RESEARCH ARTICLE

BIOREMEDIATION OF CHROMIUM FROM CONTAMINATED SOIL: OPTIMIZATION OF ISOLATES UNDER LABORATORY CONDITIONS

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Abstract: Bacterial Strains were isolated and enriched from contaminated sites of Mohkampur and Partapur industrial area in Meerut, Uttar Pradesh at regular intervals for a period of 30 months between 2018 and 2021. The strains isolated showed high efficiency towards reduction of Chromium VI variety in aerobic as well as anaerobic conditions. During the course of experimentations several varying levels of BOD and COD of isolated were observed. It was also found that strains performed better in aerobic conditions in comparison to anaerobic environment. It is safely concluded that isolated strains proved to be a successful remedy in bioremediation of toxic Chromium VI from contaminated soils.

Keywords: Bioremediation, Reduction, Chromium, BOD, COD

INTRODUCTION

The periodic table has a number of elements which have been discovered over many years. It comprises of mainly three classes viz. metals, non-metals and metalloids. The metals are further classified as light metals and heavy metals. All the heavy metals are not hazardous. They can be both beneficial as well as toxic. Generally, the elements belonging to group 3 to group 12 include the metals known as transition metals and all the heavy metals belong to this category only. The scientific world has no widely accepted definition for the term "heavy metal". Therefore, it is generally suggested that heavy metals are those elements which have a specific gravity of at least 5 times the specific gravity of water (i.e., 1 at 4^0 C or 39^0 F). Generally, those elements are considered heavy metals which have a specific gravity higher than 8g/cm^3. Most commonly encountered heavy metals which can cause trouble to the environment and living organisms include Chromium ((8.2 g/cm^3), Cadmium (8.65g/cm^3), Lead (11.34g/cm^3), and Mercury (13.546g /cm3).

Many heavy metals are beneficial to the living organisms as they form some major or minor components of the food. Many heavy metals are thus said to be essential elements as they form an important constituent of nutrition of all living organisms. Some of these heavy metals are known as "Trace Elements" as they are present in very little amounts (eg. Copper, Iron, Manganese, Zinc etc.). Some of the heavy metals are present naturally in foodstuffs like fruits and vegetables, while some other are available in commercial form as in the form of medicinal drugs.

Many heavy metals are industrially important as they are used in the manufacture of pesticides, insecticides, alloys, batteries, textile industry, medicinal industry, print media and many more. Many of these products are common utilities our day-to-day life and domestic activities and are the essential for improving the quality of life.

Heavy metals become toxic for living organisms only when they do not get metabolized by the body and accumulate in the soft tissues.

These heavy metals enter the organisms' body via food, water, air or adsorption as well as absorption through the skin. This may happen when living organisms come in contact with these heavy metals during agricultural practices, during pharmaceutical manufacturing, industrial and/or residential activities. Industrial exposure accounts for the most common route of entry of heavy metals in the human beings, animals, plants as well as micro fauna present in aquatic, terrestrial or aerial ecosystems and ingestion is regarded as the most common route of exposure in children (Roberts *et al.*, 1999).

Small children may also develop toxic levels from their normal hand-to-mouth activity after coming in contact with contaminated soil or by actually eating objects that are not food (like dirt or paint chips) (Dupler 2001). The same may happen with domestic pets also.

Other common routes of exposure are during a radiological procedure, from inappropriate dosing or monitoring during intravenous nutrition, from a broken thermometer (Smith *et al.*, 1997), or from a suicide or homicide attempt (Lupton *et al.*, 1985), and from mercury-based dental amalgams.

The current study is focused on Chromium metal bioremediation. This heavy metal is found in abundance in soil and effluents of Mohkampur Industrial area near Meerut district of Uttar Pradesh.

MATERIALS AND METHODS

Collection of Soil Samples and its Analysis

Samples of soil were taken near Partapur Industrial area, Mohkampur Industrial area, Mawana Road Industrial area and printing press. The samples were taken to the lab and dried in the shade. To

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approximate various parameters, the samples were crushed with a mortar and pestle and afterwards moved through a 2mm sieve. A 0.5mm sieve was used to analyze organic carbon, and tests were saved for physic chemical and chemical research. Standard methods were used to test soil samples for various soil parameters, as seen in Table 1.

Table 1: Soil Analysis Methods

S. No.	Test Conducted	Method	Reference
1.	pH and EC	Soil and water suspension (1:2.5) and extract	Jackson (1973)
2.	Available Nitrogen	Alkaline permanganate method	Subhash and Asija (1956)
3.	Organic carbon	Chromic acid wet digestion (Walkley and Black)	Jackson (1973)
4.	Available Potassium	1N NH4OAC method	Jackson (1973)
5.	Available Phosphorus	Olsen extract (0.5M Sodium bicarbonate)	Olsen et al. (1954)

Soil Organic Carbon

A 0.5gms of soil was taken into 500ml dry conical flask. To this, 10ml of 1 N $K_2Cr_2O_7$ and 20ml of conc. H_2SO_4 was added. The contents were little shaken, and stored around 30 minutes on an asbestos layer. Slowly pour in 200 ml sterile water as well as 10 ml ortho-phosphoric acid. After that, the diphenylamine indication (0.1ml) was applied. Unutilized potassium dichromate was estimated with 0.5 N ferrous ammonium sulphate titration until green color started appearing.

Available Nitrogen

To estimate the available N in soil, 5gm of soil was taken in a Kjeldahl tube. To this, 25ml of 0.32 percent $KMnO_4$ was added. Few glass beads were added. Followed by this, 2ml to 3ml of paraffin liquid was added. While adding, the care was taken that it should not come into contact with upper part of the neck of tube. To this, 40ml of 2.5 percent NaOH solution and 25ml H_2O was added, and the instrument was started for distillation. The distillate was titrated against 0.02 N Hi_3O_4 until pink color starts appearing. The amount of available N was calculated using the standard relationships.

Available Phosphorus

2.5gms of soil is placed in a 100ml conical flask to measure the available P.A sprinkle of Darco-G60 as well as 50ml of Olsen solvent (0.5 M $NaHCO_3$, pH 8.5) was applied. A mechanical shaker was used to shake the contents for 30 minutes. The contents were processed using filter paper of Whatman, as well as 5 ml of transparent and colorless aliquot was moved to volumetric flask of 25 ml. Furthermore, 2-3 drops of 5N H_2N_4 were applied one at a time to oxidize the Olsen substrate to pH 5, at which point the yellow color disappeared. Using distilled water, dilute the contents to around 20ml. Four ml ascorbic acid solution can be applied, shaken thoroughly, and the volume was increased to 25ml. After 10 minutes, the concentration of blue color was assessed at 730-840nrn.

Available Potassium

A sample of 5gms of soil was placed in a conical flask with 100ml, along with a solution of 25ml of neutral ammonium acetate, and the contents were shaken for 5 minutes. The contents were purified using Whatman No. 1 filter paper, then used a flame photometer to determine the K concentration in the filter paper.

PHYSIOCHEMICAL PARAMETERS OF WASTEWATER

The Standard procedures for investigation of water and wastewater were used to conduct all physiochemical analyses of effluent samples (Patricia, 2012). The biological, physical, and chemical aspects of water are examined in physiochemical parameter study of effluent samples. Pure water is colourless in general and should have a pH of 6.5 to 8.5. The following parameters may be used to determine the level of contamination in effluent samples.

pH of Effluent Samples

According to Gupta et al., the pH of a wastewater has been evaluated using a pH meter (2000). The pH meter was warmed up for 15 minutes before use. The pH was measured that used recognized buffer solutions, as well as the electrode has been immersed in an unidentified effluent sample as well as shaken for 3 minutes, only with pH of wastewater displayed on the display screen.

Total Hardness

25 ml of wastewater samples were taken in a 250 ml conical flask. A buffer solution of 1-2 ml was added. 2 drops of erichrome black T will be added to a solution as well as diluted to normal EDTA (0.01 M) till the wine red colour transformed to blue.EDTA was necessary (A) until the end point was recorded. Running reagent was used to get a blank reading, and the amount of EDTA (B) was recorded. The amount of EDTA needed by the effluent sample was calculated using C = (A-B). Formula is used to determine the hardness of the effluent sample -

Hardness in mg/l= MxCx 1000/m x 25

Turbidity

Turbidity can be measured by nepelometer and expressed as nepholometric turbidity units (NTU).

Total Dissolved Solids (TDS)

The framework described by Gupta et al. has been used to recognize dissolved solids in wastewater samples (2000). In a pre-weight evaporating dish, 100 ml of effluent sample was filtered using Whatmann filter paper No. 4. The sample was evaporated in a hot water bath until all of the water was gone. After chilling in desiccators, the final weight of the evaporating dish was recorded. The following formula was used to compute TDS as:

Total Dissolved Solids (g/L) = Final Weight-Initial weight of the evaporating dish x 1000 Volume of sample taken

Dissolve Oxygen (DO)

All types of life need oxygen to survive. For excellent water quality, a sufficient amount of dissolved oxygen is required. Aquatic life may be stressed if DO levels in water fall below 5.0 mg/1. DO is measured in milligrams per litre.

The Winkler technique with azide modification was used to quantify dissolved oxygen in effluent samples. Using a DO sampler, an effluent sample was collected in a BOD container. To a sample collected in a 250 ml vial, 1ml $MnSO_4$ was added, 1 ml alkali-iodide-azide reagent was then added. While adding these reagents, maintain its pipette tip just below the level of liquid.The pipettes would be washed before being placed in the bottles of reagent. By tilting the container 2-3 times, the solution was well mixed, and the precipitate was allowed to settle, leaving 150ml of clear supernatant. Cone with a volume of 1 ml. H_2SO_4 was further added to a solution, which was vigorously enraged till the precipitate has been dissolved. Using starch (2ml) as an indicator, 20ml of this solution was titrated against a standard $Na_2S_2O_3$ solution in a conical flask.

1 ml of 0.025N NazS^Oa = 0.2mg of 02

DO in mg/L = (0.2 x 1000) x (0.025N) ml of thiosulfate / 200

Biological Oxygen Demand (BOD)

With the aid of IN H_2SO_4 and IN NaOH, the pH of the effluent was corrected to 7. The material was placed in 250 ml BOD bottles as well as for the measurement of B.O.D. 1 ml of allyl thiourea solution was added to it. There were no air bubbles in the BOD bottles. One set dissolved oxygen was assessed using an oxygen estimation technique, whereas specimens from a wider variety were soaked for five days.Each bottles would be separated after five days, and also the oxygen in the water content was measured through a formula.

BODS (mg/L) = (D0-D05) x Dilution factor

DO = Sample immediate oxygen dissolved in water (mg/L)

DOS = No dissolved oxygen in the sample after incubation of five days(mg/L)

Chemical Oxygen Demand (COD)

COD is measured in milligrams per litre of solution and is represented as (mg/L). In a SOO ml refluxing flask, 50 ml of sample was mixed with distilled water. $HgSO_4$ was added to the sample, followed by S ml sulphuric acid reagent, the mixture was stirred, and the solution was cooled. A 25 ml solution of 0.0417 M $K_2Cr_2O_7$ was added as well as stirred. Just after flask was linked to the compressor as well as ice water was starting to flow upon this, an extra 70 ml of sulphuric acid solution were added through the use of the open end of the compressor with stirrer. After freezing, the compressor was rinsed with distilled water to half times its original volume. After adding 2 droplets of ferroin marker, the leftover potassium dichromate were determined by titration with FAS till a color transition from blue green to reddish brown were observed. Distilled water refluxed as well, and the blank was titrated with reagents. The method and solvents were evaluated using an experiment on a solution of potassium hydrogen phthalate.

Testing of Bioremediation Potential of Mutant Strains of *B. subtilis and B. Amyloliquifaciens*

Chemically induced 'suspected' mutations will henceforth be called chemical mutants. In the preceding set of tests, the mutant strains were examined for chromium bioremediation capability with B. subtilis and lead bioremediation capacity with B. amyloliquifaciens. The wild type, which were free-floating strains and fundamentally individual in character, were employed as reference cultures for comparing levels of chromium and lead bioremediation, whereas the controls were cultivated in conditions without exposure to chromium

Results

Physico-chemical characteristics of Soil

Samples of soil were taken near Pratap Industrial area, Mohkampur Industrial area, Mawana Road Industrial area and printing press. The samples were taken to the lab and dried in the shade. To approximate various parameters, the samples were crushed with a mortar and pestle and afterwards moved through a 2mm sieve. A 0.5mm sieve was used to analyze organic carbon, and tests were saved for physic chemical and chemical research. Standard methods were used to test soil samples for various soil parameters, Physico-chemical characteristics of Soil are showing in Table 2.

Table 2. Physico-chemical characteristics of Soil

Moisture %	pH 1:2.5	Conductivity (mmho/cm) (1:2)	Organic matter%	Exchangeable Na (mg/100g)	Exchangeable K (mg/100g)	Ca (mg/100g)	Mg (mg/100g)	Exchangeable Na% (mg/100g)	CEC (mg/100g)
23.90	10.58	8678	0.18	1103	16	337	146	70	73

Table 3. List of Physicochemical parameters and its permissible limits values

Parameters	Permissible limit in mg/lit
Color	Colorless
pH	6.5-8.5
Dissolved oxygen	5
CaC03	300
Biological oxygen demand(BOD)	40
Chemical oxygen demand(COD)	140
Total Dissolve Solid	1000
Sulphate	750
Sulphite	0.002
Nitrate	45
Chromium (Cr)	0.05
Lead (Pb)	0.05
Cadmium (Cd)	0.01
Zinc (Zn)	5.0
Chloride (Cf)	250

DISCUSSION

The samples were gathered from the dyeing business every two weeks for a year, and the work was completed between 2018 and 2021.Temperature (T), pH, Specific Conductance (K), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Total Hardness (THA), Temporary Hardness (HAT), Permanent Hardness (HAP), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD)The results of physiochemical analysis of all the electroplating industrial effluent were clearly indicated that samples were having appreciable amount of toxic chromium(VI).

CONCLUSION

The indigenous strains isolated from electroplating effluents (Tharannum *et al.*, 2012) have an innate ability to resist chromium. They presumably possess the chromium reductase activity because of which the chromium is successfully reduced in spiked sample as well as in the effluent. In effluents, along with chromium, there are extraneous toxic substances which the microbes have shown resistance to and reduced chromium.

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