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Short Communication

Efficient lipid production with *Trichosporon fermentans* and its use for biodiesel preparation

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Abstract

Effects of medium components and culture conditions on biomass and lipid production of *Trichosporon fermentans* were studied. The optimal nitrogen source, carbon source and C/N molar ratio were peptone, glucose and 163, respectively. The favorable initial pH of the medium and temperature were 6.5 and 25 °C. Under the optimized conditions, a biomass of 28.1 g/l and a lipid content of 62.4% could be achieved after culture for 7 days, which were much higher than the original values (19.4 g/l and 50.8%) and the results reported by other groups. *T. fermentans* could grow well in pretreated waste molasses and a lipid yield of 12.8 g/l could be achieved with waste molasses of 15% total sugar concentration (w/v) at pH 6.0, representing the best result with oleaginous microorganisms on agro-industrial residues. Addition of various sugars to the pretreated molasses could efficiently enhance the accumulation of lipid and the lipid content reached as high as above 50%. Similar to vegetable oils, the lipid mainly contains palmitic acid, stearic acid, oleic acid and linoleic acid and the unsaturated fatty acids amount to about 64% of the total fatty acids. The microbial oil with an acid value of 5.6 mg KOH/g was transesterified to biodiesel by base catalysis after removal of free fatty acids and a high methyl ester yield of 92% was obtained.

Keywords: *Trichosporon fermentans*; Microbial oil; Waste molasses; Biodiesel

1. Introduction

Biodiesel, produced from vegetable oils and animal fats, is rather an attractive alternative for its biodegradable, nontoxic and clean renewable characteristics as well as the similar properties to the conventional diesel fuels. Although biodiesel has presently been used in many countries such as European Union, the USA, Australia and some Asian countries including Japan, Malaysia and China and so on, the high cost of biodiesel has become one of the major obstacles for its further development and wide application. Besides, the use of vegetable oils as raw material for biodiesel production would compete with edible oils, thus leading to the soar of food price. Using recovered animal fats and used frying oils as feedstock can efficiently reduce the price of biodiesel, however, the amount of waste oils is limited and cannot meet the increasing needs for clean renewable fuels.

Microbial oils, namely single cell oils, produced by oleaginous microorganisms involving bacteria, yeasts, moulds and algae, are now believed as a promising potential feedstock for biodiesel production due to their similar composition of fatty acids to that of vegetable oils (Li et al., 2007). Compared with the production of vegetable oils, the culture of oleaginous microorganisms is affected neither by seasons nor by climates. In addition, oleaginous microorganisms can not only accumulate lipids within a short period of time but grow well on a variety of substrates, even inexpensive material, such as nutritional residues from agriculture and industry (Rupčić et al., 1996; Xue et al., 2006; Angerbauer et al., 2008), thus lowering the cost of oils. *Trichosporon fermentans* is a kind of yeast belonging to the family of *Cryptococcus* and could produce a large amount of extracellular lipase from olive oil or tung oil (Chen et al., 1992). Recently, it has been found that *T. fer-*

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mentans also has the ability to accumulate lipid in nitrogen-limited medium (Wang et al., 2005). To improve its lipid yield, effects of medium components and culture conditions on cell growth and lipid accumulation of *T. fermentans* were systematically studied. In order to reduce the cost of lipid production, culture of *T. fermentans* with waste molasses as substrate was also investigated. Furthermore, the fatty acid composition of lipid from *T. fermentans* was determined and the biodiesel production from the lipid was preliminarily studied.

2. Methods

2.1. Microorganism and precultivation

T. fermentans CICC 1368 was supplied by China Center of Industrial Culture Collection and kept on wort agar at 4 °C. The preculture was performed in YEPD medium (g/l, glucose 20, peptone 10, yeast extract 10) at 28 °C and 160 r/min for 24 h.

2.2. Media preparation and cultivation

Seed culture (5%) was inoculated to the culture medium. Cultures were performed in 250 ml conical flasks containing 50 ml fermentation broth. The original nitrogen-limited medium was as follows (g/l): glucose 100, yeast extract 0.5, peptone 1.8, $MgSO_4 \cdot 7H_2O$ 0.4, KH_2PO_4 2.0, $MnSO_4 \cdot H_2O$ 0.003, $CuSO_4 \cdot 5H_2O$ 0.0001. All cultures were incubated in a rotary shaker at 160 r/min.

Cane molasses was obtained from Jiangmen Sugar-refinery (Guangdong, China), and it contains 35% (w/w) sucrose, 10% (w/w) converted sugars (glucose and fructose), 2.5% (w/w) other carbohydrates, 4.3% (w/w) crude protein, 0.06% (w/w) crude fat, 9.6% (w/w) ash, 4.6% (w/w) salt, 8.9% (w/w) metal ions such as calcium, potassium, sodium, iron, magnesium, copper, etc, and 25% (w/w) water. The crude molasses was diluted with distilled water for specified total sugar concentration (w/v). The crude molasses was pretreated with sulfuric acid according to the reported method (Liu et al., 2008), followed by centrifugation at 10,000 *g* for 10 min and the supernatant was adjusted to pH 6.0 with 10 mol/l NaOH.

2.3. Analytical methods

Biomass was harvested by centrifugation and determined in its lyophilized form (Kavadia et al., 2001). The total reducing sugars were determined according to the method described by Somogy (1945).

Extraction of lipid from lyophilized biomass was performed according to the modified procedure of Bligh and Dyer (1959). Lipid was extracted with a mixture of chloroform: methanol (2:1, v/v) for 1 h. The extracted lipid was centrifuged to obtain a clear supernatant and the solvent was removed by evaporation under vacuum.

The fatty acid profile of the lipid was determined by saponifying followed by methylation for conversion of fatty acids to fatty acid methyl esters (FAMES) according to the method of Morrison and Smith (1964). The fatty acid methyl esters were determined by gas chromatography (GC-2010) with ionization detector and a DB-1 capillary column (0.25 cm × 30 m, Agilent Technologies Inc., USA). The column temperature was programmed as being upgraded from 170 °C to 220 °C at a rate of 3 °C/min and kept for 3 min. Nitrogen was used as the carrier gas at 0.80 ml/min. Split ratio was 1:50 (v/v). The injector and the detector temperatures were set at 250 °C and 280 °C, respectively. All data were averages of triplicate determinations.

3. Results and discussion

The time course of cell growth, glucose exhaustion and lipid production of *T. fermentans* were shown in Fig. 1. It is apparent that glucose was used mainly for cell growth at the beginning of the fermentation. Biomass, lipid content and utilized glucose gradually increased with time after inoculation. On the 7th day, lipid content and lipid yield reached the maximum of 57.0% and 12.3 g/l. A slight decrease was found in biomass on the day 8 while utilized glucose increased. The possible reason may be that nitrogen source was exhausted and a great deal of glucose consumption led to a decrease of pH, thus inhibiting cell growth. During the period between days 9 and 10, there was a clear increase in biomass. However, lipid content showed an apparent decrease. The similar changes were also observed in lipid content of *Yarrowia lipolytica*, *Cunninghamella echinulata* and *Mortierella isabellina* after exhaustion of the carbon source in the growth environment (Papanikolaou and Aggelis, 2003; Papanikolaou et al., 2004; Fakas et al., 2007). The use of lipid for cell prolifer-

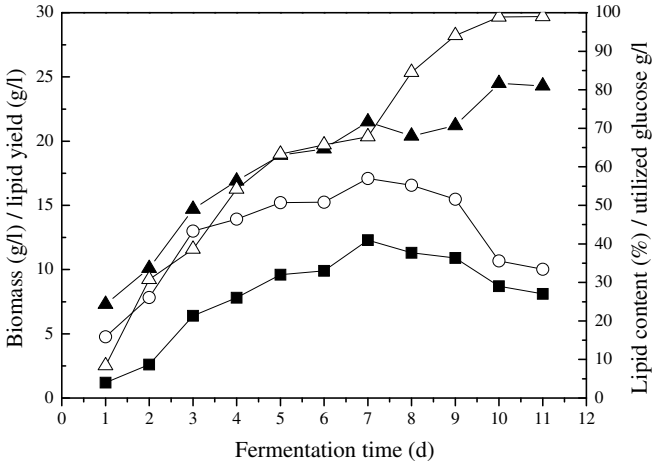

Fig. 1. Time course of cell growth and lipid accumulation with *T. fermentans*. Culture was performed in the original nitrogen-limited medium. (▲) Biomass; (■) lipid yield; (○) lipid content; and (△) utilized glucose.