

Bioremoval of phenol using immobilized cells of *B.pumilus* under batch and recycling condition and analysis of the biodegraded product

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Abstract:

Water pollution occurs when pollutants are directly or indirectly discharged into water bodies without adequate treatment to remove harmful compounds. Phenol along with other xenobiotic compounds is one of the most common organic pollutants present in effluents from chemical process industries. Due to the high toxicity of phenols, they are strictly regulated and their industrial use is increasingly avoided by substituting them with harmless compounds. Phenol contaminants are relatively soluble in water and accumulate in soil, resulting in extensive surface water, ground water and soil contamination owing to its severe toxicity.

Materials: *Bacillus pumilus* strain SCH2JF914985, a newly phenol-degrading bacterium with high biodegradation activity and high tolerance of phenol and phenolic compound was isolated from the coal industrial area of Dankuni, West Bengal, India. The present investigation made an attempt to make a comparative analysis of bio-removal of phenol by immobilized cells of *Bacillus pumilus* strain SCH2JF914985 under batch culture and Recycling condition.

Result: Bioremoval studies were conducted with immobilized cells at various phenol concentrations ranging from 50ppm to 700ppm. The treated products were then analysed by HPLC in order to get an idea about the mechanism of degradation.

Conclusion: From the analysis of the microbiologically treated phenol samples by HPLC it was concluded that there was removal of phenol.

Keywords: Phenol, *Bacillus pumilus* strain SCH2JF914985, HPLC

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I. Introduction

Industry needs water as its major utilities. Moreover it is vital for all known forms of life. Water covers 71% of the Earth's surface, but only one percent is accessible as surface fresh water. Water pollution occurs when pollutants are directly or indirectly discharged into water bodies without adequate treatment to remove harmful compounds. Phenol along with other xenobiotic compounds is one of the most common organic pollutants present in effluents from chemical process industries. Due to the high toxicity of phenols, they are strictly regulated and their industrial use is increasingly avoided by substituting them with harmless compounds. Phenol contaminants are relatively soluble in water and accumulate in soil, resulting in extensive surface water, ground water and soil contamination owing to its severe toxicity [1]. Various industries like pharmaceutical, petroleum refining, pesticide manufacturing, synthetic resin, wood pulp, coke and coal chemical plants are rich source of phenolic wastewater [2]. Also these compounds form complexes with metal ions discharged from other industries, which are carcinogenic in nature. They are water soluble and highly mobile. The Environmental Protection Agency (EPA) calls for lowering phenol and phenolic content in the wastewater to less than 1 mg/L. Among various methods available, biodegradation is environmental friendly and cost effective for pollution remediation in the environment [3] and produces no harmful end products.

Many studies have been reported on degradation of phenol and phenolic compounds by bacteria. The genus *Pseudomonas* is widely applied for the degradation of phenolic compounds [4]. However, the microorganisms suffer from substrate inhibition at higher concentration of phenol, by which the growth is inhibited. The gram positive bacteria *Bacillus pumilus* strain SCH2JF914985 [5] was found to be very efficient in removal of high concentration of phenol. Free bacterial cells can be used for treatment of industrial waste products, but at times they create problem due to wash out of the cells. These cells mostly accumulate their secondary metabolites in the stationary phase during their growth. Entrapment of the cells is one of the means to create non-growth condition under which the production of secondary metabolites can be avoided.

Immobilized microorganisms have been shown to be effective to treat phenol-containing wastewater and have been receiving increasing attention [6].

The present investigation made an attempt to make an analysis of bio-removal of phenol by immobilized cells of *Bacillus pumilus* strain SCH2JF914985 under batch culture and recycling condition. Bioremoval studies were conducted with immobilized cells at various phenol concentrations ranging from 50ppm to 700ppm and it was observed that better removal could be achieved with immobilized cells under recycling conditions. The treated products were then analysed by HPLC in order to get an idea about the mechanism of degradation. High performance liquid chromatography is a technique to separate the components of a mixture, to identify and quantify each component.

II. Material and Methods

Organism: *Bacillus pumilus* strain SCH2JF914985 earlier isolated from samples taken from Dankuni coal complex was used for removal of phenol in our studies. The bacterial strain was grown in 100 ml of modified mineral salt media having the following composition (g/L): Lactose: 15, beef extract: 3, KH_2PO_4 :1, KCl : 0.5 , NaNO_3 : 2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.5, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 0.01 supplemented with phenol up to 5 mM as the source of carbon and energy in 250 ml Erlenmeyer flasks at 37°C for 24 h.

Immobilization of the strain: The phenol degrading bacteria was harvested after 12 hr of growth from 1 L of culture medium. The cells obtained by centrifugation at 5000 rpm for 10 min at 4°C was washed with normal saline water and was used for immobilization in alginate matrix. The alginate entrapment of cells was performed according to Bettemann and Rehm [7].

Estimation of phenol: When Phenol compounds reacts with 4-amino antipyrine at pH 7.9±0.1 in the presence of potassium ferricyanide it forms a coloured antipyrine dye which can be measured spectrophotometrically. 70µl of 0.5N NH_4OH solution was added into the sample and pH was adjusted to 7.9±0.1 with phosphate buffer. 30µl of 4 amino antipyrine solution was added and mixed well and then 30µl of $\text{K}_3\text{Fe}(\text{CN})_6$ solution was added to develop the colour. After 15 min absorbances of samples were determined at 550nm by UV-visible spectrophotometer and phenol concentration was estimated from the standard curve.

Removal of Phenol by immobilized cells under batch culture: The beads were added to 250 ml conical flask containing 100 ml of MSM and various concentrations of phenol. The experiment was performed at 37°C with initial pH of 7.0 on a rotary shaker at 150 rpm for 48hour. Samples from the culture broths were taken at desired time interval for residual phenol analysis. Control flasks were incubated in parallel under the same conditions to ascertain the evaporation losses of phenol.

Removal of Phenol by Packed Bed Bioreactor of Immobilized Cell: Reactor type used in this experiment was a packed-bed bioreactor (Fig.1) with Ca-Alginate beads. It was operated at room temperature (35- 37°C) and initial pH 7. The column was partially filled by the immobilized beads at a bed height of 20cm. The initial concentration was taken as 50ppm to 700ppm. Initial concentrations were changed time to time and each set was continued for 48 hr. 100 ml of phenol solution were poured in feed tank and circulated through the bed at a flow rate of 5ml/min with a peristaltic pump. At specific intervals about 5ml of sample was collected and was centrifuged to get a clear solution. The residual phenol content was measured.

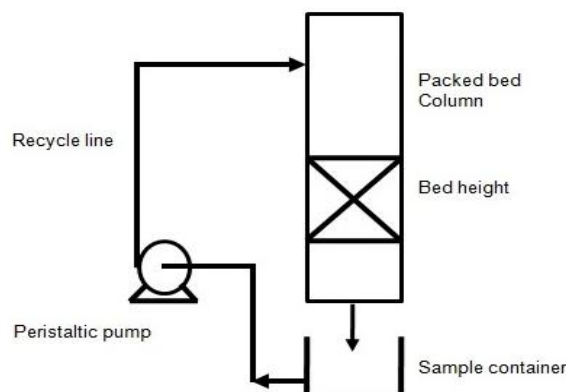


Figure1: Schematic diagram of Immobilized cell-Packed Bed column

Comparative study on the effect of using immobilized cells in packed bed and in batch culture:

Same amount of beads were used for preparing packed bed column (20 cm) and for conducting batch operation in conical flask (containing 100ml sample in 250 ml flasks). After 48 hr the samples were analyzed for residual phenol content.

The samples for which complete removal of phenol were achieved were further analysed by HPLC.

HPLC analysis: In this technique a pressurised liquid solvent containing the sample mixture is pumped through a column filled with a solid adsorbent material. Each component in the sample reacts slightly differently with the adsorbent causing different flow rate for different component and leading to separation of the components as they flow out of the column made of silica. The HPLC instrument includes sampler, pump and detector. The sampler brings the sample mixture into mobile phase stream which carries it into column. The pump controls the desired flow rate. The composition of mobile phase is governed by the column material. The detector generates a signal proportional to the amount of sample component emerging from the column.

A HPLC analysis of the control (i.e. reaction mixture with all the components except inoculum) and of the biodegraded products were done simultaneously. The sample for this analysis was prepared by taking 1ml of culture sample, which was first acidified by adding 0.1 ml of 6.0 N HCl and saturated with NaCl, and then it was extracted with 3ml ethyl ether. The ether extract was evaporated to dryness. The HPLC (Waters HPLC system) analysis was performed with a C₁₈ column (Spherisorb; 4.0x250mm; particle size, 5µm; HiQ sil C18HS no.OHS00013, Kyatech Corporation) with methanol, water and acetic acid (40:58:2) as the mobile phase with a flow rate of 1ml/min.

III. Results and Analysis

3.1 The effect of initial phenol concentration on % removal under batch culture is presented in Figure 2.

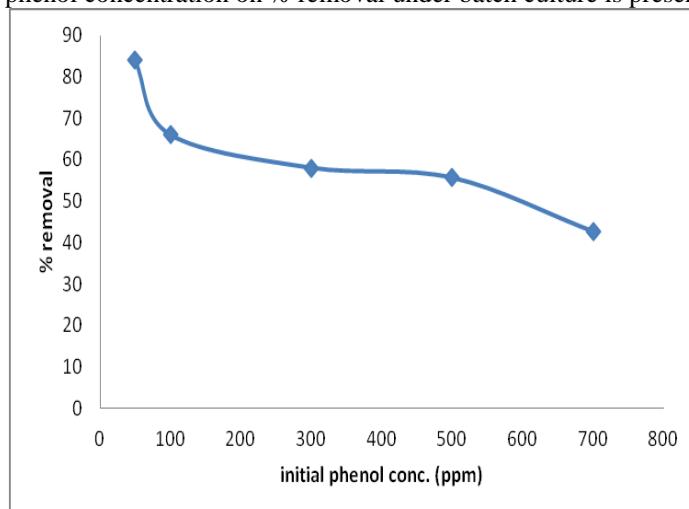


Figure 2: Effect of initial phenol conc.on removal

In the present investigation it is found that as the initial concentration of phenol increased the efficiency of removal decreased. Maximum removal of 84% is obtained when the concentration is least i.e., 50ppm within 48 hr. The carrier material of the immobilized cell act as a protective shelter against the toxicity of phenol. In immobilized beads as the cells are in non-growth condition, therefore although the concentration of phenol increases, due to lack of increasing cell numbers the immobilized biomass fail to show good consistency in removal at higher concentrations.

3.2 The effect of removal of phenol by the immobilized cells through packed bed column reactor is shown in Figure 3.

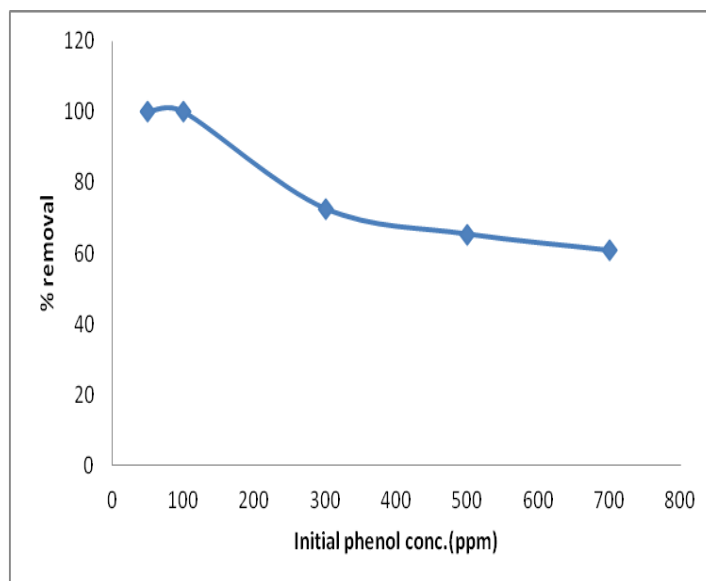


Figure 3 : Effect of initial phenol conc. on % removal in packed bed column under recycling condition

From the above figure it is observed that at the bed height of 20 cm the phenol is almost completely removed after 48 hr of recycling. This is due to the fact that the molecules of phenol remain in contact of the cells more with subsequent recycling. It is also observed that as the initial concentration is increasing the percentage removal is decreasing. This is due to the inhibition of substrate. It is found that complete removal of phenol is possible up to an initial loading of 100ppm. Accordingly if the initial concentration of the effluent is over 100ppm phenol it has to be diluted before treatment by this method.

3.3 The results of comparative study on the effect of using immobilized cell packed bed instead of immobilized batch culture is presented in Figure 4.

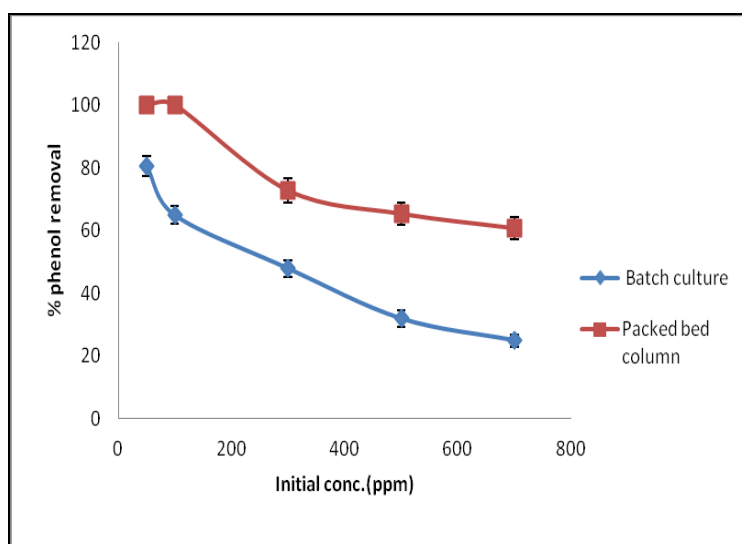


Figure 4: Effect of immobilized cells in packed bed and in batch culture on removal of phenol

Comparative study on the effect of using immobilized cell packed bed reactor instead of immobilized batch culture on removal of phenol showed that the packed bed is more effective. Sample containing 50ppm and 100ppm phenol could be completely removed within 48 hr of recycling in packed bed.

3.4 Analysis of the product in HPLC

After treatment one of the solutions (100ppm phenol) and the control were analysed by HPLC.

The extracts of control and the treated samples of phenol were injected in the mobile phase and then analysed. In case of phenol standard (The sample purchased from Merck) it gave a peak at retention time (RT) 7.7min(Fig

5), the peak was present in the control sample at the same retention time of 7.7min(Fig6) which was completely absent in the treated sample(Fig7). This indicated absence of phenol.

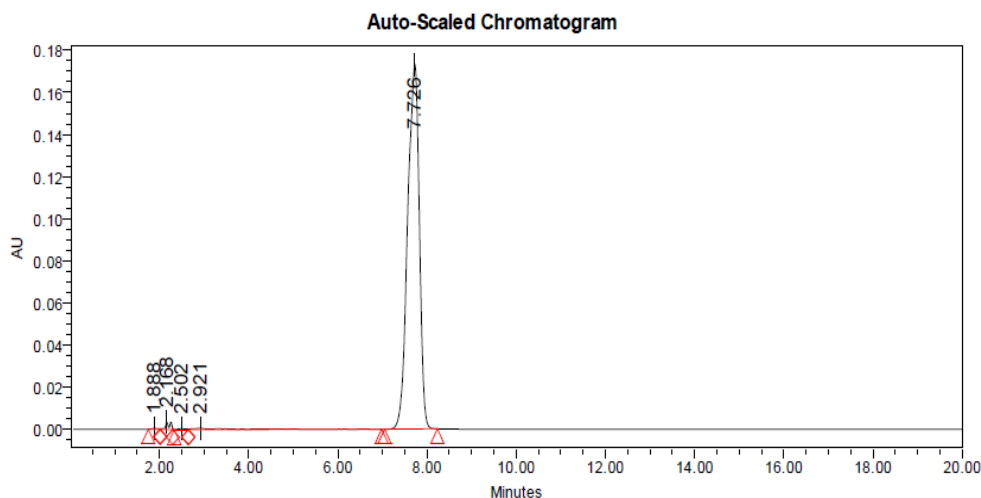


Figure 5: HPLC analysis of pure phenol solution

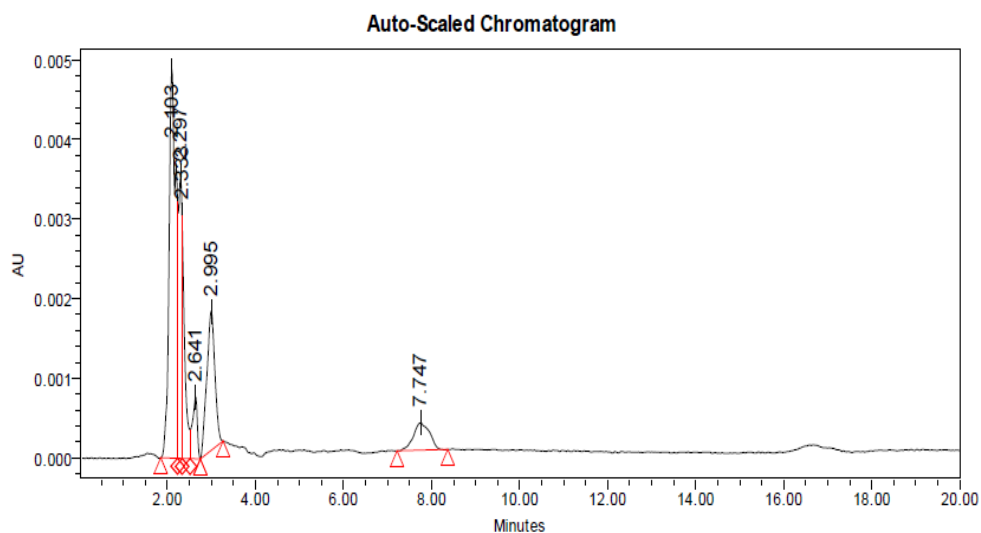


Figure 6: HPLC analysis of phenol control

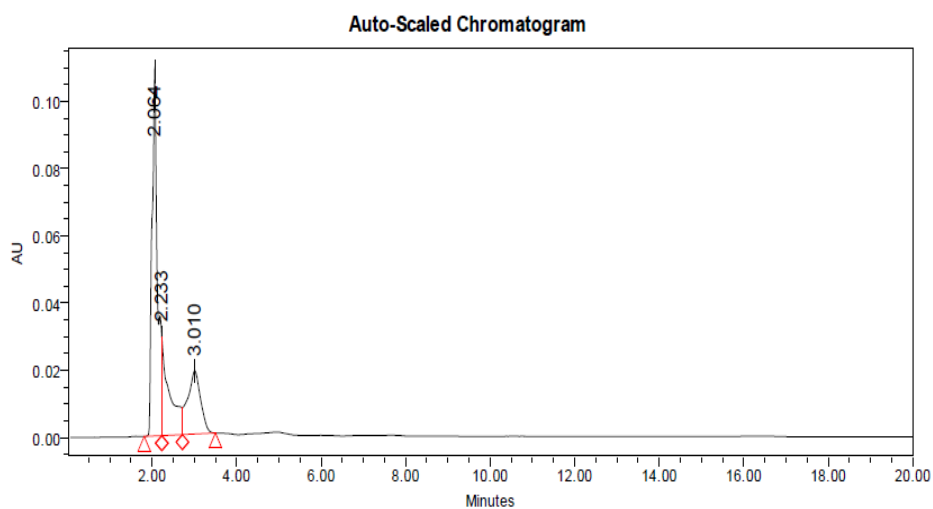


Figure 7: HPLC analysis of sample treated by *B. pumilus*

IV. Conclusion

From the above studies it can be concluded that the strain *Bacillus pumilus* strain SCH2JF914985 immobilized on calcium alginate matrix could be effectively utilized for phenol removal. It is found that as the initial concentration of phenol increased the efficiency of removal decreased. Higher concentration of phenol will require more time for complete removal. It is also observed that complete removal of phenol is possible up to an initial loading of 100ppm. Accordingly if the initial concentration of the effluent is over 100ppm phenol it has to be diluted before treatment by this method. For this reason recycling methodology in packed bed column reactor is being recommended for the removal of phenol as this method provides with the potential homeostatic condition provided by packed bed column of immobilized cells. The recycling of effluent added extra potential to the bio- removal due to the enhanced contact time.

From the analysis of the microbiologically treated phenol samples by HPLC it can be concluded that there is removal of phenol. Further study needs to be conducted with the treated samples to know the nature of the removal products.

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