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Comparative Analysis of Heavy Metal Biosorption by Natural and MTCC Bacterial Strains from the Electroplating Effluents

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Abstract:
Due to rapid urbanization, technological innovation, evolving consumption patterns, increasing population growth, and swift socioeconomic developments has unquestionably led to water pollution within the biosphere, thereby leading to environmental pollution. The main reasons are, effluents released from domestic and industries, constituting the toxic heavy metals, pesticides and chemical-based dyes. Even though Copper (Cu) is considered to be an important micro-nutrient for all living beings, it could be toxic when exposed at lower concentrations. Further, the biosorption process is considered to economical, rapid, needs minimal preparatory steps, highly efficient, requires no toxic chemicals, and permits regeneration of biosorbent material at the end of the process. The bio removal efficiency of copper ions by a natural bacterial isolate and a MTCC bacterial strain was comparatively studied for the duration of eight days by providing different copper concentrations. The samples were subjected to Atomic absorption spectrophotometric analysis. The obtained results were correlated statistically with multiple factors associated with the biosorption. The outcome from the study reveals, it may be employed to remove the toxic heavy metals released from industries in an eco-friendly manner.

Keywords: Copper; Pseudomonas fluorescens; Biosorption; Atomic absorption spectrophotometry; MTCC Strain.

Introduction

Due to the increase of industries in the cities, the wastewater produced from the industrial applications encompass anonymous organics and highly toxic metals which in turn leads to shocking environmental pollution globally. These pollutants have long been to cause the environmental and health hazards (1). The heavy metals are the elements with density higher than 5 gcm^{-3}. They are described as Chromium (Cr), Arsenic (As), Cupper (Cu), Gallium (Ga), Lead (Pb), Cobalt (Co), Cadmium (Cd), Iron (Fe), Mercury (Hg), Nickel (Ni) and Manganese (Mn) are commonly known heavy metals widely used in Industries. These metals together cover the basic metals, transitional metals, lanthanides, some metalloids, and actinides, which have high molecular weight, specific gravity and atomic number. Further, in several industrial sectors such as tanneries, paper and pulp, electroplating, metallurgies, textiles, dying industries, mining sectors, and painting extensively consume diverse heavy metals for varied purposes (2). The heavy metal exposure in the environment is considered to be the major global concerns owing to its high bioaccumulation in the food chain and human body, high toxicity, their nature of non-biodegradability in the environment, and leads to human carcinogenicity. These heavy metals which are mentioned above have also been clearly documented to pose the toxic effect in humans such as inflammatory, cardiovascular, skin allergies and respiratory diseases. In the plants they tend to interfere in the process of photosynthesis, and slow growth and in the aquatic lives they finally affect the reproduction process and in certain circumstances to even death (3). For the removal of toxic substances produced by metals released in the water bodies several waste water treatment technologies have already been documented. They can be explained as follows; membrane filtration, biofiltration, advanced oxidation process, biological activated carbon, iminodiacetic acid-carbon nanotubes and ion-exchange resins (4). Till date, adsorption-based technologies such as metal organic framework (MOF), 3D porous aerogels, chitosan-based adsorbents, porous geopolymers, covalent organic frameworks (COFs) based materials, nanomaterials usage, biosorption and phytoremediation have been extensively studied to eliminate the toxic heavy metal contaminants from water and wastewater (5). The bioremediation method such as biosorption no secondary or produce less wastes during the removal of toxic heavy metals such as the secondary wastes which might require further extensive technologies to be managed. These are the processes which use mainly the microorganisms to deal with toxic heavy metals are bioaccumulation, biosorption, and also the enzymatic oxidation/reduction. Among-st the bioremediation techniques, biosorption process by means of the microbial biomass is documented to be modest, fast, inexpensive, efficient, and also very effective at the level of industrial scales (6). Microbes deliver high surface area to the volume due to their small size nature and have the ability to assimilate the metals from the surroundings. Several microbes were identified from the industrial effluents discharging area and they were capable of defending themselves from the toxic nature of existing heavy metals in the industrial effluents. These microbes include bacteria, algae, fungi, and protozoa use variety of systems for the survival against the heavy metal toxicity, which includes the uptake of heavy metal, oxidation, adsorption, methylation, and reduction of heavy metals to the nontoxic forms (7). The bacterium *Pseudomonas fluorescens* is stained as Gram-negative, a non-pathogenic, and metabolically adaptable bacterium commonly observed in the soil and water. They employ multiple mechanisms for toxic heavy metal remediation, such as bioaccumulation, biotransformation, biosorption, and the siderophores production (8). An attempt has been made in the present study to explore and also to compare the biosorption potential of copper ions using two bacterial strains viz., one natural isolate and one procured MTCC strain.

Materials and Methods

Sample Collection

The soil samples were collected from an electroplating industry, near Ambattur, Chennai, Tamil Nadu, India for the natural isolate. The samples were collected in sterilized glass bottles avoid other contamination. The bacterial strain which was isolated from the soil sample has been serial diluted and maintained in nutrient agar slants. Amongst them one bacterial colony (strain) was selected and identified according to morphological, biochemical, finally by 16s rRNA PCR and sequencing criteria. The procured bacterial strain used in the study was *Pseudomonas oleovorans*, which was obtained from Microbial Type Culture Collection [MTCC 617], The Institute of Microbial Technology, Chandigarh, India. This organism was selected based on their metabolic versatility, particularly its ability to degrade the toxic metals from the polluted atmosphere. Its strength and adaptability make it valuable in the field of industrial and environmental microbiology.

Estimation of Metal Tolerance

The tolerance of copper by *P.fluorescens* and *P.oleovorans* were determined by the inoculation of the both the bacterial strains into the nutrient agar plates comprising varied range of copper concentrations (100, 500, 1000, 2000, and 3000 ppm). The nutrient agar plates were then incubated at 37°C and noticed for growth after 24 hours. Based on the microbial growth, 200, 400, 600 and 800 ppm of copper concentrations were considered for further processes in the study.

Bio-removal of copper

The nutrient broth containing both the culture's maintained for the overnight was used, 0.1 ml was inoculated into the minimal broth comprising the selected concentrations of copper (200, 400, 600 and 800ppm). The conical flasks were incubated at 37°C on a shaker for recurrent mixing and the samples were then used for the assessment of residual metal concentration from two to eight days.

Optical Density Estimation and pH Determination

2 ml of the samples from the culture flask were taken and optical density values were taken at 450nm using the colorimeter. It was executed from two to eight days of treatment period. The pH of the culture medium during the treatment period was calculated using pH meter and pH 7 was detected all over the treatment period.

Estimation of the Biomass

The pellet from the above step was collected and transferred in a glass petri plate. Further, the glass petri plate encompassing the pellet was dried in a hot air oven at 75°C for four hours. The dried biomass was weighed and estimated.

Atomic Absorption Spectrophotometry (AAS) Analysis

15 ml of the sample from 200, 400, 600 and 800 ppm concentrations of copper after ten minutes up to ninety minutes were centrifuged at 3000rpm for 12 minutes. Then the supernatant was taken for Atomic Absorption Spectrophotometry analysis. The obtained values by AAS analysis represent the residual concentration of copper in the solutions.

Sugars Supplementation

The competence of both the natural and MTCC bacterium for the biosorption of copper were identified by supplementing the different carbon sources like fructose, dextrose, glucose, sucrose and lactose at 10% concentration in the minimal broth containing 400 ppm concentration of copper and the inoculum (10^8 cells).

The culture flasks were then incubated at room temperature on a shaker and optical density and biomass were estimated after 2 days by doing centrifugation at 3000rpm for 15 minutes, followed by drying in a hot air oven at 75°C for four hours.

Statistical Analysis

Two-way ANOVA (analysis of variance) was executed for the various factors such as, percent removal of copper and biomass of *P.fluorescens* and *P.oleovorans* during copper treatment for the two variables namely copper concentration and treatment period. Further, analysis was also executed for the factor, percent removal of copper using sugars supplementation source through two variables such as treatment period and copper concentration, using SPSS software Package.

Results

The natural isolate sample was confirmed through serious of biochemical tests, which showed positive for citrate utilization test and catalase test. The *16s rRNA* sequencing results showed the similarity of 99% with the *Pseudomomas fluorescens*. Based on the sequencing results, phylogenetic analysis was executed and the results are presented in the Figure-1.

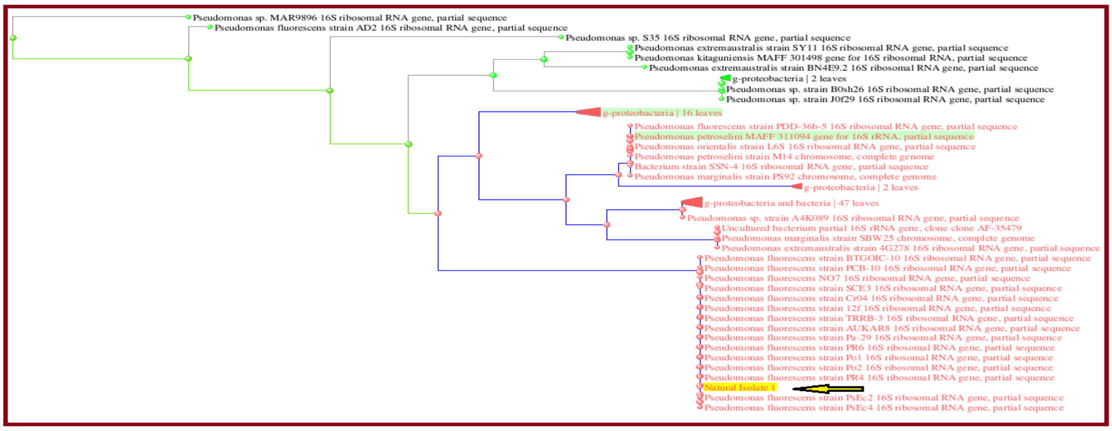
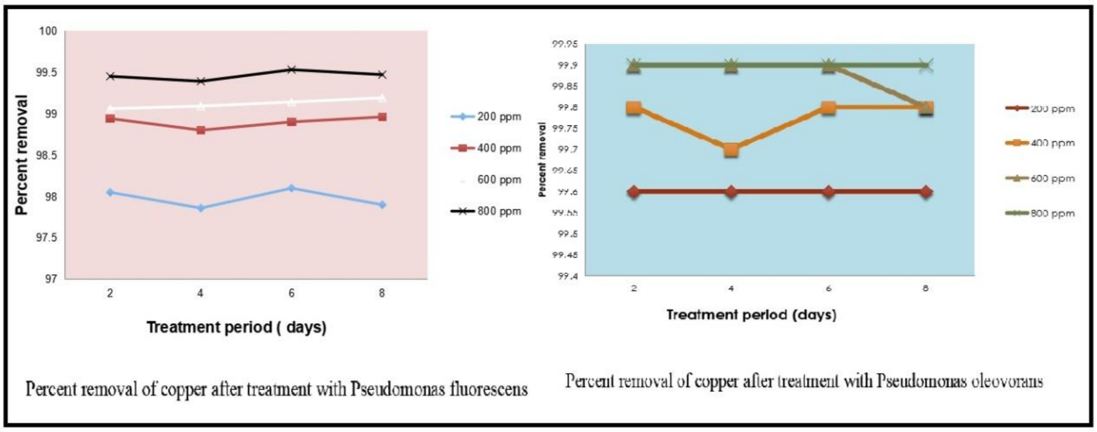


Figure 1. Phylogenetic tree showing the natural isolate along with the similar bacteria

Both the microbial strain *P.fluorescens* and *P.oleovorans* were tested for metal tolerance with wide range of copper concentrations which showed, the microbes grew well up to 1000ppm concentration of copper. Based on the tolerance level of the metal the strains were subjected to different concentrations of copper (200, 400, 600, 800ppm) for biosorption up to 8 days. The highest percent removal was observed in sixth day of treatment for both the strains.

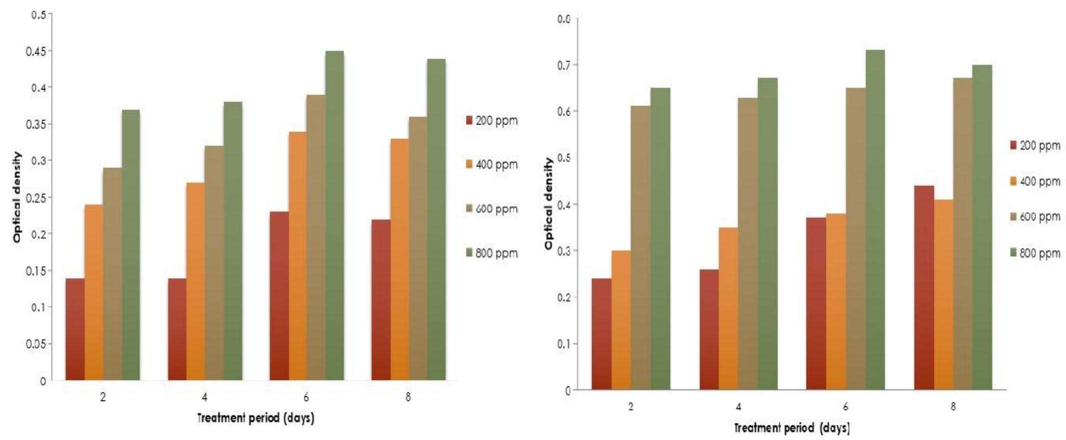


Percent removal of copper after treatment with Pseudomonas fluorescens

Percent removal of copper after treatment with Pseudomonas oleovorans

Figure 2 Percent removal of copper after treatment with both the microbial strains

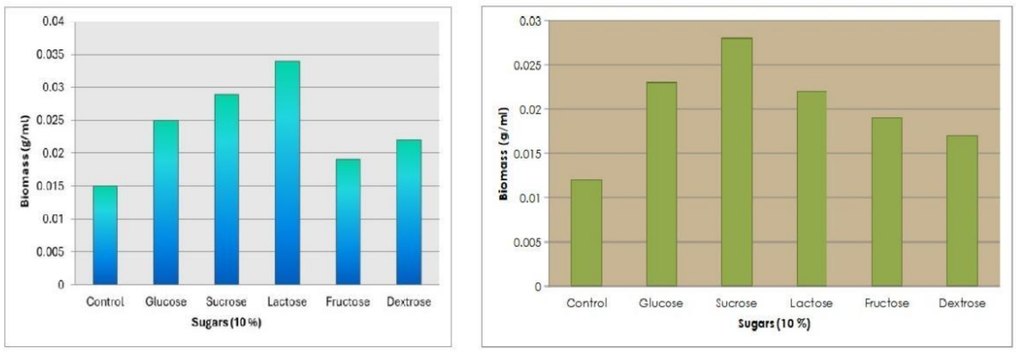
The OD values noted during the treatment of the bacterial isolates were illustrated in Figure 3. The trends in increase in the OD values during the entire treatment period was observed. This shows the growth of bacterium in the minimal broth. Highest optical density was observed after six days for 600ppm of copper concentration in both the strains.



Optical density values obtained during treatment with *Pseudomonas fluorescens*

Optical density values obtained during treatment with *P. oleovorans*

Figure 3 Optical density values obtained during treatment with both the microbial strains



Influence of sugars on the biomass (g/ml) of *Pseudomonas fluorescens* during chromium treatment (400 ppm) after two days

Influence of sugars on the biomass (g/ml) of ***Pseudomonas oleovorans*** during copper treatment (400 ppm) after two days

Figure 4 Influence of sugars with constant PPM for both the microbial strains

The consequence of 10% concentration of sugars on the biomass of the bacterial strains of *P.fluorescens* and *P.oleovorans* during (400ppm) copper treatment are exhibited in Figure 4. The results reveal us that the biomass being highest in the case of lactose for *P.fluorescens and* sucrose for *P.oleovorans* followed by other sugars respectively. The drift reveals us that the increase in the biomass may be due to the influence of disaccharides (lactose and sucrose) while compared with monosaccharide (dextrose, glucose and fructose) the biomass shows declining activity. The Two-way anova for comparing the percent removal of copper with the variables, such as treatment period and copper concentration for *P.fluorescens* and *P.oleovorans* reveals the significant association with copper concentration. Further, data compared with other factors showed both significant and insignificant association, but the results have not been shown.

Discussion

Heavy metals such as lead, cadmium, nickel, manganese, copper, arsenic, chromium due to their non-biodegradable nature, post an important threat to human health and environment which we live. This leads to continuous release of toxic heavy metals into water bodies and thereby causing various physical, chemical and biological complications (9). They inherit toxicity at low concentrations which raises fears, as they intend to accumulate in the food chain, leading to bio-magnification and severe health effects. The chronic exposure to toxic heavy metals, such as lead (0.01 mg/L), arsenic (0.01 mg/L, cadmium (0.003 mg/L), uranium (0.03 mg/L), and mercury (0.006 mg/L) interrupts the normal cellular functions and thereby triggers a variety of pathological disorders in humans (10).

Copper is widely used as an anti-microbial agent in the consumer products which includes the construction materials, e.g., wood for houses, roofing shingles and commercial structures to lessen bio-deterioration. Considerably, the copper used in the consumer products ultimately ends in the atmosphere through dumping and accompanying waste streams. They are difficult to remediate back owing to its relatively high mobility. Based on the above factors, Copper is being considered as the common environmental heavy metal pollutants (11). The microorganisms such *as Acinetobacter, Arthrobacter, Serratia marcescens, Ochrobactrum, Micrococcus luteus, Bacillus spp., Shewanella oneidensis, Cellulomonas spp, Corynebacterium spp, Enterobacter spp, Streptomyces spp, P.fluorescens, Intrasporangium spp, Enterobacter cloacae, Bacillus spp, and E. coli a*re widely used as bacterial strains for the biosorption processes *(12). P.fluorescens* are classified as harmless type of bacterium present in soil that has the capacity to transform the foreign substances biologically. This specific bacterial strain can be isolated and cultured from a population of microbes especially found in soil which are heavily contaminated with environmental pollutants. Further, they are considered as good choice for bioremediation technologies, since they look to be highly robust and effective in removing the pesticides and toxic heavy metals which are frequently observed in wastewater systems (13).

Conclusion

Bio-remediation methods are widely used to treat the municipal, industrial, automobile, electro-plating and mining waste, which includes the effects of toxic heavy metals, chemical spills and pollution in the environment. Biosorption based techniques presents numerous benefits such as highly efficient, regeneration of the biosorbents allowing for optimum metal recovery, economic viability, and finally the reduction of chemical/biological sludge. The outcome from the present study suggests that bacterial biosorbents are more effective in removing the toxic heavy metals from polluted and contaminated environments. *P.fluorescens* can act as an effective biosorbent for copper removal from industrial wastewater compared to the MTCC strain. These technologies serve as a cost-effective and eco-friendly method to protect our surroundings from toxic metal pollution. Genetic engineering approaches may further augment the biosorptive ability of bacterial strains. CRISPR-Cas tools, for instance, could be employed to up-regulate metal-binding proteins or surface functional groups, thereby tailoring biosorbents for targeted toxic heavy metals. Such advancements would be especially valuable in treating complex effluents with fluctuating metal concentrations.

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