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Studies on lipid production by *Rhodotorula glutinis* fermentation using monosodium glutamate wastewater as culture medium

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Abstract

Microbial lipid, as a raw material for biodiesel, can be produced by *Rhodotorula glutinis* with the monosodium glutamate (MSG) wastewater. The effect of adding glucose to MSG wastewater on lipid production was studied in this paper. Three different strategies, including initial addition, fed-batch addition and glucose feedback addition were attempted. The results show that addition of glucose was found favorable not only for cell growth but also for lipid synthesis. Of the three adding methods glucose feedback addition was the most effective one: about 25 g/L of biomass, 20% of lipid content and 45% of COD degradation were obtained respectively. And the components of the resulted lipid using different addition strategies were further studied.

Keywords: Biodiesel; Lipid; *Rhodotorula glutinis*; Monosodium glutamate (MSG) wastewater; Glucose addition

1. Introduction

Since 1960s, monosodium glutamate production has been increasing annually and is one of the primary fermentation industries in China (Jia et al., 2006). Wastewater from monosodium glutamate manufacturing is also one of the most intractable fermentative wastewater. It has high content of COD (10,000–40,000 mg/L), ammonium (15,000–25,000 mg/L), sulphate (15,000–30,000 mg/L) and very low pH (about 2.0). The treatment of such wastewater, if using conventional activated sludge processes, will consume a lot of energy resulting in high treatment costs (Yang et al., 2005). Therefore, developing an efficient and economical treatment approach of such MSG wastewater is necessary. On the other hand, natural resources from fossil, such as petroleum, coal and natural gas, are currently scarce and are to be exhausted in the near future. Thus, looking for alternative novel and renewable resources such as biodiesel is of vital importance (Demirbas, 2005). However, the cost of biodiesel is high due to the expensive raw materials (about 70–75% of total cost). Biodiesel is still not economically feasible, though the production of this fuel has been developed for decades (Allen et al., 1999; Ma and Hanna, 1999; Ramadhas et al., 2005; Leunc, 2001).

Microbial oils, which are renewable and potentially inexhaustible source of energy as potent as diesel fuel, have attracted much attention in recent years. Due to recent increment in petroleum prices, more concerns as shown (Papanikolaou et al., 2004; Zhao, 2005) in microbial oil fuels arise nowadays.

The yeast *Rhodotorula* produces large amounts of fats (Rose and Harrison, 1970; Maria et al., 2002), and has been used for the production of single-cell proteins from ethanol, acetic acid and acetaldehyde (Yech, 1996). Recently, *Rhodotorula glutinis* was used for wastewater treatment (Yang et al., 2005; Xue et al., 2006). MSG wastewater was used as a cheap fermentation broth, which served as the raw material for the production of biodiesel

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using lipid from *R. glutinis*. However, both the biomass and lipid content were very low as demonstrated (Xue et al., 2006). Yang et al. (2005) have recently shown the ratio of C:N:P (1:2.4:0.005) in MSG wastewater is not appropriate for microbial growth and lipid synthesis: phosphorus and carbon are obviously not sufficient while the concentration of NH^{4+}–N is too high, which will boost cell growth but inhibit the lipid synthesis. It has been stated (Feng et al., 2005) that glucose could enhance lipid production with some microorganism species, so the effect of glucose addition in the MSG wastewater on lipid production was studied in this work. The aim of this study was to examine the potential of MSG wastewater with glucose addition as substrates for the production of biodiesel lipid by the yeast strain belonging to the genus *Rhodotorula*.

2. Methodology

2.1. Materials and culture conditions

R. glutinis was a budding-reproductive yeast as observed with an ordinary microscope (BH-2, OLYMPUS, Japan). Cell density increased significantly during the early culturing stage. During the medium-term cells containing increased gradually. The release of intracellular products was found at the terminal stage ($t = 96$ h). *R. glutinis* was maintained on YPD agar slant for three days at 30 °C to get its activation and then inoculated into the seed culture medium comprising of (%, g/dL) glucose 4, $(NH_4)_2SO_4$ 0.2, KH_2PO_4 0.7, Na_2SO_4 0.2, $MgSO_4 \cdot 7H_2O$ 0.15, yeast extract 0.15, pH 5.50. The inoculum was cultured for 24 h at 30 °C with rotation speed of 140 r/min, then inoculated into 250 mL flask or a 5 L fermentor (BIOTECH-2002, Shanghai Baoxing, China) for 5 days with MSG wastewater. Culture conditions of different adding strategies were depicted in the legends of figures. The MSG wastewater provided by Hongmei Co., Ltd (China), was diluted with tap water to COD = 10,000 mg/L and adjusted pH to 5.5 prior to use. The original characteristics of MSG wastewater are listed as: COD 40,000 ± 3000 mg/L, BOD_5 30,000 ± 5000 mg/L, NH_4^+–N 165 ± 5 mg/L, TP 190 ± 10 mg/L, glutamic acid 6.85 ± 0.05 g/L, reducing sugar 2.9 ± 0.1 g/L, pH 2.0–2.5. The cells were harvested by centrifugation (3000 r/min × 10 min), washed with distilled water, fragmented by sonication and finally dried at 60–80 °C to constant weight.

2.2. Measurement of glucose, biomass and COD

Glucose concentration was detected using a glucose biosensor (SBA 40C, Biological Institute of Shandong Academy of Sciences). The cell concentration was determined by the dry cell weight. Five milliliters culture was centrifuged at 3000 r/min for 10 min. The cell was washed twice with 5 mL distilled water and then dried to constant weight at 60–80 °C. COD was measured by $K_2Cr_2O_7$ method (Xue et al., 2006).

2.3. Lipid extraction and analysis

Lipid was continuously extracted with a mixture of chloroform and methanol (2:1, v/v) for 20 min (Zhu et al., 2002). The extracted lipid was centrifuged to obtain a clear supernatant and sodium sulphate anhydrous was added to remove any residual moisture. The total lipid was estimated by gravimetric method. And the lipid components were analyzed by the GC-2010 gas-chromatograph (Shimadzu, Japan). The condition of GC analysis was as following: flame ionization detector (FID) 350 °C; column DB-1ht (J&W Scientific, USA), 30 m (length) × 0.25 mm (inner diameter) × 0.1 μm (thickness); PTV sample entrance (33 cm/s); diffluent ratio1:5; carrier gas: N_2.

3. Results and discussion

Addition of different concentrations of glucose varied the growth of *R. glutinis* and the corresponding effect on lipid content and COD degradation were studied. The result of flask culturing indicated that initial addition of glucose into the MSG wastewater broth resulted in an increase of both the biomass and the lipid content. Adding between 2% and 4% of glucose helped to reach satisfactory levels on the 5th day. And moderate COD degradation of 45% was still obtained. So in the following experiments, proper concentration of initial glucose between 2% and 4% was adopted.

3.1. Effect of different glucose addition methods on R. glutinis

3.1.1. Initial addition

Glucose of 4% (w/v) was initially added into the MSG wastewater as the fermentation broth (Fig. 1). The cell growth displayed a similar growth S-curve: a lag phase from 0 to 24 h, an exponential phase from 24 to 36 h and a relatively stationary phase after 36 h of growth. The results showed that *R. glutinis* has the ability to accumulate lipid as storage compounds. This accumulation is triggered by exhaustion of the nitrogen source (NH^{4+}–N and glutamic acid was utilized by 86% and 99%, respectively) thus preventing cell proliferation, but still allowing the conversion of substrate to lipid. The glucose was also consumed rapidly until 60 h, after which the glucose was soon exhausted. Lipid content and COD degradation, reached maximum at 72 h, then declined. The synthesized lipid was utilized as substrate after glucose was exhaustion, which caused lipid content decrease; and the release of intracellular product into the broth was responsible for the lower COD degradation. Therefore, only initial addition of glucose was not favorable enough for lipid synthesis in MSG wastewater. In addition, the results of 4% of glucose addition in 5 L fermentor were better than the results gained in 250 mL flask. That is to say, by adding glucose to treat MSG wastewater in larger scale system is more effec-