with three different microorganisms. In fermentation trials with *Lactobacillus plantarum* and *Lactobacillus brevis*, initial pH adjustment were done with HCl and lactic acid during the first 5-6 hrs of fermentation to avoid spoilage. So this acid treatment also influenced the demineralization process during fermentation with the two Lactobacillus species. But with Bacillus species, demineralization effected only due to the acid produced by the organism during fermentation.

5. SUMMARY AND CONCLUSION

Since, chemical method, which is used conventionally nowadays, deteriorates the properties of the chitin prepared, biological method of deproteinisation and demineralization for chitin preparation can be adopted as an alternative. This study is an attempt to go deeper to the beneficiaries of fermentation techniques for the preparation of chitin from shrimp shell waste. Efficiency of three different strains of bacteria for the removal of proteins and minerals from the shrimp shell was studied. *Lactobacillus plantarum, Lactobacillus brevis* and *Bacillus subtilis* were used for the study.

Fermentation parameters were optimized for the fermentation with all the three strains. Type and quantity of the sugar, quantity of the inoculum, fermentation period etc. were standardized by studying different fermentation parameters like changes in pH, total titrable acidity, changes in proteolytic activity, changes in microbial count, sensory evaluation for spoilage, extent of deproteinisation and extent of demineralization. In all the studies, it was found that pH first started decreasing with fermentation time accordingly total titrable acidity was increased. This was in line with the increase of proteolytic activity. After reaching the optimum level, proteolytic activity showed a declining trend and parallely pH started increasing as the acid production yielded to low. During fermentation, the bacterial strains utilized the sugar available in the fermenting media and at the end of fermentation; about 87% of the sugar was utilized. From the optimization studies, 20% w/v jaggery broth containing $10^8$ CFU/ml *Lactobacillus plantarum* was used for fermentation studies for 15 days. To adjust initial pH adjustment, mild HCl and lactic acid were used for the study. Lactic acid treated samples showed greater extent of demineralization and deprotenisation at the end of fermentation study than hydrochloric acid treated samples. It can be due to the effect of strong hydrochloric acid on the initial microbial count, which directly affects the fermentation process. At the end of fermentation, about 76.5% of ash was removed in lactic acid treated samples and 71.8% in hydrochloric acid treated samples; 72.8% of proteins in lactic acid treated samples and 70.6% in
hydrochloric acid treated samples. Lactic acid treated samples showed greater extent of demineralization and deprotenisation at the end of fermentation study than hydrochloric acid treated samples. The residual protein and ash in the fermented residue were reduced to permissible limit by treatment with 0.8N HCl and 1M NaOH to produce chitin. The properties of chitin and chitosan were studied.

Detailed fermentation study with *Lactobacillus brevis*, was done with 20% w/v jaggery broth for 17 days. All the parameters affecting the fermentation like pH changes, changes in microbial count, changes in proteolytic activity, sensory changes etc were studied. At the end of fermentation, protein remaining in the samples were only 32.48% in HCl treated samples and 31.85% in lactic acid treated samples. About 33.68% of residual ash was present in HCl treated samples and 32.52% in lactic acid treated ones. For conversion to chitin, mild acid and alkali treatments were standardized. The fermented residue was converted to chitin with good characteristics by treatment with 1.2M NaOH and 1N HCl in both HCl treated and lactic acid treated samples. Chitosan prepared from the chitin prepared from the fermented residue was with high molecular weight and high viscosity.

Fermentation with *Bacillus subtilis* was done for 15 days with 20%w/v jaggery broth. From the studies it was found that the proteolytic activity of Bacillus was more compared to Lactobacillus strains. The residual protein content was only 16% and residual ash content was about 28% in the fermented residue. Different trials of mild acid and alkali treatment done to standardize the chemical treatment concentration for the preparation of chitin. 0.8N HCl and 0.6M NaOH was used for the preparation of chitin.

From the studies it was found that *Bacillus subtilis* strain was the most efficient for removing the proteins from the shrimp shell than Lactic acid bacteria. But the extent of demineralization obtained was almost similar in fermentation trials with *Lactobacillus plantarum* and *Bacillus subtilis*. Protein liquor obtained from Lactic acid fermentation was also studied to study the extent of spoilage.
Chitosan with highest viscosity and molecular weight was obtained in *Lactobacillus plantarum* fermentation.