CHAPTER V

CONCLUSIONS

1. Covalent immobilization of cyclodextrin glycosyltransferase from *Paenibacillus* sp. A11 was investigated on various supports including alumina, silica, activated carbon and chitosan. Among these supports, CGTase bound to alumina was found to exhibit highest retained enzyme activity.
2. The best condition for immobilized enzyme preparation to achieve high immobilization yield was to activate the alumina with 2% γ-aminopropyltriethoxysilane, 1% glutaraldehyde, and incubate the activated alumina with enzyme solution for 6 hours at 4°C,
3. Under optimum immobilization conditions, CGTase was immobilized on 1 g of alumina approximately 4.36 units with 31.2% of immobilization yields when 14 units of CGTase was applied.
4. After immobilization, the optimum pH of immobilized CGTase was shifted from 6.0 to 7.0, whereas the optimum temperature remained unaltered (60°C).
5. Both free and immobilized CGTase were stable in the pH range of 5.0-9.0 and the thermal stability of immobilized CGTase was slightly higher than that of the free enzyme.
6. The apparent K_m of immobilized CGTase was 5.62 ± 0.20 mg/ml which was higher than the free enzyme (K_m = 0.59 ± 0.25 mg/ml). The V_{max} value of immobilized CGTase (5.82 ± 0.13 U/mg protein) was lower than the free form (9.69 ± 0.38 U/mg protein).

7. The immobilized CGTase exhibited higher stability than the soluble enzyme. When stored at 4°C, no loss of activity was seen after 30 days and only 20% loss was seen after 2 months.
8. In the production of AA-2G by batch system, the optimal conditions were to incubate 350 U/g β-CD of immobilized CGTase with 4% (w/v) β-cyclodextrin, 4% (w/v) ascorbic acid in the presence of 0.2% thiourea in the reaction pH of 5.0 for 24 hours at 40°C. The amount of AA-2G formed was 0.584 g/l with 2.92% production yield.
9. The immobilized CGTase produced AA-2G without significant loss of activity after 3 cycles of repeated use.