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Full Length Research Paper

Bioremediation of chromium in tannery effluent by microbial consortia

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Chromium is the most toxic and common among the heavy metal pollutants of industrial effluents. In the present work the chromium remediation ability of *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* in consortia and in their immobilized forms was studied and their efficiencies were compared. Flame Atomic Absorption Spectroscopy and diphenyl carbazide method was used to quantify chromium in the effluent. The chromium content of the effluent was around 770 mg/l before remediation, after which it reduced to 5.2 – 5.7 mg/l. The best activity was observed by *S. cerevisiae* - *P. aeruginosa* consortia, followed by immobilized beads of *S. cerevisiae* and *S. cerevisiae* - *B. subtilis* consortia.

Key words: Effluent, chromium, remediation, consortia, immobilization.

INTRODUCTION

Heavy metals are ubiquitous and persistent environmental pollutants that are introduced into the environment through anthropogenic activities (Teitzel and Mathew, 2003). Chromium (Cr) is a metal contaminant that exists in nature primarily as the soluble highly toxic Cr (VI) anion and the less soluble, less toxic Cr(III) species (James, 2002). Chromium VI is a transition element that is extensively used in tanning, metal finishing, petroleum refining, iron and steel industries, inorganic chemical production, and textile processing and pulp production (Srinath et al., 2002; Meriah and Tebo, 2002).

Tanneries are a major source of chromium pollution and release Cr(VI) ranging from 40 – 25,000 mg/l of wastewater. The maximum tolerance of total Cr for public water supply has been fixed at 0.05 mg/l as per Indian standards. The environmental protection agency has formulated the maximum permissible levels of Cr(VI) into water bodies at 50 µg/dm^3 and in drinking water as 3 µg/dm^3 and that of Cr(III) as 100 µg/dm^3 (Lee and Jones, 1998; Palmer and Puis, 1994).

The toxicity of Cr in various industrial effluents is well documented. Hexavalent Cr compounds pose health risks to humans, plants, animals and fishes (Srinath et al., 2002; and Lee and Jones, 1998). Due to its carcinogenicity and mutagenicity, the United States Environment Protection Agency (USEPA) has designated Cr as a "Priority pollutant" or Class A" pollutant (Srinath et al., 2002; Lee and Jones, 1998). At high levels, heavy metals like chromium damage cell membranes, alter enzyme specificity; disrupt cellular functions and damage structure of DNA (Bruins et al., 2000). Cr(VI) activates p53 by reactive oxygen species (ROS) mediated free radical reactions that occur during the oxidative reduction of hexavalent Cr within the cell. Oxidative damage is considered to be an important mechanism in the genotoxicity of Cr(VI). Hence, the need arises to remediate chromium before being discharged.

Conventional methods used for the removal of hexavalent Cr use chemical procedures, which are expensive and lack specificity (Katiyar and Katiyar, 1997). As an alternative, biological approaches utilizing microorganisms offer the potential for a highly selective removal of toxic metals coupled with considerable operational flexibility, hence they can be both *in situ* or *ex situ* in a range of bioreactor configurations (Lloyd et al., 2001; Lovely and Coates, 1997). Biological approaches may reduce Cr (VI) to Cr (III) intracellularly or by making the extra cellular environment more reducing or lowering pH to favor Cr (VI) reduction. Immobilized cells as biofilms, beads or inert supports have been found to be most effective in

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Table 1. Chromium remediation by individual cells, consortia and immobilized cells of *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Saccharomyces cerevisiae*.

Group	Test Organism	Initial Cr. conc. (mg/l)	Cr conc. after remediation (mg/l)	Efficiency (%)	Rank (group)	Overall rating
Individual cells	*Bacillus subtilis* (B)	570	2	99.6	I	-
	Pseudomonas aeruginosa (P)	570	2	99.6	III	-
	Saccharomyces cerevisiae (Y)	570	25	95.6	II	-
Consortia	*Pseudomonas aeruginosa* and *Bacillus subtilis* (P+B)	570	2	99.6	III	-
	Saccharomyces cerevisiae and *Bacillus subtilis* (Y+B)	570	16	97.2	II	III
	Saccharomyces cerevisiae and *Pseudomonas aeruginosa* (Y+P)	570	4	99.3	I	I
Immobilized Cells	Immobilized *Bacillus subtilis* (B bead)	570	2	99.6	II	-
	Immobilized *Pseudomonas aeruginosa* (P bead)	570	4	99.3	III	-
	Immobilized *Saccharomyces cerevisiae* (Y bead)	570	0	100	I	II

designing bioreactors for heavy metal degradation (James, 2002).

Chromium remediation studies have been carried out with a variety of organisms like *Pseudomonas* species, *Aeromonas* species, *Bacillus* species, *Micrococcus* species and *Microbacterium* species (Laxman and More, 2002). Of these *Pseudomonas* species were the most efficient. Fungi are being exploited for heavy metal degradation as a result of their various characteristics; the most significant are the metallothioneins (MT) and phytochelatins that are small cysteine rich polypeptides that can bind metals. Hence, the present study exploited the properties of *Bacillus subtilis, Pseudomonas aeruginosa and Saccharomyces cerevisiae* in the remediation of chromium.

MATERIALS AND METHODS

Tannery effluent was collected from leather tanneries near Erode, Tamilnadu, India. The sample was stored at 4°C to arrest any biological activity. The color and pH of the effluent was recorded. The Chrome water obtained from tanneries was filtered using Whatman No.1 filter paper and the pH was adjusted to <2 using concentrated HNO_3. The collected tannery effluent was analyzed for total chromium and hexavalent chromium using (AAS), EMerck SQ118 and "Spectragnant kit" at SITRA (South India Textile Research Association), Coimbatore. Estimation of hexavalent Chromium (Cleseari and Green, 1995) included the oxidation of trivalent chromium, color development by adding diphenyl carbazide and measurement of optical density at 540 nm using reagent water as reference; the value was plotted against the standard graph to determine the concentration of Cr in the effluent.

Microbial culture and maintenance

The microbial cultures used for the study were *B. subtilis*, *P. aeruginosa* and *S. cerevisiae*. They were maintained in their respective selective media viz; Nutrient agar, Cetrimide agar and Yeast potato dextrose agar. Immobilization of microbial cells (Champagne and Gardner, 2001) involves the mixing of equal volume of overnight microbial broth culture and 4% sterile sodium alginate. This mixture was dropped gently in 0.1 M calcium chloride solution using a sterile syringe to get even sized beads.

Bioremediation

The pH of the effluent was altered to 7.0 with NaOH and then distributed in 250 ml conical flasks (100 ml in each). 1% sucrose was added and 1% of overnight grown culture was initiated in each conical flask. The organisms were used as single, in combination and in immobilized forms (Table 1). The flasks were kept on rotary shaker at 180 rpm for *S. cerevisiae* at room temperature. *B. subtilis* and *P. aeruginosa* were maintained at 37°C for a period of 10 days. Chromium was estimated at an interval of 12 h to calculate the chromium depletion from the effluent by the different test organisms. The results were calculated and tabulated. Simple statistical analysis like coefficient of variation, regression analysis, correlation and 'T' test was performed to analyze and interpret the results.

RESULTS

The collected tannery effluent containing chromium was dark green in color. The chrome water was filtered and pre-treated to adjust the pH to <2. When subjected to FAAS, total chromium was estimated to be about 770 mg/lt

(7698 ppm). The chromium content obtained by laboratory estimation amounted to 760 mg/l, similar to the result obtained by FAAS. As set by the National Pollutant Dischargeable Elimination System, the allowed dischargeable limit for hexavalent chromium is only 11ppb. Hence, it was essential to evolve a method to remedy chromium.

Chromium remediation by individual organisms

All the organisms, consortia and immobilized cells were found to be effective in remedying chromium. There was a considerable and gradual reduction in the absorbance values and Cr concentration every hour. The concentration of total Chromium in the effluent was reduced to 2 mg/l from 570 mg/l after 192 h of treatment with *B. subtilis* (B) which was rated to be IV in efficiency and degraded 1.870 mg/l chromium every one hour and showed 99.6% efficiency but the reduction in chromium levels was not constant and fluctuations were observed as the co-efficient of variation was as high as 176.94. *P. aeruginosa* (P) removed Cr with 99.6% efficiency. The rate of reduction every hour was 1.926 mg/l but it was not constant and showed fluctuations (co-efficient of variation was 180.70). *S. cerevisiae* (Y) showed 95.6% efficiency in remedying chromium and reduced at a rate of 1.829 mg/l per hour. Yeast was found to be more constant in degradation when compared to B and P and showed a co-efficient of variation of 168.7.

Chromium remediation by consortia

P. aeruginosa and *B. subtilis* (P+B) consortia showed an efficiency of 99.6% with a reduction rate of 1.565 mg/l per hour. However, the remediation activity was found to fluctuate to the maximum (co-efficient of variation of 192.42). *S. cerevisiae* and *B. subtilis* (Y+B) combination showed an efficiency of 97.2% with a rate of 2.185 mg/l chromium reduction per hour. It was rated to be second in chromium remediation as the rate of chromium reduction was considerably constant without fluctuations and variations. *S. cerevisiae* and *P. aeruginosa* (Y+P) proved 99.3% efficiency in chromium reduction, with a rate of reduction of 2.302 mg/l per hour. This consortium was rated to be first in reducing chromium and acting effectively with minimal fluctuations (co-efficient of variation is 145.53).

Immobilized cells in chromium remediation

100% of Cr removal was observed by immobilized yeast cells that remedied Cr at a rate of 2.332 mg/l per hour and was rated to be second efficient with very high stability, whereas Cr remediation by immobilized *Bacillus* cells was similar to that of live cells. However, immobilized *Pseudomonas* beads were more efficient than the live cells.

DISCUSSION

The above organisms were found to be metal tolerant and might remedy Cr by biosorption, bioaccumulation (Teitzel and Mathew, 2003; Meriah and Tebo, 2002; Katiyar and Katiyar, 1997; Turpeinen et al., 2002) or by transformation of heavy metals, entrapment in extracellular capsules, protein DNA adduct formation, induction of stress, transformation of components by oxidation, reduction, methylation and demethylations and by binding cytosolic molecules (Lovely and Coates, 1997; Gadd, 1990; Ksheminska et al., 2003).

B. subtilis reduced chromium VI under aerobic conditions. This may be due to the presence of chromium reductase. Similarly chromium VI reduction has also been reported by *B. coagulans* (James, 2002). Accumulation of chromium VI by *B. circulans* has also been demonstrated (Srinath et al., 2002). Alkali treatment of *B. subtilis* resulted in an increase in silver and copper accumulating capacity (Katiyar and Katiyar, 1997), which is similar to the results obtained.

Removal of chromium by *P. aeruginosa* might be due to the presence of chromium reductase gene (Jin, 2000; Mellor et al., 1996). Accumulation of other heavy metals like uranium in its cells might also match with its tendency to remediate chromium (Stanberg et al., 1981). There are also reports on biosorption that mainly involve cell specific complexation, ion exchange and micro precipitation (Figveria et al., 1999) using *Pseudomonas* species.

The Chromium remediation by fungi and yeast particularly might be due to the excellent potential of metal biosorption (Gupta and Ahuja, 2002). Yeast has also shown to accumulate Uranium in its cells at a rapid rate (Stanberg et al., 1981). *S. cerevisiae* has been found to be resistant to cadmium and copper that is mediated by a cysteine rich protein- copper metallothionein (Cu-Mt) (Katiyar and Katiyar, 1997). This has been suggested to be of potential in metal recovery and remediation since it can bind other metals besides Cu including chromium (Butt and Jecker, 1987). Of the three different consortia *Saccharomyces* and *Pseudomonas* was the most efficient. The high efficiency might be contributed to the chromium reductase genes in *P. aeruginosa* and the MT genes in *S. cerevisiae*.

Immobilized cells have been reported to be very effective in heavy metal removal. Heavy metal toxicity and other extreme properties of waste effluents that may limit the use of living cell systems. Freely suspended microbial biomass has disadvantages that include small particle size and low mechanical strength (Katiyar and Katiyar, 1997). Immobilized cells appear to be of greater potential in controlling particle size, better capability of regeneration, easy separation of biomass and effluent and re-circulation, high biomass loading, minimal clogg-

ing and reduced depletion of nutrient source (Katiyar and Katiyar, 1997). It has also been reported that immobilized cells have found to be most effective in designing small and large-scale bioreactors for heavy metal degradation (James, 2002).

In summary, the study establishes the role and efficiencies of *B. subtilis*, *P. aeruginosa* and *S. cerevisiae* in the absorption, accumulation, degradation and detoxification of chromium in tannery effluents. The benefits of immobilized cells compared to whole cells have also been highlighted. This study is the basis for implementation of advanced technologies like exo-polymer and bioreactor technology for rapid and effective removal of chromium from polluted water bodies. *In situ* bioremediation with biostimulation and bioaugumentation may prove to be highly efficient in chromium remediation.

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