Biodegradation **15:** 1–8, 2004.

Effects of fungal bioaugmentation and cyclodextrin amendment on fluorene degradation in soil slurry

David Garon, Lucile Sage & Françoise Seigle-Murandi*
*UMR LECA 5553, Equipe PEX, Bâtiment D de Biologie, Université J. Fourier, BP 53, 38041 Grenoble Cedex 09, France (*author for correspondence: e-mail: francoise.seigle-murandi@ujf-grenoble.fr)*

Accepted 11 September 2003

Key words: biodegradation, cyclodextrin, fluorene, fungi, polycyclic aromatic hydrocarbons

Abstract

This study assesses the potential of fungal bioaugmentation and the effect of maltosyl-cyclodextrin amendment, as an approach to accelerate fluorene biodegradation in soil slurries. 47 fungal strains isolated from a contaminated site were tested in the biodegradation of fluorene. Results showed the greater efficiency of "adaptated" fungi isolated from contaminated soil vs. reference strains belonging to the collection of the laboratory. These assays allowed us to select the most efficient strain, *Absidia cylindrospora*, which was used in a bioaugmentation process. In the presence of *Absidia cylindrospora*, more than 90% of the fluorene was removed in 288 h while 576 h were necessary in the absence of fungal bioaugmentation. Maltosyl-cyclodextrin, a branched-cyclodextrin was chosen in order to optimize fluorene bioavailability and biodegradation in soil slurries. The results of this study indicate that *Absidia cylindrospora* and maltosyl-cyclodextrin could be used successfully in bioremediation systems.

Introduction

Polycyclic aromatic hydrocarbons (PAH) are poorly soluble, hydrophobic organic compounds which have been released into the environment on a large scale.

Their ubiquity along with their toxicity and mutagenicity, makes them priority pollutants. Although laboratory studies have revealed that virtually all PAH are biodegradable (Cerniglia 1992), the rates of PAH degradation at contaminated sites are often much lower than the rates expected on the basis of laboratory studies. It is generally admitted that a low level of bioavailability (i.e., slow release of the pollutant from the solid phase to the aqueous phase) is one of the most important factors involved in the slow biodegradation of hydrophobic organic compounds in soil (Mihelcic 1993).

Fluorene (FL), a tricyclic PAH, and its derivatives, are commonly found in environmental samples of soil, sediments, waters .... Because FL is present in most PAH mixtures and its structure is found in several mutagenic and/or carcinogenic PAH such as 2-amino-FL, 2-nitro-FL and 2-acetyl-amino-FL (Martelmans et al. 1986), it is often used as a model structure. Therefore, it is one of the 16 PAH listed as priority pollutants by the US Environmental Protection Agency (Keith & Telliard 1979).

Bacterial metabolism of FL has been extensively studied (Baldrin et al. 1993) but less is known about the fungal metabolism of FL: *Cunninghamella elegans* metabolizes FL to 9-fluorenol, 9-fluorenone and 9-hydroxy-9-fluorenone (Pothuluri et al. 1993). Various other fungi also oxidize FL (Sack & Günther 1993; Cerniglia & Sutherland 2001).

Many of the remediation technologies currently being used for contaminated soil and water involve not only physical and chemical treatment but also bioremediation of pollutants by microbial activity strategies (Cha et al. 1999; Straube et al. 1999). Fungi are present in aquatic sediments, terrestrial habitats and water surfaces and play a significant part in natural biodegradation of PAH (Atlas & Cerniglia 1995). Fungi also have advantages over bacteria since fungal hyphae can penetrate contaminated soil to reach the PAH (April et al. 2000) and that most fungi have ex-

tracellular enzymes involved in degradation of a wide range of pollutants (Aust 1995).

The efficiency of microbial degradation is often seriously impeded by 2 major facts: the poor accessibility of lipophilic compounds to microrganisms and the toxic effects of substrates exerted upon the microbe. In this study, cyclodextrins (CD) were used to significantly alleviate or remove these obstacles. CD are cyclic oligomers of 1,4α-D-linked glucose units (Szejtli 1988). The ability of the CD and CD derivatives to desorb the lipophilic xenobiotics (PAH, pesticides) and to solubilize these organics via inclusion complex formation makes them a useful additive in soil bioremediation. The solubilized pollutants are more accessible for the microorganisms. For example, hydroxypropyl-β-cyclodextrin significantly accelerated the degradation of phenanthrene (Wang et al. 1995, 1998).

The originality of this work was to combine a fungal bioaugmentation approach with the enhancement of bioavailability by use of CD.

Materials and methods

Cyclodextrins

α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD) were respectively obtained from Wacker (Munich) and Roquette (Lestrem). Hydroxypropyl-β-cyclodextrin (HPβCD) and partiallymethyl-β-cyclodextrin (PMβCD) were received from Roquette. β-cyclodextrin-sulfobutylether (SEβCD) and maltosyl-cyclodextrin (MCD) were respectively received from Orsan and Captisol, and Maruha corporation.

Isolation and identification of fungal strains

A contaminated soil containing 100 μg g^{-1} FL (2061 μg g^{-1} total PAH) was collected from a deserted industrial plant in France. Soil samples (quadruplicates) were analyzed following 2 methods: direct spreading and deep inoculation following the soil plates method of Warcup (1950). Incubations were at 22 °C and 37 °C. Isolations were made on malt extract agar solid medium (MEA) with added chloramphenicol (0.05%). Agar and malt extract were respectively purchased from Cooperative Pharmaceutique Française (Melun, France) and Difal (Villefranche-sur-Saône, France). After isolation, Zygomycetes and anamorphic fungi were identified according to general principles of fungal classification (Domsch et al. 1980). 47 fungal strains were isolated and identified from contaminated soil (Table 1).

Biodegradation test in liquid medium

Fungal strains isolated from contaminated soil were tested for FL biodegradation. GS (Galzy & Slonimski 1957) liquid medium supplemented with glucose (5 g l^{-1}) was used for biodegradation assays. Strains with a degradation capacity higher than 60% were compared with reference strains from the collection of the laboratory of Mycology (CMPG Collection Mycology Pharmacy Grenoble). These reference strains were isolated from uncontaminated environments.

Before degradation test, fungi were cultivated on MEA for 8 d without FL. Then, surface mat mycelia were filtered, washed, suspended in GS medium and fragmented in a laboratory blender. 500 μl of fungal suspension was resuspended in 100 ml Erlenmeyer flasks containing 25 ml of autoclaved (20 min, 121 °C) GS medium. Flasks were incubated at room temperature (23 °C) on a rotary shaker (180 rpm) for 2 d in order to obtain a biomass of 0.05 g $\pm$ 10% (dry weight). FL in DMSO stock solution sterilized by filtration through 0.22 μm pore size Millipore membrane, was added to the 2d-old cultures to a final concentration of 0.005 g l^{-1}. FL (purity > 98%) was purchased from Sigma-Aldrich (Sigma chemical Co, St Louis, MO) and purified by recrystallisation in acetone. Solvents [acetone, acetonitrile, dimethyl sulfoxide (DMSO), ethyl acetate, methanol, 2-propanol, tetrahydrofuran (THF) and water] were purchased from Baker (Mallinckrodt Baker BV, Holland).

The depletion of FL was evaluated after 2 d of cultivation with the PAH. Temperature was 23 °C, assays were conducted in the dark to avoid photooxydation of FL. Each series of experiment was performed in triplicate and included cell-free flasks for stability assessment. The optimal conditions of extraction were obtained after preliminary assays. Fungal mycelia and culture media were extracted 3 times with 25 ml of ethyl acetate. Flasks were shaken at 240 rpm at 23 °C for 30 min. After the third extraction the flasks were washed with 10 ml acetone and then with 10 ml ethyl acetate. By this method more than 80% of initial FL was recovered. The combined crude extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness at 4 °C under reduced pressure. The residue was dissolved in acetonitrile.

Table 1. Depletion of FL (0.005 g l^{-1}) by 47 micromycetes isolated from contaminated soil

Strain	Depletion of FL (%)[a]	Strain	Depletion of FL (%)[a]
Absidia cylindrospora	98*	*Minimedusa polyspora*	40 ± 1
Absidia spinosa	95 ± 2	*Mucor hiemalis*	69 ± 4
Acremonium murorum	68 ± 2	*Neosartorya fischeri*	43 ± 2
Alternaria alternata	46 ± 1	*Paecilomyces lilacinus*	22 ± 3
Aspergillus flavipes	58 ± 2	*Penicillium canescens*	61 ± 2
Aspergillus fumigatus	82 ± 3	*Penicillium janczewskii*	79 ± 2
Aspergillus niger	82*	*Penicillium montanense*	62 ± 1
Aspergillus oryzae	80*	*Penicillium restrictum*	50 ± 1
Aspergillus parasiticus	45 ± 4	*Penicillium simplicissimum*	72 ± 1
Aspergillus terreus	64 ± 8	*Phoma eupyrena*	0.1*
Aspergillus ustus	75*	*Phoma exigua*	18 ± 4
Botryotrichum piluliferum	49 ± 1	*Pseudallescheria boydii*	69 ± 3
Cladosporium herbarum	40 ± 1	*Seimatosporium* sp.	55*
Cunninghamella bainieri	91 ± 1	*Stachybotrys bisbyi*	20 ± 1
Cylindrocarpon lucidum	3 ± 2	*Stachybotrys chartarum*	75 ± 5
Doratomyces asperulus	26 ± 2	*Talaromyces flavus*	49 ± 4
Doratomyces stemonitis	46 ± 8	*Trichoderma hamatum*	60 ± 1
Fusarium culmorum	40 ± 2	*Trichoderma harzianum*	29*
Fusarium lateritium	3 ± 2	*Trichoderma koningii*	72 ± 2
Fusarium oxysporum	39*	*Trichurus spiralis*	34 ± 3
Fusarium solani	22 ± 1	*Verticillium tenerum*	40 ± 1
Gliocladium roseum	92 ± 2	*Westerdykella dispersa*	23 ± 1
Gliocladium virens	73 ± 1	*Zygorhynchus heterogamus*	87 ± 1
Humicola grisea	23 ± 1		

[a]The results are presented according to the formula $m \pm$ SD in which m is the mean and SD the standard deviation.
*SD < 1.

HPLC analysis

HPLC was performed with a liquid chromatography apparatus (Shimadzu) equipped with a LC 6A pump and a SIL-9A automatic injector. The separation column, Hypersil® PAH 5 μ was 4.6 mm inside diameter × 100 mm (Franklin, MA). The mobile phase was acetonitrile:water (70 : 30, v : v), flow rate 1 ml min^{-1}. Fluorescence emission was detected at 330 nm using a Shimadzu RF-30 fluorescence detector with an excitation wavelength of 280 nm. FL was identified by comparison with pure standard (retention time: 3.6 min) used as both external and internal standards. Each sample was injected at least 3 times. Quantification used a RC-6A integrator. The percentage of FL depletion was given by the formula: $[(n_I - n_R)/n_I] \times 100$, in which n_I was the initial number of extracted FL moles and n_R, the number of remaining extracted FL moles; the mean (m) and the standard deviation (SD) were calculated and presented according to the formula $m \pm$ SD.

Soil slurry system

An uncontaminated agricultural top soil (texture: 10% sand, 58% silt, 32% clay; organic matter: 2.8%; pH: 7.4) was spiked with 100 μg g^{-1} (of soil) FL. FL was previously dissolved in acetone and the solution was spread over the soil and mixed during a total time of 2 h. When all the solvent had evaporated the soil fractions were stored cold (+4 °C).

The quantification of bacteria and fungi in soil was carried out by plate counts on different media. To extract the microorganisms, 10 g soil sample was added to 90 ml sterile water with 0.05% (w/v) SDS and agitated vigorously for 1 h. The suspension was filtered (paper filter, pore size 7.4 μm) and used for enumeration. In order to estimate the number of microorganisms overall, several dilutions of the filtrate were plated (10 plates for dilution) on bacterial medium (Nutrient Broth Agar) and fungal medium (MEA with 0.05% chloramphenicol). Colonies were counted after 3 d of incubation at 30 °C for bacteria and 22 °C

for fungi. The plate counts of experimental soil determined 21.10^6 bacteria and 3.10^5 fungi CFU (colony forming units)/g (dry wt) of soil.

Soil slurries were prepared in Erlenmeyer flasks (100 ml) containing 5 g (dry wt) of spiked soil and 25 ml of GS liquid synthetic medium. A solid phase extraction (SPE) method was developed to extract and purify FL from soil samples. After centrifugation of soil slurry (10 min, 3000 rpm), FL was extracted from aqueous and solid phases by shaking using 20 ml of acetone. The vials were sealed and shaken on a rotary shaker at 240 rpm during 30 min. Then the extracts were centrifuged for 10 min at 3000 rpm and extraction solution was saved. The Bakerbond spe octyl column (Mallinckrodt Baker, BV, Deventer, Holland) was conditioned with 2 ml of methanol followed by 2 ml of water:2-propanol (9 : 1, v/v). The extraction solution was pumped (flow 2 ml min^{-1}) through the column, which was washed with 2 ml of methanol:water (1 : 1, v/v). The samples were eluted by applying 2 ml of THF and pooled before HPLC analysis. 80% of FL was recovered after this procedure. FL was analyzed by HPLC as previously described in biodegradation tests (Garon et al. 2000).

Three categories of assays were practiced in soil slurry system:

(I) Assay without bioaugmentation in order to observe the effect of indigenous microflora on FL biodegradation.

(II) Assay with fungal bioaugmentation: addition of selected strain, *Absidia cylindrospora*. For inoculum preparation, surface mat mycelia of *Absidia cylindrospora* was filtered, washed, suspended in 5 ml GS liquid medium and fragmented in a laboratory blender. Two inocula were tested: a standard inoculum of 500 μl of fungal suspension (corresponding to 0.5 g of fresh weight or 0.05 g of dry weight) and a 8-fold more concentrated inoculum.

(III) Bioavailability assay with addition of selected cyclodextrin: maltosyl-cyclodextrin (MCD).

Assays were made in a 5-fold replication and incubated in the dark at 23 °C on a rotary shaker (150 rpm). The depletion of FL was evaluated at the times 0, 24, 48, 72, 144, 288 and 576 h. Cell free flasks (subjected to chemical sterilization) were practiced for stability assessment.

Results and discussion

Efficiency of fungal strains isolated from contaminated soil

Table 1 presents the capacity of fungal strains to degrade FL, expressed as the percentage of FL depletion. Strains were qualified as "efficient" when they depleted it at least at 60% or more, in order to select strains for soil slurry applications. Efficient strains isolated from contaminated soil were compared to reference strains belonging to the laboratory collection. Thus, Figure 1 compares the rates of biodegradation of strains isolated from contaminated soil vs reference strains of the same species, originating from uncontaminated environments (soil or food without PAH contamination). Out of 22 strains, 18, isolated from PAH contaminated soil, had higher degradation efficiency than the reference strains, while the 4 remaining had an equivalent efficiency. These results can be attributed to the adaptation of the microflora to a polluted environment. The 4 other strains are Zygomycetes, a well-known efficient taxonomic group in PAH degradation (Krivobok et al. 1998; Salicis et al. 1999; Garon et al. 2000). Microorganisms contained in contaminated soils are usually found to metabolize PAH at higher rates than those from similar uncontaminated environments (Thomas et al. 1989; Madsen et al. 1992). This phenomenon was observed in bioreactor with anthracene (Richnow et al. 1999). In other studies (Carmichael & Pfaender 1997; Solano-Serena et al. 2000) the greatest potential of degradation of microorganisms originating from PAH-contaminated soil was reported.

Absidia cylindrospora, the most efficient strain was selected for practice in soil slurry. This strain degraded 98% of FL in liquid medium after a 48 h incubation period. *Absidia cylindrospora* belongs to the Zygomycetes group and had several advantages:

– tolerance and degradation of high concentrations of FL and other PAH.
– fast growth on solid media, in liquid media and in soil. Since bioremediation is generally attempted in unsterile soil, it is important to determine whether introduced fungi will survive and remain active in the presence of indigenous soil microflora (Cerniglia & Sutherland 2001).
– widespread in the environment, particularly in soil.

No commercial applications of fungi for the bioremediation of individual PAH or mixtures of PAH have been established, although there appears to be poten-

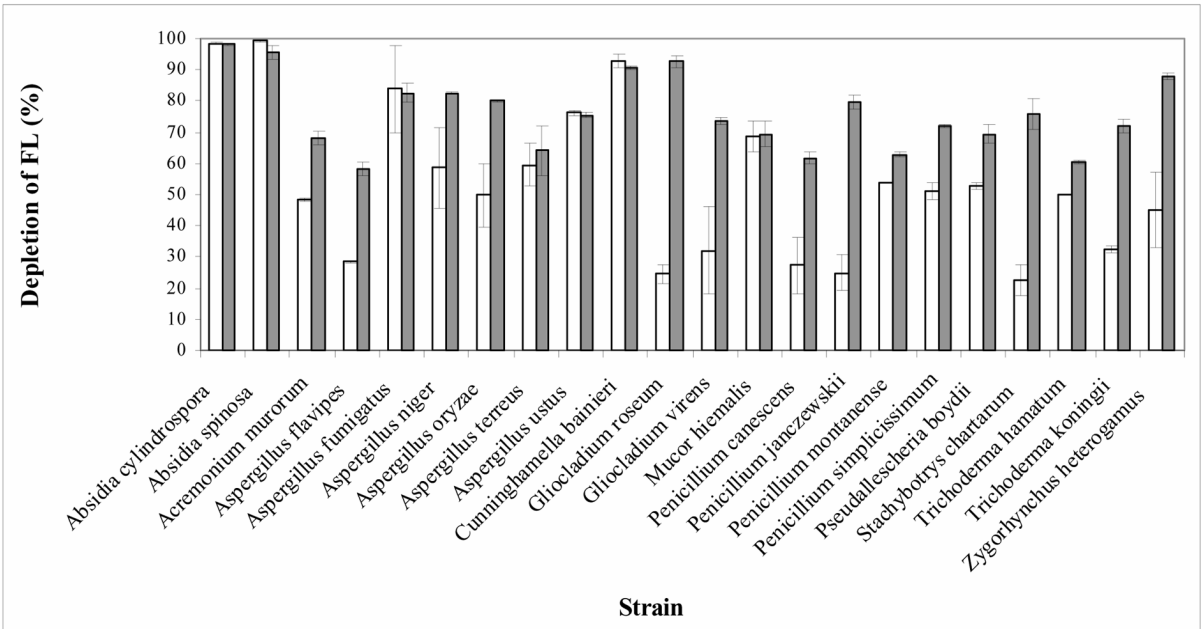

Figure 1. Comparison of FL degradation (0.005 g l^{-1}) by fungal strains isolated from a contaminated soil and reference strains isolated from uncontaminated environments: ■, strain from contaminated soil; □, reference strain.

tial for the use of *Cunninghamella elegans* (Cutright 1995), white rot fungi (Andersson 2000) and in this study *Absidia cylindrospora*.

Bioremediation assays in soil slurry

Native microflora of soil slurry was able to degrade 90% of the FL which could be associated with the presence of overgrowing Zygomycetes observed during the enumeration by CFU counts. Wischmann (1996) followed PAH degradation in soil/compost mixture: 0.1 g kg^{-1} of PAH was degraded in 40 d. Other work (Findlay et al. 1995) showed that 143 ppm of 3-ring PAH were degraded by bacteria after a 26 d period incubation.

Figure 2 showed the efficiency of fungal bioaugmentation with *Absidia cylindrospora* (principally with the most concentrated inoculum) after 288 h on FL degradation. More than 90% of the FL was degraded at time 288 h in the presence of *Absidia cylindrospora*. In contrast 90% of the FL was degraded at time 576 h in the absence of bioaugmentation. Brodkorb & Legge (1992) demonstrated in their study that the addition of *Phanerochaete chrysosporium* was synergistic with the enhancement of phenanthrene mineralization. The effect of inoculum concentration on FL degradation was observed with the addition of *Absidia cylindrospora*. Other studies showed the importance of inoculum density (Thomas et al. 1989) and also inoculum formulation in bioremediation strategy (Lestan & Lamar 1996). Our results indicate that FL degradation can be enhanced by *Absidia cylindrospora*, a reintroduced fungi isolated from PAH-contaminated soil.

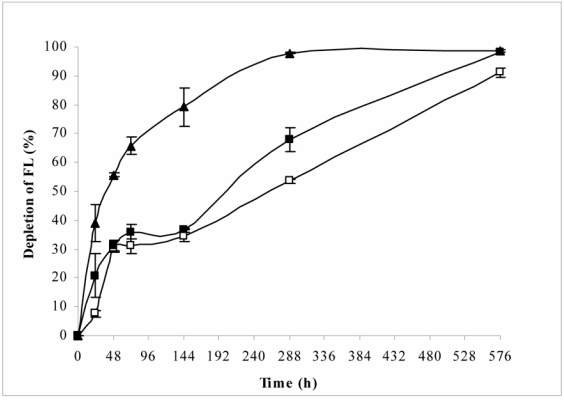

Figure 2. Effect of fungal bioaugmentation with *Absidia cylindrospora* in soil slurry (100 μg g^{-1} FL): ▲, bioaugmentation with *Absidia cylindrospora* (concentrated inoculum); ■, bioaugmentation with *Absidia cylindrospora* (standard inoculum); □, autochtonous microflore (without bioaugmentation).

Table 2. Depletion of FL (0.005 g l^{-1}) in presence of CD derivatives

Strain	Tested CD	Depletion of FL (%)[a]	Increase of biomass (factor)[b]
Penicillium italicum	Without CD	28 ± 6	1.0
	β-CD	11 ± 1	1.3
	HPβCD	46 ± 1	1.0
	MCD	47 ± 1	1.0
	PMβCD	46 ± 1	1.0
	SEβCD	41 ± 8	1.0
Phanerochaete chrysosporium	Without CD	41 ± 5	1.0
	α-CD	18 ± 1	1.4
	β-CD	19 ± 6	1.4
	HPβCD	50 ± 3	1.0
	MCD	57 ± 7	1.0
	PMβCD	39 ± 8	1.0
	SEβCD	44 ± 9	1.0

[a]The results are presented according to the formula $m \pm$ SD in which m is the mean and SD the standard deviation.
[b]Factor calculated with the formula dw_{CD}/dw in which dw_{CD} is the mycelial dry weight (g) of strain cultivated in the presence of CD and dw, the mycelial dry weight (g) of strain cultivated without of CD.

CD derivatives as MCD were less hydrolyzed by fungal amylases than natural CD (Garon et al. 2001). 2 fungal strains, *Penicillium italicum* and *Phanerochaete chrysosporium* previously observed for an inhibition of FL degradation in the presence of natural CD (Garon et al. 2001) were tested in liquid medium with 4 CD derivatives: HPβCD, MCD, PMβCD, and SEβCD. In the presence of CD derivatives, no inhibition of FL depletion and no increase of fungal biomass were observed (Table 2). MCD, the most efficient CD derivative was selected for biodegradation experiments in soil slurries.

Figure 3 presented the positive effect of MCD addition on FL degradation. The results of coupling fungal bioaugmentation approach (*Absidia cylindrospora*) and bioavailability improvement with MCD showed an enhancement of FL biodegradation during the first 144 h. This enhancement had previously been observed with surfactant (Garon et al. 2002), β-CD and hydroxypropyl-β-cyclodextrin (Schwartz & Bar 1995; Wang et al. 1998). In the presence of hydroxypropyl-β-cyclodextrin, only 0.3% of the phenanthrene remained at the end of a 48 h incubation. In contrast, 45% of the phenanthrene remained in the absence of CD (Wang et al. 1998). Other CD were efficient on various pollutants: γ-cyclodextrin (Fava et al. 1998) and methylated-β-cyclodextrin (Fava & Ciccotosto 2002) were found capable to enhancing the bioavailability and the aerobic degradation of polychlorobiphenyls.

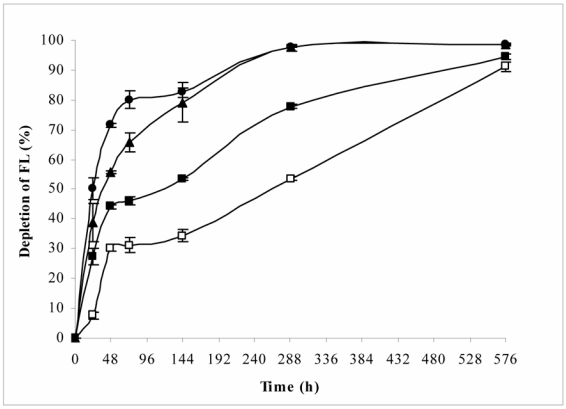

Figure 3. Effect of MCD addition in soil slurry (100 μg g^{-1} FL): □, autochtonous microflora (without bioaugmentation); ■, autochtonous microflore + MCD; ▲, bioaugmentation with *Absidia cylindrospora* (concentrated inoculum); ●, *Absidia cylindrospora* (concentrated inoculum) + MCD.

The use of MCD had also a positive effect on FL degradation by native microflora: 80% of the FL was degraded after 288 h, whereas 480 h were necessary in the absence of MCD.

Conclusion

Our results indicate that FL was degraded by native microflora. *Absidia cylindrospora*, a reintroduced fungi isolated from PAH-contaminated soil enhanced this biodegradation during a bioaugmentation process. The effectiveness of bioremediation in many systems may be constrained by low contaminant bioavailability due to low water solubility and dissolution rates of these chemicals. CD such as α-cyclodextrin and β-cyclodextrin were well tolerated and able to enhance FL solubility (Garon et al. 2001) and other PAH solubility (Wang et al. 1995). The low water solubility may limit its bioavailability and consequently its biodegradation. Thus an objective of this research was to evaluate the effect of MCD in association with fungal strains. It was hypothesized that CD could increase the apparent solubility of FL by forming inclusion complexes, thereby enhancing the bioavailability of FL and its fungal degradation. MCD, a branched cyclodextrin was found capable of enhancing the bioavailability and the microbial degradation of FL in a soil slurry system.

References

Andersson BE, Welinder L, Olsson PA, Olsson S & Henrysson T (2000) Growth of inoculated white-rot fungi and their interactions with the bacterial community in soil contaminated with the polycyclic aromatic hydrocarbons, as measured by phospholipid fatty acids. Bioresource. Technol. 73: 29–36

April TM, Foght JM & Currah RS (2000) Hydrocarbon-degrading filamentous fungi isolated from flare pit soils in northern and western Canada. Can. J. Microbiol. 46: 38-49

Atlas RM & Cerniglia CE (1995) Bioremediation of petroleum pollutants. Bioscience 45: 332–339

Aust SD (1995) Mechanisms of degradation by white rot fungi. Environ. Health. Perspect. 103: 59–61

Baldrin B, Tiehm A & Fritzsche C (1993) Degradation of phenanthrene, fluorene, fluoranthene and pyrene by a *Mycobacterium* sp. Appl. Environ. Microbiol. 59: 1927–1930

Brodkorb TS & Legge RL (1992) Enhanced biodegradation of phenanthrene in oil tar-contaminated soils supplemented with *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 58: 3117–3121

Carmichael LM & Pfaender FK (1997) Polynuclear aromatic hydrocarbon metabolism in soils: relationship to soil characteristics and preexposure. Environ. Toxicol. Chem. 16: 666–675

Cerniglia CE & Sutherland JB (2001) Bioremediation of polycylic aromatic hydrocarbons by ligninolytic and non-ligninolyic fungi. In: Gadd GM (Ed) Fungi in Bioremediation (pp 136–187). British Mycological Society, Cambridge University Press

Cerniglia CE (1992) Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3: 351–368.

Cha DK, Chiu PL, Kim SD & Chang JS (1999) Treatment technologies. Wat. Environ. Res. 71: 870–885

Cutright TJ (1995) Polycyclic aromatic hydrocarbon biodegradation and kinetics using *Cunninghamella echinulata* var. *elegans*. Inter. Biodeterior. Biodegrad. 397–408.

Domsch KH, Gams W & Anderson T-H (1980) Compendium of Soil Fungi. Academic Press, London

Fava F & Ciccotosto VF (2002) Effects of a randomly methylated-beta-cyclodextrins (RAMED) on bioavailability and aerobic biodegradation of polychlorinated three pristine soils spiked with a transformer oil. Appl. Microbiol. Biotechnol. 58: 393–399

Fava F, Di Gioia D & Marchetti L (1998) Cyclodextrin effects on the ex-situ bioremediation of a chronically polychlorobiphenyl-contaminated soil. Biotechnol Bioeng. 58: 345–355

Findlay M, Fogel S, Conway L & Taddeo A (1995) Field treatment of coal tar-contaminated soil based on results of laboratory treatability studies. In: Young LY & Cerniglia CE (Eds) Microbial Transformation and Degradation of Toxic Organic Chemicals (pp 487–513). Wiley-Liss, New York

Galzy P & Slonimski p (1957) Variations physiologiques de la levure au cours de la croissance sur l'acide lactique comme seule source de carbone. C. R. Acad. Sci. Paris. 245 sér. D: 2423–2426

Garon D, Fauvelle F, Gèze A, Wouessidjewe D & Seigle-Murandi F (2001) Effect of cyclodextrins on fungal degradation of fluorene. Ann. Pharm. 59: 366–368

Garon D, Krivobok S & Seigle-Murandi F (2000) Fungal degradation of fluorene. Chemosphere 40: 91–97. Cited in Cerniglia CE & Sutherland JB (2001) Bioremediation of polycylic aromatic hydrocarbons by ligninolytic and non-ligninolyic fungi. In: Gadd GM (Ed) Fungi in Bioremediation (pp 136–187). British Mycological Society, Cambridge University Press

Garon D, Krivobok S, Wouessidjewe D & Seigle-Murandi F (2002) Influence of surfactants on solubilization and fungal degradation of fluorene. Chemosphere 47: 303–309

Keith LH & Telliard WA (1979) Priority pollutants-IA perspective view. Environ. Sci. Technol. 13: 16–23

Krivobok S, Miriouchkine E, Seigle-Murandi F & Benoit-Guyod J-L (1998) Biodegradation of anthracene by soil fungi. Chemosphere 37: 523–530

Lestan D & Lamar RT (1996) Development of fungal inocula for bioaugmentation of contaminated soils. Appl. Environ. Microbiol. 62: 2045–2052

Madsen EL, Winding A, Malachrowsky K, Thomas CT & Ghiorse WC (1992) Contrasts between subsurface microbial communities and their metabolic adaptation to polycyclic aromatic hydrocarbons at a forested and an urban coal-tar disposal site. Microbial. Ecol. 24: 199–213

Martelmans K, Waworth S, Lawlor T, Speck W, Tainer B & Zeiger E (1986) *Salmonella* mutagenicity tests. II. Results from the testing of 270 chemicals. Environ. Mutagen. 8: 1–119

Mihelcic JR, Luehing DR, Mitzell RJ & Stapleton JM (1993) Bioavailability of sorbed-and separate-phase chemicals. Biodegradation 4: 141–153

Pothuluri JV, Freeman JP, Evans FE & Cerniglia CE (1993) Biotransformation of fluorene by the fungus *Cunninghamella elegans*. Appl. Environ. Microbiol. 59: 1977–1980

Richnow HH, Eschenbach A, Mahro R, Kaestner K, Annweiler F, Seifert R & Michaelis W (1999) Formation of nonextractable soil residues: a stable isotope approach. Environ. Sci. Technol. 33: 3761–3767

Sack U & Günther T (1993) Metabolism of PAH by fungi and correlation with extracellular enzymatic activities. J. Basic. Microbiol. 33: 269–277

Salicis F, Krivobok S, Jack M & Benoit-Guyod J-L (1999) Biodegradation of fluoranthene by soil fungi. Chemosphere 38: 3031–3039

Schwartz A & Bar R (1995) Cyclodextrin-enhanced degradation of toluene and p-toluic acid by *Pseudomonas putida*. Appl. Environ. Microbiol. 61: 2727–2731

Solano-Serena F, Marchal R, Lebeault J-M & Vandecasteele J-P (2000) Distribution in the environment of degradative capacities for gasoline attenuation. Biodegradation 11: 29–35

Straube WL, Jones-Mechan J, Pritchard P-H & Jones WR (1999) Bench scale optimization of bioaugmentation strategies for treatment of soils contaminated with high molecular weight polyaromatic hydrocarbons. Res. Conserv. Recycle. 27: 27–37

Szejtli J (1988) Cyclodextrin Technology. Kluwer Academic Publishers, Dordrecht

Thomas JM, Lee MD, Scott MJ & Ward CH (1989) Microbial ecology of the subsurface at an abandoned creosote waste site. J. Ind. Microbiol. 4: 109–120

Wang JM, Brusseau ML & Miller RM (1995) Biodegradation of nonionic organic compounds complexed with cyclodextrins. 209th ACS National Meeting, American Chemical Society, Anaheim, CA, 2–6 April

Wang JM, Marlowe EM, Miller-Maier RM & Brusseau ML (1998) Cyclodextrin-enhanced biodegradation of phenanthrene. Environ. Sci. Technol. 32: 1907–1912

Wang X & Brusseau ML (1995) Cyclopentanol-enhanced solubilization of polycyclic aromatic hydrocarbons by cyclodextrins. Environ. Sci. Technol. 29: 2346–2351

Warcup JH (1950) The soil-plate method for isolation of fungi from soil. Nature 166: 117–118

Wischmann H, Steinhart H, Hupe K, Montresori G & Stegmann R (1996) Degradation of selected PAHs in soil/compost and identification of intermediates. Int. J. Environ. Anal. Chem. 64: 247–255