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Pentachlorophenol sorption in nylon fiber and removal by immobilized *Rhizopus oryzae* ENHE

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ABSTRACT

This study describes pentachlophenol (PCP) sorption in nylon fiber in which *Rhizopus oryzae* ENHE was immobilized to remove the chemical compound. The experimental sorption data were analyzed using the Langmuir, Freundlich, and Redlich–Peterson isotherm models using non-linear error functions to fit the experimental data to the three models. Results showed that the isotherm obtained from the data fitted the three models used. However, the *g* parameter from Redlich–Peterson model showed that the isotherm obtained approaches the Freundlich model. This support reached the sorption equilibrium concentration at 3 mg PCP g^{-1} nylon. To study PCP removal capability by *R. oryzae* ENHE and to eliminate the error caused by PCP sorbed by the nylon fiber during its quantification, nylon fiber at PCP equilibrium sorption concentration was used to immobilize *R. oryzae* ENHE. It was found that this fungus grew within nylon fiber cubes in presence or not of PCP, even when PCP caused growth inhibition. Maximum biomass accumulated into nylon cubes without PCP was of 32 mg biomass g^{-1} nylon and into nylon cubes at PCP equilibrium concentration was of 18 mg g^{-1} nylon. The results showed that *R. oryzae* ENHE immobilized into nylon fiber removed 88.6% and 92% of PCP in cultures with 12.5 and 25 mg PCP L^{-1}, as initial concentration, respectively. This is the first work to report that a zygomycete, such as *R. oryzae* ENHE, immobilized into nylon fiber kept its potential to remove PCP.

1. Introduction

Pentachlorophenol (PCP) is a phenolic compound and a hazardous pollutant due to its persistence in the environment [1,2]. Throughout the last century, PCP has been used extensively and its concentration in water and soil has increased markedly. PCP is used as wood preservative, and it is discharged in wastewaters from some industries, mainly from the bleaching process in paper and pulp mills, electricity generation, etc. The widespread use of PCP and its recalcitrant nature, because it is a highly chlorinated compound, has resulted in the contamination of soils and groundwater, especially at the sites of wood treatment plants. PCP uncouples oxidative phosphorylation and alters cell membrane properties, making it a very toxic compound. PCP removal from a natural system by photolysis, volatilization, adsorption, or degradation is extremely slow, an alternative to the latter is biodegradation [3–5]. The potential of the microorganism to degrade recalcitrant compounds have been used for this purpose and the application of fungi, particularly basidiomycetes, such as *Phanerochaete chrysosporium*, has been studied widely [1,6–8]. It has been demonstrated that *P. chrysosporium* degraded and mineralized PCP from initial concentrations of 1–500 mg L^{-1} in submerged cultures. Nevertheless, immobilized fungi have several advantages over suspended biomass cultures; among them is the biomass retention within the support and its reuse, as well as an easier liquid–solid phase's separation [9,10]. In addition, immobilized cultures tend to have a higher level of activity and they are more tolerant to environmental perturbations, such as pH, or to the exposure to toxic concentrations, than suspended biomass cultures [11]. Moreover, cell immobilization diminishes the apparent broth viscosity found in liquid cultures and makes the rheological features of the liquid phase more favorable for oxygen supply and mass transfer [12]. Another advantage of cell immobilization is a reduction in the contamination risk and protease activity on other enzymes acting in the bioremediation process. Gao et al. [13] reported the use of immobilized *P. chrysosporium* to degrade the reactive dye K-2BP under non-sterile conditions. Some works report the removal of aromatic toxic compounds, such as anthracene, phenol, and PCP by filamentous fungi immobilized in different supports such as sugarcane bagasse, polyurethane, and polystyrene, but the possibility of PCP sorption has not been considered [14,15]. In our laboratory, we performed some PCP extraction assays from three inert supports using methanol–water 50:50 and we found that: no PCP was extracted from polyester; 11% was

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Table 1
Equations that describe the isotherm adsorption models used.

Model	Non-linear expression	References
Langmuir	(5)$q_e = \frac{q_m K_a C_e}{1+K_a C_e}$	Langmuir [18]
Freundlich	(6)$q_e = K_F C_e^{1/n}$	Freundlich [19]
Redlich–Peterson	(7)$q_e = \frac{AC_b}{1+BC_e^g}$	Redlich–Peterson [20]

extracted from polyurethane; and from nylon fiber 21% of initial PCP was extracted [16].

The aims of this work was to study the sorption of PCP by polyurethane and nylon fiber; and determine the growth of *R. oryzae*, a zygomycete isolated from a PCP-contaminated soil, in nylon fiber, as well as, the ability of the nylon fiber-immobilized fungus to remove PCP.

2. Materials and methods

2.1. Microorganism and culture media

R. oryzae ENHE isolated from a PCP-contaminated soil was used in this study [17]. The strain was kept in a 30% glycerol solution at −70 °C. Erlenmeyer flasks containing PDA (potato-dextrose agar) medium were inoculated with *R. oryzae* ENHE spores and incubated at 30 °C for 4–5 days to obtain asexual spores. Spore suspensions were obtained in 20 mL of sterile water containing 1.0% Tween-80. These suspensions, at an approximate concentration of 1×10^7 spores mL^{-1}, were used to inoculate the nylon fiber impregnated with culture medium for fungal immobilization. Merlin–Norkrans (M–N) medium was used to study PCP sorption by the supports and PCP removal by *R. oryzae* ENHE. The M–N medium containing (g L^{-1}): glucose, 10.0; malt extract, 2.0; yeast extract, 1.0; KH_2PO_4, 0.5; $MgSO_4$ $7H_2O$, 0.15; and $(NH_4)_2HPO_4$, 0.5; dissolved in citrate buffer at pH 5.3. This medium was used twofold concentrated (2×) to immobilize *R. oryzae* ENHE into the nylon fiber; except for glucose that had a final concentration of 25 g L^{-1}.

2.2. Synthetic support

The nylon fiber used in this study was a commercial product from 3M Company (Scotch Brite, 3M Spain, SA). Polyurethane (PUF) with a density of 20 kg m^{-3} was also used. The nylon fiber and PUF were cut in cubes of approximately 1 cm^3, and then washed twice with a 40% NaOH and 10% HCl solution. After washing, the nylon fiber and PUF were rinsed with distilled water to reach a neutral pH. Thereafter, the cubes were dried in an oven at 60 °C during 24 h.

2.3. Sorption isotherms

A sorption isotherm is a very useful tool to describe the equilibrium relationship between the amount of solute sorbed per mass unit of sorbent and the amount of solute remaining in the liquid phase. To investigate the sorption capacity of PCP by polyurethane and nylon fiber, the three most common isotherm models, i.e., the Langmuir, Freundlich, and Redlich–Peterson models were used to describe the PCP sorption by the fiber. Table 1 shows the equations describing each model.

The Langmuir isotherm is used for homogeneous sorption and describes an ideal sorption where only one molecule of solute can occupy one sorption site forming a mono layer on the sorbent. Once the saturation concentration has been reached or all available sorption sites have been occupied, sorption stops and no other layers are formed [21]. q_m and K_a are the sorption capacity to reach the saturation point and the Langmuir equilibrium constant, respectively. It is thermodynamically consistent and follows Henry's Law at low concentrations [22].

The Freundlich isotherm is an empirical model used to describe a non-ideal sorption. This model is a generalization of the Langmuir model applied to heterogeneous surfaces with an energy distribution corresponding to an exponential diminution, and it describes multilayered sorption. Theoretically, an infinite amount of sorption can occur. However, this empirical model does not follow the thermodynamic fundaments since it is not consistent with Henry's Law at low concentrations, and it does not predict saturation [21,23,24]. K_F and $1/n$ are the binding affinity of the solute with a sorbent and the heterogeneity index, respectively. The heterogeneity index ranges between 0 and 1, $1/n$ becomes closer to 1 as heterogeneity decreases, being $1/n = 1$ for a homogeneous surface system.

The Redlich–Peterson isotherm contains three parameters (A, B, g) and involves the features of both the Langmuir and Freundlich isotherms [25,26]. This equation reduces to a linear isotherm at low solute concentrations, to the Freundlich isotherm at high solute concentration, and to the Langmuir isotherm when $g = 1$ [21].

Isotherm studies were carried out by contacting 100 mL of M–N medium containing 200 mg of PCP L^{-1} (pH 5.3) in 250 mL Erlenmeyer flasks with different masses of nylon fiber and PUF, which varied from 0.04 to 4.15 g of nylon fiber and from 0.05 to 1.0 g of PUF. The flasks were shaken at 200 rpm at 30 ± 1 °C for 5 days. Two replicates of each support mass were conducted for the PCP sorption assays for both supports.

In previous studies, the sorption capacity of PCP at equilibrium concentration by the nylon fiber was reached after 5 days of exposure and by the PUF after 2 h [16]. Thereafter, solutions were centrifuged at 16,000 × g for 30 min and filtered through 0.22-μm nitro-cellulose membranes. The remaining PCP in the solution was determined through HPLC. The quantified PCP was considered the PCP concentration at equilibrium (C_e).

The amount of sorbed PCP by both supports was calculated by using the following equilibrium equation:

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (1)$$

where q_e is the equilibrium sorption capacity of PCP sorbed per unit mass of nylon fiber or PUF (mg g^{-1}), C_0 and C_e are the initial and equilibrium PCP concentrations (mg L^{-1}), respectively, V is the volume of the PCP solution (L), and m is the weight of the support (g).

2.4. Error analysis

In this study, the non-linear coefficients of determination (r^2), Chi-square test, and normalized standard deviation ($\Delta q(\%)$) were used to evaluate the fit of the experimental data to the isotherm models [25,27]. Table 2 shows the equations that describe these error functions.

Where $q_{e,m}$ is the sorption capacity at equilibrium conditions obtained from the isotherm model, q_e is the sorption capacity at equilibrium conditions obtained from the experimental data, $\bar{q}_e$ (with a line above) is the average of q_e, and n is the number of experimental data.

2.5. Immobilization of R. oryzae ENHE

Nylon fiber was used as inert support for the immobilization and growth of *R. oryzae* ENHE. Sterilized nylon cubes were impregnated to 55% humidity with M–N (2×) medium, inoculated with 1×10^7 spore mL^{-1}. Six grams of nylon cubes, of 1 cm^3 each, impregnated with medium and inoculated with *R. oryzae* ENHE spores were placed in 250 mL Erlenmeyer flasks and covered with aluminum foil and parafilm. Cultures were incubated at 30 ± 1 °C

Table 2
Error functions used to discriminate between models.

Error function	Definition	Reference
Coefficient of determination	$(2) r^2 = \frac{\sum (q_{e,m} - \bar{q}_e)^2}{\sum (q_{e,m} - \bar{q}_e)^2 + \sum (q_{e,m} - q_e)^2}$	Kumar et al. [25]
Chi-square function	$(3) x^2 \sum \frac{(q_e - q_{e,m})^2}{q_{e,m}}$	Ho et al. [26]
Normalized standard deviation	$(4) \Delta q(\%) = 100 \times \sqrt{\frac{\sum [(q_e - q_{e,m})/q_e]}{n-1}}$	Mathialagan–Viraraghavan [27]

for 5 days. Experiments were made in triplicate. Growth of *R. oryzae* ENHE (dry weight mycelium) into the nylon fiber was measured indirectly by glucosamine concentration. Glucosamine was quantified by the modified method of Tomaselli et al. [28] and Marcial et al. [14]. Simultaneously a submerged culture of *R. oryzae* ENHE was carried out and growth was determined by the dry weight of the mycelium and glucosamine concentration. Using these values, a curve that related the milligrams of dry mycelium *vs*. micrograms of glucosamine was made to obtain the correlation between the amount of mycelium and glucosamine concentrations [14]. A linear relationship was obtained and used to determine indirectly the biomass of immobilized mycelium (mg) in the samples, $y = 178.34X + 30.345$; $R^2 = 0.9884$ [16] and was referred as mycelium dry weight.

To eliminate the error induced by PCP sorption in the nylon fiber, and avoid inaccurate quantifications, the nylon fiber was impregnated with 200 mg of PCP L^{-1}, previously dissolved in M–N medium, until equilibrium conditions before immobilization. Immobilization assays were carried out using three conditions. The first, using nylon cubes with PCP at equilibrium concentration; the second, using nylon cubes with PCP at equilibrium concentration added with 14 mg PCP L^{-1} dissolved in the medium. And a third corresponded to the control culture, where nylon cubes were not previously impregnated and no PCP was added to the medium.

2.6. PCP removal

To study the PCP removal capability of immobilized *R. oryzae* ENHE in nylon fiber, experiments were performed in submerged fermentations using the M–N medium. Two initial PCP concentrations were used for removal experiments, 12.5 mg L^{-1} and 25 mg L^{-1}. Immobilized *R. oryzae* ENHE in 2.5 g of nylon fiber, previously impregnated with PCP at equilibrium concentration, were placed into Erlenmeyer flasks containing 100 mL of M–N medium at the initial PCP concentration to be assayed. The cultures were incubated at 30 ± 1 °C under constant shaking at 200 rpm. Samples were collected, in triplicate, every 24 h to quantify residual PCP in the liquid phase.

2.7. PCP quantification

To determine the PCP sorbed by the immobilized biomass [29], samples were taken from the liquid phase and immobilized material, liquid phase free of biomass was considered the direct extract. Nylon cubes with immobilized biomass were placed in a sonic bath for 30 min using a carbonate buffer, pH 11; this fraction was named biomass extract. The pH was adjusted to 7.0 in both extracts, before HPLC analysis; both fractions were filtered through 0.22-μm FP Vericel membranes (Gelman Sciences). PCP from both extracts was quantified and the sum of these values was reported as total residual PCP. HPLC quantification was conducted in a Waters System 600 solvent delivery system equipped with a 996 Photodiode Array Detector at 240 nm. A volume of 20 μL was introduced on the μBondapak™ C-18 column (inverse phase) and an isocratic elution was carried out at a flow rate of 1.5 mL min^{-1} with the mobile phase containing acetonitrile–1% acetic acid solution and water–1% acetic acid solution (75:25, v/v). Concentrations were quantified using a calibration curve obtained from external standards and analyzed using the Millenium software (Waters).

3. Results and discussion

3.1. Sorption isotherm

Fig. 1a and b shows the fit of the isotherm obtained from the Lagmuir, Freundlich, and Redlich–Peterson models, using a non-linear method adjustment (equations shown in Table 2) for PCP sorption by PUF and nylon fiber, respectively, compared with the experimental data. White circles correspond to experimental data, obtained in duplicate, as can be seen there are no difference between duplicates, the lines correspond to the adjusment obtained from the three models indicating that all three models fitted well with the experimental data.

The three isotherm model constants are shown in Table 3. The coefficient of determination, the Chi-square, and the normalized standard deviation values are very similar for the three models, which means that the PCP sorption isotherm by PUF and nylon fiber can be described by any of the models used. The value of *g*, obtained from the Redlich–Peterson model, was of 0.830 and 0.815 for PUF and nylon fiber, respectively, indicating, that probably the data of the isotherm approach the Freundlich model better than the Langmuir model. For this reason, it was not possible to achieve saturation conditions of the nylon fiber with PCP, under the experimental conditions assayed. Hu et al. [30] studied PCP sorption in PUF and used Langmuir model to describe the sorption and obtained a q_m of 76 mg PCP g^{-1} PUF.

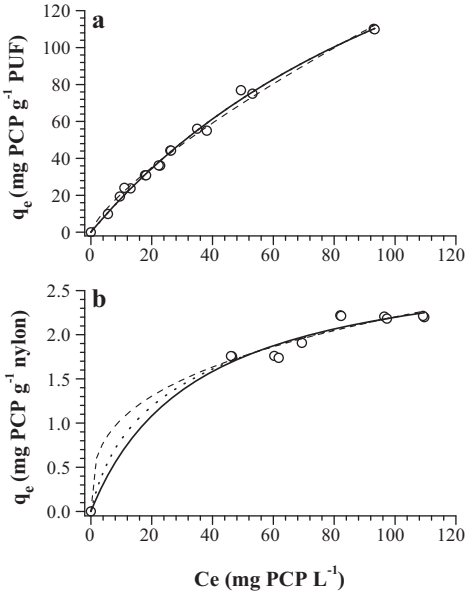

Fig. 1. Isotherms obtained using the non-linear method for the sorption of PCP into the PUF (a) and nylon fiber (b) at 30 °C and 200 rpm. (♦) Experimental data, two replicates for each point; (—) Freundlich isotherm; (–) Langmuir isotherm; (. . .) Redlich–Peterson isotherm.

Table 3
Isotherm parameters obtained using the non-linear method for the sorption of PCP into the PUF and nylon fiber at 30 °C and 200 rpm.

Model and parameters	PUF	Nylon fiber
Langmuir		
q_m (mg g^{-1})	273.12	2.9655
K_a (L mg^{-1})	0.0072	0.0287
r^2	0.9947	0.9781
χ^2	1.9331	0.0476
Δq (%)	6.2993	5.0093
Freundlich		
K_F (mg g^{-1}) (L mg^{-1})$^{1/n}$	3.2698	0.4927
$1/n$	0.7831	0.3250
r^2	0.9922	0.9781
χ^2	2.6602	0.0466
Δq (%)	8.7940	4.8463
Redlich–Peterson		
g (L mg^{-1})$^{1/n}$	0.8300	0.8154
A (L g^{-1})	2.1001	0.1599
B	0.0179	0.1466
r^2	0.9948	0.9782
χ^2	1.8686	0.0466
Δq (%)	8.7940	4.8843

The maximum sorption capacity term, q_m, determined from the Langmuir model defines the total capacity of the support to sorb PCP, which was found around 273.12 mg PCP g^{-1} PUF and 2.965 mg PCP g^{-1} nylon fiber. With 95%, confident, the q_m constant varied from 231.39 to 330.65 mg PCP g^{-1} PUF and from 2.53 to 3.55 mg PCP g^{-1} nylon fiber. We selected the nylon fiber to continue the *R. oryzae* ENHE immobilization and PCP degradation study, since the sorption capacity of the nylon fiber was approximately 100 times lower than the PUF's sorption capacity. From the data obtained we consider that the PCP concentration needed to reach sorption equilibrium was of 3.0 mg PCP g^{-1} nylon.

3.2. *R. oryzae ENHE immobilization into nylon fiber*

Few studies have reported the use of nylon fiber to immobilize fungi, Shin et al. [11] studied the ability of *Trametes versicolor* to colonize several supports and reported that this fungus did not grow on nylon fiber. Our results indicate that *R. oryzae* ENHE grew well in nylon fiber and similar results have been reported for *P. chrysosporium*, which also grows in nylon fiber [31]. Gawande and Kamat [32] reported that xylanase production was increased when using immobilized *Aspergillus* sp. in nylon bolting cloth. Rodríguez-Couto et al. [33] reported that *Trametes hirsuta* grew into the hollow spaces of nylon fiber cubes and produced lacasse, the nylon fiber used by these authors is similar to that used in this work.

R. oryze ENHE grew into nylon fiber impregnated with culture medium using the three conditions described in the methodology section: nylon at equilibrium sorption concentration impregnated with 3.0 mg PCP g^{-1} nylon; nylon at equilibrium sorption concentration impregnated with 3.0 mg PCP g^{-1} nylon plus 14 mg PCP L^{-1}, added to the culture medium used to impregnate the nylon fiber and as control nylon without PCP. Results showed that PCP inhibited growth of *R. oryzae* EHNE, as reported before by León-Santiesteban et al. [17]. In the control culture, maximal biomass obtained was of 32 mg dry biomass g^{-1} nylon and, in cultures containing PCP, growth of *R. oryzae* ENHE was lower; maximal biomasss was of 18 and 15 mg biomass g^{-1} nylon in cultures with nylon cubes that contained PCP at equilibrium concentration and nylon at equilibrium concentration amended with 14 mg PCP g^{-1} nylon, respectively (Fig. 2). In cultures impregnated with 3.0 mg PCP g^{-1} nylon, maximal growth was observed at 36 h; in cultures with 3.0 mg PCP g^{-1} nylon plus 14 mg PCP L^{-1}, maximal growth was achieved at 24 h. In order to determine if the mycelium

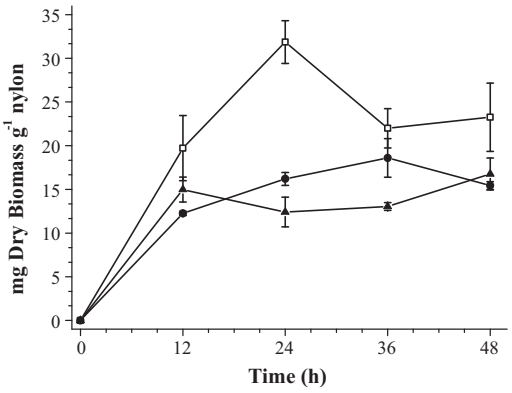

Fig. 2. Growth kinetics profile of *R. oryzae* ENHE immobilized into nylon fiber in M–N medium (2×) incubated at 30 °C with different initial PCP concentrations. (□) nylon fiber without PCP (control); (●) nylon fiber with PCP at equilibrium concentration (approximately, 3 mg PCP g^{-1} nylon); (▲) nylon fiber with PCP at equilibrium concentration supplemented with 14 mg PCP L^{-1} dissolved in the medium. Each point is the mean of three values.

was fixed inside the nylon fiber, the immobilized nylon cubes were mixed with culture medium and shaken at 200 rpm during 96 h. At 48 h, the cubes were observed under a microscope, revealing that mycelia were still immobilized in the hollow spaces inside the nylon fiber cubes. After this time, it was observed that *R. oryzae* ENHE continued growing in the nylon cubes, and, at 96 h, some mycelium was observed in the liquid phase, although the cubes were completely invaded by micelia.

3.3. *PCP removal by R. oryzae ENHE immobilized in nylon fiber*

R. oryzae ENHE immobilized in nylon fiber was assayed to determine its capacity to remove PCP. Immobilization was made using nylon cubes impregnated with PCP at equilibrium concentration that contained 18 mg biomass g^{-1} dry nylon. To eliminate the error induced by any amount of PCP sorbed by mycelia, PCP quantification was made from the liquid extract and from the biomass extract after sonication [29]. Two initial PCP concentrations of 12.5 and 25 mg PCP L^{-1} were assayed. In both cultures, PCP removal was similar at 48 h: in cultures with 12.5 mg PCP L^{-1}, 88.6% of initial PCP was removed and in cultures with 25 mg PCP L^{-1}, 85.7% of initial PCP was removed (Fig. 3). The rate of PCP removal (0.6 mg PCP L^{-1} h^{-1}) was higher in cultures with 25 mg PCP L^{-1} than in cultures with 12.5 mg PCP L^{-1} in which the rate was of 0.4 mg PCP L^{-1} h^{-1}. Perhaps this is due to the fact that immobilized fungi are not in contact with the toxic compound and, for this reason, the fungus degrade

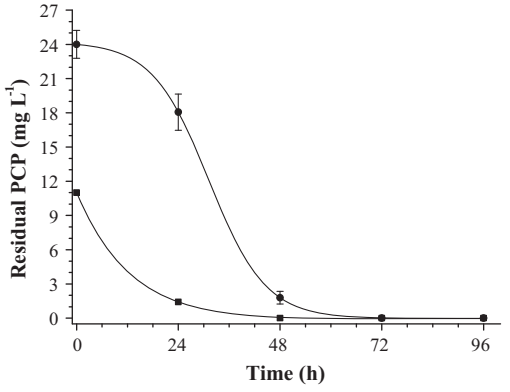

Fig. 3. PCP degradation kinetics with *R. oryzae* ENHE immobilized into nylon fiber in M–N medium incubated at 30 °C with different initial PCP concentrations: (●) 25 mg L^{-1}; (■) 12.5 mg L^{-1}. Each point is the mean of three values.

faster a higher PCP concentrations, 25 mg PCP L^{-1}, than at the lower one of 12.5 mg PCP L^{-1}.

Many studies have reported PCP removal using immobilized microorganisms, but, in these works the amount of PCP sorbed by the support was not studied. Few works report PCP sorption by support, for example O'Reilly and Crawford [34] reported that polyurethane-immobilized *Flavobacterium* degrade PCP, these authors attribute the PCP removal to biodegradation, 60% and 40% to absorption of PCP by the support. Hu et al. [30] characterized PCP adsorption in PUF and reported that PUF-immobilized *Flavobacterium* enhanced the capacity to degrade PCP since the toxicity in the medium is reduced due to sorption of the toxic by the support. Cea et al. [5] reported 76% removal of initial PCP, 250 mg PCP kg^{-1}, in 14 days from soil; they attributed the removal to the inoculated white rot-fungi, to the autochthonous microorganisms, and to PCP adsorption by the soil; a strong PCP adsorption by the type of soil used by these authors has been reported.

León-Santiesteban et al. [17] showed that *R. oryzae* ENHE removed 90% of the initial PCP (12.5 mg L^{-1}) at 48 h in submerged cultures. In this work, maximal PCP removal was 92% of the initial PCP achieved at 72 h in cultures with 25 mg PCP L^{-1}, twice the initial concentration reported by León-Santiesteban et al. [17]. Similar results have been found by Lu et al. [35], who reported that the removal rate of phenolic compounds by free fungi is lower than the removal rate observed by immobilized *P. chrysosporium* fungi. Shim and Kawamoto [36] reported PCP degradation using immobilized *P. chrysosporium* in a polypropylene packed-bed reactor, fed at a rate of 2 L d^{-1} containing 30 mg PCP L^{-1} (or 60 mg PCP d^{-1}); a continuous steady-state was observed from day 7 to 21 with 80% of PCP removal. Other aromatic toxic compounds as well as PCP degradation by immobilized fungi, such as *P. chrysosporium*, *T. hirsuta* and *Monascus kaoliang*, have been reported, using polyurethane foam, alginate, and stainless steel sponge as support materials [35–39]. Ehlers and Rose [40] reported phenol and 2,4,6-trichlorophenol degradation in a sequencing batch reactor using white-rot fungi and found that immobilized fungi presented advantages to be used in this reactor system. This is the first work reporting the immobilization of a zygomycete, *R. oryaze* ENHE, in nylon fiber for PCP removal. It was possible to remove 24 mg PCP L^{-1} in 48 h, apparently immobilization increased the ability of the fungus to remove the toxic compound compared to León-Santiesteban et al. [17] who found that the fungus removed 11 mg PCP L^{-1} in 48 h in submerged culture. Moreover, nylon fiber used as support can be biodegraded [41–43].

4. Conclusions

In this study the importance in determining toxic compound sorption capacity of the support used for cell immobilization before toxic degradation was shown, since it may contribute in some extent to an apparent removal. The amount of sorption depends on the chemical characteristics of the toxic and on the characteristics of the support material used.

In this work, results showed that *R. oryzae* ENHE can grow into the nylon fiber with increased capability to degrade PCP with the advantages of the immobilization systems. Probably because the metabolism of the fungus is stressed due to the immobilization, also due to the toxic is not in contact directly with fungus.

It could be interesting to study desorption of PCP from the support, mainly from PUF, which presented a higher sorption capacity and is one of the most used supports for cell immobilization. Also it is important to know the fate of the toxic sorbed into the support, is it degraded during the removal process of the toxic compound in the liquid phase or into the support? Or remains sorbed to the support and is not released to the environment?

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References

[1] R.T. Lamar, M.J. Larsen, K.T. Kirk, Sensitivity to and degradation of pentachlorophenol by *Phanerochaete chrysosporium* spp., Appl. Microbiol. Biotechnol. 56 (1990) 3519–3526.
[2] B.C. Okeke, A. Paterson, J.E. Smith, I.A. Watson-Craik, Comparative biotransformation of pentachlorophenol in soils by solid substrate cultures of *Lentinus edodes*, Appl. Environ. Microbiol. 48 (1997) 563–569.
[3] K. Anandarajah, P.M. Kiefer Jr., B.S. Donohoe, S.D. Copley, Recruitment of a double isomerase to serve as a reductive dehalogenase during biodegradation of pentachlorophenol, Biochemistry 39 (2000) 5303–5311.
[4] C.J. Jasper, G. Ewbank, A.J. McCarthy, M.J. Pennincxx, Successive rapid reductive dehalogenation and mineralization of pentachlorophenol by the indigenous microflora of farmyard manure compost, J. Appl. Microbiol. 92 (2002) 127–133.
[5] M. Cea, M. Jorquera, O. Rubilar, H. Langer, G. Tortella, M.C. Diez, Bioremediation of soil contaminated with pentacholorophenol by *Anthracophyllum discolor* and its effect on soil microbial community, J. Hazard. Mater. 181 (2010) 315–323.
[6] R.T. Lamar, The role of fungal lignin-degrading enzymes in xenobiotic degradation, Curr. Opin. Biotechnol. 3 (1992) 261–266.
[7] J.E. Lin, H.Y. Wang, R.F. Hickey, Degradation kinetics of pentachlorophenol by *Phanerochaete chrysosporium*, Biotechnol. Bioeng. 35 (1989) 1125–1134.
[8] G.J. Mileski, J.A. Bumpus, M.A. Jurek, S.D. Aust, Biodegradation of pentachlorophenol by the white-rot fungi *Phanerochaete chrysosporium*, Appl. Environ. Microbiol. 54 (1988) 2885–2889.
[9] M.Y. Arica, Y. Kacar, O. Genc, Entrapment of white fungus *Trametes versicolor* in Ca-alginate beads: preparation and biosorption kinetic analysis for cadmium removal from an aqueous solution, Bioresour. Technol. 80 (1993) 121–129.
[10] Y.P. Tieng, G. Sun, Use of polyvinyl alcohol as a cell immobilization matrix for copper biosorption by yeast cells, J. Chem. Technol. Biotechnol. 75 (2000) 541–546.
[11] M. Shin, T. Nguyen, J. Ramsay, Evaluation of support materials for the surface immobilization and decoloration of amaranth by *Trametes versiclor*, Appl. Microbiol. Biotechnol. 60 (2002) 218–223.
[12] N. Thongchul, S.T. Yang, Controlling filamentous fungal morphology by immobilization on a rotating fibrous matrix to enhance oxygen transfer and L(+)-lactic acid production by *Rhizopus oryzae*, Ferment. Biotechnol. 862 (2003) 36–51.
[13] D. Gao, Y. Zeng, X. Wen, Y. Qian, Competition strategies for the incubation of white rot fungi under non-sterile conditions, Process Biochem. 43 (2008) 937–944.
[14] J. Marcial, J. Barrios-González, A. Tomasini, Effect of medium composition on pentachlorophenol removal by *Amylomyces rouxii* in solid-state culture, Process Biochem. 41 (2006) 496–500.
[15] A. Mohammadi, B. Nasernejad, Enzymatic degradation of anthracene by the white rot fungus *Phaenerochaete chrysosporium* immobilized on sugarcane bagasse, J. Hazard. Mater. 161 (2009) 534–537.
[16] H. León-Santiesteban, Inmovilización de Rhizopus oryzae in cursives ENHE, MS Thesis, Biotechnology Graduate Studies, Universidad Autonóma Metropolitana-Iztapalapa, Mexico, 2010.
[17] H. León-Santiesteban, R. Bernal, F.J. Fernández, A. Tomasini, Tyrosinase and peroxidase production by *Rhizopus oryzae* strain ENHE obtained from pentachlorophenol-contaminated soil, J. Chem. Technol. Biotechnol. 83 (2008) 1394–1400.
[18] I. Langmuir, The constitution and fundamental properties of solids and liquids, J. Am. Chem. Soc. 38 (1916) 2221–2295.
[19] H.M.F. Freundlich, Over the adsorption in solution, J. Phys. Chem. 57 (1906) 385–470.
[20] O. Redlich, D.L. Peterson, A useful adsorption isotherm, J. Phys. Chem. 63 (1959) 1024.
[21] J.A. García-Calzón, M.E. Díaz-García, Characterization of binding sites in molecularly imprinted polymers, Sens. Actuators B 123 (2007) 1180–1194.
[22] S.J. Allen, G. Mckay, J.F. Poter, Adsorption isotherm models for basic dye adsorption by peat in single and binary component systems, J. Colloid Interface Sci. 280 (2004) 322–333.
[23] C. Ng, J.N. Losso, W.E. Marshall, R.M. Rao, Freundlich adsorption isotherms of agricultural by-product-based powdered activated carbons in a geosmin–water system, Bioresour. Technol. 85 (2002) 131–135.
[24] X.-S. Wang, Y. Qin, Equilibrium sorption isotherms for Cu^{+2} on rice bran, Process Biochem. 40 (2005) 677–680.
[25] K.V. Kumar, K. Porkodi, F. Rocha, Comparison of various error functions in predicting the optimum isotherm by linear and non-linear regression analysis for the sorption of basic red 9 by activated carbon, J. Hazard. Mater. 150 (2008) 158–165.
[26] Y.S. Ho, W.T. Chiu, C.C. Wang, Regression analysis for the sorption isotherms of basic dyes on sugarcane dust, Bioresour. Technol. 96 (2005) 1285–1291.
[27] T. Mathialagan, T. Viraraghavan, Biosorption of pentachlorophenol from aqueous solutions by a fungal biomass, Bioresour. Technol. 100 (2009) 549–558.

[28] S.C. Tomaselli, C. Vergoignan, G. Feron, A. Durand, Glucosamine measurement as indirect method for biomass estimation of *Cunningamella elegans* grown in solid state cultivation conditions, Biochem. Eng. J. 7 (2001) 1–5.
[29] A. Tomasini, V. Flores, D. Cortés, J. Barrios-González, An isolate of *Rhizopus nigricans* capable of tolerating and removing pentachlorophenol, World J. Microbiol. Biotechnol. 17 (2001) 201–205.
[30] Z.C. Hu, R.A. Korus, W.E. Levinson, R.L. Crawford, Adsorption and biodegradation of pentachlorophenol by polyurethane-immobilized *Flavobacterium*, Environ. Sci. Technol. 28 (1994) 491–496.
[31] C. Lauguero, J.-C. Sigoillot, S. Moukha, P. Frasse, M.-N. Bello-Fontaine, P. Bonnarme, C. Mougin, M. Asther, Selective hyperproduction of manganese peroxidases by *Phanerochaete chrysosporium* l–1512 immobilized on nylon net in a bubble-column reactor, Appl. Microbiol. Biotechnol. 44 (1996) 717–723.
[32] P.V. Gawande, M.K. Kamat, Immobilization of *Aspergillus* sp. on nylon blotting cloth for production of xylanase, J. Ferment. Bioeng. 86 (1998) 243–246.
[33] S. Rodríguez-Couto, M.A. Sanromán, D. Hofer, G.M. Gübitz, Stainless steel sponge: a novel carrier for the immobilization of the white-rot fungus *Trameste hirsuta* for decolourization of textile dyes, Bioresour. Technol. 95 (2004) 67–72.
[34] K. O'Reilly, R. Crawford, Degradation of pentachlorophenol by polyurethane-immobilized Flavobacterium cells, Appl. Environ. Microbiol. 55 (1989) 2113–2118.
[35] Y. Lu, L. Yan, Y. Wang, S. Zhou, J. Fu, J. Zhang, Biodegradation of phenolic compounds from coking wastewater by immobilized white rot fungus *Phanerochaete chrysosporium*, J. Hazard. Mater. 165 (2008) 1091–1097.
[36] S.S. Shim, K. Kawamoto, Enzyme production activity of *Phanerochaete chrysosporium* and degradation of pentachlorophenol in a bioreactor, Water Res. 36 (2002) 4445–4454.
[37] X.-Y. Jiang, G.M. Zeng, D.L. Huang, Y. Chen, F. Liu, G.H. Huang, J.B. Li, B.D. Xi, H.L. Liu, Remediation of pentachlorophenol-contaminated soil by composting with immobilized *Phanerochaete chrysosporium*, World J. Microbiol. Biotechnol. 22 (2006) 909–913.
[38] S. Rodríguez-Couto, Dye removal by immobilized fungi, Biotechnol. Adv. 27 (2009) 227–235.
[39] M.A. Quezada, J.D. Carballeira, J.V. Sinisterra, *Monascus kaoliang* CBS 302.78 immobilized in polyurethane foam using iso-propanol as co-substrate: optimized immobilization conditions of a fungus as biocatalyst for the reduction of ketones, Bioresour. Technol. 100 (2009) 2018–2025.
[40] G.A. Ehlers, P.D. Rose, Immobilized white-rot fungal biodegradation of phenol and chlorinated phenol in trickling packed-bed reactors by employing sequencing batch operation, Bioresour. Technol. 96 (2005) 1264–1275.
[41] T. Deguchi, M. Kakezawa, T. Nishida, Nylon biodegradation by lignin-degrading fungi, Appl. Environ. Microbiol. 63 (1997) 329–331.
[42] T. Deguchi, Y. Kitaoka, M. Kakezawa, T. Nishida, Purification and characterization of a nylon-degrading enzyme, Appl. Environ. Microbiol. 64 (1998) 1366–1371.
[43] M. Sudhakar, C. Priyadarshini, M. Doble, P. Sriyutha Murthy, R. Venkatesan, Marine bacteria mediated degradation of nylon 66 and 6, Int. Biodeter. Biodegr. 60 (2007) 144–151.