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Tannase: Hydrolyzing agent for tanning industries

Kannan Natrajan
Department of Biotechnology, Manipal Institute of technology, Manipal-576104, Karnataka, (INDIA)
Tel : 91-0820-24321 ; Fax : 91-0820-2571071
E-mail : biomateix.kannan@gmail.com
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ABSTRACT

Tannins are an important ingredient in the process of tanning leather that prevents decomposition and ususlaly imparts colour to the leather. Tannin is the fourth most abundant plant constituent after cellulose, hemicellulose and lignin. Tannins are water soluble poly-phenols with varying molecular weight depending on the bonds possessed with proteins and polysaccharides. Tannins occur commonly in the wastes from forestry, plant medicine, paper and leather industries. The treatment of this kind of tannin containing wastes, including adsorption, coagulation, liming, settling and biodegradation, is usually difficult because the nature of tannins are highly soluble in water and also it inhibit the growth of microorganisms in activated sludge. The objective of this present paper reviews the investigation about the biodegradability of tannin containing wastes with the help of biological treatment.

KEYWORDS

Tannins;
Biological treatment;
Biodegradation;
Effluent;
Microbial enzyme.

1. INTRODUCTION

Tannase (tannin acyl hydrolase, E.C.3.1.1.20) is an inducible, extra cellular hydrolase enzyme produced in cultures of Penicillium glaucum and found in certain tannin-forming plants; it catalyzes the breakdown of ester and depside bonds present in hydrolysable tannins or gallic acid esters, liberating glucose and gallic acid (GA). Gallotannins are plant polyphenolic compounds that can easily precipitate any protein. Tannase cleaves the ester linkages between galloyl groups present in various compounds such as epigallocatechin and epigallocatechin gallate that are present in green tea leaves[6]. Tannase find wide speared application in the field of food industries used as antioxidant and is mainly used in the production of gallic acid, instant tea, acron wine, coffee flavored soft drinks and high grade leather tannin. Tannase is also used as a clarifying agent in clarification of beer, fruit juice and various food stuffs. Act as a hydrolyzing agent in cleaning up the highly polluting tannin (polyphenols) from the effluent of leather industry[6]. Tannin rich parts of the plants such as fruits, leaves, branches and barks possess considerable amount of tannase. Plants like penduculate oak (*Quercus rubra*), myrobolano (*Terminalia chebula*) and badul were rich in tannase[7]. Tannase can be extracted from bovine intestine and ruminal mucous. The enzyme produced from microbial sources find immense application in various industries due to its higher stability and availability[6]. Among the various microbial sources for tannase production, filamentous fungi like *Ascochyta, Aspergillus, Chaetomium, Mucor, Myrothecium, Neurospora, Rhizopus, Trichothecium, Fusarium, Trichoderma* and *Penicillium* were studied extensively[6]. Tannase

producing yeasts have also been isolated but they were not extensively studied. Bacterial sources such as *Bacillus, Corynebacterium, Klebsiella, Streptococcus bovis* and *Selenomonas ruminantium*[2] have been studied for tannase production. Lactic acid bacteria play a vital role in hydrolyzing tannins present in food and intestines. High tannase activity was reported in lactic acid bacteria *Lactobacillus plantarum*[1].

The tanning processing industry is a miscellaneous industry with a variety of production methods reflecting the diversity of hides and tanneries and the individuality of tanners. In India, the tanning industry is very important for exporting leather as main industrial source. In west costal and southern region of India placed the tanning industries are around residential areas because there is no proper industrial area. These wastes are discharged directly to the main domestic sewage pipeline which adds difficulties to the sewer system and to the waste treatment plants. The discharge of tanneries effluents has the main drawback of creating pollution to air, soil, subsoil, surface, water and underground water due to the presence of toxic organic and inorganic compounds especially polyphenols and chromium which is used in tanning processes, as well as large amounts of water, which results in the generation of large flows of heavily polluted wastes. The hazardous substances pollute the surface water, the soil and the ground water becomes accumulated in food chain and therefore a special need for treatment before being discharged.

Normally the chemical precipitation process used to treat the polluted effluents by physico-chemical procedures, but during the last decade processes based on biological treatments have been increasingly studied and applied. Initial stage of physio-chemical process involves the aerobic processes were used to treat the remaining pollution present in the effluent. However, the most commonly used method is direct treatment of these effluents by high-rate biological units, such as enzymes and modern anaerobic reactors, because of their suitability and stability for the treatment of highly loaded complex wastes. Tannery effluents are characterized by their high organic load and the significant presence of different toxic, recalcitrant or slowly biodegradable substances, some of them have been successfully biodegraded anaerobically[4]. Reclamation of industrial waste is an important work for reuse purpose in developing countries suffering from water shortage. Recycling of waste is mainly depending on the consistent waste treatment that produces the good water quality requirement for the intended reuse application and also it provides the protection of public health. Reuse of treated effluent may have two different forms: water conservation and recycling internally in plants and disposal to a public sewer system in which the waste is treated and later reused for irrigation. The discharge of industrial waste to sewage system is required through effluent standards based on local permit parameters of the country environmental protection act law (EPA). High capacity trickling filters have been tested for their effectiveness in the treatment of the industrial waste such as pulp mill waste, leather industry and highly polluting sources from the heavy metals storage and production units, have proven successful results in industrial application.

Generally the tannery wastes originate from the beam house and tan yards. In the beam house, curing, fleshing, washing, soaking, de-haring, lime splitting, bating, pickling, and degreasing operations are carried out. The final stage of producing leather involving in the tan yard by various step processes, these include vegetable or chrome tanning, shaving and finishing. Here the finishing operation process includes bleaching, stuffing, fat liquoring and coloring.

1.1. Tannins

Leather industry is expecting for the metal-free tanning systems and mainly the naturally available tanning materials such as vegetable tannins are gained importance. Well known that the plants synthesis different kind of polyphenolic compounds, some of the polyphenolic substances has the ability to form the tannins. The presence of tannins in the wastes would cause many problems associated with environmental pollution and waste treatment. Owing to the presence of tannins, the wastes are usually highly colored, which is very difficult to be eliminated by common methods like dilution and adsorption In addition, tannins can inhibit growth of microorganisms and therefore, are toxic to activated sludge. This negative effect can be observed when tannin-containing wastes are biologically treated in waste treatment plants[3].

Vegetable tannins are one of the oldest materials used for tanning hides and skins. The term tannin as the substance which mainly involves the process of con-

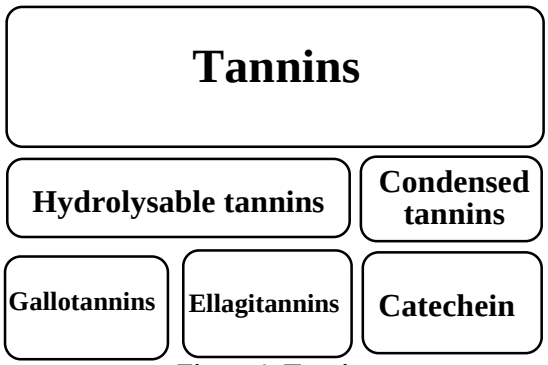

Figure 1: Tannins

verts the decayable hide or skin into imperishable leather. The simple and most acceptable definition for tannins is that of "water soluble phenolic compounds having molecular weights between 500-3000 and, besides giving the usual phenolic reactions, they have special properties such as the ability to precipitate alkaloids, gelatin and other proteins". Phenolic compounds are widely distributed in the plant kingdom and are considered to be secondary metabolites. Structurally they contain an aromatic ring bearing one or more hydroxyl groups, together with a number of other substituents. Tannins are secondary metabolites found in plants and widely distributed in the plant kingdom. Tannins are classified in to two subclasses according to their chemical nature and structural characteristics[3].

Hydrolysable tannins are composed of esters of gallic acid (gallotannins) or ellagic acid (ellagitannins) with a sugar core which is usually glucose, and are readily hydrolysed by acids or enzymes into monomeric products. The most commonly used hydrolysable tannins in the leather industries are chestnut, tara, valonea, sumach, divi divi, algarobilla and myrabolans. The polyphenolic composition of fruits varies in accordance with species, cultivar, degree of ripening and environmental conditions of growth and storage. Phenolics also contribute to colour, astringency, bitterness, and flavour in fruits. Valonea tannin, obtained from tannin rich fruits of acorn cups of *Quercus* species, have been used to tan hide and skin either alone or accompanying with other tanning materials in leather industry. The condensed tannins which are used as tanning materials in the leather industry are normally polymerised by molds and are also decomposed by soil microorganisms. It was therefore of interest to investigate the action of *Aspergillus flavus,* isolated from soils contaminated with tannery wastes, on condensed tannins and develop a method for estimating tannins. Condensed tannins, also known as polymeric proanthocyanidins, are composed of flavonoid units, and are usually more abundant in tree barks and woods than their hydrolysable counterparts. Tannery effluents discharges with an average of 66 to 100 liter of waste per kg of wet settled hide processed. The pH level of the waste has the range from 11 to 12 and also it produces a high concentration of sludge 5 to 10% due to presence of polyphenols and heavy metals such as chromium, sulphide etc., In the traditional tanning process were used the natural tannin extracts as a main compounds, nowadays being almost completely replaced by condensed tannins and chromium. However, these tannin substances are still used in modern industries for the purpose of the fabrication of shoe soles and as a complement to chromium in the retanning process. Biological anaerobic treatment has been successfully applied for the process of both simple tannin compounds[4] as well as more complex wastes such as those generated by the forest industry.

1.2. Tanneries and leather industry

The tannery sludge is a combination of hair, fleshings, shavings, splits, hide/skin trimmings, leather trimmings, buffing dust, leather finishing residues, general plant wastes, and waste water treatment sludge. Nowadays the tanneries also use chemical agents like chromium salts in a smaller quantities along with the natural tannins. According to Imamul Huq[5], various chemicals are used during the soaking, tanning and post tanning processing of hides and skins. The main chemicals used include sodium sulphite and basic chromium sulphate including non-ionic wetting agents, bactericides, soda ash, CaO, ammonium sulphide, ammonium chloride. The sole chromium salts are not of the same quality as natural tannin powders which produce the excellent, durable leather ideal for shoe soles and also it has the hazardous activity. Until the mid of 19th century, domestically produced natural plant tannin was readily available to tanneries. In particular, shrubs such as algaroba whose fruit contains up to 65 percent tannin were used.

Adsorption, membrane filtration, photocatalytic and sonochemical degradation these are the steps and methods employed for the treatment of tannin containing waste process. For the commercial treatment of tannin

containing waste process involves the sequentially procedure of chemical settling and neutralization process along with the biological degradation method used for discharging or removing the toxic compounds present in the tannery waste. As a result of above statement, a direct biological treatment technique required for the purpose of treating the tannin containing waste, without undergoing any pretreatment of settling and unhairing process. In general tannin has the ability to inhibit the microorganism growth, but some microorganism has the positive resistant to tannins and it can take tannin as a carbon source[3] in proper physic chemical conditions.

Moreover, the current development and application of cleaner technologies using enzymatic treatment to this sector implies, a step by step implementation of an improved process water management, which commonly results in the discharge of lower flows of wastes.

TABLE 1: Methods of biological treatments

Process	Treatment agent (s)	Wastes Treated
Trickling filters	Packed bed (stones or synthetic) converted by microbial film	Poly phenolic compounds, Acetaldehyde, benzene, chlorinated hydrocarbons, nylon and rocket fuel
Activated sludge	Aerobic microorganisms suspended in waste	Refinery, tannery effluent, petrochemical and Biodegradable organic wastes.
Aerated lagoon	Surface impoundment + mechanical reaction	Biodegradable organic chemicals
Waste stabilization ponds	Shallow surface impoundsments +aeration to promote growth of algae and bacterial symbiosis	Biodegradable organic chemicals, Tannery and Leather industrial waste.

2. Mechanism of biological treatment-enzymatic action

The objectives of biological treatment of wastes are to remove the non-settleable colloidal solids and to degrade other organic matter. For industrial waste, the objective is to remove or reduce the concentration of organic and inorganic compounds. Because many of these compounds are toxic to microorganisms, pretreatment may be required. With proper analysis and environmental control, almost all wastes can be treated biologically (TABLE 1).

Chemical coagulation, biological treatment and adsorption are the methods for the treatment of waste from tanneries for organic substances and heavy metals removal. Enzymes are specific, often stereo-selective, catalysts, which do not produce unwanted byproducts. Consequently, there is less need for extensive refining and purification of the target product. Compared with chemical processes, the enzyme based processes are 'environmentally friendly' as enzymes are biodegradable and there are fewer associated waste disposal problems. Certain enzymes are not restricted to aqueous environments and can operate in two-phase water-organic solvent systems and in non-aqueous organic media, particularly hydrophobic solvents. Operation under such conditions can often improve enzyme performance, especially where substrates have limited water solubility.

Nowadays many industries are dependent on mi-

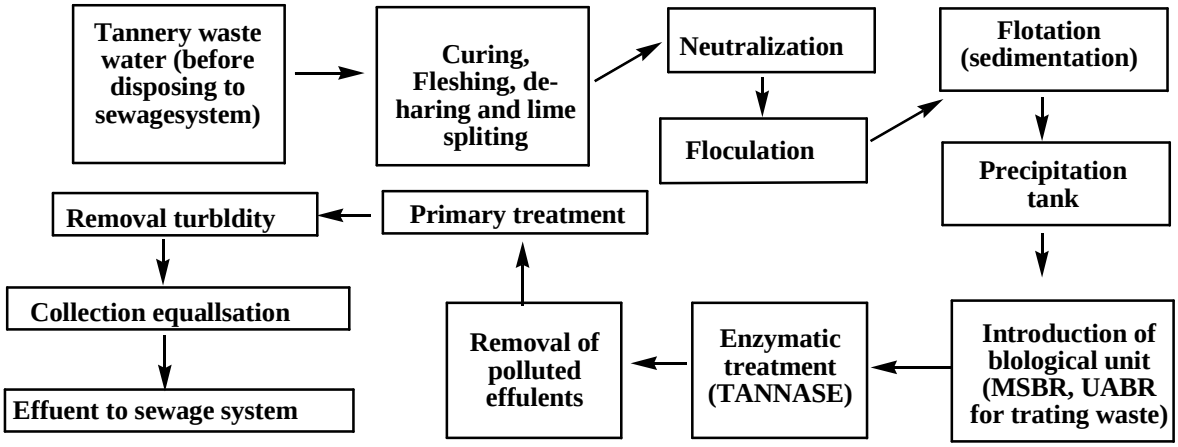

Figure 2 : Schematic diagram for treatment of tannery waste using Biological units and microbial enzymes

crobial enzymes for the production of their goods. Enzymes have been used in the tanning industry for centuries because they are efficient in degrading protein and fat. In the beginning, the enzymes were derived from animal excrement and later on from the pancreas of cattle. Nowadays, the enzymes are produced by microbial fermentation. Microbial enzymes are highly specific and fast in action and are used in the tanning industry in soaking and unhairing processes because they can replace chemicals and shorten processing time, thereby reducing production costs. Textile enzymes are the third most significant segment of this market. The major enzymes in this category are enzymes for processing cotton and cellulosic textiles, followed by enzymes for processing leather and fur.

The main agenda of this review gives the idea about enzymatic treatment for achieving the good result in the tanning industry, namely soaked and unhaired/limed bovine hides using either an entirely chemical method or using an enzymatic method. Tannase enzymes act on the final leather product to some extent and it increases the area of the final yield product. However, this increase is difficult to quantify exactly. The use of enzymes in the soaking and unhairing/liming processes does not influence other processes in leather-making and processes. The environmental friendly enzyme production and delivery to the tannery industries has the impact of saving chemicals and electricity, also it contribution to global warming. The global tanning industry generates approximately 4 million tonnes of solid waste per year. As the economic and environmental costs of tannery waste disposal is very high and the cost associated with the use of fossil fuels to generate energy continues to spiral.

The process indicates the movement of the hides and skins through the treatment tank, initially it undergoes the primary chemical treatment for the purpose of removal of unwanted heavy metals present in the sludge than it goes to the enzymatic treatment with the help of biological treatment, using biological units such as membrane sequential biological reactor, upstream biological reactors etc., Then after it reaches the recycling process of effluent and it involves the process of producing renewable energy sources and saving the electricity. The working principle of tannin degradation on the tannery effluents by different microorganisms gives the detailed account information about the biodegradation of natural environments, especially in the case of hydrolysable tannins[8]. A switching from conventional to enzyme assisted soaking and unhairing/liming are applicable worldwide, this involves global energy saving potential improvement in the environment. Currently, the most commonly used biotechnology applications cover all the stages of leather making processes and treatment of waste. An emerging technology is the use of enzymes in unhairing process, which minimizes/replaces the use of sulphide, a major pollutant in the tannery.

3. Concluding remarks

Tannase is produced by microorganisms fed with carbohydrates produced manily using the agriculture source, and the environmental gets beneficial in terms of avoided environmental impacts and pollution and also it involves the energy saving process like producing the renewable energy. The saving energy turns out to be the most important environmental effect of enzyme use. The direct biological treatment reduces the effect of pollution due to the heavy metals present in the tanning industry to the environment and human health have been affects due to the presence.

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