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Removal of Heavy Metal (Chromium) by Microbes and to Study the Impact of TDS on its Removal Efficiency

[1]Komal Kansagra, [2]Dr. Hardik Gohel*, [3]Huma Syed

[1]Dept. of Environmental Engineering, L.D. College of Engineering, Ahmedabad,komal41.kansagra@gmail.com
[2] Senior Scientist, Disha Life Science Pvt. Ltd., Ahmedabad, drhardikgohel@dishalifescience.org
[3]Asso. Prof., Environmental Engg. Dept. LDCE, Ahmedabad, huma_env@hotmail.com

Abstract

Industrial operations such as electroplating, steel manufacturing, leather tanning, wood preservation, ceramics, glass manufacturing and chemical processing and fertilizer applications release alarmingly higher amounts of heavy metals into the natural environment, which are detrimental to human health. Of the important metals Chromium (VI) is regarded as toxic; whereas, others, such as copper, nickel, cobalt and zinc are not as toxic, but their extensive usage and increasing levels in the environment are of serious concerns .Various techniques have been employed for the treatment of metal bearing industrial effluents, which usually include precipitation, adsorption, ion exchange, membrane and electrochemical technologies but these techniques are expensive, not environment friendly and usually dependent on the concentration of the waste which are ineffective in very diluted solutions. Therefore, the search for efficient, eco-friendly and cost effective remedies for wastewater treatment has been initiated. Heavy metal resistant bacteria have significant role in bioremediation of heavy metals in wastewater. Initially six microbial species Aspergillus Niger,Pseudomonas Putida,Pseudomonas Aeruginosa, Pencilium Crysogenum, Bacillus Thrunengis and Bacillus subtiluss were selected for removal of chromium. but among them Pseudomonas Putida has shown efficient removal of chromium 100% in 10 ppm,73.08% in 25ppm,34.42%in 50 ppm and various parameter has been optimized and Waste water also contains high TDS along with heavy metal. So Impact of varying TDS concentration on removal efficiency for chromium has been studied and results shows that TDS concentration does have impact on microbial removal for chromium.

Key words - Heavy Metals,Chromium (VI),TDS,Bioremediation

I. INTRODUCTION

Earth's surface comprises of 70% water is the most valuable natural resource existing on our planet. Without this invaluable compound, the life on the Earth would not exist. Although this fact is widely recognized, pollution of water resources is a common problem being faced today. Heavy metal pollution occurs directly by effluent outfalls from industries, refineries and waste treatment plants and indirectly by the contaminants that enter the water supply from soils/ground water systems and from the atmosphere via rain water[5]. Modern industry is, to a large degree, responsible for contamination of the environment. Lakes, rivers and oceans are being overwhelmed with many toxic contaminants. Among toxic substances reaching hazardous levels are heavy metals[4]. Heavy metals are the group of contaminants of concern, which comes under the inorganic division. Some strong toxic metal ions such as Hg are very toxic even in lower concentration of 0.001-0.1 mg/ L. Metals are extensively used in several industries, including mining, metallurgical, electronic, electroplating and metal finishing. The presence of metal ions in final industrial effluents is extremely undesirable, as they are toxic to both lower and higher organisms. Under certain environmental conditions, metals may accumulate to toxic levels and cause ecological damage[2]. Of the important metals Chromium (VI) is regarded as toxic; whereas, others, such as copper, nickel, cobalt and zinc are not as toxic, but their extensive usage and increasing levels in the environment are of serious concerns[1] .Various techniques have been employed for the treatment of metal bearing industrial effluents, which usually include precipitation, adsorption, ion exchange, membrane and electrochemical technologies but these techniques are expensive, not environment friendly and usually dependent on the concentration of the waste which are ineffective in very diluted solutions. Therefore, the search for efficient, eco-friendly and cost effective remedies for wastewater treatment has been initiated. It was only in the 1990s that a new scientific area developed that could help to recover heavy metals and it was bioremediation. The early reports described how abundant biological materials could be used to remove, at very low cost, even small amounts of toxic heavy metals from industrial effluents. The principle advantages of biological technologies for

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the removal of pollutants are they can be carried out in situ at the contaminated site, usually environmentally benign (no secondary pollution) and they are cost effective. Of the different biological methods, bioaccumulation and biosorption have been demonstrated to possess good potential to replace conventional methods for the removal of metals[6]

Some confusion has prevailed in the literature regarding the use of the terms "bioaccumulation" and "biosorption" based on the state of the biomass. Herein, therefore, bioaccumulation is defined as the phenomenon of living cells; whereas, biosorption mechanisms are based on the use of dead biomass. To be precise, bioaccumulation can be defined as the uptake of toxicants by living cells. The toxicant can transport into the cell, accumulate intracellularly, across the cell membrane and through the cell metabolic cycle[3].Conversely, biosorption can be defined as the passive uptake of toxicants by dead/inactive biological materials or by materials. Metal-sequestering properties of non-viable biomass provide a basis for a new approach to remove heavy metals when they occur at low concentrations.That aspect of biosorption makes the eventual recovery of this waste metal easier and economical

II. MATERIALS AND METHODS

2.1 Stock solution preparation:

To prepare a chromium metal solution, 0.69 gm of potassium dichromate were dissolved into 5 ml of distilled water. And to prepare a TDS salt solution 2.5 gm of each salts, Magnesium sulfate (MgSo4), Manganese chloride (MnCl2), Zinc Chloride (Zncl2),Calcium Carbonate(CaCo3) were dissolved in 25 ml of distilled water of four different flask.

2.2 Determining TDS tolerance for survival of microbes:

500 ml of Nutrient broth was prepared with standard composition in a conical flask. then the media was sterilized at 15 lb/in^2 pressure and 121^{0}C for 30 minutes and from sterile medium 5 ml of medium transferred to each 36 test tubes. Loop full of six microbial culture (*Aspergillus Niger*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Pencilium crysogenum*, *Bacillus thrunengis* and *Bacillus subtilus*) was taken and inoculated in 10 ml of medium and allowed for growth at 35 C for 24 hrs. After 24 hrs,Set the TDS of 3000, 6000, 9000, 12000, 15000, 30000, 45000, 60000 mg/l, aliquots of total 150, 300, 450, 600, 750, 1500, 2250, 3000 µl were added from stock salt solution and to set the chromium concentration of 100 mg/l, 20 µl of prepared potassium dichromate were added and 5 µl of inculcated culture was added, in each 30 test tubes. Then the test tubes were kept in an orbital shaker at 32^{0}C for 2 days for growth. All the experiments were carried out in triplicate.

2.3 Determining optimum TDS value for maximum accumulation of chromium

Further experiments were continued with two microbial species only *Pseudomonas Putida* and *Bacillus subtilis*. 150 ml of Nutrient broth was prepared with standard composition in a conical flask then the media was sterilized at 15 lb/in^2 pressure and 121^{0}C for 30 minutes. Loop full of bacterial strains has been inoculated. Chromium concentration was kept 25 mg/l. and selected TDS values were from 600, 1200, 1800, 2400, 3000 mg/l. and concentration were set by adding appropriate aliquots from stock solution. Cell suspension was kept at 120 rpm for 48 hrs at 35±2 ˚C. After period of incubation, medium were centrifuged at 8000 rpm for 10 minutes. Supernatant were collected and analyzed for chromium concentration by spectrophotometery at 540 nm.

2.4 Optimization of environmental parameters for maximum accumulation of chromium

2.4.1 Effect of incubation time

To examine incubation time required, 10 mL of media containing metal solution inoculated with bacterial culture kept for different time intervals(0-96 h) at 35±2 C at 120 rpm for 48 hrs, After incubation were analyzed for residual metal content. The study is realized in individual samples, and supernatant were collected after centrifugation at 8000 rpm for 10 mints and analyzed for chromium concentration by spectrophotometry. All the experiments were carried out in triplicate and results show the mean value.

2.4.2 Effect of Initial Chromium Concentration

To examine the effect of the initial metal concentration, the experiments were performed at different initial metal concentrations such as 10, 50, 75 and 100 mg/l .bacterial strain inoculated in 10 ml of liquid Nutrient broth media and incubated at 35±2˚C at 120 rpm for 48 hrs. Supernatant were collected after centrifugation at 8000 rpm for 10 mints and analyzed for chromium concentration by spectrophotometry. All the experiments were carried out in triplicate and results show the mean value.

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Table 3.1 Determining TDS tolerance for survival of microbes

TDS(mg/l)	***Pseudomonas putida***	***Pseudomonas aeruginosa***	***Bacillus mojuvensis***	***Bacillus subtilis***	***Aspergillus niger***	***Penicillium crysogenum***
3000	+++	+	+	+++	+	+
6000	++	+	+	++	+	No Growth
9000	+	+	No Growth	+	+	No Growth
12000	+	No Growth	No Growth	+	No Growth	No Growth
15000	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
30000	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
45000	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
60000	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
Initial Chromium Concentration 100 and 50 mg/l						

Table 3.2 Determining Optimum TDS Value for *Pseudomonas putida*

Pseudomonas putida	
TDS (mg/l)	Chromium Removal(mg/l)
600	4.5±0.26
1200	7.8±0.15
1800	8±0.15
2400	13.66±0.16
3000	3.7±0.24

Table 3.3 Determining Optimum TDS value For *Bacillus subtilis*

Bacillus subtilis	
TDS (mg/l)	Chromium Removal(mg/l)
600	6.75±0.11
1200	No Growth
1800	12±0.25
2400	22.4±0.13
3000	4±0.14

Table 3.4 Effect of incubation time on Cr. removal

Pseudomonas putida	
Time (hrs)	Chromium Removal (mg/l)
48 hrs	14.2±0.14
72 hrs	14.68±0.15
96 hrs	15.73±0.11

Table 3.5 Effect of Initial Chromium Concentration

Pseudomonas putida	
Initial Chromium Concentration (mg/l)	Chromium Removal (mg/l)
10 mg/l	10
50 mg/l	17.21±0.16
75 mg/l	No growth
100 mg/l	No growth

Table 3.6 Effect of Temperature on Cr. removal

Pseudomonas putida	
Temperature (˚C)	Chromium Removal(mg/l)
25 C/120 rpm	18.27±0.22
35 C˚/Static	13.65±0.23
35 C˚/120 rpm	15.15±0.21
47 C˚/120 rpm	10.43±0.15

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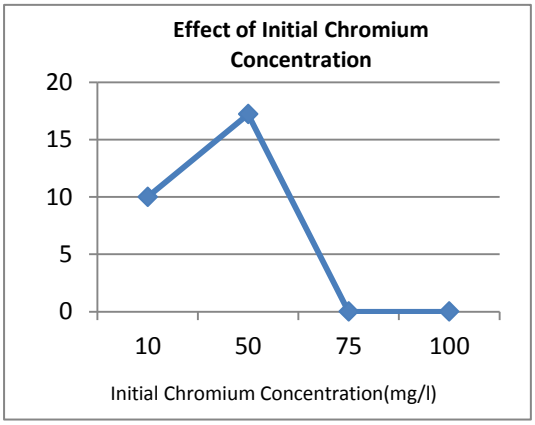

Figure 1 Effect of Initial Chromium Concentration

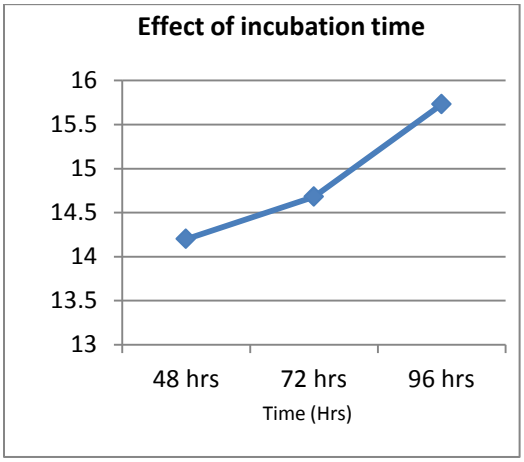

Figure 2 Effect of incubation time on Cr. removal

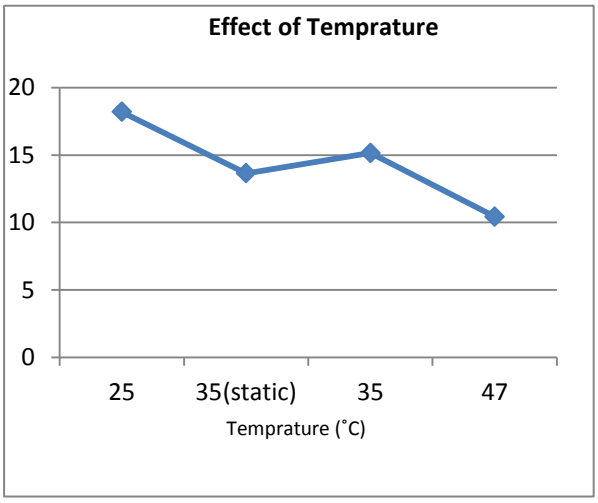

Figure 3 Effect of Temperature on Cr. removal

2.4.2 Effect of temperature

To check the optimum temperature for metal accumulation three temperature values were selected 25˚C,35˚C and 47˚C media containing 25 mg/l of chromium are inoculated with *Pseudomonas putida* and kept for 48 hrs at 120 rpm at different temperature. Supernatant were collected after centrifugation at 8000 rpm for 10 mints and analyzed for chromium concentration by spectrophotometry. All the experiments were carried out in triplicate and results show the mean value.

III. RESULT AND DISCUSSION

3.1 Determining TDS tolerance for survival of microbes

Varying concentration of TDS has been taken to check its impact on chromium removal by microbes. Cr concentration was remaining fixed at 50 and 100 mg/L. It has been observed that up to 3000 mg/l of TDS no microbial growth occurred and slight precipitates were formed. As shown in the table 1.1Selected fungal species has shown very less growth so further study has been carried out with two microbial species, *Pseudomonas putida* and *Bacillus subtilis*

3.2 Determining optimum TDS value for maximum accumulation of chromium

As shown in the Table Optimum TDS was found to be 2400 mg/l for *Pseudomonas putida* and *Bacillus subtilis* has shown very random growth pattern.

3.3 Optimization of environmental parameters for maximum accumulation of chromium

As shown in the result, *Bacillus subtilis* has shown random growth pattern and *Pseudomonas putida* has shown linear growth pattern with varying TDS concentration. So, further optimization of parameters and removal of chromium has been studied with *Pseudomonas putida* only.

After having Optimized TDS value, other environmental parameters incubation time, temperatures, initial chromium concentration was done. Maximum Chromium accumulation was found at 25 ˚C, and 96 hrs. At 10 mg/l of Chromium concentration, removal was 100% and then growth of *Pseudomonas Putida* was decreased with increase in chromium concentration. All the experiments were

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carried out in triplicate and results show the mean value.

IV. CONCLUSION

From the experiment it could be concluded that bacteria play a very important role in the removal of heavy metals from waste water. In the present study six microbial species were selected to study the removal of heavy metals by microbes. it was found that TDS concentration in medium have impact on chromium accumulation efficiency by microbes and *Pseudomonas Putid* were able to remove Cr with an average reduction of 100% in 10 mg/l, 73.08% in 25ppm,34.42%in 50 ppm. It may be concluded that *Pseudomonas Putida* has the ability to remove chromium in dilute concentration of chromium in medium

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