

Evaluation of Cr(VI) Reducing Capability of *Shewanella putrefaciens* (MTTC8410) and Optimization of Operational Parameters

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Abstract

Bioremediation is an important technology to remediate the chromium (Cr) contaminated soil and water. In this study, *Shewanella putrefaciens* (MTTC8410) was used to investigate the influence of carbon concentration, pH, and temperature on reduction of hexavalent chromium [Cr(VI)] into trivalent chromium [Cr(III)]. The increased bacterial growth rate was significantly reduced the Cr(VI) concentration. In batch mode experiments, 1% starch recorded the highest reduction of Cr(VI) (90%) followed by 1% glucose (88% reduction) and a reduction of 77% was by 1% cellulose. By using various pH conditions the maximum Cr(VI) reduction was achieved at pH 7.0. In this experiment the maximum Cr(VI) reduction (75%) was observed at 35°C, followed by 30°C with 62% of Cr(VI) reduction. Bioreactor analysis revealed the highest reduction of Cr(VI) (88%) in unsterile tannery effluent. The significant levels of physico-chemical parameters were reduced in unsterile tannery effluent, as compared to the sterile tannery effluent. The experimental results revealed that the *S. putrefaciens* (MTTC8410) could be used as a potential bacterial strain for reduction of Cr(VI) from contaminated groundwater.

Keywords: *Shewanella putrefaciens*, Bioremediation, Cr(VI) reduction, Tannery effluent, Black gram, Phytotoxicity assay

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INTRODUCTION

Chromium (Cr) is the seventh most abundant element on earth, the twenty-first metal in the earth's crust and is mined as chromites^{1,2}. The tannery industry is among the most contaminating industrial sectors and in the process of leather manufacture each tannery industry uses vast amounts of chemicals. The leather industries which widely use compounds containing Cr(III) in the tanning process generally release Cr(VI) effluents into natural water supplies, often without adequate effluent treatment, leading to anthropogenic contamination in water sources³. Chromium is one of the main contaminants in various engineering processes including tannery, electroplating, mining, fibre, metal processing, fertilizer, coloring and manufacturing of pigments⁴. Globally, Cr contamination estimates that the sector is accountable for the annual disposal into water of 300-400 million tons of heavy metals, solvents, toxic sludge and other wastes⁵. Among tannery effluent pollutants, Cr(VI) is one of the most toxic and causes various human health problems symptoms including nasal and skin irritation and lung carcinoma. While Cr(III) is harmless and insoluble, this is vital for human diet^{6,7}. Cr can be found in various oxidation forms; Cr (VI) is the most toxic and soluble and Cr (III) is the least toxic form to human beings.

The traditional Cr management method included physical techniques (reverse osmosis, soil washing, membrane separation, exchange of ions, and adsorption) to remove Cr from the environment. Similarly, various chemical methods have also been used to extract Cr from aqueous media, including graphene-coated iron oxide (GClO) nanoparticles⁸, maghemite nanoparticles⁹, iron oxide/carbon¹⁰ and mackinawite (FeS)-coated sand¹¹. Such strategies pose further drawbacks such as high costs, energy and secondary hazardous waste generation¹²⁻¹⁵. Biological removal of Cr is too good a process and option for the physical and chemical methods, as it is environmentally safe, less costly and lacks secondary emissions. Microbial bioremediation is a cost-effective and beneficial biosource for removing Cr from tannery and other industrial waste along with other harmful pollutants^{16,17}. There are many microorganisms that play a crucial role in water, soil and air. A number of researchers

previously successfully isolated a variety of possible microorganisms, minimizing effectiveness with Cr(VI). Some bacterial and fungal species such as *Escherichia coli*, *Shewanella oneidensis*, *Bacillus firmus*, *Aspergillus niger* and *Pleurotus ostreatus* were used to transform the Cr(VI) into Cr(III)¹⁸⁻²². The novelty of this study is to evaluate the ability of live cells of a bacterium namely *S.putrefaciens* (MTTC8410) to reduce the Cr(VI) into Cr(III). The batch process and bioreactor study was carried out for Cr reduction from chromium contaminated ground water.

The present study was aimed at evaluating *S.putrefaciens'* Cr(VI) reduction capability. Studies of the batch mode were performed by living bacterial cells. Under optimized conditions, the bacterial strain on Cr contaminated tannery effluent is checked via bioreactor analysis. The toxicity of treated wastewater samples from the bioreactor was examined using seed germination of on black gram.

MATERIALS AND METHODS

Bacterial strain

The *S. putrefaciens* (MTTC8410) utilized in this study was obtained from MTCC Chandigarh in India. It was sub cultured in the laboratory using Luria broth medium. The bacterial strain was maintained by nutrient agar slant. The bacterial strain was subcultured every month and preserved using a glycerol solution 20% and stored at 4°C for further studies.

Media and chemicals

The optimization of potential bacterial strain was performed by using mineral salt medium. All analytical grade chemicals and reagents were purchased from Loba Chemie Laboratories (Mumbai, India) and Hi-media Laboratories (Mumbai, India).

Influence of Cr(VI) on bacterial growth

An influence of different concentrations of Cr(VI) on the bacteria's growth rate was investigated. *Shewanella putrefaciens* strain (MTTC8410) was grown at 120 rpm and pH 7.0 in a rotary shaker (model: OrbiTech LETT, India), whereas the temperature in LB broth medium was 37°C supplemented by Cr(VI) range 10 to 30 mg/ L²³. Every 4 hrs time interval 5 ml samples were obtained from the conical flasks. Followed by the samples were collected and centrifuged

for 10 min at 3000 rpm. The pellet was then dissolved with sterile distilled water and calculated growth rate was measured at 600 nm using UV-spectrophotometer (model: Cyber lab-UV-100, USA)²⁴.

Biochemical tests of *S. putrefaciens* (MTTC8410)

The *S. putrefaciens* (MTTC8410) was qualitatively investigated for its ability to produce various enzymes viz, amylase²⁵, cellulose²⁶, chitinase²⁷, gelatinase²⁸, tryptophanase²⁹, citrase, L-asparaginase³⁰, protease³¹, tannase³², oxidase and catalase by appropriate standard method.

Effect of carbon sources on reduction of Cr(VI)

Shewanella putrefaciens (MTTC-8410) was used to evaluate the influence of various carbon sources on the reduction of Cr(VI). So far, 100 ml mineral salt medium (MSM) was prepared in 250 ml conical flasks with various concentrations of carbon sources (0.5, 1 and 1.5 per cent) and amended with 10 mg Cr(VI) and all flasks were sterilized. After the sterilization individually, 1% of *S. putrefaciens* (MTTC-8410) was transferred by aseptically³³. Flasks were then kept for 10 days in shaker at 37°C at 125 rpm. Then 5 ml of sample were taken from all conical flasks at every 24 hrs time interval. After this the collected samples were spun at 3000 rpm for 10 min. Then aqueous phase was withdrawn from the centrifuge tube and chromium was estimated by DPC (diphenylcarbazide) method using in UV-spectrophotometer at 540 nm (model: Cyber lab-UV-100, USA). The culture growth rate was measured simultaneously, at 600 nm.

Effect of different pH conditions on Cr(VI) reduction

S. putrefaciens was used to assess the effectiveness of different pH conditions on the Cr(VI) reduction. In which various pH of (5 to 9) 100 ml (MSM) medium were prepared with optimized carbon source 0.5 per cent starch in 250 ml conical flasks and adjusted with 10 mg Cr(VI) and all flasks were sterilized. After the sterilization 1% of *S. putrefaciens* (MTTC-8410) was transferred to individual conical flask by aseptically³⁴. Flasks were then kept for 10 days in shaker at 37°C with 125 rpm. Then 5 ml of sample were taken from all conical flasks at every 24 hrs time interval. After this the collected samples were spun at 3000 rpm for 10 min. Then aqueous phase was withdrawn from the centrifuge tube

and Cr(VI) was estimated by DPC method in UV-spectrophotometer at 540 nm (model: Cyber lab-UV-100, USA). Simultaneously the culture growth rate was measured at 600 nm.

Influence of temperature on Cr(VI) reduction

Shewanella putrefaciens (MTTC-8410) was used to assess the influence of different temperatures on reduction of Cr(VI). Hence, in 250 ml conical flasks 100 ml (MSM) medium was prepared with optimized carbon source 0.5% starch and amended with 10 mg Cr(VI) and all flasks were sterilized. After the sterilization 1% of *S. putrefaciens* was transferred to individual conical flask by aseptically. Then conical flasks were kept in shaker under different temperatures (25 to 45°C) at 125 rpm for 10 days³⁵. Followed by 5 ml of sample was taken from all conical flasks at every 24 hrs time interval. After this the collected samples were spun at 3000 rpm for 10 min. Then aqueous phase was withdrawn from the centrifuge tube and Cr(VI) was estimated by DPC method in UV-spectrophotometer at 540 nm (model: Cyber lab-UV-100, USA). Simultaneously the culture growth rate was measured at 600 nm.

Analytical method

The [Cr(VI)] was determined using the (DPC) procedure using a UV-spectrophotometer (model: cyber lab-UV-100, USA)³⁶. Around 0.025 g of DPC was mixed with 9.67 ml of acetone and 330 ml of 3 M H₂SO₄. The absorbance was calculated at 540 nm and all assays were performed in triplicates, and mean values were recorded.

Bioreduction of chromium from tannery effluent using lab scale bioreactors

The tannery effluent treatment method was planned and the setup was made as shown in (Fig. 1). This set up was prepared based on the pilot scale water treatment plant³⁷. The tannery effluent was obtained from tannery industry in Periyakulam, district of Erode, Tamil Nadu. The sample was collected in sterile container and was taken to the laboratory and analyzed for various physicochemical parameters³⁸. The established lab scale bioreactors have a capacity of 10 liters and are made of tarson. Mechanical stirrers were installed in the reactor tank and the settling tanks. Artificial aerator was connected to both bioreactors for aeration purpose. About 10 liters of tannery effluent containing 20 mg of Cr(VI) was subjected to primary (bioprocess) treatment

and inoculated with *S.putrefaciens*. Then 5 ml of sample was taken from all conical flasks at every 24 hrs time interval. After this the collected samples were spun at 3000 rpm for 10 min. Then aqueous phase was withdrawn from the centrifuge tube and Cr(VI) was estimated by DPC method using UV-spectrophotometer at 540 nm (model: Cyber lab-UV-100, USA). Simultaneously the culture growth rate was measured at 600 nm. Further, all physic-chemical parameters were analyzed before and after the treatment of tannery effluent.

Reactor 1 (R1)

Unsterile tannery effluent + 0.5% starch + 1% inoculum

Reactor 2 (R2)

Sterile tannery effluent + 0.5% starch + 1% inoculum

Phytotoxicity assay

Pot culture experiments were performed to evaluate the toxicity of the treated and untreated tannery effluent. The fertile soil was collected without any contamination near Omalur Taluk of Salem district. The Cr(VI) amended soil was filled in sterile plastic cups with the dimensions of 5 x 15 cm. The black gram seeds obtained from the licensed agency and surface sterilized by mercury chloride (HgCl₂) (0.1%, w/v). Four seeds were

sowed in each cup and the set up was kept for phytotoxicity assay. In each pot containing black gram (*Vigna mungo*) the treated and untreated tannery effluents at varying percentages viz 25, 50, 75 and 100% were irrigated and allowed to germinate the plants. Control pot was irrigated with tap water to compare the efficiency of the treatment. Germination of the seeds was noted after 3rd day of irrigation and calculated³⁹.

Statistical Analysis

All tests were performed in triplicates and reduction of Cr(VI) was evaluated using error bars⁴⁰. Data were statistically analyzed using the Microsoft[®] Excel (Version 2010) statistical package.

RESULTS AND DISCUSSION

Effect of Cr(VI) on bacterial growth rate

The influence of different Cr(VI) (10 and 30 mg/L) concentrations on the growth of *S.putrefaciens* has been performed and presented in (Fig.2). The interaction between cell growth and varying Cr(VI) concentration was studied at 24 hrs time of incubation. The rate of bacterial growth in LB media without Cr(VI) was very significant as compared to the LB medium containing Cr(VI) 10 and 30 mg / L containing. In which 10 mg/L of Cr(VI) has a minor effect on the development of bacterial cells. Cr(VI) at a concentration of 10 to 30 mg/L have negatively effect on the growth of the bacteria. The previous study reported that concentration of Cr(VI) in the range of 10 and 20 mg/L would have a small impact on *Bacillus sp.* growth, while 100 mg/L of Cr(VI) was greatly inhibit the bacterial growth⁴¹. The present study



Fig. 1. Bioreduction of chromium contaminated tannery effluent through lab scale bioreactor study

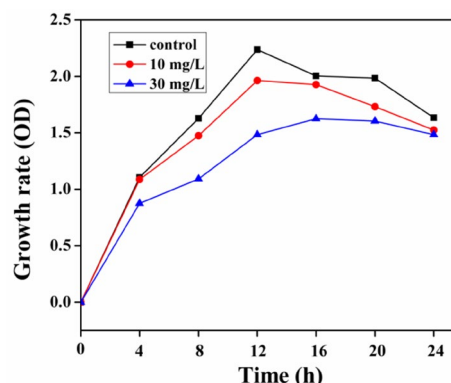


Fig. 2. Influence of Cr (VI) on bacterial growth rate

reported a decrease in *Escherichia coli* ATCC 33456 growth rate during lag phase due to the increased concentrations of Cr(VI)⁴².

Biochemical tests of *S. putrefaciens*

The enzymatic characteristic of *S. putrefaciens* was presented in Fig. 3. The strain developed a clear zone around the culture in starch agar medium, but in the case of cellulose, chitinase and pectinase enzyme assay, the culture could not develop the clear zone around the colonies due to the lack of enzyme producing gene. The *S. putrefaciens* (MTTC-8410) can produce enzymes like gelatinase, tryptophanase, citrase, L- Asparaginase, protease, oxidase and catalase and it was confirmed by the strain developed a clear zone in respective media. Similar results were reported by Cr reducing *Pseudomonas* species⁴³.

Effect of different concentrations of glucose on Cr(VI) reduction

The effect of different doses of glucose (0.5, 1 and 1.5 percent) on reduction of Cr(VI) by *S. putrefaciens* was presented in Fig. 4a. In which, by using 0.5% of glucose, no significant Cr(VI) reduction was observed from 1st to 5th day of experiment. After the 5th day of experiment, slight significant Cr(VI) reduction (82%) was observed. By using 1% of glucose, the maximum Cr(VI) reduction (88%) was observed in 8th day to 10th of day of experiment. The previous study reported that glucose was donor of electrons, which could easily be oxidized glucose⁴⁴. Some previous research reported that glucose can play a good carbon source while act as a efficient electron donor to reduce Cr(VI) by bacteria⁴⁵⁻⁴⁷. By

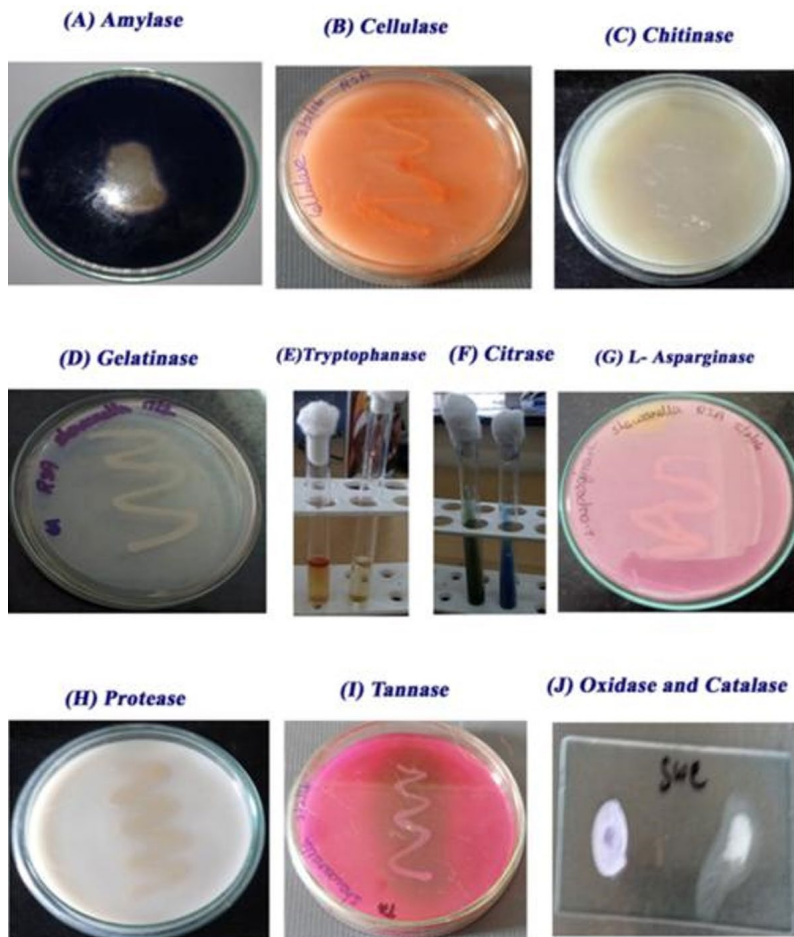


Fig.3. Biochemical tests of *Shewanella putrefaciens* (MTTC8410)

using 1.5% of glucose, 80% of Cr(VI) reduction was observed. Overall in this different concentration of glucose optimization, 1% of glucose achieved very significant Cr(VI) reduction. Previously many researchers reported that glucose is a known electron donor to several bacterial strains for Cr(VI) reduction⁴⁸. Fig. 4b showed the growth rate of *S. putrefaciens* by various doses of glucose in MSM medium containing 10 mg of Cr(VI). In this study by using 0.5% of glucose, there was no significant

growth observed from 1st day to 10th day of experiment and it does not influence the Cr(VI) reduction. But by using 1 and 1.5% of glucose the significant Cr(VI) reduction was observed. In which both 1 and 1.5% of glucose can influence the Cr(VI) reduction as well as growth rate of *S. putrefaciens*. Different concentrations of cellulose on Cr(VI) reduction.

The effect of different doses of cellulose (0.5, 1 and 1.5 percent) on reduction of Cr(VI) by

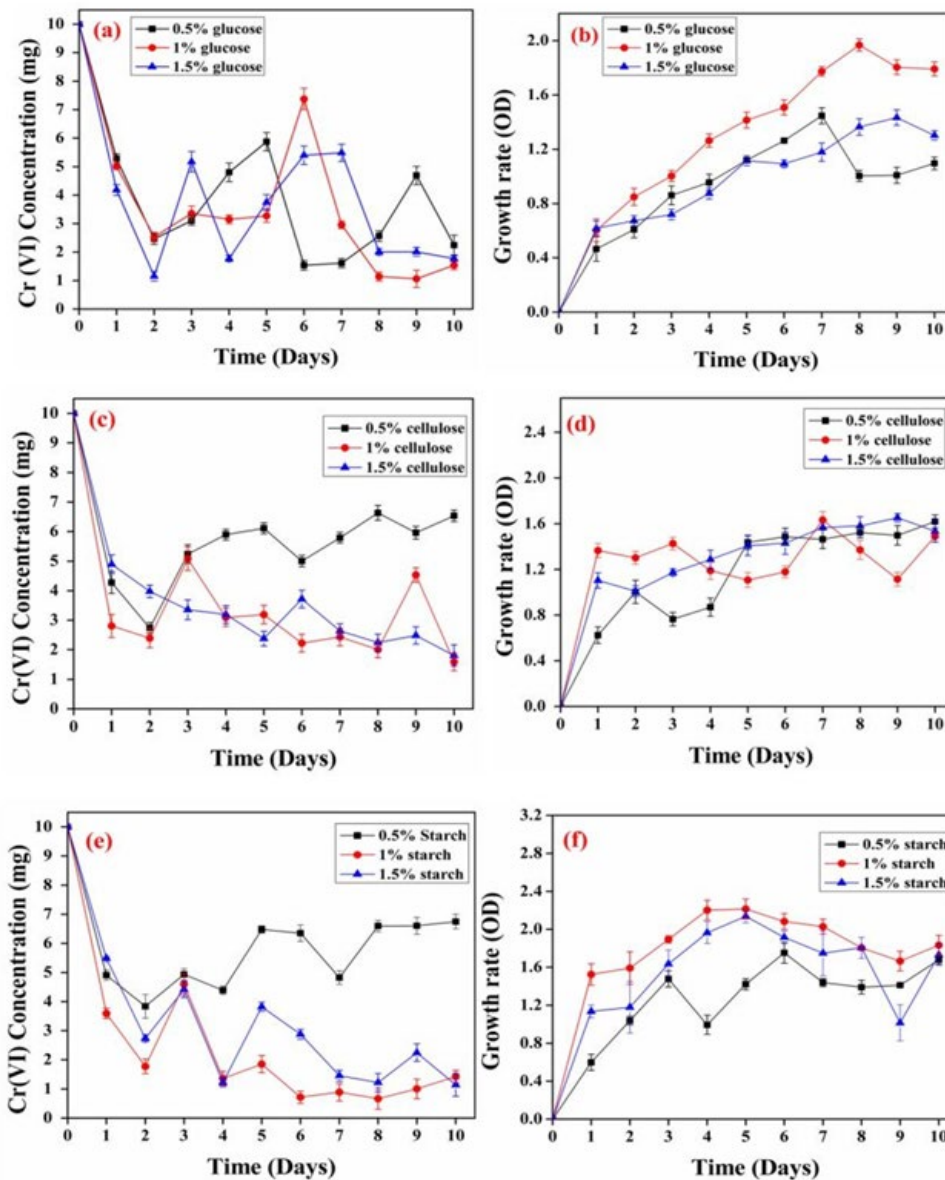


Fig. 4. Influence of various carbon sources on the reduction of hexavalent chromium

S. putrefaciens was presented in (Fig.4c). In this experiment, by using 0.5% of cellulose, there was very poor Cr(VI) reduction was observed. By using 1% of cellulose, 77% of Cr(VI) reduction was observed in 6th to 8th day of experiment. Followed by using 1.5% of cellulose, there was 75% of Cr(VI) reduction from 5th to 10th day of experiment. Fig. 4d showed the growth rate of *S. putrefaciens* under various concentrations of cellulose (0.5, 1 and 1.5%) in MSM medium containing 10 mg of Cr(VI). In this study by using 0.5% of glucose, better significant growth was observed from 1st day to 10th day of experiment and it also contribute poor influence on Cr(VI) reduction. But by using 1 and 1.5% of cellulose, significant chromium reduction was observed. In which both 1 and 1.5% of glucose can influence the Cr(VI) reduction as well as growth rate of *S. putrefaciens*. Previous study reported that a number of electron donors play an important role for the reduction of Cr(VI) by *Ganoderm lucidum*⁴⁹.

Effect of different concentrations of starch on Cr(VI) reduction

The effect of different doses of starch (0.5, 1 and 1.5 percent) on reduction of Cr(VI) by *S. putrefaciens* was presented in (Fig.4e). In this study, by using 0.5% starch, there was considerable Cr(VI) reduction was observed in first two days, after that on the 3rd day of experiment it loss the Cr(VI) capacity. Using 1% of starch, 90% of Cr(VI) reduction was observed in 6th to 9th day of experiment. Followed by using 1.5% of starch, there was 79% of Cr(VI) reduction was achieved from 7th to 8th day of experiment. The electron donors of carbon sources influenced the Cr(VI) reduction by using *Brevibacterium casei*⁵⁰. Fig.4f was showed the growth rate of *S.putrefaciens* under various concentrations of starch (0.5, 1 and 1.5%) in MSM medium containing 10 mg of hexavalent chromium. In this study, by using 1% of starch was can influenced the maximum level of Cr(VI) reduction and growth rate of *S.putrefaciens* when compared to 0.5 and 1.5% of starch.

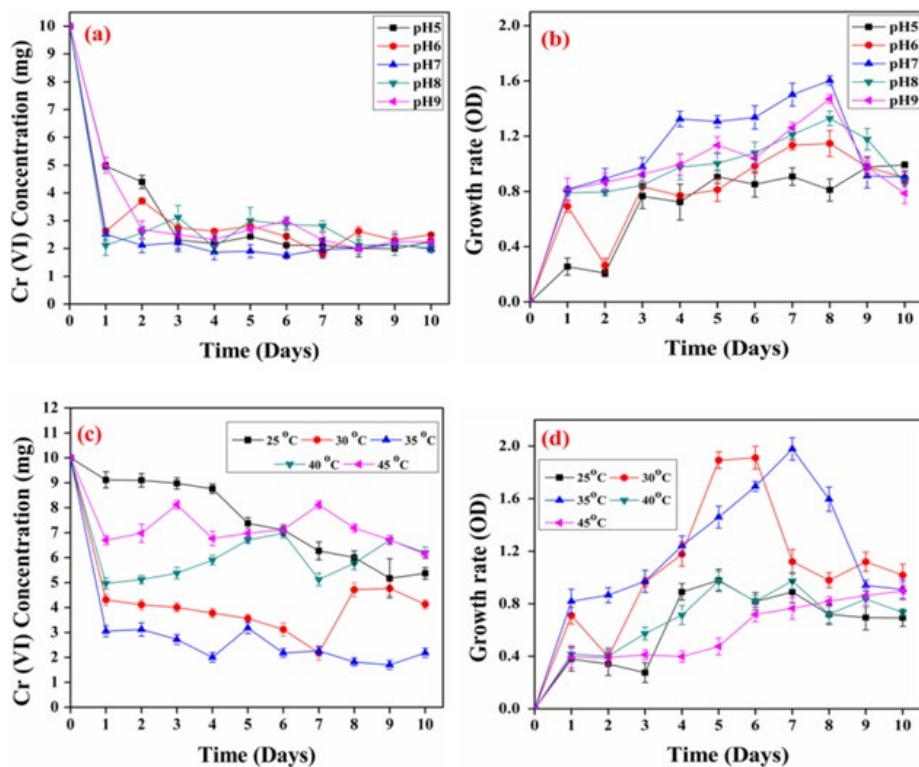


Fig. 5. Influence of various pH and temperatures on reduction of hexavalent chromium

Influence of different concentrations of pH (5,6,7,8 and 9)

The reduction of Cr (VI) under various pH conditions by using *S.putrefaciens* was shown in Figure 5a. The pH has an influence on Cr(VI)

bio-reduction. The effects of incubation period on reduction of Cr(VI) at various pH (5, 6, 7, 8 and 9) was analyzed (The operation conditions; 10 mg of Cr (VI)/100 ml medium, temperature 37°C and 1ml of one OD inoculums). In this study, all pH from

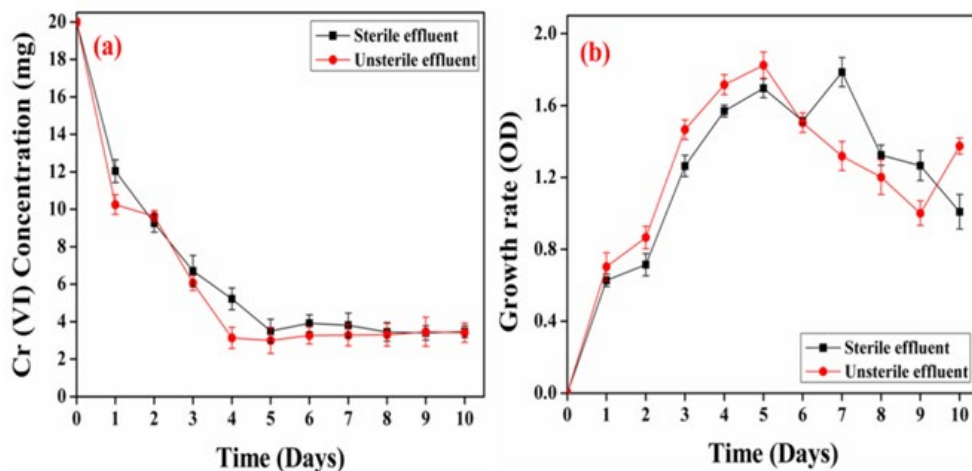


Fig. 6. Bioreduction of chromium contaminated tannery effluent through lab scale bioreactor

Table 1. Physico-chemical parameters of untreated tannery effluent, treated sterile tannery effluent and treated unsterile tannery effluent

S.No	Parameters	Untreated tannery effluent	Treated sterile tannery effluent	Treated unsterile tannery effluent
1	Colour	Dark Black	Light Black	Black
2	pH	7.86	7.54	7.24
3	Turbidity	Above 200	Above 400	Above 400
4	Electrical/Specific conductivity	7890 Micro mho/cm	4368 Micro mho/cm	4308 Micro mho/cm
5	Total dissolved solid	4385	2401	2101
6	Total suspended solid	660	330	290
7	Total Hardness	2842	1042	981
8	Chromium	20	5	2.3
9	Chloride	1124	1216	1016
10	Sulphate	390	234	211
11	Calcium	656	136	98
12	Magnesium	220	170	112
13	Sodium	118	98	83
14	Pottassium	98	73	71
15	Biological oxygen demand	223	78	74
16	Chemical oxygen demand	458	94	88
17	Nitrate	6.4	6.4	5.3
18	Phosphate	1.0	1.0	1.0
19	Manganese	1.6	1.6	1.6
20	Phenolic compound	2.1	2.1	2.1
21	Fluorides	1.8	1.8	1.8

5 to 9 was very significantly reduced the Cr(VI) from the aqueous solution. In this experiment the maximum Cr(VI) reduction was observed at pH7. The similar result was observed that the maximum removal of Cr(VI) (88%) by PCP3 strain at pH 7.0⁵¹. So the further studies were carried out at pH7. The reduction of Cr(VI) during low pH may be due to the relationship of bioaccumulation and functional group dissociation⁵². The (Fig. 5b) was showed the growth rate of *S. putrefaciens* under various pH conditions. All pH levels significantly influenced the growth rate.

Influence of different temperature of pH (25, 30, 35, 40 and 45°C)

Temperature is one of the important key for microbial growth Cr(VI) reduction. The effectiveness of various temperatures on reduction of Cr(VI) by *S. putrefaciens* were presented in (Fig. 5c). In this study, the maximum Cr(VI) reduction (75%) was observed at 35°C. Followed by 30°C was showed the 62% of Cr(VI) reduction. The previous study was reported that hat 30°C was significantly encourage the growth of *Bacillus* KCH3 and growth was inhibited by below15°C and above 40°C⁵³. The significant bacterial growth achieved by 30°C and 35°C when compared to other temperatures (Fig. 5d). The similar result was observed that

the maximum removal of Cr(VI) (85.87%) by *Brevibacillus* sp at 35°C⁵⁴.

Bioreduction of chromium contaminated tannery effluent through lab scale bioreactor

Bioreduction of chromium contaminated tannery effluent through lab scale bioreactor results were presented in (Fig. 6a). In sterile effluent, the maximum Cr(VI) reduction (84%) was observed from 4th day to throughout the study. In unsterile effluent, the maximum Cr(VI) reduction (88%) was observed from 5th day to throughout the study. The significant Cr(VI) reduction was obtained in bioreactor study, due to more biochemical reactions to convert raw materials to products through the action of biocatalyst and enzyme of microorganisms. The growth rate of *S.putrefaciens* was presented in (Fig. 6b). In unsterile effluent, growth rate was very significantly high because of unsterile effluent have native microbes, hence those native microbes also influence the Cr(VI) reduction and growth rate.

Physicochemical parameters of untreated and treated tannery effluent

The physicochemical parameters of the raw tannery effluent, treated sterile tannery effluent and treated unsterile tannery effluent were described in Table 1. The tannery effluent

Table 2. Effectiveness of various concentrations of treated and untreated tannery effluent on germination and seedling growth of black gram (*Vigna mungo*)

Reactors	Effluent con.	Shoot length (cm)/days						
		1	2	3	4	5	6	7
Reactor 1	25%	+	+	+	2.2	3.6	7.8	10.2
	50%	+	+	+	1.9	3.1	6.5	8.9
	75%	-	+	+	1.6	2.8	6.1	7.7
	100%	-	+	+	1.2	1.9	5.6	6.9
	control	-	+	+	3.4	4.1	8	10.8
Reactor 1	25%	+	+	+	2.6	4.1	8	11
	50%	-	+	+	1.9	2.8	6.2	9.3
	75%	-	+	+	1.3	2.1	5.6	8.4
	100%	-	-	+	+	1.6	3.2	7.4
	control	+	+	+	3.1	4.6	8.8	11.5
Untreated raw effluent	25%	-	-	+	+	0.8	1.3	2
	50%	-	-	-	+	0.5	0.9	1.2
	75%	-	-	-	-	NG	NG	NG
	100%	-	-	-	-	NG	NG	NG
	control	+	+	+	3.2	4.4	7.2	8.2

+ (Germination)

- (No Germination)

contained high concentrations of salts and other parameters above the permissible limits. The high concentration of Cr, organic material and salinity in the effluents released from tanneries are considered to be the major hazard^{55,56}. The collected tannery effluent contains higher concentrations of BOD (Biochemical oxygen demand) and COD (Chemical oxygen demand) along with other parameters. The similar results were reported that, tannery effluent was having high BOD and COD concentrations⁵⁷. After the treatment of sterile tannery effluent by using *S.putrefaciens*, most of the salts and other parameters were reduced up to permissible limits fixed by the BIS (Bureau of Indian Standards). In this study, because of the use of dissolved organic substances by bacterial strain⁵⁸, *S. putrefaciens* significantly decreased the BOD and COD of tannery effluent. Furthermore, after the treatment of unsterile tannery effluent by using *S.putrefaciens*, most of the salts and other parameters were reduced permissible limits fixed the BIS and other organizations. The physico-chemical parameters of unsterile treated effluent were significantly reduced as compared to the treated sterile tannery effluent.

Phytotoxicity assay by pot culture method

The data regarding phytotoxicity assay of treated and untreated tannery effluent studies has been presented in (Table 2). In treated sterile tannery effluent (25 to 100%), significant black gram seed germination was observed every day. In treated unsterile tannery effluent (25 to 100%), very significant growth was observed when compared to treated sterile effluent. The similar results were reported by using bacterial strain *pv*₂₆⁵⁹. But in the case of raw untreated tannery effluent very poor growth rate was observed by using 25 and 50% of raw untreated effluent and there was no growth observed when using 75 and 100% raw untreated tannery effluent. At higher concentrations (80 and 100%) tannery effluent inhibited germination of black gram due to toxicity and stress by using untreated effluent⁶⁰. The previous study reported that the higher tannery effluent concentration reduces the dehydrogenase activity of the enzyme, which may be changing to disrupt germination and seedling growth⁶¹. This study was revealed that after the treatment of tannery effluent by using *S.putrefaciens* the

treated effluent was did not showed toxic effects on black gram plants.

CONCLUSIONS

Shewanella putrefaciens strain (MTTC8410) was investigated for its an ability to reduce Cr(VI) into Cr(III). In this study the maximum Cr(VI) reduction was achieved at pH 7.0 and temperature 37°C. In the bioreactor test, *S.putrefaciens* maximum Cr(VI) reduction capacity was 97 %. Consecutive experiment for the toxicity of treated tannery effluent on black gram showed no toxicity in plant growth parameters. The bacterially treated tannery effluent has very less amount of Cr, due to this nature its harmless for the agricultural crops. Hence, it was recommended that the *S.putrefaciens* (MTTC8410) have potential to reduce Cr(VI) from aqueous media.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors have made a substantial, direct, and intellectual contribution to the work and approve it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies

with human participants or animals performed by any of the authors.

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