CHAPTER VII
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SUMMARY AND CONCLUSION

7.1 Summary

Lipolytic bacteria *Bacillus sp.* was isolated from oil spilled soil samples and used for crude lipase enzyme production with groundnut oilcake substrate. The application of partially purified crude enzyme on polyester fabric and its characterization using various analytical techniques were discussed in the previous chapters are summarised as follows.

Bacterial strains were isolated from oil spilled soil samples by serial dilution plating technique in butter fat agar medium. The formation of greenish blue zone confirmed the positive lipolytic colony. The isolated colonies showed clear zone of lipolysis around the tributyrin agar medium confirming the production of lipase enzyme. The screened five lipolytic isolates responded for gram positive test. Out of five, three bacterial strains were confirmed as *Bacillus sp.* by microscopic observation and other two were confirmed as *Staphylococcus aureus* by their positive response to tube coagulase test.

Lipase production by *Bacillus sp.* was identified to be high compared to *Staphylococcus aureus* using groundnut oilcake as substrate at pH 6.5 incubated at 55°C for 102 hours under shaking condition. The extracted lipase enzyme was partially purified using ammonium sulphate and then dialysed using phosphate buffer was estimated to have activity of 400 U/ml.

The lipase enzyme was characterized for optimum pH, temperature, molecular weight and protein content. The optimum pH was identified as 6.5 and temperature optimum was 50°C. The molecular weight of lipase
enzyme was 43 kDa confirmed by SDS-PAGE method. The protein content was 4.60 mg/ml.

Washing of enzyme hydrolysed polyester fabric was optimized since literature report showed that the hydrophilic property of polyester fabric was enhanced due to protein adsorption. Polyester fabric were treated with 1200 U lipase enzyme at its optimum pH 6.5 and temperature 50ºC for 20 minutes and then subjected to washings in different medium. The enzyme treated and washed fabrics were analyzed for nitrogen % using CHNS elemental analysis. The washing with 2 g/l of acetic acid resulted in efficient removal of adsorbed protein reporting 0.17 % of nitrogen which is negligible.

Optimization of lipase treatment on polyester was done at various concentration of enzyme at different pH and temperature for various durations and then washed with 2 g/l of acetic acid. The samples were then characterized for % weight loss and capillary rise test. The enzyme hydrolysed fabrics were dyed with reactive, basic and disperse dyes and their colour intensity was measured as ΔE values in computer colour matching system. An increase in % weight loss due to surface hydrolysis resulted in the release of water soluble compound and creation of new functional groups such as –OH and –COOH. These polar functional groups on the surface of enzyme treated fabric were responsible for increase in water absorption ability reported as capillary rise. An increase in ΔE values of reactive and basic dyed samples also confirmed the presence of –OH and –COOH groups respectively. The disperse dyed samples reported high ΔE values due to increase in surface hydrophilicity. The optimized conditions were concluded to be 1600 U lipase concentration, pH 6.5, temperature 50ºC and treatment time of 30 minutes.
From the results of optimization of lipase treatment on polyester, bulk treatment were carried out for best four optimized conditions and performed for instrumental characterization. The FTIR spectra clearly show the sensitivity of lipase enzyme towards the ester linkages causing major shift in these areas. Further the increase in intensity of carbonyl and hydroxyl band confirmed the surface functionalisation of polyester fabric.

The X-ray diffraction studies supports with previous work showing an increase in the degree of crystallinity for enzyme treated samples. This is because the enzymes attack the amorphous region of polymeric chain.

The temperature optima of lipase enzyme (50°C) and the $T_g$ (48.9°C) of untreated sample determined using DSC are closer. At $T_g$, the polymeric chain exhibits maximum mobility thus enhancing the hydrolytic activity of lipase enzyme.

The SEM images at high magnification shows the presence of small crack and voids which is responsible for the increase in hydrophilicity of polyester fabric and not due to protein adsorption.

The AFM images of polyester shows a decrease in particle size with regular arrangement and small rough surface as the hydrolytic activity at the surface is increased.

The Kawabata analysis for surface roughness does not show any significant differences between untreated and enzyme treated fabric and hence it is concluded that enzymatic hydrolysis has not imparted any harsh feel to the fabric.
7.2 Conclusion

1. The appearance of greenish blue zone in butterfat agar medium around the colonies confirmed the presence of positive lipolytic colonies.

2. The clear zone of lipolysis around the tributyrin agar medium confirmed the presence of lipase producing isolates.

3. The screened five isolates showed positive response towards gram positive test.

4. Rod shape of three isolates confirmed the presence of *Bacillus sp.* and positive response of two isolates to tube coagulase test confirmed the *Staphylococcus aureus*.

5. *Bacillus sp.* is the best lipase producer than *Staphylococcus aureus* which was confirmed by lipase assay.

6. The temperature optima of lipase were identified as 50°C and the pH optima was 6.5.

7. The molecular mass of lipase enzyme was 43 kDa and protein content was 4.60 mg/ml.

8. Washing of enzyme treated fabric with 2 g/l of acetic acid at 90°C for 1 hour was sufficient for protein removal than the other methods.

9. The treatment condition of 1600 U enzyme concentration, pH 6.5, 50°C temperature and 30 minutes gives a maximum weight loss.
10. The rising height measurement clearly shows the increase of water absorption ability of enzyme treated polyester fabric than untreated one.

11. Higher $\Delta E$ values of reactive and basic dyed samples show the formation of $-\text{OH}$ and $-\text{COOH}$ groups.

12. A slight increase in $\Delta E$ values of disperse dyed samples indicates the increase of hydrophilicity.

13. The peak at 1744 cm$^{-1}$ representing ester group of untreated sample has undergone shift towards lower frequency for enzyme treated sample confirms the strong activity of lipase on ester linkages.

14. The enzymatic treatment shifts the ethylene glycol moiety peak from 1120 cm$^{-1}$ to lower frequency.

15. The band associated with $\text{C}=$C benzene ring conjugated with $-\text{COO}^-$ stretching and $-\text{OH}$ in plane bending clearly appeared in the enzyme treated sample spectrum than the untreated samples.

16. The increase of XRD degree of crystallinity confirms the preferential attack at amorphous region by lipase enzymes.

17. The $T_g$ obtained by DSC for untreated polyester fabric is same as that of active temperature of lipase enzyme. This results in maximum hydrolytic activity due to the high mobility of polymeric chain.

18. The increase of crystallinity and $T_g$ of enzyme treated samples indicates, that the amorphous region may undergo hydrolysis during treatment.
19. Presence of small cracks and voids observed at higher magnification of SEM images may be one of the reasons for the improvement of wettability of fabric.

20. The AFM pictures also confirm the presence of small rough surface and reduction in particle size after enzyme treatment.

21. In the physical testing by Kawabata evaluation system, the surface roughness of the enzyme treated fabric not much affected as compared with untreated fabric.