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ISOLATION OF PHENOL BY DEGRADING MICRO-ORGANISMS FROM SLUDGE

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ABSTRACT

Degradation of Phenol compounds because of its wide range usage, is one of the most important facts that most of industries are dealing with it. Various methods including Physical, Chemical and Biological, have been used for reducing the pollution of environment. Because of economical and safty factors; Biological methods could be preferable. In this study, micro-organisms capable to use phenol as their sole source of carbon and energy, were isolated from enrichment cultures. 5 gr of industrial sludge were added to individual 250ml Erlen meyer flasks each contain 100ml of sterile Mineral Salt Medium (MSM). The pH was adjusted to 7 with NaOH and different concentrations of Phenol in the range of 0.1 ml ,0.2 ml, 0.3 ml & 0.5ml were added to flasks. Control flasks without inoculum were also prepared. Pure culture of Phenol degrading bacteria, mold and yeasts were isolated by plating out on Mineral salt Agar Medium containing 0.1% concentration of Phenol. From the result of microscopic observation and growth characteristics as well as biochemical tests and fermentative characteristics of isolated bacteria, seems to be gram-negative and gram-positive bacteria, some molds and yeasts.

Keywords: Biodegradation, bacteria, mold, phenol, yeast and sludge.

INTRODUCTION

In a non-polluted environment, bacteria, fungi, protists and other microorganisms are constantly at work of breaking down organic material. What would occur if an organic pollutant such as dyes, pesticides, plasticizer, crude oil and aromatic compounds contaminated this environment ? Some of the microorganisms would die, the behavior of pesticides in soils has been reported.(Helling *et al.* 1971, Tu, 1970) Phenols are distributed either as natural or artificial mono-aromatic compounds in various environmental sites. (Watanabe *et al.,* 1996). Phenol and its derivatives are among the most frequently found pollutants in rivers, industrial effluents and land fill runoff waters. (Prasad and Ellis, 1978)

In phenol- contaminated sites, phenol toxicity studies have shown that bacteria can adapt to low phenol concentrations, but increasing phenol concentrations appear to decrease the overall phenol biodegradation. (Dean Ross, 1989) The toxicity of phenolic compounds often results in the reduction of wastewater biotreatment even at relatively low concentrations. (Hinteregger *et al.,* 1992).

Biodegradation is a natural process carried out by soil and aquatic micro-organisms mostly bacteria and fungi. Bioremidation is a rapidly developing field of environmental restoration, utilizing natural microbial activity to reduce the concentration and/or toxicity of various chemical substances such as industrial solvents.(phenols, benzene, etc), pesticides and metals. Although aromatic compounds are rather persistent in nature, numerous micro-organisms capable of degrading PAHs have been described and the basic mechanisms of the metabolic pathways have been elucidated (Cerniglia, 1984)

Not only pure microbial cultures but also mixed cultures have been investigated concerning their ability to degrade a vast number of Organic compounds. (Bauer and Capone, 1998, Mueller *et al.*, 1990; Walter , 1991) The microbial technology is thus effective in removal of Phenol from industrial waste waters. In the present study, microorganisms capable of using Phenol as their sole source of carbon and energy were isolated from enrichment culture. Also, the effect of increasing concentration of Phenol on biodegradational activity of bacteria, and the morphological features of them have been

investigated. There are number of papers dealing with degradation of phenol by micro-organisms such as bacteria, fungi and yeast.

In future experiments, the Phenol biodegrading capability of bacteria in mixture. Cultures will be under study and Phenol-breaking characteristic of the microbes will be assayed by using GC-mass and spectrophotometer techinques. In addition to all above, the bacteria must be identified according to their morphological, biochemical and physiological features based on "Bergey's Manual of Bacteriology" and the role of plasmid in the degradation of Phenol will be investigated.

MATERIAL AND METHODS

Isolation of Phenol degrading micro-organisms from enrichment Culture, industrial sludge of Gohar-rood river(5gr) were added to individual 250 ml Erlen meyer flasks each contains 100 ml of sterile Mineral Salt Medium (MSM) Containing the following ingredients:

K_2HPO_4 2.75gr, KH_2PO_4 2.25gr, $(NH_4)_2$ SO_4 1.0 gr, $MgCl_2$. H_2O 0.2gr, NaCl 0.1gr, $FeCl_3$. $6H_2O$ 0.02 gr, $CaCl_2$ 0.01gr. per 1 litle.

And 2 ml of trace elements containing:

0.74 gr of $CaCl_2$. $6H_2O$, 0.18gr of $ZnSO_4$.$7H_2O$, 0.1gr of $MnSO_4$. H_2O, 20.1 gr of Na_2-EDTA, 16.7 gr of $FeCl_3$. $6H_2O$, 0.1 gr of $CuSO_4$, 0.104 gr of $CoCL_2$, 2ml of $MgSO_4$. $7H_2O$

per 1 litle of MSM was added. The pH was adjusted to 7 with NaOH and Phenol was added in four different concentrations including 0.1 %, 0.2%, 0.3% and 0.5 %.

These primary enrichment cultures were incubated several days at 25°C in a shaker incubator at 120 rpm. Numerous attempts have been made to obtain single colonies of phenol degrading bacteria.Micro- organisms capable of degrading phenol, containing bacteria , fungi and yeast, were isolated from enrichment cultures were streaked onto agar solidified medium containing mineral salt, trace element and phenol special concentration of 0.1 %.When the plates were incubated at 25°C, small pure cultures of Phenol degrading bacteria were achived by repeated transfer from agar plate to agar plate and agar to liquid cultures media both containing Phenol (0.1%).

Table - 1.a : Microscopic observation of the isolated bacterial strain 1

	Tests	Observations
Microscopic examination	I. Simple Staining	Rod shaped
	II. Gram's Staining	Gram- Negative

Table - 1.b : Biochemical and fermentative tests on isolated bacteria strain 1

Tests	Results
Glucose	Positive
Lactose	Positive
Sucrose	Positive
Hydrogen sulfide	Negative
Gas From Glucose	Positive
Motility	Positive
Simmons Citrate	Negative
Malonate	Negative
Urease	Negative
Indole	Positive
Methyl Red	Positive
Voges- proskauer	Negative
OF (Oxidative Fermentation)	Positive
OF- Oil	Positive

RESULTS AND DISCUSSION

Indentification of the bacteria:

Microscopic observation and growth characteristics as well as biochemical tests and fermentative characteristics of the isolated bacteria were studied and are represented in table 1-a, 1-b, 2-a, 2-b, 3-a, 3-b, 4-a, 4-b. All of tests have been perfromed according to the "Bergey's Manual of Bacteriology."

In this study, microorganisms capable of using phenol as their sole source of carbon , energy were isolated from the enrichment cultures. Pure culture of phenol degrading bacteria was isolated by plating out on mineral salt agar medium containing phenol. When the morphological, biochemical and physiological tests were done, four microorganisms were found. Two of them were

Table - 2.a : Microscopic observation of the isolated bacterial strain 2

	Tests	Observations
Microscopic examination	I. Simple Staining	Rod shaped
	II. Gram's Staining	Gram- Negative

Table - 2.b : Biochemical and fermentative tests on isolated bacteria strain 2

Tests	Results
Glucose	Negative
Lactose	Positive
Sucrose	Positive
Hydrogen sulfide	Negative
Gas From Glucose	Positive
Motility	Positive
Simmons Citrate	Negative
Malonate	Positive
Urease	Positive
Indole	Positive
Methyl Red	Positive
Voges- proskauer	Negative
OF (Oxidative Fermentation)	Positive
OF- Oil	Positive

Table -4.a: Microscopic observation of the isolated bacterial strain 4

	Tests	Observations
Microscopic examination	I. Simple staining	Rod shaped
	II. Gram's staining	Positive
	III. Spore staining	Negative

Table - 3.a : Microscopic observation of the isolated bacterial strain 3

	Tests	Observations
Microscopic examination	I. Simple staining	Rod shaped
	II.Gram's staining	Positive
	III. Spore staining	Positive

Table 3.b : Biochemical and termentative tests on isolated bacterial strain 3

Tests	Results
Catalase	Positive
OF (Oxidative Fermentation)	Positive
OF - Oil	Positive
Poly β- hydroxy buteric acid (P.H.B)	Negative

Table - 4.b : Biochemical and fermentative tests on isolated bacterial strain 4

Tests	Results
Catalase	Positive
OF(Oxidative Fermentation)	Positive
OF- Oil	Positive
Poly β – hydroxy buteric acid (P.H.B)	Negative

Gram-negative, rod -shaped and the other two bacteria were Gram-positive, rod-shaped.

As it was explained in materials and methods, foure different concentration of phenol were preparad in Erlen-meyer flasks containing 0.1%, 0.2%, 0.3% and 0.5% . The growth of bacteria in mixture forms were observed in all of phenol comcentrations. After playing out on mineral salt agar medium containing phenol in concentration of 0.1%, the growth was observed in the bacteria transtered from 0.1%, 0.2% and 0.3% phenol flasks on to 0.1% phenol plates and there was no growth observed in the bacteria transfered from 0.5% phenol flasks on to 0.1% phenol plates.

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