Jr. of Industrial Pollution Control 34(2)(2018) pp 2059-2062
www.icontrolpollution.com
Research Article

STUDIES ON BIOREMEDIATION TECHNIQUE ON HEXAVALENT CHROMIUM USING AUTOMOBILE INDUSTRIAL WASTE

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(Received 06 July, 2018; accepted 19 July, 2018)

Key words: Recycling, Environmental effects, Hazardous waste, Non-hazardous wastes.

ABSTRACT

Conventional methods for the removal of metal ions from the industrial effluents proving very expensive due to its high initial capital investment and high regenerative expenses. Hence in the present study a simple and economic technique i.e., bioremediation technique was applied for the removal of hexavalent chromium using automobile industrial waste. Bioremediation is emerging as one of the simple, economic and efficient method for the removal of metal ions from the industrial effluents. In the present study, bacteria is isolated from the root rhizoshere of *Eichhorniacrassipers* and estimated its capacity to grow in chromium containing effluents. Further its ability to remove chromium ions from industrial effluents was determined. The results revealed that bacteria can survive and multiply in both synthetic solutions and industrial waste effluents. The results revealed that the observed *Pseudomonas* bacteria can remove both trivalent and hexavalent chromium ions from the solutions efficiently.

INTRODUCTION

Water pollution with heavy metals has increased exponentially in many developed countries due to urbanization and industrialization. Industrial effluents discharged into the water sources are the major reason for the accumulation of heavy metals in water. The accumulation of heavy metals in the water is an alarming issue because of their toxicity and hazardness to human and other aquatic life. These contaminated water sources plays important role in the transfer of heavy metals to plants and groundwater. The consumption of the contaminated water by these plants and human beings pose lot of hazards to fauna and environment. From the last two decades onwards, researchers have realized the need to study the heavy metals, such as chromium, nickel, zinc etc., removal from water. Chromium is extensively used in electroplating, tannery, wood preservation industries etc. Effluents from those industries contaminate soil and water sources which pose serious threat to the living and non-living environment.

Hexavalent chromium present in industrial effluent is highly mobile, hazardous, and carcinogenic and marked as one of the significant environmental pollutant. The United States Environmental Protection Agency has stated Cr(VI) as one of the chemical posing the significant threat to humans (Rita and Ravisankar, 2014). In this work emphasis was laid on assessing the toxicity of the hexavalent chromium present in the sludge of metal plating of automobile industry and to reduce the toxic effects using microbial bioremediation technique.

The conventional methods of Cr(VI) removal from

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wastewaters includes chemical reduction followed by chemical precipitation, adsorption, coagulation, electro dialysis etc. However, these methods utilize large amounts of chemicals and generate large amount of sludge (Malaviya and Singh, 2016). Adsorption technique using activated carbon as adsorbent is industrially widely used technique for the removal of heavy metals. However due to its high initial and regeneration costs still most of the industries are not preferring to use this adsorbent which in turn resulting them unable to implement effluent treatment effectively thus necessitates the need for the use of cheap and eco-friendly method. In recent decade many scientists reported the use of bacteria, yeast, fungi etc for the removal of chromium (Kapoor and Viraraghavan, 1997; Niu H, *et al*., 1993; Nourbakhsh, *et al*., 1994; Camargo, *et al*., 2003).

Bioremediation involves the breaking down of the constituents of the effluent into simple fragments which will be either taken as a substrate by the organisms or simply remain in the system (Saranraj and Sujitha, 2013; Thatoi, *et al*., 2014). Bioremediation using microbes helps to transfer Cr(VI) to Cr(III) and makes it stable and hence reduces the toxins. The bacterial species are able to grow in the toxic conditions and are generally assumed to be resistant to chromium (Viti and Giovannettim, 2001; Gadd, 1992). Hence, the present study is focused on the isolation of hexavalent chromium resistant bacteria from the root rhizoshere of *Eichhornia crassipers*, evaluation of the chromium tolerating capacity of the bacterium and determining its potential for the treatment of automobile industrial effluents.

MATERIALS AND METHODS

Sample collection and preparation of stock culture

The *Eichhornia* plant root sample was collected in a sterile bottle at Visakhapatnam. A sterile bottle with a lid was taken near the plant. The plant was uprooted and placed in that bottle. The bottle was brought to the lab and added with sterile distilled water in a laminar air flow chamber.

The stock solutions were prepared by dissolving potassium dichromate in accurately measured distilled water in 250 ml Erlenmeyer flasks. Working solutions were obtained by diluting the stock solution to the required concentrations using distilled water. Initial pH of the solutions was adjusted using 0.1 N HCl and 0.1 N NaOH solutions.

1 ml of sample solution in the airtight bottle was taken by using a micropipette and diluted by adding it to a test tube of 10 ml sterile distilled water. Then 1 ml from this test tube was taken and diluted into another test tube of distilled water. This procedure was continued until 8 serial dilutions and diluted samples from test tubes were taken (0.1 ml) and inoculated into the solidified nutrient agar medium in the petri plates using streak and spread plate methods and then the Petri plates were incubated for 24 hr at 37 degrees centigrade. Two types of colonies were observed growing on the Petri plates following incubation. Standard procedure was followed in isolation and growth of pure culture of the colonies found in the petri plates. *Pseudomonas* thus obtained was inoculated into 100 ml of nutrient broth and also nutrient agar slants were made for further use was stored in the refrigerator.

Metal tolerance studies

Nutrient broth was prepared by adding tryptone, yeast extract, NaCl, various increasing concentration of potassium dichromate and potassium chromate (0.005-0.010) g, distilled water, to a conical flask and it was cotton plugged and sterilized. 6 conical flasks were added with potassium chromate and potassium di-chromate was added to the other 6 conical flasks. In a laminar air flow, the loop is first burnt red hot and allowed to cool and then a loop full of pure culture was taken and suspended in the test tube of sterile distilled water for preparation of microbial suspension. After sterilization, 0.1 ml of the suspension of the test tube was taken with the help of a micropipette and suspended in each of the conical flask of varying chromium concentration of potassium dichromate and chromate. The conical flasks were then again cotton plugged and incubated for 7 days at 37°C. The values of optical density were measured at 600 nm for measuring biomass concentration after each day for 7 days. The liquid suspension from the conical flasks were taken, centrifuged at 4000 rpm for 10 min and analysed for residual Chromium concentrations using atomic absorption spectrophotometer. This procedure was repeated by replacing the conical flask containing potassium dichromate and chromate with conical flasks containing different concentrations of automobile industrial sludge (0.005-0.01 g) to determine the chromium tolerance efficiency of *Pseudomonas* bacteria from industrial sludge solutions.

Bioremediation studies

The biosorption studies to evaluate the ability of the biosorbent for Chromium removal from aqueous solutions were carried out in triplicate using the batch biosorption studies. The experiments were performed by adding 0.5 g of adsorbent in 50 ml of the 0.005 g Chromium solutions maintained at 25°C and pH 5, and agitated at 150 rpm for 2 hr in orbital shaker, then the liquid suspension was taken, centrifuged at 4000 rpm for 10 min and aqueous solution was analysed for residual Chromium concentration using atomic absorption spectrophotometer. The percentage removal of metal from the solution by the biosorbents was determined by the equation (1).

$$\%\text{Removal}=\frac{(C_i - C_f)}{C_i}\times 100 \quad (1)$$

Where C_i and C_f are the initial and equilibrium metal concentrations in the solution (mg/L).

RESULTS AND DISCUSSION

Metal tolerance ability

The results of the metal tolerance ability of *Pseudomonas* species in potassium dichromate and industrial sludge solutions were shown in Tables 1 and 2 and (Fig. 1 and 2) respectively. The results revealed that the nutrient medium provided for the growth of *Pseudomonas* species is adequate for the bacteria to thrive and multiply. The only possible hindrance for the bacteria is the concentration of hexavalent chromium. However, the observed values showed in Table 1 and (Fig. 1) shows that the biomass concentration increased significantly from day-1 to day-7 which indicated that bacterium species can tolerate the presence of hexavalent chromium. It is observed that there is a significant increase in microbial biomass even in the sludge sample Table 2 and (Fig. 2) containing varying high concentrations of chromium along with other heavy metals like nickel, led etc. This infers that *Pseudomonas* species can capable of tolerating other heavy metals also.

Ability to remove chromium from aqueous solution

The results of the chromium removal ability of *Pseudomonas* species from potassium dichromate solution was shown in Table 3 and (Fig. 3) respectively in the form of percentage removal. It is observed that the chromium concentrations in the samples were decreased remarkably in the 7 day period. The results revealed that (Fig. 3) the percentage removal of chromium is increased significantly up to 5 days after that it slowed down and reached equilibrium after 6 days. This indicates that 7 days of incubation

Table 1. Biomass increase in potassium dichromate solution

No. of Days	Concentration of $K_2Cr_2O_7$ in g					
	0.005	**0.006**	**0.007**	**0.008**	**0.009**	**0.01**
1	0.01	0.04	0.05	0.06	0.07	0.07
2	0.05	0.12	0.15	0.16	0.18	0.21
3	0.11	0.17	0.19	0.21	0.28	0.31
4	0.33	0.37	0.42	0.44	0.48	0.51
5	0.78	0.82	0.87	0.89	0.88	0.89
6	0.84	0.87	0.89	0.91	0.92	0.93
7	0.86	0.88	0.91	0.92	0.93	0.94

Table 2. Biomass increase in industrial sludge solution

No. of Days	Concentration of sludge in g					
	0.005	**0.006**	**0.007**	**0.008**	**0.009**	**0.01**
1	0.11	0.13	0.16	0.18	0.21	0.33
2	0.18	0.22	0.28	0.33	0.45	0.5
3	0.32	0.36	0.42	0.52	0.61	0.67
4	0.51	0.54	0.59	0.71	0.8	0.84
5	0.57	0.59	0.69	0.81	0.86	0.91
6	0.61	0.63	0.72	0.87	0.94	0.97
7	0.63	0.65	0.76	0.89	0.95	0.97

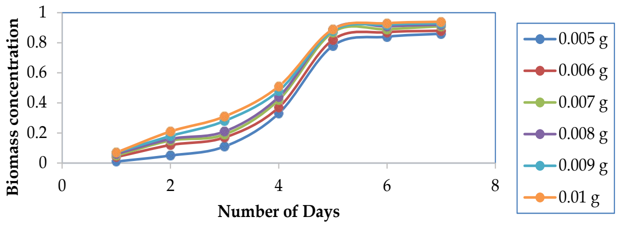

Fig 1. Biomass growth in potassium dichromate solution.

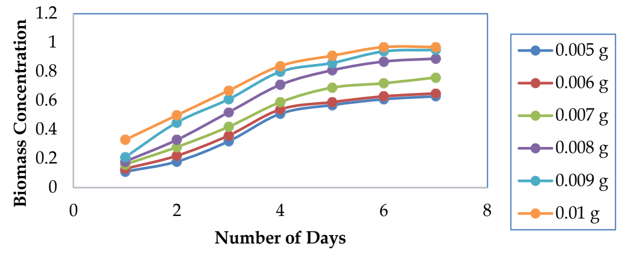

Fig 2. Biomass growth in industrial sludge.

Table 3. Percentage removal of chromium

No. of Days	Concentration of $K_2Cr_2O_7$ in g					
	0.005	**0.006**	**0.007**	**0.008**	**0.009**	**0.01**
1	4	6	8	10	11	12
2	15	17	19	21	22	24
3	21	25	31	41	43	45
4	45	48	52	56	58	59
5	75	77	82	84	86	88
6	84	85	89	90	91	93
7	86	88	91	92	93	94

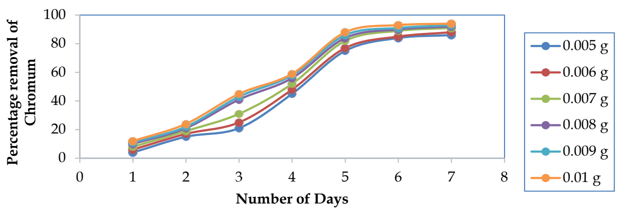

Fig 3. Percentage removal of chromium.

is sufficient is remove the 95% of chromium from the aqueous solutions. The results obtained show the ability of *Pseudomonas* to remove chromium from aqueous solutions.

CONCLUSION

In the present study of isolation of hexavalent chromium resistant *Pseudomonas* bacterial species, it is proved that the response of *Pseudomonas* to remediate hexavalent chromium under controlled laboratory conditions showed promising results. Bioremediation of chromium using the bacterial species of *Pseudomonas* provides a wide scope for further research to scientists to carry out various experiments of tolerating capacity of hexavalent chromium, rate of biomass production and also the reaction of this species of bacteria to various environmental conditions and also its ability to remediate various environmentally polluted soils with other harmful heavy metals can be tested. Increase in the biomass and the decrease in the chromium concentrations in the Chromium sources clearly indicate that this species of *Pseudomonas* can bio remediate chromium in both trivalent and hexavalent states. Which provides a wide area to focus for further research.

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