5.0. SUMMARY:

Biotechnology is gaining ground rapidly due to the various advantages that it offers over convectional chemical technologies. Industrial enzymes represent the heart of biotechnology processes. The industrial enzyme market is divided into three application segments; technical enzymes (60%). Food enzymes (32%) and animal feed enzymes (8%). Among all industrial enzymes, amylase have pivotal role in application spectra ranging from domestic to leather processing, environmental pollution abatement to Nutraceuticals applications, health care products to diagnostic kits development and value added product production to clinical applications. Global amylase market is expected to reach approximately 25 billion US $ by 2010-11 with a growth rate of 6.5%. At present 60% of the world enzyme market is driven by different types of amylases. The majority of enzymes in the current industrial use are of microbial origin. At industrial scale these enzymes are produced by the fermentation of bio-based materials.

The application web of amylase is increasing as the knowledge on structural, bio chemical and functional properties of enzymes is elucidated. Hence, scientific community is always on their toes to expedite the natural and exotic samples to isolate the specific microbial strain and study in detail for improving the productivity yields. In the present investigation too, an effort has been made to isolate a potential alpha amylase producing microbial strain, identify the strain using bio chemical properties and ribotyping methodologies, improve the enzyme productivity values using different fermentation strategies at shake flask level followed by bioreactor, characterize the enzyme for its biochemical and molecular properties and evaluate its biotechnological application potential.

The research work performed in five chapters in this thesis. The first chapter deals with the introduction of enzymes. In this chapter, information
on sources of enzymes, advantages of enzymes in different sectors, procedure for determination of enzyme activities, different enzyme production strategies including classification and mechanism of amylase and current problems and potential solution were elucidated along with aim and objectives of the present work.

Second chapter deals with review of literature especially pertaining to alpha amylase. In this chapter effort has been made to consolidate the literature review with respect to alpha amylase enzyme producing microbial strains, isolation procedure, different assays used for determination of amylase activity, strategies to improve the enzyme production in designing and optimization of submerged and solid state fermentation conditions, importance of scale up studies, methods available for enzyme purification and kinetic property evaluation, zymography technique importance and application of amylase at different industrial sectors.

While third chapter deals with materials and methods used in this investigation. Detailed isolation, purification, characterization, optimization of microbial growth and amylase production procedures were mentioned. In addition the procedures used for optimization including fermentation parameter and its concentration level selection, experimental set up conditions, data evaluation procedures, validation experimental procedures were reported. This chapter also deals with downstream processing of enzyme, purification, characterization and application procedures used in this investigation.

The fourth chapter mainly deals with results and discussion of the data obtained in this study. This chapter was sub divided into six sub units. Sub unit one deals with initial screening of different fermentation parameters and subsequent optimization of enzyme production under submerged fermentation. The second sub unit deals with amylase production scale up studies in bioreactor and model development for
effective enzyme production evaluation criterion. In sub unit three, enzyme production process under solid state fermentation was studied. In this section various low cost agro industrial materials and different solid state fermentation factors influence on amylase production was studied. In sub unit four, enzyme production process under submerged fermentation and at reactor level studies were performed. In sub unit five the amylase produced by the selected microbial strain was purified characterized for biochemical, thermal, kinetic and molecular properties in addition to its application potential evaluation at different industrial sectors with special references to textile industries. In subunit six the total research activity was summarized and concluded. An exhaustive bibliography was given at the end.

Total 30 different amylase producing microbial strains were isolated from the effluent soil samples of a local starch plant near Hyderabad. Among them one of the strains showing higher activity was selected and designated as MK 07. This strain was characterized for various tests mentioned in Bergey’s manual of bacteriology. The strain grew in the temperature range of 20° C-40° C and in the pH range of 4 to 6 with optimum growth at pH 5.0 and identified as Aspergillus niger. Hence this strain was designated as Aspergillus niger MK 07.

The impact of different fermentation parameters such as medium pH, incubation temperature, inoculum concentration, incubation time and RPM influence was studied using PDA while different carbon (glucose, starch, arabinose, ribose, xylose, sucrose, fructose and mannose) and nitrogen (soyabean meal, yeast extract, corn steep liquor, beef extract, potassium nitrate, ammonium sulphate and ammonium nitrate) sources impact was evaluated using Plackett-Burman design. The enzyme production was improved to 164 U/g at optimized environments i.e. at 30° C and pH 5.0 in the medium consisting of 3% Sucrose.
Summary

The interactive role of incubation temperature, medium volume, inoculum level, pH of the medium, synthetic media and glucose levels on enzyme production with *Aspergillus* niger species MK 07 was studied. The enzyme production varied from 55 U/ml to 85 U/ml. The enzyme production data was further optimized and only the best four conditions were further verified for enzyme production of amylase compared to conventional optimization. Analysis further suggested that the enzyme production was regulated by the selected parameter concentrations.

The growth of *Aspergillus* sp. and amylase production pattern under different sugar concentration conditions was evaluated in 2.0 litre continuous stirred tank bioreactor and the data was analysed with developed unstructured models of logistic and Luedeking-Piret equations. The results indicated that the fermentation medium regulate biomass and enzyme production in *Aspergillus* sp.

Further amylase production improvement studies were made based on microbial growth and enzyme production. Economic enzyme production was achieved with SMF and SSF. Overall evaluation criterion based optimization of enzyme production was performed by assigning the relative weightage to reusability, cell leakage and enzyme production and conducting the experiments using optimized parameter. The data suggested that carbon substrate is very significant parameter in control of above selected factors.

To understand the importance of solid state fermentation for economic enzyme production, different locally available inexpensive agro substrates such as wheat bran, green gram husk and rice bran were evaluated. Among all substrates wheat bran supported maximum production of enzyme. Various fermentation parameters like incubation time, pH of the medium, particle size, moisture content, initial inoculum concentration and different carbon and nitrogen sources were optimized. Under this conventional one-at-a-time optimization conditions, the enzyme
production improved from 106 U/g to 164 U/g after optimization of selective solid state fermentation parameters especially incubation temperature, pH of the medium, particle size, moisture content nitrogen source, inoculum level and incubation time subsequently. Statistical analysis of data suggested that the selected parameters have influence either at individual level or interactive level. Inoculum at individual level and pH interaction with particle size at interactive level showed highest positive impact on amylase production. Under optimized environment enzyme production reached to 164 U/g.

The amylase was purified to near homogeneity by ammonium sulphate precipitation, ion exchange chromatography using Sephadex G100 and gel filtration.

5.1. **CONCLUSIONS**

The present investigation on “Isolation, identification, Characterization and fermentative production of alpha amylase by *Aspergillus* sp.” revealed that:

- An efficient amylase producing microbial species belonging to *Aspergillus* sp. was isolated from soil sample collected from dump yards of a local starch processing industry near Hyderabad.

- The strain was identified based on Bergey’s manual of bacteriology and ribotyping.

- The strain of *Aspergillus* sp. MK 07 was characterized for amylase production using conventional and statistical software programmes.

- The enzyme production was improved from 55 U/ml to 85 U/ml in SmF and 106 U/g to 164 U/g in SSF upon optimization of fermentation parameters.
Solid state fermentation studies revealed that enzyme production was influenced by the type of agro material and other fermentation parameters especially medium pH, incubation temperature, inoculum level, moisture content, particle size, carbon and nitrogen supplements.

Over all enzyme production was improved from 106 U/g to 164 U/g with wheat bran as substrate materials under optimized solid state conditions.

In Fermentor study highest amylase activity of 1734 U/ml was obtained with 250 RPM and with overall optimization of all process variables it reached up to 2112 U/ml.

The enzyme is monomeric in nature with molecular weight of 38 KDa.

The enzyme revealed stability for more than 24 hrs in different pH environment and active in the pH range of 4.5 to 6.5.

The enzyme is thermostable up to 45°C and showed improved activity with increase in incubation temperature up to 35°C.

The enzyme showed high specificity for starch compared to other amylolytic materials.

The enzyme showed high stability towards oxidizing and reducing agents.

The results of the present study showed effective desizing of cloth removal property of the enzyme alpha amylase for which Aspergillus species is one of the principal sources.