Sustainability, Agri, Food and Environmental Research, (ISSN: 0719-3726), 11(X), 2023:
http://dx.doi.org/10.7770/safer.v12i1.2433

Isolation and characterization of chromium (vi) tolerant bacterial strains isolated from the tannery effluent of park circus area

Aislamiento y caracterización de cepas bacterianas tolerantes al cromo (vi) del efluente de Tannery del área de Circus Park

Soumyajit Guha[a], Sampa Debnath[b] and Saswati Gayen*

Department Of Microbiology, Vijaygarh Jyotish Ray College, Kolkata - 700032,West Bengal, India.

*Corresponding author, Assistant Professor, E-mail: saswatimicrobio@gmail.com

Research Scholar, E-mail(a): soumyajitguhas42@gmail.com; E-mail(b): sampamicrobio@gmail.com

ABSTRACT

Heavy metals contamination is a global environmental concern because it is difficult to remove these contaminants from the environment, unlike other pollutants. Chromium (Cr) is one of the most important heavy metals used extensively in various industries, out of which the tanning industry deserves special mention. There are more than 2500 tanneries in the country and nearly 80% of the tanneries are engaged in the chrome tanning process. Massive amounts of Cr waste are discharged annually in the environment as a result of industrial and manufacturing activities. Cr, a highly reactive element with an oxidation state of 6 exhibits stability as Cr (III) and Cr (VI). Hexavalent chromium is actually more toxic in effect to living organisms than its trivalent form. USEPA has classified Cr (VI) as a group A carcinogen, based on its chronic effects. Prolonged exposure to Cr (VI) is found to cause cancer in the digestive tract and lungs and in other cases epigastric pain, nausea and vomiting. Cr (VI) exposure has severe impacts on aquatic species also. In the present study chromium (VI) tolerant bacterial strains were isolated from the tannery effluent of Park Circus area. Most of the bacterial isolates were Gram-negative while few were Gram-positive. 6 bacterial strains were tested for their Cr tolerance capacity of which S6 showed maximum Cr tolerance of 0.4-0.5 mg/ml. The growth responses to different concentrations of Cr (VI) by the bacterial isolates, were also studied. The responses of the bacteria depended on the time of incubation and Cr (VI) concentration.

Keywords: Chromium, tolerance, bacteria, growth response.

RESUMEN

La contaminación por metales pesados es una preocupación ambiental mundial porque es difícil eliminar estos contaminantes del medio ambiente, a diferencia de otros contaminantes. El cromo (Cr) es uno de los metales

pesados más importantes que se utiliza ampliamente en diversas industrias, de las cuales la industria del curtido merece una mención especial. Hay más de 2500 curtidurías en el país y casi el 80% de las curtidurías se dedican al proceso de curtido al cromo. Anualmente se descargan enormes cantidades de residuos de Cr en el medio ambiente como resultado de las actividades industriales y de fabricación. El Cr, un elemento altamente reactivo con un estado de oxidación de 6, presenta estabilidad como Cr (III) y Cr (VI). El cromo hexavalente tiene un efecto más tóxico para los organismos vivos que su forma trivalente. La USEPA ha clasificado al Cr (VI) como carcinógeno del grupo A, con base en sus efectos crónicos. Se ha descubierto que la exposición prolongada al Cr (VI) provoca cáncer en el tracto digestivo y los pulmones y, en otros casos, dolor epigástrico, náuseas y vómitos. La exposición al Cr (VI) también tiene graves impactos en las especies acuáticas. En el presente estudio se aislaron cepas bacterianas tolerantes al cromo (VI) del efluente de la curtiduría del área de Park Circus. La mayoría de los aislados bacterianos fueron gramnegativos, mientras que pocos fueron grampositivos. Se analizaron 6 cepas bacterianas para determinar su capacidad de tolerancia al Cr, de las cuales S6 mostró una tolerancia máxima al Cr de 0,4-0,5 mg / ml. También se estudiaron las respuestas de crecimiento a diferentes concentraciones de Cr (VI) por los aislados bacterianos. Las respuestas de las bacterias dependieron del tiempo de incubación y de la concentración de Cr (VI).

Palabras clave: cromo, tolerancia, bacterias, respuesta de crecimiento.

INTRODUCTION

Chromium (VI) is a heavy metal. Chromium (Cr) compounds have widespread industrial uses in steel production, wood preservation, leather tanning, aluminum productions, nuclear power production, metal processing, electroplating, iron sheet cleaning, chrome plating, wood preservation, inorganic chemical production, galvanometric, electric, mining industries, water cooling, battery manufacturing, mine drainage, metal corrosion inhibition, paints and pigments, metal plating and other applications (Papp 1985, Ranjan *et al.* 2009, Gupta and Rastogi 2009, Mishara and Doble 2008) Hexavalent chromium generated from several of these industrial processes is discharged into the environment and is toxic and mutagenic to most organisms (Poopal and Laxman 2009).

Despite the fact that heavy metals are acutely toxic to most microbes, there are metal tolerant bacteria. Prolonged exposure to metals favors the proliferation of microbes that are tolerant to the metals and this has been observed by assaying habitats exposed to anthropogenic or natural metal contamination over an extended period (Hutchinson and Symington 1997). Several physical and chemical methods exist to remove heavy metals from the environment but these methods are quite impractical due to the high operational cost and subsequent generation of solid waste which is difficult to treat. Various researches in recent years have found that many microorganisms are able to accumulate noteworthy concentrations of metals (Ramteke 2000). Bioreduction of Cr (VI) can occur directly as a result of microbial metabolism (enzymatic) or indirectly, mediated by bacterial metabolic products (Losi

et al. 1994). Tolerance showed by microorganisms towards hexavalent chromium has practical significance, because it can serve as a basis for selecting the organism, that can be further used to detoxify chromium in the environment (Ganguli and Tripathi 2002). The objective of this study was to isolate and characterize Cr (VI) tolerant bacterial strains from tannery effluent of Park Circus area [Kolkata, West Bengal, India].

MATERIALS AND METHODS

Sample Collection: Sample (tannery effluent) was collected from a tannery located in Park Circus area. The sample was collected in a sterile capped Tube (50 ml capacity). The tube was then brought to the laboratory for analysis. The sample was shaken vigorously 20 times to obtain a uniform distribution of the organisms (Fig. 1).

Figure 1: Colony characteristics of the selected bacterial strains

Isolation of Bacterial Strains From The Effluent: The sample was serially diluted in 5 test tubes and marked as stock, (10^{-1}), (10^{-2}), (10^{-3}), (10^{-4}), (10^{-5}). All the samples were plated on nutrient agar plates and incubated overnight at 37^0 C. Several morphologically different colonies were randomly selected and sequentially subcultured for pure colony isolation on the same medium.**6** different bacterial strains were isolated in this process. They were stored in the refrigerator at 4^0C till further analysis.

Gram staining: Gram staining was done according to the standard protocol developed by Hans Christian Gram. Growth Responses of The Isolated Bacterial Strains Against Different Concentrations of Chromium: 6 nephelometric flasks were taken each containing 20 ml sterile nutrient broth. The flasks were marked and 6 of the isolated bacterial strains were inoculated in 6 different flasks and incubated overnight at 37^0C. A control flask was

also kept containing only 20 ml of sterile nutrient broth without any bacterial suspension. Optical density of each of the 6 different cell suspensions was measured at 600 nm using a spectrophotometer and the cell concentration for each of the 6 organisms was brought to the same value by adding sterile distilled water. Various concentrations of chromium (0.1 mg/ml, 0.2 mg/ml, and 0.3 mg/ml) were added in all 7 flasks (in all the 6 flasks containing different bacterial cell suspension as well as in the control). The optical density of the cell suspensions at different concentrations of chromium was measured at 30 minutes time interval for 3.5 hours to determine the growth responses of the bacterial strains at different concentrations of chromium.

Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration of different antibiotics was determined by standard protocol.

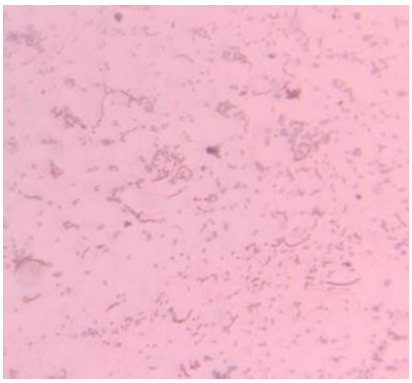
All the experiments were performed in triplicates and proper controls were kept in all the experiments.

RESULTS AND DISCUSSION

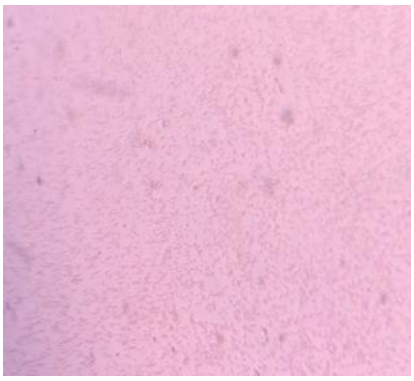
The spread plate method was followed to isolate the bacterial strains. Many different bacterial colonies were formed in all the plates of different dilutions. Among all the wide variety of colonies formed 6 were chosen primarily based on unique colony characteristics and their morphological, as well as Gram characters, were tested. Their chromium tolerant capability was also tested. Table 1 depicts the characteristic of the selected colonies. Picture of the colony characteristics of the selected strains is shown in Figure 2 and microscopic view of their morphological and Gram characteristics is shown in Figure 3.

Table 1. Colony and Morphological characteristics of the isolated bacterial strains (S1 - S6).

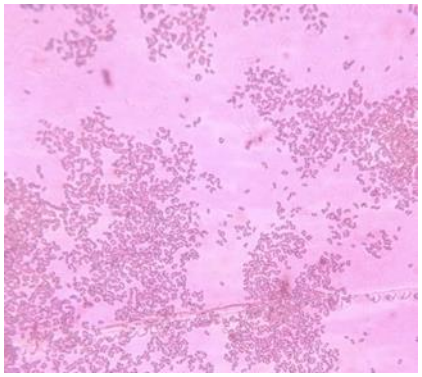
Colony number	Colony characteristics		Morphological characteristics	
	Colour	Shape	Shape	Gram character
S1	Light yellow	Rhizoid, cloudy in structure.	Short rod	Gram-positive
S2	Brown	Curled, irregular in structure.	Micrococcus	Gram-positive
S3	Light yellow	Circular, droplets like structure.	Micrococcus	Gram-negative
S4	Light yellow	Toruloid, chain like structure.	Micrococcus	Gram-negative
S5	Brown	Curled, irregular in structure.	Micrococcus	Gram-negative
S6	Light white	Toruloid, chain like structure.	Slightly oval	Gram-negative



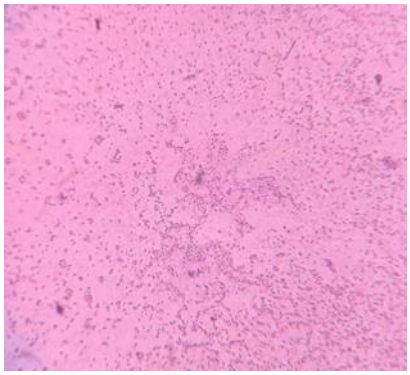
S1



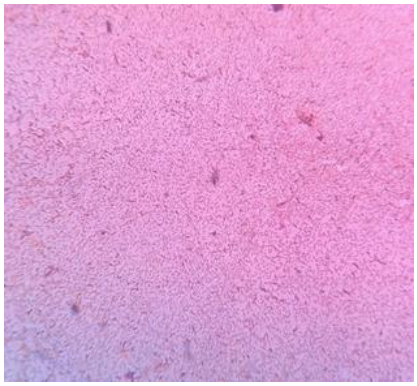
S2



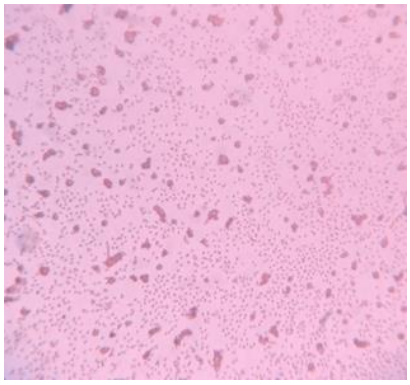
S3



S4



S5



S6

Figure 2. Microscopic view of the morphological and Gram characteristics of the isolated bacterial strains.

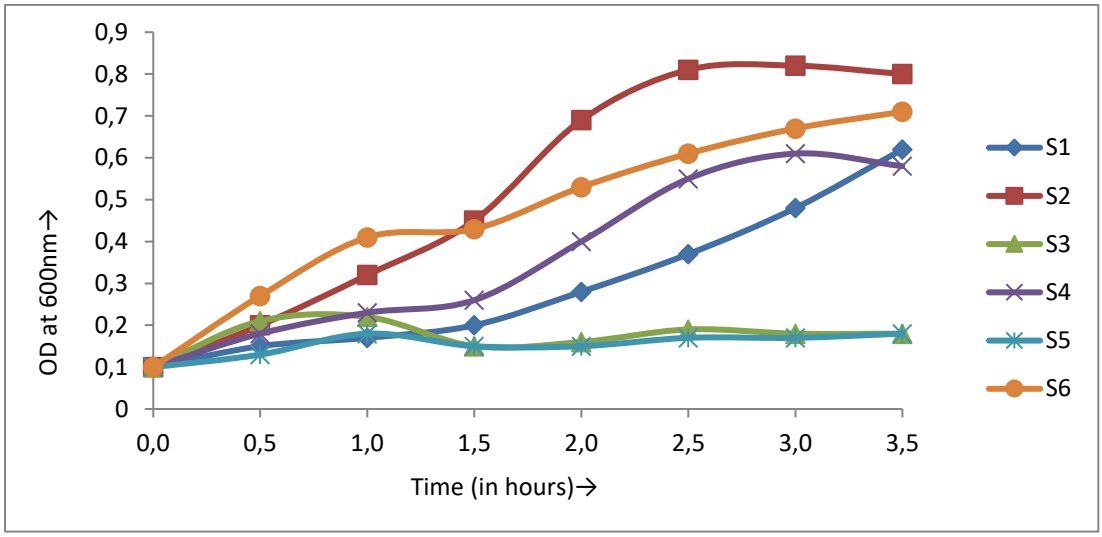


Figure 3: Growth responses of the bacterial isolates at chromium concentration of 0.1mg/ml

Growth characteristics of the bacterial strains at different concentration of chromium:

The growth responses of the 6 different bacterial isolates to different concentrations of hexavalent chromium at different time intervals were determined. The results of these studies are shown in Figures 3 to 5.

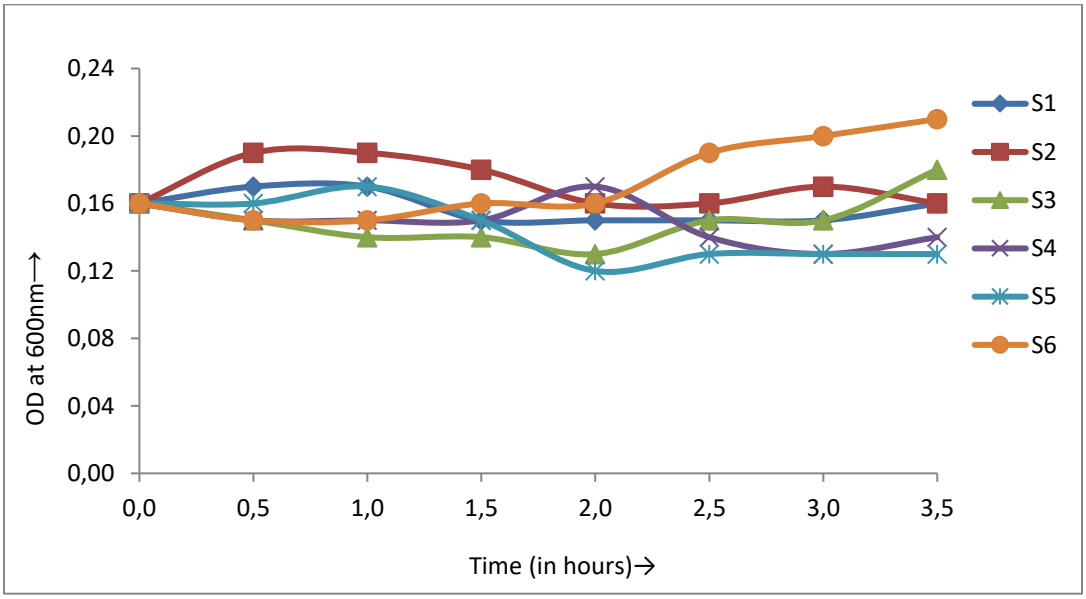


Figure.4: Growth responses of the bacterial isolates at chromium concentration of 0.2mg/ml.

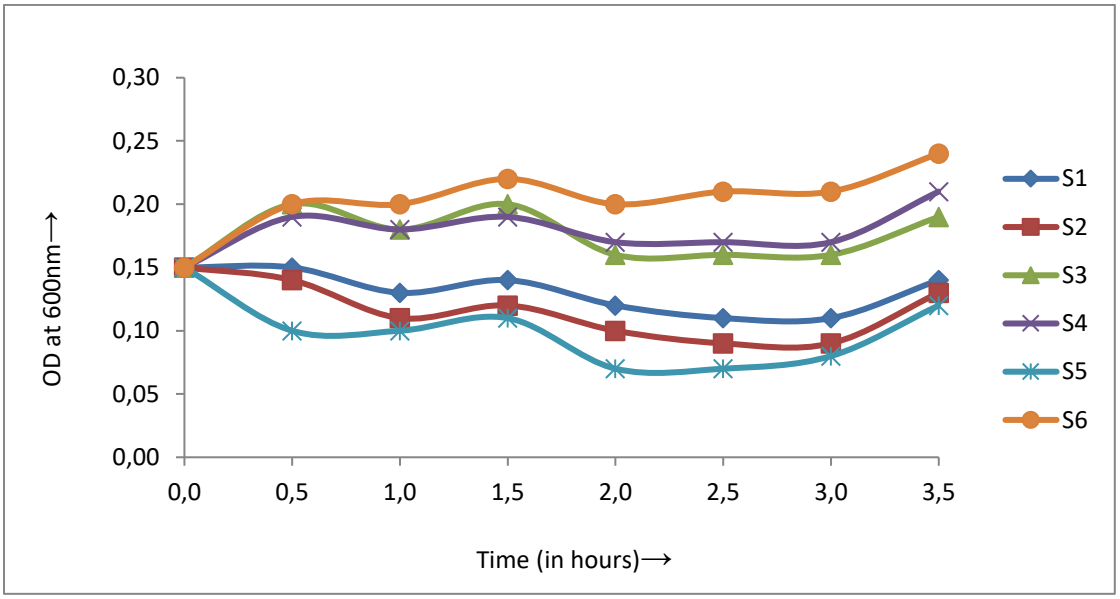


Figure 5: Growth responses of the bacterial isolates at chromium concentration of 0.3mg/ml

In Figure 3 the growth responses of the 6 bacterial isolates at Cr(VI) concentration of 0.1mg/ml showed that the growth rate of the isolate S2 increased with the increase of the incubation time and reached the stationary phase after about 3 hrs. The growth responses of S1 and S6 also increased with time and did not reach the stationary phase even after 3.5 hrs. S4 showed a similar growth curve pattern as S2 but its growth rate was lower than S2. S3 and S5 showed a minimum growth rate in response to a 0.1 mg/ml concentration of hexavalent chromium.

In Figure 4 the growth responses of the 6 bacterial isolates at Cr (VI) concentration of 0.2 mg/ml showed that the growth rate of the isolate S6 increased with the increase of the incubation time and did not reach the stationary phase even after about 3.5 hrs of incubation. The growth responses of S2 and S4 decreased with time. S5 showed a drastic decrease in the growth rate with time and reached the stationary phase after 2.5 hrs of incubation. S3 showed a very uneven growth curve pattern and the growth of S1after increasing slightly entered the stationary phase after 1.5 hrs.

In Figure 5 the growth responses of the 6 bacterial isolates at Cr (VI) concentration of 0.3 mg/ml showed that the growth rate of the isolate S6 increased with the increase of the incubation time and did not reach the stationary phase even after about 3.5 hrs of incubation. Although the growth pattern of S4 was similar to S6 but its growth rate was lower than S6. The growth responses of S1, S2, and S5 decreased with time and then began to increase slowly. The decrease in growth may be as a result of the toxic effect of the metal at this concentration whereas the subsequent increase may be as a result of the ability of the isolates

to secrete enzymes that enable them to withstand such concentration or the organisms become adapted to the metal (Anyanwu and Ezaka 2011).

The growth responses of the bacterial isolates at different concentrations of chromium were carried out in this experiment. The responses of the bacteria were dependent on the time of incubation and Cr (VI) concentration used. The highest growth was recorded at a concentration of 0.1 mg/ml which may be as a result of a less toxic effect of the metal. However, growth decreased as the concentration of chromium increased. The decrease in growth rate may be a result of the toxic effect of the high concentration of the metal which prevented bacterial multiplication (Anyanwu and Ezaka 2011). The analysis of the results showed that the isolate S6 was much more adapted to grow in the presence of chromium than the other isolates.

Minimum inhibitory concentration: Minimum inhibitory concentration of Cr (VI) which prevents the growth of the bacterial isolates showed a similar result as indicated in the previous experiment. Isolates S1 to S5 showed MIC in the range of 0.2 - 0.3 mg/ml whereas S6 showed MIC in the range of 0.4 - 0.5 which again proved that S6 has the maximum chromium tolerant capacity as compared to the other bacterial isolates [Table 2]. Verma et al reported that the majority of heterotrophs isolated from tannery effluent were resistant to chromate concentrations at 50 - 100 µg/ml whereas coliforms were resistant to higher concentrations of chromate (150 - 250 µg/ml) (Verma *et al*. 2001).

Table 2: MIC of the bacterial isolates

Strain number	Mic concentration range (mg/ml)
S1	0.2 and 0.3
S2	0.2 and 0.3
S3	0.3 and 0.4
S4	0.2 and 0.3
S5	0.2 and 0.3
S6	0.4 and 0.5

In this study, chromium tolerant bacterial strains were tried to be identified from the tannery effluent collected from the Park circus area.6 different bacterial isolates were selected for further study after initial isolation in Nutrient Agar medium. The selection of the isolates was made based on the presence of unique colony characteristics. Gram staining revealed the presence of both Gram-positive as well as Gram-negative bacteria. Three different morphological types - rod-shaped, micrococcus, and oval-shaped were observed. All the 6 bacterial isolates were found to be chromium resistant but maximum resistance was shown by the isolate S6 as its MIC value was in the range between 0.4 - 0.5 mg/ml. Cr(VI) concentration can be an important

environmental factor regulating remediation strategies for ecosystem polluted with hexavalent chromium. The native isolates which tolerate different concentrations of Cr (VI) will be highly effective in Cr (VI) bioremediation which is potentially less expensive than the chemical method in the treatment of environment contaminated with Cr (VI). The present study revealed the capacity of bacterial isolates to grow and tolerate different concentrations of chromium. Such isolates can be used to remove Cr (VI) from the environment. Further research is, therefore, needed to evaluate their ability to detoxify hexavalent chromium in the environment.

REFERENCES

Anyanwu, C.U. and Ezaka, E. 2011. Growth responses of Chromium (vi) Tolerant Bacteria to Different Concentrations of Chromium. *International Journal of Basic & Applied Sciences*. 11(5):41-44.

Ganguli, A. and Tripathi, A.K. 2002. Bioremediation of toxic chromium electroplating effluent by chromate reducing *Pseudomonas aeruginosa* A2chr in two bioreactors. *Applied Microbiology Biotechnology*. 588:416-420.

Gupta, V.K. and Rastogi, A. 2009. Biosorption of hexavalent chromium by raw and acid-treated green alga *Oedogoniumhatei* from aqueous solutions. *Journal of Hazardous Materials*. 163:96-402.

Hutchinson, T.C. and Symington, M.S. 1997. Persistence of metals stress in a forested ecosystem near Sudbury, 66yrs after closure of the O'Donnell roast bed. *Journal of Geochemical Exploration*. 58:323-330.

Papp, J.S. 1985. Chromium. In: Mineral facts and problems. Knoerr, A.W. (Ed.). Bureau of Mines Bulletin 675. U.S. Printing Office, Washington D.C.

Ranjan, D., Srivastava, P., Talat, V. and Hasan, S.H. 2009. Biosorption of Cr (VI) from water using biomass of *Aeromonashydrophila*: central composite design for optimization of process variables. *Applied Biochemical Biotechnology*. 158:524- 539.

Losi, M.E., Amhein, C. and Frankanbarger, W. T. 1994. Bioremediation of chromate contaminated groundwater by reduction and precipitation of surface soils. *Journal of Environmental Quality*. 23:1141-1150.

Mishara, S. and Doble, M. 2008. Novel chromium tolerant microorganisms: isolation, characterization, and their biosorption capacity. *Ecotoxicology and Environmental Safety*. 71:874-879.

Poopal, A.C. and Laxman, R.S. 2009. Chromate reduction by PV-alginate immobilized *Streptomyces griseus* in a bioreactor. *Biotechnology Letters*. 31: 71 – 76.

Sustainability, Agri, Food and Environmental Research, (ISSN: 0719-3726), 11(X), 2023:
http://dx.doi.org/10.7770/safer.v12i1.2433

Ramteke, P.W. 2000. Biosorption of nickel (ii) by *Pseudomonas stutzeri*. *Journal of Environmental Biology*. 21:219-221.

Verma, T., Srinath, T., Gadpayle R.U., Ramteke, P.W., Hana, R.K. and Garg, S.K. 2001. Chromate tolerant bacteria isolated from tannery effluent. *Bioresource Technology*. 78:31-35.

Received: 17th February 2021; Accepted: 13th December 2022; First distribution: 14th December 2022