

Chapter 5

General Discussion and Conclusion

The industrial use of enzymes as biocatalysts dates back to the early 1900s with the development of enzyme processes to manufacture cheese products, bread, beer and wine. Some industrial processes require highly purified enzyme preparations which can be obtained by a variety of enzyme purification procedures. The extent to which the enzyme is purified is dependent upon the cost of purification, the stability of the enzyme and whether unwanted side reactions are likely to occur. Enzymes can be used once only in any commercial process if it is used in the free form. This is uneconomical, as active enzyme is lost after each batch reaction. It is therefore necessary to immobilize these enzymes on some inexpensive solid support for continuous reuse, improved stability and economic advantage. Using an inexpensive carrier matrix and a cost effective method is also desired.

In this study, laccase was obtained from Novo Nordisk in Kuala Lumpur. Details of the microorganism was not revealed to us. The laccase was used for both the biochemical characterization and immobilization work. We followed the immobilization method published by Gianna Palmieri in 1994 which involves entrapment of laccase into copper alginate beads. Partial characterization studies was carried out. The pH optimum of the

crude enzyme extract was found to be 5.5. This enzyme was stable for a range of pH values. The enzyme lost 95% of its activity at pH 5.0, 5.5 and 6.5 at room temperature after four days. Whereas at 5°C, the enzyme appeared to lose about 70% - 80% of activity after four days. The apparent molecular weight of this single peak was around 79,000 daltons. Three peaks were obtained from isoelectric focussing. Three peaks were obtained from isoelectric focussing. The pI were estimated to be 4.7, 5.2 and 6.0. The partially purified peaks from isoelectric focussing had an affinity towards syringaldazine as a substrate by two folds.

The optimum pH for activity of the immobilized laccase by entrapment into copper alginate beads was 6.0 whereas that of the free enzyme which was 5.5. Laccase concentrations of 5mg was found to provide the optimum level of activity by entrapment method. Further, thermal stability of the laccase was considerably increased by immobilization. The optimum temperature for activity of free and immobilized laccase for both methods was found to be maximum at 30°C. At higher temperature there was a significant reduction in activity. However immobilization of laccase by entrapping it into copper alginate did not result in any increase in stability compared to the soluble enzyme. Considering the cost of immobilization it is therefore advisable to work at room temperature. Storage stability was also studied. It appears that the local concentration of 100µl laccase within the copper alginate beads did not drastically improve their storage stability in this study. Immobilization of manganese peroxidase and glucose oxidase was reported to have improved their storage stability by increasing the local concentration of the enzymes (Aken *et al.*, 2000).

One of the challenges of immobilization is that optimization is achieved by trial and error (Gerhatz, 1990). In this experimental procedure, leakage of enzyme into the copper sulphate solution was evident. The pore size of the gel which is reflected by the viscosity of the carrier can affect diffusion of the substrates, products and limit the reaction rates of the entrapped enzyme (Tanaka *et al.*, 1983). Large pores can cause problems by allowing the entrapped enzymes to leak out. Studies done by Tanaka *et al.* (1983) showed that diffusion of substrate into calcium alginate was not very much affected by increasing the calcium chloride concentration but was disturbed considerably by an increase of the calcium alginate concentration. Similar studies can be carried out for the copper alginate system. Initial cost of immobilization has to be considered if it is found to be a success in the industry. Moreover, there is a possibility of the enzyme losing its activity during the immobilization process. Advantages of this immobilization method is that the enzyme inactivation is less likely to occur as polymerization can be carried out in frozen state and various shapes of the entrapped matrix is prepared. It requires small amount of enzyme. This method doesn't involve any chemical modification of the enzyme and therefore the intrinsic properties of the enzyme are not changed.

Further studies can be conducted to compare the dye colour removal capacity of laccase with high decolourisation and degradation of dyes. These dyes of diverse chromophoric groups can be highly recalcitrant and nonbiodegradable. The textile industry is the largest user of synthetic dyes (EPA 1996),

consuming 56% of the estimated annual world production (7×10^5 tons) (Hutzinger 1980). Many of these dyes are recalcitrant to microbial degradation and they vary significantly in their in their chemical structure.

Coloured industrial effluents not only produce visual pollution but can also be a public health risk. About 50% of the industrial dyes produced in the world are azo dyes. These azo dyes may be transformed to carcinogenic compounds such as Remazol Brilliant Blue R (Schliyshake and Lonegram, 1996) or triphenylmethane dyes (Vasdev *et al.*, 1995). This suggests a potential application of laccase in bleaching or decolourization processes. Studies can be carried out to see how effective the immobilized enzyme is in decolourizing the industrial dyes and then compare it to the free enzyme system.

In this study immobilization of laccase by entrapment into copper alginate did not improve the thermal stability and storage stability significantly.