Waste Management xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

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

# Chemical and biological conversion of crude glycerol derived from waste cooking oil to biodiesel

Jiaxin Chen [a,b], Song Yan [b], Xiaolei Zhang [a,*], Rajeshwar Dayal Tyagi [b], Rao Y. Surampalli [c], J.R. Valéro [b]

[a] School of Civil and Environmental Engineering, Harbin Institute of Technology (Shenzhen), Shenzhen, Guangdong 518055, PR China
[b] INRS Eau, Terre et Environnement, 490, rue de la Couronne, Québec G1K 9A9, Canada
[c] Department of Civil Engineering, University of Nebraska-Lincoln, N104 SEC, PO Box 886105, Lincoln, NE 68588-6105, USA

ARTICLE INFO

*Article history:*
Received 16 May 2017
Revised 24 October 2017
Accepted 25 October 2017
Available online xxxx

*Keywords:*
Crude glycerol
Energy
Lipid accumulation
Biodiesel

ABSTRACT

In this study, crude, purified, and pure glycerol were used to cultivate *Trichosporon oleaginosus* for lipid production which was then used as feedstock of biodiesel production. The purified glycerol was obtained from crude glycerol by removing soap with addition of $H_3PO_4$ which converted soap to free fatty acids and then separated from the solution. The results showed that purified glycerol provided similar performance as pure glycerol in lipid accumulation; however, crude glycerol as carbon source had negatively impacted the lipid production of *T. oleaginosus*. Purified glycerol was later used to determine the optimal glycerol concentration for lipid production. The highest lipid yield 0.19 g/g glycerol was obtained at 50 g/L purified glycerol in which the biomass concentration and lipid content were 10.75 g/L and 47% w/w, respectively. An energy gain of 4150.51 MJ could be obtained with 1 tonne of the crude glycerol employed for biodiesel production through the process proposed in this study. The biodiesel production cost estimated was 6.32 US $/gal. Fatty acid profiles revealed that C16:0 and C18:1 were the major compounds of the biodiesel from the lipid produced by *T. oleaginosus* cultivated with crude and purified glycerol. The study found that purified glycerol was promising carbon source for biodiesel production.



## 1. Introduction

One of the most urgent issues in the world is to seek renewable, sustainable, and affordable energy source due to the risk of the depletion of petro energy. Biodiesel is gaining increasing attention as it can be produced by renewable and cheap materials. The dramatic increase in demand of biodiesel resulted in its increased production from various types of oils. The common method of biodiesel production is trans-esterification in which oils or fats react with short chain alcohol (generally methanol) with acid or base as catalyst. In the process, crude glycerol is generated as by-product. About 0.10 kg of glycerol is generated per kilogram of biodiesel produced. It is normally called crude glycerol and mainly contains glycerol (20–96% w/w), free fatty acids, soaps, catalyst, salts, methanol etc. (Gao et al., 2016; Hansen et al., 2009; Hu et al., 2012). The composition of crude glycerol varies from one biodiesel production plant to another and is mainly determined by the feedstock oil composition and quality, the oil and methanol molar ratio used in trans-esterification, type of catalyst used, and the detailed procedure such as with or without methanol recovery (Athalye et al., 2009; Uprety et al., 2017; Yen et al., 2012).

Crude glycerol is a complex material, and the proper utilization to attain its maximum value is desirable for its appropriate handling. Purification of crude glycerol was the most applied method before biodiesel boom. However, due to a substantial decrease in the price of purified glycerol (1.54 US $/kg before 2000 and 0.66 US $/kg after 2007), the purification is getting less attractive. Therefore, direct use or partial purification of crude glycerol is becoming promising. Due to the large demand on energy in the current world, use of crude glycerol for energy production has been widely reported (Nartker et al., 2014; Oliveira et al., 2015; Trchounian et al., 2016). Bioconversion of glycerol to biodiesel is an interesting way of utilization of original or partially purified crude glycerol. Oleaginous microorganisms such as *Schizochytrium* sp., *Yarrowia lipolytica*, *Rhodotorula* sp., and *Cryptococcus* sp. are reported capable of assimilating glycerol to produce lipid which is the raw material of biodiesel production (Deeba et al., 2016; Gao et al., 2016; Polburee et al., 2015; Ryu et al., 2013).

Current studies revealed that the composition of crude glycerol had great impact on lipid accumulation in microorganisms (Cerón-García et al., 2013; Polburee et al., 2015). Normally, high

* Corresponding author.
*E-mail address:* xiaolei.zhang2016@foxmail.com (X. Zhang).

https://doi.org/10.1016/j.wasman.2017.10.044
0956-053X/

glycerol content in crude glycerol tends to lead high lipid accumulation in microorganism. Study showed that the lipid accumulation reached around 60% w/w (lipid g/ biomass g) with pure glycerol as carbon source, which was only 20% w/w (lipid g/ biomass g) when crude glycerol was applied as carbon source (Polburee et al., 2015). The highest biomass and lipid concentration reached 26.7 g/L and 18.5 g/L when utilized crude glycerol with 85% glycerol content, respectively, but they were 18.0 g/L and 13.4 g/L when the crude glycerol with glycerol content of 33%, respectively (Xu et al., 2012).The content of impurities including methanol and soap have showed significant effect on cell growth as well as lipid accumulation (Liang et al., 2010a; Lorenz et al., 2017). It was revealed that biomass productivity of *Cryptococcus curvatus* cultivated with crude glycerol medium was only 67% of that from pure glycerol with the same carbon concentration (Liang et al., 2010a). Lipid accumulation in *Kodamaea ohmeri* with crude glycerol was only 50% of that with pure glycerol (Kitcha and Cheirsilp, 2011). Gao et al. (2016) found that the lipid productivity of *Rhodosporidium toruloides* in crude glycerol cultivation was reduced around 18% compared with that of pure glycerol cultivation (Gao et al., 2016). Overall, crude glycerol with high glycerol content and low impurity content showed advantage on lipid production from oleaginous microorganism.

Crude glycerol composition is highly related to the character of feedstock oil of biodiesel production. The crude glycerol with high glycerol content (>60%) was normally generated in the biodiesel production from plant seed oils which have low free fatty acid (FFA) content. It suggests that FFA content in the feedstock oil has great impact on the final glycerol content in crude glycerol as FFA can be transferred to soap during the transesterification in base catalyzed reaction. As the prices of plant seed oils are increasing, waste cooking oils which have high FFA content are largely employed in biodiesel production, and hence the production of crude glycerol with high soap content is gradually increasing. In order to reduce the influence on lipid production from microorganism, soap can be removed from crude glycerol prior to the utilization. By soap removal, the glycerol content will be increased in the crude glycerol, and thus the purified crude glycerol could enhance the production of biomass and lipid from oleaginous microorganisms. In addition, it would bring extra value to the process if the recovered soap could be also converted to useful product. In fact, soap in crude glycerol can be converted to FFA which can be used to produce biodiesel with acid as catalyst.

In this study, crude glycerol generated in the biodiesel production from waste cooking oil, which has low glycerol content, was used as carbon source for oleaginous yeast cultivation after FFA recovery. The obtained FFA from crude glycerol purification was then transferred to biodiesel through esterification. The study provides a way to maximize the value of crude glycerol with low glycerol content. In addition, the work increases energy efficiency as energy (biodiesel) production by-product (crude glycerol) again converted to energy (biodiesel). It provides a feasible method to manage crude glycerol and create energy. The flow diagram of the study is shown in Fig. 1.

## 2. Materials and methods

### 2.1. Materials

Crude glycerol was kindly provided by a biodiesel production plant, in Quebec, Canada. Oleaginous microorganism *Trichosporon oleaginosus* (ATCC20509) was employed in this study to produce lipid.

### 2.2. Crude glycerol characterization

Density and pH: The weight of 2 mL of crude glycerol was measured at room temperature. The density of crude glycerol was determined by dividing the weight with the volume (2mL).To determine the pH, 1.0 g of crude glycerol was dissolved in 50 mL of deionized (DI) water. The pH of the solution was measured by a digital pH meter at room temperature (Hu et al., 2012).

Glycerol content: The glycerol content was determined according to the method reported by Bondioli and Della Bella (2005). 3,5-diacetyl-1,4-dihydrolutidine, a yellow complex, was formed in a two-step reaction. In the first step, glycerol reacted with sodium periodate to form formaldehyde, following, acetyl acetone was added to generate the complex of 3,5-diacetyl-1,4-dihydrolutidine. The complex was measured by UV–Vis Spectrophotometer at 410 nm. The glycerol content was calculated according to standard curve (=0.05645 × conc.−0.07437; $R^2$ = 0.99534). The method is a well established one for glycerol determination (Lima et al., 2012; Sidnei et al., 2011). It could rapidly and accurately determine glycerol concentration in liquid. To verify the method, glycerol was also determined with High Performance Liquid Chromatography (HPLC), and results showed that Bondioli and Bella method was reliable. Thus, Bondioli and Bella method was used in this study to determine glycerol concentration.

Soap content: The soap content was estimated as reported by Liang and co-workers (Liang et al. (2010b). The pH of 50 g crude glycerol was adjusted to1.0 with 85% $H_3PO_4$. After well mixing, the solution was centrifuged at 5000 rpm for 20 min. The top red dark layer (in the centrifuged liquid) which was FFA was collected and weighed. The soap content was calculated according to soap amount = 304 × FFA amount/282; where 304 is average soap molar mass and 282 is the average FFA molar mass.

Biodiesel content: Biodiesel content was analyzed with Gas Chromatography coupled with Mass Spectroscopy (GC-MS) (Perkin Elmer, Clarus 500) to control the result quality. Helium was used as the carrier gas. The dimensions of the column used were 30 m × 0.25 mm, with a phase thickness of 0.2 μm. The calibration curve was prepared by injecting known concentrations of an external standard, mixture comprising 37 FAMEs (47885-U, 37 Component FAME Mix; Supelco, Bellefonte, PA, USA). 1,3-dichlorobenzene was also used as internal standard with a concentration of 50 ppm.

Ash content: The 10 g of crude glycerol was heated at 750 °C for 3 h (Manosak et al., 2011). After the sample was cooled down to room temperature, the residue ($W_3$) was weighed and then the ash content was calculated ($W_3/10 \times 100\%$).

Catalyst content: Crude glycerol was sampled from a company in which NaOH was used as the catalyst in the transesterification process to produce the biodiesel. To determine the content of NaOH, 10 g of crude glycerol was adjusted to pH 7 with 1 M HCl and the consumed volume of acid 1 M HCl (V) was recorded and used to calculate the NaOH content (=40 × 1 × V/10; where 40 is NaOH molar mass, 1 is HCl molar concentration, V is the volume of 1 M HCl consumed to bring the pH to 7; and 10 is crude glycerol amount) in crude glycerol.

Methanol content: The methanol content was determined with Heidolph Laborota 4011 digital evaporator. The 100 mL (107.3 g) of crude glycerol was subjected to 60 °C for 15 min. The evaporated methanol ($W_4$) was collected and the methanol content in the crude glycerol was calculated as follows: $W_4/107.3 \times 100\%$.

Water content: The 10 g of crude glycerol was heated at 105 °C until weight constant ($W_5$). The weight loss during the heating was due to the evaporation of water and methanol. The sum of water and methanol content was calculated as follows: [(10-$W_5$)/10 × 100%]. After subtracting methanol content, water content was obtained.

Please cite this article in press as: Chen, J., et al. Chemical and biological conversion of crude glycerol derived from waste cooking oil to biodiesel. Waste Management (2017), https://doi.org/10.1016/j.wasman.2017.10.044