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Research Article

Biosorption of Heavy Metal from Textile and Dye Industrial Effluent

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Abstract

Introduction: Preliminary experiments were conducted during the initial stages of this work showed high concentrations of total Chromium and Zinc in effluent samples. As a result, a specific treatment process was required, aiming to the reduction of Chromium and Zinc content in the textile and dye industry effluent.

Objective: Biosorption, the eco-friendly method to remove heavy metals from the textile and dye industry effluent.

Methodology: Isolation and identification of bacteria and fungi from textile and dye industry effluent, estimation of the heavy metal concentration in the effluent followed by effluent treatment by predominant isolate of bacteria and fungi.

Result: The heavy metals like Chromium and Zinc in the test samples were treated with the predominant bacterial and fungal organisms till 25 days and observed at the time interval of 5 days. Bacteria treated effluent reduced Cr and Zn to 0.25 mgl-1 and 0.09 mgl-1, while fungi reduced 0.36 mgl-1 and 0.11 mgl-1, respectively. Maximum removal of Chromium and Zinc heavy metals was done by Bacteria than fungal treated effluent.

Conclusion: The bioremediation of heavy metals using microorganisms has received a great deal of attention in recent years, not only a scientific novelty but also for its potential application in industry.

Keywords: Bioremediation; Biosorption; Heavy Metals; Chromium; Zinc

Introduction

Biodegradation is nature's way of recycling wastes or breaking down organic matter into nutrients that can be used and reused by other microorganisms. Bioremediation means that the decaying of all organic materials is carried out by a huge assortment of life forms comprising mainly bacteria and fungi. Bioremediation refers to process that use microorganisms or their enzymes for clean up contaminated soil or water. Whereas "Bioremediation" is the biologically mediated breakdown of chemical compounds.

Bioremediation offers a simpler, cheaper and more environment-friendly option to traditional methods of remediation [1]. Bioremediation is a process used to treat contaminated media, including water, soil and surface material by altering environmental conditions to stimulated growth of microorganisms and degrade the target material.

Industrial pollution at international and national level

Rapidity of industrialization and urbanization around the world has led to the reorganization and understanding of relationship between environmental pollution and public health. Among the most concerned environmental pollutions that threating our biodiversity water pollution is a major one where effluents from dye-based industries serve as principal source [2].

Approximately 10,000 different dyes enter the environment via wastewater different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are used annually worldwide [3]. Increasing human population has led to an increase in industrial activities one of the main sources of pollution worldwide is the textile industry and its dye-containing wastewater. The about 25% of the textile dyes are discharged as aqueous effluent in different environmental pollution [4].

Textile industry is one of the greatest generations of liquid effluent pollutants due to the high quantities of waste water dyeing process. There are more than 10 kinds of commercially available dyes with over 7×10 tonnes of dye stuff produced annually and it is estimated that 2,80,000 tons of textile dyes are discharged from such industries effluent every year [5]. In India textile is one of the oldest establishments with nearly 1569 large cotton textile industries, which gives employment to nearly 30 million people. India is the second largest exporter of dyestuffs, where ~ 80,000 tons of dyes and pigment are produced annually [6]. Small-scale industries in India contribute 3900 million litres wastewater per day. Presence of very low concentration of dyes in effluent is highly visible and undesirable. Some of these dyes are potentially mutagenic, carcinogenic and toxic [7].

Heavy metal pollution

Contamination of heavy metal in the environment is a major global concern because of their toxicity and threat to human life and environment [8]. Heavy metal is widely electroplating, chrome plating, petroleum refining, paint industries. These industries discharge large quantities of toxic waste and the untreated to the environment and cause a serious ecological contamination [9]. Discharge of effluents containing heavy metal many affect all-natural resources along with living organisms in the receiving water. The risk of heavy metal in the environment is mostly due to their non-degradability, biological affects and possible accumulation in the food chain [10]. The harmful effect of heavy metals on microorganisms depends on various abiotic factors such as concentration of chelating agent's speciation, pH and organic matter. Heavy metal like Nickel, Iron, Copper, Zinc, Mercury, sliver, Chromium, magnesium.

Heavy metal is a problem associated with areas of intensive industry. However, roadways and automobiles are considered to be one of the largest sources of heavy metals. During the precipitation, the bound metals will either become soluble (dissolved) or be swept off the roadway with dust. In either case, the metals enter the soil or are channeled into a storm drain. Where in the soil or aquatic environment, metals can be transported by several processes. These processes are governed by the chemical nature of metals, soil and sediment particles, and the pH of the surrounding environment. Health problem caused by low chronic exposure to heavy have been linked to conditions ranging from cardiovascular disease, high blood pressure, insomnia and many more. Microorganisms are nature's original recyclers, converting toxic organic compounds into harmless products, often carbon dioxide and water. Ever since it was discovered that microbes have the ability to transform and/or degrade xenobiotics, scientists have been exploring the microbial diversity, particularly of contaminated areas in search for organisms that can degrade a wide range of pollutants.

Materials and Methods

Sample collection

The effluent sample was collected directly from the outlet of Komarapalayam area, Karur. The samples were collected in large sterile bottles and brought to the laboratory for further study.

Isolation, identification and maintenance of bacteria

Ten ml of the water sample was taken in a 250 ml conical flask containing 90 ml sterile distilled water. The flask was shaken on an electric shaker to get a homogenous suspension and the serial dilution of the suspension was done to get various dilutions viz., 10^{-2}, 10^{-3}, 10^{-3}, and 10^{-5}. One ml of the 10^{-5} dilutions was plated in Petri dishes containing nutrient agar medium. The inoculated plates were incubated at 25 ± 2ºC for one or two days and bacteria appearing over the medium were picked up for identification.

After inoculation the colonies on the nutrient agar plates were observed and identification was done based on the color and morphology of the colonies. Based on Bergey's manual of systematic Bacteriology (1995) the following tests were performed to identify and confirm the organism isolated (Figure 1). The identified and characterized *Pseudomonas sp.* was further maintained as pure culture for its use in the formation of biofilms. The culture was inoculated into nutrient agar slants and incubated for 24 hrs at 37ºC (Figure 2). After incubation the slants were filled up with mineral oil and stored at 4ºC for further use.

Figure 1: Biochemical tests.

Figure 2: Pure plate and microscopic view of *Pseudomonas putida.*

Isolation, identification and maintenance of fungi

About 10 ml of the effluent water sample was taken in a 250 ml conical flask containing 90 ml of sterile distilled water. The flask was shaken on an electric shaker to get a homogenous suspension and serial dilution was done. One ml of 10^{-5} dilution was plated in Petri dishes containing Potato Dextrose Agar medium**.** Streptomycin sulphate (100 mgl^{-1}) was added to the media to prevent the bacterial growth. The plates were incubated at 25 ± 2ºC for five days and observed. After incubation the fungi appearing on the medium were mounted over a clean slide, stained with lacto phenol cotton blue (LPCB) staining observed under the microscope photomicrographs were also made. LPCB stain is excellent for examination of fungal material. Phenol kills the fungi, and the lactic acid increase preservation chino (cotton) blue is a strain chitin and cellulose. The fungi were identified by using standard manuals, such Manual of soil fungi (Gillman, 1957), *Dematiaceous hyphomycetes* (Ellis, 1971), more *Dematiaceous hyphomycetes* (Ellis, 1976), *Hyphomycetes* (Subramanian, 1971). The identified and characterized *Aspergillus* sp. was further maintained as pure culture for its use in Potato Dextrose Agar medium. The plates were incubated at 25 ± 2ºC for five days and observed (Figure 3). The plate with resulted colonies was stored at 4ºC for further use.

Figure 3: Pure plate and microscopic view of *Aspergillus niger.*

Experimental conditions

Among the bacterial and fungi isolates from the tannery effluent the predominant *Pseudomonas putita* and *Aspergillus niger* were selected for the following experimental conditions (Figure 4):

- Raw effluent without microbial inoculation as control
- Effluent inoculated with *Pseudomonas putita*
- Effluent inoculated with *Aspergillus niger*.

The experimental was conducted in batch cultures in duplicates for a total period of 20 days under controlled conditions. After 20 days, the cultured organisms were separated from the effluents. Then the effluents were analyzed for heavy metals.

Heavy metal analysis (chromium (Cr) and Zinc (Zn)

Estimation of zinc (IS 3025 (part 49) 1994) Procedure

To 50 ml of sample, 1ml of conc. HCL was added and boiled for 5 minutes. Then the solution was adjusted to 7 with NaOH. The volume of the content was made upto 50 ml. from this 10 ml was taken in Erlenmeyer flask, to which 0.5 ml of sodium ascorbate, 1ml of potassium cyanide solution reagent, 5 ml of Borate buffer solution, 3 ml of zinc solution were added in sequence with sufficient precaution. Finally, the content was made upto 500 ml with distilled water. Reagent blank was prepared by treating 50 ml of double distilled water in the same way as described above. The optimal density was measured at 620 nm using a spectrophotometer. The amount of zinc was calculated using the calibration graph prepared from standard solution.

Figure 4: Experiment Conditions.

Calculation

Zinc, mg l^{-1} = M/V×100
Where, M = Mass of zinc present in mg in the sample
V = Volume of sample in ml.

Estimation of chromium (Cr) (IS 3025, 1994)

In a platinum basin 100 ml of sample was taken along with 0.1g of sodium sulphite and evaporated to dryness. To this 0.5 ml diluted sulphuric acid and 1 ml of distilled water were added and heated until white fumes were given off. The residue obtained was dissolved in 40 ml of distilled water. To the content 1 ml of sliver nitrate solution and 4 drops of conc. Nitric acid were added and heated to boiling. The 10 ml of Ammonium per sulphate solution was added and boiled for 10 minutes. In order to remove manganese from the sample, 2 drop of conc. HCl was added, cooled transferred to Nessler tube and neutralized by adding sodium carbonate and the volume were added. The violet color was obtained and measured at 510 nm using a spectrophotometer against the reagent blank. The amount of total chromium was estimated by comparing with standard graph prepared from the stock potassium chromate solution (10mg^{-1} of chromium).

Results

Bacterial flora in the effluent

Bacteria were isolated from effluent by pure culture techniques. Then the isolated bacteria were identified through various biochemical test (Table 1). Totally 7 species of bacteria belong to 5 genera were identified from the effluent sample. Among the genus *pseudomonas* and *bacillus* were recorded with two species (Table 2).

S. No	Organisms	Indole	Methyl Red	Voges Proskauer	Citrate Utilization	Urea Hydrolysis	Cytochrome	Catalase
1	*Pseudomonas putida*	-	+	+	-	-	+	-
2	*Pseudomonas fluorescens*	-	+	+	-	-	+	-
3	*Klebsiella pneumoniae*	-	-	+	-	+	+	-
4	*Escherichia coli*	+	+	-	-	-	+	+
5	*Bacillus sp*	-	+	+	-	-	-	-
6	*Bacillus subtilis*	+	+	-		-	+	+
7	*Micrococcus sp*	-	-	+	-	+	+	-

Table 1: Biochemical characteristic of isolated bacteria.

S. No	Name of the Bacteria
1.	*Pseudomonas putida*
2.	*Pseudomonas fluorescens*
3.	*Klebsiella pneumoniae*
4.	*Escherichia coli*
5.	*Bacillus cereus*
6.	*Bacillus subtilis*
7.	*Micrococcus*

Table 2: Bacterial flora from the effluent.

Fungal flora in the effluent

Totally 11 species of fungi belong to 7 genera were recorded from the effluent. Among the genus *Aspergillus* was recorded as dominant genus with 5 species such as *A. niger, A. flavus, A. luchensis, A. nidulans* and *A. terreus.* Remaining genus such as *Helminthosporium, Trichoderma, Geotichum, Curvularia, Glicocladium* and *Verticillium* were recorded single species each (Table 3).

S. No	Name of the Bacteria
1.	*Aspergillus niger*
2.	*Aspergillus flavus*
3.	*Aspergillus terreus*
4.	*Aspergillus lichensis*
5.	*Trichoderma sp*
6.	*Helminthosporium sp*
7.	*Geotrichum sp*
8.	*Curvularia sp*
9.	*Verticillium sp*
10.	*Glioladium sp*

Table 3: Fungal members in the effluent.

Initial heavy metal analysis

The effluent was analyzed initially for heavy metals such as chromium (Cr) and zinc (Zn) (Table 4). Among the heavy metals analyzed, chromium was recorded as maximum amount (0.90 mgl^{-1}) than zinc (0.36 mgl^{-1}).

S. No	Heavy metal	Initial
1	Chromium	0.90
2	Zinc	0.36

Table 4: Level of heavy metals in the effluent (Mg l^{-1}).

Heavy metals in the treated effluents

The amount of heavy metals Cr and Zn were estimated in the test samples *Pseudomonas putida* (Table 5) and *Aspergillus niger* (Table 6) inoculate effluent on 5th, 10th, 15th, 20th and 25thday. Pseudomonas treated effluent showed a maximum removal of Cr (Figure 5) and Zn (Figure 6) when compared to Aspergillus treated effluent. Generally, the heavy metal, Cr was maximum removal than Zn by both microbes.

Heavy Metals	Treated effluent				
	5th day	10th day	15th day	20th day	25th day
Chromium	0.75	0.64	0.52	0.44	0.25
Zinc	0.29	0.26	0.18	0.15	0.09

Table 5: Effluent treatment with *Pseudomonas Putida.*

Heavy Metals	Treated effluent				
	5th day	10th day	15th day	20th day	25th day
Chromium	0.77	0.68	0.59	0.47	0.36
Zinc	0.38	0.32	0.27	0.20	0.11

Table 6: Effluent treatment with *Aspergillus niger.*

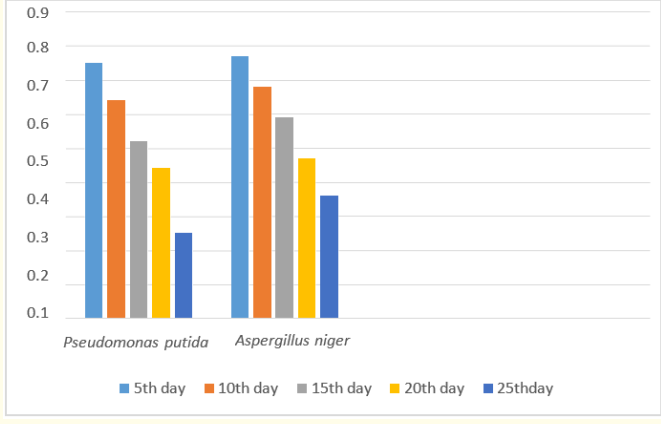

Figure 5: Optimal Density Value of the Effluent Test with *Pseudomonas putida* and *Aspergillus niger* (Chromium).

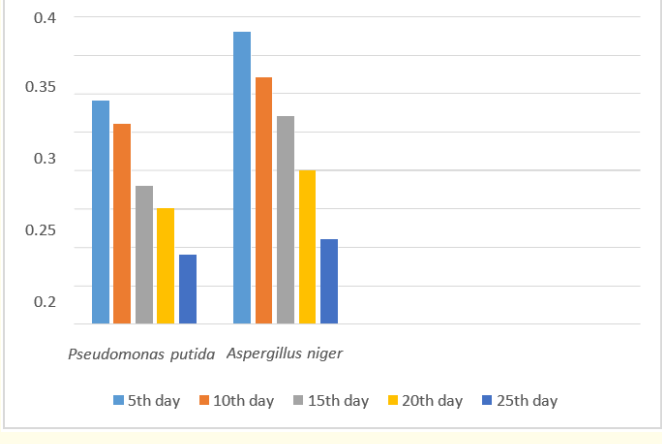

Figure 6: Optimal Density Value of the Effluent Test with *Pseudomonas putida* and *Aspergillus niger* (Zinc).

Discussion

It is well recognized that microorganisms have a high affinity for the metals and can accumulate both heavy and toxic metals by a variety of mechanisms.

Microorganisms highly effective in sequestering heavy metals include bacteria, fungi, algae and actinomycetes. These have been used to remove metals from polluted industrial and domestic effluent on a large scale [11]. And this work focused around the use of gram-negative Bactria, *Pseudomonas putida* and fungi, *Aspergillus niger* to remove heavy metal, Chromium and Zinc in Textile and Dye industrial effluent.

Hence, the present study was undertaken to know the bacterial and fungal species in textile and dye industrial effluent. The isolation of bacteria from the textile and dye industrial effluent sample was showed 5 bacterial isolates. Then the isolated bacteria were identified through number of various biochemical tests. Among this genus *Pseudomonas* and *Bacillus* were recorded with two species. It is confirmed and supported by earlier finding of [12] have suggested that the polluted habitats found mostly *Pseudomonas* sp. Because it having ability to degrade various pollutants in textile and dye industry effluent.

Totally 11 species of fungi belong to 7 genera were recorded from the effluent. Among the genus A*spergillus* was recorded as dominant genus with 5 species such as *A. niger, A. luchenis, A. niduans and A. terreus.* Remaining genus such as *Geotrichum, Trichoderma, Helminthosporium, Glicocladiuum, Curvularia* and *Verticillium* were recorded single species each. Fungi are known to colonize, multiply and survive in diversified habitats, viz., water, soil air, liter, dung, foam, etc. Fungi are ubiquitous and cosmopolitan in distribution covering tropics to poles and mountaintops to the deep oceans. The kingdom of fungi contains 1.5 million fungal species, of which 74,000 species are named. Many of the discovered species are known only as dead herbarium material and around 5% of species are isolated as pure cultures. Geographic location, climatic conditions, microhabitat, substrate type, distribution of fauna and flora are all important factors contributing to fungal distribution around the world.

Preliminary experiments were conducted during the initial stages of this work showed high concentrations of total Chromium and Zinc in effluent samples. As a result, a specific treatment process was required, aiming to the reduction of Chromium and Zinc content in the textile and dye industry effluent.

In the present study, amount of heavy metals Chromium and Zinc were estimated in the test samples Pseudomonas *putida* and *Aspergillus niger* inoculated in the effluent on 5th day, 10th day, 15th day, 20th day, and 25thday. *Pseudomonas putida* treated effluent showed a maximum removal of Chromium and Zinc when compared to *Aspergillus niger* treated effluent. Generally, the heavy metals, Chromium was maximum removal than Zinc by both microbes.

The cell wall of *Pseudomonas* contains a thin layer of peptidoglycan and a second membrane (outer membrane) within the cellular envelope which is a porous structure, rich in protein and lipopolysaccharide (LPS). The peptidoglycan, phospholipids and LPS are the components primarily responsible for the anionic character and metal binding ability of the cell walls. These characteristics of Gram-negative bacteria make them to adsorb heavy metals (Chromium and Zinc) less effectively than *Aspergillus niger.*

It is difficult to say whether the heavy metal copper, were biodegraded. However, their accumulation in the bacteria, *Pseudomonas putita* and the fungi, *Aspergillus niger* suggests that these cells are able to entrap the heavy metals as they occur in the aqueous phase. The finding of the study indicate that Biosorption is a promoting technology for removal of heavy metal. Cost-effectiveness is the main attraction of metal Biosorption. In addition, biosorbents derived from microbial biomass through a simple process are expected to be the lowest- priced and most-economical for metal removal. Our results show that bacterial cells and fungal strains have Biosorption capability, by being able to sequester substantial amounts of heavy metals from effluents [13].

Summary

Remediation of toxic metals by bacteria offers a relatively inexpensive and effective way for the decontamination of soil and aquatic environmental which has been demonstrated by several biotechnology companies employing bioaccumulation. The cell surface of most microorganisms is negatively charged owing to the presence of various anionic structures. Chromium and also Zinc as such one of the major toxic heavy metal causing carried out to investigate the adsorption of chromium by *Pseudomonas putida and* the fungi, *Aspergillus niger.* The study was carried out by inoculating the selected microbes in the textile and dye industry effluent at laboratory condition. The amount of Chromium and Zinc adsorbed by the cells were estimated by the Spectroscopic method at 620nm and 510nm in the intervals of 5th day, 10th day, 15th day, 20th and 25th day. The result showed that effective adsorption of the metal was observed in effluent treated with *Pseudomonas putida* than *Aspergillus niger*.

Conclusion

Thus, the study emphasizes on the use of an eco-friendly alternative for the reduction of Copper and Zinc from the environment. The method offers several advantages including cost effectiveness, high efficiency. In countries with lack of awareness about metal toxicity there is an urgent need for developing an economical and eco-friendly technology which satisfies these demands when other conventional methods fail.

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