Malaysian Journal of Microbiology, Vol 20(2) 2024, pp. 237-243
DOI: http://dx.doi.org/10.21161/mjm.221536

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

# Bioremediation of tannery wastewater using UV-treated bacteria in the free and immobilized form

**Basirat Jabbar[1] and Muhammad Imtiaz Shafiq[1,2]***

[1]School of Biochemistry and Biotechnology, University of the Punjab, Lahore, 54590 Pakistan.
[2]Centre for Bioinformatics and Drug Design, University of the Punjab, Lahore, 54590 Pakistan.
Email: imtiaz.ibb@pu.edu.pk

Received 30 May 2022; Received in revised form 25 September 2022; Accepted 29 January 2024

## ABSTRACT

**Aims:** Heavy metals are significant environmental pollutants and toxic to life and chromium (Cr) (VI) is one of them being discharged in the environment due to many human activities. The leather industry uses Cr(VI) salt in the tanning process, which is discharged untreated and becomes a source of many diseases. The use of microbes to remove metals is a cost-effective and clean method. The present study aims to isolate local and native microbes for Cr(VI) removal from tannery wastewater and enhance their capacity to bioremediate the tannery wastewater. Further, efficiency in free and immobilized forms was also checked.
**Methodology and results:** Microbes were isolated from a local tannery wastewater outlet and after many rounds of minimum inhibitory concentration, and concentration of 500 µg/mL was found to be that concentration at which microbes could survive, above which they died. The sequencing of 16S rRNA and its analysis showed that it was closely related to *Staphylococcus saprophyticus* and in the given study, it was named B6. At 37 °C, pH 7.5 and 120 h of incubation, it removed 77% Cr(VI) from the reaction mixture. B6 was exposed to UV to obtain mutant. Exposure of 15 min to a UV lamp gave mutant MB6, which showed a removal capacity of 77% after 72 h only. Cr(VI) removal capacity of the mutant was then analyzed in the free and attached form where coal and sodium alginate were used as solid surfaces. Mutants immobilized on coal showed 91% Cr(VI) removal after 96 h, while sodium alginate showed 58% Cr(VI) removal in 120 h, thus showing coal as a more effective surface for adsorption.
**Conclusion, significance and impact of study:** Our present study shows the use of cheap and environmentally friendly methods to remediate tannery wastewater, which is a big problem in a country like Pakistan. Pakistan is the second largest producer of leather but lacks a wastewater treatment facility. So, this method offers in-situ wastewater treatment, which can be further enhanced in different ways.

*Keywords:* Chromium removal, Cr(VI) removal by immobilized bacteria, Cr(VI) removal by wild and mutant, Cr(VI) removal using mutant, metal removal by mutants, UV treated bacteria

## INTRODUCTION

Chromium is a widely used heavy metal in different industries, including leather tanning, metallurgical processes, ceramic industry, pigment industry, paper and pulp industry (Basu *et al*., 1997; Pandit *et al*., 2013; Shakir *et al*., 2013; Jhunjhunwala, 2016) which discharge Chromium(VI) along with other heavy metals as major environmental pollutants which are toxic to life (Barrera-Díaz *et al*., 2012; Pandit *et al*., 2013; Mala *et al*., 2015; Durán *et al*., 2018; Hu *et al*., 2018).

Basic chromium sulphate and chrome liquor are widely used tanning agents and are mainly released untreated, collectively, making 40 million tons of chromium waste yearly (Saranraj and Sujitha, 2013; Gupta *et al*., 2016).

The presence of heavy metals in the environment causes many life-threatening diseases, including cardiovascular diseases, cancers and genetic diseases (Mosa *et al*., 2016). Cr(VI) is toxic to almost all life forms as it has the potential to denature proteins, which are the building blocks of life (Durán *et al*., 2018).

In trace amounts, Cr(III) is part of a balanced diet, but if exceeded, it can combine with nucleic acids, proteins and organic compounds, forming different complexes (Eastmond *et al*., 2008; Gupta, 2016; Vendruscolo *et al*., 2017), so it is necessary to remove these heavy metals from the wastewater. Old methods, including precipitation, adsorption, filtration, and others, are time-consuming and less efficient, a problem that can be overcome with biological methods (Sharma and Malaviya, 2014; Mala *et al*., 2015) using different physical adsorbents, including

*Corresponding author


ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(2) 2024, pp. 237-243
DOI: http://dx.doi.org/10.21161/mjm.221536

different fruit peels, silica gel, charcoal, alum and many other etc. (Bhatnagar and Minocha 2006; Barrera-Díaz *et al*., 2012; Elyahyaoui *et al*., 2017; Hu *et al*., 2018).

Bioremediation offers a promising tool for the removal of Cr(VI) as it is safe, cheap and does not create any by-products (Pandit *et al*., 2013; Ncibi *et al*., 2017; Durán *et al*., 2018; Prevot *et al*., 2018). Microbes, free or attached to any solid surface, also perform well for the removal of metal using different mechanisms, which may include biological reduction, physical adsorption, bioaccumulation and biotransformation (Issazadeh *et al*., 2013; Saranraj and Sujitha, 2013; Herath *et al*., 2014; Mitra and Mukhopadhyay 2016; Mosa *et al*., 2016; Fernández *et al*., 2018; Durán *et al*., 2018).

Studies show that different microbes, including bacteria and fungi, have the potential to remove Cr(VI). Some of the different bacteria which have been reported for the removal of chromium may include *Bacillus sphaericus* AND 303 (Pal *et al*., 2013), *Pseudomonas stutzeri*, *Brevundimonas diminuta*, *Achromobacter* sp., *Bacillus* sp., *Exiguobacterium* (Pandit *et al*., 2013), *Baillus megaterium*, *Pseudomonas aeruginosa* (Islam, 2016), *Acinetobacter* (Farag and Zaki, 2010) and *B. thuringienesis*. Some reported fungi for chromium removal might include *Fusarium chlamydosporium*, *Rhizopus* sp., *Curvularia* sp., some species of *Penicillium* etc. (García-Hernández *et al*., 2017), *Aspergillus niger* and *Aspergillus flavus* (David, 2016).

Using genetic engineering and some strain improving techniques, microbes can be altered in such a way that their metal-removing capacity can be enhanced, and they act as a more promising tool for bioremediation (Mosa *et al*., 2016). Different mutations in chromate reductase enzyme (ChrR), an enzyme responsible for chromium reduction, have been reported to enhance the Cr(VI) removal capability of different microbes, including *P. putida* and *E. coli* (Barak *et al*., 2006; Jin *et al*., 2012; Algamdi *et al*., 2017). Engineered ChrT gene in *E. coli* from *Serratia* sp. S2 showed an enhanced ability to bioremediate chromium. i.e., 40% high Cr(VI) reduction rate compared to wild strain after 48 h (Zhou *et al*., 2017). The present study aims to improve the bioremediation capacity of microbes isolated from tannery wastewater using a simple mutagen, i.e., UV radiation.

## MATERIALS AND METHODS

### Collection of strain

Samples were collected from a waste discharge outlet of a tannery located at Shiekhupura Road, near Lahore, namely EPCT (Pvt) Ltd, and were transferred to lab in a sterile screw-capped bottle. Three different samples were taken from different points. Colour, pH and temperature of samples were noted. LB-agar having a final concentration of $K_2Cr_2O_7$ of 10 mg/L, was prepared, autoclaved and poured into sterilized Petri plates. Isolated strains were then taken into multiple rounds of increasing $K_2Cr_2O_7$. Isolate, which could tolerate maximum $K_2Cr_2O_7$ was selected for further studies.

### Multi metal resistance study

The strain was checked for its ability to grow in the presence of different heavy metals other than chromium. LB-agar supplemented with different heavy metals in the form of their salts was prepared and autoclaved. Different heavy metal salts used included $HgCl_2$, $NiSO_4$, $FeCl_3$, $ZnSO_4$, $CuCl_2$, $CoCl_2$ and $Pb(C_2H_3O_2)_2$ having final concentration 100 mg/L. Metal-LB-agar plates were prepared, and plates were streaked with the freshly grown culture of B6, kept at 37 °C for 24 h and observed.

### Identification of bacterial strain

16S rRNA sequencing was done for the identification of the strain. Purified isolated bacterial colony grown on LB slant was sent to Macrogen for complete 16S rRNA sequencing using 785F 5'(GGATTAGATACCCTGGTA)3' and 907R 5'(CCGTCAATTCMTTTRAGTTT)3'. The sequence of bacterial strains was analyzed, and evolutionary history was inferred using the Neighbor-Joining method.

### Reduction capacity study

To determine the reducing ability of given strain, 50 mL of freshly prepared LB media was amended with $K_2Cr_2O_7$ having a final concentration 500 µg/mL. Flask was inoculated with 500 µL of fresh inoculums of B6 and was analyzed after each 24 h for 5 days. 1,5-Diphenylcarbazide method was used to determine the chromium reducing ability of strain (Rand *et al*., 1979, Batool *et al.*, 2012). For which 1 mL aliquot was taken from grown culture and spun down for 2 min at 1300 rpm to get clear supernatant to estimate remaining Cr(VI) in it. In a glass test tube, 1 mL of supernatant was added, followed by adding a few drops of Conc. $H_3PO_4$ and 2 drops of 0.2 N $H_2SO_4$. The volume was made up to 10 mL by the addition of distilled water. To this reaction mixture, 0.2 mL of 1,5-Diphenylcarbazide was added and the mixture was kept at room temperature for 10 min to allow for color development and then OD was measured at 540 nm. The Cr(VI) concentration was calculated from the standard curve.

Percentage reduction in Cr (VI) was calculated by formula as: [(Initial concentration - Final concentration)/Initial concentration] × 100.

### Production of mutant

In order to produce mutant, LB-agar was prepared and amended with Cr(VI) in the form of $K_2Cr_2O_7$, having a final concentration of 500 µg/mL. LB-agar was poured into sterilized Petri plates under aseptic conditions and left to solidify. Plates were inoculated with 100 µL of freshly grown inoculums of B6 and then were exposed to a UV lamp for 10 min, maintaining aseptic conditions. Plates were placed at 37 °C and examined after 24 h for surviving colonies.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(2) 2024, pp. 237-243
DOI: http://dx.doi.org/10.21161/mjm.221536

### Chromium reducing ability of mutants

Surviving colonies are those who withstood UV exposure and are Cr(VI) tolerant at the same time. These surviving colonies were marked on plates and each surviving colony was inoculated individually in a separate tube having 10 mL of freshly prepared LB media amended with Cr having a final concentration as 500µg/mL. To check chromium reducing ability, 50 µL of this freshly grown culture of each surviving colony was added to 50 mL of freshly prepared and sterilized LB-media having a final concentration of Cr (VI) as 500 µg/mL. Wild-type strain was also inoculated separately for comparison. After each 24 h an aliquot was taken from all mutant reaction mixtures and wild type of mixture and amount of remaining chromium was calculated as above. A percentage reduction was also calculated as above. The mutant having the highest percentage reduction ability was selected for further studies.

### Reduction ability of immobilized mutant

Chromium reducing ability of mutant was analyzed in free and immobilized form. Domestic coal and sodium alginate beads were used for the immobilization of mutant MB6.

Coal was washed with distilled water to remove any dirt from it. 1-7 g coal and 50 mL of freshly prepared LB medium was taken in 7 different flasks and autoclaved. 1 mL of $K_2Cr_2O_7$ solution at a final concentration of 500 µg/mL was added to all flasks and the volume was made up to 50 mL using distilled water. All reaction flasks were put into a rotatory shaker at 150 rpm. After each 24 h sample was drawn from the reaction mixture and was analyzed for percentage removal of Cr(VI) from the reaction mixture. 1,5-Diphenylcarbazide method was used to estimate the percentage removal of Cr(VI) from the reaction mixture. The optimum concentration of coal, at which it gave maximum reduction, was used for immobilization of Mutant MB6. 6 g of washed coal was taken in a clean flask. Approximately, 50 mL of freshly prepared LB broth was added to the flask and autoclaved. One mL of freshly prepared inoculum of MB6 was also added to the flask under aseptic conditions. Flask was kept in a rotatory shaker at 150 rpm at 37 °C for 48 h. After 48 h, excess media was drained, and the remaining coal was washed with autoclaved water to remove excessive media. MB6 is now immobilized on the surface of coal. To reaction mixture flask, 50 mL freshly prepared LB broth was added with 1 mL $K_2Cr_2O_7$ having a final concentration of 500µg/mL. A flask having 50 mL of LB-broth and 1 mL of $K_2Cr_2O_7$ solution having final concentration of 500 µg/mL was used as control. Both flasks were kept at 150 rpm and 37 °C. After each 24 h, 1 mL aliquot was taken from the reaction mixture and was analyzed for remaining Cr(VI) using 1,5-Diphenylcarbazide method.

To compare Cr(VI) reduction ability of mutant in different forms, sodium alginate beads having MB6 immobilized on its surface were also used. To prepare sodium alginate beads method described by Batool *et al.* (2012) was used. 24 h grown culture at 37 °C was transferred to sterilized falcon tubes under aseptic conditions and was centrifuged at 10,000 rpm for 5 min. The supernatant was discarded, and the cell pellet was resuspended in 10 mL of normal Saline. 10 mL bacterial culture suspension was mixed with 40 mL of 2% sodium alginate solution and mixed using a magnetic stirrer under aseptic conditions for 2 h. Using a 10 mL disposable syringe, the mixture was poured drop-wise in a freshly prepared and autoclaved solution of 2% calcium chloride. The beads were left in $CaCl_2$ solution overnight and then washed with autoclaved distilled water 3-4 times. To check Cr(VI) removing capability of MB6 beads, beads were transferred to a clean and autoclaved flask under aseptic conditions having 50 mL freshly prepared LB-broth and 1 mL of $K_2Cr_2O_7$ solution having a final concentration as 50 µg/mL. The reaction flask was incubated at 37 °C, 1 mL aliquote was taken every 24 h and the remaining Cr(VI) was estimated using 1,5-Diphenylcarbazide method.

All reactions were carried out in triplicates. The standard deviation and variance of each triplicate were calculated and recorded.

## RESULTS

### Collection of strain

Samples were collected from three different sites and their color ranged from grey to black, which showed that the contamination level in wastewater was high. The temperature of samples collected was found to be 33-35 °C. On LB-agar plates, different colonies were obtained. Based on size and color, 14 isolated colonies were marked. After multiple rounds of gradually increasing Cr(VI) concentration, the isolate which could tolerate 500 mg/L was separated, named B6 and was used for further studies.

### Multi metal resistance

B6 could tolerate Cr(VI) up to 500 mg/mL. It was further studied for multi metal resistance and out of all metals tested, and it was found to be resistant against Ni, Fe, Co and Pb. B6 was sensitive to Hg and Cu. This given strain can be used to remediate nickel, iron, cobalt and lead along with chromium. Results are shown in Table 1.

**Table1:** Multi metal resistance analysis for isolated strain B6.

| Sr. No. | Metal salt | Resistant/Sensitive |
|---|---|---|
| 1 | $HgCl_2$ | S |
| 2 | $NiSO_4$ | R |
| 3 | $FeCl_3$ | R |
| 4 | $ZnSO_4$ | R |
| 5 | $CuCl_2$ | R |
| 6 | $CoCl_2$ | S |
| 7 | $Pb(C_2H_3O_2)_2$ | R |

R = Resistant, S = Sensitive.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(2) 2024, pp. 237-243
DOI: http://dx.doi.org/10.21161/mjm.221536

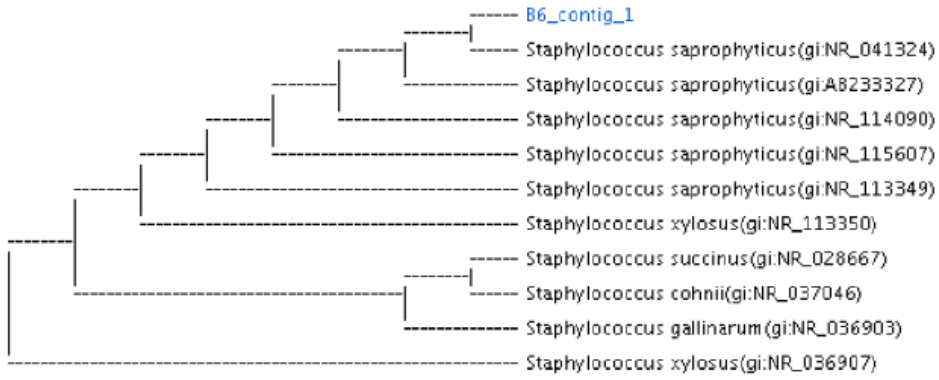


**Figure 1:** Neighbour-joining phylogenetic tree of 16S rRNA gene sequence of isolated strains.

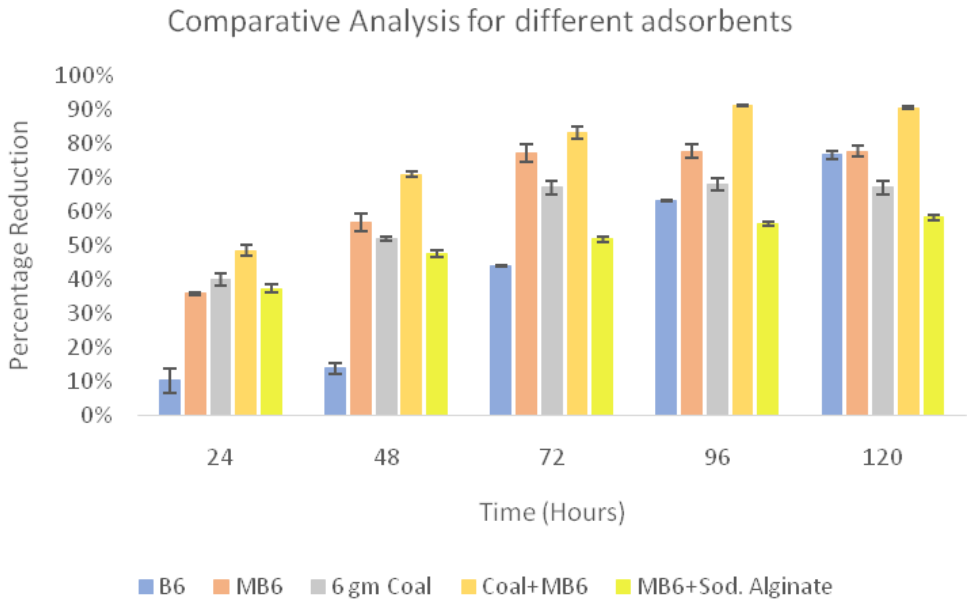


**Figure 2:** Percentage reduction comparison of chromium (VI) under different conditions.

**Identification of strain**

16S rRNA studies showed that isolate is closely related to *S. saprophyticus* (Figure 1).

**Cr reduction using B6**

Results showed that B6 has the potential to reduce Cr(VI), over a period of time. B6 reduced 10%, 14%, 44%, 63% and 77% Cr(VI) after 24, 48, 72, 96 and 120 h. After which, the culture media started to destroy and there was no further reduction in Cr (VI).

**Mutant production**

After many rounds of exposure and re-exposure of B6 to UV, finally, a mutant having a better ability to remove Cr(VI) was obtained. The given mutant was obtained under conditions of LB-agar amended with 500 µg/mL of Cr as $K_2Cr_2O_7$ and 15 min exposure to UV light under aseptic conditions (Figure 2). The reduction potential of this mutant was analyzed as wild type.

**Comparison of reducing the ability of wild and mutant**

After each 24 h, an aliquot of 1 mL was taken from the reaction media of wild and mutant, and the remaining chromium was analyzed using 1,5 DPC method as described above. Wild strain could reduce 10%, 14%, 44%, 63% and 77% after 24, 48, 72, 96 and 120 h, while mutant reduced chromium at the rate of 36%, 58%, 78% 88% and 95% after 24, 48, 72, 96 and 120 h. Mutant B6 reduced more than 50% chromium in just 2 days, while wild type reduced almost after 4 days. Simple strain improvement using simple methods like UV mutagenesis can easily enhance the ability of microbes to remediate different metals.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(2) 2024, pp. 237-243
DOI: http://dx.doi.org/10.21161/mjm.221536

**Table 2:** Percentage removal of Chromium using different concentrations of coal.

| Coal | Time in h | | | | |
|---|---|---|---|---|---|
| (g) | 24(%) | 48(%) | 72(%) | 96(%) | 120(%) |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 5 | 8 | 8 | 8 | 8 |
| 2 | 7 | 12 | 18 | 20 | 21 |
| 3 | 11 | 17 | 24 | 31 | 32 |
| 4 | 15 | 22 | 28 | 37 | 42 |
| 5 | 27 | 38 | 46 | 59 | 68 |
| 6 | 40 | 52 | 67 | 68 | 67 |
| 7 | 52 | 65 | 66 | 67 | 66 |

**Table 3:** Comparison of chromium removal under different conditions.

| Time | Percentage reduction(%) | | | | |
|---|---|---|---|---|---|
| (h) | B6 | MB6 | 6 g Coal | Coal + MB6 | MB6 + Sod. Alginate |
| 24 | 10 | 36 | 40 | 49 | 37 |
| 48 | 14 | 57 | 52 | 71 | 47 |
| 72 | 44 | 77 | 67 | 83 | 52 |
| 96 | 63 | 78 | 68 | 91 | 56 |
| 120 | 77 | 78 | 67 | 90 | 58 |

**Chromium reduction using immobilized bacteria**

1-7 g of coal was used for Cr(VI) reduction, 6 g of coal was found to reduce maximum Cr(VI) in a given period of time. At the end of 120 h incubation time, 6 g of coal reduces 68% chromium(VI). Detailed results are shown in Table 2.

As coal and MB6 can both reduce chromium individually. To analyze the combined effect of MB6 and coal, MB6 was immobilized on the coal surface and then immobilized MB6 was used to reduce Cr(VI). Results showed enhanced Cr(VI) removal ability of mutant MB6, when immobilized on the surface of 6 g coal. After 96 h almost 90% chromium was reduced by Mb6 immobilized on the surface of coal, as compared to 88% when MB6 alone was used and 68% when 6 g coal was used to reduce Cr(VI) from media. Detailed and comparative results are shown in Table 3. No remarkable change in Cr(VI) reduction after 90% can be due to no other available active sites or may be due to nutrient depletion.

**DISCUSSION**

In the present study, 14 isolates were isolated from tannery wastewater, which could grow under the presence of Cr(VI). Out of these 14, one isolate, B6, could tolerate 500 µg/mL of Cr(VI) and was used further. From reaction medium, B6 removed 10%, 14%, 44%, 63% and 77% after 24, 48, 72, 96 and 120 h respectively. No further Cr(VI) removal can be linked to depletion of nutrients and death of culture media. 16 rRNA sequencing revealed that the given strain showed a close resemblance to *S. saprophyticus.* Strain improvement can enhance the ability of microbes for Cr(VI) removal. Chromate reductase enzyme (ChrR) is an enzyme responsible for chromium reduction, in which certain mutations have been reported to enhance the Cr(VI) removal capability of different microbes, including *P. putida* and *E.coli* (Barak *et al*., 2006; Jin *et al*., 2012; Algamdi *et al*., 2017).

To enhance the Cr(VI) reducing ability of a given strain, UV exposure for strain improvement was used. After rounds of exposure and re-exposure, a mutant having more ability than wild to remediate Cr(VI) was isolated and named MB6. As compared to wild it could remove 36%, 58%, 78% 88% and 95% after 24, 48, 72, 96 and 120 h, respectively. Microbes attached to some surfaces have shown better results for metal removal (Herath, 2014). In the present study, when different concentrations of coal were used, 6 g coal showed maximum Cr(VI) removal in 96 h. mutant MB6 was immobilized on 6 g coal, so the combined effect of mutant and coal can be observed. After 96 h, 91% Cr(VI) was removed from the media as compared to alone mutant and coal, which removed 78% and 68% Cr(VI) from reaction media, respectively. Mutant was further immobilized in the form of beads, as beads have also been reported enhancing Cr(VI) removing ability (Pal *et al*., 2013) where *B*. *sphaericus* AND 303 was immobilized on different matrices, including PVA-alginate, Ca-alginate, PVA-borate, agarose and agar-agar. PVA-borate showed the best-reducing ability, i.e., after just 24 h, almost 87.5% Cr(VI) was reduced. In another study (Batool *et al*., 2014), two strains of *Exiguobacterium* sp. were immobilized on agar and sodium alginate, out of which sodium alginate showed better reducing ability, i.e., 89% and 93% by both strains. *Bacillus cereus* $M^{1}{}_{16}$ when immobilized on 3% calcium alginate, showed Cr(VI) reduction up to 92.5% at 25 °C (Bera *et al*., 2007). In the present study, MB6 was immobilized on 3% sodium alginate beads and its Cr(VI) reducing ability was analyzed. After 96 h of incubation, 56% Cr(VI) was reduced as compared to MB6

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

immobilized on coal, which reduced 91% within the same time period. The present study has confirmed the potential of a given strain to bioremediate Cr(VI) from the medium with even increased potential when treated with UV. Using microbes to remediate pollutants is a cheap and cost-effective method, and strain improvement using UV is a fast method to get microbes to have even better and improved efficiency. Strains can be further improved using genetic engineering and molecular biology techniques. Stain improvement using UV does not give stable results and results cannot be reproduced; the only drawback of using UV for strain improvement.

The strain improvement method, alone or combined with some other method, can be used efficiently to remove metals from the environment. Using improved/engineered strain reduces time and cost.

## CONCLUSION

Cr(VI) removal using *Staphylococcus* sp. has been investigated. *Staphylococcus* sp. when undergoing strain improvement, enhanced the Cr(VI) reducing capacity. It showed enhanced Cr(VI) removal capacity when attached to a solid surface. Microbes immobilized on coal showed an even higher rate of Cr(VI) removal than sodium alginate. A relatively low rate of Cr(VI) can be due to loss of rigidity of beads due to hot weather in Pakistan or Sod. alginate may not work as an excellent inert substance for adsorption. The use of other inert substances may overcome this issue. More research needs to be done to develop an even more economical, efficient and better method to remove the metal *in situ*.

## REFERENCES

**Algamdi, A. Y., Alharbi, S. A., Mainwright, M. and Al-Solaimani, S. G. (2017).** Role of the enzyme chromium reductase in detoxification of potassium dichromate ($Cr^{+6}$) by bacteria. *International Journal of Agronomy and Agricultural Research* **11(4), 63-69.**

**Barak, Y., Ackerley, D. F., Dodge, C. J., Banwari, L., Alex, C., Francis, A. J. *et al.* (2006).** Analysis of novel soluble chromate and uranyl reductases and generation of an improved enzyme by directed evolution. *Applied and Environmental Microbiology* **72(11), 7074-7082.**

**Barrera-Díaz, C. E., Lugo-Lugo, V. and Bilyeu, B. (2012).** A review of chemical, electrochemical and biological methods for aqueous Cr(VI) reduction. *Journal of Hazardous Materials* **223-224, 1-12.**

**Basu, M., Bhattacharya, S. and Paul, A. K. (1997).** Isolation and characterization of chromium-resistant bacteria from tannery effluents. *Bulletin of Environmental Contamination and Toxicology* **58, 535-542.**

**Batool, R., Qurat-ul-ain, K. and Naeem, A. (2014).** Comparative study of Cr(VI) removal by *Exiguobacterium* sp. in free and immobilized forms. *Bioremediation Journal* **18(4), 317-327.**

**Batool, R., Yrjala, K. and Hasnain, S. (2012).** Hexavalent chromium reduction by bacteria from tannery effluent. *Journal of Microbiology and Biotechnology* **22(4), 547-554.**

**Bera, D., Chattopadhyay, P. and Ray, L. (2007).** Chromium (VI) biosorption by immobilized biomass of *Bacillus cereus* $M116^{1}_{16}$. *Journal of Hazardous Substance Research* **6(2), 1-20.**

**Bhatnagar, A. and Minocha, A. K. (2006).** Conventional and non-conventional adsorbents for removal of pollutants from water - A review. *Indian Journal of Chemical Technology* **13, 203-217.**

**David, E. (2016).** Isolation and characterization of Cr (VI) reducing bacteria and fungi their potential use in bioremediation of chromium containing tannery effluent (Ambur and Ranipet, Vellore dist, Tamilnadu). *Journal of Life Sciences* **2(1), 1-4.**

**Durán, U., Coronado-Apodaca, K. G., Meza-Escalante, E. R., Ulloa-Mercado, G. and Serrano, D. (2018).** Two combined mechanisms responsible to hexavalent chromium removal on active anaerobic granular consortium. *Chemosphere* **198, 191-197.**

**Eastmond, D. A., MacGregor, J. T. and Slesinski, R. S. (2008).** Trivalent chromium: Assessing the genotoxic risk of an essential trace element and widely used human and animal nutritional supplement. *Critical Reviews in Toxicology* **38(3), 173-190.**

**Elyahyaoui, A., Ellouzi, K., Al Zabadi, H., Razzouki, B., Bouhlassa, S., Azzaoui, K. *et al.* (2017).** Adsorption of chromium (VI) on calcium phosphate: Mechanisms and stability constants of surface complexes *Applied Sciences* **7(3), 222.**

**Farag, S. and Zaki, S. (2010).** Identification of bacterial strains from tannery effluent and reduction of hexavalent chromium. *Journal of Environmental Biology* **31(5), 877-882.**

**Fernández, P. M., Viñarta, S. C., Bernal, A. R., Cruz, E. L. and Figueroa, L. I. C. (2018).** Bioremediation strategies for chromium removal: Current research, scale-up approach and future perspectives. *Chemosphere* **208, 139-148.**

**García-Hernández, M. A., A. G., Villarreal-Chiu, J. F. and Garza-González, M. T. (2017).** Metallophilic fungi research: An alternative for its use in the bioremediation of hexavalent chromium. *International Journal of Environmental Science and Technology* **14(9), 2023-2038.**

**Gupta, A., Joia, J., Sood, A., Sidhu, C. and Kaur, G. (2016).** Microbes as potential tool for remediation of heavy metals: A review. *Journal of Microbial & Biochemical Technology* **8, 4.**

**Herath, H. M. L. I., Rajapaksha, A. U., Vithanage, M. and Seneviratne, G. (2014).** Developed fungal-bacterial biofilms as a novel tool for bioremoval of hexavelant chromium from wastewater. *Chemistry and Ecology* **30(5), 418-427.**

**Hu, Q., Sun, J., Sun, D., Tian, L., Ji, Y. and Qiu, B. (2018).** Simultaneous Cr(VI) bio-reduction and methane production by anaerobic granular sludge. *Bioresource Technology* **262, 15-21.**

**Islam, H. S. (2016).** Bioremediation of Chromium. *Research Journal of Chemistry and Environment* **20(1), 34-42.**

**Issazadeh, K., Jahanpour, N., Pourghorbanali, F., Raeisi, G. and Faekhondeh, J. (2013).** Heavy metals resistance by bacterial strains. *Annals of Biological Research* **4(2), 60-63.**

**Jhunjhunwala, P., Roy, R., Roy, S. and Chaudhuri, A. N. (2016).** Bioremediation of chromium by unique chromium reductase activity in *Proteus* sp. isolated from wastewater. *International Journal of Applied Research* **2(11), 249-256.**

**Jin, H., Zhang, Y., Buchko, G. W., Vamum, S. M., Robinson, H., Squier, T. C. *et al.* (2012).** Structure determination and functional analysis of a chromate reductase from *Gluconacetobacter hansenii*. *PLoS ONE* **7(8), e42432.**

**Mala, J. G. S., Sujatha, D. and Rose, C. (2015).** Inducible chromate reductase exhibiting extracellular activity in *Bacillus methylotrophicus* for chromium bioremediation. *Microbiology Research* **170, 235-241.**

**Mitra, A. and Mukhopadhyay, S. (2016).** Biofilm mediated decontamination of pollutants from the environment. *AIMS Bioengineering* **3(1), 44-59.**

**Mosa, K. A., Saadoun, I., Kumar, K., Helmy, M. and Dhankher, O. P. (2016).** Potential biotechnological strategies for the cleanup of heavy metals and metalloids. *Frontier in Plant Science* **7, 303.**

**Ncibi, M. C., Mahjoub, B., Mahjoub, O. and Sillanpaa, M. (2017).** Remediation of emerging pollutants in contaminated wastewater and aquatic environments: Biomass-based technologies. *CLEAN - Soil, Air, Water* **45(5), 1700101.**

**Pal, A., Datta, S. and Paul, A. K. (2013).** Hexavalent chromium reduction by immobilized cells of *Bacillus sphaericus* AND 303. *Brazilian Archives of Biology and Technology* **56(3), 505-512.**

**Pandit, R., Patel, B., Kunjadia, P. D. and Nagee, A. (2013).** Isolation, characterization and molecular identification of heavy metal resistant bacteria from industrial effluents, Amala-khadi-Ankleshwar, Gujarat. *International Journal of Environmental Sciences* **3(5), 1689-1699.**

**Prevot, A. B., Ginepro, M., Peracaciolo, E., Zelano, V. and De Luca, D. A. (2018).** Chemical vs bio-mediated reduction of hexavalent chromium. An in-vitro study for soil and deep waters remediation. *Geoderma* **312, 17-23.**

**Rand, M. C., Arnod, E. and Michel, J. (1979).** Standard Methods for the Examination of Water and Wastewater, Edn. 14th. American Public Health Association, Washington, DC.

**Saranraj, P. and Sujitha, D. (2013).** Microbial bioremediation of chromium in tannery effluent: A review. *International Journal of Microbiology Research* **4(3), 305-320.**

**Shakir, H. A., Shakeel, S. and Qazi, J. I. (2013).** Bioremediation of some frequently encountered non essential heavy metals: A review. *Punjab University Journal of Zoology* **28(1), 45-54.**

**Sharma, S. and Malaviya, P. (2014).** Bioremediation of tannery wastewater by chromium resistant fungal iolate *Fusarium chlamydosporium* SPFS2-g. *Current World Environment* **9(3), 721-727.**

**Vendruscolo, F., da Rocha Ferreira, G. L. and Filho, N. R. A. (2017).** Biosorption of hexavalent chromium by microorganisms. *International Biodeterioration and Biodegradation* **119, 87-95.**

**Zhou, S., Dong, L., Deng, P., Jia, Y., Bai, Q., Gao, J. *et al.* (2017).** Reducing capacity and enzyme activity of chromate reductase in a ChrT-engineered strain. *Experimental and Therapeutic Medicine* **14(3), 2361-2366.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538