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Hexavalent Chromium Bioreduction Using Microbes in Cooum River Water and Sediments

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A B S T R A C T

In the present study, an effort was made to investigate the current condition of heavy metal concentration on the bank of the Coovum River in the city of Chennai. According to the findings of this study, both industrialization and urbanisation have been major contributors to the massive magnitude of pollution that has recently been observed. People living in this area are exposed to a major risk to their health as a result of breathing in chromite dust brought on by pollution caused by hexavalent chromium in this area. Because of this, it is imperative that the hexavalent chromium be eliminated from the environment. The use of microbes to bring about a reduction in hexavalent chromium (VI) serves as the basis for this study. The discovered strain has a maximum Cr VI tolerance of 100 mg/100 ml when exposed to the element. The samples that included hexavalent chromium were biodegraded with the help of these cultures, which were also employed for future research. According to the findings, an isolated culture has the potential to be exploited in an effort to increase the efficacy of biological treatment methods for effluents that include greater concentrations of Cr (VI). Carried done in order to ascertain the concentration of heavy metal ions present in this area and its surrounding areas.

Keywords: Biodegradation, Chromium, Heavy Metals, Hexavalent, Pollution

Introduction

In the context of economic growth, mining is one of the industries that substantially contributes to the pollution of air, water, land. This deterioration of the natural environment is a direct result of the development process. At the beginning of the planning phase of a mining project, environmental protection measures have to be implemented so as to fulfil the goal of sustainable development. The nation of India is blessed with a diverse range of mineral deposits, the production of minerals in the country has maintained an upward trend. India is responsible for the production of up to 90 different minerals, including fuel minerals. Water pollution, which may be defined as a deterioration in the quality of water as a result of numerous human activities that render water unsuitable for drinking and for use in home settings, is now the most significant challenge that humanity faces. Heavy metals that are known to be toxic have been released into the environment as a waste of industrial processes, leading to severe soil and water pollution. Growing demand from a variety of industries, including residential, agricultural, industrial, as well as hydropower, among others, is increasing to an increase in water scarcity on a global scale. This is increasing strain on the world's limited water resources. (Ayres.R.U1992) Water is a component that is human for the survival of all living things, including humans, plants, animals. According to Eneji.I.S, Sha'Ato.R and Annune.P.A. (2011), the primary causes of water pollution are the introduction of chemical fertilisers and pesticides into sewage that has not been treated, the dumping of waste and industrial effluents into rivers and streams that run close to cities and lowlands, the improper disposal of waste. In the presence of precipitation, flocculation or coagulation takes problem, one of the most major issues is the formation of vast quantities of sediments that are rich in heavy metal ions (Gibbs,1995). It is caused on by activities such as the excavation of pits, the dumping of solid waste, other linked processes. The disposal of solid waste such as overburden, waste rock, sub-grade ore, mineralized refuse has the potential to result environmental pollution, which in turn may disrupt both the water level and the water quality (Dhar, 1993). When minerals are caused to the elements of air and water, a natural oxidation process takes place, which is what causes the problem of heavy metal pollution. Sulfide-oxidizing bacteria have a significant role in the formation of age-related macular degeneration. (Kleinmann et al., 1985).

Chromium is an essential industrial metal that finds use in a wide variety of goods and processes (Nriagu,1988). Leakage caused by incorrect disposal practises or inadequate storage conditions might result in it being discharged into the environment in many different areas (Palmer and Wittbrodt, 1991; Calder, 1988). Chromium (VI) species in aqueous solutions are generally soluble and have relatively low adsorption capacity; as a result, they have the potential to be mobile in groundwater (Kent et al.,1995). If it is not complexed, Cr (III) should experience a significant rate retardation in groundwater due to the considerable adsorption or insolubility of its aqueous species (Kent et al.,1994; Anderson et al., 1994).

Multiple ground water samples taken in the city of Kanpur were found to contain hexavalent chromium. In most cases, the flow of contaminants was parallel to the flow of ground water, although this does to a large extent rely on the hydrological characteristics of the region. It also suggested that hand pumps located at shallow depths were to a significant extent polluted (Panday, 1997).

Near the chromium-rich ultramafic terrain in Pakistan, it has been reported that the soil and plants in the surrounding area have an increased content of chromium in their tissues (Kafay yatullah). Previous research has shown that several locations throughout the globe have seen high levels of heavy metal pollution in the soil and plants, which may be connected to the presence of ore resources in certain locations (Gough et al.,1989). Chromite has been found in both the ground water and the surface water, previous research shows that both are polluted with hexavalent chromium (R.K.Tiwary et al., 2005). Chromium is released into the environment and stays in its Cr (III) and Cr oxidation states, which are its two most stable forms (VI). The trivalent form of chromium is mostly harmless and immobile, while the virial form, Cr (VI), is very mobile and may travel through soils and aquatic environments. Hexavalent compounds are "acute toxic, mutagenic" (Bianci et al., 1983; Beyersmann et al., 1984), "teratogenic" (Abbasi and Soni, 1984), "carcinogenic" (Mancuso and Hueper., 1951; Waterhouse 1975; Yassi and Nieboer, 1988).

The most toxic toxin that may be found in water is chromium (Cr^{+6}). A value in drinking water greater than 0.05 may be associated with adverse effects on human health. A more concentrated form of Cr^{+6} has the concentration to cause disorders, such as those caused by corrosive effects on the skin and mucous membranes. In addition to being an irritant to both plants and animals, it is also a powerful oxidising agent that may be absorbed via the skin (Nriagu and Nieboer, 1988). Trivalent compounds, on the other hand, have a toxicity level that is considered to be rather low (Van Weerelt et al., 1964), they are immobile in environments ranging from mildly alkaline to slightly acidic.

During the course of this investigation, we were successful in resisting a locally present bacterial strain from the Coovam wastewater that was indigenous to chromium. Establishing a remediation route for the detoxification of Cr (VI) utilising this indigenous microbe with all of the process parameters and circumstances being optimised is the primary goal of this research.

Material and Method

Samples of water and soil were taken from the Coovam River so that the research could be carried out. The first concentration of hexavalent chromium in water samples as well as a research on the characteristics of the soil have both been completed. Sample Identifying features of In several locations along the Coovam River, screw-capped sterile bottles contained soil and water samples that had been contaminated with chromium. An ion-specific electrode, also known as a pH metre, was used to measure the pH of the samples, the results showed that the pH of the soil samples ranged from 7.3 to 7.8 while the pH of the waste water samples ranged from 8.3 to 8.5. This suggested that the chromite-contaminated areas had a somewhat alkaline composition in their natural state. The concentration of chromium (VI) in the contaminated soil sample ranged from 2 to 6 mg/l, while the concentration of chromium (VI) in the effluent varied from 36 to 44 mg/ l. The Cr_2O_3 content in the water body ranges from 25.31 to 55.525 percent, but the waste material only contains 5-9 percent Cr_2O_3, according to the results of an XRF analysis of the dump material. The sample also has a high iron percentage in terms of its composition. Sample collection, isolation of microbes, checking the minimum inhibitory concentration, growth of microbes, strain identification, diphenyl carbazide assay, initial measurement of chromium (VI), bioreduction of chromium (VI), measurement of Cr (VI) after reduction are the steps involved in the bioremediation process for hexavalent chromium. These procedures are broken out in further depth below.

Isolation of Microbe

Microbes that are able to live in soil that is contaminated with chromium must have built up a resistance to the chromium in order to survive there. Because of this, it is assumed that these microbes are capable of reducing Cr (VI) to Cr (III). Therefore, these soil and water samples were used in the cultivation of microbes resistant to chromium. In order to make culture medium, peptone and yeast extract (also known as PYE) were used. It is necessary to test the microbes that have been cultivated on the Petri plate for the greatest possible concentration of chromium that they are able to withstand while still exhibiting apparent growth on the Petri plate. In order to accomplish this goal, petri plates were prepared with increasing levels of $K_2Cr_2O_7$ concentrations. After then, the plates were smeared. Following an incubation period of twenty-four hours, the microbes that had developed on the plates were moved to a new plate that had a greater concentration of $K_2Cr_2O_7$. Following the determination of the MIC, a single strain that was able to propagate at a greater concentration was chosen for the further research.

Following the MIC test, the chosen strain was put through its paces to provide the basis for the culture. The process that was discussed before was prepared in order to prepare the culture. Additionally, the plates were prepared beforehand. Incubation was carried out for a whole day at a temperature of 37 degrees Celsius. In order to complete the identification process, the chosen strain was sent to the “Centre for Cellular and Molecular Biology” (CCMB) in Hyderabad.

Initial Measurement of Chromium (VI)

Mix together 50 millilitres of acetone and 250 milligrammes of 1, 5-diphenylcarbazide. Keep it in a bottle of dark colour. When solutions change hue, they should be discarded. After combining one gramme of the solid sample with twenty-five ml of distilled water, the mixture was strained through filter paper. We use 25 ml of it for the water sample, 5 drops of phosphoric acid are added to both the water and the filtered soil samples in order to acidify them. After that, two millilitres of 0.2N H_2SO_4 and two millilitres of diphenyl carbazide are put into the mixture. The development of a pink colour occurs. After that, a spectrophotometer examination will take place.

Bioreduction of Chromium (VI)

The components of the media are combined in a flask that is 250 millilitres in volume and contains 50 millilitres of either a water or soil sample that has been filtered. It also has an additional 0.5g of lactose supplemented to it. After the process of sterilising, 2 2ml of culture that had been developed for 24 hours was added to it. The inoculation procedure was performed under the laminar flow of air. Following that, the incubation process was carried out in the incubator at a temperature of 37 hours Celsius for a duration of both 24 and 72 hours. After what seemed like a standard amount of time had passed, the assessment of chromium (VI) was carried out in accordance with the established process. Controls were prepared, but no culture was added to them throughout the preparation process.

Final Measurement of Chromium (VI)

After preculturing the bacterial strain for one night in PYE broth, the cell pellets were recovered by centrifugation (6000g for 10 minutes), this was followed by washing the cell pellets in phosphate buffer. Following two washes in phosphate buffer, the cells were resuspended in the same buffer. This was done in order to remove any remaining phosphate buffer. Culture flasks containing minimum salts media that had been supplemented with Cr (VI) from the filtered water and soil sample were inoculated with equal quantities of culture species. The flasks had 40 ml of each medium, 0.5g of lactose was also added. Bacteria were then introduced into media that did not include Cr (VI), the uninoculated medium that did contain Cr (VI) was used as a control. At a temperature of 37 degrees Celsius, all of the cultures, including the controls, were incubated for a total of 72 hours. The optical density of the cultures was measured at 540 nm at regular intervals in order to track the growth of the bacteria during the time of the study. A three ml sample of culture from each of the aforementioned flasks was centrifuged at 6000 revolutions per minute for fifteen minutes in order to determine the reduction of Cr (VI) that was removed by the developing cells (VI). In order to identify the chromium-resistant bacteria, several soil samples and water samples were taken from various locations, which are detailed in Table 1 below. The Cr (VI) analysis was performed using the diphenyl carbazide assay, the pH of the samples was determined. The soil sample and the water sample that will be used for the isolation of chromium-resistant bacteria are chosen from among these samples. Because of the high chromium content found in both the soil sample and the waste water sample, these two samples will be used in present research.

Result and Discussion

Isolation of Bacterial Strains Resistant to Chromium and a Study of Their Tolerance to Chromium (VI)

A very high number of different kinds of microorganisms may be found in soils, some of which include bacteria that are capable of utilising Cr (VI). Through the use of culture-based procedures, a solitary chromium-resistant bacterial strain was isolated from the samples. In a medium consisting of solid agar, the isolated strains are tested using chromate tolerance testing at various dosages, ranging from 25 to 200 mg/100 ml. Figure 1 and Figure 2 show microbes that grew on 125 mg $K_2Cr_2O_7$ per 100 ml of distilled water, respectively.

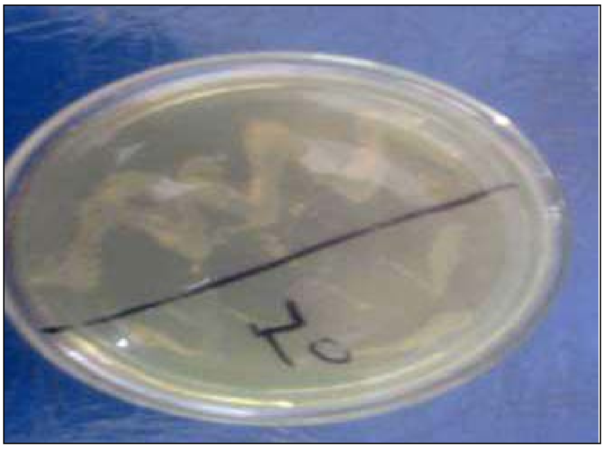

Figure 1.Showing growth of microbe at 125mg $K_2Cr_2O_7$/100ml distilled Water

Bioreduction of Chromium

The initial Chromium (VI) concentration of the water sample that was taken was 0.755 parts per million (ppm). A representative sample of the water was collected from the Coovam River. After waiting for 24 hours, samples were collected, 3 millilitres of each sample was centrifuged before being filtered using What man filter paper number 42. The filtration process resulted in a supernatant that was measured at 540 nm. The sample had a lowered concentration of 0.261 ppm after being processed. Another set of samples was filtered after 72 hours, 3 millilitres of each of those samples was centrifuged and taken through Whatman filter paper number 42. The filtration process resulted in a supernatant that was measured at 540 nm. The new concentration, after the reduction, was 0.022 ppm.

A representative sample of the soil was collected from the same area.

Initially, there was a concentration of 0.998 ppm of chromium (VI). The first set of the soil sample was measured after 24 hours, the concentration was found to be 0.58 ppm. The concentration of the second set of soil samples, which had been left for 72 hours, was found to be 0.43 ppm after being measured. The results of the reduction after the various time periods are shown in Table 6.

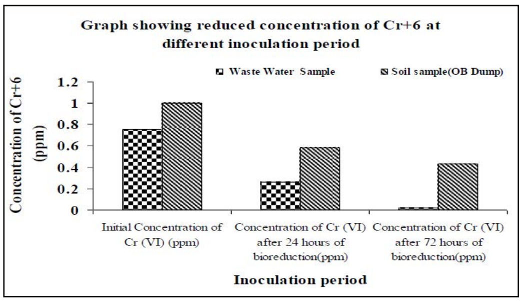

Figure 2.Showing reduced concentration of Cr+6 after different period of time

Conclusion

Hexavalent chromium is a very toxic substance. As a result, the present inquiry has looked at whether or not the contaminated soil and water include any indigenous species that are resistant to Cr(VI). The microorganism that has been identified has a high resistance to Cr (VI) and possesses a significant power to totally breakdown Cr (VI) in waste water. According to the findings, the bioreduction of Cr (VI) in the soil sample was 47 percent and 97 percent in the waste water sample after 72 hours, while the degradation of the soil sample and the water sample was 42 percent and 66 percent in 24 hours correspondingly. Therefore, it is possible to draw the conclusion that the microorganism that was identified as being resistant to chromium does a better job of cleaning waste water. The groundwater supply will be fully polluted and rendered unsuitable for drinking as well as other uses if the present situation is allowed to persist into the foreseeable future. This is despite the fact that the situation is already in a very poor state at the moment. This finding provides evidence that the environment has been contaminated. Therefore, the disposal of waste items that include pollutants should be prevented, these materials should not be allowed to enter the river. As a result, a great deal of care should be taken to prevent the consequences.

Table 1.Bioreduction of samples after different time intervals

Samples	Initial Concentration of Cr (VI) (ppm)	Concentration of Cr (VI) after 24 hours of bioreduction (ppm)	Concentration of Cr (VI) after 72 hours of bioreduction (ppm)
Waste Water Sample	0.755	0.261	0.022
Soilsample (OB Dump)	0.998	0.58	0.43

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