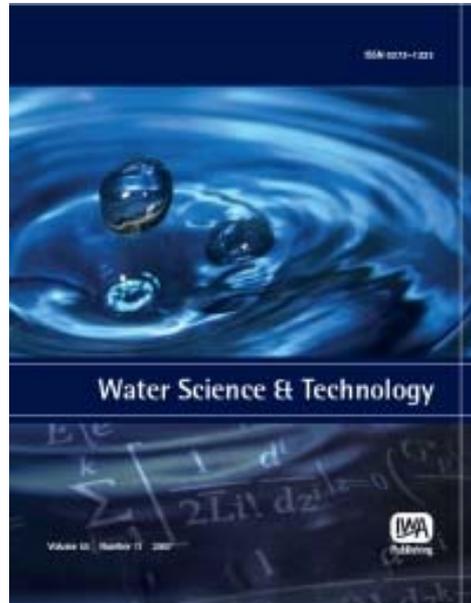


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Biodegradation of 2,4-dichlorophenol in a fluidized bed reactor with immobilized *Phanerochaete chrysosporium*

Xiao-ming Li, Qi Yang, Ying Zhang, Wei Zheng, Xiu Yue, Dong-bo Wang and Guang-ming Zeng

ABSTRACT

The performance of a fluidized bed reactor using immobilized *Phanerochaete chrysosporium* to remove 2,4-dichlorophenol (2,4-DCP) from aqueous solution was investigated. The contribution of lignin peroxidase (LiP) and manganese peroxidase (MnP) secreted by *Phanerochaete chrysosporium* to the 2,4-DCP degradation was examined. Results showed that LiP and MnP were not essential to 2,4-DCP degradation while their presence enhanced the degradation process and reaction rate. In sequential batch experiment, the bioactivity of immobilized cells was recovered and improved during the culture and the maximum degradation rate constant of $13.95 \text{ mg (Ld)}^{-1}$ could be reached. In continuous bioreactor test, the kinetic behavior of the *Phanerochaete chrysosporium* immobilized on loofa sponge was found to follow the Monod equation. The maximum reaction rate was $7.002 \text{ mg (Lh)}^{-1}$, and the saturation constant was 26.045 mg L^{-1} .

Key words | 2,4-DCP, degradation, immobilization, loofa sponge, *Phanerochaete chrysosporium*

Xiao-ming Li (corresponding author)

Qi Yang
Ying Zhang
Wei Zheng
Xiu Yue

Dong-bo Wang
Guang-ming Zeng

College of Environmental Science and Engineering,
Hunan University,
Changsha 410082,
P.R. China
E-mail: xmli@hnu.cn

Xiao-ming Li (corresponding author)

School of the Environment,
Guangxi University,
Nanning 530004,
P.R. China

Xiao-ming Li (corresponding author)

Qi Yang
Ying Zhang
Wei Zheng
Xiu Yue

Dong-bo Wang
Guang-ming Zeng

Key Laboratory of Environmental Biology and
Pollution Control (Hunan University),
Ministry of Education,
Changsha 410082,
P.R. China

INTRODUCTION

2,4-Dichlorophenol (2,4-DCP) is extensively used for the production of germicides and soil sterilizants and also in the manufacture of methylated chlorophenols, that are used in antiseptics and disinfectants (Vroumsia *et al.* 2005). Because of this great ubiquity, 2,4-DCP, was rated as a priority pollutant by the US Environmental Protection Agency (Keith & Telliard 1979). The commonly used procedures for removing 2,4-DCP from wastewater include flocculation, adsorption, filtration, oxidation and biological treatments, among which biological methods using microorganisms are preferable because of their low cost, effectiveness and the low production of byproducts (Quan *et al.* 2003a,b).

Many researchers have studied 2,4-DCP degradation, either by using several different microorganisms, including *Aspergillus awamori* (Stoilova *et al.* 2006), *Pseudomonas putida* (Kargi & Eker 2005), *Phanerochaete chrysosporium* (Choi *et al.* 2002; Wu & Yu 2008), *Saccharomyces cerevisiae* (Cabral *et al.* 2003), etc. or mixed cultures (Sahinkaya & Dilek 2006; Sahinkaya & Dilek 2007). Among them, *Phanerochaete chrysosporium* has been used to a higher extent in biodegradation processes, comparing to other bacterial species. *Phanerochaete chrysosporium* is a kind of white rot fungus and is able to degrade lignin, one of the main components of woods, when other substrates are exhausted or limited

(Leontievsky *et al.* 2002; Wu & Yu 2007). Moreover, the lignin biodegradation system, including extracellular peroxidases, is very non-specific to the substrates so that this system can attack some recalcitrant chemicals, which have a structural similarity to lignin, such as chlorinated phenols, polychlorinated biphenyls and kraft bleach-plant effluents (Kennedy *et al.* 1990; Li *et al.* 2008; Wang *et al.* 2009). Certain achievement has been reached on the remediation of soil with PCP contaminated by *Phanerochaete chrysosporium* and recently the interest of its 2,4-DCP degradation ability is growing. However, *Phanerochaete chrysosporium* was employed as free cells in previous studies and the problems of cells re-usage and partial or complete inhibition of the biodegradation effectiveness due to the toxicity of the recalcitrants hasn't been solved satisfactorily. The disadvantages of free cell system gave rise to the idea that a new biodegradation system using immobilized cells may be possible and several supports for immobilization of *Phanerochaete chrysosporium* have been tried such as polyurethane foam, porous ceramic, porous poly and fibrous nylon sponge (Kirkpatrick & Palmer 1987; Capdevila *et al.* 1989; Cornwell *et al.* 1990; Sayadi *et al.* 1996; Couto *et al.* 2002; Quan *et al.* 2003a,b). Loofa sponge is considered to be one of the most suitable supports for application because it is renewable and biodegradable, and could offer ideal size, mechanical strength, rigidity and porous characteristics (Iqbal & Edyvean 2004). The microbial immobilization method is also very simple. Moreover, Loofa grows widely in many developing countries so that it is affordable. But so far the ability of *Phanerochaete chrysosporium* immobilized on loofa sponge to degrade 2,4-DCP in aqueous system hasn't been reported yet; we hope to develop a simple, economic and efficient degradation system using loofa sponge as supporting material for treatment of wastewater containing 2,4-DCP.

This research thus focuses on examining the ability of loofa sponge immobilized *Phanerochaete chrysosporium* to degrade 2,4-DCP. The role of extracellular enzymes (Lip, Mnp) was determined in 2,4-DCP degradation. The bioactivity of immobilized cells along with the culture was studied. Furthermore, the kinetic data in continuous immobilized fungal bioreactor experiment was modeled.

MATERIALS AND METHODS

Chemicals and strain

The white rot fungus *Phanerochaete chrysosporium* was purchased from the China Center For Type Culture Collection (CCTCC) and the stock cultures were maintained by periodic subculture on Potato Dextrose Agar (PDA) slants at 4°C. Veratryl alcohol (VA) was purchased from Sigma Chemical Co USA. All other inorganic chemicals were of analytical grade and were obtained from Changsha Chemical Company, China. Conidial suspensions for immobilization were prepared from 1 week-old cultures grown on PDA slants at 35°C. The number of spores was determined under a cell counter under the fluorescence microscope.

Medium composition and the immobilization

The liquid medium used for *P. chrysosporium*'s growing consists of a salt solution of (mg L^{-1} , the concentration unit of the following substances is as same as this) glucose, 10,000; KH_2PO_4 , 200; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 500; CaCl_2 , 100; NH_4Cl , 120; thiamine, 1; and 60 ml of trace element solution (containing mg L^{-1} : nitrilotriacetate, 1,500; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 500; NaCl , 1,000; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 100; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 100; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 100; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 10; $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, 10; H_3BO_3 , 10; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 10) (Kirk *et al.* 1978). The medium pH was adjusted to 4.5 with 1.0 mol L^{-1} acetic and 1.0 mol L^{-1} NaOH.

Loofa sponge, used as the supporting matrix for immobilization, was obtained from the matured dried fruit of *Loofa cylindrica*. For experimental use, the loofa discs were prepared as previously described (Iqbal & Edyvean 2004). Firstly, the sponge was cut into segments of approximately 2–3 mm thick and 2.5 cm diameter. The loofa discs were then soaked in boiling water for 30 min, thoroughly washed under tap water, and left for 24 h in distilled water, repeated 3–4 times. At last, the sponge discs were dried at 60°C and stored in desiccators before further use.

The immobilization of *Phanerochaete chrysosporium* within loofa discs was carried out as follows: 2 mL conidial suspension was inoculated in 250 cm^3 Erlenmeyer flasks containing 100 mL of the liquid medium. Pre-weighted loofa sponge discs were placed in flasks above and

autoclaved at 115°C for 30 min. The incubation was taken with shaking (125 rpm) at 35°C. The cultures were flushed with pure oxygen for 1 min once a day.

Mycelium dry weight determination

The dry weight of fungal biomass immobilized on loofa discs, expressed as grams of biomass per litre of culture, was calculated as the difference between the total dry weight of sponge discs before and after fungal growth after 48 h drying at 60°C in each experimental set.

Analytical methods

The concentration of 2,4-DCP was measured using HPLC (Agilent 1,100, Ca., U.S.). The HPLC was equipped with a Zorbax Eclipse XDB column (C18, 4.6 × 150 mm, 5 μm, Agilent) and UV detection set at 280 nm. The mobile phase was a mixture of methanol/2% acetic acid water solution (77:23, v/v) and the flow rate was 1 ml min⁻¹. The sample was filtered through a Millipore membrane (pore size 0.45 μm) and the injection volume was 20 μL.

Enzyme assay

Lip was assayed according to the method of Tien and Kirk (Tien & Kirk 1988) by monitoring the oxidation of VA to veratrylaldehyde at 310 nm while Mnp was determined by monitoring oxidation of Mn²⁺ to Mn³⁺ at 290 nm. One unit was defined as the amount of enzyme that oxidized 1 mmol of substrate per minute and the activities are reported as U L⁻¹.

Fluidized bed bioreactor

For continuous studies, a fluidized bed reactor with 1 L working volume was used. The internal diameter was 18 cm and the height was 35 cm. The design of the fluidized bed bioreactor system was similar to that described elsewhere (Lewandowski *et al.* 1990). The schematic diagram of the fluidized bioreactor system is shown in Figure 1. The reactor temperature of 30°C was controlled by water bath with a temperature controller. The laboratory air was introduced

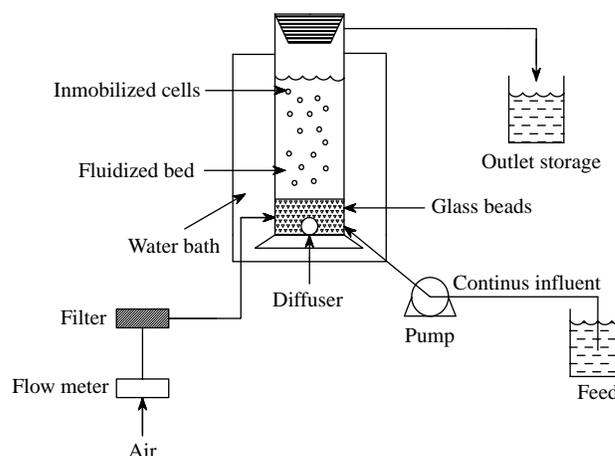


Figure 1 | The schematic diagram of fluidized bed bioreactor system.

into the bioreactor through a regulated air diffuser at the bottom of the inside tube at the rate of 3 mL min⁻¹.

Batch studies using mycelial pellets of *Phanerochaete chrysosporium*

Effect of 2,4-DCP on *Phanerochaete chrysosporium* growth

In order to determine the range of 2,4-DCP concentration which *Phanerochaete chrysosporium* could withstand, 1 ml conidial suspension (1.5 × 10⁶ cells mL⁻¹) was inoculated and cultivated in the liquid medium supplemented with 0, 25, 50, 75, 100, 200 and 300 mg L⁻¹ of 2,4-DCP respectively. The biomass was measured.

Role of extracellular enzymes on removal of 2,4-DCP

The activity of Lip and Mnp were tested during the *Phanerochaete chrysosporium* growth in 250 mL flask containing 100 mL liquid medium. 3 mL 10 mmol L⁻¹ veratryl alcohol and 0.05 g Tween 80 were added and 2 ml conidial suspension was inoculated at the beginning of the culture. This culture was incubated at 120 rpm and 35°C and it was flushed with pure oxygen for 2 min once a day.

Then fungus *Phanerochaete chrysosporium*, grown in the form of mycelial pellets for 6 days, was used in the following test. The washed mycelia were averagely divided into three parts (biomass dry weight in each flask is about 0.65 g L⁻¹). In flask 1#, only the medium of 6 d old which contained the extracellular fluid was used. While fresh

medium was used in flask 2# and 3# and the divided mycelia mentioned above was added into flask 2# and 3# respectively. The contents of three flasks were dosed with 2,4-DCP stock solution to yield a 10 mg L^{-1} of final 2,4-DCP concentration respectively. 3# was autoclaved for 30 min at 115°C . Then the three flasks were all placed in a shaker at 125 r/min, 35°C . The concentrations of 2,4-DCP in each flask were measured daily.

Sequential batch studies using loofa sponge immobilized *P. chrysosporium*

To examine the bioactivity variability of the immobilized cells with the prolonging of time, semi-continuous cultures which contained 6 runs were carried out in a 250 mL flask containing 100 mL liquid medium. 2 mL conidial suspension was incubated and four loofa discs were placed in the flask. The flakes were sealed with foam stoppers, placed in a shaker at 125 rpm, 35°C , and was sparged with pure oxygen once a day for 1 min. At the end of each culture, the medium was replaced by fresh medium. The 2,4-DCP concentration in solution was measured daily.

Continuous immobilized fungal bioreactor study

In order to determine the degradation kinetic data for reactor design, the 2,4-DCP degrading activity of immobilized *Phanerochaete chrysosporium* in a fluidized bed reactor was examined at different reactor residence times. The bioreactor was fed with contaminated water with a 2,4-DCP concentration of 20 mg/L . It should be mentioned that generally the 2,4-DCP concentration in the contaminated water is about $5\text{--}20\text{ mg L}^{-1}$ (Quan *et al.* 2004). 20 loofa sponge discs (approximately 2.5 cm diameter and 2–3 mm thick) were incubated in liquid medium for three days, and then transferred into the reactor. The bioreactor was filled with a feed solution of 20 mg L^{-1} 2,4-DCP and operated in batch mode for three days. At the fourth day the operation was switched to a continuous pattern. Under different reactor retention times, continuous operation of the bioreactor was carried out for at least five days to allow the reactor to reach biologically stable (steady state) condition. The 2,4-DCP concentration in outlet was measured daily.

RESULTS AND DISCUSSION

Batch studies using mycelial pellets of *Phanerochaete chrysosporium*

Effect of 2,4-DCP on *Phanerochaete chrysosporium* growth

The growth curve of the *Phanerochaete chrysosporium* supplemented with 0, 25, 50, 75, 100, 200 and 300 mg L^{-1} of 2,4-DCP respectively was obtained (Figure 2). After 1 day incubation, lower fungal growth was observed with 50 mg L^{-1} of 2, 4-DCP. After 7 days of incubation at 36°C , biomass production gradually decreased as the concentration of 2,4-DCP initially increased. No growth of the fungal cells in the 75 mg L^{-1} 2,4-DCP solution was observed.

The role of extracellular enzymes on removal of 2,4-DCP

The enzyme activity curve in batch culture was shown in Figure 3

As shown in Figure 3, it was demonstrated that the maximum activity of Lip and Mnp were both reached in day 6, which were 112.88 U L^{-1} and 943.87 U L^{-1} respectively. Therefore fungus *Phanerochaete chrysosporium*, grown in the form of mycelial pellets for 6 days, were used in the following tests. Mycelial pellets grown for 6 days were harvested by vacuum filtration with glass fiber filters (GF/C, Whatman) and rinsed with deionized water. The concentrations of 2,4-DCP in each flask were measured daily and the result was shown in Table 1.

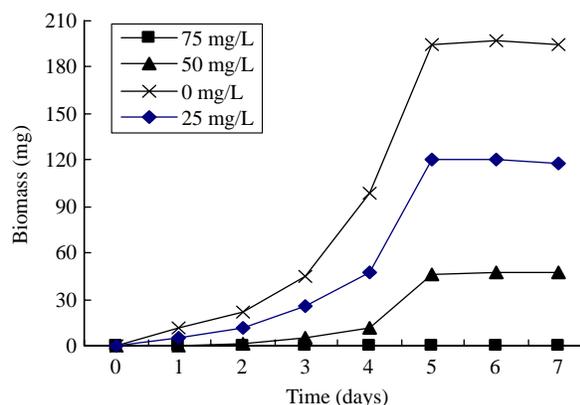


Figure 2 | The growth curve of *Phanerochaete chrysosporium* supplemented with 2,4-DCP.

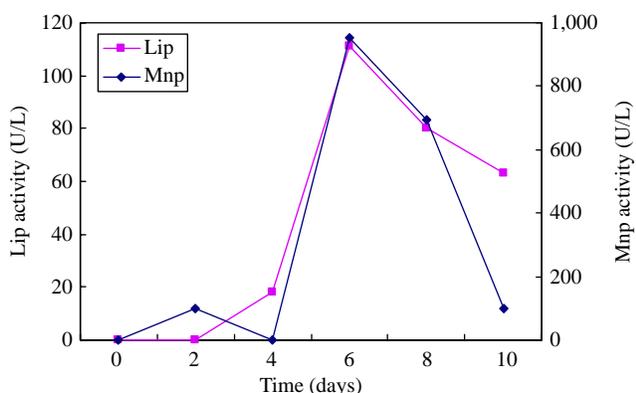


Figure 3 | The enzyme activity curve in batch culture.

From Table 1, we could see that the largest overall reduction of 2,4-DCP (49.0%) was achieved in 1# flask. 2# flask yielded slightly lower reduction levels as compared with 1# flask, although the overall amount of reduction was quite large (44.8%). In flask 3#, containing autoclaved mycelia, the 2,4-DCP concentration dropped rapidly during the first 24 h due to the uptake by the mycelia. However, from the second day onwards the 2,4-DCP concentration of the solution increased, indicating that the 2,4-DCP uptake by the mycelia without subsequent metabolism is temporal and reversible. Experiment with unpurified extracellular fluid without any mycelia present, however, showed negligible 2,4-DCP degradation. The best studied mechanism of degradation by *Phanerochaete chrysosporium* is associated with the lignin degradation system. The lignin degradation system of *Phanerochaete chrysosporium* consists of Mnp, Lip, H₂O₂-producing enzymes, veratryl alcohol (3,4-dimethoxybenzyl alcohol, VA), manganese and oxalate. Related studies described the important role of LiP in the dechlorination of polychlorinated phenols (Hammel *et al.* 1986; Linko *et al.* 1986; Blondeau 1989). However, Yadav and Reddy (Yadav & Reddy 1992)

Table 1 | 2,4-DCP uptake and removal in batch cultures of *P. chrysosporium*

Time (days)	1# (mg L ⁻¹)	2# (mg L ⁻¹)	3# (mg L ⁻¹)
0	10.0	10.0	10.0
1	6.63	8.65	8.22
2	5.89	7.23	7.03
3	5.09	5.51	8

reported that LiP and MnP were not involved in 2,4,5-trichlorophenoxyacetic acid degradation by *Phanerochaete chrysosporium*. Zouari also reported that Lip and Mnp were not essential for the 4-CP degradation (Zouari *et al.* 2002). From the results obtained in the present study, we could conclude that the Mnp and Lip were not essential for the 2,4-DCP degradation while the presence of extracellular enzymes indeed enhanced the degradation process and reaction rate (Table 1 and Figure 5).

Sequential batch studies using loofa sponge immobilized *P. chrysosporium*

The 2,4-DCP degradation rates under 6 different initial concentrations ranging from 5 to 100 mg L⁻¹ are depicted in Figure 4.

From Figure 4, it was found that biodegradation rate of 2,4-DCP increased obviously with feeding cycle. In run 1, 4 d was needed for complete degradation of 5 mg L⁻¹ 2,4-DCP, while it only took 3 d for complete removal of 40 mg L⁻¹ 2,4-DCP in run 4. Results showed that bioactivity of the immobilized cells was recovered and improved in the process of semi-continuous test. The immobilized cells microbes gradually adapted to high concentration of 2,4-DCP and became more tolerable in shock load. It was also observed from the Figure 4 that, in the first day in each run, the removal rate of 2,4-DCP was a little faster than the following 4 d. This could be attributed to the adsorption on the surface of the immobilized cells.

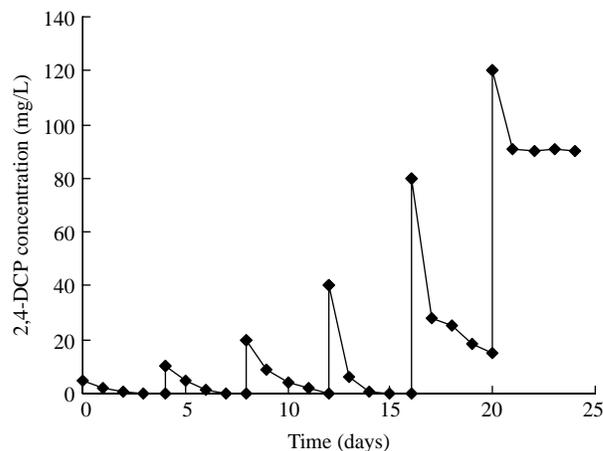


Figure 4 | Biodegradation of 2,4-DCP by immobilized fungus in semi-continuous test.

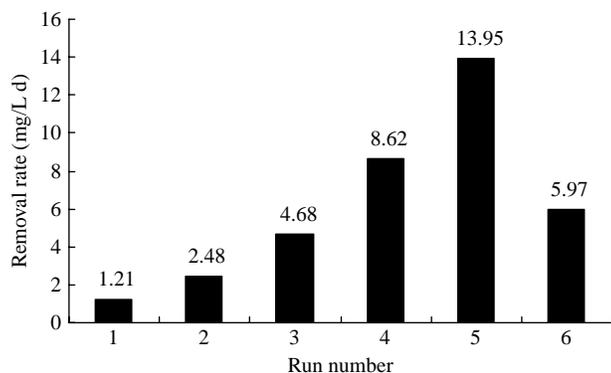


Figure 5 | Variation of biodegradation rate of 2,4-DCP in the semi-continuous test.

The effect of starvation during the semi-continuous operation was also believed to be associated with the faster removal in the first day.

The degradation rates were obtained by zero order regression of the degradation data, and the results were shown in [Figure 5](#). A gradual increase trend for the kinetic rate constant was observed from run 1 to run 5. In the run 1, the rate constant was only $1.21 \text{ mg (Ld)}^{-1}$, while it increased to $13.95 \text{ mg (Ld)}^{-1}$ in the run 5, which may be due to microbial growth through degrading 2,4-DCP and increased biomass in the reactor. The other reason would be that the bioactivity of the immobilized cells was inhibited because of the space limitation and it was recovered and improved in the semi-continuous culture. However, the constant decreased in the sixth run. It could be possible that the cell activity was inhibited by 2,4-DCP of such high concentration. [Ettayebi *et al.*](#) reported that the cells's support matrix protects the cells from the toxic effects of 2,4-DCP by reducing the number of attack sites and the entrapped cells may receive flow of 2,4-DCP solution at lower rate ([Ettayebi *et al.*](#) 2003). It could be concluded that immobilization could protect cells from losing activity, which is accordant with the previous study 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 ([Roy-Arcand & Archibald](#) 1999).

Continuous immobilized fungal bioreactor study

The fungal biomass in the reactor was measured twice. The biomass concentration was $2,970 \text{ mg L}^{-1}$ after 3 days in

the reactor column, and the fungal biomass concentration in steady state was $4,340 \text{ mg L}^{-1}$. During the reactor operation period, the concentration of 2,4-DCP in the outflow was continuously monitored to determine steady-state conditions, as discussed in the results below. The attainment of steady state was confirmed when there was no observed change in the outflow concentration of 2,4-DCP. After steady state has been reached, the sorption of 2,4-DCP into mycelium or sponge discs is assumed to be at equilibrium because 2,4-DCP is already saturated in the mycelium and sponge discs. All reported results were obtained when the steady state was reached in the reactor tests. [Table 2](#) shows the steady-state effluent concentrations of 2,4-DCP from the fluid bed reactor at different reactor retention times.

The general mass balance for the fluid bed reactor can be expressed using the following relation ([Tchobanoglous *et al.*](#) 2003):

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

where F , flow rate (mL min^{-1}); S , 2,4-DCP concentration in the effluent when reaching steady state (mg L^{-1}); S_0 , 2,4-DCP concentration in the feed (mg L^{-1}); V , reactor volume (L); r_s , reaction rate (mg (Lh)^{-1}).

At steady state, $dQ/dt = 0$. Hence, the substrate balance in the fluidized bed fungal bioreactor is given by:

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

The reaction rate, r_s are described in terms of the Michaelis-Menten model after the microorganism has grown up to a steady state mycelial concentration

Table 2 | Effluent concentrations of 2,4-DCP at different reactor retention times in fluid bed reactor

Flow rate (mL min^{-1})	Residence time (h)	Average outlet 2,4-DCP concentration (mg L^{-1})	Rate (mg (Lh)^{-1})
0.69	24	3.03	0.71
1.19	14	4.81	1.09
1.39	12	5.37	1.22
1.78	6	9.12	1.81

Table 3 | Kinetic parameters obtained in the present study using the Monod equation

V_m (mg(Lh) ⁻¹)	K_m (mgL ⁻¹)	R	$Rsqr$
7.0027	26.045	0.9992	0.9985

(Lewandowski *et al.* 1990).

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

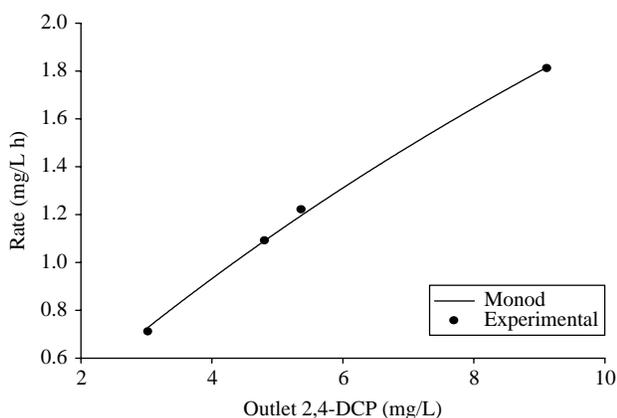
where V_m , maximum reaction rate (mg(Lh)⁻¹); K_m , saturation constant (mgL⁻¹).

Rates were calculated by applying the data presented in Table 2 in Equation (2). The experimental data were modeled by nonlinear-regression analysis in Sigma Plot 10.0 which yielded a maximum reaction rate of 7.003 mg(Lh)⁻¹ and a saturation constant of 26.045 mgL⁻¹. The results are shown in Table 3.

A comparison between the experimentally determined 2,4-DCP at different reactor-retention times and the corresponding curve obtained from the regression of Equation (3) is given in Figure 6. Figure 6 shows good agreement between the measured data and the kinetic model developed for the biodegradation kinetics at 20 mgL⁻¹ of 2,4-DCP. Therefore, in the loofa sponge immobilized fungus reactor system, the biodegradation kinetics at common concentrations of 2,4-DCP (20 mgL⁻¹) can be explained by using the Michaelis-Menten equation and the mass-balance equation for a fluid bed reactor.

The results showed that the biodegradation rate was affected by the retention times. The transfer of the necessary

nutrients to the microorganisms, which are immobilized on the loofa sponge matrix, is governed by a combination of convection and diffusion. The retention time affects mass transfer and thus influences the biodegradation rate. Stoodley (Stoodley *et al.* 2001) reported that the biofilms grown under high shear tended to form filamentous streamers, whereas those grown under low shear formed an isotropic pattern of moundshaped microcolonies. In addition to structural differences, their data suggested that biofilms grown under high shear stress were more strongly attached and were cohesively stronger than those grown under lower shears (Stoodley *et al.* 2001). Thus, a relatively higher flow velocity is favorable in that a shorter retention time would be required to formate better biofilm. Therefore, a moderate retention time which could balance the mass transfer and the bilfilm grown is essential for the reactor performance. Furthermore, ambient laboratory air, rather than pure oxygen, and large size sponge discs were used for fluid bed reactor tests and a relatively low nitrogen concentration (0.002 molL⁻¹) was supplied in the nutrient solution. Lewandowski reported that the rate of lignin degradation by extracellular enzymes in pure oxygen condition is 2 to 3 times greater than that in the air conditions. (Lewandowski *et al.* 1990). Therefore, a more impressive performance of the sponge immobilized fungus reactors is worth expecting under more favorable conditions such as those of pure oxygen, more moderate retention time and smaller size sponge discs with greater surface area.

**Figure 6** | Applicability of Monod model for 2,4-DCP biodegradation in an immobilized bioreactor.

CONCLUSIONS

The results demonstrate that loofa sponge is an effective carrier for *Phanerochaete chrysosporium* immobilization and the immobilized fungal system could be effectively used for the degradation of 2,4-DCP from aqueous solution. The extracellular enzymes were not essential in 2,4-DCP degradation while their presence indeed enhanced degradation process and reaction rate. The bioactivity of immobilized cells was recovered and improved along with the semi-continuous culture. The Monod model describe 2,4-DCP degradation kinetics in the immobilized fluidized bed bioreactor very well.

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REFERENCES

- Blondeau, R. 1989 Biodegradation of natural and synthetic humic acids by the white rot fungus *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* **55**, 1282–1285.
- Cabral, M. G., Viegas, C. A., Teixeira, M. C. & Sá-correia, I. 2003 Toxicity of chlorinated phenoxyacetic acid herbicides in the experimental eukaryotic model *Saccharomyces cerevisiae*: role of pH and of growth phase and size of the yeast cell population. *Chemosphere* **51**, 47–54.
- Capdevila, C., Corrieu, G. & Asther, M. 1989 A feed-harvest culturing method to improve lignin peroxidase production by *Phanerochaete chrysosporium* INA-12 immobilized on polyurethane foam. *J. Fermentation Bioeng.* **68**, 60–63.
- Choi, H. C., Moon, S. H. & Gu, M. D. 2002 Biodegradation of chlorophenols using the cell-free culture broth of *Phanerochaete chrysosporium* immobilized in polyurethane foam. *Chem. Technol. Biotechnol.* **77**, 999–1004.
- Cornwell, K. L., Tinland-butez, M. F., Tardone, P. J., Cabasso, I. & Hammel, K. E. 1990 Lignin degradation and lignin peroxidase production in cultures of *Phanerochaete chrysosporium* immobilized on porous ceramic supports. *Enzyme Microb. Technol.* **12**, 916–920.
- Couto, S. R., Barreiro, M., Rivela, I., Longo, M. A. & Sanromán, A. 2002 Performance of a solid-state immersion bioreactor for ligninolytic enzyme production: evaluation of different operational variables. *Process Biochem.* **38**, 219–227.
- Ettayebi, K., Errachidi, F., Jamai, L., Tahri-jouti, M. A., Sendide, K. & Ettayebi, M. 2005 Biodegradation of polyphenols with immobilized *Candida tropicalis* under metabolic induction. *FEMS Microbiol. Lett.* **223**, 215–219.
- Hammel, K. E., Kalyanaraman, B. & Kirk, T. K. 1986 Oxidation of polycyclic aromatic hydrocarbons and dibenzo(p)dioxin by *Phanerochaete chrysosporium* ligninase. *J. Biol. Chem.* **261**, 16948–16952.
- Iqbal, M. & Edyvean, R. G. J. 2004 Biosorption of lead, copper and zinc ions on loofa sponge immobilized biomass of *Phanerochaete chrysosporium*. *Miner. Eng.* **17**, 217–223.
- Kargi, F. & Eker, S. 2005 Kinetics of 2,4-dichlorophenol degradation by *Pseudomonas putida* CP1 in batch culture. *Int. Biodeter. Biodegrad.* **55**, 25–28.
- Keith, L. H. & Telliard, W. A. 1979 Priority pollutants: a prospective view. *Environ. Sci. Technol.* **13**, 416–423.
- Kennedy, D. W., Aust, S. D. & Bumpus, J. A. 1990 Comparative biodegradation of alkyl halide insecticides by the white rot fungus, *Phanerochaete chrysosporium* (BKM-F-1767). *Appl. Environ. Microbiol.* **56**, 2347–2353.
- Kirk, T. K., Schultz, E., Connors, W. J., Lorenz, L. F. & Zeikus, J. G. 1978 Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*. *Arch. Microbiol.* **117**, 227–285.
- Kirkpatrick, N. & Palmer, J. M. 1987 Semi-continuous ligninase production using foam-immobilized *Phanerochaete chrysosporium*. *Appl. Microbiol. Biotechnol.* **27**, 129–133.
- Leontievsky, A. A., Myasoedova, N. M., Golovleva, L. A., Sedarati, M. & Evans, C. S. 2002 Adaptation of the white-rot basidiomycete *Panus tigrinus* for transformation of high concentrations of chlorophenols. *Appl. Microbiol. Biot.* **59**, 599–604.
- Lewandowski, G. A., Armenante, P. M. & Pak, D. 1990 Reactor design for hazardous waste treatment using a white rot fungus. *Water Res.* **24**, 75–82.
- Li, Q. B., Wang, H. T., He, N., Wang, Y. P., Sun, D. H. & Lu, Y. H. 2008 High efficiency of batch operated biofilm hydrolytic-aerobic recycling process in degradation of 2,4-dichlorophenol. *J. Hazard. Mater.* **152**, 536–544.
- Linko, Y. Y., Leisola, M., Lindholm, N., Troller, J., Linko, P. & Fiechter, A. 1986 Nitrogen and carbon regulation of lignin peroxidase and enzymes of nitrogen metabolism in *Phanerochaete chrysosporium*. *J. Biotechnol.* **4**, 283–291.
- Quan, X. C., Shi, H. C., Wang, J. L. & Qian, Y. 2003a Biodegradation of 2,4-dichlorophenol in sequencing batch reactors augmented with immobilized mixed culture. *Chemosphere* **50**, 1069–1074.
- Quan, X. C., Shi, H. C., Zhang, Y. M., Wang, J. L. & Qian, Y. 2003b Biodegradation of 2,4-dichlorophenol in an air-lift honeycomb-like ceramic reactor. *Process Biochem.* **38**, 1545–1551.
- Quan, X. C., Shi, H. C., Zhang, Y. M., Wang, J. L. & Qian, Y. 2004 Biodegradation of 2,4-dichlorophenol and phenol in an airlift inner-loop bioreactor immobilized with *Achromobacter sp.* *Sep. Purif. Technol.* **34**, 97–103.
- Roy-Arcand, L. & Archibald, F. S. 1991 Direct dechlorination of chlorophenolic compounds by laccases from *Trametes (Coriolus) versicolor*. *Enzyme Microb. Technol.* **13**, 194–203.
- Sahinkaya, E. & Dilek, F. B. 2006 Biodegradation of 4-CP and 2,4-DCP mixture in a rotating biological contactor (RBC). *Biochem. Eng. J.* **31**, 141–147.
- Sahinkaya, E. & Dilek, F. B. 2007 Biodegradation kinetics of 2,4-dichlorophenol by acclimated mixed cultures. *J. Biotechnol.* **127**, 716–726.
- Sayadi, S., Zorgani, F. & Ellouz, R. 1996 Decolorization of olive mill wastewaters by free and immobilized *Phanerochaete chrysosporium* cultures-effects of high molecular weight of polyphenols. *Appl. Biochem. Biotechnol.* **56**, 265–276.
- Stoilova, I., Krastanov, A., Stanchev, V., Daniel, D., Gerginova, M. & Alexieva, Z. 2006 Biodegradation of high amounts of phenol, catechol, 2,4-dichlorophenol and 2,6-dimethoxyphenol by *Aspergillus awamori* cells. *Enzyme Microb. Technol.* **39**, 1036–1041.
- Stoodley, P., Wilson, S., Hall-stoodley, L., Boyle, J. D., Lappin-stott, H. M. & Costerton, J. W. 2001 Growth and detachment of cell clusters from mature mixed-species biofilms. *Appl. Environ. Microbiol.* **67**, 5608–5613.

- Tchobanoglous, G., Burton, F. L. & Stensel, H. D. 2003 *Wastewater Engineering: Treatment and Reuse*, 4th edition. McGraw-Hill Companies, Inc, Chicago, Illinois.
- Tien, M. & Kirk, T. K. 1988 Lignin peroxidase of *Phanerochaete chrysosporium*. *Methods Enzymol.* **161**, 238–249.
- Vroumsia, T., Steiman, R., Seigle-murandi, F. & Benoit-guyod, J. L. 2005 Fungal bioconversion of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4 dichlorophenol (2,4-DCP). *Chemosphere* **60**, 1471–1480.
- Wang, H. T., Li, Q. B., Wang, Y. P., He, N. & Sun, D. H. 2009 Effect of recycling flux on performance and characteristics of activated sludge hydrolytic-aerobic recycling process in degradation of 2,4-dichlorophenol. *J. Hazard. Mater.* **168**, 203–209.
- Wu, J. & Yu, H. Q. 2007 Biosorption of 2,4-dichlorophenol by immobilized white-rot fungus *Phanerochaete chrysosporium* from aqueous solutions. *Bioresour. Technol.* **98**, 253–259.
- Wu, J. & Yu, H. Q. 2008 Biosorption of 2,4-dichlorophenol from aqueous solutions by immobilized *Phanerochaete chrysosporium* biomass in a fixed-bed column. *Chem. Eng. J.* **138**, 128–135.
- Yadav, J. S. & Reddy, C. A. 1992 Non-involvement of lignin peroxidases and manganese peroxidases in 2,4,5-trichlorophenoxyacetic acid degradation by *Phanerochaete chrysosporium*. *Biotechnol. Lett.* **14**, 1089–1092.
- Zouari, H., Labat, M. & Sayadi, S. 2002 Degradation of 4-chlorophenol by the white rot fungus *Phanerochaete chrysosporium* in free and immobilized cultures. *Bioresour. Technol.* **84**, 145–150.