

Bioremediation of Hexavalent Chromium by Chromium Resistant Bacteria Isolated from a Polluted Soil

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ABSTRACT: Tannery effluents from the textile industry contain various organic chemicals used in manufacture, including dyes, chrome dyes, and other chemicals. These can result in large-scale liquid waste discharges that contain heavy metals, including hexavalent chromium. Chromium-resistant bacteria were isolated from polluted soil in order to address these heavy metals biologically as environmental contaminants. Using the atomic absorption spectrophotometer AAS, the concentration of chromium in the soil sample was determined to be 0.0863 mg/l. The effects of pH and different chromium concentrations on the bacterial tolerance to hexavalent chromium were also studied for a duration of 72–96 hours. *Pseudomonas* spp., a gram-negative rod-shaped bacterium, was isolated from the soil using a combination of microscopic identification (Gram staining) and other biochemical testing. The greatest biotransformation capability was found to be 90.65% and 90.99% at pH 6 and 7, respectively. The results of this study demonstrate that *Pseudomonas* species are capable of withstanding hexavalent chromium.

Keywords: *tannery effluents, bio-transformation, hexavalent, chromium, resistant bacteria, atomic absorption spectrophotometer (AAS), gram-negative bacteria*

Introduction

The industrial revolution has led to environmental contamination. It has grown to be a significant issue for several centuries and is among the world's biggest issues. It is growing daily as a result of industrialization. Many human activities, like the widespread, large-scale use of chemicals, have increased dramatically in order to support and meet the demands of an overpopulation (Hazarika, 2016). Large liquid waste discharges comprising hexavalent chromium, salts of zinc, sulfates, copper, sodium, potassium, and other organic chemicals can result from the manufacturing of dyes, chrome dyes, and other chemicals in tanning companies. Due to their toxicity, including mutagenicity and carcinogenicity. The survival of organisms within an ecosystem is dependent upon their handling prior to release into the environment (Deepali, 2011). NVF5.

The United States Environmental Protection Agency (USEPA) states that chromium is an elemental substance recognized as an environmental contaminant that endangers human health. It is a naturally occurring heavy metal and transition element in the periodic table in soil, rocks, vegetation, animals, gasses, and volcanic ash. While chromium can exist in various oxidation states, trivalent and hexavalent are the most stable. While hexavalent chromium is very hazardous to biological systems, trivalent chromium is essential for the metabolism of proteins, lipids, and glucose in mammals. Because of its high solubility and permeability through the biological barrier, it is easily disseminated through the soil and water (Arshad et al., 2019). It is required in a trace amount as it is an essential micronutrient, but above the permissible level, it is more toxic, carcinogenic, and mutagenic to the plant and animal system. It is therefore recommended that the hexavalent chromium concentration should not exceed 0.005 mg/L in drinking water. Their compound can cause nasal irritation, breathing problems, allergic reactions, skin rashes, reproductive problems, anemia, and occasionally intestinal and stomach ulcers, cancer, gastrointestinal tract tumors, and lung cancer (Deepali, 2011). If the concentration of chromium is higher than 2 ppm, it can inhibit plant growth and seed germination.

Additionally, in fruits, vegetables, and trees growing next to a discharging factory, necrosis resulting in changes in the structure of the chloroplast causes reduced photosynthesis (Arshad et al., 2019). Upon cellular entry, hexavalent chromium [Cr (VI)] undergoes spontaneous reactions with the intracellular reductants such as ascorbate and glutathione, generating the short-lived intermediates Cr(V) and/or Cr (IV), free radicals and the end-product Cr (III). In the cytoplasm, Cr (V) is oxidized to Cr (VI) and the process produces a reactive oxygen species, referred to as ROS, that easily combines with DNA–protein complexes. On the other hand, Cr (IV) interferes with the normal physiological functions of the cells by binding to the cellular organelles. It is known that Cr (VI) species and hydroxyl radicals cause DNA lesions in vivo (SHANKER et al., 2005). The intermediates that originated from the action of Cr (VI) are harmful to cell organelles, proteins and nucleic acids. Cr (VI) is known to have several dangerous effects on biological systems as it has mutagenic, carcinogenic and teratogenic effects. Moreover, Cr (VI) can induce oxidative stress in cells, damaging their DNA (Sylvia, *et al.*, 2005).

For all parties involved, managing and treating chromium-contaminated soil presents a problem in this case. Bioremediation utilizing microorganisms is a very expensive technology that may be crucial for plant growth. Certain heavy metals can be readily reduced or built up by some microbes. Because of their enormous surface area-to-volume ratio and ability to interact with nearby metals, these microorganisms are advantageous to the environment since they simultaneously improve other factors of importance, such as the growth of plants. The application of microorganisms to remove environmental pollutants that pose a risk to human health is known as bioremediation. Bioremediation is the biological process of treatment of environmental contaminants into less hazardous forms using living organisms such as bacteria, fungi, or plants.

It reduces contaminants to harmless levels under controlled conditions, often utilizing microorganisms that may be native or introduced to the contaminated site (Dahl, *et al.*, 2010). The organisms use metabolic reactions to detoxify pollutants, and when external microorganisms are added to enhance degradation, it is called bioaugmentation. For bioremediation to be effective, microorganisms must transform

pollutants (biotransformation) or use them as carbon sources (biodegradation). The process often involves manipulating environmental conditions to promote microbial activity and accelerate degradation (Dahl, *et al.*, 2010).

Bioremediation techniques are categorized into: In-situ bioremediation (addresses contaminants on-site without extraction) and ex-situ bioremediation (entails the removal of contaminated material for treatment at a different location). Bioremediation on site (treatment takes place at a facility close to the pollution site) Off site bioremediation: the material is taken to a different location to be treated. Techniques like bioaugmentation and biostimulation (adding nutrients or electron donors/acceptors to enhance natural microbial activity) are applied to both in-situ and ex-situ methods for improved results (Microbewiki, 2014). Numerous distinct microorganisms, particularly fungi and bacteria, work in tandem or sequentially to finish the breakdown processes in bioremediation. (Arshad *et al.*, 2019). Microorganisms transform chemicals in ecosystems through various mechanisms. Pollutants can serve as either a carbon and energy source for microbial growth or as terminal electron acceptors. Their ability to degrade and convert toxic molecules highlights their diverse capabilities. (Sylvia, *et al.*, 2005). Primary substrate utilization involves an electron acceptor, either aerobic or anaerobic, to facilitate chemical conversions (Harms *et al.*, 2011). Oxygen accelerates the reaction but is not always necessary. This biodegradation process is commonly observed in the breakdown of hydrocarbons and certain pesticides (Sylvia, *et al.*, 2005). Cometabolism, also known as secondary substrate usage, is the process by which an organism uses various substances as its major source of energy or metabolites (a supply of carbon) when undergoing a chemical change. This process often involves toxic intermediates and provides no net energy or carbon gain to the organism. The degradation of Trichloroethylene (TCE) is an example, requiring organic growth substrates like propane or butane for enzymatic transformation (Microbewiki, 2014).

Microorganisms, particularly bacteria, exhibit remarkable diversity, making them valuable agents in biodegradation and environmental remediation. Due to the limited presence of universally toxic substances to all bacterial species, it is probable that, under suitable environmental conditions—such as the presence or absence of oxygen and the availability of appropriate electron donors or acceptors—a bacterium exists

that can degrade virtually any substrate. *Pseudomonas putida*, a Gram-negative soil-dwelling bacterium, plays a significant role in detoxifying environments contaminated with petroleum derivatives such as toluene and hexavalent chromium (Sylvia et al., 2005). Another example, *Dechloromonas aromatics*, a rod-shaped microbe, facilitates the oxidation of aromatic compounds like benzoate, chlorobenzoate, and toluene, using oxygen, chlorate, or nitrate as terminal electron acceptors. Additionally, bacteria such as *Nitrobacter hamburgensis* and *Nitrosomonas europaea* are integral to nitrogen cycling, particularly in the conversion of ammonium to nitrite during nitrification processes. *Deinococcus radiodurans*, recognized for its extreme resistance to ionizing radiation, has been genetically modified to enhance its efficiency in removing solvents and heavy metals (Nordberg H. et al., 2024).

While bacterial species dominate current bioremediation strategies, the potential of fungi has gained increasing recognition. Mycoremediation—the use of fungi in environmental clean-up—relies on the inherent role fungi play in nutrient cycling through the decomposition and transformation of various organic and inorganic substances. This metabolic capacity enables fungi to degrade hazardous organic compounds and immobilize or detoxify heavy metals. Notably, fungi often demonstrate superior environmental adaptability compared to bacteria, tolerating arid conditions and high pollutant levels. Such resilience, along with their broad enzymatic repertoire, makes them promising candidates for soil decontamination. Fungal species such as *Phanerochaete chrysosporium*, known for its ligninolytic activity in degrading organic pollutants, and *Aspergillus oryzae*, are commonly applied in bioremediation contexts (Nordberg H. et al., 2024). This study seeks to isolate and identify bacterial strains from contaminated soil that will demonstrate resistance to hexavalent chromium. Furthermore, it aims to assess the influence of varying concentrations of Cr (VI) and the role of pH in modulating the growth and activity of these chromium-resistant isolates.

Material and Methods

The soil samples were collected from three different locations within the Gombe Dumping Site, along Bajoga Road in Gombe State. Soil was extracted from a depth

of 0–15 cm (surface layer) and 15–30 cm (subsurface layer). The samples were stored in soil collection bags during transportation to the laboratory. Physicochemical parameters, such as pH, were measured by mixing soil with water in a 1:5 ratio. The concentration of chromium was initially measured using an atomic absorption spectrophotometer (AAS) to determine the total chromium content in the soil samples. (Arshad, *et al.*, 2019).

Growth Media

Nutrient broth and Agar were prepared according to the supplier's instruction and sterilized at 121⁰C for 15 minutes. 100ml of nutrients agar was placed in Petri dishes and allowed to cool.

Isolation of the Chromium Resistant Bacteria

Preparation of the Growth Media:

For the isolation of bacteria, a serial dilution technique ranging from 10⁻¹ to 10⁻⁵ was employed (Arshad *et al.*, 2019). An aliquot from each dilution was streaked onto separate nutrient agar plates, which were then incubated at 37°C for 24 hours. After incubation, three plates labeled 10⁻¹, 10⁻³, and 10⁻⁵ were selected. Among these, the 10⁻³ plate was chosen, and bacterial cultures were purified through repeated streaking (Arshad *et al.*, 2019).

Preparation of Chromium Stock Solution

Synthetic solutions were prepared by dissolving 0.283g of potassium dichromate (K₂Cr₂O₇) in 100ml of distilled water (Yoganand and Umapathy, 2017).

Effect of Chromium Concentrations on Bacterial Growth and Bacterial Resistance to Chromium

Nutrient broth was dispensed into three separate test tubes and supplemented with varying concentrations of chromium (50, 100, and 150 mg/L). To assess bacterial growth, 2 mL of Cr (VI) solution was added to each tube, and optical density (OD) was measured at 600 nm against a blank at 24-hour intervals, in accordance with standard procedures. A test tube without chromium served as the control. The total

chromium concentration was determined using an atomic absorption spectrophotometer (AAS) (Arshad et al., 2019).

Effect of Various pH on Chromium Degradation Ability of the Bacteria

The bacterial isolate was inoculated into separate test tubes containing nutrient broth adjusted to different pH levels (4, 5, 6, 7, 8, 9, 10, and 11) and incubated at 37°C for 24, 48, and 72 hours. The initial concentration of chromium used in the experiment was 100 mg/L (Arshad et al., 2019).

Identification of the Bacteria

The methods adopted during bacterial identification were Gram Staining and Biochemical tests (Oxidase, Catalase, Urease, Indole, Citrate utilization, and Methyl Red Test).

Results and Discussion

Identification of Chromium Resistance Bacterial Isolate

Macroscopic methods and gram staining techniques were the first approaches used to identify the morphology of the bacteria. As illustrated in figure 4.1, the isolate was found to be gram-negative bacteria with pink-coloured cells and a rod shape. This may be due to the reaction between the safranin dye and the bacterial cell wall. Gram-negative bacteria do not pick up the primary stain (Crystal violet) (Ruiz *et al.*, 2017).

Biochemical Test Result

Urease Test

Some bacteria have a urease enzyme. The urease can decompose the urea by hydrolysis to give ammonia and carbon dioxide. Change in the alkaline medium which is shown by the change in colour of the indicator to red-pink indicates a positive result (Ruiz *et al.*, 2017). Based on the test and the result found (Table 4.1), there is no colour change which indicates negative results.

Some bacteria deaminate the amino acid tryptophan in order to produce Indole, which is found in peptone water. The test showed a negative result (Table 4.1).

Citrate Utilization

Some groups of bacteria can utilize citrate as their carbon source and ammonia as a source of nitrogen. The citrate is converted to acetoin and Carbon dioxide (Shoaib *et al.*, 2020). The result of this reaction was found to be positive simply because there is a colour production on Simon's citrate agar (Table 4.1).

Oxidase Test

Some aerobic bacteria produce enzyme oxidase as part of their respiratory mechanism. Pseudomonaceae and negative Enterobacteriaceae families can be differentiated using this test. This enzyme oxidizes a redox dye called TMPPDH, Tetra methyl Paraphenyldiamine Dihydrochloride to deep purple colour (Shoaib *et al.*, 2020). Because of the colour production, this test was also found to be positive (Table 4.1).

Methyl red test

Different microorganisms ferment glucose at varying pH levels and produce distinct end products. Some bacterial species can tolerate and function at pH levels below 4.5. During glucose fermentation, bacteria convert glucose to pyruvic acid, which is subsequently metabolized into various organic acids depending on the bacterial species (Shoaib *et al.*, 2020). The production of these acids is indicated by a color change in the methyl red indicator, which is added at the end of the incubation period. In this study, the test result was negative, as no color change was observed (Table 4.1).

Effect of Various Chromium Concentrations on Chromium Resistance Bacteria

Pseudomonas spp. shows higher growth at 50 mg/L (Figure 4.3). However, at elevated Cr (VI) concentrations, the growth of *Pseudomonas* spp. decreased continuously from 50 mg/L to 200 mg/L. It was also reported by some scientists that

higher concentrations of Cr (VI) showed an inhibitory effect on the growth of bacteria. This may be due to the toxic effect of hexavalent Cr (Deepali, 2011).

Chromium Biotransformation Observed after 72hrs of Incubation under Various Chromium Concentrations

The data revealed that at neutral pH, metal removal efficiency increased over a 72-hour period. A time-dependent increase in the percentage of chromium (VI) degradation by *Pseudomonas* spp. was observed, with removal efficiencies recorded as 97.34% at 50 mg/L, 90.68% at 100 mg/L, 86.46% at 150 mg/L, and 80.30% at 200 mg/L (Figure 4.4). *Pseudomonas* spp. have demonstrated a high tolerance to Cr (VI) and are highly effective in its reduction (Arshad *et al.*, 2019). However, the efficiency of biotransformation declined at higher concentrations of Cr (VI), which may be attributed to limited enzyme active sites and increased competition among metal ions for the available binding sites. The reduction in Cr (VI) biotransformation by bacterial cells may also be associated with the mutagenic and toxic effects of hexavalent chromium (Deepali, 2011).

Effect of Various pH on Chromium Biotransformation Ability of Chromium Resistance Bacteria

According to the literature on bioremediation processes, the pH range of 6-8 was found to be optimal for bacterial growth and biotransformation rate (Mishra *et al.*, 2012). The pH ranges from 4–11 was selected for this research. From above, eleven pH values (4–11) were investigated. As the pH value increased above the optimum level, the biotransformation ability of the *pseudomonas* spp. Decreased (figure 4.5). Based on the data observed, the optimum pH level for the biosorption ranges from 6–7. At pH4, the percentage biotransformation was 83.66%, pH5 was 86.60%, pH6 was 90.65%, pH7 was 90.99%, pH8 was 86.82%, pH9 was 86.76%, pH10 was 81.91%, and pH11 was 73.11%. Therefore, the highest percentage was observed at pH 7 and 6. A low percentage is observed because the change in pH of the medium is toxic to the bacterial cells. (AY *et al.*, 2006).

***Pseudomonas* spp Growth curves in Nutrient broth at 24, 48 and 72hrs at Varying pH conditions**

It was shown that variations in pH values influence both the percentage of biotransformation and the growth of *Pseudomonas* spp. This may result from the

denaturation of DNA caused by the hydrolysis of hydrogen bonds that maintain the integrity of the DNA strands at elevated pH levels. Figure 4.6. Consequently, alterations in pH affect the ionization of amino acid functional groups and disrupt hydrogen bonds, thereby inducing modifications in protein folding, leading to denaturation and loss of activity (AY *et al.*, 2006).



Figure 4.1: Shows the morphology of the bacteria on the Nutrient agar.

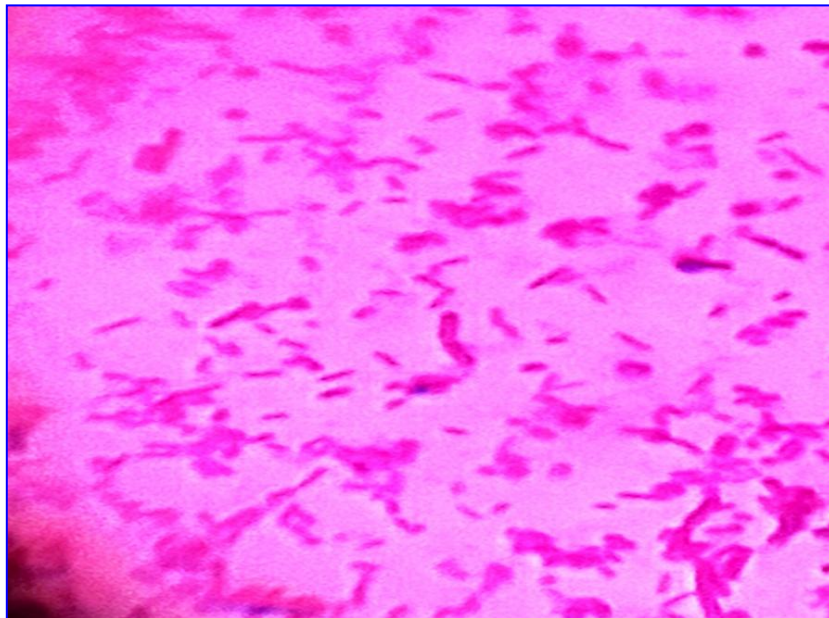
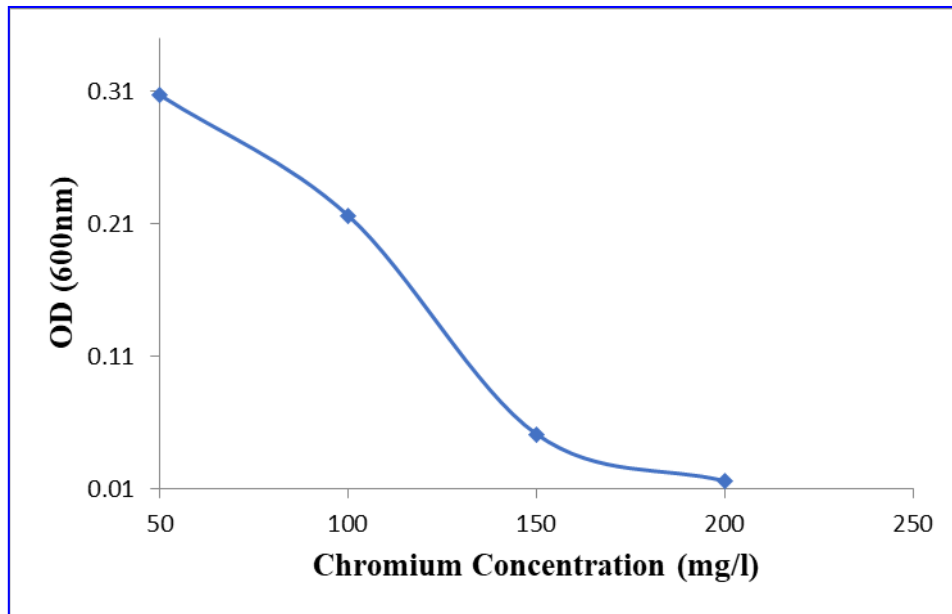


Figure 4.2. Gram stains smear of the isolate observed under a compound light microscope using an oil immersion (100x objective lens).

To study the effect of chromium-on-chromium resistance bacteria, the experiment was carried out using $K_2Cr_2O_7$ as a source of chromium 50mg/l, 100mg/l, 150mg/l and 200mg/l.



OD = Optical density

Figure 4.3: Growth of the bacterial isolate (*Pseudomonas* spp) in Nutrient broth observed after 48hrs of incubation under various chromium concentrations

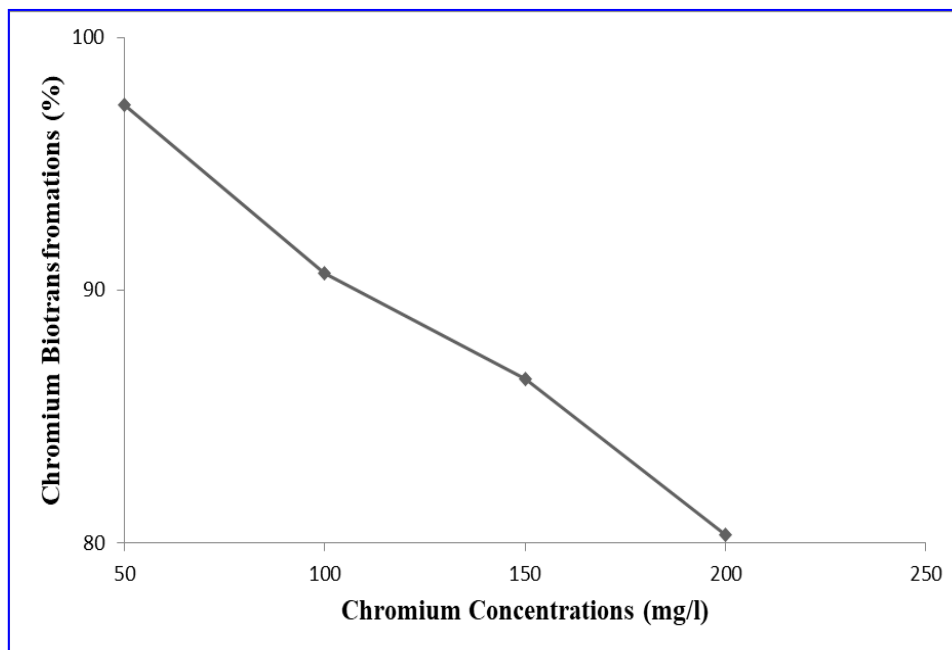


Figure 4.4 Chromium biotransformation observed after 72hrs of incubation under various chromium concentrations

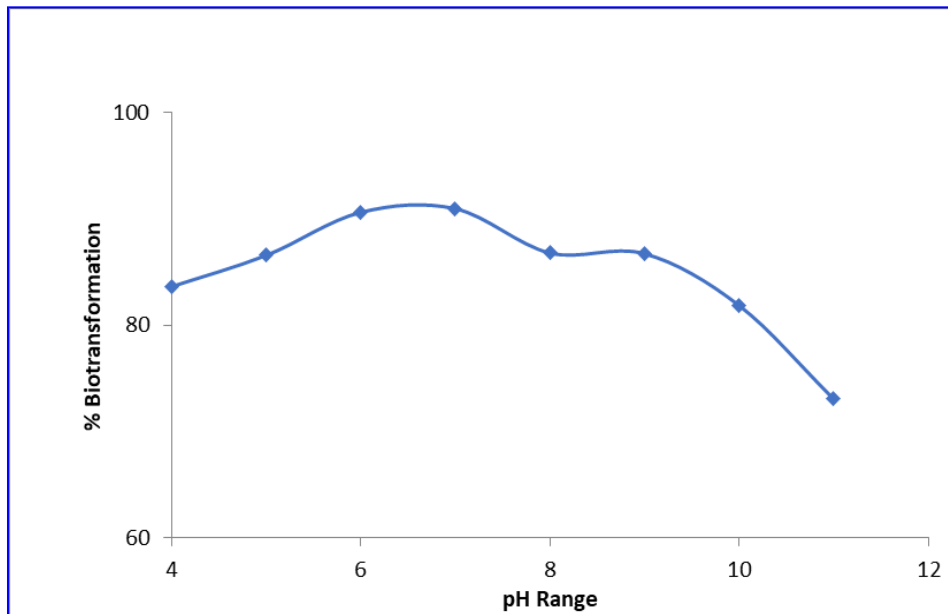


Figure 4.5: Effect of pH on Biotransformation ability of the Pseudomonas spp

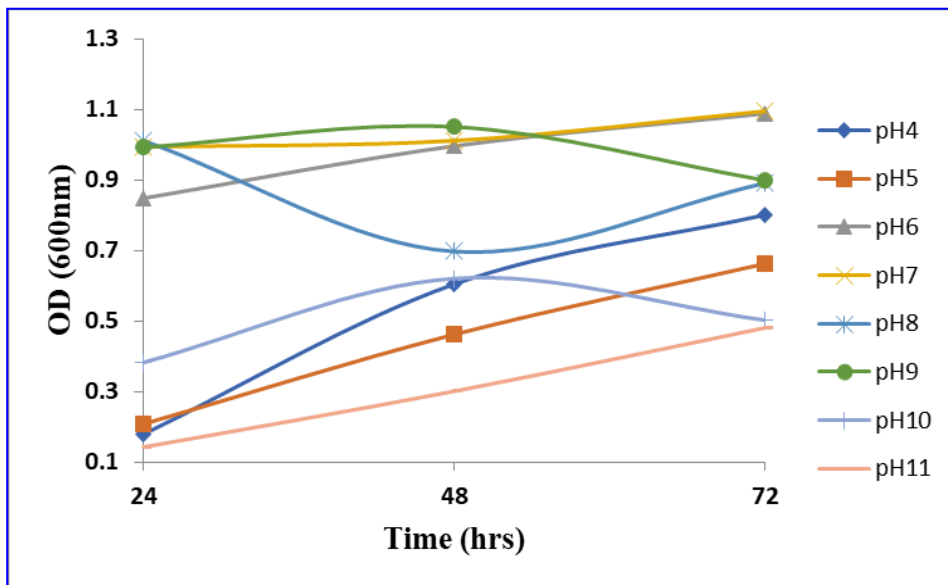


Figure 4.6: Pseudomonas spp Growth curves in Nutrient broth at 24, 48 and 72hrs at varying pH conditions

Conclusion and Recommendations

According to the current study, gram-negative bacteria known as pseudomonas spp. were identified as chromium-resistant bacteria and have a significant capacity to remove hexavalent chromium from soil and chromium aqueous solutions. According to the study's findings, Pseudomonas species that were isolated were found to be effective biosorbents that could be used to remove heavy metals from industrial waste. With particular care, more research is required for this technology's

implementation. When the technology is improved, it will help industrialists address the issue of mining and wastewater contamination.

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REFERENCES

1. B. Dahl, Abhilash, K.D. Mehta, B.D Pandey. (2010). Microbial Bioremediation of Chromium. A Promising Approach of Environmental Microbiology. 131- 133
2. Bioremediation, Biostimulation and Bioaugmentation: (2016), A Review. (n.d.). Retrieved March 13, from <http://pubs.sciepub.com/ijebb/3/1/5/>
3. Deepali (2011). Bioremediation of chromium from textile industry's effluents and contaminated soil using pseudomonas putida. Punjabi State Council for Science and Technology
4. Nordberg H , Grigoriev IV , Smirnova T , Dubchak I, Cantor M D, Dusheyko S, Hua S, Poliakov A, & Shabalov I. (2024). *Home - Dechloromonas aromatica RCB*. Doe.gov. <http://genome.jgi.doe.gov/decar/decar.home.html>
5. Harms, H., Schlosser, D., & Wick, L. Y. (2011). Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nature Reviews Microbiology*, 9(3), 177-192
6. Hazarika Samiul Islam (2016). Bioremediation of chromium. Division of microbiology, Annamalai University, Annamalai Nagar, Chidambaram 608002 India.

7. M.U. Khan, A. Sessitsch, M. Harris, K. Fatima, A. Imran, M. Arslan, M. Afzal (2015). Cr-resistant rhizo- and endophytic bacteria associated with *Prosopis juliflora* and their potential as phytoremediation enhancing agents in metal-degraded soils. *Front. Plant Sci.*
8. Muhammad Arshad, Anza Javaid, Maria Manzoor, Kiran Hina, Muhammad Arif Ali, and Iftikhar Ahmed, (2019). Isolation and identification of chromium-tolerant bacterial strains and their potential to promote plant growth. *E3S Web of Conferences* **96** 01005 (2019). <https://e3sconf/20199601005>.
9. Patricia Ruiz – Garbajosa and Rafael Cantón. (2017). Epidemiology of antibiotic resistance in *Pseudomonas aeruginosa*. Implications for empiric and definitive therapy.
10. Sylvia, D. M., Fuhrmann, J.F., Hartel, P.G., and D.A Zuberer (2005). "Principles and Applications of Soil Microbiology." New Jersey, Pearson Education Inc.
11. Muhammad Shoaib, Iqra Muzammil, Zeeshan Ahmad, Bhutta , Ishrat Yaseen. (2020). A Mini-Review on Commonly used Biochemical Tests for Identification of Bacteria. *International Journal of Research Publications* Volume-54, Issue-1, June 2020 ISSN number 2708-3578 (Online).
12. Microbewiki. (2014). *Bioremediation - microbewiki* (Kenyon college, Ed.). Kenyon.edu. <https://microbewiki.kenyon.edu/index.php/Bioremediation>
13. SHANKER, A., CERVANTES, C., LOZATAVERA, H., & AVUDAINAYAGAM, S. (2005). Chromium toxicity in plants. *Environment International*, *31*(5), 739–753. <https://doi.org/10.1016/j.envint.2005.02.003>
14. Yoganand, K. S., & Umopathy, M. J. (2017). Green methodology for the recovery of Cr (VI) from tannery effluent using newly synthesized quaternary ammonium salt. *Arabian Journal of Chemistry*, *10*, S1227–S1234. <https://doi.org/10.1016/j.arabjc.2013.02.022>