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Short Communication

Lipid fermentation of corncob residues hydrolysate by oleaginous yeast *Trichosporon cutaneum*

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HIGHLIGHTS

- CCR was used as the feedstock to produce microbial lipid for the first time.
- *Trichosporon cutaneum* was selected for lipid production using CCR hydrolysate.
- Highest lipid titer of 12.3 g/L and DCW of 38.4 g/L were obtained finally.

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ABSTRACT

Corncob residues (CCR) are cellulose residues of corncob after xylan (hemicellulose) is extracted for production of xylitol. Here, an oleaginous yeast *Trichosporon cutaneum* ACCC 20271 was screened for lipid fermentation using CCR hydrolysate. The initial carbon-to-nitrogen molar ratio (C/N ratio) and the initial sugar concentration of the CCR hydrolysate were investigated in the lipid fermentation of *T. cutaneum* ACCC 20271. A C/N ratio gradient was generated by changing the corn steep liquor (CSL) addition and an optimal C/N ratio of 49.3 was obtained. The different initial sugar concentration was obtained by changing the cellulase amount and the lipid titer was enhanced by the increased sugar concentration. To our knowledge, this is the first report on using CCR as the feedstock for lipid fermentation. The lipid titer of 12.3 g/L and dry cell weight (DCW) of 38.4 g/L were the highest values among the studies using lignocellulose for lipid production.

1. Introduction

The lipid feedstock of biodiesel includes vegetable oils, animal fats, waste cooking oil etc., and accounts for 70–80% of the total production cost of biodiesel (Pizarro and Enoch, 2003; Han et al., 2005). Therefore, a cheap alternative of lipid feedstock is crucially important for the further development of biodiesel industry. Microbial lipid produced by oleaginous microorganism fermentation is a suitable feedstock for biodiesel synthesis because of its similar fatty acid composition to that of vegetable oils (Papanikolaou and Aggelis, 2011). Utilization of cheap and abundant lignocellulose feedstocks for microbial lipid, such as rice straw (Huang et al., 2009), sugarcane bagasse (Tsigie et al., 2011), wheat straw (Yu et al., 2011), corn stover (Huang et al., 2011; Hu et al., 2011; Liu et al., 2012), and corncob (Huang et al., 2012; Chen et al., 2012), had been investigated in the previous studies.

Corncob residues (CCR) are cellulose residues of corncob after xylan (hemicellulose) is extracted using dilute acid hydrolysis for the production of xylose and xylitol. In China, xylitol production from corncob has become a large industry and, as the negative outcome, approximately 400,000 tons of CCR was generated annually as solids wastes (Cheng et al., 2010). CCR contains high cellulose content (more than 50% of its dry weight), and more importantly, CCR has two major advantages than virgin lignocellulose biomass such as corncob and corn stover: (1) CCR can be enzymatically hydrolyzed directly without pretreatment, because the upstream dilute acid hydrolysis deeply deconstructed its lignocellulose recalcitrance, and (2) the water soluble inhibitors such as furan aldehydes, aliphatic acids and some phenolic compounds were removed in the multiple washing steps of xylitol production. For these merits, CCR had been applied for production of ethanol and other value-added products (Zhang et al., 2012; Liu et al., 2010; Cheng et al., 2011). However, no microbial lipid production study using CCR as the feedstock was reported before.

In this study, the feasibility of CCR as the feedstock of microbial lipid production was investigated. First, CCR was enzymatically hydrolyzed into its monosaccharides form of glucose and xylose. Then, the CCR hydrolysate was used as carbon source for lipid fermentation by an oleaginous yeast *Trichosporon cutaneum* ACCC 20271 screened using the CCR hydrolysate as the culture medium. The lipid fermentation performance of *T. cutaneum* ACCC 20271 using CCR hydrolysate was investigated by examining two

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important parameters, the initial carbon-to-nitrogen molar ratio (C/N ratio) and the initial sugar concentration of the hydrolysate. The results showed that high lipid production performance with the lipid titer and content of 12.3 g/L and 32.1%, respectively, was obtained using the CCR hydrolysate. To the best of our knowledge, this is the first report on using CCR as the feedstock for lipid fermentation. The lipid titer of 12.3 g/L and dry cell weight (DCW) of 38.4 g/L were the maximum on using lignocellulose derived biomass for lipid production. This study provided not only an economical feedstock option for microbial lipid production, but a practical method for CCR utilization also.

2. Methods

2.1. Raw materials and chemicals

Corncob residues (CCR) materials were provided by KDN Biotech Co., Qingdao, China. The water content was measured to be 71.6% using the method in Zhao et al. (2012). The composition of CCR was analyzed using ANKOM 220 Cellulose Analyzer (ANKOM Technology, Macedon, NY, USA). The original CCR contained 52.2% cellulose and 3.0% hemicellulose (w/w, dry weight base). The low xylose content in CCR was due to almost a complete extraction of hemicellulose from corncob in xylose production steps.

Cellulase enzyme Youtell #6 was kindly provided by Hunan Youtell Biochemical Co., Yueyang, China. The activity was 135.0 FPU/g DM in filter paper unit using the method in Adney and Baker (1996).

2.2. CCR hydrolysate preparation

CCR was hydrolyzed at pH 4.8, 50 °C for 48 or 72 h, depending on the cellulase dosage used. The CCR solids were added to 15% (w/w, dry basis) in the hydrolysis, and the cellulase dosage was 7 FPU/g DM unless mentioned elsewhere. The CCR hydrolysate was centrifuged at 10,000 rpm for 10 min, and the clear supernatant was collected and autoclaved at 115 °C for 20 min, and then filtered through filter paper before use.

2.3. Strains and lipid fermentation

T. cutaneum ACCC 20271 was purchased from Agricultural Culture Collection of China, Beijing, China; *Rhodotorula glutinis* CGMCC 2.703, *R. glutinis* CGMCC 2.704, and *Rhodosporidium toruloides* CGMCC 2.1609 were purchased from China General Microbiological Culture Collection, Beijing, China; *Lipomyces starkeyi* DSM 70295 was purchased from German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany. These yeast strains were cultured in YPD medium (10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose) at 30 °C, 180 rpm for 24 h. Then 10% of the culture was inoculated into the CCR hydrolysate with the inorganic salt ions supplements of 0.5 g/L $MgSO_4 \cdot 7H_2O$, 1.0 g/L KH_2PO_4, 0.5 g/L $(NH_4)_2SO_4$, and appropriate organic nitrogen source corn steep liquor (CSL). The lipid fermentation was carried out in a 250 mL conical flask containing 50 mL medium at 30 °C, 180 rpm for 120 h, with the initial pH of 5.0. Lipid fermentation in the 3-L fermenter (Baoxing Biotech, Shanghai, China) was performed at 30 °C and constant pH control. All experiments were performed for 3 times and the error ranges were given in the tables and figures.

2.4. Determination of dry cell weight (DCW), lipid content and lipid composition

Fermentation broth was collected, centrifuged, washed, dried at 80 °C for 24 h, and then measured gravimetrically for DCW determination. The lipid was extracted using chloroform–methanol method according to Huang et al. (2011). Lipid yield indicates the lipid produced from one gram of sugar (g/g).

The fatty acid composition was measured using gas chromatograph–mass spectrometer (GC–MS). The GC was Clarus 500, PerkinElmer with PE-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 for 3 min and then with the increase gradient of 16 °C/min, to the final of 280 °C, and the MS was Clarus 500, PerkinElmer with electron impact at 70 eV, charge-mass ratio range at 33–500. The extracted lipid was saponified for 1 h with 5 mL 4 M KOH at 60 °C, and then methyl esterified for 30 min by adding 4 mL methanol and boron trifluoride, then mixed with 2 mL n-hexane and purified H_2O, centrifuged and injected the upper hexane layer to the GC–MS. The composition analysis system is NIST MS Search 2.0.

2.5. Determination of total nitrogen and total phenolic content in the hydrolysate

The total nitrogen content was measured by the alkaline potassium persulfate digestion-UV spectrophotometric method described in Huang et al. (2011). The initial C/N ratio of the hydrolysate was calculated using the equation:

$$C/N = \frac{[Glu] \times 6/180 + [Xyl] \times 5/150}{([N]_{CCR} + [N]_N)/14}$$

where [Glu] and [Xyl] were the initial glucose and xylose concentrations in the hydrolysate (g/L), respectively; $[N]_{CCR}$ and $[N]_N$ were the total nitrogen concentrations in the CCR hydrolysate and added nitrogen source at the beginning of the fermentation, respectively. The nitrogen content of nutrients were measured to be $(NH_4)_2SO_4$ 0.212 g/g and CSL 0.069 g/g.

For total phenolic content determination, 500 μL hydrolysate and 1 mL foline-phenol regent were mixed with 4 mL 1 M Na_2CO_3 for reaction 2 h at room temperature, then the absorbance was detected at 765 nm (Ainsworth and Gillespie, 2007). The calibration curve was drawn using the standard solutions of 100 mg/L gallic acid at five diluted concentrations.

2.6. Sugar and inhibitor analysis

The components of the hydrolysate including glucose, xylose, 5-hydroxymethylfufural (HMF), furfural, formic acid, acetic acid and levulinic acid were determined using HPLC (LC-20AD, refractive index detector RID-10A, Shimadzu, Japan) with the sulfuric acid as the eluent (5 mM, flow rate at 0.6 mL/min) and a Bio-rad Aminex HPX-87H column at the column temperature 65 °C (Huang et al., 2011).

3. Results and discussion

3.1. Screening for the suitable oleaginous yeast in the CCR hydrolysate

The growth performance of five selected oleaginous yeasts, *T. cutaneum* ACCC 20271, *R. glutinis* CGMCC 2.703, *R. glutinis* CGMCC 2.704, *R. toruloides* CGMCC 2.1609, and *L. starkeyi* DSM 70295 were culture in the CCR hydrolysate for screening a most suitable strain. The results showed that only *T. cutaneum* ACCC 20271 was survived in the CCR hydrolysate and no cell growth was observed for the other four strains. The reason might be due to the better tolerance of *T. cutaneum* ACCC 20271 to the CCR hydrolysate environment. Although most of the water soluble inhibitors from the corncob residues hydrolysis such as HMF, furfural, formic acid, acetic acid, levulinic acid were removed during the xylitol production process, some inhibitor residues and the

water insoluble phenolic derivatives still existed (1.0 g/L of acetic acid and 0.15 g/L of phenolic compounds) and these phenolic inhibitors negatively affected the microbial growth and metabolism (Huang et al., 2011).

The growth curves of *T. cutaneum* ACCC 20271 were shown in Fig. 1. The growth behavior of *T. cutaneum* ACCC 20271 was very similar in the synthetic medium and the CCR hydrolysate. The lipid fermentation of *T. cutaneum* ACCC 20271 in the CCR hydrolysate was carried out in the fermentor at constant pH of 5.0. Fig. 2 showed that glucose was almost completely consumed after 120 h and xylose consumption started at this time point; the dry cell weight (DCW) and lipid content inside the cells increased within in the fermentation period. The fatty acid composition of the lipid accumulated in *T. cutaneum* ACCC 20271 cells was 72.1% of oleic acid (C18:1), 12.5% of palmitic acid (C16:0), 6.6% of stearic acid (C18:0), 5.6% of linoleic acid (C18:2), 1.1% of margaric acid (C17:0), and several minor components of lignoceric acid (C24:0), arachidic acid (C20:0) and pentadecanoic acid (C15:0) in less than 1% each. The fatty acid compositional profile was similar to that of vegetable oil (Ratledge and Wynn, 2002), indicating that microbial lipid produced from the CCR hydrolysate was a suitable feedstock for biodiesel production.

3.2. Nitrogen limitation on lipid fermentation performance in the CCR hydrolysate

Nitrogen limitation is the essential requirement for lipid accumulation in oleaginous yeast cells (Ratledge and Wynn, 2002). In this study, the initial carbon-to-nitrogen molar ratio (C/N ratio) in the CCR hydrolysate was adjusted by changing the addition of organic nitrogen source, corn steep liquor (CSL), for searching a suitable C/N ratio. Table 1 showed the lipid production in the CCR hydrolysate at a changing C/N ratio with different concentration of CSL added. Without the CSL adding, the lipid yield reached the highest value of 0.144 g/g, but the DCW and lipid titer were all very low and it was not benefit for the lipid production. The lipid content in cells and lipid titer in the hydrolysate increased with increasing CSL concentration and reached a maximum at CSL of 6 g/L, then both lipid content and lipid titer decreased with the further increase of CSL addition although sugar consumption and cell growth continued to increase. The maximum lipid content, lipid titer, and the relatively high lipid yield were 29.1%, 7.8 g/L, 0.132 g/g, respectively, at the CSL concentration of 6 g/L and the corresponding C/N ratio of 49.3.

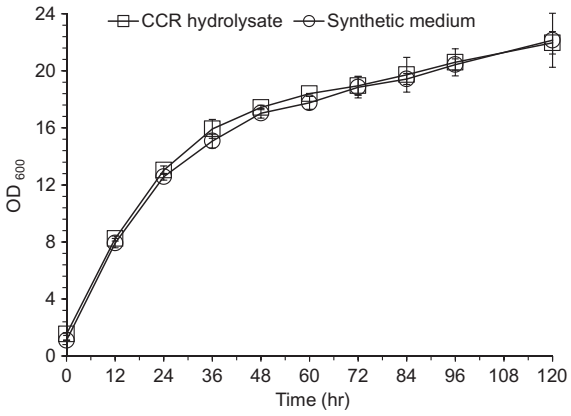

Fig. 1. Time courses of cell growth of *T. cutaneum* ACCC 20271 in the CCR hydrolysate and synthetic medium. Cell growth in conical flask at 30 °C, 180 rpm, and the initial pH of 5.0. CCR was hydrolyzed at the solids content of 15% (w/w), the cellulase of 7 FPU/g DM, pH 4.8, 50 °C for 48 h, adding KH_2PO_4 1.0 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L and $(NH_4)_2SO_4$ 0.5 g/L. The synthetic medium contained the same sugar and salts concentration as in the CCR hydrolysate.

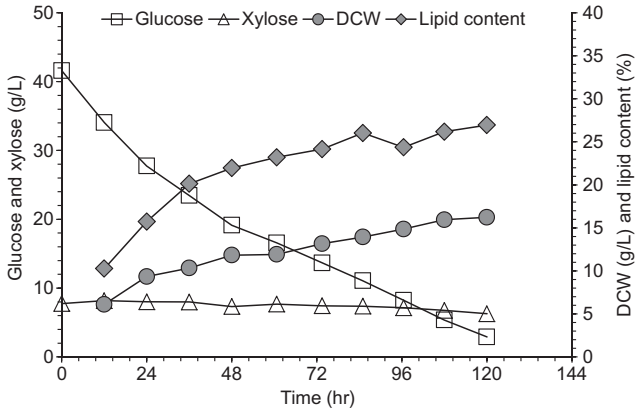

Fig. 2. Lipid fermentation of *T. cutaneum* ACCC 20271 in the CCR hydrolysate. Lipid fermentation in 3-L fermentor at 30 °C and the constant pH of 5.0. CCR was hydrolyzed at the solids content of 15% (w/w), the cellulase of 7 FPU/g DM, pH 4.8, 50 °C for 48 h, adding KH_2PO_4 1.0 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L and $(NH_4)_2SO_4$ 0.5 g/L.

This optimal initial C/N ratio around 49.3 should be considered as a specific value for the present CCR hydrolysate, the strain *T. cutaneum* ACCC 20271 used, and the medium nutrients used. The change of nitrogen nutrient components may lead to a change of optimal C/N ratio. Nevertheless, the results indicated that the lipid fermentation using CCR as the feedstock behaved a typical nitrogen limitation phenomenon and the change of C/N ratio was an important method for improving the lipid fermentation performance.

3.3. Initial sugar concentration on lipid fermentation performance in the CCR hydrolysate

High sugar concentration generally leads to a high lipid titer in the lipid fermentation (Huang et al., 2011). In this study, a sugar concentration gradient in the hydrolysate was generated by enhancing the CCR hydrolysis yield. The cellulase was added at different dosages of 3, 7, 10, 15, and 20 FPU/g DM to create a glucose and xylose concentration gradient in the hydrolysate as shown in Table 2. The initial C/N ratio of the hydrolysate was maintained at the optimal value of 49.3 by adjusting the addition of $(NH_4)_2SO_4$ and CSL into the hydrolysate at different sugar concentrations. The results showed that under the constant C/N ratio, the DCW and the lipid titer increased with increasing sugar concentration, but the cell lipid content kept constantly at approximately 30%. The maximum lipid concentration of 12.3 g/L was achieved when the cellulase was 15 FPU/g DM, with the lipid content of 32.1% and lipid yield of 0.131 g/g. The results demonstrated that lipid production was apparently enhanced by increasing sugar concentration of the CCR hydrolysate under the same initial C/N ratio.

Results using different lignocellulose feedstocks for lipid fermentation were summarized in Table 3. Comparing to rice straw hydrolysate (Huang et al., 2009), rice hull hydrolysate (Economou et al., 2011), wheat straw hydrolysate (Yu et al., 2011), sugarcane bagasse hydrolysate (Tsigie et al., 2011), corn stover hydrolysate (Huang et al., 2011; Hu et al., 2011), or corncob hydrolysate (Huang et al., 2012; Chen et al., 2012), the present corncob residues (CCR) hydrolysate demonstrated the significant advantage as the feedstock to other lignocellulose feedstocks on lipid fermentation. Although the cell lipid content was moderate comparing to other strains (32.1%), the cell mass growth (DCW) and the lipid titer of *T. cutaneum* ACCC 20271 using the CCR hydrolysate were the highest values: 38.4 g/L in DCW and 12.3 g/L in lipid titer, respectively. Furthermore, considering the advantages of CCR on collection availability (already in bulk accumulation in xylitol production plants) and process simplicity (without pretreatment), lipid

Table 1
Lipid fermentation of *T. cutaneum* ACCC 20271 in the CCR hydrolysate under different C/N ratio.

CSL (g/L)	C/N ratio (mol/mol)	Sugar consumption (g/L)	DCW (g/L)	Lipid content (%)	Lipid titer (g/L)	Lipid yield (g/g)
0	130.1 ± 6.8	20.8 ± 1.0	11.7 ± 0.7	25.6 ± 0.8	3.0 ± 0.1	0.144 ± 0.003
4	62.2 ± 2.1	44.5 ± 2.6	19.9 ± 0.9	27.4 ± 0.5	5.5 ± 0.1	0.123 ± 0.004
5	55.0 ± 1.7	53.6 ± 1.1	24.0 ± 0.2	27.9 ± 0.5	6.7 ± 0.1	0.125 ± 0.000
6	49.3 ± 1.4	58.9 ± 1.8	26.7 ± 0.7	29.1 ± 0.7	7.8 ± 0.0	0.132 ± 0.004
8	40.9 ± 1.0	63.5 ± 1.5	26.9 ± 0.5	28.8 ± 2.1	7.7 ± 0.4	0.122 ± 0.004
10	34.9 ± 0.7	63.9 ± 2.9	28.6 ± 1.8	23.8 ± 0.4	6.8 ± 0.6	0.107 ± 0.004

In conical flasks 30 °C, 180 rpm for 120 h, and the initial pH of 5.0.

Table 2
Lipid fermentation of *T. cutaneum* ACCC 20271 in the CCR hydrolysate under different initial sugar concentration.

Cellulase dosage (FPU/g DM)	Hydrolysate composition			Fermentation parameters				
	Glucose (g/L)	Xylose (g/L)	Total nitrogen (g/L)	Sugar consumption (g/L)	DCW (g/L)	Lipid content (%)	Lipid titer (g/L)	Lipid yield (g/g)
3	44.4 ± 0.9	8.9 ± 0.1	0.098 ± 0.000	46.6 ± 2.7	21.7 ± 0.5	29.7 ± 0.4	6.4 ± 0.2	0.139 ± 0.013
7	59.6 ± 0.2	10.9 ± 0.2	0.147 ± 0.020	59.1 ± 0.0	24.6 ± 1.1	28.7 ± 0.2	7.1 ± 0.3	0.119 ± 0.004
10	89.8 ± 0.3	13.3 ± 0.1	0.187 ± 0.003	89.3 ± 1.0	35.1 ± 1.7	26.6 ± 1.9	9.3 ± 0.2	0.103 ± 0.001
15	98.9 ± 0.7	16.6 ± 1.2	0.269 ± 0.003	94.1 ± 0.7	38.4 ± 0.3	32.1 ± 0.4	12.3 ± 0.3	0.131 ± 0.002
20	102.6 ± 0.5	14.9 ± 0.5	0.303 ± 0.030	92.6 ± 0.8	37.6 ± 0.3	31.2 ± 0.1	11.7 ± 0.1	0.127 ± 0.000

All cultures incubated in the conical flask at 30 °C, 180 rpm, and the initial pH of 5.0. Fermentation time for the different CCR hydrolysates were 3 FPU/g DM for 5 days, 7 FPU/g DM for 6 days, and 10, 15, 20 FPU/g DM all for 8 days, respectively.

Table 3
Lipid fermentation performance with different lignocellulose feedstocks and microorganisms.

Strains	Feedstocks	DCW (g/L)	Lipid content (%)	Lipid titer (g/L)	References
T. fermentans CICC1368	Rice straw hydrolysate	28.6	40.1	11.5	Huang et al. (2009)
M. isabellina ATHUM 2935	Rice hull hydrolysate	5.6	64.3	3.6	Economou et al. (2011)
C. curvatus ATCC 20509	Wheat straw hydrolysate	17.2	33.5	5.8	Yu et al. (2011)
Y. lipolytica Po1 g	Sugarcane bagasse hydrolysate	11.4	58.5	6.7	Tsigie et al. (2011)
T. dermatis CH007	Corncob hydrolysate	24.4	40.1	9.8	Huang et al. (2012)
T. dermatis CH002	Corncob hydrolysate	22.9	35.9	10.4	Chen et al. (2012)
T. cutaneum CX1	Corn stover hydrolysate	10.2	30.4	3.1	Huang et al. (2011)
T. cutaneum AS 2.571	Corn stover hydrolysate	19.3	39.2	7.6	Hu et al. (2011)
T. cutaneum ACCC 20271	Corncob residues hydrolysate	38.4	32.1	12.3	This study

production from CCR feedstock could become a favorite technology option for both CCR utilization and microbial lipid production.

4. Conclusion

T. cutaneum ACCC 20271 was screened as the most suitable oleaginous yeast in the CCR hydrolysate to microbial lipid fermentation. The optimal C/N ratio of 49.3 in the CCR hydrolysate was obtained. The lipid yield was enhanced by increased sugar concentration in the CCR hydrolysate. The lipid titer and lipid content using the CCR hydrolysate by *T. cutaneum* ACCC 20271 were 12.3 g/L and 32.1%, respectively, and the lipid titer of 12.3 g/L and DCW of 38.4 g/L were the highest values on using lignocellulose derived biomass for lipid production.

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References

Adney, B., Baker, J., 1996. Measurement of Cellulase Activities. NREL LAP-006, Golden, CO.
Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat. Protoc. 2, 875–877.
Chen, X.F., Huang, C., Xiong, L., Chen, X.D., Ma, L.L., 2012. Microbial oil production from corncob acid hydrolysate by *Trichosporon cutaneum*. Biotechnol. Lett. 34, 1025–1028.
Cheng, K.K., Zhang, J.A., Erik, C., Li, J.P., 2010. Integrated production of xylitol and ethanol using corncob. Appl. Microbiol. Biotechnol. 87, 411–417.
Cheng, K.K., Wang, W., Zhang, J.A., Zhao, Q., Li, J.P., Xue, J.W., 2011. Statistical optimization of sulfite pretreatment of corncob residues for high concentration ethanol production. Bioresour. Technol. 102, 3014–3019.
Economou, C.N., Aggelis, G., Pavlou, S., Vayenas, D., 2011. Single cell oil production from rice hulls hydrolysate. Bioresour. Technol. 102, 9737–9742.
Han, H.W., Cao, W.L., Zhang, J.C., 2005. Preparation of biodiesel from soybean oil using supercritical methanol and CO_2 as co-solvent. Process Biochem. 40, 3148–3151.
Hu, C., Wu, S., Wang, Q., Jin, G., Shen, H., Zhao, Z.K., 2011. Simultaneous utilization of glucose and xylose for lipid production by *Trichosporon cutaneum*. Biotechnol. Biofuels 4, 25.
Huang, C., Zong, M.H., Wu, H., Liu, Q.P., 2009. Microbial oil production from rice straw hydrolysate by *Trichosporon fermentans*. Bioresour. Technol. 100, 4535–4538.
Huang, X., Wang, Y.M., Liu, W., Bao, J., 2011. Biological removal of inhibitors leads to the improved lipid production in the lipid fermentation of corn stover hydrolysate by *Trichosporon cutaneum*. Bioresour. Technol. 102, 9705–9709.
Huang, C., Chen, X.F., Xiong, L., Chen, X.D., Ma, L.L., 2012. Oil production by the yeast *Trichosporon dermatis* cultured in enzymatic hydrolysates of corncobs. Bioresour. Technol. 110, 711–714.

Liu, K., Lin, X.H., Yue, J., Li, X.Z., Fang, X., Zhu, M.T., Lin, J.Q., Qu, Y.B., 2010. High concentration ethanol production from corncob residues by fed-batch strategy. Bioresour. Technol. 101, 4952–4958.

Liu, W., Wang, Y.M., Yu, Z.C., Bao, J., 2012. Simultaneous saccharification and microbial lipid fermentation of corn stover by oleaginous yeast *Trichosporon cutaneum*. Bioresour. Technol. 118, 13–18.

Papanikolaou, S., Aggelis, G., 2011. Lipids of oleaginous yeasts. Part II: Technology and potential applications. Eur. J. Lipid Sci. Technol. 113, 1052–1073.

Pizarro, A.V.L., Enoch, Y.P., 2003. Lipase-catalyzed production of biodiesel fuel from vegetable oils contained in waste activated bleaching earth. Process Biochem. 38, 1077–1082.

Ratledge, C., Wynn, J.P., 2002. The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. Adv. Appl. Microbiol. 51, 1–51.

Tsigie, Y.A., Wang, C.Y., Truong, C.T., Ju, Y.H., 2011. Lipid production from *Yarrowia lipolytica* Po1g grown in sugarcane bagasse hydrolysate. Bioresour. Technol. 102, 9216–9222.

Yu, X.C., Zheng, Y.B., Dorgan, K.M., Chen, S., 2011. Oil production by oleaginous yeasts using the hydrolysate from pretreatment of wheat straw with dilute sulfuric acid. Bioresour. Technol. 102, 6134–6140.

Zhang, W., Liu, Z., Liu, Z., Li, F.L., 2012. Butanol production from corncob residue using *Clostridium beijerinckii* NCIMB 8052. Lett. Appl. Microbiol. 55, 40–246.

Zhao, K., Qiao, Q.A., Chu, D.Q., Gu, H.Q., Dao, T.H., Zhang, J., Bao, J., 2012. Simultaneous saccharification and high titer lactic acid fermentation of corn stover using a newly isolated lactic acid bacterium *Pediococcus acidilactici* DQ2. Bioresour. Technol. 135, 481–489.