Topic: Biocatalytic Production of Biodiesel and Bioethanol from Palm-Oil Feedstock

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ABSTRACT

Palm oil is a potent feedstock for the production of biodiesel, while the cellulosic waste from palm oil processing represents an underused biomass for valorization. In this study, conversion of oil palm-derived feedstock to biodiesel and bioethanol using novel biocatalysts and genetically modified ethanolgen has been investigated. In this part, the work is focused on development of an efficient biocatalytic process for biodiesel production from palm oil using novel biocatalyst designs i.e. crosslinked protein-coated microcrystalline lipase (CL-PCMC lipase) and magnetic nanoparticle lipase with modified covalent linkage. The zwitterionic glycine (Gly) was found to be a superior core matrix component from the screening of various organic/inorganic solid-state buffer compared to the conventional insert K_2SO_4 for synthesis of immobilized *Thermomyces lanuginosus* lipase with the highest catalytic performance towards esterification of palmitic acid, transesterification of refined palm oil and co-ester/transesterification of crude palm oil with the fatty acid methyl ester (FAME) yield of $\geq$ 95% (mol/mol) from the optimized reaction containing at 4:1 [MtOH]/[FFA] with 20% of the CL-PCMC-lipase/Gly in the presence of tert-butanol as a co-solvent and incubated at 50 °C for 6 h. The high performance of the biocatalyst could be related to the control of enzyme ionization state by glycine which can act as solid-state buffer. Esterification of palm fatty acid distillate using ethanol as a nucleophile led to the highest fatty acid ethyl ester yield of > 95% based on molar basis. The reaction kinetics on esterification followed the Ping-Pong Bi-Bi model and key kinetic parameters were obtained for further reaction up-scaling. Immobilization of the lipase on magnetic nanoparticle was investigated as another potent biocatalyst design with high reactive surface area and simple separation by magnetization. Modification of covalent linkage between the iron core matrix and the enzyme in the form of Fe_3O_4-AP-EN-lipase showed the superior catalytic performance toward transesterification of

RPO. The optimal reaction contained 23.2% w/w enzyme loading and 4.7:1 methanol to oil molar ratio with 3.4% water content in the presence of 1:1 (v/v) *tert*-butanol to oil obtained from experimental design optimization using central composite design (CCD) approach. Under the predicted optimal condition, the maximal FAME yield of 97.2% was achieved after incubation at 50 °C for 24 h, which was slightly lower than the predicted yield. Both microcrystalline lipase and magnetic nanoparticle lipase showed high operational stability and could be used in consecutive batches or continuous process.

Another part of the study was focused on the conversion of palm kernel cake (PKC), a mannnan rich by-product to ethanol by using genetically engineered thermophilic bacterium *Geobacillus thermoglucosidasius* TM242. The highest fermentable sugars of 28.3 g/L was obtained from 5% PKC pretreated conditions at 4.5 bar (148 °C) for 15 min using 1.67 %v/w of Advanced Enzymes Technology mannanase (AET mannanase) and 10.38 FPU/g glucan of Cellic Ctec2 cellulase. Fermentation of the PKC hydrolysate led to the maximal ethanol concentration with 9.9 g/L (92.4% of theoretical yield based on the total sugars in 5% PKC) after incubation at 60 °C for 48 h whereas a lower ethanol concentration was obtained using *S. cerevisiae*. The higher ethanol yield from TM242 was due to the ability of the bacteria on converting both hexose and pentose sugars together with the short-chain glucose and mannose oligomers to ethanol. The *G. thermoglucosidasius* TM242 is thus a promising alternative microorganism for improvement of bioethanol production from lignocellulosic biomass from PKC and other lignocellulosic biomass. Overall the work provides efficient approaches for improvement of biodiesel and bioethanol production from oil palm-derived products by biotechnological processes using novel biocatalysts and ethanologen which can lead to increasing economic competitiveness of the biofuel industry.

Keywords: Biodiesel, Bioethanol, Cross-linked protein coated microcrystalline (CL-PCMC), *Geobacillus thermoglucosidasius*, Immobilized lipase, Magnetic nanoparticle, Palm oil, Palm kernel cake