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TRANSGLUTAMINASE APPLICATION FOR CARRIER-FREE ENZYME IMMOBILIZATION BY CLEA METHOD

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Enzymes are widely used in industry, and they can be used in two different ways. The first approach involves using soluble enzymes, which are used only once and can be affected by environmental conditions such as temperature or pH, causing them to lose activity[1]. In addition, usually separating the enzymes from the reaction mixture is necessary. The second approach involves immobilization methods, which results in higher stability of the enzymes. There are two categories of enzyme immobilization methods: those involving support materials and carrier-free immobilization. Carrier-free immobilization, such as CLEA (cross-linked enzyme aggregates), is more attractive than immobilization with support materials because it is simple, cost-effective and does not require a matrix. Furthermore, pure enzymes are not necessary for CLEA.

The CLEA process consists of two phases: enzymes are first precipitated, and their aggregates are formed, and then these aggregates are cross-linked, typically using glutaraldehyde, a toxic and impure reagent[2]. Our project proposes using transglutaminase in CLEA preparation to eliminate the precipitation step and avoid using glutaraldehyde. Transglutaminase is an enzyme that catalyses the formation of cross-link isopeptide bonds between the glutamine γ-carboxyamide group and the lysine ε-amino group, which are highly resistant to proteolysis, preventing the loss of enzyme activity and leaching from CLEA derivatives[3].

To reach the project goal, considerable amounts of clean protein were needed. The initial investigation started from a desk study to identify the microorganisms that produce sufficient quantity of transglutaminase and found that S. mobaraensis is the bacterium capable of producing the highest amount of active enzyme. Four different growth media composition were tested to cultivate S. mobaraensis. The transglutaminase activity, protein concentration and specific activity were followed for 144 h. The influence of polypeptone, glucose and starch for transglutaminase expression were also examined. The output of transglutaminase production experiments will be presented in more detail during the poster session.

[1] Velasco-Lozano et al. Editorial: Designing Carrier-Free Immobilized Enzymes for Biocatalysis, Front. Bioeng. Biotechnol., vol. 10, 2022.
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