Energy Conversion and Management 172 (2018) 306–315

Contents lists available at ScienceDirect

Energy Conversion and Management

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

Microbial lipid production from food waste saccharified liquid and the effects of compositions

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ARTICLE INFO

Keywords:
Food waste saccharified liquid
Rhodosporidium toruloides
Fermentation
Microbial lipid
Biodiesel

ABSTRACT

The residues of food waste after removing waste cooking oils could be converted to microbial lipid by oleaginous yeasts and microbial lipid could be used as a raw material of biodiesel with waste cooking oils. This process would have positive environmental and economic benefits. However, the composition of food waste is extremely complex. Many types of organic acids, such as lactic acid and acetic acid, could be accumulated during the collection, transportation and storage processes. The effects of these substances on microbial lipid production remained unknown. In this study, food waste saccharified liquid (FWSL) without any additional nutrients was fed to *Rhodosporidium toruloides* 2.1389 for microbial lipid production. Total biomass concentration, lipid concentration and lipid yield increased by 87.4%, 69.4% and 69.3%, respectively, compared with the YPD medium. Remarkably, increasing the concentration of lactic acid in the medium promoted the total biomass concentration of yeast. Furthermore, lipid concentration decreased slightly when single lactic acid or single acetic acid concentration was below 5 g/L or 1 g/L in the culture media. Metal elements that were present in the medium had considerable synergistic effects on lipid production. When four metal elements (i.e. Mg, Ca, Na and K) were present in the medium, total biomass concentration, lipid concentration, lipid content and lipid yield increased by 22.01%, 33.51%, 9.43% and 33.33%, respectively, compared with those in the control group. The interaction among the FWSL compositions that led to the promotion of microbial lipid production was beyond the inhibition effect. The engineering implication analysis evidently suggested that the process of microbial lipid production from FWSL was economically feasible and had potential industrial application prospects.

1. Introduction

The mounting shortage and environmental issues of traditional energies has resulted in the people's awareness of the importance of developing alternative and clean energies. Biodiesel has attracted increasing attention because it can be applied to any diesel engine without requiring modifications, particularly with its safety and excellent environmental characteristics, including minimal sulfur oxide and carbon oxide emissions and biodegradability [1–3]. As a type of a promising and green fuel, biodiesel can be obtained from vegetable oil, animal fat and waste cooking oil esterification or transesterification with a few short chain alcohols [4]. The major hurdle of a large-scale commercialised production of biodiesel is its high production cost, including high raw material costs and limited provision of raw materials.

Waste cooking oil is demonstrated as a good raw material for biodiesel production [5–7]. However, how to use residual food waste efficiently after waste cooking oil removal is an important question. To the best of our knowledge, food waste after waste cooking oil removal is still rich in organic compounds. Various studies had reported that certain biofuel productions, such as bioethanol and methane, are based on food waste as a substrate [8,9]. But if a factory only uses food waste to produce ethanol or methane, waste cooking oils cannot be recycled efficiently. And while this factory uses waste cooking oils to produce biodiesel besides, capital construction investment of the factory will

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https://doi.org/10.1016/j.enconman.2018.07.005
Received 19 April 2018; Received in revised form 17 June 2018; Accepted 3 July 2018

increase substantially, and the cost related to workers will be considerably higher. However, if all food wastes (including waste cooking oils) could be used to produce biodiesel, then the cost could be reduced. At present, only a few studies of biodiesel production from residual food waste (after waste cooking oil removal) have been conducted. These studies showed that food waste hydrolysate contains glucose, protein, fat and other organic materials [10–12]. Moreover, this waste contained a few metal elements, such as K, Ca and Mg. Nearly no heavy metals and toxic substances were found in food wastes. Thus, food waste is a suitable fermentation material that can promote the growth of microorganisms and the production of microbial lipids. Microbial lipids are ideal raw materials for biodiesel preparation because their main constituent are triglycerides, which are extremely similar to those in vegetable oils [13]. Oleaginous microorganisms are efficient in biodiesel production because of their numerous advantages, such as short growth cycle, high lipid productivity, low labour costs and small land coverage [14,15]. Thus, these microorganisms are potential workshops for lipid production.

In recent years, microbial lipid production by oleaginous yeasts has been a heavily researched topic. *Rhodosporidium toruloides,* which can accumulate lipids up to 70% of their biomass dry weight, has been widely investigated. Compared with other oleaginous yeasts, such as *Yarrowia lipolytica, Candida tropicalis, Rhodotorula glutinis* and *Trichosporon cutaneum, R. toruloides* shows a strong capability to convert low-cost feedstocks into lipid products in batch culture [16]. Canonico et al. [17] utilised *Y. lipolytica* to convert crude glycerol into microbial lipids, and the maximum lipid concentration only reached 2.6 g/L. Xiong et al. [18] used acetone-butanol-ethanol fermentation wastewater to feed *T. cutaneum* and lipid content was approximately 14%. In addition, the lipid content of *R. glutinis* was approximately 36% when corncob hydrolysate was used as feedstocks [19]. *R. toruloides* 2.1389 was reportedly capable of converting acetic acid and distillery wastewater into microbial lipids [20,21]. Therefore, food waste saccharified liquid (FWSL) is feasible for using as a low-cost fermentation substrate for *R. toruloides* for biolipid production. Although there is a little research on microbial lipids from food waste hydrolysate, the effects of the compositions of food waste hydrolysate (lactic acid, acetic acid and metal elements) have yet to be completely investigated.

In the current study, food waste residues after removing waste cooking oils were initially saccharified into FWSL. Thereafter, FWSL was used as feedstock for *R. toruloides* 2.1389. Then the improvement of lipid production from FWSL by investigating fermentation parameters was studied. The feasibility analysis was presented from different aspects, including total biomass concentration, lipid concentration, lipid content, lipid yield and fatty acid profiles of microbial lipid compared with YPD as culture media. The positive or negative effects of main organic acids (lactic acid and acetic acid) and the main metal elements (Mg, Ca, Na and K) of FWSL on lipid production were eventually analysed. Lastly, the environmental and economic benefits of microbial lipid from FWSL were discussed through engineering implication analysis. Consequently, this study could provide a scientific basis for the industrial production of microbial lipid.

2. Materials and methods

2.1. Preparation of FWSL

Food waste was collected from a canteen in the University of Science and Technology Beijing, China. After pretreatment (removing sundries, grinding and mixing), food waste was mixed with water at a ratio of 2:1 (w/v) and 100 U/g glucoamylase (Beijing Ordered Star Biological Technology Co., LTD, China). The mixed liquor was then saccharified at 60 °C for 6 h with stirring. After which, the liquid was harvested by centrifugation at 4000 rpm for 10 min. Then the cooking oil was removed and the remaining liquid was stored at −20 °C for further use.

2.2. Yeast strain

R. toruloides 2.1389 purchased from the China General Microbiological Culture Collection Center was maintained on slants of YPD agar (20 g/L glucose, 20 g/L peptone, 10 g/L yeast extract and 20 g/L agar, pH 6.0) at 4 °C. To prepare inocula, the organism was precultured for 24 h in the media that contained 20 g/L glucose, 20 g/L peptone and 10 g/L yeast extract at 30 °C and 200 rpm in an air-bath shaker. The media were sterilised by autoclaving at 121 °C for 20 min before the experiment. Glucose and agar were obtained from Shanghai Aladdin Reagent Co., Ltd. (Shanghai, China). Yeast extract and peptone were obtained from Beijing Lang Bridge Technology Co., Ltd. (Beijing, China).

2.3. Fermentation experiments with FWSL as substance

Fermentation experiments with FWSL as substrate were conducted in 250 mL conical flasks that contained 100 mL of liquid medium. In the present experiment, pH was adjusted by adding NaOH solution (1 mol/L) or HCl solution (1 mol/L). Different C/N ratios were obtained by adding $(NH_4)_2SO_4$, and different substrate concentrations were prepared through dilution. Through sterilisation at 121 °C for 20 min, 5 mL of inocula was added to the medium, and the culture was conducted at 30 °C in an air-bath shaker at 200 rpm for 6 days. After fermentation, total biomass concentration, lipid content and lipid concentration were measured. All experiments were conducted in triplicate. The related reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.4. Comparative experiments between FWSL and YPD as substance

Comparative experiments with FWSL and YPD medium as substrates were conducted in 250 mL conical flasks that contained 100 mL of liquid medium. FWSL was diluted in 50 g/L total sugar, in which C/N = 73 g/g and pH was adjusted to 4.0 by using HCl solution (1 mol/L). Meanwhile, the YPD medium was prepared, with concentrations of 50 g/L glucose, 1.14 g/L peptone (18% nitrogen content) and 0.57 g/L yeast extract (12% nitrogen content), C/N ratio of 73 g/g, and pH of 4.0 by adjustment using HCl solution (1 mol/L). After all media were sterilised at 121 °C for 20 min, 5 mL of inocula was added to the medium, and the mixture was cultured at 30 °C in an air bath shaker at 200 rpm for 6 days. All experiments were conducted in triplicate. When the experiments were conducted at the optimal pH, C/N and substrate concentrations, the fermentation broths were sampled every 2 days to measure residual total sugar concentration, residual lactic acid and acetic acid concentrations and residual glucose concentration.

2.5. Fermentation experiments with simulated FWSL as substance

Experiments on the effects of the main compositions of FWSL were conducted in 250 mL flasks with 100 mL of liquid medium of different compositions. The optimal fermentation conditions were simulated with 50 g/L glucose, 1.14 g/L peptone (18% nitrogen content) and 0.57 g/L yeast extract (12% nitrogen content), in which C/N = 73 g/g, and pH was adjusted to 4.0 by HCl solution (1 mol/L). For the effect of FWSL compositions, experiments were designed by adding different concentrations of lactic acid, acetic acid and metal elements. All media wrapped in tinfoil were autoclaved at 121 °C for 20 min and taken out of the steriliser after the temperature of the steriliser decreased to room temperature. Then, a total of 5 mL of inocula were added to every medium, and all cultures were conducted at 30 °C in an air-bath shaker at 200 rpm. All experiments were conducted in triplicates. After fermentation, total biomass was collected and microbial lipid was extracted. Lactic acid and acetic acid were obtained from Shanghai Aladdin Reagent Co., Ltd. (Shanghai, China). Other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai,