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STUDY ON GROWTH PERFORMANCE OF METAL TOLERANT *PSEUDOMONAS* SP IN NICKEL EFFLUENT – BASED CULTURE MEDIUM

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Abstract

In nature, microorganisms play a vital role in biosorption of heavy metals from contaminated water and soil environments. Hence, an attempt was made in this study to investigate the metal tolerant capacity of microbial strains isolated from electroplating effluent contaminated soil. The studies include physicochemical characterization of effluent and isolation, identification & characterization of metal resistance bacteria. The physicochemical parameters such as pH, temperature, hardness, electrical conductivity, total suspended solids, total dissolved solids, total solids, sodium, potassium, calcium, chloride biological oxygen demand, chemical oxygen demand and nickel in the electroplating effluent were analyzed using standard procedures. Nickel metal tolerant bacteria were isolated from electroplating effluents contaminated soil and identified as *Pseudomonas* sp based on morphological and biochemical characteristics. The selected bacterial strain *Pseudomonas* sp tested for the metal tolerant property in different pH (pH 5, pH 7 and pH 9), various temperatures (20°C, 30°C and 40°C) and various metal concentrations (15%, 20% and 25%). The better bacterial growth performance by *Pseudomonas* sp was recorded in 20% nickel effluent based - medium with pH 7.0 at 30°C.

Keywords: Electroplating effluent, heavy metals, Nickel, *Pseudomonas* sp.

Introduction

Toxic heavy metal containing industrial wastewater can cause serious environmental pollution. The main industrial sources of toxic metal contamination in wastewaters includes electroplating, metal finishing, metallurgical, tannery, chemical manufacturing, mining and battery manufacturing industries, etc. Some heavy metals are necessary in small amounts for normal development of biological cycles; however, most of these heavy metals are becoming toxic at high concentrations. Effluents from the electroplating industries are the cause of serious ground water and soil contamination in vicinity area which pose a significant threat to human health and ecology [1]. The toxicity of heavy metals is apparent in reducing growth and development in microorganisms and plants and seriously harming the health of animals and humans. The long-term action of heavy metals may cause the development of cancer, allergy, dystrophy, physical and neurological degenerative processes, Alzheimer's and Parkinson's diseases. However, in small amounts, heavy metals are indispensable for many organisms but their enhanced doses induce acute or chronic poisoning [2]. The removal of toxic contaminants and organic pollutants from industrial wastewaters is one of the most important environmental issues [3].

Several methods such as reverse osmosis, ion exchange resin, solvent extraction, electrolytic and precipitation processes, electrolysis and membrane technology have been used to remove heavy metals from industrial effluents. However, one of the best methods was the use of adsorbents since it outperforms other techniques due to its low cost, high efficiency, simplicity and insensitivity to toxic substances [4,5,6,7]. These methods are often expensive and difficult to maintain due to high capital and operational costs as well as extra cost of treating the resultant sludge generated before disposal [8]. Hence, there is a need for cost-effective and efficient alternatives to the conventional methods [9].

Bioremediation constitutes are an attractive alternative to physiochemical methods of remediation, mainly due to its reputation of low cost, ecofriendly and publically acceptable treatment technology. Microganisms have been used in a number of biological treatment processes for metal remediation [10]. The microbes based technologies can provide an alternative to convention method for metal removal [11]. Bioremidiation of heavy metals could be brought about by employing methods such as bioaccumulation, biosorption, bioprecipitation and uptake by purified biopolymers from microbial cell [12].

The technique of biosorption utilizes the characteristics of microorganisms to adsorb metals in a commercial manner. Microorganisms uptake metal, either actively (bioaccumulation) and/or passively (biosorption) [13]. This is due to affinity of bacterial surfaces for heavy metal leading to their adsorption and precipitation. The biosorption is passive non-metabolic process of binding various chemicals on biomass [14].

In view of the above, the investigation was made an isolation of metal resistant *Pseudomonas* sp and to evaluate the growth performance in nickel – based culture medium against various environmental conditions.

Materials and Methods

Physicochemical characteristics of the nickel electroplating effluent

The nickel electroplating effluent sample was collected from the direct outlet of Meena electroplating industry, Madurai, Tamil Nadu India and the sample was immediately transported to the laboratory, Department of Biology, GRI, Gandhigram for the analysis of various physicochemical parameters such as pH, temperature, hardness, electrical conductivity, total suspended solids, total dissolved solids, total solids, sodium, potassium, calcium, chloride biological oxygen demand and chemical oxygen demand were analyzed from electroplating industrial effluent using standard methods [15].

Isolation and Identification of nickel resistant *Pseudomonas* sp

The electroplating effluent contaminated soil sample was collected from the electroplating effluent contaminated lagoons near Meena Electroplating Industry, Madurai, Tamil Nadu, India. The soil sample was diluted with distilled water up to 10^{-9} dilutions and 0.1 ml each from 10^{-5} and 10^{-6} dilutions were spread on the nutrient agar plates and incubated at 37°C for 24 hrs. Two predominant colonies grown on nutrient agar medium were selected and screened for metal tolerant with Nutrient Agar (NA) plate supplemented with 5 mg/l concentration of nickel metal [16]. One potential bacterial isolate was selected based on their better growth performance in the screening medium incorporated with known metal ions and maintained as pure culture.

The selected strain was identified through morphological and biochemical characteristics such as Gram staining, motility test, indole production, methyl red reaction, voges proskauer reaction, citrate utilization, urease hydrolysis, starch hydrolysis and catalase reaction and authenticated with Bergey's manual of determinative bacteriology [17].

Growth studies of metal resistant *Pseudomonas* sp

The growth study was carried out using the nickel effluent - based culture medium in a 100ml Erlenmeyer flask and inoculated with *Pseudomonas* sp. The flasks were later placed on rotary shaker with an operating speed of 150 rpm at a temperature of 20°C, 30°C and 40°C for 24 hours [18]. Bacterial growth in the nickel containing medium was assessed by reading the culture broth at 540 nm using Spectrophotometer (Version Spectronic 200).

Result and Discussion

The phenomenon of microbial resistance and the understanding of mechanism of microbial tolerance against various pollutants are of ecological importance [19]. Therefore, in the present work an attempt was made to isolate and study the growth performance of nickel resistant *Pseudomonas* sp. The nickel - based effluent sample was collected from the electroplating industry and analyzed for various physicochemical characteristics using standard procedures and the values are presented in Table 1. One predominant bacterial colony was isolated from electroplating effluent sediments and identified as *Pseudomonas* sp based on morphological and biochemical characteristics as given in Table 2.

[20] Mahalingam *et al.*, (2014) have isolated metal (zinc) tolerant *Bacillus* sp and suggested that this isolate could be used effectively in biosorption process for the treatment of effluent from industries handling heavy metals.

To survive under metal stressed conditions, bacteria have evolved several types of mechanisms to tolerate the uptake of heavy metal ions. These mechanisms include the efflux of metal ions outside the cell. Accumulation and complexation of the metal ions inside the cell and reduction of the heavy metal ions to a less toxic state [21]. In this study, the result of metal resistance by bacterial isolate, *Pseudomonas* sp in different concentration of electroplating effluent contain nickel (15%, 20% and 25%) against different temperature conditions (20°C, 30°C and 40°C) with various pH (5, 7 and 9) are shown in Figures 1 and 2.

The better growth performance of *Pseudomonas* sp was recorded in 20% nickel effluent based medium with pH 7.0 at 30°C. Thus, the ability of microbial strains to grow in the presence of heavy metals would be helpful in the wastewater treatment in the treatment of effluent in various electroplating and other metal based industries.

Table 1: Physicochemical characteristics of nickel - based electroplating effluent

S.No	Physicochemical parameters	Values
1.	Temperature (°C)	32±3
2.	pH	7.3±1.3
3.	Electrical conductivity (µS/cm)	3.9±0.6
4.	Hardness (mg/l)	1789±40
5.	Total Solids (mg/l)	11900±18
6.	Total Dissolved Solids (mg/l)	10863±16
7.	Total Suspended Solids (mg/l)	1037±8
8.	Calcium (mg/l)	13.4±4.1
9.	Sodium (mg/l)	10.27±0.4
10.	Potassium (mg/l)	22.7±0.2
11.	Chloride (mg/l)	39±5
12.	Biological oxygen demand (mg/l)	61.9±1.1
13.	Chemical oxygen demand (mg/l)	390±12

± (Values are mean of three replicates ± standard error)

Table 2: Morphological and biochemical characteristics of nickel tolerant *Pseudomonas* sp

S.No	Characteristics of Pseudomonas	Result
Morphological characteristics		
1.	Gram's staining	Negative
2.	Shape	Bacilli (Rod)
3.	Motility	Motile
Biochemical characteristics		
4.	Indole Production	Negative
5.	Methyl Red Reaction	Negative
6.	Voges Proskauer Reaction	Negative
7.	Citrate Utilization	Positive
8.	Catalase Reaction	Positive
9.	Oxidase Reaction	Positive
10.	Starch Hydrolysis	Negative
11.	Urease Hydrolysis	Negative

Figure 1: Growth performance of *Pseudomonas* sp in different concentrations of Nickel containing electroplating effluent medium with various pH on 24 hours.

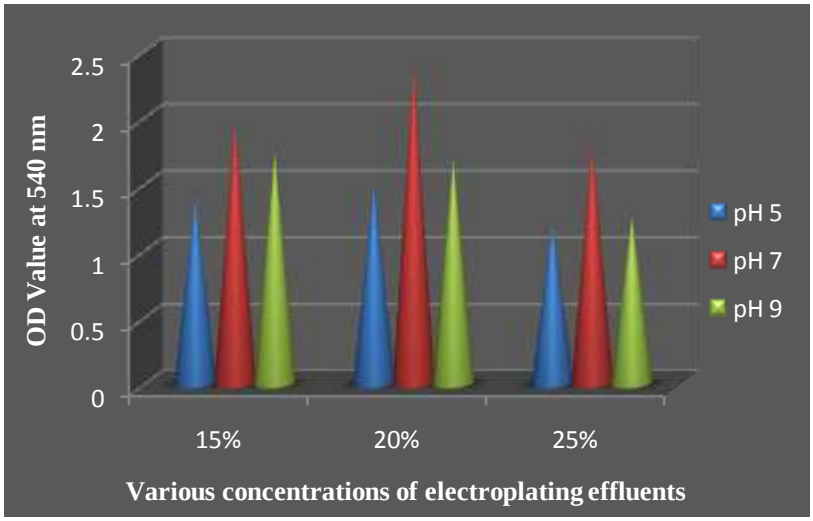
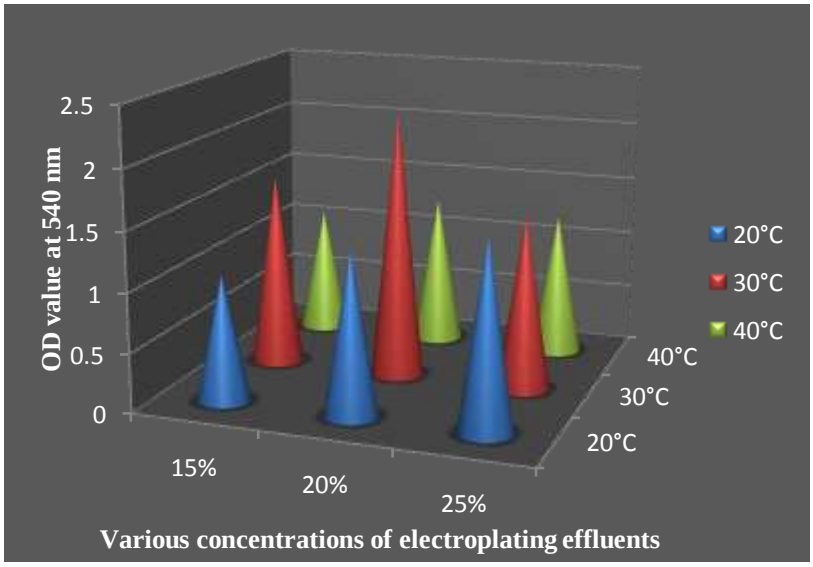


Figure 2: Growth performance of *Pseudomonas* sp in different concentrations of Nickel containing electroplating effluent medium with various temperatures on 24 hours.



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