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Optimization of growth conditions for maximum hexavalent chromium reduction by the microbial consortium isolated from chromite mines

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Hexavalent chromium (Cr(VI)) contamination is one of the important threat to the environment. Detoxification of Cr(VI) can be achieved with the use of chromium resistant bacteria. Three chromium resistant organisms were isolated from the soil of chromite mines and identified as *Serratia nematodiphila*, *Bacillus cereus* and *Bacillus* sp. SDIP3 using 16S rRNA sequencing. A consortium was developed with the isolated bacterial strains after the acclimatisation. The effect of environmental factors, such as temperature, pH and nutrient sources were studied for the maximum chromium reduction by the consortium at the Cr(VI) concentration of 25 µg/mL. The reduction of Cr(VI) was optimum at the temperature 35ºC and pH 7. The Cr(VI) reduction was more effective with the glucose as carbon source, inoculum age of 24 h and 4% of inoculum volume. The statistical optimisation of parameters such as pH, temperature and carbon source concentration for the maximum reduction of Cr(VI) was done by Response surface methodology using Box-Behnken design (BBD). The consortium has shown 92% reduction of Cr(VI) under the optimised conditions. This consortium was further used in continuous reactor system to reduce the Cr(VI) to less toxic trivalent chromium.

Keywords: Bioremediation, Box-Behnken design (BBD), Heavy metal pollution, Response surface methodology (RSM), *Serratia nematodiphila*

Heavy metals have been used for numerous industrial applications due to their importance in industries. Heavy metals are evolved as a huge threat to humans and environment due to their contamination in ecological system[1]. Health effects such as carcinogenesis, growth and developmental abnormalities, mental retardation and neuromuscular control defects caused by the accumulation of heavy metals have been reported[2].

Among the heavy metals, hexavalent chromium (Cr(VI)) is one of the most common water pollutant and is threat to most organisms while exceeds its permissible limit of 0.05 mg/L[3]. Hexavalent chromium is the most stable form of chromium and highly soluble in nature[4]. Typically, it is profoundly available in water discharges from the industrial and industrial mining activities. In order to continue with both the activities, it is essential to treat Cr(VI) from the discharge water.

In India, and particularly in Odisha, there are few chromite mines located in the Sukinda area of Jajpur district. The presence of Cr(VI) in fresh water bodies beyond the permissible limit is the potential threat to the residents of the area[5]. The conventional method of treating Cr(VI) contaminated water is reported to have adverse environmental consequence since high sulphur content is left in the effluents. Besides, disposal of sludge in long run may render the land unusable[6]. Removal of heavy metals, especially chromium using microorganisms is an eco-friendly alternative and cheaper than chemical methods[7].

In recent years, many research works has been carried out to detoxify Cr(VI) by the reduction into Cr(III) using microorganisms. Few Chromium resistant microorganisms capable of reducing Cr(VI) to nontoxic Cr(III) such, *Cellulomonas sp.*[8], *Arthrobacter sp.*[9], *Desulfovibrio vulgaris*[10], *Providencia sp.*[11], *Serratia marcescens*[12], *Pseudomonas stutzeri*[13] and *Cellulosimicrobium sp.*[14] have been reported. Reduction of Cr(VI) to Cr(III) can be achieved by the enzymatic activity, accumulation and absorption of the microorganisms[9, 15]. To enhance the Cr(VI) reduction efficiency of microorganisms, the acclimatization and consortium development could be effective techniques. Literature regarding consortium based detoxification of Cr(VI) is limited. The potential for the reduction of Cr(VI) by using the consortium of *Bacillus endophyticus*, *Microbacterium paraoxydans*

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and *Bacillus simplex* have been reported[16]. Similarly the consortium developed with *Bacillus endophyticus*, *Paenibacillus macerans*, and *Bacillus pumilus*[17] was also reported for their potential of Cr(VI) detoxification. The synergistic relationship between the microorganisms in the consortium would increase the activity against the pollutant and provide the stability in wide range of environmental influencing factors such as temperature and pH.

In the present study, A consortium was developed with the organism *Serratia nematodiphila* which is one of the rarely explored organisms for its potential for Cr(VI) reduction. A consortium with the organism *Serratia nematodiphila* can be unique combination to treat Cr(VI) and can increase the activity of organisms by the synergistic effect. The growth parameters of the acclimatised organisms were optimised to achieve the maximum Cr(VI) reduction. Optimization by classical methods which involves the change of one variable at a time, is extremely time consuming and expensive when a large number of variables are considered[18]. To reduce the number of experimentations, it is desired to use the statistical tools such as RSM, Plackett Burman, Taguchi methods. In this study, Box-Behnken Design (BBD), one of the Response Surface Methodology (RSM) method was used for statistical optimization of parameters, due to the advantages of BBD such as: (i) estimation of the quadratic model factors (ii) detection lack of fit of the model, (iii) avoids the extreme treatment combinations. Statistical method also used to validate the results of single parameter optimisation method[19].

The present study is a site specific bioremediation of Cr(VI) using microbial consortium. The optimization of parameters for the maximum reduction of Cr(VI) by the microbial consortium, developed from the indigenous organisms isolated from chromite mine area was studied. Under the optimized conditions, the potential of consortium for Cr(VI) reduction was studied in a stirred tank reactor.

Materials and methods

Sample collection

Soil and water samples from the chromite mines of South Kaliapani (21°02'52.0"N 85°47'23.0"E), Jajpur district of Odisha, were collected in sterile polypropylene containers and stored at 4°C until further microbial analysis.

Isolation of chromium resistant bacteria

Collected soil samples were dissolved in sterile distilled water and serially diluted. To isolate the chromium resistant organisms, serially diluted samples were plated in M9 minimal salt media supplemented with 100 mg/L of Cr(VI). M9 minimal salt media contained m9 salts (Na_2HPO_4.7H2O, KH_2PO_4, NaCl, NH_4Cl) along with $MgSO_4$ and $CaCl_2$. Glucose was used as the carbon source.

Acclimatization of organisms

To enhance the Cr(VI) reduction potential of isolated organisms, each organism were grown at 10,20,40,60,80,100 and 120 mg/L of Cr(VI) serially. The acclimatization period varied sequentially with the increasing concentration of Cr(VI) until the maximum growth observed in the media. Acclimatized organisms were maintained at 100 mg/L concentration of Cr(VI).

Identification of organisms

The genomic DNA was extracted from the isolated organisms (CRB5, CRB8 and NITRKLR1). The bacterial 16S rRNA gene (1500 bp) was amplified by the polymerase chain reaction in a thermal cycler (Clarridge, 2004) and purified with Exonuclease I Shrimp Alkaline Phosphatase[20]. Sequencing was done by Sanger method with the purified amplicon. Basic Local Alignment Search Tool (BLAST) was used for the analysis of sequences with closest culture sequence retrieved from the National Centre for Biotechnology Information (NCBI) database. RDP database was used to generate distance matrix using and the phylogenetic tree was constructed using MEGA5 software.

Development of consortium

Three isolates were grown separately in nutrient broth media containing 100 mg/L of Cr(VI). After 4 h of incubation period, 100 µL of *Serratia nematodiphila* culture broth was pour plated on nutrient agar plate supplemented with 100mg/L of Cr(VI), and a loop full of microbial culture of the other two isolated cultures were streaked on the same plate. The plate was observed after the incubation at 37 °C for 24 h. The absence of the zone of inhibition between the three isolated organisms showed the lack of competitive inhibition between the organisms. The synergistic effect of the organisms is vital for consortium development. Equal volume of 16h cultures of each organisms was inoculated in a sterilized nutrient broth and incubated at 35°C for 24

hours. Further Cr(VI) reduction studies were carried out with the developed consortium (CRC589) based on its maximum Cr(VI) reduction potential.

Chromium estimation

Cr(VI) was estimated using 1,5-diphenylcarbazide (DPC) method[21]. Fifty µL of DPCZ solution (0.6 g of 1,5- diphenylcarbazide diluted to a final volume of 1000 mL by the addition of 200 mL of 95% ethanol and 800 mL 3.6 N sulphuric acid) was added to 1 ml of appropriately diluted sample. DPCZ reacts with acidified chromate forming a colored complex with the maximum absorbance at 540 nm. Suspended microbial cells were removed by centrifugation.

Optimization of Cr(VI) reduction conditions

To achieve the maximum Cr(VI) reduction by the consortium, the environmental conditions such as Temperature and pH were varied and the optimum condition was observed. Inoculum size and inoculum age were the other important parameters optimized by single parameter optimization method. Temperature was varied (25, 30, 35, 40 and 45°C), when the other parameters were fixed (Inoculum size 2% and Inoculum age 18 and pH 7). Similarly pH was varied (4.0, 5.5, 7.0, 8.5 and 10) at temperature 30°C. Inoculum size (1, 2, 3, 4 and 5%) and Inoculum age (6, 12, 18, 24, 36 and 48h) also varied when the rest of the parameters were constant. Temperature 30°C, pH 7, inoculum age 18 h and inoculum size 2 % were constant when the other parameters were varied. Cr(VI) reduction percentage was calculated by the following equation,

$$\text{Percentage reduction of Cr(VI)} = \frac{C_0 - \mathrm{C}}{C_0} * 100\%$$

Where, C_0 is initial concentration of Cr(VI) and C is final concentration of Cr(VI). 100ml of M9 Minimal salt media supplemented with 25 mg/L of Cr(VI) was used for optimization studies.

Response Surface Methodology

The influencing parameters such as Temperature, pH and Carbon source concentration were optimized by using Response Surface Methodology (RSM). Among the many methods, Box-Behnken design (BBD) is a widely used design method for the optimization and validation. Design- Expert Software, Version 7.0 (Stat-Ease, Minneapolis, USA) was used to determine the total experimental runs (N). The number of experiments (N) needed for BBD is defined as

$$N = 2k\,(k - 1) + C_0$$

where k is the number of factors and C_0 is the number of central points.

The predicted response (Y) is a second order polynomial regression model equation, expressed as a function of independent variables,

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} (x_i)^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} x_i x_j$$

Where β_0, β_i; β_{ii}; β_{ij} are the regression coefficients.

Statistical analysis

The statistical software package Design-Expert 7.0 (StatEase, Minneapolis, MN) was used for regression analysis of experimental data. Analysis of variance (ANOVA) is used to estimate the statistical significance of interactions, residues and coefficients which provides an overall summary for the full model [19, 22]. Values of "Prob > F" less than 0.0500 indicate model terms are significant.

Studies in continuous flow system

The continuous removal of Cr(VI) from the media by the consortium CRC589 was studied in a Continuous Stirred Tank Reactor (CSTR) of 12 L working volume. The schematic diagram of the bioreactor assembly is shown in Fig. 1. CSTR consists of a cylindrical vessel of volume 16 L which was connected to media holding tank. Media was continuously fed from the media holding tank by using peristaltic pump which was used to maintain the flow rate of media. The outlet of the system was connected to effluent collection tank. The pH and Temperature were monitored with pH sensor and temperature sensor which were fixed on the top of the reactor. pH was adjusted by diluted acid and base. The reactor system had an impeller connected with the motor on the top. This provides the agitation for the reactor to distribute the media. Sterile air was supplied through the air filter since the process was aerobic. The process was operated in continuous mode at the working volume of 12 L, pH 7 and temperature 35 ºC with the inoculum size of 4%. The samples were periodically withdrawn and analyzed for the residual chromium concentration. System was operated at different dilution rates (0.01, 0.02 and 0.03 h^{-1}). The flow rates (2, 4 and 6 mL/min) were maintained with the peristatic pumps. The dilution rate is calculated by dividing the flow rate by the culture volume.

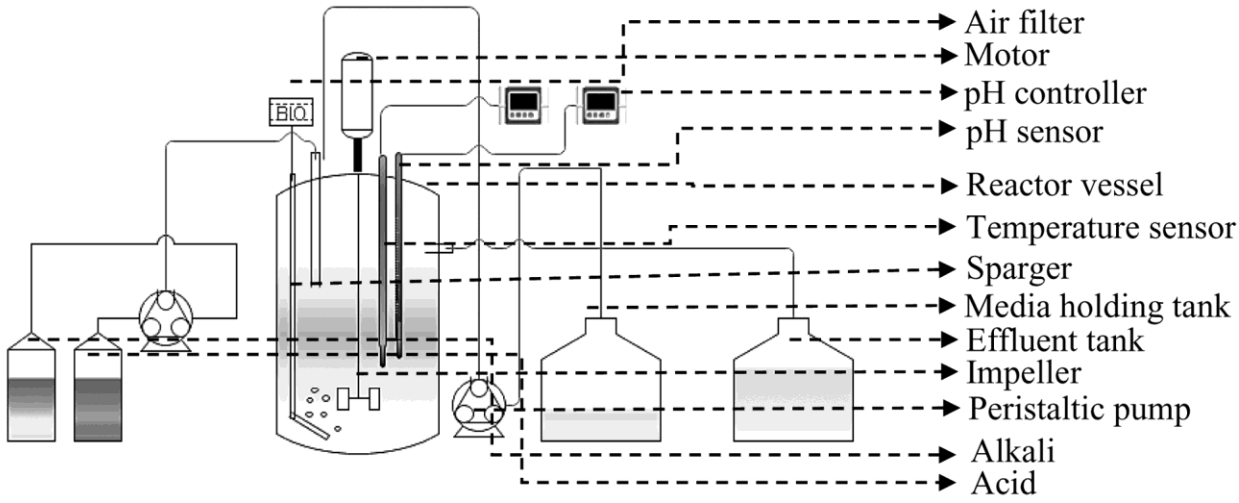

Fig. 1 — Schematic diagram of CSTR assembly.

Results and Discussion

Identification of Chromium resistant organisms

The isolated Chromium resistant organisms were identified using 16s rRNA sequencing. Three bacterial isolates along with the BLAST search attributes were shown in Table 1.

The phylogenetic trees were constructed using the MEGA5 tool. The phylogenetic trees shown in Fig. 2 represents the closest relatives of CRB5, CRB8 and with accession numbers and names. Based on 16s rDNA homology match , CRB5 showed the closest relation with *Serratia nematodiphila* and has not been reported for chromium reduction activty. Similarly, CRB8 showed the closest relative with *Bacillus cereus.* Cr(VI) reduction by *Bacillus cereus* was reported by other researchers[23,24]. NITRKLR1 showed the closest relative with *Bacillus sp.*, SDIP3 with 100% match.

Table 1 — BLAST search with query attributes at NCBI GenBank

	CRB 5	CRB8	NITRKL R1
Query ID	NC150319b	NC151119	lcl|Query_8285
Query length	1276bp	1349bp	1397 bp
Maximum score	1267	1347	2580
Total score	1276	1351	2580
Maximum identity	99%	99%	100%
Identified strain with accession number	*Serratia nematodiphila* NR_044385	*Bacillus cereus* MN326684	*Bacillus* sp., SDIP3 KU291425.1

Effect of temperature on Cr(VI) reduction

The effect of temperature on the reduction of Cr(VI) is shown in Fig. 3A. Cr(VI) reduction activity of the consortium CRC589 was found to be significant at 30-35ºC. The maximum reduction of Cr(VI) was achieved at 35ºC. Despite the organisms were surviving in wide range of temperature, the activity of Cr(VI) reduction was highly influenced by the temperature. At the lower temperature Cr(VI) reduction by the consortium was not significant, since the metabolic activity of the organisms was suppressed at lower temperature. The optimum temperature for the maximum reduction (57%) of Cr(VI) by *Bacillus cereus* was reported as 37ºC[23]. The maximum Cr(VI) reduction by *Serratia sp*. also reported as at 37 ºC[25]. The reduction percentage was enhanced by the development of consortium with synergistic organisms.

Effect of pH on Cr(VI) reduction

The activity of consortium was checked in different pH ranging from 4 to 10 and shown in Fig. 3B. Most of the organisms cannot perform metabolic activities below pH 4 and above pH 9 and believed to perform in the approximately neutral environment. The consortium CRC589 exhibited maximum chromium reduction of 92% at pH 7. The consortium showed significant activity at acidic and neutral pH. Other research works[23,26] also have similar observations and reported the maximum Cr(VI) reduction at neutral pH. When pH was higher than 8 or less than 5, the enzyme activity by the organism was affected which lead to reduction in Cr(VI) removal efficiency[27].The maximum Cr(VI) reduction by *Serratia* sp. was reported in 7-8 pH range[25]. The optimum pH for the Cr(VI) reduction by *Bacillus subtilis* is reported to be 5[28]. The presence of two bacillus strains in the consortium could favor the better activity in acidic pH over the alkaline pH.

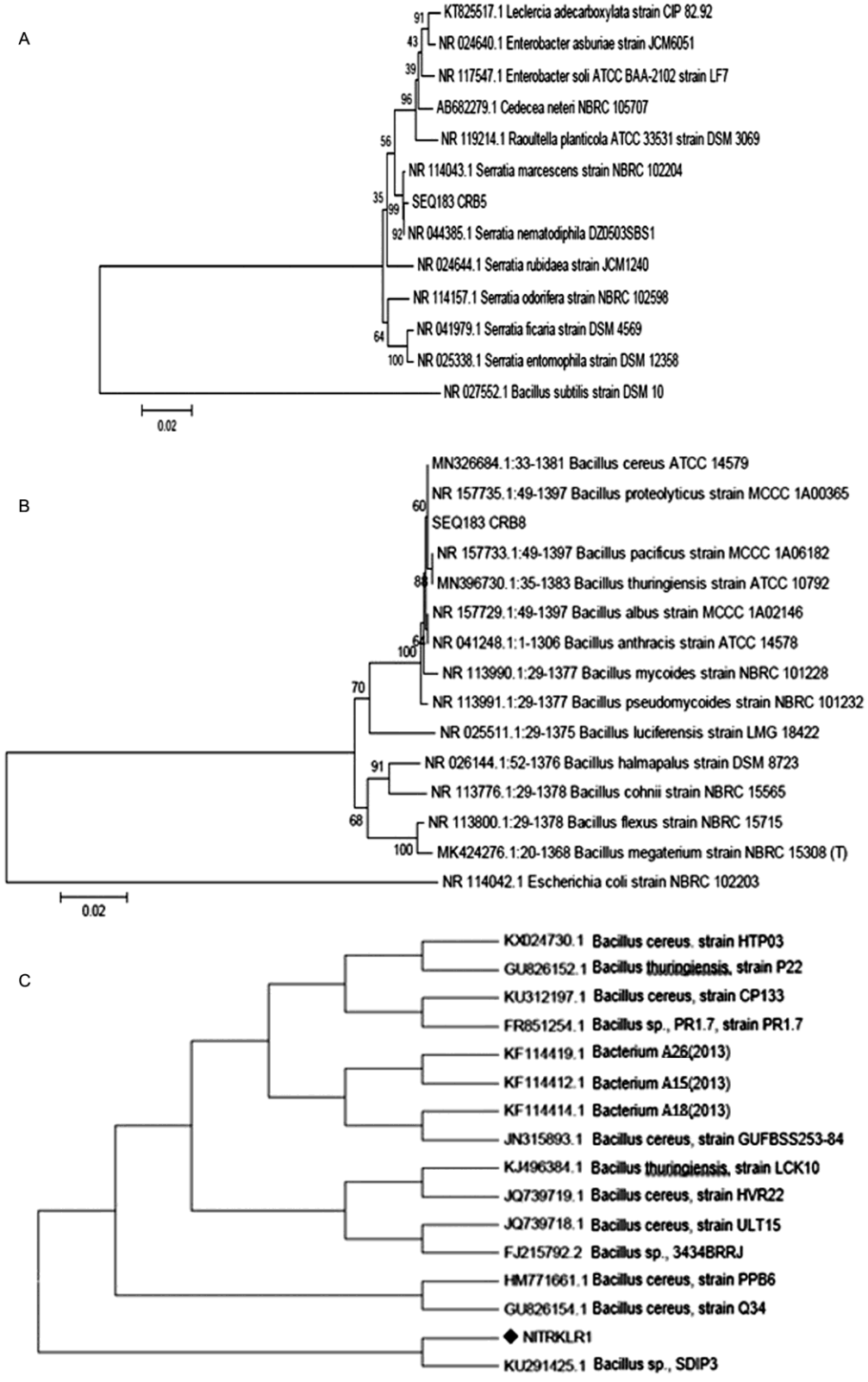

Fig. 2 — Phylogenetic trees showing the similarity of isolated organisms CRB5, CRB8, NITRKLR1 with (A) *Serratia nematodiphila*, (B) *Bacillus cereus* (C) *Bacillus* sp., SDIP3, respectively

Effect of inoculum size on Cr(VI) reduction

Inoculum size was varied from 1 to 5% and the response on chromium reduction was observed. Fig. 3C shows the percentage of Cr(VI) reduction increases with the increase of inoculums size up to a threshold level. After 4% of inoculums size, the chromium reduction was stagnant. The maximum percentage of chromium reduction was achieved by CRC589 at the inoculums size of 4%. At the lower inoculum size, the time for maximum Cr(VI) reduction was prolonged due to less number of active microbial cells. With increase in inoculum size the active cells also increased which is evident from Fig. 3C. It is also shown that at inoculum size beyond 4% reduction in Cr(VI) was not affected due to saturation of cells at definite concentration of Cr(VI). Similar effects of inoculum size on Cr(VI) reduction activity by *Bacillus subtilis* was reported by other researchers also[28].

Effect of inoculum age on Cr(VI) reduction

The effect of inoculum age on the reduction of chromium was observed by varying the inoculum age from 6 to 48 h as shown in Fig. 3D. The significant chromium reduction was achieved by the consortium at the inoculum age of 12-24 hours range due to the exponential phase of the organism was lying under this time range. The percentage of Cr(VI) reduction was reduced with the increase of inoculum age after 24 hours. After the inoculum age of 24 hours the inoculum would be in the stationary phase where the growth rate is equal to death rate. The number of organism alive in the inoculum after stationary phase is lesser than the inoculum in the log phase. Hence the efficiency decreases with the further increase of inoculum age. It has been reported that the maximum Cr(VI) reduction at the inoculum age of 24 h with *bacillus subtilis*[28].

Effect of carbon source on Cr(VI) reduction

Cellulose, Glucose, Starch and Sucrose were the carbon sources used for study and their effect on the chromium reduction by organism was shown in Fig. 3E. The amount of carbon source used for the experiments was 6 g/L. The maximum chromium reduction (95%) was achieved with the glucose which is the simple sugar used as carbon source. The other carbon sources such as starch and cellulose are more complex in nature than glucose. The utilisation of glucose was comparatively more for the organisms as compared to other carbon sources.

Statistical optimization of Parameters

Three independent variables namely temperature, pH and carbon source concentration were chosen as the significant parameters which influences the Cr(VI) reduction. Experiments were performed with different combinations of parameters to study the synergic effect of those factors. The 17 experimental runs including 12 trials and 5 mid points trials according to the Box-Behnken design were carried out to find a quadratic model for Cr(VI) reduction with the consortium developed from the isolated

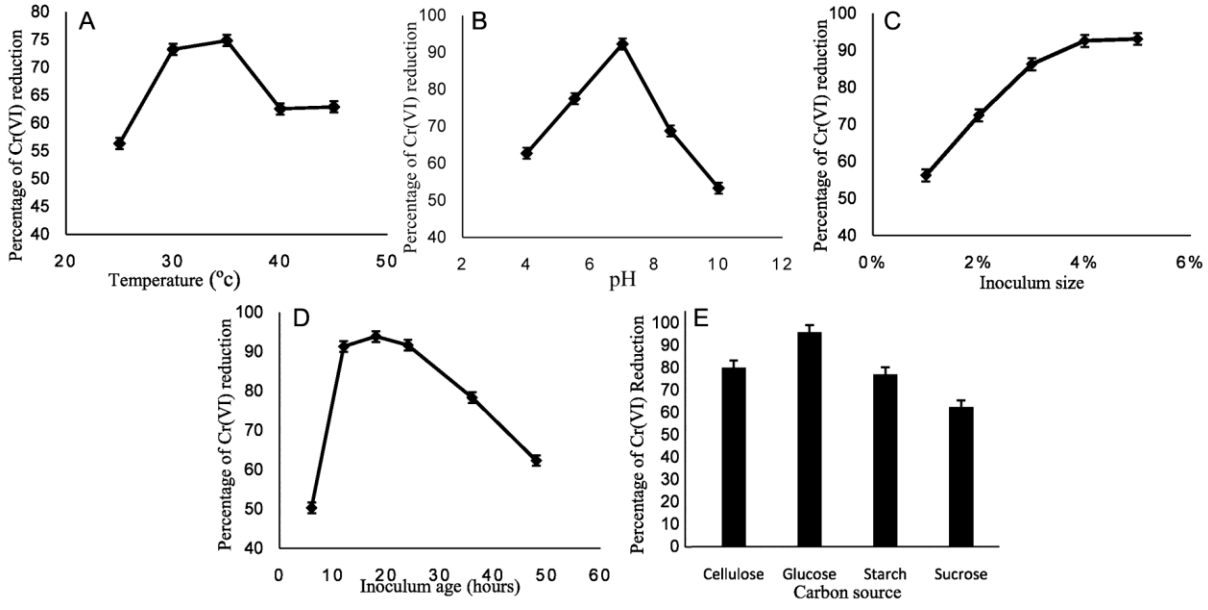

Fig. 3 — Effect of (A) temperature (B) pH (C) inoculum size (D) inoculum age (E) Carbon source on Cr(VI) reduction by consortium CRC589

organisms. The reported second order polynomial was fitted to the obtained data by using the multiple linear regressions for the determination of the optimum conditions which resulted in the maximum reduction of chromium. The effects of temperature, pH and carbon source concentration were evaluated with the use of response surface curves. Experimental design variables for Box-Behnken design for the consortium CRC589 was shown in Table 2. The observed and predicted response for the experimental runs were tabulated in Table 3.

The predicted response, Y was determined by the following equation,

$$Y = +88.94 + 0.97A - 4.93B + 3.35C - 1.24AB - 0.53AC + 1.04BC - 8.56A^2 - 8.62B^2 - 9.74C^2$$

where Y is the predicted response and A, B and C are the independent variables viz temperature (°C), pH and Carbon source concentration (g/ L) respectively.

Analysis of variance (ANOVA) shown in table 4 expresses the significance of the fit of the second order polynomial for Cr(VI) reduction percentage. A p-value lesser than 0.05 specifies that the model is significant. The insignificance of the model is indicated by the p-value above 0.1000. From the ANOVA table 4, the model terms B, C, $A^{2,}$ B^2 and C^2 were observed to be statistically significant. However, the model terms A, AB, BC and AC were statistically insignificant. The interaction effects between the parameters were not significant. pH and carbon source concentrations were significantly affecting the Cr(VI) reduction.

Perturbation plot for percentage of Cr(VI) reduction was shown in Fig. 4. The curvature of the plot revealed the significance of the individual parameter effect on the percentage of Cr(VI)

Table 2 — Experimental design variables for Box-Behnken design for the microbial consortium CRC589

Independent variables	Symbols	Actual and coded levels		
Coded levels		Low (-1)	Middle (0)	High (+1)
Temperature (ºC)	A	25	35.00	45
pH	B	5	7.50	10
Carbon source Conc. (g/L)	C	5	12.50	20

Table 3 — Experimental and predicted responses of Box Behnken design for Cr(VI) reduction using microbial consortium CRC589

Run	Response: Independent variables			% of Cr(VI) reduction		Residual
	Temp. (°C)	pH	Carbon source conc. (g/L)	Observed	Predicted	
1	45	10	12.5	68.47	66.543375	1.926625
2	35	5	5	73.675	73.191875	0.483125
3	25	5	12.5	72.545	74.471625	–1.926625
4	45	7.5	5	69.756	68.78325	0.97275
5	25	7.5	5	67.223	65.7795	1.4435
6	45	7.5	20	72.98	74.4235	–1.4435
7	35	10	20	69.545	70.028125	–0.483125
8	45	5	12.5	77.442	78.897875	–1.455875
9	35	5	20	80.725	77.825625	2.899375
10	35	7.5	12.5	87.324	88.9388	–1.6148
11	25	10	12.5	68.546	67.090125	1.455875
12	25	7.5	20	72.575	73.54775	–-0.97275
13	35	7.5	12.5	91.215	88.9388	2.2762
14	35	7.5	12.5	89.362	88.9388	0.4232
15	35	7.5	12.5	87.563	88.9388	–1.3758
16	35	7.5	12.5	89.23	88.9388	0.2912
17	35	10	5	58.354	61.253375	–2.899375

Table 4 — ANOVA for response surface quadratic model of percentage of Cr(VI) reduction using microbial consortium CRC589

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	S/NS
Model	1444.79	9	160.53	25.00	0.0002	S
A-Temperature	7.53	1	7.53	1.17	0.3149	
B-pH	194.75	1	194.75	30.33	0.0009	
C-Carbon source	89.89	1	89.89	14.00	0.0072	
AB	6.18	1	6.18	0.96	0.3592	
AC	1.13	1	1.13	0.18	0.6872	
BC	4.29	1	4.29	0.67	0.4408	
A^2	308.86	1	308.86	48.10	0.0002	
B^2	313.11	1	313.11	48.76	0.0002	
C^2	399.50	1	399.50	62.22	<0.0001	
Residual	44.95	7	6.42			
Lack of Fit	35.00	3	11.67	4.69	0.0847	NS
Pure Error	9.95	4	2.49			
Cor Total	1489.73	16				

[S, Significant; NS, Nono-significant]

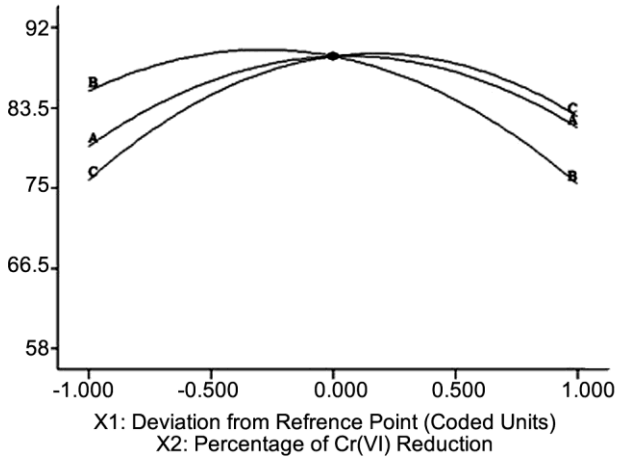

Fig. 4 — Perturbation plot for percentage of Cr(VI) reduction (A) Temperature (°C); (B) pH; and (C) Carbon source concentration (g/L)

reduction. It is evident from the figures that the Cr(VI) reduction was significantly high in temperature range of 30 °C to 35 °C. Further increase in temperature lead to decrease in chromium reduction efficiency. This shows that the organisms in the consortium are like to be mesophilic and hence grow efficiently at normal atmospheric temperature. In case of temperature, maximum reduction of chromium was achieved in 35ºC. Many researchers reported the similar range of temperature was optimum Cr(VI) reduction[23,25] due to the favorability for enzymatic activity of organisms. The organisms were showing significant Cr(VI) reduction activity in acidic and neutral pH. Alkaline pH greatly affects the chromium reduction ability of the organisms. *Bacillus sp*. which were reported to favor the acidic pH over alkaline pH for Cr(VI) reduction[28]. However all three organisms which were the part of consortium were showing the highest Cr(VI) reduction activity at neutral pH similar to the many of the reports[28-30]. The lowest Cr(VI) reduction was observed at highly alkaline condition. The activity of enzymes responsible for Cr(VI) reduction by the organisms were not active at pH over 8 and pH below 5[27].

Cr(VI) reduction activity increased with the increase of concentration of carbon source. This shows the necessity of nutrient source over the inhibitory effect of Cr(VI) especially carbon source. Both Statistical and experimental optimization were showing maximum Cr(VI) reduction at temperature of 35ºC and pH 7.

The predicted versus actual plot was shown in Fig. 5. The data obtained from the experimental runs is the actual value whereas the predicted value is evaluated from the prediction equation by the software. From the plot, it was observed that the most of the data points were distributed near to the straight line which evidently suggested a significant relationship between the experimental and predicted values of the responses.

Continuous stirred tank reactor study

The reduction in residual Cr(VI) concentration in the reactor vessel with time at initial Cr(VI) concentration of 25mg/L at three different dilution rates 0.01 h^{-1}, 0.02 h^{-1} and 0.03 h^{-1} was shown in Fig. 6. The different dilution rates used for the reactor study were 0.01 h^{-1}, 0.02 $^{-1}$ and 0.03 h^{-1} which were lesser than the specific growth rate of consortium to avoid the cell wash out. The reactor was operated with the optimized conditions in batch study.

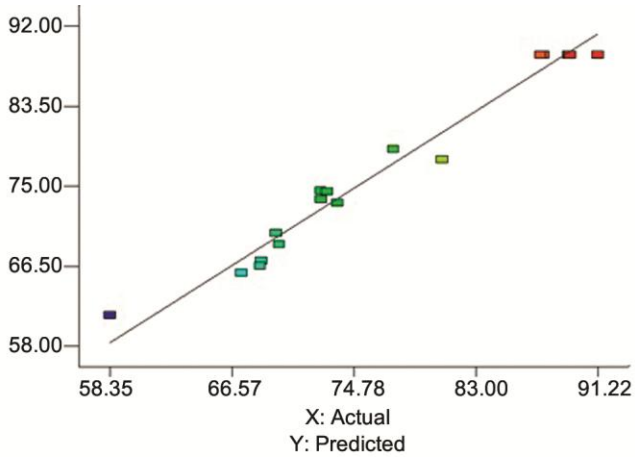

Fig. 5 — Predicted response *vs*. actual response for percentage of Cr(VI) reduction

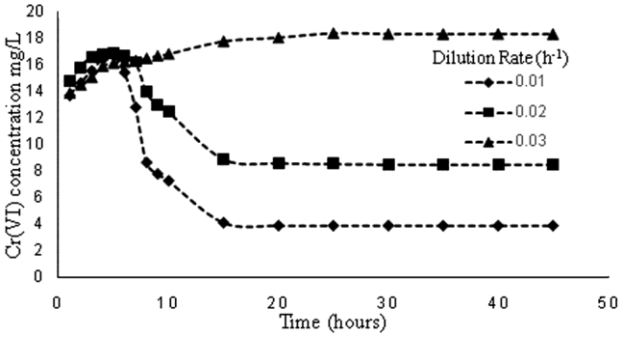

Fig. 6 — Change of residual Cr(VI) concentration in CSTR with time at different dilution rates at initial Cr(VI) concentration of 25 mg/L in M9 minimal salt media

Dilution rate 0.03 h^{-1} was nearly equal to the maximum specific growth rate of the consortium CRC589 at the chromium concentration of 25 mg/L. hence at the dilution rate of 0.03 h^{-1} there was no significant reduction of chromium was observed. The least dilution rate of 0.01 h^{-1} resulted in the maximal reduction of Cr(VI). 85% reduction of chromium achieved from the initial chromium concentration of 25 mg/L at the dilution rate of 0.01 h^{-1}.

Conclusion

Using the consortium developed with the indigenous bacterial strains (*Serratia nematodiphila*, *Bacillus cereus* and *Bacillus sp*., SDIP3) isolated from chromite mines, the maximum percentage of Cr(VI) reduction (92.6%) was achieved at the optimum temperature of 35°C and pH 7 at 25 mg/L of Cr(VI). Statistical optimisation was done by RSM using Box Behnken Design. Polynomial equation was fitted and also its predicted values were verified by carrying out actual experiments. The acclimatization and

consortium development enhanced the activity of microorganisms. Under the optimised conditions Cr(VI) was treated with microbial consortium in Continuous stirred tank reactor at different dilution rate. The maximum reduction of Cr(VI) (85%) achieved at the dilution rate of 0.01 h^{-1}. The potential of the organism *Serratia nematodiphila* in the consortium as well as an individual organism can be explored more in future works. The optimised parameters can be used to scale up the Cr(VI) reduction process. In order to reduce the process cost, further organic wastes can be explored as an alternative nutrient source.

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Conflict of interest

Authors declare no competing interests.

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