ABSTRACT

Abdurrahman Dzaki Muhammad Al Faathir, 240202221400184. ***Optimisation of the Partial Purification of Isolates A3 and C4 Using a Combined Ammonium Sulphate–Deep Eutectic Solvent (DESs) System and Characterisation of Extracellular Lipase.*** *Under the guidance of Agung Supriadi and Titin Haryati.*

The high national import rate of biocatalysts, which reaches 99%, underscores the urgent need to explore extracellular lipases from local lipolytic bacteria, specifically isolates A3 and C4, derived from waste cooking oil. This research aimed to optimize partial purification using a combination system of ammonium sulfate and Deep Eutectic Solvents (DESs) as stabilizing green solvents, while simultaneously mapping their physicochemical characteristics. This experimental study was designed using a Completely Randomized Design (CRD) with a Factorial Pattern. The workflow comprised sterilization, preparation of lipid-inducer media, bacterial reactivation, growth curve mapping (OD600) to determine harvest time, crude extract centrifugation, choline chloride-based DESs synthesis, partial purification optimization, dialysis, and enzyme characterization. The two-way ANOVA statistical analysis revealed that the ammonium sulfate fraction (levels: 20–40% and 40–60%), DESs formulation type (levels: ChCl+Ethylene Glycol (ChCl+EG), ChCl+Glycerol (ChCl+Gly), ChCl+Urea (ChCl+Urea), and ternary (ChCl+EG+Gly)), and DESs concentration (levels: 10%, 20%, and 30% v/v) exerted a highly significant effect ($p < 0.001$) on the increase of specific enzyme activity, with the optimum combination achieved at the 40–60% fraction, ChCl:Urea DESs, and 30% (v/v) concentration. Furthermore, characterization using ANOVA proved that variations in p-nitrophenyl ester substrate types (C4, C8, C12, C14, C16, and C18), pH (range of 6–11), and temperature levels (range of 30–80°C) had a highly significant impact ($p < 0.001$) on hydrolysis rate of the biocatalyst. In conclusion, this research demonstrates that the combination method successfully enhances the partial purity value of lipase significantly without compromising its functional integrity. Characterization confirmed both enzymes as true lipases, exhibiting the highest affinity toward the long-chain substrate p-nitrophenyl stearate (C18). Lipase from isolate A3 was characterized as alkaline-thermostabilized, functioning optimally at pH 9.0 and 50°C, whereas lipase from isolate C4 was acid-mesophilic, with optimal conditions at pH 6.0 and 40°C. This hybrid procedure successfully provides an efficient and stable alternative for biocatalyst preparation in the biotechnology industrial sector

Key words: *Deep Eutectic Solvent (*DESs*), Partial purification, Enzyme characterization, Extracellular lipase.*