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RESEARCH ARTICLE

BIODEGRADATION OF PHENOL BY BACTERIAL STRAIN D4, ISOLATED FROM PETROLEUM CONTAMINATED SOIL

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ABSTRACT

Petrochemical, textile and coal industries contains phenolic compounds in very high concentration are released from effluent. The release of such industrial byproducts into environment increases the environmental pollution by phenolic substances. Biodegradation is an environment friendly and cost effective method for the removal of phenolic compounds. This paper describes about isolation and screening of microorganisms involved in the biodegradation of phenol. Isolate D4 shows maximum removal of phenol amongst various microorganisms tested. The optimum pH and temperature for phenol degradation was found to be pH 7 and 30 C respectively. The best carbon source was found to be glucose for the degradation of phenol at a concentration of 1 gm/lit. Upon optimization of phenol degradation process, 93 % phenol degradation was obtained after 48 hrs of incubation at 30 C.

INTRODUCTION

Phenol is an organic compound having formula C_6H_5OH. The molecule consists of a phenyl group ($-C_6H_5$) and a hydroxyl group (-OH). It is a white crystalline solid and molecular weight is 94.14g/mol. It is volatile and mildly acidic in nature. Due to its propensity to cause burns, it requires careful handling (Srideviet *al.,* 2012).

Phenol is a basic structural unit for a variety of synthetic organic compounds including agricultural chemicals and pesticides. Phenol is naturally found in decaying dead organic matters like rotting, vegetables and in coal. Phenol (hydroxy benzene) is both a synthetically and naturally produced aromatic compound and was first used in the raw state to prevent the weathering of railway ties and ships timber, and to reduce the odor of decomposition in sewage (Basha *et al*., 2010).

At room temperature phenol is a translucent, colorless, crystalline mass, white powder or syrupy liquid when mixed with water. The crystals are hygroscopic and turn pink to red in air. Phenol has a sweet tar like odor and is soluble in alcohol, glycerol, petroleum and water to a lesser extent (Basha *et al*, 2010).

Phenol is made from 1-methylethylbenzene and obtained by fractional distillation of coal tar. Phenol can also be made by synthetic processes such as oxidation of toluene, fusion of sodium benzene sulfonate with sodium hydroxide or heating mono-chlorobenzene with sodium hydroxide under high pressure (Basha *et al*., 2010).

MATERIALS AND METHODS

Media and Reagents

Phenol (Sigma-chemicals, Mumbai); $CaCl_2$ (LOBA Chem, Mumbai); K_2HPO_4 (Rankem, Mumbai); KH_2PO_4 (Thomas Baker, Mumbai); $MgSO_4$ (LOBA Chem, Mumbai); $NaNO_3$ (Rankem, Mumbai); and all the other media components and chemicals used in the studies were of high purity and analytical grade. All the media and reagents were prepared in distilled water.

Sample Collection

Soil sample was collected from contaminated site of Aarti chemical waste effluent, Daman Ganga waste effluent, KBS College garden and petroleum soil, Vapi, Gujarat (India).

Isolation and Screening of Phenol Degrading Microorganisms

Isolation of phenol degrading microorganisms was carried out by inoculating 1g of soil sample in 100 ml of BHM medium (Bushnell Haas medium containing (g/l); $MgSO_4$. 7 H_2O 0.2; $CaCl_2$ 0.02; KH_2PO_4 1; K_2HPO_4 1; NH_4NO_3 1; $FeCl_3$ 0.05) containing 100ppm of phenol into 250ml of Erlenmeyer flask. The inoculated medium was incubated at 30°C for 72 hrs and observed for the phenol degradation.

The enriched media is serially diluted and plated on phenol containing plate. The isolates obtained were purified by sub culturing on nutrient agar plates.

Phenol Degradation Experiment

Phenol Degradation under Submerged Cultivation

The preserved culture was transferred in 100 ml Erlenmeyer flask containing 50 ml nutrient broth. The flasks were incubated at 30°C for 24 hrs. The freshly grown 24 hrs old culture with 1.0 O.D. at 600 nm is used as inoculum for phenol degradation study.
The sterilized BHM medium was inoculated with 100 ppm phenol and 1% (v/v) of 24 hrs old culture. The inoculated flask was allowed to incubate at 30°C for 72 hrs under static condition. The sample was withdrawn at regular time interval and subjected to centrifugation at 5,000 rpm for 20 min. The cell free supernatant was used to determine phenol concentration.

Estimation of Phenol

To5ml sample, 0.3 ml of 2% aqueous 4-amino antipyrinesolution and 1ml of 2N NH_4OH were added. After mixing the content thoroughly, 1ml of 2% K_3FeCN_6 is added. Absorbance ofred color produced is measured at 520 and compared with standard curve of phenol to determine phenol concentration.

Phenoldegradation was quantitatively analyzed by measuring the phenol concentration of the supernatant of respective samples anddegradation was calculated by using the equation: %Degradation = (A B)/ A×100; Where, A is initial concentration ofphenol (Zero hrs sample) B is concentration of phenol in sample taken at various time intervals.

Optimization of Culture Condition

To enhance the phenol degradation various process parameters: incubation period, pH, temperature, carbon source and nitrogen source were optimized.

Effect of Incubation Period on Phenol Degradation

In present study the effect of incubation period was determined in a 250 ml Erlenmeyer flask with 100 ml BHM medium and 100 ppm phenol. The flasks were inoculated with 1% inoculums and incubated at 30°C under static condition. A sample of 5ml was withdrawn from the inoculated medium at different incubation periods (0 to 48hrs) and subjected to centrifugation at 5,000 rpm for 20 min and supernatant was used for the determination of phenol degradation.

Effect of pH on Phenol Degradation

The effect of pH on phenol degradation was studied by adjusting medium pH in the range of 4-11. The respective flasks were inoculated with 1% inoculum, 100 ppm phenol and incubated for 48hrs under static condition at 30°C. The samples were withdrawn after 48 hrs of incubation, centrifuged at 5,000 rpm for 20 min and percent degradation was determined.

Effect of Temperature on Phenol Degradation

The effect of temperature was studied by incubating inoculated medium at a temperature range of 15°C - 60°C. The samples were withdrawn after 48 hrs of incubation, centrifuged at 5,000 rpm for 20 min and percent degradation was determined.

Effect of Carbon Source on Phenol Degradation

The degradation was done by taking different carbon sources such as sucrose, glucose, fructose, maltose, lactose, and mannitol in the medium. The respective flask were then inoculated with 1% inoculum, 100 ppm phenol and incubated for 48hrs under static condition at 30°C. The samples were withdrawn after 48 hrs of incubation, centrifuged at 5,000 rpm for 20 min and percent degradation was determined.

Effect of Nitrogen Source on Phenol Degradation

Nitrogen sources such as peptone, ammonium acetate, urea, ammonium chloride, yeast extract and beef extract were used to determine the effect of various nitrogen sources on phenol degradation process. The degradation flask with respective nitrogen source in BHM medium was inoculated with 100 ppm phenol and 1% inoculum and incubated for 48hrs under static condition at 30°C. The samples were withdrawn after 48 hrs of incubation, centrifuged at 5,000 rpm for 20 min and percent degradation was determined.

RESULTS AND DISCUSSION

Isolation and Screening of Phenol Degrading Microorganisms

Hydrocarbon pollution is known to cause a shift in microbial community with the emergence of new bacterial species having elevated PAH degrading capacity because of assimilation and adaptation of micro-organisms toward organic pollutants. In present study, a total of 9 potent phenol degrading bacterial strains were isolated from various environment samples. All 9 isolates were further purified and stored after streaking on BHM agar plate. Bacterial isolate D4 showed maximum degradation (63.12%) of phenol within 48 hrs of incubation (Fig. 3.1) at 30°C under static condition. Followed by D4, bacterial isolate B7 & B2 shows 52 % & 48% phenol degradation, respectively. Thus, isolate D4 was selected for further study as it exhibit highest degradation of phenol.

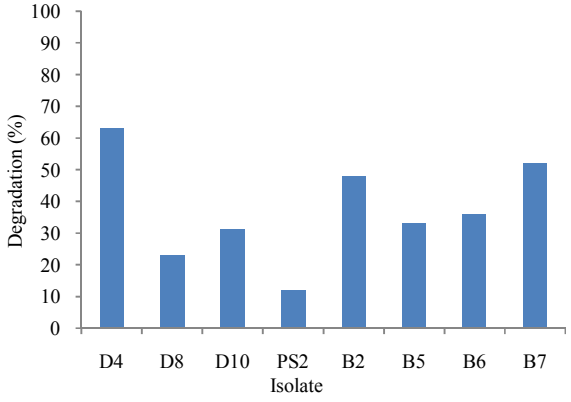

Figure 3.1 Screening of phenol degrading isolates

Optimization of Parameters for Enhanced Biodegradation of Phenol

Effect of Incubation Period

The degradation of phenol was determined at various incubation time (i.e. 0-96 hrs). The result obtained shows that degradation of phenol was increased as incubation time increased and maximum degradation of phenol was obtained

at 48 hrs of incubation (66.33%), however, further incubation of degradation flask does not enhances the degradation of phenol process. The growth profile shows that the degradation of phenol was concomitant with the growt of microorganisms.

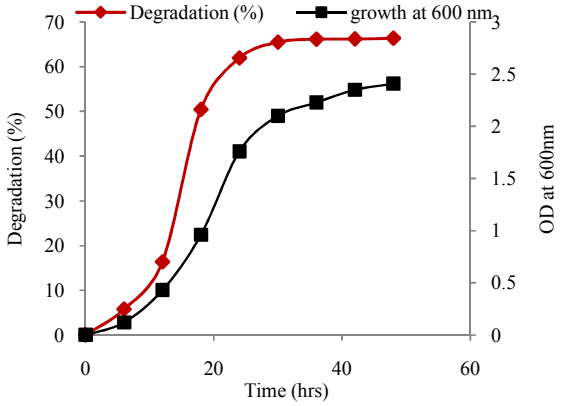

Figure 3.2 Time Course Study of Phenol Degradation

Effect of Medium pH on Phenol Degradation

Medium pH is a selective environmental factor affecting microbial diversity, controlling enzyme activity, transport process and nutrient solubility, thus affecting the whole microbial process (Abdel wahab, 2009). The effect of pH on degradation was studied by adjusting the medium (BHM) pH in the range of pH 5-12 using 1N NaOH and 1N HCL after sterilization. Each flask was inoculated with 1% inoculum and incubated at 30 C for 24 hrs. The incubated flask was analyzed for phenol degradation. The result observed shows that as medium pH increased from pH 4 to 7, phenol degradation increased simultaneously. Medium pH 7 was found to be most effective for the degradation of phenol (78%). However, further increase in pH results in slower phenol degradation rate. Similar results had been shown by Ahamad *et al*., (1996) and Anselmo *et al*., (1984).

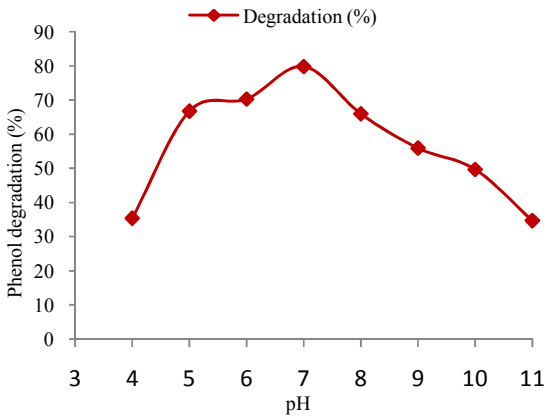

Figure 3.3 Effect of Ph on Phenol Degradation

Effect of Incubation Temperature on Phenol Degradation

Temperature is one of the important factors affecting the growth and activity of microorganisms. Effect of varying temperature on phenol degradation was studied by incubating the inoculated experimental flask in the temperature range of 15- 60 C. The sample was with drawn after 48 hrs of incubation, centrifuged at 5000 rpm for 20 min and supernatant was used to determine phenol degradation. The optimum temperature for maximum degradation of phenol was recorded at 30 C. Atlas *et al*., (1991) and Basha *et al*., (2010) reported 30 C as optimum temperature for PAH degradation.

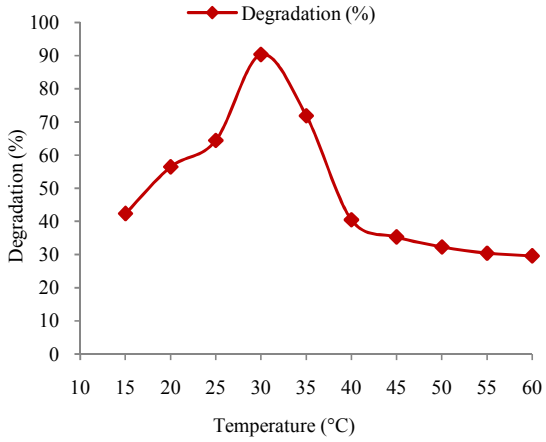

Figure 3.4 Effect of Temperature on Phenol Degradation

Effect of Carbon source on Phenol Degradation

The addition of carbon and nitrogen sources has increase the growth of microorganisms which resulted in enhanced PAH degradation (Marrot *et al*., 2006).Various carbon sources such as sucrose, glucose, fructose, maltose, lactose and mannitol, were usedto study the phenol degradation. Each flask containing 100ml of medium with respective carbon source were inoculated with 100 ppm phenol, 1% inoculum and incubated at 30°C for 24 hrs. After incubation of 48 hrs, sample were withdrawn from each flask and centrifuged at 5,000 rpm for 20 min and supernatant were used to determine degradation of phenol. Maximum phenol degradation (90%) obtained in medium supplemented with glucose as carbon source. Medium supplemented with fructose also showed comparable amount of phenol degradation (82%). However, medium without any carbons source also showed significant phenol degradation after 48hrs of incubation. Ehrharat *et al*., (1985) reported biodegradation of phenol by mutated *Candida tropicalis* in medium supplemented with glucose as carbon source.

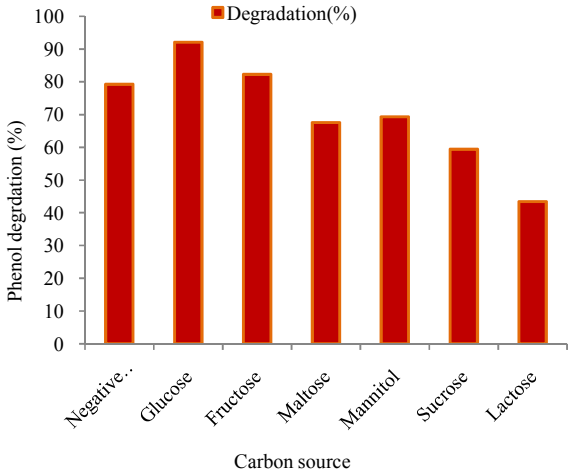

Figure 3.5 Effect of Carbon Source on Phenol Degradation

Effect of Nitrogen Source on Phenol Degradation

Hydrocarbons are weak sources of nutrients such as nitrogen and phosphorus which are essential building blocks of structural macromolecules like proteins and nucleic acids, enzymes and coenzymes, these nutrients are therefore limiting in hydrocarbon containing medium environments (Feryal Akbal., 2003). Nitrogen such as peptone, ammonium acetate, urea, ammonium chloride, yeast extract and beef extract were used to determine the effect of nitrogen source on phenol degradation. Each flask containing 100ml of medium with respective nitrogen source were inoculated with 100 ppm phenol and 1% inoculum and incubated at 30°C for 48 hrs. A 5ml of sample were withdrawn after 48 hrs of incubation from each flask and centrifuged at rpm 5,000 for 20 min. The supernatant was used to analyze for determination of phenol degradation.

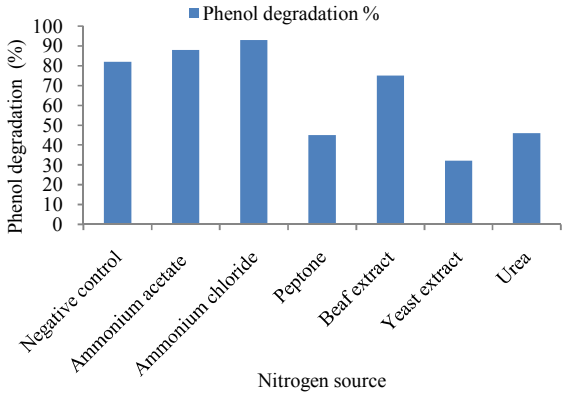

Figure 3.6 Effect of Nitrogen Source on Phenol Degradation

Phenol degradation was maximum (93%) when ammonium chloride was used as the nitrogen source. Degradation medium supplemented with ammonium acetate and beaf extract also showed significant phenol degradation after 48 hrs of incubation at 30 C. While other nitrogen source does not shows significant role in biodegradation of phenol.

CONCLUSION

In present study the growth and phenol biodegradation study was carried out in BHM media with phenol as the sole carbon source and energy. A total of 9 different bacterial strains were isolated as phenol degrading microorganisms and isolate D4 was found to be highly effective for the removal of phenol. Maximum phenol degradation was obtained at 48 h of incubation at pH 7.0 (78%) by isolate D4. The phenol degradation was maximum in presence of glucose and ammonium chloride as carbon and nitrogen source. Thus present study, evaluate the ability of Isolate D4 for the bioremediation of phenol and related compound.

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