BIODEGRADATION OF PHENOL BY BACTERIAL STRAIN ISOLATED FROM PAPER SLUDGE

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In partial fulfillment of the requirements

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By

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CERTIFICATE

This is to certify that the thesis on **"Biodegradation of phenol by bacterial strain isolated from paper sludge"** is submitted by **Manju Lata (109CH0508)** to National Institute of Technology, Rourkela under my supervision and is worthy for the partial fulfillment of the **degree of Bachelor of Technology (Chemical Engineering)** of the Institute**.** She has fulfilled all the prescribed requirements and the thesis, which is based on candidate's own work, has not been submitted elsewhere.

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Manju Lata

ABSTRACT

The objective of this project work is to study the degradation of phenol by bacterial strain isolated from paper sludge. Biodegradation is one of the cheapest methods without any production of hazardous by-products. The growth and phenol biodegradation study was carried out in MSM broth with phenol as the sole carbon source and energy. The strains were designated as S_1, S_2 and S_3 and examined for colony morphology, Gram staining and biochemical tests. Phenol degrading performance of all the strains was evaluated initially. One of the strains namely S_2 was found to be highly effective for the removal of phenol. The effect of temperature, pH and phenol concentration on the rate of phenol degradation by that particular strain was carried out. Observations revealed that the rate of phenol biodegradation was significantly affected by pH, temperature of incubation and phenol concentration. The optimal conditions for phenol removal were found to be pH of 8, temperature of 30°C and concentration of phenol of 200 ppm.

KEY WORDS: Phenol degradation, bacterial strain, Biodegradation and temperature.

LIST OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER-1

INTRODUCTION

INTRODUCTION:

Phenol is an organic compound having formula C6H5OH. The molecule consists of a phenyl group (-C6H5) bonded to a hydroxyl group (-OH). It is a white crystalline solid .It is volatile and mildly acidic in nature. Due to its propensity to cause burns, it requires careful handling. Phenol was first extracted from coal tar, but today is produced on a large scale (about 7 billion kg/year) from petroleum.

Phenol is used to make pharmaceuticals, synthetic resin, dyes, perfumes, pharmaceuticals, pesticides, synthetic tanning agents, lubricating oils and solvents. In the manufacture of industrial and agricultural products, phenols are used as common starting materials and often it is produced as waste products. Especially phenolic compounds are often found in wastewaters from coke-oven batteries, coal gasification refinery and petrochemical plants and other industries, such as herbicides, synthetic chemicals, pesticides, pulp-and-paper, photo developing chemicals, antioxidants, tannery and foundries (washing of the gas effluents) etc. Thus, the presence of phenols in water generally comes from this industrial pollution. The increasing presence of phenols represents a significant environmental toxicity hazard.

Phenol is very toxic substance. Acute exposure of phenol can result in myocardial depression and central nervous system disorders. Exposure to phenol may result in irritation of the eye, conjunctional swelling, corneal whitening and finally blindness. It leads to collapse and coma. A reduction in body temperature is resulted and this is known as hypothermia. Mucus membrane is highly sensitive to the action of phenol. Muscle weakness and tremors are also observed. Phenol has an anesthetic effect and causes gangrene. Renal damage and salivation may be induced by continuous exposure to phenol. Chronic exposure may result in anorexia, dermal rash, dysphasia, vomiting, weakness, weightlessness, muscle pain and hepatic tenderness .Other effects include frothing from nose and mouth followed by headache. Phenol can cause hepatic damage also. It is also suspected that exposure to phenol may cause paralysis, cancer and genetofibre striation. Phenol and its derivatives are toxic and classified as hazardous materials. These phenolic compounds possess various degrees of toxicity.

Even when it is present in waste water in low concentrations can be toxic to some aquatic species and causes taste and odour problems in drinking water. Ingestion can cause serious

gastrointestinal damage and severe skin damage while inhalation and dermal contact of phenol causes cardiovascular diseases and oral administration into laboratory animals has also induced muscle tremors and death. Even short-term application of phenol to the skin can produce blisters and burns in animals. That's why; the removal of such chemicals from industrial effluents is necessary.

There are different methods available for phenol removal such as solvent extraction, adsorption, chemical oxidation, incineration, hybrid process and electro catalytic degradation.

All these non-biological methods have serious drawbacks such as high cost and formation of hazardous byproducts, whereas biological degradation (biodegradation) is generally preferred due to lower costs and possibility of complete mineralization.

Natural way of recycling wastes or breaking down organic matter into nutrients that can be used by the organisms is called biodegradation. There are two types of biodegradation aerobic which takes place in presence of oxygen and anaerobic biodegradation which takes place in absence of oxygen. There are basically two generalized category of biodegradation- mineralization and biotransformation. Mineralization involves total degradation of the organic matter. In case of biotransformation organic matter is not degraded totally while a part of it is degraded; another part is converted into other smaller chain organic compounds. . Because of widespread occurrence of phenol in the environment many microorganisms utilizes phenol as the sole carbon and energy source which includes both aerobic and anaerobic microorganisms.

CHAPTER-2

LITERATURE REVIEW

2.1 BIOLOGICAL TREATMENT:

For the treatment of effluents biological method is a practical and not very expensive solution compared to chemical method (no need to add chemicals); because various populations of microorganisms in the activated sludge are able to degrade organic compounds and most of effluents can be degraded biologically. Biological treatment of the phenolic compounds is not an easy task because it is highly toxic for microorganisms. Even when phenolic compound is present in low concentrations (lower than 200 mg L−1), can cause the inhibition of the growth of microorganism.

2.2 MICROORGANISMS CAPABLE OF DEGRADING PHENOL:

The species most often present are pseudomonas, flavobacterium, achromobacter, rhomobacterium, azobacter, micrococcus, bacillus alkaligenes, arthrobacter, ycobacterium, aeromonas, nocardia and lophomonas. Their respective propor-tion will depend mainly on the specific substrate concentration and the potentiality of growth. When microorganisms are cultivated, they consume substrates for their growth and for their energy.

Buitron et al.,Isolated and identified the microorganisms responsible for the phenol degradation. Four types of Gram-negative unicellular bacteria were obtained from the acclimated consortium: Chryseomonas luteola, Pseudomonas sp., Flavomonas oryzihabitans and Aeromonas sp.There are other microorganisms able to degrade phenol like: Alacaligenes sp., Sarcinas, Desulfovibrio sp., Bacillus alkaligenes, Acinetobacter and more. Alexievaa et al. demonstrated that T. cutaneum R57 has all the properties of an efficient phenoldegrading microorganism. Two groups of degrading bacteria are mainly specified and used: the Rhodococci (Rhodococci shows considerable morphological) like Rhodococcus spp. and Pseudomonads like Pseudomonas putida. P. putida seem to have the highest degradative potential. That is why a great number of studies upon the degradation of phenols by these bacteria have been done (A. Mordocco et.al.,) But opinions are divided, it seems that acclimated activated sludge degrades the phenolic compounds more efficiently than the pure strains by one to two orders of magnitude faster and Annadurai et al.,showed that the mixed liquors had a best ability for phenol degradation than pure activated sludge.

In 2006, B. Marrot, A. Barrios-Martinez, P. Moulin, and N. Roche carried out biodegradation of high phenol concentration by activated sludge in an immersed membrane bioreactor. The effect of adaptation of mixed culture in the phenol biodegradation has been studied. Biological treatment has been shown to be practical and economical; it leads to a complete removal of phenol. High concentrations of phenol are limited for growth; so it is for the rates of substrates utilization that are greater at low initial concentrations. From the experimental results it was observed that it is possible to treat effluents containing high phenol concentration (up to 1.0 g L−1) by activated sludge at typical biomass concentrations of a membranes bioreactor (around 10 g L−1). It was observed that the optimal temperature for degradation is 30°C.However the rate and the extent of degradation is relatively sensitive to deviations outside the optimal range 5°C variation in temperature may cause a decrease in phenol degradation rate of at least 50% at the lower end and almost 100% at the higher end (A. Mordocco et.al.,). The difference between phenol removal efficiency at 30 ◦C is probably due to the higher production of metabolites at this temperature (C.S.A. S´a et,al 2001). Moreover, at this temperature, the degradation rate seems better for free than immobilized cell system (1.45 times higher).

Chung et al. 2003, found an optimal temperature of 30 ◦C for the two processes but different optimal pH values: 6.8 for immobilized cells and 8.0 for free cells.

A slight reduction was observed as biomass grows and pH variation increases when the initial phenol concentration increases. The decrease in pH suggests that biological degradation of phenol occurs and phenol was successfully degraded at pH of 7.

In 2008 C. Indu Nair, K. Jayachandran and Shankar Shashidhar were carried out their study on biodegradation of phenol. It was found that large numbers of microbes co-exist in almost all natural environments, particularly in soils. Many synthetic and natural organic chemicals are readily biodegradable in natural environment. They observed that biodegradation of materials involve allowing adsorption or physical access to the substrate, initial proximity, secretion of extra cellular enzymes to degrade the substrates or uptake via transport systems followed by intracellular metabolism. They observed that the efficiency of biodegradation of organic compounds is influenced by the type of the nature of the organism, the organic pollutant, the enzyme involved, the nature of the influencing factors and the mechanism of degradation. Phenolic compounds are toxic at relatively low concentration and also they are hazardous

pollutants. Accumulation of phenol creates toxicity both for plants and animals. Since phenol is a toxic substance and also it cause pollution, therefore it must be removed from the environment.

It was found that K. oxytoca degraded phenol at elevated concentration where 75% of initial phenol concentration at 100 ppm was degraded within 72 h (Shawabkeh et al., 2007). Phenol was degraded by Actinobacillus species (Khleifat and Khaled, 2007). They found that optimal conditions for maximum degradation of phenol are at pH of 7, the incubation temperature of 35 to 37°C, and the agitation rate of 150 rpm.

Succinic acid and glycine as respective carbon and nitrogen source were found to be the most efficient co-substrates for the removal of phenol. Immobilized Alcaligenes sp d2 was successfully used for the effective treatment of phenolic paper factory effluent (Nair and Shashidhar, 2007).

Khazi Mahammedilyas Basha, Aravindan Rajendran, and Viruthagiri Thangavelu were carried out their study on recent advances in the biodegradation of phenol. They described about the various reactors used for biodegradation. Fluidized, activated sludge, packed-bed and moving bed biofilm reactors were studied as biological treatment processes. Phenol degradation using an anaerobic packed-bed reactor was reported by Holladay et al.1978, and also compared phenol degradation in stirred tank, packed bed and fluidized bed reactors. They conclude that the efficiency for degrading phenolic liquid among the three types of bioreactors increased in the following order: stirred tank bioreactor, packed bed fluidized-bed bioreactor. The degradation rate depends on the state of biomass development, feed concentration, liquid and air flow rate.

Most organisms cannot tolerate pH values below 4.0 or above 9.0 (Hill et.al. 2001). At low (4.0) or high (9.0) pH values acids or bases can penetrate into cells more easily, because they tend to exist in undissociated form under these conditions and electrostatic force cannot prevent them from entering cells (Robertson et.al, 1992). The optimum pH for phenol degradation is 7.0 for Pseudomonas putida NICM 2174. According to Pakula et al. phenol biodegradation was significantly inhibited at 30°C. Most laboratory studies on phenol degradation have been carried out at an optimum temperature of 30°C Annadurai et.al, (2004). Annadurai et al. and Chitra et.al, described that when the temperature increased from 30°C to 34°C no phenol degradation

was observed due to cell decay, which shows that the phenol degradation is a temperature dependent process.

In 2009, S. Chakraborty, T. Bhattacharya, T.N. Patel and K.K. Tiwari were carried out their study on biodegradation of phenol by native microorganisms isolated from coke processing wastewater. It was observed that maximum degradation of 33.46% occurred in cultures placed at 30°C for 6hr. At 35°C also degradation occurred significantly but less than at 30°C.It was observed that degradation was hampered both at low as well as high temperatures. Similar trend was observed in 12, 18 and 24 hr with slightly higher values of removal rate. At the end of 24 hr. 76.69% of phenol was degraded at 30°C and 69.90% at 35°C. While at extreme temperatures of 20°C and 45°C it was only 48.62 and 27.63% respectively.

It was observed that by increasing the pH of the media at 30°C from 5 to 7 rate of phenol degradation also increases. On increasing the pH further it had reserved effect on phenol removal potentiality. In 6 hr 39.85% phenol was removed at pH 7, while the rest of the pH conditions could not degrade phenol more than 8.42%. It was observed that both acidic and alkaline pH had a marked inhibition on phenol removal efficiency. After 12, 18 and 24 hr also analogous result was seen with only 84.63% removal till end at pH 7 at 30°C.At pH 8 phenol removals was maximum up to 14.41%. Neutral pH (pH-7) could degrade phenol at higher rates as compared to the other pH at 30°.

Phenol removal efficiency was determined at different glucose concentrations at a neutral pH of 7 and 30°C temperature. It was observed that the data collected after 24 hr. shows the maximum phenol removal efficiency of 97.88% at 0.25% of glucose concentration. But it was decreased to 55.36% with increasing glucose concentration to 0.5% and also in the absence of the glucose. The optimal conditions for phenol removal were found to be pH of 7 (84.63% removal), temperature, 300C (76.69% removal) and 0.25% supplemented glucose level (97.88% removal)

2.3 REMARKS

From the literature review it was found that biodegradation is one of the most efficient methods for removal of toxic organic material like phenol from industrial effluent. Phenol and its derivatives are toxic and classified as hazardous materials. The phenolic compounds possess

various degrees of toxicity. Even when it is present in waste water in low concentrations can be toxic to some aquatic species and causes taste and odour problems in drinking water. That's why; the removal of such chemicals from industrial effluents is necessary. It was found that the degradation mainly depends on bacterial growth and the growth of bacteria highly dependent on concentration of carbon source, pH and incubation temperature. My study was based on biodegradation of phenol, and found out the optimum condition for biodegradation process.

2.4 OBJECTIVE

- To isolate and characterize microorganism capable of degrading phenol.
- Study of spectrophotometric phenol degradation and bacterial growth kinetics.
- To optimize physiological parameters that affects the biodegradation of phenol.

2.5 SCOPE

- Study of effects of various physiological parameters on degradation of phenol like
    - Effect of incubation temperature
    - Effect of pH
    - Effect of concentration of phenol (used as source of carbon and energy)
- Bacterial growth kinetic study

CHAPTER-3

MATERIALS

AND

METHODS

3. MATERIALS AND METHODS

3.1 MATERIAL REQUIRED:

1). Sample of paper sludge.

2). Flask (different size), Pipette, Petri disk, Test tube, hot-air oven, and chemicals for different tests.

3). Autoclave, laminar flow hood, Biological Incubator and Orbital shaker

3.2 SAMPLE COLLECTION:

Paper sludge was collected from **JK Paper mill, Rayagada Odisha.**

3.3 SAMPLE PREPARATION:

Sample was prepared by adding 1 gram sludge in 100 ml of water.

3.4 PREPARATION OF PHENOL STOCK:

Phenol stock was prepared by adding 50 mg of phenol in 500 ml of water.

3.5 ISOLATION AND CHARACTERIZATION OF BACTERIA:

The process consists of the following steps:

MSM Preparation ➡ Sterilization ➡ Culturing ➡ Inoculation

MSM preparation: MSM composition was added in 100 ml of distilled water in a conical flask.

Table.3.1 MSM Composition

Chemical composition	g/L (Distilled water)
KH_2PO_4	1.5
K_2HPO_4	0.5
NH_4Cl	0.5

$CaCl_2$	0.02
$MgSO_4.7H_2O$	0.2
NaCl	0.5
Na_2SO_4	3.0
Yeast Extract	2.0
Nutrient Agar (for Streaking on Petridis)	2.0

Heat Sterilization: It is the process, in which foreign contamination is removed by means of heat, in lab Auto-clave was used for heat sterilization.

- After the preparation of MSM, it was kept in auto-clave. A pressure of 15-20 psi and temperature of 120-121^{0}C was maintained in autoclave. It was sterilized for 15-20 minutes and then cooled at room temperature.

Culturing: Culturing is a process in which a nutrient media is provided for micro-organism growth; the media is called culture media.

Culture media: Mineral salt media

- After the cooling takes place, by using laminar flow hood (Product protection from microbial contaminants) 2ml of phenol as a carbon source and 1 ml of paper sludge was added to the sterilized MSM solution.

Inoculation: Inoculation is the process in which some living microbes are added that will grow or reproduce.

- After the addition of phenol and paper sludge in the conical flask it was kept in shaker at 115 rpm and incubation temperature of 30°C.
- After 48 hours sufficient growths of bacteria was found that further sub cultured five times in same media and same condition to isolate the phenol degradable bacteria, after five consecutive sub-culturing, the media was transferred to sterile petri plates and over that 20ml of nutrient agar was poured,1ml cultured medium serial dilution(10^{-6}). It was kept inverted in incubator for 48hrs and at 30^{0}C.

- After the incubation different colony was selected for further studies.

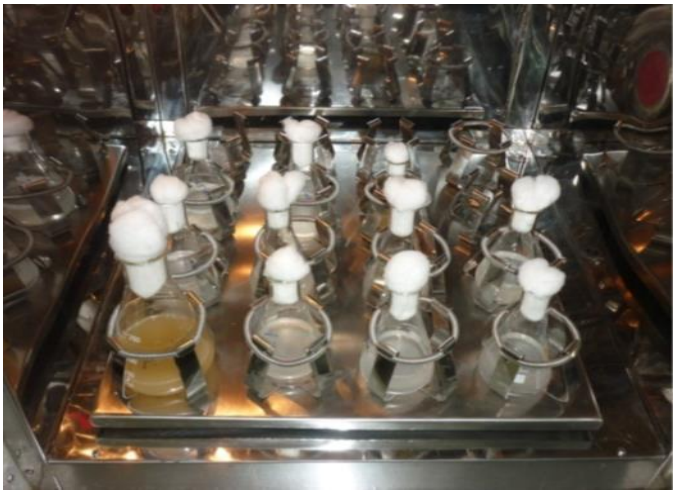


Fig.3.1 Shaker

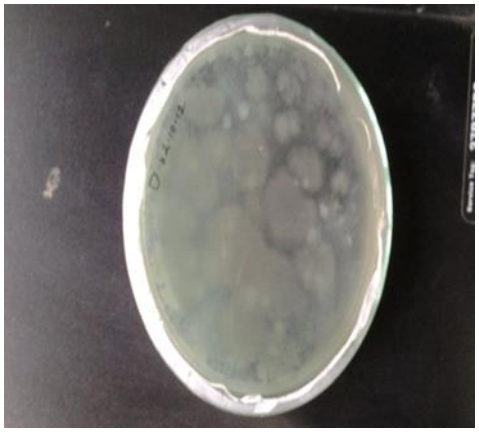


Fig.3.2 Bacterial colony

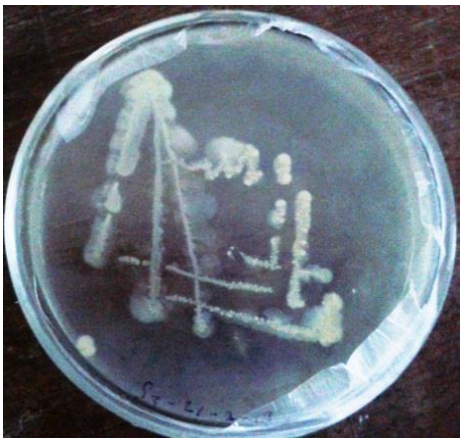


Fig.3.3 Isolated bacterial strain

3.5.1 BIOCHEMICAL CHARACTERIZATION:

a) Catalase test:

This test determines whether the bacteria are able to produce catalase enzyme or not.

Procedure:

1). Firstly a drop of 3% H_2O_2 was placed on microscope slide.

2). Secondly a sample of 24hrs old pure bacterial culture was collected.

3). Then the loop was placed in the H_2O_2.

4). Instant bubbling or foaming occurs if the result is positive.

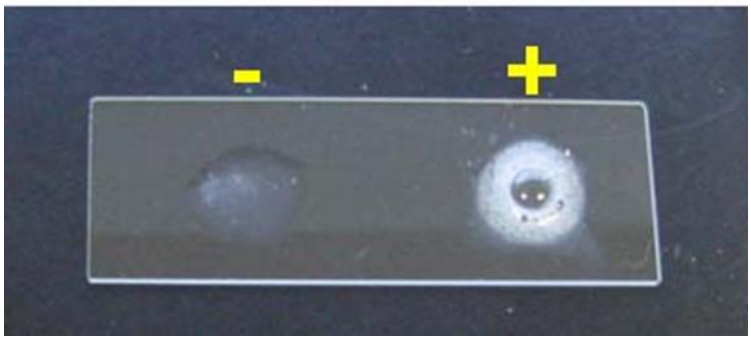


Fig.3.4 Catalase test

b) Citrate test:

This test detect whether an organism use citrate as the sole source of carbon and energy or not.

Procedure:

1).The agar was inoculated by making a streak onto the surface of the slant with a 24hrs old pure culture.

2). If the test is positive then color change to blue-green or royal blue, else remain dark green color.

20ml of sample was prepared that contain following compositions.

Table 3.2Materials required for preparation of citrate agar

Citrate Agar	Quantity
NaCl	5g
Sodium Citrate	2g
Ammonia di-hydrogen	1g
K_2HPO_4	1g
Distill water	1ltr

c) Urease Test:

This test determines whether the microorganisms are able to degrade urea by means of the enzyme urease.

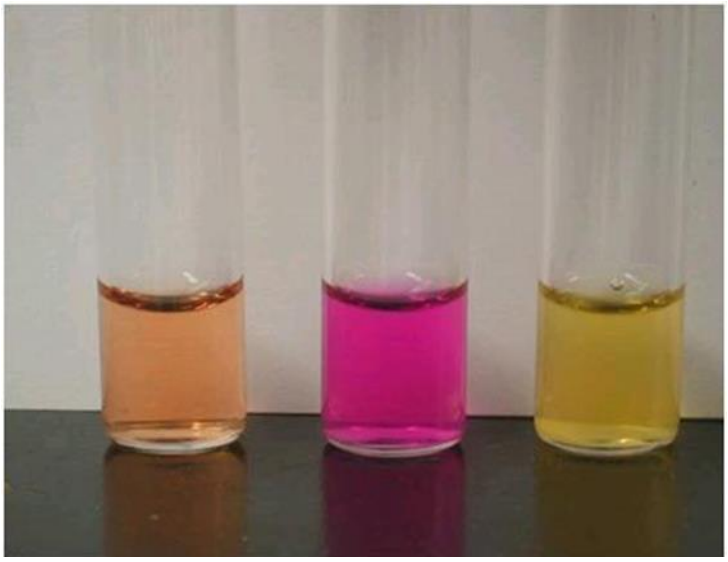
Procedure:

1). Culture was inoculated in broth for 48hrs and temperature of 37^0C was maintained.

2). Then the broths was inoculated from the incubator and color was observed.

Requirement: Christense's Urea-Agar

Peptone	1g
Dextrose	1g
NaCl	1g
Pot.(P)	2g
Urea	20g
Phenol	0.012g
Agar	15 to 20g



Uninoculated Positive Negative

Fig.3.5 Result of urease test.

d) Methyl-Red Test:

Some bacteria perform MIXED-ACID FERMENTATION. This test determines the ability of bacteria to perform mixed-acid fermentation.

Procedure:

1). Two MR-VP broths were taken.

2).One broth was inoculated using aseptic technique and the other broth was leaved uninoculated (this will be a control).

3). Then broth was incubated at appropriate 37°C for two to five days.

4). A drop of Methyl Red was added to each broth.

5).Lastly the color was observed (which should develop within a few minutes).

Fig.3.6 Methyl Red Test

a) Indole test:

This test determines whether the microbe produces indole from the amino acid tryptophan. If indole is produced, it will react with a chemical reagent added after incubation to produce a color change. Tryptone broth medium is used. If the result is positive then red layer will form at the top, yellow or amber ring forms if test is negative.

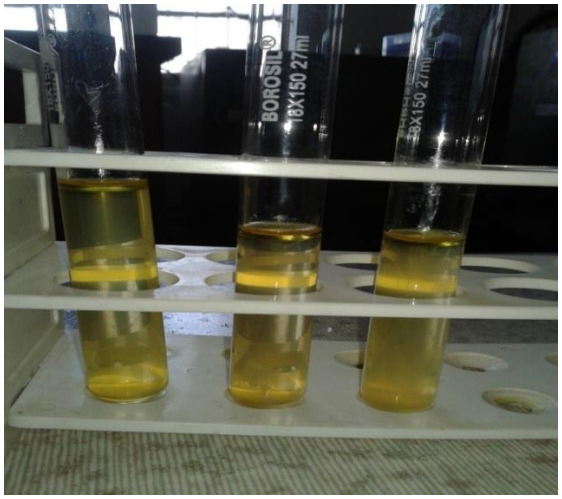


Fig 3.7 Indole test

f) Gelatin liquefaction test:

Some bacteria produce gelatinase, enzyme that hydrolyzes gelatin. This test is used to differentiate between organisms that produce the exoenzyme gelatinase and those that do not.

Procedure:

1) 2000 mg gelatin agar was mixed in 100ml distilled water conical flask. After that it was poured in plates and sterilized.

2) Incubated for 3 days at 30°C, after incubation the plates were flooded with 12 percent $HgCl_2$ solution and allowed to stand for 20 mins.

3) Then it was observed for clear zone around the growth of organism to indicate gelatin liquefaction.

3.5.2 MORPHOLOGICAL CHARACTERS: Isolated bacterial strains were examined for colony morphology; cell shape and size of the isolated bacteria's were studied using the microscope.

a) Gram staining test:

This test detects a fundamental difference in cell wall composition of Bacteria.

Gram negative: Cells are decolorized by the alcohol-acetone solution and take on a pink to red color when counterstained with safranin.

Gram positive: Crystal violet was retain by the cells and remain purple to dark blue.

Colony morphology: The shape, size, elevation, margin and color of the colony were observed.

Cell morphology: The Gram stained cells were observed under the light microscope under 100x using oil immersion. The shape and color of the cells were determined.

b) Motility test:

Hanging drop slides were prepared from overnight grown cultures and were observed under the microscope

3.6 STUDY OF GROWTH KINETICS:

Growth study of phenol was studied; freshly inoculated culture was taken on 6 hours interval and then centrifuged at 14000 rpm for 2 minutes. Supernatant was collected and sample was prepared for measurement of optical density of phenol. It was measured at 510 nm. For growth kinetics of phenol degradation graph of OD with time was plotted.

a) Buffer solution: 16.4 gram of NH_4Cl was dissolved in 143 ml conc.NH_4OH and diluted to 250 ml with distill water .2 ml stored and just 100 ml of distillate to pH 10.

b) 4-Aminoantipyrine (AAP) solution: 2 gram of 4 AAP was dissolved in distilled water and diluted to 100 ml.

c) Potassium ferricynide solution: 8 gram of $K_3Fe\ (CN)_6$ was dissolved in distill water and diluted to 100 ml.

Sample mixture contains:

0.9 ml distill water + 0.1 ml supernatant sample + 50 µl of 2N NH4OH + 25µl of 2% 4 AAP + 25 µl 8% of $K_3Fe\ (CN)_6$

CHAPTER-4

RESULTS AND DISCUSSION

4.1ISOLATION AND CHARACTERIZATION OF BACTERIAL STRAINS:

Three bacterial strains were isolated from paper sludge. The strains were designated as S1, S2 and S3 and examined for colony morphology, Gram stain characters and biochemical tests.

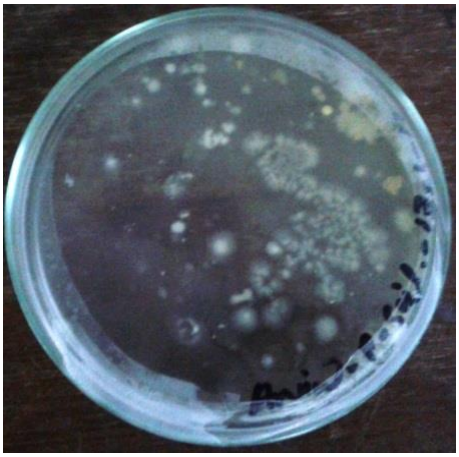


Fig 4.1 Isolated colonies of bacterial strains

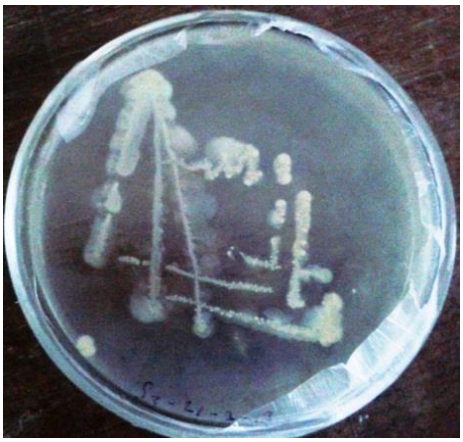


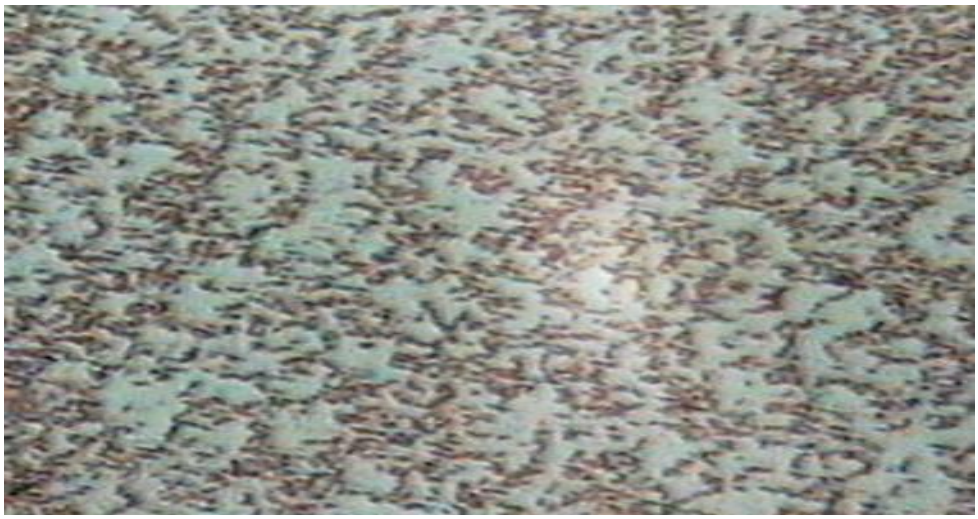
Fig 4.2 Isolated pure culture of bacterial strain

Table.4.1 Biochemical characterization of isolated strains

Tests	S_1	S_2	S_3
Gram Staining	Gram negative	Gram negative	Gram negative
Cell shape	Rod shaped Bacilli	Rod shaped Bacilli	Rod shaped Bacilli
Motility	Motile	Motile	Motile
Colony shape	Circular,smooth,wet, convex	Circular,smooth,wet, convex	Circular,smooth,wet, convex
Pigment	Colorless	Colored	Colorless
Oxygen requirement	Aerobic	Aerobic	Aerobic
Indole test	-ve	-ve	-ve
Gelatin liquefaction	-ve	-ve	-ve
Catalase test	+ve	+ve	+ve

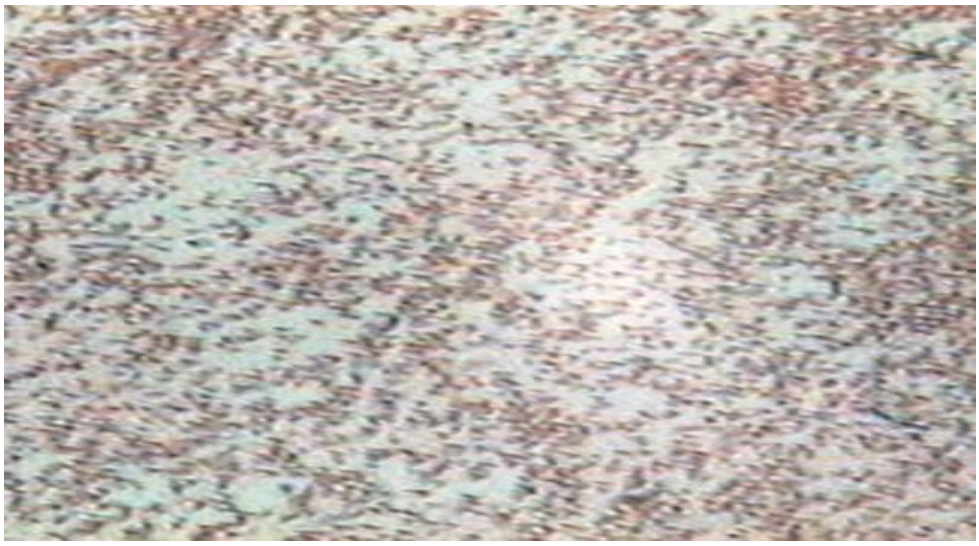
Citrate test	-ve	-ve	-ve
Urease test	+ve	+ve	-ve
Methyl red test	-ve	-ve	-ve

From results of various tests in biochemical characterization and morphological characterization of bacteria, it may be pseudomonas bacteria.



(a) Strain S1

(b) Strain S2



(c) Strain S3

Fig 4.3 Microscopic view of isolated bacteria

4.2 OPTIMIZATION OF PHYSIOLOGICAL PARAMETERS (TEMPERATURE, pH AND CONCENTRATION) OF THE ISOLATE BACTERIA (PSEUDOMONAS) FOR ENHANCED BIODEGRADATION OF PHENOL:

Growth and biodegradation of any microorganism depends on various physiochemical parameters. Aim of this project is to optimize the parameters like temperature of incubator, pH of the medium and concentration of phenol which is used as a sole of carbon source and energy. Study on growth of bacteria at different temperature, pH and concentration of phenol were carried out and optimize conditions was found out.

As biomass increases the optical density also increases or with increase in cell growth optical density increases .Increase in cell growth indicates increase in degradation rate.

4.2.1 EFFECT OF TEMPERATURE ON GROWTH OF MICROORGANISM:

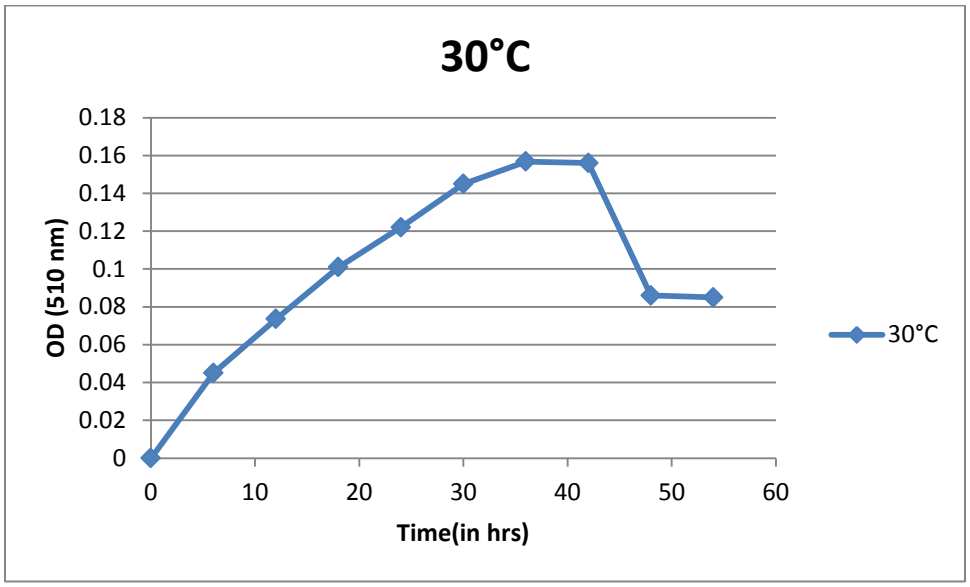


FIG 4.4 GROWTH PROFILE OF THE ISOLATED PSEUDOMONAS AT 30°C, 7 pH AND 200 ppm OF INITIAL CONCENTRATION OF PHENOL.

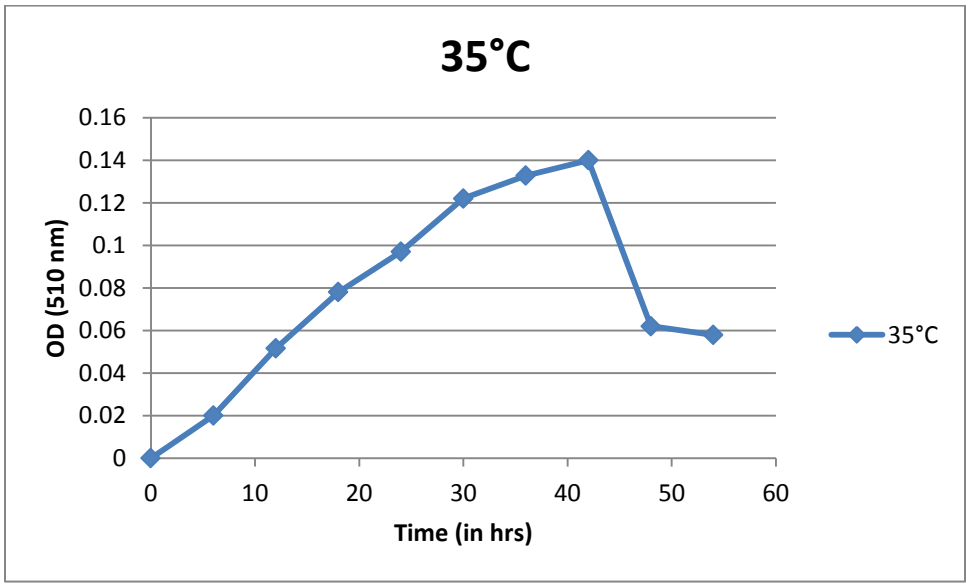


FIG 4.5 GROWTH PROFILE OF THE ISOLATED PSEUDOMONAS AT 35°C, 7 pH AND 200 ppm OF INITIAL CONCENTRATION OF PHENOL

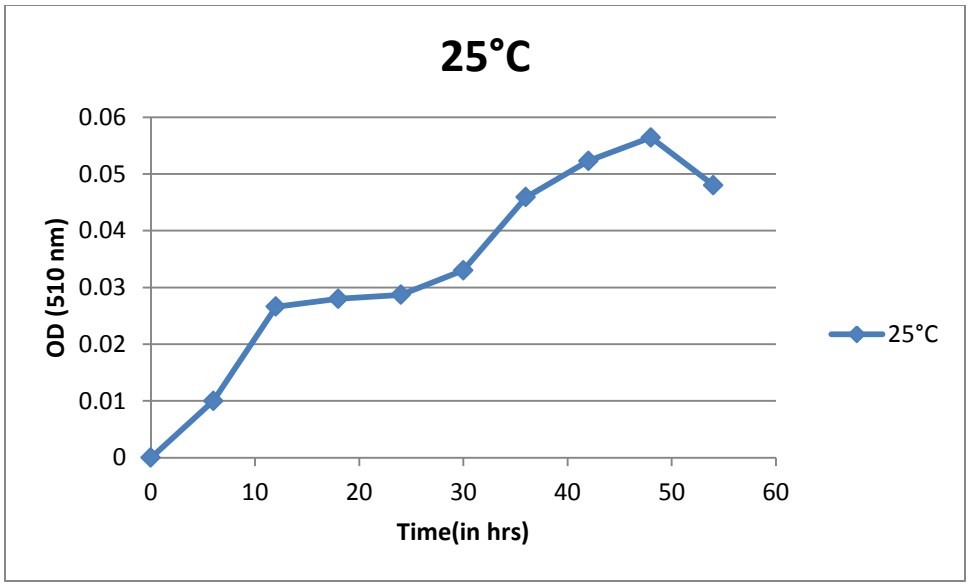


FIG 4.6 GROWTH PROFILE OF THE ISOLATED PSEUDOMONAS AT 25°C, 7 pH AND 200 ppm OF INITIAL CONCENTRATION OF PHENOL

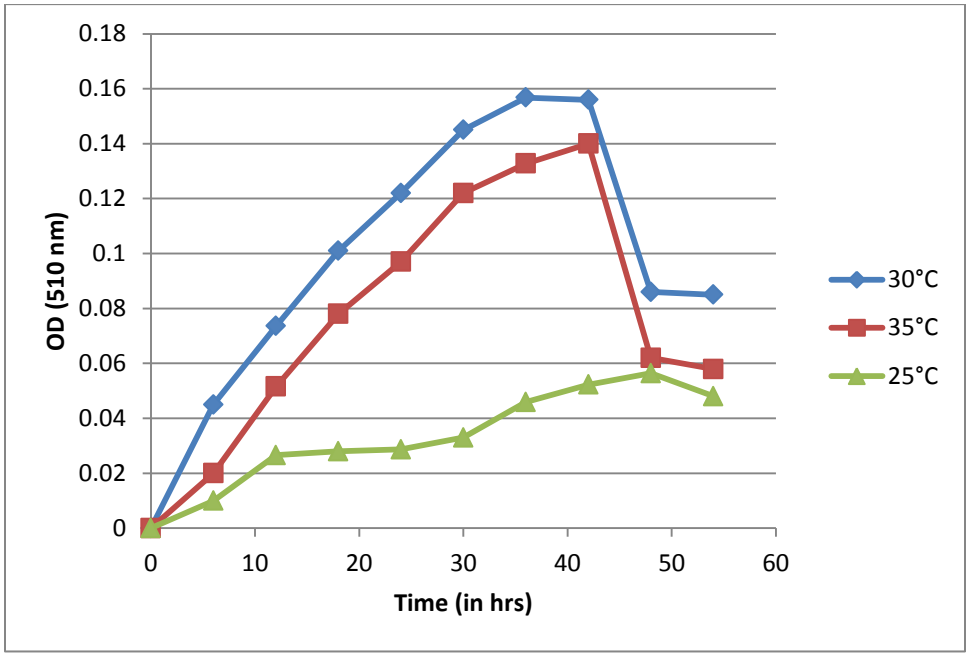


FIG 4.7 GROWTH PROFILE OF THE ISOLATED PSEUDOMONAS AT VARIOUS TEMPERATURES AT 200 ppm OF INITIAL CONCENTRATION OF PHENOL AND 7 pH

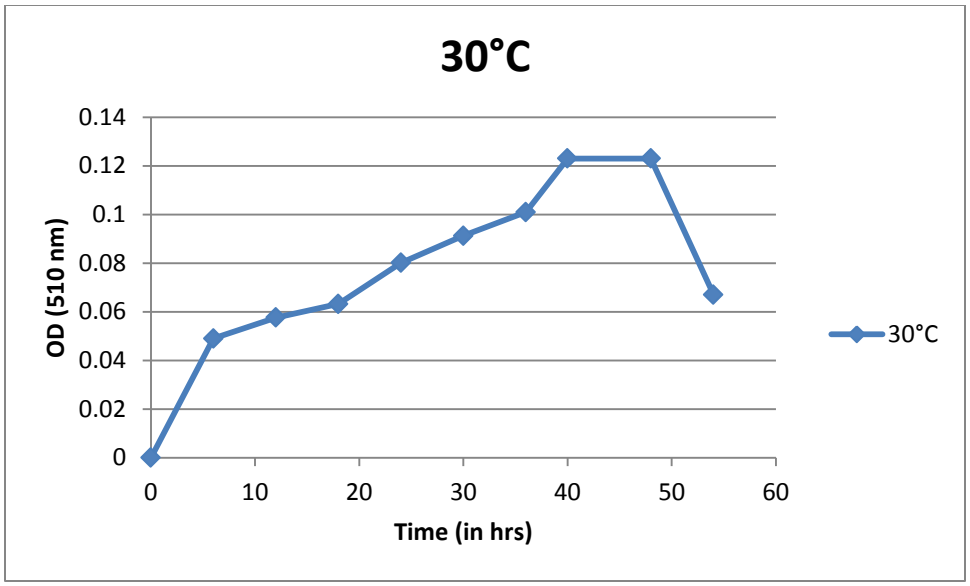


FIG 4.8 GROWTH PROFILE OF THE ISOLATED PSEUDOMONAS AT 30°C, 7 pH AND 400 ppm OF INITIAL CONCENTRATION OF PHENOL

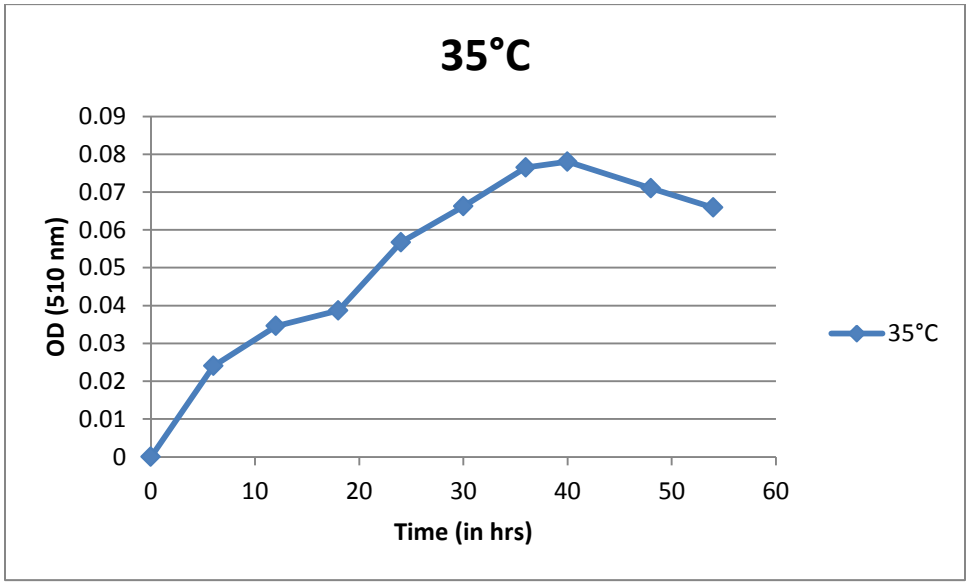


FIG 4.9 GROWTH PROFILE OF THE ISOLATED PSEUDOMONAS AT 35°C, 7 pH AND 400 ppm OF INITIAL CONCENTRATION OF PHENOL

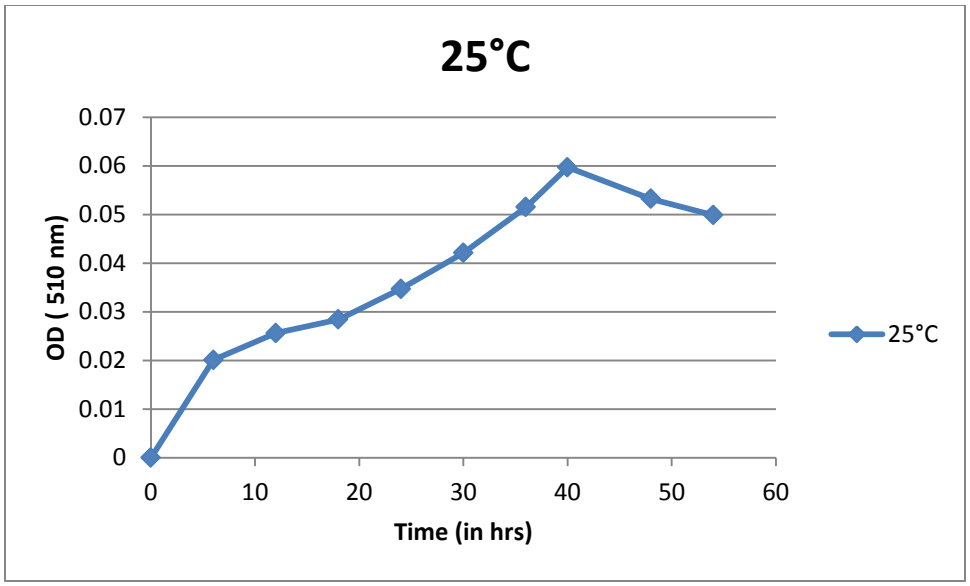


FIG 4.10 GROWTH PROFILE OF THE ISOLATED PSEUDOMONAS AT 25°C, 7 pH AND 400 ppm OF INITIAL CONCENTRATION OF PHENOL

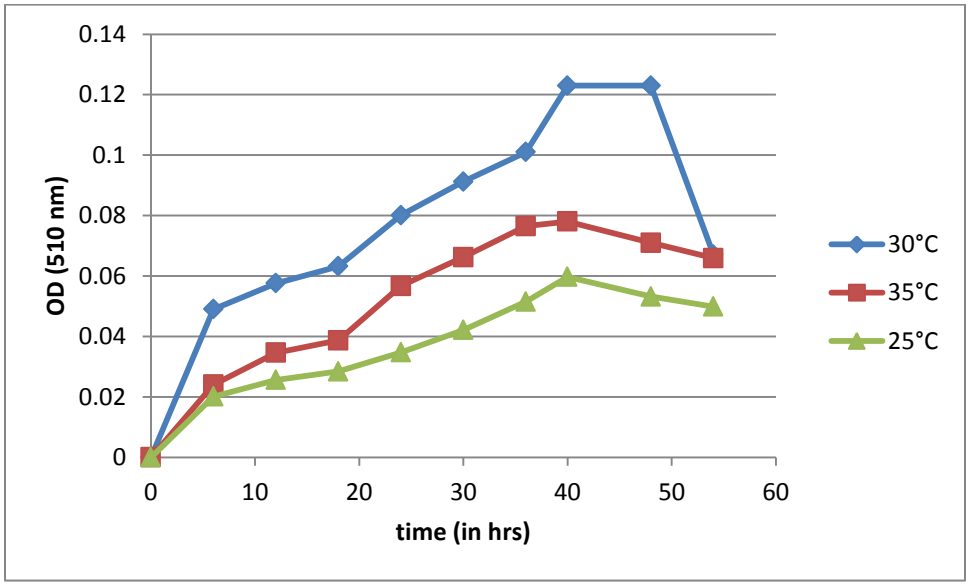


Fig 4.11 GROWTH PROFILE OF THE ISOLATE PSEUDOMONAS AT VARIOUS TEMPERATURES AT 400 ppm OF INITIAL CONCENTRATION OF PHENOL

From the graph maximum degradation was observed at 30°C.In case of 25°C and 35°C growth is reduced which in turn reduced the rate of degradation.

4.2.2 EFFECT OF PH ON GROWTH OF MICROORGANISM:

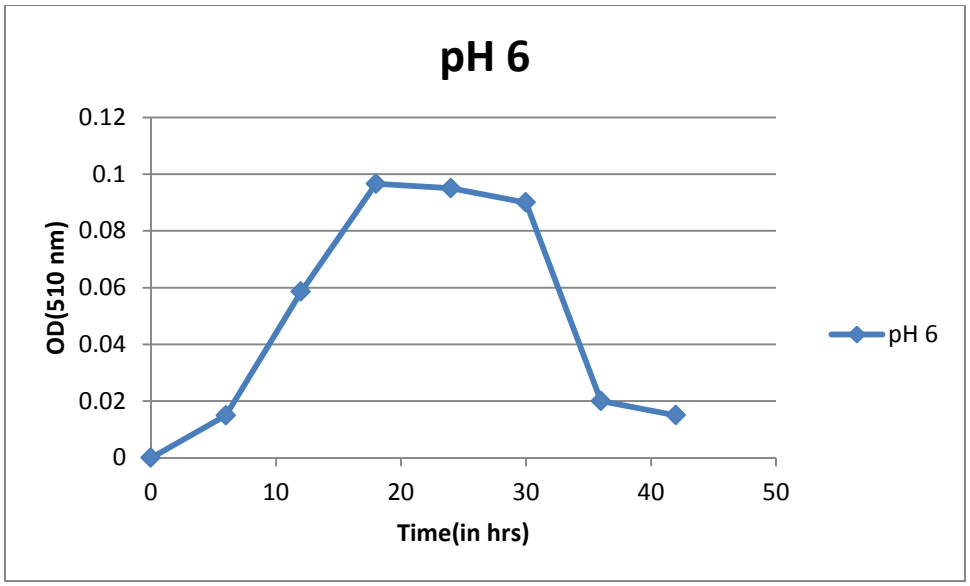


FIG 4.12 GROWTH PROFILE OF ISOLATED PSEUDOMONAS AT pH 6(30°C AND 200 ppm OF INITIAL CONCENTRATION OF PHENOL)

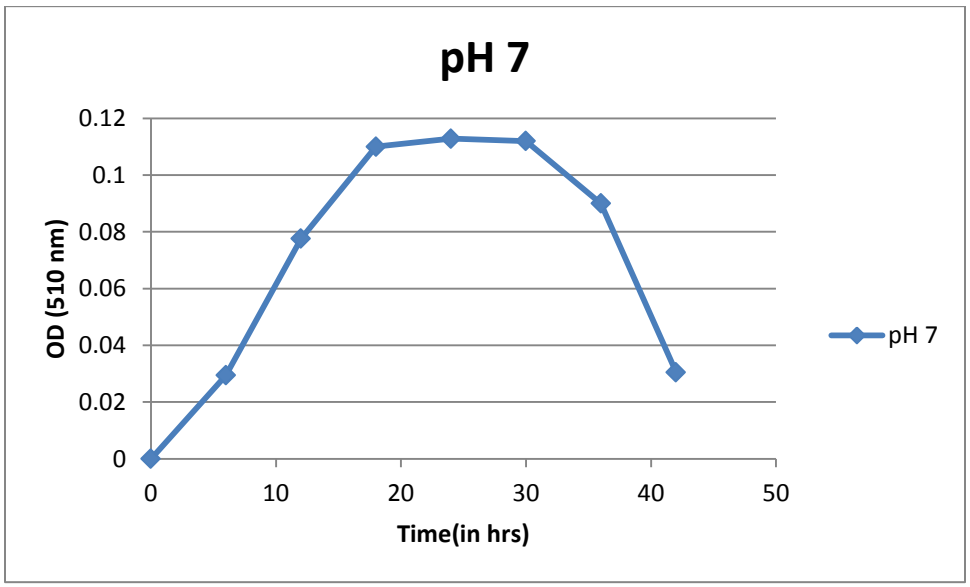


FIG 4.13 GROWTH PROFILE OF ISOLATED PSEUDOMONAS AT pH 7 (30°C AND 200 ppm OF INITIAL CONCENTRATION OF PHENOL)

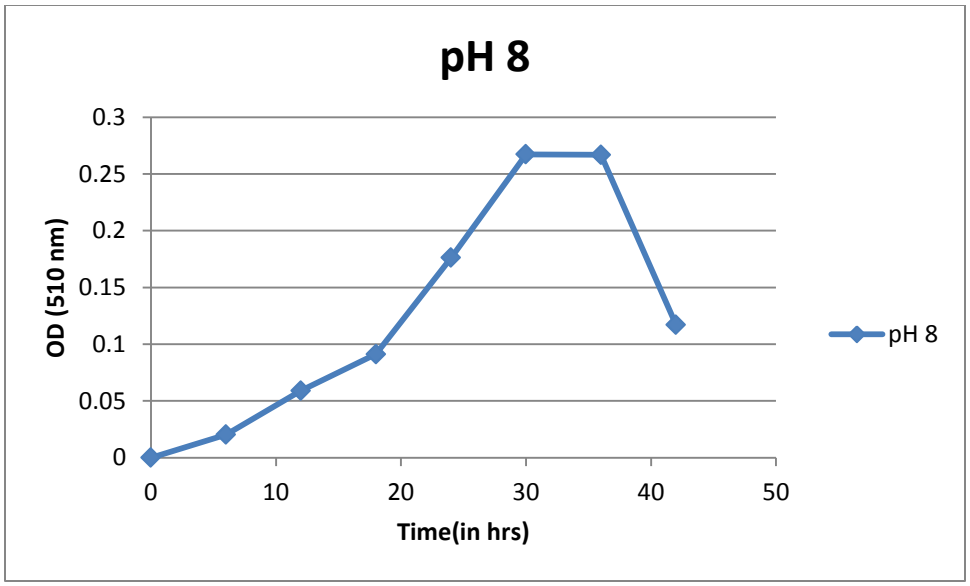


FIG 4.14 GROWTH PROFILE OF ISOLATED PSEUDOMONAS AT pH 8 (30°C AND 200 ppm OF INITIAL CONCENTRATION OF PHENOL)

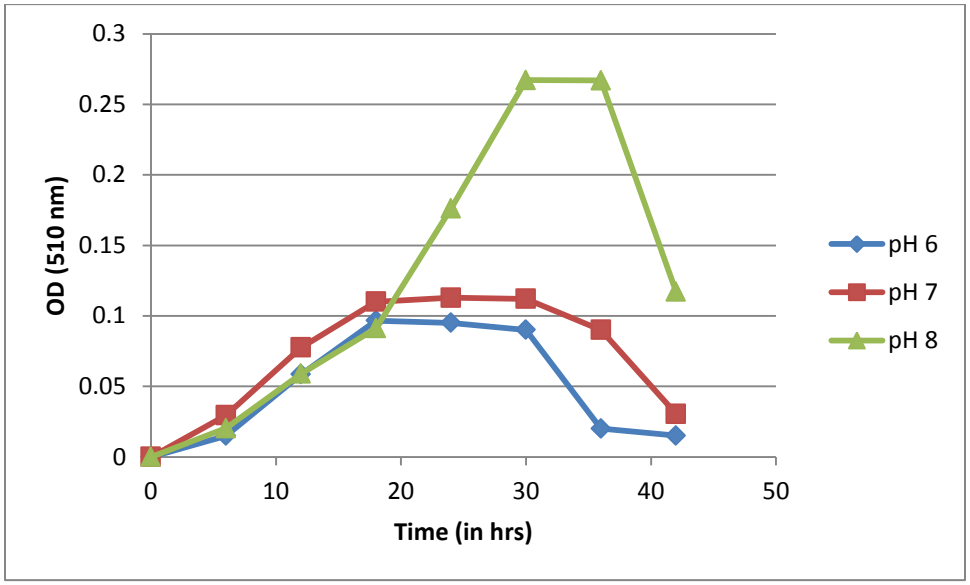


Fig 4.15 GROWTH PROFILE OF PSEUDOMONAS AT VARIOUS pH CONDITIONS (30°C, 200 PPM OF INITIAL CONCENTRATION OF PHENOL)

From the graph maximum growth was observed at pH 8. In case of pH 6 and pH 7 growths is less which ultimately gives rise to less degradation.

4.2.3 EFFECT OF CONCENTRATION ON GROWTH OF MICROORGANISM:

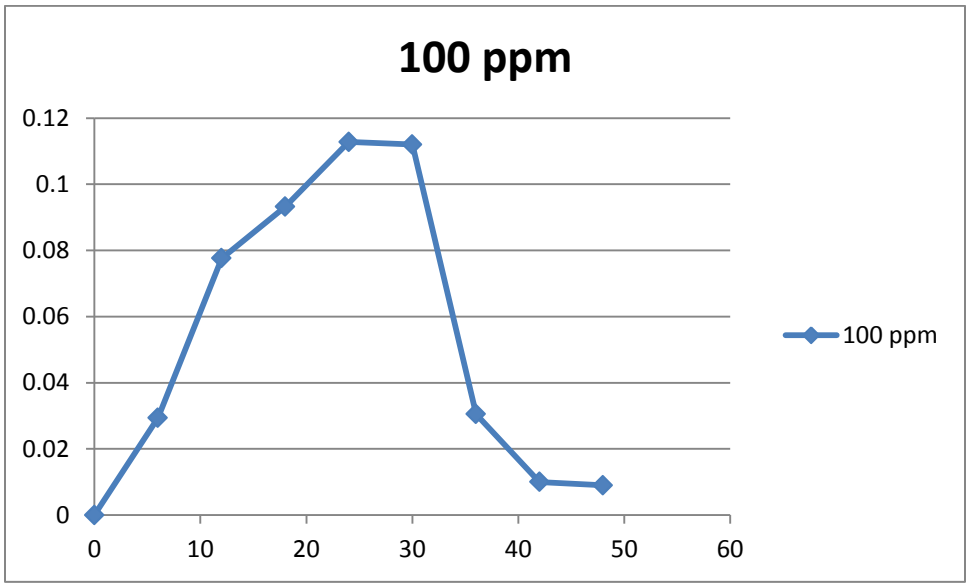


FIG 4.16 GROWTH PROFILE OF ISOLATED PSEUDOMONAS AT 100 ppm OF INITIAL CONCENTRATION OF PHENOL (pH 7 AND 30°C)

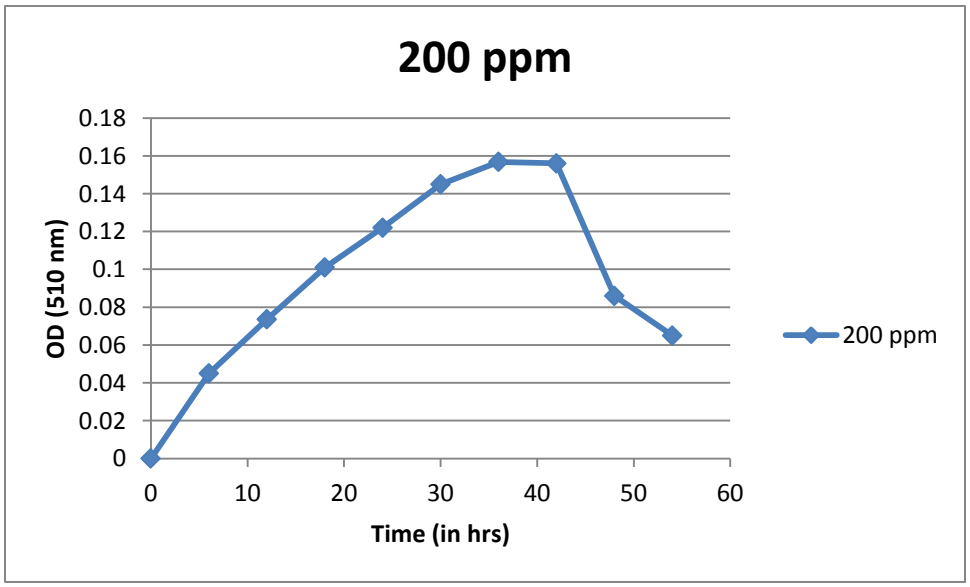


FIG 4.17 GROWTH PROFILE OF ISOLATED PSEUDOMONAS AT 200 ppm OF INITIAL CONCENTRATION OF PHENOL (pH 7 AND 30°C)

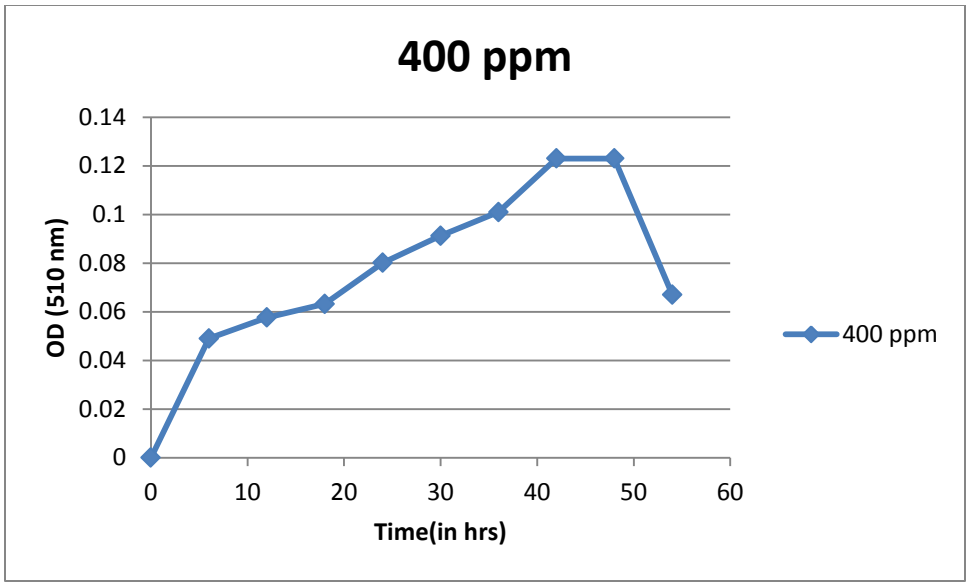


FIG 4.18 GROWTH PROFILE OF ISOLATED PSEUDOMONAS AT 400 ppm OF INITIAL CONCENTRATION OF PHENOL (pH 7 AND 30°C)

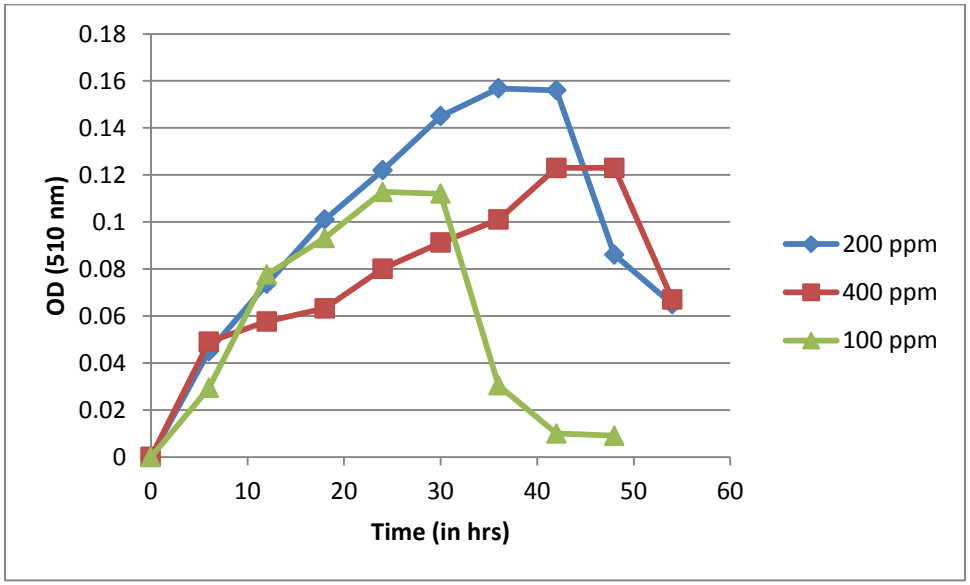


Fig 4.19 GROWTH PROFILE OF PSEUDOMONAS AT VARIOUS INITIAL CONCENTRATION OF PHENOL (30°C, pH 7)

From the graph maximum degradation was observed at 200 ppm. In case of 100 ppm and 400 ppm the cell growth was less than that in 200 ppm, which in turn gives rise to less degradation.

CHAPTER-5

CONCLUSION

Biodegradation is one of the cheapest methods with no production of hazardous by-products. This method is generally preferred due to lower costs and possibility of complete mineralization. The growth and phenol biodegradation study was carried out in MSM broth with phenol as the sole carbon source and energy. The strains were designated as S1, S2 and S3 and examined for colony morphology, Gram stain characters and biochemical tests. The phenol biodegradable bacteria were isolated. From the results of various tests in biochemical characterization and morphological characterization of bacteria, it may be pseudomonas bacteria.

Phenol degrading performance of all the strains was evaluated initially. One of the strains namely S2 was found to be highly effective for the removal of phenol. The effect of temperature, pH and phenol concentration on the rate of phenol degradation by that particular strain was carried out.

It was observed that the rate of phenol biodegradation was significantly affected by pH, temperature of incubation and concentration of phenol which is used as a source of carbon and energy. The process parameters were optimized and it was observed that as pH increases the growth of microorganism increases. The optimal conditions for phenol removal were found to be pH of 8, temperature of 30°C and concentration of phenol of 200 ppm.

REFRENCES:

- S. Chakraborty, T. Bhattacharya, T.N. Patel and K.K. Tiwari , May 2010. Biodegradation of phenol by native microorganisms isolated from coke processing wastewater. Journal of Environmental Biology, pp. 293-296.
- A. Mordocco, C. Kuek, R. Jenkins, 1999 Continuous degradation of phenol at low concentration using immobilized Pseudomonas putida, Enzyme Microb. Technol. Vol. 6, pp.530–536.
- C. Indu Nair, K. Jayachandran and Shankar Shashidhar, 29 December, 2008. Biodegradation of phenol. African Journal of Biotechnology Vol. 7.
- Agarry, S. E., Solomon, B. O., Audu, T. O. K., 2010. Substrate utilization and inhibition kinetics: Batch degradation of phenol by indigenous monoculture of Pseudomonas aeruginosa. International Journal for Biotechnology and Molecular Biology Research Vol.1, pp.22-30.
- Khazi Mahammedilyas Basha, Aravindan Rajendran, and Viruthagiri Thangavelu, 2010. Recent advances in the Biodegradation of Phenol. ASIAN J. EXP. BIOL. SCI., Vol 1.
- B. Marrot, A. Barrios-Martinez, P. Moulin, N. Roche, 2006. Biodegradation of high phenol concentration by activated sludge in an immersed membrane bioreactor. Biochemical Engineering Journal, Vol.30,pp.174–183
- Busca, G., Berardinelli, S., Resini, C., Arrighi, L., 2008. Technologies for the removal of phenol from fluid streams: A short review of recent developments. Journal of Hazardous Material, Vol.160, pp.265- 288.
- El-Sayed, W.S., Ibrahim, M.K., Abu-Shady, M., El-Beih, F., Ohmura, N., Saiki, H., Ando,A., 2003. Isolation and characterization of phenol catabolizing bacteria from a coking plant. Bioscience Biotechnology and Biochemistry, Vol. 67, pp.2026-2029.
- Holladay, D.W., Chilcote, D.D., Hancher, C.W. & Scott, C.D. (1978). Biodegradation of phenolic waste liquors in Stirred Tank, Packed-Bed and Fluidized-Bed Bioreactors. J. Water Poll. Control Federation.,Vol 50, pp.2573-2578.

- Kim, N.W. & Armstrong, M.E. (1981). Acomprehensive study on the biological treatabilities of phenol and methanol II. The effects of temperature, pH, salinity and nutrients. Water Res.,Vol.15, pp.1233-1247.

- Robertson, B.K. & Alexander, M. (1992). Influence of calcium iron and pH on phosphate availability for microbial mineralization of organic chemicals. Appl. Environ. Microbiol., Vol.58, pp.38-41.

- Paraskevi, N.P. & Euripides, G.S. (2005). Effect of temperature and additional carbon sources on phenol degradation by an indigenous soil Pseudomonad. Biodegradation., J.Appl. Environ. Microbiol Vol.16, pp.403-413.