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CONTINUOUS FLOW WASTE WATER PURIFICATION WITH IMMOBILIZED *CELLS*

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ABSTRACT. An efficient procedure using a fixed bed column reactor for phenolic wastewater purification with immobilized *Pseudomonas putida* cells is presented. The influence of several parameters like, temperature, pH and the size of the immobilized cell particles upon the efficacy of the phenol remove is discussed.

Keywords: *Pseudomonas putida, optimal phenol degradation, immobilized cells, optimal temperature and pH, fixed bed column reactor*

INTRODUCTION

One of the methods involving low costs in maintaining the bioremediation activity by prolonging the life of microorganisms without nutrient supplementation is based on the use of immobilized cells. In the process of immobilization, the biological stability of microorganisms increases when facing toxic compounds in nutrient-poor environments. By immobilization, cells increase their tolerance for organic environments and a number of chemical materials like phenols or halogenophenols[1-3]. Using immobilized cells has several advantages over against processes using cells not immobilized:

- retention of a larger quantity of microorganisms in the reactor;
- protection of cells from toxic substrates;
- separation of suspended biomass from wastewater effluents.

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Cells can be immobilized in various materials, including alginates, agar, agarose, chitosan, activated carbon, polyacrylamide, polyurethane, cellulose, collagen and polymeric membranes. Using immobilized cells allows the realisation of continuous processes and an increasing productivity of industrial installations [4-6].

There is a relatively wide range of industrial bioreactors in which reactions are carried out with immobilized biocatalysts like stirred-tank reactors, continuously operated stirred-tank reactors, fixed bed reactors, fluidised bed reactors and membrane reactors.

In the industrial application of the process of phenol degradation, the immobilization of the biocatalyst is the most advantageous method. Immobilising biomass in solid structures makes it possible to retain a material with the necessary characteristics depending on the type of equipment used in the technological procedure [7-9]. These solid structures, with the necessary dimensions and mechanic resistance, are rigid and porous enough to be used in conventional unit operations. Another advantage of using immobilized cells is that they allow the regeneration of the biocatalyst, confirming once again that the adopted method allows the elaboration of an optimal technological procedure.

RESULTS AND DISCUSSIONS

The objective of the current study was to investigate the capacity of *Pseudomonas putida,* cells, immobilized in alginate gel, in the biodegradation of the phenol, in a continuously operating system. The effects of several key parameters that can influence the biodegradation process were also studied: temperature, the pH-value of the solution, as well as the dimension of the alginate beads. Phenol degradation tests were made using both immobilized and non-immobilized cells for a comparative study.

The influence of temperature

Knowing that both the process of the phenols metabolism by the microorganism and the process of cellular growth is highly influenced by the temperature, one has to determine the optimal value of this parameter.
The experiment was made in suspension, as well as with immobilized cells in a vessel with magnetic stirring (at low speed of 150 rpm to avoid the mechanic degradation of the cells or the alginate beads), thermostated at temperatures in the range of 25-50 ^{o}C. The initial phenol concentration was 8 mM in all cases. The efficiency of the process was assessed by determining the residual phenol concentrations after 24 hours. It can be noticed that dephenolation reaches the maximum degree at 37 ^{o}C both in cellular suspension and with cells immobilized in alginate gel. At temperatures higher than 40 ^{o}C, the decrease of phenol concentration is almost insignificant, which is a sign that cells are less viable at such temperature levels (Figure 1).

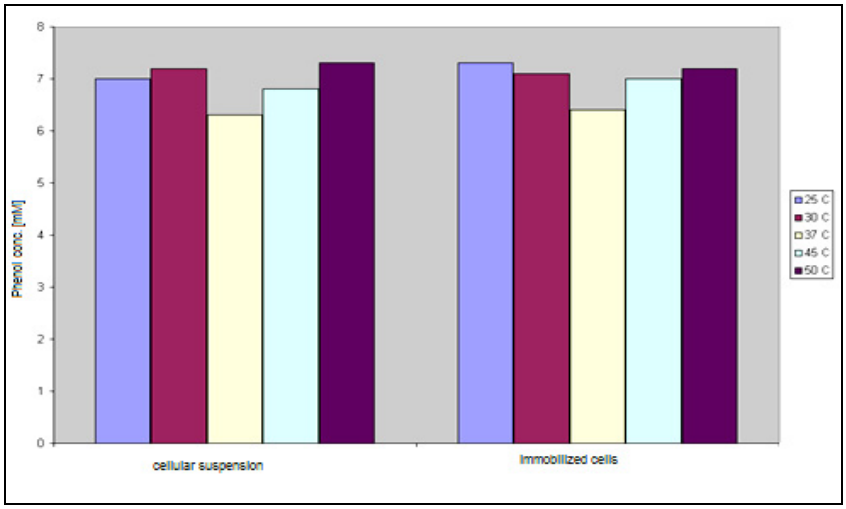

Figure 1. The influence of temperature on phenol degradation

Influence of pH

The influence of pH was studied in five series of experiments, with *Pseudimonas putida* cells in suspension (free form) with pH-values between 4 and 8. In order to reach the required parameters, phosphate buffers were used in preparing the culture environment. After adding all nutritive components to the environment, pH values were corrected as applicable with diluted solutions of either NaOH or HCl. Phenol concentration was determined by spectrophotometric method after 24 and 48 hours. The results are presented in Figure 2.

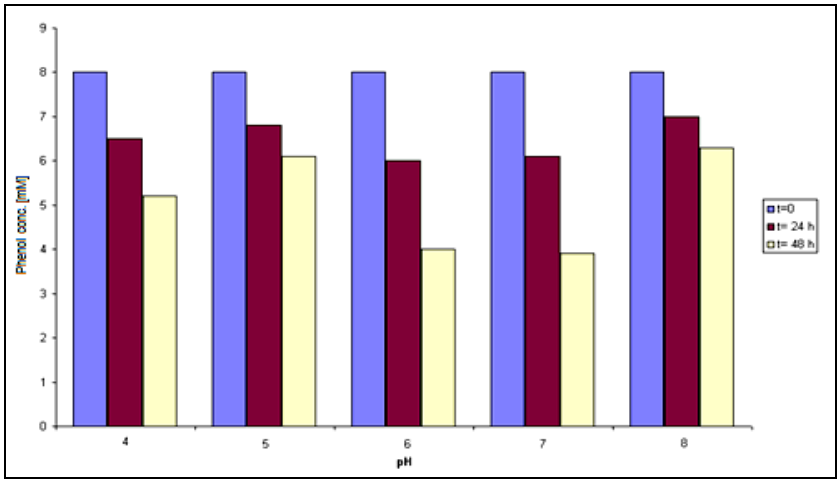

Figure 2. The influence of pH on phenol degradation

As it can be observed, at pH values of 6 and 7 one reaches a maximum decrease of concentration after the first day (ca. 24%), and after two days phenol concentration decreased to about 50%. At more acidic or basic values of the pH, the concentration decrease in the solution was lower.

The influence of the dimension of the alginate beads

As in all processes taking place in a heterogeneous system, this process is strongly influenced by mass transfer. When immobilising the cells in alginate gel, the dimension of the alginate beads determines the size of the surface of contact between the biocatalyst and the phenol, which influences significantly the efficiency of the degradation process. The alginate beads of different dimensions can be prepared with syringe needles of different diameters. The prepared beads were then sorted and used in the dephenolation experiments.

It can be observed that the efficiency was low at diameters of 5 mm and below, as well as for beads of 7 mm and above. Small alginate volumes possibly include insignificant quantities of biocatalyst even while contact surface is at maximum value. In case of beads of large dimensions, mass transfer processes are limited by the reduced contact surface size as shown in Figure 3.

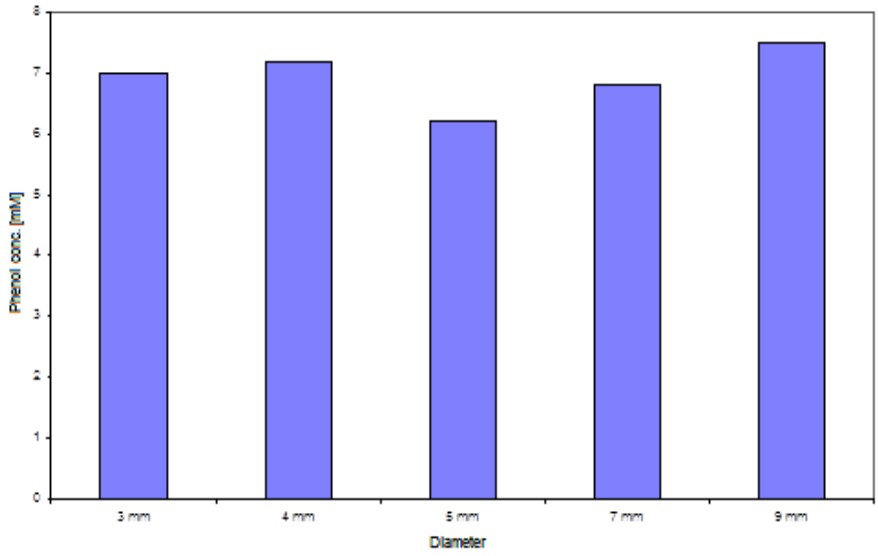

Figure 3. The influence of the diameters of the alginate beads on phenol degradation

Continuously operated fixed bed reactor for phenol degradation

Based on these experiments aiming to determine the optimal conditions under which phenol degradation takes place in wastewaters, a continuously operated fixed bed reactor was constructed for the degradation process of the phenol in presence of immobilized *Pseudomonas putida* cells in alginate gel in a packed column reactor.

The equipment was relatively simply constructed from a column in which the immobilized biocatalyst is introduced and a peristaltic pump was used to recirculate a certain volume of the phenolic solution.

Experiments with the installation for optimisation

The column reactor was continuously supplied with phenol solution with 18 mM concentration. Depending on the flow rate used, a certain degree of microbial dephenolation of the water was reached (Figure 4). At a flow rate of 0.2 mL/s, phenol concentration decreases with ca. 50%. If a further reduction of concentration level needs to be attained, the effluent is recirculated into the column. In practice, by recirculating the phenolic solution three times through the biocatalyst, the concentration of the phenol falls below the maximum level admitted by current regulations. Due to the long duration of the process and implicitly the low productivity of the proposed equipment, a flow rate below 0.2 mL/s is disadvantageous from an economic point of view. On the other hand, at higher flow rates, the higher energy consumption of the pump increases significantly the costs of operation of the installation.

CONCLUSIONS

The optimal pH, temperature and immobilized particle size for the efficient phenol remove was set up. The continuous flow procedure was superior in the phenol degradation process.

EXPERIMENTAL SECTION

Materials

- 100 g of immobilized cells
- 350 mL phenol solution, with an initial phenol concentration of 18 mM (the used peristaltic pump has a flow rate of 0.2 mL/s).

After putting the equipment into operation, solution samples were taken and their concentration established to illustrate the evolution in time of the biosorption process.

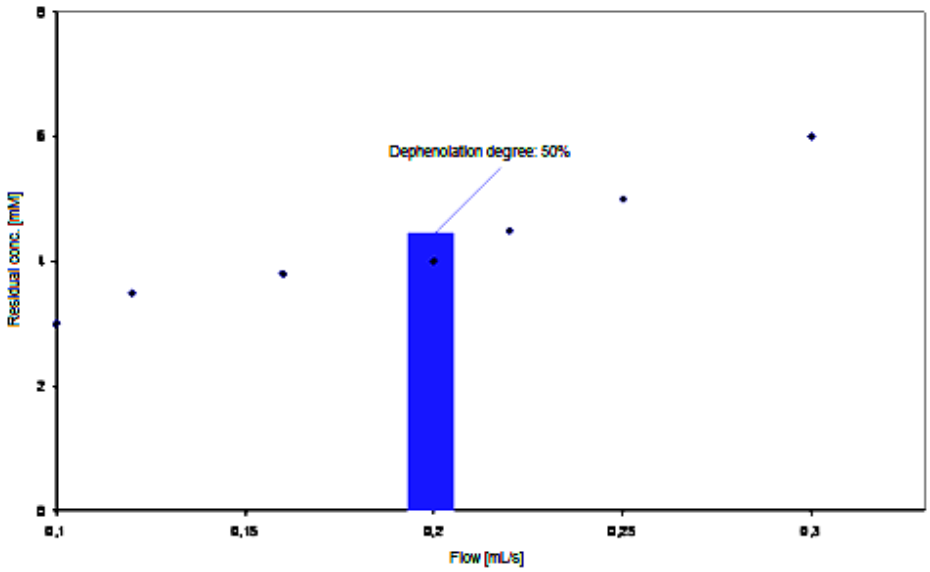

Figure 4. The influence of flow rate on the dephenolation degree

Culture media

The microorganism *Pseudomonas putida, was* cultivated on a minimal culture media containing (g/l) 3.4 K_2HPO_4, 4.3 KH_2PO_4, 0.3 $MgCl_2 \cdot 2H_2O$, 1 $(NH_4)_2SO_4$, glucose, lactate, succinate and benzoate were added as carbon sources. The cultures were incubated at 30 ^oC for 120 hours.

Inoculation

Colonies grown for 72 hours on agar plates with minimal culture media were inoculated in Erlenmeyer flasks containing 500 mL of the same media, and were grown for 48 hours in an incubator at 30 ^oC. Afterwards, cells were separated by centrifugation (at 1500 rpm) at 5 ^oC for 10 minutes and washed with sterilised water to eliminate small residues and intermediary components. The residue was suspended in a small volume of sterilised distilled water. Samples from this suspension were used as inoculum for the experiments of phenol degradation with both immobilized and non-immobilized cells.

Immobilization in alginate gel

2 g of sodium alginate was dissolved in 80 mL of distilled water in order to obtain a polymeric alginate solution. After sterilising the alginate (for 15 minutes at 120 ^oC), 20 mL of cellular suspension was added and homogenised by stirring. The mixture of cells and sodium alginate was put into a syringe. The solution of alginate and yeast was added slowly from a distance of 20 cm

to a vessel containing 100 mL solution of 1M $CaCl_2$. Beads of various diameters were formed, depending on the diameter of the needle and the applied pressure. The beads were preserved in $CaCl_2$ for 0.5-3 hours. Subsequently they were decanted and washed several times with distilled water.

Analytic methods

Phenol concentration was determined with spectrophotometer at 270 nm. For each phenol concentration used, the degradation speed corresponding to the degraded phenol quantity (gl^{-1}) was calculated after 24 hours. The maximal volumetric degradation speed corresponds to the highest value obtained for each phenol concentration. All experiments and determinations were carried out three times, reporting the medium of the obtained measurements.

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