COMPARATIVE STUDY OF PHENOL BIODEGRADATION BY FREE AND IMMOBILIZED CELLS

A Thesis submitted to the

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In partial fulfillment of the requirements

Of the degree of

Bachelor of Technology (Chemical Engineering)

BY

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CERTIFICATE

This is to certify that the thesis on **"Comparative study of phenol biodegradation by free and immobilized cells"** is submitted by **Rapaka Soumita Siri (111CH0610)** 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.

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ABSTRACT

The objective of this project work is comparative study of the biodegradation of phenol by bacterial strain isolated from Petroleum Contaminated Soil sample and immobilized on commercial activated carbon by simultaneous adsorption and biodegradation. Biodegradation is one of the cheapest methods without any production of hazardous by-products. The biodegradation study of phenol is carried out in MSM broth where phenol is provided as a source of carbon and energy. The effect of temperature, pH and phenol concentration on the rate of phenol biodegradation by a particular strain is carried out. Activated carbons are the most broadly utilized adsorbents because of their incredible adsorption capacities for organic pollutants. The cells are immobilized on activated carbon and the effect of temperature, pH and phenol concentration on the rate of phenol biodegradation by immobilized cells is carried out. Observations uncovered that the rate of phenol biodegradation is essentially influenced by pH, temperature of incubation and phenol concentration and immobilized cells are more effective than free cells.

KEY WORDS: Phenol, Biodegradation, Bacterial strain, Adsorption, Activated Carbon

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CHAPTER-1
INTRODUCTION

1.1 PHENOL

Phenol is a solid white crystalline organic compound having formula C_6H_5OH. The molecule consists of a phenyl group ($-C_6H_5$) bonded to a hydroxyl group (-OH). It is volatile and mildly acidic in nature. In the manufacture of pharmaceuticals, lubricating oils and solvents, pesticides, synthetic tanning agents, synthetic resin, dyes, perfumes, phenol is used. It is the common starting material used in the manufacture of many agricultural and industrial products and is often produced as a waste product. The major industries that discharge phenolic wastewater include petroleum refineries, dye manufacturing, petrochemicals, textile, glass fiber units, phenolic resin manufacturing, varnish industries and smelting related to metallurgical operations. Thus, the increasing amount of phenols in the water is mostly due to these industrial effluents, which represents a significant environmental toxicity hazard.

Phenol is a toxic substance and classified as hazardous materials. Even low concentrations of it in waste water can be toxic to some aquatic species and causes taste and odor problems in drinking water. Minute exposure to phenol can result in central nervous system disorders and myocardial depression. Exposure to phenol may result in irritation of the eye, corneal whitening, conjunctional swelling and finally blindness. It leads to collapse and coma. Severe exposure may result in dysphagia, dermal rash, muscle pain, vomiting, weakness, anorexia, weightlessness, and hepatic tenderness. Other effects include frothing at nose and mouth followed by headache. Even exposure of phenol to the skin can produce burns and blisters in animals. So, it is essential to remove such chemicals from effluent streams.

Several methods are available for treating the phenolic wastewater like adsorption, reverse osmosis, stripping-oxidation, distillation processes, solvent extraction, chemical oxidation, in cineration, hybrid process and electrocatalytic degradation. Most of these methods have some

drawbacks, such as high capital and operational cost, regeneration cost, and problems of residual disposal. All these non-biological methods have a serious drawback of forming of hazardous byproducts. However, microbial degradation is an environmental friendly method and widely accepted as cost effective, more efficient to detoxify phenol contaminants. Microorganisms can adapt to wide range of different environmental conditions, have rapid population growth and high metabolism which is the main reasons for the use of microorganisms in bioremediation.

1.2 BIODEGRADATION

A natural process of recycling wastes or breaking down complex organic matter into nutrients or simple matter that can be used by the microorganisms is called biodegradation. There are two types of biodegradation - aerobic which takes place in the presence of oxygen and anaerobic biodegradation which takes place in the absence of oxygen. Biodegradation is generally categorized into two ways - mineralization and biotransformation. Mineralization is the total degradation of the organic matter and biotransformation is organic matter is partially degraded and the remaining is converted into other smaller chain organic compounds. Because of the widespread occurrence of phenol in the environment, numerous microorganisms use phenol as the sole carbon and energy source that incorporates both aerobic and anaerobic microorganisms.

1.3 ADSORPTION

Adsorption is a process in which atoms, molecules, or ions adhere from a gas, liquid, or dissolved solid to a solid surface where they bond with the solid surface or are held there by weak intermolecular forces. The solid material is referred to as adsorbent and adsorbed solutes are known as adsorbate. Activated carbon acts as excellent adsorbent and is most widely used. Activated carbon is an amorphous, carbon-based material with high porosity and an extended inter particulate surface area that can be used for the removal of liquids and gaseous pollutants as

well for the gas storage application. The main advantage of using this process is the adsorbents can be reused several times and thus reduces waste disposal problems.

1.4 IMMOBILIZATION

Enzyme immobilization is the constrainment of enzyme to a phase (matrix/support) not the same as the one for substrates and products. It gives an amazing base to the expanding accessibility of enzyme to the substrate with more prominent turnover over a significant time. Inert polymers and inorganic materials are commonly utilized carrier matrices. These days, immobilized enzymes are favored over their free counterpart due to their prolonged availability.

The present study is aimed to describe the phenol biodegradation by bacteria isolated from the petroleum contaminated soil and immobilized on Activated carbon. The combined process of adsorption and biodegradation is applied. The growth characteristics, phenol biodegradation potential and effect of various physiological parameters were studied separately for biodegradation (free cells) and adsorption and compared the result. The combined study of biodegradation and adsorption (immobilized cells) is also carried out and reported.

CHAPTE2

LITERATURE REVIEW

2.1 MICROORGANISMS CAPABLE OF DEGRADING PHENOL

Jiang et al. (2004) isolated ten bacterial strains from aerobic granules that degrade phenol.

The phenol degrading microbes most often present are pseudomonas, flavobacterium, achromobacter, zhomobacterium, azobacter, micrococcus, bacillus alkaligenes, arthrobacter, ycobacterium, aeromonas, nocardia and lophomonas. Based on the specific substrate concentration and the potentiality of growth, their respective proportion will depend. When microbes are cultured, they consume substrates for their growth and energy. Phenol is provided as sole carbon source and energy and degradation rate is known.

Kuo-Ling Ho et al. (2009) has isolated Corynebacterium sp. DJ1, a functional phenol-degrading strain from wastewater sludge and utilized this stain to cultivate single-culture granules. Two groups of degrading bacteria are used mainly: the Rhodococci (Rhodococci shows considerable morphological) like Rhodococcus spp. and Pseudomonads like Pseudomonas putida. P. putida seems to have the highest degradation potential. That is the reason degradation of phenols by these bacteria is immensely studied (A. Mordocco et.al.)

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

Abdul Haleem Shah et al. (2013) Reported that isolates obtained from different soils were grown on nutrient agar plates, mineral salt media and nutrient broth. MSM was used for the degradation of phenol. Phenol degradation was examined along with dry cell weight. The bacteria which were isolated from local soil sample were made potent up to 2.5 g/100 ml phenol concentration. 35˚C was found to be the optimum temperature for the degradation of phenol when these bacteria were examined for their degrade ability under different temperatures and 20˚C or 50˚C were found to be minimum. The ability of bacteria to degrade phenol at different pH was also observed. It was examined that maximum degradation was recorded at pH 7 and minimum was recorded at pH above 9 or below 4. Phenol degrading ability was examined by locally isolated soil bacteria by trying different shaking speeds. The optimum speed was found to be 120 rpm. No effect was observed at shaking speed 80 rpm and 160 rpm.

By increasing the pH of media from 5 to 7at 30^0C rate of phenol degradation also increases. By increasing the pH of the media further it had reverse effect on phenol removal potentiality. In 6 hr. at pH 7, 39.85% phenol was removed, while the rest of the pH conditions could not degrade phenol more than 8.42%. After 12, 18 and 24 hr. also analogous result was seen with only 84.63% removal till end at pH 7 at 30°C. A maximum of 14.14% of phenol was removed at pH 8. Maximum degradation is possible at neutral pH (pH-7) at 30°C.

2.2 METHODS OF IMMOBILIZATION

There are several methods available for immobilization of a cell. The various factors that influence the performance of immobilized enzymes are characteristics like inertness, physical strength, stability, regenerability. Apart from being affordable, an ideal matrix must possess ability to increase activity of enzyme and reduce product inhibition, microbial contamination and nonspecific adsorption.

Rosa et al. (2002); Wu and Lia (2008); Cordeiro et al. (2011) Reported that adsorption/carrier-binding method uses water-insoluble carriers such as glass, polysaccharide derivatives and synthetic polymers.

Singh (2009) reported that in cross-linking/covalent method, multipurpose reagents such as glutaraldehyde, hexamethylene diisocyanate and bisdiazobenzidine are used.

Chen et al. (2011); Klein et al. (2011) reported that polymers like collagen, cellulose and κ-carrageenan are employed by entrapment method, while the membrane confinement method includes formulation of liposomes and microcapsules.

Gonzalez et al. (2001); Pazarlioglu et al. (2005); Wu et al. (2005) have proved that phenol-containing wastewater is effectively treated by immobilized microorganisms little sludge production.

WANG Ying et al. (2006) isolated a new phenol-degrading bacterial strain PD12 with high biodegradation activity and high tolerance of phenol, from the activated sludge of Tianjin Jizhuangzi Wastewater Treatment Facility in China. The isolated strain could remove 500 mg phenol/L in liquid minimal medium by 99.6% in 9 h and metabolize phenol at concentrations up to 1100 mg/L. To immobilize *Acinetobacter* sp. strain PD12, polyvinyl alcohol (PVA) was used as a gel matrix by repeated process of thawing and freezing.

2.3 IMMOBILIZATION BY ADSORPTION

Alkan et al. (2009) stated that lately, mesoporous activated carbon particles with large contact sites is used for immobilizing acid protease and acidic lipases where catalytic efficiency was significantly maintained even after 21 cycles of reuse (Kumar et al. 2010; Ramani et al. 2011).

Jun Jiang et al. (2013) treated the phenolic water high-concentrations (_10,000 mg/L) directly by the Pseudomonas putida which is immobilized on the activated carbon fiber (ACF) by

adsorption–synergic biodegradation. Within 120 hr. at 30˚C, pH = 7, the cells-immobilized ACF performed the sequential adsorption–biodegradation process from the phenol concentration of 10,000–0 mg/L. Based on the cells-immobilized ACF, the whole process was investigated in detail. The adsorption process played a major role and phenol concentration of 2000 mg/L could be decreased under lesser duration.

C.S.A. S et.al (2001) reported that the difference between phenol removal efficiency at 30 ◦C is higher as production of metabolites is high at this temperature. It is also reported that at this temperature, the degradation rate seems better for free than immobilized cell system (1.45 times higher).

Chung et al. (2003) found optimum conditions for both the processes i.e both by free and immobilized cells. Temperature of 30˚C is found to be optimum for both processes but different optimal pH values: 6.8 for immobilized cells and 8.0 for free cells. As biomass grows and variation in the pH takes place when the initial phenol concentration increases, a slight reduction in biomass is seen. The decrease in pH suggests that biological degradation of phenol occurs and phenol was successfully degraded at pH of 7.

2.4 REMARKS

From the literature review, it can be stated that biodegradation is one of the preferable methods for removal phenol from industrial effluents. Phenol is a toxic substance and classified as hazardous materials. Phenol and its derivatives possess various degrees of toxicity. It harmful to both animals and plants and also pollute the environment. So, it is essential to reduce the amount of such chemicals from effluents streams. It is found that the degradation is mainly dependent on growth of bacterial which inturn depends on the concentration of carbon source, pH and incubation temperature. My study is based on degrading performance of phenol by free and

immobilized cells where the strain is isolated from petroleum contaminated soil and parameters which effect the process are found and optimized.

2.5 OBJECTIVE

- To isolate and characterize microorganism capable of degrading phenol.
- To immobilize the isolated microorganism on activated carbon
- Study phenol biodegradation by free cell and immobilized cell
- To optimize physiological parameters that affect the biodegradation process and adsorption process of phenol

CHAPTER-3
MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 MATERIALS REQUIRED

i. Sample of Petroleum Contaminated Soil collected from **Haldia Petrochemicals.**

ii. Commercial Activated Carbon sample was collected from **Kalpaka chemicals P.Ltd, Rourkela, Odisha.**

iii. Autoclave, laminar flow hood, Biological Incubator, hot-air oven and Orbital shaker.

3.2 SAMPLE PREPARATION

- Sample is prepared by adding 1 gram soil in 100 ml of water.
- Activated carbon is sieved and particles of size IC-70 (<720 microns) are separated and then powdered and dried in an oven.
- Phenol stocks of concentration 100ppm, 200ppm,300ppm, 400ppm, 500ppm are prepared by adding 0.1g/L, 0.2g/L, 0.3g/L, 0.4gL, 0.5g/L and diluted to 100ml.

3.3 ISOLATION AND CHARACTERIZATION OF BACTERIA

The process consists of the following steps:

MSM Preparation→ Sterilization→ Culturing→ Inoculation

Table 3.1 MSM composition:

Chemical composition	**g/L (Distilled water)**
KH_2PO_4	0.3
K_2HPO_4	6.0
NH_4Cl	1.0
$CaCl_2$	0.001
$MgSO_4.7H_2O$	0.001
NaCl	0.5

Nutrient Agar (for Streaking on Petridis)	2.0

3.4 PROCEDURE

- The MSM media is prepared and it is kept in an autoclave. It is maintained at a pressure of 15-20 psi and temperature of 120-121^{0}C. After sterilizing for 15-20 minutes it is cooled at room temperature.
- After the cooling, 1 ml of petroleum contaminated soil and 2ml of phenol which is the carbon source is added to the sterilized MSM solution in laminar flow hood (Product protection from microbial contamination)

Fig 3.1 Laminar flow hood

- This prepared media is kept in an orbital shaker at 115 rpm and incubation temperature of 30°C.
- Sufficient growth of bacteria is found after 48 hours which is further again sub-cultured five times in same media and same conditions to isolate the phenol degradable bacteria. After five consecutive sub-culturing, the media is transferred to sterile petri plates over a 20ml of solidified nutrient agar. 1ml cultured medium of serial dilution (10-6) is poured

over it and left for some time in the laminar hood. It is sealed and kept inverted in an incubator for 48hrs and at 30^0C.

- After the inoculation, different colonies were selected for further studies

Fig 3.2 Orbital shaker

3.4.1 MORPHOLOGICAL CHARACTERS

The bacterial strains are isolated and examined for colony morphology. Cell shape and size of the isolated bacteria are studied using the microscope.

a) Gram staining test:

This test is used to detect the fundamental difference in cell wall composition of bacteria.

Gram-negative: Using alcohol-acetone solution, cells are decolorized and obtain pink colour when counterstained with safranin.

Gram-positive: The cells retain the crystal violet and remain purple to dark blue color.

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

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

b) Motility test:

Using a microscope, hanging drop slides which are prepared from overnight grown cultures are observed.

3.5 DEGRADATION STUDY OF PHENOL

Degradation of phenol is studied. Freshly inoculated culture is taken on 6 hours interval and then centrifuged at 10000 rpm for 10 minutes. Supernatant is collected and sample was prepared by dilution (4 times) for measurement of optical density of phenol. It is measured at 270 nm. For degradation study of phenol graph of final concentration with time is plotted.

3.6 ADSORPTION

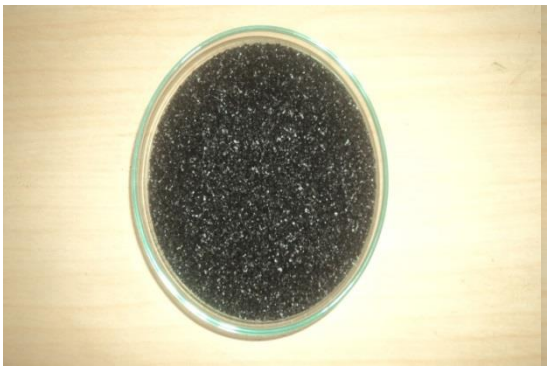

Fig 3.3 Granular activated carbon

Fig 3.4 Powdered activated carbon

3.6.1 ADSORPTION STUDY OF PHENOL

The samples are prepared by adding activated carbon of dosage 0.1gm – 0.5gm with different phenol concentrations of 100ml each and put in an orbital shaker at 25˚C. Samples are collected after regular time interval and centrifuged at 10000rpm for 10 min. Supernatant is collected and OD is obtained at 270 nm. Concentration, pH and temperature are optimized and adsorption study is carried out. Amount adsorbed is calculated by the following formula

$$Q = \frac{(C_0 - C_t)V}{M}$$

Where C_0 is initial concentration (mg/L) and C_t is concentration at time t, V is volume taken (Lit) and M is amount of activated carbon (g)

For the adsorption of phenol study, %removal versus concentration is plotted.

3.7 COMBINED EFFECT

3.7.1 PROCEDURE

- Microbial culture of 1ml is added to 100ml of nutrient broth and put in shaker at 35˚C for 48hrs.
- 0.1gm of activated carbon is taken in 5 different flasks. MSM media of 100ml each is prepared 5 different flasks. All of them are autoclaved for 15 minutes at 120-121˚C and then cooled. 3-4ml Phenol of different concentrations which were prepared is added to this MSM media along with activated carbon and 1ml of microbial culture.

3.7.2 DEGRADATION STUDY

- The prepared samples are put in an orbital shaker at 35˚C. Samples were collected after every 4hrs and centrifuged at 10000rpm for 10 min. Supernatant is collected and OD is obtained at 270 nm. For the combined study of adsorption and biodegradation of phenol, concentration versus time is plotted.
- Effect of degradation of phenol by both free cells and immobilized cells is compared.

CHAPTER-4
RESULTS AND DISCUSSION

4.1 ISOLATION AND CHARACTERIZATION OF BACTERIAL STRAINS:

There are 6 isolated bacterial strains which are designated as S1, S2, S3, S4, S5, S6. The performance of each of these bacterial strains is evaluated and their biodegradation rate is observed. Among the isolated strains, S4 is proved to be more efficient in degrading phenol. That stain S4 is examined for colony morphology and Gram stain characters.

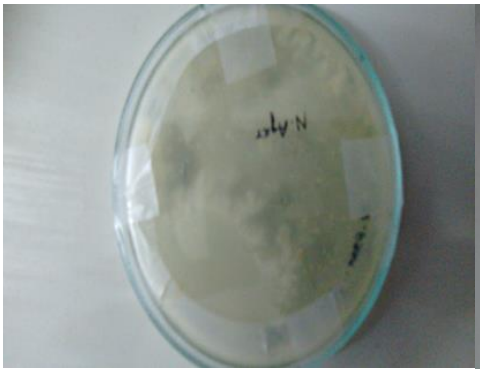

Fig 4.1 Bacterial colony

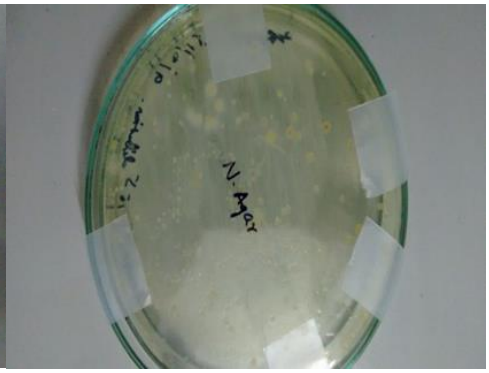

Fig 4.2 Isolated bacterial strain

Table.4.1 Biochemical characterization of isolated strain S4

Tests	**Strain**
Gram Staining	Gram negative
Motility	Motile
Colony shape	Circular, wet, smooth, convex
Cell shape	Rod shaped Bacilli
Pigment	Colored

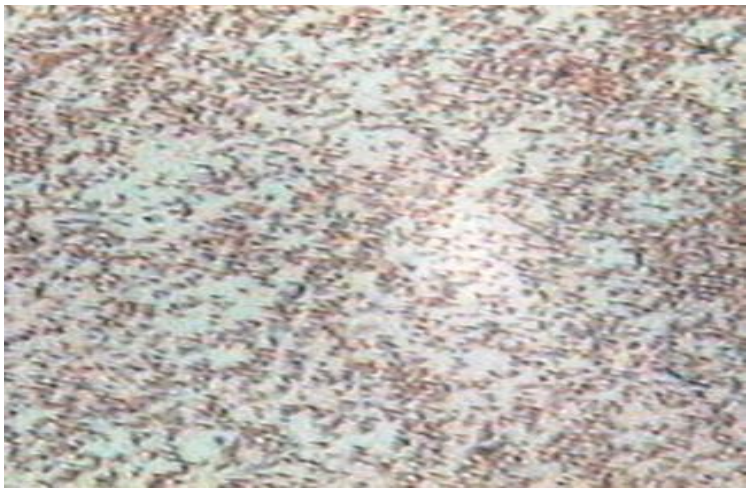

Fig 4.3 Microscopic view of isolated bacteria S4

4.2 OPTIMIZATION OF PHYSIOLOGICAL PARAMETERS (TEMPERATURE, pH AND CONCENTRATION)

Growth and biodegradation of any microorganism depends on various physiochemical parameters like temperature of incubator, pH of the medium and concentration of phenol which is used as a sole of carbon source and energy. Adsorption of any substance is also influenced by various parameters like dosage of carbon, temperature, pH, and concentration of phenol. Aim of this project is to optimize these parameters.

Study on phenol biodegradation and adsorption at different temperature, pH and concentration of phenol are carried out and optimize conditions are found out.

4.2.1 BIO DEGRADATION:

Table 4.2 Effect of temperature on degradation of phenol (OD values):

TEMP\ TIME	**6hrs**	**12hrs**	**24hrs**	**48hrs**
25	0.128	0.11	0.03	0.026
30	0.145	0.173	0.023	0.038
35	0.15	0.12	0.098	0.117

Table 4.3 Effect of pH on degradation of phenol (OD Values):

pH \ TIME	6hrs	12hrs	24hrs	48hrs
6	0.982	0.94	0.904	0.99
7	0.97	0.921	0.887	0.98
8	0.98	0.94	0.83	0.85

Table 4.4 Effect of concentration on degradation of phenol (OD Values):

CONC \ TIME	6hrs	12hrs	24hrs	48hrs
100 ppm	0.83	0.79	0.667	0.482
200 ppm	0.957	0.922	0.878	0.89
400 ppm	3.59	3.49	3.29	3.396

TEMPERATURE EFFECT:

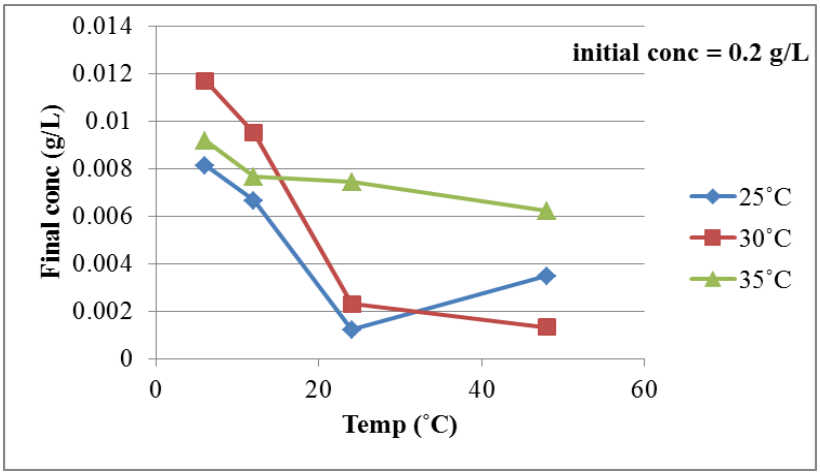

Fig 4.4 Degradation profile of phenol at various temperatures at 0.2g/L of initial concentration of phenol and 7pH

From the graph maximum degradation is observed at 30°C. In case of 25°C and 35°C less degradation is observed, which implies that growth of microbes has reduced.

pH EFFECT:

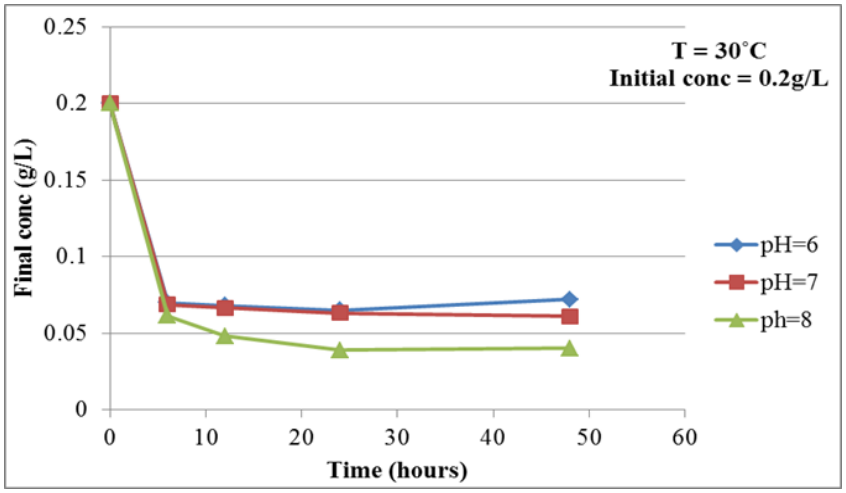

Fig 4.5 Degradation profile of phenol at various pH conditions (30°C, 0.2g/L of initial concentration of phenol)

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

CONCENTRATION EFFECT:

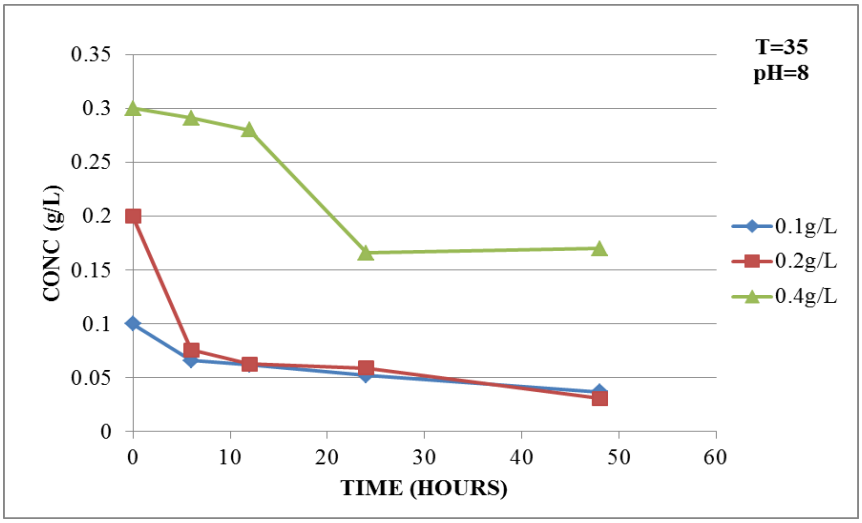

Fig 4.6 Degradation profile of phenol at various initial concentration of phenol (30°c, pH 7)

From the graph maximum degradation is observed at initial phenol concentration of 0.2g/L. In case of 0.1g/L and 0.4g/L of initial concentrations the degradation is less.

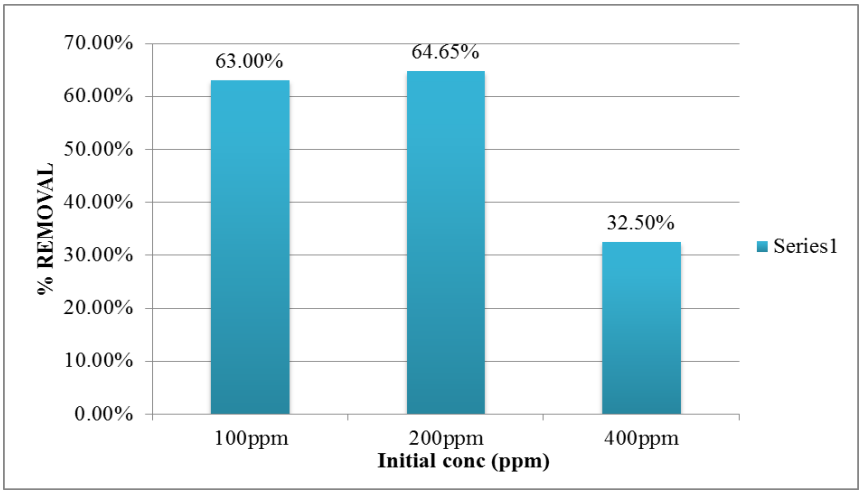

Fig 4.7 %Removal profile of phenol at optimized conditions:

The maximum percentage of phenol removed by biodegradation by free cells is 64.65 under optimized conditions.

4.2.2 ADSORPTION:

Table 4.5 Effect of temperature on adsorption of phenol (OD values):

TEMPERATURE (˚C)	OD
25	5.0190
30	4.9162
35	4.5877
40	4.7322
45	4.8359

Table 4.6 Effect of pH on adsorption of phenol (OD values):

pH	OD
2	5.1131
3	5.0128
4	4.8609
5	5.1131
6	5.2198
7	5.3315
8	5.3368
9	5.3613

Table 4.7 Effect of concentration on adsorption of phenol (OD values):

TIME \ CONC	100ppm	200ppm	300ppm	500ppm
5min	0.76569	1.83191	3.05692	5.83916
10min	0.67926	1.73513	2.7944	5.48
20min	0.56851	1.48302	2.5857	5.276
30min	0.51596	1.38206	2.5234	5.206
60min	0.45745	1.44051	2.608	5.09256
90min	0.44623	1.4326	2.576	5.0732
120min	0.4392	1.4105	2.524	5.006

TEMPERATURE EFFECT:

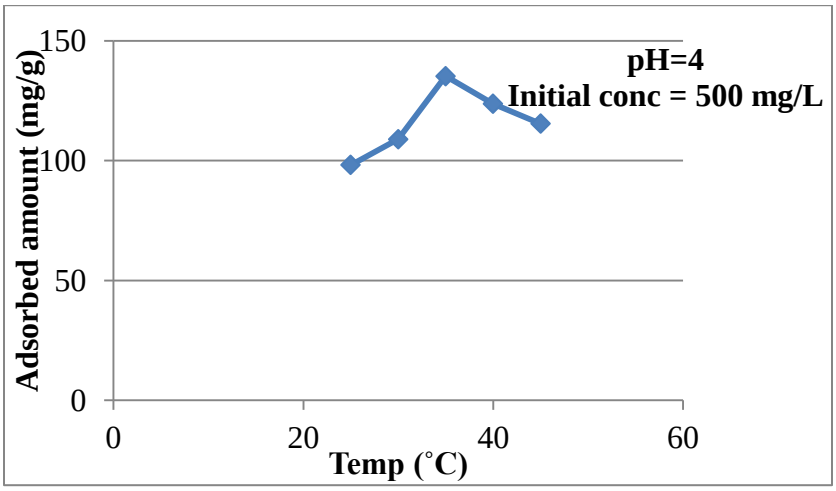

Fig 4.8 Adsorption profile of phenol at various temperatures at 0.5g/L of initial concentration of phenol

From the graph maximum adsorption is observed at 35°C.

pH EFFECT:

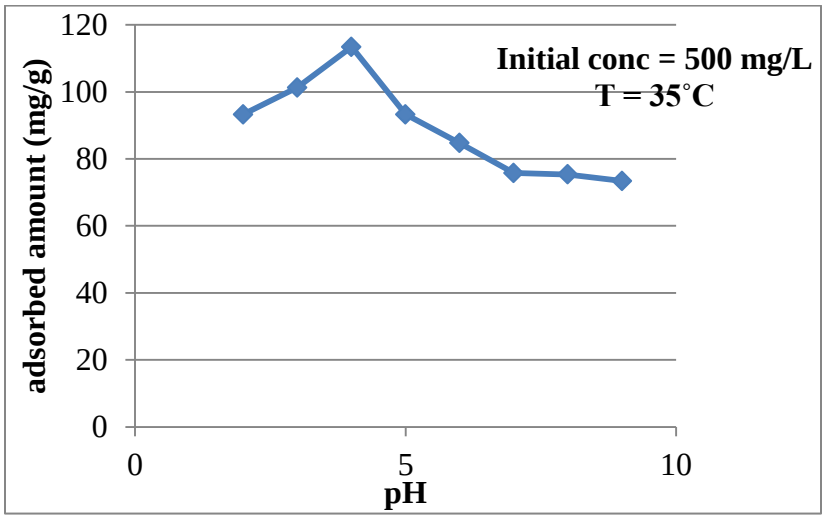

Fig 4.9 Adsorption profile of phenol at various pH conditions (35°C, 0.5g/L of initial concentration of phenol)

From the graph maximum adsorption is observed at pH 4. In other cases adsorption is reduced.

CONCENTRATION EFFECT:

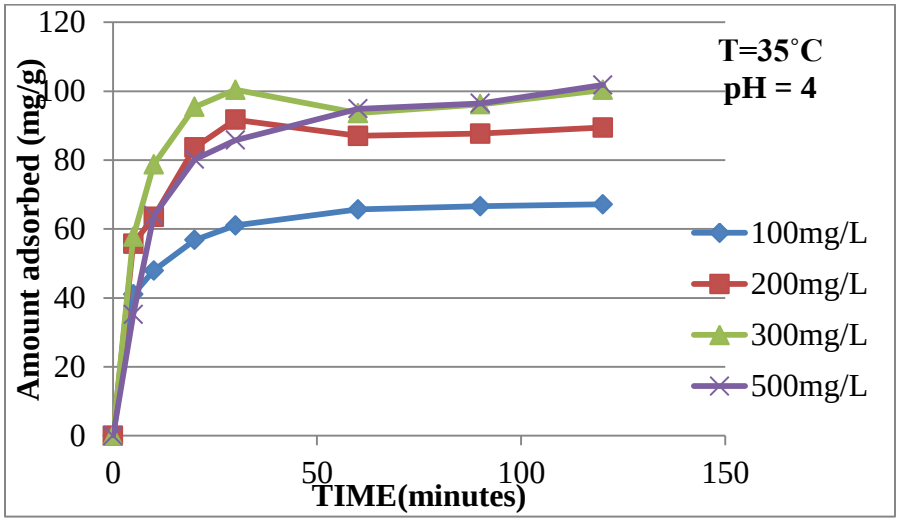

Fig 4.10 Adsorption profile of phenol at various initial concentration of phenol (35°C, pH 4)

From the graph maximum adsorption is observed at 100 mg/L of initial concentration. In other cases adsorption is less.

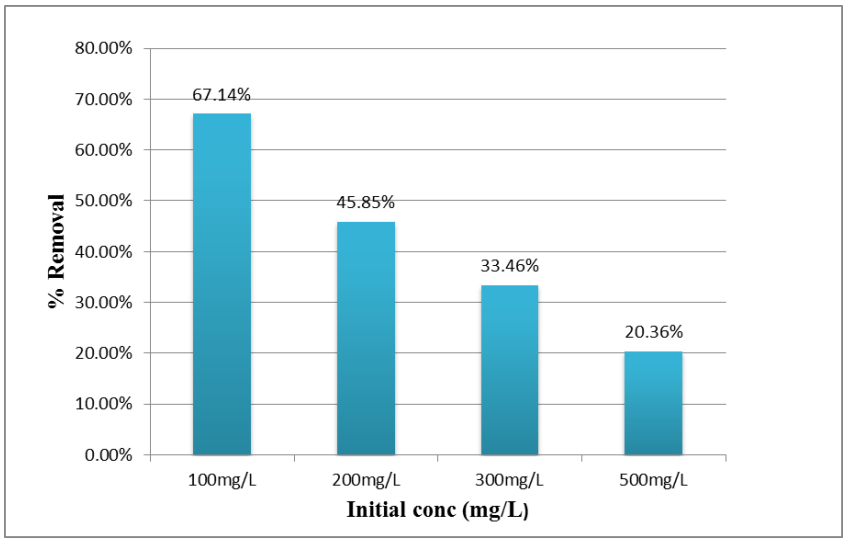

Fig 4.11 %Removal profile of phenol at optimized conditions:

The maximum percentage of phenol removed by adsorption is 67.14 under optimized conditions.

4.2.3 COMBINED EFFECT:

Table 4.8 Effect of concentration on degradation of phenol by immobilized cell at 7pH and 35˚C

CONC\TIME	4hrs	8hrs	12hrs	16hrs	20hrs	24hrs
100ppm	0.13387	0.1202	0.11468	0.10684	0.09468	0.07684
200ppm	0.7494	0.74368	0.71210	0.69284	0.64224	0.6302
300ppm	1.11096	1.10716	1.0982	1.0851	1.07436	1.06488
400ppm	1.9653	1.5438	1.37811	1.187	1.0672	1.0311
600ppm	3.76304	3.4310	3.1101	2.87605	2.543	2.1120

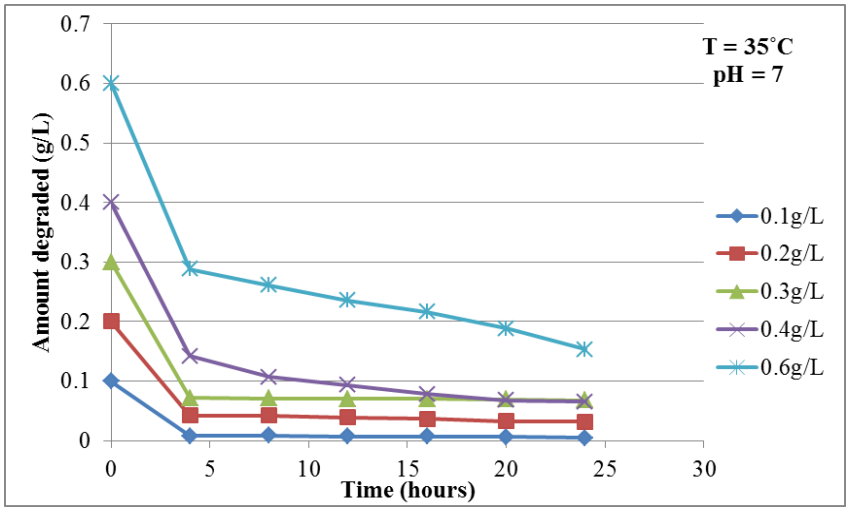

Fig 4.12 Degradation profile of phenol at various initial concentration of phenol by immobilized cells (35°C, pH 7)

It is found that maximum degradation occurs at 0.1g/L of initial phenol concentration under optimized conditions for immobilized cells.

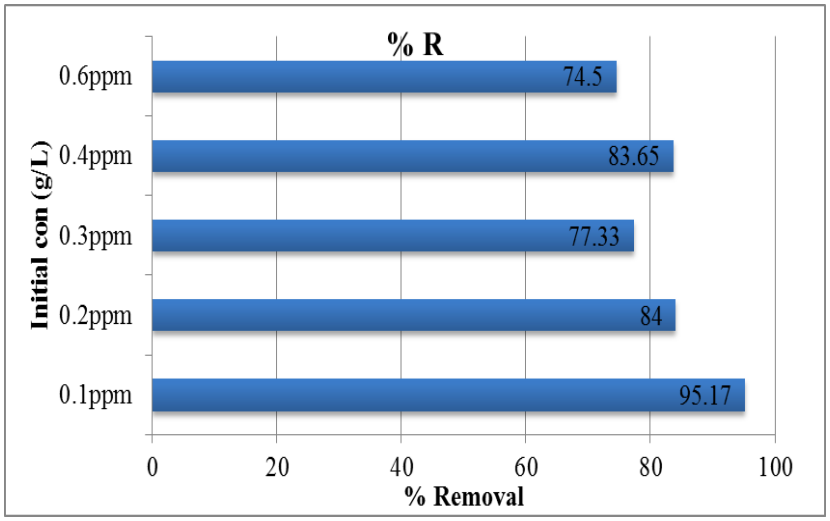

Fig 4.13 %Recovery profile of phenol under optimized conditions for simultaneous adsorption and immobilization:

The maximum percentage of phenol recovered by immobilization of bacterial strain S4 is 94.23% with initial phenol concentration of 0.1g/L under optimum conditions.

COMPARISION:

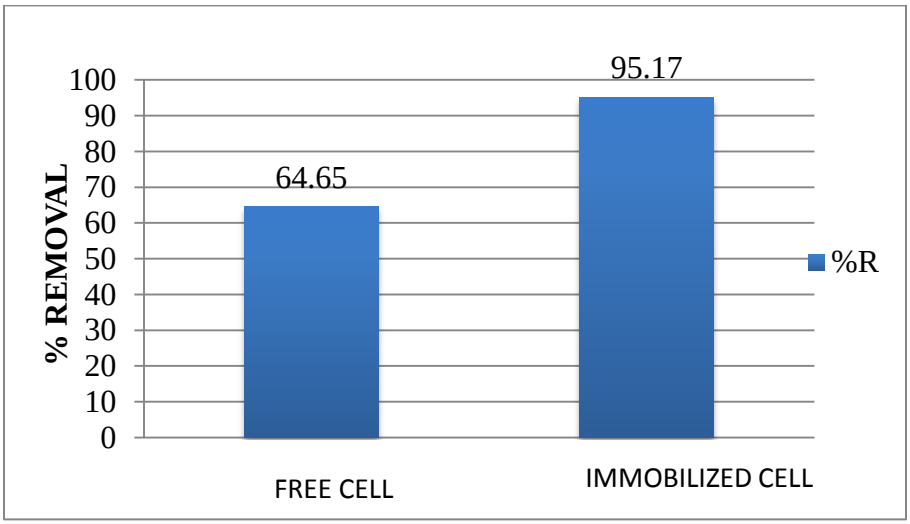

Fig 4.14 %Removal profile of phenol under optimized conditions by free cell and immobilized cell

From the graph it is observed that biodegradation by immobilized cell removes phenol by 95.17% and that by free cell is 64.65%. The phenol removal efficiency of immobilized cells is 1.47 times than that of free cells.

CHAPTER 5

CONCLUSION

Biodegradation is one of the most economical methods with no production of toxic by-products. Generally, this method is preferred due to lower costs and possibility of complete mineralization. The biodegradation study of phenol is carried out in MSM broth with phenol as the sole carbon source and energy. The strain which is isolated is named S4 and phenol degrading capacity of the strain is initially evaluated. The effect of temperature, pH and phenol concentration on the rate of degradation of phenol by that particular strain is carried out. The optimal conditions for phenol removal are found to be incubation temperature of 30°C, pH of 8, and concentration of phenol of 200 mg/L.

Adsorption is one of the efficient methods for phenol removal where the adsorbent can be reused for several times thus increasing the efficiency of removal and reducing waste disposal problems. The effect of temperature, pH and phenol concentration on the rate of adsorption is carried out. The optimum conditions for adsorption are found to be pH of 4, temperature of 35°C and concentration of phenol of 100 mg/L.

The immobilization technique used is adsorption. The phenol degrading bacteria is immobilized on activated carbon by simultaneous adsorption and biodegradation. Thus it removes phenol more efficiently as both adsorption and biodegradation processes are involved. The optimal conditions for removal of phenol are found to be pH of 7, temperature of 35°C and concentration of phenol of 100 mg/L.

Immobilized cells are found to be more efficient in removing phenol than free cells. The removal efficiency is 64.65% for free cells at 200ppm, 8pH and 30˚C as compared to that of 95.17% for immobilized cells at 100ppm, 7pH and 35˚C. The efficiency is 1.47 times more for immobilized cells than free cells.

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