Physicochemical Characteristics, Identification of Bacteria 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 bacteria. The results of the parameters analysis 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 Since the effluent had high organic load, microbes (bacteria) present within the effluent was identified and isolated. The results of the study revealed the occurrence of 4 species of bacteria namely Escherichia coli, Klebsiella sp., sp., Pseudomonas sp. and Staphylococcus aureus. 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 bacteria for comparing their degrading efficiency. The results of the degradation study shows that native bacteria E.coli was found to be very much successful in reduction of toxic substances at the percentage range of 54-91% and the biotreated water can be reused for the agricultural and aqua cultural purposes.*

INTRODUCTION

Nature has given gifts like air, water, land, forest, minerals, fossil fuels and several resources to man. These gifts given to improve his living standards. But unbridled exploitation gradually resulted in the release of pollutants into the environment. (Sarala Thambavani et al., 2009). Pollution is a major environmental issue in the world due to its adverse effect on living organism. In the past few decades, uncontrolled urbanization has caused a serious pollution problem due to the disposal of sewage and industrial effluents to water bodies (Tamil Selvi et al., 2012). Majority of industries are water based and a considerable volume of waste water emanates from them which are generally discharged into water courses either untreated or inadequately treated causing water pollution (Pandey and Carnay, 1998).

Tannery is one of the important industry causing water pollution. There are about 2161 tanneries in India excluding cottage industries, which processes 500,000 tonnes of hides and skins annually. A total annual discharge of waste water from these tanneries is 9,420,000m³, which generates about 100,000m³ of waste water per day (Mohan et al., 2005) and these industries spread mostly across Tamilnadu, West Bengal, Utttar Pradesh, Andhra Pradesh, Karnataka, Rajasthan and Punjab (Lefebvre et al., 2005). In Tamil nadu alone there are about 1120 tanneries concentrated in Vellore, Ranipet, Tiruchy, Dindugal, Erode and pallavaram in Chennai.The effluent generated in the tanneries has high amounts of organic substances as well as high concentration of chloride, chromium, sulphide and ammonium salts used during the process .

IMPACT OF TANNERY EFFLUENT ON THE ENVIRONMENT:

The tannery industries are considered as polluting due to the inherent manufacturing processes as well as type of technology employed in the manufacture of hides and skin into leather. During the tanning process atleast 30 kg of chemicals are added per ton of hides. (Indu SekharThakur et al., 2011).Tannery effluent when discharged into water bodies alter the physical, chemical and biological characteristics of water and depletes the dissolved oxygen, in-creases alkalinity, suspended solids and sulphides which are injurious to fish and other aquatic lives.

Apart from organic materials which release valuable nutrients for decomposition, tannery effluent contains chromium and pathogens mainly of faecal origin and toxic organic components, all of which pose of serious threat to the environment. (Indu Sekkhar Thakur et al., 2011). Heavy metals in the tannery effluent is one of the most hazardous environmental pollutants. Human beings and cattles and plants are affected when these toxic metals like Cr, Cu, Zn, Pb and Cd are incorporated into the food chain (Iman Khasim and Nandakumar, 1989). Hence tannery effluent with high pollutional load should be treated before its disposal.

The treatment of industrial waste varies with its character, quantity and the nature of receiving media and the dilution available. The different methods are: Physical method, Chemical Method and Biological method. Physical and chemical methods of waste water treatment are invariably cost intensive and cannot be employed in all industries especially in developing and under developed countries. Compared with chemical/physical methods, biological processes have received more interest because of their cost effectiveness, lower sludge production and environmental friendliness. Bioremediation of tannery effluents is an attractive environment friendly, safe and cost effective alternative technology to conventional methods. Microbes in the environment play an important role is cycling and destroying them through bio-degradation. (Aneez Mohammed et al., 2011).

Taking into consideration of all the above said investigation carried out by many researchers pertaining to degradation of various industrial effluents using microbes especially bacteria, an attempt has been made to degrade the untreated tannery effluent using native bacteria E.coli sp.

AIMS AND OBJECTIVES:

To analyse the physicochemical parameters of industrial effluent as a means of monitoring the pollution.

To isolate and identify the microbes 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 microbes isolated from industrial effluent for its treatment.

To study the native microbe 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 physicochemical 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 PRESENT IN INDUSTRIAL EFFLUENT

Industrial effluent was collected in sterile bottles for microbial analysis, brought to the laboratory and bacterial identification was carried out by pour plate method, following the procedure of Powar and Daginwala (1995) and Sundararaj (1995).

BIODEGRADATION OF INDUSTRIAL EFFLUENT USING NATIVE MICROBES

Isolates of Native bacteria- E.coli obtained from industrial effluent was 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 unpleasant odour. This colour and odour could be due to decomposition of organic and inorganic matter (Singh et al., 1998). A large number of pollutants can impart colour, taste and odour to the receiving water, thereby making them anaesthetic and unfit for domestic consumption (Goel, 2000 and Tamil Selvi et al., 2012).

pH ranged from 7.14±0.0187 (August 2011) to 7.27± 0.0187 (May 2011) during the period of study. pH 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 Singh et al. (1998), highly alkaline water if consumed would affect the mucous membrane and may cause metabolic alkalosis. In addition, the toxicity of certain substances present in water may be enhanced due to their interaction with high or low levels of pH prevailing which may further be detrimental to aquatic organisms (Goel, 1997 and Jerin, 2011).

Electrical conductivity of the effluent has a minimum range of 8354µmhos/cm±1.8708 (August 2011) and maximum range of 9148 µmhos/cm ±1.877 (July 2011) . Untreated tannery effluent showed higher level of Electrical conductivity which could reflect the presence of organic and inorganic substances and salts that would have increased the conductivity (Marwaha et al., 1998 and Krishna Priya, 2010).

TSS ranged from 1138 mg/l ±1.8756 (June 2011) to 6908 mg/l ±1.8708 (May 2011) which was found to be beyond the permissible limits (100mg/l) of CPCB (1995) 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 slitting in ponds during rainy season (Goel, 2000 and Karthikeyan, et al., 2010).

TDS ranged from 5768 mg/l ±1.8794 (August 2011) to 6712 mg/l ±1.8755 (June 2011) which surpassed the CPCB (1995) permissible limits (2100mg/l). 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 may be due to high salt content ad also renders it unsuitable for irrigation hence further treatment or dilution would be required (Goel, 1997). Singh et al. (1998) cautioned that if the TDS level of water exceeded 500 mg/l, it becomes unsuitable for bathing and drinking purposes for animals as it could cause distress in cattle and livestock.

BOD has a minimum range of 600 mg/l ±1.8759 (August 2011) and maximum range of 1722 mg/l ±1.8906 (May 2011) which was beyond the permissible limit (30mg/l) of CPCB (1995).). 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 (Sukumaran, et al., 2008). Further the presence of organic matter will promote anaerobic action leading to the accumulation of toxic compounds in water bodies (Goel, 1997). Oxygen depletion could be followed by anaerobic conditions which would result in reduced diversity and distribution of aquatic fauna.

COD ranged from 2386 mg/l ± 1.8708 (August 2011) to 9600 mg/l ± 2.908 (May 2011) which has exceeded the permissible limit (250mg/l) of CPCB (1995) . 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. This indicates that the effluent is unsuitable for the existence of aquatic organisms due to the reduction in DO content (Goel, 1997).

Total Hardness has a minimum value of 1080 mg/l ± 1.8708 (May 2011) and maximum value of 3750 mg/l ± 1.8708 (September 2011) which was beyond the CPCB (1995) permissible limit of 1000 mg/l. Chloride ranged from 1184 mg/l ±1.8720 (July 2011) to 1890 mg/l ± 1.8895 (June 2011) which surpassed the permissible limit (1000 mg/l) of CPCB (1995). Sodium has a minimum range of 1200 mg/l ± 1.672 (October 2011) and maximum range of 2100 mg/l ± 1.8708 (August 2011) which was found to be beyond the permissible limit (600 mg/l) of CPCB (1995) whereas Calcium ranged from 257 mg/l ± 1.7605 (May 2011) to 560 mg/l ±1.8062 (October 2011).

Heavy metals - chromium ranged from 0.01 mg/l ± 0.04319 (August 2011) to 44.5 mg/l ± 0.1870 (May 2011). Copper has a minimum level of 0.00012 mg/l ±0.00063 (October 2011) and maximum level of 6.40 mg/l ± 0.0187

(May 2011) whereas zinc ranged from 0.010 mg/l (September 2011) to 0.57 mg/l ± 0.0125 (June 2011).). The presence of heavy metals in the tannery effluent produces several adverse effects on living organisms as reported by Chukwu (2006).

Thus the analysis of physicochemical parameters of untreated industrial effluent for a period of 6months (May 2011 – October 2011) confirms that the effluent released from the industry was black in colour with unpleasant odour,   pH was alkaline with high pollution load such as EC, TSS, TDS, BOD, COD, Chloride, Sodium, Calcium and Chromium which surpassed the permissible limits of CPCB (1995) for its disposal indicating high pollution potential of the effluent.

ISOLATION AND IDENTIFICATION OF MICROBES PRESENT IN INDUSTRIAL EFFLUENT

Microbes especially bacteria act as bio indicator of high polluted effluents as reported by Soha Farag and Sahar Zaki (2010), which prompted to analyse the native bacterial population in tannery effluent and to use it for biodegradation. The results of the analysis of isolation and identification of microbes (bacteria) present in untreated industrial effluent for a period of 6 months (May 2011 – October 2011) are represented Table 2.The results of the study revealed the occurrence of 4 species of bacteria namely Escherichia coli, Klebsiella sp., sp., Pseudomonas sp. and Staphylococcus aureus. Escherichia coli were found to be a dominant bacterial species due to the growth of maximum number of colonies (30) on the medium compared to other bacterial species.

The presence of 4 bacterial species in the tannery effluent as reported in the present study has significance in their utility as biological indicators (Rae 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.

BIODEGRADATION OF INDUSTRIAL EFFLUENT USING NATIVE MICROBES

Pure culture of native bacteria, E.coli were used for biodegradation of industrial effluent. Results of analysis of degradation of effluent using native E.Coli are presented in Table 3.The colour of industrial sample is blackish before degradation but after degradation using native E.Coli there is a change in colour from blackish to light brown of the industrial effluent .The odour of effluent was offensive in nature before degradation but after degrading the effluent using native E.Coli samples shows odourless condition. This may be due to the action of microbe – E.coli 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).

pH of effluent before degradation is alkaline in nature but after degrading the sample using native E.Coli for 96 hrs, alkaline nature of pH has changed to the neutral state 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 8615 (μmhos/cm) ± 3.16227 which is beyond the permissible limit (400 μmhos/cm) of CPCB (1995) but after degradation using native bacteria E.Coli and EC degraded to 3920 μmhos/cm ± 1.9235 and the percentage change of EC is 54.5%. TSS of

Industrial effluent before treatment is 184 mg/l ± 3.03315 which is beyond the permissible level (100 mg/l) of CPCB (1995) for disposal, but after degradation, native bacteria E.Coli degraded TSS to 24 mg/l ± 1.5811 and the percentage change is 89.9%. TDS of effluent before treatment is 5950 mg/l ± 3.1622 which is beyond the permissible limit (2100 mg/l) of CPCB (1995) but after degradation, native bacteria E.coli degraded TDS to 2740 mg/l ± 1.5811 and the percentage change is 53.9%. 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 in agreement with the work of Karthikeyan, et al.,(2010).

BOD of industrial effluent before treatment is 700 mg/l ± 1.5811 which is beyond the permissible limit (30 mg/l) of CPCB (1995) for disposal but after degradation, native bacteria E.coli degraded BOD to 70 mg/l ± 1.5811 and the percentage change is 90%. COD of effluent before treatment is 2399 mg/l ± 1.5811 which is beyond the permissible limit (250 mg/l) of CPCB (1995), but after degradation, native bacteria E.coli degraded COD to 200 mg/l ± 1.5811 and the percentage change is 91.6%. This is supported by the work of Soha Farag and Sohar zaki (2010).

Chromium of industrial effluent before treatment is 0.0724 mg/1 ± 0.00015 which is within the permissible (3 mg/l) of CPCB (1995) but after degradation , native bacteria E.coli degraded chromium to 0.535 mg/l ± 0.0015 and the percentage change of chromium is 63.8%. Copper of industrial effluent before treatment is 0.00124 mg/l ± 3.1622 which is within the permissible limit (1.5 mg/l) of CPCB (1995) but after degradation for 96 hours, native bacteria E.coli degraded copper to 0.0094 mg/l ± 0.00158 and the percentage change of copper is 40.37%. This is in agreement with the work of Karthikeyan etal., (2010). Thus from the above results, it can be inferred that the maximum reduction of toxic substances was recorded in biotreated sample using native bacteria E.coli (54-91%) compared to untreated effluent.

Thus from the foregoing discussion it is very clear that microbes play an important role in the biodegradation of organic and inorganic matter. During biodegradation the key element is the micro-organisms. They have enzymes that allow them to use environmental contaminants as food and hence make them ideal for biodegradation. Besides their characteristics like rapid growth, metabolism and a remarkable ability to adjust to a variety of environments make them very useful in biodegradation. How successful are the micro organisms in degrading the environmental contaminants depends on the type of microbes, contaminant and on the nature of contaminated site.

From the present study, native E.coli showed efficient degrading capabilities by degrading the contaminants as they use it for their growth and reproduction. Organic compounds are a source of carbon which forms one of the basic building blocks of new cell contaminants. In addition to the carbon source, they require nitrogen and phosphorus asimary nutrient and traces of inorganic salts through a series of complex enzymatically catalysed reaction, the toxic organic contaminant is converted to innocuous chemical compound, obtain energy by catalysing energy producing chemical reactions and this energy is used in the production of new cells (Goudar and Subramanian, 1996) finally resulting in carbon-di-oxide and water.

Thus degradation by microbes seems to be most promising technique for 100% untreated tannery effluent as evidenced in the present investigation It is well known that water of good quality and free of pollutants are primary requirements for agricultural and piscicultural practice. After degradation the treated water could be used for crop cultivation or irrigation and aquaculture purpose.

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	7.27±0.018	7.25±0.018	7.21±0.018	7.14 ±0.018	7.19±0.018	7.21 ±0.07
4	Electrical Conductivity (EC) (µmhos/cm)	400	8990±1.87	9080±1.87	9148 ± 1.87	835 ±.87	8615±1.87	8799± 2.31
5	Total Suspended Solids (TSS)(mg/l)	100	6908±1.87	1138±1.87	1176±1.87	1192±1.87	1184±1.87	1260 ±1.87
6	Total Dissolved Solids (TDS)(mg/l)	2100	6672±1.87	6712±1.87	6428±1.87	576±1.87	5950±1.87	6166 ±1.87
7	Biochemical Oxygen Demand (BOD) (mg/l)	30	1722±1.89	633±1.87	900±1.87	600± 1.87	70 ±1.80	620± 1.87
8	Chemical Oxygen Demand (COD) (mg/l)	250	9600±2.90	2920±1.87	2637±1.87	238±1.87	2399±1.87	2679± 1.87
9	Total Hardness (mg/l)	1000	1080±1.87	1426±1.16	1550±1.87	162±1.87	3750± 1.87	2300 ±1.87
10	Chloride (mg/l)	1000	1455±1.87	1890±1.88	1184±1.87	124±1.87	1373 ±6.19	1485 ±1.76
11	Sodium (mg/l)	600	1318±1.87	1300±1.87	1900±1.87	210±1.87	2040 ±1.41	1200± 1.67
12	Calcium (mg/l)	100	257±1.76	300±1.76	340±1.76	360± 1.76	390 ±1.76	560± 1.80
13	Total Chromium (mg/l)	2	44.5±0.18	36±0.187	5.47±0.018	0.01±0.043	7.24± 0.017	9.20± 0.01
14	Copper (mg/l)	3	6.40±0.018	3.89±0.018	0.001±0.0001	0.013±0.001	0.001±0.01	0.0001±0.0006

± Standard Deviation

Table 2 : Isolation and Identification of Bacteria 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	Escherichia coli (Gram negative)	+	+	+	-	+	+	30
2	Klebsiella sp (Gram negative)	+	+	+	-	-	-	16
3	Pseudomonas sp (Gram negative)	+	+	-	+	+	-	20
4	Staphylococcus aureus (Gram Positive)	+	+	+	+	+	-	20

+ Present   - Absent

TABLE – 3 Analysis of physico chemical parameters of industrial effluent before (control) and after degradation using native E.coli.

S. No.	Parameters	CPCB (1995)	Control (Untreated)	Bio-treated Native E.coli
1.	Colour	Colourless	Blackish	Light Brown
2.	Odour	Odourless	Offensive	Odourless
3.	pH	5.5 - 9.0	8.01 ± 0.5205	7.0 ±0.19235 (12.6%)
4.	Electrical Conductivity (µmhos/cm)	400	8615 ± 3.1622	3920 ±1.9235 (54.5%)
5.	Total Suspended Solids (mg/l)	100	184 ± 3.03315	24 ± 1.5811 (89.9%)
6.	Total Dissolved Solids (mg/l)	2100	5950 ± 3.16227	2740 ± 1.5811 (53.9%)
7.	Biochemical Oxygen Demand (mg/l)	30	700 ± 1.5811	70 ± 1.5811 (90%)
8.	Chemical Oxygen Demand (mg/l)	250	2399 ± 1.5811	200 ± 1.5811(91.6%)

| 9. | Chromium (mg / l) | 3 | 0.0724± 0.000158 | 0.535 ± 0.0015(63.8%) |
| 10. | Copper (mg / l) | 1.5 | 0.00124 ± 3.16228 | 0.0094 ± 0.00158 (40.3%) |

± = Standard Deviation;
% = Percentage Change

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