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 °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 °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 °C compared to free lipase which had the highest activity at 30 °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 80μmol and above for Lipase - CAB. In the study of effect of acetic acid, it was found that increasing concentration of acetic acid above160 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 Tekmologi 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.

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CHAPTER 1

INTRODUCTION

Enzymes have gained considerable importance as catalyst in many reactions because of their high selectivity under mild reaction conditions at near ambient temperature and pressure. Furthermore, enzymes are environmentally friendly, as they are generally nontoxic and biodegradable.

Lipases also known as glycerol ester hydrolyses (EC 3.1.1.3) belong to the hydrolase enzyme class and were originally employed for hydrolysis of ester bond (Balco *et al.*,1996). Lipases have been employed for direct esterification and transesterification in organic solvent to produce aliphatic alcohols (Welsh *et al.*, 1990), esters of glycerol (Akoh *et al.*, 1992) and terpenic alcohols (Cloan *et al.*,1994). However, esterification of short chain fatty acids and alcohols has not received much attention (Abbas and Comeau, 2003). In this project, the synthesis of short chain ester formed by esterification of n-butanol and acetic acid catalysed by immobilized lipase in calcium alginate beads (Lipase - CAB) and chitosan coated calcium alginate beads (Lipase - CCAB) was reported. A study was also conducted using free lipase for comparison. The most commercially important field of application for hydrolytic lipases is their addition to detergents, which are used mainly in household and industrial laundry and in household dishwashers. Another application is the use of lipases in the dairy industry, oleochemical industries, paper industry, pharmaceutical industry, cosmetic industry as well as in medical applications. Lipases are indeed one of the most versatile enzymes in terms of industrial application. It is anticipated that in the coming years there will be large-scale commercial exploitations of these enzymes.

Free enzyme is not always sufficiently stable under operational conditions and provides one time usage as catalyst is costly. Therefore, the method to immobilize enzyme is developed, whereby the immobilized enzyme can be removed afterwards and can be used again. Furthermore, immobilization is important to maintain