7. SUMMARY

In the present study, marine sediment samples were collected from Tiruchendur coastal area of Tamil Nadu, India. The actinomycetes were isolated by serial dilution method using starch casein nitrate and actinomycetes agar medium. The marine isolates were identified as Streptomyces indiaensis ACT 7 (JQ801298.1) and Streptomyces hygroscopicus ACT 14 (JQ801299.1) using morphological and genotypic analysis. *S. indiaensis* ACT 7 and *S. hygroscopicus* ACT 14 were screened for lipase, protease production using the zone formation due to hydrolysis of tributyrin and casein.

In the present study, *S. indiaensis* ACT 7 exhibited maximum lipase activity on 4\(^{th}\) day for submerged and solid state fermentation. *S. hygroscopicus* ACT 14 showed maximum lipase activity on 3\(^{rd}\) day for submerged and 4\(^{th}\) day for solid state fermentation. The maximum protease production was exhibited on 3\(^{rd}\) day and 4\(^{th}\) day for submerged and solid state production using *S. indiaensis* ACT 7. Similarly *S. hygroscopicus* ACT 14 showed highest protease production on 4\(^{th}\) and 5\(^{th}\) day for submerged and solid state production. The optimum pH for lipase and protease production was pH 9.0 for *S. indiaensis* ACT 7 and pH 10.0 for *S. hygroscopicus* ACT 14 using submerged and solid state fermentation. *S. indiaensis* ACT 7 showed maximum enzyme production at 55°C for lipase and 45°C for protease. *S. hygroscopicus* ACT 14 exhibited maximum activity at 50°C for lipase and protease production using submerged and solid state fermentation. *S. indiaensis* ACT 7 showed maximum lipase activity with fructose (1.0 %) and yeast extract (1.0 %) as carbon and nitrogen sources respectively. Metal salt, MgCl\(_2\) (0.1 mM) increased the lipase activity of *S. indiaensis* ACT 7. *S. hygroscopicus* ACT 14 showed highest lipase activity for glucose (1.5 %), peptone (1.5 %) as carbon and nitrogen sources and metal salt, CaCl\(_2\) (0.15 mM). *S. indiaensis* ACT 7 utilized sucrose (0.5 %) as carbon and Tryptone (1.5 %) as nitrogen source, metal ion CaCl\(_2\) (0.1 mM) for maximum protease activity. *S. hygroscopicus* ACT 14 showed glucose (0.5 %), beef extract (1.0 %) as carbon and nitrogen sources and FeSO\(_4\) (0.1 mM) for maximum enzyme activity.
The particle size of 0.82 mm was favourable for both the *Streptomyces* spp. to increase the production of lipase and protease. Coconut oil cake with soya meal and wheat bran increased the lipase activity for *S. indiaensis* ACT 7. For *S. hygroscopicus* ACT 14 groundnut oil cake with soya meal and wheat bran showed maximum lipase activity. For protease production the agro waste bengal gram, black gram amended with soya meal and wheat bran exhibited maximum activity for *S. indiaensis* ACT 7 and *S. hygroscopicus* ACT 14 respectively. The lipase and protease production increased with 10.0 per cent inoculum, 1 mm particle size, 10.0 per cent of substrate concentration, 70.0 to 80.0 per cent of moisture content and 3.0 to 4.0 per cent of sodium chloride concentration. Solid state fermentation enhanced lipase and protease enzyme activities than submerged fermentation for both *Streptomyces* spp. Lipase and protease enzymes were purified from the culture filtrates by acetone precipitation, dialysis and Sephadex G-100 column chromatography. *S. indiaensis* ACT 7 showed active fractions at 4.0, 7.0 and *S. hygroscopicus* ACT 14 at 6.0, 9.0 for lipase. *S. indiaensis* exhibited active fractions at 6.0, 10.0 and *S. hygroscopicus* ACT 14 showed at 5.0 and 9.0 for protease.

Lipase by *S. indiaensis* ACT 7 showed the recovery yield of 13.63 per cent and purification factor of 61.35 for 4th fraction. 7th fraction exhibited the total activity of 5800 U and the purification factor of 42.55 with 10.50 per cent of recovery yield. In *S. hygroscopicus* ACT 14 6th and 9th fractions of column chromatography exhibited the recovery yield of 13.66 and 11.25 per cent with purification factor of 63.99 and 49.77 respectively. For protease by *S. indiaensis* ACT 7, 6th and 10th fractions showed the recovery yield of 5.88 and 5.32 per cent with purification factor of 57.93 and 48.70 respectively. In *S. hygroscopicus* the 9th fraction showed the purification factor 35.42 with recovery yield of 5.01 per cent. The optimum pH and temperature for lipase and protease enzymes were in the range of 9.0 to 10.0 and 45 to 55°C. The $V_{\text{max}}$ and $K_m$ values for lipase enzyme by *S. indiaensis* ACT 7 and *S. hygroscopicus* ACT 14 were 702, 627 U/mg protein and 194, 192 μM respectively. Similarly, the $V_{\text{max}}$ and $K_m$ values for protease enzyme produced by *S. indiaensis* ACT 7 and *S. hygroscopicus* ACT 14 were observed as
811, 800 U/mg protein and 190, 212 μM respectively. The molecular weight for lipase isoenzyme was 60.0 kDa, 36.0 kDa for *S. indiaensis* ACT 7 and 58.0 kDa, 37.0 kDa for *S. hygroscopicus* ACT 14. For protease, the molecular weights of 56.0 kDa, 47.0 kDa were exhibited by *S. indiaensis* ACT 7 and 48.0 kDa, 40.0 kDa were obtained by *S. hygroscopicus* ACT 14.

The isolates *Streptomyces* *indiaensis* ACT 7 and *Streptomyces* *hygroscopicus* ACT 14 and their enzymes were studied for the following environmental applications. The dairy industry effluent was biologically treated with *S. indiaensis* ACT 7, *S. hygroscopicus* ACT 14 and mixed consortium in batch mode for 20 days. In dairy effluent treatment, the mixed consortium showed maximum removal of BOD, COD, oil content, chloride, TS, TDS, and TSS from the effluent. Among single sp. *S. hygroscopicus* ACT 14 showed maximum reduction potential of BOD than *S. indiaensis* ACT 7. Similarly *S. indiaensis* ACT 7 reduced maximum COD level than *S. hygroscopicus* ACT 14. The oil content was reduced maximum by *S. hygroscopicus* ACT 14. *S. indiaensis* ACT 7 showed maximum reduction potential of TS, TDS and TSS in dairy industry effluent. The impact of treated and untreated effluent was studied using germination studies of *Vigna radiata*. The treated and untreated effluent showed 100 and 70 per cent germination in *V. radiata* respectively.

The effluent from tannery industry was treated with single and mixed consortium of *Streptomyces* spp. The mixed consortium showed efficient removal of BOD, COD, oil content, Cr metal, chloride, TS, TDS and TSS. *S. indiaensis* ACT 7 showed maximum reduction potential of COD, oil content, TS and TSS. *S. hygroscopicus* ACT 14 reduced BOD, chloride, TDS and Cr content from the tannery effluent. The germination studies were conducted using *Vigna radiata* before and after the treatment. It showed 100 and 23 per cent of germination in treated and untreated effluent. The treated dairy and tannery effluent showed highest germination percentage and growth of seedlings than untreated effluents.

In the present study, oil cake was used as feed stock for biogas production for 30 days HRT. *Streptomyces* spp. was used for the initial treatment of feed stock.
*S. hygroscopicus* ACT 14 showed maximum biogas production of 686.1 ml than *S. indiaensis* ACT 7. The mixed consortium showed highest gas production of 829.5 ml on 18th day. In enzymatic treatment, enzymes from mixed consortium showed the highest rate of total biogas yield. Among individual sp. the enzymatic treatment by *S. hygroscopicus* ACT 14 showed high rate of gas production. The lipolytic *Streptomyces* spp. were used for the enhancement of biogas production in latex bearing plant *Pedilanthus tithymoloides*. The mixed *Streptomyces* spp. showed highest bioenergy production of 810.6 ml on 20th day than control of 445.3 ml. *S. indiaensis* exhibited maximum rate of gas production of 761.6 ml than *S. hygroscopicus* with gas production of 640.00 ml.

The crude oil waste (5.0 g) was degraded by single and mixed consortium of *Streptomyces* spp. with pH 8.0 to 10.0 at 40 to 50°C. The increased pH and temperature showed the highest degradation rate of crude oil waste. The mixed consortium showed maximum degradation rate of 96.0 per cent at pH 10.0 and 50°C on 28th day than single sp. and control. Among single sp. *S. hygroscopicus* ACT 14 exhibited maximum degradation rate of crude oil comparing to *S. indiaensis* ACT 7.

The protease and lipase enzymes were used to remove grease and blood stains from cloth along with detergents (Rin, Aerial, Surf excel, Tide, Henko). Lipase from *S. indiaensis* ACT 7 showed maximum compatibility with Surf excel detergent and *S. hygroscopicus* ACT 14 exhibited maximum compatibility with Henko detergent. Similarly for protease, *S. indiaensis* ACT 7 achieved maximum compatibility with Ariel detergent and *S. hygroscopicus* ACT 14 showed maximum compatibility with Rin detergent. Lipase from *S. hygroscopicus* ACT 14 showed highest residual activity with Henko detergent. Protease from *S. indiaensis* ACT 7 exhibited 6.5 hrs for the complete dehairing process. The lipase and protease enzymes were used as an alternative for chemical based dehairing using sodium sulphide. The lipase and protease enzymes from *S. indiaensis* ACT 7 showed the complete dehairing rate within 6.0 hrs where as the chemical treatment needed 9.0 hrs for this. The enzymes from *S. hygroscopicus* ACT 14 exhibited 6.5 hrs for the complete dehairing process. The lipase and protease...
enzymes from the mixed consortium had the highest capability of dehairing within 5 and 5.5 hrs. The results revealed that the combination of lipase and protease could be used effectively for dehairing of goat skin. Among single sp. enzymatic treatment \textit{S. indiaensis} ACT 7 exhibited maximum dehairing rate than \textit{S. hygroscopicus} ACT 14.

In the present study, maximum lipase production was obtained by \textit{S. hygroscopicus} ACT 14 and highest protease production was shown by \textit{S. indiaensis} ACT 7. The solid state fermentation of agro waste with inducers soya meal, wheat bran increased the enzymes activities for \textit{Streptomyces} spp. Among single sp. treatment of effluents, the following observations were obtained. In biotreatment of dairy and tannery effluent \textit{S. indiaensis} ACT 7 showed maximum reduction of toxicants. \textit{S. hygroscopicus} ACT 14 showed the efficiency for maximum reduction of BOD and \textit{S. indiaensis} ACT 7 reduced maximum COD level in both the effluents. \textit{S. hygroscopicus} ACT 14 reduced maximum chromium level from tannery effluent. \textit{S. indiaensis} ACT 7 increased the biogas production from oil cake and latex plant. The enzymes from \textit{S. hygroscopicus} ACT 14 showed maximum rate of biogas yield. \textit{S. hygroscopicus} ACT 14 represented highest degradation of crude oil waste. The combination of lipase and protease enzymes from the isolates showed efficient stain removal of grease and blood from the cloth. Lipase from \textit{S. hygroscopicus} ACT 14 represented maximum compatibility with Henko detergent and proteases from \textit{S. indiaensis} ACT 7 showed highest compatibility with Ariel detergent. The combination of enzymes from \textit{S. indiaensis} ACT 7 showed maximum dehairing rate.

From the present study, it was concluded that the marine \textit{Streptomyces} spp. were well exploited to various environmental applications due to their distinctive features of tolerance to high pH, temperature and salt concentrations. \textit{S. indiaensis} ACT 7 was recommended for maximum protease production using agro waste, dairy, tannery industry effluent treatment and biogas production from oil cake and latex plants. Similarly \textit{S. hygroscopicus} ACT 14 was suggested for maximum lipase production using agro waste, crude oil waste degradation and maximum removal of chromium from tannery effluent. The results confirmed that mixed consortium and
enzymes showed better results than individual sp. and single enzyme treatment. The effluent treated by *Streptomycyes* is used for irrigation purpose in the agricultural field that enhanced the conservation of water resources and sustainable development. The *Streptomycyes* spp. and their respective enzymes could be used for the pre treatment of agro waste and favoured more biogas production. This alternative energy is safer to the environment without causing the pollution effects. The crude oil waste was efficiently degradated by *Streptomycyes* spp. The enzymes extracted from the *Streptomycyes* spp. are utilized in detergent industry as an additive and proved to be an effective alternative for the chemical formulated detergents. The lipase and protease from *Streptomycyes* spp. could be utilized as an ecosafe alternative for the chemical dehairing of hides in tannery industry. The observations revealed that the marine *Streptomycyes* spp. and their enzymes are well exploited for the better waste management and bioremediation of toxicants from the environment in an ecofriendly way.