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Bacterial Treatment For Removal of Chromium (Vi) Containing Electroplating Waste Waters

KEYWORDS

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ABSTRACT *A bacterial isolate (HN03) with high Cr(VI)-removinging capacity was isolated from Cr(VI)-contaminated soil and identified as Serratia rubidaea. The isolate was a Gram-positive, aerobic rod. The hexavalent chromium-removinging capability of the isolate was investigated under 1, 5 Diphenyl carbazide (DPC) method, Atomic absorption spectrophotometer (AAS), UV-Visible scanning spectroscopy, Inductively coupled plasma - optical emission spectrometer (ICP-OES) and phytotoxicity study. Under these conditions, the isolate tolerated chromium concentrations up to 1,200 mg/l. The strain reduced Cr(VI) over a wide range of pH (6.5–10.0) and temperatures (30-37°C) with optimum performance at pH 8.5 and 35 °C. The various other parameters such as agitation speed (80- 200) and various carbon source with optimised conditions 150 rpm agitation speed and sucrose as carbon source were studied one at a time the experiments were conducted in 250 mL Erlenmeyer flask containing 100 mL LB broth medium. This is the novel report of a bacterial growth and Cr(VI)-removal process under aerobic growth conditions. Further Laboratory scale model microaerophilic, down flow type, batch fixed film bioreactor (BRF) was operated. The study suggested that the isolate possesses a distinct capability for Cr(VI) removal which could be harnessed for the detoxification of chromium contaminated wastewaters.*

Introduction

Environmental pollution is an important consequence of industrial processes and human activity. Different industries, including mining and electroplating, release aqueous effluents containing a variety of toxic heavy metals. Wastewaters from these industries have permanent toxic effects to human and the environment. Accumulation of toxic metals, e.g., Cd, Cr, Cu, Hg and Zn, in humans has several consequences such as growth and developmental abnormalities, carcinogenesis, neuromuscular control defects, mental retardation, renal malfunction and wide range of other illnesses.

Chromium is a chemical element discovered in 1797 by Louis Nicolas Vauquel in which has the symbol Cr. Its atomic number is 24. It is a hard metal of steely grey colour and also it has a high melting point of 1907°C. It is odourless and tasteless metal. Many of its compounds are intensely coloured. Chromium is an important metal due to its high corrosion resistance and hardness. Chromium is a naturally occurring element found in many foods and drinking water, thus it makes its way into the body mainly from dietary intake. In addition, intake of chromium results from airborne dusts and mists, and cigarette smoke as well as from industrial and occupational exposures.

Trivalent chromium, Cr (III) is an essential micronutrient for humans and is relatively less soluble than the hexavalent chromium, The trivalent chromium Cr (III) is required in trace amounts for sugar and lipid metabolism in humans and its deficiency causes disease. Whereas the high valence chromium is toxic, mutagenic and carcinogenic. Environmental cleanup strategies for Cr (VI) removal involve physicochemical or biological detoxification. Major limitations of physicochemical processes are the high energy inputs, different chemical treatments and generation of unnecessary sludge, reactive chemical species as secondary wastes. These problems can be overcome by biological Cr (VI) detoxification which is more ecofriendly and an economically feasible technology [1].

Chromium is an essential micronutrient required for the growth of many microorganisms for the maintenance of normal glucose, cholesterol and fatty acid metabolism. The deficiency of, chromium has been implicated in impaired insulin action, which can cause glucose intolerance, elevated glucose, blood levels, diabetes, elevated cholesterol levels, obesity and heart diseases, as well as other conditions not yet documented. Chromium is considered the cofactor for all the actions of the hormone insulin, primarily the regulation of carbohydrate, protein and fat metabolism. Signs of chromium deficiency are widespread; they tend to be associated with aging, and are consistent with the progressive decline in body and organ content of chromium from birth onward.

Traditional metal removing methods from industrial effluents include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies and evaporation recovery [2]. These processes may be ineffective or extremely expensive, especially when the metals in solution are in the range of 1-100 $mg.L^{-1}$ [3].

Recently microbial systems like fungus, bacteria and algae have been successfully used as absorbing agents for removal of heavy metals. In the present investigation, the ability of isolated bacterial strains towards removal of chromium was evaluated. Optimization study, reactor study and phytotoxicity study were carried out.

MATERIALS AND METHOD

Isolation and screening of chromium removing bacterial isolates

Soil and sludge samples were collected from electroplating industry, Rakhiyal, Ahmedabad, Gujarat, India for isolation of potent chromium removal bacterium. Isolation was carried through enrichment culture techniques, using LB broth medium amended with $K_2Cr_2O_7$, 2.5 $mg.L^{-1}$. The flask showing chromium removals were acclimatized with increasing the chromium concentration up to 800 $mg.L^{-1}$ in 7 days.

From the culture broth the needed inoculum is taken by the wire loop on LB agar plates amended with 800 $mg.L^{-1}$ of chromium incubated at 37 °C for 24 to 48 hrs. The different colonies found on the plates after incubation was checked for the chromium removal ability by individual culture

Chromium estimation

The chromium removal potential of individual isolate was

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determined by 0.25 % (w/v) 1, 5 Diphenyl carbazide (DPC) method [4], based on coloration Cr (VI) reacts with diphenylcarbazide to give violet red coloration.

% chromium removal was computed by this formula

$$\% \text{ chromium removal} = \frac{\text{Initial chromium} - \text{Residual chromium}}{\text{Initial chromium}} \times 100$$

Identification of potent chromium removing bacterial isolates

Identification was done by the morphological, culture characteristics and

biochemical analysis.

Effect of various physicochemical parameters on chromium removal by potent bacterial isolate

The effect of various parameters such as effect of pH (6.5 - 10), effect of temperature (30- 37), effect of agitation speed (80- 200), effect of chromium concentration (200- 1200), effect of various carbon source effect of nutrient concentration were studied one at a time the experiments were conducted in 250 mL Erlenmeyer flask containing 100 mL LB broth medium and incubated in rotary shaker (120 rpm) at 37 °C.

Removal studies

Atomic absorption spectrophotometer (AAS), UV-Visible scanning spectroscopy and Inductively coupled plasma - optical emission spectrometer (ICP-OES)

Bio reactor study on chromium removal by HN03-02

Laboratory scale model was carried out by using microaerophilic, down flow type, batch fixed film bioreactor (BRF). The reactor was built from borosilicate glass column of 60 cm length and 5 cm internal diameter. The pottery material pieces of 2.0-3.0 cm size were used as support material. The reactor was operated 6664.00 $mg.L^{-1}$ of chromium with HRT of 10 days. The effluent of the bioreactor collected after 24 hrs was used for further analytical studies.

Analytical methods

Feed and reactor effluent samples were routinely analysed for physicochemical parameters such as pH, O/R potential (mV) was measured by using a digital pH meter (Systronics MK VI), total Solids (TS), total volatile solids (TVS), total acidity, chemical oxygen demand (COD) (close reflux method) and % removal of chromium (1,5 Diphenyl carbazide method) [4].

Phytotoxicity study

Tests were carried out using 0.8% agar plates. Total 10 healthy seed of green gram (Gujarat 4) was sawn in each plate. A test was carried out in two sets at room temperature by adding a sample of reactor influent in their respective plates. Control set was run using distilled water at the same time in another plate. Germination (%), length of the pumule (shoot), radical (root) length and breadth of leaf were recorded at regular interval up to 10 days.

RESULTS AND DISCUSSION

Isolation and screening of chromium removing bacterial culture

Bacterial cultures were isolated by enrichment culture technique from various sites contaminated with wastewater from electroplating industry. They grew efficiently in LB broth medium containing 250 $mg.L^{-1}$ chromium (VI), 5 $mL.L^{-1}$ wastewater. Gradually increase the chromium concentration up to 800 $mg.L^{-1}$.

Identification was done on the basis of morphological characteristics, Gram's staining and biochemical test. Results of biochemical test for HN03-02 was identified as a Serratia rubidaea (Burgess manual).

Effect of physicochemical parameters on Cr (VI) removal

The culture exhibited growth as well as removal activity over the entire selected pH range (6.5 - 10). Maximum removal (95 ± 0.9 %) was observed at pH 8.5. Our results are in good agreement with the work carried out by Long et al., [5] by Pseudochrobactruma saccharolyticum maximum chromium removed at pH 8.5. LB broth medium kept at different temperatures (30 - 40 °C) 95 ± 0.6 % removal of chromium peaked at 35 °C temperature. At 35 °C temperature more suitable for the maximum chromium removal [6]. Agitation was a key factor for living cell the maximum 99 ± 1% removal of chromium at 150 rpm. [7] observed satisfactory growth and higher chromium removal at 150 rpm. The removal ability of the developed and highly efficient culture were studied at various concentrations of chromium (200-1200 mg.L-1) in the medium and results are shown 97 ± 0.9 % removal of chromium. The highest hexavalent chromium uptake was found to be at 800 ppm concentration by Pseudomonas fluorescens [8]. In other hand LB Broth medium supplemented with carbon source as fructose, glucose, lactose, maltose, sucrose and xylose were tested for the influence of carbon sources at 0.1 gm% concentration. Serratia rubidaea HN03-02 showed maximum 99.04 ± 0.6% chromium removal in sucrose supplemented LB broth medium.

Graph 1.1 Effect of physicochemical parameters on Cr (VI) removal

Comparison between unoptimized and optimized conditions

To evaluate the effect of optimization on chromium removal by Serratia rubidaea HN03-02 in unoptimized condition, The result obtained after optimization was higher than unoptimized condition therefore it clearly indicates the importance of optimization study in chromium removal study.

Removal study

Inductively coupled plasma-optical emission spectrometer (ICP-OES)

Inductively coupled plasma-optical emission spectrometer (ICP-OES) was used to evaluate the removal activity of chromium. At the time of inoculation, the chromium concentration of optimization study control was 1009.00 mg.L-1and reactor influent chromium was 6624.00 mg.L-1. However after treatment with the Serratia rubidaea HN03-02 the chromium level was reduced to 449.00 mg.L-1and 3184.00 mg.L-1 respectively after 17 hrs inoculation.

UV-Vis Scanning spectrophotometer

The removal of chromium analysis was confirmed by performing UV-Vis scanning of the optimization study and reactor effluent showed a decrease in pick as compare to the control.

Graph 1.2 UV-Vis Scanning spectrophotometer

Reactor study

Table: 1.1 Values of down flow, Batch Fixed Film Reactor (BFR) effluent operated at room temperature

Phytotoxicity study

The relative sensitivity towards the chromium containing reactor effluent and its removing products in relation to green gram was studied. The mean of pumule and radical length of green gram was 15.00 and 9.5 cm respectively, of 10 seeds in tap water as a control with 95 ± 1 % germination. The germination of plant seeds 65 ± 0.5% inhibited when seeds treated with reactor influent the pumule and radical length was found 5.00 ± 0.1cm and 2.00± 0.1 cm respectively, whereas reactor effluent the pumule and radical length was found 13.5± 0.4 and 7.00± 0.2 cm respectively with 85 ± 0.9% germination when treated with the reactor effluent

Conclusion

Present investigation successfully showed chromium (VI) removal by potent bacterial isolate Seratia rubidaea HN03-02 was identified and showed very good chromium removal in shake flask condition as well as in fixed film down flow bio-

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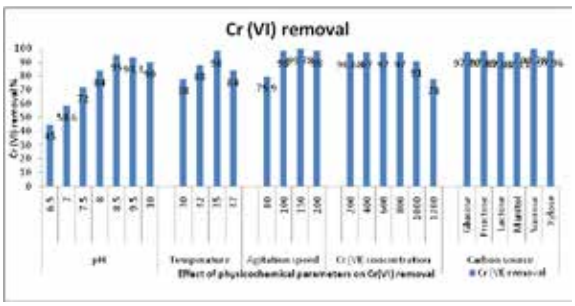
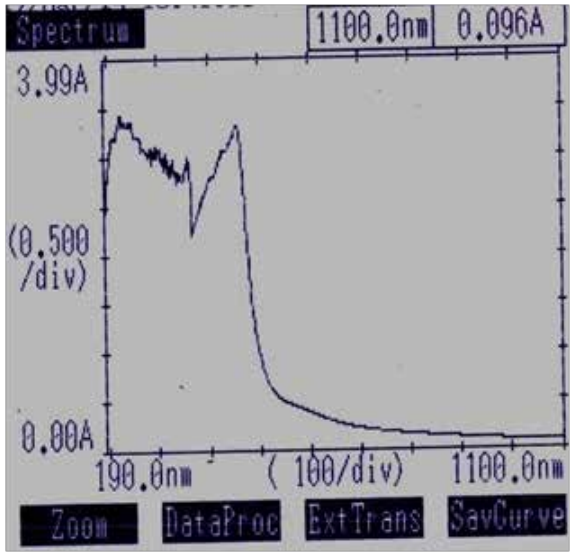
reactor. Present investigation successfully showed chromium (VI) removal by potent bacterial isolate Seratia rubidaea HN03-02 was identified and showed very good chromium removal in shake flask condition as well as in fixed film down flow bioreactor. The study demonstrated that the newly isolated Serratia rubidaea strain has potential application for the removal of chromium from electroplating wastewaters.

Graph 1.1 Effect of physicochemical parameters on Cr (VI) removal





Graph 1.2 UV-Vis Scanning spectrophotometer

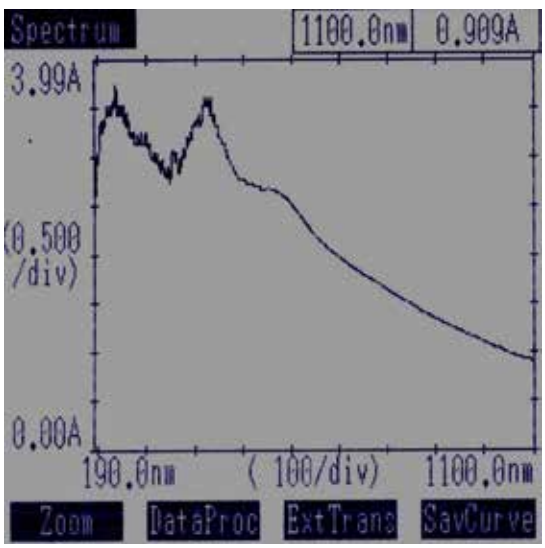


Table: 1.1 Values of down flow, Batch Fixed Film Reactor (BFR) effluent operated at room temperature

Sr No.	Parameters	10 days HRT	9 days HRT	8 days HRT	7 days HRT	5 days HRT
1	pH	7.3± 0.3	8.49± 0.3	8.5± 0.4	8.51±0.3	8.49± 0.4
2	ORP	-015± 0.003	-065± 0.002	-067± 0.007	-066± 0.002	-067± 0.004
3	OLR(kg COD m-3 d-1)	11.8± 0.4	2.42± 0.16	3.36± 0.1	4.9± 0.2	6.4± 0.2
4	COD removal (%)	92.02± 0.9	94.98±1	92.09± 0.9	62± 0.7	48± 0.4
5	TS (mg.L-1)	11000± 0.3	13000±22	12000±22	14000±24	15000±25
6	TVS (mg.L-1)	950±13	10000±20	12000± 21	14000±21	15000± 24
7	Chromium removal (%)	89.00± 0.2	75± 0.6	62± 0.55	41± 0.3	35± 0.2

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