BIODEGRADATION OF TANNERY EFFLUENT BY *ASPERGILLUS* SPP

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Abstract: Tannery industry is reputed globally as a major industry, which contributes to water pollution. The tannery effluent wastes are ranked as one of the high polluting waste due to a heavy load of pollutants. The collected tannery effluent showed very high BOD and COD values. An indigenous fungal isolate from soil near the effluent discharged area with potential tannase activity was selected for degradation study and was identified as *Aspergillus spp*. This study also focused on optimization of conditions that are necessary before any degradation experiment. The microbiological and enzymatic degradation of tannery effluent showed a decline in all the initial physicochemical parameters after degradation. The biodegradation of tannery effluent by *Aspergillus spp* decreases its tannin content and represents a valuable source of tannase for potential application in various industries.

Keywords: Tannery effluent, Tannin and *Aspergillus spp*.

Introduction: The environment is always under constant and continued pressure from solid and liquid wastes emanating from the tanning industry. The tannery effluents are ranked as high pollutant wastes among all other industrial wastes due to a heavy load of pollutants like Tannins, chromium, chlorides, sodium, dissolved solids, BOD, COD and suspended solids.

Tannery effluents containing large amount of wastes especially tannins when discharged into watercourse affects physical, chemical and biological characteristics of water and deplete the dissolved oxygen, which in turn affects the aquatic life [1]. As the tannins enter the soil organic matter pool, they affect several aspects of ecosystem functioning by reducing the rate of decomposition of soil organic matter by inhibiting the biodegradative enzymes of the attacking organisms and nitrogen availability in the soil possibly because of their protein binding properties [2]. Hence, it is important to give destructive treatment to tannins. Tannase, an inducible enzyme is quite versatile and efficient in degrading tannins [3].

The present study dealt with the isolation of potent tannase producer and optimization of the conditions for degradation of tannery effluent.

Materials And Methods:

All the reagents and chemicals required were obtained from Hi Media, Mumbai.

Sample collection: The tannery effluent was collected from the discharge left by leather processing unit in Dharavi, Mumbai. Effluent sample was collected from the unit's outlet which eventually flowed into the nearby reservoir in clean plastic container. Immediately after collection, the effluent was brought to the laboratory and stored at 4^{o}C.

Isolation and Selection of potent tannase producer:

Tannase producing fungi were isolated by enrichment culture technique from soil collected from the nearby area where the tannery effluent was being discharged. Isolation was carried out

using Sterile Mineral salt medium with tannic acid. Tannase producers were observed for zone of clearance around the colony after the addition of $FeCl_3$ solution. The morphologically distinct fungi were selected and then studied for their tannase activity quantitatively [4]. The isolate showing highest tannase activity was selected for further study and was identified.

Optimization of degradation conditions:

The tannery effluent degradation parameters were optimized with respect to Incubation period and condition (static or shaker), temperature (30°C, 37°C and 55°C). Effect different carbon sources (1% concentration) and Nitrogen sources (0.3% concentration) on degradation of tannin were also studied.

Production and extraction of tannase:

Tannase enzyme was produced under all optimized conditions and extracted by ammonium salt precipitation method.

Degradation of the tannery effluent for safe disposal:

Degradation of tannery effluent was carried out in Erlenmeyer flask for 8 days under static condition by microbiologically (isolated tannase producer) and enzymatically (extracted tannase by ammonium precipitation method). The collected effluent was analyzed for various physicochemical characters like pH, colour, the total suspended solids, total dissolved solids, BOD (biological oxygen demand) and COD (Chemical Oxygen demand) as per standard methods of APHA [5] and the tannin content [4].

Results And Discussion:

The native microflora can be acclimatized for degradation of specific types of wastewaters [6]. The mechanism of acclimatization includes factors such as enzymatic, multiplication of specialized microorganisms, genetic exchanges, inorganic nutrient limitation and toxicity. The tannase producers were isolated from the soil contaminated with tannery effluent using tannic acid as sole source of carbon by enrichment culture technique. The fungal isolate with highest tannase activity was selected on the basis of qualitative and quantitative tannase assay and identified to as *Aspergillus* spp. on the basis of its morphological and physiological characteristics. Fungus like *Aspergillus species, Penicillum species* have been reported as potent producer of tannase [7].

The selected fungal isolate was found to grow upto 10% tannic acid concentration but tannase activity was observed only upto 4% concentration of tannic acid. Some species of *Aspergillus* has been reported to tolerate upto 20% tannic acid [8] & [9].

Tannin degradation by *Aspergillus* spp was more under shaker condition after 4 days of incubation (Fig. 1). The results are in agreement with that of Rana and Bhat [10] who performed submerged fermentation using *Aspergillus niger van Tieghem MTCC 2425* for tannase production.

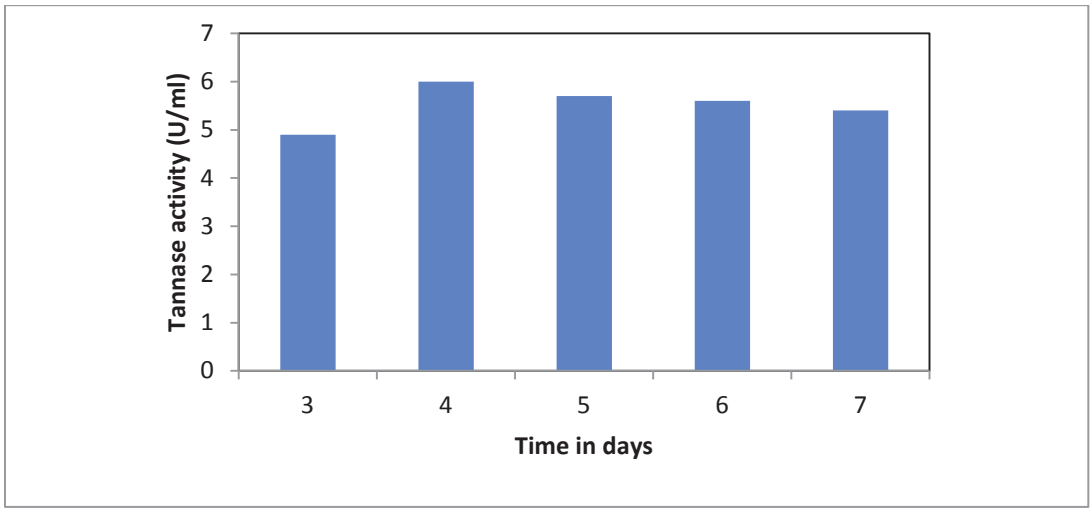

Fig 1: Tannase activity with respect to incubation period of isolated *Aspergillus* spp.

Incubation temperature of 30°C gave maximum tannnase activity. Higher incubation temperature had an adverse effect on the tannase activity. Similar results have been reported by Sabu *et al.*, [11] and Banerjee *et al.* [12]. Supplementation of the effluent sample with different **carbon source** showed a negative effect on tannase production except cellulose (Fig. 2). Costa *et al.* [13] showed that tannase of *A. tamari* was produced only in presence of tannic acid, methygallate and gallic acid. $NaNO_3$ was found to be the most suitable nitrogen source for tannase production by isolated *Aspergillus.* However, other nitrogen sources showed lower values of tannase activity. Similar results have been reported by Paranthaman *et al.* [14] and Bradoo *et al.* [15].

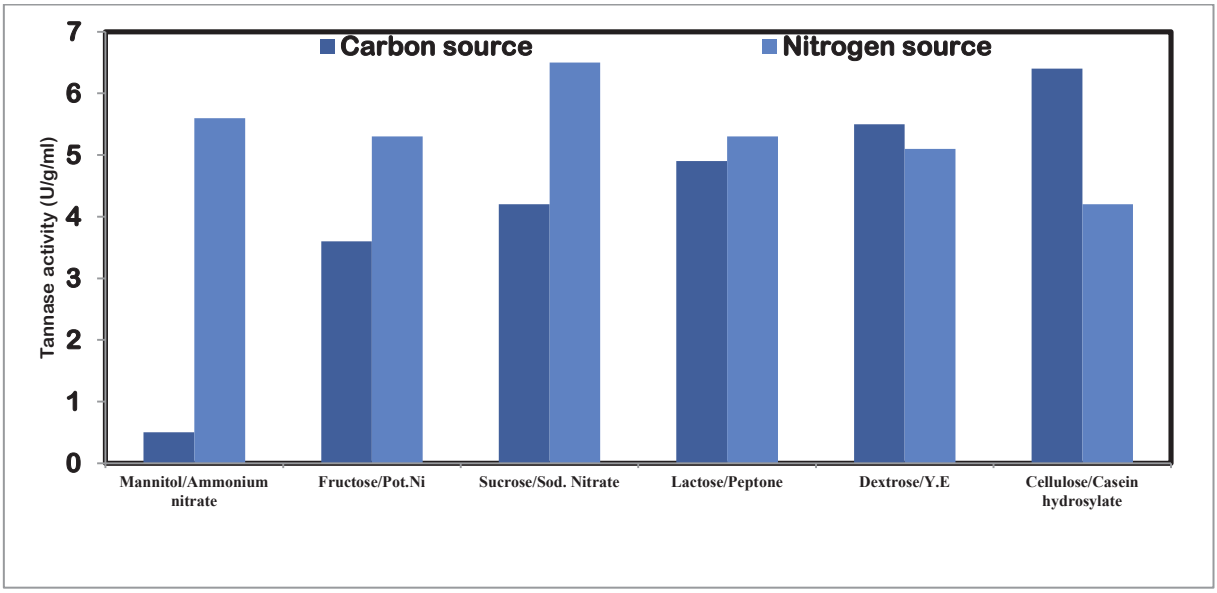

Fig: 2 Effect of different carbon and nitrogen sources on tannase activity of *Aspergillus* spp.

The tannery effluent collected from leather processing unit in Dharavi, Mumbai was analyzed for various physiochemical characteristics (Table 1). The colour of the collected effluent sample was dark grey which is indicative of the high degree of pollution caused

by humus materials, peat, chemicals and weeds. The treatment of the sample with isolated *Aspergillus species* showed the prominent reduction in the extent of grey color compared to extracted enzyme. This might be due to the conversion of tannin from the effluent into gallic acid and glucose. The results are in agreement with that of Murugan and Al Sohaibani, [16]. The pH of waste water could vary due to the presence of various tanning and colouring materials. The isolated *Aspergillus species* showed reduction in the pH of the effluent to 6.5 from initial pH of 8.8. The isolated *Aspergillus species* showed prominent reduction in the pH of the effluent compared to extracted tannase enzyme (7.2) over a period of 8 days.

Table 1: Analysis of the collected tannery effluent.

Characteristics	Observed values (mg/L)
TS	11270
TSS	780
TDS	10490
Tannin content	4320
BOD	940
COD	4800

Total solids give an idea about the amounts of solids present and pose severe problems of water pollution. Suspended solids impart turbidity to water and make water unfit for use. The degradation results showed significant reduction in the TS of the effluent sample over a period of 4 days by *Aspergillus spp* (Fig. 3 and 4).

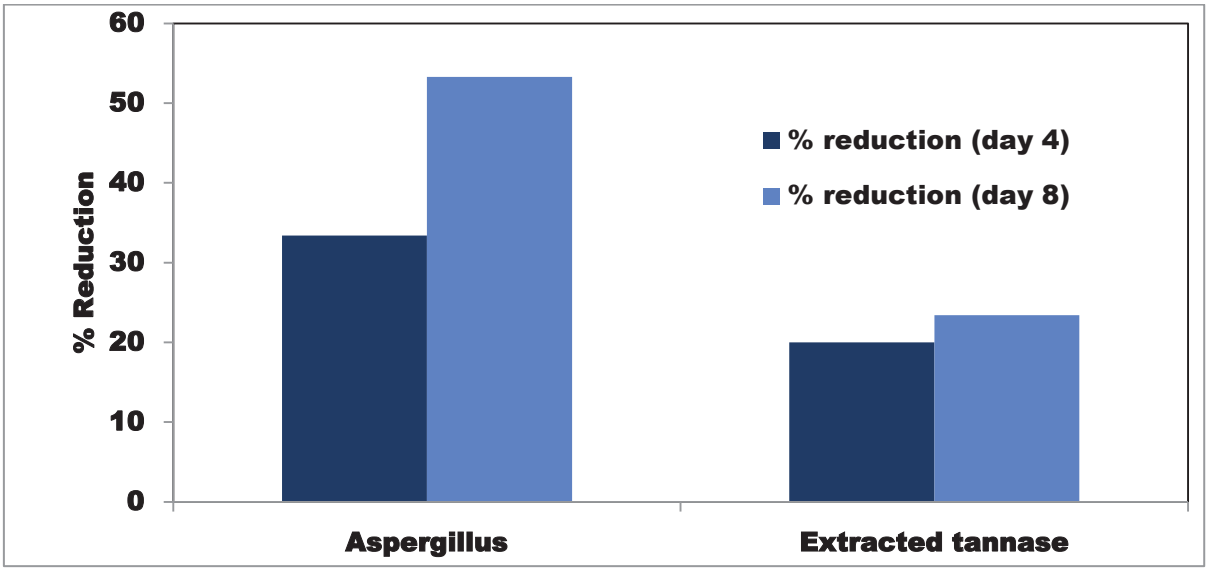

Fig: 3 Percentage reduction in the total solids during degradation of the tannery effluent.

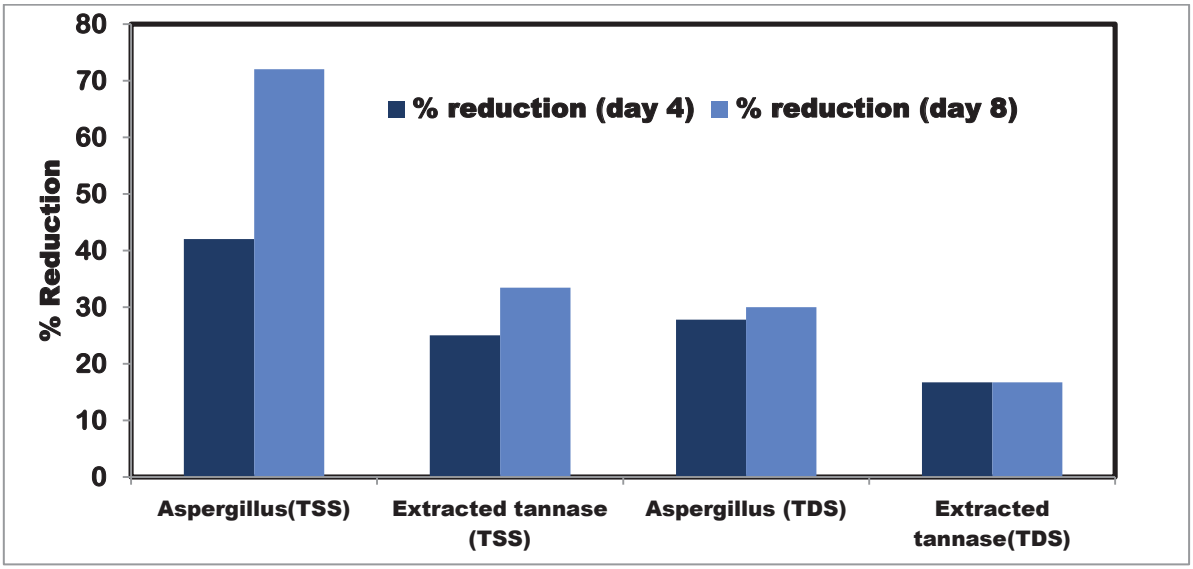

Fig: 4 Percentage reduction in the total suspended solids and the total dissolved solids during degradation of the tannery effluent.

Tannins cause various health hazards like interfering with iron absorption and damaging of mucosal lining of the GIT. Tannins are considered to be one of the major pollutants of tannery effluent. Hence biodegradation of tannins was done by treating the tannery effluent sample with isolated *Aspergillus species* and was found to be highly efficient in the removal of tannin content of the effluent (Fig. 5). Rajakumar and Nandy [17] and Raaman *et al.*, [18] have recorded the hydrolysis of tannic acid and other tannin into glucose-gallic acid intermediates. Complete hydrolysis of tannic acid to gallic acid by *A. japonicus* has been noticed after 48h incubation [15].

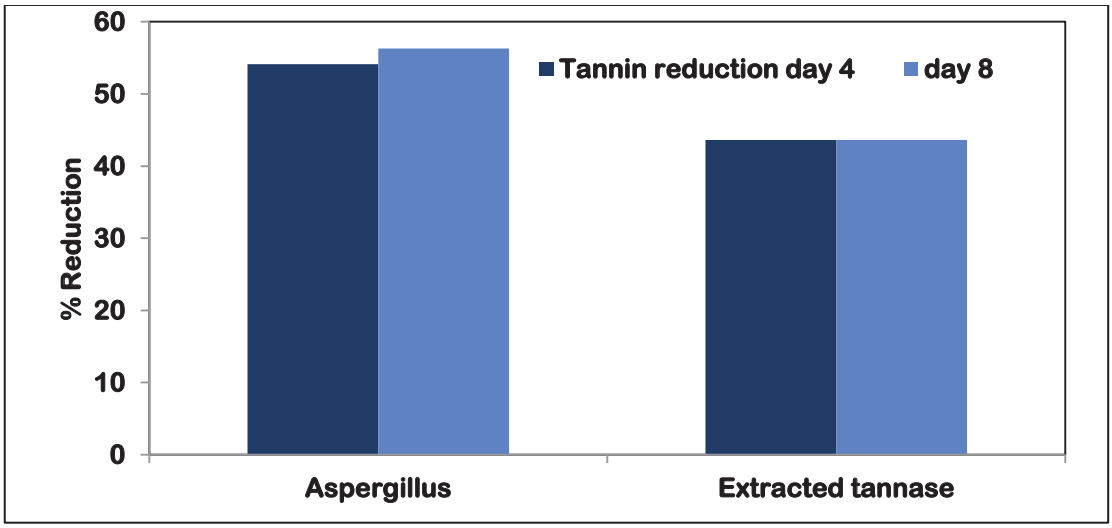

Fig: 5 Degradation of tannin content of the tannery effluent.

BOD and COD determine the efficiency of the treatment process. The marked reduction in BOD and COD by isolated *Aspergillus sp* indicates its degradation potential (Fig 6). The microbial degradation of tannery effluent was better than enzymatic degradation. The mycelial growth habitat and battery of extracellular enzyme makes filamentous fungi a good candidate for biodegradation of polluted waters. The biodegradation of tannery effluent by the selected *Aspergillus spp* reflects its potential in the degradation of tannins and thereby decreasing the consequences associated with its toxicity in the environment and also represents a valuable source of tannase for application in food, pharmaceutical and chemical industries.

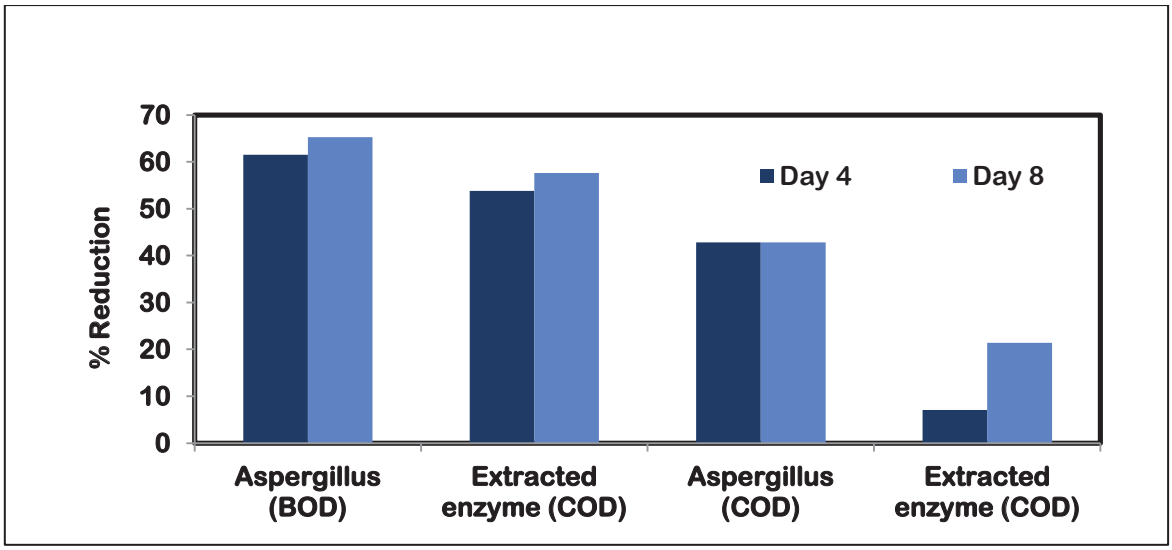

Fig: 6 Reduction in BOD and COD of the tannery effluent.

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