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Use of Potential Bacterial Strains to Remove Hexavalent Chromium from Aqueous Medium and Sediments of the Periyar River

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ABSTRACT

Chromium is one of the most abundant metals in the earth's crust mined as chromites. Industries that widely use compounds containing chromium as Cr(III) in the tanning process generally release effluents with high chromium levels into natural water resources, frequently without appropriate effluent treatment. Though physical and chemical methods of chromium removal are adopted, the processes are expensive, use a lot of energy and generate secondary toxic waste. The removal of chromium by biological resources on the other hand is more environmentally friendly, less expensive, and lacks the release of secondary pollutants. The present study is aimed towards the assessment of the Cr(VI) reduction capability of two bacterial strains, *Alcaligenes* sp. (S11) and *Listeria* sp. (S18) used as a consortium. These bacterial strains were isolated from the Periyar river of Kerala, India. Batch mode studies were carried out by live bacterial cells and an integrated study was carried out to test the bacterial strains on chromium-contaminated water and sediments through bioreactor and column study respectively under optimized conditions. The phytotoxicity of the treated water was studied on black gram. In the bioreactor study, the maximum Cr(VI) reduction capacity of bacterial consortium (S11 and S18) was found to be 97%. The successive experiment for the toxicity of treated water on the black gram indicated that there was no toxicity in the growth parameters of the plant.

Key words: Bacterial consortia, Reduction, Biotransformation, Growth rate, phytotoxicity

INTRODUCTION

Chromium is the earth's seventh most abundant element and the 21st most abundant metal in the earth's crust mined as chromites (Cervantes and Campos-Garcia 2006). The tannery industry and chrome-plating industries are the most polluting industrial sectors and every tannery industry uses large amounts of chemicals in the process of leather manufacturing. The metal alloys and leather industries that widely use compounds containing chromium Cr(III) in the tanning process generally release effluents with high chromium levels into natural water resources, frequently without any appropriate effluent treatment, resulting in anthropogenic contamination (Cheung and Gu 2007). Among the various heavy metals, chromium is one of the main pollutants resulting from various industrial processes such as leather tanneries, electroplating, mining, textile, metal processing, fertilizer, dyes and pigment manufacturing industries (Sathvika et al. 2016). Worldwide, the estimated chromium contamination is believed to be from industries that are responsible for dumping 300-400 million tons of heavy metals, solvents, toxic sludge, and other wastes into waters each year (UNEP 2010). Cr(VI) is one of the more toxic metals causing various effects in humans, including nasal and skin irritation, eardrum perforation and lung carcinoma. Cr(III) is harmless and insoluble and is essential for the human diet (Narayan et al. 2016, Nduka et al. 2019).

Conventional management option for chromium includes physical techniques (reverse osmosis, soil washing, membrane separation, ion exchange and adsorption) that have been used to remove the

chromium from the environment. Likewise, some chemical methods have been used to remove the chromium from an aqueous medium which include, Graphene Coated Iron Oxide (GCIO) nanoparticles (Khare et al. 2018), maghemite nanoparticles (Seraj et al. 2018), iron oxide/carbon (Neto et al. 2019), mackinawite (FeS)-coated sand (Park et al. 2018). These methods have more disadvantages such as high cost and energy and they generate secondary toxic waste (Chen et al. 2012). The removal of chromium by biological resources is also a promising method and a better choice in comparison to the physicochemical method because it is environmental friendly, less expensive and lacks the release of secondary pollutants. Microbial bioremediation is cost-effective and a beneficial bioresource for removing Cr along with other hazardous contaminants from the tannery and other industrial wastes (Garg et al. 2012, Putro et al. 2017). Certain microorganisms play a vital role in soil, water and air. Previously, several researchers have successfully isolated a variety of potential microorganisms with Cr(VI) reducing efficiency, with some effective bacterial and fungal species such as *Escherichia coli, Shewanella oneidensis, Bacillus firmus, Aspergillus niger* and *Pleurotus ostreatus* (Carol et al. 2012). The present study is aimed toward the assessment of the Cr(VI) reduction capability of the bacterial strains *Alcaligenes* sp. and *Listeria* sp. The batch mode studies were carried out by live bacterial cells. An integrated study was carried out to test the bacterial strains on chromium-contaminated water and sediments through bioreactor and column study respectively under optimized conditions.

MATERIALS AND METHODS

Bacterial strains

The bacterial strains *Alcaligenes* sp. (S11) and *Listeria* sp. (S18) as a consortium utilized in this study were isolated from the Periyar river of Kerala, India. It was sub-cultured in the laboratory using a nutrient agar medium. The bacterial strains were maintained in the same medium as described by Bergey's manual of determinative bacteriology, having the composition of sodium chloride (5.0 g/L), peptone (5.0g/L), beef extract (3.0 g/L), yeast extract (3 g/L) and agar (20.0 g/L) in 1.0 L distilled water. The bacterial strain was sub-cultured every week and preserved by using glycerol solution having a concentration of 20 % (v/v), followed by storage at 4°C for further studies.

The bacterial strain was cultivated in a mineral salt medium (pH 8 ± 0.2) containing (g/l) 1.17 g of NaCl, 0.30 g of KCl, 0.15 g of NH_4Cl, 0.41 g of $MgCl_2.6H_2O$, 0.11 g of $CaCl_2$, 0.20 g of KH_2PO_4, 0.07 g of Na_2SO_4, 2 g of $NaHCO_3$ and 0.4 g of yeast extract.

Effect of Cr(VI) on bacterial growth

The effect of various concentration of Cr(VI) on bacterial growth (S11 and S18) were investigated, in which, the S11 and S18 were grown in a rotary shaker (OrbiTech LETT) at 120 rpm in nutrient broth medium supplemented with Cr(VI) range from 10 to 30mg/L at pH 7.0 and temperature 37°C (Marzan et al. 2017). At every 4 hours time interval, 5 ml samples were collected from the conical flasks, followed by the collected samples being centrifuged at 3000 rpm for 10 minutes. The pellets were dissolved with sterile distilled water and the culture growth rate was measured by UV-spectrophotometer at 600 nm (Cyber lab-UV-100) (Thacker et al. 2007).

Effect of various carbon sources, pH and temperature on the reduction of hexavalent chromium in synthetic medium

Effect of carbon sources on chromium reduction

The effect of various carbon sources on the reduction of hexavalent chromium (10mg/l) was investigated by using bacterial consortium (S11 and S18). In 250 ml conical flasks, 100 ml mineral salt medium was prepared with various concentrations of carbon sources (0.5%, 1% and 1.5%) and amended with 10 mg/l constant hexavalent chromium. All the flasks were sterilized. After the sterilization individually, 1% of the bacterial consortium (S11 and S18) was transferred aseptically (Sundari 2017). All the conical flasks were then kept in a shaker at 37°C at 125 rpm for 10 days. At every 24 hours time interval, 5 ml samples were collected from all conical flasks, following which the collected samples were centrifuged at 3000 rpm for 10 minutes. The supernatant was collected from the centrifuge tube and chromium was estimated using the DPC method in UV-VIS spectrophotometer at 540 nm (Cyber lab-

UV-100). Simultaneously the culture growth rate was also measured at 600 nm.

Effect of various pH conditions on hexavalent chromium reduction

The effect of various pH conditions on the reduction of hexavalent chromium was investigated by using bacterial consortium (S11 and S18), in which, in 250 ml conical flasks, 100 ml mineral salt medium was prepared with optimized carbon source 0.5% starch, and amended with 10 mg/L constant hexavalent chromium and maintained at pH 5, 6, 7, 8 and 9 accordingly. All flasks were sterilized. After the sterilization, 1% of bacterial consortium (S11 and S18) were transferred to individual conical flasks aseptically (Essahale et al. 2012). The conical flasks were then placed in the shaker under an optimized temperature of 35°C at 125 rpm for 10 days. At every 24 hours time interval, 5 ml samples were collected from all the conical flasks. The collected samples were then centrifuged at 3000 rpm for 10 minutes. The supernatant was collected from the centrifuge tube and chromium was estimated by the DPC method in UV-VIS spectrophotometer at 540 nm. Simultaneously the culture growth rate was also measured at 600 nm.

Effect of various temperatures on hexavalent chromium reduction

The effect of various temperatures on the reduction of hexavalent chromium was investigated by using a bacterial consortium (S11 and S18). In 250 ml conical flasks, 100 ml mineral salt medium was prepared with optimized carbon source 0.5% starch and amended with 10 mg/l constant hexavalent chromium. All flasks were sterilized. After the sterilization, 1% of bacterial consortium (S11 and S18) was transferred to an individual conical flask aseptically. The conical flasks were then placed in the shaker under temperatures of 25, 30, 35, 40 and 45°C at 125 rpm for 10 days (Verma et al. 2013). At every 24 hours time interval, 5 ml samples were collected from all the conical flasks. The collected samples were then centrifuged at 3000 rpm for 10 minutes. The supernatant was collected from the centrifuge tube and chromium was estimated by the DPC method in UV-VIS Spectrophotometer at 540 nm. Simultaneously the culture growth rate was measured at 600nm.

Analytical method

The hexavalent chromium [Cr(VI)] was determined with UV-VIS spectrophotometrically using S-diphenylcarbazide (DPC) method (Pattanapipitpaisal et al. 2001). About 0.025 g of S-diphenylcarbazide was dissolved in 9.67 ml acetone and 330 ml of 3 M H_2SO_4. 1 ml of the reaction mixture was containing 200 µl of the sample, 400 µl of 20 mM MOPS-NaOH, buffer (pH 7.0), 33 µl of 3M H_2SO_4, 40 µl of 0.25% (w/v) S-diphenylcarbazide, and 327 µl of distilled water. The absorbance was measured at 540 nm and all assays were carried out in triplicates and the mean values were recorded.

Bioreduction of chromium in the aqueous medium through lab scale bioreactor study

The treatment of the aqueous medium with Cr^{6+} (10 mg/L) method was planned and the setup was based on the pilot scale water treatment plant (Ayyasamy et al. 2008). The aqueous medium with Cr^{6+} was taken in a sterile container and was taken to the laboratory and analyzed for chromium concentrations (APHA 2005). The lab scale setup consists of a reactor tank with 10 liter capacities made up of tarson. The reactor tank and settling tanks were fitted with mechanical stirrers. An artificial aerator was connected to both the bioreactors for aeration purposes. Aqueous medium with 10 mg/l of hexavalent chromium (Cr^{6+}) was subjected to primary (bioprocess) treatment inoculated with the bacterial consortium (S11 and S18). At every 24 hours time interval, 5 ml samples were collected from both the bioreactors, following which the collected samples were centrifuged at 3000 rpm for 10 minutes. The supernatant was collected from the centrifuge tube and chromium was estimated by DPC method in UV-VIS spectrophotometer at 540 nm. Simultaneously the culture growth rate was measured at 600 nm.

Reactor 1 (R1): Unsterile mineral salt medium + 0.5% starch + 1% Inoculum (S11 and S18)

Reactor 2 (R2): Sterile mineral salt medium + 0.5% starch + 1% Inoculum (S11 and S18)

Removal of Cr^{6+} from Periyar river sediments using different treatment columns through biotransformation process

This study was a modified setup of bio-leaching of a heavy metal process as reported by Ayyasamy et al. (2009). The removal of Cr^{6+} (10mg/L) through the transformations from sediments was examined using

fixed bed columns. Core columns approximately 45 cm in length and 4 cm in diameter were used in this study. Before its use, each column was sterilized 4–5 times with absolute alcohol (99.9%). The sediments collected from the Periyar river were synthetically contaminated with Cr^{6+} (10mg/L) before packing them in the entire column. Approximately 5 kg of sediment was packed in each column under aseptic conditions and closed tightly with holed caps. Each hole was connected to silicon tubes. The tube from the bottom of the collection vessel was connected to the reservoir containing 500 ml of synthetic water while the tube from the top of the column was inserted into the same collection vessel. Approximately 250 ml/day of synthetic water was prepared in a 1 L Erlenmeyer flask. The synthetic water used in the column study consisted of the following ingredients:

Column Treat 1: 250ml water + 1% glucose + 1% containing 10 x 10^7 CFU/ml of S11 inoculum

Column Treat 2: 250ml water + 1% glucose + 1% containing 10 x 10^7 CFU/ml of S18 inoculum

Column Treat 3: 250ml water + 1% glucose + 1% containing 10 x 10^7 CFU/ml of bacterial consortium (S11 + S18)

Column Treat 4: 250ml water + 1% glucose + 1% containing 10 x 10^7 CFU/ml of MTCC 447

To compare the efficiency of bacterial strains on Cr(VI) removal, a reference strain MTCC 447 was used in the study of treatment 4. A Cr(VI) reducing *Lactobacillus acidophilus* (MTCC 447) reported by Mishra et al. (2012) was acquired from CSIR-IMTECH, Chandigarh, India and maintained in the laboratory. For the above-said treatment, synthetic water containing 1% glucose and 1% potential bacterial strains was prepared. After preparing the synthetic water, the Erlenmeyer flasks were kept on the platform of a magnetic stirrer to distribute the bacterial inoculum and nutrients consistently. The water was passed continuously through the soil column (upwards in the column) for up to 30 days using a peristaltic pump at a flow rate of 10 ml/h. Every 24 h, the effluents were collected from each column and the amount of Cr^{6+} and Cr^{3+} were analyzed by standard methods using UV-VIS spectrophotometer. In the end, the column set-ups were dismantled and treated sediment samples were carefully collected. The collected sediment samples were then subjected to microwave oven digestion to find out the presence of total chromium.

Statistical analysis

All experiments were conducted in triplicates, and the rate of Cr(VI) reduction from the aqueous medium was calculated with error bars (Steel et al. 1992). Data were statistically analyzed using the statistical package within Microsoft® Excel (Version 2010).

RESULTS AND DISCUSSION

Effect of Cr(VI) on bacterial growth rate

The effect of varying concentrations of Cr(VI) (10 and 30 mg/L) on the growth of bacterial consortium (S11 and S18) are studied and presented in Figure 1**.** The relationship between cell growth and varying Cr(VI) concentration was studied at 24 h of incubation. The rate of bacterial growth was very significant in nutrient broth media without Cr(VI) when compared to the nutrient broth medium containing Cr(VI) at 10 and 30 mg/L. The medium with 10 mg/L of Cr(VI) showed a small effect on the growth of bacterial cells. Further, the increasing concentrations of Cr(VI) from 10 to 30 mg/L exhibited a negative influence on bacterial growth. Previous studies have reported that Cr(VI) concentration at 10 and 20 mg/L has a small impact on the growth of *Bacillus* sp., but in the concentration of 100 mg/L of Cr(VI), it was found to greatly inhibit the bacterial growth (Liu et al. 2006). The existing

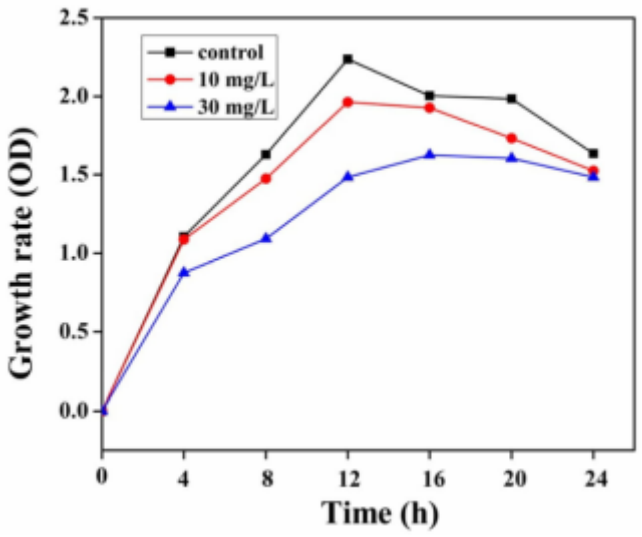

Figure 1. Effect of Cr(VI) on bacterial growth rate

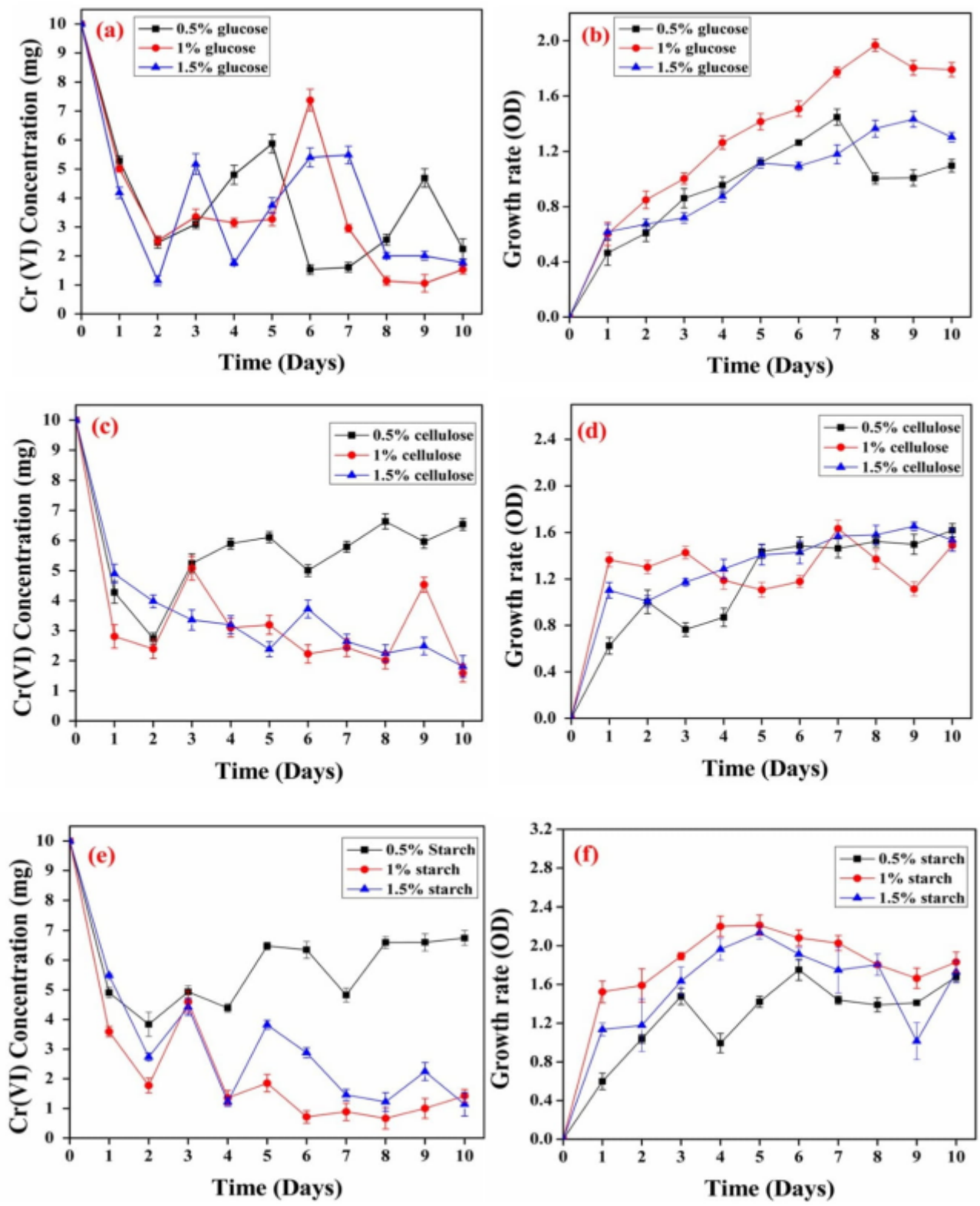

Figure 2. Effect of various carbon sources on the reduction of hexavalent chromium in synthetic medium

study reported that the growth rate of *Escherichia coli* ATCC 33456 was decreased and the lag period increased with the increase of Cr(VI) concentrations (Bae et al. 2000).

Effect of various concentrations of glucose

The effect of various concentrations of glucose (0.5, 1 and 1.5%) on the reduction of hexavalent chromium Cr(VI) by the bacterial consortium (S11 and S18) are presented in Fig. 2a. By using 0.5% of glucose,

there was no significant chromium reduction observed from 1st to 5th day of the experiment. After the 5th day of the experiment, there was a slight significant chromium reduction (82%) observed. By using 1% of glucose, the maximum chromium reduction (88%) was observed from the 8th day to the 10th day of the experiment. The previous study reported that glucose was chosen as the most appropriate electron donor as the glucose could be easily oxidized (Murugavelh et al. 2013). And it has been previously reported that glucose can act as the correct carbon source and electron donor for Cr(VI) reduction by bacteria (Das et al. 2013). By using 1.5% of glucose, 80% chromium reduction was observed. Overall, in these different concentrations of glucose optimization, 1% of glucose achieved very significant chromium reduction. Previously many researchers reported that glucose is a known electron donor for Cr(VI) reduction by several bacterial strains (Zakaria et al. 2007). Figure 2b shows the growth rate of bacterial consortium (S11 and S18) under various concentrations of glucose in mineral salts medium containing 10 mg of hexavalent chromium. In this study by using 0.5% of glucose, there was no significant growth observed from the 1st day to the 10th day of the experiment and it does not influence the hexavalent chromium reduction. But by using 1 and 1.5% of glucose, significant chromium reduction was observed, in which both 1 and 1.5% of glucose influenced the hexavalent chromium reduction as well as the growth rate of bacterial consortium (S11 and S18).

Effect of various concentrations of cellulose

The effect of various concentrations of cellulose (0.5, 1 and 1.5%) on the reduction of hexavalent chromium Cr(VI) by the bacterial consortium (S11 and S18) was studied and the results are given in Figure 2c. In this study, by using 0.5% of cellulose, there was very poor hexavalent chromium reduction observed. By using 1% of cellulose, 77% chromium reduction was observed from the 6th to the 8th day of the experiment. Using 1.5% of cellulose, there was 75% of hexavalent chromium reduction achieved from the 5th day to the 10th day of the experiment. Fig. 2d shows the growth rate of bacterial consortium (S11 and S18) under various concentrations of cellulose in mineral salts medium containing 10 mg of hexavalent chromium. In this study by using 0.5% of glucose, there was better significant growth observed from the 1st day to the 10th day of the experiment and it also contributed to the poor influence of hexavalent chromium reduction. But by using 1 and 1.5% of cellulose, significant chromium reduction was observed, in which both 1 and 1.5% of glucose influenced the hexavalent chromium reduction as well as the growth rate of bacterial consortium (S11 and S18). One of the studies reported shows that a variety of electron donors are responsible for Cr(VI) reduction using *Ganoderm lucidum* (Krishna et al. 2005).

Effect of various concentrations of starch

The effect of various concentrations of starch (0.5, 1 and 1.5%) on the reduction of hexavalent chromium Cr(VI) by the bacterial consortium (S11 and S18) is presented in Figure 2e. In this study, by using 0.5% starch, there was considerable hexavalent chromium reduction observed in the first two days, after which from the 3rd day of the experiment, the chromium reduction capacity was lost. Using 1% of starch, 90% chromium reduction was observed from the 6th to the 9th day of the experiment. Using 1.5% of starch, there was 79% chromium reduction achieved from the 7th to the 8th day of the experiment. The electron donors of carbon sources influenced chromium reduction by using *Brevibacterium casei* (Das and Mishra 2010). Figure 2f shows the growth rate of bacterial consortium (S11 and S18) under various concentrations of starch in mineral salts medium containing 10 mg of hexavalent chromium. In this study, the use of 1% starch showed the maximum level of chromium reduction and growth rate of bacterial consortium (S11 and S18) when compared to 0.5 and 1.5% of starch.

Effect of pH

The effect of various pH conditions on the reduction of hexavalent chromium Cr (VI) reduction by the bacterial consortium (S11 and S18) is presented in Figure 3a. The pH affects the bioreduction of hexavalent chromium Cr(VI). In the present study, the effects of incubation time on hexavalent chromium Cr(VI) reduction at different pH levels of 5, 6, 7, 8 and 9 were studied. (The operation conditions; 10 mg of Cr (VI)/100 ml medium,

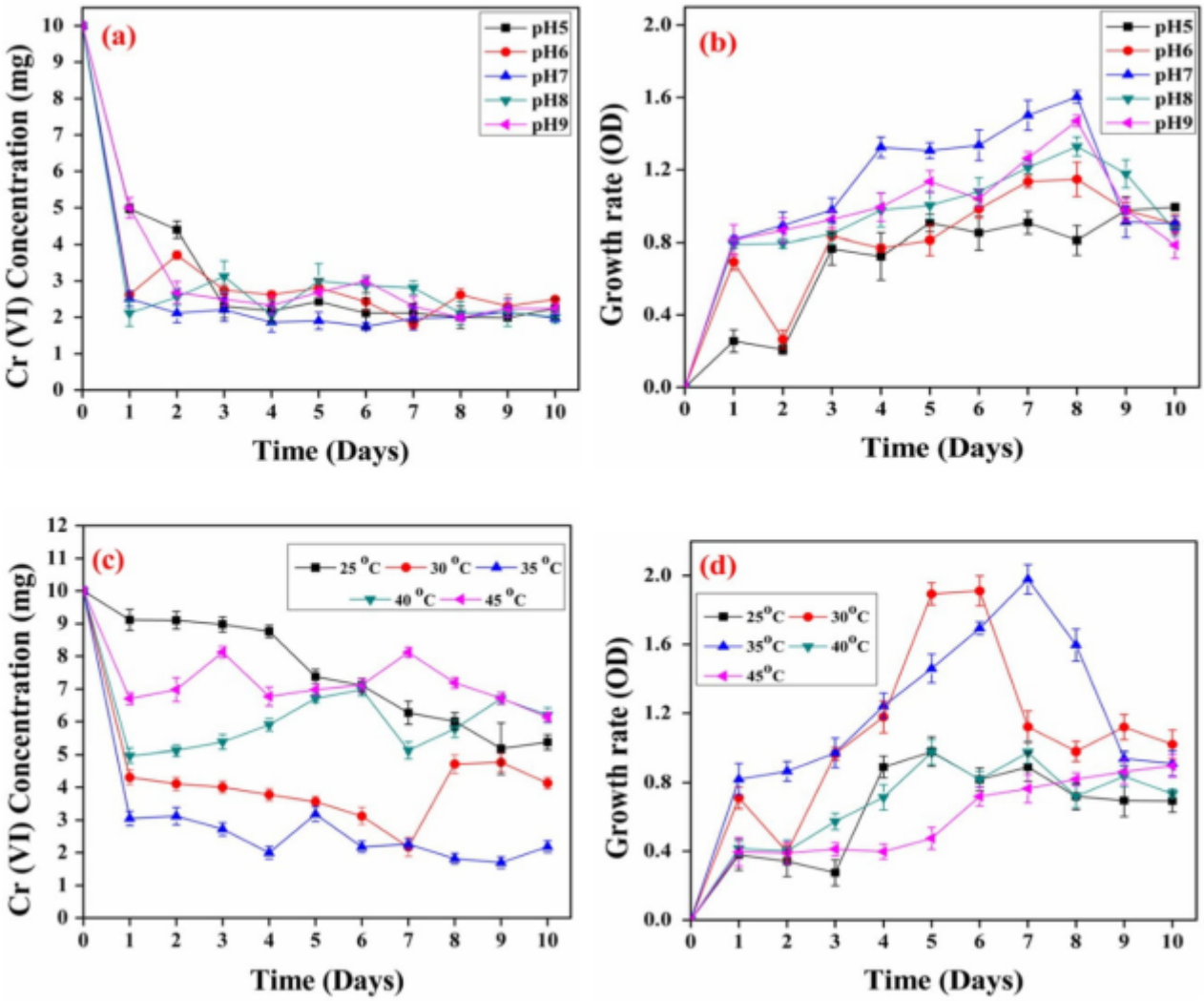

Figure 3. Effect of pH and temperatures on Cr(VI) reduction of hexavalent chromium in synthetic medium

temperature 37°C and 1ml of one OD inoculums). pH from 5 to 9 was observed to significantly reduce the hexavalent chromium Cr(VI) from the aqueous solution, with the maximum hexavalent chromium reduction observed at pH7. A similar result was observed in another study with the maximum removal of chromium (88%) by PCP3 strain at pH 7.0 (Srivastava et al. 2007). So further studies were carried out at pH7. The decreased chromium reduction at low pH levels might be due to the relation between bio-accumulation and dissociation of functional groups (Arica et al. 2004). The growth rate of bacterial consortium (S11 and S18) under various pH conditions was very significant as presented in Figure 3b. All pH parameters were found to significantly influence the growth rate.

Effect of various temperatures

Temperature is one of the chief factors for bacterial growth and microbial Cr(VI) reduction. The effect of various temperatures on the reduction of hexavalent chromium Cr(VI) by the bacterial consortium (S11 and S18) is presented in Figure 3c. Temperature is an important factor that affects microbial Cr(VI) reduction. In the present study, the effect of incubation time on hexavalent chromium reduction by the bacterial consortium (S11 and S18) at different temperatures (25, 30, 35, 40 and 45°C) was studied at the operation conditions; 10 mg of Cr (VI)/100 ml medium, pH at 7.0 and 1ml of one OD inoculum. Maximum chromium reduction (75%) was observed at 35°C, followed by 30°C (62%). Previous studies also found that *Bacillus KCH3* growth was encouraged at a temperature of 30°C and inhibited

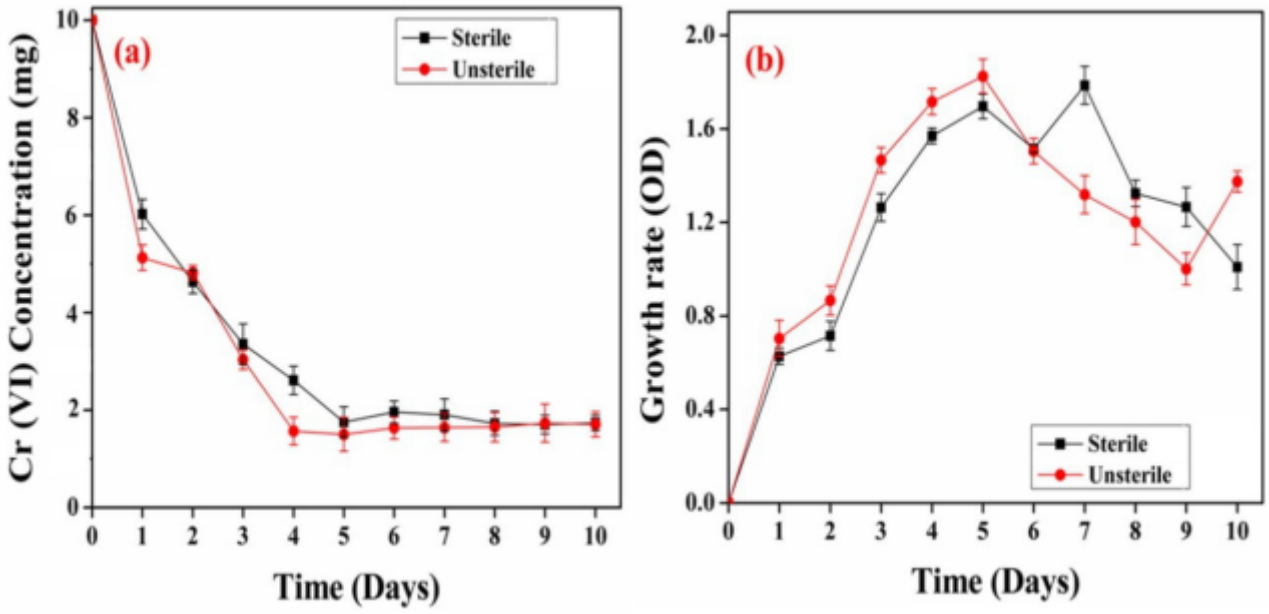

Figure 4. Bioreduction of chromium contaminated aqueous medium through lab scale bioreactor

at a temperature below 15°C and over 40°C (Sarangi et al. 2008). The significant bacterial growth rate was observed at 30 and 35°C when compared to other temperatures (Fig. 3d). A similar result was observed with the maximum removal of chromium (85.87%) by *Brevibacillus* sp at 35°C (Chatterjee et al. 2018).

Bioreduction of chromium aqueous medium through lab scale bioreactor

Bioreduction of chromium contaminated aqueous medium through lab scale bioreactor results are presented in Figure 4a. In a sterile medium, the maximum hexavalent chromium Cr(VI) reduction (84%) was observed from the 4th day throughout the study. In the unsterile medium, the maximum hexavalent chromium Cr (VI) reduction (88%) was observed from the 5th day throughout the study. The significant chromium reduction was obtained in the bioreactor study due to more biochemical reactions that convert raw materials to products through the action of biocatalysts and enzymes of microorganisms. The growth rate of bacterial consortium (S11 and S18) is presented in Figure 4b. In an unsterile medium, the growth rate was very significantly high because of the unsterile medium having native microbes which also influence the chromium reduction and growth rate.

Removal of Cr^{6+} from Periyar river sediments using different treatment columns through biotransformation process

The removal of Cr^{6+} (10mg/L) through the transformations from sediments was examined using fixed bed columns. The synthetic water containing 1% glucose and 1% containing potential bacterial strains was processed for the removal of chromium. Every 24 h, the effluent was collected from each column and the amount of Cr^{6+} and Cr^{3+} was analyzed by standard methods using the UV-VIS spectrophotometer. At the end, the column setups were dismantled and treated sediment samples were collected carefully. The collected sediment samples were subjected to microwave oven digestion to find out the presence of total chromium. Initially, Cr^{6+} was raised slightly in each treatment from 1 to 4. It was recorded until the 5th day of the study and thereafter it disappeared (Fig. 5a). Removal of Cr^{6+} was shown to be negligible in treatment 4 performed with MTCC 447. In the case of Cr^{3+}, a gradual increase in each treatment was observed to convert Cr^{6+} by the bacterial species (Fig. 5b). However, the formation of Cr^{3+} was recorded at only trace level.

The proposed mechanism of Cr(VI) reduction

The diagrammatic representation of the cellular mechanism of bacterial consortium (S11 and S18)

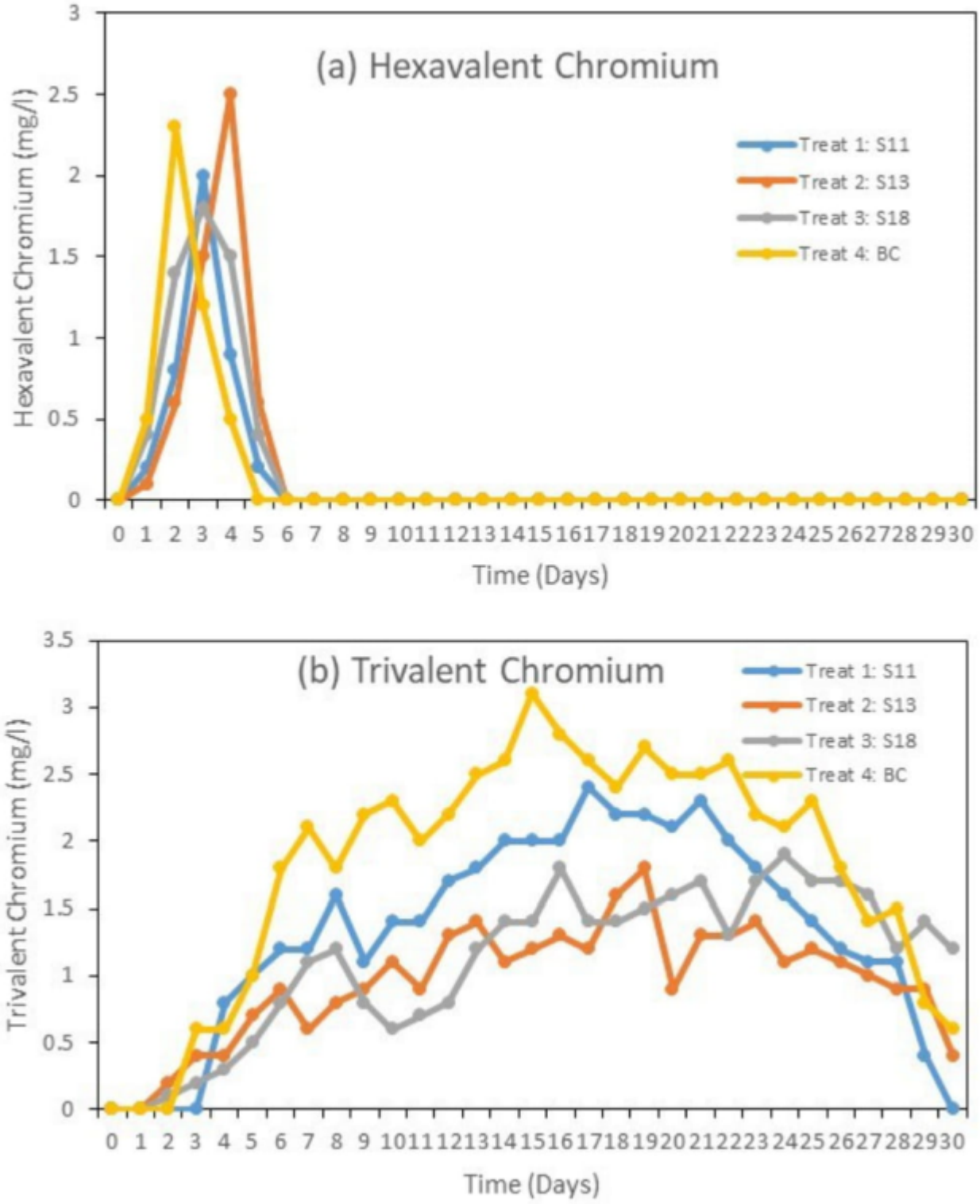

Figure 5. Removal of Cr^{6+} from Periyar river sediments using different treatment columns through biotransformation process

on reduction of Cr(VI) is presented in Figure 6. The number of existing research works suggested that the Cr(VI) reduction mechanism of bacterial consortium (S11 and S18) might be divided into extracellular reduction and intracellular reduction. In the phenomenon of extracellular Cr(VI) reduction, the Cr(VI) reducing enzymes are synthesized consciously by the bacterial cell and exported to the outside of cells to reduce Cr(VI) to Cr(III) (Singh et al. 2008). Intracellular Cr(VI) reduction takes place

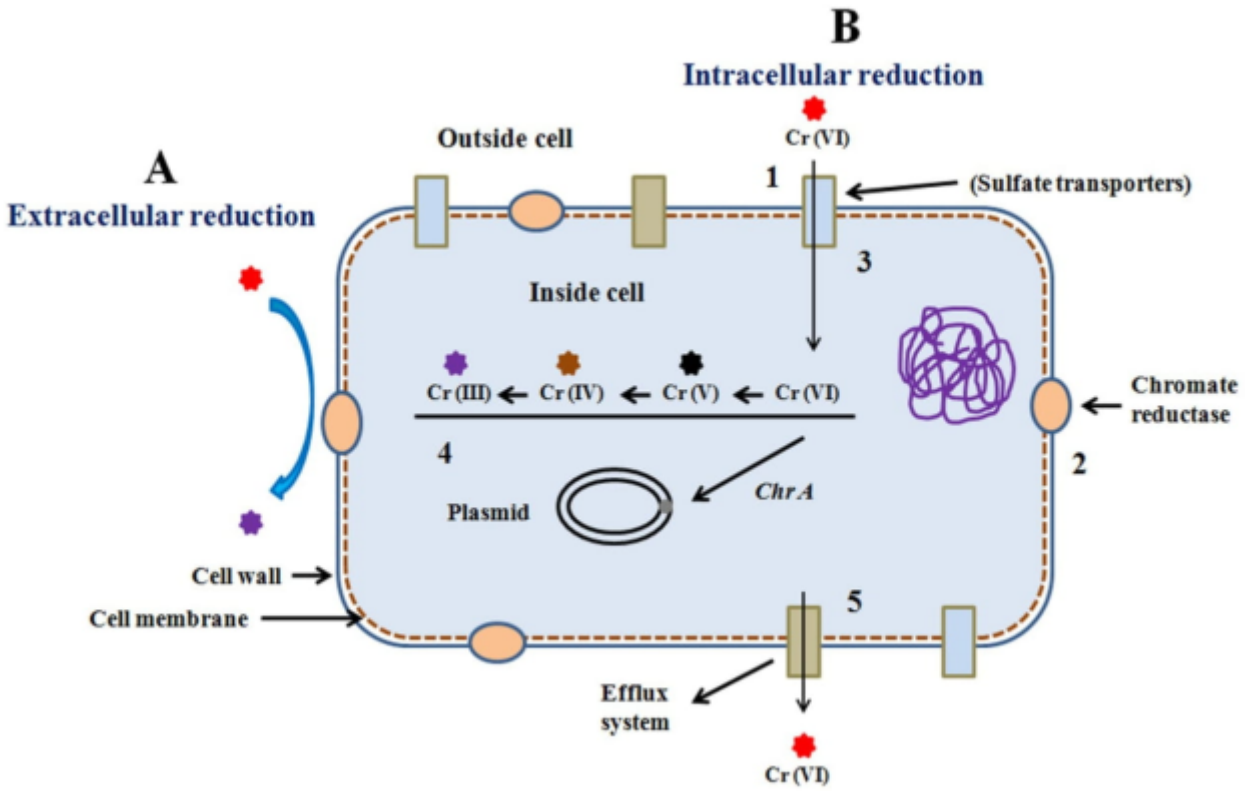

Figure 6. The proposed mechanism of Cr(VI) reduction

through the following main steps: (i) Cr(VI) in an aqueous medium combines by an organization with the cell surface functional groups and the Cr(VI) complex generated, (ii) Cr(VI) is transported into the cells through sulfate/phosphate transporters (Karthik et al. 2017), (iii) Cr(VI) in cytosolic solution is reduced to water-insoluble Cr(III) by chromate reductase of bacterial consortium (S11 and S18).

Phytotoxicity assay by pot culture method

The phytotoxicity of treated and untreated water was studied and the results are presented in Table 1. In treated sterile water (25 to 100%), there was significant black gram (*Vigna mungo*) seed germination observed every day. In treated unsterile water (25 to 100%), there was very significant growth observed when compared to treated sterile water. Similar results were reported when using bacterial strain pv_{26} (Vijayanand et al. 2014). But in the case of raw untreated water, a very poor growth rate was observed using 25% and 50% and there was no growth observed when using 75% and 100% raw untreated water. In one of the studies, at higher concentrations of 80% and 100%, chromium inhibited the germination of black gram due to higher concentration of total solids and heavy metals stress on the seed germination when using untreated effluent (Mythili et al. 2011). Another study reported that the higher concentration of tannery effluent reduces the enzyme dehydrogenase activity, which might disrupt germination and seed growth (Murkumar et al. 1987). This study revealed that after the treatment of chromium by using bacterial consortium (S11 and S18), the treated water did not show toxic effects on black gram plants.

CONCLUSIONS

The bacterial strains *Alcaligenes* sp. (S11) and *Listeria* sp. (S18) as a consortium utilized in this study were isolated from the Periyar river of Kerala, India. The optimal pH for Cr(VI) reduction was 7.0 and the optimal temperature was recorded as 37°C. In the bioreactor study, the maximum Cr(VI) reduction capacity of bacterial consortium (S11 and S18) was 97%. The successive experiment for the toxicity of treated water on black gram indicated that there was no toxicity in the growth parameters of the plant. The bacterially treated water, which was established safe for the plantation, might be used at

Table 1. Phytotoxicity assay by pot culture method with black gram (*Vigna mungo*)

Reactors	Water Concentrations	Shoot length (cm)/days						
		1	2	3	4	5	6	7
Reactor 1	25%	G	G	G	2.2	3.6	7.8	10.2
Unsterile MSM	50%	G	G	G	1.9	3.1	6.5	8.9
	75%	NG	G	G	1.6	2.8	6.1	7.7
	100%	NG	G	G	1.2	1.9	5.6	6.9
	Control	G	G	G	3.4	4.1	8	10.8
Reactor 2	25%	G	G	G	2.6	4.1	8	11
Sterile MSM	50%	NG	G	G	1.9	2.8	6.2	9.3
	75%	NG	G	G	1.3	2.1	5.6	8.4
	100%	NG	NG	G	G	1.6	3.2	7.4
	Control	G	G	G	3.1	4.6	8.8	11.5
Aqueous solution	25%	NG	NG	G	G	0.8	1.3	2
(Untreated)	50%	NG	NG	NG	G	0.5	0.9	1.2
	75%	NG	NG	NG	NG	NG	NG	NG
	100%	NG	NG	NG	NG	NG	NG	NG
	Control	G	G	G	3.2	4.4	7.2	8.2

G- Germination; NG- No Germination

least for crop irrigation. Hence, the bacterial consortium (S11 and S18) is recommended for the reduction of chromium from an aqueous medium.

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Conflict of interest: The authors declare no conflict of interest.

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