Isolation and identification of Chromium-reducing bacteria from Challawa industrial area Kano State, Nigeria

Abstract

Chromium (Cr) is toxic, mutagenic, carcinogenic, and teratogenic and much more toxic to many plants, animals, and bacteria inhabiting aquatic environments because its motile, highly toxic, soluble in water and it is a strong oxidizing agent that causes severe damage to cell membranes. Chromium remediation through microorganisms is accepted as the best and economically affordable technology at present to clean-up Cr contamination, some chromium resistant bacteria possessed the property of reducing chromium (VI) to (III) with the help of reductase enzymes. This research was aimed to isolate and identify bacteria capable of reducing chromium from tannery effluent. The level of chromium from different tannery industries were analyzed using MPAES and compared to EPA standard which shows that all the samples were above the EPA standard with the exception of one sample, a pour plating method was used to isolate the bacteria on Luria bertani (LB) amended with $K_2Cr_2O_7$ after serial dilution, the isolate was identified morphologically, biochemically and molecularly as Acetanaerobacter sp. *based on 16s rRNA gene sequencing and molecular phylogenetic analysis, Atomic absorption spectrophotometry was carried out to determine the level of chromium from the bio-reduced sample. A reduction of 18.32% was observed in broth treated with the isolate within 48 hours of incubation at 37 ˚C.*

Key words: Bioreduced,chromium, tannery industries, *Acetanaerobacter* sp.

Introduction

Leather tanning and its related industrial pollution is one of the major industrial as well as environmental pollution problems in developing countries (Kabir *et al.,* 2018). Kano has several tannery industries in Challawa, Sharada and Bompai industrial estates. These areas are well known for its tannery industry. Hence, resolving environmental pollution from these industries have been a challenging issue, Because their operations could generates large amount of organic and inorganic wastes known as Sludge (Nigam *et al.,* 2015) and the quantity continually increases because of the high demand in leather materials and its status (as a second non-oil source of foreign exchange) in the nation's economic development FME, 2012,(Garba and Mohammed, 2017). Leather tanning industries use chromium compounds extensively in the tanning process and release the untreated effluents and solid wastes containing higher level of chromium in the natural environments which results to severe anthropogenic chromium pollution (Cheung and Gu, 2007; Garg *et al.,* 2012).

Among heavy metals presents in tannery waste, Chromium is one of the most common pollutant. Chromium is found in several oxidation states ranging from -2 to +6, among which chromium (VI) and chromium (III) are the most significant as of their persistence and stability. Chromium (VI) catches its place in the priority list arranged by the Agency of Toxic Substances and Diseases Registry (ATSDR) and also Cr(VI) has been listed as one of the seventeen chemicals causing harm to human beings by the United States' Environmental Protection Agency (US EPA) (US Dept. of Energy) and the limited concentrations of Cr(VI) into the inland surface water and potable water have been set as 0.1 mg•kg-1 and 0.05 mg•kg-1 respectively (Malairajan *et al.,* 2008) and the permissible limit of 2 mg/L for wastewater discharge (Belay, 2010). Currently, a maximum value is accepted of 10 µg/L for the general population and 20 µg/L for the occupationally exposed population (Baldiris *et al.,* 2018).

Chromium is discharged into the environment through various industrial wastewaters, such as leather tanning, electroplating, paints, pigment production, steel manufacture. Others industrial processes using catalysts discharge worldwide huge amounts of chromium every year and it has become a serious health problem (Farag and Zaki, 2010)

Chromium compounds can cause mutation and cancer, and hinder enzymes and nucleic acid synthesis(Belay, 2010). At high concentrations chromium is toxic, mutagenic, carcinogenic, and teratogenic. Chromium exists in oxidation states of +2, +3, and +6. The trivalent oxidation state is the most stable form of chromium and is essential to mammals in trace concentration and relatively immobile in the aquatic system due to its low water solubility. The hexavalent chromium is much more toxic to many plants, animals, and bacteria inhabiting aquatic environments. Most microorganisms are sensitive to Cr (VI) toxicity but some groups possess resistance mechanisms to tolerate high levels. A relationship was found between the total chromium content of soil and the presence of metal tolerant/resistant bacteria. In natural waters two stable oxidation states of Cr persist (III and VI), which have contrasting toxicities, motilities, and bioavailability. Cr (VI) is motile and highly toxic and soluble in water and it is a strong oxidizing agent that causes severe damage to cell membranes (Belay, 2010; Garba and Mohammed, 2017)

One of the major emerging environmental problems in the tanning industry is the disposal of chromium contaminated sludge produced as a by-product of wastewater treatment. it is important to treat these wastewaters before their final discharge (Sharma and Malaviya, 2016).There are several physicochemical methods (electrocoagulation, ozonation, reverse osmosis and ultrafiltration) available for the treatment of tannery wastewater, but they have one or more shortcomings. For instance, coagulation-flocculation by Fe and Al salts have shown reasonable results in terms of total suspended solids (TSS), chromium, COD, and turbidity removals, albeit at the expense of additional sludge formation. Likewise, electrochemical oxidation proved uneconomic when applied to raw tannery wastewaters due to the very high energy requirement and also due to corrosive effects of effluent on the electrodes. Similarly, main drawback of membrane application is a significant fouling due to the clogging, adsorption and cake layer formation by the pollutants onto the membrane (Lofrano *et al.,* 2013) Bioremediation, which includes the utilization of microorganisms or their enzymes is a potential technique for detoxification of industrial wastewaters (Sanjay *et al.,* 2018).

Bioremediation using bacteria is regarded as the most suitable technique since bacterial populations can show resistance to Cr (VI). Chromium remediation through microorganisms is accepted as the best and economically affordable technology at present to clean-up Cr contamination. It was noted that some chromium resistant bacteria possessed the property of chromium reduction with the help of reductase enzymes. The chromate reductases found in chromium resistant bacteria are known to catalyze the reduction of chromium reduction (Murad *et al.,* 2018). This property may be due to the presence of chromate reductase gene or induced protein (Sanjay *et al.,* 2018).

Materials and Methods

Sample collection

Effluent samples from the tannery industry was collected on August 28th, 2019 from Challawa industrial area Kano located on latitude 11˚58-11˚50 N and longitude range of

8˚31- 8˚40 E at an average elevation of 430m above the mean sea level in sterile bottles and transported to Microbiology laboratory, Bayero University Kano.

Detecting Chromium level

The samples were digested and chromium level was analyzed using Microwave Plasma Atomic Emission Spectroscopy (MPAES).

Isolation of chromium-reducing bacteria

The microbes were isolated from tannery effluent (semi solid) by pour plating method. About 1 ml of the effluent was mixed with 9 ml of sterile physiological saline (0.85%) and serially diluted. Dilutions from 10^{-2} to 10^{-8} were pour plated on Luria Bertani (LB) medium amended with $K_2Cr_2O_7$ as hexavalent chromium compound and incubated for 24 h at 37 °C. The selected colonies were then grown on tryptic soy broth throughout the study (Sundari, 2017)

Pure culture of isolates

Out of the various colonies that were obtained from serial dilution by pour plating method, unique colonies were then selected based on the colony morphology and were purified by repeated streaking on Luria Bertani (LB) medium amended with $K_2Cr_2O_7$.

Screening for hexavalent chromium tolerant bacteria

The selected colonies labelled A, B, C, and D were then separately grown on tryptic soy broth (TSB) amended with different concentration of $K_2Cr_2O_7$ as source of hexavalent chromium 20, 40, 60, 80 and 100 mg/L along with control (without $K_2Cr_2O_7$) and incubated at 37 °C for 24 h.

Identification of Cr(VI)-reducing bacteria

The isolate that tolerated the highest concentration of Cr(VI) was selected and further identified based on morphology, biochemical and molecular identification (Smrithi and Usha, 2012).

Gram staining

A smear was prepared on a clean grease free slide and gram staining was carried out according to (Cheesbrough, 2006).

After Gram Staining biochemical test was then carried out.

Catalase test

A portion of the bacteria was transferred with a sterilized wire loop to a drop of H_2O_2 on a clean glass slide. The presence of catalase was observed by effervescence of O_2 and no effervescence indicate negative result.

Urea Utilization Test

A sterilized wire loop was used to transfer the inoculum on to a medium containing urea as a sole source of nitrogen and phenol red and was incubated for 24 h at 37 °C. The development of pink red indicates positive result and initial yellow indicate negative result

Oxidase Test

p-phenylenediamide dihydrochloride solution and placed over a colony and then observed for colour change after 10-15 seconds. Dark purple indicates the presence of cytochrome oxidase in the test colony.

Methyl Red Test

A portion of the media was inoculated into a Glucose phosphate broth and incubated at 37 °C for 24 – 48 h. After 24 hours few drops methyl red reagent was added color change indicate positive result.

Voges-Prauskauer (VP) test

Glucose phosphate broth was inoculated with culture and incubated at 37 °C for 24 – 48 h. After 24 h few drops of 40% KOH, mix vigorously and alpha naphthol was added and results were observed immediately.

H_2S production test

Peptone broth was inoculated with test culture. A white filter paper strip was soaked in saturated lead acetate solution. Lead acetate paper strip was placed in neck of the tube such that ¼ of the strip projects below the cotton plug and then broth was incubated at 37 °C for 24 h. After 24 hours result was observed based on color change.

Nitrate reduction test

The test culture was inoculated in Peptone Nitrate Broth and incubated at 37 °C for 24 h. After 24 h, 0.5 ml of alpha napthylamine was added, followed by addition of 0.5 ml sulphanilic acid and observed for development of red colour.

Citrate utilization test

Simmon citrate agar slant was streaked with the test culture and incubated at 37 °C for 24 – 48 h. Slant was observed for the color change, color change from green to blue indicates positive result.

Indole production test

Tryptone broth was inoculated with test culture and incubated at 37 °C for 24 h. After 24 h, 1 ml of Kovac's reagent was added on top of the medium and observed for formation of pink ring or cherry red ring.

Starch utilization test

The nutrient agar plate having 2% starch was streaked at the center as a spot with test culture and incubated at 37 °C for 24 – 48 h. After 24 h, the plate was flooded with iodine solution and checked for the zone of hydrolysis (Brahmbhatt, 2012).

Molecular Identification

Genomic DNA was extracted from pure bacterial culture grown on tryptic soy broth according to (Livak, 1984). The PCR reaction was carried out using KAPATaq DNA polymerase. and 16S rRNA gene fragment analysis was carried out by PCR amplification using forward primer Bact1442-F (AGAGTTGATCCTGGCTCAG) and reverse primer Bact1492-R (GGTTACCTTGTTACGACTT). PCR products were separated in 1.5% agarose gel stained with ethidium bromide, visualized under UV light, and photographed. The 16S

rRNA gene sequences of the isolates obtained in this study were aligned and compared with the known 16S rRNA gene sequences in Genbank database using the BLAST at the National Center for Biotechnology Information (http://www.ncbi. nlm.nih.gov/BLAST/) to determine the closest available database sequences.

Assessment of Cr VI) bio-removal efficiency

Atomic Absorption Spectroscopy (AAS) was carried out in order to analyze the level of chromium in the bioreduced (cultured) samples relative to control (uncultured). Both samples were digested and taken for AAS at the central laboratory complex Bayero University, Kano.

Results and Discussion

Level of Chromium in Tannery Effluent

The level of chromium from discharged tannery effluent was assessed using MPAES and compared with EPA standard as presented in **Figure** I, the result revealed that out of the six samples only one is below EPA standard for chromium discharged in industries

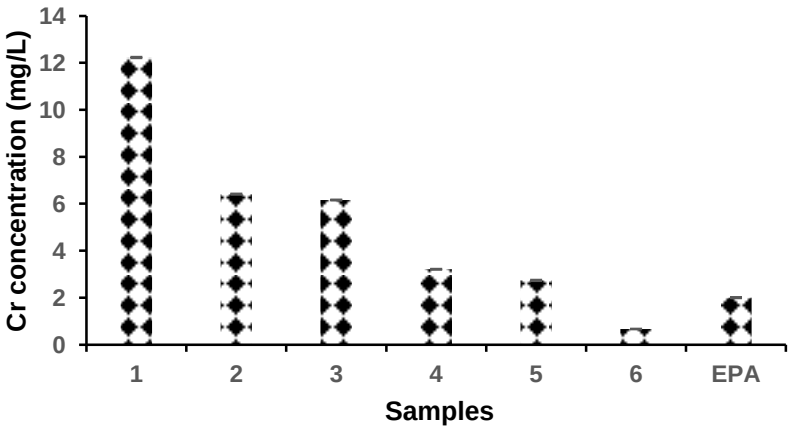

Figure I: Level of Chromium from discharged tannery effluent. Data are mean ± SD of triplicate determinations

Following serial dilution and a successful pour plating on LB media and incubated at 37 °C for 48 h, a round milky colonies appeared, from which a distinct colony was isolated and streaked on a freshly prepared sterile media to obtain pure culture.

Screening for Cr(VI) tolerant bacteria

Isolated bacteria (4) labelled A, B, C and D were screened for their tolerance to chromium salt by growing them on tryptic soy agar supplemented with various concentrations of Cr compound and incubated for 24 h at 37 °C. Of the four isolates, only isolate B was able to tolerate the highest concentration tested, therefore was chosen for the study (**Table** I).

Table I: Screening of various isolates from tannery effluent for tolerance to various Cr concentrations on tryptic soy broth (TSB) media at 37 °C for 24 h

Isolate	20	40	60	80	100
		(mg/L)			
A	+	+	+	−	−
B	+	+	+	+	+
C	+	+	−	−	−
D	+	+	+	−	−

Key: + = present, - = absent of growth

Identification hexavalent chromium-reducing bacteria

The morphology of the isolates from the tannery effluent grown on tryptic soy agar at 37 °C for 24 h was visualized under microscope following gram staining. The result shows that all the isolates (A, B, C and D) were gram positive with different morphology (**Table** II).

Table II: Morphological identification of the isolates following gram staining

Isolate	Gram staining	Shape
A	Positive	Rod shaped
B	Positive	Rod shaped
C	Positive	Rod shaped
D	Positive	Cocci

Biochemical identification of hexavalent chromium-reducing bacterium

Biochemical test for the bacterial isolates from tannery effluent was presented in **Table** III. It was observed that the candidate isolate was positive to Voges-prauskauer, catalase, oxidase, nitrate production, H_2S production, indole production, citrate utilization, starch utilization and negative to methyl red, and urea utilization.

Table III: Biochemical identification for the candidate isolate

Biochemical Test	Result
Methyl Red	−
Catalase	+
Oxidase	+
Voges-Prauskauer	+
Nitrate Reduction	+
H_2S Production	+
Indole Production	+
Citrate Utilization	+
Starch Utilization	+
Urea Utilization	−

Key: +Positive -Negative

Molecular identification of hexavalent chromium reducing bacteria

It was found that after subjecting the PCR product to sequencing using cycle sequencing kit genetic analyzer from both forward and reverse directions. The 16S rRNA gene sequences of the bacterium obtained was compared with GenBank database using Blast Server at NCBI. The analysis shows that the DNA sequences obtained were closely related to the partial sequence of several *Acetanaerobacter* sp. Molecular phylogenetic tree studies using the neighbor joining method is presented in **Figure** II

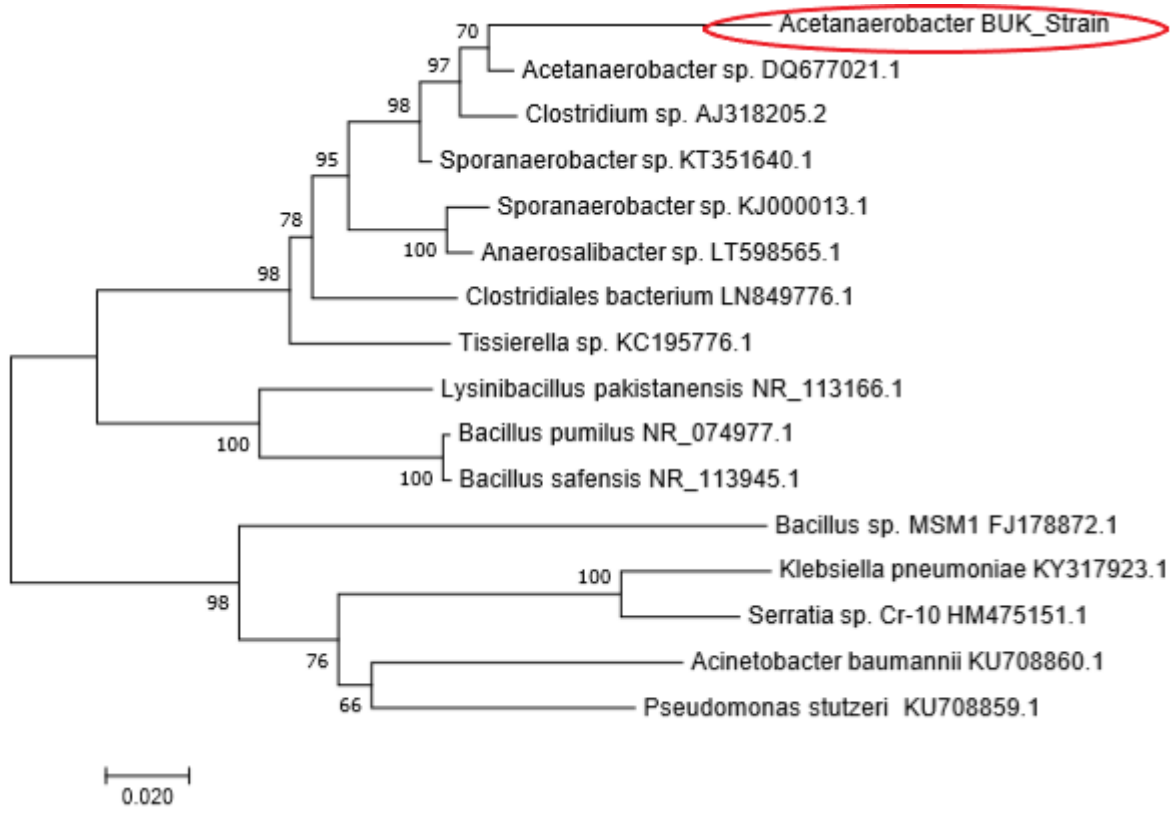

Figure II: Cladogram (neighbour-joining method) indicating the genetic relationship between unknown and referenced related microorganisms based on 16s rRNA gene sequence analysis. Accession numbers are accompanied by the specie names

Assessment of Cr(VI) bio-removal efficiency

To determine the Cr bio-removal efficiency of the isolate, inoculated and non-inoculated Cr containing culture media was subjected to Atomic Absorption Spectrophotometry (AAS) to determine the concentration of Cr, following 48 h incubation at 37 °C. From the result (**Figure** III) it was found about 18.32% of the Cr was removed from the cultured sample within 48 h of incubation.

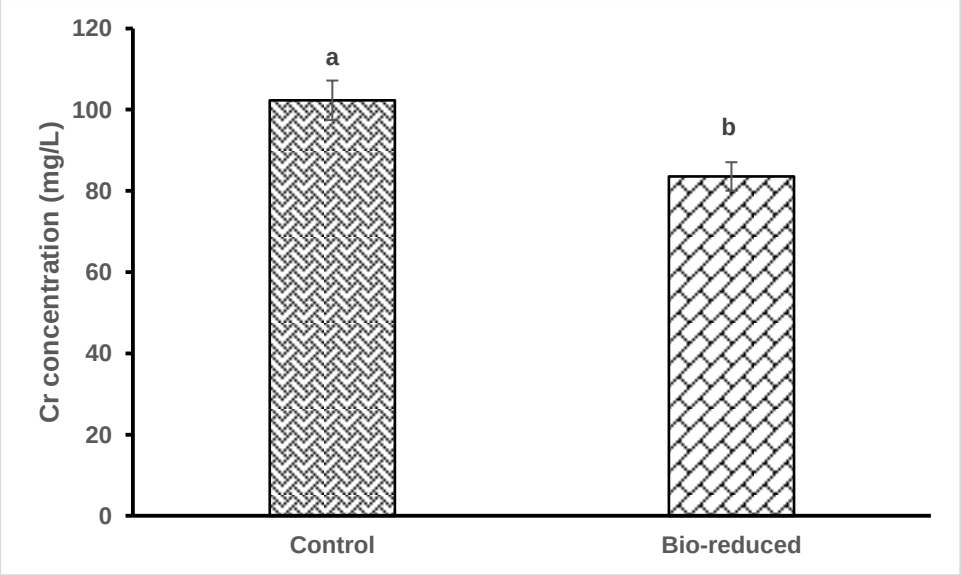

Figure III: Chromium concentration in inoculated (Bioreduced) and uninoculated (control) samples incubated at 37 °C for 48 h. Data are mean ± SD of triplicate determinations. Different letters over the bar indicates significant difference ($p<0.05$).

Discussion

Microbial reduction of Cr is a green technology that is more cost effective compared with chemical processes (Xiao *et al.,* 2012). To develop an efficient process for Cr bio reduction, microbes that are native to Cr-contaminated sites can be utilized. Since the site is their natural habitat, we hypothesize that they have developed a potential detoxifying process that allows them to cope with the otherwise toxic Cr. Indeed, a significant relationship was found between soil Cr content and presence of Cr-tolerant and -resistant bacteria (Das *et al*., 2013).
Since the first report of anaerobic Cr^{6+} reduction by Romanenko and Koren'Kov (1977) in uncharacterized *Pseudomonas* sp., worldwide researchers have isolated both aerobic and anaerobic Cr^{6+} reducing bacteria belonging to a range of genera from diverse environments (Alam and Ahmad, 2012) Up to now, a number of Cr-reducing bacteria have been reported, including *Bacillus* (Pan *et al*., 2014), *Enterobacter cloacae, Desulfovibrio vulgaris, Pseudomonas aeruginosa, Cupriavidus metallidurans, Ralstonia metallidurans, P. putida, Escherichia coli, Caulobacter crescentus, Shewanella oneidensis, Bacillus firmus* and *Burkholderia cepacian* (Zhang and Li, 2011). *Enterobacter* (Panda and Sarkar, 2015), *Escherichia* (Lin, 2014), *Achromobacter* sp., *Shewanella* sp., *Pseudomonas* sp. and others (Geoffrey *et al*., 2005; Pal and Paul, 2004; Sultan and Hasnain, 2007), *Nesterenkonia* sp. strain MF2, *Achromobacter* sp. strain Ch1, *Sphaerotilus natans* (Caravelli *et al*., 2008), *Escherichia* (Shen and Wang, 1993), and *Arthrobacter* (Avudainayagam *et al*., 2003), *Enterobacter cloacae* HO1 (Wang *et al*., 1989), *Bacillus* sp. (Tin and Changsong, 1995) and so on, indicating an important approach in bioremediation of Cr contamination. Utilization of microbes to bioremediate metal contaminated environment is an economical and environmentally friendly approach. Thus, chromate-reducing microbes with inherent ability for chromium detoxification is considered to be suitable for on-site and in *situ* bioremediation (Elahi *et al*., 2019).

Conclusion

The bacterium was isolated from tannery effluent, it was found to be gram positive and identified as *Acetanaerobacter* sp. by 16S rRNA gene sequencing and molecular phylogeny

and was capable of tolerating up to 100 mg/L of K_2CrO_7.The isolate was found to reduce Cr(VI) concentration by 18.32% within 48 hours. Thereby the isolate can be considered as a candidate for chromium bio-reduction

REFERENCES

Alam, M. Z., and Ahmad, S. (2012). Toxic chromate reduction by resistant and sensitive bacteria isolated from tannery effluent contaminated soil. *Annals of Microbiology*, **62**(1): 113–121. https://doi.org/10.1007/s13213-011-0235-4

Avudainayagam, S., Megharaj, M., Owens, G., Kookana, R. S., Chittleborough, D., & Naidu, R. (2003). Chemistry of chromium in soils with emphasis on tannery waste sites. *Reviews of Environmental Contamination and Toxicology*, **178**: 53–91. https://doi.org/10.1007/0-387-21728-2_3

Baldiris, R., Acosta-tapia, N., Montes, A., Hern, J., & Vivas-reyes, R. (2018). Reduction of Hexavalent Chromium and Detection of Chromate Reductase (ChrR) in Stenotrophomonas maltophilia. *Molecules*, **23**(406): 1–20. https://doi.org/10.3390/molecules23020406

Belay, A. A. (2010). Impacts of Chromium from Tannery Effluent and Evaluation of Alternative Treatment Options. *Journal of Environmental Protection*, **01**(01): 53–58. https://doi.org/10.4236/jep.2010.11007

Brahmbhatt, D. (2012). molecular identification of bacteria using 16s rdna sequencing a Dissertation Submitted to the In Partial Fulfilment of the Requirements for the Degree of Master of Science in Microbiology *:* (Issue May). https://doi.org/10.13140/2.1.4412.6406

Caravelli, A. H., Giannuzzi, L., and Zaritzky, E. (2008). Reduction of hexavalent chromium by Sphaerotilus natans a filamentous micro-organism present in activated sludges. *Journal of Hazardous Materials*, **156**: 214–222. https://doi.org/10.1016/j.jhazmat.2007.12.014

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries.

Cheung, K. H., & Gu, J. (2007). Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential : A review. *International Biodeterioration and Biodegradation*, **59**: 8–15. https://doi.org/10.1016/j.ibiod.2006.05.002

Das, S., Mishra, J., Kumar, S., Pandey, S., Srinivas, D., Chakraborty, A., Sudarshan, M., Das, N., and Thatoi, H. (2013). Investigation on mechanism of Cr (VI) reduction and removal by Bacillus amyloliquefaciens , a novel chromate tolerant bacterium isolated from chromite mine soil. *CHEMOSPHERE*. https://doi.org/10.1016/j.chemosphere.2013.08.080

Elahi, A., Ajaz, M., Rehman, A., Vuilleumier, S., Khan, Z., and Zajif, S. (2019). Isolation , characterization , and multiple heavy metal-resistant and hexavalent chromium-reducing Microbacterium testaceum B-HS2 from tannery effluent. *Journal of King Saud University - Science*, **31**: 1437–1444. https://doi.org/10.1016/j.jksus.2019.02.007

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.

Garba, M. ., and Mohammed, M. . (2017). Environmental Pollution Potential of Tannery Sludge from Challawa Industrial Area of Kano State, Nigeria. *IOSR Journal of Applied Chemistry (IOSR-JAC*, **10**(7): 28–34. https://doi.org/10.9790/5736-1007022834

Garg, S. K., Tripathi, M., and Srinath, T. (2012). *Strategies for Chromium Bioremediation of Tannery Effluent*. https://doi.org/10.1007/978-1-4614-2329-4

Geoffrey, P., Kramer, D. M., Roberts, A., and Xun, L. (2005). Formation of Soluble Organo - Chromium (III) Complexes after Chromate Reduction in the Presence of Cellular Organics. *Environmental Science Technology*, **39**(8): 2811–2817.

Kabir, M. M., Fakhruddin, A. N. M., Chowdhury, M. A. Z., Pramanik, M. K., and Fardous, Z. (2018). Isolation and characterization of chromium(VI)-reducing bacteria from tannery effluents and solid wastes. *World Journal of Microbiology and Biotechnology*, **34**(9). https://doi.org/10.1007/s11274-018-2510-z

Lin, Y. (2014). Modeling chromium (VI) reduction by Escherichia coli 33456 using ceramic pearl as a supporting medium. *International Journal of Environmental Science*

Technology, **11**: 1887–1896. https://doi.org/10.1007/s13762-013-0392-z

Livak, K.J. (1984) Organisation and mapping of sequence on the Drosophila melanogaster X and Y chromosomes that is transcribed during spermamatogenis. *Genetics journal* **107**:611-634

Lofrano, G., Meriç, S., Zengin, G. E., and Orhon, D. (2013). Chemical and biological treatment technologies for leather tannery chemicals and wastewaters: A review. *Science of the Total Environment*, **461–462**: 265–281. https://doi.org/10.1016/j.scitotenv.2013.05.004

Malairajan, S., Mengistie, A. A., and Rajamanickam, V. (2008). Biosorption of Cr (III) from aqueous solutions using indigenous biomaterials. *International Journal of Environmental Reasearch*, **2**(2): 177–182.

Murad, A. B. M. W., Mia, A. S., and Rahman, M. A. (2018). Studies on theWaste Management System of a Tannery : An overview. *International Journal of Science and Engineering Technology*, **7**(4).

Nigam, H., Das, M., Chauhan, S., Pandey, P., and Swati, P. (2015). Effect of chromium generated by solid waste of tannery and microbial degradation of chromium to reduce its toxicity : A review. *Advances in Applied Science Research*, **6**(3): 129–136.

Pal, A., & Paul, A. K. Ã. (2004). Aerobic chromate reduction by chromium-resistant bacteria isolated from serpentine soil. *Microbiological Research*, **159**: 347–354. https://doi.org/10.1016/j.micres.2004.08.001

Pan, X., Liu, Z., Chen, Z., Cheng, Y., Pan, D., Shao, J., Lin, Z., and Guan, X. (2014). Investigation of r (VI) reduction and Cr (III) immobilization mechanism by planktonic cells and biofilms of Bacillus subtilis ATCC-6633. *Water Research*. https://doi.org/10.1016/j.watres.2014.01.066

Panda, J., and Sarkar, P. (2015). Biosorption of Cr (VI) by Calcium Alginate-Encapsulated Enterobacter aerogenes T2 , in a Semi-Batch Plug Flow Process. *Water Air Soil Pollution*, **226**(2157). https://doi.org/10.1007/s11270-014-2157-9

Romanenko, V.I., and Koren'Kov,V.N. (1977). Pure culture of bacteria using chromates and bichromates as hydrogen acceptors during development under anaerobic conditions. *Mikrobiologiia* **46**(3): 414-417

Sanjay, M. S., Sudarsanam, D., Raj, G. A., and Baskar, K. (2018). Isolation and identification of chromium reducing bacteria from tannery effluent. *Journal of King Saud University - Science*, 265–271. https://doi.org/10.1016/j.jksus.2018.05.001

Sharma, S., and Malaviya, P. (2016). Bioremediation of tannery wastewater by chromium resistant novel fungal consortium. *Ecological Engineering*, **91**: 419–425. https://doi.org/10.1016/j.ecoleng.2016.03.005

Shen, H. A. I., and Wang, Y. (1993). Characterization of Enzymatic Reduction of Hexavalent Chromium by Escherichia coli ATCC 33456. *Applied and Environmental Microbiology*, **59**(11): 3771–3777.

Smrithi A, and Usha K. (2012). Isolation and Characterization of Chromium Removing Bacteria from Tannery Effluent Disposal Site. *International Journal of Advanced Biotechnology and Research*, **3**(3): 644–652. http://www.bipublication.com

Sultan, S., and Hasnain, S. (2007). Reduction of toxic hexavalent chromium by Ochrobactrum intermedium strain SDCr-5 stimulated by heavy metals. *Bioresource*

Technology, **98**: 340–344. https://doi.org/10.1016/j.biortech.2005.12.025

Sundari, N. S. (2017). Characterization of chromium bioremediation by Stenotrophomonas maltophilia SRS 05 isolated from tannery effluent. *International Research Journal of Engineering and Technology*, **4**(2): 403–429. www.irjet.net

Tin, W. Y., and Changsong, X. (1995). Factors Affecting hexavalent chromium reduction in pure cultures of bacteria. *Water Research*, **29**(11): 2467–2474.

Wang, P., Mori, T., and Masanori, K. T. (1989). Isolation and Characterization of an Enterobacter cloacae Strain That Redqces Hexavalent Chromium under Anaerobic Conditions. *Applied and Environmental Microbiology*, **55**(7): 1665–1669.

Xiao, W., Zhang, Y., Li, T., Chen, B., Wang, H., He, Z., and Yang, X. (2012). Reduction Kinetics of Hexavalent Chromium in Soils and its Correlation with Soil Properties. *Journal of Environmental Quality*, **41**. https://doi.org/10.2134/jeq2012.0061

Zhang, K., and Li, F. (2011). Isolation and characterization of a chromium-resistant bacterium Serratia sp . Cr-10 from a chromate-contaminated site. *Applied Microbiological Biotechnology*, **90**: 1163–1169. https://doi.org/10.1007/s00253-011-3120-y