Summary and Conclusions
5. SUMMARY AND CONCLUSIONS

*Beauveria bassiana*, isolated from the marine sediment and available in the culture collection of the Department was used in the present study for evaluation of its potential for L-glutaminase production, as an extra cellular enzyme. Enzyme production was carried out under three fermentation conditions namely submerged, solid state and immobilized.

Initially the various process parameters that influence the rate of enzyme production under SmF were optimized using distilled water as basal medium. pH of the medium, incubation temperature, additional nitrogen source, additional carbon source, aminoacid requirement, and NaCl concentration in the medium were optimized. pH 9.0, 27°C, sorbitol (1% w/v), yeast extract (2% w/v), potassium nitrate (1% w/v), methionine (0.2% w/v), 9.0% NaCl concentration were observed as optimal conditions for maximal enzyme production. After optimization a time course study was conducted and found that incubation for 108 hours of incubation was required for maximal enzyme production under submerged fermentation.

Solid state fermentation production of L-glutaminase was carried out using polystyrene as inert solid support, using aged sea water as basal medium. Initially the various process parameters that influence the rate of enzyme production under SSF were optimized. pH of the medium, incubation temperature, additional carbon source, additional nitrogen source, aminoacid requirement, L-glutamine concentration, initial
moisture content, inocula concentration, sea water concentration and additional NaCl concentration in the medium were optimized. Two pH optima one at 6.0 and another at 9.0, 27°C, D-glucose(0.5%), Glutamine concentration(0.25% w/v), 80% moisture content, spore inocula (3.7 x 10^8 spores /ml) and 100% sea water concentration were observed as optimal conditions for maximal enzyme production. Absence of additional nitrogen source and NaCl in the medium were observed as ideal condition for maximal enzyme production. After optimization a time course study was conducted and found that incubation for 96 hours of incubation was required for maximal enzyme production under submerged fermentation.

*B. bassiana* could produce L-glutaminase both in distilled water medium as well as in sea water based medium. Sea water could act as an ideal fermentation medium without any additives.

A comparison was made between marine and terrestrial strains of *B. bassiana* using a standard strain *B. bassiana* NCIM 1216. It was observed that marine strain produced higher levels of L-glutaminase compared to terrestrial strain. Further marine strain required sea water as ideal fermentation medium for maximal enzyme production under both submerged and solid state fermentation conditions, whereas terrestrial strain required distilled water based medium as ideal fermentation medium for maximal enzyme production under both submerged and solid state fermentation conditions.

Spores of *B. bassiana* were immobilized in calcium alginate beads. Optimal conditions for preparation of stable immobilized spore beads were standardized. 3% sodium alginate as support concentration, 12 x 10^8 spores /gram bead, 0.1 M CaCl_2, 3 hours of curing time, 15 hours activation time, 18 hours retention, pH 9.0, 27°C were
identified as optimal conditions for preparation of stable beads. After optimization L-glutaminase production under continuous mode in a packed bed reactor was carried out at different flow rates (20ml/hour - 60ml/hour), substrate concentration, aeration and bed heights. 20ml/hour flow rate, 0.25% glutamine concentration and 15cm bed height were observed as ideal conditions for maximal glutaminase production. No aeration of the packed bed reactor was required.

Of the three fermentation systems, immobilized system supported maximal level of enzyme by the selected fungal strain compared to submerged and solid state fermentation.

**CONCLUSIONS**

Based on the results obtained in the present study the following conclusions are drawn. *Beauveria bassiana* isolated form marine sediment has immense potential as an industrial organism for production of L-glutaminase as an extracellular enzyme employing either submerged fermentation or solid state fermentation. Spores of this fungus are capable of synthesising L-glutaminase in abundant quantity at high rate, under immobilized condition entrapped in calcium alginate beads. High rate of enzyme synthesis could be achieved by immobilized spores under continuous mode of operation in a packed bed reactor. Further it was observed during the course of the present study that aged sea water could be used as an ideal fermentation medium for L-glutaminase synthesis using polystyrene as inert solid support system.

The results obtained for the media optimization studies and use of sea water alone as media for L-glutaminase synthesis indicate the possible role of nutrients present in aged
sea water in induction of L-glutaminase compared with distilled water based enzyme production medium, which is almost chemically defined. Further studies are warranted to substantiate the exact role of constituents of sea water in extra cellular enzyme synthesis, and to understand the differential nutritional requirement shown by this strain under submerged and solid state fermentation.