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Direct transesterification of oleaginous yeast lipids into biodiesel: Development of vigorously stirred tank reactor and process optimization

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ABSTRACT

Oleaginous yeast *Yarrowia lipolytica* is considered as an environmental friendly oil source due to its ability to convert industrial wastes into microbial oils and their fatty acid compositions similar to those of plant oils. After cultivation in 20 L bioreactor, the yeast cells with lipid content >30% were directly used in transesterification process. A 2-L vigorously stirred tank reactor (VSTR) with balloon-whisk impeller was designed for simultaneously disrupting the yeast cells and converting the yeast lipids into biodiesel in one reactor. Operating conditions for direct transesterification in VSTR were: the use of glass beads as cell disrupting agents at a bead:biomass ratio of 1:1 (w/w), methanol as lipid extracting solvent and feedstock at a methanol:biomass ratio of 4:1 (v/w), sulfuric acid as catalyst at a concentration of 2% (v/v), agitation speed at 1000 rpm and reaction temperature at 50 °C. Under these conditions, the highest biodiesel yields of 94.99% and 80.91% were obtained when using dried and wet yeast cells, respectively. The cell debris left after the reaction contained carbohydrate at 63.41% and protein at 15.76% indicating its potential use as nutrient sources for further fermentation process.

1. Introduction

The raw materials for biodiesel production now mainly are plant oils and some recovered animal fats and oils which their amounts might not meet the continuously increasing demand of biodiesel. Microbial oils are considered to be a promising next generation of oil sources for biodiesel production because their production have short life cycle, less labor required, less affection by venue, and easier to scale up [1]. Among available microbial oil producers, oleaginous yeasts have advantages over other oleaginous species such as faster growth rate, higher lipid content and ability to use low-cost nutrient sources [2]. Traditional techniques for biodiesel production from microbial oils require two steps of lipid extraction step and transesterification step. Recently, a direct process which combines both steps has been developed [3–5]. The direct process not only simplifies the production step but also improves the biodiesel yield compared with the conventional method because it can avoid the lipid loss during the extraction step. The reaction is simple and requires mainly biomass, alcohols and catalysts. The direct process with chloroform-free was also attempted for production of biodiesel from wet microalgal biomass using supercritical alcohol [6–8]. However, the appropriate reactors for these direct processes have not been demonstrated.

This study aimed to develop direct process for biodiesel production from yeast biomass. The vigorously stirred tank reactor (VSTR) was designed and the important parameters such as the effectiveness of cell disruption by bead milling, reaction temperature and catalyst dosage were investigated. The lipid extraction yield and biodiesel yield using either wet or dried yeast biomass by this direct process were compared. The fuel properties of biodiesel produced by this process were estimated based on the fatty acid compositions. In addition, the composition of cell debris after the reaction was analyzed for evaluation of their prospect use.

2. Materials and methods

2.1. Microorganism and culture condition

Oleaginous yeast *Yarrowia lipolytica* TISTR 5151 from the Thailand Institute of Scientific and Technological Research was used in this study. It was cultured on industrial wastes based on the optimal conditions that have been previously reported [9]. A

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20 L bioreactor contained 10 L of non-sterile palm oil mill effluent added with 2% crude glycerol. The cultures were initiated with 24-h-old seed cultures (approximately 10^7 yeast cells/mL) and pH was controlled at 6.0 by addition of 1 M of NaOH or 1 M of HCl. The aeration rate was maintained at 6.0 vol of air per volume of medium per minute (vvm) with an agitation speed of 200 rpm for 48 h.

2.2. Configuration of vigorously stirred tank reactor

In this study, the vigorously stirred tank reactor (VSTR) was designed in order to simultaneously disrupting the yeast cells, extracting and converting the yeast lipids into biodiesel in one reactor. The configuration of VSTR is shown in Fig. 1. Glass vessel has a working volume of 2 L with 12 cm inner diameter and 20 cm height (Fig. 1b); it is equipped with stainless steel airtight lid which has a diameter of 15 cm (Fig. 1c) and stainless steel building base which has a diameter and a height of 19 cm and 26 cm, respectively (Fig. 1d). Condenser is fitted on the lid and circulated with cooling water and a pressure gauge is fitted on the lid. Heat jacket is fitted on the external surface of the vessel to heat and to maintain the feedstocks at the required temperature and thermocouple is introduced into the reactor through a hole on the lid. A hole is drilled on the lid used to insert stainless steel tap for sampling through threaded steel adapters and rubber stoppers to avoid leakage. Finally, motor with the speed range of 100–1800 rpm is fitted on the lid and connected with a balloon-whisk impeller which has a diameter and height of 6 and 17 cm, respectively (Fig. 1e) to allow vigorously mixing of glass beads with the feedstocks.

2.3. Direct transesterification of yeast lipids into biodiesel

The direct transesterification process was performed in 2-L VSTR with a liquid volume of 0.5 L. After cultivation on industrial wastes in bioreactor, the yeast cells were harvested by self-flocculation for 1 h and then centrifugation at 1585×*g* for 15 min. The wet yeast cells were washed and transferred into VSTR and then added with glass beads as cell disrupting agents at a bead:biomass ratio of 1:1 (w/w) and methanol as a lipid extracting solvent and feedstock at a methanol:biomass ratio of 4:1 (v/w). The total volume of the mixture was 0.6–0.7 L. The agitation speed was set at 1000 rpm and the reaction temperature was set at 50 °C. Sulfuric acid was used as catalyst and its concentration in methanol was varied at 0.4, 0.8 and 1.2 M (2, 4 and 8% v/v, respectively). As the water in the wet cells might affect the yield of biodiesel, the process using dried cells was performed and the results were compared with those using wet cells. To determine the biodiesel yield, the transesterified lipids were extracted by adding hexane to the reactants at the volume ratio of 2:1 and then vigorously mixed at 1000 rpm in an eppendorf vortex (Daihan Scientific, Korea) for 30 min. Solvent was recovered by evaporation and the extracted lipids were weighed and analyzed for fatty acid methyl esters (FAMEs) content. The biodiesel yield was calculated based on the amount of known FAMEs (C8-C24) in total extracted lipids.

2.4. Analytical methods

To determine dried biomass of the yeast, the yeast cells were harvested by centrifugation at 1585×*g* for 15 min and dried at 60 °C to constant weight in vials [10]. In order to classify the lipids in non-polar and polar fractions, two solvents with different polarities, hexane and methanol, were used to extract the lipids from the dried biomass [11]. The total lipids were extracted using a mixture of chloroform:methanol (2:1, v/v) and then sonicated (Transsonic model 460/H, Elma, Singen, Germany) for 1 h [12]. Carbohydrate, protein, crude fat, moisture and ash contents of yeast biomass were measured according to the method of AOAC [13]. Fatty acid methyl esters with 8–24 carbon atoms (FAMEs) of transesterified products were analyzed using gas chromatography (Hewlett Packard Plus 6850 series, Agilent, USA) equipped with capillary column, 30 m length, 320 μm I.D., 0.25 film thickness (Selected Biodiesel for FAME, Varian, USA) and a flame ionization detector (FID). The column temperature was maintained at 210 °C for 12 min and increased up to 250 °C at a rate of 20 °C min^{-1}, and then held for 8 min. The detector temperature was set at 300 °C. Quantitative analysis of the biodiesel yield was performed using methyl heptadecanoate as internal standard. The statistical significance of the results was evaluated by one-way ANOVA (analysis of variance) and Duncan's multiple range tests ($P < 0.05$) using the SPSS version 22.0 software.

Table 1
Fatty acid composition of lipid from *Yarrowia lipolytica* TISTR 5151.

Parameters	Relative amount of total fatty acids (%)		
	Total lipids	Non-polar lipids	Polar lipids
Content (g/g-total lipids)	–	0.51 ± 0.08	0.47 ± 0.01
Lauric acid (C12:0)	0.15	0.10	0.14
Myristic acid (C14:0)	0.57	0.63	0.78
Palmitic acid (C16:0)	32.78	26.28	21.77
Stearic acid (C18:0)	6.36	5.61	2.70
Oleic acid (C18:1)	34.11	38.39	31.38
Linoleic acid (C18:2)	1.46	8.42	5.23
Eicosenoic acid (C20:1)	2.89	1.87	2.65
Erucic acid (C22:1)	nd.	6.70	2.10
Lignoceric acid (C24:0)	19.00	11.25	32.85
Others	2.69	0.75	0.41
Saturated fatty acids	61.55	44.62	58.65
Unsaturated fatty acids	38.46	55.38	41.36

Note: nd. is the data was not detected. Total lipids, nonpolar lipids and polar lipids were extracted by chloroform: methanol, hexane and methanol, respectively and then converted into FAMEs for GC analysis. The total lipids in the biomass was 0.25 ± 0.01 (g/g-biomass).

3. Results and discussion

3.1. Characterization of yeast lipids

The microbial lipids can be classified in non-polar fraction (storage lipids) and polar fraction (structural lipids), according to their polarity. Non-polar lipids mainly comprise monoacylglycerol, diacylglycerol, triacylglycerol, free fatty acids, and alkyl chain while polar lipids mainly comprise sphingolipids, phospholipids, glycolipids, lipoproteins, sulfolipids and acylglycerides. Both are sponifiable lipids which can be converted into biodiesel [14,15]. The lipids from *Y. lipolytica* TISTR 5151 were extracted using conventional mixed solvents of chloroform and methanol which could extract both non-polar and polar forms of lipids [12]. In order to classify the yeast lipids, two solvents with different polarities, hexane and methanol, were separately used to extract non-polar lipids and polar lipids, respectively [11]. The extracted lipid yields were compared to those extracted by conventional mixed solvents. It was found that the non-polar and polar lipids accounted for 51% (0.51 g/g-total lipids) and 47% (0.47 g/g-total lipids), respectively (Table 1). The ratios between non-polar and polar lipids differ in different species of the yeasts. For example, the lipids of yeast *Trichosporon oleaginosus* contained polar lipids as high as 75.7% [11], while the lipids extracted from yeast *Candida* sp. LEB-M3 contained only 5.5% polar lipids [14].

The fatty acid compositions in each fraction of *Y. lipolytica* TISTR 5151 lipids are shown in Table 1. Both non-polar and polar fraction of yeast lipids contained long chain saturated and unsaturated fatty acids with 12–24 carbon atoms. Three main fatty acids found in the yeast lipids were oleic acid, palmitic acid and lignoceric acid at 34.11%, 32.78% and 19% of total fatty acids, respectively. In non-