co., in Shanghai, China. Methanol was purchased from Sinopharm Chemical Reagent Co., in Shanghai. Trichloromethane was purchased from Shanghai Chemical Reagent Co.

2.3. Pretreatment and biodetoxification of CS

The dilute sulfuric acid pretreatment of CS was performed in the pretreatment reactor described in Zhang et al. (2011). The feedstock was presoaked with diluted sulfuric acid solution with the solid (the dry material) to the liquid (sulfuric acid solution) ratio of 2:1 (w/w) and then fed into the reactor. The hot steam was jetted directly into the reactor to 190 °C, 1.2 MPa for 3 min at the sulfuric acid usage of 3.0 g/100 g of the dry solids. All the sulfuric acid solution and the steam condensed water were absorbed into the solids to give a dry solids content of 50% (w/w) of the pretreated CS.

For detoxification operation, the moisture and pH value of the pretreated CS were adjusted to 60% (w/w) and 6.0, respectively, using 5 M calcium hydroxide slurry and distilled water. *A. resinae* ZN1 spores on the PDA slant were washed by 20 mL sterilized water, and then the spore suspension was incubated into 200 grams pretreated CS and cultured at 25 °C for 3 days as inoculum. The pretreated CS was inoculated with 10% (v/v) and maintained at 25 °C for 7 days. After being autoclaved at 121 °C for 20 min, the biodetoxified CS was stored at −20 °C before feeding into the bioreactor. (Zhang et al., 2010b)

2.4. CS hydrolysate preparation

The biodetoxified CS was hydrolyzed using the commercial cellulase enzyme Accellerase 1000 (55.0 FPU/ml Genencor International, Rochester, NY, USA) with the enzyme dosage of 7 FPU/g DM at 50 °C, pH 4.8 for 48 h at 10% (w/w) solids loading. The solids in the hydrolysate were centrifuged at 10,000 rpm for 5 min, then autoclaved at 115 °C for 20 min in the same procedures as in Huang et al. (2011).

2.5. Lipid fermentation operation

A three-step adaptation procedure of *T. cutaneum* CX1 was carried out as follows: first, 1 mL inoculum of *T. cutaneum* CX1 as above paragraph 2.1 was inoculated into a 100 mL Erlenmeyer flask containing 20 mL of the sterilized CS hydrolysate at pH 5.0 and cultured at 30 °C, 180 rpm for 24 h; then, 20 mL of the culture was inoculated into a 1 L flask containing 250 mL of the sterilized CS hydrolysate at pH 5.0, cultured at 30 °C, 180 rpm for 18 h; finally, the culture was incubated into the 5 L fermentor (Baoxing Biotech, Shanghai, China) containing 2.5 L of sterilized CS hydrolysate to start the separate hydrolysis and fermentation (SHF). The precultures used in both the SHF and the SSF were exactly the same. The SHF operation was proceeded at 30 °C, pH 5.0 for 65 h with addition of 0.5 g/L $(NH_4)_2SO_4$ and 0.5 g/L $MgSO_4{\cdot}7H_2O$. 35 mL fermentation broth as the sample was taken every 8 h.

The simultaneous saccharification and fermentation (SSF) of the pretreated and biodetoxified CS was performed in a 5 L bioreactor.

The SSF process was operated at two stages: in the prehydrolysis stage, the cellulase enzyme was fed into the tank at the dosage of 3.0–15.0 FPU/g DM and pre-hydrolyzed for 0–24 h pH 4.8, 50 °C; then the SSF started by inoculating 250 mL of the *T. cutaneum* CX1 preculture into the prehydrolysis slurry (2.5 L) at the sterilized air rate of 0.2 vvm with the inlet pressure of 1.2 bar, the dissolved oxygen (DO) of 10–40%, $(NH_4)_2SO_4$ of 0–5.0 g/L at pH 5.0 and 30 °C. 35 mL fermentation broth as the sample was taken every 8 h. The scale-up of the SSF was carried out in a 50 L bioreactor with the inoculum of 10% (v/v) at the same conditions with that in the 5 L bioreactor.

2.6. Analysis on HPLC

Glucose and xylose were analyzed using HPLC (LC-20AD, refractive index detector RID-10A, Shimadzu, 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, and then filtered through a 0.22 μm filter before analysis.

2.7. Lipid content determination

Total lipid in the *T. cutaneum* cells was extracted using chloroform–methanol method, and estimated gravimetrically with modification on solvent use and removal (Folch et al., 1957). Briefly, the cell was disrupted in 10 mL of 4 mol/L hydrochloric acid for 30 min, then heated in boiled water for 10 min, and quenched in ice water. The cell lysate were homogenized with 20 mL chloroform–methanol (2:1, v/v) for 1 h and the solvent was removed using rotary evaporation method at 80 °C to remove all the residual solvent. If only the vacuum condition was applied to remove the solvent, the residual solvent in the lipid would add a significant error, because the lipid content in the microbial oleaginous cells was significantly lower than that of the animal lipid extraction used in Folch's method (Folch et al., 1957). Then, the residual was mixed with 20 mL petroleum ether for 30 min and then the mixture was centrifuged to remove the impurities, and the petroleum ether was removed by rotary evaporation at 80 °C. The total lipid was measured by gravimetric method.

2.8. Total nitrogen determination

Total nitrogen in the fermentation broth was determined according to alkaline potassium persulfate digestion-UV spectrophotometric method (D'Elia et al., 1977). First, 40 μL sample was diluted to 10 mL and then 5 mL alkaline potassium persulfate solution (40 g/L of $K_2S_2O_8$, 15 g/L of NaOH) was added. The solution was sterilized at 121 °C for 30 min and then 1 mL HCl (10%, v/v) was added. The absorbance was measured at 220 nm and 275 nm, and calculated by the formula $A = A_{220} - 2 \times A_{275}$. The total nitrogen was determined according to the standard curve using KNO_3 as the standard at the nitrogen concentration from 0 to 80 μg.

3. Results and discussion

3.1. Effect of the SSF parameters on the lipid production

The effects of several fermentation parameters on the microbial lipid production in the SSF of the pretreated and detoxified CS under the 10% solid loadings were investigated. The parameters included the prehydrolysis time, the cellulase enzyme dosage, the nitrogen content, and the dissolved oxygen (DO) level.

Table 1 shows the effects of nitrogen addition and DO level on the lipid accumulation of *T. cutaneum*. The lipid concentration increased from 2.86 g/L to 3.27 g/L with the DO increased from 10% to 20%, but then decreased to 2.75 g/L with the further increase of DO to 30%. When the DO increased to 40%, the SSF operation could not be processed after 38 h SSF because the foam formed on the top of the fermentation broth was unable to be eliminated. The DO was maintained mainly by adjusting the stirring rate under the constant air flow rate. For the present lipid fermentation operation, the greater DO requirement would exceed the upper limit of the stirring rate of the fermentor system. Therefore, the moderate DO value was selected for the lipid fermentation.

Nitrogen limitation is the most important requirement for lipid formation in the oleaginous yeast cells (Ratledge and Wynn, 2002). Table 1 shows that the minimum addition of $(NH_4)_2SO_4$ at 0.5 g/L did not make any change to the lipid accumulation, and the greater increase of the $(NH_4)_2SO_4$ addition from 0.5 to 1.0 g/L and 5.0 g/L led to the drop of lipid concentration from 3.03 to 2.70 and to 1.36 g/L, respectively. The result was in agreement with our previous results that the nitrogen content in the pretreated and biodetoxified CS might be sufficient and the further addition of $(NH_4)_2SO_4$ may lead to the decrease of lipid accumulation (Huang et al., 2011). Although the minimum addition of $(NH_4)_2SO_4$ showed no difference on lipid accumulation, the utilization rate and the lipid fermentation time of glucose were prolonged (data not shown) thus 0.5 g/L of $(NH_4)_2SO_4$ was added for maintain the proper fermentation period.

Fig. 1 shows the effect of prehydrolysis time on the lipid accumulation at different initial sugar concentrations created by the different prehydrolysis time. The result indicates that the initial glucose increased significantly with the prolonged prehydrolysis time, but the lipid concentration did not the observable change with the increasing prehydrolysis time. Therefore the minimum prehydrohysis time to generate the initial soluble sugar for cell growth at the starting time of SSF should be sufficiently enough.

Fig. 2 shows the effect of cellulase enzyme dosage on the lipid accumulation and the results indicates that although the initial glucose release increased with the increased cellulase dosage, the lipid concentration change was complicated. The lipid concentration increased from 1.95 g/L to 3.03 g/L when the cellulase dosage increased from 3.0 to 7.0 FPU/g DM, then decreased with the further increase of cellulase dosage from 7.0 to 15.0 FPU/g DM, although the initial glucose concentrations increased with the increasing cellulase dosage. The nitrogen consumption was almost constant at different cellulase enzyme dosage: 0.28, 0.28, 0.31, and 0.31 g/L at the cellulase of 3.0, 7.0, 11.0, and 15.0 FPU/g DM, respectively. The results might indicate that the nitrogen consumption at the different cellulase enzyme addition was not affected by the different cellulase addition. The results indicate that the lipid accumulation preferred a certain hydrolysis level generated by cellulase catalysis, and the high cellulase dosage may give negative effect for lipid accumulation. The substrate inhibition for the lipid accumulation was occurred in very low substrate (glucose) concentration (Economou et al., 2011).

Fig. 3 shows the SSF operation at different solids contents on the lipid accumulation. When the solids content in the SSF was increased to 15% (w/w), the lipid concentration increased to 4.02 g/L, almost 30% increased compared to that at the 10% (w/w) solids content (3.03 g/L). Meanwhile, the theoretical substrate (glucose) concentration was increased about 50% with the solid content from 10% to 15%. This makes clear that the increase of 30% is less than the expected 50% and therefore the process with the 15% loading performs less good than the 10%, although a higher lipid concentration is reached. At the same time, the operation time at the 15% (w/w) solids content was also increased to 134 h, almost doubled than that at the 10% (w/w) solids content (66 h). The result indicates

Table 1
Effects of dissolved oxygen level and nitrogen addition on the lipid accumulation in SSF.

	Dissolved oxygen (%)[a]				$(NH_4)_2SO_4$ (g/L)			
	10	20	30	40	0.0	0.5	1.0	5.0
Initial glucose (g/L)	18.22	16.16	17.66	16.28	16.10	16.92	17.12	16.56
Xylose (g/L)	7.32	6.72	7.50	7.02	6.46	6.23	7.21	7.05
Lipid concentration (g/L)	2.86	3.27	2.75	/	2.98	3.03	2.70	1.36

[a] At the 40% of dissolved oxygen saturation, the SSF could not continue because of the foam formation from the 38 h. Prehydrolysis conditions: 50 °C, pH 4.8, prehydrolysis time 6 h, Accellerase 1000 dosage at 7.0 FPU/g DM. SSF conditions: $(NH_4)_2SO_4$, 0–5.0 g/L; $MgSO_4$, 0.5 g/L; 30 °C, DO 10–40%, pH 5.0.

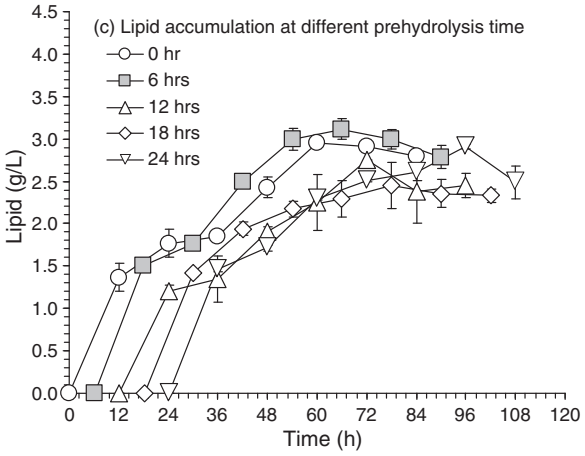

Fig. 1. Effect of prehydrolysis time on the lipid accumulation in SSF. (a) Glucose concentration; (b) xylcose concentration; (c) lipid accumulation. Prehydrolysis conditions: 50 °C, pH 4.8, the Accellerase 1000 at 7FPU/g DM. SSF conditions: $(NH_4)_2SO_4$, 0.5 g/L; $MgSO_4$, 0.5 g/L; 30 °C, DO 20%, pH 5.0.

that the SSF operation of lignocellulose material such as corn stover for lipid accumulation was different from cellulosic ethanol fermentation (Zhang et al., 2010a). In the SSF for ethanol production, the higher solids loading was preferred for obtain the high ethanol titer without considering the oxygen transfer factor because ethanol fermentation was an anaerobic process. However, the lipid fermentation was an aerobic process and the high solids loading may not apply because the high viscosity caused by the high solids loading severely limited the efficient oxygen transfer. Therefore a moderate solids loading of 10% (w/w) was selected for the SSF in the lipid fermentation.

3.2. Comparison of the operation modes: SHF and SSF

The separate hydrolysis and fermentation (SHF) and the simultaneous saccharification and fermentation (SSF) operation modes were compared using the pretreated and biodetoxified CS as the feedstock for microbial lipid fermentation. The same saccharification conditions was carried out for both the SHF and SSF at pH 4.8 and 50 °C at the 10% solids loading of the CS; the same fermentation conditions was also maintained at pH 5.0, DO 20% of dissolved oxygen (DO) saturation, the addition of $(NH_4)_2SO_4$ at 0.5 g/L and 30 °C. Fig. 4 shows that the lipid yield in SSF after 72 h was 3.03 g/L, three folds greater than that in SHF (0.97 g/L). The SSF process provided many advantages over the SHF on lipid fermentation in general, such as lessened product (sugar) inhibition to the cellulase enzyme, reduced equipment number, simplified solid/liquid separation step, and the contamination was avoided (Kim et al., 2008). For lipid fermentation, the inhibitions of the end product (glucose) of the hydrolysis to cellulase and the substrate (glucose) inhibition to the fermenting organisms may also the reasons leading to a lower amount of total released sugar in SHF.

The solutions for the two technical difficulties for microbial lipid SSF, the limited oxygen transfer and the complicated lipid extraction, were investigated. The results showed that the oxygen transfer from the gas phase into the liquid phase was strongly limited by the viscosity of the SSF fermentation broth when the solids content was as high as that in the ethanol fermentation (30% or above). However, the result also showed that the DO level could be maintained more than 20% of the oxygen saturation, which was sufficient for microbial lipid fermentation, if a reasonable high

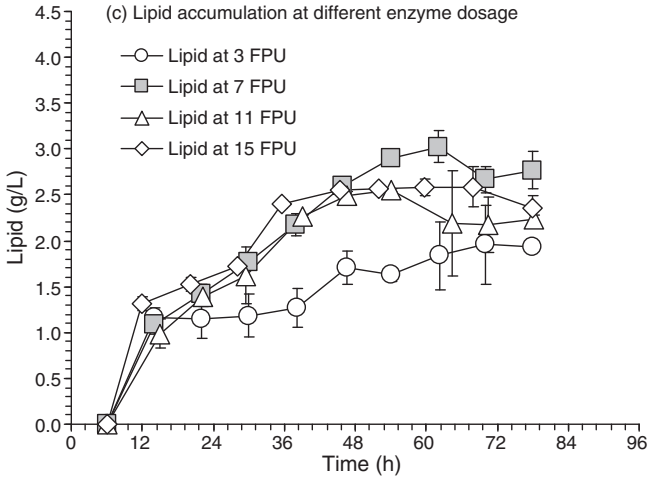

Fig. 2. Effect of cellulase enzyme dosage on lipid accumulation in SSF. (a) Glucose concentration; (b) xylcose concentration; (c) lipid accumulation. Prehydrolysis conditions: 50 °C, pH 4.8, prehydrolysis time 6 h. SSF conditions: $(NH_4)_2SO_4$, 0.5 g/L; $MgSO_4$, 0.5 g/L; 30 °C, DO 20%, pH 5.0.

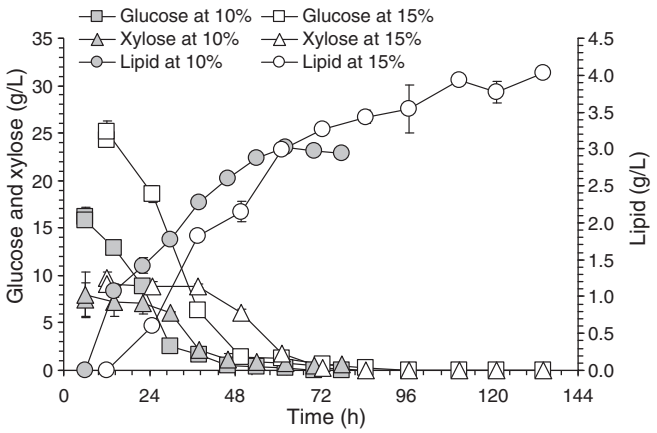

Fig. 3. Effect of solids loading on lipid accumulation in SSF. (a) Substrate concentration; (b) lipid accumulation. Prehydrolysis conditions: 50 °C, pH 4.8, prehydrolysis time 6 h, Accellerase 1000 dosage at 7.0 FPU/g DM. SSF conditions: $(NH_4)_2SO_4$, 0.5 g/L; $MgSO_4$, 0.5 g/L; 30 °C, DO 20%, pH 5.0.

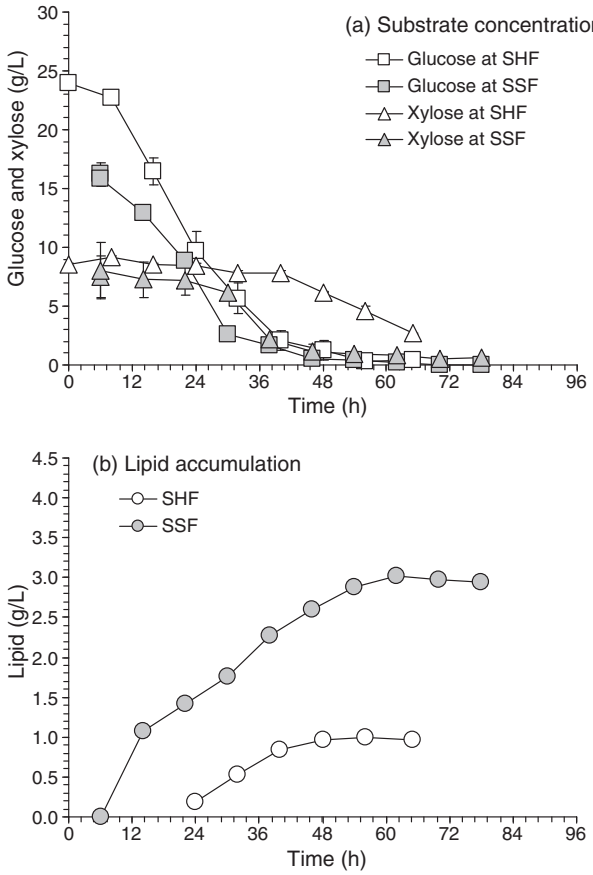

Fig. 4. Comparison of SHF and SSF for microbial lipid fermentation. (a) Substrate concentration; (b) lipid accumulation. SHF conditions: $(NH_4)_2SO_4$, 0.5 g/L; $MgSO_4$, 0.5 g/L; 30 °C; DO 20–30%; pH 5.0. Prehydrolysis conditions: 50 °C, pH 4.8, prehydrolysis time 6 h, Accellerase 1000 at 7.0 FPU/g DM. SSF conditions: $(NH_4)_2SO_4$, 0.5 g/L; $MgSO_4$, 0.5 g/L; 30 °C, DO 20%, pH 5.0.

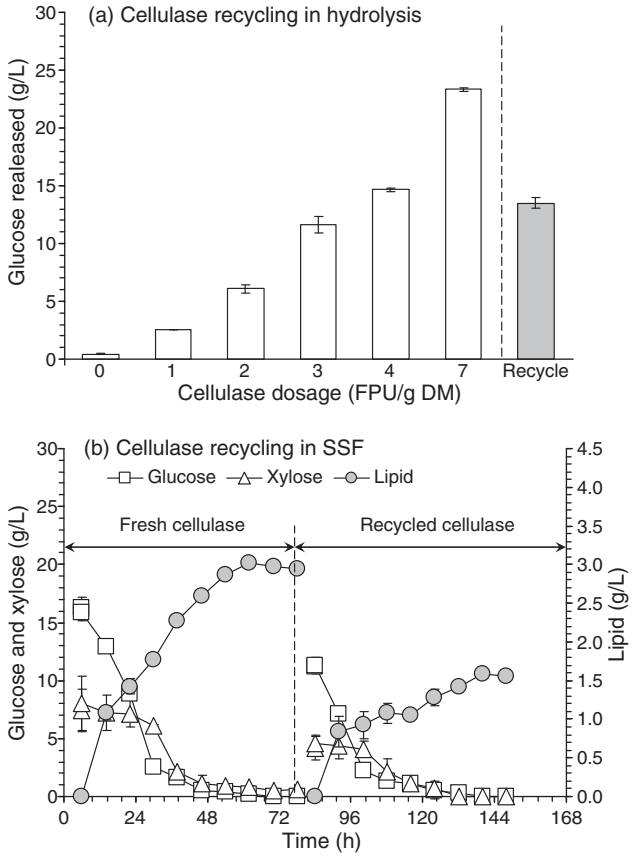

Fig. 5. Cellulase enzyme recycling in SSF. (a) Cellulase recycling in hydrolysis; (b) Cellulase recycling in SSF. Conditions: (a) CS at 10% (w/w), Accellerase1000 in 0–7.0 FPU/g DM at 50 °C, initial pH 4.8 for 48 h in 250 mL shake flask. (b) Prehydrolysis conditions: 50 °C, pH 4.8, prehydrolysis time 6 h. The initial cellulase addition was Accellerase 1000 dosage at 7.0 FPU/g DM. The supernatant containing the residual cellulase was used for the consequent second round of SSF at the same solids loading of 10% (w/w) but without new cellulase enzyme addition. SSF conditions: $(NH_4)_2SO_4$, 0.5 g/L; $MgSO_4$, 0.5 g/L; 30 °C, DO 20%, pH 5.0.

solids content (10–15% solids, w/w) was selected together with the reduced air flowrate (0.2 vvm) and the enhanced air dispersing by stirring.

In the lipid extraction step, since the cells were mixed with the solids residues in the SSF operation, the lipid extraction using the modified chloroform–methanol method (Folch et al., 1957) contained considerable impurities. Therefore, one more extraction operation using petroleum ether solvent was applied to sediment the floating fragments in the chloroform layer, followed by the centrifugation to remove the fragments. The solvent was completely removed using rotary evaporation at 80 °C to leave the purified lipid.

These solutions practically worked well and were effective to overcome the two difficulties for the microbial lipid SSF, the oxygen transfer and the lipid extraction. Therefore SSF could be considered as the suitable and better process option than SHF.

3.3. Cellulase recycling in the SSF

The cellulase recycling in the SSF is very important because of the high enzyme cost in lignocellulose bioconversion. The low temperature of the SSF operation for microbial lipid fermentation provided an opportunity for retaining the cellulase activity in the fermentation broth. After the *T. cutaneum* cells and the CS residues were separated from the SSF fermentation broth, certain amount of cellulase enzyme may still exist in the fermentation supernatant with high activity and this liquid may be recycled in the next round of the SSF operation. Thus the soluble cellulase enzyme in the fermentation liquid may be re-used in the consequent SSF operation. Fig. 5 shows the SSF operation with the cellulase recycling in the two consequent SSF operation.

Fig. 5(a) shows the hydrolysis of the pretreated and biodetoxified CS at 10% (w/w) solids loading for 48 h using the fresh cellulase and the recycled cellulase in the supernatant of the first round of the SSF. Different cellulase dosage at 0, 1.0, 2.0, 3.0, 4.0, 7.0 FPU/g DM was used and the supernatant of the first round SSF after the removal of cells and CS residues was also tested at the same conditions. The glucose released by the cellulase enzyme in the supernatant was 13.51 g/L, which approximately corresponded to the enzyme dosage of 3–4 FPU/g DM.

Fig. 5(b) shows that the first round SSF at 10% (w/w) solids and 7.0FPU/g DM of cellulase dosage received the lipid concentration of 3.03 g/L, then the cells and CS residues were removed from the fermentation broth for lipid extraction. The supernatant containing the residual cellulase was used for the consequent second round of SSF at the same solids loading of 10% (w/w) but without new cellulase enzyme addition. The lipid concentration reached 1.57 g/L at 62 hours SSF operation in the second round SSF, indicating the cellulase remaining in the supernatant could be utilized for the hydrolysis of the CS then used for lipid accumulation. The result indicates that the cellulase enzyme activity in the second round of SSF operation decreased and the lipid accumulation decreased correspondingly.

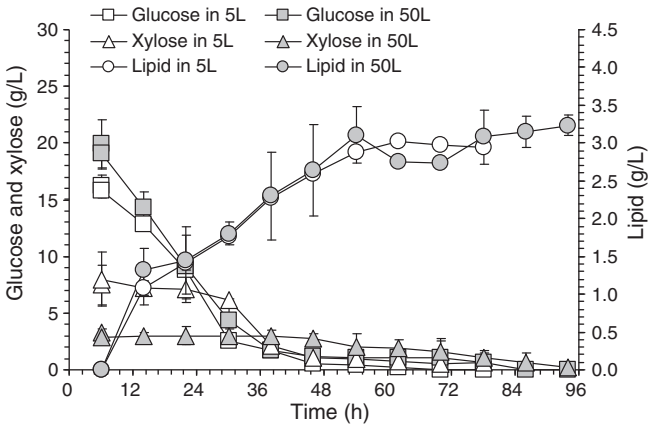

Fig. 6. SSF of microbial lipid fermentation in the 50 L bioreactor. Prehydrolysis s conditions: 50 °C. pH 4.8, prehydrolysis time 6 h, Accellerase 1000 at 7.0 FPU/g DM. SSF conditions: $(NH_4)_2SO_4$, 0.5 g/L; $MgSO_4$, 0.5 g/L; 30 °C, DO 20%, pH 5.0.

3.4. Scale-up of the SSF operation

The scale-up of the SSF of the pretreated and biodetoxified CS for microbial lipid production was carried out in the 50 L bioreactor and the result was shown in Fig. 6. The initial glucose and xylose concentrations in the 5 L and 50 L bioreactors showed t some differences on the hydrolysis, however, the maximum lipid accumulation in the two scales were almost the same, 3.23 g/L in the 50 L SSF and 3.03 g/L in the 5 L bioreactor. The result indicates that at the solids content of 10% (w/w) and the DO level of 20% oxygen saturation, the SSF operation may not cause major changes on the mass and heat transfer in the scale-up of the bioreactor, thus could be scaled up safely in a certain range.

4. Conclusion

In this study, the SSF process for microbial lipid was tested using the diluted acid pretreated and biodetoxified corn stover. The process parameters were optimized and the lipid yield obtained were 3.03 g/L in the 5 L, and 3.23 g/L in the 50 L, respectively. SSF was found to be an effective and better method than SHF in both the small (5 L) and enlarged (50 L) bioreactors. The result also shows that the cellulase enzyme could be partially recycled in the SSF. The present study provided a practical and efficient way for lipid production from lignocellulose material.

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