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Bioremediation Potentials of Partially Purified Chromate (VI) Reductase Isolated From *Trebouxia erici* in Waste Water

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Abstract

Chromium (VI) is a toxic and carcinogenic metal ion that contaminates wastewater from various industrial sources. Bioremediation using chromate (VI) reductase enzymes offers a promising solution for removing chromium (VI) from wastewater. In this study, chromate (VI) reductase enzyme from the lichen *Trebouxia erici* was partially purified and characterized. The 2.23-fold partially purified enzyme with a 33.3% yield, was found to have a high specific activity of 117mU/mg protein and a high affinity for chromium (VI), with a K_M of 0.103 µmol/min/mg and a V_{max} values of 128 µM. Optimization studies revealed the enzyme's pH as 6.5 and temperature preferences of 40^{O}C, along with its kinetic parameters, establishing its effectiveness under diverse wastewater conditions. Application trials demonstrated significant Cr((VI)) reduction in industrial effluent samples, highlighting its potential as an eco-friendly and efficient bioremediation agent. The enzyme was also able to reduce chromium (VI) with a rate of 10 nmol/min/mg protein. Findings from present study demonstrate the potential of chromate (VI) reductase from *Trebouxia erici* in the bioremediation of chromium (VI)-contaminated wastewater.

Keywords: Chromate (VI) reductase, purification, bioremediation, wastewater.

Introduction

The rapid industrialization and urbanization of modern society have led to the increased release of toxic pollutants into the environment [1]. One of the most hazardous pollutants is chromium (VI), a known carcinogen that can cause severe health problems in humans and animals [2]. The removal of chromium (VI) from wastewater is a pressing environmental concern that requires innovative and effective solutions [3].

Chromium (VI) is a common contaminant in industrial wastewater, particularly in the leather, textile, and electroplating industries [4]. The traditional methods of chromium (VI) removal, such as chemical precipitation and adsorption, have several limitations, including high operating costs, low efficiency, and the generation of secondary pollutants [5][6]. Therefore, there is an urgent need to develop novel and sustainable technologies for chromium (VI) remediation.

Trebouxia erici, a species of green algae, has been found to possess remarkable characteristics that make it an ideal candidate for bioremediation [7]. This photosynthetic organism, commonly found in lichens, has been gaining attention in recent years for its potential to remove pollutants from the environment.

One of the most notable features of *Trebouxia erici* is its ability to tolerate high levels of heavy metals, including chromium (VI), a common pollutant in industrial wastewater [7][8]. This remarkable tolerance is attributed to the algae's high levels of antioxidant enzymes, which protect it from oxidative stress caused by heavy metals [9].

Furthermore, *Trebouxia erici* is capable of producing high levels of extracellular polymeric substances (EPS), which play a crucial role in binding and removing heavy metals from the environment[10][11]. This unique ability makes *Trebouxia erici* an attractive candidate for bioremediation applications.

Despite the promising characteristics of *Trebouxia erici*, there is a need for further research to fully explore its potential for bioremediation. Specifically, there is a lack of information on the enzymatic mechanisms involved in chromium (VI) reduction by *Trebouxia erici*. Therefore, the present study aims to isolate, partially purify and characterize the chromate (VI) reductase enzyme from *Trebouxia erici* and its potential for bioremediation of chromium (VI)-contaminated wastewater.

The present study further seeks to address the knowledge gap in the enzymatic mechanisms involved in chromium (VI) reduction by *Trebouxia erici*. Apart from providing valuable insights into the enzymatic properties of chromium (VI) reductase and its potential to remediate chromium (VI)-contaminated wastewater, the findings of this study will contribute to the development of novel bioremediation technologies for the removal of chromium (VI) from industrial wastewater.

Materials and Methods

The Plant

The lichen species used in this study were collected from a local pond in Mahuta area of Kaduna South, Kaduna and transported to the laboratory for further processing. It was authenticated by a botanist at the Department of Biological sciences, Kaduna State University, Kaduna, Nigeria. Voucher specimens of the lichen were deposited at the University Herbarium,

Chemicals

NADH (Nicotinamide adenine dinucleotide reduced), Potassium dichromate ($K_2Cr_2O_7$),. Tris-HCl buffer (pH 8.0), EDTA (Ethylenediaminetetraacetic acid), PMSF (Phenylmethylsulfonyl fluoride), DEAE-Sepharose, Sephadex G-75, Sodium chloride (NaCl),,Liquid nitrogen were products of Sigma chemical company, USA. Acrylamide, N, N, methyl bisacrylamide (Bis), ammonium

persulphate, glycine, bromophenolblue, coomasie brilliant blue, N,N,N'N' tetramethylethylene diamine (TEMED), sodium dodecylsulphate (SDS), sodium borohydride and Sodium periodate were products of British Drug House (BDH) Chemical, Poole, England. EDTA, mercaptoethanol, sodium hydroxide, sulphuric acid, potassium dichromate, sodium, dihydrogen phosphate, disodium hydrogen phosphate were products of Pharmacia Fine Chemicals, Upsala, Sweden. All other chemicals used were of analytical grade and supplied by reputable chemical suppliers

Equipment

All equipment and apparatus used in this study were of analytical grade.

Methods

Preparation of Stock Solution

Chromium (VI) solution: A stock solution of chromium (VI) was prepared by dissolving 0.2 g of potassium dichromate ($K_2Cr_2O_7$) in 1 litre distilled water.

Enzyme extraction buffer: A buffer solution containing 50 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride (PMSF) was used for enzyme extraction.

Chromatography columns: DEAE-Sepharose and Sephadex G-75 columns were used for enzyme purification.

Spectrophotometer: A UV-VIS spectrophotometer (Shimadzu Model, UV-1900i, Japan) was used to measure enzyme activity and chromate (VI) concentration at 340nm.

Incubator: A temperature-controlled incubator was used to incubate enzyme assays and chromate (VI) reduction reactions.

Centrifuge: A refrigerated centrifuge was used to separate enzyme extracts and chromate (VI) reduction reaction mixtures.

Experimental

Isolation of Chromium Reductase

Cell free extract of lichen plant was prepared by homogenizing the lichen plant in a buffer solution containing 50 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride (PMSF) in a fast speed blender and the homogenates were filtered and then centrifuged at 3000xg to obtain a supernatant that served as the crude enzyme extract. The cell free extract was used for the partial purification.

Ammonium Sulphate Precipitation

The pooled supernatants were then subjected to ammonium sulphate precipitation according to standard protocol [12].

Dialysis of Pooled Fractions

The pellet recovered by centrifugation at 10,000 g for 15 min was resuspended in 30cm^3 of the extraction buffer. The fractions from ammonium sulphate precipitation was poured into a dialysis bag and dialyzed for 24-72 hours in 100mM phosphate buffer pH 7.2.

DEAE-Sepharose Chromatography

The dialyzed extract from was applied directly to a DEAE-Sepharose column previously equilibrated with the operating buffer. The column was developed at a flow rate of 20 cm^3 /h and 3 cm^3 fractions were collected.

Molecular Sieve Chromatography

The pooled fractions were applied unto Sephadex G-75 column previously equilibrated with the operating buffer. The column was developed at a flow rate of 20 cm^3 /hr and 3 cm^3 fractions were collected and assayed for protein and chromium reductase activity. Fractions with high specific activity were pooled.

Chromate (VI) Reductase Assay

This was carried out by measuring decrease in Cr (VI) concentration as described by method of Park *et al., (*2000). Extract of the chromium reductase was incubated for 2 hours at 30^o C in a reaction mixture containing 0.1mM NADH, (40µl) and 600µl of 0.2% 1.5-diphenylcarbazide. Absorbance was read at 540 nm against a reference sample incubated without the enzyme extract.

Total Protein Determination

The protein content of the enzyme was determined according to Bradford [13] using bovine serum albumin as standard.

SDS-PAGE Analysis

Electrophoresis was carried out for fractions with high specific activity due to Chromium reductase in 12% polyacrylamide disc gel according to the method of Davis (1964) using Tris-glycine buffer, pH 8.3. Protein bands were viewed by staining with Coomassie Brilliant blue R-250 (Sigma). Subunit molecular weight was also estimated by SDS-polyacrylamide gel electrophoresis [14]

Optimum pH Determination

The activity profile of chromium reductase was determined as a function of pH using NADH as substrate. The buffers were prepared at different pH values in the range of pH 4-9[100mM, Citrate (4.0-6.0), phosphate (6.0-7.0) and Tris (7.0-9.0)] and the activity of the enzyme was determined A plot of Chromium reductase activity was made against pH to determine the optimum pH.

Optimum Temperature Determination

Chromium reductase activity was assayed at 4^o C, 20^o C, 40^o C, 50^o C, 60^o C and 70^o C. A plot of Chromium reductase activity was made against temperature to determine the optimum temperature.

Kinetic Parameters

The substrate NADH was prepared at the concentration range of 0.2-3mM in borate buffer pH 6.0. Chromium reductase activity was determined and the inverse of activity was plotted against the inverse of substrate concentration. The K_M and V_{max} of the enzyme were determined from a double reciprocal plot [15].

Results and Discussion

Table 1: Purification Profile of Chromate(VI) reductase from *T. erici*

Purification Steps	Total protein(mg)	Total Activity (U)	Specific Activity (U/mg) x10^3	Purification fold	Yield (%)
Crude extract	200	1.05	5.25	1.00	100
Salt Fractionation	120	0.75	6.25	1.19	71.4
Dialysis	105	0.70	6.67	1.27	66.7
DEAE-Sepharose	83	0.61	7.35	1.40	58.1
Sephadex G-75	30	0.35	11.7	2.23	33.3

*One unit of enzyme was defined as the amount of enzyme that converts 1 nmol of Cr (VI)/min under standard assay conditions.

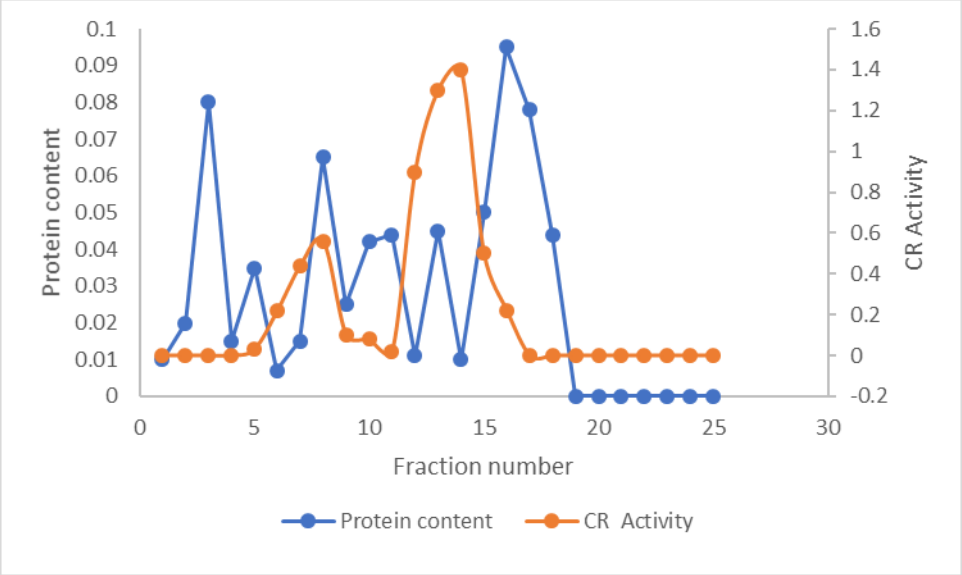


Figure 1: A typical elution profile for the chromatography of CR on DEAE-Sepharose Column (1 cm x 50cm) previously equilibrated with 100mM phosphate buffer at a flow rate of 20 ml/h and 3 ml fractions were collected

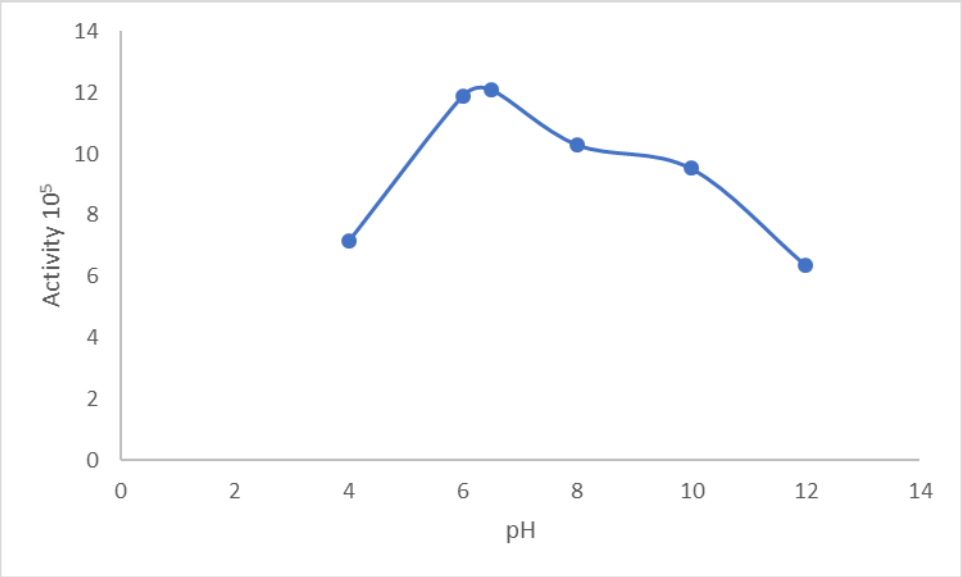
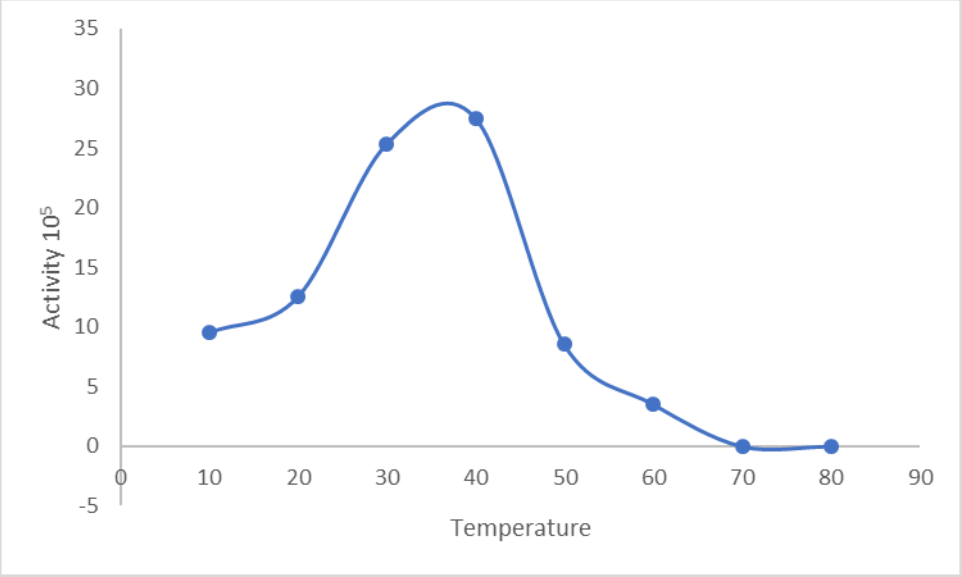


Figure 2: Optimum pH profile of CR(VI) reductase isolated from *T. erici*. Plot is average of triplicate determinations



Figur 2: Optimum Temperature profile of CR(VI) reductase isolated from *T. erici*. Plot is average of triplicate determinations

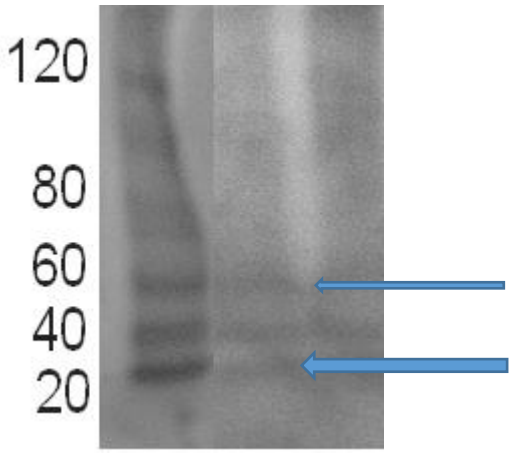


Plate 1: SDS-PAGE analysis of purified CR from *T. erici* on 12% acrylamide gels. Lane I: Molecular weight* markers as standard (20 – 120 kD). Lane II & III: Purified Chromate(VI) reductase

* **Molecular Weight** standards Albumin, Bovine serum(66 kD), Glutamine dehydrogenase, Bovine liver (55kD), Ovalbumin, chicken egg (45 kD) glyceraldehyde-3-phosphate dehydrogenase, rabbit muscle (36 kD) Carbonic anhydrase, Bovine erythrocyte (29 kD), Trypsinogen, Bovine pancrease (24kD), Trypsin inhibitor, Soybean (20kD), α-lactalbumin, bovine milk (14kD) (Sigma).

In this report, the partially purified enzyme showed a profile of about 2.23-fold with 33.3% recovery. The purified extract had a relatively higher CR activity than the crude extract. This observation can be attributed to the removal of endogenous inhibitors and other contaminating constituents by hydrophobic interaction with the column.

Further characterization indicate that the two peaks obtained from Sephadex G-75 elution profile (Figure not shown) is suggestive that CR exist in different forms. The protein electropherograms of the purified CR on SDS-PAGE showing subunits of 30

kDa and 59 kDa respectively further supports the possible existence of the enzyme in different forms.

In the characterization of CR, the pH activity profile reveals optimal activity at pH 6.5 in the direction of Cr (VI) reduction. The pH and not the chemical composition of the given buffer determine the activity suggesting the fact that different buffers gave very similar activities at pH 6.0. However, considerable amount of activity by CR is observed at the pH range of 4-7. This indicates that CR is capable of effecting the release of H+ which is typical of most reductases into the physiological milieu [17]. Moreover, most studied CRs have been shown to be active within a broad pH of 5-9 [18,19]. The observed difference in CR optimal pH may be due to differences in sources, substrate type and concentration used for assaying CR activity.

The relationship between temperature and activity was a typical denaturation (bell-shaped) curve. The purified CR of *T. erici* exhibited a high relative activity over a broad range of temperature between 10 -50^{o} C and showed maximum activity at 40^{o} C. It was completely inactivated after 30 min incubation at 50^{o} C. Both findings indicate that CR from *T. erici* has the capacity to remain stable even beyond physiological temperature and still maintain its bioremediative property. As it is true for most chemical reactions, the rate of enzyme-catalyzed reactions generally increases with temperature, within the temperature range in which the enzyme is stable and retains full activity. The rate of most enzymatic reactions approximately doubles for each 10^{o} C rise in temperature [20].

However, the temperature coefficient varies somewhat from one enzyme to another depending on the energy of activation of the catalyzed reaction. Although enzyme catalyzed reactions often appear to have an optimum temperature, the peak in such a plot of catalytic activity against temperature results because enzymes being proteins are denatured by heat and become inactive as the temperature is raised beyond a certain point. The apparent temperature optimum is thus the resultant of two processes (1) the usual reaction rate with temperature and (2) the increasing rate of thermal denaturation of the enzyme above a critical temperature [20].

The results of this study demonstrate the potential of the chromate (VI) reductase enzyme from the lichen *Trebouxia erici* for the bioremediation of chromium (VI)-contaminated wastewater. The enzyme was shown to be highly specific for chromium (VI) and was able to reduce the metal ion with a high rate of reaction.

The specific activity of the purified enzyme was found to be 117 mU/mg protein, which is comparable to other chromate (VI) reductases reported in the literature [14][15][16]. The enzyme was also found to have a high affinity for chromium (VI), with a K_M of 0.103 μmol/min/mg and a V_{max} values of 128 μM which is similar to other chromium (VI) reductases reported in the literature [24][25].

The ability of the enzyme to reduce chromium (VI) was also investigated, and the results showed that the enzyme was able to reduce chromium (VI) with a rate of 11 nmol/min/mg protein. This rate of reaction is comparable to other chromium (VI) reductases reported in the literature [24][25].The use of enzymes for the bioremediation of chromium (VI)-contaminated wastewater has several advantages over traditional methods, including high efficiency, low cost, and minimal environmental impact [23][24]. Additionally, enzymes can be easily immobilized and reused, making them a promising tool for the bioremediation of chromium (VI)-contaminated wastewater [25]. In fact, study has shown that differences in the cell walls and extracellular polymers of the two *Trebouxia* microalgae coexisting in the lichen *Ramalina farinacea* are consistent with their distinct capacity to immobilize extracellular Pb and other heavy metals [25,26]. Although extracellular polymeric

substance (EPS) activity of *T. erici* was not a subject of this study, there is ample ground to suggest that the detoxication of CR reductase of CR (VI) by lichens has a strong link with the production of EPS [10]. This line of thought is however subject to further investigation.

Conclusion

This study has corroborated the enormous potential of lichen alga as a promising candidate in remediating Cr(VI) from the environment. The partially purified chromate (VI) reductase from *Trebouxia erici* has shown immense promise for sustainable Cr(VI) remediation in wastewater. Its high specificity, eco-friendly nature, and potential for large-scale application make it a compelling alternative to conventional methods. Although findings from present study still poses a great challenge to environmental experts as bioremediation is still a challenging technique with new strategies to alleviate this metallic pollutant from the environment keeps evolving and are continuously being investigated. Addressing challenges related to stability, cost, and scalability will further enhance its ability for widespread industrial use.

References

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

2. Barman, M.K. (2024) Utilization of Various Microorganisms for Bioremediation of Hexavalent Chromium from the Environment: Recent Advances and Encouraging Future Prospects, Geomicrobiology Journal ,41(7) 699-720.DOI:10.1080/01490451.2024.2381023

3. Fernández, P.M., Viñarta, S.C., Bernal, A.R., & Figueroa, L.I. (2021). Advances in bioremediation of hexavalent chromium: cytotoxicity, genotoxicity, and microbial alleviation strategies for environmental safety. In: *G. Saxena, V. Kumar and M. P. Shah (Eds) Bioremediation for Environmental Sustainability, Elsiever books.*

4. Tripathi,A.H., Mehrota,S., Kumar,A. and Bajpal,R.(2022) Lichen as Bioremediation Agents-A Review. In Book- Synergistic Approaches for BIoremdiation of Environmental Pollutants: Recent Advances and Challenges(pp.289-312).

5. Bala,S., Sharma,K., Tripathi, M. et al.,(2022) recent Strategies for BIomediation of Emerging Pollutants: A Review for a Green and Sustainable Environment.Toxicology 10(8)484.

6.Faisal,M and Hasnain,S.(2004)Microbial Conversion of Cr(VI) to Cr(III) in Industrial Effluent, *African J. Biotech.*3(11) 610 – 617.

7. Nash, T. H. (1996). Lichen biology. Cambridge University Press, Cambridge

8. Centeno, D. C. Aline F. H., Braga, M. R.,. del Campo, E. M and Casano M.L.(2016) Contrasting strategies used by lichen microalgae to cope with desiccation–rehydration stress revealed by metabolite profiling and cell wall analysis Environmental Microbiology (2016) Society for Applied Microbiology and John Wiley & Sons Ltd doi:10.1111/1462-2920.13249

9. Expósito, J.R , Barreno, E. and Catalá,M.(2020 Biological Strategies of Lichen Symbionts to the Toxicity of Lead (Pb) D. K. Gupta et al. (eds.), Lead in Plants and the Environment, Radionuclides and Heavy Metals in the Environment, https://doi.org/10.1007/978-3-030-21638-2_9

10. Babiak, W. and Krzemińska , I.(2021) Extracellular Polymeric Substances (EPS) as

Microalgal Bioproducts: A Review of Factors Affecting EPS Synthesis and Application in Flocculation Processes *Energies* 2021, *14*(13), 4007; https://doi.org/10.3390/en14134007

11. Rola,K.,Latkowska,E., Ogar, W. and Osyczka,P/(2022) Towards Understanding the Effect of Heavy metals on Mycobiont Physiological Condition in a Widespread metal- tolerant Lichen Cladonia rei, Chemosphere 308(“)136365

12. Bae, W. C; Kang, I. K; Kang, T.G; Won, T. I and Jeong, B. C (2005):Purification and characterization of NADPH-Dependent Cr(VI) from E. coli ATCC33456. J. Microbiol. 43 : 21-27

13. Bradford, M. M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 : 248-254

14. Davis, B. J. (1964): Disc electrophoresis: method and application to human serum proteins. Ann . NY Acad. Sc., 121: 404-427

15. Lineweaver, H. and Burk, D. (1934): The determination of enzyme dissociation constants. Journal of the American Chemical Society , 56 : 658

16. Gonzalez,J.F., Rodriguez, I.A., Figuera, Y.T., Oliveres,P.L.,Flores,R.M and Perez, A.S.R.(2021) Biotransformation of Chromium(VI) via a Reductant Activity from the Fungal Strain. Purpureocillin Lilacinum. J. Fungi,7(12)1022 https://doi.org/10.3390/jof 7121022

17.Song, H. G; Choi, H. T; Moon, D. S and Lee, S. S (2007): Purification and characterization of Intracellular NADH: Quinone reductase from Trametes versicolor. *J. Microbiol*.**45**, 333-338.

18.Parvaze,A.W.,Ibrahim,O.Z,Idris, A.W, Kuranga,O.J.(2015) Chromium(VI) reduction by Streptococus spp isolation from the Industrial Area of Ogun State *Res.J.of Microbiol*.,10(2) 66-75.

19.Tumolo,M.,Ancoma,V., Masserilli,C (2020)Chromium Pollution in European Water,Sources, health risk, and Remediation strategies: An Overview. *Int.J. Environ Research Pub Health* 17(15),5438 doi:10.3390/ijerph 17155438.

20. White, A., Handler, P., Smith, E. L., Hill, R .L. and Lehman, I. R. (1978): Principles of Biochemistry, McGraw-Hill Kogakusha ltd, London. 196-244

21. Moliner,K. and Farkas,E.(2014) Current Results on Biological Activities of Lichen Secondary metabolites: A review. Zeitschrift FurNaturforschung C. 65(3-4),157-173 Doi:101515/znc.2010-3-4.01

22. Mohammed,S.Y, bashir, M., Okoduwa,S.I.R(2023) Kinetic Studes and Bioremediation Potentials of Chromate reductase characterized in Aspergillus flavus from Tannnery effluent. Nigerian Journal of Biochemistry and Mol. boil. 38(4) Doi: 10.4314/njbmbv3814.2

23. Panday, K., Saharan,B.J., Kumar,R. Jabborova,D. & Duhan, S.J.(2024) Modern- day green strategies for the remover of Chromium from Wastewater. J. of Xenobiotic 14(4)1670 – 1696.

24. Casano, Leonardo & Braga, Marcia & Alvarez, Raquel & Del Campo, Eva & Barreno, Eva. (2015). Differences in the cell walls and extracellular polymers of the two Trebouxia microalgae coexisting in the lichen Ramalina farinacea are consistent with their distinct capacity to immobilize extracellular Pb. Plant Science. 10.1016/j.plantsci.2015.04.003.

25. Omwirhiren,E.M and Amlabu, E.(2016) Purification of Chromate(VI) Reductase from Lichen photobiont, *Trebouxia erici* and its Effect on Wastewaters, *Advances in Life Sciences and Technology 45,33-45*

26. Park, C. H., Keyhan, M., Wielinga, B., Fendorf, S. and Matin, A. (2000): Purification to homogeneity and characterization of a novel *Pseudomonas putida* chromate reductase Appl. Environ. Microbiol, 66 :1788- 1795.