Physicochemical Characteristics, Identification of Fungi and Biodegradation of Industrial Effluent

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Abstract

An investigation was carried out to analyse Physico-chemical parameters like colour, odour, pH, EC, TSS, TDS, BOD, COD, chromium and copper of untreated tannery effluent and to degrade the effluent using native and non – native Fungi. The results of the analysis of parameters revealed that untreated tannery effluent was black in colour with offensive odour. pH was alkaline with high organic load such as EC, TSS, TDS, BOD and COD which were higher than the permissible limits of CPCB. Since the effluent had high organic load, microbes (Fungi) present within the effluent was identified and isolated. The results of the study revealed the occurrence of 10 species of fungi namely *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus sydowii, Aspergillus terreus, Aspergillus versicolor, Fusarium moniliformis, Paecilomyces variotii, Penicillium citrinum, Penicillium frequentos and Trichoderma harzianum.* The presence of bacteria indicates the pollutional status of the untreated tannery effluent suggesting that it should be treated before its disposal using the biological method particularly native and non native fungi for comparing their degrading efficiency. The results of the degradation study shows that native fungus, *A. niger* was found to be very much successful in reduction of toxic substances at the percentage range of 52-96% whereas non native fungus, *Aspergillus flavus* showed reduction of 53-96% and the biotreated water can be reused for the agricultural and aquacultural purposes.

Key Words : Untreated tannery effluent, physicochemical parameters, Fungal identification, degradation, native *A. niger* and non – native *A. flavus.*

INTRODUCTION

Environmental pollution is becoming the global problem in which water pollution is an important issue as water is used directly for various purposes. (Vidya and Usha, 2007). The major sources of water pollution are industrial effluents which is being discharged to the common drainage. The effluent pollute not only the nearby soil but may cause the pollution of drinking water also (Lokhande and Vaidya, 2004). It is difficult to put a price tag on the cost of pollution (Arora, 2001).Untreated industrial effluent discharged on the surface cause severe ground water pollution in the industrial belt of the country. This poses a problem of supply of hazard-free drinking water in the rural parts of the country (Vidya and Usha, 2007). Pollution is the greatest threat posed to humanity and even to the whole biosphere. (Shivakumar and Thippeswamy, 2012).

The tanning industry is one of the major consumers of water and most of it is discharged as waste water, which contains high amounts of heavy metals such as Fe, Cr, Zn, Mn, Cu etc. (Devi *et al.*, 2011).The enormous pollution load along with the toxic nature of waste water makes the tanneries a potential threat to the areas in the vicinity of their location which when consumed causes serious health hazards (Panwar *et al.*, 2002).

Though tanneries are revenue and job generating sector, the pollution from their effluent is of major concern. The objectionable constituents present in large amounts in the effluents are suspended solids, chlorides, sulfides, chromium, tannins and organic wastes.

1.1.1 Impact of Tannery Effluent on the Environment

Tannery effluent with high pollutional load when discharged into water bodies alter the physical, chemical and biological characteristics of water and depletes the dissolved oxygen, increases alkalinity, suspended solids and sulphides which are injurious to fish and other aquatic lives. Tannery effluent with high TDS would produce undesirable taste and gastrointestinal irritations, chrome ulcers, corrosive reaction on the nasal septum, acute irritative dermatitis and allergic eczematous dermatitis are caused due to chromium toxicity (Gokulakrishnan & Pandurangan, 2004).Tannery effluent contains chromium and pathogens mainly of faecal origin and toxic organic components, all of which pose a serious threat to the environment.

Heavy metals in the tannery effluent is one of the most hazardous environmental pollutants, toxic heavy metals like Cr, Cu, Zn, Pb and Cd are mostly absorbed and get accumulated in various plant parts as free metals which may adversely affect the plant growth and metabolism . Major diseases of cattle and human beings produced by Chromium and Nickel are bronchitis and cancer. (Sivakumar and Thippeswamy, 2012).

Disposal of the wastes with high pollution load into water course or onto land, with or without prior treatment, creates a great problem in the environment in the vicinity. So, it has become essential to treat the waste before its disposal. Though there are physical, chemical and biological means of waste treatment are available, scientists have found that in managing certain wastes, the best option is microbiological treatment which is more efficient and consumes no energy. Since the complete degradation of organic chemicals in the natural ecosystem is primarily carried out by micro-organisms, bio technological application using microbes or their enzymes for waste treatment are efficient (Devi *et al.*, 2011). Degradation is defined as stimulation of micro-organism to degrade rapidly hazardous organic contaminants to environmentally safe levels in soils, waters sludges and residues. The key element in degradation are enzymes which uses environmental contaminants as source of food and hence make them ideal for degradation. (Vijayaraghavan *et al.*, 2007). Taking into consideration of all the above said investigations carried out by many researchers pertaining to degradation of various industrial effluents using microbes especially fungi, an attempt has been made to degrade the untreated tannery effluent using fungi.

Aims and objectives:

- To analyse the physicochemical parameters of industrial effluent as a means of monitoring the pollution.
- To isolate and identify the microbes (fungi) present in the industrial effluent which provide clues on the ability of the microbes to survive, adapt and colonise in the polluted environment.
- To ascertain the bioremediation potential using native microbe - fungus isolated from industrial effluent for its treatment.
- To degrade the industrial effluent using non-native microbe fungus which is not present in industrial effluent and obtained from Microbiological Laboratory Centre, India
- To study the comparison between the native and non-native microbes - fungi for the degrading efficiency of the effluent.

Materials and Methods

Analysis of physicochemical parameters of industrial effluent

Untreated industrial effluent was collected in polythene containers from an industry located in Chennai, Tamil nadu, India, were brought to the laboratory, and stored for further analysis. The sample was collected for a period of 6 months (May 2011 to October 2011). The physico-chemical parameters of the effluent - pH, Electrical Conductivity (EC), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Total Hardness, Chloride, Sodium, Calcium and heavy metals were estimated by following the Standard methods suggested by APHA (1995).

Isolation and identification of microbes – fungi present in industrial effluent

Industrial effluent was collected in sterile bottles for microbial analysis, brought to the laboratory and fungal identification was carried out by pour plate method, following the procedure of Onions *etal.*, (1981).

Biodegradation of Industrial Effluent using Native Microbe – fungus

Isolates of Native *Aspergillus niger* obtained from industrial effluent was used for biodegradation of industrial effluent by following the procedure of Aftab Begum and Noorjahan (2006).

➢ **Biodegradation of Industrial Effluent using Non-Native Microbe - fungus**

Isolates of Non-Native fungus , *Aspergillus flavus* which was not present in the industrial effluent was obtained from Microbiology Laboratory Centre,,,India and used for biodegradation of industrial effluent by following the procedure of Aftab Begum and Noorjahan (2006).

Results and Discussions

Analysis of physicochemical parameters of industrial effluent

Results of the analysis of the physico chemical parameters of untreated industrial effluent collected for a period of 6 months (May 2011 to October 2011) are depicted in Table. 1. Statistical analysis was also carried out. The results of the study revealed that colour of the untreated industrial effluent was blackish in colour with offensive odour. This colour and odour could be due to decomposition of organic or inorganic matter (Singh *et al.*, 1998). A large number of pollutants can impart colour, taste and odour to the receiving water, thereby making them unaesthetic and unfit for domestic consumption (Jamal *et al.*, 2011). P^H of the tannery effluent was found to be alkaline. Discharge of such effluent with alkaline pH into ponds, rivers, etc for irrigation may be detrimental to aquatic biota such as zooplankton and fishes. According to Saxena and Shrisvastava (2002), alkaline nature of the tannery effluent may be due to the presence of carbonates and bicarbonates present in the effluent.

Untreated effluent showed higher level of Electrical conductivity(8344-9138 µmhos/ cm) which could reflect the presence of organic and inorganic substances and salts that would have increased the conductivity (Marwaha *et al.*, 1998). It may be due to high concentration of acid base and salt in the effluent (Jamal *et al.*, 2011).

Level of suspended solids was found to be higher in the effluent (5174-6908 mg/l) when compared to the permissible limit (100 mg/l) prescribed by CPCB (1995) for effluent discharge. High amounts of suspended particles has detrimental effects on aquatic flora and fauna and reduce the diversity of life in aquatic system and promote depletion of oxygen and sliting in ponds during rainy season (Goel, 2000).

The composition of solids present in a natural body of water depends on the nature of the area and the presence of industries nearby. High levels of TDS (5758 - 6672 mg/l) may be due to high salt content and also renders it unsuitable for irrigation hence further treatment or dilution would be required (Goel, 1997). The presence of high level of TSS and TDS may be due to the insoluble organic and inorganics present in the effluent (Nagarajan *et al.*, 2005).

Determination of BOD is one of the important parameters used in water pollution to evaluate the impact of wastewaters on receiving water bodies. The present study revealed high levels of BOD (600 - 1622 mg/l) in the effluent due to the presence of considerable amount of organic matter. High BOD levels have also been reported for effluent discharged from tanneries (Kulkarni, 1992) and dairy effluent (Noorjahan *et al.,* 2004). Increase in BOD which is a reflection of microbial oxygen demand leads to depletion of DO which may cause hypoxia conditions with consequent adverse effects on aquatic biota (Jerin, 2011).

COD test is the best method for organic matter estimation and rapid test for the determination of total oxygen demand by organic matter present in the sample. The present investigation revealed high levels of COD (2286 - 9600 mg/l) which surpassed the standards limit for COD (250 mg/l) prescribed by CPCB (1995) for effluent discharge into inland surface waters. This indicate that the effluent is unsuitable for the existence of aquatic organisms due to the reduction in DO content (Goel, 1997).

Total hardness were found to be high (1416-3850 mg/l) than the permissible limit of CPCB(1995). Chloride (1244-1895mg/l), Sodium (1200-2100 mg/l) and Calcium (257-560 mg/l) were higher compared to permissible limit of CPCB(1995).

Chromium and copper levels were within the permissible limits of CPCB (1995), the physicochemical properties and heavy metals concentration of the effluent varies depending on the process of tanning adopted in various industries (Vidya and Usha, 2007).

Thus the analysis of physico chemical parameters of untreated effluent for the period of 6 months (May 2011 - Oct. 2011) confirms that the wastewater released from the tannery industry has higher concentration of EC, BOD, COD, TSS, TDS, which surpassed the permissible limits prescribed by CPCB (1995) for discharge of industrial effluent into inland surface water as well as an land for irrigation.

Alkaline pH, high TSS, TDS, BOD and COD of the effluent reveals that the effluent is highly polluted and it has to be treated before disposal. Hence it is imperative to adopt technologies to reduce or degrade the effluent using microbes.

The most reliable way seems to be the biological treatment in which microorganisms serve as efficient detoxifiers of pollutants. Microorganisms degrade organic contaminants as they use it for their growth and reproduction. The microorganism obtains energy by catalyzing energy producing chemical reactions and this energy is used in the production of new cell (Goudar and Subramanian, 1996). Further biological treatment process is a cost effective method for hazardous substance disposal.

The researchers have clearly indicated that aquatic fungi play a key role in the productivity of streams, estuaries and oceans (Jamal, 2002). There are many fungi occurring naturally in conditions ecologically substrate i.e. the wastes from the industries. The present study indicates the possibilities of employing such organisms under controlled conditions to degrade the waste. The spectacular array of fungal populations adorning aquatic habitats has been reviewed by Noorjahan *et al.* (2003), and Jerin, (2011), which prompted to analyse the native microbial population in the effluent and to use it for biodegradation instead of introducing other microbes.

The results of the microbial analysis from the effluent are represented in Table . 2. The results of the microbial analysis of the effluent revealed the occurrence of 10 species of fungi such as: *Aspergillus fumigatus, Aspergillus niger, Aspergillus sydowii, Aspergillus terreus, Aspergillus versicolor, Fusarium moniliformis, Paecilomyces variotii, Penicillium citrinum, Penicillium frequentos and Trichoderma harzianum.* This finding would help to unreveal 4 major outcomes namely :

- Isolation & identification of new isolates of microbes particularly fungi from the effluent.
- Provides clues on the ability of microbes to survive, adapt and colonize in the polluted environment.
- To study the capacity of the mycoflora to bioaccumulate contaminants.
- To assess the biodegradation potential of the mycoflora present in the effluent.

The effluent is rich in organic and inorganic nutrients which would have supported the growth of fungal population. Prabakar (1999) also reported the occurrence of various fungi in sugar and distillery effluent, which contains high organic load. Noorjahan *et al.* (2003) identified 7 species of fungi in both untreated and industry treated dairy effluent. Adekunle and Oluyode (2005) reported the presence of 21 fungal species in melon and soyabeans. Kannagi (2007) reported the presence of 6 species of fungi in brewery effluent. 6 fungal species were identified in Tannery effluent (Krishnapriya, 2010 and Jerin, 2011).

The occurrence of 10 species of fungi in the effluent, as reported in the present study has significance in their utility as biological indicators (Rao and Rao, 2000). Further as pointed out by Radha (1995), the presence of native microbes in tannery effluent would be successfully exploited to remove the pollutants, a technique which is more economically and industrially effective.

Based on the above suggestions, biodegradation of effluent was planned and executed using microbes - fungus. Biodegradation technology is potentially useful, it is important to document enhanced biodegradation of the pollutant under controlled conditions, this cannot be accomplished in sites and thus must be accomplished in laboratory experiments before the technology is transferred to field.

According to Goudar and Subramanian (1996), laboratory experiments which provide real environmental conditions are most likely to produce relevant results with indigenous microbial population.

Native fungus, *Aspergillus niger* is used to biodegrade the tannery effluent. Since *Aspergillus* species were documented by many workers for their capacity in degrading the effluent, it was selected and were used in biodegradation of tannery effluent. Non native fungus *Aspergillus flavus* was selected to biodegrade the tannery effluent in order to compare the degrading efficiency of native fungus *Aspergillus niger* and non native fungus *Aspergillus flavus*.

Degradation of 100% untreated effluent using Native fungus, *Aspergillus niger* and non - native fungus, *Aspergillus flavus* :

Results of analysis of degradation of industrial effluent using native fungus, *Aspergillus niger* and non native fungus, *Aspergillus flavus* are presented in Table.3. The colour of industrial effluent is black before degradation but after degradation using native fungus *Aspergillus niger* and non native fungus *Aspergillus flavus* there is a change in colour from black to almost colourless nature of the effluent. The odour of the effluent changed from offensive to odourless after degradation using native fungus *Aspergillus niger* and non native fungus *Aspergillus flavus* .This may be due to the action of microbes – *E.coli* and *Bacillus sp.*, which decomposed the toxic pollutants present in the effluent and made the change in colour and odour of the effluent. This is supported by the work of Sukumaran *et al.*, (2008).

The pH was alkaline in nature but after degrading the sample using native fungus *Aspergillus niger* and non-native fungus *Aspergillus flavus* , alkaline nature of pH has changed to the neutral state. pH of untreated tannery effluent changed to almost neutral pH which may be due to accumulation of organic acids and also indicating the efficiency of the microbes to biodegrade the effluent. This is in agreement with the reports of Noorjahan *et al.* (2004)

EC of effluent before treatment is 9148 (µmhos/cm) ± 1.8708 which is beyond the permissible limit (400 µmhos/cm) of CPCB (1995) but after degradation. using native fungus *Aspergillus niger* EC degraded to 4350 µmhos/cm ± 1.5811 and the percentage change of EC is 52.4% and using non-native fungus *Aspergillus flavus* EC degraded to 4260 µmhos/cm ± 3.1622 and the percentage change of EC is 53.43%.

TSS of the effluent before treatment is 176 mg/l ± 1.8708 which is beyond the permissible level (100 mg/l) of CPCB (1995) for disposal, but after degradation, native fungus *Aspergillus niger* degraded TSS to 32 mg/l ± 1.8708 and the percentage change is 93.27% and non-native fungus *Aspergillus flavus* degraded TSS to 24 mg/l ± 3.1622 and the percentage change is 94.53%.

TDS of the effluent before treatment is 6428 mg/l ± 3.3115 which is beyond the permissible limit (2100 mg/l) of CPCB (1995) but after degradation for 96 hrs, native fungus *Aspergillus niger* degraded TDS to 2045 mg/l ± 1.5811 and the percentage change is 68.17% and non-native fungus *Aspergillus flavus* degraded TDS to 2986 mg/l ± 1.5811 and the percentage change is 53.54%. Since TSS and TDS are the major pollutants, the above biodegradation results are encouraging and scale up studies for continuous treatment of wastewater at pilot scale is required. The information generated would help to scale up the process and assess the economic feasibility of the technology. This is supported by the work of Srivastava and Thakur(2006).

BOD of the effluent before treatment is 900 mg/l ± 2.1602 which is beyond the permissible limit (30 mg/l) of CPCB (1995) for disposal but after degradation, native fungus *Aspergillus niger* degraded BOD to 36 mg/l ± 1.5811 and the percentage change is 96% and non-native fungus *Aspergillus flavus* degraded BOD to 33 mg/l ± 4.3846 and the percentage change is 96.33%.

COD of effluent before treatment is 2637 mg/l ± 2.3166 which is beyond the permissible limit (250 mg/l) of CPCB (1995), but after degradation, native fungus *Aspergillus niger* degraded COD to 297 mg/l ± 1.5811 and the percentage change is 88.73% and non-native fungus *Aspergillus flavus* degraded COD to 380 mg/l ± 3.1622 and the percentage change is 85.5%. This is in agreement with the work of Karthikeyan *et.al.(*2010).

Chromium of effluent before treatment is 5.149 mg/1 ± 0.0034 which is within the permissible (0.05 mg/l) of CPCB (1995) but after degradation, native fungus *Aspergillus niger* degraded chromium to 0.829 mg/l ± 0.00258 and the percentage change of chromium is 83.8% and non native fungus *Aspergillus flavus* degraded chromium to 0.756 mg/l ± 0.00316 and the percentage change of chromium is 85.3%.

Copper effluent before treatment is 0.00168 mg/l ± 2.804 which is within the permissible limit (3 mg/l) of CPCB (1995) but after degradation , native *Aspergillus niger* degraded copper to 0.00296 mg/l ± 1.58114E-05 and the percentage change of copper is -76.19% and non native fungus *Aspergillus flavus* degraded copper to 0.00242 mg/l ± 1.5811 and the percentage change of copper is -65%. This is in agreement with the work of Karthikeyan *et.al.(*2011).

Thus from the above results it can be inferred that the maximum reduction of toxic substances was recorded in biotreated sample using native fungus *Aspergillus niger* (52-96%) and non native *Aspergillus flavus*(53-96%) compared to untreated effluent. Thus it may be concluded from the above study that untreated effluent with high pollutants can be reduced by using native fungus, *Aspergillus niger* and non native fungus, *Aspergillus flavus* and this treated water can be reused for agricultural purpose as evidenced in the present work.

Acknowledgement

This work was supported by the fund provided by University Grants Commission (UGC) Bahadur Shah Zafar Marg, New Delhi, Pin:110 002,India

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Table 1: Analysis of physicochemical parameters of Industrial effluent for a period of six months from May 2011-Oct. 2011

S.No.	Parameters	CPCB 1995	May 2011	June 2011	July 2011	August 2011	Sept. 2011	Oct. 2011
1	Colour	Colourless	Blackish	Blackish	Blackish	Blackish	Blackish	Blackish
2	Odour	Odourless	Unpleasant	Unpleasant	Unpleasant	Unpleasant	Unpleasant	Unpleasant
3	pH	5.5-9.0	7.2±0.0187	7.2±0.0187	7.2±0.0187	7.1±0.0187	7.1±0.0187	7.21 ±0.0769
4	Electrical Conductivity (EC) (mhos/cm)	400	8890±1.8708	9080±1.877	**9138 ± 1.877**	**8344 ± 1.8708**	8415 ±1.8708	8699± 2.3166
5	Total Suspended Solids (TSS)(mg/l)	100	**6908±1.8708**	6128±1.8756	6076±1.8756	5192 ±1.8708	**5174 ±1.8708**	5260 ±1.8708
6	Total Dissolved Solids (TDS)(mg/l)	2100	**6672±1.8748**	6612±1.8755	6528±1.8794	**5758 ±1.8794**	5952 ±1.8765	6166 ±1.8793
7	Biochemical Oxygen Demand (BOD) (mg/l)	30	**1622±1.8906**	613±1.8760	910±1.8765	**600± 1.8759**	710 ±1.8062	620± 1.8759
8	Chemical Oxygen Demand (COD) (mg/l)	250	**9600±2.908**	2820±1.8780	2637±1.8780	**2286 ±1.8708**	2299 ±1.8708	2679± 1.8708
9	Total Hardness (mg/l)	1000	2080±1.8708	**1416±1.1690**	1550±1.8708	1635 ±1.8708	**3850± 1.8708**	2300 ±1.8708
10	Chloride (mg/l)	1000	1455±1.8725	**1890±1.8895**	1284±1.8720	**1244 ±1.8708**	1373 ±6.1913	1485 ±1.7692
11	Sodium (mg/l)	600	1218±1.8708	**1200±1.8708**	1900±1.8708	**2100 ±1.8708**	2040 ±1.4142	1250± 1.672
12	Calcium (mg/l)	100	**257±1.7605**	310±1.7607	320±1.7612	360± 1.7615	380 ±1.7625	**560± 1.8062**
13	Total Chromium (mg/l)	2	**43.5±0.1870**	35±0.1870	5.47±0.0187	**0.01±0.04319**	7.14± 0.01769	9.20± 0.01871
14	Copper (mg/l)	3	**6.40±0.0187**	3.79±0.0187	0.00158±0.0001	0.0133 ±0.0016	0.00124 ±0.0187	**0.00013 ±0.00063**

± Standard Deviation

Table 2: Isolation and Identification of Fungi from Industrial effluent for a period of six months from May 2011 – October 2011

S.No.	Name of the Organisms	May 2011	June 2011	July 2011	August 2011	Sept. 2011	Oct. 2011	No. of Colonies
1	*Aspergillus fumigatus*	+	+	+	+	+	+	19
2	*Aspergillus niger*	+	+	+	+	+	+	22
3	*Aspergillus sydowii*	-	-	+	+	-	-	2
4	*Aspergillus terreus*	+	+	-	-	+	+	4
5	*Aspergillus versicolor*	+	+	-	-	-	-	2
6	*Fusarium moniliformis*	-	-	-	+	+	-	2
7	*Paecilomyces variotii*	+	+	-	-	-	-	2
8	*Penicillium citrinum*	+	-	-	+	-	-	2
9	*Penicillium frequentos*	+	-	-	-	-	+	2
10	*Trichoderma harzianum*	+	+	-	-	-	-	4

\+ = Present - = Absent

TABLE – 3

Analysis of physico chemical parameters of industrial effluent before (control) and after degradation using native fungus *Aspergillus niger* and non-native fungus *Aspergillus flavus*

S.No.	Parameters	CPCB (1995)	Control (Untreated)	Bio treated native fungus *Aspergillus niger*	Bio treated non-native fungus *Aspergillus flavus*
1.	Colour	Colourless	Blackish	Almost Colourless	Almost Colourless
2.	Odour	Odourless	Offensive	Odourless	Odourless
3.	pH	5.5 - 9.0	8.19 ± 0.08	7.21 ± 0.0207	7.32 ± 0.0286
4.	Electrical Conductivity (µmhos/cm)	400	9148 ± 1.8708	4350 ± 1.5811 (52.44%)	4250 ± 3.1622 (53.43%)
5.	Total Suspended Solids (mg/l)	100	176 ± 1.8708	32 ± 1.8708 (93.27%)	24 ± 3.1622 (94.53%)
6.	Total Dissolved Solids (mg/l)	2100	6428 ± 3.3115	2045 ± 1.5811 (68.17%)	2986 ± 1.5811 (53.54%)
7.	Biochemical Oxygen Demand (mg/l)	30	900 ± 2.1602	36 ± 1.5811 (96%)	33 ± 4.384 (96.33%)
8.	Chemical Oxygen Demand (mg/l)	250	2637 ± 2.3166	297 ± 1.5811 (88.73%)	380 ± 3.1622 (85.58%)
9.	Chromium (mg/l)	3	5.149 ±0.0034	0.829 ± 0.00258 (83.89%)	0.756 ± 0.00316 (85.3%)
10.	Copper (mg/l)	1.5	0.00168 ± 2.804	0.00296 ± 1.5811 (76.19%)	0.0024 ± 1.5811 (65%)

± = Standard Deviation   % = Percentage Change