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RESEARCH ARTICLE

# THE ROLE OF CHROMATE REDUCTASE ENZYME ACTIVITY FOR EFFECTIVE REDUCTION OF CHROMIUM BY *PSEUDOMONAS AERUGINOSA*

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**ABSTRACT**

In the recent investigation, chromium tolerant strains were isolated from tannery effluent collected from Dindigul district. Six chromium tolerant strains were isolated from potassium dichromate amended Nutrient agar plates and their maximum tolerance were determined. The strain TE-2 and TE-6 exhibited maximum tolerance upto 500 ppb. The two chromium tolerant strains were identified as *Bacillus subtilis* and *Pseudomonas aeruginosa* on the basis of phenotypic and biochemical characteristics. In the present investigation, the chromium reduction for *Pseudomonas aeruginosa* was performed two different concentration of potassium dichromate (200 ppb and 400 ppb). The AAS analysis was performed to analyze hexavalent chromium and role of microorganisms in biodegradation process. *Pseudomonas aeruginosa* revealed a reduction in total chromium of 0.016 ppb when compared with control which revealed 0.019 ppb. On analysis with 400 ppb after 15 days of exposure a remarkable reduction of 0.020 ppb rather compared with compared (0.024ppb). The extraction of chromate reeducates enzyme was performed which revealed that the crude enzyme had a total chromate reeducates activity of 0.014 µ moles $min^{-1}$ and the total protein concentration of 0.25 mg respectively. The specific activity of µ moles $min^{-1}$ $mg^{-1}$ was recorded for crude chromate reeducates enzyme. The effect of temperature on chromate reductase was performed with temperature ranging from 20°C to 70°C. *Pseudomonas aeruginosa* exhibited an optimum temperature of activity of 50°C with 0.106 µ moles $min^{-1}$. *Pseudomonas aeruginosa* was found to be a versatile microorganism which can tolerate up to 500 ppb concentration and can be used for bioremediation process.



## INTRODUCTION

Environmental pollution is becoming the global problem in which water pollution is an important issue as water is used directly for various purposes. (Vidya and Usha, 2007). The major sources of water pollution are industrial effluents, which is discharged to the common drainage. The effluent pollute not only the nearby soil but may cause the pollution of drinking water also (Lokhande and Vaidya, 2004).

Tanning Industry is considered to be a major source of pollution and tannery wastewater in particular, is a potential environmental concern (Ros and Ganter 1998).

The tannery effluents were rich in large concentration of nutrients including inorganic nutrients, inorganic nitrogen (N), N-rich organic residues (Goyal *et al*., 2003). Tannery effluent with high pollution load when discharged into water bodies alter the physical, chemical and biological characteristics of water and depletes the dissolved oxygen, increases alkalinity, suspended solids and sulphides which are injurious to fish and other aquatic lives. (Gokulakrishnan and Pandurangan, 2004). Chromium is a transition metal present in group VI-B of the periodic table. Although it can exist in nine valence states, from -2 to +6 (Smith *et al*., 2002). Traditionally, by the use of various expensive chemical and physical processes high concentrations of Cr(VI) are reduced from the industrial wastewater. Those methods include ozonization, adsorption, ion exchange, membrane filtration, chemical oxidation etc. (Arutchelvan *et al*., 2006). Many microorganisms have been reported to reduce the highly soluble and toxic Cr (VI) to the less soluble and less toxic Cr (III), e.g., *Acinetobacter* and *Ochrobactrum*, *Arthrobacter*, *Pseudomonas sp.* (Rajkumar *et al*., 2005).

In the present study the isolation of tannery effluent resistant bacteria were performed and evaluation of hexavalent chromium toxicity and detoxification mechanism by flask culture method were carried out. The characterized and resistant bacterial isolate was found as *Pseudomonas*

*aeruginosa* and *Baacillus subtilis*. In order to study the role of detoxifying enzymes such as chromate reductase and its effect on chromium uptake were being investigated. Bacterial transformation is a process designated to isolate and to study the role indigenous microbes in removing hexavalent chromium in laboratory conditions.

## MATERIAL AND METHODS

### *Collection of sample*

The effluent sample was collected from the tannery industry, Dindigul district and it was transported into laboratory condition at room temperature.

### *Enrichment culture technique*

The sample collected from the tannery industry was enriched with potassium dichromate (chromium solution) and kept in room temperature for 15 days.

### *Isolation of Bacteria for chromium degradation studies*

Chromium degrading microorganisms were isolated from enrichment culture by spreading the turbid culture onto nutrient agar containing chromium (100 PPB) and incubated at $28^0$C for 24 hrs. The microorganisms with different morphological characteristics were selected and purified to a single colony by repeated streaking under the same conditions and maintained at $4^0$C for further analysis.

### *Determination of Minimum Inhibitory Concentration (MIC) for chromium tolerant bacteria*

Minimum Inhibitory Concentration (MIC) was carried out to determine the resistant capacity of chromium degrading bacteria, at different concentrations of chromium. The nutrient agar medium was prepared with various concentrations of chromium ranging from 200 to 600 PPB, and the isolates were streaked. The inoculated plates were incubated at $35^o$C for 24 hrs, and the visible growth of the isolates after incubation was monitored.

### *Morphological and Biochemical characterization*

The selected chromium degrading bacterium was identified using morphological and biochemical characterization methods.

### *Biodegradation of chromium by Pseudomonas aeruginosa*

In the present study, the chromium reducing capabilities of Pseudomonas aeruginosa were studied with different concentration of chromium 200ppb - 400ppb).

The present study was carried out to determine biodegradation of chromium by *Pseudomonas species*. The bacterial strain was cultured overnight in nutrient broth and cetrimide broth. The culture flask containing nutrient broth medium was supplemented in Cr (VI) in different concentration of 200ppb and 400ppb control was maintaining. The control and experimental treated cultures were maintained with continuous shaking in shaker. The chromium reduction was analyzed by Atomic Absorption Spectrophotometry.

### *Atomic Absorption Spectrometry*

2ml of the sample from 200ppb and 400ppb concentration of hexavalent chromium along with nitric acid mix with equal ratio was digested and the supernatant was collected which was used for Atomic Absorption Spectrophotometry (AAS) analysis. The values obtained by Atomic Absorption Spectrophotometry (AAS) represent the residual concentration of Cr (VI) in the solutions.

### *Synthesis of chromate reductase*

The 100ml media contained 1g glucose, 0.165g Ammonium sulfate, 0.067g ammonium chloride, 0.01g magnesium sulfate, 0.25g potassium dihydrogen phosphate, 0.15g di sodium hydrogen phosphate, 0.003g sodium sulfate, 0.008g potassium sulfate, 0.0075g potassium dichromate and 0.01g magnesium chloride was also added to the media and then sterilized. After sterilization 5ml of inoculum containing *Pseudomonas aeruginosa* was added into the flask and kept on a rotary shaker for 15 days.

### *Extraction of chromate reductase enzyme*

2ml of the sample collected from the production medium was centrifuged at 7,500 rpm for 15 minutes. The clear supernatant was collected.

### *Assay for chromate reductase activity*

Chromate reductase activity was assayed by measuring the decrease in concentration of chromate. The activity was assayed after containing 200µl of enzyme extract, 300µl of 0.1mM NADH, 200µl of 50mM Tris buffer pH 7.0 and 250µl of 0.05 mM potassium dichromate for 1 hr. 1 ml of 0.2% 2,4-dinitro phenyl hydrazine reagent was then added, allowed to stand for 10 minutes and absorbance was read at 540 nm using a colorimeter.

### *Enzyme unit-calculation*

One unit of chromate reductase activity was defined s the amount of enzyme required to reduce 1 µ mole of chromate VI to chromate III under defined assay conditions.

Enzyme unit (µ moles $min^{-1}$)-$\mathbf{R}$ X $\eta_s$ X $\mathbf{A_{540}}$ / **0.01** X **T**

R – Final reaction volume

$\eta_s$ – Dilution factor

$A_{540}$– Absorbance at 540 nm

0.01 – constant

T– Time of incubation in minutes

### *Effect of temperature on chromate reductase enzyme activity*

To determine this, chromate reductase activity was assayed at varying temperatures ranging from $20^O$C to $50^O$C to establish the optimum temperature of the enzyme activity.

## RESULTS

In the present study, biosorption of chromium by microorganisms obtained from tannery effluents from Dindigul district were investigated (Plate 1). The tolerant bacteria were isolated by serial dilution and amended with known concentration of chromium and six predominant colonies were isolated (Plate 2, 3, 4 and 5). The obtained six isolates were chosen to determine the Minimum Inhibitory Concentration (MIC) of chromium.

The Minimum Inhibitory Concentration (MIC) of chromium were performed in Nutrient agar plates supplemented with different concentration of chromium (200 ppb, 300 ppb, 400 ppb, 500 ppb, 600 ppb) and were inoculated aseptically with the culture of bacterial isolates in exponential growth phase. Minimum Inhibitory Concentration (MIC) was evaluated to determine the maximum tolerance limit of chromium. Among the six isolates, the stains TE - 2, TE - 4 and TE - 6 executed

maximum resistance to chromium up to 500 ppb. On the basis of these experiments the maximum chromium tolerant strains were found to be TE - 2, TE - 4 and TE - 6. Of them TE - 2 and TE - 6 were selected for further analysis (Plate:6).

***Identification of the efficient bacterial isolates***

The selected potential chromium tolerant isolates TE-2 and TE-6 were identified by morphological and biochemical characterization methods. The bacterial isolate TE-2 was found to be gram positive short rods and spore forming organisms. On nutrient agar plate TE-6 produce cream colored rod shaped colonies. The organism was gram negative non sporulating rod shaped bacteria.

***Biochemical characterization***

The biochemical characterization of isolate TE-2 was confirmed as *Bacillus sp.* on the basis of biochemical investigation. The organism did not produce indole, methyl red and voges proskauer test. It revealed negative results. Citrate was utilized by the organism which indicated positive result. The nitrate utilization test was found to be negative. It utilized gelatin and starch. The sugars like dextrose, fructose, mannitol and sucrose were fermented by TE-2. From the biochemical observation, the bacteria TE-2 was identified as *Bacillus subtilis*. The results were compared in accordance with the Bergeys Manual of determinative bacteriology. The results were depicted in Table-1.

Similarly, the TE-6 was also found to be a potential chromium tolerant bacteria were identified morphologically and biochemically. In nutrient agar medium the TE-6 produced mucoid whitish grey colored colonies. The bacterial isolate TE-6 was found to be gram negative rod shaped organism. Biochemical investigation of indole production test and methyl red test revealed negative results. Citrate was utilized by the organism. Nitrate was not reduced to nitrites. So it is indicated negative results.

The strain TE-6 utilized gelatin hence result was found to be positive whereas it does not utilize starch. The sugars like dextrose, fructose, mannitol, and sucrose were tested where in this strain fermented dextrose hence it is considered to be positive. Whereas the other sugars were not fermented by the TE-6.

From the biochemical observation the bacterial isolate TE-6 was identified by *Pseudomonas aeruginosa*. The results were compared in accordance with the Bergeys Manual of determinative bacteriology. The results were depicted in Table-1.

***Analysis of chromium by Atomic Absorption Spectrophotometry***

Atomic Absorption Spectrophotometry is a rapid and sensitive technique to analyze hexavalent chromium and role of microorganisms in biodegradation process were performed. Table: 2 depict the total chromium concentration and its absorbance between control and experimental treated *Pseudomonas aeruginosa* revealed a reduction in total chromium of 0.016 ppb for 200 ppb whereas on exposure to chromium as control revealed 0.019 ppb. Simultaneously when chromium was treated with *Pseudomonas aeruginosa* and it was found elevated to 400 ppb revealed a remarkable reduction of 0.024 as control and followed by experimental treatment exhibited in 0.020 ppb. Similarly, the total chromium concentration and its absorbance between control and experiment were studied.

On analyzing the experimental results by Atomic Absorption Spectrophotometry, the control treated sample revealed 0.019 ppb exposed for 30 days. Whereas, hexavalent chromium inoculated with *Pseudomonas aeruginosa* revealed 0.018 ppb observed for 30 days. Simultaneously, an enhanced concentration of chromium with 400 ppb exposed for 30 days. whereas analysis for control treated sample exhibiting 0.024 ppb. Whereas, the experimental treated sample that is hexavalent chromium inoculated with *Pseudomonas aeruginosa* for 30 days of absorption of exposure revealed 0.021ppb. From the above results, it is observed that hexavalent chromium can be degraded by chromate reductase rended by versatile microorganism known for its ubiquitous presence play a pivotal role in reducing hexavalent chromium fore into different compounds leading to release of $CO_2$ and $H_2O$. the results were recorded in Table:3 and Plate:7.

***Assay of chromate reductase***

*Pseudomonas aeruginosa* was grown in submerged culture for chromate reductase extraction. The extraction of chromate reductase enzyme was performed after 7 days of incubation. The results for chromate reductase enzyme activity was presented in Table-4.

From the table it was clear that the crude enzyme had a total chromate reductase activity and total protein concentration of 0.014 µ moles $min^{-1}$ and 0.25 mg respectively. A specific activity of 0.56 µ moles $min^{-1}$ $mg^{-1}$ was recorded for the crude chromate reductase enzyme.

***The effect of temperature on chromate reductase enzyme***

The effect of temperature on chromate reductase on temperature was depicted in Figure: 1. The enzyme obtained from *Pseudomonas aeruginosa* exhibited an optimum temperature of activity of 50°C with 0.106 µ moles $min^{-1}$. Above the optimum temperatures (60°C and 70°C) the enzyme stability and chromate reductase activity was found to be decreased to 0.08 µ moles $min^{-1}$ and 0.06 µ moles $min^{-1}$. At 20°C and 30°C the chromate reductase activity was found to be 0.016 µ moles $min^{-1}$ and 0.024 µ moles $min^{-1}$ respectively.

**Table 1** Identification of the Chromium Tolerant Bacteria

| S. No | Characterization | TE-2 | TE-6 |
|---|---|---|---|
| | **Morphological characterization** | | |
| I | Gram's staining | Positive (short rod) | Negative (rod) |
| | Spore staining | Presence of endospore | Negative |
| | **Biochemical characterization** | | |
| II | Indole production test | Negative | Negative |
| | Methyl red test | Negative | Negative |
| | Citrate utilization test | Positive | Positive |
| | Triple sugar iron test | Alkaline slant | Alkaline slant, Acid Butt and $H_2S$ Production |
| | Vogesproskauer test | Negative | Negative |
| | Nitrate utilization test | Negative | Negative |
| | Gelatin hydrolysis test | Positive | Positive |
| | Starch hydrolysis test | Positive | Negative |
| | **Carbohydrate fermentation test** | | |
| III | Dextrose | Positive | Positive |
| | Fructose | Positive | Negative |
| | Mannitol | Positive | Negative |
| | Sucrose | Positive | Negative |

**Table 2** Analysis of total chromium by Atomic Absorption Spectrophotometry on treatment with *Pseudomonas aeruginosa* after 15 days of exposure

| S. No | Name of the sample | Duration of exposure | Concentration of chromium exposed in ppb | Absorption |
|---|---|---|---|---|
| 1 | Control | 15 days | 200 | 0.019 |
| 2 | Experiment | 15 days | 200 | 0.016 |
| 3 | Control | 15 days | 400 | 0.024 |
| 4 | Experiment | 15 days | 400 | 0.020 |

**Table 3** Analysis of total chromium by Atomic Absorption Spectrophotometry on treatment with *Pseudomonas aeruginosa* after 30 days of exposure

| S. No | Name of the sample | Duration of exposure | Concentration of chromium exposed in ppb | Absorption |
|---|---|---|---|---|
| 1 | Control | 30 days | 200 | 0.019 |
| 2 | Experiment | 30 days | 200 | 0.018 |
| 3 | Control | 30 days | 400 | 0.024 |
| 4 | Experiment | 30 days | 400 | 0.021 |

**Table 4** Assay of chromate reductase enzyme from *Pseudomonas aeruginosa* after 7 days

| Extraction step | Total activity μ moles $min^{-1}$ | Total protein (mg) | Specific activity μ moles $min^{-1}$ $mg^{-1}$ |
|---|---|---|---|
| Crude enzyme | 0.14 | 0.25 | 0.56 |

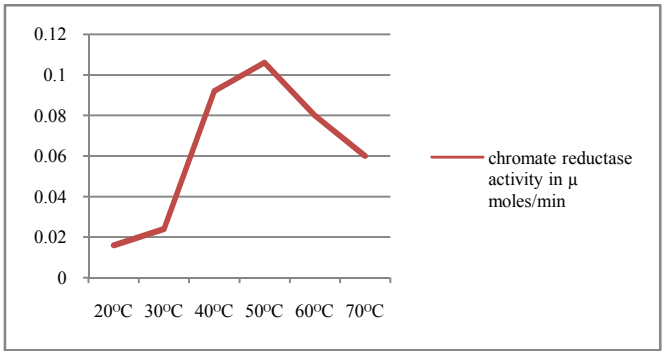



**Figure 1** Effect of temperature on the activity of chromate reductase from *Pseudomonas aeruginosa*

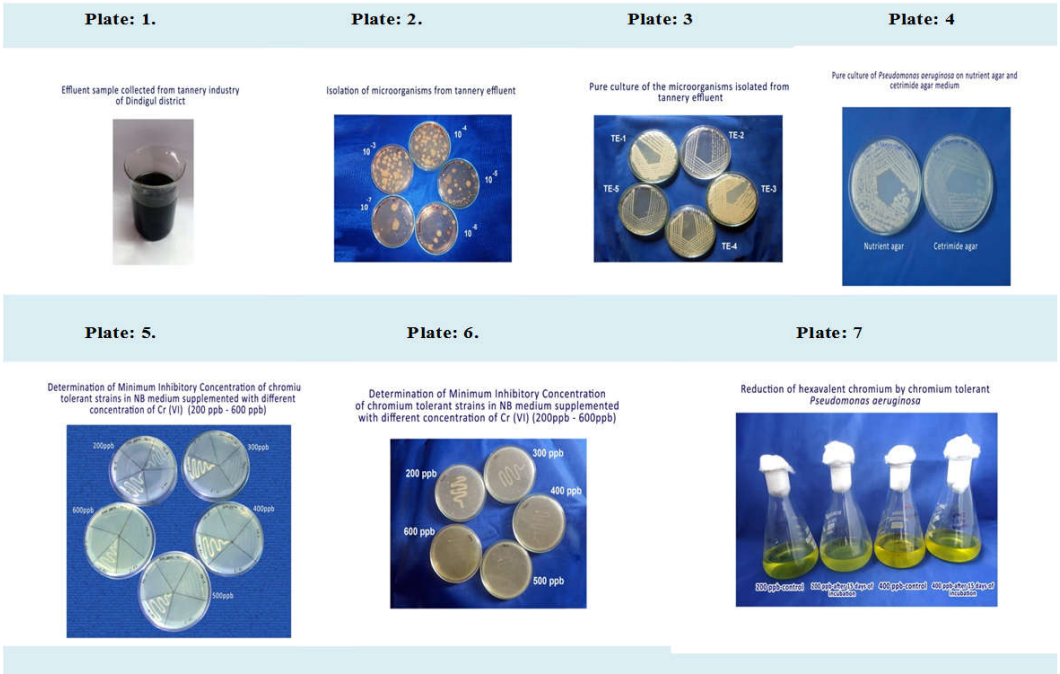
## DISCUSSION

Chromium is one of the major pollutants released from tanneries, dyes and textile industries were highly toxic and carcinogenic in nature. The Cr (VI) is associated with various forms of cancer, particularly pancreatic cancer (Alguacil *et al.,* 2002) and respiratory tract (Kuo *et al.,* 2004).

The chemical methods for the bulk treatment of these effluents often fail to reduce the level to meet the environmental regulations. Bioremediation is considered a better alternative to chemical treatment for this purpose as the chemical agents add to the environmental pollution. A wide variety of mechanisms exist for the removal of heavy metal from aqueous solution by bacteria, fungi, algae and higher plants (Pattanapipitpaisal *et al*., 2001).

The human exposure to such toxic substance (Cr) is both unavoidable and prevalent. The health implications are quite serious and devastating especially for children and pregnant women. These rising levels of heavy metal such as chromium contaminated the aquatic as well as terrestrial environments. Such toxins tend to persist in the environment, accumulating in the biota, thereby entering the food chain causing dheleterious harm to higher animals and human (Zhang *et al*., 1998).

The microbial reduction of hexavalent chromium has practical importance because biological strategies provide grain technology that is cost effective (Prabakaran, 2004). Subsequent studies with *Pseudomonas* a gram negative rod shaped bacteria and by employing sporulating microorganisms like *Bacillus subtilis* have shown that it can reduce hexavalent chromium discharged as effluent from tannery industry. Chromium is one of the major pollutant releases from tanneries, dyes and were highly toxic, carcinogenic in nature bioremediation is considered as a successful because it relies on management of soil microbial population catabolizing the contaminants like chromium a wide variety of mechanism exist for removal of heavy metal from aqueous solution by bacteria, fungi, algae and higher plants (Rahman *et al*., 2007).



In the present study, the chromium reducing capability of *Pseudomonas aeruginosa* were studied with different concentration of chromium that 200 ppb and 400 ppb exposed for control as well as in experimental study rate of chromium reduction increased with increase in cell density. The strain *Pseudomonas aeruginosa* exhibited increased cell growth up to 96 hrs of incubation. The findings of the study revealed that *Pseudomonas aeruginosa* removed chromium effectively under optimum conditions within 96 hrs of incubation. Our results are in total conformity with the findings of Terry *et al*., 2008 wherein they reported that gram negative bacterial isolate from chromium contamination site that was capable of reducing chromium to an insoluble precipitate.

In our study the control a known concentration of hexavalent chromium with 200 ppb revealed an absorbance limit of 0.019 analyzed with Atomic Absorption Spectrophotometry. whereas on treatment with experimental samples with 200 ppb of chromium inoculated with *Pseudomonas aeruginosa* revealed a drastic reduction in residues of chromium due to the degradative nature of bacteria *Pseudomonas aeruginosa* observed for 15 days chromium reduction is not observed in uninoculated control. This indicates the ability of *Pseudomonas aeruginosa* to reduce Cr (VI) although cell morphology is damage and bacterial growth is inhibited by Cr (VI). This indicates a small amount of chromium is adsorbed on the cells and decrease of chromium is caused by reducing activity of enzymes present in the *Pseudomonas aeruginosa.*

In the present investigation, the analysis of experimental results were performed after 30 days of exposure by Atomic Absorption Spectrophotometry. The results revealed a drastic difference between control and experimentally treated samples. On analyzing the control treated sample of 200 ppb revealed 0.019 ppb of chromium whereas on treatment with *Pseudomonaa aeruginosa* exhibited the accumulation of chromium with 0.018 ppb. This may be due to the presence of chromate reductase enzyme. It was secreted by *Pseudomonas aeruginosa*. The Atomic Absorption Spectrophotometry analysis of 400 ppb chromium control exhibited 0.024 ppb. Whereas, on treatment with *Pseudomonas aeruginosa* reduces the hexavalent chromium to the concentration of 0.021 ppb. The total chromium analysis result indicated the chromium reduction. Our results are in total conformity with the work of Srivatsava and Thakur (2008). Benazir *et al*., 2009 also estimated total chromium by Atomic Absorption Spectrophotometry to know the total chromium present in tannery effluent after degradation by the microbial consortia.

On treatment with an increased concentration Cr (VI) to 400 ppb, the results revealed an absorbance limit of 0.024 was recorded by Atomic Absorption Spectrophotometry. Simultaneously, when chromium was inoculated with *Pseudomonas aeruginosa* a reducing level of chromium as 0.020 was recorded by Atomic Absorption Spectrophotometry. *Pseudomonas aeruginosa* advocate for heavy metal degradation.*Pseudomonas aeruginosa* play an important role in the bioremediation of hexavalent chromium ion. This is due to the presence of enzymes secreted by the microorganism.

Chromate reductases are a group of enzyme that convert $Cr^{6+}$ to $Cr^{3+}$. (Garcia, 2004). In the present study, the chromate reductase activity was assayed after 7 days of incubation. A specific activity of 0.56 µ moles $min^{-1}$ $mg^{-1}$ was recorded for the crude enzyme. Our present work coincides with the work of Bae *et al.,* 2005. Wherein they purified and characterized NADPh-Dependent Cr (VI) reductase from *Escherichia coli*. Park *et al*., 2000 also purified and characterized chromate reductase enzyme from *Pseudomonas putida* which coincide with our present work.

Temperature plays a significant role in the production and stability of the enzyme. In the present study, the enzyme produced from *Pseudomonas aeruginosa* recorded an optimum enzyme activity at 50$^{o}$C. Similar work was carried out by Suzuki *et al.,* 1992. Wherein they emphasized 50$^{o}$C was optimum for chromate reductase activity of *Pseudomonas ambigua.*

## CONCLUSION

From the present study, *Pseudomonas aeruginosa* a versatile microorganism showed excellent ability to reduce toxic hexavalent chromium to nontoxic trivalent chromium. Hence, this potential isolate can be used for removing chromium from the tannery effluent. In addition the role of chromate reductase present in *Pseudomonas aeruginosa* advocate for biodegradation of Xenobiotic compunds by enzymatic detoxification pathway.

## Reference


Alguacil,F.J., Villegas,M.A., 2002. Liquid membranes and the treatment of metal-bearing wastewaters. *Rev. Metal. MADRID* Vol.No38, PP: 45-55.

Arutchelvan,V., Kanakasabai,V., Elangovan,R., Nagarajan,S. and Muralikrishnan V. (2006). Kinetics of high strength phenol degradation using Bacillus brevis, *Journal of Hazardous Materials*, Vol.No; 129, PP:216-222.

Bae,W., Lee,H., Choe,V., Jahng,D., Lee,S. and Jeong,B. (2005). Purification and characterization of NADPH-Dependent Cr (VI) Reductase from *Escherichia coli* ATCC 33456, *J. Microb.,* Vol.No. 43, PP: 21-27.

Benazir,J., Fathima, Suganthi,R., Rajvel,D., Pooja,M., Padmini and Mathithumilah,B. (2010). Bioremediation of chromium in tannery effluent by microbial consortia, *African journal of biotechnology*, Vol.No.9(21), PP: 3140-3143.

Gokulakrishnan and Pandurangan,S.A. (2004). Contamination of ground water by tannery-A case study. *IJEP*, Vol.No:24(1): PP:18-24.

Goyal,N., Jain,S.C. and Banerjee,U.C. (2003). Comparative studies on the microbial adsorption of heavy metals. *Advances in Environmental Research* Vol.No:7, PP:311-319.

Kuo,H.W., Chang,S.F. Wu,K.Y. and Wu, F.Y. (2004). Cr (VI) induced oxidative damage to DNA; increase of urinary 8-hydroxdeoxyguanosine concentration (8-OHdG) among electroplating worker. *Occup. Environ.* Vol.No. 60, PP:590-594.

Lokhande,R.S. and Vaidya,S.S. (2004). Physiochemical studies of the industrial effluents from the mid area of Kalyan-Dombivali. *Journal of Ecotoxicology and Environmental Monitoring*, Vol.No:14(3): PP:199-202.

Park,C.H., Keyhan,M., Wielinga,B., Fendorf ,S. and Matin,A. (2000). Purification to Homogeniety and Characterization of a novel *Pseudomonas putida* Chromate Reductase, *APP:l.*and*Env. Microb.,*Vol.No:66:PP:1788-1795.

Pattanapipitpaisal,P., Brown,N.L. and Macaskie,L.E. (2001). Chromate reduction and 16S rRNA identification of bacteria isolated from a Cr(VI)-contaminated site.

Applied Microbiology & Biotechnology Vol.No:57, PP:257-261.

Prabakaran,V. (2004). Impact of microbial fauna on biodegradation and bioaccumulation of pesticides by aquaphytes in an agro eco system. Ph.D. Thesis, Madurai Kamaraj University.PP:35-98.

Rahman, M.U., S. Gul and Z.U. Haq 2007. Reduction of chromium by locally isolated Pseudomonas sp. C-171. *Turk. J. Biol.* Vol.No.311, PP:61-66.

Rajkumar,M., Nagendran,R., Lee,K.J. and Lee,W.H. (2005). Characterization of a novel Cr(VI) reducing *Pseudomonas* sp. with plant growth-promoting potential. *Current Microbiology*, Vol.No:50: PP:266–271.

Ross,M. and Ganter,A. (1998). Possibilities of reduction of recipient loading of tannery waste Slovenia. *Water Sci Tech* Vol.No:37: PP:145–152.

Smith,W.A., Apel,W.A., Petersen,J.N. and Peyton,B.M. (2002).Effect of carbon and energy source on bacterial chromate reduction. *Bioremediation Journal*. Vol.No:6, PP:205-215.

Srivatsava and Thakur (2008). Evaluation of biosorption potency of *Acinetobacter species* for removal of hexavalent chromium from tannery effluent, Iran, *J. Environ. Health. Sci. Eng*., Vol.No. 3, PP: 195-200.

Suzuki,T., Miyata,N., H,Horitsu,H., Kawai,K., Takamizawa,K., Tai,K. and Okazaki,M. (1992). NAD(P)H-dependent chromium (VI) reductase of *Pseudomonas ambigua* G-1; a Cr VI intermediate is formed during the reduction of Cr (VI) to Cr (III). *J. Bacteriol.,* Vol.No. 174, PP: 5340-5345.

Terry,N. and Zayed.A. (2008). Chromium in the environment: factors affecting biological remediation. Plant Soil 249: 139-156.

Vidya,S. and Usha,K. (2007). Remediation potential of ocimum Basilicum against Tannery wastes. *Poll. Res.,* Vol.No:26(3): PP: 421-425.

Zhang,L., Zhae,L., Yu,Y. and Chen,C. (1998). Removal of lead from aqueous solution by non living *Rhizopus nigricans*. *Water Res*., Vol.No. 32, PP: 1437-1444.

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