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Comparison of fermentation by mono-culture and co-culture of oleaginous yeasts for ABE (acetone- butanol- ethanol) fermentation wastewater treatment

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ABSTRACT

ABE (acetone- butanol- ethanol) fermentation wastewater was treated by mono-culture or binary co-culture of three oleaginous yeasts belonging to genus *Trichosporon* (*Trichosporon cutaneum*, *Trichosporon dermatis*, and *Trichosporon coremiiforme*). Considering biomass and lipid content, co-culture showed little beneficial effect possibly due to the earlier lipid turnover. The treatment by co-culture of *T. dermatis and T. cutaneum* showed no beneficial effect for lipid yield, but the ratio of oleic and linoleic acid in microbial oil increased obviously. For co-culture, the COD degradation rate of batch mode was higher than that of fed-batch mode, but the biomass of batch mode was lower, indicating that the degraded COD did not totally convert into biomass. Lipid turnover existed in the treatment of ABE fermentation by mono-culture of yeast or co-culture of yeasts, and the lipid turnover was much obvious for co-culture mode. The evolution of polysaccharide was complex that lipid and polysaccharide might convert into each other.

1. Introduction

Recently, the technology of ABE (acetone- butanol- ethanol) production by the fermentation of *Clostridium acetobutylicum* has attracted much attention for its great potential as alternative energy in future [1–6]. For this technology, ABE is usually recovered by distillation after fermentation. However, the residual sugars and some kinds of organic acids (the by-products of ABE fermentation) will still be present in the fermentation broth after distillation and this remaining fermentation broth so called ABE fermentation wastewaters commonly has high COD (Chemical Oxygen Demand) value and requires treatment before drainage [7].

Biological treatment with certain equipment such as UASB (Up-flow Anaerobic Sludge Blanket) or EASB (Extended Anaerobic Sludge Blanket) is usually used for the treatment of fermentation wastewaters [8,9]. Recently, new technology of using oleaginous yeast for fermentation wastewaters treatment has attracted much attention not only for its simple fermentation technology and common fermentation equipment, but also for its potential to generate valuable by-products such as microbial oil [10]. In previous researches, ABE fermentation wastewater could be treated efficiently by oleaginous yeasts belonging to genus *Trichosporon* including *Trichosporon dermatis, Trichosporon coremiiforme*, and *Trichosporon cutaneum* with mono-culture (fermentation by individual microorganism) [7,11,12].

Besides mono-culture, co-culture of different microorganisms is also used commonly in the field of biochemical engineering [13,14]. In fact, traditional fermentation wastewaters treatment by activated sludge is also co-culture of various microorganisms. By the cooperation of different microorganisms, the COD degradation could be greater due to their different metabolism property. However, up to date little is known about the effect of co-culture on the COD degradation and lipid production using fermentation wastewaters as substrate.

For ABE fermentation wastewater treatment, *T. dermatis, T. coremiiforme*, and *T. cutaneum* showed different metabolic capacity on sugars and organic acids, thus co-culture of these oleaginous yeasts might be one potential mode for ABE fermentation wastewater treatment. In this study, the binary co-culture of

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three oleaginous yeasts (*T. dermatis, T. coremiiforme*, and *T. cutaneum*) was carried out and compared to the mono-culture to evaluate the effect of co-culture on the COD degradation and products accumulation in ABE fermentation wastewater.

2. Material and methods

2.1. Wastewater preparation

Wastewater after ABE fermentation was obtained from Laboratory of Energy and Biochemical Engineering, Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences (GIEC CAS). The ABE fermentation was carried out in the medium containing a mixture of various sugars (g/L, glucose 20.2, xylose 23.2, and arabinose 1.4). After fermentation, more than 99% of butanol, ethanol, and acetone in the fermentation broth were recovered by distillation. The remaining fermentation broth containing the residual sugars and organic acids was further filtrated by Whatman® qualitative filter paper (Hangzhou Wo-Hua Filter Paper Co. Ltd., China) to remove the sediments and the filtrate was the wastewater used in this work. The pH value of wastewater was adjusted to 7.0 by 4 M NaOH.

2.2. Microorganism and microbial oil production on wastewater

Oleaginous yeasts *Trichosporon cutaneum* CH002, *Trichosporon dermatis* CH007, and *Trichosporon coremiiforme* CH005 (Laboratory of Energy and Biochemical Engineering, GIEC CAS) were used for microbial oil production. Yeasts were previously grown on the pre-cultivation medium (g/L, glucose 20, peptone 10, yeast extract 10) at 28 °C and 150 rpm for 24 h. The wastewater after ABE fermentation without adding other nutrients (nitrogen sources or trace elements) was used as the medium for microbial oil production by different microorganisms. Ten percent (10%) seed culture was inoculated to the culture medium. After inoculation into ABE fermentation wastewater (0 h of fermentation), the biomass and lipid content of *T. dermatis, T. coremiiforme*, and *T. cutaneum* were 0.4 ± 0.0 g/L and $8.7 \pm 1.5\%$, 0.4 ± 0.2 g/L and $7.3 \pm 2.7\%$, 0.3 ± 0.0 g/L and $8.2 \pm 1.0\%$, respectively. Then, cultivation was performed in a 250 mL conical flask containing 50 mL fermentation broth in a rotary shaker at 28 °C and 150 rpm. Generally, the co-culture ratio of seed culture was 1:1 except in experiments to compare the effect of different co-culture ratio. In these cases of binary co-culture, 5% of seed culture of each yeast was used. To have a clear evaluation on the evolution of different composition in yeast biomass and compare with previous studies using oleaginous yeasts for ABE fermentation wastewater treatment, the fermentation period was set as five days [7,11,12]. Two different modes of co-culture (batch mode and fed-batch mode) were used for ABE fermentation wastewater treatment. For batch mode of co-culture, both oleaginous yeasts were inoculated into the medium simultaneously at the beginning of fermentation. For fed-batch mode of fermentation, one oleaginous yeast was inoculated into the medium firstly at the beginning of fermentation, and then the other oleaginous yeast was inoculated into the medium on different times of the fermentation process. All the experiments were performed in duplicate and the results were expressed as the averages.

2.3. Analytical methods

Biomass was harvested by centrifugation and the dry cell weight of biomass was determined [15]. Extraction of lipid from dry biomass was performed using the modified procedure of previous study, with a mixture of chloroform: methanol (2:1, v/v) [16]. The extracted lipid was centrifuged to obtain a clear supernatant and the solvent was removed by evaporation under vacuum. Lipid yield is expressed as the amount of lipid extracted from the cells in per liter of fermentation broth (g/L) and lipid content is defined as the percentage of lipid to dry biomass (%, w/w).

The fatty acid composition was measured by converting fatty acids into fatty acid methyl esters and the fatty acid methyl esters which in turn were determined by gas chromatography (GC) (GC-7890, Agilent, USA) with ionization detector and an HP-INNOWAX polyethylene glycol column ($30\,m \times 250\,\mu m \times 0.25\,\mu m$). The column temperature was maintained at 170 °C for 1 min and upgraded from 170 °C to 200 °C at a rate of 10 °C min^{-1} and kept for 1 min. Then, it increased to 230 °C with a temperature gradient of 3 °C min^{-1} and held for 15 min. Argon was used as the carrier gas at 1.0 mL min^{-1}, with split ratio was 1:10 (v/v). The injector temperature and detector temperature were both set at 240 °C, respectively.

The concentration of sugars and organic acids in the wastewater were analyzed by High Performance Liquid Chromatography (HPLC) (Waters 2685 systems, Waters Corp., USA), with a RI (Refractive Index) detector (Waters 2414), and on Aminex HPX-87H column (300 mm × 7.8 mm, Bio Rad Corp., USA) using 5 mM H_2SO_4 solution at 0.5 mL/min. COD in the wastewater was evaluated by Hach DR2700 Water Quality Analyzer (Hach Company, USA). The polysaccharides content in yeast biomass was evaluated by phenol–sulfuric acid method [17].

3. Results and discussion

3.1. Comparison for the effect of co-culture and mono-culture on yeast growth

The fermentation period of both mono-culture and co-culture by oleaginous yeasts was five days. After five days of fermentation, the biomass of both mono-culture and co-culture was compared as shown on Fig. 1. In this study, the initial biomass of three oleaginous yeasts (*T. dermatis, T. coremiiforme*, and *T. cutaneum*) was almost the same (about 0.3–0.4 g/L at the 0 h of fermentation), thus, the inoculation (10% of seed culture) showed little influence on the final biomass. Namely, the biomass obtained by different fermentation modes (fermentation by mono-culture and co-culture) depended mostly on the metabolic capacity of different oleaginous yeasts.

The mono-culture of *T. coremiiforme* had the lowest biomass yield after fermentation using ABE fermentation wastewater as substrate, showing that its biomass production capacity was lower

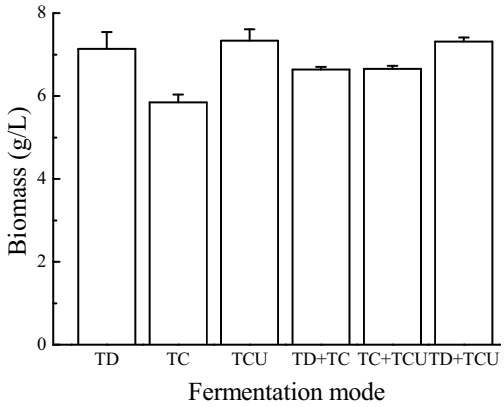

Fig. 1. Biomass after 5 days' fermentation with different fermentation modes. The data represent the means ± standard errors of the means for duplicate samples. Abbreviations: TD: *T. dermatis*; TC: *T. coremiiforme*; TCU: *T. cutaneum*.