

**IMMOBILISATION AND BIOCHEMICAL CHARACTERISATION
OF A SELECTED COMMERCIALY AVAILABLE LACCASE**

By

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ABSTRACT

In this study, laccase from Novo Nordisk was chosen for biochemical characterization and immobilization investigation. The specific aims of this work was to develop optimum conditions for immobilization of laccase by entrapment method using copper alginate and characterize the laccase biochemically.

The pH optimum of the crude enzyme extract was 5.5 and was found to be stable over a wide range of pH values. The apparent molecular weight of this enzyme was around 79,000 Daltons. Three peaks of activity (isoenzymes) were obtained from isoelectric focussing of pI values 4.7, 5.2 and 6.0.

The optimum pH of the immobilized laccase by entrapment into copper alginate beads was 6.0. Laccase amount of 5mg was found to provide the optimum level of activity by entrapment method. The optimum temperature of activity for the free and immobilized laccase was found to be 30^oC. At higher temperature there was a significant reduction in activity. Thermal stability of immobilized laccase 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 amount of 100 µl laccase within the copper alginate beads did not drastically improve their storage in this study.

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ABBREVIATIONS

BSA	Bovine serum albumin
cm	centimeter
DEAE	diethylaminoethylemine
E.C	enzyme commission
<i>et al</i>	et alia (and others)
g	gravity force
gm	gram
hr	hour
IEF	isoelectric focussing
L	litre
M	Molar
mA	milliampere
mg	milligram
min	minute
ml	millitres
mM	millimolar
NaCl	sodium chloride
N	normality
μ M	micromolar