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BIODEGRADATION OF PHENOL USING *ACTINOMYCETES* SPECIES, ISOLATES OF CORINGA MANGROVE FOREST

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Abstract

Most of the industrial effluents containing phenols and its derivatives, leading to pollution in water bodies i.e. marine, river, lake and ground waters. We have adopted ecologically friendly process- Biodegradation, since conventional treatment technologies are not economical. It has been proved more successful in removal of different toxic chemical substances by using various micro organisms. The immobilization of the cells on a suitable support can simplify the treatment of liquid waste as the entrapment of living cells increases the retention time of cells in contaminated water. In the present study, the free and immobilized cells of Actinomycetes species, isolates of Coringa Mangrove forest were used as biological agents for the removal of phenol from the aqueous solutions. The major influencing factors such as pH, incubation time, initial concentration of biomass and temperature were verified to optimize the process.

Key words: Water pollution, Phenol, Actinomycetes species, Biodegradation

I. INTRODUCTION

The rapid development of industrial sector leading to the problem of pollution and hazardous waste water. Systematic procedure for the minimization of these problems is one of the important challenges for the industries(Singleton*etal*.,1994, Lanouette *et al*., 1977, Klein *et al*., 1978, Arutchelavan *et al*., 2004). Phenolic compounds, the aromatic compounds with hydroxyl groups have widespread contribution in plant kingdom owing to their color and flavor. Phenols offer excellent resistance to diseases and pests in plants (Cirelli, 1978). Presence of higher amounts of polyphenols increases the resistance of plants to bird attack. However, as a consequence of their widespread use and subsequent introduction into the environment causing the phenolic compounds as a priority class of pollutants. The phenolic compounds enter the environment through waste water discharges from a variety of industries like leather, textiles, phenol-formaldehyde resin, oil refinery, pharmaceutical etc (Arutchelavan *et al*., 2006, Babitch *et al*., 1981). The discharged phenolic compounds into the waste water have both direct and indirect effects on the society as the water is the prime necessity of life and extremely needed for the survival of living organisms. Phenolic compounds are strong skin irritants and are responsible for toxic effects in the brain, lungs, kidneys, liver, pancreas and spleen (Brett *et al*., 1986, EPA. 1996, ASTDR 1997). Due to the greater toxic nature of phenol and its derivatives, the effective removal of these compounds from the industrial aqueous effluents is very important for the protection of environment.

Several physico-chemical methods are under implementation for the removal of phenol and its derivatives from industrial effluents (Lanouette *et al*., 1977, Singleton *et al*., 1994, Tareq *et al*., 2004). Biodegradation techniques have been found potential and economical to mineralize phenolic compounds to maximum extent and without possibility of secondary pollution (Klein *et al*., 1978, Hamed *et al*., 2004). Many aerobic phenol degrading microorganisms have been isolated and the clear mechanisms for the aerobic degradation of phenol are established (Hill *et al*., 1975, Park *et al*., 2003, Kumar *et al*., 2004). Halophiles are also found as one of the classes of bacteria for biodegradation of phenolic compounds under aerobic conditions (Woolard C R *et al*., 1994, Hinteregger *et al*., 1997, Rosa M *et al*., 2001, Carla *et al*., 2004, Muhammad Afzal *et al*., 2007). Biodegradation of industrial waste waters can also be improved if the microorgan is mis adapted to the to xic chemical (Zilli *et al*., 1993, Gonzalez *et al*., 2001). Studies are also

available on the biodegradation of phenol from model solutions with free and immobilized solutions in spite of toxic properties of phenol under aerobic conditions as the sources of carbon and energy (Paller *et al*., 1995, Kar *et al*., 1997, Paraskevi *et al*., 2005, Chandana Lakshmi *et al*., 2008).

The prime objective of the present investigation was to examine the capability and mechanism of free cells of *Actinomycetes* in biodegradation of phenol.

II. MATERIALS AND METHODS

Microorganisms and cultivation conditions

Actinomycetes species is the microbial mass used for the biodegradation of phenol . The *Actinomycetes species* isolates were obtained from the culture collection of Coringa Mangrove forest, Kakinada. The *Actinomycetes species* were grown in 250 mL Erlenmeyer flasks containing 100 mL medium consists of Peptone, Yeast Extract, Malt Extract, Glucose at a constant pH of 7, an optimal pH.

The Erlenmeyer flask was inoculated with freshly prepared homogenous culture suspensions of *Actinomycetes species.* The suspensions were incubated at 37^{O}C for one week under constant rotatory conditions (120 rpm). The bacterial mass was harvested by centrifugation at 12000rpm for 10 min. The resultant was washed thoroughly with 15% sodium chloride solution and dried at room temperature. The stock solution of microbial culture was prepared by dissolving 100 mg of *Actinomycetes species* in 100 ml of 15% sodium chloride solution. The exact dilutions of the respective stock solutions were used for the biodegradation studies. Phenols

All the chemicals used were of A R Grade obtained from Loba Chem. Pvt. Ltd. and S. D. Fine Chemicals Ltd. The stock solutions of phenols were prepared by dissolving 100 mg of the phenol in 100 ml of 1% sodium carbonate and 15% sodium chloride solution. The exact dilutions of the respective stock solutions were used for their biodegradation studies.

Biomass Characterization

In order to study the nature and capacity of the microbial mass for the present studies, the *Actinomycetes species* was characterized. The presence of phosphate content on the cell wall of the microbial biomass was determined spectrophotometrically by using Molybdenum Blue Method (Bassett, *et al*., 1978, Suryanarayana Raju *et al*., 2008).

Methods

For biodegradation studies, 100 mg of dried bacterial biomass was suspended in 100 mg of X solution contained in 100 mL Erlenmeyer flasks and the pH of the solution was adjusted to 7.4, optimal PH for *Actinomycetes species* (Thomas, 1978, Suryanarayana Raju *et al*., 2008). The flasks were agitated on a rotary shaker (120 rpm) at 35^{O} C. Samples were withdrawn at definite intervals, centrifuged at 12,000 rpm for 15 min. 0.1 ml of supernatant was collected in test tubes and 0.2 ml of Folin-Coicatteau reagent, 2 ml of sodium carbonate and 2 ml of distilled water were added to the test tube. The samples were incubated at 37 ^{O}C for 2 hrs. The concentrations of X were estimated by measuring the absorbance at 650 nm. The percentage degradation of X have been calculated by using FC reagent standard curve methods (Malick *et al*., 1980, AOAC, 1984). All the experiments were triplicate for the confirmation of the results.

Effect of Biomass concentration on degradation

Different biomass concentrations 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mg of dried bacterial pellet were added to 10 mL of Phenol solution taken in 100 mL Erlenmeyer flasks to verify the influence of initial concentration of biomass on degradation of Phenol. The resulting mixtures were kept in an orbital incubator shaker for incubation for 24hrs under similar conditions along with their corresponding reference solutions.

Effect of pH on degradation

The pH effect on the biodegradation of **Phenol** was examined by adding 10 mL of each **Phenol** stock solution to the 10 mg of bacterial mass taken in 50 mL of Erlenmeyer flasks at different pH values, 5, 6,

7, 8 and 9. The resulting mixtures were then kept in an orbital incubator shaker for incubation for 24hrs. The studies were carried out at constant temperature.

III. RESULTS AND DISCUSSION

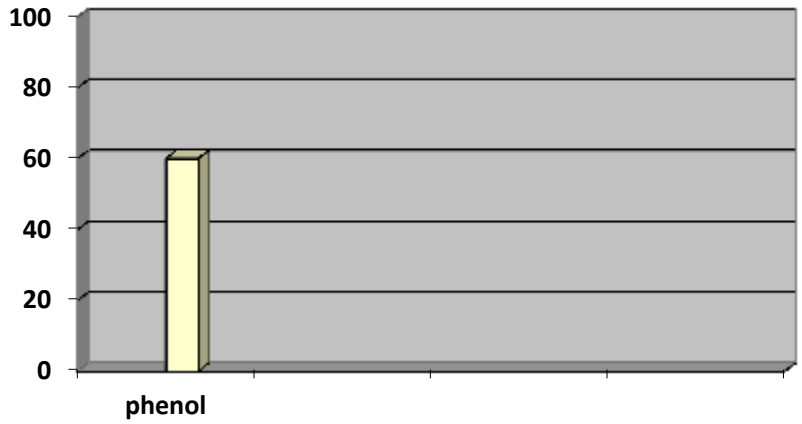
The biodegradation of **Phenol** by free cell of *Actinomycetes species*, have been carried out under similar experimental conditions in order to compare the biodegradation capability of selected microorganisms for the identified phenols. At each trial, the exact amounts of bacterial mass were added to the Phenol solution taken in standard well cleaned and dried Erlenmeyer flask. The reaction mixtures were incubated for a span of 10 days to verify the degradation of Phenol. From the data, it is clear that free cells of *Actinomycetes species* are capable of degrading the Phenol with in the stipulated period. The kinetic data of Phenol estimated by F.C. reagent method is furnished in Table 1. The % degradation of Phenol is furnished in Table 2 and pictorially represented in Fig. 3. From the data it is clear that though *Actinomycetes species* is able to degrade Phenol, the percentage degradation is 60% which indicates that degradation is highly structural dependant under similar experimental conditions (Rosa, M. *et al*., 2001, Jiyang *et al*., 2006).

Biodegradation Mechanism

The typical structure of Actinomycetes species implies that there may be different ways for the degradation. Our studies reveal that dihydroxylation is necessary to break the aromatic ring of the phenolic compounds (Rebecca et al., 2000, Sharma, 2005 Weijie, 1999). Based on the reports, Catechol and protocatechuic acid are the substrates for the aromatic ring cleavage during biodegradation (Dagley et al., 1964, Hayaishi, 1966, Gibson, 1968). We propose that catechol is very important intermediate compound which generates fast and will undergo further cleavage either at ortho/meta positions based on the neighboring groups present on the molecule and also on the microbial system in the biodegradation of our compounds. Further, we propose that the degradation is highly influenced by the substituent groups present on the system. From the data, we propose that, the increase in the hydroxyl groups are leading to the fast generation of intermediate catechol and subsequent degradation to $CO_2$and H_2O.

Table 1. Kinetic data of the biodegradation of phenol by F.C.reagent method at 35^oC.

Days	%Degradation of Phenol
0	0
1	5
2	10
3	18
4	23
5	30
6	38
7	42
8	49
9	54
10	60



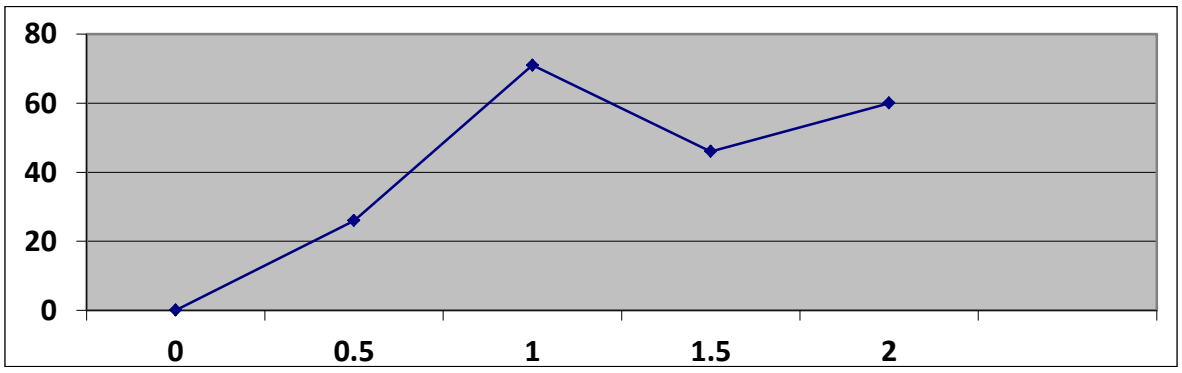
% of Phenol Degradation

Effect of biomass concentration on biodegradation

The reaction mixtures were incubated with varied initial concentrations of biomass to verify the change in the degradation capacity of the bacterial mass if any. From the data it is clear that the initial concentration of biomass has considerable effect on the degradation. Interestingly, the degradation of all the mentioned compounds exhibits same trend. i.e., increases gradually with increase in the initial concentration of biomass till it get its equilibrium, where highest cell density persists (Erika *et al*., 2006). Interestingly, the optimum biomass concentration for all the X is 1 mg / ml.

Table 2. The effect of biomass concentration on degradation of phenol at 35°C.

Biomass Concentration (mg/ml)	%Degradation of Phenol
0	0
0.5	26
1.0	71
1.5	46
2.0	60

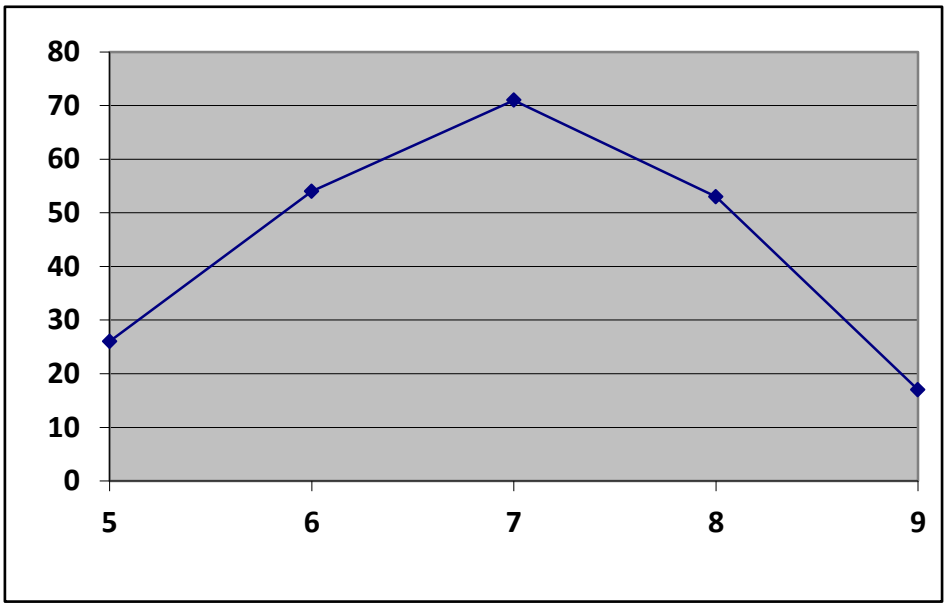


Effect of pH

Since, pH is one of the very important factors in most biological processes process (Galun *et al*., 1987, Lezcano *et al*., 2001, Alaa H *et al*., 2006) the optimum pH value for the degradation of X was determined. The pH dependence of degradation is highly related to functional groups present in the biomass and also on the other reactive moieties present in solution (Suryanarayana Raju *et al*., 1996, Vinoj Kumar *et al*., 2006, Suryanarayana Raju *et al*., 2007). The results obtained in our investigations confirmed it. The data are shown in below Tables is the pictorial representation of the same. From the figure, it is obvious that under highly acidic conditions, the degradation was very low for all the X due to the interference of H^+ ions, protonation of the substrate (Fourest *et al*., 1992, Suryanarayana Raju *et al*., 1996). There was a significant increase in degradation with increase in pH from 6 to 8 beyond which no considerable changes were observed. The possible explanation for this case is that the though the sustainment of *Actinomycetes* is good at basic conditions (Rosa Margesin *et al.*, 2001,Thomas**, 1978,** Suryanarayana Raju *et al*., 2008), the conversion of phenolic compounds to their corresponding salts decelerate the attack of microbial culture. Further, from the studies it is to be noted that the optimum pH range for degradation of Phenol is 6 to 8.

Table 3. The effect of pH on degradation of phenol at 35°C.

pH	%Degradation of Phenol
0	0
5.0	26
6.0	54
7.0	71
8.0	67
9.0	17



pH

IV. CONCLUSION

The prime idea of this work was to verify the biodegradation of certain phenolic compounds by using *Actinomycetes* and to optimize the conditions of the process. A series of experiments for biodegradation were conducted by using free cells of *Actinomycetes*. The studies confirmed that the degradation is highly considerable in all the conditions of study and structural dependant. The optimized parameters like biomass concentration, pH in free cells were reported. The experimental evidence shows the strong influence of operating conditions on biodegradation capability of *Actinomycetes* and can be implemented practically for the treatment of wastewater containing phenolic compounds as discharges.

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