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Estimation and Removal of Phenol in Pharmaceutical Industrial Effluents from Paracetamol and Aspirin Manufacturing Units

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Abstract: Phenol is one of the most common pollutants present in the environment. It enters into the soil and water through different pathways like cooking, plastic manufacturing, oil purification, pharmaceutical and timber industries. Good solubility of phenol in water and its high contents in industrial effluents testify to a high probability of phenol acting as a water pollutant. The industrial waste were collected from (AWT) Army Welfare Trust, Bhi Phareu at different locations and were analyzed spectrophotometrically for phenol concentration by aqueous method. The highest concentration of phenol was observed in the samples. In order to establish bioremedial process of phenolic compounds, bacteria were screened for their ability to utilize phenolic compounds. *Pseudomonas putida* strain CEMB 10124 degraded and utilized phenolic compounds as carbon and energy source. This strain could degrade 0.8 g l^{-1} phenol with in 40 h. The growth of bacteria was monitored spectrophotometrically at 600 nm. The disappearance of phenol was scanned between 220-320 nm. The decrease in phenol's peak at 269 nm depicted the decrease of phenol in the growth medium and it was further analyzed by GC-ECD.

Key words: Estimation and removal of phenol, catabolic strain, *Pseudomonas putida*, aqueous method, phenol biodegradation

Introduction

Phenol is one of the most common pollutants of the environment. It is the simplest member of a class of organic compounds possessing a hydroxyl group attached to benzene ring or to a more complex aromatic ring system. It was isolated from coal tar and named carbolic acid (Mark, 1986). Large quantities of phenolic compounds are present in food. It is thought that some may be broken down by microbiological action in the gastrointestinal tract to phenol itself. There is no accurate data for exposure to phenol by this route and therefore average daily intake is difficult to assess. Exposure to phenol may also occur through the use of some mouthwashes or skin preparations disinfections. Good solubility of phenol in the water and its high content in sewage water testify to a high probability of phenol acting as a water pollutant, deteriorating the organoleptic qualities of water (Izmerov, 1984). Small concentration of phenol stimulates photosynthesis of *Chlorella vulgaris* with the production of oxygen by *Chlorella*, a decrease of phenol in the water could be simultaneously observed (Bandakov and Krooni, 1972). The 1.0 μg ml^{-1} and higher concentration of phenol inhibited the photosynthesis of diatoms and blue green algae. Phenol concentrations in the range of 100-400 μg ml^{-1} caused the complete inhibition of photosynthesis (Kostyeav, 1973). Phenol vapours acting as air pollutant precipitate from the air into the soil like other substances. In the soil *Theromophyllite bacilli* use phenol as a source of energy and carbon (Alexeyev, 1973). Several microorganisms have been isolated and characterized which possess the ability to act on aromatic hydrocarbon (phenol & substituted phenol). It is recognized that these bacteria harbor specialized genes, which are capable of detoxifying many of the organic compounds (Rheinwald, 1973). Some *Pseudomonas* species have been identified efficient for degradation of aromatic compounds including phenolic compounds. It has been identified that *Pseudomonas* DNA bear the specialized genes that produced catabolic enzymes responsible for hydrocarbon degradation. Rheinwald (1973) studied the large number of degradation plasmids in pseudomonoids. Chakrabarty (1976) and Holloway (1978) described the degradative plasmids in *P. putida* strains. The degradative plasmids comprise a rather unique group of plasmids each of which contains specialized genes involved in the biodegradation of organic compounds (Gunsalus, 1975). The first step in the oxidation of many aromatic compounds is carried out by the action of oxygenase enzyme that incorporates molecular oxygen directly into the chemical structure of organic compounds. Oxygen is the absolute requirement for the oxidation of these compounds. The oxygenases of *Pseudomonas* have been studied in great detail (Hayaishi, 1969) and several of them have been crystallized. The first enzyme to be crystallized was the metapyrocatechase, which incorporate two atoms of oxygen across the double bond between the one hydroxylated carbon atom and an unsubstituted carbon atom of the catechol with the formation of alpha-hydroxymuconic semialdehyde after ring fission. The meta catechol dioxygenase from fluorescent *Pseudomonas* cleaves a number of ring substituted alkyl derivatives of catechol (Ribbons; 1970; Sala-Trepart, 1972; Bayly and Wigmore, 1973). In the light of these information's different types of *Pseudomonas* strains were used in biodegradation research. *Pseudomonas* strains beared degradative plasmids that give these bacteria their diverse catabolic potential.

Materials and Methods

Samples collection: Samples of phenolic compounds were collected from (AWT) Army Welfare Trust, Bhi Phareu where during the production of paracetamol, phenolic compounds (p-amino phenol) were used as a raw material. They treated substituted phenol with

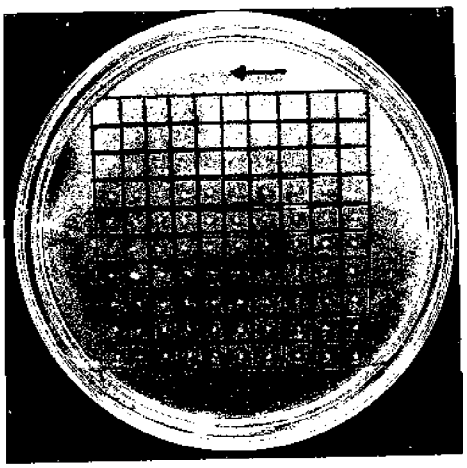

Fig. 1: Bacterial growth *Pseudomonas putida* strain CEMB 10124 in the presence of phenol 100 μM spreading on LB agar plate.

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acetic anhydride or acetic acid and then finally converted into paracetamol. The samples were stored in plastic bottles at room temperature and analyzed spectrophotometrically for phenol concentration by aqueous method.

Aqueous method: Standard solutions containing 100-800 μg cm^{-3} of phenol were prepared. Buffer solution of pH 10.0 was added. To the above solution 2% aqueous 4-aminoantipyrine (photosensitive and kept into dark) and 2% aq. potassium ferricyanide were added and thoroughly mixed. The red colored complex was developed depending upon the concentration of phenol. The solutions were then diluted with deionized water. The industrial sample or blank was also prepared similarly by adding all the reagents in the same order and amount except phenol. Absorbance of each phenol solution was measured by spectrophotometer at 510 nm. The results were read off the calibration curve established under the same conditions. According to Lambert's Beer law to estimate the unknown concentration of any substance in the sample were determined by spectrophotometer.

Identification of catabolic strain: Different *Pseudomonas* strains were used in the biodegradation of phenolic compounds. These *Pseudomonas* strains have shown different degradation potential. The *Pseudomonas putida* strain CEMB 10124 was selected as the best degrader for phenolic compounds and used for degradation of different concentrations of phenol. The bacterial growth *Pseudomonas putida* strain CEMB 10124 in the presence of phenol 100 μM spreading on LB agar plate shown in Fig. 1.

Biodegradation of phenol: *P. putida* strain CEMB 10124 was first grown in M-9 medium; the medium contained the following (per liter) Na_2HPO_4, 6g; KH_2PO_4, 3g; NaCl, 10g; NH_4Cl, 1g; after autoclaving added 1ml 1M $MgSO_4.7H_2O$, 10ml of 0.01M $CaCl_2$ and 5ml of 20% glucose to check their growth behavior on individual carbon source (0.5 % glucose). The growth was measured by using spectrophotometer at 600nm and by spreading the growing culture on LB agar plates with specific intervals of time. These *Pseudomonas* strains *(P. putida, P. aerogenosa and P. floroscence)* were then exposed to specific concentration of phenol to check their degradation potential.

Results

Phenol gave peak at wavelength of 269 nm in M-9 medium when scanned on spectrophotometer. It was observed that only *P. putida* strain CEMB 10124 utilized phenol as a source of carbon and energy and converted phenol into its metabolites. The peak of phenol increased gradually as the bacterial strain utilized phenol and increased its cell mass. But there was no growth observed in *Pseudomonas aerogenosa and P. floroscence* strains and phenol peak remain unchanged. In this experiment we succeeded to identify that only *P. putida* strains have ability to degrade phenolic compounds. The spectrophotometric analysis of degradation of phenol in the bacterial culture of *P. putida* strain CEMB 10124 (Fig. 2).

For this purpose the identified *P. putida* strain were then exposed to different concentrations of phenol (0.2, 0.3, 0.5, 0.6 and 0.8 g l^{-1}) one by one and analyzed the phenol

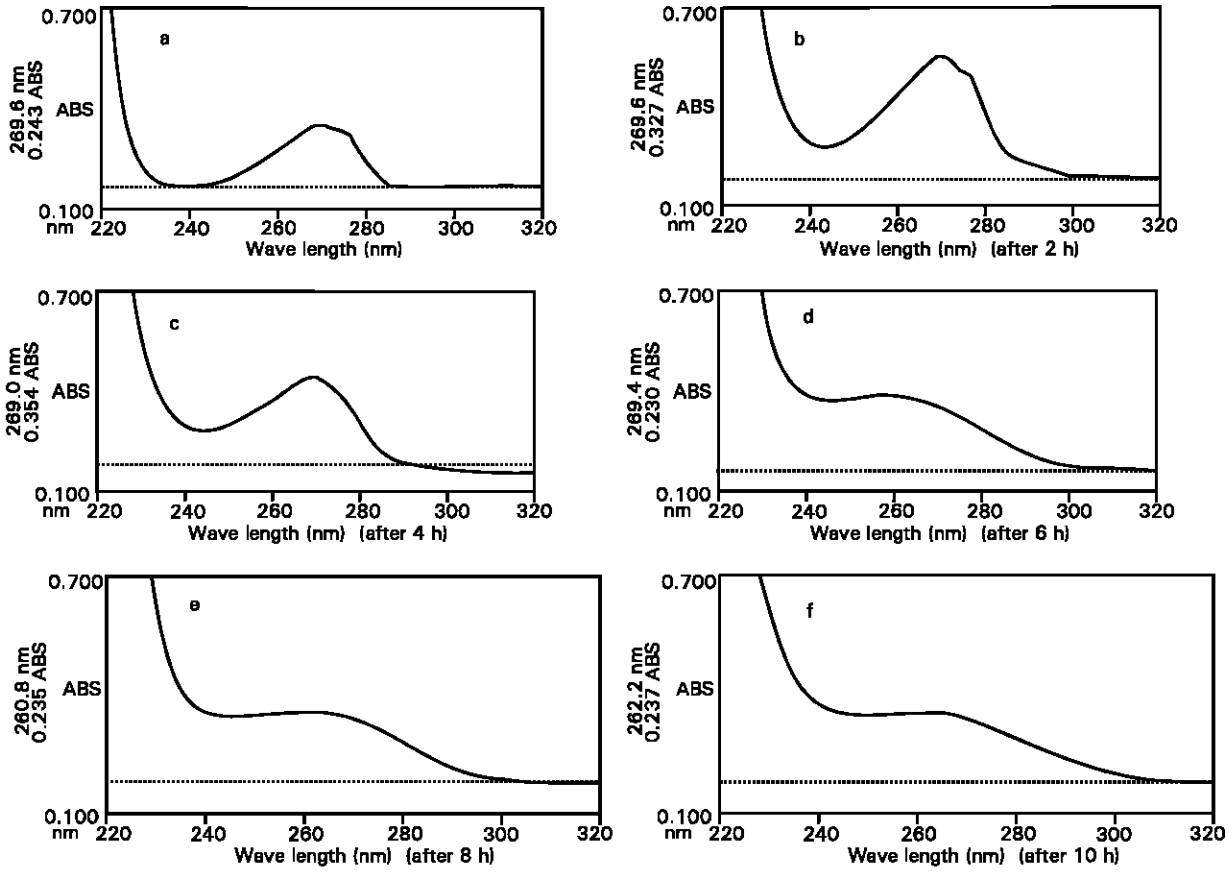

Fig. 2(a-f): Spectrophotometric analysis of degradation of phenol in the bacterial culture of *Pseudomonas putida* strain CEMB 10124. M-9 medium with a) 0.5% glucose+phenol (100 μM), b-f) 0.5% glucose + bacterial culture + phenol (100 μM)

Table 1: Quantitative estimation of phenol in pharmaceutical industrial effluent

Concentration in $\mu g\ cm^{-3}$	Absorbance at 510 nm
100	0.176
200	0.291
300	0.412
400	0.578
500	0.718
600	0.922
700	1.002
800	1.130
Pharmaceutical effluent	0.480

Concentration in $\mu g\ cm^{-3}$ can also be expressed as (parts per million) ppm, mg dm^{-3} and mg kg^{-1}.

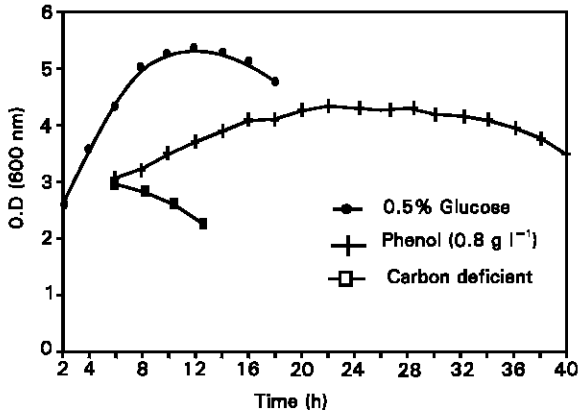

Fig. 3: Biodegradation of (0.8 g l^{-1}) phenol

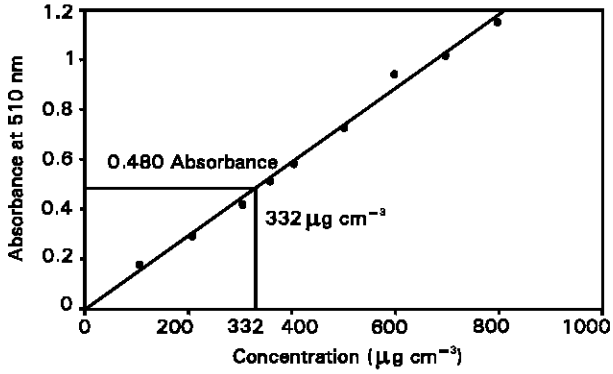

Fig. 4: Quantitative estimation of phenol in pharmaceutical effluent

degradation by measuring optical density of cell free medium at 269 nm. The growth curve was to identify that the mid log phase for the addition of pollutants, because at the mid log phase (which comes after 6 h) the cells were metabolically very active. The bacterial strain was then exposed to one by one of each dose of phenol concentration and observed the tolerance period. It was observed that 0.2 g l^{-1} of phenol was utilized by bacterial strain with in 26 h. Similarly, 0.3 g l^{-1} of phenol was utilized by bacterial strain with in 28 hrs. 0.5 g l^{-1} of phenol with in 30 h, 0.6 g l^{-1} of phenol within 34 h. and 0.8 g l^{-1} of phenol was utilized within 40 h by the *Pseudomonas putida* strain CEMB 10124 (Fig. 3). After 40 h a stage reached when there was no further increase in bacterial growth observed and the bacterial density started to decrease.

Discussion

Linear relationship was observed between concentration of phenol and absorbance in aqueous method as shown in Fig. 4. Various oxidizing agents like $Na_2S_2O_3$, $K_2Cr_2O_7$ and $K_3[Fe(CN)_6]$ were tried but only K_3 $[Fe(CN)_6]$ produced a red colored complex. By varying the pH it was observed that the colored complex always formed at pH range of 9.5–10.5 with maximum absorbance at pH 10.0. The aqueous red complex formed by the reaction of phenol with 4-aminoantipyrine in the presence of oxidizing agent at pH 10.0 was not stable and colour diminished gradually with time and showed absorbance just like blank after 90 min. The quantitative estimation of phenol make the different concentrations (100-800 $\mu g\ cm^{-3}$) of standard phenol, where the highest concentration (332 $\mu g\ cm^{-3}$) of phenol was found in the unknown sample of pharmaceutical industrial effluent Army Welfare Trust (AWT). The water pollution is affecting the quality of environment and creating one of man's biggest problems. The greatest problem concerning the water pollution is the problem of maintaining a supply of clear, pure and fresh water. Contaminations of water in rivers, streams and lakes or any other reservoir by various pollutants have posed serious problems not only in non-developed countries but also in many advanced countries. Almost a similar situation is encountered in Pakistan (Saleem, 1993). Especially in Lahore and Karachi because major industries like fertilizers, food, sugar, paper and pulp, cement, tanneries, electroplating and textile etc are located near such cities, which are discharging large number of pollutants. So the degradation of aromatic compounds such as phenolic compounds, *Pseudomonas* species have been identified that can degrade phenolic compounds. Different *Pseudomonas* strains and especially the *Pseudomonas putida* strains are capable of using a wide variety of organic compounds as a growth substrate (Den doorn de Jung, 1927; Stanier, 1966). The genes which were responsible for the degradation of aromatic compounds i.e. phenolic compound was found on NAH plasmid and have been localized and identified (Gunsalus, 1975). For the phenolic compound degradation, firstly phenol oxidized into catechol, when catechol accumulated into the bacterial cells, the gene catechol 2, 3-dioxygenase was turned on that cleaves catechol to muconate require molecular oxygen as a second substrate (Bird and Cain, 1969). Dioxygenases are concerned at several stages of the pathway for the catabolism of aromatic compounds especially in the reaction which give rise to catechol or its derivatives or convert these compounds into the next intermediates of the pathways (Bayly and Wigmore, 1973). Studied the metabolism of phenol and p-cresol by species of *Pseudomonas*. The use of *Pseudomonas* species in the present studies is a good choice because these bacteria are typically found in soil. In the present studies it has been found that *Pseudomonas putida* strain CEMB 10124 successively to degrade 0.8 g l^{-1} phenol with in 40 h. This experiment was repeated for several times to introduce induction with different concentrations of phenol. There was a possibility that this decline in bacterial growth may be due to the accumulation of some toxic metabolites in the culture media, which was not suitable for the bacterial growth.

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