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ORIGINAL PAPER

# Biodegradation of phenol by immobilized *Aspergillus awamori* NRRL 3112 on modified polyacrylonitrile membrane

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**Abstract** Covalent immobilization of *Aspergillus awamori* NRRL 3112 was conducted onto modified polyacrylonitrile membrane with glutaraldehyde as a coupling agent. The polymer carrier was preliminarily modified in an aqueous solution of NaOH and 1,2-diaminoethane. The content of amino groups was determined to be 0.58 mgeq $g^{-1}$. Two ways of immobilization were used—in the presence of 0.2 g $l^{-1}$ phenol and without phenol. The capability of two immobilized system to degrade phenol (concentration—0.5 g $l^{-1}$) as a sole carbon and energy source was investigated in batch experiments. Seven cycles of phenol biodegradation were conducted. Better results were obtained with the immobilized system prepared in the presence of phenol, regarding degradation time and phenol biodegradation rate. Scanning electron micrographs of the polyacrylonitrile membrane/immobilized *Aspergillus awamori* NRRL at the beginning of repeated batch cultivation and after the 7th cycle were compared. After the 7th cycle of cultivation the observations showed large groups of cells. The results from the batch experiments with immobilized system were compared to the results produced by the free strain. Phenol biodegradation experiments were carried out also in a bioreactor with spirally wound membrane with bound *Aspergillus awamori* NRRL 3112 in a regime of recirculation. 10 cycles of 0.5 g $l^{-1}$ phenol biodegradation were run consecutively to determine the degradation time and rate for each cycle. The design of the bioreactor appeared to be quite effective, providing large membrane surface to bind the strain.

**Keywords** Biodegradation · Phenol · *Aspergillus awamori* NRRL3112 · Immobilization · Polymer membrane

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## Introduction

One of the problems attracting highest attention in modern ecology is related to the biodegradation of xenobiotics. Typical representatives of this group of contaminants are phenol and its derivatives. The environmental clean-up of phenol by adsorption, solvent extraction, chemical oxidation, incineration and abiotic treatment procedure suffers from serious drawbacks such as economic issues and the production of hazardous byproducts. Biodegradation is generally preferred, due to the lower costs and the complete mineralization.

Extensive studies revealing the potential of microorganisms in phenol biodegradation have been

carried out. Major part of the research in the field of phenol decomposition have been performed using bacterial strains, such as *Alcaligenes europhus* (Hudges et al. 1984), and *Pseudomonas sp.* (Allsop et al. 1993). Other publications report biodegradation of phenol by yeast strains *Candida* (Yan et al. 2005) and *Trichosporon* (Chtourou et al. 2004). Chitra et al. (1995) have studied the removal of phenol using a mutant strain of *Pseudomonas*. The use of fungal strains for the degradation is relatively untouched area. Mycelial fungi such as *Fusarium floccieferum* (Anselmo and Novais 1992), *Aspergillus fumigatus* (Jones et al. 1995) and *Graphium* sp. (Santos et al. 2003) have been cited for their potential of phenol degradation. Little is known about phenol metabolism in mycelial fungi. They have some advantages, because of their resistance to a great number of xenobiotics, toxic to the most of the microorganisms. The fungal strain *Aspergillus awamori* NRRL 3112 is capable of assimilating a wide range of carbon substrates, including phenol and its derivatives (Sundar et al. 2005; Yordanova et al. 2006). This feature makes them a valuable research object for a future development of industrial-water cleaning technology.

There has been an increasing interest in the immobilization of microorganisms in recent years (Abd-El-Haleem et al. 2003; Bolaños et al. 2001; Gonzalez et al. 2001; Fiest and Hegeman 1969; Mordocco et al. 1999; Pazarlioglu and Telefoncu 2005; Shetty et al. 2007a, b). The immobilized cells have advantages over the native cells due to the possibility for multiple applications for a extended period of time. Different carriers have been used for microbial cell immobilization, but there aren't many reports for cell immobilization on polymer membranes (Uzunova et al. 2002; Marrot et al. 2006; Hua et al. 2005; Godjevargova et al. 2006). Synthetic polymers constitute the largest group of supports in use. Polyacrylonitrile-based ultrafiltration (UF) membranes obviously turn up to be more advantageous over other conventional membranes in various aspects, such as thermal stability, resistance to most organic solvents, commercial availability, etc. (Wang et al. 2007). PAN-based membranes can offer some special promises as cradle for cell immobilization, which is mainly because different reactive groups can be easily generated on the PAN membrane surface for the covalent immobilization (Godjevargova et al. 2006; Kowalska et al. 1998).

The membranes are very suitable for immobilization of microbial cells, when a spiral membrane module is used. There is no common strategy for preparing such integrated systems and opportunities for using are still at hand. Due to their high surface area per unit module volume, spiral membrane modules serve as better immobilization supports than alginate beads, activated carbon, sintered glass, and polymer beads (Jose et al. 2002; Karel et al. 1985). On the other hand, polymer membranes can act as barriers limiting the movement of cell mass, thus providing restricted and/or regulated passage of one or more species through the pores. From this point of view, membrane bioreactors are promising for practical applications for wastewater treatment (Loh et al. 2000; Stephenson et al. 2000).

The present paper investigates the possibility of immobilizing *Asp. awamori* NRRL 3112 spores on modified polyacrylonitrile membrane and their ability to biodegrade phenol. Spiral membrane module with immobilized spores was created and phenol biodegradation was also investigated.

## Materials and methods

### Microorganism and growth medium

A strain of *Aspergillus awamori* NRRL 3112, obtained from US Department of Agriculture, Illinois, USA was used throughout this study. The organism was grown on slants immersed in a medium having the following composition (g $l^{-1}$): malt extract 3.0, yeast extract 3.0, peptone 5.0, glucose 10.0 and agar 20.0. The organism on the slants was allowed to grow for 72 h at 30°C and then stored at 4 ± 1°C for further use.

### Medium for degradation studies

The studies on the biodegradation of phenol were carried out in the Czapekdox medium, which had the following composition (g $l^{-1}$): sodium nitrate 2.0, potassium phosphate (dibasic) 1.0, potassium chloride 0.5, magnesium sulfate heptahydrate 0.5 and ferrous sulfate heptahydrate 0.01. The initial pH of the medium was adjusted to 5.5 (the optimal pH for this strain) using 1 N NaOH or 1 N HCI (Sundar et al. 2005). The minimal medium contains phenol as a sole carbon source with 0.5 g $l^{-1}$ concentration.

## Chemical modification of polyacrylonitrile membrane

The ultrafiltration polyacrylonitrile (PAN) membrane (average pore size of selective layer 0.02 μm) was produced by Ecofilter Co (Bourgas, Bulgaria). PAN membrane was treated with 10% aqueous solution of NaOH at 50°C for 60 min. After thorough washing with distilled water, the membrane was treated with a 10% aqueous solution of 1,2-diaminohexane for 60 min at 40°C. The membrane was once again thoroughly washed with distilled water. At the end of the procedure the modified carrier was immersed in 0.5% aqueous solution of glutaraldehyde for 60 min at room temperature. Excess glutaraldehyde was removed by washing the membranes with distilled water until there was a complete absence of glutaraldehyde in the rinse waters.

## Phenol biodegradation in batch experiments

### *Spore immobilization for batch experiments*

The 14-day culture in spore form in concentration of $3.33 \times 10^7$ ml$^{-1}$ were introduced by flushing 5 cm$^3$ sterile water in 500 cm$^3$ sterile flask containing 50 cm$^3$ Czapekdox medium and sterile modified carrier (40 cm$^2$ PAN flat membrane). Sterilization of the modified membrane was carried out by ethanol. Two ways of immobilization were used—in the presence of 0.2 g l$^{-1}$ phenol and without phenol. The two flasks were placed on a shaker for 24 h at 30°C. Then the liquid medium was decanted and the immobilized systems were washed several times with sterile water to remove the unbound spores. After that the immobilized spores were used for phenol biodegradation.

### *Batch experiments*

The immobilized spores onto the polymer membrane (single flat piece—40 cm$^2$) with concentration 0.158 g cm$^{-2}$ were introduced in 500 cm$^3$ sterile flask containing 50 cm$^3$ Czapekdox medium, as well as phenol as the only carbon source adjusted to the appropriate concentration. Then they were cultivated on a shaker (220 rpm) at 30°C until the complete phenol degradation. At every 24 h, samples were withdrawn and analyzed for phenol concentration. Thus, seven cycles were carried out with the same immobilized system.

Simultaneously, parallel experiments with free cells were also performed for comparison. The flasks containing 50 cm$^3$ liquid medium, as well as 0.5 g l$^{-1}$ phenol as the only carbon source were inoculated with equal volume of inoculum ($3.33 \times 10^7$ conidia cm$^{-3}$ medium) and agitated on a shaker (220 rpm) at 30°C. Samples were taken at every 24 h interval for determination of phenol concentration. After the biodegradation of the entire phenol, the cultural medium was decanted and the biomass was recultivated in fresh liquid medium containing 0.5 g l$^{-1}$ phenol. Thus, four cycles were carried out in the same manner.

Each experiment for phenol degradation was repeated twice, the reproducibility being always within 2.5%.

## Phenol biodegradation in recycle reactor with spirally wound membrane

### *Spore immobilization for experiments with membrane bioreactor*

The bioreactor was 40 mm in diameter and 100 mm high, it was made of glass and equipped with thermostatic bath (Fig. 1). The modified and activated with glutaraldehyde polyacrylonitrile membrane with

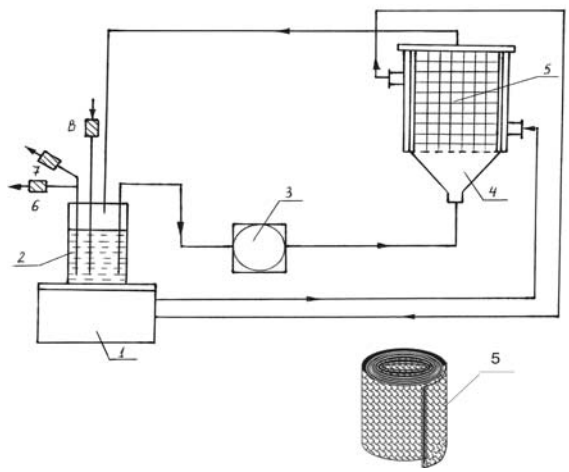


**Fig. 1** Scheme of recycle system: thermostatic bath (1); feed reservoir (2); peristaltic pump (3); thermostatic reactor (4); membrane with immobilized cells (5); sampling (6); air outlet (7); and air inlet (8)

surface area of 100 $cm^2$ was spirally wound in the glass bioreactor using supporting polyethylene mesh. In this case, the immobilization was carried out by flowing spore suspension ($5.47 \times 10^7$ conidia $cm^{-3}$ medium) through the membrane module using peristaltic pump at rate of 1 ml $min^{-1}$ for 24 h. Then the membrane spiral was thoroughly washed out with sterile water, fed by peristaltic pump to remove the non-bound spores.

*Experiments with membrane bioreactor*

Further, the liquid medium (300 ml) containing phenol at certain concentration was fed to the membrane bioreactor using peristaltic pump at rate of 1.5 ml $min^{-1}$. The temperature required for the strain growth (30°C) was maintained using the thermostat and the heat-exchanging coating. After the full degradation of phenol in the nutrient medium, the reservoir was charged with fresh nutrient medium containing phenol. Samples were taken at every 24 h interval for determination of phenol concentration. Ten cycles were carried out in the same manner.

Each experiment was repeated twice, the reproducibility being always within 2.5%.

Analytical methods

Phenol concentration was measured spectrophotometrically in the presence of *p*-nitroaniline at 495 nm (Gonzalez et al. 2001).

The amount of spores immobilized on the support was estimated by the difference between the wet weight of spores with membrane at the beginning (immediately after the immobilization) and the wet membrane weight. The amount of free cells was determined by the measuring of the wet weight of the cells and by the counting of the number of conidia in the samples using a standard microscope method.

Scanning electron microscopy (SEM)

The immobilized system *Aspergillus awamori* NRRL 3112/polyacrylonitrile membrane was washed with distilled water and then they were dehydrated in 30–100% water ethanol series. The membrane were coated with 120–130 Å of gold in argon medium in an Edwards apparatus (model 150 A). The SEM observations were done on a scanning device attached to a Zeiss electron microscope (model 10C) at 20 kV accelerating voltage with a 5–6 nm electron beam.

## Results and discussions

Modification of membranes

The aim of the present studies is to obtain an efficient immobilized system of *Aspergillus awamori* NRRL 3112/polyacrylonitrile membrane for biodegradation of phenol. The immobilization of spores was carried out with glutaraldehyde. The polymer membrane was preliminarily treated by using two different modification procedures. The first procedure introduced –COOH and $–NH_2$ groups on the membrane surface by using NaOH solution. In order to increase the amount of amine groups on the surface of the carrier, the latter was treated with 1,2-diaminoethane. The optimal conditions of modification were defined in a previous research work (Godjevargova et al. 2000). The superficial carboxyl groups on the carrier surface reacted with one of the amine groups of 1,2-diaminoethane, leaving the other one free. The results showed that the PAN membranes had been modified in a greater extent and the content of amino groups was 0.58 mgeq $g^{-1}$. The latter was determined titrimetrically (Dimov et al. 1983). Further, the modified membrane was treated with an aqueous solution of glutaraldehyde. The bifunctional agent bound the strain to the membrane surface.

Batch experiments

Spores concentration used for the immobilization was $3.33 \times 10^7$ $ml^{-1}$. The first task was to determine which immobilization method would serve better for these experiments—in the presence or in the absence of phenol (0.2 g $l^{-1}$) in a nutrient medium containing spore suspension. The amount of spores bound to the membranes in both cases was 0.158 g $cm^{-2}$ (wet weight). Then the spores immobilized on the polymer membrane by both methods were cultivated in a nutrient medium containing 0.5 g $l^{-1}$ phenol. The results obtained for phenol biodegradation by both systems are presented in Fig. 2a, b. The rate of phenol biodegradation was determined after the end of each cycle for both immobilization systems (Table 1). As can be seen from Table 1 the lowest

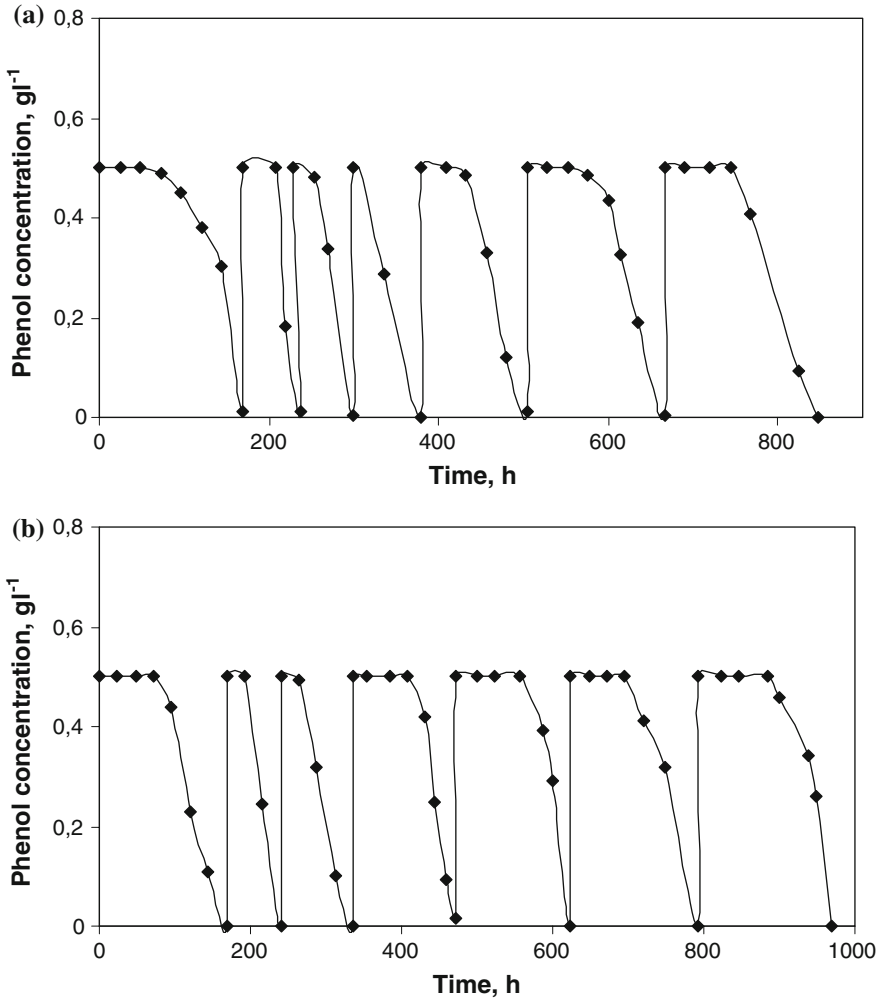


**Fig. 2** Phenol biodegradation (0.5 g $l^{-1}$) by immobilized system: *Aspergillus awamori* NRRL 3112/modified PAN membrane obtained by immobilization in presence of 0.2 g $l^{-1}$ phenol (**a**) and without phenol (**b**)

biodegradation rate is observed at the first cycle, then it increases 3 times at the second and the third cycles, and from the fourth to the seventh cycle the rate decreases continuously. This trend of the biodegradation rate is observed for both of the immobilized systems. The deceleration of the phenol biodegradation could be explained with the increasing of biomass and mycelia formation on the membrane surface after the fourth cycle, which intensified at the final cycles. An obvious inhibition of the biodegradation process at the final cycles was observed, which was due to the hindrance of the substrate diffusion to the cells. When comparing the results presented on Fig. 2a and b and Table 1 it becomes obvious that the rate of phenol degradation was higher throughout all cycles in the presence of the system where the spore immobilization was conducted with addition of 0.2 g $l^{-1}$ phenol. For better comparison, the biodegradation times of each cycle with the two immobilized systems are presented in Fig. 3. As results suggest better degradation times were

**Table 1** Phenol biodegradation rate (mg $h^{-1}$) of free and immobilized *Aspergillus awamori* NRRL 3112 on polymer PAN membrane at batch cultivation with 0.5 g $l^{-1}$ phenol

| Number of cycles | Free cells | Immobilized cells without 0.2 g $l^{-1}$ phenol | Immobilized cells in the presence of 0.2 g $l^{-1}$ phenol |
|---|---|---|---|
| 1 | 2.97 | 3.00 | 3.00 |
| 2 | 2.01 | 7.14 | 6.94 |
| 3 | 1.49 | 8.06 | 5.43 |
| 4 | 1.16 | 6.25 | 3.67 |
| 5 | | 4.03 | 3.29 |
| 6 | | 3.08 | 3.00 |
| 7 | | 2.75 | 2.70 |

obtained with the immobilized system prepared in the presence of phenol. This can be explained with the fact that the strain could have adapted to the substrate during the immobilization process. All further experiments were carried out with the immobilized system prepared in presence of phenol.

The biodegradation ability of the immobilized system onto polyacrylonitrile membrane towards phenol in a concentration range from 0.2 to 0.8 g $l^{-1}$ was studied. As can be seen from Fig. 4, the increase of phenol concentration resulted in increased biodegradation time. For instance, the biodegradation cycle at concentration of 0.4 g $l^{-1}$ was 66 h while at 0.7 g $l^{-1}$ the cycle lasted for 135 h.

The results obtained with the selected immobilized system were compared to those with the free strain. For this purpose, the spores ($0.8 \times 10^7$ $ml^{-1}$) were cultivated in Czapekdox medium containing 0.5 g $l^{-1}$ phenol (Fig. 5). Four cycles were carried out in the same manner. The amount of non-bound spores in the first cycle of cultivation is equivalent to the amount of immobilized spores on PAN membrane with surface 40 $cm^2$ in the first cycle (wet weight—6.32 g). As can be seen from Fig. 5 the phenol degradation time was

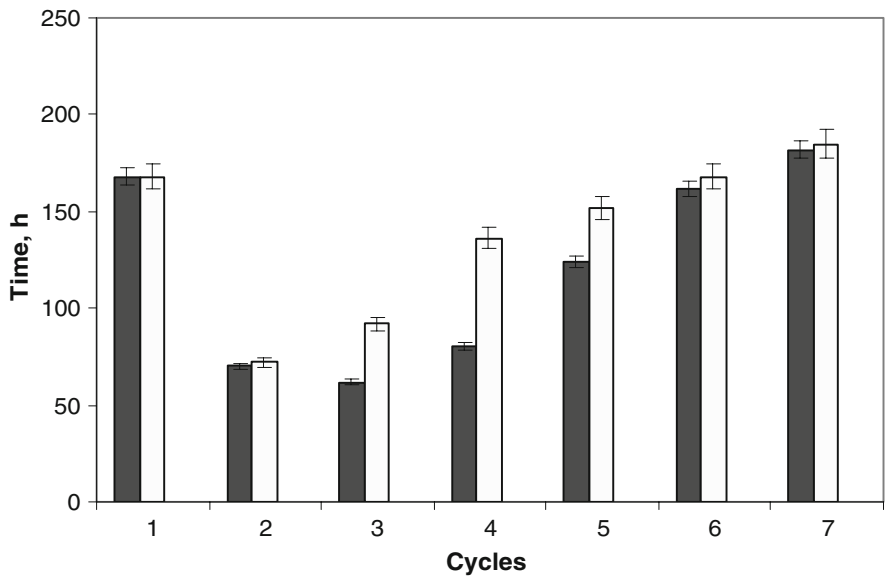


**Fig. 3** Cycles duration of phenol biodegradation (0.5 g $l^{-1}$) by two immobilized systems: *Aspergillus awamori* NRRL 3112/modified PAN membrane obtained by immobilization in presence of 0.2 g $l^{-1}$ phenol (□) and without phenol (■)

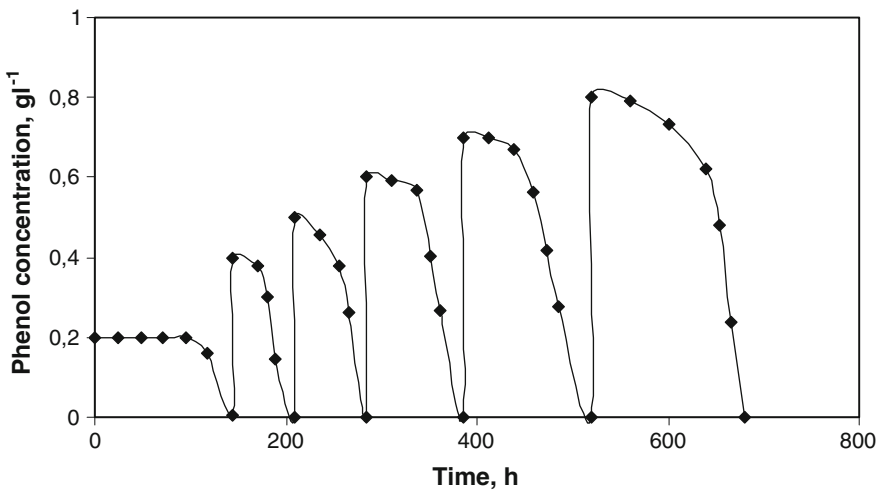


**Fig. 4** Phenol biodegradation (from 0.2 to 0.8 g $l^{-1}$) by immobilized system: *Aspergillus awamori* NRRL 3112/ modified PAN membrane

considerably greater for the four cycles in these experiments. This time extension was confirmed by the calculated rate of biodegradation (Table 1). When comparing the rates of phenol biodegradation by the free and the immobilized cells, the greater stability of the immobilized systems over the free cells becomes more prominent after multiple cultivation in media containing phenol with concentration 0.5 g $l^{-1}$. Obviously the free cells were inhibited to a greater extent by phenol (biodegradation rate at Cycle 4—1.16 mg $h^{-1}$) in comparison with the immobilized cells (biodegradation rates at Cycle 4 are 6.25 and 3.67 mg $h^{-1}$, respectively for immobilized system obtained in the presence of 0.2 g $l^{-1}$ phenol and the system without phenol).

In order to distinguish between phenol adsorption on the membrane surface and phenol degradation by microorganisms, the pure carrier was also studied for phenol adsorption. For this purpose, 40 $cm^2$ pure membrane was immersed in 0.5 g $l^{-1}$ phenol solution for 6 h. The results are presented in Fig. 6. Since the phenol concentration didn't change significantly, the only possible reason for the decrease of the phenol content was the degradation activity of the immobilized cells. This simplifies the kinetic studies when membrane is to be used as a support material for the immobilization of *Asp. awamori* for the purpose of phenol degradation.

The amount of cells (wet weight) on the membrane of 40 $cm^2$ was measured after the sixth cycle of

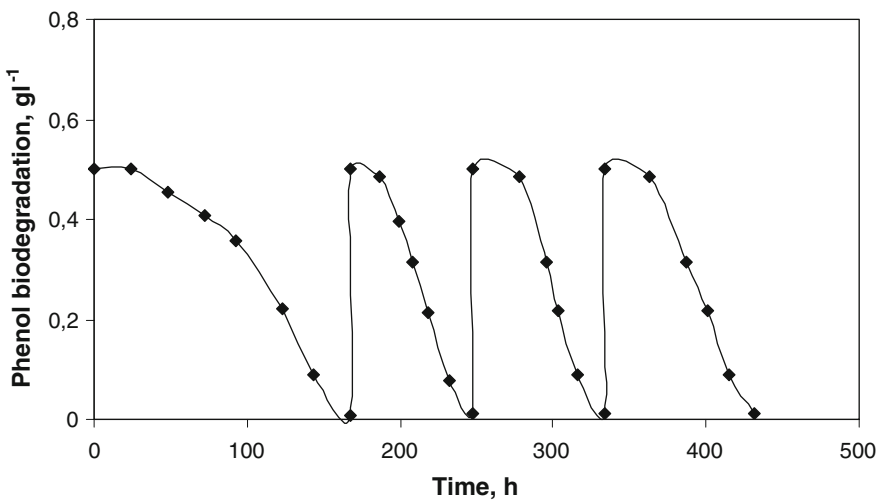


**Fig. 5** Phenol biodegradation with concentration 0.5 g $l^{-1}$ by strain *Aspergillus awamori* NRRL 3112

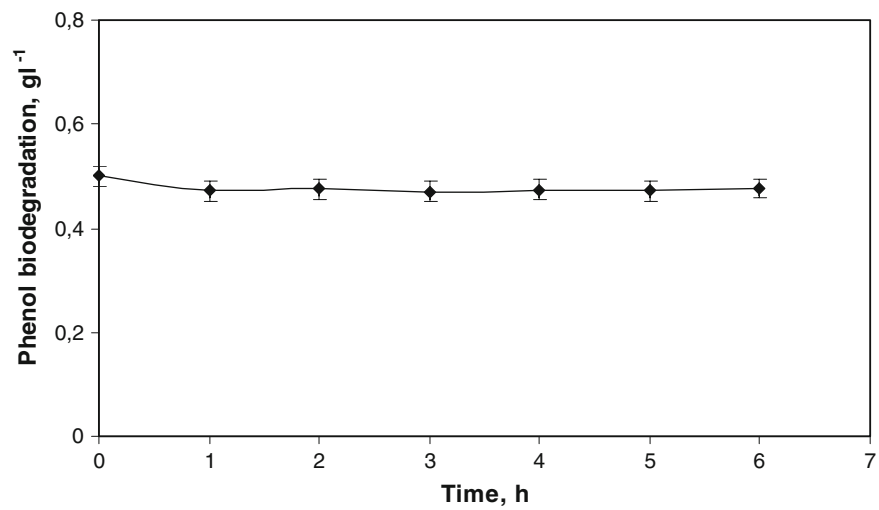


**Fig. 6** Adsorption of phenol by modified PAN membrane

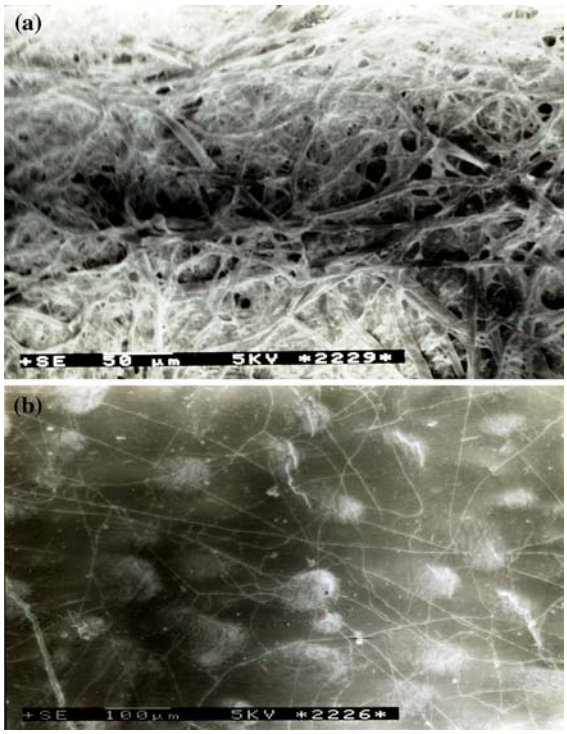


**Fig. 7** Scanning electron micrographs of immobilized system *Aspergillus awamori* NRRL 3112/modified PAN membrane after 7th cycle (**a**) and before 1st cycle (**b**)

phenol biodegradation—1.104 g $cm^{-2}$. The significant increase of the biomass with the increased number of cycles is obvious (after immobilization—0.158 g $cm^{-2}$). Thus, the biomass accumulated on the membrane was approximately ten times higher after six cycles of phenol biodegradation. The high operational stability of investigated biocatalysts was confirmed by the scanning electron micrographs of the polyacrylonitrile membrane/immobilized *Aspergillus awamori* NRRL at the beginning of repeated batch cultivation and after the 7th cycle. After the 7th cycle of cultivation the observations showed large groups of cells (Fig. 7a). The significant increase of biomass and mycelia formation on the membrane surface after the 7th cycle of cultivation was inevitably followed by deceleration of the phenol degradation rate.

Experiments with membrane bioreactor

The next step of the research work involved experiments, which were carried out in a bioreactor with spirally wound membrane in a regime of recirculation. The spores were immobilized onto the membrane directly in the system as described in "Materials and methods" section. Phenol biodegradation was carried out under aerobic conditions. The operation parameters of the bioreactor are presented in Table 2. Fresh nutrient medium, containing 0.5 g $l^{-1}$ phenol were added after each cycle. Phenol concentration was monitored for degradation. At the end of a cycle, the system was washed with sterile distilled water and charged with nutrient medium and phenol for the next cycle. Thus, 10 cycles of 0.5 g $l^{-1}$ phenol biodegradation were run consecutively to determine the degradation time for each cycle (Fig. 8). No significant difference was observed between the degradation times of the cycles from the second to the seventh (about 50 h) and between the biodegradation rates (10.0 mg $h^{-1}$). After the 7th cycle the biodegradation rate began to decrease gradually and at 10 cycles it was 4.0 mg $h^{-1}$. These results confirmed the advantages of the recycled reactor with spirally wound membrane. The design of the bioreactor appeared to be quite effective providing large membrane surface to bind the strain cells. Besides, phenol would flow tangentially which prevents membrane from a decrease of the flow velocity.

## Conclusion

A novel immobilized system—*Aspergillus awamori* NRRL 3112/polyacrylonitrile membrane was obtained for the purpose of phenol biodegradation. It was established that the immobilized system prepared in the presence of 0.2 g $l^{-1}$ phenol was more effective than the system prepared in the phenol absence. It was revealed that the immobilized systems were more stable and more effective when biodegrading 0.5 g $l^{-1}$ phenol in comparison with the

**Table 2** Operation conditions of bioreactor

| Parameter | Value |
|---|---|
| Rate flow | 1.5 ml $min^{-1}$ |
| Volume | 300 ml |
| Initial phenol concentration | 0.5 g $l^{-1}$ |
| Membrane surface area | 100 $cm^2$ |
| Immobilized spores | 0.174 g $cm^{-2}$ (wet weight) |

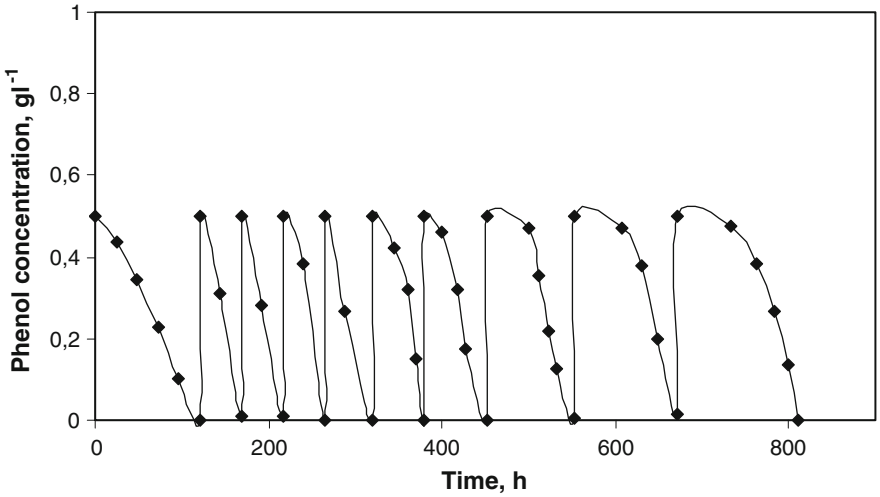


**Fig. 8** Concentration profile of phenol in a recirculating system with immobilized system *Aspergillus awamori* NRRL 3112/modified PAN membrane

free cells. A bioreactor was constructed containing spirally wound membrane with immobilized spores of *Aspergillus awamori* NRRL 3112 in a regime of recirculation, which was successfully applied for phenol biodegradation. The obtained results confirmed the advantages of the recycled reactor with spirally wound membrane. Thus the immobilized microbial technology is an extremely versatile approach that can be used for degradation of toxic pollutants from the industrial effluents.

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## References

Abd-El-Haleem D, Beshay U, Abdelhamid AO, Moawad H, Zaki S (2003) Effects of mixed nitrogen sources on biodegradation of phenol by immobilized *Acinetobacter* sp. strain W-17. Afr J Biotechnol 2(1):8–12

Allsop PJ, Chisti Y, Moi-Youngand M, Sullivan GR (1993) Dynamics of phenol degradation by *Pseudomonas putida*. Biotechnol Bioeng 41:572–579

Anselmo AM, Novais JM (1992) Degradation of phenol by immobilized mycelium of *Fusarium floccieferum* in continuous culture. Water Sci Technol 25:161–168

Bolaños ML, Varesche MBA, Zaiat M, Foresti E (2001) Phenol degradation in horizontal-flow anaerobic immobilized biomass (HAIB) reactor under mesophilic conditions. Water Sci Technol 44(4):167–174

Chitra S, Sekaran G, Padmavathi S, Gowri C (1995) Removal of phenolic compounds from wastewater using mutant strain of *Pseudomonas pictorum*. J Gen Appl Microbiol 41:229–237

Chtourou M, Ammar E, Nasri M, Medhioub K (2004) Isolation of a yeast, *Trichosporon cutaneum*, able to use low molecular weight phenolic compounds: application to olive mill waste water treatment. J Chem Technol Biotechnol 79:869–878

Dimov K, Samardjieva V, Pavlov P (1983) Laboratory practice on technology of synthetic fibers. Technica, Sofia, BG

Fiest CF, Hegeman GD (1969) Phenol and benzoate metabolism by *Pseudomonas putida*. J Bacteriol 100:869–877

Godjevargova T, Aleksieva Z, Ivanova D (2000) Cell immobilization of *Trichosporon cutaneum* strain with phenol degradation ability on new modified polymer carries. Process Biochem 35:699–704

Godjevargova T, Ivanova D, Aleksieva Z, Burdelova G (2006) Biodegradation of phenol by immobilized *Trichosporon cutaneum R57* on modified polymer membranes. Process Biochem 41:2342–2346

Gonzalez G, Herrera MG, Pena MM (2001) Biodegradation of phenol in a continuous process: comparative study of stirred tank and fluidized-bed bioreactors. Bioresour Technol 76:245–251

Hua L, Sun ZH, Leng Y (2005) Continuous biocatalytic resolution of DL-pantolactone by cross-linked cells in membrane bioreactor. Process Biochem 40:1137–1142

Hudges EJL, Bayly RC, Skurray RA (1984) Evidence for isofunctional enzymes in the degradation of phenol, *m*- and *p*-toluate, and *p*-cresol at catechol meta-cleavage pathways in *Alcaligenes eutrophus*. J Bacteriol 158:79–83

Jones KH, Trudgill PW, Hopper DJ (1995) Evidence of two pathways for the metabolism of phenol by *Aspergillus fumigatus*. Arch Microbiol 163:176–181

Jose G, Sanchez M, Theodore T (2002) Catalytic membranes and membrane reactors. Wiley-VCH Verlag GmbH, Weinheim. *Source*: IEM (M. Didier Cot and Jose G. Sanchez Marcano)

Karel S, Libichi S, Robertson C (1985) The immobilization of whole cells: engineering principles. Chem Eng Sci 40: 1321–1354

Kowalska M, Bodzek M, Bohdziewicz J (1998) Biodegradation of phenols and cyanides using membranes

immobilized microorganisms. Process Biochem 33: 189–197

Loh K, Chung T, Ang W (2000) Immobilized-cell membrane bioreactor for high-strength phenol wastewater. J Environ Eng ASCE 126:75–79

Marrot B, Barrios-Martinez A, Moulin P, Roche N (2006) Biodegradation of high phenol concentration by activated sludge in an immersed membrane bioreactor. Biochem Eng J 30:174–183

Mordocco A, Kuek C, Jenkins R (1999) Continuous degradation of phenol at low concentration using immobilized *Pseudomonas putida*. Enzyme Microb Technol 25:530–536

Pazarlioglu NK, Telefoncu A (2005) Biodegradation of phenol by *Pseudomonas putida* immobilized on activated pumice particles. Process Biochem 40:1807–1814

Santos VL, Heilbuth NM, Braga T, Monteiro AS, Linardi VR (2003) Phenol degradation by a *Graphium* sp. FIB4 isolated from industrial effluents. J Basic Microbiol 43:238–248

Shetty KV, Kalifathulla I, Srinikethan G (2007a) Performance of pulsed plate bioreactor for biodegradation of phenol. J Hazard Mater 140:346–352

Shetty KV, Ramanjaneyulu R, Srinikethan G (2007b) Biological phenol removal using immobilized cells in a pulsed plate bioreactor: effect of dilution rate and influent phenol concentration. J Hazard Mater 149:452–459

Stephenson T, Judd S, Jefferson B, Brindle K (2000) Membrane bioreactors for wastewater treatment. IWA Publishing, London, UK, pp 129–136

Sundar P, Daniel D, Krastanov A (2005) Biodegradation of phenol by immobilized *Aspergillus awamori* cells. Bulg J Agric Sci 11:61–71

Uzunova K, Vassileva A, Ivanova V, Spasova D, Tonkova A (2002) Thermostable exo-inulinase production by semi-continuous cultivation of membrane-immobilized *Bacillus* sp. 11 cells. Process Biochem 37:863–868

Wang Z, Wan L, Xu Z (2007) Surface engineerings of polyacrylonitrile-based asymmetric membranes towards biomedical applications: an overview. J Membr Sci 304:8–23

Yan J, Jianping W, Hongmei L, Suliang Y, Zongding H (2005) The biodegradation of phenol at high initial concentration by the yeast *Candida tropicalis*. Biochem Eng J 24(3):243–247

Yordanova G, Ivanova D, Godjevargova T, Krastanov A (2006) Biodegradation of phenol by immobilized *Aspergillus awamori* NRRL3112 cells. Food science, engineering and technologies conference. Scientific works, Plovdiv, pp 260–265