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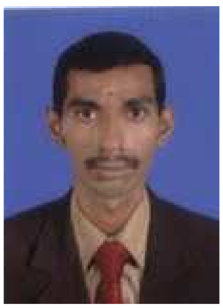
EFFECT OF MICROBIALLY TREATED INDUSTRY EFFLUENT ON THE GROWTH OF GREEN GRAM (PHASEOLUS AUREUS-ROXB)



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ABSTRACT

Biosurfactant are surface-active substances synthesized by living cells-in the majority of cases by microorganisms. Metallic contaminants in the soil are poisonous and their accumulation in plants and water may be dangerous. Microorganisms are known to absorb; adsorb and accumulate heavy metals and they can be made use of in solving major problems associated with metals. The extensive use of chromium in different industries such as leather, textile, metal electro plating etc has resulted in the discharge of chromium compounds into aquatic system. Among the fungal isolates, *Mucor sp.* ATS 5 was predominant using inactive cells about 84% chromium was removed, whereas in resting cells it was found to be 79%. In the case of bacterial population, *Pseudomonas sp.* ATS-08 was predominant about 86% chromium was removed using inactive cells followed by resting cells (75%). Among the actinomycetes isolates, *Nocardia sp.* ATS-2 was predominant. 90% chromium was removed using inactive cells of *Nocardia* sp. followed by resting cells (84%). From the above study, it is concluded that the actinomycetes was the best effective followed by bacteria and fungi and could be used for treating industrial effluents having high amount of chromium.

KEY WORDS

Biosurfactant, metals, chromium, leather, effluents

INTRODUCTION

Heavy metal is a prevalent pollutant in the soil, water and air. Heavy metal pollution has arised from the rapid urbanization, evolution of metal based industries and other developmental activities. Some of the heavy metal acts as a harmful environmental pollutant. The sources of these metals is an important factor that include metal plating, mining by product, pesticide waste, chemical waste, coal based waste, industrial waste, gasoline, nuclear waste and mineral leaching (Brady *et al*., 1994; Horvat, 2007). Cadmium occurs in higher concentration in the waste from electroplating, paints, dyes, chrome tanning, paper industries etc (Ansari and Malik, 2007). Lead is also a toxic element and is obtained from industrial wastewater such as printing, dyeing and oil refineries. Tanning industry is one of the major industries, which contributes to water pollution owing to the usage of mineral tanning agents. Katsoyiannis and Zouboulis *et al*., (2004) have reported heavy metal removal by oxidation processes which were mediated by specific bacteria, namely the *Leptothrix ochracea* and *Gallionella ferruginea*, which belong to the general category of manganese and iron oxidizing bacteria. Biosurfactant are surface-active substances synthesized by living cells-in the majority of cases by microorganisms. A number of microorganisms are known to synthesize surface-active agents (Cooper and Zagic, 1980; Banat, 1993). Biosurfactant production by several hydrocarbon-degrading microorganisms is growth associated (Rosenberg *et al*., 1979; Banerjee *et al.,* 1983). Several microorganisms are known to synthesise surface-active agents, most of them are bacteria and yeasts (Banat, 1995; Kim *et al.,* 2000). There are reports of surfactants produced by *Corynebacterium sp.* (Haferburg *et al*., 1986; Cooper *et al*., 1989; Banat, 1995), *Bacillus* sp. (Cooper *et al* 1981; Banat, 1993), *Pseudonomas* sp. (Guerra Santos *et al* 1986), *Micrococcus* sp. (Gutnick, 1984) and *Mycobacterium sp*. (Cooper *et al*., 1989).

Greater understanding of the physiology, genetics and biochemistry of biosurfactant producing strains and improved process technology can reduce the production costs (Fiechter, 1992). The efficient removal of copper by terrestrial plants like *Elsholtzia splendens* and *Silene vulgaris* (Jing Song *et al*., 2004). There are reports on genotoxicity on plants test systems by effluents. Also, in the present investigation, an attempt has been made to study the effect of microbially treated and untreated tannery effluent on the growth of green gram.

MATERIALS AND METHODS

Collection of samples

The tannery industry effluent and effluent contaminated soil were collected from tannery industry located in Erode District. The tannery effluent was collected in a sterile glass bottle from the point of release. The effluent contaminated soil was also collected in a sterile polythene bag from near by canal. The samples were transported to the laboratory within two to three hours in an ice bar. The microbiological (bacteria, fungi and actinomycetes) of the samples were estimated within two to three hours after reaching the laboratory.

Estimation of microbial population

Pour plate technique and spread plate technique were employed for the enumeration of bacteria, fungi and actinomycetes. One ml aliquot of 10^{-1} to 10^{-4}

sample dilutions were pipetted out into sterilized petridishes and 20ml of the medium was poured (Nutrient agar for bacteria, Czapeks-Dox agar for fungi, Glucose asparagine agar for actinomycetes). After incubation, morphologically different colonies were isolated and purified by repeated streaking on agar plates. Isolates were stored in respective agar slants for further studies.

Identification of microbes

The bacterial cultures were grouped to various genera based on their morphological and biochemical characters as given in Bergy's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). Fungal hyphae spores and fruiting structures by lactophenol cotton blue. The genera of actinomycete were identified by Microbial type cell culture collection and gene bank (MTCC).

Chromium (Cr^{3+}) resistance

The organisms were determined for resistance to various concentrations of chromium. Metal solutions of filter sterilized chromium was added to sterile agar medium (Chromium + NA for bacteria); Chromium + CDA for fiungi); chromium +GAA for actinomycetes). The test isolates were spot inoculated against various concentration of chromium and incubated at room temperature (30^0C). Growth indicated resistance of the organism to that concentration of chromium.

Oxidation of trivalent chromium (Cr III)

10 ml of the sample was taken in 100ml conical flask and 3 drops of methyl orange and 1ml of ammonium hydroxide was added. It was then neutralized with 1:1 H_2SO_4 until permanent pale pink colour persists. Twenty drops excess of 1:1 H_2SO_4 was added. It was heated to warm temperature over sand bath. To this hot solution one drop of 4 % $KMNO_4$ solution was added and heated continuously. Potassium permanganate solution was added drop wise until the colour of $KMNO_4$ persisted. Sodium azide solution was added drop wise until the solution turned colourless and again heated to 30 sec. Cool it to make upto 50ml in a SMF with 1ml of diphenyl carbazide. After 10 min, the absorbance was measured at 540nm (Standard methods for the examination of water and waste water, Manivasakam, 1987).

Removal of chromium (Cr^{3+}) by fungal biomass

Batch mode studies were carried out to determine the adsorption of various concentration of chromium (Cr^{3+}). For efficient removal of chromium from aqueous solution, the agitation time, adsorbent dosage, temperature and pH were determined.

Effect of agitation time

About 50ml of different concentrations (4, 5, and 6ppm) of the synthetic solution were taken to which adsorbent was added. It was then agitated in a rotary shaker at 120 rpm for predetermined time intervals. Then the adsorbate and the adsorbent were separated by filtration or centrifugation at 10000 rpm for 20 min. The remaining chromium in aqueous solution was analyzed spectrophotometrically.

Effect of adsorbent dosage

For determining optimum adsorbent dosage for chromium removal, the batch mode study was carried out with different adsorbent dosages (0.25, 0.50, 0.75, 1.0, 1.25, 1.50 and 1.75g/50ml for an equilibrium time.

Effect of pH

To determine the optimum pH level, the adsorption experiments were conducted at various pH (2, 4, 6, 8 and 10) with predetermined optimum adsorbent dosage and agitation time. For pH adjustment 0.1N HCL and 0.1N NaOH were used.

Effect of temperature

To determine the optimum temperature, the adsorption experiments were conducted at various temperatures (25, 30, 35, 40, and 50^0C) with predetermined optimum adsorbent dosage, agitation time and pH .

Removal of chromium (Cr^{3+}) using bacteria

After 48hours (log to stationary phase) the bacterial biomass was harvested by centrifugation method (10000rpm for 120 min). The harvested biomass was used for adsorption studies. The harvested biomass was washed 3 to 4 times with sterile distilled water and used as adsorbent for chromium removal.

Immobilized bacterial cells

Batch mode studies were carried out to determine the adsorption of various concentration of chromium (4, 6 and 8ppm) with immobilized bacterial cells. For efficient removal, the agitation time, adsorbant dosage, temperature and pH were determined.

Removal of chromium (Cr^{3+}) using actinomycetes

The various concentration of trivalent chromium (4, 6 and 8ppm) in aqueous solution using actinomycetes biomass as adsorbent. The effects of agitation time, adsorbant dosage, pH and temperature on chromium removal were optimized.

Removal of chromium (Cr^{3+}) using biosurfactants

After the cultivation, the biomass was harvested by filtration or centrifugation. Surface-active compounds were extracted by liquid – liquid extraction method (Rajashree, 2001). The supernatant was mixed with equal volume of Chloroform : Methanol (2 : 1) ratio in a separating funnel and shaken for five minutes. After the extraction the biosurfactant was used as an adsorbent for the adsorption studies for the removal of chromium.

Tannery effluent on the growth of green gram (phaseolus aureus co-4)

Above all optimization was applied to remove chromium from tannery industry effluent under aerobic condition. Red soil was collected from the field of agriculture area without any contamination by tannery industry effluent, made into powder and sieved (2 mm mesh). The prepared soil was taken in separate pots (30cm height x 30cm width). Each pot contains 4kg of soil. Five different concentrations (20, 40, 60, 80 and 100%) of effluents (treated and untreated) was prepared and poured into each pot. Control case was also maintained, irrigated with tap water. Then, four seeds already sterilized with 0.1% mercuric chloride and the effluent was irrigated periodically at 48 hrs interval. The shoot length of the plants was recorded at every 48 hrs for 20 days.

RESULTS AND DISCUSSION

Tannery industry plays an important role in the economic development of the country. In most of the industries large quantities of liquid effluents are generated during various industrial processes. The tannery effluents contain high suspended solids, dissolved solids, BOD and some inorganic compounds such as chlorides, sulphides, sulphates, sodium and some toxic heavy metals, which affect the environment (Manivasakam, 1986). Extensive research works have been conducted on heavy metal contamination in soils from various anthropogenic sources such as industrial wastes (Gibson ad Farmer, 1983). Chromium ions, one of the more toxic ions under hexavalent form are potentially carcinogenic for humans. The interaction of metals and microorganism are diverse and can be considered in three major categories. *Pseudomonas* sp. ATS – 18 was resistant, *Mucor* sp. ATS - 5 was selected as chromium resistance. *Nocardia* sp. ATS – 2 were found to be predominant.

Chromium uptake by *Pseudomonas* sp, *Mucor* sp. and *Nocardia* sp. differ in many respects. The surface associated accumulation of chromium exhibited by *Pseudomonas*, *Nocardia* and *Mucor* sp. is consistent with the view that metal biosorption occurs by the complexation of positively charged metal ions with the negatively charged sites (R-COO^-) on the cell surface. As expected, metal uptake was affected by environmental parameters such as agitation time, adsorbant dosage, pH and temperature. Not only can environmental changes affect reactive metal binding sites, but also the solution chemistry of chromium is quite complex.

The biosorbent property of *Pseudomonas* resides in the hyphal cell wall. These walls can be characterized as a system of biopolymers fibres interwoven to form an ion exchange and coordination surface. The uptake of chromium by *Pseudomonas* is highly pH sensitive, with a pH optimum at about 5.5 and sometimes varies little in rate between 3 and 9.5. Among the three isolates, *Pseudomonas* sp. (ATS 18), *Mucor* sp. (ATS 5) and *Nocardia* sp. (ATS 2) was found to be predominant with a maximum percentage_removal of 92% with dead cells followed by 89% reduction using the live cells at an agitation time of about 60min (Table 1). In the case of fungal species, about 84% of chromium was utilized after 70 min whereas in bacteria only 78% reduction was observed in the dead cells.

Table 1
Chromium resistant microorganism

S. No	Concentration (ppm)	Percentage		
		Bacteria	Fungi	Actinomycetes
1	100	100	100	87
2	200	79.3	86	55
3	300	32.4	40	34
4	400	6.5	22.2	12.5
5	500	0	2	1.0

Bacterial (*Pseudomonas* sp) immobilized cells were found to be an effective adsorbant for chromium removal having a percent removal of about 78% using dead cells. The bacterial genera *Bacillus*, *Corynebacterium*, *Micrococcus*, *Pseudomonas*, *Alcaligenes*, *Vibrio* and members of *Enterobactericeae* were isolated in effluent and effluent irrigated soil. Among the isolates, *Pseudomonas* sp. (78%) have been found to predominant followed by *Bacillus* sp. (72%). Similarly the fungal genera *Aspergillus* sp. (84%) and *Mucor* sp. (88%) were found to be most important. Among the actinomycetes used, *Nocardia* sp. (92%) was found to more crucial.

Majority of the microbial isolates showed resistance upto 200 ppm of chromium solutions and were able to grow. At 400 ppm concentration of chromium, the percentage resistances were found to be 6.5%, 22.2% and 12.5% for bacteria, fungi and actinomycetes respectively (Table 2). Based on the resistance study the isolates were selected for the adsorption study. Metallic ion uptake by the living microorganisms is dependent on different parameters such as adsorbent dosage, temperature, pH, and agitation time.

Table- 2
Chromium resistant microbial genera

Sl. No	Strain No.	*Microbial genera*
		Bacteria
1.	ATS - 01	*Alcaligenes* sp.
2.	ATE – 02	*Alcaligenes* sp.
3.	ATS – 03	*Bacillus* sp.
4.	ATS – 04	*Bacillus* sp.
5.	ATE – 05	*Bacillus* sp.
6.	ATS – 06	*Bacillus* sp.
7.	ATS – 07	*Corynebacterium* sp.
8.	ATE – 08	*Corynebacterium* sp.
9.	ATS – 09	*Enterobacteriaceae* sp.
10.	ATE – 10	*Enterobacteriaceae* sp.
11.	ATE – 11	*Micrococcus* sp.
12.	ATS – 12	*Micrococcus* sp.
13.	ATS – 13	*Moraxella* sp.
14.	ATE – 14	*Pseudomonas* sp.
15.	ATE – 15	*Pseudomonas* sp.
16.	ATE – 16	*Pseudomonas* sp.
17.	ATS – 17	*Pseudomonas* sp.
18.	**ATS – 18**	***Pseudomonas* sp.**
19.	ATS – 19	*Pseudomonas* sp.
20.	ATS – 20	*Pseudomonas* sp.
21.	ATE – 21	*Vibrio* sp.
22.	ATS – 22	*Vibrio* sp.
		Fungi
23.	ATE – 01	*Rhizopus* sp.
24.	ATE – 02	*Mucor* sp.
25.	ATS – 03	*Mucor* sp.
26.	ATS – 04	*Mucor* sp.
27.	**ATS – 05**	***Mucor* sp.**
28.	ATE – 06	*Penicillium* sp.
30.	ATE – 07	*Trichoderma* sp.
31.	ATS – 08	*A.flavus* sp.
32.	ATE – 09	*A.niger* sp.
33.	ATS – 10	*A.fumigatus* sp.
		Actinomycetes
34.	ATS – 1	*Streptomyces* sp.
35.	**ATS –2**	***Nocardia* sp.**
36.	ATE –3	*Nocardia* sp.

Removal of chromium

The interaction of the metal cations with the electron rich functional groups located on the biomass may be strongly sensitive to the pH values of the environment. The optimum pH for adsorption of chromium by mycelial by-products of *Nocardia* was 6, and the optimal pH for adsorption of same metal ion was around 8 for biomass of *Mucor* sp. An optimal pH of 7 – 8 for the adsorption of chromium was found using *Pseudomonas* as the biosorbent. These results seem to suggest that, the adsorption of chromium to biomass is mainly due to ionic attraction. In contrast, higher pH results in facilitation of the metal uptake, since the cells surface is negatively charged.

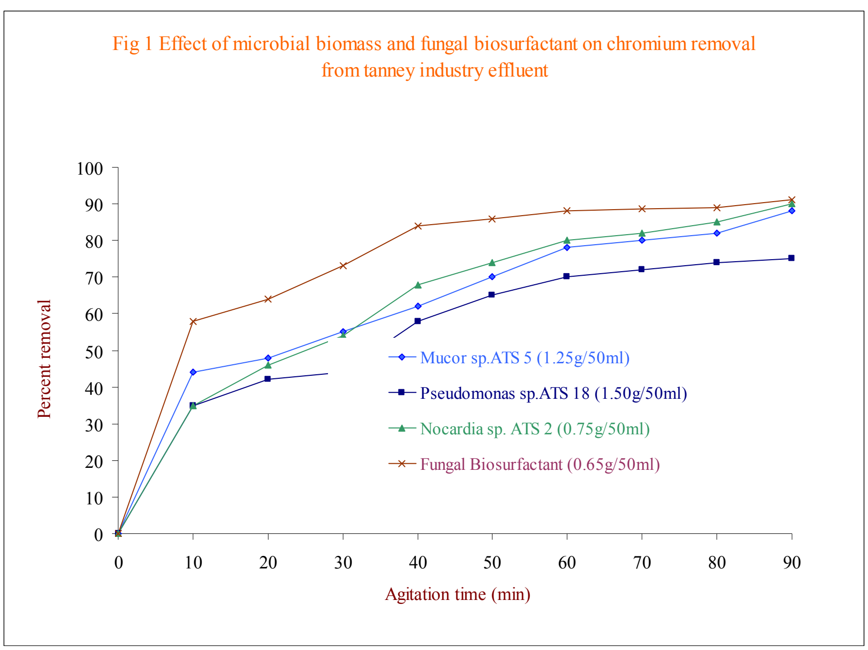
Adsorption studies of chromium were also performed with agitation time. An agitation time of 70 – 90 min was found to be effective in removal of chromium. The biosorptive capacity of dead fungal cells has been studied extensively in comparison to living cells. System using living cells is likely to be more sensitive to metal ion concentration and adverse operating conditions.

The effect of temperature on chromium removal was determined. Temperature had a masked effect on chromium removal. More amount of chromium are to be removed at temperature of 50^{0}C. Transport of metal ions into microbial cells is inhibited by low temperatures metabolic inhibitors and the absence of an energy source.

Different adsorbent dosages were applied to the chromium solution and an optimum adsorbent dosage was determined. Adsorbant dosages with both dead cells and live cells were treated and were noticed that the dead cells (90%) were more effective than live cells (85%). Due to the smaller particle size, low mechanical strength, the live cells were replaced by the dead cells. *Nocardia* sp.(89%) was found to be more effective followed by *Mucor* sp. (87%) and *Pseudomonas* sp. (78%) (fig. 1).

Fig.1

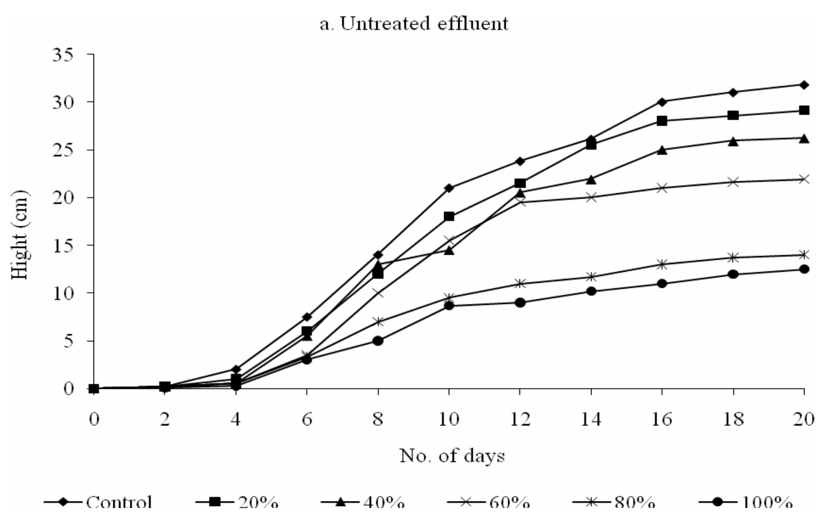
Effect of microbial biomass and fungal biosurfactant on chromium removal from tannery industry effluent

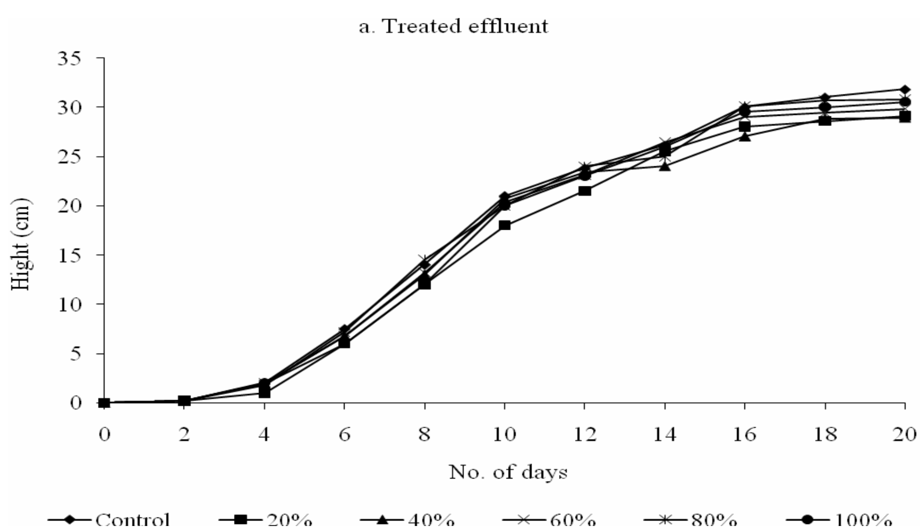


Removal of chromium in tannery industry effluent

By keeping the optimal conditions obtained from the aqueous solutions, these were applied on the sample. Positive result was given with all of the three isolates and the fungal biosurfactant. Among the three, the *Nocardia* sp. was found to be more active in chromium removal followed by fungal biosurfactant, *Mucor* sp. and *Pseudomonas* sp. The sample containing 5.9ppm concentration was reduced to 0.75 after the treatment of the microbial biomass and biosurfactant. (fig. 2)

Fig. 2
Effect of treated and untreated tannery industry effluent on the growth of green gram





CONCLUSION

From the study it is inferred that untreated effluents possibly lead to soil pollution, deterioration and low productivity. Even the plant ecosystem\m are affected. This can be averted by proper treatment of effluent by suitable conventional methods. There was a gradual decrease of shoot length when the concentration of effluent increased. Whereas the reverse results were noted in the soil irrigated with various concentration of microbially treated tannery industry effluent.

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