

# Bioremediation of chromium(VI) by *Stenotrophomonas maltophilia* isolated from tannery effluent

N. M. Raman<sup>1</sup> · S. Asokan<sup>1</sup> · N. Shobana Sundari<sup>1</sup> · S. Ramasamy<sup>1</sup>

Received: 21 July 2016/Revised: 5 April 2017/Accepted: 20 June 2017/Published online: 10 July 2017  
© Islamic Azad University (IAU) 2017

**Abstract** Bioremediation of chromates using bacteria primarily involves the removal/reduction of heavy metals in effluent using indigenous micro-organisms such as chromium reducing bacteria as biosorbents for cleaner and healthier environment. In the present study, the removal of hexavalent chromium by micro-organisms isolated from acclimatized tannery effluent was investigated. Biochemical assays and molecular sequencing revealed strain SRS05 to be *Stenotrophomonas maltophilia*. Resistance to chromium was determined by agar and broth dilution assays followed by determination of minimal inhibitory concentration. Strain SRS05 was able to resist 400 mg/ml of chromium which reflects that the heavy metal could be utilized by the micro-organism for its growth. Results by atomic absorption spectroscopy, Fourier transform infrared spectroscopic analysis and scanning electron microscopy revealed effective biosorption of chromium by *S. maltophilia* SRS05 with no intracellular changes morphologically indicating the stability of the organism in the presence of chromium. It is therefore recommended that this bacterium can be used widely for remediation of hexavalent chromium although the genetic basis for observations concluded in this study is to be confirmed.

**Keywords** Acclimatization · Chromium reduction · Chromium uptake · Diphenylcarbazide · Hexavalent chromium · Industry · Spectroscopy

## Introduction

The indiscriminate release of heavy metals from various industries has led to its increased accumulation in water bodies and soil. The waters released from various industries as a result of industrial revolution in the country have affected soil and plants, thus directly and indirectly having an enormous medical implication on humans (Salas et al. 2000). Various procedures such as chemical precipitation, oxidation, reduction, reverse osmosis and membrane technology are now being utilized for treatment of contaminated waters and for the removal of toxic heavy metals (Dixit et al. 2015). Other detoxifying mechanisms include biotransformation and biomineralization that can be used either in situ or ex situ (Malik 2004).

Chromium is a refractory metal that exhibits two inorganic states namely trivalent (Cr(III)) and hexavalent (Cr(VI)). Despite being an essential mineral directly involved in carbohydrate, fat and protein metabolism, the hexavalent state of chromium has largely been accepted to be toxic due to its solubility in water, interaction with cellular proteins and biological membrane permeability (Sultan and Hasnain 2005). These processes are initiated with chromate acting as an electron acceptor leading to severe environment-related gastrointestinal problems. The waters in and around Coimbatore, Tamil Nadu, India, have seen an empirical increase in the amount of heavy metals dispersed mainly through industrial effluents (Mohankumar et al. 2016). Chromium has been indicated to be a pollutant in various sites and waters including tannery effluents (Alhossny and Avudainayagam 2009).

The biological approach to the degradation of heavy metals has been an important case study for bioremediation via biosorption of these metals using indigenous micro-organisms (Katiyar and Katiyar 1997; Xie et al. 2010) due

Editorial responsibility: Tanmoy Karak.

✉ S. Ramasamy  
sugantham2000@gmail.com

<sup>1</sup> Department of Biotechnology, Dr. G. R. Damodaran College of Science, Coimbatore, Tamil Nadu 641 014, India

to the inherent benefits with respect to social, economic and environment (Kumar and Gopal 2015). The application of resistant bacteria for this purpose has been involved with its growth and morphological parameters. The present study deals with isolation and identification of bacteria acclimatized in tannery effluent to be used in remediation of chromium during the period of September 2012–April 2014 at Department of Biotechnology, Dr. G. R. Damodaran College of Science, Coimbatore, Tamil Nadu, India.

## Materials and methods

### Effluent sample collection and analysis

Tannery effluent was collected from leather tanneries near Erode, Tamil Nadu, India. The sample was stored at 4 °C to arrest biological activity, and the colour and pH of the effluent were recorded. The water sample was filtered using Whatman No. 1 filter paper and given for water analysis at South India Research Association, Coimbatore.

### Isolation and identification of chromium resistant bacteria from tannery effluent

All the agar media used in the present study were procured from HiMedia Laboratories, India. The tannery effluent sample was diluted in sterile distilled water, and the dilutions from  $10^{-2}$  to  $10^{-8}$  were plated on Luria–Bertani (LB) medium and incubated at 37 °C for 3 days. The selected yellow-pigmented colonies were then grown and maintained on tryptone soy agar medium. A presumptive identification was performed by biochemical tests (Bernardet and Bowman 2006) and confirmed by 16s rRNA sequencing.

### Antibiotic sensitivity assay

The antibiotic sensitivity assay of the identified strain under the study was examined by disc diffusion method (Bauer et al. 1966).

### Minimal inhibitory concentration (MIC) of chromium against *Stenotrophomonas maltophilia* SRS05

Minimal inhibitory concentration of chromium against *S. maltophilia* SRS05 is determined by tube dilution method. A set of twelve sterile test tubes were taken, nine of which were marked and the rest three were assigned as  $T_M$  (Medium),  $T_{MC}$  (Medium + Compound),  $T_{MI}$  (Medium + Inoculum). Serial dilution of the chromium from the stock solution was carried out in the nine tubes, and all tubes were incubated at 37 °C for 24 h.

## Evaluation of chromium tolerance

The isolated bacterium *S. maltophilia* SRS05 was tested for their resistance to chromate by agar dilution method and broth dilution method. Overnight culture of *S. maltophilia* SRS05 was aseptically streaked into freshly prepared TSB amended with potassium dichromate at various concentrations ranging from 5 to 400 mg/ml. The agar plates and broth tubes were incubated at 37 °C for 72 h.

### Determination of chromium uptake and reducing activity by the bacterium

Chromium uptake and reducing activity was examined using 1,5-diphenylcarbazide (DPC) method at 540 nm in live and killed cells.

Determination of chromium uptake and reducing activity by the bacterium *S. maltophilia* SRS05 (24-h-old culture) was carried out in TSB broth. To 100 ml of TSB, 100 µg/ml of potassium dichromate was added and sterilized. To the first set, 10 ml of overnight broth culture (live cells) was added, whereas 20 ml of 24 h culture autoclaved at 121 °C for 15 min (killed cells) was added to the second set. Chromium uptake and reducing activity were examined using DPC method at 540 nm (Saranraj et al. 2010).

Reduction of chromium was determined by growing the *S. maltophilia* SRS05 in TSB broth supplemented with potassium dichromate at a concentration of 50 µg/ml. Supernatant obtained after centrifugation of cells was grown on a shaker incubator (150 rpm) for 24 h. Chromate reduction activity was estimated by DPC method with 5% (w/v) prepared in acetone. To 1 ml of supernatant, 1 ml of phosphate buffer (pH 7.2) with chromium was added and incubated for 1 h. After incubation, 1 drop of 0.1 M sulphuric acid and 0.4 µl of 1,5-diphenylcarbazide were added and measured colorimetrically at 540 nm (Saranraj et al. 2010).

### Statistical methodology

The Cr(VI) reduction potential of *S. maltophilia* SRS05 was assessed in a liquid medium containing (g/l): lactose 10, soy bean meal 10, NaCl 5,  $K_2HPO_4$  2.5,  $K_2Cr_2O_7$  1000 µg, pH 7, temperature 35 °C, time and inoculum volume. The composition of reduction of medium varied according to the design matrix. Plackett–Burman design (Plackett and Burman 1946) was used to screen and evaluate the important medium components that influence the response. Four independent variables termed to have a positive effect according to Plackett–Burman design were studied at different levels to obtain the response surface methodology for bioremediation of Cr(VI) ions. The present study involves four-level, three-factorial Box–Behnken experimental design (Box and Behnken 1960) which constituted of 29 experiments.



## Atomic absorption spectrometry (AAS)

For the determination of hexavalent chromium in tannery effluent, 100 ml of filter sterilized tannery effluent was taken in a conical flask to which the killed cells were added. The sample was then submitted at Chemical laboratory, SITRA (South Indian Textile Research Association), Coimbatore, for the analysis of chromium content. Similar samples were prepared for the determination of chromium reduction in tannery effluent. Live cells were added to the tannery effluent without autoclaving the culture and incubated in orbital shaker at 150 rpm with 37 °C. Chromium reduction was assessed on atomic absorption spectroscopy method for 20 days at 5-day interval.

## FT-IR spectrum acquisition and scanning electron microscopy (SEM)

In this study, a cumulative effect of untreated (*S. maltophilia* SRS05 grown on TSB for 24 h); *S. maltophilia* SRS05 treated with 100 µg/ml of chromium and effluent containing 245 µg/ml of chromium treated *S. maltophilia* SRS05 was observed. Finely ground lyophilized bacterial cell samples were pressed by a manual hydraulic press into spectroscopic quality KBr pellet with a sample/KBr ratio of about 1/100 and scanned using an FT-IR spectrometer (FT-IR 1600, PerkinElmer). For SEM analysis, the cells were harvested by centrifugation at 6500g for 15 min and fixed using 2.5% (v/v) aqueous glutaraldehyde for 2 h. These cells were dehydrated using a gradient of ethyl alcohol (10–100%) and a final wash was done with absolute ethyl alcohol. The dried cells were lyophilized and subjected to scanning electron microscopy (HITACHI S—4500).

## Result and discussion

### Physiochemical analysis

Industrial effluent was collected and its physiochemical parameters are analysed (Table 1). Specific detailing reveals 490 mg/l of chromium to be present in the effluent which is more than 200% higher than the permissible discharge limit of 2 mg/l. The pH in the effluent is towards the lower value indicating acidic conditions that render metals to solubilize in water creating a hazardous environment. The measure of the inorganic salts and other substances discharged into the effluent indicated by the TDS value reflects salinity problems that generate carcinogenic effects to the aquatic and human populations residing in the specified area. The high levels of BOD and COD indicate high strength of pollution in the waters that generate lower

**Table 1** Physicochemical analysis of tannery effluent

| S. no. | Property                              | Sample analysis |
|--------|---------------------------------------|-----------------|
| 1      | Colour                                | Hazen (17075)   |
| 2      | pH at (30 °C)                         | 5.88            |
| 3      | Dissolved oxygen                      | 4.56            |
| 4      | Biological oxygen demand (BOD) (mg/l) | 992             |
| 5      | Chemical oxygen demand (COD) (mg/l)   | 9869            |
| 6      | Total dissolved solid (TDS) (mg/l)    | 10,448          |
| 7      | Temperature (°C)                      | 27              |
| 8      | Electrical conductivity               | 14.18           |
| 9      | Total carbon (mg/l)                   | 3700.88         |
| 10     | Chromium with effluent control (mg/l) | 490             |

oxygen supply to the microflora. The higher concentration of chemical oxygen demand also indicates the presence of large amount of resistant organic and inorganic substances in the effluent.

### Characterization of *S. maltophilia*

The bacteria isolated from the tannery effluent were mostly Gram-negative rods. Among all the strains, yellow-pigmented colonies showing positive reactions for citrate, catalase, KOH assay, DNase, decarboxylation, esculin, chitin hydrolysis and chromium tolerance capability, one of the isolates SRS05 was selected for further study. Sequencing and phylogenetic analysis of the strain SRS05 revealed similarity to *S. maltophilia* strain (IAM 12423). The next closest homologue was found to be *S. maltophilia* R551-3 strain. The sequenced strain SRS05 was found to be *Stenotrophomonas maltophilia* and was submitted to GenBank and obtained accession number KF558319.

### Antibiotic sensitivity assay

The strain of *S. maltophilia* SRS05 analysed in this study were resistant to antibiotics such as tetracycline, polymyxin, vancomycin, methicillin, amikacin, gatifloxacin, gentamycin, tobramycin, ampicillin, rifampicin, streptomycin, kanamycin, ofloxacin, enrofloxacin. However, *S. maltophilia* SRS05 was sensitive to chloramphenicol, ciprofloxacin, trimethoprim and imipenem (Table 2). The breakpoints of each antibiotic was taken and confirmed with respect to the resistance and susceptibility values of the zone of inhibition (a clear halo).

### Minimum inhibitory concentration of the strains

Resistance of *S. maltophilia* SRS05 to chromium was determined by tube dilution method with three different

**Table 2** Antibiotic sensitivity assay of *Stenotrophomonas maltophilia* SRS05

| S. no. | Antibiotics     | Disc concentration (mcg) | Susceptibility |
|--------|-----------------|--------------------------|----------------|
| 1      | Tetracycline    | 30                       | R              |
| 2      | Chloramphenicol | 10                       | S              |
| 3      | Tobramycin      | 10                       | R              |
| 4      | Gentamycin      | 10                       | R              |
| 5      | Streptomycin    | 10                       | R              |
| 6      | Ciprofloxacin   | 5                        | S              |
| 7      | Ampicillin      | 2                        | R              |
| 8      | Rifampicin      | 5                        | R              |
| 9      | Ofloxacin       | 5                        | R              |
| 10     | Imipenem        | 10                       | R              |
| 11     | Enrofloxacin    | 5                        | R              |
| 12     | Penicillin      | 2 units                  | R              |
| 13     | Kanamycin       | 30                       | R              |
| 14     | Polymyxin       | 50                       | R              |
| 15     | Piperacillin    | 100                      | R              |
| 16     | Vancomycin      | 30                       | R              |
| 17     | Methicillin     | 5                        | R              |
| 18     | Trimethoprim    | 10                       | S              |
| 19     | Amikacin        | 30                       | R              |
| 20     | Gatifloxacin    | 5                        | R              |

R resistance, S sensitive

concentration of chromium (200, 400 and 600 µg/ml). Good growth was seen in concentration up to 400 µg/ml beyond which growth was inhibited. The MIC of *S. maltophilia* SRS05 against chromium was determined to be 400 µg/ml (Table 3).

**Table 3** MIC of the *Stenotrophomonas maltophilia* SRS05 against different concentration of chromium

| S. no.   | Inoculum added in 1 ml of nutrient broth (µl) | Concentration of chromium (600 µg/ml) |                            | Concentration of chromium (400 µg/ml) |                            | Concentration of chromium (200 µg/ml) |                            |
|----------|---|---------------------------------------|----------------------------|---------------------------------------|----------------------------|---------------------------------------|----------------------------|
|          |   | Dilution of chromium (µg/ml)          | Growth observed against Cr | Dilution of chromium (µg/ml)          | Growth observed against Cr | Dilution of chromium (µg/ml)          | Growth observed against Cr |
| 1        | 10  | 600                                   | –                          | 400                                   | +                          | 200                                   | +                          |
| 2        | 10  | 300                                   | +                          | 200                                   | +                          | 100                                   | +                          |
| 3        | 10  | 150                                   | +                          | 100                                   | +                          | 50                                    | +                          |
| 4        | 10  | 75                                    | +                          | 50                                    | +                          | 25                                    | +                          |
| 5        | 10  | 37.5                                  | +                          | 25                                    | +                          | 12.5                                  | +                          |
| 6        | 10  | 18.5                                  | +                          | 12.5                                  | +                          | 6.25                                  | +                          |
| 7        | 10  | 9.325                                 | +                          | 6.25                                  | +                          | 3.175                                 | +                          |
| 8        | 10  | 4.687                                 | +                          | 3.175                                 | +                          | 1.562                                 | +                          |
| 9        | 10  | 2.34                                  | +                          | 1.562                                 | +                          | 0.781                                 | +                          |
| $T_{MC}$ | 0   | 600                                   | –                          | 400                                   | –                          | 200                                   | –                          |
| $T_{MI}$ | 10  | –                                     | +                          | –                                     | +                          | –                                     | +                          |
| $T_M$    | 0   | –                                     | –                          | –                                     | –                          | –                                     | –                          |

+ indicates growth, – indicate no growth

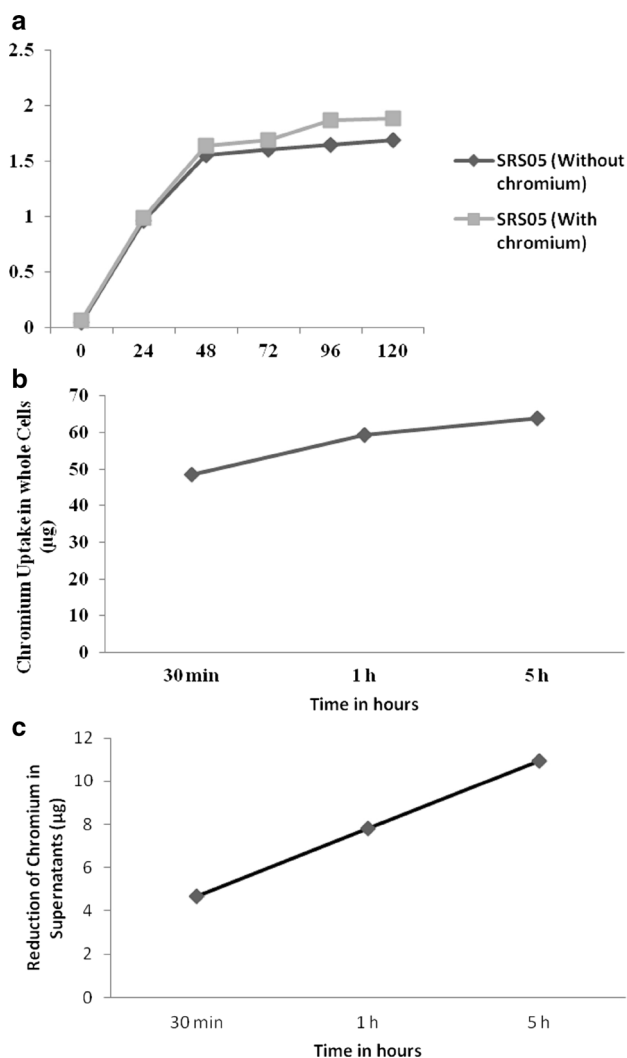
## Evaluation of chromium tolerance

In agar dilution method, the bacterial isolate *S. maltophilia* SRS05 was resistant to chromium at 200 µg/ml, whereas growth was found to be inhibited at concentration higher than the 400 µg/ml in broth dilution assay. The results indicated that *S. maltophilia* SRS05 had a higher degrading capacity of chromium. The wide difference in these results might be attributed to the fact that the growth conditions are more favourable in the broth due to better aeration and agitation conditions when compared to an agar plate. Similar studies undertaken by Saranraj et al. (2010) showed a uptake of chromium by enterococci although their reduction results were not significant. Such assays involving agar- and broth-based reduction were also discussed in aerobic heterotrophic bacteria (Satarupa and Paul 2013) and Lactobacilli (Mishra et al. 2012).

## Chromium uptake and reduction

The growth of the isolates was studied in the presence and the absence of Cr(VI). The optical density of *S. maltophilia* SRS05 was determined at 600 nm. The growth of the isolate is much better in the presence of Cr(VI) in the medium which is clearly shown in Fig. 1a.

Bioadsorption studies were done to test the ability of *S. maltophilia* SRS05 to accumulate chromium at different time intervals 30 min, 1 and 5 h. These studies were determined by deducing the levels of chromium uptake and reduction in the cellular biomass and media supernatant as described in “Materials and methods” section. The rate of chromium accumulation was rapid indicating better chromium uptake in a short period of time (Fig. 1b). The ability



**Fig. 1** Bioadsorption studies indicating the effect of Cr(VI) on the growth of *Stenotrophomonas maltophilia* SRS05. **a** Growth of *Stenotrophomonas maltophilia* SRS05 in chromium containing medium; **b** chromium uptake at different time intervals; **c** chromium reduction at different time intervals

of *S. maltophilia* SRS05 to reduce chromium at various time intervals from 30 min, 1 and 5 h was 5, 7.8, 10.9 µg/ml of chromium, and the chromium reduction is shown in Fig. 1c indicating increase in chromium reduction with increasing time intervals. These results indicates *S. maltophilia* SRS05 could be used as a promising agent for the removal of hexavalent chromium in effluents as the higher presence of chromium in the cellular pellet when compared to the supernatant indicates the utilization of chromium for its metabolic activities.

In the present study, the chromium levels were found to be higher in the cell biomass when compared to media supernatant. These results indicated that the uptake of chromium was higher than its reduction. The higher presence of chromium in cell biomass indicates that the

organism utilizes the chromium for its metabolic activities, and hence, it is present in higher quantities in the cell pellet. Kader et al. (2007) showed that the rate of chromium accumulation by active cells was also faster compared to chromium reduction. Shahida and Thakur (2007) found that *Brevibacterium* sp. showed higher chromium uptake than the pellet of killed cells when time intervals increased. The chromium remediation might be due to excellent potential of metal biosorption as the potential in metal recovery and remediation may be due to binding of other metals along with chromium. The bioremediation process of the above study concluded that the *S. maltophilia* SRS05 could be used as a promising agent for the removal of Cr(VI) in effluents.

**Evaluation of culture conditions affecting Cr(VI) reduction by *S. maltophilia* SRS05**

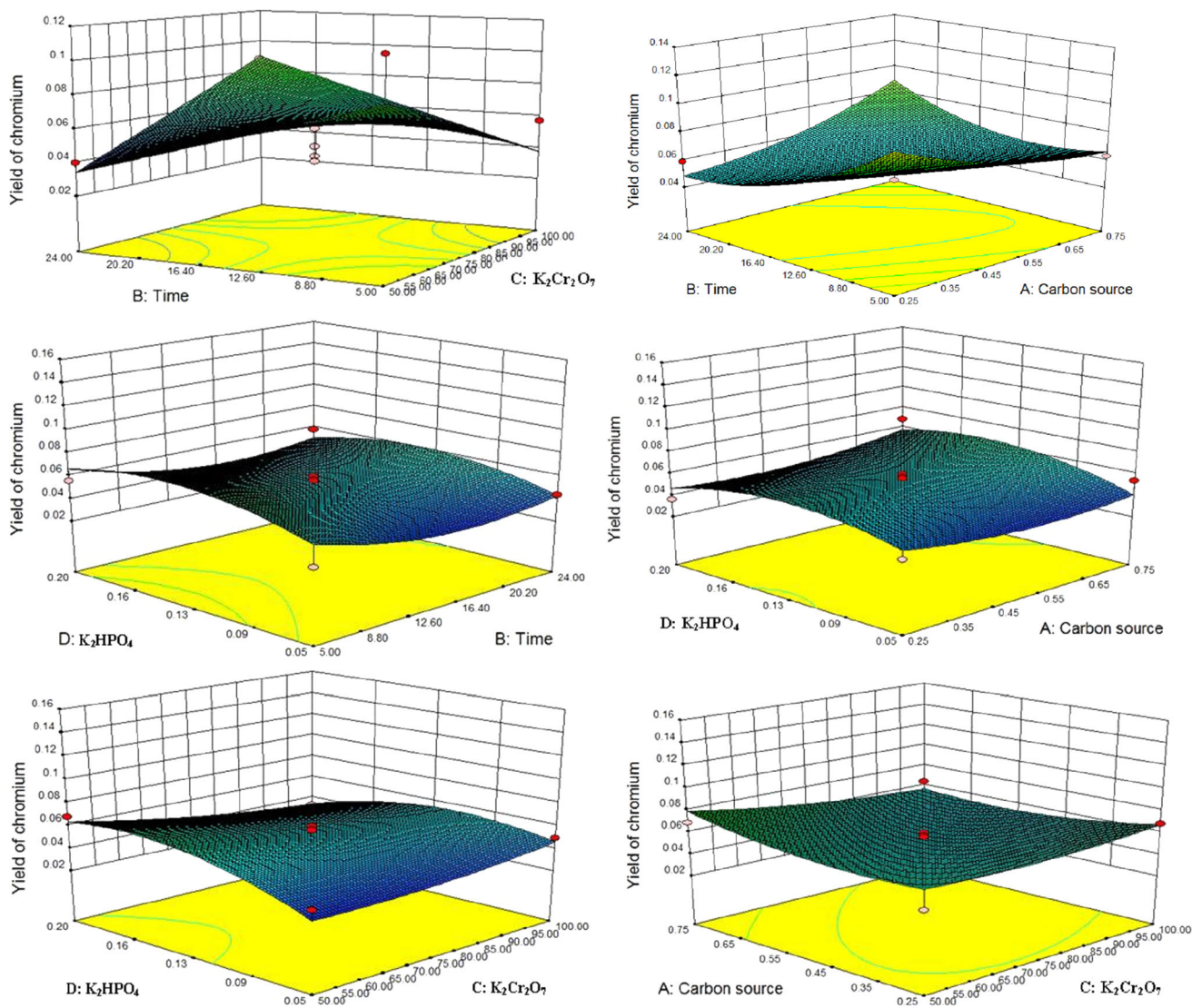
The analysis showed that carbon source (lactose), K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, time and K<sub>2</sub>HPO<sub>4</sub> within the test range had a positive effect on Cr(VI) reduction, whereas nitrogen source (soy bean meal), NaCl and temperature contributed negatively. Some researchers thought that the variables with confidence level above 80% (Pujari and Chandra 2000) or 85% (Xiong et al. 2004) were significant. Box–Behnken design matrix and RSM experiments were used to optimize the process of bioremediation of Cr(VI) ions using *S. maltophilia* SRS05. Four significant parameters carbon source, time, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and K<sub>2</sub>HPO<sub>4</sub> were studied. The domain factor and level selected for designing the Box–Behnken design are presented in Fig. 2.

The regression equation coefficients were calculated, and the result revealed that the response, i.e. Cr(VI) bioremediation fitted to the second-order polynomial equation. Significance of each coefficient was determined by Student’s *t* test and *p* values.

The independent variables carbon source, time, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and K<sub>2</sub>HPO<sub>4</sub> are coded as *A*, *B*, *C* and *D*, respectively, in the present study. The second-order polynomial function was fitted to correlate the relationship between independent variables and the response for prediction of the optimum point conditions.

$$Y = ao + aiA + aiB + aiC + aiD + aiA^2 + aiB^2 + aiC^2 + aiD^2 + aiA * B + aiA * C + aiA * D + aiB * C + aiB * D + aiC * D$$

The quality of polynomial model equation was expressed statistically by the coefficient of determination (*R*<sup>2</sup>), and its statistical significance was determined by using *F* test. Each experimental design was carried out in triplicates. *T* test was used to find the significance of the regression coefficients. The residual error, pure error and



| Bacterial strain | Source          | Sum of Squares | d.f. | Mean square | F-Value  | P-Value | Probe>F         |
|------------------|-----------------|----------------|------|-------------|----------|---------|-----------------|
| SRS 05           | Model           | 0.008481       | 10   | 0.000848    | 3.098125 | 0.0178  | Significant     |
|                  | Residual        | 0.004928       | 18   | 0.000274    |          |         |                 |
|                  | Lack-of-fit     | 0.004489       | 14   | 0.000321    | 2.922762 | 0.155   | not Significant |
|                  | Pure error      | 0.000439       | 4    | 0.00011     |          |         |                 |
|                  | residual square | 0.632512       |      |             |          |         |                 |
|                  | Adj -R square   | 0.428353       |      |             |          |         |                 |

**Fig. 2** 3D contour plot shows the interactive effect of time, chromium, carbon source and phosphates on Cr(VI) biodegradation by *Stenotrophomonas maltophilia* SRS05

lack of fit were calculated from repeated measurements (Myers and Montgomery 2002; Reddy et al. 2009). The desirable response was selected as maximum percentage

Cr(VI) bioremediation at optimum carbon source, time,  $K_2Cr_2O_7$  and  $K_2HPO_4$  concentration. The relationship between response and experimental levels for each of the



**Table 4** Bioremediation of Cr(VI) in tannery effluent analysed by AAS

| Initial Cr(VI) concentration (mg/l) in tannery effluent | Days of incubation | Hexavalent Cr(VI) concentration after remediation (mg/l) |
|---|--------------------|--|
| 490   | –                  | –  |
| 490   | 5                  | 396  |
| 490   | 10                 | 317  |
| 490   | 15                 | 260  |
| 490   | 20                 | 260  |

factors could be observed as fitted polynomial equation in form of surface plots. The interactive effects of  $K_2Cr_2O_7$  (C: 50–100  $\mu$ g) and carbon source (A: 0.25–0.75 g) on biodegradation of Cr(VI) using *S. maltophilia* SRS05 show increased concentration of  $K_2Cr_2O_7$  and carbon source yield decreased the biodegradation of chromium as the bacterial growth in the culture medium was decreased with increasing concentration of  $K_2Cr_2O_7$  and carbon source. Therefore, the bacterial metabolic uptake of  $K_2Cr_2O_7$  is less.

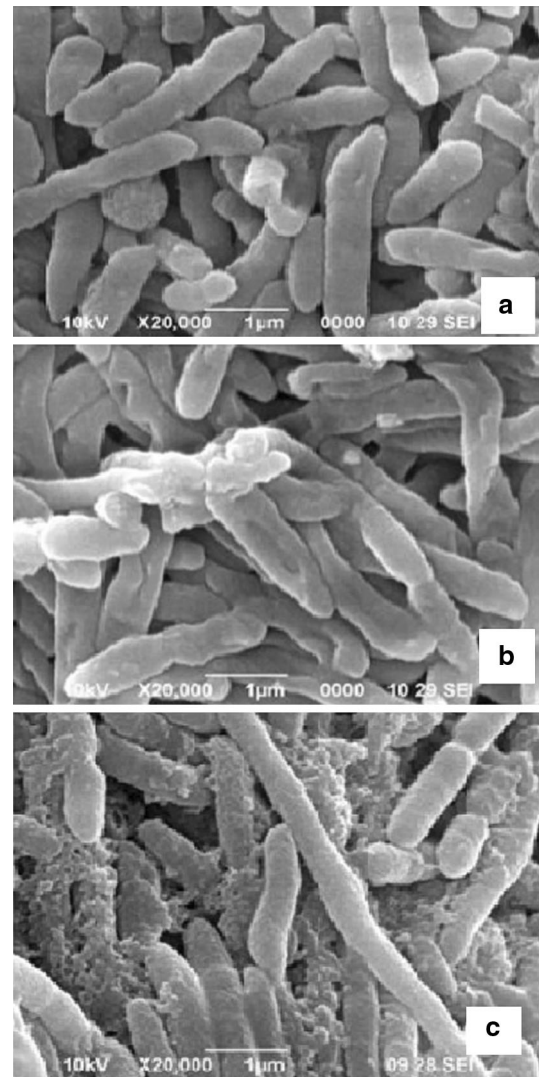
### Bioremediation of tannery effluent

Over the period of time, the bacterial growth and bioremediation of Cr(VI) by *S. maltophilia* SRS05 were maximum in 9th day in the dilution of 9.5 ml TSB medium +0.5 ml effluent, because the organism utilized the minimum volume of effluent as a nutrient supplement (i.e. 0.5 ml effluent in 9.5 ml TSB medium). The 3rd day sample showed bacterial growth and reduction in the concentration of Cr(VI) as compared with control. There was difference in the bacterial growth and chromium(VI) concentration in the 6th day sample when compared to the 9th day sample. The 3rd day to 9th day sample showed bacterial growth and reduction in Cr(VI) concentration with a high growth and reduction in concentration in the 9th day sample when compared with the control. These observations therefore confirm Cr(VI) reduction was growth related (Sultan and Hasnain 2007).

### Atomic absorption spectrometry (AAS)

Processed tannery effluent was submitted to SITRA, Coimbatore, for the determination of chromium reduction in atomic absorption spectrophotometer. *S. maltophilia* SRS05 reduced chromium(VI) under aerobic conditions (Table 4).

The bioremediation of Cr(VI) was analysed by atomic absorption spectroscopy comparing the degradation in the test sample (tannery effluent + live cells) against the control (tannery effluent + killed cells) at regular intervals.



**Fig. 3** SEM of untreated *Stenotrophomonas maltophilia* SRS 05 in TSB (a) and the bacterium in the presence of chromium (b) and effluent (c)

The 5th day sample showed reduction in the concentration of Cr(VI) as compared with control. There was difference in the Cr(VI) concentration in the 10th day sample when compared to the 15th day sample. The 5–15th day sample showed reduction in Cr(VI) concentration with a high reduction in concentration in the 15th day sample when compared with the control. There was no difference in the Cr(VI) concentration when compared to the 20th day sample because the bacteria attained stationary phase. The number of new cells created is limited by the growth factor, and as a result, the rate of cell growth matches the rate of cell death. The result is a smooth, horizontal linear part of the curve during the stationary phase. Damodaran et al. (2011) also evaluated the other heavy metals and chromium before and after treatment of indigenous micro-organisms using AAS.

## Scanning electron microscopy (SEM)

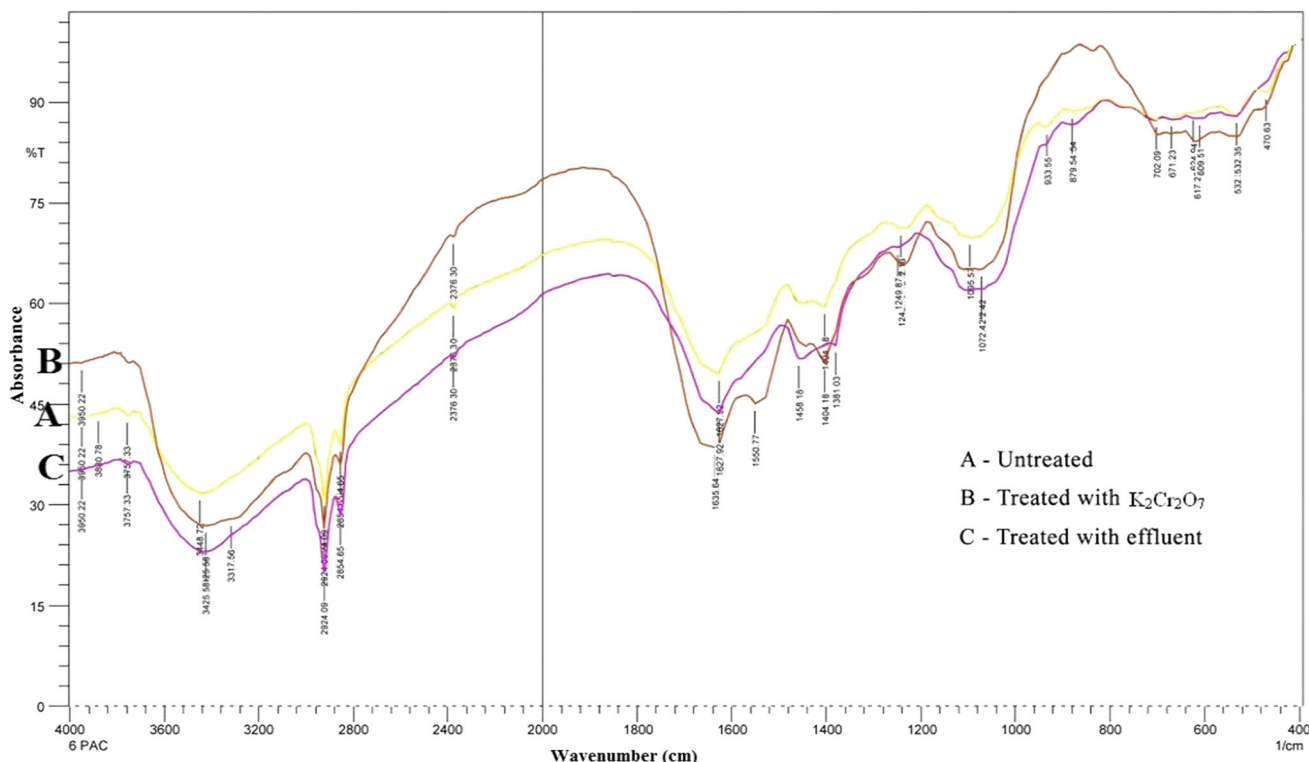
The growth characteristics of *S. maltophilia* SRS05 indicated rod or slightly curved rod-like features with smooth-walled cells. In this study, untreated *S. maltophilia* SRS05 grown on TSB for 24 h (control); *S. maltophilia* SRS05 treated with 100 µg/ml of chromium and effluent containing 24.5 µg/ml of chromium treated *S. maltophilia* SRS05 were observed by SEM. The SEM analysis conducted at a magnification of X3500, X8000, X15000 and X20,000, respectively, revealed intense layering of biofilm (Fig. 3).

After 18 h, the cells treated as control is observed as rod-shaped, smooth intact cell surface with no damages. However, in the bacteria treated with chromium, there are some irregular fragments on the adjoining cell surfaces indicating slight morphological changes on cellular surface. Degradation of the effluent in the presence of the bacterial culture was further confirmed using scanning electron microscope visually by rough cell surface although the rod-shaped characteristic of the cells remained intact. These results are in accordance with the findings of other reports (Sethuraman and Balasubramanian 2010; Thakur and Srivastava 2011) and slightly vary with the previous studies of Feng et al. (2000) and Kim et al. (2005) where morphological changes were observed on the bacterial cell.

## Changes in bond formations observed by FT-IR spectroscopy

The technique of FT-IR spectroscopy was chosen for this study to indicate any changes between treated and untreated bacteria during the process of biosorption with respect to functional groups. Significant proof of protonic exchange ( $H^+$ ) between amines, carboxyl and hydroxyl groups between the metal ions and cellular biomass was observed in the process of biosorption of hexavalent chromium using the isolated and identified bacterium (Fig. 4).

A detailed observation of the FT-IR spectra indicates a slight stretch corresponding to amide bond ( $4000\text{--}3500\text{ cm}^{-1}$ ), among  $-NH_2^+$  as well as  $-NH_3^+$  groups ( $2000\text{ cm}^{-1}$ ), carboxylic groups ( $1200\text{ cm}^{-1}$ ), ether and hydroxyl C–O between  $1700$  and  $900\text{ cm}^{-1}$  in accordance with Dean and Tobin (1999) and Yee et al. (2004). Strong and sharp adsorption bands were also observed pertaining to the presence of N–H bond ( $3600\text{--}3200\text{ cm}^{-1}$ ); C–H stretching of  $CH_2$  groups ( $2900\text{--}2850\text{ cm}^{-1}$ ); aliphatic ( $-CH_2$ ) groups ( $2925\text{ cm}^{-1}$ ). In addition to this, band observed between  $1650$  and  $1620\text{ cm}^{-1}$  indicates the protein peptide bond in the biomass in accordance with Chhikara et al. (2010). Peak intensities have been observed to be slightly different



**Fig. 4** Fourier transform infrared spectra of treated and untreated *Stenotrophomonas maltophilia* SRS05





between the untreated and treated populations especially in the region of 2400–2000  $\text{cm}^{-1}$ .

Wave numbers lower than 1500  $\text{cm}^{-1}$  show vibrations of the C=O of COO<sup>-</sup> from a amino acid at 1400  $\text{cm}^{-1}$  and lipid-based C=O bending vibrations at 530–460  $\text{cm}^{-1}$ . Further, polysaccharide-based C–O bond shift from 1030 to 1000  $\text{cm}^{-1}$  due to the biosorption process was observed along with a new peak formation in the region of 900–800  $\text{cm}^{-1}$  due to oxidated hexavalent chromium in accordance with Han et al. (2007) and Holman (2002), respectively.

## Conclusion

The present study indicated the isolated organism evolved a mechanism to grow and survive in contaminated waters. The response of other members of genus *Stenotrophomonas* has indicated variations in the heavy metal composition and its concentration in the analysis. The bioremediation process of the above study concluded that the *S. maltophilia* SRS05 could be used as a promising agent for the removal of Cr(VI) in effluents although this should also be validated on the basis of molecular characterization.

**Acknowledgements** This study has not been financed by any government or private funding agencies. We gratefully acknowledge Progen Biotech Pvt. Ltd, Salem, for their assistance in sequencing of the organism used in the study.

## References

- Alhossny AA, Avudainayagam S (2009) The kinetics of Cr(III) oxidation in dominant soils of Coimbatore district, Tamilnadu, India. *J Environ Res Dev* 4(2):417–420
- Bauer AW, Kirby WM, Sherris JC et al (1966) Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Pathol* 45:493–496
- Bernardet JF, Bowman JP (2006) The genus *Flavobacterium*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) *The prokaryotes: a handbook on the biology of bacteria*, 3rd edn, vol 7. Springer, New York, NY, pp 481–531
- Box GE, Behnken DW (1960) Some new three level designs for the study of quantitative variables. *Technometrics* 2(4):455–475
- Chhikara S, Hooda A, Rana L et al (2010) Chromium(VI) biosorption by immobilized *Aspergillus niger* in continuous flow system with special reference to FTIR analysis. *J Environ Biol* 31(5):561–566
- Damodaran D, Suresh G, Raj Mohan B (2011) Bioremediation of soil by removing heavy metals using *Saccharomyces cerevisiae*. *Int Conf Environ Sci Technol* 6(2):22–27
- Dean SA, Tobin JM (1999) Uptake of chromium cations and anions by milled peat. *Resour Conserv Recycl* 27:151–156
- Dixit R, Wasiullah Malaviya D et al (2015) Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. *Sustainability* 7:2189–2212
- Feng QL, Wu J, Chen GQ et al (2000) A mechanistic study of the antibacterial effect of silver ions on *E. coli* and *Staphylococcus aureus*. *J Biomed Mater Res* 52(4):662–668
- Han X, Wong YS, Wong MH et al (2007) Biosorption and bioreduction of Cr(VI) by a microalgal isolate, *Chlorella miniata*. *J Hazard Mater* 146(1–2):65–72
- Holman HYN (2002) Real time characterization of biogeochemical reduction of Cr(VI) on basalt surfaces by SR-FTIR imaging. *Geomicrobiol J* 16:307–324
- Kader PS, Othman O, Ismail BS et al (2007) Removal of Cr(VI) from aqueous solutions by growing and nongrowing populations of environmental bacterial consortia. *Glob J Environ Res* 1(1):12–17
- Katiyar SK, Katiyar R (1997) Microbes in control of heavy metal pollution. *Adv Microb Biotechnol* 19:330–344
- Kim JY, Sungeun K, Kim J et al (2005) The biocidal activity of nano-sized silver particles comparing with silver ion. *Korean Soc Environ Eng* 27:771–776
- Kumar BL, Gopal DS (2015) Effective role of indigenous microorganisms for sustainable environment. *3 Biotech* 5(6):867–876
- Malik A (2004) Metal bioremediation through growing cells. *Environ Int* 30:261–278
- Mishra R, Sinha V, Kannan A, Upreti RK (2012) Reduction of chromium-VI by chromium resistant lactobacilli: a prospective bacterium for bioremediation. *Toxicol Int* 19(1):25
- Mohankumar K, Hariharan V, Rao NP (2016) Heavy metal contamination in groundwater around industrial estate vs residential areas in Coimbatore, India. *J Clin Diag Res* 10(4):BC05
- Myers RH, Montgomery DC (2002) *Response surface methodology: process and product in optimization using designed experiments*. Wiley, New York
- Plackett RL, Burman JP (1946) The design of optimum multifactorial experiments. *Biometrika* 33(4):305–325
- Pujari V, Chandra TS (2000) Statistical optimization of medium components for improved synthesis of riboflavin by *Eremothecium ashbyii*. *Bioprocess Biosyst Eng* 23(3):303–307
- Reddy LVA, Young-Jung W, Jong-Sun Y et al (2009) Optimization of alkaline protease production by batch culture of *Bacillus* sp. RKY3 through Plackett Burman and response surface methodological approaches. *Bioresour Technol* 99(7):2242–2249
- Salas BV, Duran EIG, Wiener MS (2000) Impact of pesticides use on human health in Mexico: a review. *Rev Environ Health* 15(4):399–412
- Saranraj P, Stella D, Reetha D et al (2010) Bioadsorption of chromium resistant *Enterococcus casseliflavus* isolated from tannery effluents. *J Ecobiotechnol* 2(7):17–22
- Satarupa D, Paul AK (2013) Hexavalent chromium reduction by aerobic heterotrophic bacteria indigenous to chromite mine overburden. *Braz J Microbiol* 44(1):307–315
- Sethuraman P, Balasubramanian N (2010) Removal of Cr(VI) from aqueous solution using *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Enterobacter cloacae*. *Int J Eng Sci Technol* 2(6):1811–1825
- Shahida H, Thakur IS (2007) Evaluation of biosorption potency of *Acinetobacter* sp. for removal of hexavalent chromium from tannery effluent. *J Earth Environ Sci* 18(5):637–646
- Sultan S, Hasnain S (2005) Isolation of hexavalent chromium-reducing Cr-tolerant facultative anaerobes from tannery effluent. *J Gen Appl Microbiol* 47:307–312
- Sultan S, Hasnain S (2007) Reduction of toxic hexavalent chromium by *Ochrobactrum intermedium* strain SDCr-5 stimulated by heavy metals. *Bioresour Technol* 98(2):340–344
- Thakur IS, Srivastava S (2011) Bioremediation and bioconversion of chromium and pentachlorophenol in tannery effluent by microorganisms. *Int J Tech* 3:224–233



- Xie X, Jin F, Wang H et al (2010) Heavy metal resistance by two bacteria strains isolated from a copper mine tailing in China. *Afr J Biotechnol* 9(26):4056–4066
- Xiong YH, Liu YH, Song HY et al (2004) Enhanced production of extracellular ribonucleic from *Aspergillus niger* by optimization of culture conditions using response surface methodology. *Biochem Eng J* 21:27–32
- Yee N, Benning LG, Phoenix VR et al (2004) Characterization of metal-cyanobacteria sorption reactions: a combined macroscopic and infrared spectroscopic investigation. *Environ Sci Technol* 38(3):775–782

