Chapter 9
9.0 Summary and Conclusions

Citric acid is the most produced organic acid measured in tonnage. Its production has now reached 1.4 million tonnes per year and continues to increase more every year. The main reason for constant increase is the large number of applications that can be found for citric acid, mainly in the food and pharmaceutical industries. Traditional processes, such as the submerged fermentation using the fungus *Aspergillus niger*, dominate the global production. However, different techniques of production are continuously being studied showing new perspectives for the production of citric acid. In this context, submerged fermentation appears to be the best for citric acid fermentation using malted grains which are in abundant in agro-based countries like India, where agro-industrial residues can be used as substrate-supports to the filamentous fungi like *Aspergillus niger* for citric acid production. In recent years significant improvement of citric acid processes can be achieved with genetic amelioration of producer strains, which is the powerful tool of the citric acid industry.

- In the present study three fungal isolates belonging to *Aspergillus*, *Penicillium* and *Trichoderma* respectively, were isolated from the sugar factory waste and screened for their suitability of the citric acid production. Among these, *Aspergillus niger* produced highest yield of citric acid.

- To compare the yield of citric acid, standard cultures were procured from MTCC Chandigarh and another culture was gifted from Prof. B. Rajasekhar Reddy, Dept. of Microbiology, S.K. University, Anantapur (A.P), India.

- The conditions of malting and mashing were optimized for maximum yield of reducing sugars in the malts. Steeping time, temperature, germination time and mashing temperature were optimized by conventional methods.

- Maximum yield of reducing sugars were obtained at 18 h of time 31°C temperature and 72 h of germination time.
The steeping is performed with distilled water and diluted alkalis like NaOH and KOH for paddy and sorghum malts respectively. Alkali steeping induces the alpha amylase but the effect is more with NaOH than KOH because NaOH induced amylases are more stable.

Mashing of malted sorghum and paddy were performed at different range of temperatures and in the presence (or) absence of commercial enzymes like Bio-glucanase. All these conditions were optimized for maximum yield of reducing sugars.

Malting and mashing is the safe method of choice for the conversions of complex sugars into simple sugars rather than hazardous chemical methods.

The fermentation parameters such as temperature, pH, nitrogen sources, substrate concentration, and incubation period were optimized.

In order to compare the yield of the isolate wild type, standard MTCC cultures were also used for citric acid production. The isolate wild strain produced the highest yield of citric acid on par with the MTCC strains 662 and 282.

The yield of citric acid was higher with the paddy malt than the sorghum malt. Highest yield of citric acid was produced with 20% substrate concentration in both the malt media.

The yield of citric acid was further enhanced with the addition of suitable inorganic and organic nitrogen sources such as ammonium sulphate and cooked Soya beans, though there is an increase in the yield with the mixed malts at different ratios.

The parent strain was further improved by mutagenesis technique using UV mutagenesis to develop a potent and stable mutant.

UV-mutagenesis produced a stable mutant which gave 10-20% higher yield than the parent strain.
Further the fermentation parameters were optimized with Response Surface Methodology (RSM). The interactions between the different fermentation parameters were studied and critical values of the various parameters were generated.

The RSM was studied with sorghum and paddy malts with the parent strain. The isolated mutant strain was better optimized with paddy malt substrate only.

In an agro-based country like India the damaged grains can be diverted to the production of value-added product like citric acid, which has multi uses in various fields of life.