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Removal and recovery of some phenolic pollutants using liquid membranes

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Abstract

In this work, the data obtained using bulk liquid membranes for removing mono and disubstituted phenolic nitro derivatives (p-nitrophenol, m-nitrophenol, 2,4-dinitrophenol) from a source of waste water are presented. Based on the speciation diagrams of the phenolic compounds the optimum operation parameters (as pH values) of feeding and receiving phases for the transport efficiencies were established. When the waste water source (feeding source) had an acidic pH, the efficiency to transfer the studied phenolic nitro derivatives through a chloroform liquid membrane into an alkaline receiving phase (pH = 12) was greater than 90%. The fortified samples from the residual sources, in a concentration range of 0.8 – 250 mg/L resulting after the nitrophenols removal, were tested at pH=2 and pH =7 for their biocide properties using Pseudomonas aeruginosa bacteria. The toxicity tests were realized on residual sources at pH=2 and pH=7. The experiments performed proved that the tested compounds do not have any bactericide effect within the studied concentration range against the Pseudomonas aeruginosa strain.

Keywords: nitrophenols removal, bulk liquid membranes, *Pseudomonas aeruginosa*

Introduction

Nitro aromatic compounds are present at a large scale in environment as herbicides, fungicides, insecticides, explosives, being also used as precursors for dyes and plasticizers [1, 2]. Due to their large presence and their effects, being ecotoxic [3], mutagenic [4], immunotoxic [5] and causing urinary tract infections [6], p-nitrophenol, m-nitrophenol and 2, 4-dinitrophenol are the most common nitrophenols. In such circumstances, United States Environmental Protection Agency (US EPA) introduced on the priority list of pollutants p-nitrophenol, m-nitrophenol and 2, 4-dinitrophenol [7], as they are harmful for plants, microorganisms, animals and human beings.

Nitrophenols can be removed from the environment by applying various techniques such as: thermal decomposition and oxidation [8], liquid membrane separation [9-11], liquid–liquid extraction [12-13].

Due to the high risk posed by the widespread of the nitrophenolic pollutants in the environment, new methods of decontamination and cleaning-up are required. Singh et al. [14] presented an extensive review over the conventional remediation techniques stressing that the bioremediation [15] is a promising method due to its lower price [15].

Most nitrophenols are resistant to microbial degradation, especially at high concentrations. In order to understand the biodegradability and after that to formulate a strategy for bioremediation, studies have been performed on the isolation of the bacteria able to degrade the nitrophenol.

Most of studies were realized using *Achromobacter xylosoxidans* [16] *Rhodococcus opacus* [17] *Pseudomonas stutzeri* [18] *Pseudomonas aeruginosa* [19-21] *Haloanaerobium praevalens* and *Sporohalobacter marismortui* [22].

There is little information regarding the biodegradability of the phenolic compounds by a microorganism in wet environments and with traces of chloroform. In this paper, a study on the phenol removal from nitrophenolic residual sources using liquid membranes was realized. Also, in order to establish the minimum inhibitory concentration, toxicity tests were achieved on residual sources containing traces of chloroform after removing the phenols.

Materials and methods

Reagents

All reagents were analytical grade and were used without further purification. The reagents: p-nitrophenol, m-nitrophenol, 2, 4-dinitrophenol were supplied by Merck, while the chloroform, used as membranary solvent, was supplied by Sigma Aldrich. The pH of the feeding source (pH = 2) and of the receiving phase (pH = 12) was adjusted using HCl (Merck) and NaOH (Merck). The pH was measured with a glass combined electrode using a Seven Multi Metler Toledo pH-meter.

Transport experiments

The transport experiments were performed in a wall in wall transport cell as presented in figure 1. The feeding source (FS) consisted of 10^{-3} mol/L either aqueous or acidic nitrophenol solution. The volume of the feeding source was 20 cm^3. The receiving phase (RP) was 10^{-2} mol/L NaOH solution with a volume of 7 cm^3. The two aqueous phases were separated by a chloroform membrane with a volume of 50 cm^3. The working temperature was of 25°C±1°C and the transport time was 3 hours. In order to obtain the feeding source and the receiving phase, the used distilled water was saturated with chloroform, while the chloroform membrane was saturated with distilled water.

Toxicity tests (the minimum inhibitory concentration determination), MIC

A *Pseudomonas aeruginosa* - strain ATCC 15445 was used as bacteria strain to test the microbial activity of the residual source obtained after the transport of the feeding source. The tests were realized on fortified samples in the concentration range 0.8 – 250 mg/L. It was used the diffusion disk method. According to this method, the standardized inoculum of the tested strain is inoculated in an agar medium with a discontinuous concentration gradient of the microbial substance.

The agar growth medium for *Pseudomonas aeruginosa* was incubated at 35°C for 24 to 48 hours.

Procedure to obtain the inoculum

The young culture of the testing strain was prepared as a suspension in sterile distilled water. Then, the microbial culture is triturated in 3mL sterile distilled water containing 10 - 15 glass pearls. The macerating flask is stirred until an opalescent suspension is obtained. After 10 minutes of rest, necessary for the air separation, the aseptic opalescent supernatant was collected and it was used for the dilution of inoculum.

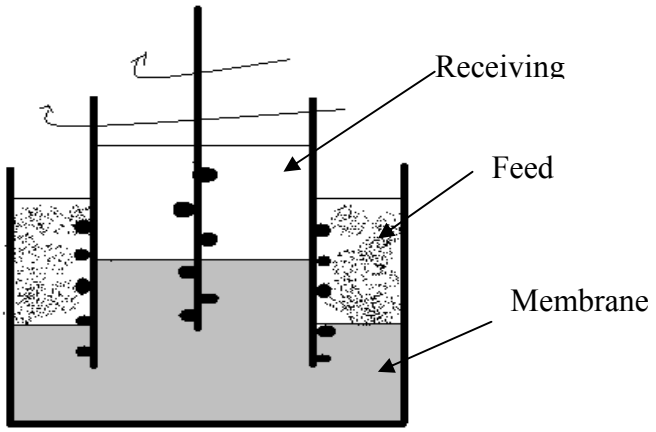


Figure1. Experimental device used for the transport experiments.

The inoculum needed to correspond to a 0.5 McFarland standard, meaning that there was assured $1{,}8 \cdot 10^7$ CFU/mL bacteria concentration.

For obtaining the antifungigram on the standardized subculture the preparation of the inoculum was similar to the method of dilution in agar. The disks with the substance were done on Watman 1 sterile filter paper and the gradient was the same as before mentioned in the procedure.

The inoculum was inoculated uniformly on the surface of the Petri dishes with agar medium and then it was allowed the absorption of inoculum for 3-5 minutes.

The disks with the testing antimicrobial substance were put at a distance of 15 mm from the margins of the dish and at 30 mm between them by pressing them firmly on the growth medium.

The Petri dishes were then incubated at 35°C for 24 to 48 hours for bacteria growth. Afterwards, the inhibition area was measured.

Results and Discussions

The neutral molecules of the phenolic compounds cross the liquid membrane due to either a concentration gradient or a chemical potential. Due to their acidic character in the aqueous solution, the phenolic compounds (ArOH) are partially dissociated according to the equilibrium:

$$ArOH + HOH \leftrightarrow ArO^- + H_3O^+ \quad (1)$$

The forming of the neutral molecules is pH controlled. At $pH<pK_{a,phenol}$ the uncharged form susceptible of crossing the chloroform membrane predominates. Based on the speciation diagrams $\alpha_c = f(pH)$ for the couple phenol-phenolate, where α_c is the formation degree:

$$\alpha_c = [H_cA^{(n-c)-}]/C_{H_nA} \quad (2)$$

it was established for which pH range the uncharged species were formed.

Relationship (2) gives the formation degree for the $H_cA^{(n-c)-}$ form which resulted from the ionization of the acid H_nA. The H_nA concentration was C_{H_nA} .

From this general formula for the couple phenol/phenolate, it follows:

$$\alpha_0 = [ArO^-]/C_{ArOH} = 1/1+10^{pK_a - pH} \quad (3)$$

$$\alpha_1 = [ArOH]/C_{ArOH} = 1/1+10^{pH-pK_a} \quad (4)$$

where: C_{ArOH} is the total concentration of the phenolic compound,
[Ar-O$^-$] - concentration of phenolate,
[Ar-OH] - concentration of phenol,
α_0 - formation degree for the dissociated form,
α_1 - formation degree for the undissociated form,

The speciation diagrams are presented in figures 2 - 4.

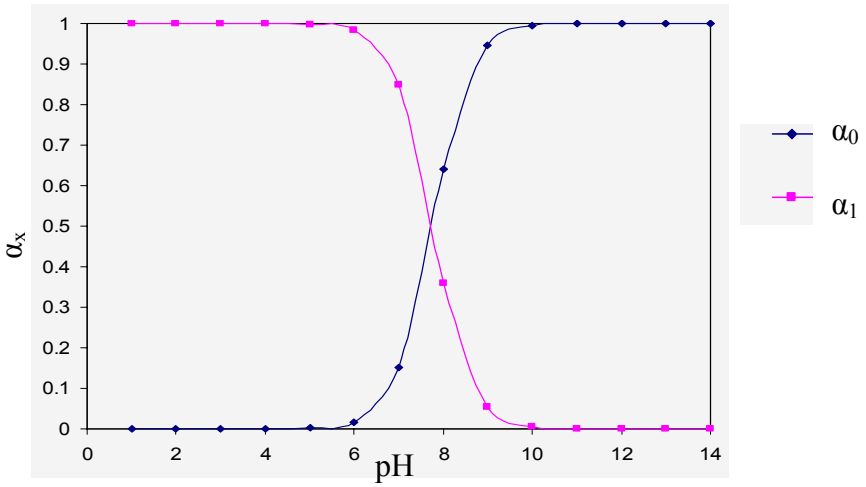


Figure 2. Speciation diagram $\alpha_c = f(pH)$ of the couple m-nitrophenol- m-nitrophenolate; α_x- formation degree; α_0- formation degree for the dissociated form- m-nitrophenolate; α_1- formation degree for the undissociated form- m-nitrophenol

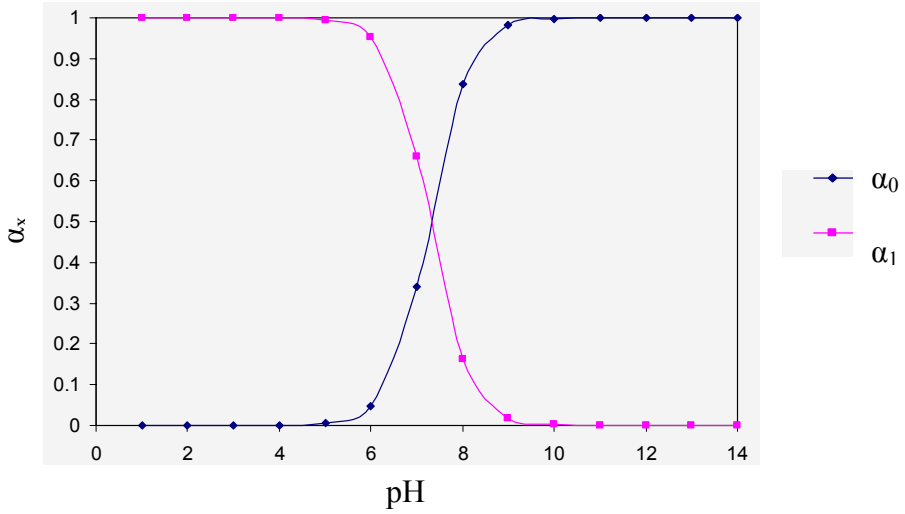


Figure 3. Speciation diagram $\alpha_c = f(pH)$ of the couple p-nitrophenol- p-nitrophenolate; α_x- formation degree; α_0- formation degree for the dissociated form- p-nitrophenolate; α_1- formation degree for the undissociated form- p-nitrophenol

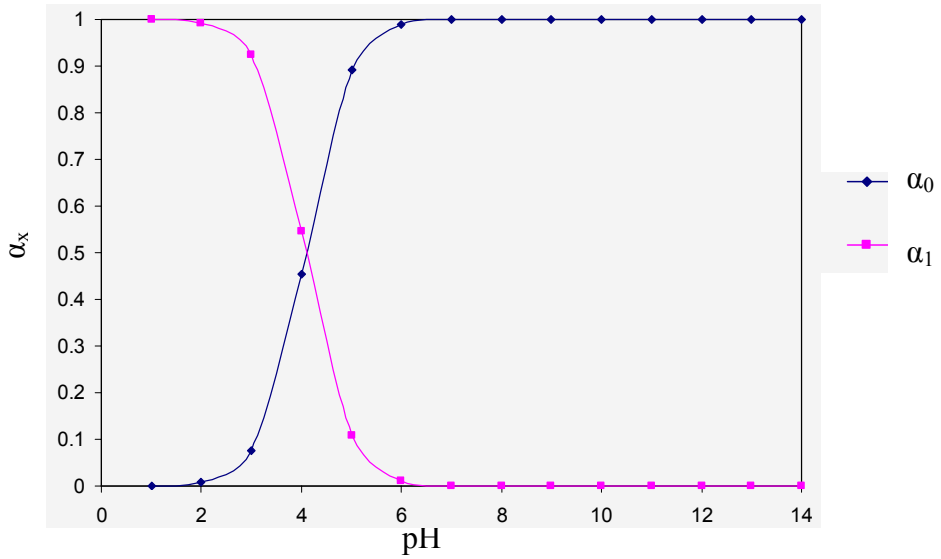


Figure 4. Speciation diagram α_c = f(pH) of the couple 2,4-dinitrophenol- 2,4-dinitrophenolate; α_x- formation degree; α_0- formation degree for the dissociated form- 2.4-dinitrophenolate; α_1- formation degree for the undissociated form- 2,4-dinitrophenol

It can be observed that for a pH between 2 and 3 all the phenolic compounds are quantitatively formed in their uncharged form. This is the reason for which the transport experiments for the three mentioned phenolic compounds were done using either acidic feeding sources, pH=2, or aqueous solutions without pH correction. The results of the transport experiments are presented in table 1.

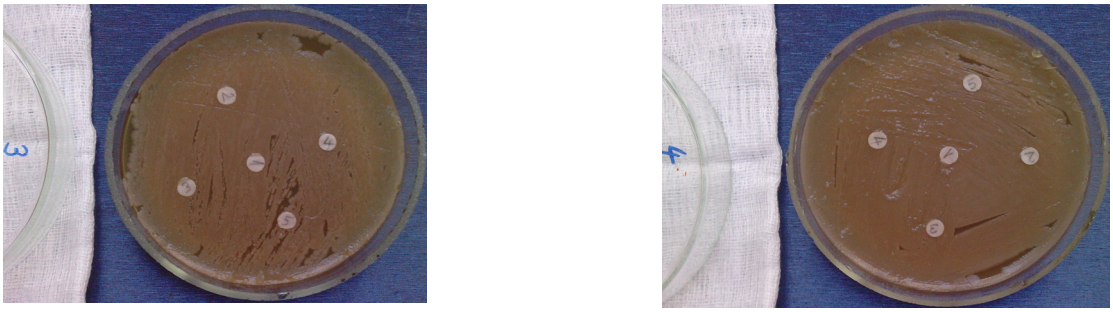
Table 1. Transport through liquid membrane of the studied nitrophenols.

Substrate	**pH**	**Feeding source composition (mg/L)**		**Efficiency (%)**
		Before transport	***After transport***	
p-nitrophenol	without pH correction	139	4.32	89.26
	pH = 2.01	139	2.05	97.78
m-nitrophenol	without pH correction	139	3.82	87.28
	pH = 2.03	139	2.19	96.17
2.4-dinitrophenol	without pH correction	184	2.04	84.54
	pH = 2.00	184	2.32	96.72

Additional data regarding the transport through liquid membranes of the specified nitrophenols were presented in our previous work [23].

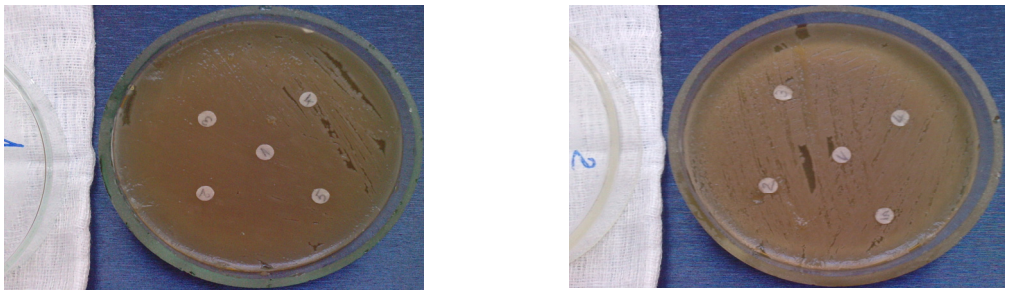
Toxicity tests

After transport, the feeding source solutions were microbiological tested in order to establish the biocide properties. The tests were realized on *Pseudomonas aeruginosa* bacteria type, strain ATCC 15442. The experimental results pointing out the growth characteristics are presented in figures 5 - 7. The concentration range was between 0.8 – 250 mg/L for m-nitrophenol, p-nitrophenol and 2, 4-dinitrophenol.



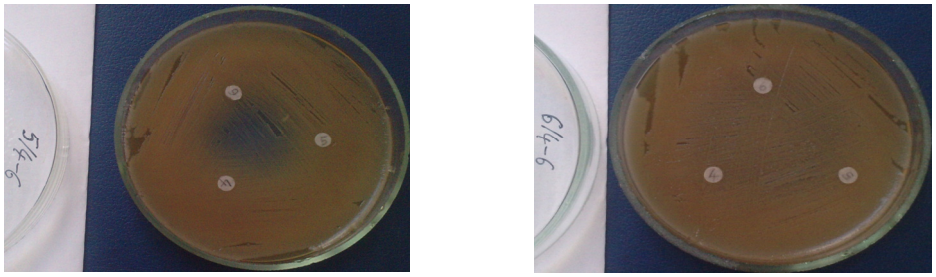
a **b**

Figure 5. *Pseudomonas aeruginosa* growth characteristics for m-nitrophenol (50-250mg/L)
a. residual source without pH correction; b. residual source at pH=2.03



a **b**

Figure 6. *Pseudomonas aeruginosa* growth characteristics for p-nitrophenol (50-250mg/L)
a. residual source without pH correction; b. residual source at pH=2.01



a **b**

Figure 7. *Pseudomonas aeruginosa* growth characteristics for 2.4-dinitrophenol (50-250mg/L)
a. residual source without pH correction; b. residual source at pH=2.00

From the antifungigram it was observed that the *Pseudomonas aeruginosa* strain was not inhibited by the concentrations of the active tested substances.

Conclusions

The usage of bulk liquid membranes permits the recovery of phenolic derivatives: m-nitrophenol, p-nitrophenol, 2,4-dinitrophenol with efficiencies of over 90% if the pH of the feeding source is acidic.

The residual sources saturated on chloroform, obtained after the removal of the nitrophenols of interest, do not present toxicity against *Pseudomonas aeruginosa* microorganism cells. This could be a first stage for adapting and forming the specific biomass within the biological wastewater treatment plants, as it could allow the biodegradation of the considered pollutants in biological treatment installations with *Pseudomonas aeruginosa* strain. Thus, the separation through bulk liquid membrane represents a viable procedure to treat the waste waters with content of nitrophenols.

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