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INVESTIGATION OF LIPASE IMMOBILISATION BY CROSS-LINKED ENZYME AGGREGATE (CLEA) METHOD

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Biocatalysts are a more environmentally friendly alternative to chemical catalysts with significantly higher reaction rates and substrate specificity. Some of the most employed biocatalysts in industrial applications are enzyme lipases, which catalyse a plethora of reversible carboxyl-nucleophile hydrolysis reactions. Nonetheless, free-state lipases exhibit limitations such as sensitivity to the reaction medium or low operational stability[1]. Enzyme immobilisation extends the application of lipases by enhancing their stability and allows enzyme recycling. Yet, currently most applied immobilisation techniques for lipases (absorption or covalently binding to carriers) produce insufficient increase in stability, carriers are expensive and often make up most of enzyme-carrier mass[2]. One of the strategies for solving these issues could be to use carrier-free enzyme immobilisation[3].

One of the most promising carrier-free immobilisation methods is based on the cross-linking enzyme aggregates (CLEA). This method involves two steps: protein aggregation followed by cross-linking (Fig. 1).

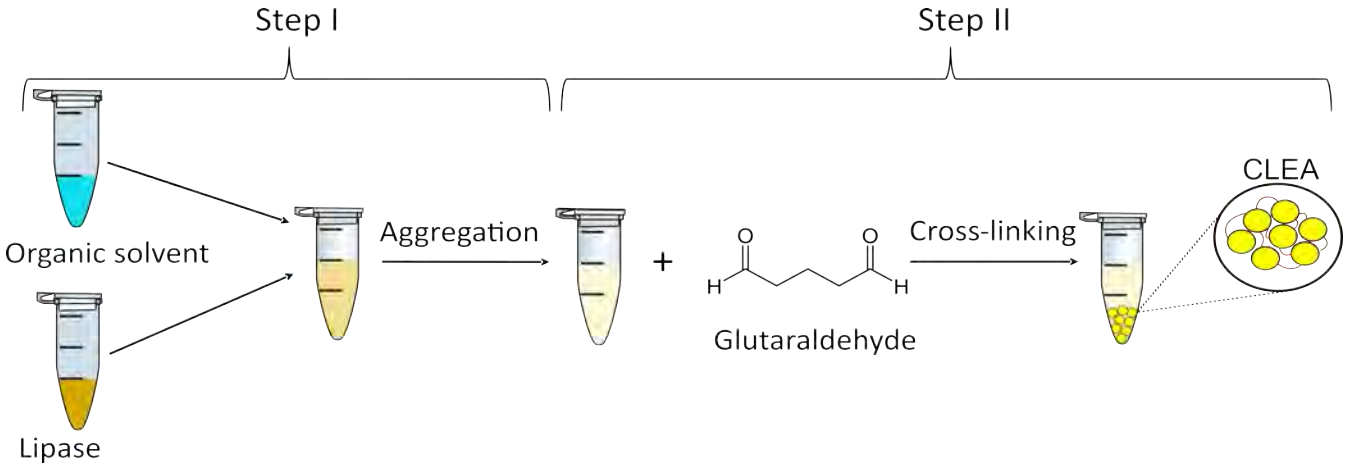

Fig. 1. Workflow of enzyme immobilisation by CLEA method.

First, enzyme aggregation is initiated by adding salts or organic solvents. Then, the enzyme aggregates are cross-linked with a bi-functional linking agent, i.e. glutaraldehyde[4]. It reacts with amino groups of lysine and arginine residues, forming imino groups. The main advantages of CLEA are that it is a relatively fast and straightforward method and does not require expensive reagents, carriers or purified enzymes. However, each step of the process must be optimised for the immobilised enzyme to retain its catalytic activity.

In this project, we investigated lipase Lipolase 100L (Novozymes) aggregation by different concentrations of four organic solvents (acetone, ethanol, isopropanol and 2-methoxyethyl ether) and optimised aggregation time. Furthermore, we optimised cross-linking reaction time and concentration of glutaraldehyde. The obtained results will be presented in more detail during the poster session.

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