ВІДНОВЛЕННЯ ТА ОХОРОНА ҐРУНТІВ

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MICROBIAL DEGRADATION OF PHENOL BY A SPECIES OF *ALCALIGENES* ISOLATED FROM A TROPICAL SOIL

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A species of *Alcaligenes* capable of degrading phenol at a higher concentration was isolated from a tropical soil by enrichment culture technique. The growth studies revealed that the specific growth rate (μ) decreased with increase in the concentration of phenol when phenol was used as the only carbon source in mineral medium. Even a low biomass content at 60 mM phenol concentration could bring 100 % phenol degradation. However, the subsequent increase in phenol concentration diminished both cell growth and degradation. The inhibitory concentration of phenol was 120 mM. For the biodegradation process the optimum p^H was 6 and the optimum incubation period was 32 hours.

Keywords: Phenol biodegradation, Soil enrichment, *Alcaligenes* sp., Substrate inhibition.

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МІКРОБНЕ ЗНИЩЕННЯ ФЕНОЛУ ВИДОМ *ALCALIGENES*, ВИДІЛЕНИМ ІЗ ТРОПІЧНОГО ҐРУНТУ

Вид *Alcaligenes*, виділений у культуру з тропічного ґрунту, здатний руйнувати високі концентрації фенолу. Дослідження росту цієї культури показали, що навіть невеликий уміст *Alcaligenes* повністю знищує фенол з концентрацією 60 ммоль, однак підвищені концентрації фенолу знижують рост клітин *Alcaligenes*.

Ключові слова: біодеградація фенолу, ґрунтове збагачення, субстратне інгібування.

Phenolic pollutants can enter the environment from several sources, viz, the partial degradation of phenoxy herbicides, the use of wood preservatives and the generation of wastes by petroleum related industries. These phenolic compounds have various degrees of toxicity and their fate in the environment is important, (Bollag *et al,* 1988). However, some microorganisms can utilize phenol as a sole carbon and energy source (Yoshitoshi Nakamura and Tatsuiro Sawada, 2000). Many mesophiic microorganisms including *Pseudomonas* sp, *Streptomyces* sp, yeast (Bollag, 1988), *Alcaligenes* sp (Baek *et al* 2001) and *Acinetobacter* sp (Abd-El_Haleem *et al* 2003) have been reported to degrade phenol at low concentrations. The present study reports degradation of phenol at higher concentrations by a recently found species of *Aicaligenes* isolated from a tropical soil. The effect of phenol on cell growth was studied as cell growth in phenol has been observed to display substrate inhibition phenomena at high phenol concentrations (Si-Jim Wang and Kai chu Loh, 1998). The different factors such as p^H, incubation period, and substrate concentration affecting the biodegradation of phenol by this organism were optimised in this study.

MATERIALS AND METHODS

Isolation of Microorganism

Phenol degrading bacteria was isolated from detergent contaminated area by soil enrichment culture technique (Saigu alui *et al*, 1988). The basal medium used for the enrichment culture contains 1g KH_2PO_4, 1g $(NH)_4SO_4$, 0.5 g $MgSO_47H_2O$ and 0.01 g $CaCl_2$ in 1 litre of the medium at pH 7. At each step, phenol was added at a concentration of 10mM after sterilization of the medium. Enrichments were incubated with shaking at 30 ± 2^0 C for 24 hours at 180 rpm before plating on mineral salt phenol agar medium. Pure cultures were stored in 50mM KH_2PO_4: K_2HOP_4 buffer at pH 7.2 containing 20 % (v/v) glycerol -20^0 C. To prevent possible plasmid loss, working cultures were maintained by subculturing every two weeks on mineral salt agar plate (Saiqa Ali, *et al.* 1998).

Phenol assay

Phenol concentrations were determined by a modified spectrophotometric technique, based on a standard method for phenol estimations (Angela Mordocco *et al,* 1999).

Selection and Identification of the strain:

The selection of the potential strain among the five bacterial isolates obtained through soil enrichment culture technique was done on the basis of maximum phenol degradation at a constant incubation period. The bacterial strains were subjected to various morphological and biochemical tests such as colony morphology, Grams reaction, Spore test, motility, fluorescence test, growth at different temperatures / p^H / salt, indole production, MR/VP test, citrate utilization, casein / starch / urea / fat / gelatin hydrolysis, nitrite / nitrate reduction, H_2S production, oxidase test, catalase test, oxidation fermentation test, lysine decarboxylase / ornithine decarboxylase test, Dnase test, phenyl alanine deamination test and acid production test from various carbohydrates. The identification was done as per Bergey's manual of systematic bacteriology.

Growth studies

The growth studies were conducted in mineral salt phenol medium containing phenol at various concentrations of 20, 40, 60, 80, 100 and 120 mM. The cell growth at various time intervals was measured as optical density at 650 nm.

Optimisation of the substrate concentration

Phenol was added as the substrate to the basal medium having at a concentration range from 20mM to 120mM and was inoculated with selected strain. The highest concentration of the phenol which showed maximum percentage of phenol removal, was selected as the optimum substrate concentration for phenol degradation process.

Optimisation of the incubation period

The basal medium containing 60 mM phenol was inoculated with the selected strain and was incubated for 50 hour. Samples were drawn at 2 hours interval and the percentage of phenol degradation was calculated. The minimum incubation period required to effect maximum phenol degradation was selected as the optimum incubation period.

Optimisation of p^H

Basal medium containing phenol at concentration of 60mM was maintained at a p^H range 3.5 – 10.5 and inoculated with the selected strain. The p^H which gave maximum percentage of phenol degradation was considered as the optimum pH.

Results and discussion

Mineralisation of the organic molecules by microbes is essential for the carbon cycle to operate. The massive mobilization of compounds in natural resources or the introduction of xenobiotics into the biosphere leads to unidirectional fluxes, which results in the persistence of a number of chemicals in the biosphere and thus constitute a source of

pollution. Since phenol is toxic and causes pollution it must be removed from the environment(H.Kadhim *et al,* 1999). Among the different treatment methods available for this biological treatment is attractive because it is able to degrade wastewater resulting in lower concentrations of organic molecules(Yoshitoshi, 2000). The present study explores the probability of obtaining a potential strain capable of phenol degradation and optimisation of the various conditions of biodegradation. The process can later be modified for the safe treatment of phenolic compounds in the industrial effluents.

Enrichment method is usually applied to isolate microorganisms which degrade complex aromatic compounds (Seung-HunBaek *et al*). In an attempt to isolate phenol degrading bacteria by soil enrichment technique, five bacterial strains were found to be resistant at a phenol concentration of 10mM and were isolated and purified. The phenol degrading capability of each strain was evaluated in the basal medium at 10mM concentrations and the d2 strain was found to have maximum percentage of phenol degradation (Table 1). The d2 strain was later identified as *Alcaligenes* sp based on Bergey's manual of systematic bacteriology. (Baek *et al,* 2001).

Table 1

Percentage of phenol degradation by the different bacterial isolates from soil

Microorganism	Percentage of phenol degradation
Allcaligenes sp d2	99.7*
Acinetobacter sp d3	16.4
Pseudomonas sp d5	95.6
Pseudomonas sp d6	91.0
Pseudomonas sp d7	92.5

* Selected strain

Phenol as a sole source of carbon at different concentrations from 20 mM to 120 mM showed that there was a steady declination in the growth along with increase in the concentration of phenol occurs. 120mM phenol concentration, completely inhibited the growth of *Alcaligenes* sp. The fig 1 clearly indicates the linear relationship between the decrease in specific growth rate with the increase in phenol concentration from 20 to 120 mM which is the characteristic feature of a substrate inhibition phenomenon (David and Nicholas, 1999). At the same time newly isolated *Alcaligenes* sp is comparatively more tolerant towards a higher concentration of phenol. The effect of substrate concentration on the phenol degradation (fig. 2) shows that upto 60mM concentration there was 100 % degradation and any further increase in the phenol concentration from 60mM, resulted in the respective decrease in the percentage of phenol degradation.

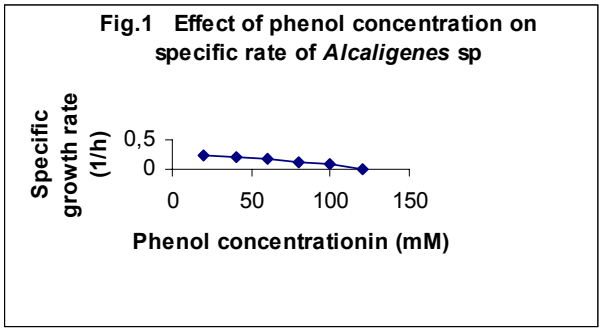

Fig.1 Effect of phenol concentration on specific rate of *Alcaligenes* sp

The fig. 3 indicates a steady increase in the degradation of phenol along with the increase in the incubation time and shows 100% degradation after 32 hours which was considered as the optimum condition for the biodegradation of phenol. Optimum incubation period was the minimum contact time for 100 % degradation. The optimum pH was found

to be 6 (fig 4). However the organism showed more than 97 % phenol degradation in the pH range from 5 to 8.5.

The species of *Alcaligenes* recently isolated from our soil could degrade phenol to a significant extent that is up to 60 mM. The time taken for degradation is 32 hrs which is also significantly less. The present study evidently suggests that the *Alcaligenes* sp isolated by soil enrichment culture technique is an efficient strain for the degradation of the phenol. These results clearly give scope of utilizing this strain for the possible treatment of phenolic effluents.

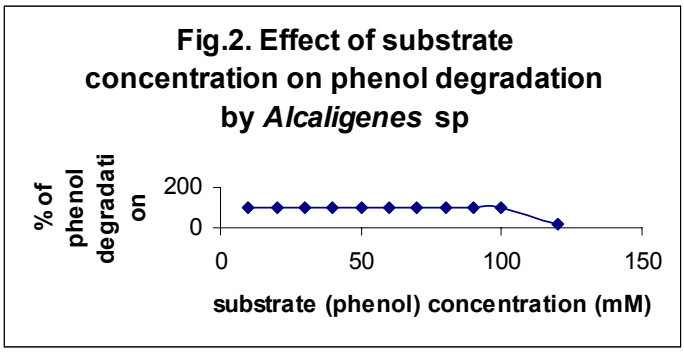

Fig.2. Effect of substrate concentration on phenol degradation by *Alcaligenes* sp

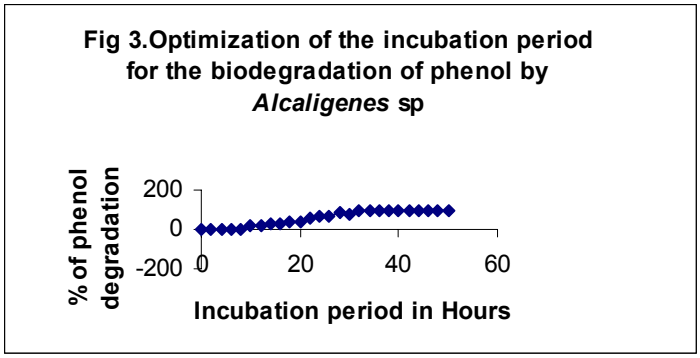

Fig 3.Optimization of the incubation period for the biodegradation of phenol by *Alcaligenes* sp

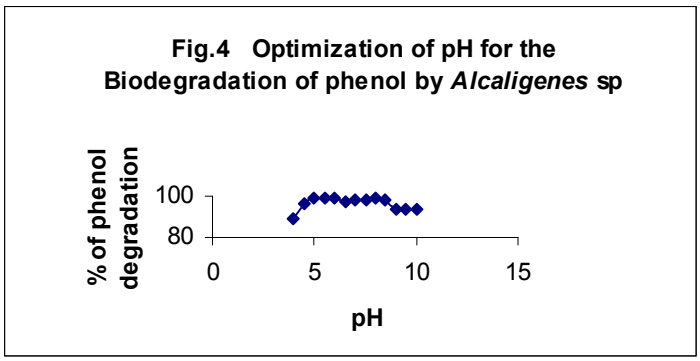

Fig.4 Optimization of pH for the Biodegradation of phenol by *Alcaligenes* sp

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