Indian Journal of Geo Marine Sciences
Vol. 48 (04), April 2019, pp. 528-534

Reduction of hexavalent chromium to trivalent chromium from tannery effluent using bacterial biomass

G.[1] Venkatesan & T.[2] Subramani

[1]Department of Civil Engineering, Priyadarshini Engineering College, Vaniyambadi, India

[2]Department of Mining Engineering, CEG, Anna University, Chennai, India

[E-mail: peccivilvenkat@gmail.com]

Received 11 September 2017; revised 20 November 2018

Bioremediation is the most promising and cost-effective biological method widely used to clean up both contaminated soil and wastewater containing organic and inorganic pollutants. Ambur in Tamil Nadu is included in top ten Cr (VI) contaminated placeS in the world rated by Blacksmith Institute, U.S., in September 2006. Cr (VI) pollution in this area causes major health issues among the settled people, destruction of waterbodies, and pollutes the groundwater and agricultural lands. This work involves the biological treatment of Cr (VI) without producing any by-product. Bacterial culture tolerating high concentration isolated from the contaminated soil samples was collected from Cr (VI) contaminated sites of Ambur. The bacterial isolate was identified as 16s rRNA sequencing for Pseudomonasputida. Under the optimum parameter condition, reduction of Cr (VI) by isolated Pseudomonas putida was found to be 90% and Cr (VI) reduction by consortium of microbe to be 92%. Finally, reduced chromium was separated from the tannery effluent by chemical precipitation method.

[**Keywords***:* Hexavalent chromium; Trivalent chromium; Tannery effluent; Bacterial biomass]

Introduction

Effluents from textile, leather, tannery, electro-plating, galvanizing, dyes and pigment, metallurgical and paint industries and other metal processing and refining operations at small and large-scale sectors contain considerable amount of toxic metal ions[1-2]. These metal ions from the above-mentioned industries pose problems to the water environment by discharging it into nearby streams, rivers and open pits[3], Potential impact of these metals on the environment most likely to be experienced as change to surface and groundwater quantity[4]. These toxic materials not only causes potential human health physical discomfort and sometimes life-threatening illness including kidney damage and cancer to vital body system[6]. Whereas it's reduced trivalent form, (Cr^{3+}) is less toxic, insoluble and a vital nutrient for humans. Due to its toxicity stringent regulations are imposed on the release of Cr into surface water bodies to below 0.05 mg/l by the U.S. EPA (Environmental Protection Agency)[7] and the European Union, while total Cr forms to below 2 mg/l. Since the industrial revolution, the anthropogenic inputs of chromium have increased rapidly[8]. Chromium is extensively used in electroplating and leather tanneries[9].

In Tamil Nadu, Ambur (Vellore district) is the major source for small and large-scale tanneries[10]. Large amount of effluents produced from these industries are directly discharged into nearby rivers, streams and open pits. It is directly affecting the nature of surface and groundwater[11]. Palar is the major affected river in Tamil Nadu due to direct discharge of tannery effluents from Ambur tanneries[12].

Palar is a south Indian river, originated from Nandi hills of Karnataka state and flows through Karnataka (93 km), Andhra Pradesh (33 km), Tamil Nadu (222 km) and finally confluences into the Bay of Bengal near Vayalur. Palar is one of the major rivers flowing [illegible] length with 4710 area of river basin)[13]. Elevated chromium concentration in the effluents from tanneries poses a serious environmental concern in Vellore district, home of innumerable small and large-scale tanneries. Palar River is the source of drinking water for 30 towns and 50 villages on its banks and also used for the cultivation purposes[14]. The tanneries present on the banks discharge effluents in the Palar River, which has become more polluted recently and no longer useful for drinking or agricultural purposes. Due to pollution, people around the river are suffering from a number of diseases such as asthma, skin disease and stomach

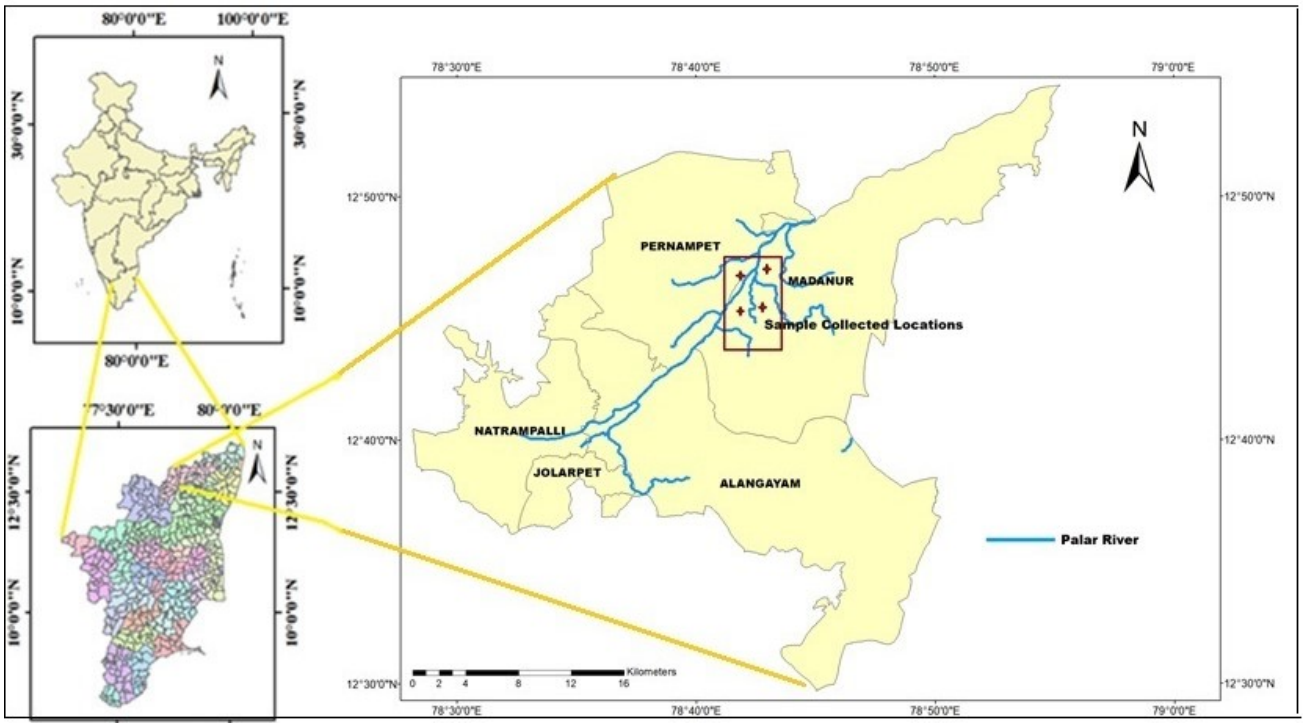

Fig. 1 — Study area map showing the locations of sampling sites

ailment. Nearly thousand acres of fertile land have become a wasteland and no more useful for cultivation. Ranipet an industrial area present in the bank of Palar River has been reported as one of the maximum polluted spaces of world by Blacksmith Institute, U.S, in 2006 (Top ten worst polluted places in the world rated by Blacksmith Institute, U.S, in September 2006).

Study area

The samples were collected from the Eastern Chrome Tanning Company, Periyakuppam in Ambur, Vellore district (Fig. 1). The soil samples were collected at a depth of 0-15 cm from different locations (2-3 m interval) of the chromium-contaminated site located at Ambur.

Need for the present study

Chromium (VI) toxic heavy metal present in the tannery effluents is harmful to both human and environment. Bioremediation is the most promising and cost effective biological method commonly used to clean up both soil and wastewater containing organic or inorganic contaminants. This investigation is done to introduce the remedial route for the detoxification of Cr (VI) present in the tannery effluents by using indigenous microorganism isolated from the contaminated soil.

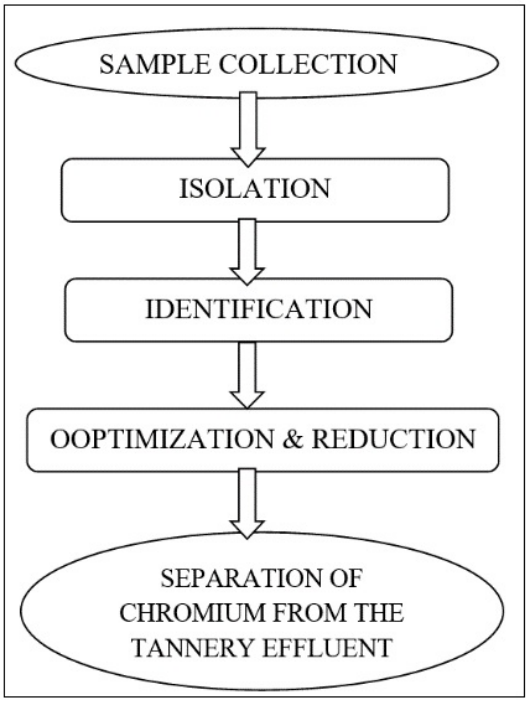

Fig. 2 — Methodology flowchart

Methodology

Materials and methods used in the present work and the outline of the experimental design for biodegradation of hexavalent chromium through batch process are given in the Figure 2.

Materials and Methods

All the glassware were sterilized in a hot air oven at 180°C for three hours and autoclaved at 121°C for 15 minutes at 15 lbs. All the bore water blanks and growth media were sterilized in an autoclave at 1 atm pressure for 15 minutes. Isolation, purification, inoculation and other microbiological procedures were carried out in the laminar flow chamber.

In order to ascertain the microbial bio-system for chromium reduction, the bacterial species has been isolated from the contaminated soil samples and same were collected from Ambur. The acclimatized microorganisms were isolated by employing standard serial dilution and plating techniques. The quantity of 10 gm of soil sample with 10 ml double distilled water was taken in 90 ml sterile water blank and serially diluted for 10-6 times using sterile water blank. After thorough shaking 1ml of aliquot from 10-6 dilutions had been drawn pour plated in PYE agar media. The plates were incubated at 30°C. The isolated microorganism was further purified by subsequent sub-culturing and maintained in the slant culture at 4°C.

The media used to isolate the effective metal resistant strain is peptone yeast extract (PYE).

- The pure culture of the isolated strains were inoculated in 10 ml of sterile luria- bertani broth and left for 24 hours.
- From the 24 hours old culture 1.5 ml - 3 ml had been withdrawn in Eppendorf tubes. The aliquot had been spun at 12000 rpm for 15 minutes to get cell pellets.
- The cell pellets were taken in Eppendorf tubes and tapped gently. In these tubes 150-200 µl of STE lysis buffer was added and tapped gently.
- Then 10 µl of lysozyme enzyme (10mg/100ml) had been added and the tubes were tapped gently.
- They were kept for incubation at 37°C for 2hrs. Intermittent tapping was done for every half an hour to enhance the reaction of lysozyme.
- Then about 150-200 µl of 10% SDS had been added and tapped gently and incubated at 37°C for 30 minutes.
- After incubation, the tubes were taken and sodium acetate (pH-5.2) of about 150 µl had been added.
- The tubes were then centrifuged at 12000 rpm for 15-20 minutes.
- After centrifugation, the aqueous phase had been transferred into a fresh Eppendorf tube. Equal volume of 100% ice cold Ethanol had been added and kept at -20°C for 1hr.
- After an hour, the tubes were taken from -20°C and centrifuged at 10000 rpm for 15 minutes.
- After centrifugation, the pellet had been held twice with 70% Ethanol and again centrifuged at 10000 rpm for 15 minutes.
- Then the ethanol had been discarded and the tubes were allowed to dry.
- After drying, about 60 µl of sterile water had been added and stored at refrigeration temperature for further use.

PCR amplification using genus specific primers

Rhizobacterial isolates (250) were screened for Pseudomonas by PCR amplification using Pseudomonas genus specific primer. Primers used are ps for 20 mer (5'- GGTCTGAGAGGATGATCAGT-3') and ps rev 18 mer (5'-TTAGCTCCACCTCGCGGC-3').

Minimum inhibitary concentration

Minimum inhibitory concentration (MIC) in microbiology is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. MIC of chromate for the isolated metal resistant strain has been determined by preparing various range of dichromate solution ranges from 100 to 400 mg/l from the stock solution. Stock solution has been prepared by dissolving appropriate amount of potassium dichromate in double distilled water. To produce a stock solution of 1000 mg/l in the prepared chromium solution growth of isolated strain has been monitored at regular time interval, and optical density has been measured at 600 nm in UV visible scanning spectrophotometer.

Estimation of hexavalent chromium reduction in tannery effluent done by diphenyl carbazide method

i) Analysis of Chromium Reduction in Tannery Effluent

Chromium reduction has been monitored by means of colour at varying predetermined time intervals from 1 to 144 hrs. The reaction mixture has been separated from biomass by centrifuging the sample at 2000rpm for 15min, and the residual chromium concentration has been determined using the standard diphenylcarbazide method and a calibration curve prepared at the corresponding optimum wavelength in UV-visible scanning spectrophotometer.

ii) Finding the Microbial Reduction of Hexavalent Chromium in Tannery Effluent

The chromium solution has been scanned in UV spectrophotometer to ascertain the wavelength, and

the maximum absorbance has been observed at 540 nm. Chromium reduced content has been assessed as the reduction in Cr concentration in supernatant with time consuming hexavalent chromium specific colorimetric reagent S-diphenylcarbazide (DPC) by spectrophotometric measurement, which are made immediately at 540 nm in the rate of reduction has been monitored at this respective wavelength. Ingredient for samples preparing and estimation of chromium reduction at 540 nm are given in Table 1.

Estimation of chromium reduction percentage

The presence of chromium reduction has been measured using the following formula[9]

$$Chromium\ removal(\%) = \frac{Initial\ absorbance - final\ absorbance}{Absorbance\ of\ uninoculated\ of\ fluent} x\ 100$$

Results and Discussion

Characteristics of collected wastewater

Chromium contaminated tannery effluents were collected in screw capped sterilized bottles and the wastewater samples have been characterized. The pH of the samples have been determined with an ion-specific electrode. The pH of the wastewater samples are in the range of 7.4 - 7.8. The temperature range of the collected wastewater is between 28 and 30ºC. This indicates that chromium-contaminated wastewater is slightly alkaline in nature. Chromium (VI) in the collected wastewater sample is 28 mg/l, and the total chromium concentration is 30 mg/l. Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) in the effluents are 485 mg/l and 580 mg/l respectively. COD of the wastewater is 1180 mg/l (Table 2).

Isolation of chromium resistant bacterial strain

Soil contains very large number of microorganisms, which can include a number of chromium (VI) utilizing bacteria. Single chromium resistant bacterial strains were isolated from the soil samples by serial dilution technique. Within that 10-6 dilution help to isolate the bacterial strain and the consortium of bacteria. The isolated microorganisms were further purified by subsequent sub-culturing and maintained in a slant culture at 4º C.

Chromium resistant strains by 16s rRNA sequencing alignment and phylogenetics

Genomic DNA has been isolated from the pure culture pellet using consequence primer. PS 20 mer and RED 18 mer were used to amplify the 16S rDNA fragment using high fidelity PCR polymerase. The PCR product has been bi-directionally sequenced using the forward reverse and an internal primer. Sequenced data has been aligned and analysed for finding the closest homologous for the microbe. Based on the nucleotide homology and phylogenetic analysis, the microbe has been detected as Pseudomonasputida.

Table 1 — Preparing the samples for the estimation of chromium reduction at 540 nm

Amount of sample	Amount of D.P.C	Amount of sulfuric acid	Distilled water
0.4(ml)	0.4(ml)	0.4(ml)	0.8(ml)

Table 2 — Characterization of the wastewater samples

Parameters	Value
pH	7.4 - 7.8
Temperature	28 - 30ºC
TSS	580 mg/l
TDS	485 mg/l
COD	1180 mg/l
Total chromium	30 mg/l
Chromium (VI)	28 mg/l

Table 3 — Minimum inhibitory concentration of *Pseudomonas putida*

Time (hr)	Growthat 100 ppm	Growthat 200 ppm	Growthat 300 ppm	Growth at 400 ppm
0	3.011	2.344	1.278	0.33
6	3.967	2.245	1.265	0.231
12	2.705	2.365	1.208	0.295
18	2.657	2.225	1.185	0.265
24	2.388	1.950	1.105	0.241
30	2.118	1.856	1.026	0.204
36	2.004	1.658	0.942	0.187
42	2.887	1.455	0.875	0.176
48	1.745	1.222	0.779	0.154
54	1.612	1.010	0.645	0.138
60	1.428	0.878	0.547	0.125
66	1.112	0.658	0.412	0.112
72	0.956	0.456	0.327	0.107

Minimum inhibitory concentration (MIC)

The identified strain Pseudomonas putida showed the survival limit up to 100-400 mg/l hexavalent chromium concentration. The minimum inhibitory concentration of selected Pseudomonas putida strain showed high-level resistance against potassium dichromate in peptone-yeast extract agar media (Table 3).

Effect of temperature on the reduction of hexavalent chromium

For the effective degradation of hexavalent chromium present in the tannery effluent, optimum

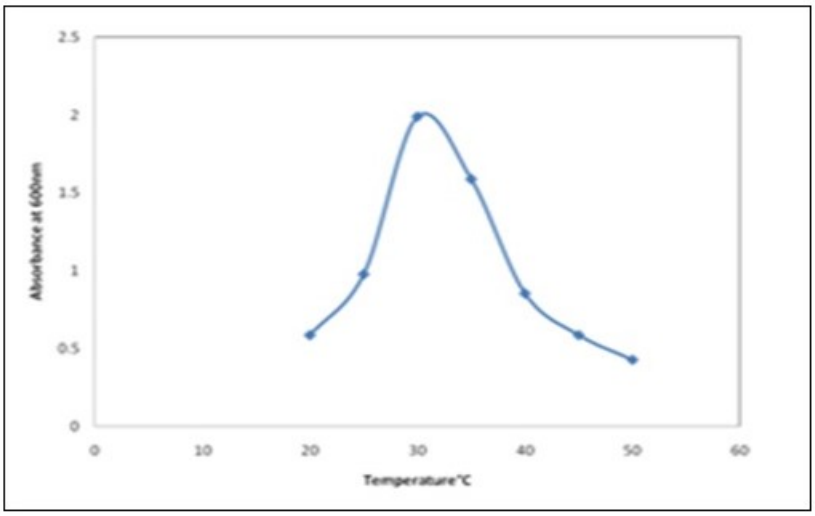

Fig. 3 — Effect of temperature on the reduction of hexavalent chromium

temperature is found to be 30°C for the Pseudomonas putida and consortium of bacteria. It shows maximum growth at 30°C for overnight incubation. Growth of microorganisms in the tannery effluent is estimated at 600nm in UV spectrophotometer (Figure. 3).

Effect of pH on the reduction of hexavalent chromium

For the effective degradation of hexavalent chromium present in the tannery effluent, optimum pH is found to be 7 for the Pseudomonas putida and consortium of bacteria. It shows maximum growth at 7 for overnight incubation. Various pH value is set by using NaOH and HCl solutions, and pH is maintained by using phosphate buffer (The phosphate buffer has been prepared by combination of mono potassium hydrogen phosphate and di hydrogen phosphate). Growth of microorganisms in the tannery effluent is estimated at 600nm in UV spectrophotometer (Figure. 4).

Effect of agitation velocity on the reduction of hexavalent chromium

For the effective degradation of hexavalent chromium present in the tannery effluent, optimum agitation velocity value is found to be 100 rpm for the Pseudomonas putida and consortium of bacteria. It shows maximum growth at 100 rpm for overnight incubation. Agitation velocity was varied by using incubating shaker. Growth of microorganisms in the tannery effluent is estimated at 600nm in UV spectrophotometer (Figure. 5).

Effect of time on the reduction of hexavalent chromium

For the effective degradation of hexavalent chromium present in the tannery effluent, optimum time is found to be 12 hrs for the Pseudomonas putida and consortium of

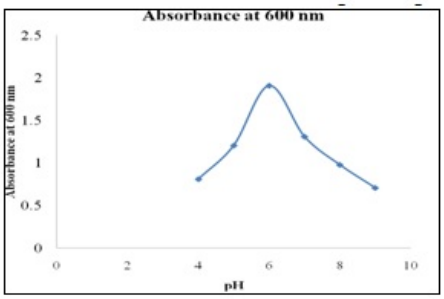

Fig. 4 — Effect of pH on the reduction of hexavalent chromium

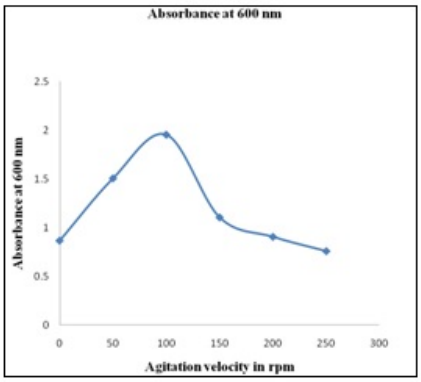

Fig. 5 — Effect of agitation velocity on the reduction of hexavalent chromium

bacteria. It shows maximum growth at 12 hrs for overnight incubation. Growth of microorganisms in the tannery effluent is estimated at 600nm in UV spectrophotometer (Figure. 6).

Reduction of hexavalent chromium present in tannery effluent by pseudomonas putida

Reduction efficiency of hexavalent chromium by Pseudomonous Putida is found to be 90.412 % under

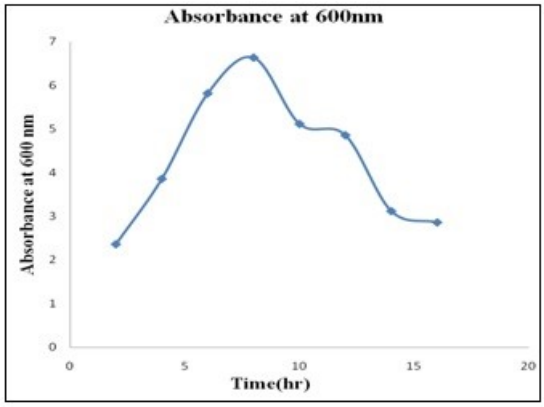

Fig. 6 — Effect of time on the reduction of hexavalent chromium

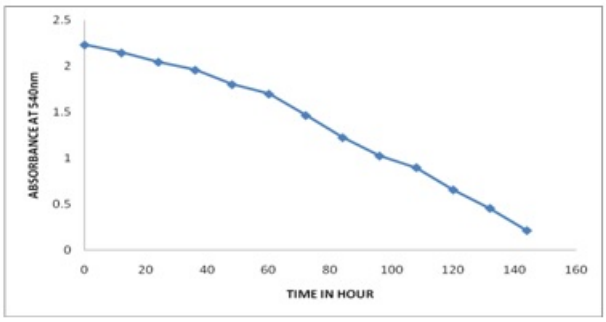

Fig. 7 — Graphical representation for the reduction of Cr (VI) by Pseudomonas Putida at 540 nm

Table 4 — Reduction of Cr(VI) by *Pseudomonas putida*at 540nm

Time (hr)	Absorbance at 540 nm
0	2.232
12	2.145
24	2.045
36	1.958
48	1.801
60	1.698
72	1.465
84	1.224
108	0.898
120	0.657
96	1.025
132	0.456
144	0.214

the optimum parametric conditions. Hexavalent chromium reduction has been estimated by diphenylcarbazide method at 540 nm (Table 4) in a UV spectrophotometer (Figure. 7).

Estimation of chromium reduction percentage by pseudomonas putida

Reduction percentage of hexavalent chromium is estimated by using the following formula

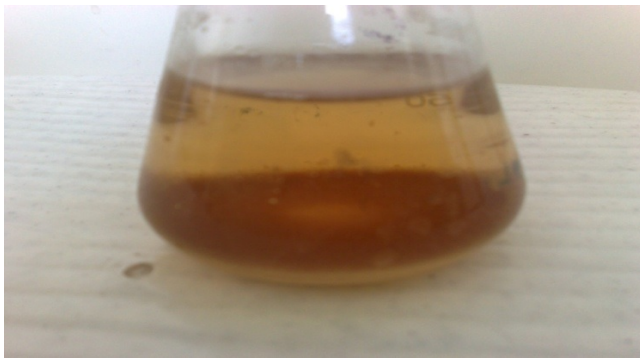

Fig. 8 — Chromium in the treated tannery effluent is settled at the bottom by chemical precipitation

Table 5 — Concentration of Hexavalent Chromium in the effluent treated by *Pseudomonas putida*

Element	Untreated Effluent (mg/l)	Treated Effluent (mg/l)	Permissible Limit (mg/l)
Hexavalent Chromium	28	2.8	4

$$Chromium\ removal(\%) = \frac{Initial\ absorbance - final\ absorbance}{Absorbance\ of\ uninoculated\ of\ fluent} x\ 100$$

$$Chromium\ removal(\%) = \frac{2.323 - 0.214}{2.232} = 90.412\%$$

The concentration of hexavalent chromium present in treated and untreated effluent by Pseudomonas putida is given in Table 5.

Separation of chromium from tannery effluent

Reduced chromium in the tannery effluent has been separated by adding alkali (sodium hydroxide and potassium hydroxide) in to it. Precipitated chromium is settled at the bottom over a period of time (Fig. 8).

Conclusion

Bioremediation is the cost-effective method for the detoxification of hexavalent chromium while compared to the other conventional methods like membrane filtration, ion exchange and bio-sorption.The bacterial isolate has been identified using 16s rRNA sequencing as Pseudomonas putida. Isolated Pseudomonas putida shows the minimum inhibitory concentration of 400 mg/l of Cr (VI). The effective degradation ofCr (VI) present in the tannery effluent takes place at the optimum conditions of (i) Temperature -30 °C, (ii) pH - 7, (ii) Time - 12 hrs, (iv) Agitation velocity - 100 rpm. Under these optimum parametric conditions, reduction of Cr (VI) by isolated Pseudomonas putida has been found to be 90 %. Finally, reduced chromium is separated from the tannery effluent by chemical

precipitation method. The chromium reduction percentage has been observed maximum in the isolate pure culture Pseudomonas putida. Treated tannery wastewater contains Cr (VI) concentration below 4 mg/l. According to the Indian Standard (IS), the treated tannery wastewater is well within the permissible limit.

References

1 Seema, S. Hexavalent chromium reduction in tannery effluent by Bacterial species Isolated from Tannery effluent contaminated soil. Journal of Environmental Science and Technology 5(3): (2012), 142-154.

2 Addis, M. Determination of Cr(VI) and Cr(III) in the tannery effluents of awash and addisababa leather industries, Addisababa university, department of chemistry, (2006).

3 Javed, A. Pollution Level Analysis in Tannery Effluents Collected From Three Different Cities of Punjab. Journal of industrial pollution control board, (2004).

4 Yangianchen, G. Bioremediation of Cr(VI) and Immobilization as Cr(III) by Ochrobactrumanthropi. Journal of Environental Science and Technology, 44: (2010), 6357–6363.

5 Durga, Devi, B. Bioremeoval of hexavalent chromium, using Pseudomonas Fluorescens. Journal of biotechnology and microbiology research, (2012).

6 Abhipsa, S. Enzymatic reduction of hexavalent chromium in bacteria, ENVIS CENTRE Newsletter, 7(2): (2009).

7 Ganguli, A. Chrome Reducing Ability of Pseudomonas Aeruginosain Indus-trial Effluents, Lettersin Applied Microbiology, 28(1): (1999).

8 Lovely, D.R. and Phillips E.J. Reduction of Chromate by Desulfovibrio Vulgaris and Its C3 Cytochrome. Applied and Environment Microbiology, l (2): (1994).

9 Ohtake, H. and Fujii, E. Reduction of Toxic Chromate in Industrial Effluent by Use of Chromate Reducing Strain of Enterobacter Cloacae. Environmental Science and Technology, 11(1): (1990).

10 Johnstone, C. Bacterial Reduction of Hexavalent Chromium by Enterobacter Cloacae Strain HO1Grownon Sucrose, Biotechnology letters. 19(7): (1997).

11 Wang, P.C. Isolation and Characterization of En-terobacter Cloacae Strain that Reduces Hexavalent Chromium under Anaerobic Conditions. Applied and Environment Microbiology, 55(7): (1989).

12 Garbisu, C. Aerobic Chromate Reduction by Bacillus Subtilis. Biodegra-dation. 9(2): (1998).

13 Philip, L. Cr (VI) Reduction by Bacillus Coagulans Isolated from Contaminated Soils. Journal of Environmental Engineering. 124 (12): (1998).

14 Venkatesan, G. & Subramani, T. Environmental degradation due to the Industrial Wastewater discharge in Vellore District, Tamil Nadu, India. Indian Journals of Geo Marine Sciences 47(11): (2018), 2255-2259.