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Cocoa pod husk: A new source of CLEA-lipase for preparation of low-cost biodiesel: An optimized process

Soofia Khanahmadi[a,*], Faridah Yusof[a], Hwai Chyuan Ong[b,*], Azura Amid[a], Harmen Shah[a]

[a] Department of Biotechnology Engineering, Faculty of Engineering, International IslamicUniversity Malaysia, P.O. Box 10, 50728 Kuala Lumpur, Malaysia
[b] Department of Mechanical Engineering, Faculty of Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia

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ABSTRACT

Enzymatic reactions involving lipases as catalyst in transesterification can be an excellent alternative to produce environmental-friendly biodiesel. In this study, lipase extracted from Cocoa Pod Husk (CPH) and immobilized through cross linked enzyme aggregate (CLEA) technology catalysed the transesterification of *Jatropha curcas* oil successfully. Face centered central composite design (FCCCD) under response surface methodology (RSM) was used to get the optimal conditions of 3% (w/w) enzyme loading, 4 h reaction time and 1:6 oil/ethanol ratio to achieve the highest conversion of free fatty acid and glycerides into biodiesel (93%). The reusability of CLEA-lipase was tested and after seven cycles, the conversion percentage reduced to 58%. The results revealed that CLEA lipase from CPH is a potential catalyst for biodiesel production.

1. Introduction

In recent years, fatty acid alkyl esters (FAAEs), also known to be biodiesel has been deemed as clean, nontoxic, biodegradable and renewable fuel and one of the best candidates in diesel fuel class to address the emerging environmental issues and unstable demands of the market. Many renewable and non-renewable sources are explored by scientists for alternative of crude oil in last couple of decades. Among them, fuel cell started taking shape into reality by delivering electric cars and biodiesel showed a promising start in many countries of Europe and Brazil.

Biodiesel is synthesized by esterification of fatty acids with short chain alcohols or transesterification of triacylglycerol (TAG) with short chain alcohols. The transesterification reaction gives biodiesel as major product and glycerol as bi-product (Hoekman et al., 2012). The advantages of biodiesel as diesel fuel includes portability, ready availability, renewability, higher combustion efficiency, lower sulfur and aromatic content, higher cetane number and higher biodegradability (Demirbas, 2009). In contrary, the main disadvantages are higher viscosity, lower energy content, higher cloud point and pour point, higher nitrogen oxide emission, lower engine speed and power, injector coking, engine compatibility, high price, and higher engine wear (Demirbas, 2009). In general, biodiesel feedstock can be categorized into four groups: vegetable oils (edible or non-edible oils), animal fats, waste cooking oil including triglycerides (Balat and Balat, 2010) and Microalgae (Halim et al., 2012). Vegetable oils are potential source of energy, with energy content contiguous to that of diesel fuel (Ashraful et al., 2014). Soybean oil was utilized as common feedstock material for the biodiesel production in Brazil (Castanheira et al., 2015) while rape seed oil in Europe (Abdelradi and Serra, 2015) and palm oil in Malaysia and Indonesia (Jaafar et al., 2015; Mukherjee and Sovacool, 2014).

Due to the overwhelming debates on food versus fuel prompted application of non-edible oils such as jatropha, keranja, neem as feedstock for biodiesel industry. *Jatorpha curcas* L. is a small perennial tree of 3–5 m tall which belongs to Euphorbiaceous family. The oil content of seeds is from 30 to 50% by weight and in kernels it ranges from 40 to 60%. Some anti-nutritional factors such as curcin and phorbol esters are present in this plant. Therefore J. *Curcas* oil is toxic and unsafe for cooking purposes, but remains an attractive option as non-edible vegetable feedstock in oleo chemi-

Abbreviations: CPH, cocoa pod husk; FCCCD, face centered central composite design; RSM, response surface methodology; CLEA, cross-linked enzyme aggregates; FFA, free fatty acids; FAEE, fatty acid ethyl ester; GC/MS, gas chromatography/mass spectrometry.

* Corresponding authors.
E-mail addresses: Sophia.khan.ahmadi@gmail.com (S. Khanahmadi), onghc@um.edu.my (H. Chyuan Ong).

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cal industries such as fatty acids, soap, surfactants, detergents and above all biodiesel production (Negm et al., 2013). In lieu of its various characteristics like hardiness, easy propagation, drought endurance, high oil content, rapid growth, adaptation to wide agro-climatic conditions, and multiple uses of plant as a whole, *J. curcas* is not just limited to its centre of origin but has gained attention in tropical and sub-tropical countries as well (Divakara et al., 2010). *Jatropha* is a-branched triglycerides. The properties of alkyl esters in this oil are similar to fossil diesel. The direct use of *Jatropha* oil has caused some problems on account of its viscosity and chemical structure and thus, needs to be modified through chemical and enzymatic approaches by using different types of catalyst.

Cocoa pod husk (CPH) is a by-product obtained after the removal of cocoa beans from the cocoa fruit. For each tonne of dry beans produced, ten tonnes of cocoa pod husks are generated, which represents a serious challenge for waste management. This by-product is a source of hydrolase enzymes in which one of them is lipase. In our previous work a high active CLEA-lipase from cocoa pod husk was prepared successfully. The stable CLEA-lipase is efficient to use as catalyser for biodiesel production (Khanahmadi et al., 2014).

Commercial biodiesel production from different vegetable oil relies on different catalysts of transesterification reaction. In terms of catalysis, transesterification reaction is classified into two categories: chemical and enzymatic. Out of these, the chemical transesterification gives high biodiesel yield (>96%) but it is still considered as a disadvantages in terms of high energy consumption. Moreover, the most conventional basic catalysts (NaOH, methoxide etc.) are not applicable at high content of FFA in oils (Shah et al., 2004). In contrary, enzyme catalyzed transesterification that involves lipases could be an excellent alternative for biodiesel production and has several advantages such as: less energy consumption and is environmental-friendly with respect to emissions. Moreover, the separation of major product from the by-product, glycerol is convenient (Bajaj et al., 2010). The tendency of enzyme catalysts to prevent soap formation have made them a better and more attractive option in biodiesel industry (Leung et al., 2010).

Lipase produced by microorganisms can be used to catalyze the biodiesel production but the process is costly and requires a complete understanding of the molecular mechanisms controlling lipase folding and secretion. The lipase extracted from plant sources has been used in biodiesel industry because it can be prepared easier than the lipase from microorganisms. Moreover, plants are wildly available and lipase can easily be extracted from them at low cost. No genetic technology is needed to produce these plants.

However, use of free enzyme as biochemical catalysts has some drawbacks such as, high cost, low stability and hindered reusability. Immobilized enzyme has been used in biodiesel industry to overcome these issues. It is reported that immobilized enzyme can be more efficient than free enzyme in production of biodiesel (Zhang et al., 2012).

Among the different methods employed in the immobilization of enzyme, cross linked enzyme aggregate (CLEA) method has attracted many researchers in recent years because of its simplicity and benefits. Immobilization of enzyme with CLEA technology is considered as a low cost method with respect to other immobilization methods as it does not need expensive carrier beads and the final product is pure due to the use of ammonium sulphate as substrate (Garcia-Galan et al., 2011). In the CLEA method, enzyme is precipitated to obtain aggregates. In the next step, the aggregate is cross linked with glutaraldehyde. In the CLEA preparation, the aldehyde group of glutaraldehyde is reacted with the amino groups of the protein. Sometimes the amine content of enzyme is low and the cross-linking might not be very effective. To overcome this issue, the aggregation can be prepared in the presence of certain additives such as Bovine Serum Albumin which has a large number of amine groups.

The current research is new and deals with the enzymatic biodiesel production from *J. curcas* oil and ethanol using cocoa pod husk (CPH) as a source of lipase for the preparation of cross linked enzyme aggregate. To the best of knowledge of the authors, biodiesel production with CLEA-lipase from CPH is the first biodiesel from a non-microbial source. Lengthening the reused cycles of immobilized lipase can reduce production cost of biodiesel significantly. It is anticipated that this study could add more to the knowledge and information available on the utilization of low cost waste oil (*J. curcas* oil), ethanol and using low-cost catalyst source (CPH) for the production of renewable biodiesel.

2. Material and methods

2.1. Raw materials and chemicals

The *J. curcas* oil was purchased from Bionas Sdn Bhd. Malaysia. Fresh cocoa fruit was collected from Malaysian Cocoa Board, Jengka, Pahang. All the chemicals and reagents were obtained from Sigma–Aldrich and Merck.

2.2. Preparation of CLEA-lipase

Enzyme solution from CPH in a volume of 0.5 ml was poured into a 15 ml Falcon tube. To the enzyme solution, 20% saturated ammonium sulfate, 60 mM glutaraldehyde as cross-linker and 0.17 mM bovine serum albumin as additive was added simultaneously to bring the final volume of the solution to 4 ml. The solution was agitated at 200 rpm for 17 h at room temperature. Then 3 ml of water was added and the mixture was centrifuged at 4000 rpm at 4° C for 30 min. The supernatant was poured out and the residue was washed three times with water, then it was centrifuged and decanted. The final CLEA preparation was kept in 5 ml of water to measure activity assay of CLEA-lipase.

2.3. Lipase activity assay

Lipase enzyme activity was conducted with *p-nitro* phenyl palmitate as substrate. To make a stock solution, 28 mg of *p-nitro* phenyl *palmitate* was dissolved in 100 ml of v/v Triton 100-X and 1.7 ml of 1% sodium dodecyl sulphate was added while stirring. The solution became quite turbid at the beginning of the heating, but cleared after a while. To initiate the reaction, 1 ml of *p-nitro* phenyl *palmitate* stock solution was incubated with 1 ml of 0.1 M Tris-HCl at pH 8.2 and 0.5 ml purified sample in a water bath for 30 min at 37 °C and 1 ml of 1 M NaOH was finally added to stop the reaction. The absorbance of the incubation product, *p-nitro* phenol, was measured by reading the absorbance at 410 nm. The absolute amount of *p-nitro* phenol produced was calculated from a calibration graph constructed with known amounts of *p-nitro* phenol. One unit of enzyme activity is defined as the amount of enzyme required to release 1.0 μmol of *p-nitro* phenol per minute under assay conditions.

2.4. Protein concentration

Protein content of the extract was determined with Bradford method (Bradford, 1976) taking BSA as standard and then scanning by Magellan Data Analysis Software at an absorbance wavelength of 595 nm.

2.5. Study of operating condition of transesterification reaction (OFAT Analysis)

In this study, the individual effect of five selected parameters (catalyst loading, temperature, agitation, ratio of oil/alcohol and