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EFFICIENT DEGRADATION OF PHENOL WITH *PSEUDOMONAS PUTIDA* CELLS FOR THE PRODUCTION OF PURE WATER

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ABSTRACT. An efficient procedure of phenolic wastewater purification with immobilized *Pseudomonas putida* cells is presented. The selection of the proper microorganism is described. The influence of the initial phenol concentration upon the efficacy of the phenol remove using free and immobilized cells is discussed.

Keywords: *Pseudomonas putida, phenol degradation, immobilized cells, alginate, wastewater treatment, human health*

INTRODUCTION

The contamination of the environment with toxic chemical materials is considered one of the major problems of industrialisation in our days. The phenol, which is found in industrial- and wastewaters, is regarded by the National Environmental Protection Agency as a primary compound of contamination. Due to the highly toxic nature of this substance, removing it with the help of microorganisms is of critical importance. Various types of microorganisms, including bacteria, yeast, algae and fungi evolved metabolically for degrading different concentrations of phenol [1-3]. The versatility and adaptability of microorganisms in degrading phenols from wastewaters shows their technological usefulness to dephenolate soil and waters.

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The process of the microbial degradation of phenol is a result of the combination of enzymatic and cellular activities, as well as the activities of cellular communities in microorganisms [4-6]. These activities depend on environmental factors, including the level of nutrients, the availability of alternative substrates, microbial population density, the concentration of contaminated substrates, and bioavailability.

Widespread use of toxic phenols has led to their accumulation in large quantities in the environment. This phenomenon calls for a scientific assessment of the impact of phenols on the environment and their influence on microorganisms, the development of analytical methods for a qualitative and quantitative evaluation, the study of processes of phenol degradation to find the most optimal methods for environmental remediation. Water pollution and implicitly the contamination of aquatic organisms with various types of organic pollutants such as phenols has stimulated research in developing technologies to depollute and especially degrade contaminants from natural and wastewaters.

Using microorganisms for dephenolating polluted waters is a simple and inexpensive method, almost as efficient as the enzymatic technology. By making use of the entire enzymatic equipment of the cells, we can reach complete degradation up to the formation of CO_2, H_2O, halogen ions (for halogenophenols) or non-toxic organic molecules, such as acetic acid in case of acetobacteria, or methane in case of methanogenic bacteria [7, 8].

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 [9-13]. 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.

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.

RESULTS AND DISCUSSIONS

Selecting the microorganism

A comparative study of phenol degradation was made with immobilized and free cells of *Pseudomonas putida, Acinetobacter sporulens* and *Rhodotula glutinis,* cultured with an initial concentration (0.176 g of dry biomass / flask) in both systems. In order to identify the optimal microorganism, a preliminary study was made only with free cells. The residual concentration of phenol in the water was determined after 24, 48, 72, 96 and 120 hours. The results obtained are presented in Figure 1.

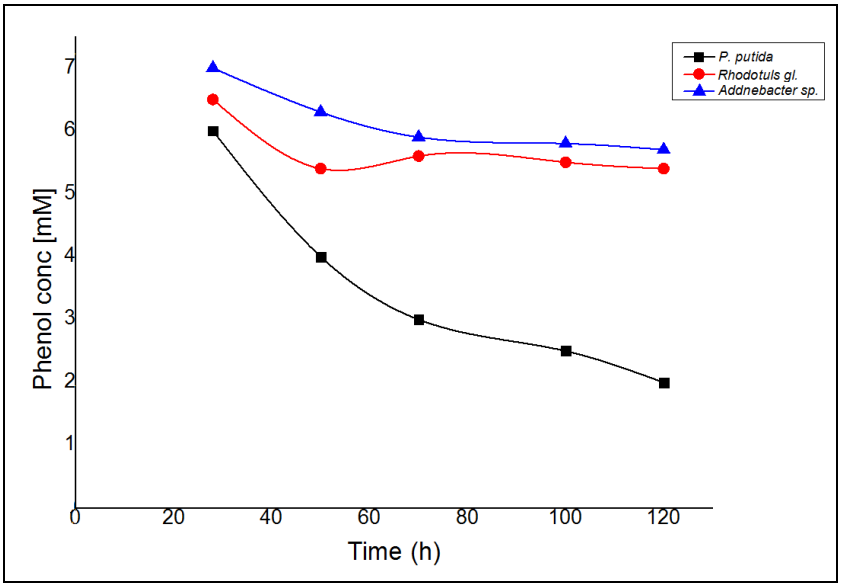

Figure 1. The study of phenol degradation with *Pseudomonas putida, Acinetobacter sporulens* and *Rhodotula glutinis* cells.

The influence of the initial concentration of the phenol solution

As it can be observed, maximal efficiency in phenol removal was obtained with the *Pseudomonas putida* culture. Therefore further experiments were made with cultured cells of *Pseudomonas putida* in both suspended and immobilized form in calcium alginate gel.

Simultaneously, in order to distinguish between phenol concentration decrease resulting from biodegradation and alginate adsorption respectively, an identical experiment was made with alginate beads without biomass, using the same recipe.

The experiments were made in Erlenmeyer flasks of 250 ml containing 40 mL of saline solution with different phenol concentrations (2, 6, 10, 16, 20, 26 and 30 mM). 10 g of biomass immobilized in alginate or non-immobilized biomass was put into the flasks and incubated at 30 ^{o}C for 120 hours. After complete mixture, samples were taken from the cultures at regular intervals (14, 24, 36, 48, 72 and 96 hours) in order to analyse the phenol and determine cellular density. In parallel, in other Erlenmeyer flasks control samples were incubated under the same conditions, but without inoculum to exclude phenol loss through evaporation.

The degradation capacity of phenol was studied and compared with the help of suspended and immobilized cells of *Pseudomonas putida* (Figures 2 and 3). The degree of phenol degradation and the time of degradation depends on the initial concentration of the phenol in the environment. An increase in the time of biodegradation was observed with the increase of the initial phenol concentration.

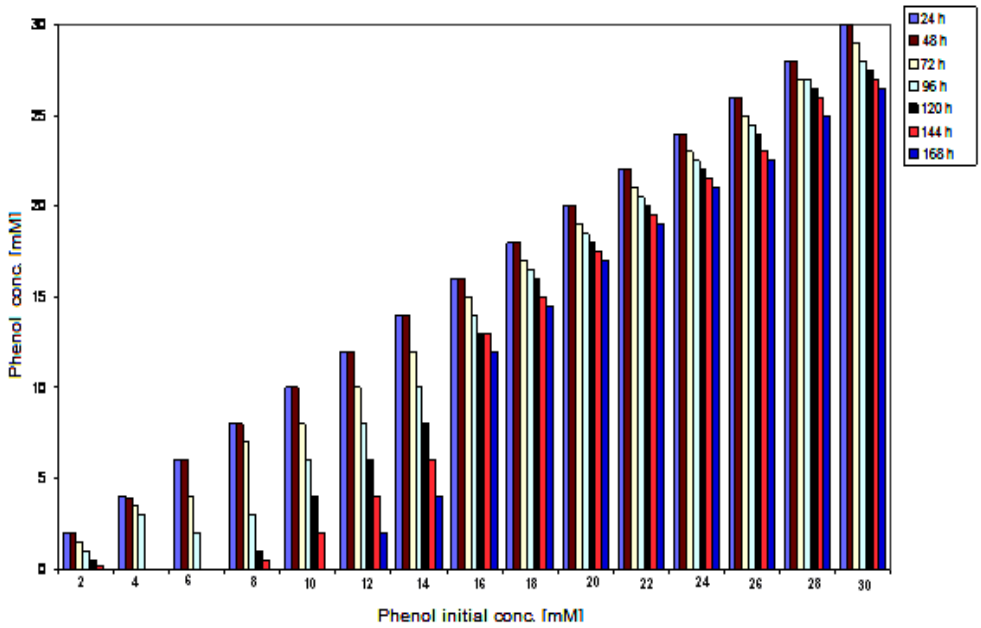

Figure 2. Phenol degradation in *Pseudomonas putida* suspension

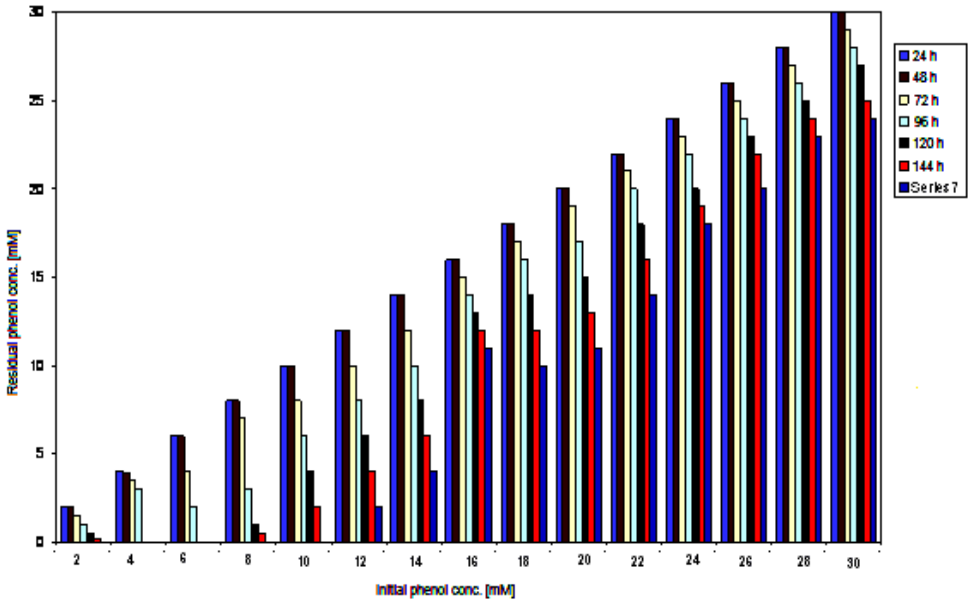

Figure 3. Phenol degradation with *Pseudomonas putida* cells immobilized in alginate

The phenol was completely removed in the experiments where the solution's initial phenol concentration was 16 mM using non-immobilized cells, and 18 mM using immobilized cells. For these initial phenol concentrations, more than 87% of the total phenol quantity was degraded in less than 72 hours.

For larger phenol concentrations, one can observe a decrease in the degree of phenol degradation and incomplete degradation in all experiments. This can probably be explained by the toxic effect of large concentrations of phenol upon the cells. Larger phenol concentrations presumably inhibit cell growth. In case of immobilized cells, the effect of this inhibition is smaller in comparison with its effect upon non-immobilized cells. In the control samples, no phenol degradation or decrease of phenol concentration was observable, which shows that no physical or chemical degradation had taken place. Concerning the alginate-sample without biomass, no significant decrease of phenol concentration was discernible.

The variations of the phenols volumetric degradation speed with initial concentrations between 2-30 mM for free and immobilized cells is presented in Figure 4.

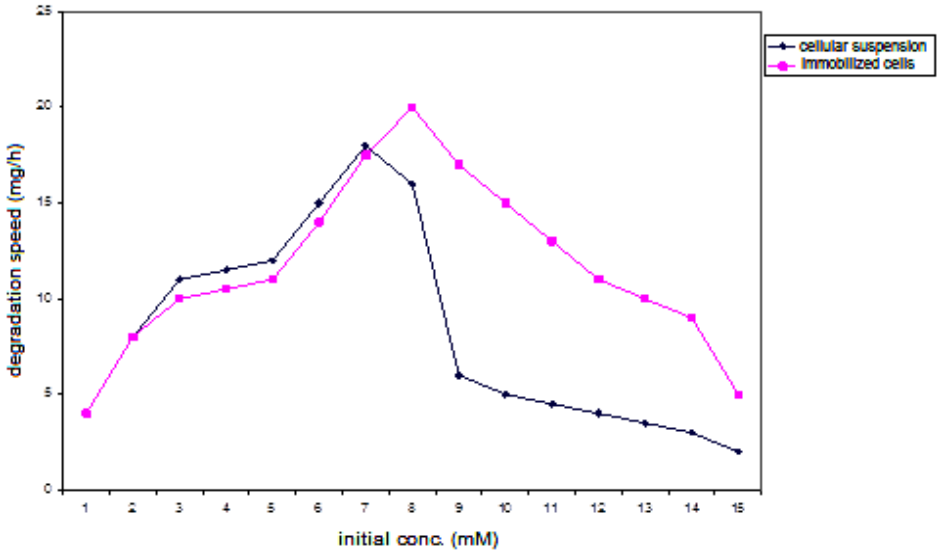

Figure 4. The phenol degradation velocity with *Pseudomonas putida* cells

As it is recognisable, with the increase of the phenols initial concentration, the phenols degradation speed also increases, reaching a maximum value of 20.45 mg/L*h (immobilized cells) and 18.35 mg/L*h (non-immobilized cells), with initial concentrations of 18 mM (immobilized cells) and 16 mM (non-immobilized cells). After this, the degradation speed decreases progressively. For initial concentrations smaller than 16 mM, the values of volumetric degradation speed show no variation between immobilized and non-immobilized cells (Figure 5). However, for initial concentrations of 20-28 mM, the specific degradation speed of immobilized cells was 2.8-3.5 times higher than in case of non-immobilized cells, while for an initial concentration of 30 mM, the specific degradation speed of immobilized cells was 1.7 times higher than in case of non-immobilized cells.

The microencapsulation mechanism using natural polysaccharides as encapsulating material presupposes polyelectrolyte complexation. Polyelectrolyte complexes form ionic interactions between two polyelectrolytes charged differently in aquatic solutions, which characterise a hydrophilic microenvironment with a large water content and electronic density. Hydrophilic polymers such as the alginate retain water. This hydrophilic capacity of the alginate can reduce the phenol-concentration around the alginate beads as a result of the partition effect.

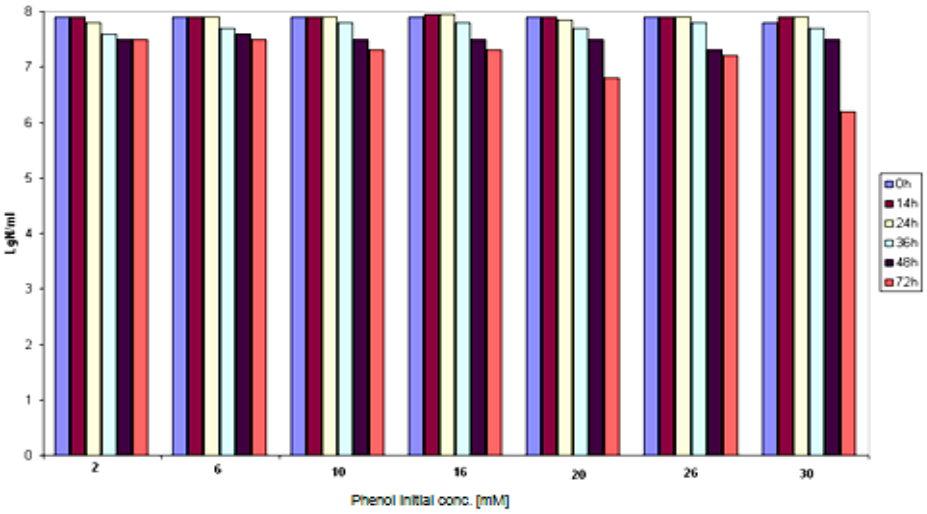

Figure 5. The viability of cellular suspension at different phenol concentrations

The viability in time of free and immobilized cells at different phenol-concentration levels is graphically presented in Figure 6. As it can be observed, enzymatic activity depends on the phenol-concentration and the used inoculum type (free or immobilized cells). The viability of free cells suspended in phenol-concentrations of 16 mM increases in the first 48 hours, followed by a decrease in time. For solutions with higher concentrations, cell activity is reduced even within the first 14 hours. The stability of immobilized cells increases in time even with higher phenol-concentrations suggesting that the process of enzyme release is continuous.

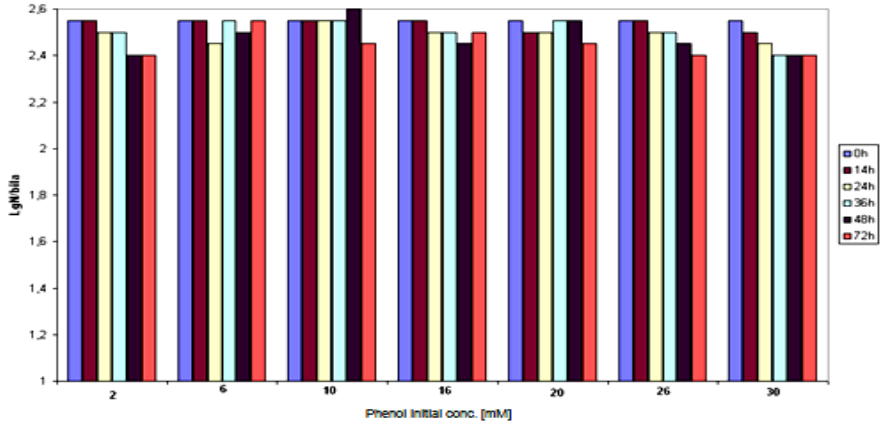

Figure 6. The viability of immobilized cells at different phenol-concentrations

CONCLUSIONS

It can be concluded that *Pseudomonas putida* proved to be the most efficient microorganism for the dephenolation of phenolic wastewaters. The initial phenol concentration is crucial upon the efficacy of the process. By immobilization the efficacy of the dephenolation process was increased.

EXPERIMENTAL SECTION

Materials

The microorganisms *Pseudomonas putida, Acinetobacter sporulens* and *Rhodotula glutinis* were obtained from Sigma Life Science Research. The phenol and the sodium alginate were acquired from Sigma-Aldrich.

Culture media

The microorganisms *Pseudomonas putida, Acinetobacter sporulens* and *Rhodotula glutinis* were 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.

REFERENCES

[1] V.V. Vel'kov, *Biotekhnologiya*, **1995**, 3−4:20.

[2] F.M. Khabibulina, A.A. Shubakov, I.B, Archegova, G.G. Romanov. *Biotekhnologiya*, **2002**, 6:57.

[3] Anokhina, T.O., Kochetkov, V.V., Zelenkova, N.F., Balakshina, V.V., Boronin, A.M. *Prikladnaia Biokhimia Mikrobiolgiya*, **2004**, 40:654.

[4] F. Bux, B. Akkinson, K. Kasan. *Water Science Technology*, **1999**, 39:127.

[5] A. Zumriye, A. Derya, R. Elif. *Process Biochemistry*, **1999**, 35:301.

[6] A, Fialova´, E. Boschke, T. Bley. *Biodegradation*, **2007**, 18:719.

[7] A.R. Bielefeldt, H.D. Stensel. *Biodegradation,* **1999**, 10:1.

[8] V. Kavitha, K. Palanivelu. *Chemosphere* **2004**, 55:1235.

[9] S.H. Yuan, X.H. Lu. *Journal of Hazardous Materials*, **2005**, 118:85.

[10] Z. Ai, P. Yang, X.H. Lu. *Journal of Hazardous Materials*, **2005**, 124:147.

[11] Z. Aleksieva, D. Ivanova, T. Godjevargova. *Process Biochemistry*, **2007**, 37:1215.

[12] Y.J. Liu, A.N. Zhang, X.C. Wang, *Biochemical Engineering Journal*, **2009**, 44:187.

[13] A. Banerjee, A.K. Ghoshal, *International Biodeterioration & Biodegradation,* **2011**, 65:1052.