P-021	**Immobilisation of porcine pancreatic lipase in oil-core calcium-alginate capsules**	

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INTRODUCTION AND OBJECTIVES

In this study, porcine pancreatic lipase was immobilised in oil-core calcium-alginate capsules by inverse gelation technique, where calcium chloride-in-oil emulsion was dropped into alginate solution. It was hypothesised by incorporating porcine pancreatic lipase in the calcium chloride-in-oil emulsion droplets, the lipase would be immobilised at the outer membrane of the oil-core capsules during cross-linking between calcium ions and alginate matrix.

MATERIALS AND METHODS

To immobilise porcine pancreatic lipase, calcium chloride of 1.08 g was added to 9 ml of 50 mg/ml lipase solution to make $CaCl_2$-lipase solution. Next, $CaCl_2$-lipase solution was dispersed into 30 ml paraffin oil containing a mixture of Tween20 and Span80 (1%v/v) with an overhead stirrer (IKA, Germany) at 500 rpm for 3 min to produce water-in-oil emulsion. The emulsion was added dropwise into 10g/L of alginate solution, stirred at 400 rpm for 30 min. Then, the capsules were filtered, washed with demineralised water and underwent a reinforcement process to further harden the membrane by putting them into 15g/L $CaCl_2$ solution for 1 h. The capsules were washed with demineralised water and gently dried with tissue papers to remove excess water. Finally, the capsules were let to dry openly at ambient temperature until constant weight. Control capsules without lipase were similarly prepared, upon which blank capsules were obtained.

Encapsulation efficiency of lipase in calcium-alginate capsules was determined by the difference between the initial amount of protein introduced in the immobilisation reaction process and the bounded protein in capsules. Protein content of lipase in calcium-alginate capsules was estimated using Lowry method (Dulley 1975).

Hydrolytic activities of free and immobilised lipase were assayed by olive oil emulsion as described in Soares 2003. One unit (U) of enzyme activity was defined as the amount of enzyme that liberates one µmol of free fatty acid per minute under standard assay conditions (37°C, pH 7.7, 200 rpm).

The effects of pH and temperature on activity of free and immobilised lipase were assayed in pH ranging from 6 to 9.5 and temperature between 25°C and 60°C using standard assay conditions, respectively.

Reusability of the immobilised lipase was determined using hydrolytic reaction under standard assay conditions after removal from reaction medium and compared with the first run (activity defined as 100%). The reaction medium was replaced with fresh medium in each cycle.

Michaelis constant, K_m, and the maximum reaction velocity, V_{max}, for free and immobilised lipase were determined by measuring initial reaction rates at 5 min with an emulsion substrate containing varying amounts of olive oil in the range of 0.8-2.5 M.

RESULTS AND DISCUSSION

The encapsulation efficiency of lipase was averagely 60%. The loss of lipase during immobilisation process might be due to the large network space formed during cross-linking between calcium ions and alginate polymer, resulting in the water-soluble lipase to diffuse out. The loss of lipase also might occur during washing process. Washing process could separate the weakly entrapped lipases at the surface of the capsules.

Both activities of free and immobilised lipase show similar trends, where their activities steeply increased from pH 6 until optimum pH 8.5, then sharply dropped between pH 9 and 9.5 (Fig. 1). The optimum value of lipase activity was at pH 8.5 for both free and immobilised lipase, indicating that the optimum pH of lipase was not altered by immobilisation.

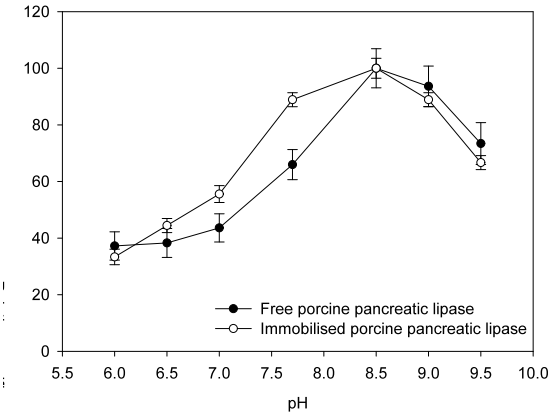

Figure 1: Effect of pH on activity of free and immobilised lipase

The optimum reaction temperatures for free and immobilised lipase were 30°C and 37°C, respectively. The activity of free lipase ascended steeply from 25°C to 30°C, and then descended gradually at higher temperatures. The activity of immobilised lipase shows a

similar profile, however it decreased at 37°C. Results suggested that the immobilisation matrix protected the lipase against denaturation at high temperature by providing a lower temperature in the gel micro-environment compared to the bulk environment. This increases the potential of immobilised lipase as a biocatalyst in industries because higher temperatures reduce the viscosity of the reaction medium, minimises the mechanical energy in agitation and other industrial operations (De Queiroz 2006).

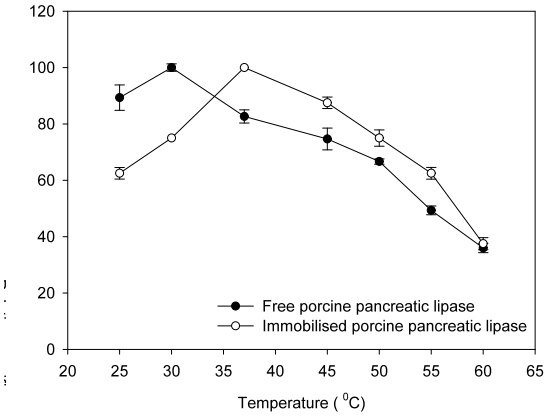

Figure 2: Effect of temperature on activity of free and immobilised lipase

Immobilised lipase shows a drastic decrease in activity with increasing number of cycles. The immobilised lipase retained 63% of the activity after the first cycle, dropped to 38% after the second cycle, and finally dropped to 13% before completely lost the activity (Fig. 3). The main reason for the loss of activity may be due to enzyme leakage from the alginate matrix capsules resulted from loosened polymer network.

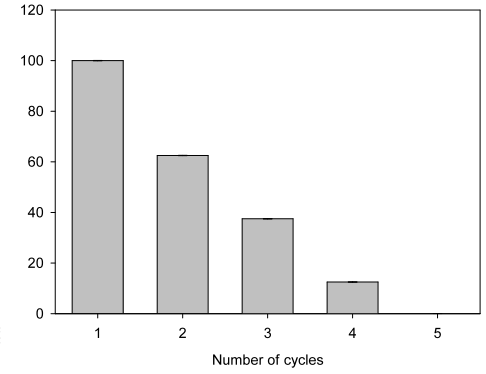

Figure 3: Recycling stability immobilised lipase

Michaelis constant, K_m, and the maximum reaction rate, V_{max}, of free and immobilised lipase were calculated from the Lineweaver-Burk plot are summarised in Table 1.

Table 1: Kinetic parameters of free and immobilised lipase

Catalyst	Michaelis constant, K_m (M)	Maximum reaction rate, V_{max} (mmol/mg protein.min)
Free lipase	3.8	17.3
Immobilised lipase	10.6	25.9

K_m shows an increase on immobilisation, indicates a lower affinity towards the substrate. This shows that diffusional limitation to mass transfer occurs in the immobilised lipase. It is interesting that V_{max} value of immobilised lipase was higher by 1.5-fold than free lipase. A definite rise in the biocatalytic activity of the immobilised lipase could be attributed to some conformational modification of lipase during immobilisation procedure. An exposure to paraffin oil (*log P* = 5) should initiate conformational changes to more hydrophobic amino acids at the surface and alters the availability of the individual amino acid residues. The combined effect of change in availability and in reaction rate of the various amino groups by paraffin oil gave a higher degree of immobilisation via tyrosine and cysteine residues and lower degree of coupling via lysine residues (de Oliveira 2000). Thus, it could be inferred that the use of paraffin oil was able to create around the enzyme a specific microenvironment that might enhance its activity.

CONCLUSION

The immobilisation method used is simple, cheap, and safe. Though these calcium-alginate capsules cannot be efficiently used in aqueous solutions as a result of leaching, it is anticipated that they can be used in water-immiscible organic solvents for synthetic reactions.

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