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## Bioresource Technology

journal homepage: [www.elsevier.com/locate/biortech](http://www.elsevier.com/locate/biortech)

## Biological removal of inhibitors leads to the improved lipid production in the lipid fermentation of corn stover hydrolysate by *Trichosporon cutaneum*

Xiao Huang, Yumei Wang, Wei Liu, Jie Bao\*

State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

## ARTICLE INFO

## Article history:

Received 25 April 2011

Received in revised form 1 August 2011

Accepted 4 August 2011

Available online 10 August 2011

## Keywords:

Microbial lipid

Corn stover hydrolysate

*Trichosporon cutaneum* CX1

Biodetoxification

Carbon-to-nitrogen molar ratio (C/N ratio)

## ABSTRACT

Corn stover (CS) hydrolysate was used as the fermentation feedstock of *Trichosporon cutaneum* CX1 for production of microbial lipid as the potential raw material of biodiesel. Two major technical barriers of the lipid fermentation were investigated: one was the strong inhibition of lignocellulose degradation compounds generated in the CS pretreatment; the other was the low carbon-to-nitrogen molar ratio (C/N ratio) of the CS hydrolysate. The newly established biodetoxification method was applied to remove the inhibitors in the pretreated CS. The enhancement of the pretreatment severity and the biodetoxification intensity on the lipid fermentation was investigated. The results show that the biodetoxification not only efficiently removed the inhibitor substances, but also led to the reduction of nitrogen content and the increase of C/N ratio. The cell lipid content of *T. cutaneum* CX1 using the biodetoxified CS hydrolysate reached 23.5%, which was doubled than that using the non-detoxified value.

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### 1. Introduction

Microbial lipid by oleaginous microorganisms is regarded as an ideal feedstock for biodiesel production, because of its shorter life cycle, less labor requirement, less affection by venue and climate, easier to scale up and non-arable land usage (Li et al., 2008; Chen et al., 2009). Corn and wheat are no longer the future option for biofuel feedstock due to the high price and food supply safety. On the other hand, if the cheap and abundant lignocellulose biomass is used as the carbon source for microbial lipid fermentation, the cellulosic lipid production will supply the sufficient lipid raw material for biodiesel industry with sound economic advantage. For example, in China, if the total 0.7 billion tons of agricultural residues were transformed into lipid according to the ratio of 10 tons of lignocellulose to 1 ton of lipid and then used for biodiesel production, biodiesel would replace most of the petroleum-based diesel on the market (Tan et al., 2010). Ethanol production from lignocellulose has been reported by several research groups, but few studies have been conducted on lipid production from lignocellulose. Huang et al. (2009) carried out the lipid fermentation of rice straw hydrolysate by *Trichosporon fermentans* and got promoted cell growth and cell lipid content by the means of hydrolysate detoxification treatment; Yu et al. (2011) screened one inhibitor-tolerant strain *Cryptococcus curvatus* in non-detoxified and detoxified wheat straw hydrolysate. However, both of these two works removed the inhibitors by overliming method, which

was accompanied with energy consumption, wastewater generation, and significant sugar loss (Dong and Bao, 2010).

In our previous experiments, the oleaginous yeast *Trichosporon cutaneum* CGMCC 2.1374 was selected for lipid fermentation using lignocellulose material because of its robust inhibitor tolerance property, and a mutant strain *T. cutaneum* CX1 was isolated from the UV-LiCl<sub>2</sub> mutation experiment (Chen et al., 2009). However, the inhibitor existence was still the major problem affecting the lipid fermentation performance. In this present research, two key technical barriers were targeted when corn stover (CS) hydrolysate was used as the carbon source of lipid fermentation by *T. cutaneum* CX1: one was the strong inhibition of lignocellulose degradation compounds, and the other was the low carbon-to-nitrogen molar ratio (C/N ratio).

The lignocellulose degradation compounds such as furfural, 5-hydroxymethylfurfural (HMF), formic acid, and acetic acid generated in the pretreatment step strongly inhibited the cell growth and lipid accumulation of oleaginous yeast (Chen et al., 2009; Hu et al., 2009). Thus the detoxification step was an inevitable step to remove these inhibitors from the pretreated lignocellulose material. Recently, a new biodetoxification method for the removal of the inhibitors was developed in our laboratory with almost zero energy input and zero wastewater generation (Zhang et al., 2010). In this study, the biodetoxification method was applied to the lipid fermentation of CS hydrolysate for improving cell growth and lipid accumulation.

Nitrogen limitation is required for lipid accumulation in cells, thus the medium with high C/N ratio is generally maintained for high cell lipid content (Ratledge and Wynn, 2002). However, the

\* Corresponding author. Tel.: +86 21 64251799; fax: +86 21 64252250.

E-mail address: [jbao@ecust.edu.cn](mailto:jbao@ecust.edu.cn) (J. Bao).

agricultural residues are perceived in rich nitrogen due to the sufficient nitrogen fertilizer utilization in the modern agriculture farming. The high nitrogen content leads to the low C/N ratio and further limits the lipid accumulation. In this study, the effect of C/N ratio on the lipid fermentation of *T. cutaneum* CX1 using CS hydrolysate was investigated. It was determined that the biodegradation of the pretreated CS showed denitrogenation, which led to the increase of C/N ratio and the improved lipid production.

## 2. Methods

### 2.1. Strains and medium

The oleaginous yeast strain *T. cutaneum* CX1 was the mutant strain obtained by our laboratory and stored in the China General Microbiological Culture Collection (CGMCC) No. 2527, Beijing, China. *T. cutaneum* CX1 was cultured in YPD medium containing 20 g/L glucose, 10 g/L peptone, and 10 g/L yeast extract at pH 6.0 for 24 h, and then the culture was aliquoted into 1.0 mL vials containing 30% (w/w) glycerol and stored at  $-80^{\circ}\text{C}$  freezer. One vial was inoculated into the medium containing 20 g/L glucose, 0.5 g/L yeast extract, 5 g/L  $(\text{NH}_4)_2\text{SO}_4$ , 1 g/L  $\text{KH}_2\text{PO}_4$ , and 0.5 g/L  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  with the pH of 5.8–6.0 at each culture to keep all the seeding condition at the same level. The biodegradation strain *Amorphotheca resinae* ZN1 was isolated and stored in our laboratory (Zhang et al., 2010) and maintained at  $4^{\circ}\text{C}$  on potato dextrose agar (PDA) slants (potato 200 g/L, glucose 20 g/L, agar 20 g/L).

### 2.2. Dilute acid pretreatment and biodegradation

CS was harvested in fall, 2007 from Northeast province of Jilin, China. It was milled, screened through a mesh with a circle diameter of 5 mm, washed, and dried at  $105^{\circ}\text{C}$ ; then it was presoaked with diluted sulfuric acid solution, and pretreated at  $190^{\circ}\text{C}$  for 3 min. The detailed procedure was described in Zhang et al. (2011). The pretreatment severity was regulated by different amount of  $\text{H}_2\text{SO}_4$  addition: 1.25%, 2.5%, 3.0%, and 3.5% (g/g dry CS). The detailed procedure of biodegradation toward the pretreated CS was described in Zhang et al. (2010). The pretreated CS was adjusted to the pH at 5.5–6.0 and the water content at 60%. The *A. resinae* ZN1 spores were inoculated onto it and cultured at  $25^{\circ}\text{C}$  for certain days depending on the required inhibitor removal percentage. 5 g sample was withdrawn every 24 h, washed with 10 mL water, and centrifuged to get the supernatant. The biodegradation was ended when both the concentration of furfural and HMF were measured to be zero. The biodegradation time depended on the pretreatment conditions of the CS and was listed in Tables 2 and 3. The detoxified CS was autoclaved and used as the feedstock for hydrolysate preparation.

**Table 1**  
Cell lipid content under different fermentation conditions.

Parameters	Constant DO (%) <sup>a</sup>				Varied DO (%) <sup>b</sup>				Temperature ( $^{\circ}\text{C}$ ) <sup>c</sup>			pH <sup>d</sup>				Initial C/N ratio (mol/mol) <sup>e</sup>			
	0–10	10–20	20–30	0	0–10	10–20	20–30	30–40	25	30	35	4.5	5.0	5.5	6.0	30.6	58.6	96.7	158.6
DCW (g/L)	7.76	9.42	9.76	6.46	9.89	10.33	9.76	9.38	11.29	11.14	8.04	10.24	10.71	11.29	11.46	13.25	17.35	16.06	15.29
Cell lipid content (g/100 g)	11.2	11.9	15.1	14.6	14.9	12.8	15.1	16.2	17.6	15.6	17.9	21.8	19.8	15.0	14.4	10.5	16.2	28.1	23.1

<sup>a</sup> Hydrolysate from the detoxified CS pretreated with  $\text{H}_2\text{SO}_4$  at 3.0% (g/g dry CS); pH 5.0;  $30^{\circ}\text{C}$ .

<sup>b</sup> Hydrolysate from the detoxified CS pretreated with  $\text{H}_2\text{SO}_4$  at 3.0% (g/g dry CS); pH 5.0;  $30^{\circ}\text{C}$ ; fixed DO at 20–30% before 24 h then changed after 24 h.

<sup>c</sup> Hydrolysate from the detoxified CS pretreated with  $\text{H}_2\text{SO}_4$  at 3.0% (g/g dry CS); pH 5.0; DO 20–30%.

<sup>d</sup> Hydrolysate from the detoxified CS pretreated with  $\text{H}_2\text{SO}_4$  at 3.0% (g/g dry CS);  $30^{\circ}\text{C}$ ; DO 20–30%.

<sup>e</sup> Hydrolysate from the non-detoxified CS pretreated with  $\text{H}_2\text{SO}_4$  at 2.5% (g/g dry CS); pH 5.0;  $30^{\circ}\text{C}$ ; DO 20–30%; an initial C/N ratio gradient formed by adding glucose with the concentration of 30.1, 70.7, 127.1, 217.9 g/L, respectively.

### 2.3. CS hydrolysate preparation

The original and the biodegraded CS were hydrolyzed using Accellerase 1000 (Genencor International, Rochester, NY, USA) at  $50^{\circ}\text{C}$  for 48 h with the solids loading of 15% (w/w) and enzyme dosage of 7 FPU/g dry CS. The activities of Accellerase 1000 were 65.8 FPU/mL in filter paper activity and 152.0 IU/mL in cellobiase activity. The water insoluble solid in the CS hydrolysate was separated by centrifugation at 10,000 rpm for 10 min, and the liquid part was autoclaved at  $115^{\circ}\text{C}$  for 20 min and filtered through qualitative filter paper. Then the composition of the hydrolysate including glucose, xylose, furfural, HMF and acetic acid was determined using HPLC (LC-20AD, refractive index detector RID-10A, Shimadzu, Japan) with a Bio-rad Aminex HPX-87H column at the column temperature  $65^{\circ}\text{C}$  (Chen et al., 2009).

### 2.4. Cell culture and lipid fermentation

A three-step adaptation procedure of *T. cutaneum* CX1 in the non-detoxified CS hydrolysate was as follows: (1) a vial of *T. cutaneum* CX1 was inoculated into a 100 mL Erlenmeyer flask containing 20 mL of sterilized inoculum medium, and cultured in a shaking incubator at  $30^{\circ}\text{C}$ , 180 rpm, for 24 h; (2) 2 mL of the culture was inoculated into a 100 mL flask containing 10 mL of sterilized CS hydrolysate and 10 mL of inoculum medium, and cultured at  $30^{\circ}\text{C}$ , 180 rpm for 12 h; (3) 10 mL of the culture was inoculated into a 500 mL flask containing 100 mL of sterilized CS hydrolysate at pH 5.0, and cultured at  $30^{\circ}\text{C}$ , 180 rpm for 15 h. The culture was inoculated into the 3-liter fermenter containing 1000 mL of sterilized CS hydrolysate to start the lipid fermentation. For biodegraded CS hydrolysate, the culture procedure skipped the second step described above.

All the lipid fermentations were carried out in a 3-liter fermenter with pH, temperature, and dissolved oxygen (DO) control (Baoping Biotech, Shanghai, China). Only inorganic salts were added into the CS hydrolysate as the nutrient supplements including 0.5 g/L  $(\text{NH}_4)_2\text{SO}_4$ , 1 g/L  $\text{KH}_2\text{PO}_4$  and 0.5 g/L  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , without the addition of any expensive ingredients such as yeast extract and peptone. In the first 24 h 5 mL and after 24 h 35 mL samples were withdrawn for lipid extraction at regular intervals. The samples were centrifuged at 10,000 rpm for 5 min. The supernatant was analyzed using HPLC; the precipitation was used for the determination of cell growth and cell lipid content.

### 2.5. Determination of dry cell mass (DCM), lipid concentration and lipid composition

DCM was determined by sampling 30 mL culture. The samples were centrifuged, washed, dried and measured gravimetrically. The total lipid was extracted using chloroform–methanol method

**Table 2**  
Lipid fermentation using CS hydrolysates under different pretreatment and detoxification conditions.

Feedstock	Hydrolysate composition (g/L)					Fermentation parameters			
	Glucose	Xylose	Acetic acid	HMF	Furfural	DCW (g/L)	Lipid concentration (g/L hydrolysate)	Cell lipid content (g/100 g cells)	C/N ratio (mol/mol)
CS-1	23.89	11.63	2.99	0	0	16.34 ± 0.21	1.87 ± 0.21	11.5 ± 1.4	32.3 ± 1.0
CS-2	31.87	13.02	3.54	0.28	0.49	12.87 ± 0.21	1.39 ± 0.12	10.8 ± 1.1	32.7 ± 4.4
CS-3	42.29	14.86	5.12	0.73	0.69	Not grow	Not grow	Not grow	Not grow
Biodetoxified CS-2	36.38	12.23	1.08	0	0	15.27 ± 0.26	2.88 ± 0.10	18.9 ± 0.3	40.0 ± 5.2
Biodetoxified CS-3	44.20	3.92	2.35	0	0	15.44 ± 0.51	3.10 ± 0.05	23.5 ± 0.6	47.4 ± 4.2
Biodetoxified CS-4	44.56	12.31	1.91	0	0	13.28 ± 0.43	2.71 ± 0.33	20.4 ± 0.8	48.5 ± 3.9

CS pretreated with varying H<sub>2</sub>SO<sub>4</sub> concentration: CS-1, 1.25% (g/g dry CS); CS-2, 2.5% (g/g dry CS); CS-3, 3.0% (g/g dry CS); CS-4, 3.5% (g/g dry CS). Biodetoxification time: CS-2, 5 days; CS-3, 7 days; CS-4, 9 days. All the fermentations were performed in duplicate and averaged.

**Table 3**  
Lipid fermentation under different biodetoxification intensity.

Biodetoxification time (day)	Hydrolysate composition (g/L)					Fermentation parameters			
	Glucose	Xylose	Acetic acid	HMF	Furfural	DCW (g/L)	Lipid concentration (g/L hydrolysate)	Cell lipid content (g/100 g cells)	C/N ratio (mol/mol)
0	42.29	14.86	5.12	0.73	0.69	<sup>a</sup> –	–	–	–
2	41.36	14.74	4.20	0.42	0.28	–	–	–	–
5	44.53	15.20	2.16	0.15	0	–	–	–	–
7	43.64	5.98	0.63	0	0	10.20	3.11	30.4	43.1
9	37.98	5.14	0.86	0	0	13.90	2.70	19.4	25.7

CS was pretreated with H<sub>2</sub>SO<sub>4</sub> at 3.0% (g/g dry CS).

<sup>a</sup> The fermentation experiment did not carry out in the hydrolysate.

(Folch et al., 1957). Briefly, the dry cells were disrupted and homogenized in 6 mL of 4 M hydrochloric acid for 30 min, heated in the boiling water for 10 min, and quenched in the ice water. The cell lysates were stirred with 20 mL methanol–chloroform mixture (1:2 by volume ratio) for 30 min and centrifuged at 10,000 rpm for 5 min to get the lipid-containing solvent. It was then evaporated at 80 °C and measured gravimetrically.

The fatty acid composition was measured using gas chromatography (Clarus 500, PerkinElmer with HP-5 column of 30 m × 0.25 mm × 0.25 μm in size; He at 1 mL/min; the initial oven temperature of 80 °C with the increase gradient of 16 °C/min, and the final of 300 °C), and mass spectrometry (Clarus 500, PerkinElmer with electron impact at 70 eV; charge–mass ratio range at 33–500) (GC–MS). The lipid was transesterified with methanol into its ester form using 10% boron trifluoride as the catalyst; then the ester was heated to 60 °C for 10 min and injected into the GC–MS. The fatty acid composition was determined using NIST MS Search 2.0.

## 2.6. Determination of total nitrogen and C/N ratio calculation

The total nitrogen content of the CS hydrolysate was determined using the alkaline potassium persulfate digestion–UV spectrophotometric method (D'Elia et al., 1977). 10 mg/L KNO<sub>3</sub>–N solution was used as nitrogen standard solution. 40 μL sample was diluted into 10 mL, added with 5 mL alkaline potassium persulfate solution (40 g/L K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> and 15 g/L NaOH) was added and autoclaved at 121 °C for 30 min. Then 1 mL HCl (10%, v/v) was added and the absorbance was measured at 220 nm (A<sub>220</sub>) and 275 nm (A<sub>275</sub>), respectively. The total nitrogen contents of the samples were determined based on their (A<sub>220</sub> – 2 × A<sub>275</sub>) values. The calibration curve was drawn using the standard solutions. The C/N ratio of the CS hydrolysate was calculated using the following equation:

$$C/N = \frac{[Glu] \times 6/180 + [Xyl] \times 5/150}{([N]_0 - [N]_1)/14}$$

where [Glu] and [Xyl] were the initial glucose and xylose concentrations in the CS hydrolysate (g/L), respectively; [N]<sub>0</sub> and [N]<sub>1</sub> were the total nitrogen concentrations at the beginning and the end of the fermentation, respectively.

## 3. Results and discussion

### 3.1. Effect of the fermentation parameters on the lipid accumulation in the CS hydrolysate

The effects of pH, dissolved oxygen (DO), and temperature on the lipid fermentation of CS hydrolysate by *T. cutaneum* CX1 were investigated. Table 1 shows that both the cell growth (DCW) and the cell lipid content increased with the increase of DO value. A two-step DO supply test was also carried out by maintaining DO at 20–30% of the saturation level in the first 24 h and setting different DO levels thereafter. The result shows that the DCW decreased significantly if the DO dropped to zero after 24 h, but it made no significant difference to the cell lipid content when the DO was set at different levels after 24 h. DCW decreased from 11.29 g/L, 11.14 g/L, to 8.04 g/L when the temperature increased from 25 °C, 30 °C, to 35 °C, but the cell lipid content did not change too much. When the pH of the hydrolysate increased from 4.5 to 6.0, the cell lipid content decreased significantly from 21.8% to 14.4% but the DCW increase was limited from 10.24 g/L to 11.46 g/L.

High nitrogen content exists in the agricultural residues, which is difficult to reduce by simple methods. In this study, the C/N ratio gradient in the CS hydrolysate was created by adding extra glucose as shown in Table 1. The result shows that the effect of the C/N ratio on lipid accumulation was much greater than that of DO, pH, and temperature. The cell lipid content increased by 168% (from 10.5% to 28.1%) when the C/N ratio increased from 58.6 to 96.7;

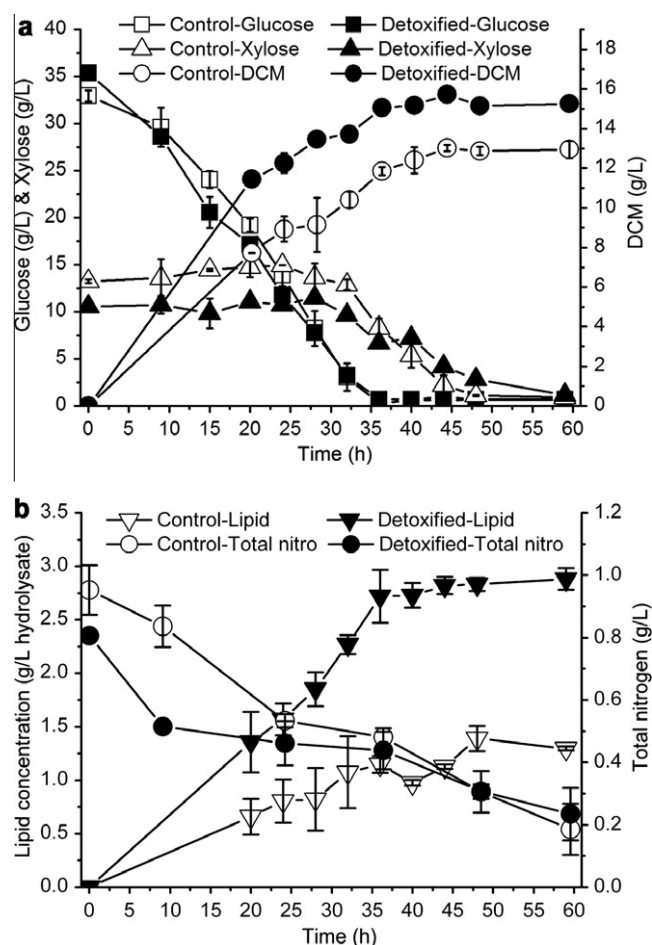
then it decreased (23.1%) with the further increase of the C/N ratio to 158.6, probably due to the cell growth repression and the ATP-citrate lyase inhibition at the high glucose concentration (Beopoulos et al., 2009; Li et al., 2007). The results indicate that the increase of the C/N ratio was critically important for efficient lipid accumulation using the agricultural residue feedstock such as CS.

The effect of lignocellulose degradation compounds on lipid fermentation of CS hydrolysate was investigated by pretreating the CS with different  $H_2SO_4$  usage. Table 2 shows that the sugar and inhibitor (acetic acid, furfural, and HMF) concentrations increased with the increasing pretreatment severity indicated by the  $H_2SO_4$  usage in the pretreatment. Both the cell growth (DCW) and the lipid concentration decreased with the intensification of pretreatment severity until the cell growth was ceased at the most severe pretreatment condition. It indicates that the inhibition of lignocellulose degradation compounds was another key barrier for lipid fermentation of CS hydrolysate other than the low C/N ratio.

The fatty acid composition of lipid obtained were mainly oleic acid (18:1) (54.2%), palmitic acid (16:0) (18.9%), linoleic acid (18:2) (9.3%), and palmitoleic acid (16:1) (7.2%); these four fatty acids accounted for about 90% of the total fatty acids. The fatty acid compositional profile was similar to that of vegetable oil such as soybean oil (Ratledge and Wynn, 2002), indicating that microbial lipid produced from the CS hydrolysate was a potential feedstock for biodiesel production.

### 3.2. Lipid fermentation in the CS hydrolysate after the biological removal of inhibitors

To improve the cell growth and lipid accumulation, the biodegradation method was introduced for quick and mild removal of inhibitors (Zhang et al., 2010). Table 2 shows that the inhibitors were either significantly reduced (acetic acid), or completely removed (furfural and HMF) after the biodegradation. The fermentation result of the biodegradation toward CS pretreated with  $H_2SO_4$  at 2.5% (g/g dry CS), both of the lipid concentration in hydrolysate and the cell lipid content almost doubled comparing to the non-detoxified CS hydrolysate, and also the C/N ratio was increased. Fig. 1 shows the time courses of the glucose and total nitrogen consumption, the cell growth, and the lipid concentration in the hydrolysates of the non-detoxified and biodegraded CS pretreated with  $H_2SO_4$  at 2.5% (g/g dry CS). The result shows that the total utilizable nitrogen in the hydrolysate decreased from 0.77 g/L to 0.57 g/L and the initial glucose concentration increased from 31.9 g/L to 36.4 g/L between the two hydrolysates, thus the initial C/N ratio was increased from 32.7 to 40.0. Fig. 1 also shows that the total nitrogen consumption rate in the biodegraded CS hydrolysate was greater than that of the non-detoxified CS hydrolysate, but their glucose consumption rates were almost the same. This result indicates that more carbon source (glucose) was used for lipid accumulation in detoxified CS hydrolysate, instead of the cell growth. Fig. 1 also indicates that the lipid accumulation continued to use xylose and other carbon sources after glucose was consumed completely. On the other hand, the main reasons affecting the lipid accumulation in this experiment were the C/N ratio and the effects of inhibitors. Therefore, although the glucose consumption was approximately the same between the detoxified and non-detoxified CS hydrolysate, the lipid yield for the detoxified CS hydrolysate increased after the increase of C/N ratio and the removal of inhibitors, and thus led to the increase of the lipid accumulation. It is estimated that the biodegradation by *A. resinae* ZN1 is accomplished through a specific kind of denitrogenation function. The nitrogen removal during biodegradation and its positive effect on the lipid accumulation were observed as an experimental



**Fig. 1.** Time course of lipid fermentation using CS hydrolysate. (a) Glucose and xylose consumption and DCM; (b) Lipid production and total nitrogen consumption. Pretreatment: 190 °C; 3 min;  $H_2SO_4$  at 2.5% (g/g dry CS). Detoxification: pH 5.0–5.5; 25 °C in a static incubator for 5 days.

phenomenon. The mechanism is not clear and still under investigation. These on-going investigations included the genome sequencing for mining the genes responsible for the nitrogen removal, the possible metabolic pathway related to the phenomenon. Up to our knowledge, the nitrogen removal might be caused by the utilization of these nitrogen-containing compounds into nitrogen gases in the metabolic activities of *A. resinae* ZN1, or by the change of the nitrogen-containing compounds into the different forms that were unable to be utilized by *T. cutaneum* CX1.

Table 2 also shows that intensified pretreatment of CS with  $H_2SO_4$  usage of 3.0% (g/g dry CS) combined with the biodegradation increased the C/N ratio from 40.0 to 47.4 and also promoted the cell lipid content from 18.9% to 23.5%; but the further intensified pretreatment of CS with  $H_2SO_4$  usage of 3.5% (g/g dry CS) deteriorated the lipid accumulation, although the inhibitors were almost completely removed by biodegradation. This result indicates that the intensive pretreatment on CS had its limitation for lipid accumulation, even accompanied by the biodegradation treatment. Table 3 shows that, with further intensified biodegradation by prolonging the processing time to 9 days, the cell growth (DCW) was increased from 10.20 to 13.90 g/L; the glucose concentration was decreased from 43.64 to 37.98 g/L and the utilizable nitrogen was increased from 0.54 to 0.78 g/L in the hydrolysate using the corresponding detoxified CS. The change of the glucose concentration and the utilizable nitrogen in the hydrolysate led to the sharp decrease of C/N ratio from 43.1 to 25.7, and the decrease of lipid accumulation in cells from 30.4% to 19.4%. The result

indicates that the biodetoxification time must match the pretreatment severity and the over-biodetoxification was not always positive.

The typical C/N ratio of the medium for lipid accumulation in the pure medium was around 500 (Li et al., 2006). With the optimized combination of the biodetoxification and the pretreatment severity in this study, the C/N ratio in the CS hydrolysate was increased to 47.4 from the original value of 30.6, and the cell lipid content reached 23.5%, which doubled the value determined at the starting point. This value is still far from the optima of lipid accumulation. The future study of lipid fermentation using agricultural residues feedstock is expected to focus on the further increase of C/N ratio by either increasing carbon content, or reducing nitrogen content of the feedstock through practical and economic methods.

#### 4. Conclusions

Biodetoxification of the pretreated CS not only efficiently removed the inhibitor substances, but also led to the reduction of nitrogen content and the increase of C/N ratio. The cell lipid content of *T. cutaneum* CX1 using the biodetoxified CS hydrolysate reached 23.5%, which more than doubled the non-detoxified value. Thus the biodetoxification method essentially helped to overcome the two major technical barriers of the lipid fermentation when CS was used as the feedstock: strong inhibition and low C/N ratio.

#### Acknowledgements

This research was supported by National Basic Research Program of China (2011CB707406), Natural Science Foundation of China (20976051), Ministry of Education of China (20090074110013), Shanghai Leading Academic Discipline Project (B505), Fundamental Research Funds for the Central Universities of China (WF0913005),

and China National Special Funds for the State Key Laboratory of Bioreactor Engineering (2060204).

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