SOIL COMPOSITION AND SUPPRESSION OF PYRENE MINERALIZATION BY PHANEROCHAETE CHRYSOSPORIUM

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ABSTRACT

Soils from four different geographic locations with different compositions all reduced mineralization of pyrene by P. chrysosporium when compared to a non-soil system. Two clays, bentonite and kaolinite, differentially decreased the mineralization of pyrene. Bentonite sorbed pyrene to a greater extent and was more inhibitory to mineralization than kaolinite. Sand had little effect on mineralization. Four artificial soils, made with different proportions of sand, silt and clay, free of organic matter and microbes, inhibited mineralization by P. chrysosporium. Inhibition increased with greater proportions of silts. These findings suggest that soil type may influence the bioremediation potential of P. chrysosporium.

KEY WORDS

pyrene, Phanerochaete chrysosporium, bentonite, kaolinite, mineralization.

INTRODUCTION

The wood decay fungus Phanerochaete chrysosporium utilizes an extracellular complex of lignin peroxidase enzymes to degrade the lignin content of the wood cell walls [1, 2]. This same complex is implicated in the degradation of pollutants of diverse structures [3, 4]. Such degradation is readily demonstrated in the laboratory in defined media conditions [3, 5]. Consequently, the potential of P. chrysosporium to degrade pollutants in the environment is being investigated [3, 6-9].

Both abiotic and biotic factors may influence the ability of P. chrysosporium to degrade pollutants in soil [10, 11]. We observed that a native soil suppressed the mineralization of pyrene by P. chrysosporium [11]. Because suppression of mineralization of the native soil was in part relieved by sterilization of the soil, antagonism of the bioremediation potential of the fungus by soil microbes seemed likely [11]. Microbes that in vitro reduced the growth of P. chrysosporium were isolated from the native soil [11]. However, mineralization with the sterilized soil and P. chrysosporium was still below that of the inoculum placed directly onto the pyrene-agar surface [11]. Consequently, in this paper we searched for abiotic features of the soil that could explain the suppression in the sterilized soil.

It seemed possible that the reduction in pyrene mineralization by sterilized soil could be both physical and chemical in nature. Inhibition by physical barriers was studied using the individual soil components, sand, silt and clays, alone and in combination to provide soils with different textures. Two different types of clays were used, the expanding clay bentonite and the non-expanding clay kaolinite. These clays were examined for their relative abilities to sorb pyrene and to determine whether sorption of the pollutant was a significant feature. Soils from diverse locations in the United States of America were also used to see whether inhibition was a generalized or

variable trait or was related to such features as soil texture and pH.

PROCEDURES

Maintenance of P. chrysosporium

P. chrysosporium BKM-F-1767 ATCC #24725 was maintained at 26°C by subculturing at two weekly intervals on potato dextrose agar (PDA) (Difco, Detroit, Michigan) amended with 1% agar. Stocks were stored as plugs of mycelia frozen at -80°C in 15% glycerol.

P. chrysosporium was grown on sterilized ground corn cobs for one month to provide an inoculum for mineralization studies as described by Tucker et al. [11]. Both spores and mycelia were present on the corn cobs at the time of inoculation into the microcosms.

Soils

The soils used in these studies were from gardens in College Park (Maryland), and Logan (Utah), a semi-industrial site from Chicago (Illinois) and from agricultural fields in Parma (Idaho) and Kaysville (Utah).

The soils were sieved through a 2 mm sieve and stored at room temperature for less than one year. Soil characteristics (Table 1) were determined by the Utah State University Analytical Laboratories Soil Testing Laboratory, Logan, Utah. Sterilized soils were prepared by autoclaving for 4 h on two consecutive days.

The microcosms

Microcosms to examine mineralization of pyrene by P. chrysosporium were established in Corning polystyrene tissue culture flasks (Corning Co. 25110-75, Corning, NY). The flasks were laid flat and contained 40 ml of 1% water agar with no added nutrients. Pyrene (^{14}C-labeled [500,000 dpm]) from Sigma Chemical Company, St. Louis, MO, in 5 µl benzene was applied to the agar surface. The benzene was removed by passing air through the flasks for 18 h using the sterile gas exchange system fitted to the flasks as described by Tucker et al. [11]. The agar layer was overlaid with a 'soil' layer that was inoculated with the P. chrysosporium-colonized corn cobs. The microcosms were incubated at 26°C and sampled for CO_2 evolution using the sterile gas exchange system as described [11].

Amendments used in the 'soil' layer

The 'soil' layer consisted of native and sterilized soils, or matrixes of defined texture. Sand (particle size < 355 µm) was purchased and autoclaved at 121°C for one

Soil origin	Exchangeable Ca^{2+}	pH	% O.C.[a]	CEC[b]	Texture		
					Sand	Silt	Clay
Chicago	50	6.4	1.78	25.4	11	49	40
Logan	142	7.6	3.95	22.1	51	35	14
College Park	102	6.6	4.10	17.1	55	31	14
Timpanogos	58	4	1.44	20.4	25	57	18
Kaysville	89	7.8	0.68	12.1	52	32	16
Parma	119	7.8	1.46	13.0	29	53	18

[a]Percent organic carbon.
[b]Cation exchange capacity (meq/100 g).

Table 1. Soil characteristics.

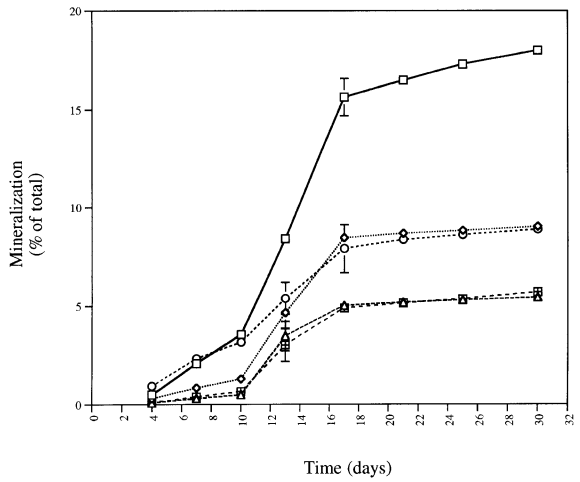


Figure 1. Suppression of mineralization of pyrene by P. chrysosporium by native soils. Microcosms were established without soils as a control (--□--) or with soils from College Park (--○--), Chicago (--◇--), Parma (--⊞--) and Logan (--△--). Mineralization of pyrene was measured as $^{14}CO_2$ evolution from ^{14}C-labeled pyrene as described in Procedures. Data are from duplicate flasks of a single experiment. Error bars represent standard deviation. Each study was replicated twice.

h on two consecutive days before use. Silt (particle size < 45 mm) was obtained by sieving and sedimenting Timpanogos soil that has a high silt content [11]. To remove organic materials, a suspension of silt (50 g) in 20 ml 30% hydrogen peroxide was heated to boiling for five min and cooled, and the process was repeated after addition of another aliquot of hydrogen peroxide [12]. The hydrogen peroxide was removed by extensive washing with sterile distilled water. Kaolinite and bentonite clays were obtained commercially and were sterilized by autoclaving at 121°C for one h on two consecutive days. The sodium-saturated bentonite clay was suspended in 10 M $MgCl_2$ and rinsed in sterile distilled water to partially exchange the cations to minimize swelling from water. Artificial soils were produced by mixing the sand, silt and commercial kaolinite clay in known proportions.

The pH of the soil and corn cob layers were determined throughout a time course in a study performed with the Kaysville soil. Soil and corn cobs (15 ml) were removed separately from the microcosms and mixed with 20 ml sterile distilled water, and the pH of the suspension was measured after 15 min.

Sorption of pyrene

The soil-water sorption coefficient, k_d, was measured for Kidman soil and compared with the k_d value for kaolinite and bentonite. The EPA protocol for sediment and soil isotherm (CFR SEC 796.2750) was followed. Clay (4 g) was mixed with 40 ml 0.01 M Ca $(N0_3)_2$. A mixture of pyrene and ^{14}C-pyrene (0.1 to 7 mg/L) was added to 50 ml Teflon tubes filled with 0.01 M $Ca(NO_3)_2$ to capacity. The tubes were sealed and weighed. The solutions were shaken at 20 ± 1°C in a rotary mixer at 30 rpm for 24 h. Samples were centrifuged at 7000 g for 30 min. A 1 ml aliquot of supernatant was counted using a Beckman 1400 Liquid Scintillation Counter in Ready Safe® cocktail. The sorbant were dried overnight and two 1 g portions each for the soil and two clays were combusted in a Harvey Biological Oxidizer. The resulting $^{14}CO_2$ was trapped in 10% monoethanolamine, 40% methanol and 50% Ready Gel (Beckman) prior to counting. The study was repeated in triplicate. K_d values were calculated by plotting S mg/kg versus C_e mg/L where S is the amount of pyrene sorbed by the clay (mg/kg) and C_e is the equilibrium solution concentration (mg/L). The slopes of the line were calculated from three separate studies for the bentonite and kaolinite and the means calculated.

RESULTS

Suppression of pyrene mineralization by native soils

Mineralization of pyrene by P. chrysosporium was suppressed by the inclusion of a layer of native soil from four different geographic locations into the microcosms

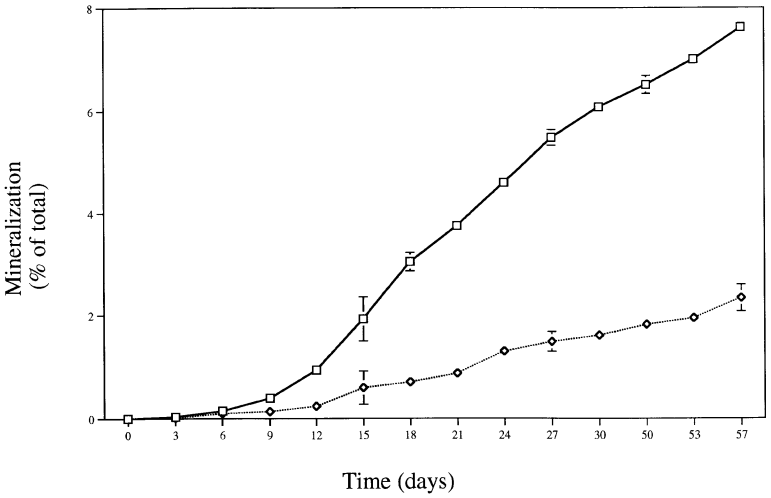


Figure 2. Suppression of mineralization of P. chrysosporium by sterilized Kaysville soil. Mineralization of pyrene was measured as $^{14}CO_2$ evolution from ^{14}C-labeled pyrene as described in Procedures. Microcosms were established with no soil layers (--□--) or with sterilized Kaysville soil (--◆--). Data are from duplicate flasks of a single experiment. Error bars represent standard deviation. Each study was replicated twice.

(Figure 1). Two of the soils, a garden soil from Logan and an agricultural soil from Parma, were more inhibitory than the soils from College Park and Chicago. A sterilized sandy-loam soil, Kaysville, also inhibited mineralization (Figure 2).

The native soils were of different textures (Table 1). The Chicago soil was high in silt and clay. Both the Logan and the College Park soils were sandy and low in clay. The Logan, Parma and Kaysville soils were the most alkaline (pH 7.6 to 7.8). All other soils were slightly acidic (pH 4 to 6.6). The organic carbon content was highest for the College Park and Logan soils. These soils and the Parma soil also had the highest exchangeable Ca^{2+} levels.

The ability to buffer acidity may be an important feature in determining the time of onset of mineralization. In studies with sterile Kaysville soil, acidification of the soil matrix occurred in the time frame (10-12 days) that is required for onset of mineralization (Figure 3). The pH of the corn cob matrix was not altered from the initial value of about pH 4.5.

Effects of artificial soils on mineralization

Soils of different textures were achieved by mixing fractions of purified sand, silt and kaolinite clay. These systems were free of organic material and microbes. The soil mixtures all decreased mineralization of pyrene by P. chrysosporium (Figure 4). The extent of inhibition increased as the amount of silt in the soil mixture increased. No mineralization occurred in microcosms without the P. chrysosporium inoculum.

Effects of clay on mineralization

Bentonite decreased the mineralization of pyrene by P. chrysosporium to a greater extent than did kaolinite (Figure 5a). A layer of sand, used as a control to provide a physical barrier of the same depth (5 mm) between the pyrene and the fungal inoculum, had little effect on the extent and rate of mineralization (Figure 5b).

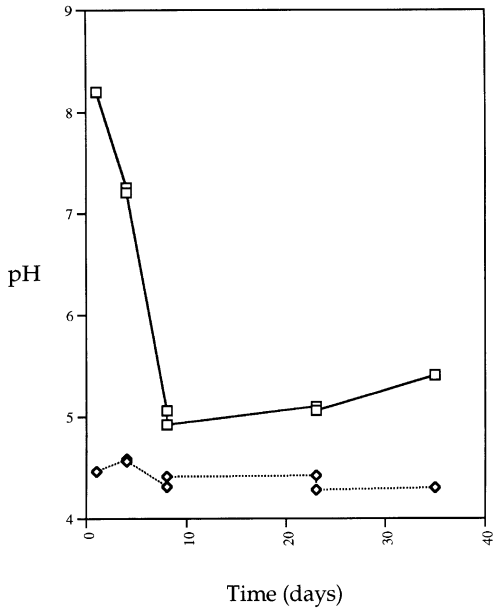


Figure 3. Timing of acidification of soil matrix and corn cobs by P. chrysosporium. Microcosms were established with a layer of corn cob inoculum over the layer of sterilized Kaysville soil as described in Procedures. The pH of the soil (--□--) and the corn cobs (--◇--) was determined with time after inoculation as described in Procedures. Data are from duplicate flasks of a single experiment. Error bars represent standard deviation. Each study was replicated twice.

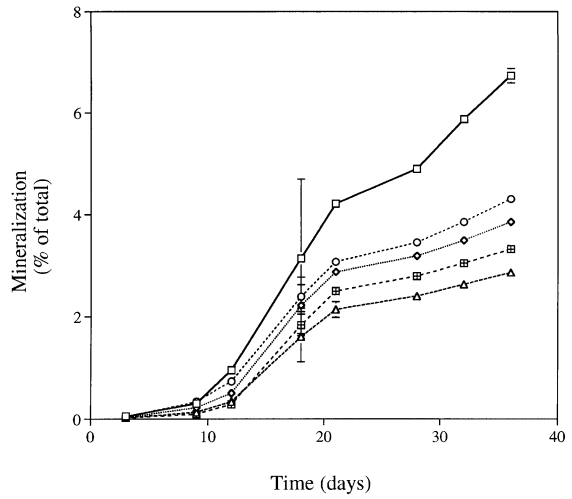


Figure 4. Effects of artificial soil mixtures on mineralization of pyrene by P. chrysosporium. Microcosms were established without soils (--□--) or with a layer of artificial soils composed of a loam (40% sand, 20% silt and 40% clay) (--○--), a sandy loam (60% sand, 30% silt and 10% clay) (--◇--), a silty clay (10% sand, 45% silt and 45% clay) (--⊞--), and a silt loam (20% sand, 60% silt and 20% clay) (--△--). Data are from duplicate flasks of a single experiment. Error bars represent standard deviation. Each study was replicated twice. Mineralization of ^{14}C-pyrene to $^{14}CO_2$ was measured as described in Procedures.

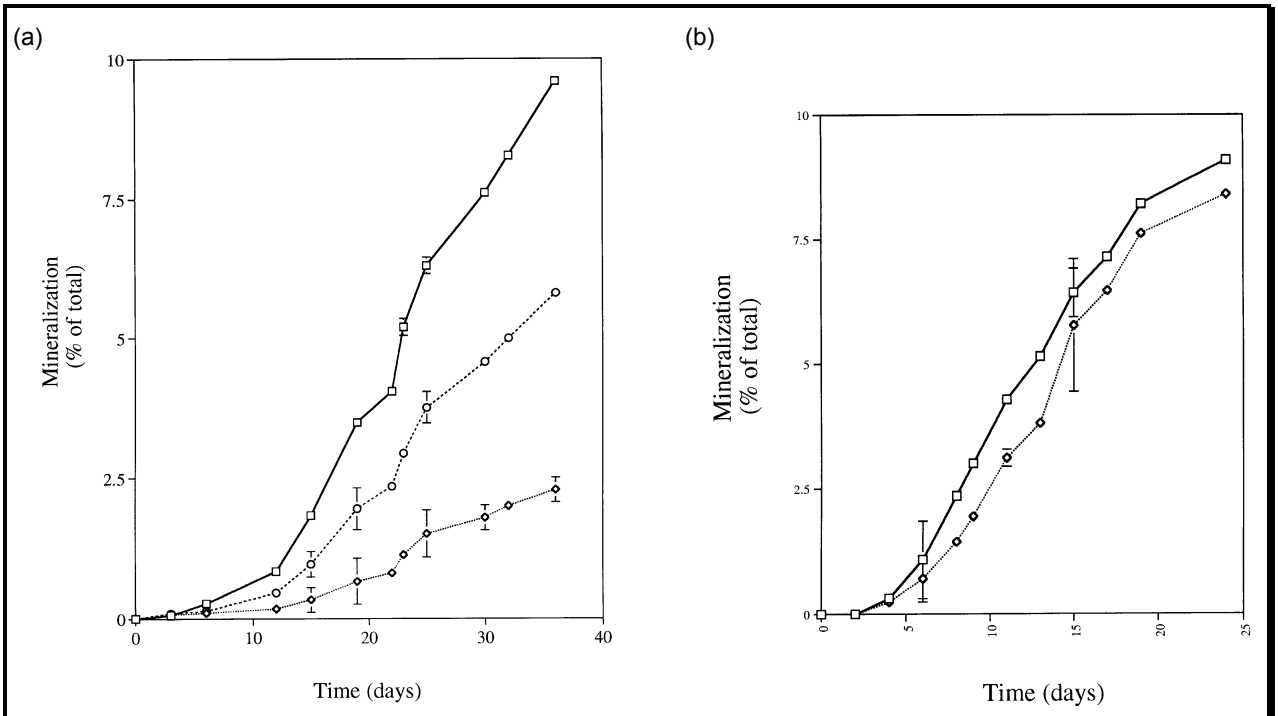
Sorption of pyrene onto bentonite and kaolinite

Pyrene was sorbed to bentonite (K_d = 5,635 [r^2 = 0.943]) to a greater degree than kaolinite (K_d = 9.83 [r^2 = 0.926]).

DISCUSSION

The chemical and physical component of soils affected the mineralization of pyrene by P. chrysosporium. Inhibition was observed to increase as the proportion of silt increased in artificial soil mixes free of microbes and organic materials. Isolated clean clays were also inhibitory but sand was without effect. The differential inhibitory effects of clays, bentonite and kaolinite could be related to differences in the sorption of the pyrene to the matrix. In other systems, sorption of pollutants to soil materials has been demonstrated to reduce bioremediation potential [13, 14, 15]. The bentonite clay, which caused the greater extent of inhibition of mineralization, had a far greater affinity for the pyrene, with a K_d value for pyrene 500-fold higher than the value for kaolinite. However, because mineralization was not different to a five hundred fold extent between the two clays, sorption of pyrene must not be the only factor to govern mineralization. In the natural soil, partitioning into organic materials may be affecting sorption of pyrene. Sorption of extracellular fungal materials that are essential for activity towards pyrene may be occurring. For example, the peptide toxins from Bacillus thuringiensis adsorb and bind to pure and soil clays (16).

The buffering capacity of the soil may be another abiotic factor influencing mineralization. It is likely that the fungus establishes an acidic environment to optimize the



Figures 5a and 5b. Effects of clay (a) and sand (b) on mineralization of pyrene by P. chrysosporium. Microcosms are established without clays (--□--) or with layers of bentonite (--◇--) and kaolinite (--○--) in (a) or sand (--◇--) in (b). Mineralization of ^{14}C-pyrene to $^{14}CO_2$ was assayed as described in Procedures. Data are from duplicate flasks of a single experiment. Error bars represent standard deviation. Each study was replicated twice.

activity of the peroxidase complex involved in degradation [17]. In in vitro assays, maximum veratryl oxidase activity of the lignin peroxidase from P. chrysosporium is about pH 2.5 [17]. An ability to pump protons into the extracellular environment has been demonstrated with P. chrysosporium in connection with the transformation of TNT [18]. Previously, we found that onset of mineralization of pyrene by P. chrysosporium was correlated with the onset of lignin peroxidase production [11]. In this study we found that the fungus acidifies the soil to pH between 4 and 5 at the same time that mineralization of pyrene commences. Presumably soils with greater buffering capacity may be more suppressive to permitting bioremediation by P. chrysosporium. Indeed we found that the calcareous Logan garden soil effectively suppressed pyrene mineralization.

CONCLUSIONS

Our finding that four native soils examined in this paper were suppressive to mineralization confirms the observation made previously [11] with the Timpanogos soil. We find that abiotic features of the soil, especially the extent of silt and clays, contribute to suppression of the bioremediation potential. These data suggest that the use of P. chrysosporium augmentation in soils for bioremediation may need to be finely tuned. Pre-acidification of the soil may be one technique to be considered to enhance efficacy.

REFERENCES

1. T.K. Kirk, E. Schultz, W.J. Connors, L.F. Lorenz and J.G. Zeikus, Influence of Culture Parameters on Lignin Metabolism by Phanerochaete chrysosporium, Arch. Microbiol., 117 (1978) 277-285.

2. C.D. Boyle, B.R. Kropp and I.D. Reid, Solubilization and Mineralization of Lignin by White Rot Fungi, Appl. Environ. Microbiol., 58 (1992) 3217-3224.

3. D.P. Barr and S.D. Aust, Mechanisms White Rot Fungi Use to Degrade Pollutants, Environ. Sci. Techno., 29 (1994) 79-87.

4. K. Valli, H. Warishi and M.H. Gold, Degradation of 2,7-dichlorobenzo-p-dioxin by the Lignin Degrading Basidiomycete Phanerochaete chrysosporium, J. Bacteriol., 174 (1992) 2131-2137.

5. M.H. Gold and M. Alic, Molecular Biology of the Lignin-Degrading Basidiomycete Phanerochaete chrysosporium, Microbiol. Rev., 57 (1993) 605-622.

6. G.J. Elias and R.D. Neufield, Degradation of Fluorene in Soil by Fungus Phanerochaete chrysosporium, Biotechnol. Bioeng., 33 (1989) 1306-1310.

7. T.P. Ryan and J.A. Bumpus, Biodegradation of 2,4,5-trichlorophenoxyacetic Acid in Liquid Culture and in Soil by the White Rot Fungus Phanerochaete chrysosporium, Appl. Microbiol. Biotechnol., 31 (1989) 302-307.

8. T.S. Brodkorb and R.L. Legge, Enhanced Biodegradation of Phenanthrene in Oil-Tar Contaminated Soils Supplemented with Phanerochaete chrysosporium, Appl. Environ. Microbiol., 67 (1992) 3117-3121.

9. R.T. Lamar and D.M. Dietrich, In situ Depletion of Pentachlorophenol from Contaminated Soil by Phanerochaete spp, Appl. Environ. Microbiol., 56 (1990) 3093-3100.

10. C.W. Radtke, S. Cook and A. Anderson, Factors Affecting the Growth Antagonism of Phanerochaete chrysosporium by Bacteria Isolated from Soils, Appl. Microbiol. Biotechnol., 41 (1994) 274-280.

11. B. Tucker, C. Radtke, S.-I. Kwon and A.J. Anderson, Suppression of Bioremediation by Phanerochaete chrysosporium by Soil Factors, Journal of Hazardous Materials, 41 (1995) 251-265.

12. P.R. Day, Particle Fractionalization and Particle-size Analysis, In: C.A. Black (Ed), Methods of Soil Analysis, Agronomy No. 9, Part 1, American Society of Agronomy, Madison, WI, 1965, pp. 545-567.

13. R.C. Sims, Subsurface Contamination Guide, Office of emergency and remedial response, U.S. EPA, Washington D.C., 20460, 1990.

14. G. Weissenfels, H. Klewer and B. Langhoff, Adsorption of Polycyclic Aromatic Hydrocarbons (PAHs) by Soil Particles: Influence on Biodegradability and Biotoxicity, Appl. Microbiol. Biotechnol., 36 (1992) 689-696.

15. F. Volkering, A.M. Breure, J.G. Van Andel and W.H. Rulkens, Influence of Nonionic Surfactants on Bioavailability and Biodegradation of Polycyclic Aromatic Hydrocarbons, Appl. Environ. Microbiol., 61 (1995) 1699-1705.

16. H. Tapp and G. Stotzky, Insecticidal Activity of the Toxins from Bacillus thuringiensis Subspecies Kurstaki and Tenebrionis Adsorbed and Bound on Pure and Soil Clays, Appl. Environ. Microbiol., 61 (1995) 1786-1790.

17. M. Tien and T.K. Kirk, Lignin Peroxidase of Phanerochaete chrysosporium, Methods Enzymol., 161 (1988) 238-249.

18. J.D. Stahl and S.D. Aust, Plasma Membrane Reduction of 2,4,5-

trinitrotoluene by Phanerochaete chrysosporium, Biochem. Biophys. Res. Comm., 192 (1993) 471-476.