Thesis Title	Studies on Immobilisation of β-Glucosidase/β-Fucosidase from *Dalbergia cochinchinensis* Pierre (Thai Rosewood) Seeds
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ABSTRACT

A crude preparation of β-glucosidase/β-fucosidase from the seeds of Thai Rosewood (*Dalbergia cochinchinensis* Pierre) was obtained by ammonium sulphate precipitation, a good step in removing a large amount of proteins, with a good recovery of β-glucosidase and β-fucosidase. The fold purification of β-glucosidase and β-fucosidase in the 35-75% ammonium sulphate fraction was 4.73 and 4.33 with yield of 60.85% and 55.75% respectively.

The supports used for immobilisation were activated carbon (Merck) and celite (Fluka). The enzyme was also cross-linked with bovine serum albumin (BSA) by 0.3% (w/v) glutaraldehyde. Four-hundred milliunits of crude β-glucosidase could be immobilised on 12.5 mg of activated carbon with about 50 % immobilisation. For celite, only 100 mU of crude β-glucosidase could be immobilised on 12.5 mg support with 60%

immobilisation. The percent immobilisation of crude enzyme cross-linked with 5% (w/v) bovine serum albumin (BSA) using glutaraldehyde was about 3%.

Enzyme immobilised on activated carbon and on celite were less stable at 50°C than free enzyme, and this may be due to the alterations of structure upon immobilisation. The pH optimum of crude enzyme immobilised on activated carbon was similar to that of free enzyme, namely about pH 4-5. K_m of crude enzyme immobilised on charcoal for pNP-β-D-glucopyranoside was 4.57 ± 0.03 mM and for pNP-β-D-fucopyranoside was 0.94 ± 0.08 mM. The K_m of free enzyme for both substrates were similar to immobilised enzyme (5.47 ± 0.4 mM for pNP-β-D-glucopyranoside and 0.73 ± 0.05 mM for pNP-β-D-fucopyranoside).

Enzyme concentrations were varied (0.1, 0.2 and 0.4 U) for both free and immobilised enzyme for use in oligosacchaide synthesis. For free enzyme, 0.2 U β-glucosidase was the most appropriate concentration since it gave a moderate rate of synthesis and with highest yield of oligosaccharide at equilibrium. For immobilised enzyme, 0.2 U enzyme was also the most appropriate concentration, since it gave the highest yield per unit enzyme. From the time course of synthesis in the first 8 h for both free and immobilised enzyme, gentiobiose (glucose-β-1-6-glucose) was the first and major product being synthesised. The results from thin-layer chromatography show that gentiobiose can be readily seen at day 5 of synthesis for both free and immobilised enzymes.

Immobilised enzyme of 0.2 U was re-used twice to synthesise oligosaccharide over periods of 5 days. The results show higher total disaccharide products than free enzyme that has been used to synthesise oligosaccharide for 15 days but the amount of trisaccharides were the same for both free and immobilised enzyme. Each usage of immobilised enzyme

showed no dramatic difference in oligosaccharide products, which were almost the same as free enzyme. After two re-uses, immobilised enzyme was washed with distilled water twice, followed by 5% and 10% ethanol. The elution profile of oligosaccharide from activated carbon shows a detectable quantity of disaccharide and other higher oligosaccharides eluted out.