INTRODUCTION
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Every human society be it rural, urban disposes all kinds of byproducts and waste products into the biosphere in large quantities. No doubt it affects the normal functioning of ecosystems as well as have adverse effect on plants, animals, man and microbes. They are collectively called as pollutants (Smith, 1977). Industrial effluents are the major pollutants that pollute not only the water bodies but also the entire biosphere.

Biotechnology has made rapid changes in tannery effluent treatment and the pace of developments in recent years; several methods leading to a thrust in for the treatment of the effluents have arrived. Biodegradation technology is a recent method most successfully employed in the treatment of several kinds of industrial wastes. Biodegradation of organic compound is the partial simplication or complete destruction by microbes of their molecular structure by physiological reaction. Biodegradation is routinely measured by applying chemical and physiological assays to laboratory incubations of flasks containing pure culture of microorganisms, mixed cultures or environmental samples. Biodegradation methodologies are designed to confirm, demonstrate, and explore both the net chemical changes and the associated intracellular details pertinent to how microorganisms influence the fate of organic contaminants. Biological treatment is also recognized as most desirable, easiest and cost effective in pollution abatement programmes. Moreover, it is free from sludge accumulation a serious problem of disposal as in the case of chemical treatment. Microorganisms are
more effective in avoiding such problems. Hence biological treatment of effluents with microorganisms is a most desirable one.

Almost without exception, microbial biochemists are interested in biodegradation and initiate their studies with classic procedures for isolation of an organism that can grow on a desired compound. Normally these procedures demand some kind of preliminary enrichment for boosting the number of organisms capable of utilizing the compound in the initial sample. A selection process, conventionally using solidified media is designed to isolate a pure culture with the capacity to use the required compound as the growth substrate, follows this. The fact that these simple methods are universally applied asserts their validity as excellent starting point for biodegradation studies, at least so for as the microbial biochemists are concerned. The primary aim is to obtain an easily cultivable microorganism in sufficient quantities to determine the biodegradation mechanisms by elucidating the catabolic sequence, purifying and identifying intermediate metabolites, assaying and characterizing the enzymes involved in the pathway, and determining the factors controlling the regulation of the pathway expression.

Industries are significant points of pollution sources from an environmental point of view and pollution characteristics are different from each other. Hence industries are to be characterized depending on their production types and pollution loads. During industrial processes, different pollutants are produced; each production stage depending on the type of industry, technologies utilized and raw and support materials with a direct relationship between products and pollution loads.

The leather industry is one of the oldest and fast paced industry in India. Tanning industry stands among the top five export oriented industries. India
occupies a predominant position in the world production of hides and skin. There are about 3000 tanneries scattered all over the country. Leather industry in India is mainly concentrated in states like Tamilnadu, UtterPradesh, West Bengal, Rajasthan, Mahararastra, Karnataka, Andhra Pradesh and Bihar. In Tamilnadu, clusters of industries are located mainly in the districts of North Arcot, Dindugul and Erode. Erode district has a large number of 112 tanneries with 3 of them being major industry. In India tanneries, excluding cottage industries which process 500,000 tones of hides and skin annually. Minimum effluent produced is 3,000 liters per 100 kg hide. Total annual discharge of wastewater is 9,420,000 M³ and 18840-28260 M tons of BOD is the organic load.

Tanning is the act of converting animal skins and hides into leather. In India, there is no single process for producing leather. Skins and hides, the raw material of tannery is classified on the basis of weight and size of the animals into two groups (Heavy and light leather respectively). The skins and hides after removal of flesh and fat, are treated with chemicals to form a stable, durable material. After tanning, the hides will usually be further processed according to their intended end use [UNEP/IEO, 1991]. The individual wastes from each step in tanning procedures are numerous and varied depending on the process employed and the type of leather produced. These include the vegetable tanning and chrome tanning. Vegetable tanning is employed in the manufacture of sole belting, upholstery bag and novelty leather. It is also used for shoe upper and lining leather to some extent, but this is usually handled in plants where majority of the tanning is done with chrome and where the character of the waste is determined by the process. Raw skin is readily putricible in the wet condition, hence upon drying, the collagen fibers become glued together, and the skin gets very stiff. The dried skins will not putrefy but it will restart as soon as skin becomes moist again. The combination of tannin with skin is called vegetable
tanning and resultant product of the combination of skin protein with tanning is leather.

Tanning has been recognized as the cause of serious environmental pollution in many developed countries. The tanning industries with their high specific demand for water and with added unfavorable composition and concentration of its effluents, have come to be regarded as one of the most water polluting industries. The individual waste from each step in tanning procedures is numerous and varied depending on the type of leather produced and process employed. When tannery effluents are discharged into aquatic system, it greatly affects the aquatic life and thereby poses a threat to aquatic system (Laurance, 1971).

1.1.1 THE TANNING PROCESS

As soon as the animal is slaughtered, the proteolytic bacteria quickly attack the skin and reduce its value and utility if left unchecked. To stop these damaging changes, the skin is to be cured by partially dehydrating it by treatment with salt (sodium chloride or sodium sulphate) or drying in air or by both. Minimum curing time in packs is 3-4 weeks. Fleshing is the removal of areolar tissues from the flesh side of hides or skins. Attached fat, connective tissues, blood vessels, nerves, voluntary muscles and left over meat are also removed. Fleshing is done before or after soaking, unhairing or re-liming. Brining and green salt curing reduce the water content of raw hides from 70% to 80% and dry-salting and flint-drying reduces the water content to as low as 10%.

The salted skins brought to the tanneries contain dirt, salts, blood, muscles, non-fibrous proteins and undissolved sodium chloride in addition to the dissolved salt. The object of washing and soaking is to remove the extraneous
matter and to restore the moisture lost during preservation and storage. There is no standardized procedure for washing and soaking.

Removal of hair and epidermis from hides and skin is achieved by employing machine, enzymes or manual pulling. In some cases, lime is used for dissolution or by loosening followed by machine or manual pulling. Lime has been used for both hair loosening and destruction. Some get by immersing unhairy hides for several minutes in 16% sodium hydroxide and neutralized in 14% hydrochloric acid. The sodium chloride formed was enough to prevent acid swelling. Sodium sulphide is the most important and widely used unhairing agent in the industry. Dimethylamine sulphate is also used to a limited extent for unhairing purposes. Some tanners prefer the painting process for hair removal, wherein a paint is made by dissolving sulphide or sulphhydrate to the desired strength and it is added to hydrated lime to form a thin paste.

After unhairing, the hide may be silted through the middle of its thickness to provide two distinct layers. The upper is called green layer and the lower layer or flesh side is called a split. When the hide has been soaked, unhaired, relimed and fleshed, it still remains swollen. Bating is the term applied to the process, which prepares hide for tanning. Its objectives are to regulate the pH (delime), to provide swelling (fetting), to poptise the fibers and to remove protein degradation products. Ammonium salts, particularly ammonium sulphate, are universally used to provide proper pH regulation, deliming action and enzyme activation. Ammonium chloride, sodium bisulphite, sulphur dioxide, and carbon dioxide have also been used. The hides are washed for 10-30 min at 70°F, followed by 10-30 min at 80-100°F. A deliming salt or acid is added. The bate is added and the hides are paddled. Bates contains deliming salts. By this time, the porosity of the skin is increased. Pickling is the term applied for the treatment of hides with salt and acid. Pickling is done in paddles or drums. Degreasing is done by
emulsification with an aqueous solution of a synthetic detergent. Solvent extraction by organic solvents or pressure degreasing is done when skin greases are squeezed out by mechanical action. For the determination of pollution discharged from a tannery, processing procedures of the plant should be outlined. The processing chemicals added and the quantity of hide being processed should be recorded. Sengul & Gurel (1993) determined chemicals used in leather industry with regard to different production technologies utilized and the water consumption and pollution loads.

1.1.2 CHROME TANNING

Vacquelyn discovered the element chromium in 1797, and in 1884, Schultz patented the first chrome-tanning process: two-bath process-bichromate followed by hypo. In 1893 Dennis patented the use of basic chrome salts-the one bath process. The new tannage is 100 years old and was developed by Philadelphia glazed kid tanners. It is a short time process (15-24 hours). It had two major advantages over the existing vegetable tannage. There was rapidity of tannage and high resistance to heat, which means the leather stood up to the lasting, mulling and steaming operations in shoe factories, and in general resists any mechanical abuse in going through quick, vigorous shoemaking techniques. Chrome tannage was well established in the decade 1900-10 and came into its own during world war I and even today it is, by far, the principal tannage for leather, the greatest use being in shoe upper leather.

1.1.3 VEGETABLE TANNING

Vegetable tannage, although of great antiquity, is still the principal tannage of the world. In United States 90% of all leather produced is either all or partly vegetable tanned. The unique qualities of vegetable leather plus the reasonable cost have not been realized by other tanning materials.
Raw skin is readily putricible in the wet condition and hence upon drying, the collagen fibers become glued together and the skin is very stiff. The dried skin will not putrify, but, it will restart as soon as skin becomes moist again. The combination of tannin with skin is called vegetable tanning. Sources of tannin are barks, woods, leaves, twigs, pods and roots of plants. In 1960, in the United States, the following important vegetable tans: on a tan basis, 27,640 tonnes of quebracho, 8,000 tonnes of wattle extract and bark, 5,100 tonnes of chesnut and 1,760 tonnes of mangrove were used. The theoretical approach of tanning was from the viewpoint of the neutralization of opposite charges on the colloidal system of collagen, and vegetable tans were regarded as high molecular weight colloids (Turley, 1993).

A combination of the advantages of both processes is obtained by tanning hides by both methods and is called chromeretan.

1.2.1 WASTE WATER FROM TANNERIES

One of the most important inputs of the leather industry is water, which is used in the entire wet processing units. By the use of classical production techniques, and thoughtless washing process, large amount of water is lost and production of wastewater amount is increased. There are more than 20 stages from soaking to finishing process. Pollution from these stages varies quantitatively and qualitatively (Hekimoglu, 1983). The composition of wastewater from the tanning industry contains pollutants from hides, products formed from their decomposition, and chemicals and various solutions used for preservation of hides during the tanning process.

'Beam house' operation in a typical tannery commences of soaking, liming, unhairing and deliming operations which together contribute wastewaters of considerable quantities of lime, sulphides, dissolved and particulate protein,
Introduction

Effluent from beam house operation is characterized by high \(p^H\), high oxygen demand, high organic content, oil and grease and sulphide (Bwig, 1987).

Soak liquor contain soluble proteins, urea, proteolytic and other bacteria, dirt, dung and blood adhering to hides and skins. Lime liquors are highly alkaline and contain suspended and dissolved lime, sodium sulphide, ammonical nitrogen and organic matter. Unhairing and fleshing effluent contains fatty and fleshy matter in suspension of hair and sulphides. Spent deliming liquors carry significant BOD load. Spent bate liquors on account of presence of soluble skin proteins and ammonium salts contain high organic matter. Pickling liquors comprise of high amount of salt and acids. After beam house operations, spent vegetable liquor contains significant amount of organic matter whereas, the spent chrome liquor exhibits less concentration of organic contents though it has toxic chromium. The spent vegetable tan liquor is probably the strongest fraction in a composite wastewater. It has permanent color, acidic in nature and contains tannins. It carries BOD load in the range of 0.34 to 1.37 kg/hide processed. The spent chrome tan liquor is acidic, greenish in color and contains trivalent chromium from 100 to 5500 mg/L. BOD of the waste is usually low about 1000mg/L. The composite effluents from a tannery are highly colored and are foul smelling. It is alkaline with high amount of suspended and dissolved impurities. BOD of the effluent varies from 2000 to 3000 mg/L.

1.2.2 EFFECT OF TANNERY WASTE WATER ON ENVIRONMENT

The tannery effluent wastes are ranked as high pollutants among all other industrial waste. (Eye and Lawrance, 1971). The tannery wastewaer contains vegetable tannins, high amounts of proteins that exert BOD, Chlorides, trivalent chromium, nitrogen, phosphorus, sulphates and sulphides are the inorganic constituents present. The presence of color, oil, and turbidity of tannery
wastewater does not allow their use for domestic, industrial, recreational and other purposes. Indiscriminate discharge of treated and untreated tannery waste into surface and underground water bodies cause pollution. The receiving bodies of water slowly increase in chlorides and hardness. Presence of ammonia, sulphides, tannins and chromium in the water bodies are considered as toxicants. These wastewaters when discharged into watercourses will affect physical, chemical and biological characteristics of water and will deplete the dissolved oxygen. High pH, excessive alkalinity, suspended solids, sulphides are injurious to fish and other aquatic lives. The freshwater being the very important medium for the production of protein rich fishes, prawns and crabs etc, gets ecologically deteriorating due to the discharge of industrial effluents. The physico-chemical characteristics and the impact of tannery effluent on water bodies were analyzed by Eye and Lawrance (1971), Kothandaraman et. al., (1972), and Guruprasada Rao and Nandha Kumar (1981). Saravanababu et. al., (1990) found that the population of phytoplankton and zooplankton decreased both in the tannery effluent discharge point and the downstream of Kalingarayan canal of Erode district to a certain extent. This study showed the impact of tannery effluent under certain concentration on cereal cultivation. They found that Cajanus cajan could be cultivated using treated effluent. Effects of effluent contaminants on the microbial flora of the tannery well waters in the nearby area were studied (Padmini Ramasamy and Krishnamoorthy, 1980). The well waters recorded high bacterial count, sodium chloride content and hardness because of their location near the tannery effluent disposal area. The wastewater from vegetable tanning process imparts color and consists of non-biodegradable matter, which persists for long. Salts and sulphides cause taste and odor problems. The discharge of tannery waste into municipal sewers can cause incrustation of sewers, sewer clogging and other forms of interference with sewage treatment.
1.2.3 TREATMENT OF TANNERY WASTE WATER

The treatment of tannery wastewater faces complications on account of intermittent dumping of strong liquors. Usually soak waters, liming waters and spent vegetable tan liquor were discharged intermittently. Spent deliming and bating liquors are usually discharged once a day. Individual processes vary from tannery to tannery. It is observed that in India, the tanneries are installed away from human habitation but as the urban limits expanded horizontally these tanneries at many places have now been surrounded by human habitation. It is observed that when the volume of wastewater discharged to the sewer is small as compared to the volume of the municipal sewage, it can be discharged with pretreatment so as to protect the sewers and the municipal treatment plant. In many cases, separate treatment facilities are required.

1.2.4 BIOLOGICAL TREATMENT

The conventional method of treatment of tannery waste consists of screening, flow equalization, primary sedimentation and/or chemical flocculation, aerobic activated sludge treatment and secondary sedimentation. The Environmental technology group of Central Leather Research Institute, Chennai has come up with modified tannery effluent treatment process successfully Rajamani et al., (1987). Sengul (1993) have also recommended a treatment system for light leather. Much work has been done on process modification aiming pollution reduction in tanning of hides and skins (Turley, 1993). Prasad et al., (1983) have carried out studies on the characterization and treatment of wastewater produced during various process modifications for pollution reduction in tanneries.
1.3.1 TANNINS AND TANNERY EFFLUENTS

The important pollutant present in vegetable tanning industry effluent is tannin. The spent tan liquor is dark brown in color and acidic in nature. This is due to the presence of tannin. Moreover, the treated tannery effluent from effluent treatment plant carries residual color contributed by undegraded vegetable tannins and certain dye stuffs due to chemical and biological oxidation systems having their own limitations towards the waste water treatment (Gupta et. al., 1992).

The treated wastewater from a common effluent treatment plant catering service to 152 tanneries in Chennai contained residual organics besides visible color due to undegraded tannins and dye stuffs (Sankar et. al., 1997).

These compounds are less amenable to biological oxidation (Ajmal khan, 1985) and they demand the inclusion of tertiary treatment system for the purpose of meeting discharging standards.

Coagulation and flocculation are a widely used technique for the removal of color and residual organics. However, the consumption of coagulants and sludge production are considerably high enough to manage. For instance, to reduce COD of biologically treated wastewater in common effluent treatment plant (CETP) from 320 mg/L to 250mg/L, 0.4 g/L of sulphate was needed. The TDS was increased by 200 mg/L and volume of sludge produced was 120 mg/L. Most of the fungal species that have been used for biodegradation of tannery effluent belong to the genera *Aspergillus* and *Penicillium*. Other fungi including *Chaetomium, Fusarium, Rhizoctonia, Cylindrocarbon* and *Trichoderma* are capable of degrading tannery waste constituents (Mahadevan and Muthukumar1980).
1.3.2 CLASSIFICATION AND OCCURRENCE OF TANNINS

The term ‘tannin’ has been used in a wider sense in botanical literature. Tannins are defined as water-soluble phenolic compounds with molecular weight ranging from 500 to 3000 and can combine with proteins, cellulose, gelatin and pectin, to form an insoluble complex. These are water-soluble polyphenolics differing from most other natural phenolic compounds in their ability to precipitate proteins such as gelatins (Spencer et. al., 1998). This property (sometimes called astringency) is the reason for their past and present use in the tanning of animal skins. The astringency property of polyphenols was studied by Tetsuo Osawa et.al., (1987). Natural plant tannins are widely distributed throughout the plant kingdom (Halthocoay, 1962; Jurd, 1962). Tannins are considered plant secondary substances, as they are not involved in metabolic pathways. After lignin, they are the second abundant group of plant phenolics. The presence of large number of phenolic hydroxyl group enables them to form large complexes, mainly with proteins and to a lesser extend with other macromolecules like cellulose and pectin. The theoretical approach of tanning was from the viewpoint of the neutralization of opposite charges on the colloidal system of collagen, and vegetable tans were regarded as high molecular weight colloids. The chemistry of the constitution of the vegetable tannins was examined early by some of the greatest organic chemists of the day, viz: Emil Fischer, Max Bergman, and Carl Freundenberg. The chemistry of the vegetable tannins is exceedingly difficult because they are all polyphenols of mixed type (Turley, 1993). Tannins are classified into condensed tannins and hydrolysable tannins. The former are polymers of catechin or similar flavones that are connected by carbon-to-carbon linkage. The latter are composed of a molecule of carbohydrate, generally glucose to which gallic acid or similar acid is attached by ester linkages.
They are widespread in the plant kingdom (pteridophytes, gymnosperms, and angiosperms), found often in leaves, fruits, barks and wood and can also accumulate in large amount in particular organs or tissues of plants (Haslam et. al., 1989).

Hydrolysable tannins are composed of esters of gallic acid (Gallo tannins) or ellagic acid (ellagitannins) with a sugar core, which is usually glucose, and are readily hydrolyzed by acids or enzymes into monomeric products. The major commercial hydrolysable tannins are extracted from Chinese gall (*Rhus semialata*) Sumac (*Rhus coriara*), Turkish gall (*Quercus infectoria*), Tara (*Caesalpina spinosa*) Myrobalan nuts (*Terminalia chebula*) and Chestnut (*Castanea sativa*). Condensed tannins also known as polymeric proanthocyanidins are composed of flavanoid units, and are usually more abundant in tree barks and woods than their hydrolysable counterparts. The important commercial condensed tannins are extracted from wattle (*Acacia mollissima*, and *A. mearnsii*), Quebracho (*Schinopsis lorentzii*) and *S. balansae*) and tree barks. A group, which occupies an intermediate position in the tannin hierarchy, is the family of catechin tannins combining elements of hydrolysable and condensed tannins. These tannins are quite common in tropical shrub legumes and tea leaves.

1.3.3 TOXICITY OF TANNINS

Toxicity studies have involved various fields of research involving food science, wood science, soil science, plant pathology, pharmacology and human and animal nutrition. Tannins inhibit the growth of microorganisms, resist microbial attack and are recalcitrant in the environment (Field and Lettinga, 1992). It is beyond doubt that tannin-containing plants have had a significant evolutionary advantage over the other organisms, which attack them.
Tannins may deter herbivores from predation. They may also deter microorganisms, either by increasing resistance against pathogens, or by protecting essential tissues such as wood against decay. Man has not ignored these remarkable properties of tannin rich materials. Many of the timbers selected for their high durability e.g.: European oak and chestnut, black locust and Eucalyptus, are rich in tannins. Rennerfelt (1948) proved the fungicidal qualities of phenolic compounds isolated from the wood of different conifers. Hans Nienstaedt (1953) demonstrated the protective role of tannins in chestnut Castanea spp against the chestnut blight fungus Endothia parasitica. The phenolic acid dilactone ellagic acid, was isolated and identified from hot methanolic extracts of five species of semi-arid plants as an insect growth inhibitor active against the polyphagus herbivore Heliothis virescens (tobacco budworm) by Klocke et. al., (1986). Now a days increasing attention is also being paid to the use of tannins as an antimicrobial agents for example in wood preservation, or prevention of dental caries (Kawamura, 1989). For grazing herbivores, tannins present in plants can, in general, adversely affect their nutrition by reducing intake, protein digestibility, inhibiting digestive enzymes or by direct systemic toxicity (Kumar and Singh, 1984).

Tannin toxicity for fungi, bacteria and yeast is reviewed and compared to toxicity of related lower molecular weight phenols. Many microorganisms detoxify tannins through synthesis of tannin-complexing polymers, oxidation, tannin biodegradation or synthesis of siderophores (Scalbert, 1991).

1.4.1 DEGRADATION OF TANNINS

Concerted efforts are in progress world wide to improve tannery waste degradation, to enrich tannin rich food and fodders by biodegradation of tannins, obtain strong tannin degraders, products like gallic acid by microbial strains. A number of reviews on tannin biodegradation have appeared in the past, providing
a general idea of the biodegradation of these polyphenols (William et. al., 1986; Bhat, 1998). A lot of work has been published on the industrial and agricultural application of tannin biodegradation. (Archambault et. al., 1996; Hatamoto et. al., 1996; Lekha and Lonsane 1997) warranting a fresh appraisal of the present scenario.

Despite the antimicrobial properties of tannins many microorganisms can grow and develop on tannin rich materials. Several microorganisms have however evolved to withstand these high concentrations of tannins. Some of them have succeeded very specifically.

1.4.2 DEGRADATION OF TANNINS BY FUNGI

The first report on the degradation of tannic acid by strains of Aspergillus niger was observed by Knudson (1913). Filamentous fungi, especially species of Penicillium and Aspergillus have been implicated in tannin degradation. Lewis and Starkey (1969) reported that pure cultures of some soil fungi grew on media containing tannins as sole carbon source. Different sources of tannins were compared and both condensed and hydrolysable tannins were used as substrates. Aspergillus, Penicillium, Fomes, Polyporus, and Trametes were shown to grow better on tannic acid (gallotannin) than on chesnut tannin (ellagitannin) or wattle tannin (condensed tannin). Ganga et. al., (1971) found that A.niger and Pencillium spp. grew profusely in a medium containing glucose and wood apple tannin. With wattle tannin at 0.3% and glucose at 166.7 mm concentration, growth of A niger improved. A tannin degrading strain of Aspergillus niger van Tieghem MTCC 2425 was isolated from faeces of hill cattle and studied by Bhat et.al., (1997). It was shown to grown at pH 5.0 and 30°C in a defined medium were tannins were the sole source of carbon. This isolate was shown to posses the ability of tannin protein degradation by the same authors previously.
Degradation of hydrolysable tannins particularly gallotannins is best understood in fungal systems (Nishira, 1961). The oxidative degradation of hydrolysable tannins has been studied in details in Aspergillus spp. and the pathways of gallic acid degradation have been determined (Watanabe et. al., 1965; Mahadevan and Sivasamy, 1985). Whereas, the pathways leading to the degradation of hexahydroxydiphenoyl moiety-related biphenyl and biaryl ether structures, and the biodegradation of proanthocyanidins are slowly being unravelled. A lot of information is now available on the tannin biodegradation pathways used by fungi. (Watanabe, 1965; William et. al., 1986; Suseela Rajakumar, 1985; Patel et. al., 1990). There are only a few reports on tannin-degrading yeasts. Initially six strains of yeasts were isolated from tannery liquors and xylophagous insects, which showed growth and hydrolytic action on tannins in culture media containing various concentrations of gallotannins. The tannin degrading enzymatic system of Candida was found to utilize gallotannins as substrates (Aoki et. al., 1976 a, b). Tannase produced by this yeast hydrolyzed the ester and depside linkages of tannic acid. Later, a number of yeasts which could degrade condensed tannins were reported (Otuk and Deschamps, 1983; Vennet et. al., 1986). The strains isolated and studied were of Candida guillermondii, C. tropicalis, and Torulopsis candida. Most yeasts were efficient degraders of quebracho tannins and reduced the tannin content of pine and gaboon wood bark extracts up to 80% in five days (Otuk and Deschamp 1983). Although, numerous studies have been conducted on tannin degradation by various yeasts, not much is known about the pathways and enzymes involved in breaking down the tannins and their intermediates to simple compounds.

1.5.1 TANNIN ACYL HYDROLASE(TAH) AND TANNERY EFFLUENTS

Tannery effluents contain higher quantities of tannins, mainly polyphenols, which are major pollutants. These tannins are hydrolyzed by the
enzyme tannase into non-toxic gallic acid and glucose. The series of events that led to the discovery and an understanding of tannase, provides interesting insight into the economical and technological factors involved in the development of a new product. Teighem (1867) first reported the formation of gallic acid by *Penicillium glaucum* and *Aspergillus niger*, which occurs naturally when an aqueous solution of tannin or a filtered infusion of gallnut solution is exposed to air. These organisms were able to grow with tannic acid as a sole source of carbon, hydrolyzing it to gallic acid and glucose. Fernback (1901) reported that the hydrolysis of tannin is brought about with a help of a particular enzyme tannase.

### 1.5.2 SOURCES OF TANNASE

Tannase can be obtained from plant, animal and microbial sources. From plant sources, the enzyme is present in tannin-rich vegetables mainly in their fruits, leaves, branches and barks of trees like Konnam, Myrobolon and badul (Madhavakrishna *et. al.*, 1960; Pourrat *et. al.*, 1985; Lekha and Lonsane, 1997). As for as animal sources are concerned, tannase can be extracted from bovine intestine and from the ruminal mucous (Begovie and Duzic, 1976, 1977). Also, it has been reported that some insects produce these enzyme during larval stage (Nierenstein, 1930). The most important source to obtain the enzyme is by microbial way, as the enzymes produced are more stable than from other sources. (Lekha and Lonsane, 1997). Additionally microorganisms can undergo new techniques such as genetic manipulation, which may result in an increase in the TAH activity titer values (Hatamoto *et. al.*, 1996).

### 1.5.3 SCREENING OF TANNASE PRODUCING FUNGI

Screening processes among a large heterogenous population may result in the rapid identification of organism of interest by allowing discarding of
unwanted organism within one or two steps. Enrichment cultures are classical microbiological technique commonly used for finding a specific microbe to degrade a certain toxic waste. Enrichment cultures favour the growth of a particular species based on its nutritional requirements. In most common application of this technique, aliquots of water, soil or effluent are placed into a medium containing the targeted compound as the sole carbon source. In liquid culture, competition for the substrate will lead to enrichment of the microbial strain that grows fastest. On petriplate colonies, colonies representing many species are usually isolated; these are then subcultured and tested further. From the literature reviewed, it was found that there was no plate assay reported in earlier works. Sapna Bradoo et al. (1996) developed the first plate assay method using Czapek Dox’s minimal medium containing commercial tannic acid as the sole carbon source for screening tannase producing laboratory isolates

1.5.4 TANNASE ASSAY

Tannase acts on the ester and depside linkages present in tannic acid liberating glucose and gallic acid. A number of methods are available to evaluate tannase activity. Some of which are titrimetric assays (Freudenberg et al., 1927; Nishira 1961, Haslam and Stangroom 1966, Yamada et al., 1967) a photometric assay (Chen, 1969) a colorimetric technique (Haslam and Tanner, 1970) U.V spectrophotometric methods, (Iibuchi et al., 1967; Sanderson et al., 1974; Aoki et al., 1976; Rajakumar and Nandy; 1983; Bajpai and Patil 1996) and two chromatographic assays (Jean et al., 1981; Beverini and Metche, 1990). All these methods have been based on the release of gallic acid from tannic acid by the action of tannase enzyme. Methods to evaluate tannase activity have been briefly reviewed by Lekha and Lonsane (1997) and severely criticized by Jean et al., (1981) and Bajpai and Patil (1996). Recently, Aguilar et al., (1999b) reported a comparative study of above such six methods to determine tannase
activity. Assay method based on the formation of chromogen between gallic acid and rhodonine is reported by Shweta Sharma et al., (2000). More recently, a new colorimetric method of tannase assay has been developed using its specific substrate tannic acid (Mondal et al., 2001). All these methods have both advantages and disadvantages over other methods in one way or another.

1.5.5 PURIFICATION OF TANNASE

In the 20th century, numerous hydrolytic enzymes involved in the degradation of relatively simple biopolymers such as starch and protein have been purified, characterized and utilized within industrial settings. These include fungal amylases, glucosamylases, lipases, pectinases, and proteases. The study of tannase enzyme, which has wide applications and value, assumes great importance in enzymatic studies. Though produced in vast amount, the information regarding structure and other functional features of the enzyme were scarce. Aoki et al., (1976) reported purification of tannase from plant and microbial sources. Tannase has been purified from a variety of fungi namely A. flavus (Yamada et al., 1968) A. oryzae (Libuchi et al., 1968; Fumihiko and Kiyoshi, 1975; Beverini and Metche 1990), Candida (Aoki et al., 1976) P. crysogenum (Rajkumar and Nandy, 1983) and A. niger (Barthomeuf et al., 1994). In all these studies the starting material was either culture filtrate (Fumihiko and Kiyoshi 1975) or mycelial extract obtained by sonication of the mycelial cells (Yamada et al., 1968) depending on the localization of the enzyme. Tannase purification schemes have generally used standard column chromatographic techniques, mainly ion-exchange and gel filtration.
1.5.6 MOLECULAR CHARACTERIZATION OF THE TANNASE ENZYME

The advent of recombinant DNA technology has revolutionized research in the field of enzymology. Although tannase was produced and purified from a number of microbial sources, isolation and characterization of the tannase genes would be essential to understand the molecular biology of tannase biosynthesis. Tannase is a high molecular weight enzyme whose molecular weight is reported to vary from 186,000 to 300,000 Daltons. The native enzyme consists of two different polypeptide chains of similar molecular size (Adachi et al., 1968; Aoki et al., 1976). Amino acid analysis of tannase from Candida revealed that the enzyme consisted of 786 amino acids per molecule (Aoki et al., 1976). The internal amino acids sequence of the Aspergillus oryzae tannase enzyme was determined and the tannase gene using three oligonucleotide probes synthesized according to tannase N-terminal and an internal amino acid sequence was cloned (Hatamoto et al., 1996). Number of authors reported the isozymes of tannase (Aoki et al., 1976; Hatamoto et al., 1996). It is suggested that the carbohydrate coating probably may protect the tannase enzyme from the inhibitory effects of tannin. The carbohydrate contents of the fungal tannases, have been determined by number of authors (Adachi et al., 1968; Aoki et al., 1976, Rajkumar and Nandy 1985). Tannases from many fungi have been extensively characterized. Numbers of reports are available on the pH optimum, temperature optimum, enzyme inhibition and stability of the enzymes. These results have led not only to the understanding of how these enzymes operate and regulated, but also an appreciation of their vastly different physico-chemical properties. The total concentration of proteins does not exactly determine the quantity of the enzyme, as, much of impurities, may still be present. Hence, for the exact idea of enzyme production, the specific activity has been calculated.
1.6.1 OPTIMIZATION OF CULTURAL CONDITIONS

Optimization is of great importance in the production of any enzyme in industrial scale. Different parameters have to be optimized to get high yield and the economic feasibility. Various factors affecting microbial enzyme production are concentration of the substrate, pH, aeration, temperature, composition of the medium, co substrate etc. There are two main fermentation types that are generally used for the production of commercial enzymes. These are submerged fermentation (SmF) and solid-state fermentation (SSF) (Frost and Moss, 1987). A literature survey indicates that tannase has been produced by liquid-surface, submerged, and solid-state fermentation, though production of tannase has been most extensively carried out in a submerged fermentation system. Different environmental factors influences that production of tannase. But an extensive study exploring all the aspects of tannase production has not been made. Details such as media, fermentation time, temperature, and the location of tannase produced by different microorganisms in submerged fermentation are reported by number of authors (Dhar and Bose 1964; Yamada et. al., 1967; Yamada et. al., 1968; Aoki et. al., 1976; Deschamps et. al., 1983; Barthomeuf et. al., 1994). As no details on optimization of the processes are available, it seems likely that fairly extensive optimization will be required.

1.7.1 SUBMERGED FERMENTATION USING BIOREACTOR

Submerged fermentation involves the growth of the microorganism as a suspension in a liquid medium in which various nutrients are either dissolved or suspended (Frost and Moss, 1987). Submerged fermentation is the preferred method for production of most of the commercially important enzymes or sterilization and process control are easier to engineer in these systems (Anunstrup et. al., 1979). Tannase production in the commercial scale is through
submerged cultures, using filamentous fungi, in which the tannase enzyme is expressed extracellularly due to its location in the cell periplasm. Beverini (1987) demonstrated that tannase is caught in the cell wall of the fungus. For this reason, it is very important to extract tannase in active form. Some studies have reported that extraction process employing hydrophilic proteins can favour the recovery process (Golden and Hatton, 1987; Grovenio and Vergegehem, 1987; Yokoyama et. al., 1988). In addition, Yamada et. al., (1968) reported that tannase is always expressed extracellularly in low levels. Tannase production is induced and generated during the first step of microbial growth because it is a primary metabolite. (Doi et. al., 1973; Garcia-Perra, 1996). General conditions for producing tannase by submerged culture systems are: temperature of 30-33°C, initial pH of 4.4 to 5.8 and agitation between 169 and 250 rpm (Adachi et. al., 1968; Doi et. al., 1973; Rajakumar and Namdy, 1983; Lekha and Lonsane 1994; Barthomeuf et. al., 1994; Bajpai and Patil, 1997).

1.7.2 PRODUCTION OF TANNASE BY SOLID STATE FERMENTATION (SSF)

Solid state fermentation is generally defined as the growth of the microorganism on moist substrates in the absence or near absence of free water. (Ashok Pandey, 1994) Solid-state fermentation offers number of economic advantages over conventional submerged fermentation for enzyme production (Mudgett, 1986). The essential feature of solid-state fermentation is the growth on an insoluble substrate without a free liquid phase. The production medium is often simple: using agro industrial by products like wheat bran, rice bran or wheat straw as substrates (Mitchell and Lonsane 1992). As the moisture level is low, the volume of medium per unit weight of substrate is low, hence, the enzyme activity is usually very high (Deschamps and Huet, 1985). Laboratory scale studies on the production of tannase by solid State fermentation
showed several advantages over conventional submerged fermentation (Lekha et. al., 1994). The literature on microbial production of tannase by solid-state fermentation is meagre. Except for a few exploratory report (Nishira and Mugibayashi, 1960) on production of solid state using wheat bran, there are no data available on the effect of media parameters on tannase production by solid-state fermentation. In India work on tannase production by solid-state fermentation was initiated at the Central Food Technological Research Institute (CFTRI) in Mysore. Extensive screening of fungal cultures from a culture collection as well as those isolated from soil and different tannin-rich plant material was carried out for tannase production. Aspergillus niger, a potent culture for tannase production was isolated. The physico-chemical parameters for tannase production by this culture in solid-state fermentation was optimized by a response surface methodology (Lekha et. al., 1994).

Aguilar (2002) showed that SSF could be an interesting alternative for the production of tannase enzymes without the interference of unwanted proteolysis. Tannase enzyme SSF production was carried out with different substrates by number of authors. The support materials like wheat bran (Nishira and Mugibayashi 1960) sugar cane baggase (Lekha and Lonsane, 1994) low-density polyurethane foam (Aguilar et. al., 2001) were used for tannase enzyme production. Conventional processes mainly utilize substrates of plant origin. The agriculture byproducts rice straw and sorghum straw was abundant in rural Tamil Nadu. Incorporation of rice straw into the soil as a fertilizer is common practice in rice cultivation. Wetland rice fields are flooded for at least the major part of the crop-growing season. As the water at the surface drastically reduces the diffusion of oxygen into the soil the rice paddy turn anoxic. Under these conditions the final step in mineralization of organic matter is the conversion of organic carbon to CH₄ and CO₂ after nitrate, ferric iron and sulphate have been
reduced up to 42% of the carbon in methane production is derived from rice straw. So the rice straw are the major contributors of green house gases (Glissmann et. al., 2001).

1.8.1 EFFLUENT TREATMENT BY ENZYMES

Tannase plays an important role in the treatment of tannery effluents (libuchi et. al., 1968). The application of tannase enzyme on tannery effluent can be carried out in two ways.

1. Direct contact of enzymatic extracts with the material to treat the hydrolyzing polyphenols and avoiding their unpleasant polymerization or.
2. growing tannase producing fungal strains on tannin rich effluents.

Both these approaches are called as bioaugmentation. Bioaugmentation is the process of introducing microorganisms selected to perform a desired task, such as degrading the contaminants. The practice of bioaugmentation is not new, it is commonly used by the wastewater industry at system start-ups, following an upset or to improve treatment. Bioaugmentation is useful one when the concentration of contaminants is toxic to indigenous flora.

An enzymatic method for removal of phenols from industrial water, using Turimp peroxidase has been developed Singh and Singh (1999). The fungal enzymes Coprinus peroxidase (CIP) can also be used for the removal of toxic phenols from water (Kauffman et. al., 1999). Zouboulis et. al., (2001) studied the effectiveness of applying enzymes (bioaugmentation) for enhancement of biological treatment ability of leachates generated in a typical municipal solid waste sanitary landfill. It was found, that, the enzymatic process was able to remove organic matter effectively and expressed as BOD and COD and nitrogen content, color and turbidity. The mass production of enzymes for effluent
treatment may in future pave way for the eco-friendly low cost treatment of the highly toxic tannery effluent.

1.9.1 RESEARCH APPROACH

In nature, fungi do much of the dirty work. They are particularly efficient at degrading the major plant polymers, cellulose and lignin. But they also decompose a huge array of other organic molecules also. Although industrial microbiologists regularly harness fungal metabolism for brewing, baking, cheese production, and best known for their dirty work paradoxically, the use of fungi in bioremediation has been limited compared with the use of bacteria. The attributes that distinguish filamentous fungi from other life forms determine why they are good biodegraders. First, their mycelial growth habit gives a competitive advantage over single cells of bacteria and yeast especially with respect to colonization on insoluble substrates; they literally digest their way by secreting a battery of extracellular degradative enzymes. Hyphal penetration provides a mechanical adjunct to the chemical breakdown effected by secreted enzymes. The high surface to cell ratio characteristic of filaments maximizes both mechanical and enzymatic contact with the environment. Second by the extracellular nature of the degradative enzymes enables fungi to locate higher concentrations of basic chemicals than would be possible if these compounds had to be brought into the cell. In addition, insoluble compounds that cannot cross a cell membrane are also susceptible to attack. Finally, since the relevant enzymes are induced by nutritional signals independent of the target compound during secondary metabolism, they can act independently of the concentration of the substrate, and their frequently non-specific nature means that they can act on chemically diverse substrates. Further, genetic engineering provides the opportunity of transfer of these fungal genes for degradative enzymes can be added to bacteria; alternatively, competent fungi can be modified to grow in an
extended range of area in nature. Common saying tells us that in the real world, complete pathways of degradation are more likely to occur through the combined effects of many organisms. Judicious combination of chemical and physical process with biological schemes also offers promising results.

In lieu of above mentioned discussion the following attempts were made in this research work:

1. The physico-chemical characterization of the vegetable tannery effluent.
2. Screening of tannase producing fungi from the effluent isolates.
5. Molecular characterization of enzymes.
7. Optimization of cultural conditions for enhanced production by isolates.
8. Production of Tannase through submerged fermentation.
9. SSF production of tannase using agriculture byproducts.
10. Studies on the impact of enzymatic treatment on the physico-chemical characters of the effluent.