

**Exploration of telluric fungi biodiversity  
for developing soft remediation technologies  
of hydrocarbons polluted soils**

**Catherine RAFIN**

**Etienne VEIGNIE**

**Université du Littoral Côte d'Opale**

**Laboratoire de Synthèse Organique et Environnement (EA 2599)**

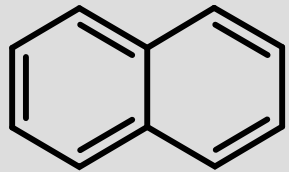
**Dunkerque - France**



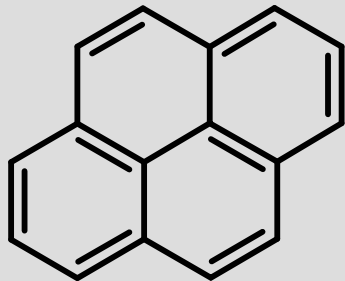
# Introduction

## Polycyclic Aromatic Hydrocarbons

Low molecular weight



**Naphthalene  
(NA)**



**Pyrene  
(PYR)**

+

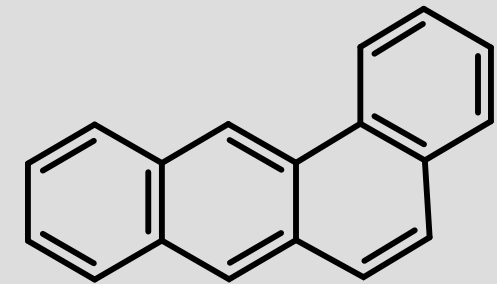
Biodegradation

↓

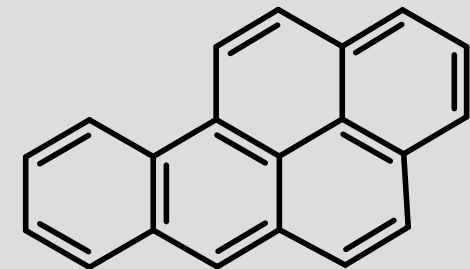
+

PAH	Molecular Weight g/mol	Solubility µg/l	Toxicity
NA	128,2	31700	-
PYR	202,3	135	+
BA	228,3	2,0	+
BAP	252,3	3,8	++

High molecular weight



**Benzo(a)anthracene  
(BA)**

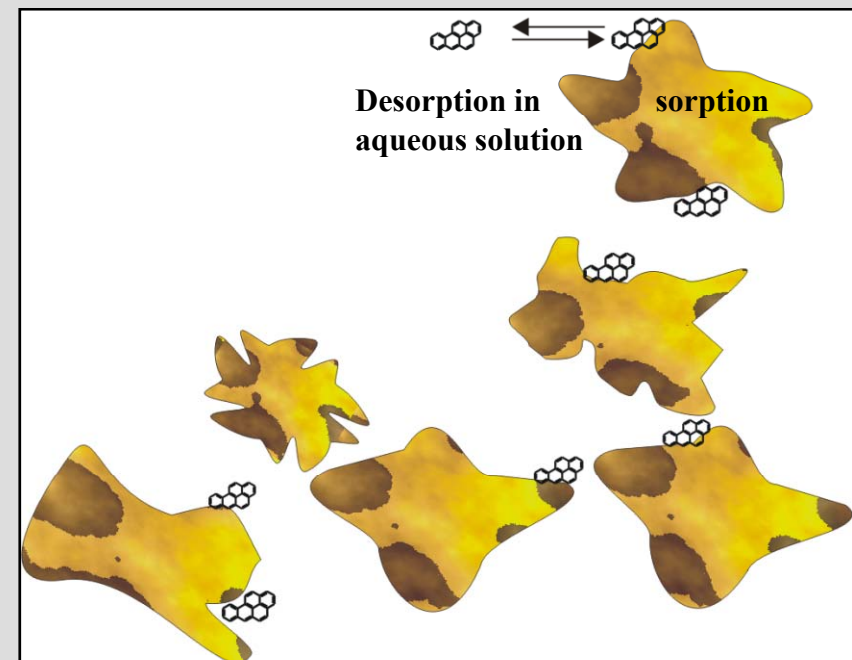


**Benzo(a)pyrene  
(BaP)**

# Introduction

## Remediation technologies of PAHs contaminated soils

- ✓ **Physical:**  
e.g. excavation, soil washing, Pump-and-treat
- ✓ **Chemical:**  
e.g. chemical extraction, chemical oxidation
- ✓ **Thermal:**  
e.g. incineration, thermal desorption
- ✓ **Biological treatments:**  
e.g. bioremediation, phytoremediation

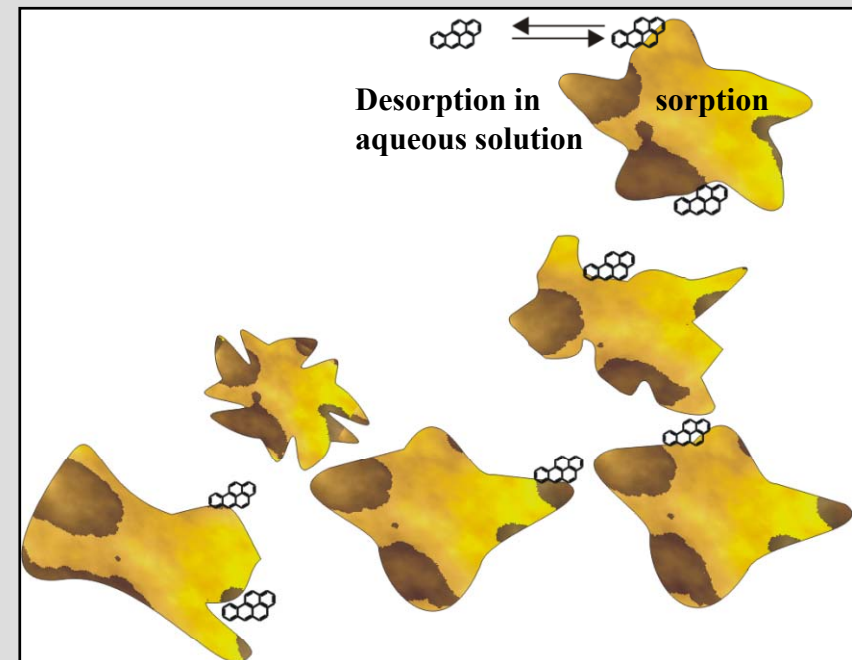


# Introduction

## Bioavailability of Organic Pollutants in soil

**Bioavailability is a complex and dynamic variable influenced by :**

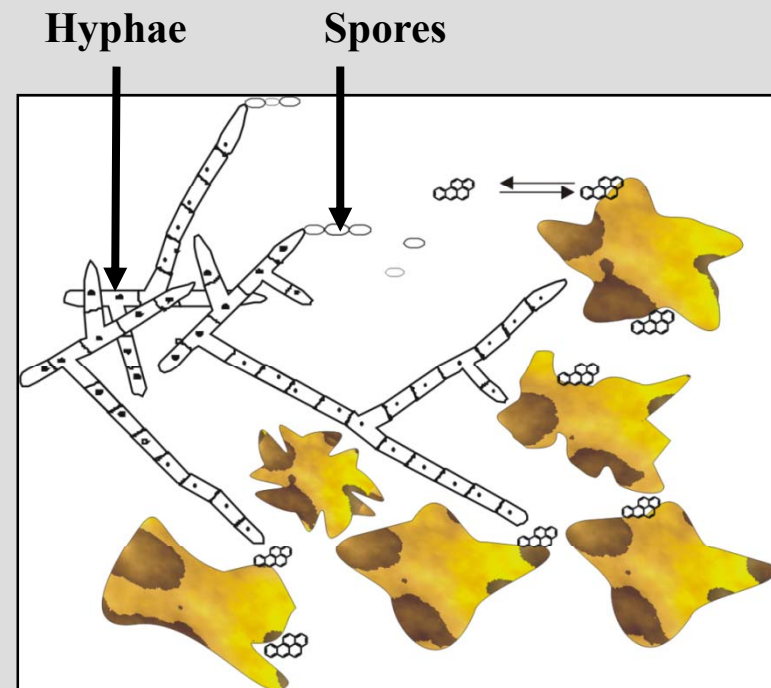
- ✓ The interactions and physical properties of the contaminant,
- ✓ The soil matrix,
- ✓ The microorganisms present within a given environment.



# Introduction

## Some important fungal characteristics

- ✓ Eucaryotes
- ✓ Ubiquitous in terrestrial ecosystems
- ✓ Filamentous branching growth habit
- ✓ Chemoheterotrophic metabolism
- ✓ Reproduction: sexual and asexual (spores)
- ✓ Diversity of fungal communities: free-living, symbiotic, pathogens
- ✓ Interactions with other partners (bacteria, plants, fauna...)

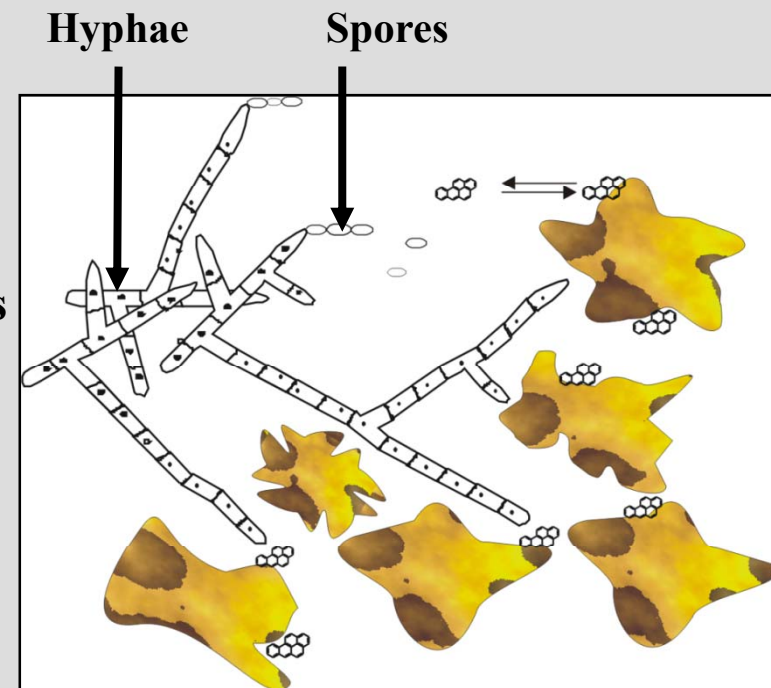


# Introduction

## Exploration of telluric fungi biodiversity for cleaning up contaminated soils

### Interest for soil remediation:

- ✓ Explorative or exploitative growth strategies
- ✓ Colonization of heterogeneous environments (soil)
- ✓ Adaptive capabilities to disturbed environments
- ✓ Xenobiotic degradation



**In aerobic environments, fungi are of great importance.**

**Nevertheless, they are frequently neglected.**

# I. Research objectives : Bioremediation

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- **Isolation of saprophytic telluric fungi from PAHs polluted soils**
- **Evaluation of the fungi degradative ability on PAHs**
- **Elucidation of the biochemical mechanisms involved in BaP degradation**

# I. Bioremediation

## Isolation of saprophytic telluric fungi from polluted soils

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DIVISION	GENRE
Zygomycotina	<i>Mucor</i>
Ascomycotina	<i>Penicillium</i>
Deuteromycotina	<i>Trichoderma</i> <i>Fusarium</i> <i>Coniothyrium</i> <i>Cladosporium</i>

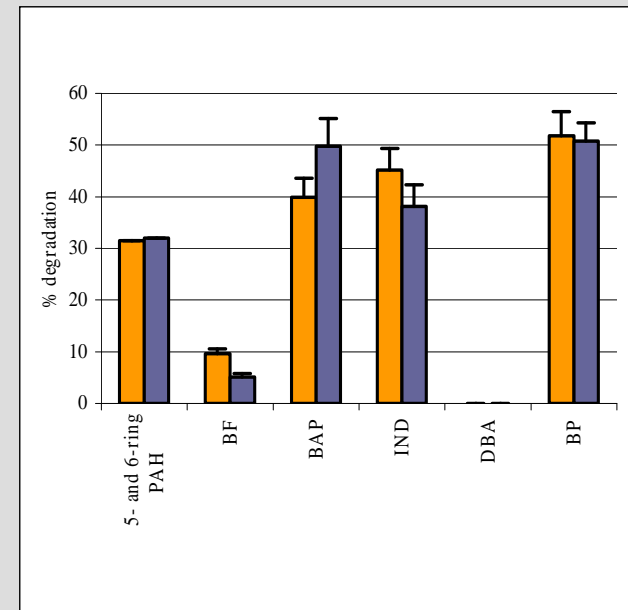
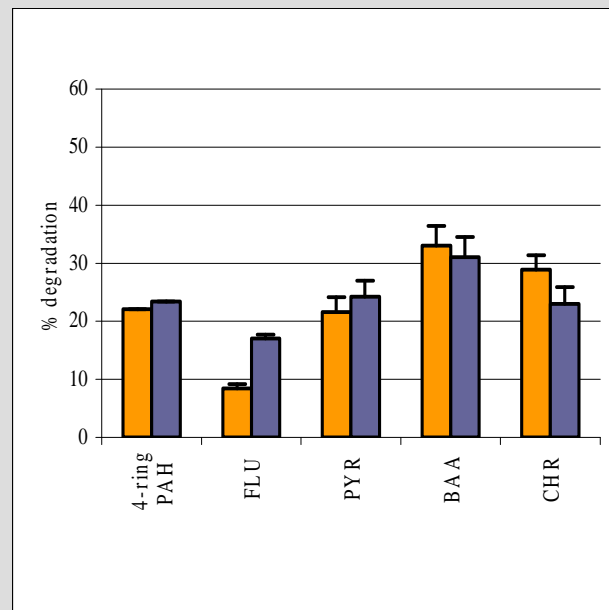
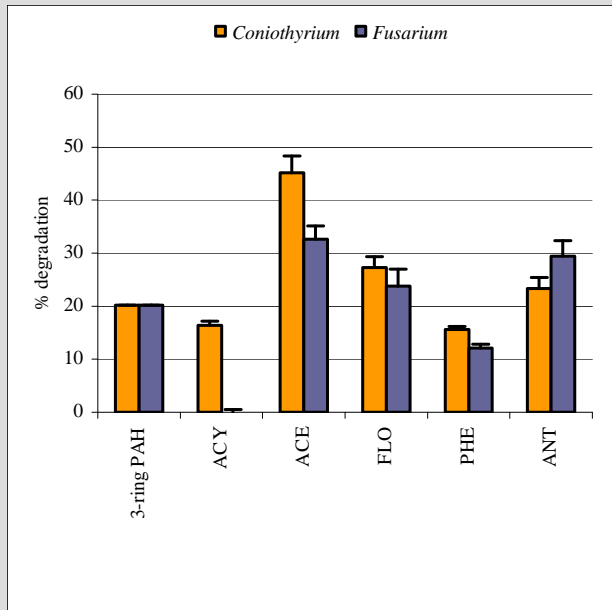
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# I. Bioremediation

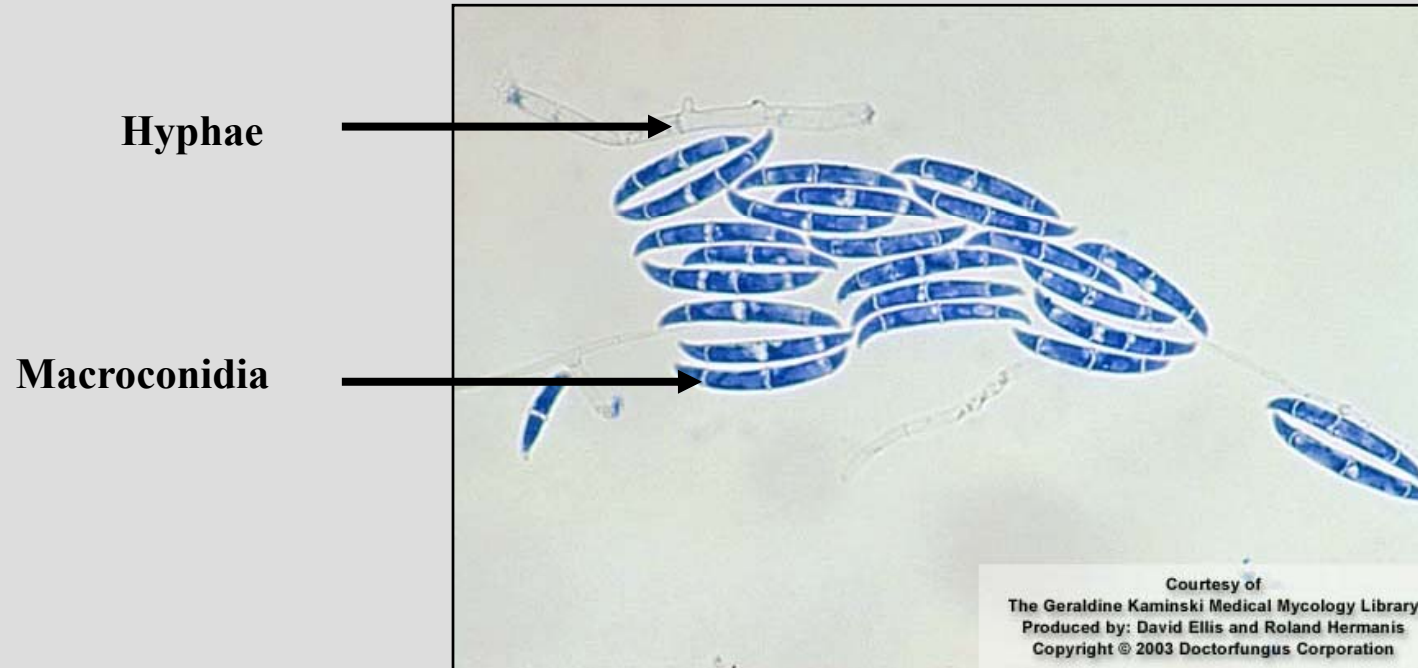
## Evaluation of PAHs degradative ability of two strains



- ✓ Study conducted in aged polluted soils
- ✓ 30 days of incubation
- ✓ Inoculation: with mycelium of *Coniothyrium* (■) and *Fusarium* (■)

# I. Bioremediation

## Microscopic morphology of a Deuteromycete fungus *Fusarium solani*



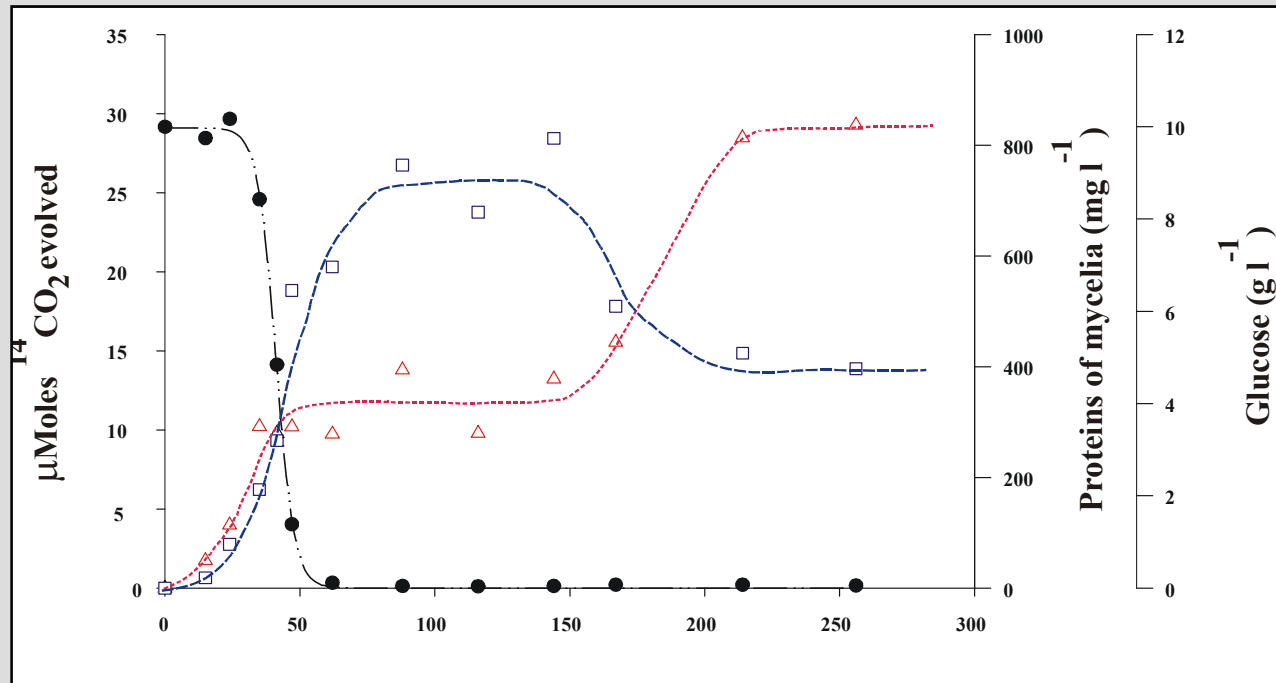
**Microconidia:** usually abundant, cylindrical to oval, 1- to 2-celled, formed from long lateral phialides.

**Macroconidia:** formed after 4-7 days from short multi-branched conidiophores, 3- to 5-septate (usually 3- septate), fusiform, cylindrical, often moderately curved.

**Chlamydoconidia:** hyaline, globose, smooth to rough walled.

# I. Bioremediation

## Mineralization of BaP by *Fusarium solani*



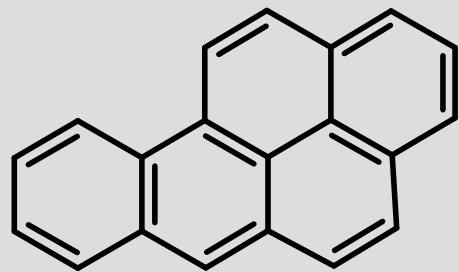
Kinetic of growth of *F. solani* in a batch fermentor in Mineral Medium (MM) supplemented with [7,10- $^{14}\text{C}$ ]benzo[*a*]pyrene

$\triangle$   $^{14}\text{C}$   $\text{CO}_2$  evolved;  $\square$  mycelium proteins;  $\bullet$  concentration of glucose

# I. Bioremediation

## Biochemical mechanisms involved in BaP degradation

### RESEARCH STRATEGY



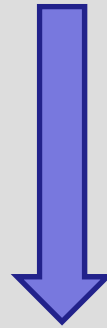
**Benzo(a)pyrene**



**Identification of  
Produced metabolites**



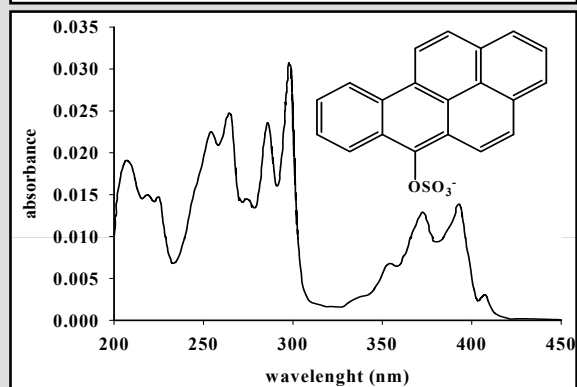
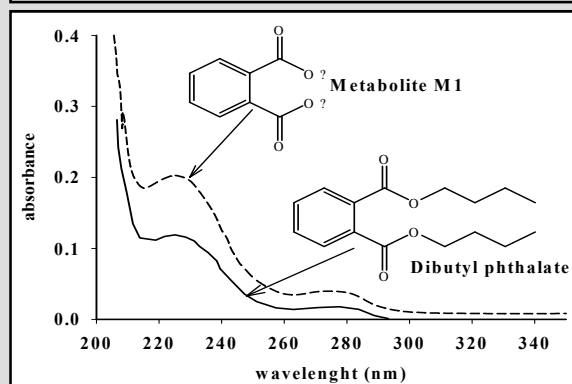
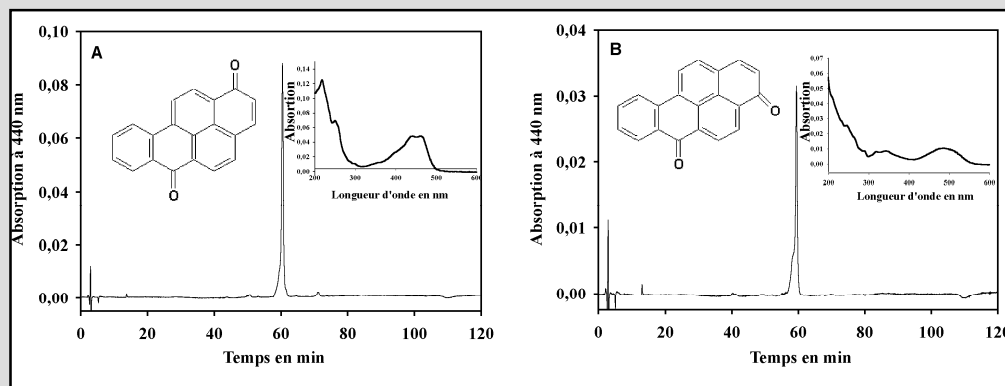
**CO<sub>2</sub>**



- ✓ **Cultures: liquid MM (batch fermentor, erlens)**
- ✓ **Characterization: HPLC, UV Spectra , MS**

# I. Bioremediation

## Identification of some produced metabolites



**A:** 1,6-benzo[a]pyrene quinone

**B:** 3,6-benzo[a]pyrene quinone

**M1:** UV spectrum similar with  
Dibutylphthalate

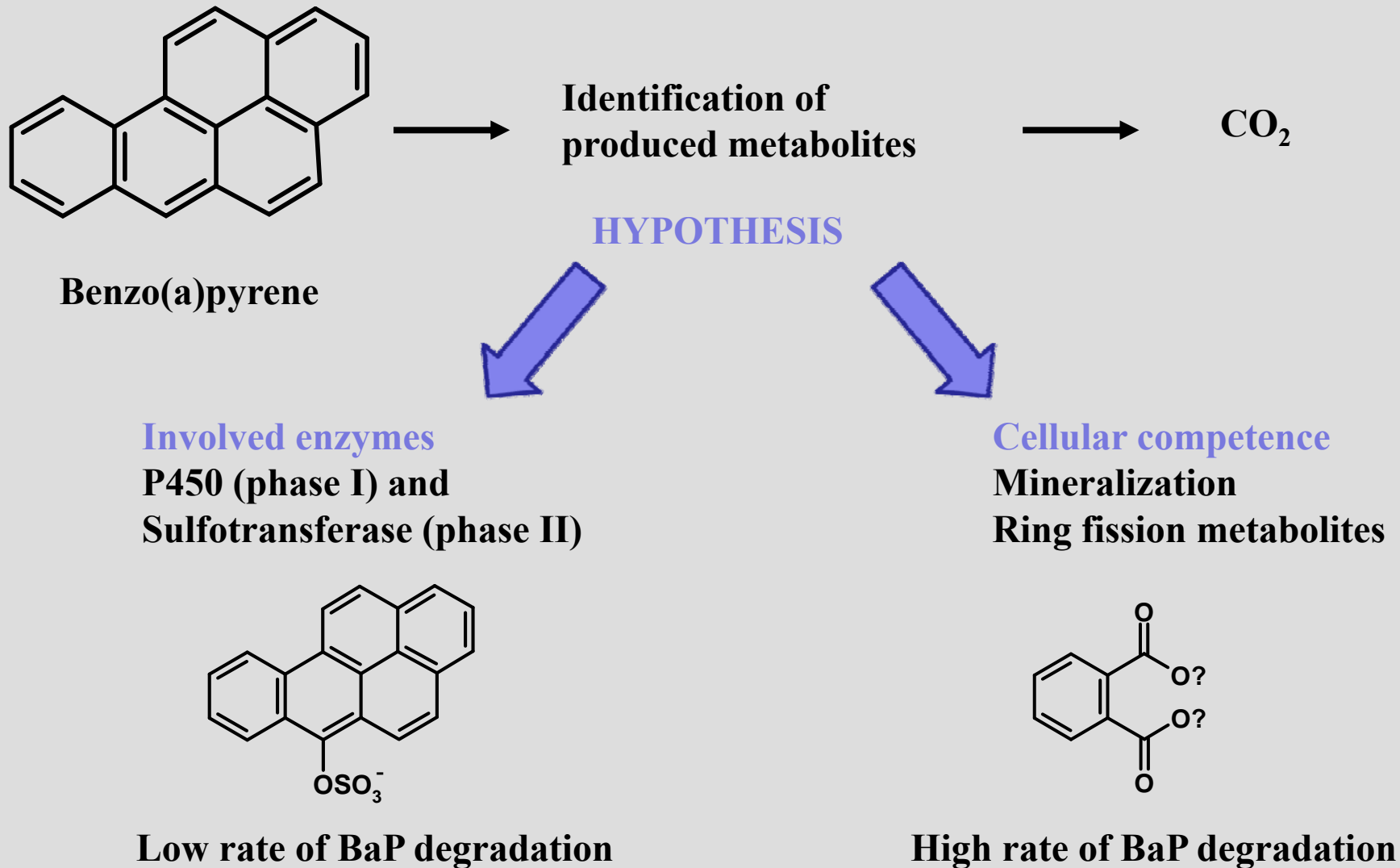
**M2:** 6-hydroxybenzo[a]pyrene sulfate

Veignie *et al.* 2002 Polycyclic Aromatic Compounds

Rafin *et al.* 2006 New Frontiers in Environmental Research

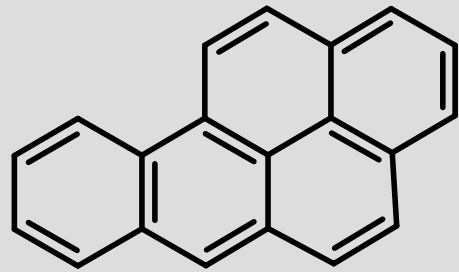
# I. Bioremediation

## Biochemical mechanisms involved in BaP degradation



# I. Bioremediation

## Biochemical mechanisms involved in BaP degradation



Benzo(a)pyrene



### ECOPHYSIOLOGICAL HYPOTHESIS

Exponential fungal growth

Detoxification via P450  
Classical protection of  
Eucaryotes against xenobiotics

Low rate of BaP degradation

Germination  
Fungal cell death (autolysis)

BaP ring fission  
Via ROS production occurring  
at oxydative burst

High rate of BaP degradation

# I. Bioremediation

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## Conclusion

- **Diversity of fungi isolated from polluted soil and able to degrade high molecular weight PAHs**
- **Fungi different from ligninolytic, wood-decaying fungi which are well known and extensively investigated (e.g. *Phanerochaete chrysosporium*)**
- **The use of ROS producing fungi, well adapted to contaminated soil, could be an alternative potential for soil bioremediation**



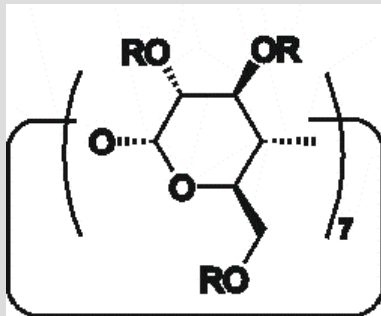
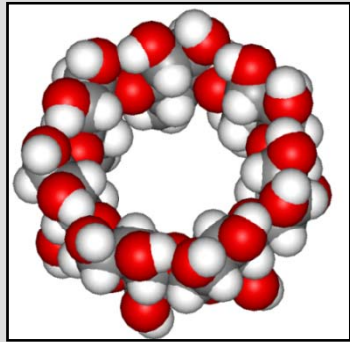
## II. Research objectives : Chemical and Biological oxidation

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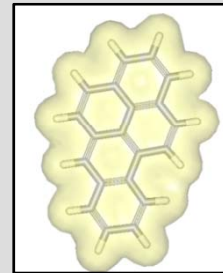
- **Use of cyclodextrins to enhance BaP bioavailability**
- **Fenton's reaction (generating ROS)**
- **Evaluation of the efficiency of Fenton's reaction on BaP degradation**
- **Interest of combining simultaneously chemical oxidation with biological treatment for BaP degradation**

## II. Chemical and Biological oxidation

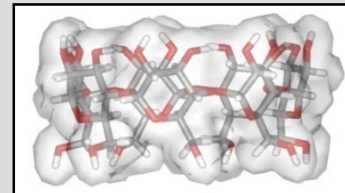
### Use of cyclodextrins to enhance BaP bioavailability



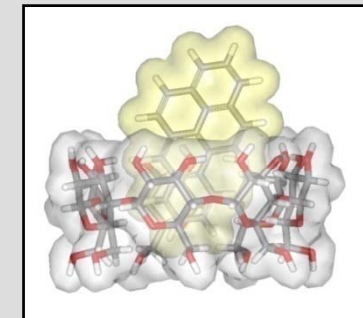
BaP



+



CD



CD/BaP

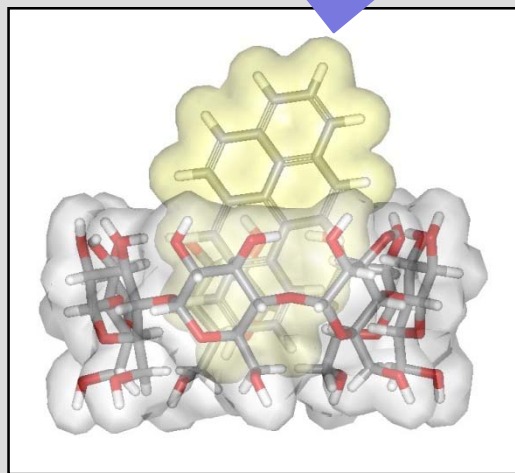
BCD: R=H

RAMEB: R=H or CH<sub>3</sub> (mean degree = 13)

HPBCD: R=H or CH<sub>2</sub>CHOHCH<sub>3</sub> (mean degree = 5)

## II. Chemical and Biological oxidation

### Fenton's reaction on BaP degradation



CD/BaP

Fenton reaction

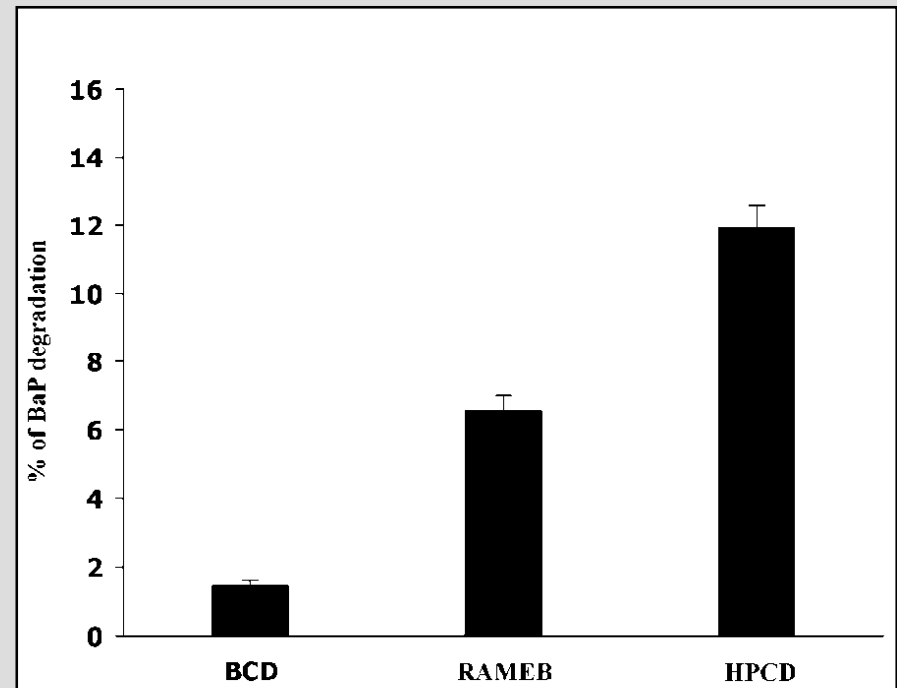
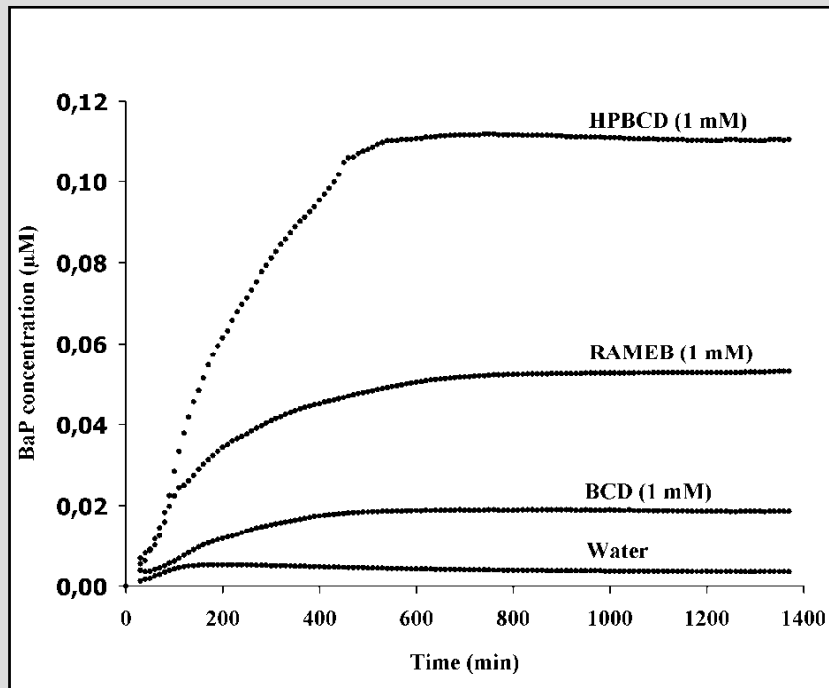


$$\text{H}_2\text{O}_2 / \text{Fe} = 5$$

$$[\text{H}_2\text{O}_2] = 10^{-2} \text{ M}$$

## II. Chemical and Biological oxidation

### Kinetics of BaP solubilisation      BaP Fenton degradation



Incubation overnight in Mineral Medium

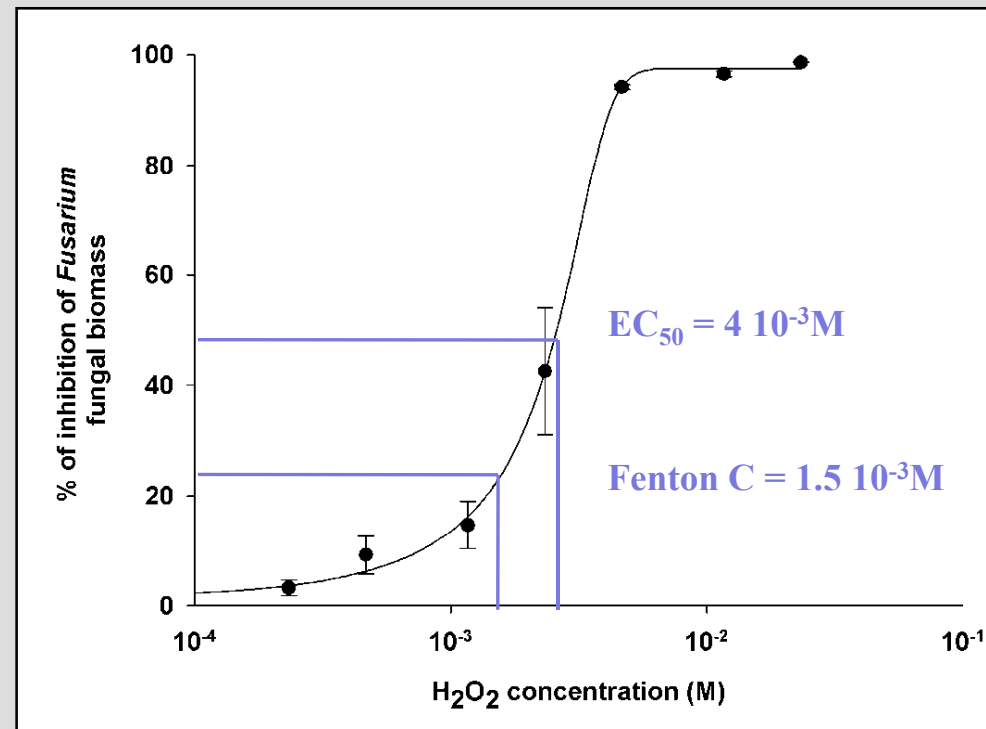
H<sub>2</sub>O<sub>2</sub>                    10<sup>-2</sup> M

FeSO<sub>4</sub>                    2.10<sup>-3</sup> M

Cyclodextrine        5.10<sup>-3</sup> M

## II. Chemical and Biological oxidation

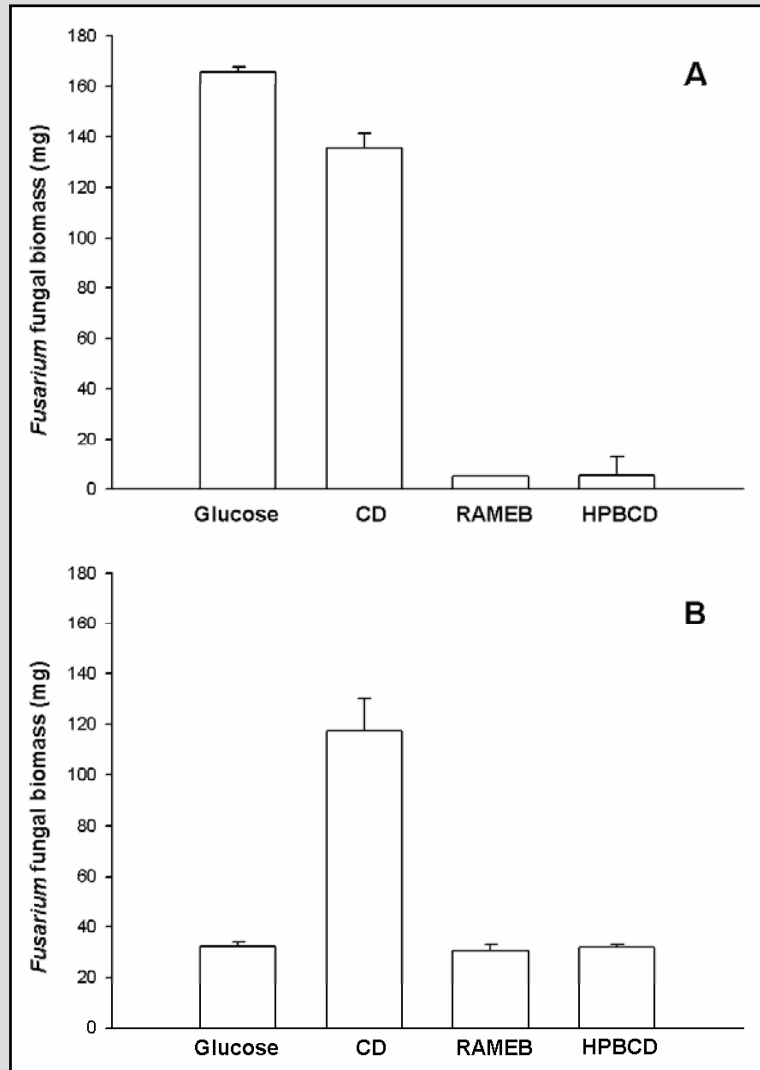
### Inhibition percentage of *Fusarium solani* biomass in presence of increased H<sub>2</sub>O<sub>2</sub> concentrations



5 days of incubation in Mineral Medium

## II. Chemical and Biological oxidation

### Biodegradation of cyclodextrins by *F. solani*

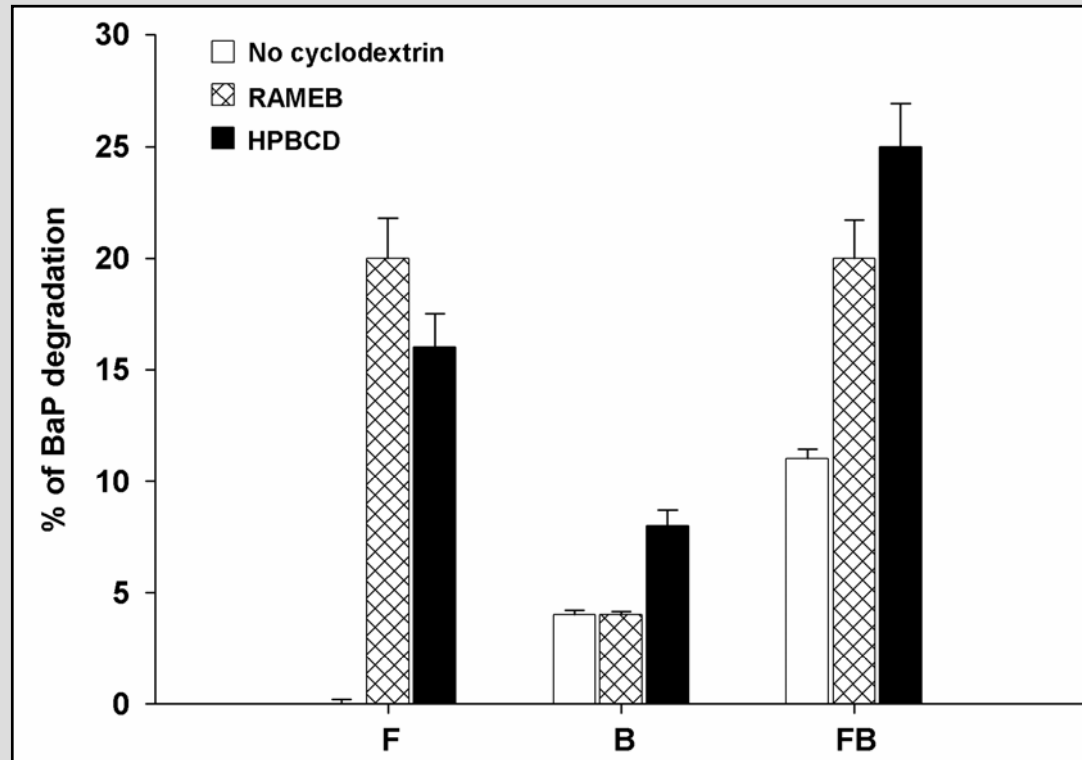


5 days of incubation in Mineral Medium

- A. In presence of glucose or cyclodextrin as sole carbon source (5.82 carbon eq g l<sup>-1</sup>)
- B. Glucose was added (1.42 carbon eq g l<sup>-1</sup>) in each cyclodextrin treatment to induce fungal growth

## II. Chemical and Biological oxidation

### Degradation of benzo[a]pyrene



12 days of incubation in Mineral Medium  
 $\text{H}_2\text{O}_2$   $1.5 \cdot 10^{-3}$  M,  $\text{FeSO}_4$   $5 \cdot 10^{-4}$  M, Cyclodextrine  $5 \cdot 10^{-3}$  M

**F:** Fenton's reaction, **B:** Biological degradation by *Fusarium solani*,  
**FB:** Combined Fenton and biological degradation

## II. Chemical and Biological oxidation

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### **Conclusion**

- **High efficiency of HPBCD to solubilise BaP**
- **Fenton degradation conducted at low H<sub>2</sub>O<sub>2</sub> concentrations compatible with fungal growth**
- **Degradation study conducted for 12 days**
- **Strategy based on two simultaneous complementary remediation approaches**



# References

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Veignie, E., Rafin, C., Landy, D., Fourmentin, S., Surpateanu, G., 2009. Fenton degradation assisted by cyclodextrins of a high molecular weight polycyclic aromatic hydrocarbon benzo[a]pyrene. *J. Haz. Mat.* doi:10.1016/j.jhazmat.2009.03.012.

## Keywords

Polycyclic Aromatic Hydrocarbons, benzo[a]pyrene, biodegradation, saprophytic telluric fungi, Reactive Oxygen Species

# Ecology and Biotechnology of Fungi (EBF Team)

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**C. RAFIN & E. VEIGNIE**

## **EXPLORATION OF FUNGAL BIODIVERSITY:**

- ✓ **Collection of fungal strains**
- ✓ **Non exhaustive bibliography**
- ✓ **Study of fungal metabolism involved in xenobiotic biodegradation (PAHs)**
- ✓ **Bioremediation of polluted soils**

**Possibility to transfer our knowledge and our know-how on:**

- ✓ **On other Persistent Organic Pollutants**
- ✓ **In other polluted environments (wastewaters, wastes, atmosphere)**

**ULCO, LSOE, Dunkerque**

**Tél bureau : 00 (0)3 28 65 82 78**

**rafin@univ-littoral.fr, veignie@univ-littoral.fr**