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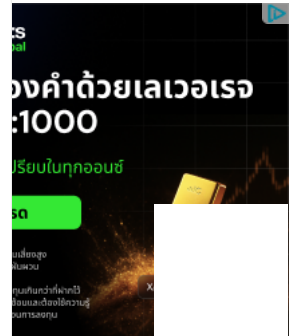
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Immobilized Ancient Lipase for Waste-to-Fuel Biocatalysis: Toward Sustainable Biodiesel Production

By Saad, AHM (Saad, Afifah Husna Mat) ; Kamarudin, NHA (Kamarudin, Nor Hafizah Ahmad) ; Leow, ATC (Leow, Adam Thean Chor) ; Mohamed, MS (Mohamed, Mohd Shamzi) ; Ali, MSM (Ali, Mohd Shukuri Mohamad)

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Source APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY
DOI: 10.1007/s12010-025-05392-0

Early Access NOV 2025

Indexed 2025-11-25

Document Type Article; Early Access

Abstract Developing thermally stable, reusable enzymes is key to eco-friendly biodiesel production. Here, we report the first immobilization of a sequence-reconstructed ancestral lipase from family 1.3 lipase, last universal common ancestor (LUCA) onto Seplite LX120, a cross-linked styrene-divinylbenzene resin, via a simple physical adsorption strategy. The immobilized LUCA exhibited broad operational stability, maintaining over 80% activity from 20-100 degrees C and above 50% across pH 4-9, with optimal performance at 70 degrees C (565.48 U/g) and pH 9 (534.52 U/g). Enhanced solvent and storage stability were

observed compared to the free enzyme, with over 50% residual activity after 12 weeks at 4 degrees C and excellent tolerance in polar solvents. Notably, the biocatalyst retained 95.35% activity after 10 reuse cycles. Characterization by Scanning Electron Microscopy (SEM) revealed enzyme coverage on the Seplite LX120 surface, while Brunauer-Emmett-Teller (BET) analysis confirmed suitable surface area and porosity for effective immobilization. Both free and immobilized LUCA catalyzed efficient transesterification of waste cooking oil (WCO), yielding 95% and 100% biodiesel, respectively, within 3 h under optimized conditions: methanol-to-oil molar ratio of 6:1, shaking at 150 rpm, and 70 degrees C. These findings highlight the promise of ancestral enzyme immobilization as a novel route to high-performance, sustainable biocatalysts for biodiesel synthesis from low-cost feedstocks.

Graphical Abstract Summary of biodiesel transesterification using immobilized and free LUCA lipase (Created with BioRender.com)

Keywords

Author Keywords: Lipase; Immobilization; Biodiesel; Waste cooking oil; Transesterification

Keywords Plus: COOKING OIL; TRANSESTERIFICATION; CATALYST; ENZYMES; STABILIZATION; OPTIMIZATION; PREDICTIONS; PERFORMANCE; ADSORPTION; STRATEGIES

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