CHAPTER-4

CONCLUSION

The application of enzymes in organic chemistry is growing exponentially in recent years. Enzymes find several applications in the aminoacid production. They may be used for the hydrolysis of amino acyl derivatives such as esters, carbohydrates, hydantoins etc. and when specific enzymes are employed to hydrolyse a racemic mixture it selectively hydrolyses only one enantiomer depending on its specificity. As L- aminoacids form the major part of the aminoacid industry, many enzymatic processes for their production from DL- aminoacids by optical resolution have been reported. Moreover, D-aminoacids have generally been recovered by chemical hydrolysis. The D-aminoacids are commercially important synthons and are important for the synthesis of semi-synthetic pencillins. D-hydantionases (E. C. 3.5.2.2) are commercially important enzymes involved in the production of the D- ammoniac's. However, commercial exploitation of biological process is rare mainly on account of insufficient
details available on the efficient production of these enzymes by microorganisms.

In the present study *Agrobacterium radiobacter* NRRL B 11291 was the source of D-hydantoinase and its production was optimized with inexpensive carbon and nitrogen source with the help of an empirical modelling technique (response surface method). The maximum level of enzyme and biomass obtained in shake flask study was 35 U/mL and 1.69 mg/mL respectively.

The hydantoinase from *A. radiobacter* was found to be inducible and a nearly two-fold increase in enzyme production was achieved with DL-phenylhydantoin as an inducer. Though the enzyme production is high with DL-phenylhydantoin, it is not economical to add an inducer for a large volume low value product. Hence, fed batch cultivation was resorted to produce more of biomass and thereby increase the enzyme production. In a fed batch with csi and maltose a three fold increase in biomass and nearly six fold increase in hydantoinase was achieved over batch cultivation. This increase in hydantoinase and biomass is very significant for its commercial application.

The optimum temperature and pH of the hydantoinase was 50° C and pH 10.0 in glycine-NaCl-NaoH buffer, but is more stable in pH 8 to 9
in Tris HCl buffer. Manganese dependence for the activity and production was also noticed and was inhibited by heavy metals like Hg and Pb. p-Chloromercuribenzoate, N-acetyl succinamide, N-ethylmaleimide, DEPC and PMSF inhibited hydantoinase activity thus indicating the involvement of sulfhydryl, histidyl, tyrosyl groups or serine residue in the catalysis of ring opening reaction or in the stabilization of the enzyme.

The biotransformation studies were carried out using whole cells of *A. radiobacter*. Under optimal conditions 59mg/mL of D-HPG was produced from 80mg/mL of DL-PHPH with a molar yield of 88%.