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ORIGINAL ARTICLE

Physico-chemical treatment of tannery effluent by using Halophilic Bacterial strain *Bacillus subtilis* and Protease Enzyme

Aji R and Raja Jeya Sekar R
Department Of Zoology, S.T. Hindu college, Nagercoil,
[Manonmanium Sundaranar University, Tirunelveli.]

ABSTRACT
Production of untreated effluents which cause the pollution of air, water, soil and soil solid waste were the results of noval industries or enlargement of existing industries. In recent years, industrial effluents have been regarded as common source of pollution due to inappropriate disposal methods by industries. Water pollution is the major pollution that effects the environment much and one of the sources of this pollution is tannery industrial effluents. Effluents are so toxic that fishes cannot survivein it even for two hours and also effects the drinking water and hence it should be treated effectively before release. Bioremediation is the only way to tackle these xenobiotics and reduce the pollutants which is eco-friendly. The present work is an attempt which has been made to analyze the effectiveness of Bacillus subtilis against tannery effluents. The substrate can be degraded by specific protein breakdown enzymes secreted by some microbes. In several industries like pharmaceuticals, textiles and leather, proteases can be app lied. The production and activity of other available enzymes have to be studied for applying in various cost effective process.
Key words: *Bacillus subtilis, Bioremediation, Tannery effluents, Xenobiotics, protease, waste degradation.*

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INTRODUCTION

Tanneries are responsible for environmental pollution as they use huge amounts of chromium sulphate, Cr(III) in the leather tanning processes[1,2,3]. Indian progress and elevated life styles of individuals are the indicators of industrialization and economic development. However, industrialization brought many environmental hazards like land, water, soil and soil solid pollution[4,5,6].All the sectors like industry, agriculture, construction, transportation, mining and consumers of our society generate waste materials[7,8,9]. Industries have been regarded as a major source of pollution due to inappropriate treatment of waste disposal methods. Waste materials secreted above a limit of the environment, pollution can be resulted. According to particular process of industry, the amount of waste and its toxicity are secreted. In industry waste, tannery effluents are first pollutants in rank list[10,11,12]. Water pollution is a major pollution that effects the environment much and one of the main sources is industrial effluents [13,14]. The inherent nature of the tanning process is such that large quantities of water are consumed. Tannery waste is a major hazard that affects the drinking water and hence it should be treated effectively before disposing. Chrome tanning is the major pollution by leather industry. Chrome tanning is the most common type of tanning in world[15,16,17]. *Bacillus subtilis* are a great interest for the biotechnology. *Bacillus subtilis* is a gram positive organism found in soil and GIT of ruminants and humans. These were popular worldwide before the introduction of antibiotics. By using the *Bacillus subtilis,* we are converting the harmful tannery effluents to less harmful[18, 19, 20].

The enzyme proteases is one of the all rounder, because it involves in the different biological reactions like cellular and organism level and also in nitrogen circulation naturally [21,22]. Exoprotease (cleaves at the end of the polypeptide chain) and endoprotease (cleaves inside the chain) are the two classes of protease enzyme, based on the site where cleavage happens (hydrolytic enzyme)[23,24]. This enzyme can be divided into seven groups; serine, cysteine, threonine, aspartic proteases., glutamic proteases, metallo proteases and asparagine peptide lyases depends on the sequence of amino acid at active sites of enzyme

[25,26]. On the basis of sequence similarities and evolutionary relationships between the proteolytic enzyme sera of sequences are introduced for upgrading system of classification[27]. The enzyme proteases are the only one group of enzyme that will conduct the breakdown of complex and recalcitrant proteins completely, with having wide range of temperature and pH. The ability to bind to the complex and insoluble substrates(feathers, wool, silk, collagen, elastin, horns, stratum corneum, hair, azokeratin and nails) is the only one difference between protease from other proteases. Mechanism of enzyme adsorption is not studied well till now, but there is a known point that, if there is adsorption capacity is higher, there is also higher hydrolysis of keratin. Confirmation changes of keratin and its exposes to multiple sites for hydrolytic action of enzymes because of the binding of enzyme and breakdown of disulphide bonds [28,29].Proteases belong to serine and metallo-proteases or serine metallo-proteases based on the nature of active site. At the active site of enzyme, various group of proteases having nucleophilic serine residues (Ser) are situated. Intermediate of acyl enzyme is formed by the latter attacks carbonyl part of the peptide bond[30,31]. Proteases are robust and having various biochemical properties. Most of the protease are monomeric in nature and also there are some information about multimeric enzymes [32,33]. The range of molecular weight of bacterial protease from 18 (enzyme SK1-02 from *Streptomyces albidoflavus*) to 200 kDa (enzymes from Kocuriarosea and *Fervido bacterium islandicum*), at the same time the pathogenic fungi enzymes can reach up to 440 kDa (for example keratinase II of Trichophytonmentagrophytes)[34,35]. Metalloproteases having high molecular mass and it derived from thermophilic microbes. Keratinsdoes not assembled naturally, despite of the resistance to proteolytic enzymes, which shows the existence of keratinolytic microbes occurs naturally[36,37].

Multistep procedure of keratin degradation having following steps: (i) using electrostatic and hydrophobic interactions, protease adsorption to the macromolecule surface, and (ii)catalysis. Keratin breakdown possess two main procedures: disulfide bond reduction(sulfitolysis) and protein degradation [38,39,40]. With the help of some compounds that are reducing; like sodium sulfide, dithiothreitol or DTT, mercaptoethanol, glutathione, cysteine, thioglycolic acid or disulfide reductases, disulfide bond reduction can occurred[41,42]. The reducing compounds can incorporate with protease in keratin degradation. According to the growth stage of microorganisms, microbial synthesis of proteolytic enzymes is highly regulated and complex process. Most of the protease enzymes are inducible, while some of them are constitutive. One of the important is that, proteases expression constitutively with the help ofcaseinolytic and not with the activity of keratin degradation. At the end of the exponential and/or stationary phase the synthesis may be intensive, it is connected with the lack of nutrients adaptation. It shows that the synthesis of proteases may be controlled by stress of nutrients, like absence of carbon and nitrogen sources[43,44]. The cellular GTP concentrations can varies by the absence of amino acids in medium. Maintains a repressor of transcription, Cod Y in activated form, when availability of higher nutrient followed by the higher GTP concentration[45,46]. For specific enzyme, transcription of the gene encoding were prevented by the association of repressor with operator. GTP concentrations decreases and then inactivates CodY by nutrients limitation [47,48].

The serine proteases producing strains are included in the Bacillus genus. In white biotechnology, the above genus has a role for multiple reasons –most of the species are non-pathogenic (for example *B.* cereus*, B. stearothermophilus, B. licheniformis, B.* subtilis, etc.) and multiple functional extracellular proteins are produced into the culture medium[49]. Other producers are representatives of the genus Streptomyces, Actinomycetes, Nocardiopsis and Oerskovia. Pseudoalteromonas, Colwellia, Flavobacterium and Shewanella are the main psychrotrophic bacterial genera. Due to adaptive structural flexibility, the enzymes derived from those organisms are active under low temperature ranges [50]. For applications of biotechnology and for research activities, archaeaplays a major source of proteases that is extremophilic. New strains isolation that secreting better products of excellent performance by target enzymes is required for the growthof a viable industrial fermentation procedures. Unlimited source of noval protein degrading strains for the execution of new industrial procedures and enhancement of the already existing ones [51].

Sodium sulfide, lime and solid wastes are the chemicals, which are the byproducts of preliminary tanning, enhance biochemical oxygen demand or BOD, chemical oxygen demand or COD and total quantity of dissolved solids or TDS in wastewater secreted in these plants. The activity of enzyme will helps in reducing environmental pollution and also enhance the leathers[52]. Nowadays protein degrading enzymes are mainly utilized for conceal softening to enhance pliability of the conceals and manufacture them for tanning[53]. For the preparations of different keratin degrading enzyme, for the removal of animal hair are used without affecting the skin (collagen). They will specifically breakdown the soft keratin tissue present in the follicle, follows by without influencing tensile strength of the leather carry out entire hair. The enzymes, are mainly derived from Bacillus sp., Pseudomonas stutzeri,

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Caldicoprobacter algeriensis, *Acinetobacter* sp., *Paenibacillus woosongensis*, Vibrio metschnikovii and various fungi from *Aspergillus tamari*, *Penicillium chrysogenum* and *Trichoderma harzianum* have been identified to be utilized in leather industries[54].

In leather industry, protease application will enhances the final product quality, and decreases pollution in environment by chemicals, gives safe climate for working. Protease used in textile industry for wool fiber processing[55]. Structural proteins having high range of cross-linked disulfide bonds is known as wools, it provide mechanical strength and degradation resistance to fiber. Absence of wool character, material yellowing, organically bound halogens that is adsorbable (AOX) containing polluted waste water, biodegradability of fabric, high energy and time consumption were the demerits of the process. Natural alternatives for the chemicals is made up of proteases and lipases, which is utilized in preparations of enzyme. The roughness of wool can be decreased by removing coarse fiber layer outer layer with the help of protease.It shows that, any of the process is not depended on enzymes, currently[56].Protease specifically affects on layer of keratin in wool, while it doesnot causes any negative action on other fiber parts.

MATERIAL AND METHODS

Collection of Tannery Effluent Waste Water

The tannery effluent waste water was collected freshly from Common effluent treatment plant, pallavaram, Tamilnadu. It stored in a brown bottle. Prior to the collection, the sample water bottle was rinsed with sterile water. The samples were taken to the laboratory as early as possible; it has to be protected from the direct sunlight during transportation. The samples were stored in a refrigerator.

Collection of Microbial Strain

Bacillus Subtilis were used for the effluent treatment. The above strains were obtained from Microbial Type of Culture Collection (MTCC), Institute of Microbial technology (IMTECH),Chandigarh. The cultures were maintained as per required norms.

Growth and Maintenance of the Culture

Primary culture of *Bacillus subtili* was prepared by inoculating the primary culture in nutrient broth and incubated at30^{o}C for 24 hours. The cultures were maintained by subculture process regularly done for once in 30 day.The halophilic bacterial strain *Bacillus subtilis* strain streaked at the centre of the sterile skimmed milk agar plate showed a dumb bell shaped zone around the bacterial colonies at 37 °C and 24 hours of incubation.

Tannery Effluent Water Treated with Microbes

Take the both water samples as 80ml, 70ml, 60ml and 50ml in a conical flask and add the *bacillus Subtilis* culture. For the80ml effluent add 20ml of culture, 70ml effluent and 30 ml of culture,60ml effluent and 40ml of culture and for 50ml effluent 50ml of culture was added respectively and incubated. At the same time blank was also inoculated with culture and incubated. After 5 days of incubation, the physical and chemical parameters were evaluated and tabulated.

Evaluation of Physicochemical Properties

The *Bacillus subtilis* treated water samples were tested by following properties as per the standard procedure prescribed by Bureau of standards (BIS) i.e., Appearance, Color, Odor, turbidity, total dissolved solids, pH, total alkalinity, hardness, Calcium, Barium,Chromium, Magnesium, residue free chlorine, Copper, Nitrate, Nitrite, Manganese, Aluminium, Ferrous, Fluoride, Sulphate and Zinc.

RESULTS

Screening of *B. Subtilis* Strain AJ for Protease Activity

Thehalophilic bacterial strain *Bacillus subtilis* strain streaked at the centre of the sterile skimmed milk agar plate showed (Fig:1) a dumb bell shaped zone around the bacterial colonies at 37 °C and 24 hours of incubation.

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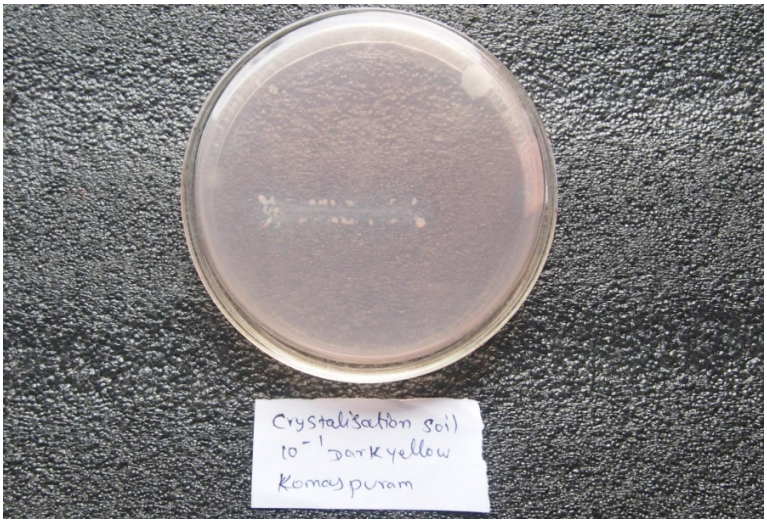

Fig:1Screening of *B. Subtilis* strain for protease activity

Influence of pH and Temperature

Ph 8, Temp. 35 °C

Influence of NaCl Concentration on *B. subtilis* Strain AJ for Protease Production and Biomass

Max production - 4 % , Enzyme production 71.4± 0.05U / ml. Biomass 26.4± 0.05mg / ml

Min production - 12 % , Enzyme production 12.9 ± 0.02U / ml. Biomass 3.2 ± 0.01 mg / ml

Influence of Incubation Time on *B. subtilis* Strain AJ for Protease Production

Max production -72 hr, Enzyme production 73.9 ± 0.02U / ml. Biomass 27.4± 0.04 mg / ml

Min production -24 hr, Enzyme production 12.8 ± 0.03U / ml. Biomass 3.5 ± 0.05 mg / ml

Influence of Carbon Sources on *B. subtilis* Strain AJ for Protease and Biomass

Glucose, sucrose, fructose, xylose and starch

Max production -Fructose, Enzyme production 67.3 ± 0.04U / ml. Biomass 23.1 ± 0.04 mg / ml

Min production- xylose, Enzyme production 46.3 ± 0.01U / ml. Biomass 12.2 ± 3.03 mg / ml

Influence of Nitrogen Sources on *B. subtilis* Strain AJ for Protease and Biomass Production

Beef extract, yeast extract, casein, peptone, gelatin

Max production- Yeast extract, Enzyme production 75.1 ± 0.05U / ml. Biomass 29.7 ± 0.03 mg / ml

Min production - gelatin, Enzyme production 41.6 ± 0.04U / ml. Biomass 10.5 ± 3.01 mg / ml

Influence of Metal Ions on *B. Subtilis* Strain AJ for Protease and Biomass Production

Beef extract, yeast extract, casein, peptone, gelatin

Max production - manganese chloride, Enzyme production 78.1 ± 0.02U / ml. Biomass 30.3 ± 0.03

Min prodcution- ammonium sulphate, Enzyme production 25.0 ± 0.01U / ml. Biomass 5.7 ± 0.01 mg

Physicochemical Parameters

The *Bacillus Subtilis* treated water samples were tested by following properties like pH, temperature(Fig:2), BOD, COD, TDS, Chlorides, hardness, chromium(Fig:3) and percentage reduction by above parameters(Fig:4)

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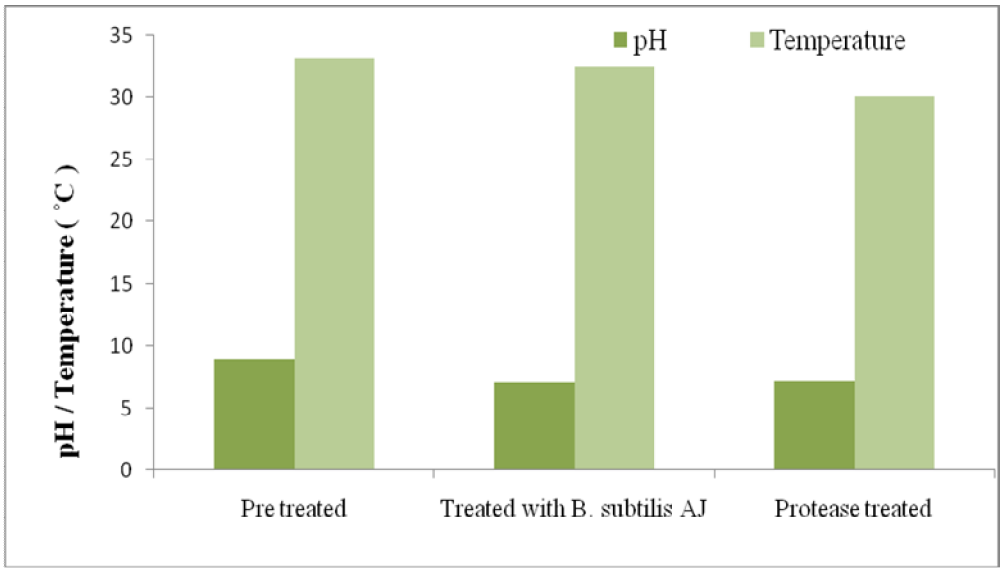

Fig:2 Influence of pH and temperature

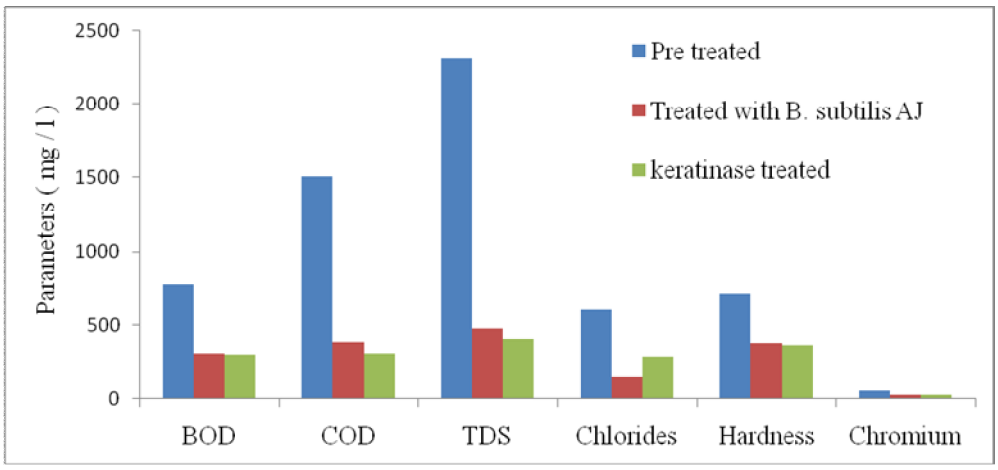

Fig:3 Influence of different parameters

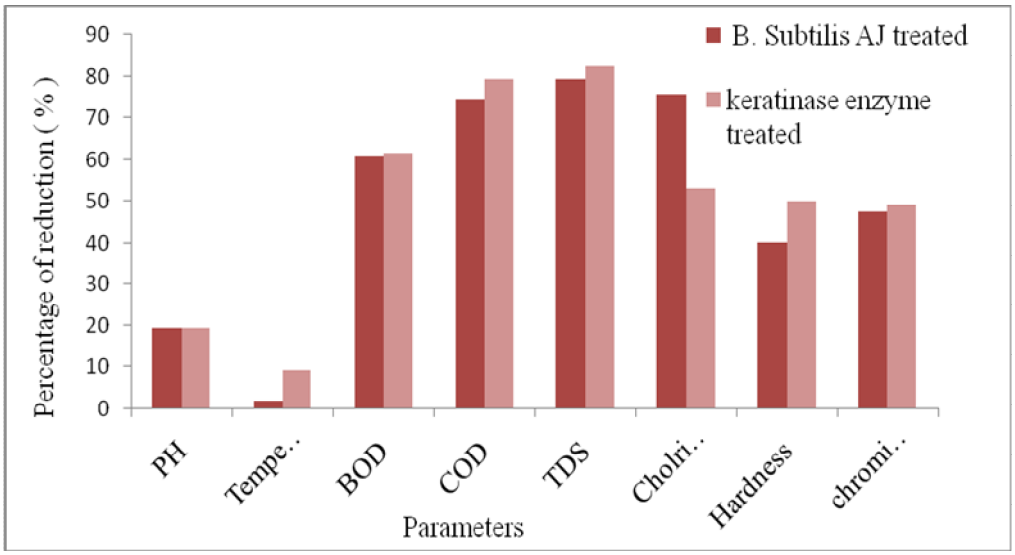

Fig: 4Percentage of reduction by the effect of different parameters

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CONCLUSION

Bioremediation is a process of clearing waste materials, having low solubility, moving capacity and toxic effects. It may be due to the concurrent process of absorption and metabolism properties of the microbial consortia, includes the heavy metal tolerance by permeability barrier, intracellular and extra-cellular sequestration, active transport efflux pumps, enzymatic methods and also reduction in the sensitivity of targeted cellular organelles to metal ions. It is evident that *Bacillus subtilis* is very effective against tannery effluents. The obtained physicochemical values compared with standards for safe drinking water prescribed by Indian Bureau of Standards (BIS). The role of proteases in agriculture and waste management of food industry have to be well studied. Complex substrates efficiency, decreased stability and production expensive are the main issues of enzymes. To avoid these issues using various processes to prepare cost effective applications. For increasing affordability of production of protease can be done by developing new tool or genome editing of available and new strains. Replacing of current practices using enzyme depended treatments of wastes of keratin possess major role and also it provide helps in humanity welfare, change of climate and species survival by future techniques.

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