Sciencia Acta Xaveriana
ISSN. 0976-1152

Volume 1
No. 1
pp. 65–72

Biological Treatment of the Tannery Effluent Using the Cellulolytic Fungi

A. Cholarajan[1*] **and R. Malathi**[2]

[1]P.G Department of Microbiology, Meenakshi Chandrasekaran College of Arts and Science , Pattukottai, Thanjavur (DT)–614626. India.

[2]Department of Biotechnology, Bharathidasan University College of Arts and Science, Orathanad, Thanjavur (DT). India.

Abstract. Water is one of the abundantly available substances in nature. It is an essential ingredient of animal and plant life. Ground water is required for industrial processes. Pollution of land, water and air through water generated as result of increasing population, urbanization and industrialization is a challenge of serious dimensions. Pollution of natural water by industrial waste is objectionable and damaging for many varied reasons. Primary importance is the possible hazard of public health by the contamination of stream with disease producing microorganisms. The disposal of textile wastewater without treatment is the most common practice in the country, which adversely affects the environment, resulting in serious environmental problems. Environmental pollution is caused predominantly by the industrial effluent disposal in water bodies. To screen and identify different cellulolytic fungal isolates and determine the degradation activity of cellulolytic fungal species. To determine the BOD and COD of the tannery effluent by cellulolytic fungi after various incubation periods. The bioremediation or treating the tannery effluents using various cellulolytic fungi Trichoderma, Pencillium, Aspergillus Sp.

Keywords: Effluents, Trichoderm sp, Pencillium sp, BOD, COD and Environmental pollution.

(Received: 09 December 2009)

1 Introduction

Water is one of the abundantly available substances in nature. It is an essential ingredient of animal and plant life. Ground water is required for industrial processes. Pollution of land, water and air through water generated as result of increasing population, urbanization and industrialization is a challenge of serious dimensions. The tannery waste water is a mixture of bioorganic matter of bides and a large variety of organic and inorganic chemicals waste water from tanneries, usually contains high levels of salinity, organic loading, inorganic matter, color matter, dissolved and suspended solids, ammonia, organic nitrogen and specific pollutants such as sulphide, chromium and other metal salt residues. Industrial wastewater contains a wide variety of toxic inorganic and synthetic organic pollutants, most of which are not readily susceptible to biodegradation. Packing plant, sewage is rich in nitrogenous dry organic matter as manure, blood, flesh, grease and hair [1]. Sewage of wood pulp is rich in cellulose, lignin and bisulphates. Almost all industries (dairy, tannery, cannery, distilleries, oil refineries, textile, coal and coke, synthetic rubber, steel etc.,) produce their own characteristic sewage. There are known to be highly toxic to living organisms including wild life [2]. The ability of fungi, especially, wood rotting fungi to transform a wide variety of hazardous chemicals has aroused great interest in using them for bioremediation. Some species of fungi like *Aspergillus flavus, Aspergillus niger, Fusarium*sp.,*Trichoderma reesei*etc. In general bacterial cellulases are constitutively produced only in the presence of cellulose [3]. Filamentous fungi particularly *Aspergillus* and *Trichoderma*are well known efficient producers of cellulases [4]. Several studies were carried out to produce cellulolytic enzymes from bio waste degradation process by many microorganisms including fungi such as *Trichoderma, Pencillium, Aspergillus sp* Many environmental problems such as hepatitis, jaundice, enteric fever, anthrax, etc. are great threats for the living beings due to the industrial effluents [5]. Bioremediation may provide an apt remedy

for treating the effluent using microbes to control the pollutions by reducing the BOD and COD [2].

2 Materials and methods

Sample collection: The tannery effluents from traditional silk weaving area were collected in a sterile container. The sample was then transported to the laboratory aseptically and stored at 4 ˚ C before processing.

Isolation and identification of fungal pathogens: The soil sample was subjected to serial dilution. 0.1ml of the aliquots of 10^{-4}, 10^{-5} and 10^{-6} dilutions were spread plated on the Rose Bengal agar plates. The predominant isolates were then pure cultured individually on Rose Bengal agar medium.

Screening for cellulose activity: On a sterile Czapek Dox medium, 1% CMC (carboxy methyl cellulose) was incorporated and the fungal isolates were placed on the wells which were cut in the individual plates. The plates were then incubated at 25 ˚ C for two days. After incubation, 0.2% of congo red solution was added to the plates and then destained with 0.1M of NaCl solution.The resulting zones were then measured and tabulated [3].

Degradation activity of cellulolytic fungi: Different cellulolytic fungal isolates were inoculated on the sterilized tannery effluent. The initial optical density value before inoculation, at inoculation and at various time intervals about 7, 15, 21 days were recorded spectroscopically at 510 nm.The degradation rate was calculated using the standard formula.

$$\text{Percentage of degradation} = 100 \times \frac{\text{(A initial - A final)}}{\text{A initial}}$$

where,

A initial = initial observant

A final = final observant after inoculation period.

Determination of Biological Oxygen Demand (BOD)

Reagents:

Sodium thiosulphate (0.025N): Dissolve 24.82 g of $Na_2S_2O_3$ $5H_2O$ in boiled distilled water and make up the volume to 1 L.This was 0.1N stock solution. Dilute it to 4 times

with boiled distilled water to prepare 0.025N solution (250 g–1000 ml) kept in a glass-stoppered bottle.

Alkaline potassium iodide solution: Dissolve l00 g of KOH and 50 g of potassium iodide in 200 ml of boiled distilled water.

Manganous sulphate solution: Dissolve l00 g of $MnSO_4$ in 200 ml of boiled distilled water and filter it.

Starch solution: Dissolve 1 g of starch in 100 ml of warm distilled water.

Concentrated sulphuric acid: One or two drops of concentrated sulphuric acid were added.

Procedure

The distilled water is filled (300 ml) carefully. Avoid any kind of bubbling and trapping of the air bubbles in the bottle after placing the stopper. 1 ml of dye effluent and 1ml of MnSO4 were pipettedout and 1ml alkaline potassium iodide solutions well below the surface from the walls. A precipitate formed. The stopper was placed and the content was shaken well by inverting the bottle repeatedly. The bottle was kept for sometime for the precipitate to settle down. 1ml of concentrated sulphuric acid was added and shaken well to dissolve the precipitate. From this 25 ml was pipetted out into a conical flask for titration. The contents were titrated against sodium thiosulphate solution using starch as an indicator. At the end point initial light blue colour changes to colourless. The BOD is calculated using the standard formula,

$$\text{Dissolved oxygen} = \frac{M \times V \times E}{\text{Volume of the sample}} \times 1000 \text{ mg/l}$$

where,

$M =$ the molarity of the sodium thiosulphate used in titration.

$V =$ the volume of sodium thiosulphate required.

$E =$ the equivalent weight of oxygen.

The results were then tabulated.

Determination of Chemical Oxygen Demand (COD)

Reagents:

Potassium dichromate (0.25N): 3.676 g of potassium dichromate was dissolved in

100 ml of distilled water.
Sodium thiosulphate: 2g of sodium thiosulphate were dissolved in 100 ml of distilled water and concentrated sulphuric acid.
Potassium iodide solution (10%): 10g of potassium iodide were dissolved in 100 ml of distilled water.
Starch indicator (1%): One or two drops of starch indicator were added.

Procedure

100 ml conical flask was cleaned with tap water and sterilized in autoclave.10 ml of dye effluent and 1 ml of 0.25N potassium dichromate was taken in a 100 ml conical flask and then 15 ml of concentrated sulphuric acid was added to it. It was allowed for 30 min incubation. After 30 min 20ml distilled water with 2 drops of starch indicator were added to the conical flask. Then the conical flask was titrated against 2% sodium thiosulphate to give a wine red colour. The COD was calculated using standard formula,

$$\text{COD} = \frac{8 \times C \times (B - A)\ \text{mg/l}}{50}$$

where, C =concentration of titrant (mg/l)
A =volume of titrant used for blank (ml)
B =volume of titrant used for sample (ml)
S =volume of water sample taken (ml)
The results were the recorded and tabulated.

3 Result and discussion

Isolation and identification of cellulolytic fungi: The cellulolytic fungi were isolated. The different fungal isolates obtained by spread plate method were pure cultured on Rose Bengal agar medium. Based on the macro and microscopic appearance of the fungal isolates, they were identified using the manual of soil fungi. The following fungal species were isolated [2]. The different cellulolytic fungal isolates include *Aspergillus niger, Pencillium camemberti, Aspergillus fumigatus, Pencillium chrysogenum, Aspergillus flavus, Penicillium javanicum, Fusarium nivale, Penicillium expan-*

sum, Trichoderma reesei, Penicillium citrinum and *Trichoderma koningi*.

Degradation activity of cellulolytic fungi: The degradation assay is found high of about 88.2% by *Apergillus niger* and of about 87% by *Trichoderma reesei* and *Aspergillus fumigatus.*

Screening of cellulose activity: The different fungal isolates were screened for their cellulolytic activity. A clear yellow zone obtained after incorporating 2% Congo red and 0.1M sodium chloride solution on the inoculated plates were measured and recorded individually. (Table 1)

Table 1: Cellulolytic activity of fungi.

Sl.no.	Name of the organism	Zone of clearance
1.	*Aspergillus niger*	+ + + + (Very maximum growth)
2.	*Penicillium camemberti*	+ + (Moderate growth)
3.	*Aspergillus fumigatus*	+ + + (Maximum growth)
4.	*Penicillium chrysogenum*	+ + (Moderate growth)
5.	*Penicillium javanicum*	+ + (Moderate growth)

Determination of biological oxygen demand (BOD): The Biological Oxygen Demand was determined for all the cellulolytic fungal isolates individually. The effective reduction of the BOD value was observed in the organisms. (Table 2)

Table 2: Determination of BOD using cellulolytic Fungal isolates.

Name of the organism	Initial value of BOD	Final value of BOD	Percentage of BOD reduction
Aspergillus niger	1.3	1.6	81.2 %
Penicillium camemberti	1.3	1.8	72.2 %
Aspergillus fumigatus	1.3	1.5	82.6 %
Aspergillus flavus	1.3	1.5	82.6%
Penicillum javanicum	1.3	1.5	92.85%

Determination of chemical oxygen demand (COD): The Chemical Oxygen Demand was determined for all the cellulolytic fungal isolates individually. The effective reduction of the COD value was observed in the organisms. (Table 3)

Table 3: Determination of cod using cellulolytic fungal isolates.

Name of the organism	Initial value of COD	Final value of COD	Percentage of COD reduction
Aspergillus niger	3.9	6	65%
Penicillium camemberti	3.9	5.4	72.2%
Aspergillus fumigatus	3.9	9	43.33%
Aspergillus flavus	3.9	5.5	70.9%
Penicillum javanicum	3.9	5.8	67.24%

The present investigations are aimed at the bioremediation of the tannery effluent using the cellulolytic fungal isolates [2]. The different cellulolytic fungal isolates include *Aspergillus niger, Pencillium camemberti, Aspergillus fumigatus, Pencillium chrysogenum, Aspergillus flavus, Penicillium javanicum, Fusarium nivale, Penicillium expansum, Trichoderma reesei, Penicillium citrinum*and *Trichoderma koningi*. Here are a number of cost/efficiency advantages to bioremediation, which can be employed in areas that are inaccessible without excavation. For example, hydrocarbon spills (specifically, petrol spills) or certain chlorinated solvents may contaminate groundwater, and introducing the appropriate electron acceptor or electron donor amendment, as appropriate, may significantly reduce contaminant concentrations after a lag time allowing for acclimation. This is typically much less expensive than excavation followed by disposal elsewhere, incineration or other *ex situ* treatment strategies, and reduces or eliminates the need for ”pump and treat”, a common practice at sites where hydrocarbons have contaminated clean groundwater.

References

[1] C.G. Boer, C.D. Souza and R.M. Peralta, *Decolouration of synthetic dyes by soild state culture method*, Bioresource Techol., **94(2)** (2004) 104–112.

[2] R. Rohella, S. Choudhury, M. Manthan, and N. Sahoo, *Decolorization of craft paper Mill Effluent by white-rot fungi*, Indian Journal of Microbiology, **38** (1998) 221–224.

[3] M.A. Aboulhassan, S. Souabi and A. Yaacoubi, *Pollution reduction and biodegradability index improvement of Tannery fflulents*, Int. J. Environ. Sci. Tech., **11** (2007) 11–16.

[4] S. Cing, G. Asma, E. Apoham and O. Yesilade, *Decolorisation of textile dyeing waste water by fungi. Folia microbial (praha)*, **48(5)** (2003) 9–42.

[5] N. Rajamohan and C. Karthikeyan, *Kinetic studies of Dye Effluent degradation by Pseudomonas stutzeri.* (2006) `Eco-web.com`.