Fuel 226 (2018) 93–102

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

Full Length Article

Lipid accumulation from *Trichosporon oleaginosus* with co-fermentation of washed wastewater sludge and crude glycerol

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ARTICLE INFO

Keywords:
Lipid
Biodiesel
Sludge
Crude glycerol
Sludge washing
Inhibition

ABSTRACT

In this study, two types of organic waste including sludge and crude glycerol were employed for lipid production from oleaginous yeast *Trichosporon oleaginosus*. As wastewater sludge contains metals and other substances, which could inhibit growth of the microorganisms, sludge washing was performed to reduce/remove the substance in order to enhance the lipid production in this study. The results revealed that the lipid production by *Trichosporon oleaginosus* was higher in the medium prepared using washed sludge fortified with glycerol compared to that of the unwashed sludge fortified with glycerol. The maximum biomass and lipid concentrations were 44.48 ± 2.34 g/L and 17.37 ± 1.22 g/L, respectively in the fermentation with washed sludge and glycerol and 39.17 ± 2.06 g/L and 14.26 ± 1.17 g/L in the fermentation with unwashed sludge and glycerol. The study showed that sludge washing had improved lipid production from sludge.

1. Introduction

Lipid droplets accumulated in microorganisms has been considered as potential feedstock of biodiesel production as it has a similar composition as plant seed oils and animal fats, which are traditionally employed in the biodiesel formation [1,2]. The microorganism, which is capable of storing lipid (energy source) up to 20% cell dry weight is called oleaginous microorganism. Some of the oleaginous microorganisms could accumulate up to 60% lipid in the cell body (dry weight); however, generally, the oil content of plant seeds or the fat content of animals are less than 40% w/w [3,4]. In addition, microorganisms have shorter life cycles and faster growth rate compared to that of plants and animals. It suggested that microorganism could be a promising feedstock for biodiesel production.

According to the different demand on the energy source, oleaginous microorganisms can be divided into autotrophic and heterotrophic ones. Most of microalgae are autotrophic microorganisms, which can assimilate carbon dioxide for cell growth. Oleaginous microalgae have been extensively studied in biodiesel production. Microalgae farms were even built to produce lipid as the process didn't require substrate investment and could sequestrate CO_2 (greenhouse gas) [5–8]. Heterotrophic microorganism relies on organic carbon. Unlike carbon dioxide, the utilization of organic carbons needs much more costlier investment. It hindered the application of lipid production from heterotrophic oleaginous microorganisms. In fact, compared to an autotrophic oleaginous microorganism, the heterotrophic oleaginous microorganism is a more competitive alternative to plant seeds and animals in biodiesel production as they grow faster, could reach higher biomass concentration, are not impacted by lights, and have less land requirement [9–11].

The major obstacle of utilizing heterotrophic oleaginous microorganism for biodiesel production is the high cost demand due to the consumption of organic carbon, which could take up 70% of the total cost of biodiesel production from heterotrophic oleaginous microorganism [12–15]. Currently, increasing interest has been given to the use of organic wastes as a carbon source for oleaginous microorganism cultivation. Many organic wastes, including starch production industry wastewater, sugarcane bagasse, corn Stover hydrolysates, sweet sorghum, wastewater sludge, and crude glycerol, have been employed in oleaginous microorganism cultivation for lipid accumulation [2,14,16–19]. Crude glycerol is the by-product of biodiesel synthesis through trans-esterification [20]. It provides a way of the process integration when crude glycerol is assimilated by microorganism for accumulating lipid, which will be further converted to biodiesel. Studies have found that crude glycerol performed better than pure glycerol in lipid accumulation with oleaginous microorganism due to the presence of trace elements and salt in the crude glycerol [21,22]. It indicates that crude glycerol is a promising replacement to the costly carbon source such as pure glycerol and glucose.

Apart from carbon source, other nutrients such as nitrogen and

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https://doi.org/10.1016/j.fuel.2018.04.013
Received 29 November 2017; Received in revised form 31 March 2018; Accepted 2 April 2018

phosphorus are also essential in microorganism cultivation. Wastewater sludge is rich in carbon, nitrogen, phosphorus and many other elements, which are required for microbial growth. Our previous studies revealed that wastewater sludge was a potential source of substrate and nutrients for oleaginous microorganism cultivation [18,23], and similar results have been reported by other researchers as well [24–26]. Wastewater sludge is naturally generated during wastewater treatment and cost free. Employing wastewater sludge in lipid production could mitigate the sludge disposal pressure. The co-fermentation of crude glycerol (carbon source) and wastewater sludge (carbon and nutrient sources) for lipid production from microorganisms provides the solution to highly reduce the cost of biodiesel production using heterotrophic microorganisms and hence would advance the process to be practical. Our previous results showed that biomass concentration and lipid accumulation of *Trichosporon oleaginosus* had been improved with the addition of crude glycerol to sludge; however, they were still lower than that of *T. oleaginosus* cultivated with glucose [19,27]. It indicates that the capacities of cell growth and lipid accumulation by *T. oleaginosus* were not fully developed with sludge and crude glycerol as a medium. Wastewater sludge is generated during wastewater treatment. Wastewater composition is getting more and more complex as the daily used products in human life become more diverse. Emerging contaminants, including nanomaterials, fragrances, antibiotics, and perfluorinated hydrocarbon have been detected in wastewater [28–30]. These contaminants could stick on sludge, and hence impact on microorganism growth [31].

In this study, sludge washing was performed before co-fermenting with crude glycerol for lipid production from *T. oleaginosus*. The aim of sludge washing was to remove inhibitors present in sludge and thus to enhance lipid accumulation in *T. oleaginosus*. The fermentation of *T. oleaginosus* with unwashed sludge and crude glycerol was conducted as a control.

2. Materials and method

2.1. Materials

As in our previous studies [27,32], in this study, the sludge was collected from the secondary sedimentation tank of a local municipal wastewater treatment plant, Communauté Urbain de Québec (CUQ) in Québec, Canada. The sludge was first undergone the gravity settling at 4 °C for 24 h, and then the suspended solids concentration (SS) of the resulting solution was measured (around 23 g/L). The concentrated sludge was then stored at 4 °C for further utilization. The sludge characterization was given in Table 1. It can be seen that the sludge properties were similar as that used in our previous study. It suggested that the sludge of CUQ was not significantly variable in its composition and characteristics during the years.

Crude glycerol was obtained from a biodiesel production industry in Québec. The crude glycerol was generated in the alkali-catalyzed transesterification of plant seed oils without methanol recovery. Crude glycerol was characterized before utilization (Table 2). Glycerol content was determined by the method reported by Bondioli and Della [33].

Table 1
The sludge characterization.

Properties	Concentration (this study)	Concentration (previous studies) [18,23,27,32]
TS (g/L)	30.11 ± 1.77	24.13–30.64
TSS (g/L)	22.89 ± 1.50	20.06–23.76
VSS (g/L)	18.94 ± 1.04	15.50–17.64
TC (g/kg TS)	453.24 ± 11.67	419.03–455.17
TN (g/kg TS)	57.19 ± 3.86	47.64–57.52
TP (g/kg TS)	31.23 ± 0.98	28.76–32.18
pH	6.23 ± 0.01	6.20–6.50

Table 2
Crude glycerol composition.

Items	Concentration (w/v)
Glycerol content (% w/w)	78.22 ± 1.36
Soap content (% w/w)	2.63 ± 0.00
Ash (% w/w)	2.52 ± 0.08
Methanol (% w/w)	12.15 ± 0.32
Water (% w/w)	1.56 ± 0.17
pH	10.81 ± 0.02
Density (g/mL)	1.16 ± 0.00

Methanol content was measured by evaporating 100 mL of crude glycerol at 60 °C for 15 min, and the weight loss was used to calculate the methanol content. Ash content was obtained by subjecting the crude glycerol at 750 °C for 3 h. The pH of crude glycerol was adjusted to 1 with 85% H_3PO_4, and the free fatty acid (FFA) obtained after centrifugation at 5000 rpm for 15 min was used to calculate the soap content (Eq. 1):

$$\text{Soap amount(g)} = 304 \times \text{FFA amount}/282 \quad (1a)$$

$$\text{Soap content(\%w/w)} = \text{g of soap}/100\text{g of glycerol solution} \times 100\% \quad (1b)$$

where 304 is average soap molar mass and 282 is average FFA molar mass.

2.2. Microorganism

Oleaginous yeast *Trichosporon oleaginosus* (ATCC 20509) was used in this study. It was maintained in malt extract agar plates at 4 °C.

2.3. Medium

2.3.1. Sludge medium

The sludge resulted from gravity settling was centrifuged at 5000 rpm for 15 min to concentrate the SS from around 23 g/L to around 125 g/L. The supernatant was collected and stored at 4 °C for further utilization. According to our previous study, lipid accumulation by *T. oleaginosus* with sludge medium was improved when the sludge SS concentration was over 30 g/L. In addition, alkaline pre-treated on sludge could highly enhance lipid accumulation [18,23]. Therefore, in this study, sludge with SS concentration of 30, 35, and 40 g/L after alkaline pre-treated was used to cultivate *T. oleaginosus*. To obtain SS concentration of 30, 35, and 40 g/L, the concentrated sludge with SS concentration of 125 g/L was diluted with the supernatant generated during the centrifugation. The obtained sludge solution was then subjected to the alkaline pre-treatment (pH 12 and 121 °C for 15 min). The pH of the solution was adjusted back to 6.5 after cooling down to 28 °C and then used as fermentation medium. Additionally, a medium was also prepared by diluting sludge with tap water to SS concentration of 35 g/L, pH adjusted to 12 and then sterilized at 121 °C for 15 min. Similarly, the medium pH was adjusted back to 6.5 after cooling down to 28 °C before utilization in fermentation.

2.3.2. Crude glycerol medium

Fermentation with crude glycerol medium was conducted as reference to compare with other fermentations. According to our previous study results, glycerol concentration of 40 g/L provided high lipid accumulation in *T. oleaginosus* [34]. Hence, in this study, the composition of crude glycerol medium was as: per liter medium contains 0.95 g Na_2HPO_4, 1.616 g NH_4Cl, 0.1 g EDTA, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.4 g peptone, 0.04 g $CaCl_2{\cdot}2H_2O$, 0.0055 g $FeSO_4{\cdot}7H_2O$, 0.001 g $ZnSO_4{\cdot}7H_2O$, 0.00076 g $MnSO_4{\cdot}H_2O$, and 51.14 mL crude glycerol (glycerol of 40 g). The crude glycerol and the nutrient solution was separately sterilized (121 °C for 15 min) and then mixed before fermentation.