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Research Article

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Bio-removal of toxic Cr (VI) by marine bacteria, *Halomonas sp.*VITP09

Sangeetha Subramanian*, Vikas Dhaka, Vipul Ranjan and Vivek Pratap Sigh

School of Bio Sciences and Technology, VIT University, Vellore, Tamil Nadu, India

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ABSTRACT

Environmental contamination is also caused due to extensive use of hexavalent chromium in various industrial applications. Halomonas sp. VITP09, obtained from Kumta Coastal region of Karnataka, India was examined for their tolerance and ability to remove Cr (VI). The influence of different factors such as pH, NaCl concentration, Initial inoculum concentration and initial Cr(VI) concentration on Cr(VI) removal and growth were studied. Halomonassp.VITP09 showed complete removal of 50,100 and 300 mg/l in 16, 24 and 72 h of time duration. The results indicated that the Halomonassp.VITP09 has high potential for Cr (VI) remediation under saline condition.

Keywords: Hexavalent chromium, marine bacteria, *Halomonas* sp., chromium removal, waste water.

INTRODUCTION

Chromium and its derivative have wider application in metallurgy, leather, steel and electroplating industries, chromic acid manufacturing and many other speciality chemicals. The extensive use of chromium increases the concentration of soluble Cr (VI) in the soil and water that leads to various health hazards, which are carcinogenic and mutagenic to living organism[10].Other common health disorder due to chromium contamination includes nausea, vomiting, epigastria pain and haemorrhage in human[8]. 40 % of total use of chromium was contributed through tanning process and thus incorporating the chromium into the biosphere. According to World Health Organization(WHO) guide lines for drinking water, permissible limit for hexavalent chromium and total chromium are .05 and 2 mg/l respectively [10]. On comparison with Cr(VI), Cr(III) is less toxic, insoluble and impermeable to cell membranes [3,4] and hence removal of Cr(VI) is significantly important. Conventional method such as precipitation, ion exchange, electro chemical treatment has various disadvantages. Detoxification of Cr(VI) by bacteria is an alternative method, as they have ability to tolerate, bio accumulate, precipitate, adsorb or reduce toxic hexavalent chromium[5,7]. Remediation of metal in saline condition become highly important as the waste water produced by most of the industries, especially leather industry contains higher concentration of salt such as NaCl [11]. Halophilic and halotolerant microbes are capable of growing in higher salt concentration and these types of microbes have also been found to exhibit resistant towards many toxic metals. Hence the present study investigates the chromium removal potential by marine bacteria, *Halomonas* sp.VITP09.

EXPERIMENTAL SECTION

Chemicals

Chemicals and culture media were purchased from Himedia, India. Analytical reagent (AR) grade chemicals were used for all the experiments and all the glasswares were washed with nitric acid (1:1) and rinsed with distilled water before use.

Microorganism and culture conditions

The halotolerant bacteria, *Halomonas* sp.VITP09 (Accession No: JN657266), used for the present study was taken from in- house culture collection. *Halomonas* sp.VITP09 was grown in Luria-Bertani (LB) medium with 40 g / l

NaCl (Temperature35°C, pH 7.0 and agitation rate- 140 rpm) under aerobic condition, unless otherwise stated.Cr (VI) was added as potassium dichromate in the media for all the experiments.

Effect of initial Cr (VI) concentration on Cr (VI) removal and biomass growth

Halotolerant bacterial cells from agar plates were sub cultured in 25 ml culture media in a 100 ml flask and the experimental flask was inoculated using this culture. The 100 ml medium in 250 ml flask with different initial Cr concentrations (0, 50,100,300,400 and 500 mg/l) was inoculated using 1% v/v overnight grown exponential culture (1.0 OD at 600 nm). At regular intervals, the samples were collected and the OD was measured at 600 nm for biomass growth. Further samples were centrifuged at 8000 rpm for 10 min and the concentration of chromium was determined in the supernatant by Diphenylcarbazide method. Each experiment was performed for a period of time until the residual concentration of chromium (VI) and biomass concentration was found to be same with time for all experiments under aerobic condition(Temperature35°C, pH 7.0,4 % w/v NaCl and agitation rate- 140 rpm).

Effect of pH on chromium removal and biomass growth

By varying the initial pH (5, 6, 7, 8, 9 and 10) in LB media, the effect of pH on chromium removal and growth was examined for *Halomonas* sp.VITP09in the presence of 200 mg/l of initial Cr (VI) concentration. Biomass growth was monitored both in the presence and absence of Cr (VI) under saline condition (4 % w/v NaCl).pH was adjusted using 1N HCl or NaOH. After 24 hours of incubation at 140 rpm and 35°C, growth and chromium concentration were analysed.

Effect of salt concentrations on chromium removal and biomass growth

The effect of different salt (NaCl) concentration (1, 2, 3, 4 and 5% (w/v)) on growth and chromium removal was investigated. The growth was monitored in different salt concentration in the presence and absence of Cr (VI). All the experiments were carried out for 24 hours of incubation at 35°C and 140 rpm with 200 mg/l of initial Cr (VI) concentration in LB media. Growth at 600 nm and Cr (VI) concentration by DPC method was analysed in all the experimental flasks.

Effect of different inoculum concentration on Cr (VI) removal and biomass growth

The effect of different inoculum concentration on biomass growth and Cr (VI) removal was investigated. Different inoculum concentrations (1, 2, 3, 4, and 5 % (v/v)) were inoculated in the flasks in the presence and absence of 200 mg/l of Cr (VI) at 35° C, 7 pH, 4% w/v NaCl and 140 rpm in LB media. After 24 hrs of incubation, Cr(VI) concentration and biomass growth was recorded.

Analytical methods

In all the experiments, Cr (VI) was determined by diphenylcarbazide method [13]. The Cr (VI) in the supernatant was determined spectrophotometrically by reaction with diphenylcarbazide in acid solution (6 M H_2SO_4). Cell free supernatant was made upto 1 ml using distilled water followed by addition of 330 µl of 6 M H_2SO_4 and 400 µl of diphenylcarbazide (0.25 % w/v in acetone). The solution was diluted to 10 ml using distilled water. The biomass concentration was inferred from the optical density value at 600 nm (Shimadzu UV 2401PC)

RESULTS AND DISCUSSION

Effect of pH

The effect of initial pH on biomass growth and chromium removal was studied in the presence 200 mg/l of initial Cr (VI) concentration under aerobic conditions (4% NaCl and 35 °C). Figure 1 shows the results of Cr(VI) removal and biomass growth for different initial pH. The results showed that the maximum percentage removal of Cr (VI) was at pH 8 (82.20%) followed by pH 7 (80.27%) and pH 9 (78.60%) and the least percentage removal was obtained at pH 5. Similar results were reported for *Achromobacter* sp. strain Ch1 [14].The effect of different pH on growth in the absence of Cr(VI) showed moderate growth in all the pH range investigated, whereas in the presence of Cr(VI), optimum growth were observed in the range from 7 to 9 pH. The biomass growth in the presence of Cr (VI) was negligible at pH 5. Similar results were reported in chromium resistant bacteria[6,15].

Effect of NaCl concentration

Figure 2 depicts the biomass growth and Cr (VI) removal results for different initial salt concentration (1, 4, 8, 12 and 16 % (w/v) NaCl) in the presence of 200 mg/l of Cr (VI) under aerobic conditions (7pHand 35°C). The effect of different salt concentration on Cr (VI) removal revealed that the maximum removal was observed at 4 % (w/v) NaCl with 81.9 % removal. At 1 and 8 % (w/v) NaCl, the Cr (VI) removal was found to be 72 and 68 % respectively. However significant removal was not observed at 12 and 16 % (w/v) NaCl. The effect of different salt concentration on the growth of biomass in the absence and presence of Cr(VI) showed maximum growth at 1% (w/v) NaCl followed by 4 and 8 % (w/v) NaCl. However growth was lowest in the presence of 12 and 16 % (w/v)

NaCl. The halotolerance characteristics of organism facilitated growth upto 8 % NaCl (w/v) but the growth was restricted at very high salt concentration at 12 and 16%. Decrease in Cr (VI) removal potential was observed with increase in NaCl concentration and optimum removal was found at 4 % (w/v) NaCl[2].

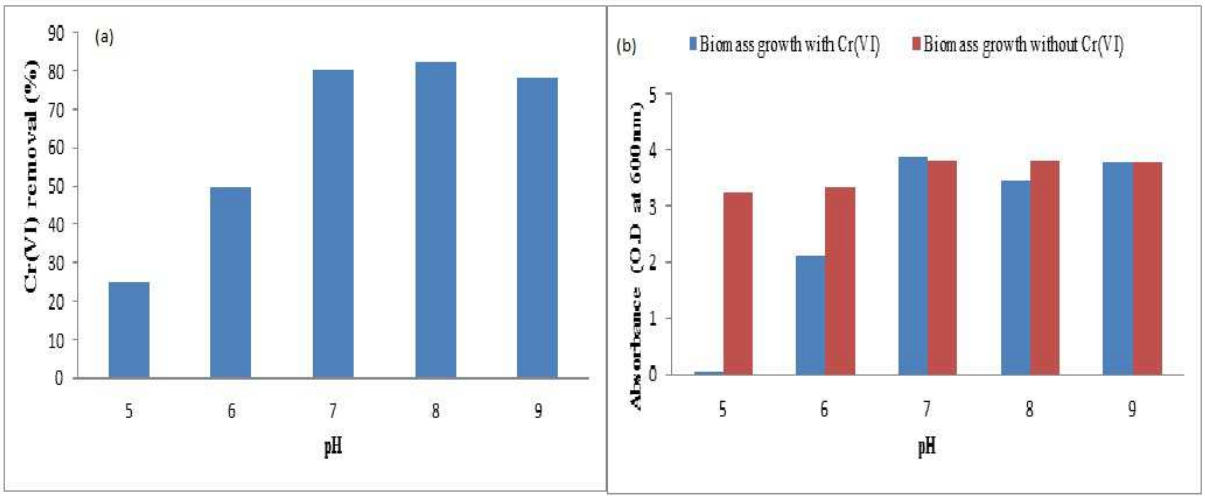

Figure 1 (a) Effect of pH on Cr(VI) removal by *Halomonas*sp.VITP09 (b) Effect of pH on the growth of *Halomonas*sp.VITP09 in the presence and absence (Cr(VI) concentration = 200mg/l, Incubation time : 24 hr, 35°C, 4 % (w/v) NaCl and 140 rpm)

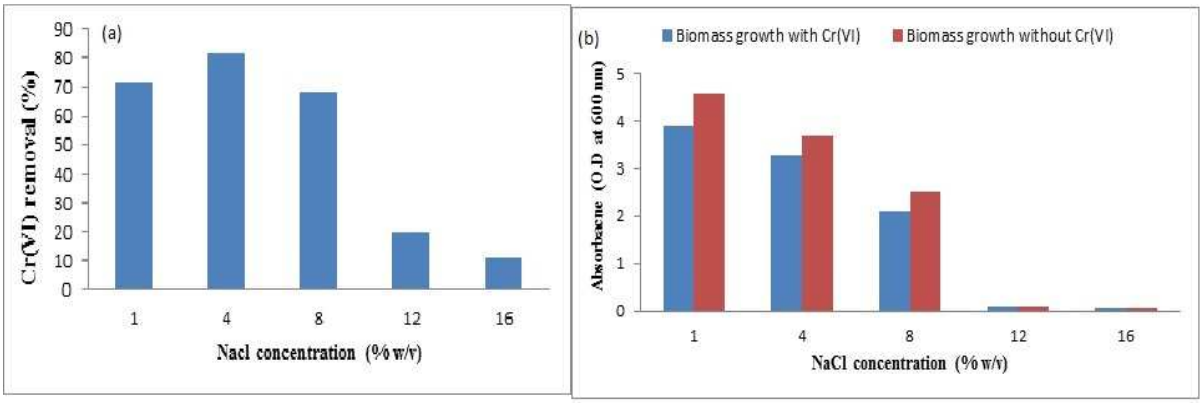

Figure 2 (a) Effect of different NaCl concentration (%w/v) on Cr(VI) removal by *Halomonas*sp.VITP09 (b) Effect of different NaCl concentration (%w/v) on the growth of *Halomonas*sp.VITP09in the presence and absence 200 mg/l of Cr(VI) concentration (Incubation time : 24 hr, 35°C, 7 pH and 140 rpm)

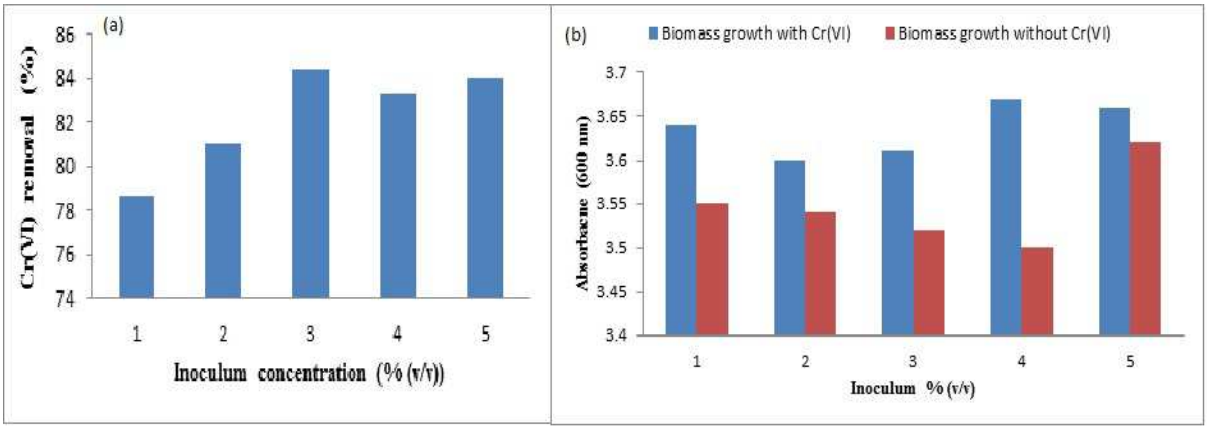

Figure 3 (a) Effect of Inoculum concentration (%v/v) on Cr(VI) removal by *Halomonas*sp.VITP09 (b) Effect of Inoculum concentration (%v/v) on the growth of *Halomonas*sp.VITP09 in the presence and absence of 200 mg/l of Cr(VI) (Incubation time : 24 hr, 7 pH, 35°C, 4 % (w/v) NaCl and 140 rpm)

Effect of different inoculum concentration

Biomass growth and Cr (VI) removal was studied for different initial inoculum concentration (1 to 5 % (v/v)) in the presence of 200 mg/l of Cr (VI) under aerobic conditions (7pH, 4% w/v NaCl and 35°C). Figure 3 shows the experimental results. At 1 % (v/v) inoculum, slight decrease in Cr (VI) reduction potential (79 %) was observed, whereas for 2 to 5% v/v greater than 80 % Cr (VI) removal was observed. The biomass growth in the presence and absence of Cr (VI) exhibited growth greater than 3 OD at 600nm for all the inoculum concentration (1 to 5 %(v/v)). The study reveals that inoculum concentration is not a significant factor to affect chromium removal [1].

Effect of initial Cr (VI) concentration

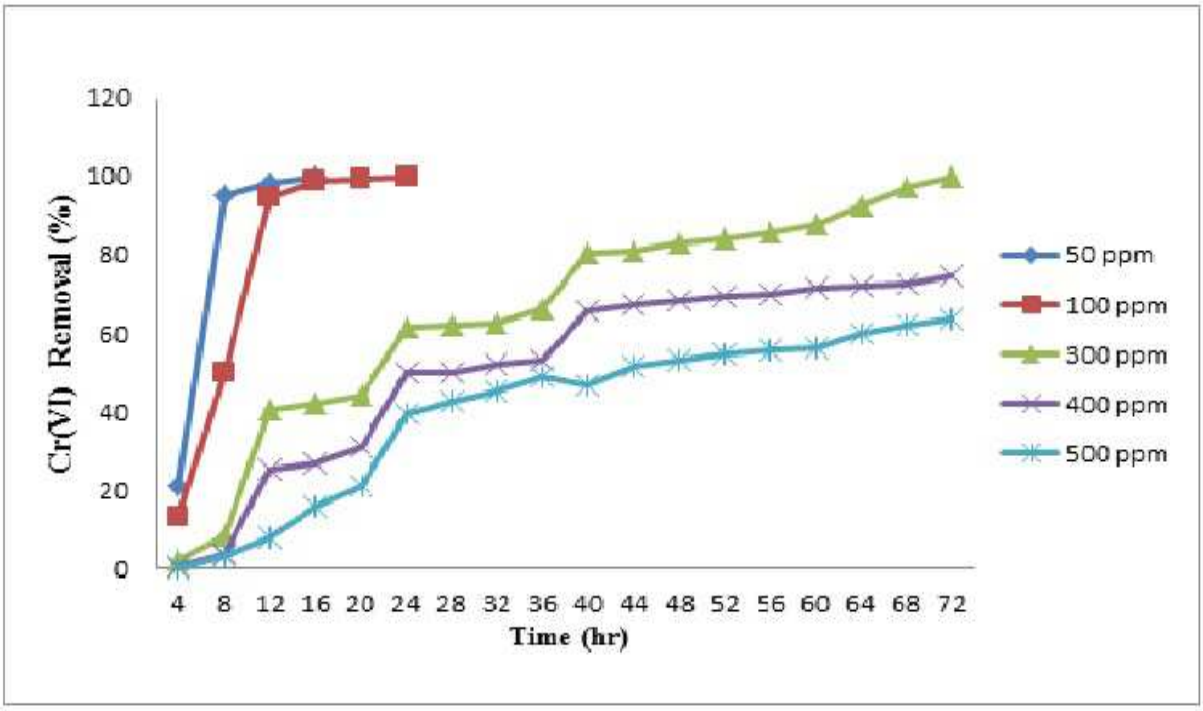

Figure 4 Effect of different Cr (VI) concentration on Cr (VI) removal by *Halomonas* sp. VITP09(35°C, 4 % (w/v) NaCl, 7 pH and 140 rpm)

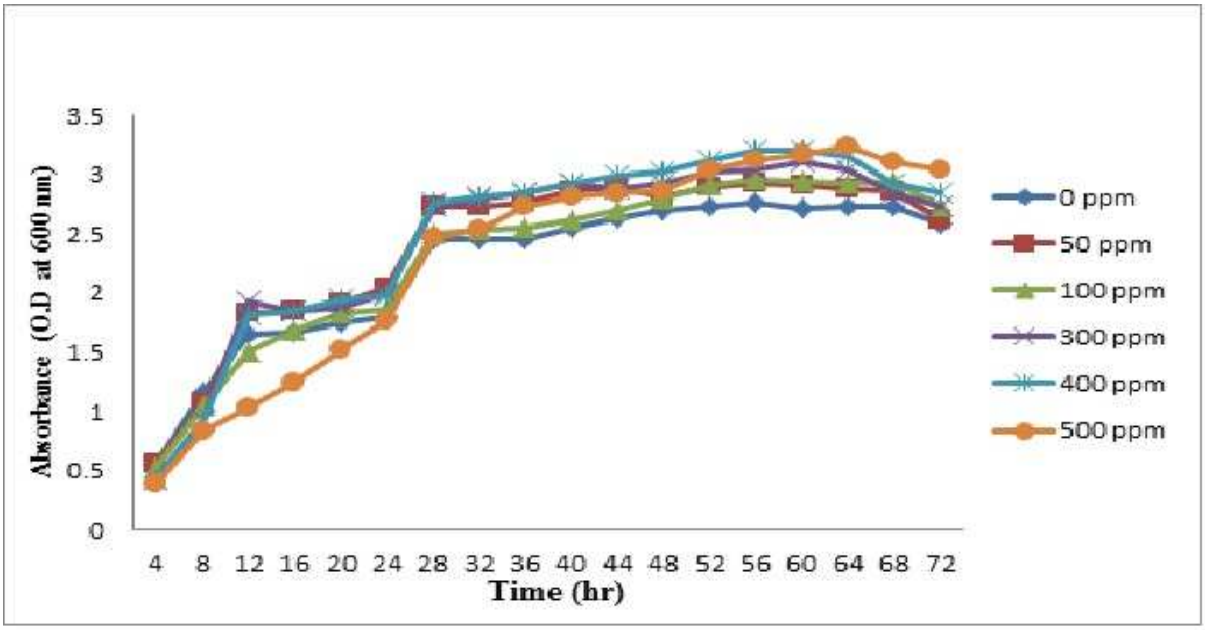

Figure 5 Effect of different Cr (V) concentration on the growth of *Halomonas* sp. VITP09(35°C, 4 % (w/v) NaCl, 7 pH and 140 rpm)

Cr (VI) removal (Figure 4)and biomass growth (Figure 5) was studied for different initial Cr (VI) concentration (0, 50,100,300,400 and 500 mg/l) under aerobic conditions (7pH, 4% w/v NaCl and 35°C). Complete removal of Cr (VI) was observed for 50,100 and 300 mg/l in 16, 24 and 72 h of time duration. But for the higher Cr (VI) concentration, incomplete removal was observed and removal was 74.6% (400 mg/l) and 63.4 % (500 mg/l) in 72 h of incubation. From the results it was observed that increase in Cr (VI) concentration resulted in increase in time duration and decrease in Cr (VI) removal potential [12]. Biomass growth at different Cr (VI) concentration (0, 50,100,300,400 and 500 mg/l) obtained its maximum biomass concentration and the concentration approximately reached the same for all different Cr (VI) concentration. However at higher Cr (VI) concentration (500mg/l) increase in lag phase was observed [9]. The halotolerant bacteria exhibited resistance even in the higher Cr (VI) concentration thus showing the potential for the treatment of saline waste water with higher Cr (VI) concentration.

CONCLUSION

Halomonas sp. VITP09 was investigated for Cr(VI) removal potential under different operating conditions. Different factors such pH, NaCl concentration, Initial inoculum concentration and initial Cr(VI) concentration affecting Cr(VI) removal and growth were studied. Complete removal of 50,100 and 300 mg/l of Cr(VI) was observed in 16, 24 and 72 h of time duration under optimum aerobic condition at 35°C, 4 % (w/v) NaCl ,7 pH and 140 rpm.The results indicated that the *Halomonas* sp.VIT09 has high potential for Cr (VI) remediation under saline condition.

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