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Isolation and Characterization of the Phenol Degrading Organisms from the Soil Sample Containing Traces of Petrol and Diesel

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Abstract: *This study examines the isolation and the characterization of the phenol-degrading-bacteria, two isolates named as P4 and O3 from the effluent of petrochemical (P4) and the crude oil (O3). These isolates were characterized depending on their morphological, and the biochemical characteristics, one isolate (Pseudomonas sp.: (P4) was recogniced as a Gram-negative, strictly aerobic, motile and short rod-shaped bacterium and the other isolate (Bacillus sp.: (O3) was identified as a Gram-positive, strictly aerobic, motile and long rod- shape bacterium. The two tolerant bacterial isolates were able to grow at the higher concentrations of the phenol and they were investigated for their ability to grow and degrade the phenol. The Mineral salt media was used for the degradation of the phenol. The efficiency and the resistivity of this biomass was checked for the different concentrations of the phenol containing compounds with the maximum of 1500 ppm. The Three different microbes were identified and then isolated which could resist this high concentration of the phenol. The different characterization tests were performed on these three microorganisms. The ability of these microorganisms to degrade the phenol at the different pH was also observed. These microbes were also examined for their degrade ability by revealing them to the different temperatures. The characterization tests and the degradation study could give an identified microorganism able to degrade the phenol containing compound.*

Keywords: Phenol degrading-bacteria, petrochemical effluent, crude oil effluent

I. INTRODUCTION

This methods are getting more and more attention and are equally more very important because all of the pollutants which are released in the atmosphere are one or by the other way are very harmful to the human in the form of some or the other disease or the disorder. From the list of the many pollutants one of the pollutant is phenol. The phenol is the 11th most toxic compound out of the 126 toxic compounds given by the EPA (1) (Environmental Protection Agency). Also the limit for the inhalation of the phenol vapour is set to around 0.04ppm by the EPA. This phenol released from the industries in the free form or in the form of the phenol derivative is the main problematic cause for the pollution of the soil and the water from the phenol release. The Phenol which is released in the soil pollutes the soil but the microorganism in this soil can degrade the phenol using the phenol as their components of the food. Hence the concept of the degradation of the phenol by using the microorganism comes into the picture(2). The Degradation of the phenol by the utilization of the microbial method is the most important methods because it does not produce any of the by products or any of the toxic waste. The Microorganism is found to degrade the phenol by the two pathways mainly known as the ortho-pathway or by the meta-pathway and produces the intermediates of the tri carboxylic acids(3).

The growing rate of the industries is in such a pace that that the pollution level is rising up with the very higher rates than its control measures. The industrial waste water is not only contaminating the surface water bodies but also the continuous dumping of this wastes in an open land leads to the toxic pollutants from the waste to get seeps into the ground. As these pollutants seeps through the ground they also affect the ground water. The People residing in the plateau region who depend on the tube wells for the drinking ground water get mostly affected by the contaminated ground water(4). This contamination contains the solid and the liquid contaminants that are present in the present water. The contaminants may be biological, chemical or may be very radioactive in nature. These contaminants have a very devastating effects on both our health and the life of all the other species which are in their vicinity. They also have an

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adverse effect on the ecological system and lead to the poisoning of the food chains of the terrestrial and the aquatic life.

The Phenol is degraded by so many types of the microbes which utilizes the phenol as the sole carbon source for their growth. Several microbes both the anaerobic and the aerobic microorganisms degrading phenol are isolated and characterized, while the microorganisms capable of the aerobic phenol degradation were defined as early as in the year 1908. The Microorganisms like *Pseudomonas putida* is the most widespread organism for the degradation of the phenol amongst the other various microorganisms. Along with the bacteria, the fungi are also known for their multiplicity and also the notable ability in the degrading phenolic compounds(5). Contrary to the bacteria, the fungi can also grow under the ecologically stressed environments such as the very low nutrient availability, low water activity and at the low pH values where the bacterial growing might be very inadequate. There are various parameters for example the contaminant concentrations, feasible biomass, concentrations, temperature, pH, microbial completion and the adaptation are the most important parameters that affect the phenol degradation rate and it also depends on the period during which the culture was adapted to the phenol.

II. MATERIALS AND METHODS

2.1 Collection of the Samples

The Soil samples were collected from the different petrol pump station and also from chemical industries backyard. The Isolation process of the microorganisms was then carried out in the microbiology laboratory.

2.2 Media Composition and the Cultural Condition

Following media were used for the growth of the microorganisms.

Mineral Salt Medium:

Following components are the basic ingredients in the mineral salt medium(6,7):

1) K_2HPO_4 (1.5g/L)
2) KH_2PO_4 (0.5g/L)
3) NaCl (0.5g/L)
4) NH_4NO_3 (1g/L)
5) $MgSO_4.7H_2O$ (0.5g/L)
6) $FeSO_4.7H_2O$ (0.01g/L)
7) $CaCl_2.2H_2O$ (0.01g/L)
8) NH_4SO_4 (0.5g/L).

Luria Bertani (LB) medium composed of the tryptone 10 g, yeast extract 5 g and the NaCl 5 g per liter. Deionized distilled water was also used for the experiments.

The pH of the media was adjusted to 6.8–7.0 and supplemented with the varying amounts of the phenol up to a final concentration of 500– 2000 ppm. Phenol was added directly from the stock solution (50,000 mg l^{-1}phenol) through the 0.45-µm syringe filters. The Cells were grown in the flasks on a rotary shaker at the room temperature (25°C) and 120 rpm. The Samples were withdrawn periodically for the bacterial growth and the phenol concentration or the determinations were monitored(8).

2.3 Isolation of the bacteria degrading phenol

We have to isolate the microorganisms for the degradation of the phenol for which we need to first grow the microbes with the help of the nutrient broth which is food for the microbes. The process of providing the microbes with a specific media for their growth is called as the enrichment. This process will then be followed by the survival of the microorganisms in the different concentration of the phenol to check the maximum concentration up to which the microbes can survive.

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Phenol Biodegradation and Cell Growth Performance:

To clarify the phenol degradation potentials of the bacterial isolates, batch experiments were performed in the duplicate manner. Prior to the inoculation for each experiment, one loop of the stock culture maintained on the nutrient agar slants was transferred aseptically into the 250-ml flasks with 100 ml of the sterilized MPsupplemented with the 500 mg/L phenol. Five milliliters of the inoculum from the late exponential growth phase of the each culture were then transferred aseptically to the each flask with the 95 ml of the MS- medium. The bacterial isolates (P1-5 and O1-5) were tested in the 500 mg/L phenol concentration then all the bacterial isolates were selected for the phenol removal assay at the different phenol concentrations (0.0, 500, 1000, 15000 and 2000 mg/L)(9).

2.3.1 Enrichment Process:

The Sterilization of the water contained in a conical flask is done in the autoclave to avoid any type of the contaminants to be present in the conical flask. The autoclave heating is done rightly at heating at 121°C for around 15 minutes. 1 g of the soil sample was mixed with the 9 ml of the sterilized water and was mixed thoroughly. The Nutrient Broth media was prepared by dissolving the 1.3 g of the nutrient broth in the 100 ml of the water and was sterilized in the autoclave. 1 ml of the soil sample was added to the nutrient broth media after cooling in the laminar flow hood which offers the cooling free of any of the contamination in the presence of the UV light. This media was then sealed with the cotton plug and the paraffin tape to avoid any type of the contamination. It was then kept in an incubator at a temperature of around 30°C and shaker at an rpm for the 24 hours(10). This was done for both the samples of the soil. It was observed that there was sufficient amount of growth of the biomass in the nutrient broth which could be inferred by the turbidity of the sample.

2.3.2 Microorganisms Resisting Phenol:-

100 ml distilled water was taken in the 250 ml flask and the different salts as per the minimal salt media composition were added to make the 100 ml media. The stock solution of around 5000 ppm phenol solution was prepared and stored. 100 ppm solution was prepared by adding the 10 ml of the stock solution to the 90 ml of the media solution. This solution was then sterilized and was kept in the laminar flow hood for the cooling process.1 ml of the culture was added to the 100 ppm solution and this is called as the inoculation process(11).This solution was then sealed and was then incubated for around 24 hours at the same condition mentioned above. The growth of the biomass was seen the next day (turbidity) which conclude that the microorganisms could resist around 100 ppm of the phenol as they used the phenol as their main carbon source. The same procedure was repeated for the concentration from the 100 ppm to 1000 ppm with an increment of the 100 ppm each day and at the different incubation temperatures (25°C, 30°C, and the 40°C)(12,13).

III. RESULT AND DISCUSSION

Ten bacterial cultures (P1 to P5, O1 to O5) were screened for the growth on the different phenol concentrations (Table 1). Out of the 10 bacterial isolates which isolated from the effluents of the, petrochemical soil (P) and crude oil (O), only two isolates were more active on the growth on the varied initial phenol concentrations of the 500 - 2000 mg/L phenol. The study was carried out at temperature of 35 °C and pH 7. Growth of the bacterial isolates on the treated media and the control was taken and expressed by the streaking growth degree as follows: no growth (-), moderate growth (+), good growth (++), very good growth (+++) andthe excellent growth (++++). All the cultures showed fast growth and activity at 500 mg/L at 35°C and pH 7 as indicated by the plate growth and the turbidity. However, only 2 plates named P4,and O3 showed the fast growth in up to 2000 mg/L phenol concentration.

The Phenol and its derivatives have shown surprising capability in the phenol elimination with the bacteria having fast reproduction after the acclimatization. In the present study, two bacterial isolates P4, and O3, showed the luxuriant growth on the phenol-amended minimal salt medium (MSM) in the presence of the 1% glucose (w/v), whereas the growth was absent in the absence of the glucose. Also, the bacterial isolates P4,and O3 can tolerate the phenol up to a phenol concentration of 2000 mg/L.

However, no growth has been observed in the other bacterial isolates (the rest of the 10 isolates) at a phenol concentration of 1500 and 2000 mg/L (Table 1).

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Table 1: Capability of bacterial isolates collected from different sources used to grow on different concentrations of phenol.

SOURCES OF ISOLATES	Concentrations of phenol mg/L				
	Control	500	1000	1500	2000
P1	++++	++	-	-	-
P2	++++	+	-	-	-
P3	++++	-	-	-	-
P4	++++	+++	+++	+++	+++
P5	++++	-	-	-	-
O1	++++	++	+	-	-
O2	++++	+	+	-	-
O3	++++	+++	+++	++	++
O4	++++	+	+	-	-
O5	++++	+	+	-	-

Identification and Characterization of Phenol-Degrading Bacterial Isolates

The samples collected from aneffluent of petrochemical (P),andeffluents of the crude oil (O) were inoculated in the MPmedium containing phenol for the enrichment and the isolation of the phenol-degrading bacteria.The isolates utilized the phenol as the sole carbon source and the energy. The outstanding isolate was named as phenol-isolates P4,and O3. After three weeks of the enrichment and one week of the bacterial isolation, a total of the 10 isolates were obtained after the 24 hrs., growth on the LB- agar plates with 100 µL of a 103 –106 -fold dilution of enrichment culture. Identification of the isolates based on their morphology, microscopic and biochemical characteristics are shown in Table 2.

Table 2: Characteristics of isolates

Characteristics	Bacterial isolates	
	P4	O3
Colonial	Circular, white	Circular, white
Gram's reaction	-	+
Shape	Rod	Bacilli
Spore staining	-	+
Indole	-	-
Methyl red	-	-
Catalase	-	+
Oxidase	-	+
Urease test	-	-
fermentation of Glucose	+	+
Lactose	+	+
Maltose	+	-
Sucrose	+	+

The bacterial isolate could grow within a range of the pH 6–8, and the degradation of the phenol was ranged between the 33-90 % in the range of the pH 6–8. The optimum pH for the phenol degradation was 7.0.

IV. CONCLUSION

In conclusion, the bacterial isolates capable of degrading the phenol were isolated from an effluent of petrochemical company (P4)and effluent of crude oil (O3). These bacterial isolates have the ability to grow in a liquid medium with phenol at different concentrations as the sole carbon and energy source. The optimal growth conditions for phenol degradation of the strain were at 35°C and pH 7.0. Regarding that native microbial species were more adaptive than

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non-indigenous microorganisms in polluted environments, their predominance facilitated the bioremediation of the phenol-contaminated environments.

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