

UNIVERSITI TEKNOLOGI MARA

**IMMOBILIZATION OF LIPASE IN CALCIUM
ALGINATE BEADS FOR THE STUDY OF
BUTYL ACETATE SYNTHESIS**

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ABSTRACT

Literature about lipase (EC 3.1.1.3), immobilized enzyme, alginate and chitosan are presented in this thesis. This study involved the use of free and immobilized lipases to catalyse esterification reaction of short chain fatty acid and alcohol to produce short chain ester. Generally, it involved the optimization of conditions of esterification reaction of acetic acid and n-butanol and followed by comparison of properties of immobilized enzyme with those of free enzyme. Results showed that 14.3 mg lipase, 80 μmol n-butanol, 160 μmol acetic acid and 3.0 days reaction time at a temperature of 40 $^{\circ}\text{C}$ were the optimum conditions for lipase - CAB in terms of enzyme loading, immobilized enzyme concentration, temperature, substrate concentration and reaction time respectively. Meanwhile, 0.8% w/v of chitosan solution was chosen for the stabilized calcium alginate beads. Results showed that product conversion increased by increasing the temperature up to 50 $^{\circ}\text{C}$ for Lipase - CAB and Lipase - CCAB but not for free lipase. Thermal stability test showed that Lipase - CAB and Lipase - CCAB remained stable against temperature up to 60 $^{\circ}\text{C}$ compared to free lipase which had the highest activity at 30 $^{\circ}\text{C}$. The studies of effects of n-butanol concentrations showed that increased in concentration of n-butanol above 40 μmol decreased the conversion of product for Lipase - CCAB and free lipase. Meanwhile, conversion of product was affected by increasing concentration of n-butanol to 80 μmol and above for Lipase - CAB. In the study of effect of acetic acid, it was found that increasing concentration of acetic acid above 160 μmol decreased the product conversion for Lipase - CAB and free lipase. However, Lipase - CCAB was not affected by high concentration of acetic acid up to 200 μmol . Kinetic parameters, K_m & V_{max} of immobilized lipases for n-butanol were lower in values when compared with K_m & V_{max} values for acetic acid. Results showed that there were no statistically significant different specific activities among the three systems studied. Operational stability test was important for repeated applications in batch or in a continuous reactor. It was demonstrated that the enzyme was still active for at least 5 cycles. Thus it was proven that immobilized lipase and free lipase were able to catalyse synthesis of short chain esters under the conditions studied. Continuous processes studies showed immobilized lipase had potential for such synthesis but need further studies. Several recommendations for further studies have also been suggested.

Candidate's Declaration

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This topic has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

In the event that my thesis found to violate the conditions mentioned above, I voluntarily waive the right of conferment of my degree and agree to be subjected to the disciplinary rules and regulations of Universiti Teknologi MARA.

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