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Microbial Removal of High Concentration Heavy Metal in Tannery.

T Sivaruban[1], S Barathy[2], S Sivakumar[3], Mukesh Goel[3]*, Ashutosh Das[3], K Ravi Kumar[3] and S Maran[4].

[1]Department of Zoology, The American College, Madurai, Tamilnadu, India - 625002,
[2]Department of Zoology, The Madura College, Madurai, Tamilnadu, India - 625002,
[3]Centre for Environmental Engineering, PRIST University, Thanjavur, Tamil Nadu, India – 613403
[4]Department of Civil Engineering, PRIST University, Puduchery, India

ABSTRACT

This work was carried out to study the remediation ability of *B. subtilis*, *P. aeruginosa* and *S. cerevisiae* in consortia and in their immobilized forms for the treatment of tannery effluents. The efficiencies of the microbes were compared towards the improvement of physic-chemical parameters as well as for Chromium (VI) remediation capacity. It is noted that all the three species were tolerant of high dosage of Cr and have been successful in remedying the Cr. However, immobilization of the species has shown the greatest potential in treating the Cr containing wastewaters.

Keywords: Pollution, waste management and sustainability

Corresponding author

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INTRODUCTION

Environmental pollution is one of the major problems of the world and it is increasing day by day due to urbanization and industrialization. Over the last few decades large scale usage of chemicals in various human activities has grown very fast, particularly in a country like India which has to go for rapid industrialization in order to sustain over growing large problem of population.

In recent years, heavy metal pollution has become one of the most serious environmental problems. Presence of heavy metals even in traces is toxic and detrimental to both flora and fauna. With the rapid development of many industries (mining, surface fishing, energy and fuel producing, fertilizer, pesticide, metallurgy, iron and steel , leather, metal surface treating) and aerospace and atomic energy installations, wastes containing metals are directly or indirectly being discharged into the environment causing serious environmental pollution and even threatening human life [1].

Tannery effluent is a major source of aquatic pollution in India with high chemical oxygen demand (COD), biological oxygen demand (BOD), and hexavalent chromium. There are a large number of tanneries scattered all over the country. Chromium is one of the most toxic heavy metals discharged into the environment through various industrial wastewaters, such as leather tanning, electroplating, paints, pigment production, steel manufacture. The effluents of these industries contain chromium at concentrations ranging from tenths to hundreds of milligrams per liter.

Several microorganisms have the exceptional ability to adapt to and colonize the noxious metal polluted environments, which are uninhabitable by higher organisms. These microorganisms have developed the capabilities to protect themselves from heavy metal toxicity by various mechanisms such as adsorption, uptake, methylation, oxidation, and reduction. The reduction of Cr(VI) to less toxic Cr(III) is an important step in the remediation of Cr(VI)-contaminated environments. The traditional treatment methodologies for soils and groundwater contaminated with Cr(VI) are based on excavation and pumping of contaminated material followed by the addition of chemical reductants, resulting in the precipitation and sedimentation of reduced Cr [2]. These processes are expensive and energy intensive. Recently, bioremediation is emerging as a safe and cost-effective technology as an alternative to the expensive traditional physico-chemical methods. However, the availability of effective Cr(VI)-reducing organisms is an essential prerequisite for the bioreduction-based remediation of Cr(VI)-contaminated water/soil. Chromate-reducing bacteria have been isolated and characterized mostly from chromium-contaminated soil, wastewater and industrial effluents [3].

Bioreduction of Cr(VI) can occur directly as a result of microbial metabolism (enzymatic) or indirectly, when mediated by bacterial metabolite (such as H_2S) [4]. A number of Cr(VI) reducing micro-organisms have been reported including *Escherichia* [5], *Pseudomonas* [6], *Enterobacter* [7] and *Arthrobacter* [8]. However, the potential for the biological treatment of

Cr(VI) contaminated waste is limited because some micro-organisms lose viability in high concentrations of chromate or require continuous supply of expensive nutrients for the desired activity. Isolating chromate reducing bacteria from contaminated soil, that could grow and reduce chromate in the contaminated environment without any amendment, could, therefore, be useful.

Immobilized microbial cells are used in organic synthesis clinical and chemical analysis, food industries, medicine, and environmental applications as well. The expansion of biotechnology and the expected developments has encouraged effects to immobilize enzymes and cells for applied purpose. For a particular application it is necessary to find an immobilization procedure that would be simple and inexpensive. Immobilization of the biomass within a suitable matrix provide a physical support for cells, ideal size, mechanical strength, rigidity and porous characteristics to the biological material [9].

The aim of this research was to isolate the microbial isolates from tannery effluent and to access the Cr reduction capacity of these species. Besides, the work also explores reduction capacity of immobilized bacterial isolate for removing Cr (VI) from aqueous solution.

MATERIALS AND METHODS

Tannery effluent was collected from leather tanneries near Tirupur. The sample was stored at 4^oC 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 and all physic-chemical parameters (Table 1)

Table 1: Physico-chemical parameter of raw tannery effluent

Physico-chemical parameter	Values
pH	9
Acidity	60
Alkalinity	250
Calcium	98.7
Magnesium	110.3
Hardness	235
D.O	1.75
COD	1470
TDS	2840
TS	139

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 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. 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 12hrs to calculate the chromium depletion from the effluent by the different test organisms.

Analysis

Physico-chemical parameters were analyzed by Systronics water analyzer kit. COD was analyzed using Hach colorimeter. Ca and Mg estimation was done using Flame photo-meter. Colorimeteric method using diphenyl carbazide was used for the estimation of Chromium.

RESULTS AND DISCUSSION

In the present study, the raw effluent from the tanning industry near Tirupur was collected and their physico-chemical character was analyzed. Generally it was found that the values were above the tolerant limit. The mean pH value of the raw effluent was 9, which is slightly alkaline in nature. The dissolved solids and suspended solids and suspended solids were high and the mean value was 2840 mg/l and 139mg/l respectively. The Calcium, Magnesium and Hardness values are found to be 98.7, 110.3 and 235 mg/ml respectively. Treatment of raw effluents with individual organisms resulted in uptakes of Ca and Mg (figures 1 and 2). It can be seen from the graph that all the three microbial species resulted in almost 15% removal of Ca. on the other hand, Mg removal was on higher side. Pseudomonas aeruginosa could remove up to 20% of Mg from the wastewater. Other physic-chemical parameters were also noted and the results indicate minor improvement when tannery wastewater was subjected bioremediation by individual species (Table 2).

Table 2: Physico-chemical parameter of treated effluent by microbes

Parameter	Treated effluents		
	Bacillus subtilis	***Pseudomonas aeruginosa***	***Saccharomyces cerevisiae***
pH	7.5	8	8.3
Acidity	55	45	47
Alkalinity	230	225	233
Hardness	101	95.2	97.3
COD	220.3	170	195
TDS	2560	2210	2570
TS	121	110	98.5

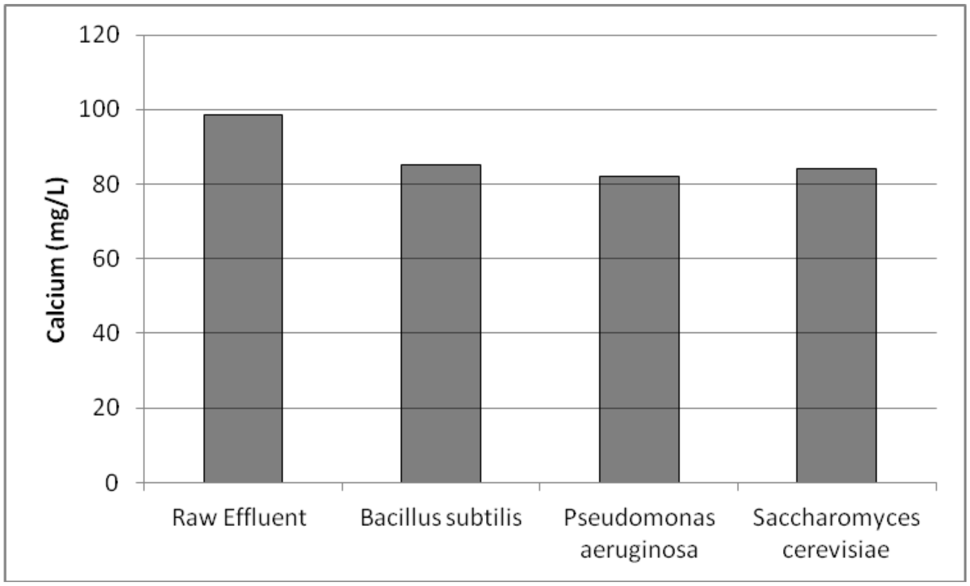

Figure 1: Application of microbes in removal of Ca from tannery wastewater

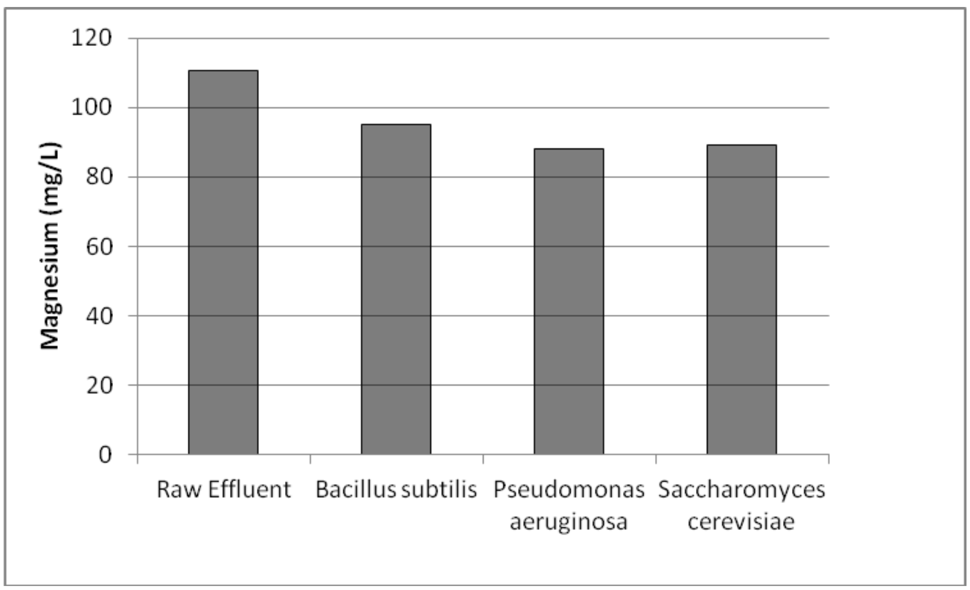

Figure 2: Application of microbes in removal of Mg from tannery wastewater

Bioremediation of Chromium

The bacterial colonies were isolated from the tannery effluent and also from the sludge at dilutions ranging from 10^{-4} to 10^{-6}. The growth of the colonies decreases when the dilution factor increases. The three major isolates such as *Bacillus subtilis, Pseudomonas aeruginosa*, *Saccharomyces cerevisiae* are found to be dominant. The analysis of the treated effluent with bacterial strain was carried out.

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 30 mg/l from 630 mg/l after 192 h of treatment with *B. subtilis* (B) which showed 95.2% efficiency in remediation of chromium. *P. aeruginosa* (P) removed Cr with 91.2% efficiency. *S. cerevisiae* (Y) showed 95.6% efficiency in remedying chromium.

Chromium remediation by consortia

P. aeruginosa and *B. subtilis* (P+B) consortia showed an efficiency of 99.7%. *S. cerevisiae* and *B. subtilis* (Y+B) combination showed an efficiency of 96.4%. 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 99.5% efficiency in chromium reduction. This consortium was rated to be first in reducing chromium and acting effectively with minimal fluctuations.

Chromium remediation by Immobilized cells

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 and immobilized *Pseudomonas* beads were more efficient than the live cells. Detailed results of chromium remediation are given in Table 3.

Table 3: Heavy metal remediation by individual cells and microbes

Group	Test organism	Initial Cr. Conc(mg/l)	Cr conc. after remediation (mg/l)	Efficiency (%)
Individual cells	*Bacillus subtilis(B)* *Pseudomonas aeruginosa(P),* *Saccharomyces cerevisiae(Y)*	630 630 630	65 55 32	89.2 91.2 94.3
Consortia	*Pseudomonas aeruginosa* and *Bacillus subtilis* (P+B)	630	3	99.7
	Saccharomyces cerevisiae and Bacillus subtilis (Y+B)	630	16	96.4
	Saccharomyces cerevisiae and *Pseudomonas aeruginosa* (Y+P)	630	4	99.5
Immobilized cells	Immobilized *Bacillus subtilis* (B bead)	630	3	99.4
	Immobilized *pseudomonas aeroginosa* (P bead)	630	5	99.2
	Immmobilized saccharomyces cerevisiae (Y bead)	630	0	100

The above organisms were found to be metal tolerant and might remedy Cr by bioaccumulation [10] 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 .

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* [11]. Accumulation of chromium VI by *B. circulans* has also been demonstrated [12]. Alkali treatment of *B. subtilis* resulted in an increase in silver and copper accumulating capacity, which is similar to the results obtained. Removal of chromium by *P. aeruginosa* might be due to the presence of chromium reductase gene. Accumulation of other heavy metals like uranium in its cells might also match with its tendency to remediate chromium. The Chromium remediation by fungi and yeast particularly might be due to the excellent potential of metal biosorption [13]. Yeast has also shown to accumulate Uranium in its cells at a rapid rate. *S. cerevisiae* has been found to be resistant to cadmium and copper that is mediated by a cysteine rich protein- copper metallothionein (Cu-Mt).

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 [14]. 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 clogging and reduced depletion of nutrient source [14]. 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 [11].

CONCLUSIONS

The major components of the tannery effluent include sulfide, chromium, volatile organic compounds, large quantities of solid waste, suspended solids etc. Tannery wastewater is difficult to treat because of complex characteristics like high BOD, COD, suspended solids, sulfide and chromium. This work was carried out to study the remediation ability of *B. subtilis*, *P. aeruginosa* and *S. cerevisiae* in consortia and in their immobilized forms for the treatment of tannery effluents. The efficiencies of the microbes were compared towards the improvement of physic-chemical parameters as well as for Chromium (VI) remediation capacity. It is noted that all the three species were tolerant of high dosage of Cr and have been successful in remedying the Cr. From the results of this work it can be concluded that the role of microbes in bioremediation of heavy metals is an ideal method without disturbing the environmental health.

REFERENCES

[1] Acevedo-Aguilar FJ, AE Espino-Saldana, IL Leon-Rodriguez, ME Rivera-Cano and M Avila-Rodriguez. Can J Microbiol 2006; 52: 809-815.

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[2] Al-Saraj M, MS Abdel-Latif, I El-Nahal and R Baraka. J Non-Crystalline Solids 1999;. 248: 137-140.
[3] Dermou E, A Velissariou, D Xenos and DV Vayenas. J Hazard Mater 2005; 26, 78-85
[4] Sultan M and Hasnain S. J Gen App Microbiol 2005; 47: 307- 312.
[5] Shen H and YT Wang. J Environ Eng 1994; 120: 560-560.
[6] Ishibashi Y, C Cervantes and S Silver. App Environ Microbiol 1990; 56: 2268-2270.
[7] Rege MA, JN Petersen, DL Johnstone, CE Turick, DR Yonge and WA Apel. Biotechnol Lett 1997; 19: 691-694.
[8] Asatiani NV, MK Abuladze TM, Kartvelishvili NG, Bakradze and NA Sapojnikova. Curr Microbiol 2004; 49: 321-326.
[9] Teitzel M, Mathew R. Appl Environ Microbiol 2003; 69: 2313-2319.
[10] Meriah Y, Tebo Bradley M. J App Environ Microbiol 2002; 86: 1847-1853.
[11] James Bruce R. J Chem Environ 2002; 6(2): 46-48.
[12] Srinath T, Garg S, Ramteke K. Chromium VI accumulation by Bacillus circulans: Effect of growth conditions. Indian J. Microbiol. 2002;42: 141-146.
[13] Gupta R, Ahuja P Manage. Ind Pollut 2002; 4: 151.
[14] Katiyar SK, and Katiyar R. Adv Microb Biotechnol 1997; 19: 330-344.