Microbial Reduction of Chromium (VI) in Tannery Waste Contaminated Soil through Optimum Concentrations of Biomass, Molasses and Mineral Medium using Miniature Reactor

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ABSTRACT: Soil pollution by metals is essentially different from air or water pollution because the persistence of heavy metals in soil is reportedly much longer than in other compartments of the biosphere. Removal of heavy metals from polluted soil is difficult. Once deposited on the soil certain metals such as chromium may be virtually permanent. Tannery wastewater is mainly characterized by high pH, high salinity, high oxygen demand, high organic loading and specific pollutants such as chromium which pollute the soil by the continuous irrigation onto the soil. High levels of heavy metals can damage soil fertility and may affect productivity. The conventional treatment methods for removal of metals caused secondary pollution and adverse effects on biological activities, soil structures and fertility. In recent years there has been an increased interest in alternative and innovative technologies which will be of low cost, low maintenance and energy efficient. The search for new and cost effective and eco-friendly innovative technology has focused the attention on biotransformation of contaminated soil by microbes. The present investigation focused the effect of pH, Electrical conductivity, total organic carbon and hexavalent chromium of tannery contaminated soil using different concentrations of biomass (10%, 15%, 20%, 25% and 30%), molasses (10ml, 15ml, 20ml, 25ml and 30ml) and mineral medium (10ml, 15ml, 20ml, 30ml and 40ml) on 10th and 20th day. The results inferred that the significant reductions were observed the pH, Electrical Conductivity and Total Organic carbon in all the concentrations of biomass, molasses and mineral medium except control. Among all the various concentrations of reactors, the maximum reductions of all the parameters were observed in 15% concentration of biomass, 25ml concentration of molasses and 40ml concentration of mineral medium.

Key words: Tannery contaminated soil, molasses, mineral medium and bioremediation

1 INTRODUCTION

Indian leather industry occupies an important position in foreign exchange earnings. This phenomenal growth obviously calls for the processing of enormous amounts of hides and skins. The tanning process involves pre-tanning, tanning and post tanning operations. During the processing of leather, substantial amount of solid and liquid wastes are generated (Chakraborty, 2003), especially toxic metal chromium passing to wastewater and are not easily eliminated by ordinary treatment process (Franco *et al.*, 2005).

Tannery wastewaters are mainly characterized by high salinity, high oxygen demand, high organic loading and specific pollutants such as chromium (Bajza and Vrcek, 2001; Colak *et al.*, 2005). Among the oxidation states of chromium, the hexavalent Chromium (Cr (VI)) and trivalent Chromium (Cr (III)) forms are of significance in aqueous systems. The hexavalent form is water soluble, toxic, mutagenic and carcinogenic to humans and mammals whereas the trivalent form is less soluble and less mobile and 100 times less toxic than Cr (VI) (DeFlora *et al.*, 1990; Ramakrishna and Philip, 2005).

A number of small scale and large scale leather processing industries are situated on the banks of river Cauvery. There are more than 110 leather processing units in Erode (TN) alone, causing serious water pollution and spoil the agricultural land and underground water. These industries have their own effluent treatment units but industries which treat the effluent before let out are very few. Most of the time, the industries let the untreated effluent in the river during the night time or during heavy rains. Frequent litigations between these units and several Green Peace Organizations lead to closure of these units temporarily. The Government and the Court are unable to find a permanent solution for the pollution free operation of these tannery units.

Various conventional methods for cleaning up the contaminated sites with hexavalent chromium is excavation

or pumping of the contaminated material, addition of chemical reductants, precipitation followed by sedimentation, or ion exchange and/or adsorption, etc. These are practiced both in situ and ex situ systems. These chemical and physical removal techniques suffer from high costs associated with energy and chemical consumption and they produce solid residues (Zahir, 1996; Davies *et al.*, 2002; Ramakrishna and Philip, 2005; Mohan *et al.*, 2006).

Bioremediation is one of the promising technologies that are expected to play an important role in waste site cleanup. Biological reduction of Cr (VI) using indigenous microorganisms offers a new cost-effective and environmentally compatible technology (Camargo *et al.*, 2005).

The present study focused to evaluate the optimum conditions of different concentrations of microbial biomass, molasses and mineral medium using miniature reactors to hexavalent chromium reduction in tannery waste contaminated soil.

2 MATERIALS AND METHODS

2.1 Collection and Characterization of Tannery Waste Contaminated Soil

Soil samples were collected from tannery waste contaminated site located nearer to the tannery industry. Sampling was done from areas within one kilometer radius of effluent discharge site. The collected soil sample was mixed well, dried in the oven at 150^{o}C for 2 hours and ground well. Parameters such as pH, Electrical Conductivity (EC), Total organic carbon (TOC) and Hexavalent chromium were characterized in the laboratory as per standard methods. The pH was determined potentiometrically in a 1:10 suspension of the sample in de-ionized water (APHA, 2005). Electrical Conductivity (EC) was measured on water extracts obtained at a sample: water ratio of 1:10 using conductometry (APHA, 2005). Total Organic Carbon was determined by wet oxidation with potassium dichromate (Walkley and Black, 1934). Hexavalent chromium were analysed by Atomic Absorption Spectrophotometric method and diphenyl carbazide assay respectively (APHA, 2005).

2.2 Evaluation of Optimum Conditions Using Miniature Reactors

To evaluate the optimum conditions for bioremediation, miniature reactors were employed. Soil used for all the biotransformation studies were sterilized in a hot air oven keeping it at 150^{o}C for 2 hours. The miniature reactors were of 1 L capacity plastic beakers containing 500 g of contaminated soil. There was no provision for leachate collection.

2.3 Preparation of Mineral Media

The mineral medium consisted of K_2HPO_4 2.12g, KH_2PO_4 2.12g, NaCl 2g, $MgSO_4.7H_2O$ 1g, $CaCl_2$ 0.1g and KNO_3 4g in 1 L of distilled water. All media were autoclaved at 120^{o}C and 15 psi for 15 minutes and stored at room temperature until use. The pH was maintained at 7±0.2 by using HCl or NaOH.

2.4 Collection of Molasses

Molasses was collected from Sakthi Sugars Factory, Erode District, Tamil Nadu. It was used as a carbon source. Molasses was added to the soil to promote microbial activity.

2.5 Optimum Concentration of Bacteria, Molasses and Mineral Medium

A set of six small reactors (1 L capacity) were operated with 500g of tannery waste contaminated soil, 40ml of mineral medium and 20ml of molasses. The first five reactors were inoculated with different concentrations of bacteria like 10, 15, 20, 25 and 30% respectively. The sixth reactor served as control (0%) without inoculation of bacteria. The same procedures followed for optimization of molasses (10, 15, 20, 25 and 30ml with 40ml of mineral media with 15% concentrations of biomass) and mineral medium (10, 15, 20, 30 and 40ml with 15% concentration of bacteria and 25ml of molasses). The performance of the reactors was maintained at same condition and monitored regularly. The top of all the reactors were covered with wet cotton to maintain the moisture content in the range of 55-60%. On 10th and 20th day, the samples were collected in sterile plastic bottles and stored in the refrigerator at 4^{o}C for analyses of physico-chemical properties.

3 RESULTS AND DISCUSSION

3.1 Physico-Chemical Parameters of Tannery Contaminated Soil with Different Concentrations of Biomass, Molasses and Mineral Medium

The experiments were carried out to evaluate the optimum conditions for soil bioreactors. After the completion of experiment, soil samples were subjected to physico-chemical analysis and the results are given below.

3.1.1 pH

The pH was analyzed in different concentrations of biomass, molasses and mineral media with tannery contaminated soil on 10th and 20th days. The pH was slightly alkaline on 10thday. This was due to the chemicals such as sodium carbonate, sodium bicarbonate, sodium chloride and calcium chloride based in tanning causes the alkalization of that soil resulting in the increase in pH of the soil as suggested by Babyshakila and Usha (2009). Subsequently it got reduced almost to the neutral except control. Significant changes (near neutral) were observed in all the concentrations of biomass, molasses and mineral medium on 20th day (Table 1). It was established that near neutral pH generally results in the largest diverse bacterial population by Rabah and Ibrahim (2010). They reported that the high counts of microorganisms obtained from all the sampling sites since most microorganisms thrive well in such

neutral pH. This pH could also further enhance microbial degradation of the contents. Mythili and Karthikeyan (2011) reported that the optimum pH was 7 for reduction of hexavalent chromium by *Bacillus* sp. and *Staphylococcus* sp.

TABLE 1 pH OF DIFFERENT CONCENTRATIONS OF BIOMASS, MOLASSES AND MINERAL MEDIUM WITH TANNERY CONTAMINATED SOIL

Experimental days	pH in different concentrations of biomass						P value (< 0.05)
	Control (0%)	10%	15%	20%	25%	30%	
0 day	8.5±1.0	8.5±1.0	8.5±1.0	8.5±1.0	8.5±1.0	8.5±1.0	0.00081*
10days	7.69±0.8	7.88±1.2	7.97±1.2	7.86±1.0	7.80±1.2	7.84±1.1	
20days	7.47±0.9	7.21±1.2	7.11±1.2	7.16±1.2	7.15±1.2	7.14±1.3	
	pH in different concentrations of molasses						
	Control (0ml)	10ml	15ml	20ml	25ml	30ml	
0 day	8.5±1.0	8.5±1.0	8.5±1.0	8.5±1.0	8.5±1.0	8.5±1.0	0.028605*
10days	7.93±1.0	7.80±1.1	7.69±1.2	7.79±1.2	7.92±1.0	7.01±1.0	
20days	7.46±1.1	7.24±1.2	7.33±1.3	7.24±1.2	7.15±1.1	7.22±1.2	
	pH in different concentrations of mineral medium						
	Control (0ml)	10ml	15ml	20ml	30ml	40ml	
0 day	8.5±1.0	8.5±1.0	8.5±1.0	8.5±1.0	8.5±1.0	8.5±1.0	0.00507*
10days	7.75±0.06	7.71±0.1	7.69±0.2	7.65±0.1	7.93±0.3	7.44±0.02	
20days	7.25±0.1	7.05±0.12	6.98±0.11	7.44±0.3	7.03±0.02	7.21±0.01	

Two way ANOVA of pH * Significance (P< 0.05) ** Insignificance (P>0.05)

3.1.2 Electrical Conductivity (EC)

Electrical conductivity of the initial contaminated soil was 1.836±0.1dSm^{-1}. This was due to the tannery effluent is rich in salts, particularly sodium chloride, which on continuous irrigation increased the concentration in soil and reflected in increased electrical conductivity. Similar observations were made by Thangavel *et al.* (2003) and Mehdi (2005). This was reduced on 10th day and on 20th day in different concentrations of biomass, molasses and mineral medium. However, no significant reductions were observed in all the control reactors (Table 2). The study showed that the electrical conductivity got reduced could be due to the utilization of soluble salts by microorganisms for the synthesis of microbial biomass (Sridevi *et al.,* 2007).

TABLE 2 ELECTRICAL CONDUCTIVITY OF DIFFERENT CONCENTRATIONS OF BIOMASS, MOLASSES AND MINERAL MEDIUM WITH TANNERY CONTAMINATED SOIL

Experimental days	EC (dSm^{-1}) in different concentrations of biomass						P value (< 0.05)
	Control (0%)	10%	15%	20%	25%	30%	
0 day	1.836±0.1	1.836±0.1	1.836±0.1	1.836±0.1	1.836±0.1	1.836±0.1	0.06925**
10days	1.089±0.1	0.986±0.2	0.910±0.1	0.926±0.3	0.911±0.1	0.821±0.2	
20days	1.026±0.2	0.840±0.1	0.846±0.4	0.700±0.2	0.754±0.1	0.893±0.2	
	EC (dSm^{-1}) in different concentrations of molasses						
	Control (0ml)	10ml	15ml	20ml	25ml	30ml	
0 day	1.836±0.1	1.836±0.1	1.836±0.1	1.836±0.1	1.836±0.1	1.836±0.1	0.81855**
10days	0.669±0.2	0.683±0.3	0.887±0.1	0.720±0.1	0.807±0.2	0.905±0.2	
20days	0.699±0.1	0.671±0.2	0.668±0.1	0.671±0.2	1.056±0.3	0.998±0.1	
	EC (dSm^{-1}) in different concentrations of mineral medium						
	Control (0ml)	10ml	15ml	20ml	30ml	40ml	
0 day	1.836±0.1	1.836±0.1	1.836±0.1	1.836±0.1	1.836±0.1	1.836±0.1	0.718311**
10days	0.714±0.01	0.669±0.2	0.656±0.1	0.682±0.1	0.711±0.02	0.977±0.3	
20days	0.687±0.1	0.831±0.2	0.755±0.03	0.680±0.1	0.620±0.2	0.926±0.1	

Two way ANOVA of EC * Significance (P< 0.05) ** Insignificance (P>0.05)

3.1.3 Total Organic Carbon (TOC)

Initially TOC was found to be higher (440±11.1mg/kg) in contaminated soil. It got decreased significantly on 10th day and on 20th day when experimental days are extended in all the concentrations of biomass, mineral medium and molasses (except in 20, 25 and 30ml concentrations) (Table 3). This is in accordance with the findings of Mathur *et al.* (1980) and Gundappa (1999), who reported that the total organic carbon reduced with time during experimental days. This decreasing trend was due to more efficient oxidation of organic carbon by the microorganisms present in the microbial population. The total organic carbon is lost as carbon di-oxide through microbial respiration and mineralization of organic matter causing proportional increase in total nitrogen. Part of the carbon released as CO_2 and a part was assimilated by the microbial biomass (Fang *et al.*, 2001; Cabrera *et al.*, 2005). On the contrary, in 20, 25 and 30ml concentrations of molasses, the total organic carbon was increased with concentration of molasses increased on 10th day afterwards it was reduced on 20th day. Tripathi and Bhardwaj (2004) explained that the loss of carbon due to the enzymatic activity of microbes.

TABLE 3 TOTAL ORGANIC CARBON IN DIFFERENT CONCENTRATIONS OF BIOMASS, MOLASSES AND MINERAL MEDIUM WITH TANNERY CONTAMINATED SOIL

Experimental days	TOC (mg/kg) in different concentrations of biomass						P value (< 0.05)
	Control (0%)	10%	15%	20%	25%	30%	
0 day	440±11.1	440±11.1	440±11.1	440±11.1	440±11.1	440±11.1	0.003348*
10days	400±15.2	410±14.2	420±13.2	408±12.8	427±14.0	425±14.3	
20days	394±17.0	318±15.7	312±15.8	324±15.6	321±15.6	332±15.8	
	TOC (mg/kg) in different concentrations of molasses						
	Control (0ml)	10ml	15ml	20ml	25ml	30ml	
0 day	440±11.1	440±11.1	440±11.1	440±11.1	440±11.1	440±11.1	6.98E-05*
10days	390±16.8	420±16.4	432±14.2	445±15.6	452±16.7	460±15.4	
20days	284±17.0	212±17.6	244±14.6	267±17.4	265±15.3	268±14.1	
	TOC (mg/kg) in different concentrations of mineral medium						
	Control (0ml)	10ml	15ml	20ml	30ml	40ml	
0 day	440±11.1	440±11.1	440±11.1	440±11.1	440±11.1	440±11.1	0.000457*
10days	383±15.6	320±15.6	330±12.1	335±12.5	338±14.5	346±15.9	
20days	344±16.5	240±16.8	245±16.4	244±16.2	288±16.7	280±15.5	

Two way ANOVA of TOC * Significance (P< 0.05) ** Insignificance (P>0.05)

3.1.4 Hexavalent Chromium

The level of hexavalent chromium was found to be 52.4±3.1mg/kg in initial contaminated soil. In various concentrations of biomass, molasses and mineral medium, tested for hexavalent chromium, significant reductions were observed when experimental days are increased. From the results it was inferred that the hexavalent chromium got reduced significantly on 10th and 20th day in all the three sets of reactors (Table 4; Fig. 10, 11 & 12). Among all the various concentrations of reactors, the maximum reductions of hexavalent chromium were observed in 15% concentration of biomass, 25ml concentration of molasses and 40ml concentration of mineral medium (20.6±5.6mg/kg; 18.5±5.5mg/kg and 16.0±4.2mg/kg respectively) on 20th day of analysis (Table 4; Fig. 10).

TABLE 4 HEXAVALENT CHROMIUM CONTENT IN DIFFERENT CONCENTRATIONS OF BIOMASS, MOLASSES AND MINERAL MEDIUM WITH TANNERY CONTAMINATED SOIL

Experimental days	Cr (VI) (mg/kg) in different concentrations of biomass						P value (< 0.05)
	Control (0%)	10%	15%	20%	25%	30%	
0 day	52.4±3.1	52.4±3.1	52.4±3.1	52.4±3.1	52.4±3.1	52.4±3.1	0.006835*
10days	50.4±4.8	36.2±5.6	31.0±5.9	35.7±5.4	34.1±5.5	36.4±5.6	
20days	50.3±4.4	26.1±5.6	20.6±5.6	21.1±5.8	26.2±5.6	23.0±5.9	
	Cr (VI) (mg/kg) in different concentrations of molasses						
	Control (0ml)	10ml	15ml	20ml	25ml	30ml	
0 day	52.4±3.1	52.4±3.1	52.4±3.1	52.4±3.1	52.4±3.1	52.4±3.1	0.003083*
10days	47.6±4.2	40.2±3.3	32.0±4.2	30.5±5.9	31.1±0.5	35.8±5.7	
20days	45.4±3.1	24.9±3.2	21.6±4.2	20.1±5.1	18.5±5.5	18.8±5.8	

	Cr (VI) (mg/kg) in different concentrations of mineral medium						
	Control (0ml)	**10ml**	**15ml**	**20ml**	**30ml**	**40ml**	
0 day	52.4±3.1	52.4±3.1	52.4±3.1	52.4±3.1	52.4±3.1	52.4±3.1	
10days	48.0±4.2	35.9±3.2	36.2±2.2	32.5±2.8	38.2±4.0	36.2±3.2	0.005822*
20days	46.4±3.2	24.5±3.1	21.5±2.8	22.0±3.0	18.3±4.5	16.0±4.2	

Two way ANOVA of Cr (VI) * Significance (P< 0.05) ** Insignificance (P>0.05)

A higher hexavalent chromium reduction by indigenous microbes under aerobic condition especially when the initial Cr (VI) concentration was higher and also depended on the amount of carbon source available to the *Pseudomonas fluorescens* (Ganguli and Tripathi, 1999; Tseng and Bielefeldt, 2002). Similar results were observed by Jeyasingh and Philip (2005). They reported that the 97% reduction of hexavalent chromium was achieved within 20 days under optimum concentrations and also reported that the Cr (VI) reduction rate increased with increase in molasses concentration. Fathima *et al.* (2010) reported that hexavalent chromium was reduced significantly by *Bacillus subtilis* under aerobic conditions in the presence of chromium reductase.

In conclusion, it was shown that the aged tannery contaminated soil has high concentrations of pH, Electrical conductivity and Total organic carbon which affected soil physicochemical properties. The optimum conditions of different concentrations of molasses, microbial biomass and mineral medium were evaluated using miniature reactors to hexavalent chromium reduction in contaminated soil. Growth and activity of indigenous microorganisms in miniature could be enhanced by optimum concentrations of molasses and mineral medium, which may further increase bioconversion of hexavalent chromium in to trivalent form in the soil for microbial degradation.

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