6. SUMMARY

Biodegradation can be defined as the decomposition of a substance through the action of biological agent, especially microorganisms. In general, then biodegradation is the process of decay initiated by microorganisms (Bollag and Bollag, 1992). In stricter sense, however biodegradation has come to signify the complete microbial breakdown or mineralization of complex material into simple organic constituent such as CO₂, water and mineral components. In the present study on attempt has been made to study the impact of tannase enzyme, produced by different fungal forms on tannins, which is a major pollutant in the tannin industry.

Tannin industry is one of the most significant pollutional industries and the composition of the wastewater from the tanning industry contains pollutants from hides etc.

The tannins, a polyphenols are a major pollutant in the effluent. In the present study, the tannase enzyme was isolated and an attempt was made to study the impact of tannase on tannin.

The serial dilution followed by plating of the tannery effluent on PDA yielded 10 isolates and 5 isolates out of 10 was found to produce zone of hydrolysis indicating tannase production. The tannase producing isolates were pure cultured and identified as A. flavus, A. niger, Fusarium spp. Penicillium spp. and Trichoderma spp.
Among the five types of fungal forms along with the reference culture *A. niger* MTCC 2425, *A. niger* showed high rate of enzyme production (2.27 U/ml), colony diameter 14mm and biomass 0.17g / 50ml which is followed by reference culture and *A. flavus*.

The protein content was estimated in crude and purified extract of both intracellular as well as extracellular enzymes. The protein values of the extracellular crude extract seem to be far higher than that of intracellular. The highest concentration of extracellular crude protein seen in isolates *Penicillium* spp. and in the case of crude enzyme, it is by reference culture *A. niger*, MTCC 2425.

The specific activity of the tannase enzyme produced by the isolate *A. niger* was found to be highest.

The enzyme sample was analysed for the amino acids using paper chromatography and identified that histidine, tyrosine, phenylalanine, isoleucine, tryptophan, serine and valine. The sugars, which are present in enzyme hydrolysates, are glucose, maltose and sucrose.

The molecular weight of purified tannase enzyme was determined as that the molecular weight of *A. niger* and *A. niger* MTCC 2425 was 155 KD, *Trichoderma* 120 KD, *Fusarium* 110 KD. *Penicillium* 130 KD and *A. flavus* 130 KD. The tannase enzyme activity and growth of different isolates has been studied under various cultural conditions such as different substrate concentration, pH, temperature, incubation time and aeration. It was reported that 2.5% substrate concentration is the optimum one for growth. Similarly the
optimum temperature for the growth and tannase activity for the fungus is 30°C. Similarly the optimum growth pH for the secretion of tannase enzyme is pH 5.0.

Between the two types of fermentation such as SSF and SmF, the kinetics of enzymes produced by *Aspergillus niger* in the extracellular production, the production is high in SSF. It was found that extracellular enzyme production is significant in Sorghum substrate by SSF process. When the tannase enzyme and the organism where inoculated into the effluent, it was found that the inoculated organism performed very well than the enzymes. Incubation at the temperature 30°C, the enzyme significantly reduces the tannin content in an effective way.

Among all the isolated fungi *A. niger*, played a significant role in the degradation of physico-chemical character of the raw effluent and at the same time, the tannase enzymes produced by this fungus is quite significant in terms of reducing the tannin value.

This study leads to recommend to that biodegradation of tannery effluent by the whole organism in mold form followed by secondary degradation by enzyme tannase extracellular of production the organism. So the effect on the effluent is significance.