Biotechnology Letters **21:** 147–154, 1999.

Diuron degradation by *Phanerochaete chrysosporium* BKM-F-1767 in synthetic and natural media

Lidy E. Fratila-Apachitei, Jennifer A. Hirst, Maarten A. Siebel* & Huub J. Gijzen
International Institute for Infrastructural, Hydraulics and Environmental Engineering (IHE), Delft, The Netherlands
**Author for correspondence (Fax: +31(0)152122921; E-mail: mas@ihe.nl)*

Received 13 November 1998; Accepted 5 January 1999

Key words: diuron, degradation, *Phanerochaete chrysosporium*, ashwood

Abstract

When incubated in synthetic (N-limited) medium and on ashwood chips, *Phanerochaete chrysosporium* BKM-F-1767 degraded 14 and 10 mg/l diuron, respectively. The wood chips were used as support and sole nutrient source for the fungus. A higher degradation efficiency was found in ashwood culture as compared to the liquid culture, probably as a result of the synergetic effect of attached fungal growth, presence of limiting-substrate conditions and the microenvironment provided by ashwood, all favorable for production of high extracellular enzyme titres. Diuron degradation occured during the idiophasic growth, in the presence of manganese peroxidase, detected as dominant enzyme in both cultures.

Introduction

Phanerochaete chrysosporium is one of the most studied white rot fungi for its ability to degrade xenobiotics such as: chlorinated aromatic compounds, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pesticides, dyes, explosives, cyanides and carbon tetrachloride (Bumpus *et al.* 1985, Hammel 1989, Kirk *et al.* 1992, Field *et al.* 1993, Barr & Aust 1994, Paszczynski & Crawford 1995). The unusual biodegradation capacity of white rot fungi is attributed mainly to the action of their extracellular enzymes involved in lignin biodegradation (Bumpus *et al.* 1987, Hammel 1989, Kirk *et al.* 1992, Barr & Aust 1994). The ligninolytic system is produced under idiophasic conditions, during secondary metabolism, as a consequence of nutrient (nitrogen, carbon or sulphur) limitation (Tien & Kirk 1988, Gold & Glenn 1988). The ability to degrade lignin, a very complex and heterogeneous polymer, indicates a low substrate specificity of these extracellular enzymes, enabling degradation of compounds which resemble lignin structure.

Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] is a pre-emergence herbicide used mainly in non-crop areas (Howard 1991). Run-off to surface waters can threaten drinking water supplies and has presented a real problem in The Netherlands, where up to 10 μg/l (100 times the EC standard limit in drinking water) has been found in the river Meuse, a major source of the countries' drinking water (Huijser 1994, 1996).

Diuron belongs to the substituted phenylurea herbicides susceptible to degradation by soil microorganisms (Weinberger & Bollag 1972, Tillmans *et al.* 1978, Madhun & Freed 1987, Vroumsia *et al.* 1996, Esposito *et al.* 1998). Enriched cultures of aquatic microorganisms from pond water could also degrade diuron to 3,4-dichloroaniline as major metabolite (Ellis & Camper 1982). Esposito *et al.* (1998) used three different actinomycete strains in soil to degrade diuron *in vitro* and the strain which produced manganese peroxidase showed the highest degradation efficiency (37%) suggesting a possible role of this enzyme in diuron degradation.

Optimization studies carried out with *P. chrysosporium* for maximizing extracellular peroxidase activity and pollutant biodegradation rates revealed that attached growth of the fungus in packed-bed reactors (Lewandowski *et al.* 1990, Pal *et al.* 1995a,b) or

biofilm reactor systems (Venkatadri & Irvine 1993) resulted in higher degradation rates and LiP titres as compared to suspended growth reactors. In addition, the use of wood chips as support and (co)-substrate for the fungus was found to sustain fungal growth (Lewandowski *et al.* 1990) and even increase the degradation efficiency of the target compound (Yum & Peirce 1998b).

In most biodegradation studies using *P. chrysosporium* in batch or continuous wood chip reactors, buffers, mineral nutrients and sometimes an extra carbon source were added to the wood culture (Lewandowski *et al.* 1990, Yum & Peirce 1998a,b). The objective of this study was to determine the ability of *P. chrysosporium* BKM-F-1767 to degrade diuron in a defined liquid medium and when incubated on wood chips as sole nutrient source.

Materials and methods

Microorganism

Phanerochaete chrysosporium BKM-F-1767 from Wageningen Agricultural University, Division of Industrial Microbiology (The Netherlands), was grown on malt extract plates (per liter, 15.0 g agar, 5 g glucose and 3.5 g malt extract) for inoculum preparation (Mester *et al.* 1995). The plates were incubated at 37 °C for 2–4 days. Both liquid and wood cultures were inoculated with one 6 mm agar plug from the leading edge of the mycelium.

Natural substrates

As natural substrates, two types of hardwood were used: ash (*Fraxinus excelsior*) and beech (*Fagus sylvatica*) in the form of wood chips. The wood represented the support and the sole nutrient source for the fungus.

Culture media

Synthetic liquid cultures. The fungus was grown in N-limited conditions. The standard medium (Mester *et al.* 1995) contained 2.2 mM NH_4^+ – N in the form of diammonium tartrate, 10 g glucose/l, and B III mineral medium (100 ml/l) in 20 mM 2,2-dimethylsuccinate buffer (pH 4.5). After sterilization at 120 °C for 20 min, a filter-sterilized thiamine solution (400 mg/l) was added (10 ml/l). For diuron degradation experiments 5 ml autoclaved liquid medium was placed in 25 ml sterilized serum bottles and inoculated with fungus. For analyses of biomass production, nutrient depletion and enzyme activities, aliquots of 10 ml autoclaved liquid medium were placed in 100 ml sterilized Erlenmeyer flasks and inoculated with fungus.

Wood cultures. For diuron degradation experiments, 0.5 g air dry wood chips was autoclaved in 25 ml serum bottles with 5 ml demineralized water. Diuron solution and the fungus were added on day zero. For enzymatic assays and fungal decay of ashwood, 5 g air dry milled ashwood ($\leq$1 mm; 4.64% moisture content) was placed in 250 ml serum bottles and autoclaved with 2 ml demineralized water (resulting in unsubmerged conditions). After sterilization, the bottles were inoculated as described above.

Culture conditions

Unless otherwise specified, inoculated samples were incubated statically at 37 °C in complete darkness. All bottles/flasks were loosely capped with cotton plugs for passive aeration. Moisture content of ashwood cultures in the experiments of decay rate and enzymatic assays was corrected twice a week with sterilized demineralized water. For diuron degradation and wood decay experiments, abiotic controls which did not receive the inoculum were incubated under the same conditions.

Enzyme assays

The activity of manganese peroxidase (MnP) and lignin peroxidase (LiP) was determined spectrophotometrically at 20 °C. Extracellular fluids from the liquid cultures, centrifuged at 1000 g for 10 min were used for enzyme assays. For the solid cultures, 50 ml of KPi buffer pH 6 was added to each serum bottle and the substrates were extracted for 30 min with 100 rpm at 20 °C. The extracellular fluid extracts were centrifuged at 1000 g for 10 min before enzyme assays. LiP activity was measured based on veratryl alcohol oxidation to veratryl aldehyde ($\epsilon = 9300\ M^{-1}\ cm^{-1}$) at 310 nm, according to Tien and Kirk (1988). MnP assay was based on 2,6-dimethoxyphenol oxidation to the corresponding dimer ($\epsilon = 49600\ m^{-1}\ cm^{-1}$) at 468 nm and the activity was corrected prior for laccase (Mester *et al.* 1995). Enzymatic activity for the liquid and wood cultures was expressed as nmol/mlAmin (U/l).

Determination of diuron

Diuron was analysed by HPLC using a C-18 reverse phase column (4.5 × 250 mm) and acetonitrile/water (40:60 v/v) as mobile phase, at 1500 ml/min. Before analysis, diuron was extracted with acetonitrile. Each sample was ultrasonicated (15 min) and then shaken (1 h) for diuron to be desorbed from the mycelia or wood support.

Determination of mycelium dry weight in liquid cultures

The mycelium was separated from the liquid cultures by filtration through tared glass fiber filters (GF/C Whatman). Mycelia were rinsed with demineralized water and their weights were determined after drying for 2 h at 105 °C.

Determination of total weight loss of wood colonized by the fungus

The total weight loss was determined from the change in the dry weight of the milled wood (dried in tared aluminium cups overnight at 105 °C).

Scanning electron micrographs

The scanning electron micrographs of the ashwood colonized by the fungus were obtained using a multipurpose digital equipment JSM-5400 JEOL and a JFC-1100E-JEOL ion sputter for samples coating.

D-*Glucose analysis in liquid cultures*

D-Glucose was analysed using an UV method provided by Boehringer Manheim (Cat. No. 716251).

Nitrogen determination in liquid cultures

NH_4^+-N was measured using an Aquatec autoanalyser, at 590 nm.

Statistical procedures

All the experiments were performed with triplicate parallel cultures. The values reported are means with standard deviations.

Experiments overview

Diuron degradation experiments were batch experiments carried out in liquid and wood cultures for a period of 14 days at 30 and/or 37 °C. *Fungal growth, glucose and nitrogen depletion*, and *LiP and MnP activity in liquid cultures* were assessed in submerged cultures incubated at 37 °C for 14 days in the absence of diuron. The experiments for *fungal growth* and *MnP activity in ashwood culture* were performed in attached cultures incubated at 37 °C for 42 days in the absence of diuron.

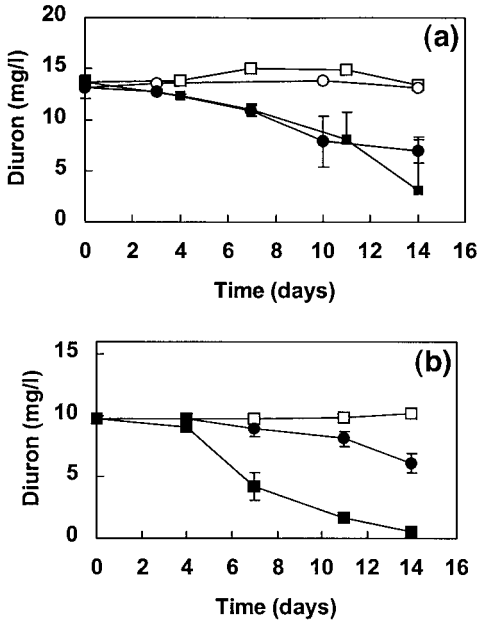

Fig. 1. (a) Diuron progression in the presence of *P. chrysosporium* incubated in N-limited liquid medium at two different temperatures. Diuron was added at time zero together with the medium and the inoculum ($n = 3$). ■ synthetic culture, 30 °C; ● synthetic culture, 37 °C; □ abiotic control, 30 °C; ○ abiotic control, 37 °C. (b) Diuron progression in the presence of *P. chrysosporium* incubated on two different types of wood as sole nutrient source (30 °C). Diuron was added at time zero together with the inoculum ($n = 3$). ■ ashwood culture; ● beechwood culture; □ abiotic control.

Results

The experiments of this study focused both on diuron degradation and on fungus physiology in the two types of culture media investigated. The latter were performed in the absence of diuron.

Diuron degradation in liquid medium

The results of batch experiments carried out in defined liquid medium at two different temperatures (30 and 37 °C) are presented in Figure 1a.

The experiment showed that up to 75% of the initial diuron could be degraded by the fungus after 14 days under the specified culture conditions. No significant differences were found between the two incubation temperatures investigated. Abiotic controls indicate a good desorption of diuron from the mycelium by extraction with acetonitrile. Also, controls using dead inoculum showed no decrease in diuron concentration.

Diuron degradation on natural substrates

In this experiment, the fungus was grown on wood chips as support and sole nutrient source. Two different temperate hardwoods, non-resistant to fungal decay were used: ashwood (*Fraxinus excelsior*) and beechwood (*Fagus sylvatica*). Progression of diuron over a 14-day incubation period is shown in Figure 1b.

Both curves show the same trend, in which after a lag phase of about 4 days, diuron concentration decreased continuously until the end of the experiment. However, in ashwood cultures, diuron was more efficiently degraded (95%) than in beechwood cultures where about 70% of the initial diuron was still present on day 14. Since the same amount of wood chips (0.5 g), both originating from hardwoods, and same operational conditions were used in this experiment, comparable degradation efficiencies were expected. The results suggest however, that other physicochemical properties of the wood (e.g. pH, permeability, porosity, chip size and shape) might have influenced nutrient up-take and degradation ability of the fungus. For further studies in wood culture, ashwood was selected as it resulted in highest degradation efficiency.

The experiments on fungus physiology were performed at 37 °C since no significant differences were observed for the degradation efficiencies at 30 and 37 °C in liquid medium.

Fungal growth and nutrient depletion in liquid medium

Biomass production over a 14-day incubation period, measured as mycelium dry weight in defined liquid medium is presented in Figure 2. The exponential growth phase covered days 1 to 3 after which biomass production stabilised at around 16 mg dry weight/flask, indicating the onset of the idiophasic growth.

The nutrients monitored in liquid medium were nitrogen and glucose. Their progression during the 14-day incubation period is shown in Figure 3. Nitrogen is almost completely depleted in the first 3 days while

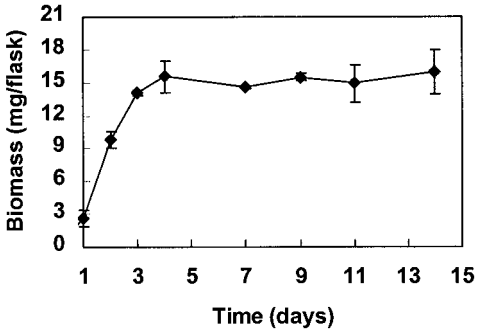

Fig. 2. Progression of biomass production by *P. chrysosporium* at 37 °C in N-limited liquid medium ($n = 3$).

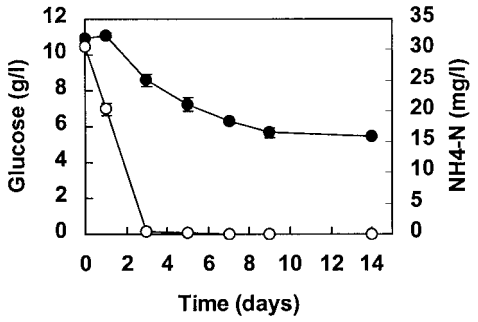

Fig. 3. Progression of glucose and nitrogen depletion by *P. chrysosporium* at 37 °C in N-limited liquid medium ($n = 3$). ● glucose; ○ NH4-N.

glucose was still present at the end of the incubation period. It was consumed at about half of the initial concentration with a highest rate during the exponential growth phase (day 2 and 3). Thus, under the liquid culture conditions used in this study, the medium was nitrogen limited but never became carbon limited.

Lignin peroxidase (LiP) and manganese peroxidase (MnP) activity in liquid cultures

The activity of the two extracellular enzymes produced by the fungus in synthetic medium at 37 °C is presented in Figure 4. Both enzymes could be detected spectrophotometrically during the idiophasic growth. However, MnP was detected first (day 4) and exhibited higher activities compared to LiP during the entire incubation period. The MnP peak value (58 U/l) was reached at day 10. LiP peak value was about 10 U/l at day 11. Preliminary investigations conducted in the presence of diuron resulted in higher ligninolytic activity than in its absence, indicating a possible substrate specificity of these enzymes for diuron (results not shown).

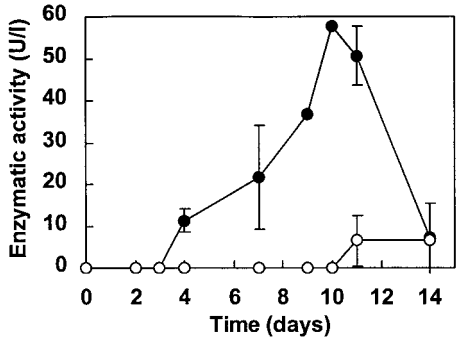

Fig. 4. Progression of peroxidase activity produced by *P. chrysosporium* at 37 °C in N-limited liquid medium ($n = 3$). ● MnP; ○ LiP.

Table 1. Ashwood decay in the presence of *P. chrysosporium* at 37 °C.

Time (days)	Dry weight (g)	Weight loss (%)
0	4.78(0)	0
14	4.56(0.04)	4.6
28	4.30(0.02)	10
42	4.00(0.10)	16.32

Values between brackets represent standard deviation of three replicates.

Fungal growth on ashwood chips

The experiment was carried out to examine fungal growth and ligninolytic activity over a longer incubation period (42 days), in the absence of any other nutrient source except ashwood chips.

The total wood weight loss at different times during the incubation period is presented in Table 1. The fungus colonized and decayed the woodchips as can be observed in the scanning electron micrographs (SEM) presented in Figures 5a–5b). The total wood weight loss at the end of the incubation period was 16.32%. However, the net wood weight loss will be slightly higher taking into consideration that the measurements included the gain in fungal biomass. A specific decay rate of 3.88×10^{-3} day^{-1} was found indicating an expected life time of about 270 days for the 5 g sample of ashwood used in this experiment (<1 mm size). The abiotic controls (without the fungus) showed no significant change in the dry weight after a period of 42 days.

MnP activity in ashwood cultures

Only MnP activity could be detected in ashwood cultures, as shown in Figure 6. The pattern presents fluctuations over the incubation period reaching a maximum of 564 U/l at day 17.

Discussion

This study aimed to determine the ability of *P. chrysosporium* BKM-F-1767 to grow and degrade diuron when incubated on synthetic (N-limited liquid medium) and natural substrates (wood chips). Wood chips were used as support and sole nutrient source for the fungus. In this preliminary study, no attempts were made to investigate the physicochemical characteristics of the wood used.

The results of the degradation experiments in liquid medium indicate that diuron could be degraded by the fungus with a maximum efficiency of 75% within 14 days. When incubated on two different species of hardwood chips in the presence of diuron, the fungus could grow and degrade the target compound to certain extents depending on the wood type. Figure 7 represents the relationship between the progression of diuron and enzymatic activity (MnP) in both media investigated. From Figures 2, 3 and 7, a temporal coincidence seems to exist between the onset of idiophasic growth, nitrogen depletion, production of extracellular enzymes (MnP) and diuron degradation. This finding suggests that diuron degradation involves the ligninolytic system of the fungus represented by MnP as dominant enzyme.

The ability of this fungus to degrade xenobiotics (i.e. chlorophenols) to a larger extent when grown on wood chips in the absence of glucose, than in the presence of glucose, was recently reported by Yum & Peirce (1998b). Unlike their batch culture medium, which contained extra mineral nutrients and thiamine, the results reported in this study for diuron degradation on ashwood, were obtained in the absence of any external addition of nutrients and buffer. However, for a better comparison of the degradation efficiency in the liquid and ashwood cultures, specific degradation rates should be used since different culture media (nutrients source and concentration) were provided, leading possibly to different biomass concentration and consequently, to different degradation efficiencies of the target compound (at this stage of research no attempts were made).

Lignin metabolism by *P. chrysosporium* is a slow process even in optimized culture conditions (Kirk *et al.* 1978) which may ensure a long life time of the wood support. The percentage of total weight loss af-

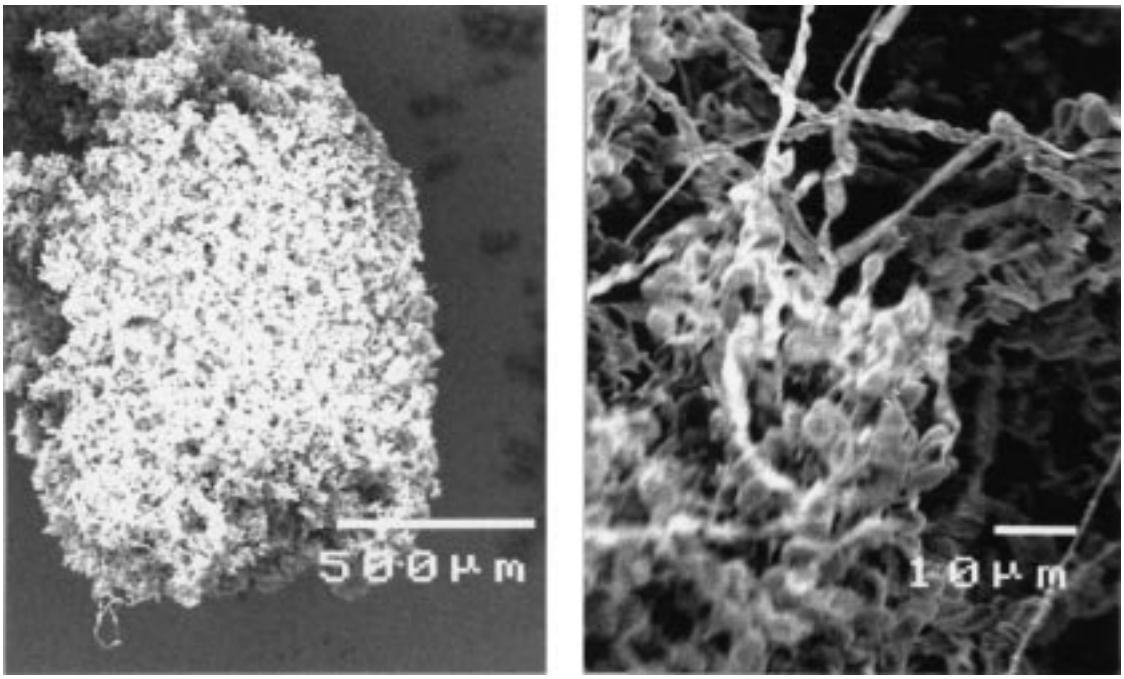

Fig. 5. SEM of the fungi-covered woodchips (a) and fungus cells (b) after 6 weeks of incubation. Scale bar and magnification: (a) 500 μm, $\times$35; (b) 10 μm, $\times$750.

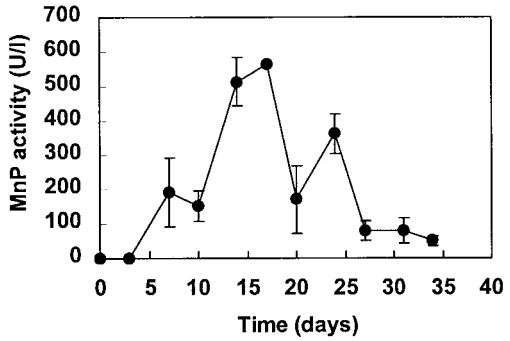

Fig. 6. Progression of MnP activity produced by *P. chrysosporium* at 37 °C on ashwood chips ($n = 3$)

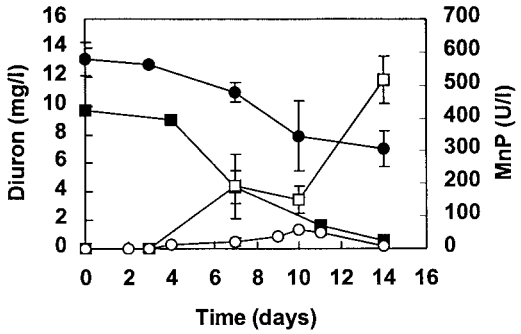

Fig. 7. Diuron and MnP progression in liquid and ashwood cultures ($n = 3$). ■ diuron-ashwood culture; ● diuron-liquid culture; □ MnP-ashwood culture; ○ MnP-liquid culture.

ter 14 incubation days (4.60%) is comparable to that of birchwood (4% after 16 days) found by Yum & Peirce (1998a) indicating that the fungus is able to obtain from its wood support sufficient nutrients required for both growth and production of extracellular enzymes (Figures 5 and 6). Specific decay rates as found for ashwood can be considered attractive for a continuous degradation process using fungi-activated wood chips.

In general, wood is known for its high C/N ratio (Rayner & Boddy 1988) which may lead to nitrogen limitation of the medium and so, to the induction of extracellular enzymes. Lignin degradation products may serve as precursors for the biosynthesis of secondary metabolites involved in the regulation of extracellular enzyme activities (Mester *et al.* 1997). Previous biodegradation studies reported that, when a readily available carbon source, like glucose, is present in the culture medium, the secondary metabolic activity of *P. chrysosporium* is decreased, as is the degradation ability towards the target compound (Kirk *et al.* 1978, Pal *et al.* 1995a). On the other hand, in a substrate depleted medium, the production of extracellular enzymes stops (Armenante *et al.* 1994). These aspects

may have contributed to the observed high degradation efficiency of diuron in ashwood culture, and also point to the benefits of using fungi-activated wood chips for continuous biodegradation processes.

The fact that only MnP activity was detected in ashwood culture does not exclude the existence of LiP activity. The spectrophotometrical method applied has limitations in detecting this enzyme in the extracts from solid cultures (Vares *et al.* 1992, Orth *et al.* 1993, de Jong *et al.* 1994, Sayadi *et al.* 1996). Vares *et al.* (1995) could not measure LiP activity produced by *Phlebia radiata* during incubation on wheat straw using the veratryl alcohol assay, even after enzyme purification, although the fungus is known to produce LiP in liquid cultures. Therefore, they suggest that new methods should be established for LiP detection in extracts from solid substrate cultures.

On the other hand, an increasing number of studies have reported on the role of MnP in the degradation of lignin model compounds and others (Paice *et al.* 1993, Harazono *et al.* 1996, Hofrichter *et al.* 1998). Recently, Jensen, Jr. *et al.* (1996) reported that MnP can also oxidize non-phenolic lignin structures (specific for LiP) via a lipid peroxidation mechanism. All these findings support the idea that MnP, which was the dominant enzyme detected in cultures investigated in this research, could be responsible for diuron degradation.

In this study, diuron could be degraded by *P. chrysosporium* in synthetic, N-limited liquid medium with a maximum efficiency of 75%. Furthermore, the fungus could grow, produce MnP and degrade diuron with an efficiency of 95% when grown on ashwood chips as sole nutrient source. This indicates that a wood chip reactor inoculated with the fungus can potentially be used as a self-controlling system (no addition of nutrients and buffers is required) to degrade diuron. In addition, a low risk of microbial contamination is expected as no readily available nutrients are present. However, wood characteristics may greatly influence fungal degradation of diuron. The ligninolytic system of the fungus seems to be involved in diuron degradation as in both type of cultures investigated, a relationship was observed between the onset of ligninolytic activity and diuron disappearance.

Acknowledgements

The financial support from NOVEM (The Netherlands; Project No. 351210/4410) is greatly acknowledged. Also, many thanks to Jim Field for his valuable input to this research and to Tünde Mester for critically reading the manuscript.

References

Armenante PM, Pal N, Lewandowski G (1994) *Appl. Environ. Microbiol.* **60**: 1711–1718.
Barr DP, Aust SD (1994) *Env. Sci. Technol.* **28**: 79A–87A.
Bumpus JA, Tien M, Wright D, Aust SD (1985) *Science* **228**: 1434–1436.
Bumpus JA, Aust SD (1987) *BioEssays* **6**: 166–170.
de Jong E, Field JA, de Bont JAM (1994) *FEMS Microbiol. Rev.* **13**: 153–188.
Ellis PA, Camper ND (1982) *J. Environ. Sci. Health* **B17**: 277–289.
Esposito E, Paulillo SM, Manfio GP (1998) *Chemosphere* **37**: 541–548.
Field JA, de Jong E, Feijoo-Costa G, de Bont JAM (1993) *TIBTECH* **11**: 44–48.
Gold MH, Glenn JK (1988) *Methods Enzymol.* 161: 258–264.
Hammel KE (1989) *Enzyme Microb. Technol.* 1: 776–777.
Hofrichter M, Scheibner K, Scheneegaß I, Fritsche W (1998) *Appl. Environ. Microbiol.* **64**: 399–404.
Howard PH (1991) *Handbook of Environmental Fate and Exposure Data for Organic Chemicals*. Lewis Publishers, Inc., pp. 319–326.
Harazano K, Kondo R, Sakai K (1996) *Appl. Environ. Microbiol.* **62**: 913–917.
Huijser PJ (1994) *Verontreiniging van de Maas door Diuron*. RIZA, Nota nr.: 94.014, pp. 10–16.
Huijser PJ (1996) *Verontreiniging van de Maas en Zijrivieren in 1994 en 1995 door Diuron*. RIZA, Nota nr.: 96.018, pp. 40–41.
Jensen KA Jr, Bao W, Kawai S, Srebotnik E, Hammel KE (1996) *Appl. Environ. Microbiol.* **62**: 3679–3686.
Kirk TK, Schultz E, Connors WJ, Lorenz LF, Zeikus JG (1978) *Arch. Microbiol.* **117**: 277–285.
Kirk TK, Lamar RT, Glaser JA (1992) The potential of white-rot fungi in bioremediation. In: Mongkolsuk S, Lovett PS, Trempy JE, eds. *Biotechnology and Environmental Science: Molecular Approaches*. New York: Plenum Press, pp. 131–138.
Lewandowski GA, Armenante PM, Pak D (1990) *Wat. Res.* **24**: 75–82.
Madhun YA, Freed VH (1987) *Chemosphere* **16**: 1003–1011.
Mester T, de Jong E, Field JA (1995) *Appl. Environ. Microbiol.* **61**: 1881–1887.
Mester T, Swarts HJ, Romero i Sole S, de Bont JAM, Field JA (1997) *Appl. Environ. Microbiol.* **63**: 1987–1994.
Orth AB, Royse DJ, Tien, M (1993) *Appl. Environ. Microbiol.* **59**: 4017–4023.
Paice MG, Reid ID, Bourbonnais R, Archibald FS, Jurasek L (1993) *Appl. Environ. Microbiol.* **59**: 260–265.
Pal N, Lewandoski G, Armemante PM (1995a) *Biotechnol. Bioengg.* 46: 599–609.
Pal N, Chritodoulatos C, Kodali S (1995b) *Proc. 27th Ind. Waste Conf.*: 284–293.
Paszczynski A, Crawford RL (1995) *Biotechnol. Prog.* **11**: 368–379.

Rayner ADM, Boddy L (1988) *Fungal Decomposition of Wood. Its Biology and Ecology*. New York: Wiley-Interscience, pp. 14–58.

Sayadi S, Zorgani F, Ellouz R (1996) Role of lignin peroxidase and manganese peroxidase of *Phanerochaete chrysosporium* in the decolorization of olive mill wastewaters. In: Moo-Young M, Anderson WA, Chakrabarty AM, eds. *Environmental Biotechnology: Principles and Applications*. Dordrecht: Kluwer Academic Publishers, pp. 511–523.

Tien M, Kirk TK (1988) *Methods Enzymol.* **161**: 238–249.

Tillmanns GM, Wallnofer PR, Engelhardt G, Olie K, Hutzinger O (1978) *Chemosphere* **1**: 59–64.

Vares T, Lundell TK, Hatakka AI (1992) *FEMS Microbiol. Lett.* **99**: 53–58.

Vares T, Kalsi M, Hatakka A (1995) *Appl. Environ. Microbiol.* **61**: 3515–3520.

Venkatadri R, Irvine RL (1993) *Wat. Res.* **27**: 591–596.

Vroumsia T, Steiman R, Seigle-Murandi F, Benoit-Guyod J-L, Khadrani A (1996) *Chemosphere* **33**: 2045–2056.

Weinberger M, Bollag J-M (1972) *Appl. Microbiol.* **24**: 750–754.

Yum K-J, Peirce JJ (1998a) *J. Env. Engg.* **124**: 184–190.

Yum K-J, Peirce JJ (1998b) *Water Env. Res.* **70**: 205–213.