Summary and Conclusion
Lipases have attracted scientific attention in terms of its versatile properties and much interest lies in the prospect for novel lipases. This calls for extensive screening and isolation of potent lipase producing microorganisms, characterization of lipases, their optimization, bulk production and application. In view of the present scenario, the thesis entitled “Characterization of lipase from lactic acid bacteria isolated from fish processing waste” details on the lipases produced by *E. faecium* MTCC5695 (MTCC5695) and *E. durans* NCIM5427 (ED-27) and respective roles both in the food industry and waste management.

Chapter-wise summary as well as conclusions are listed as follows:

- **Chapter-1** details upon the general introduction and the main genesis behind the study. It also enlists the major objectives of the study for which the research work was carried out.

- **Chapter-2** reviews the available literature in the publication domain that includes waste generated from fish processing, its potential uses and occurrence of lipolytic LAB in FPW. It also discusses the methods adopted for isolation of lipase producing LAB, optimization of conditions for their growth and lipase production, characterization and application. The chapter ends with the scope of investigation wherein the significance of the study is implicated.

- **Chapter-3** focuses on the methods adopted for extensive screening, isolation and characterization of lipolytic LAB. Enterococci was found to be the predominant microflora amongst other LAB in FPW and 2 potent lipolytic LAB isolates *E. faecium* MTCC5695 (MTCC5695) and *E. durans* NCIM5427 (ED-27) were isolated and characterized by both phenotypic and genotypic means. Since the
lipase production by the isolates was comparatively low they were subjected to optimization.

*Chapter-4* accounts for the various statistical methodologies adopted for maximal lipase production by the isolates. Box-Behnken design was used and 3.15 fold and 3-fold increase in lipase production was obtained in the case of MTCC5695 and ED-27, respectively. Moreover, the fermentation kinetics was quantitatively measured and lipase production by both the isolates was found to be growth-associated. Furthermore, a cost-effective media was designed wherein the carbon and nitrogen sources were extracted from FPW. The growth and lipase production of the isolates grown in this particular media was almost equivalent to the growth when grown in the commercial media MRS. This is a very crucial finding as the media is cheap, can be easily constituted and thereby used for microbial growth and metabolite production on an industrial scale.

*Chapter-5* details on the two-step procedure applied for extraction and purification of lipases. ATPS coupled with ultrafiltration was used leading to a 5.99-fold and 4.98-fold purification of MTCC5695 and ED-27 lipase with a recovery of 82.09% and 87.93% respectively. Even though, most of the industrial processes do not require enzymes in high purity, a large amount of contaminants in the crude sample affects the process as a whole. ATPS helped in the partial purification of lipases with better recovery which is a feasible method on the industrial scale. In addition, characterization of lipases was carried out implying that MTCC5695 produces an alkaline lipase (pH 10.8, temp 40°C) whereas ED-27 lipase is of acidic nature (pH 4.6, temp 30°C). Based on their characteristics, they were subjected to 2 different fields of application.
Chapter-6 contains the major applications of MTCC5695 and ED-27 lipases. MTCC5695 was applied as starter culture for the preparation of curd. Additionally, sensory and rheological property of the final product was analyzed. The final product so obtained was well-set, consistent and palatable probiotic curd. Moreover, this application holds high significance since MTCC5695 was active against a broad range of Gram-negative bacteria which pose a serious threat to the food industry as they are the major food borne pathogens affecting the keeping quality of the food. On the contrary, ED-27 lipase with respect to its acidic nature was subjected to enzymatic treatment of the lipids present in slaughter house waste effluent. The conditions for enzymatic hydrolysis were determined statistically for better performance of the treatment process. This strategy culminated in a quantitative approach towards problems associated with biodegradation and helps in optimizing the process thereby increasing the overall process efficiency. Validation of the experiment at the optimal conditions proved that this method is advantageous over other conventional methods of treatment and could be adopted by the slaughter house industries. In addition, enzymes like lipases have huge potential for treatment of wastewaters and other hazardous wastes and may be of great interest in solving problems in biological wastewater treatment processes related to high fat content and suspended solids.