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BIODEGRADATION OF NUTRIENT RICH TANNERY EFFLUENT USING CELL SUSPENSION OF *PSEUDOMONAS PUTIDA*

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ABSTRACT

Tannery effluent samples rich in nutrient were collected from Ranipet, North Arcot District, Tamilnadu and analyzed for the physico-chemical parameters such as colour, pH, TDS, TSS, Chloride, Sulphide, Sulphate, Phosphorus and Nitrogen. The results showed that the above parameters exceeded the permissible limits prescribed by CPCB(1995). Biodegradation involves the use of microorganisms to degrade environmental contaminants, as microorganisms requires nitrogen and phosphorus as primary nutrients to degrade toxic compounds. In the present study *Pseudomonas putida* is used in biodegradation of tannery effluent and the results are discussed.

INTRODUCTION

Tanning industries in India are classified as high polluting and growth oriented industries. There are about 3000 tanneries in India (Sekaran *et al*. 1999). According to Buljan and Sahasranaman (1999), the tanning operations world wide have used almost 4 million tonnes of various chemicals and water produced possibly over 3000 m.cubic of waste water and 8 million tonnes of solid waste (sludge). Tannery waste waters contain high amount of proteins, BOD, chloride, hexavalent and trivalent chromium, nitrogen, phosphorus, sulphate and sulphides as inorganic constituents present in significant amount (Manivasakam, 1997). Due to indiscriminate discharges of treated and untreated tannery waste into land and water bodies get contaminated. This waste water when discharged into water course will affect physical, chemical and biological characteristics of ground water and will deplete the dissolved oxygen, High pH, excessive alkanity, suspended solids and sulphides (Gokulakrishnan 2004).

According to Covington (2000) new processing technologies are developed to meet the standards for effluent discharge. Further biological treatment processes are cost effective method for hazardous substance disposal (Ajbar 2001). The most reliable way seems to be the biological treatment in which microorganisms serve as efficient detoxifiers of pollutants (Saravanan *et al*. 1999) as microorganisms degrade organic contaminants as they use it for their growth and reproduction. The microorganisms obtain energy by catalyzing energy producing chemical reactions and this energy is used in the production of new cells (Goudar and Subramanian, 1996). According to Emmanuel (1997), nitrogen and phosphorous are the

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prime nutrients required for the biological reaction which are present in the effluents after industrial treatment. Generally, tannery effluent has been found to contain these nutrients in appropriate ratio and therefore, practically no nutrients required for aerobic and anaerobic processes through different stages in which they utilize convert the organic matter into its basic components.

Pseudomonas putida is a unique soil microorganism which can adversely effects of organic solvents, such as toluene and poly aromated hydrocarbon (PAH'S). *P.putida* was able to degrade the compounds other than phenol, present in the industrial waste water (Gonzalez *et al*. 2001). Bacterial chromate reductase can convert soluble and toxic chromate to the insoluble and less toxic Cr III can be reduced by *Pseudomonas putida* (Park *et al*. 2000). *P. putida* CA-3 possesses a styrene mono oxygenese enzyme activity and is capable of growth with the aromatic alkene styrene as a sole source of carbon and energy (Okamoto *et al*. 2003). According to Mikoczy *et al*. (1996), *P.putida* ENV 2030 efficiently degrades a model organophosphate , paraxon and use it as a carbon, energy and phosphorous source. Pure culture of *P.putida* KP-T 201 can achieve a high efficiency of TNT (Park *et al*. 2005). Strain of *P.putida* with dominant quinone ubiquinone -9 was considered to be the dominant species of the microbes contributing to the biological degradation of compounds contained in the coke oven waste water (Manohar *et al*. 2001). According to Kaech and Egli, 2001, nitrogen and phosphorus was always added to the media as NH^{+4} and phosphate buffer as sole energy. Hence, no selection pressure was applied for *Pseudomonas putida* in tannery as it utilizes energy from the effluent itself. Hence in the present study biodegradation using *Pseudomonas putida* was carried out to meet the permissible limits for the biotreated effluent.

MATERIALS AND METHODS

For the present study, the tannery effluent was collected from a tannery in Ranipet, Tamilnadu, which has an industrial treatment plant. The effluent samples were collected from initial and final discharge points.

The pure culture of *Pseudomonas putida* (MTCC 2445) was obtained from the Institute of Microbial Technology, Chandigarh, India.

The physico-chemical parameters such as pH, EC, colour, COD, BOD, TSS, TDS, Sulphide, Chloride, Sulphate, Phosphorous and Nitrogen of the effluents were analyzed following the Standard Methods of APHA (1989).

MASS Culture of *Pseudomonas putida*

The viability of *Pseudomonas putida* from pure culture was maintained by regular subcultures on the nutrient agar slant to have the stock through out the year. *Pseudomonas putida* was inoculated in the nutrient agar medium (Nwachukwu *et al*. 2001), the pH was adjusted at 7.4 ± 2°C, for mass culture the inoculum was taken from the nutrient agar medium and inoculated in 200mL nutrient broth in flask. The flasks were kept in orbit shaker at 180 rpm for 24hrs. The cell number was calculated using haemocyto-meter before subjecting it for experimental studies.

Biodegradation of Tannery Effluent Using Cell Suspension Method

400mL of tannery effluent (both untreated and industrial treated) were taken in 500mL autoclaved serum bottles. To this *Pseudomonas putida* was added at a cell population of (10-4 cells/mL). Controls were in abiotic medium with 400mL of untreated and industrial treated effluent. The experiments were conducted at 30°C ± 2 at 180 rpm. The physico-chemical parameters were studied on 5th, 10th and 15th day of experiments.

RESULTS AND DISCUSSION

Analysis of physico-chemical parameters in tannery effluent from untreated and industrial treated is shown in Table1. The investigation reveals that the colour of the untreated effluent was black and industrial treated was greyish yellow and the odour was pungent in both the sample may be due to large amount of total dissolved solids and chromium (Jawahar *et al*. 1998). The pH of the untreated effluent and industrial treated was highly acidic (3.9 and 6.7) and did not meet the general standards recommended by CPCB (1995) which is due to sub process such as bathing and fat liquoring in which acetic acid and formic acid are used as the source for the acidic pH (Manivasakam 1997 and Mohamed 2002). The EC of tannery effluent and industrial treated effluent was 12222 µmhos/cm and 8633 µmhos/cm respectively high level of conductivity may be due to the presence of inorganic substances and salts (Jawahar *et al*. 1998).

BOD (5 days) of the untreated and industrial

Table 1. Physico-chemical Characteristics of tannery effluents

Parameters	Untreated effluent	Industrial treated effluent	Permissible limits of CPCB (1995) mg/L
Colour	Grey colour	Slightly grey	*
Odour	Disagreeable smell	Disagreeable smell	Odourless
pH	3.90 ± 0.51	6.7 ± 0.42	5.5-9.0
EC (µm.hos/cm)	12222 ±1119	8633±966	**
TSS (mg/L)	1015 ±115	528 ± 134	200
TDS (mg/L)	11556 ±909	7496 ± 794	2100
COD (mg/L)	4857 ±563	3112 ± 108	250
BOD (mg/L)	1019 ±163	705 ± 148	100
Sulphide (mg/L)	906 ±119	508 ± 119	**
Chloride (mg/L)	3419 ±671	2415 ± 704	650
Sulphate (mg/L)	2274 ± 430	1387 ± 445	400
Phosphorous (mg/L)	9.2 ± 2.7	7.7 ± 3.3	0-0.02
Nitrogen (mg/L)	61 ± 10	30.3 ± 10.9	0-0.02
Chromium (mg/L)	39.1 ± 11.8	20.8 ± 6.3	2

*-All efforts should be made to remove colour and unpleased odour as far as possible.
**-Permissible limit should be placed as Nil or agreeable.

treated were 1019 mg/L and 705 mg/L respectively which is due to presence of organic matter and toxic compounds (Trivedy, 2002) while the COD was 4857 mg/L and 3112 mg/L, these results found to be higher than the prescribed limits of CPCB, 1995 indicating the highly polluting nature of the tannery effluent. TDS of the untreated and industrial treated was 11556 mg/L and 7496 mg/L respectively due to substantial contribution made by sodium chlorides used during the preservation of raw hide skins (Manivasakam 1997) high contents of TDS would produce undesirable taste, gastro intestinal irritation, corrosion or incrustation(Gokulakrishnan and Pandurangan 2004) and the TSS content of untreated was 1015 mg/L and 528 mg/L. Sulphide content of untreated and industrial treated was 906 mg/L and 508 mg/L sulphide is usually present in excess concentration its toxic to fish and other aquatic life (Manivasakam 1997).

Chloride was 3419 mg/L and 2415 mg/L which make the water unsuitable for irrigation. Sulphate was found to be 2274 mg/L and 1387 mg/L which was found to be higher and impact hardness to the water and exceed the permissible limits of CPCB (1995). Phosphorus level of untreated was 9.2 mg/L and industrial treated was 7.7 mg/L. Nitrogen level of untreated and industrial treated was 61 mg/L and 30.3 mg/L. The results of all parameters showed values greater than the limits prescribed by CPCB (1995) after industrial treatment which prompted us to find ways and means to biodegrade the tannery effluent.

Pseudomonas putida were used to biodegrade as they degrade organic contaminants for their growth and reproduction (Park *et al*. 2000). In addition the microorganisms requires nitrogen and phosphorus as primary nutrients, the microorganisms obtain energy by catalyzing energy producing chemical reaction and this energy is used in the production of new cells (Goudar and Subramaniam, 1996). The results showed that both untreated and industrial treated effluent has been found to contain these nutrients, practically no nutrient addition is required for microbial growth (Emmanuel, 1997) addition of phosphorus as phosphate buffer and nitrogen as NH+4 again increase the total dissolved solids and other organic solvents.

Culture of *Pseudomonas putida* were suspended in 100% untreated and industrial treated effluent for biodegradation. The biotreated effluents (untreated and industrial treated effluent) were analyzed for physico-chemical parameters at 5th, 10th, &15th day. According to of Ghosh *et al*. 2002, *Pseudomonas putida* was used to reduce colour as it is known to produce hydrogen peroxide, a strong decolourizing agent. These colour reduction from the results satisfy the CPCB (1995) prescribed standards for effluent discharge into land surface waters and irrigation. While Park *et al*. 2003 has demonstrated that *P.putida* KP-T202 could degrade TNT at various pH. The biotreated effluent after biodegradation was found to be de-

Table 2. Physico-chemical characteristics of untreated and industrial treated effluent before (control) and after biotreatment using cell suspension culture of *P.putida*

Parameters	Control	Biotreatment Untreated Effluent			Control	Biotreatment Industrial Treated		
		5th Day	10th Day	15th Day		5th Day	10th Day	15th Day
Colour	Grey	Slightly grey	Slightly grey	Slightly grey	Slightly grey	Yellow	Yellow	Yellow
Odour	Disagreeable	Agreeable	Agreeable	Agreeable	Disagreeable	Agreeable	Agreeable	Agreeable
pH	3.90 ± 0.51	6.3 ±0.43***	7.29 ± 0.47***	6.4 ± 0.47***	6.7 ± 0.42	6.4 ± 0 .66 NS	7.0 ± 0.41 NS	7.0 ±0.41*
EC (µmhos/cm)	12222 ±1119	9981 ±1080***	8209 ± 915***	9066 ± 920***	8633±966	8223 ± 923 NS	8303 ± 934 NS	8434 ± 878 NS
TSS (mg/L)	1015 ±115	797 ± 87***	584 ± 103***	690 ± 117***	528 ± 134	498 ± 131 NS	505 ± 137 NS	536 ±119 NS
TDS (mg/L)	11556 ±909	8496 ± 774***	6767 ± 832***	7166 ± 621***	7496 ± 794	7141 ± 755 NS	7219 ± 767 NS	7242 ± 735 NS
COD (mg/L)	4857 ±563	3955 ± 615 NS	3227 ± 715*	3507 ± 652*	3112 ± 108	2966 ± 103 NS	2991 ± 104 NS	3025 ± 104 NS
BOD (mg/L)	1019 ±163	817 ± 113**	578 ± 95***	689 ± 92***	705 ± 148	667 ± 143 NS	680 ± 142 NS	685 ± 144 NS
Sulphide (mg/L)	906 ±119	729 ± 99**	601 ± 74***	690 ± 73**	508 ± 119	473 ± 84 NS	490 ± 83 NS	503 ± 84 NS
Chloride (mg/L)	3419 ±671	2655 ± 480**	2049 ± 394***	2201 ± 324***	2415 ± 704	2301 ± 670 NS	2321 ± 675 NS	2373 ± 687 NS
Sulphate (mg/L)	2274 ± 430	1656 ± 390***	1163 ± 310***	1432 ± 303***	1387 ± 445	1294 ± 426 NS	1333 ± 427 NS	1348 ± 432 NS
Phosphorous (mg/L)	9.2 ± 2.7	7.9 ± 2.2 NS	7.1 ± 1.8 NS	7.7 ± 2.7 NS	7.7 ± 3.3	7.4 ± 3.2 NS	8.1 ± 3.3 NS	8.2 ± 3.4 NS
Nitrogen (mg/L	61 ± 10	54.8 ± 10.3 NS	46.5 ± 8.7**	51 ± 10.5*	30.3 ± 10.9	29.0 ± 10.5 NS	30.1 ± 10.6 NS	31.1 ± 11.0 NS
Chromium (mg/l)	39.1 ± 11.8	32.2 ± 10.7 NS	27 ± 6.6*	21.1 ± 8.5**	20.8 ± 6.3	20.3 ± 6.0 NS	18.1 ± 5.9 NS	16.9 ± 5.6 NS

***Significant at P< 0.001, **Significant at P<0.01, *Significant at P<0.05 and NS- Not Significant

creased in all physico chemical parameters and this work was supported with the work of Nisha (2002), according to whom there is a reduction of 60% TSS, TDS, Sulphate during biodegradation of petrochemical effluent using cell suspension *P.putida*. Biodegradation of black liquor by P.putida emphasizes its biodegrading efficiency 70-80% of COD removal (Srivastava *et al*. 1995). The results showed that *Pseudomonas putida* reduced the concentration of pollutants in 5th and 10th day (untreated and industrial treated effluent) like chloride, sulphide, TDS. During 15th day of study there is no drastic changes occurred due to increase of phosphorus and nitrogen which the microorganisms obtain less amount of phosphorus and nitrogen has considerably indicating the degradation of organic matter without any sludge formation. This work has been supported by Ishibashi *et al*. 1990 by reduction of hexavalent chromium (chromate) to less toxic trivalent chromium using cell suspension of Pseudomonas putida.

Results of the present study also reveals that the biodegradation of untreated effluent was more efficient than the industrial treated effluent this may be due to the reason that the growth and activity of fungus was inhibited in high nitrogen concentration (Keyser *et al*. 1978; Jeffries *et al*. 1981 and Leatham and Kirk, 1983). High nitrogen concentrations in the industrial treated effluent may be due to the increase in nitrogen and phosphorous compounds present in the industrial treated effluent which may releases from the chemical compounds used for the treatments such as alum and calcium carbonate, these chemicals may precipitate the organic and inorganic compounds which may not be available to the microorganisms.

From the foregoing results it is evi-

dent that a beneficial association of *Pseudomonas putida* as biodegraders in the tannery effluent decreased the parameters like colour, odour, TDS, sulphide and chloride which help as in reduction of organic pollutants.

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