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Microbial conversion of mixed volatile fatty acids into microbial lipids by sequencing batch culture strategy

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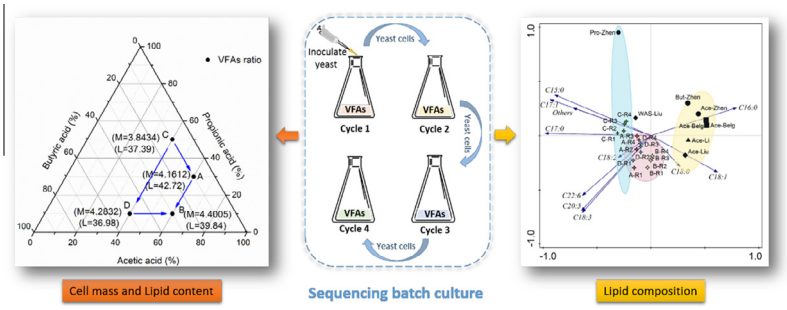
HIGHLIGHTS

- The highest lipid yield was achieved when acetic:propionic:butyric acid was 6:3:1.
- Utilization and conversion of VFAs were different during sequencing batch culture.
- Lipids produced from mixed VFAs were ideal raw materials for biodiesel.
- High content of propionate in VFAs lead to high yield of odd-numbered fatty acids.

GRAPHICAL ABSTRACT



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ABSTRACT

Four mixed volatile fatty acids (VFAs) were used as sole carbon source to culture oleaginous yeast *Cryptococcus curvatus* by sequencing batch culture strategy. The highest lipid content (42.7%) and concentration (1.77 g/L) were achieved when the ratio of VFAs (acetic, propionic, and butyric acids) was 6:3:1. The oleaginous yeast favored to use VFAs for lipid biosynthesis rather than cell proliferation. With regard to the utilization ratio of VFAs, acetic acid reached over 99%, whereas propionic acid was barely 35%. The produced lipids contained nearly 45% of monounsaturated fatty acids, which can be the ideal raw materials for biodiesel production. Additionally, the produced odd-numbered fatty acid content reached 23.6% when the propionate acid content of VFAs was 50%. Further analysis showed that increasing the ratio of acetic acid was most beneficial to cell mass and lipid production, whereas propionic acid and butyric acid were more conducive to lipid and cell mass synthesis, respectively.

1. Introduction

Microbial lipids existing in the form of lipid drops inside oleaginous microbes (such as bacteria, microalgae, yeast, and molds) are currently recognized as the most promising feedstock for biodiesel production. The conversion of organic waste into available substrate for synthesis of microbial lipids is an ideal way to reduce the production cost of microbial lipids and promote its application. In particular, the use of waste-derived volatile fatty acids (VFAs) to cultivate oleaginous yeast for microbial lipids can achieve the transformation of organic waste to high-value-added products (Chang et al., 2010). Researchers found that VFAs can be used to substitute common carbon sources, such as glucose, for microbial oils owing to its shorter metabolic pathway and higher theoretical conversion efficiency (Lian et al., 2012). Economic calculation results showed that the use of waste-derived VFAs to produce biodiesel via oleaginous yeast demonstrated both technical and economic feasibility (Gaeta-Bernardi and Parente, 2016). Common organic wastes, such as food waste (Wang et al., 2014), organic

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wastewater (Fernandes et al., 2016), and waste-activated sludge (Jin et al., 2016), can produce VFAs through acidogenic fermentation. Waste-derived VFA solutions are mixtures of various acids including acetic acid, propionic acid, isobutyric acid, n-butyric acid, isovaleric, and n-valeric acid, with total VFA concentrations ranging from 2 g/L to 34 g/L (Ma et al., 2016; Yin et al., 2014). The composition ratio of various acids can be affected by the substrate and fermentation conditions. Generally, the ratio of acetic acid was the highest with the content of 43–69% (Yuan et al., 2011), the ratio of propionic acid was 10–54% (Khiewwijit et al., 2015), and the ratio of butyric acid was 9–46.9% (Chang et al., 2010).

Nevertheless, the utilization of low-concentration VFAs produced from waste fermentation to produce microbial lipids was rarely studied. In terms of culture strategies, fed-batch cultivation method was applied to obtain high lipid yield in previous studies (Chi et al., 2011; Christophe et al., 2012). However, the aforementioned strategy is not suitable for the application of waste-derived VFAs because of the requirement of a concentrated solution. To directly utilize the actual waste-derived VFAs, Gong et al. (2015) applied continuous culture to use acetic acid (5 g/L) for lipid production by *Cryptococcus curvatus*; the maximal lipid content was 56.7%, but the cell mass was only 1.34 g/L. Huang et al. (2016) developed a sequencing batch culture (SBC) to use low-concentration acetic acid (4 g/L) as carbon source for cell mass and lipid production with *Rhodosporidium toruloides* AS 2.1389. The cell mass and lipid content reached 4.21 g/L and 38.6%, respectively, after a four-cycle cultivation (Huang et al., 2016). Thus, continuous and SBC strategies may be more suitable for the use of actual VFA solutions from waste fermentation.

In terms of lipid production, the composition of different waste-derived VFAs can significantly influence the lipid production. Vajpeyi and Chandran (2015) used VFA solutions (VFA concentration was 6.2 ± 3.9 g/kg; the ratio of acetic, propionic, and butyric acids was about 5:2:3) from the fermentation of food waste as media to cultivate *C. curvatus*; the cell mass and lipid concentration were only 0.96 g/L and 14.9%, respectively. Xu et al. (2014) utilized VFA supernatant from macroalgae fermentation with VFA content of 13 g/L and VFA ratio of 7:2:1 to produce lipids with content of 48.3% by using *C. curvatus*. Gong et al. (2016) used corn stover hydrolysates containing 17.7 g/L acetate, 17.9 g/L glucose, and 17.2 g/L xylose to cultivate *C. curvatus* and acquired a higher lipid content at 60.8%. Pure VFA solution derived from waste-activated sludge (VFA content was 3.2 g/L, and VFA ratio was about 6:3:1) was used to cultivate *C. curvatus* via SBC strategy in our previous study (Liu et al., 2016), and this process yielded 39.6% lipid content.

Previous research shows that the utilization and conversion efficiency of VFAs are discrepant when different VFAs with different carbon-chain lengths was used as carbon sources. Generally, propionic acid and butyric acid are less favorable to be used by yeast than acetic acid (Fei et al., 2015; Vajpeyi and Chandran, 2015). The microbial lipids produced with propionic acid as carbon sources contained more odd-numbered fatty acids; in particular, the contents of heptadecanoic acid (C17:0) and heptadecenoic acid (C17:1) increased significantly (Kolouchova et al., 2015). Therefore, the effects of the ratio of mixed VFAs on the synthesis and composition of microbial lipids should be further revealed. In the present study, four ratios of VFAs (6:3:1, 6:1:3, 5:4:1, and 5:1:4) based upon the representative waste products were set to reveal the effects of different ratios of mixed VFAs on the accumulation of cell mass and lipid by using oleaginous yeast via SBC strategy, which was developed in our previous research (Huang et al., 2016). Furthermore, we explored the utilization and transformation of VFAs, as well as the composition of microbial lipids.

2. Materials and methods

2.1. Strain and media

Cryptococcus curvatus MUCL 29819 was obtained from the Biological Resource Center, National Institute of Technology and Evaluation (Tokyo, Japan). Before cultivation in liquid media, the yeasts were maintained at 4 °C on yeast malt (YM) agar slants and activated at 30 °C on YM agar plates for 4 days to 7 days. First, a loopful of cells were inoculated into 50 mL of yeast extract-peptone-dextrose (YPD) medium in a 250 mL flask and incubated in a gyratory shaker at 200 rpm and 30 °C for 36 h as seed culture. Afterward, 5 mL of the seed culture was inoculated into 45 mL of fermentation media in a 250 mL flask. The fermentation media were incubated under the same condition as that of the seed culture. The YPD medium contained 20 g/L glucose, 10 g/L fish peptone, and 10 g/L yeast extract, and the initial pH was adjusted to 6.0.

The fermentation media were prepared with 4 g/L VFAs, 0.4 g/L KH_2PO_4, 1.5 g/L $MgSO_4{\cdot}7H_2O$, and different concentrations of yeast extract and $(NH_4)_2SO_4$ because of the differences in proportion of VFAs in the experiment. The C/N ratio was 100. The concentrations of yeast extract and $(NH_4)_2SO_4$ differed with a ratio of 7.5:1.0 (w/w). Acetate, propionate and butyrate were mixed together in four different ratios, which was used as the sole carbon source. The four VFA ratios (6:3:1, 6:1:3, 4:5:1, and 4:1:5) were controlled as the mass ratio of acetic acid, propionic acid and butyric acid. The initial pH was adjusted to 6.0 by using 2 mol/L HCl and NaOH solutions. The media were autoclaved under 115 °C for 30 min before use. All cultivations were performed under the condition of 200 rpm and 30 °C in a gyratory shaker.

2.2. Sequencing batch cultivation

Cryptococcus curvatus MUCL 29819 was cultured in 250 mL flasks containing 50 mL of fermentation media. During the cultivation, OD_{600} and the concentration of VFAs (acetic, propionic and butyric acids) were monitored. Once the yeast reached the stationary growth phase, the media and cells were separated by centrifugation. After that, the separated yeast cells were resuspended in the fresh fermentation media for the next growth cycle. Four cycles were performed in this study. According to a previous research (Liu et al., 2016), the growth times of the first to fourth cycle were 20 h, 15 h, 15 h and 14 h, respectively.

2.3. Analytical method

2.3.1. Determination of cell mass

At the end of the cultivation, samples were centrifuged (3600×*g*, 4 °C) for 5 min, and then pellets were rinsed with a normal saline solution. After being dried by vacuum lyophilization at −50 °C for 24 h, cell mass accumulation was determined by weighing the samples.

2.3.2. Lipid extraction and determination of fatty acid methyl esters (FAMEs)

Dried yeast cells were weighed and suspended in 1 mL of methanol with glass beads (diameter 0.5 mm) and then disrupted by the MonoLyser™ lysing system (RotaPrep Inc., USA) before extraction with chloroform/methanol (1:1, vol/vol) twice. The extracts were washed using the same volume of 0.1% NaCl solution and dried by nitrogen blowing method at 50 °C (Gong et al., 2012).

The lipid composition was determined as FAMEs by transesterification catalyzed using methanolic BF_3 solution. FAMEs were detected through gas chromatography by using an Agilent 6890N