CHAPTER 5

CONCLUSION

The presented technique for immobilization of lipase in carboxymethyl cellulose (CMC), polyvinyl alcohol (PVA) and PVA: CMC blend have shown to be very efficient as it have improved the biocatalytic properties of *Candida rugosa lipase* such as activity, stability, reusability and kinetic reaction for esterification process of butanol and oleic acid to produce butyl oleate ester. The study continued with immobilization of CRL onto the natural supports by entrapment method, where protein loadings and immobilization percentages were determined. With regards to the practicality of the immobilization method and improved lipase catalytic properties, entrapment is the best method for these kinds of supports, that were implemented in the immobilization of enzyme and showed high percentage conversion of ester, means it is applicable for this reaction. Interestingly, the use of the newly immobilized CRL as a biocatalyst showed a better result than the used of free lipase. The effectiveness of an immobilization process depends on the type of supports used. Through the enzyme preliminary procedure, *candida rugosa lipase*, (CRL) exhibited the most applicable enzyme in carboxymethyl cellulose (CMC) as a support one give highest yield of ester produced (28.3%) as compared to the other supports. The results showed that 50g CRL/0.5g CMC is sufficient to obtain the product in quantitative yield, and the optimum temperature for immobilized enzyme films storage condition is about -20 ^{o}C for PVA-CRL (28.9%).

Esterification reaction between butanol and oleic acid in hexane was used to assay the catalytic efficiency of lipase to produce butyl oleate ester. The characterizations of supports physicochemical properties have been performed through instrumental analysis using SEM-EDX, XRD, FTIR, DSC and SEM cross section. Enzyme-support attachment was characterized by implementation of instrumental and experimental analyses, as mentioned earlier. In immobilized lipases procedure, they were clearly demonstrated higher catalytic activity. An optimization study of butyl oleate synthesis with reference to native lipase, showed that butyl oleate (>28%) optimal production was achieved using CRL-CMC after 6 hours of reaction. Immobilized of CRL-CMC, CRL-PVA and CRL-PVA:CMC also showed high thermal stability (>30% relative activity) after incubation at 37 ^{o}C in water bath shaker for 6 hours of reaction as compared to native. The immobilized enzymes were also able to retain half-life (50% ester production) of over 10 cycles respectively.

The kinetic study for the enzymatic reaction of native CRL, CMC-CRL, PVA-CRL and PVA:CMC –CRL were found to follow Michaelis Menten kinetics with inhibition by the water and were further described by the Ping Pong Bi Bi model mechanism. A kinetic model based on a Ping Pong Bi Bi mechanism was developed that takes into account the effects of both butanol and oleic acid inhibition on the enzymatic production of butyl oleate showing excellent agreement with the experimental profiles for various substrate concentrations. The kinetic behaviours of CMC-CRL, PVA-CRL and PVA:CMC-CRL on their enzymatic esterification rates were investigated with focus on the effect of various types of substrate concentrations using Michaelis Menten kinetic model and the value of K_m and V_{max} were estimated by means Lineweaver-Burk plot. In this research, we can define that the lower K_m suggested that the CMC-CRL indicated the higher affinity toward butanol (But) than

oleic acid (Ol), so that, K_m(Ol) > K_m(But). In contrast, native CRL exhibited higher affinity towards oleic acid than the butanol, K_m(Ol) < K_m(But). On the other hand, the value of $V_{max(But)}$ showed that the initial rate catalyzed by PVA-CRL in reaction containing different concentrations of butanol was higher than those catalyzed by immobilized enzymes (Native-CRL, CMC-CRL and PVA:CMC-CRL), while the PVA:CMC-CRL indicated the lowest reaction rate compared to the others. The immobilized lipase of PVA-CRL demonstrated considerably highest $V_{max(Ol)}$ compared to the native CRL and others in reaction containing different concentrations of oleic acid. The Ping Pong Bi-Bi mechanism with one substrate (CMC-CRL, PVA-CRL and PVA:CMC-CRL) and two substrates (native CRL) competitive inhibition were best suited the experimental result. The kinetic parameter determined from the proposed models was used to stimulate the initial rate data which further gave excellent correlation with the experimental value.

In industrial applications, the cost is main factor that should be considered. As for immobilization technique, in addition to provide an active and stable biocatalyst, it also should be simple operation to decrease the cost of operation. For that reason, CMC and PVA seemed to meet the requirement of suitable support because of an inexpensive materials for lipase immobilization with CMC-CRL, PVA-CRL and PVA:CMC-CRL showing promising actions as biocatalysts.

5.1 Recommendations for Further Studies

From the present study, there are some recommendations which can be considered to further improve enzyme catalytic performance, for future development in enzyme technology:

1. Determination of the suitability of the CMC/PVA thin films for immobilization of whole cell. This can be a useful method to immobilize enzyme producing microorganisms isolated from a source, without having to isolate the desired enzyme before its use.

2. Alteration of the functional groups available on the CMC/PVA thin films for increased suitability as support for enzyme / whole cell.

3. Optimization of esterification processes by the present enzyme system using Response Surface Methodology method instead of the conventional one-at-a-time optimization approach.

    - Using related software such as Response Surface Method (RSM) to study the interactive effect of important parameters in large scale processing.