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The production of biodiesel using residual oil from palm oil mill effluent and crude lipase from oil palm fruit as an alternative substrate and catalyst

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HIGHLIGHTS

- High biodiesel was obtained from POME oil and crude lipase under optimal conditions.
- The properties of POME biodiesel are acceptable, according to ASTM standards.
- The production of biodiesel can be done at a low investment cost.

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ABSTRACT

Biodiesel production using residual oil from palm oil mill effluent (POME) and crude lipase from oil palm fruit as the substrate and catalyst had a high biodiesel yield (92.07 ± 1.04%) under optimal conditions. POME is considered as an alternative source for oil because it contained high oil and grease content (5569.82 mg/L). Oil was extracted from POME by the soxhlet method using a mixture of hexane, methanol and acetone. Eighty percent of residual oil (4455 mg/L) was recovered from POME. Biodiesel production from crude lipase catalyst is an alternative method that is simple to perform and can be done at a low investment cost. In addition, biodiesel from residual oil using crude lipase catalyst was characterized according to ASTM standards. Most properties of biodiesel from crude lipase are acceptable, according to Thai biodiesel and ASTM standards. Low free fatty acid (0.07%) content was observed in enzymatic biodiesel. A high cloud point (10–13 °C) and cetane number (59.0–60.0) were also illustrated since a high cetane number is an important property used to qualify high quality biodiesel, POME biodiesel may possibly be used as a sole biofuel or blended with fossil fuels.

1. Introduction

Palm oil mills are abundant in Southern Thailand. Currently, more than 72 palm oil industries are in operation in Thailand. Most of them are oil palm crushing mills (>60 mills) and the others are palm refineries mills. In 2010, production of crude palm oil (CPO) in Thailand reached 1.29 million tons [1]. In the process of palm oil milling, effluent is mainly generated through the sterilization and clarification process. Due to an increase in palm oil productions in the last few years, palm oil waste have increased rapidly. According to Borja and Bank [2], more than 3.86 million tons of palm oil mill effluent (POME) is obtained annually. POME contains a high amount of organic matter, oil and grease, total solids and suspended solids. Therefore, a suitable treatment should be utilized to keep the surrounding environment safe from damage. In addition, looking for an alternative method for utilizing and treating POME at the same time is still the main challenge. POME from oil palm crushing mills (Thailand) contained COD and oil in the range of 45000 and 6000 mg/L, respectively. After oil recovery, more than 70% of COD was significantly decreased and 78% of oil was easily recovered from POME using *n*-hexane [3]. Therefore, the recovery of oil from POME shows a great potential to reduce high COD from POME and an adequate amount of oil from POME may be utilized as substrate for valuable products. Due to the reduction of fossil fuel, many countries encourage and support projects which produce energy from renewable resources. Therefore, POME shows a potential to be utilized as a novel material for bio-

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diesel production due to its high content of oil and grease which could be converted into biodiesel via chemical and bio-catalytic transesterification.

Currently, sodium hydroxide is mostly utilized as a chemical catalyst for biodiesel production. However, high FFA and acid value in oil from POME are considered drawbacks of chemical biodiesel [3,4]. Due to the limit of FFA, content for chemical method is 1% or 2.5% [5,6]. The chemical catalyst can easily promote soap formation. Thus, it reduces the yield of biodiesel and complicates the separation process [6,7]. Hayyan et al. [5] reported the production of biodiesel from sludge palm oil (SPO). SPO, a byproduct from palm oil mill using distillation method, also contained high FFA. Therefore, both acid- and alkali-catalyst were suggested for biodiesel production from SPO. Two steps of biodiesel production began with acid catalyst to reduced FFA content in SPO. Afterwards, biodiesel was produced via the transesterification process. High yield of biodiesel (76.62%) with 96% ester content was obtained from SPO. But, it consumed more energy and effort for the separation process. Therefore, the utilization of enzyme instant of chemical transesterification was considered in this study. Lipase is biodegradable, nontoxic, helps to ease recovery of product and glycerol and requires moderate alcohol and mild reaction conditions [8–10]. It can also catalyze both esterification and transesterification [9]. Thus, it is suitable for high FFA feedstock like POME. However, lipase-catalyzed biodiesel production is still less attractive for commercialization because commercial lipase is very expensive [11–13]. From our knowledge, crude lipase from oil palm fruit after 120 h of harvesting contained high lipase activity (1.38 Unit/mg protein) using Tris-HCl (pH 8) extraction. Afterwards, lipase activity increased significantly to 4.76 Unit/mg protein after the separation process using PEG and NaH_2PO_4. Palm lipase was already evaluated for its feasibility for biodiesel production using cooked palm oil [12]. The results indicated that biodiesel from palm lipase were not significantly different from commercial biodiesel and it also passed Thailand's fuel standards. The objective of this research was to investigate the potential of residual oil from POME and crude palm lipase as a novel substrate and catalyst for biodiesel production. The optimal conditions for biodiesel production including molar ratio, enzyme ratio, incubation temperature, mixing speed and reaction time were also determined. Biodiesel from crude palm lipase was characterized and compared with the production from NaOH, partial purified lipase and commercial lipase. In addition, the properties of biodiesel from crude lipase were also compared with Thai biodiesel and ASTM standards.

2. Materials and methods

2.1. Raw materials and chemicals

POME was collected from the Krabi Oil Palm Farmers Cooperatives Federation Limited (Krabi, Thailand) and stored at 4 °C to avoid any decomposition, oxidation and changes to the FFA content. All the chemicals such as organic solvents and reagents of laboratory and analytical grades were employed in this study. Lipase (EC 3.1.1.3) from *Candida rugosa* was obtained from Sigma-Aldrich. The *Candida* sp. lipase was prepared in 0.1 M Tris-HCl buffer followed the instruction from Kimtun et al. [12].

2.2. Oil extraction from POME

POME was firstly centrifuged at 5000 rpm for 60 min before use. Only sediment was collected and utilized. Furthermore, oil was extracted from the sediment by the soxhlet extraction technique using an organic solvent containing hexane:methanol:acetone (6:2:2). The sample was mixed with the solvent under an optimal ratio at 1:6 (w/w). The process was maintained at 100 °C for 1 h, afterward oil was collected and recovered by evaporation [14]. Afterward, the residual oil was utilized as a substrate for biodiesel production.

2.3. Lipase extraction from oil palm fruit

2.3.1. Plant collection

Ripen palm fruit (*E. guineensis*) were collected randomly according to ARDA (Thailand) standards and left for 120 h after harvesting under room temperature (28 ± 2 °C). Afterward, 1 g of lipase was extracted from 50 g of ripen palm fruit using 0.1 M Tris-HCl buffer (pH 8.0) as described by Kimtun et al. [12]. The supernatant was freeze-dried and kept at 4 °C until used. The enzyme after buffer extraction was called "crude lipase".

2.3.2. Partial purification of lipase from oil palm fruit

Crude lipase from oil palm fruit was purified by using the aqueous two-phase systems (ATPS) method, 20% of polyethylene glycol (PEG)-1000 and 15% of NaH_2PO_4. Lyophilized lipase (1 g) was firstly mixed with 5 g of distilled water. Thereafter, the sample was loaded into the ATPS system. A bottom layer, an enzyme containing layer, was collected and characterized [12]. The supernatant was dialyzed with 12000 Da membrane and left overnight against distilled water. The protein and protease activity after dialysis were determined. Afterwards, the supernatant was freeze-dried and kept at 4 °C until used. The enzyme after ATPS was called "partial purified lipase".

2.3.3. Determination of protein and lipase activity

The determination of protein was followed by the Lowry method. The activity of lipase was determined by adding 200 μL samples in 0.1 mM Tris-HCl buffer (pH 8.0) 2.45 mL containing 0.15 M NaCl and 0.5% Triton X-100. The determination was operated under 40 °C for 5 min, then 50 mM *p*-nitrophenylpalmitate 200 μL was added. *p*-nitrophenol was used as a standard and the sample was analyzed at 410 nm [12].

2.4. Enzymatic biodiesel production from POME and optimization study

All experiments were determined in a 250 mL screw-capped flask under batch condition. Crude lipase from palm oil fruit was used as the catalyst. The investigation and optimization of enzymatic biodiesel from POME was varied using one-factor-at-a-time (OFAT) method. Ten grams of residual oil from POME were added to the flask with a methanol-to-oil ratio from 2:1 to 12:1 and enzyme loading at 14 to 91 U/10 g of oil were varied and tested. The process conditions of these experiments including incubation temperature (35–70 °C), mixing speed (100–400 rpm) and reaction time (6–72 h) were also varied. At the end of the reaction, centrifugation was used to remove glycerol and lipase before separating the product in a separating funnel. The sample was left for 1–2 h to separate in a separating funnel. Biodiesel (upper layer) was taken, washed twice with 20 mL of deionized water at 50 °C and dried in an oven (105 °C, 24 h) to get rid of the remaining water and alcohol. Fatty acid methyl ester (FAME) content was evaluated by GC/MS [16]. The yield of biodiesel and the conversion of FFA into biodiesel were calculated by weight and the final FFA content in the product. Biodiesel yield from crude lipase was also compared with NaOH, partial purified and commercial lipase.

2.5. Analytical analysis

Table 1 shows the full list of parameters for the characterization of POME, while the methodologies for each of the analyzed