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Reactor development for biodegradation of pentachlorophenol

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Abstract

Biodegradation of pentachlorophenol (PCP) by the free-cell and polyurethane immobilized white-rot fungus *Trametes versicolor* was investigated in batch and continuous bioreactors. Results with both active and deactivated fungus showed that the degradation process proceeds intracellularly after initial uptake by the mycelia. However, unpurified extracellular fluid alone showed negligible PCP degradation. The polyurethane immobilized *T. versicolor* fungal bioreactor, previously demonstrated for the treatment of kraft bleach plant effluents, yielded greater than a 99% removal within 12 h for inlet PCP concentrations ranging from 20 to 25 mg/l. A substrate inhibition model adequately described PCP degradation kinetics in the prepolymer immobilized fluidized bed bioreactor system. © 1998 Elsevier Science B.V.

Keywords: Fungi; *T. versicolor*; Biodegradation; Bioreactor; Pentachlorophenol; Enzymes; Kinetics; Reactor design

1. Introduction

Pentachlorophenol (PCP) has been extensively used as a wood preservative, pesticide, and fungicide and is rated as a priority pollutant by the EPA. The wood preserving industry is a primary source of PCP containing waste waters and ground waters. Several studies have reported on the biodegradation of pentachlorophenol by some bacteria and fungi. However, although PCP undergoes biodegradation, the process has been observed to be very slow.

Many reports show that white-rot fungi can degrade a variety of persistent aromatic organopollutants such as PCP, PCBs, DDT, PAHs and phenanthrene [1,2]. Lignin peroxidase (LiP) and manganese peroxidase (MnP), secreted by the fungi, are implicated in the initial degradation of chlorinated aromatic compounds [2]. *Phanerochaete chrysosporium* has received almost exclusive attention among white-rot fungi for the PCP degradation in contaminated water and soil. Recently, studies have shown that another white-rot fungus *Trametes versicolor* also has potential as a PCP degrader [3,4]. Current interest in using *T. versicolor* in bioreactor studies for aqueous waste treatment and bioremediation of contaminated soil is growing because this microorganism has been found to degrade many persistent pollutants very efficiently, and it is known to have a greater resistance to PCP toxicity than other species of fungi [5]. Although Logan et al. [3] and Alleman et al. [4] have explored the ability of this species along with other species of the fungi for PCP biodegradation, their studies were limited to static batch cultures which required long residence times to observe appreciable degradation levels. It is important that the degradation studies

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focus on realistic bioreactor operating conditions and information on biodegradation mechanisms and kinetic data capable of allowing an engineered reactor design using *T. versicolor.*

The primary objective of our research was to examine the ability of *Trametes versicolor* (ATCC 20869) to degrade PCP in batch and continuous cultures. Another objective was to examine the specific functions of mycelia and extracellular enzymes in PCP degradation. Finally, PCP degradation was evaluated with a polyurethane immobilized fluidized bed bioreactor previously developed for the treatment of paper mill bleach plant effluents [6]. A series of batch and continuous experiments were conducted using free-cell and polymer immobilized *T. versicolor* which allowed us to determine PCP degradation kinetics, mechanisms, and biological behavior of the reactor for PCP degradation.

2. Materials and methods

2.1. Feed composition

0.5 g of PCP (Sigma) was dissolved in 100 ml of 0.25 N NaOH solution to make a 5000 ppm stock solution. The bioreactor feed solutions of different PCP concentrations were prepared from this stock. The feed solution was supplied with a vitamin and mineral stock solution, and a carbon, nitrogen, and phosphorous source solution containing 0.6% glucose, 0.16% $(NH_4)_2SO_4$, 0.3% K_2HPO_4 and 0.2% KH_2PO_4 (w/v). The composition of the vitamin supplement (final concentration) was: 0.2 mg/l of each D-pantothenic acid, riboflavin, folic acid, pyridoxal, thiamine-HCL, nicotinic acid, and biotin. The composition of a mineral salt supplement was: 0.1 μM $NiCl_2$, 5 μM $CoCl_2$, 20 μM $MnCl_2$, 5 μM $ZnCl_2$, 1 μM $CuSO_4$, 40 μM Na-citrate, 20 μM $FeSO_4$ and 0.5 μM $(NH_4)_6Mo_7O_{24}$. The final pH of the mixture was adjusted to 5.0. with 1 N HCl.

2.2. Gas chromatography

The concentration of PCP in samples was determined by gas chromatography. Quantification was carried out after derivatization (acetylation). The sample (1 ml), 9 ml of distilled water, and 25 μl of a 0.05 mg/ml solution of 2,6-dibromophenol as an internal standard were added to a 50 ml beaker, and the pH was adjusted to 7.0 using NaOH. Then potassium carbonate (0.26 ml of 0.6 g/ml) was added and the pH was adjusted 11.6. Finally, the mixture and 0.5 ml of acetic anhydride were added to a separating funnel. The funnel was shaken vigorously, venting frequently to mix the contents, and extracted with 2 ml of hexane. A 0.5 μl extract was analyzed on a DB-1 fused silica capillary column (length 15 m; ID 0.25 mm; J&W) installed in a Varian 3400 gas chromatograph equipped with an electron capture detector (ECD). Split injection (1:100) and programmable temperature was used with nitrogen as the carrier gas. The oven temperature was 45°C for 1 min, increased to 100°C at a rate of 15°C/min, increased to 165°C at a rate of 2°C/min, increased to 230°C at a rate of 20°C/min, and maintained at 230°C for 5 min. The injector temperature was 260°C and the detector temperature was 325°C. Standards were run and used for identification of the unknown concentrations. For the determination of PCP concentration in the biomass, the mycelium was suspended in 0.25 N NaOH and homogenized in a Waring Blender and then centrifuged. The centrate was used for analysis using the above described method.

2.3. Mycelia concentration

The biomass concentration in the immobilized foam cubes was determined by the protein content method. Protein content was determined by the method described by Lowry et al. [7]. For calculation of the biomass concentration, it was assumed that 50–60% of the mycelial dry weight is cell protein [7]. The biomass concentration in case of free-cell experiments was estimated by dry weight.

2.4. Determination of enzyme activities

Laccase activity was measured in 3 ml cuvette containing 2.2 ml of 100 mM potassium phosphate buffer, pH 6.5 at 30°C, 0.3 ml of 0.216 mM syringaldazine solution, and 0.5 ml of the sample. The increase in $A_{530\ nm}$ was recorded for 10 min. The rate of $\Delta A_{530\ nm}$ was measured from slope. One unit will produce a $\Delta A_{530\ nm}$ of 0.001 per minute at pH 6.5 and

at 30°C in a 3 ml reaction volume using Syringaldazine as substrate [8].

Manganese peroxidase activity was measured as described by Paszczynski et al. [9]. Briefly, the reaction mixture contained 0.1 M sodium tartarate (pH=5.0), 0.1 mM H_2O_2, and 0.1 mM $MnSO_4$. Reactions were initiated by the addition of H_2O_2. Increase in absorbance was monitored during the first 5–30 s of reaction. One unit of peroxidase oxidizes 1 μmol of Mn(II)/min. Lignin peroxidase activity was measured by the procedure of Tien and Kirk [10]. All the chemicals for enzyme assay were obtained from Sigma, St. Louis, MO.

2.5. Fluidized bed bioreactor

For continuous studies, a fluidized bed bioreactor with a 600 cm^3 working volume was used. The fungus immobilization technique and reactor system was similar to that described elsewhere [6]. The bioreactor was aerated with air using a glass dispersion tube (Ace Glass). The bioreactor was operated continuously. The inlet air and inlet feed to the reactor were passed through an in-line air purifying membrane filter (pore size 0.2 microns, ACRO 37 TF, Gelman Sciences) and a micro-filter (pore size 0.2 microns, SUPORCAP, Gelman Sciences), respectively. The schematic diagram of the fluidized bioreactor system is shown in Fig. 1. PCP degradation was evaluated based on PCP removal from solution.

2.6. Experiment approach

Experiments were conducted to investigate degradation of PCP by *Trametes versicolor* in lab scale batch and continuous bioreactor systems. Experiments performed include: (1) batch reactor studies using free cells of *T. versicolor*, (2) batch studies with polyurethane immobilized fungus, (3) continuous fluidized bioreactor studies employing mycelial pellets of *T. versicolor*, and (4) continuous polyurethane immobilized fungal fluidized bed bioreactor (IFFBB) that was previously developed for the treatment of bleach plant effluents. All batch experiments were conducted in foam-stoppered 500 ml Erlenmeyer flasks containing 150 ml PCP feed solution and fungus.

3. Results and discussions

3.1. Batch studies using mycelial pellets of T. versicolor

Fungus *T. versicolor*, grown in the form of mycelial pellets for three days, was used to examine the role of extracellular enzymes and removal of PCP. Controls

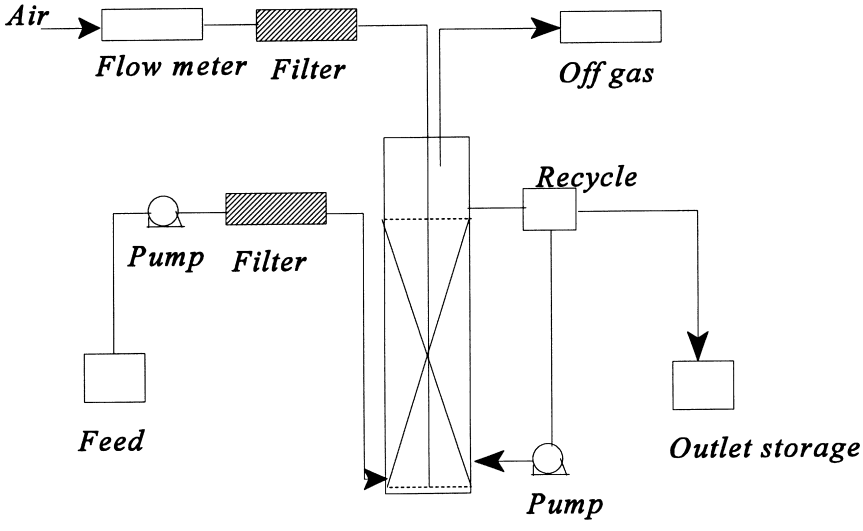

Fig. 1. The schematic diagram of fluidized bed bioreactor system.

Table 1
PCP uptake and removal in batch cultures of *T. versicolor*

Time (days)	Batch-1 (mycelia+extracellular fluid)	Batch-2 (washed mycelia+feed solution)	Batch-3 (autoclaved mycelia+feed solution)
0	10.0 mg/l	10.0 mg/l	10.0 mg/l
1	0.593	1.979 (80%)	1.747 (82%)
2	0.00026	0.984 (90%)	3.064 (69%)
3	ND[a]	0.941 (91%)	6.217 (38%)

( ) – % reduction.
[a]ND: not detected by GC.

were performed using autoclaved (225°F, 20 min) fungal cultures. Grown mycelial pellets were separated from extracellular fluid by vacuum filtration with glass fiber filters (GF/C, Whatman) and rinsed with deionized water. The washed mycelia were weighed and added to the 500 ml Erlenmeyer flasks (Batch-2 and Batch-3) containing 150 ml of 10 mg/l PCP feed solution (Table 1). Mycelia used in Batch-1 was not separated from the extracellular fluid. The contents of Batch-1 (mycelia+extracellular fluid) were directly dosed with PCP stock solution to yield a 10 mg/l of final PCP concentration. The extracellular fluid was characterized for lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase enzymes. Under the growth media conditions of this study, laccase was the only enzyme secreted at high levels (479 units/ml), together with low levels of manganese peroxidase (MnP). Similar results were obtained in the past by Archibald et al. [11] and Roy-Arcand and Archibald [12] in their studies that dealt with the quantification of extracellular enzymes in *T. versicolor* cultures.

The largest overall reduction of PCP ($\geq$99%) was achieved from Batch-1 which contained both the mycelia and the extracellular fluid (see Table 1). Batch-2, which contained washed mycelia and feed solution, but without the initial extracellualr fluid, yielded slightly lower reduction levels as compared with Batch-1, although the overall amount of reduction was quite large (90% in 48 h). Experiments with unpurified extracellular fluid without any mycelia present showed the presence of laccase at a concentration typically secreted by *T. versicolor* cultures (479 units/ml). However, negligible PCP degradation was observed in those experiments. These observations suggest that the PCP degradation process proceeds intracellularly and the presence of extracellular enzymes enhances the degradation process and reaction rate. In Batch-3, containing deactivated mycelia (autoclaved), the PCP concentration dropped rapidly during the first 24 h due to the uptake by the mycelia. However, from day two onwards the PCP concentration in the solution increased suggesting that the PCP uptake by the mycelia without subsequent metabolism is temporal and is reversible.

3.2. PCP removal in sequential batch studies

The purpose of this study was to determine the PCP concentration that the fungus can withstand and degrade efficiently. Sequential batch experiments were carried out in 500 ml flasks containing 150 ml feed using three-day-old cultures of *T. versicolor* grown as pellets (2.37 g/l of mycelia as dry wt). The flasks were sealed with foam stoppers and placed in a shaker (Labline) at 180 rpm. The flasks were sparged with pure oxygen once a day for 1 min. The incubating feed solution was decanted and replaced with a fresh media daily. The PCP concentration in solution was measured daily. PCP degradation levels at initial concentrations ranging from 5 to 10 mg/l are shown in Table 2. During the first batch cycle, all three flasks yielded $\geq$98% reduction in PCP due to rapid uptake by the mycelia. For subsequent four batch cycles, flasks containing up to 7.5 mg/l PCP concentrations showed significant reductions (over 80%). However, the flask containing 10 mg/l PCP concentration showed marked decline in reduction for each cycle of operation. This clearly showed that the 10 mg/l PCP levels exhibited toxicity to the free-cell mycelia. It can be concluded from these results that the

Table 2
Degradation of PCP in sequential batch experiments (batch time=1 day) using free-cells of *T. versicolor*

Batch	PCP concentration in feed solution (mg/l)		
	5	7.5	10
1	0.094 (98%)	0.1109 (99%)	0.204 (98%)
2	0.250 (95%)	0.294 (96%)	2.82 (72%)
3	0.386 (94%)	0.704 (91%)	3.146 (69%)
4	0.940 (81%)	1.523 (80%)	5.288 (47%)
5	0.921 (82%)	1.427 (81%)	5.828 (42%)

fungus *T. versicolor* can withstand the inhibiting nature of PCP up to a level of 7 mg/l when operated in batch cultures. Many reported studies show that PCP can inhibit the activity of the fungi at higher concentrations, particularly in batch cultures. Though some studies have shown that PCP can be toxic to fungi at higher concentrations [13,14], Alleman et al. [5] reported that the PCP toxicity could be better described by the chemical doses, expressed as the ratio of the mass of chemical to the mass of the mycelium rather than by the solution concentration. The 10 mg/l experiments showed PCP toxicity was at a rate of 5.5 mg of PCP per g mycelia (dry wt basis).

Other information can be drawn from the data presented in Table 2 by calculating degradation rates at each initial concentration. The activity of the mycelia exposed to 5–10 mg/l of PCP on a daily basis was calculated for five days (Fig. 2). The rate of PCP degradation was determined by finding the slope of the linear fit for each concentration. The rates of PCP degradation over the five days of the study were 0.52, 0.35 and 0.33 mg (g. mycelia)$^{-1}$ day^{-1} at an initial PCP concentration of 5, 7.5, and 10 mg/l, respectively. From Fig. 2, it can also be seen that the total amount of PCP degraded was similar from both 7.5 and 10 mg/l batch experiments. This similarity indicates that there may be an upper limit on PCP removed by a specified amount of mycelia. Two possibilities may be suggested that may limit the amount of PCP removal [14,15]. The first would be the buildup of intermediates which inhibit PCP transformations. In the present study since the feed solution was replaced daily by fresh medium, the possibility of intermediates is limited to intermediates retained by the mycelia. The second explanation for a limit on the amount of PCP removed may be the inactivation of mycelia and/or some of the degrading enzymes during the course of those experiments.

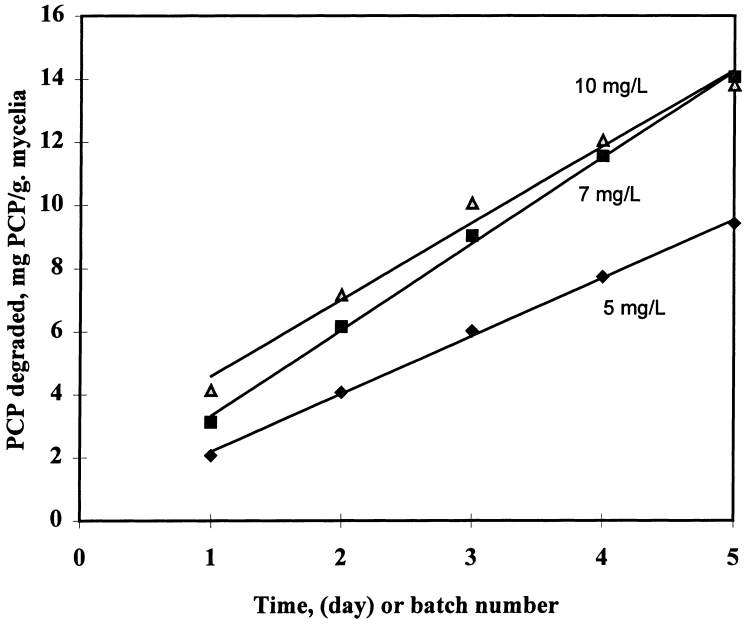

Fig. 2. Total mass of PCP degraded in batch reactors. Degradation rates (slope) are: 0.52, 0.35 and 0.33 mg PCP/g.myc.d.

Table 3
PCP levels in the aqueous and biomass phases: 48 h batch study

Feed PCP (mg/l)	Percent PCP remaining (mass%)	
	Liquid phase	Biomass phase
10 (1.5 mg)	1.5	12
15 (2.25 mg)	0.6	9
20 (3.0 mg)	1.4	17.7
10 (1.5 mg)[a]	24	70

Note: bracketed numbers in column 1 represent amount of PCP in each flask.
[a]Control experiments with deactivated mycelia.

3.3. Role of PCP uptake on removal

The specific function of mycelia in the PCP degradation was determined in a 48 h batch study conducted at different initial PCP concentrations (Table 3). After 48 h, the amount of PCP in the liquid phase and the biomass phase were determined. The data listed in Table 3 show that most of the PCP in the feed solution was degraded by active fungus and not simply taken up by the mycelia. In control batch experiments using deactivated mycelia, about 70% of the initial PCP was found to be in the biomass phase. While in other studies using live mycelia the amount of PCP associated with mycelia was ranged from 7% to 12% only. As it can be expected there was similarly a large difference in percent PCP detected from liquid phase in experiments with live and deactive mycelia. These results clearly suggest that the active live mycelia was able to degrade the initial adsorbed PCP. It is believed that the sorbed pollutant was diffused/transported through cell membrane/wall and degraded by intracellular enzymes.

3.4. Continuous reactor study

3.4.1. Continuous free-cell bioreactor

A continuous free-cell experiment was conducted to measure the PCP removal capacity of *T. versicolor* grown in the form of pellets. From three day batch cultures, mycelial pellets were transferred to the reactor and the reactor was fed with PCP feed and operated in batch mode for one day initially to allow the mycelia to acclimate to PCP. To examine the effect of higher PCP concentrations, the bioreactor was fed with a feed containing PCP at concentrations varying from 5 to 15 mg/l. The continuous operation of the reactor was initiated with a lower (5 mg/l) PCP concentration and later once the bioreactor attained a biologically stable operating condition, the bioreactor feed PCP concentration was increased stepwise to 15 mg/l. Normally, it required about three days at each concentration. For the range of initial PCP concentrations studied, the free-cell bioreactor yielded more than 99% PCP reduction (results not shown).

3.4.2. Continuous immobilized fungal bioreactor

The primary objective of this part of the study was to evaluate the potential application of polyurethane prepolymer immobilized *T. versicolor* fluidized bed bioreactor for the biodegradation of PCP. The PCP degradation capacity of immobilized fungus at various initial PCP concentrations and longevity of PCP-degrading activity was examined. It has been observed that the catalytic stability of immobilized cells can be greater than for free cells, and some immobilized microorganisms tolerate higher concentrations of toxic compounds than their non-immobilized counterparts [12].

The PCP degrading activity of immobilized *T. versicolor* in a continuous reactor was examined for 240 h at different inlet PCP concentrations and reactor residence times. The bioreactor was fed with a PCP concentration of as high as 25 mg/l (Table 4). It should be mentioned that the solubility of PCP in water is 20–25 mg/l at room temperature [2]. About 360 fungus immobilized foam particles (approximately $6.4\times4.8\times3.3$ mm) were incubated in mycological broth for one day, rinsed with sterile water and transferred into the reactor. The initial mycelia concentration was about 4.89 mg/l (measured as protein). The bioreactor was filled with a feed solution of 25 mg/l PCP and operated in batch mode for one

Table 4
Summary of the results and operation conditions for PCP removal in the immobilized fluidized bed fungal bioreactor

Run	Inlet PCP (mg/l)	Residence time (h)	Outlet PCP (mg/l)	Reduction (%)	Biomass (g/l)
1	25	24	0.186	99	6.44
2	20	24	0.173	99	6.65
3	20	12	0.165	99	6.21
4	25	12	0.179	99	5.00

day. From the second day onwards the operation was switched to a continuous pattern. At each condition of the variable (either inlet PCP concentration or reactor residence time) studied, continuous operation of the bioreactor was carried out for at least three days to allow the reactor to reach biologically stable (steady-state) condition.

Table 4 shows the reactor effluent PCP levels and inlet PCP concentrations at different residence times. More than 99% of the inlet PCP was removed during all experiments. About 175 mg of PCP was degraded in the immobilized bioreactor over the 240 h of continuous operation. This corresponds to degradation of 99% of the PCP added to the reactor. At steady state, the rate of PCP removal was determined by a PCP material balance: $V_t = F(S_i - S_e)$, where V_t is the removal rate (mg/d), F the flow rate (l/d), S_i the reactor inlet PCP concentration (mg/l) and S_e is the reactor outlet PCP (mg/l). The removal levels were similar at all initial PCP concentrations. The degradation rate ranged from 198 to 497 mg of PCP/(l foam) day with a maximum observed rate during Run4 (see Table 4). Mycelia concentration increased from 4.89 to 6.44 g/l over first two days of a continuous study. However, during later two runs (Run2 and Run3) mycelia present in the bioreactor was essentially constant. During Run4 mycelia concentration decreased to 5.0 g/l. It is believed that this decrease is caused by the random changes in the influent PCP concentration and flow rate that may have caused the inactivation of part of the mycelia. These results showed that even higher PCP feed concentrations can be degraded by the fungus in continuous immobilized fungal bioreactor by maintaining the low PCP concentration levels within the reactor.

3.5. Reactor design

The dynamic PCP balance around the fluidized bed bioreactor is

$$V\frac{dS}{dt} = F(S_0 - S) + r_s V, \quad (1)$$

where F is the volumetric flow rate through the reactor (l/h), V the reactor volume (l), S_0 the bioreactor feed PCP concentration (mg/l), S the bioreactor outlet PCP concentration (mg/l), and r_s is the rate of PCP biodegradation (mg/l h).

For most engineering applications, for example the biotechnological production of chemicals or the treatment of waste water, only steady-state conditions are of importance. In biological processes where steady state is very much a condition of continuous variation around the average, steady-state conditions (quasi-steady-state) are often assumed [16].

At steady state, $dS/dt=0$. Hence, the substrate balance around the fluidized bed fungal bioreactor (FBBR) is given by

$$0 = F(S_0 - S) + r_s V. \quad (2)$$

After the microorganism has been grown up to a steady-state mycelial concentration, the reaction rate can be described by the Michaelis–Menton model [17]:

$$r_s = -\frac{V_m S}{K_m + S}. \quad (3)$$

Since PCP has been known to be toxic to microorganisms, a substrate inhibition model given as follows can also be used for rate expression involving PCP:

$$r_s = -\frac{k_1 S}{K_2 + S + S^2/K_i}, \quad (4)$$

where k_1 is the model parameter (mg/l h), K_i the inhibition constant (mg/l), K_2 the Monod type constant (mg/l), and S is the PCP concentration (mg/l).

Another study was conducted to determine the degradation kinetic data for reactor design. The reaction rate constants were determined using a continuous immobilized fluidized bed reactor. Residence time was used as an evaluating parameter.

Table 5 shows the steady-state effluent concentrations of PCP from fluidized bed bioreactor at different

Table 5
Performance of immobilized fungal fluidized bed bioreactor (inlet PCP concentration=20 mg/l)

Flow rate (ml/min)	Residence time (h)	Average outlet PCP concentration (mg/l)	Rate (mg/l h) (Eq. (2))
0.42	24	0.173	0.82
0.58	18	0.185	1.10
0.86	12	0.334	1.63
1.66	6	1.579	3.07
2.12	5	3.457	3.30

Table 6
Parameter estimates for the Monod model and substrate inhibition model used to describe the PCP degradation

Parameter	Monod (SSR=0.0604)		Substrate inhibition (SSR=0.0326)		
	V_m (mg/l h)	K_m (mg/l)	k_1 (mg/l h)	K_2 (mg/l)	K_i (mg/l)
Estimate	3.878	0.491	4.703	0.658	14.78
L95	3.293	0.266	1.298	−0.107	−44.01
U95	4.463	0.716	8.108	1.424	73.57

L95: Lower 95% confidence interval limit, U95: Upper 95% confidence interval limit, SSR: sum of squares of residuals.

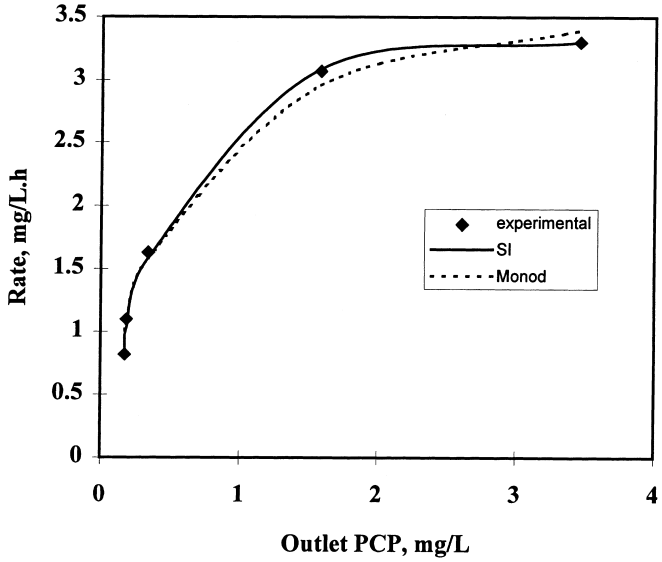

Fig. 3. Applicability of substrate inhibition model and Monod model for PCP biodegradation in an immobilized bioreactor.

Table 7
Comparative illustration of reported results with significantly higher concentrations and present study on PCP biodegradation using *T. versicolor*

Fungi	Conditions	PCP (mg/l)	Degradation (%)	Reference
Trametes versicolor	Shake cultures	10	90 (2 days)	Present study
	IFFBB	25	>99 (12 h)	
	Static cultures	8	50 (1 day)	[3]
	Rotating tube fixed film	10	>99 (1 day)	[4]
	Static cultures	40	>99 (2 weeks)	[5]
	Liquid cultures	6.4	82 (3 h)	[12]
Phanerochaete chrysosporium	Liquid Batch cultures (LBC), N-limited	1.1	97 (1 day)	[2]
	Liquid	25	16	[13]
	Liquid	5.05	70	[12]
	Bench-scale bioreactors	2–5	37–72 (5–90 min)	[18]

flow rates. Rates were calculated by applying the data presented in Table 5 in Eq. (2), and regressed to obtain the parameters in Eqs. (3) and (4). For the range of conditions employed in this study, a substrate inhibition model (Eq. (4)) described (SSR=0.0326) PCP degradation kinetics in the fluidized bed bioreactor very well. The Michaelis–Menton equation also performed satisfactorily (SSR=0.0604). The parameter estimates for the Monod model and substrate inhibition model are shown in Table 6. The predicted values for rates from both models are shown in Fig. 3.

A brief comparison of PCP degradation results from reported studies and present study using *T. versicolor* under different culture conditions is shown in Table 7. One advantage of using *T. versicolor* is its ability to withstand higher concentrations of PCP in fluidized bioreactor. The higher degradation levels obtained in the present study can be attributed to the advantages of the immobilized fluidized bed bioreactor such as better PCP and oxygen transfer, retention of higher mycelial loading and possibly greater enzyme activities.

4. Conclusions

The white-rot fungus *T. versicolor* degraded pentachlorophenol (PCP) efficiently after initial uptake by the mycelia. Results showed that the degradation process proceed intracellularly because unpurified extracellular fluid showed negligible PCP degradation. The polyurethane immobilized fungal bioreactor yielded more than 99% PCP reduction with a residence time of 12 h. The substrate inhibition model described PCP degradation kinetics in the immobilized fluidized bed bioreactor very well.

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