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

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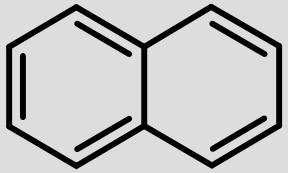
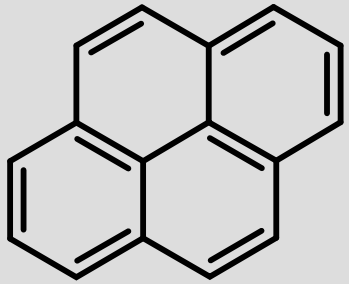
Introduction

Polycyclic Aromatic Hydrocarbons

Low molecular weight



Naphthalene (NA)



Pyrene (PYR)

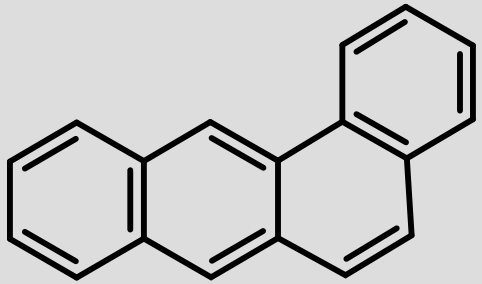
+

Biodegradation

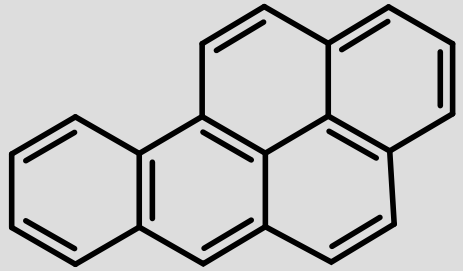
−

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)

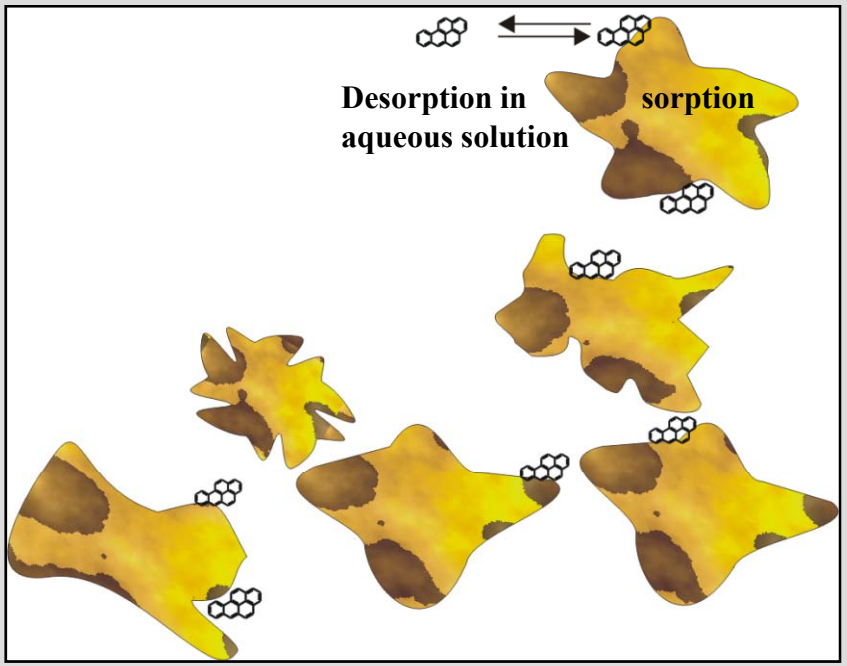
Remediation technologies of PAHs contaminated soils

✓ **Physical:**
e.g. excavation, soil washing, Pump-and-treat

✓**Chemical:**
e.g. chemical extraction, chemical oxidation

✓**Thermal:**
e.g. incineration, thermal desorption

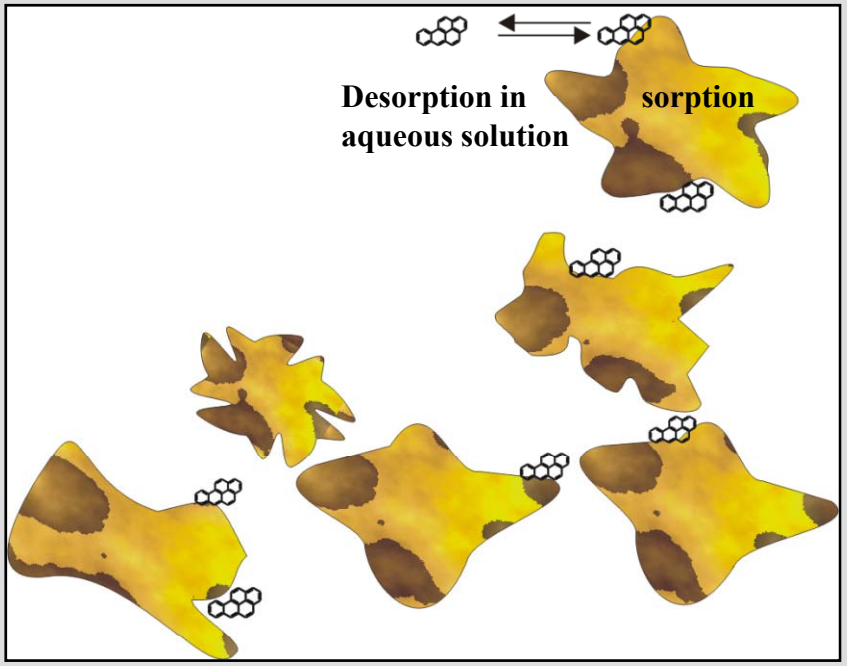
✓**Biological treatments:**
e.g. bioremediation, phytoremediation



Bioavailability of Organic Pollutants in soil

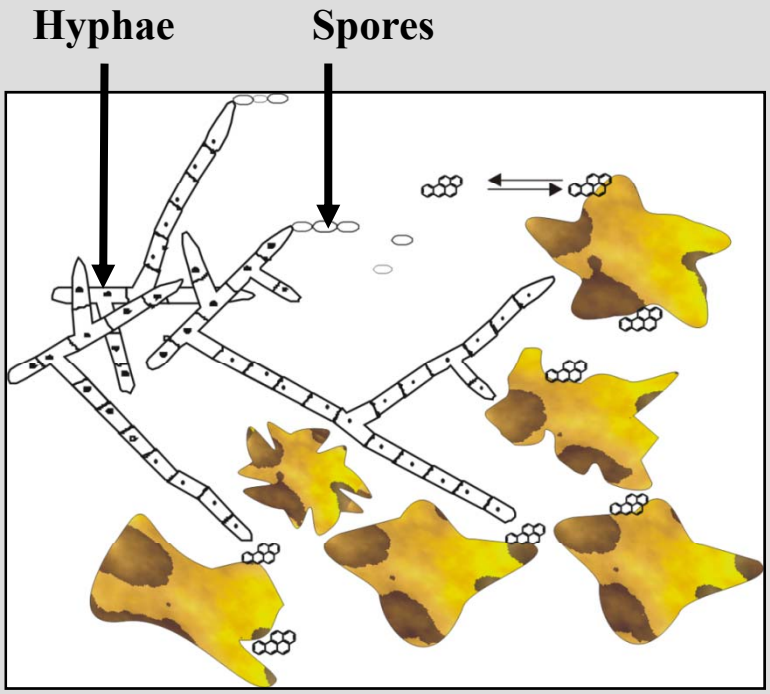
Bioavailability is a complex and dynamic variable influenced by :

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



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…)

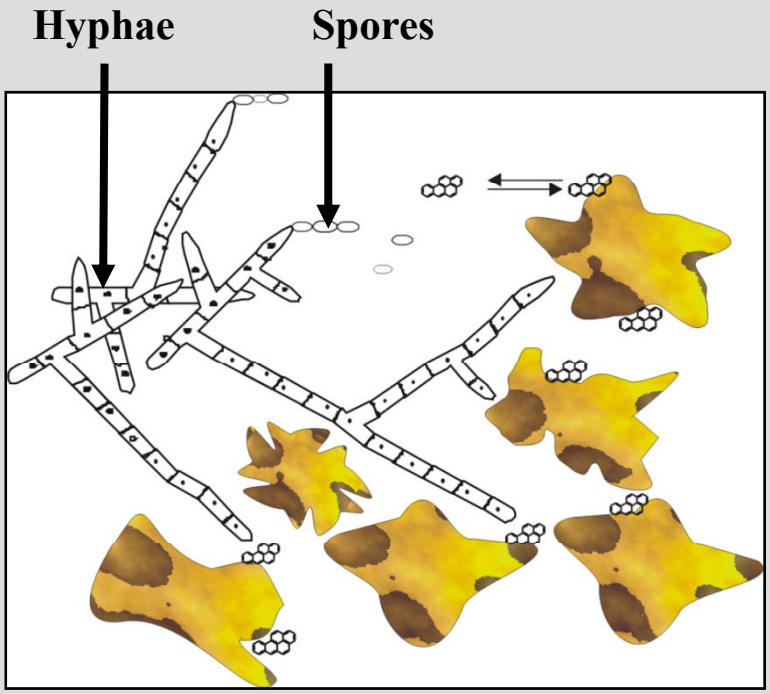


Introduction

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

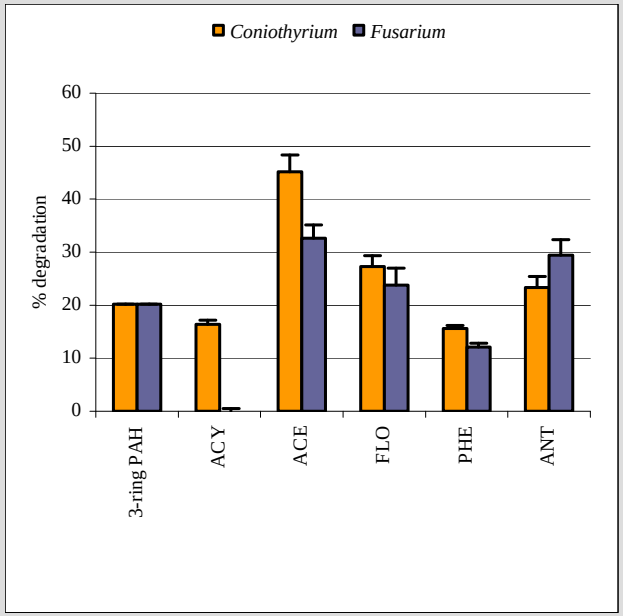
- **Isolation of saprophytic telluric fungi from PAHs polluted soils**
- **Evaluation of the fungi degradative ability on PAHs**
- **Elucidation of the biochemical mechanisms involved in BaP degradation**

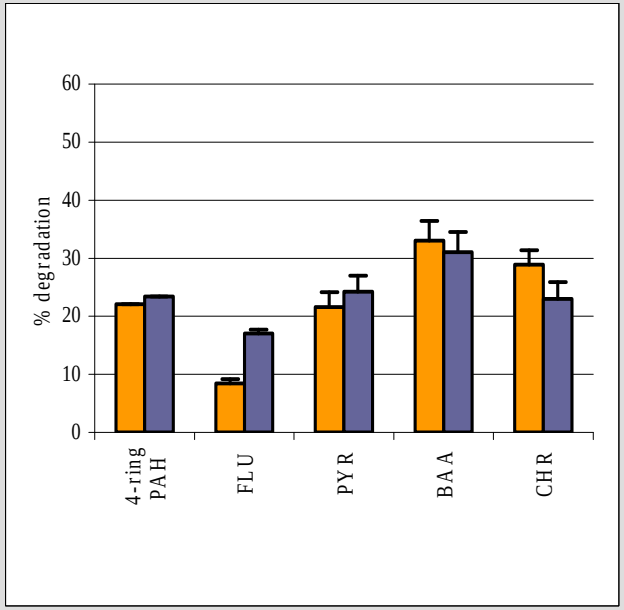
Isolation of saprophytic telluric fungi from polluted soils

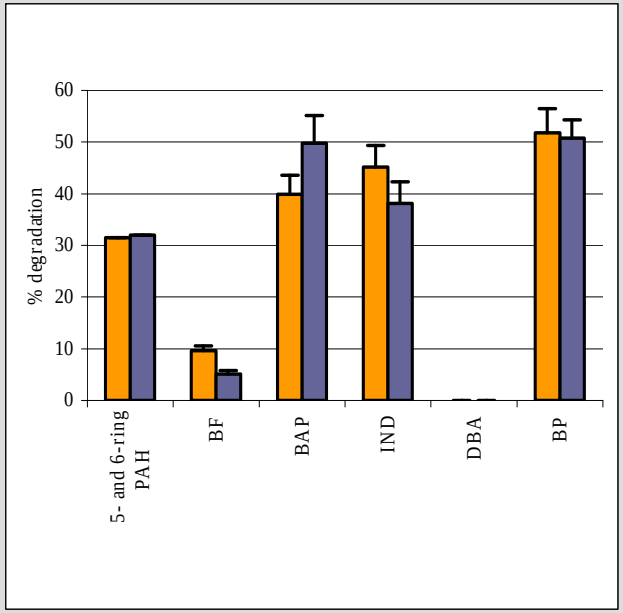
DIVISION	GENRE
Zygomycotina	*Mucor*
Ascomycotina	*Penicillium*
Deuteromycotina	*Trichoderma* *Fusarium* *Coniothyrium* *Cladosporium*

Potin *et al.* 2004 International Biodeterioration & Biodegradation

Evaluation of PAHs degradative ability of two strains



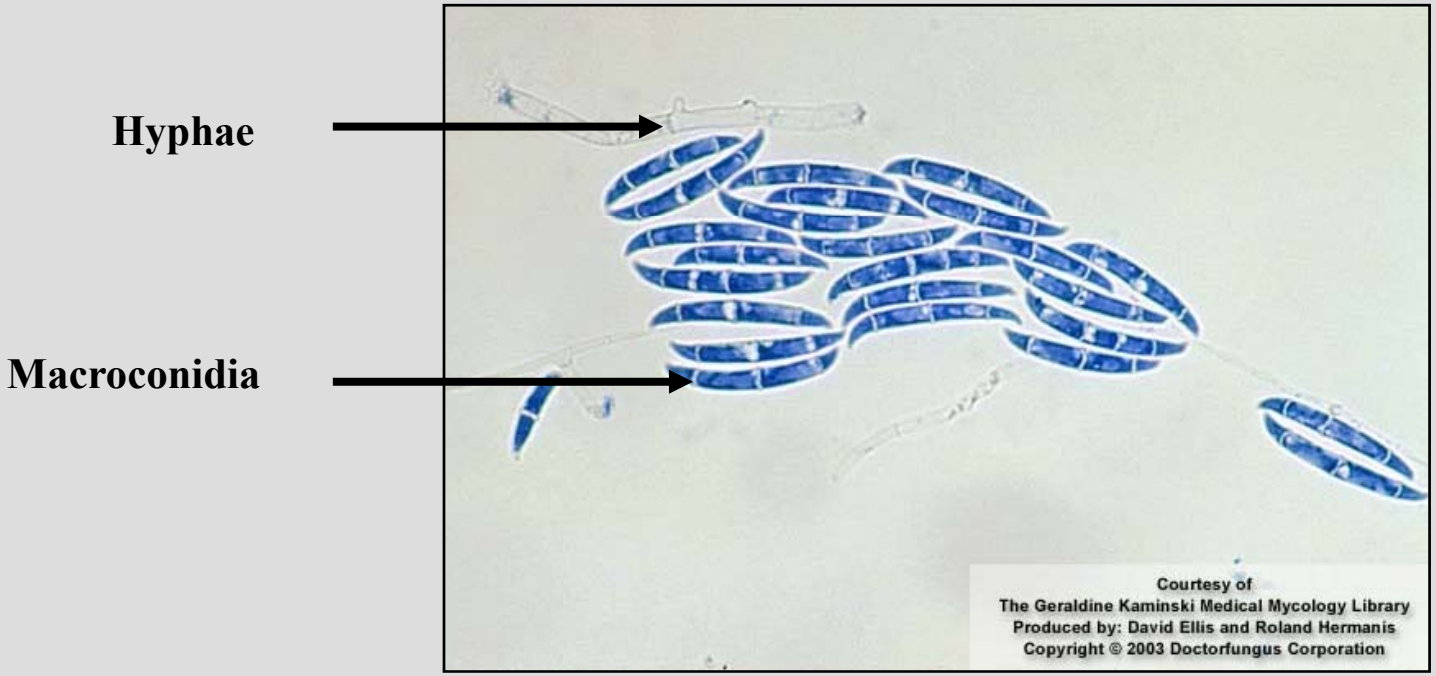




- ✓**Study conducted in aged polluted soils**
- ✓**30 days of incubation**
- ✓ **Inoculation: with mycelium of *Coniothyrium* (■) and *Fusarium* (■)**

Potin *et al.* 2004 International Biodeterioration & Biodegradation

Microscopic morphology of a Deuteromycete fungus *Fusarium solani*

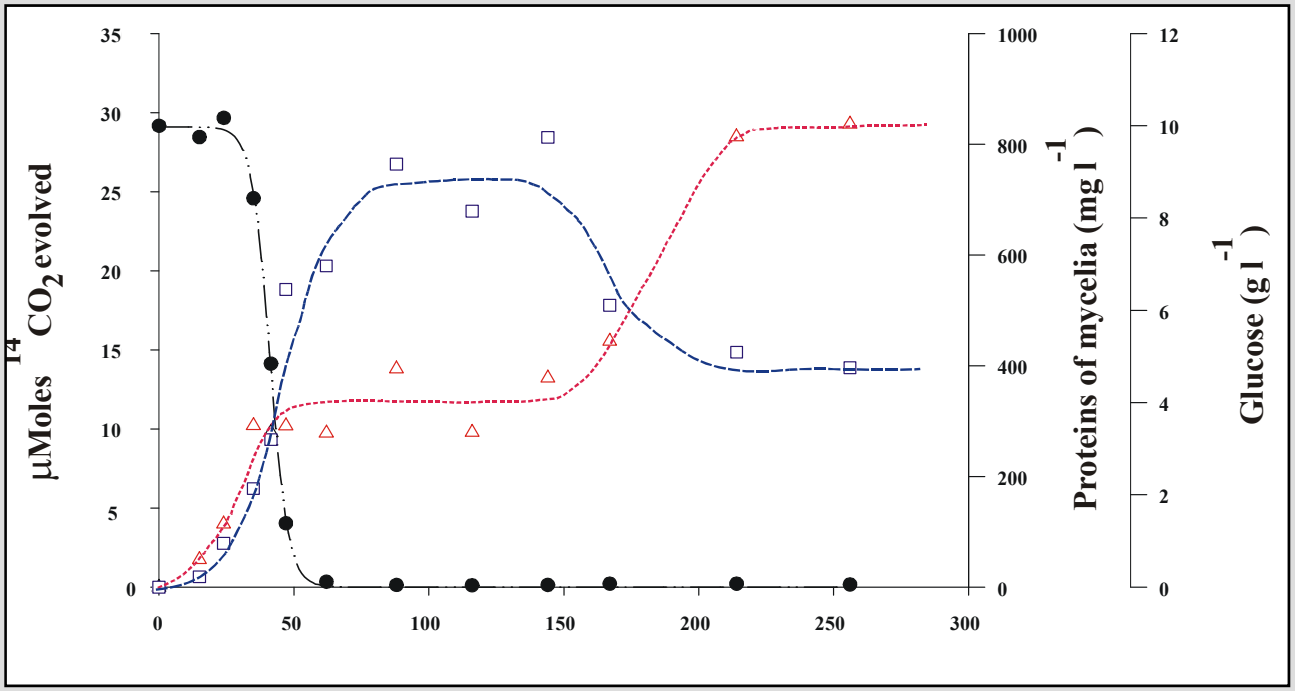


Microconidia: usually abundant, cylindrical to oval, 1- to 2-celled, formed from long lateral phialides.

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.

Mineralization of BaP by *Fusarium solani*



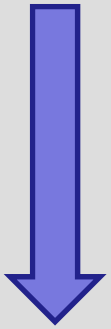
Kinetic of growth of *F. solani* in a batch fermentor in Mineral Medium (MM) supplemented with [7,10-^{14}C]benzo[*a*]pyrene

△ $^{14}CO_2$ evolved; □ mycelium proteins; • concentration of glucose

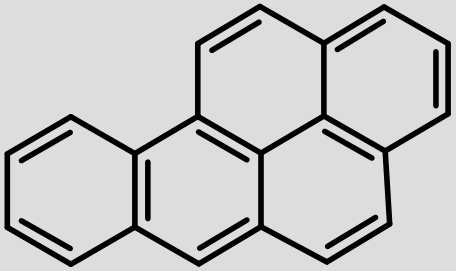
Veignie *et al.* 2002 Polycyclic Aromatic Compounds

Biochemical mechanisms involved in BaP degradation

RESEARCH STRATEGY



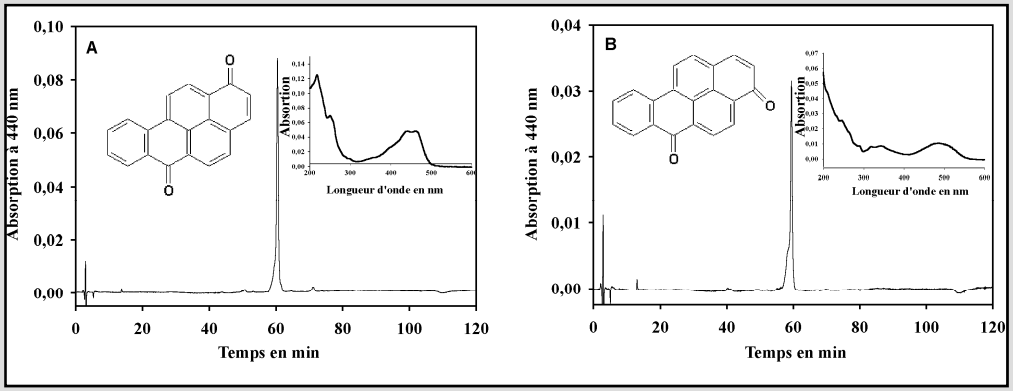
- ✓ Cultures: liquid MM (batch fermentor, erlens)
- ✓ Characterization: HPLC, UV Spectra , MS

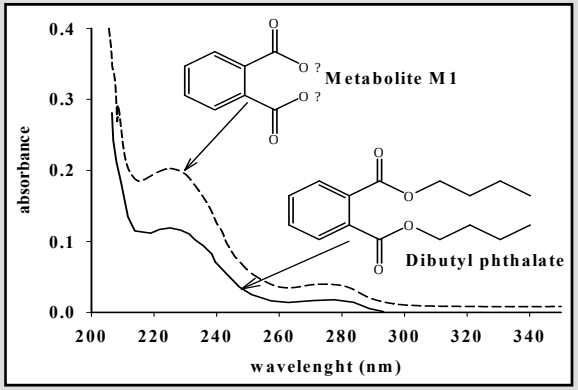


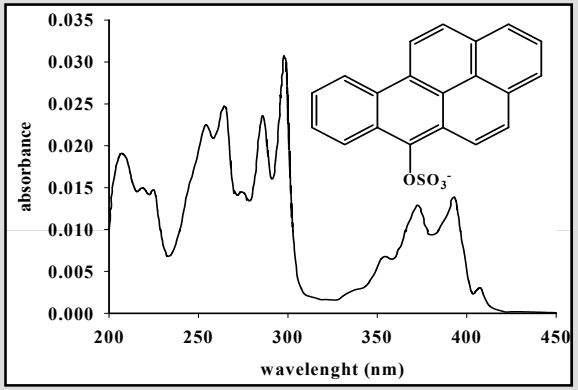
Benzo(a)pyrene

→ Identification of Produced metabolites → CO_2

Identification of some produced metabolites







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

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

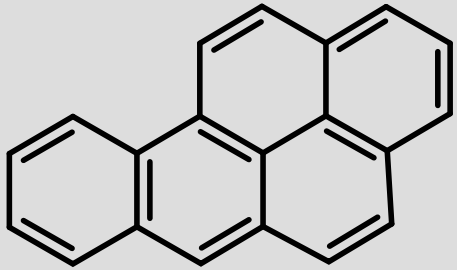
M1: UV spectrum similar with Dibutylphthalate

M2: 6-hydroxybenzo[a]pyrene sulfate

Veignie *et al.* 2002 Polycyclic Aromatic Compounds

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

Biochemical mechanisms involved in BaP degradation



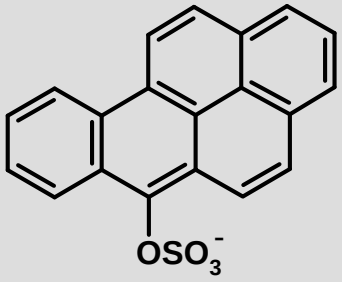
Benzo(a)pyrene

Identification of produced metabolites

CO_2

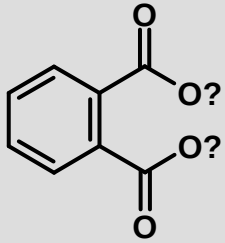
HYPOTHESIS

Involved enzymes
P450 (phase I) and
Sulfotransferase (phase II)



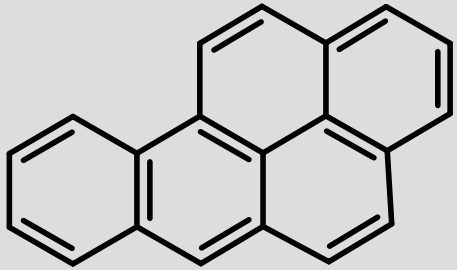
Low rate of BaP degradation

Cellular competence
Mineralization
Ring fission metabolites



High rate of BaP degradation

Biochemical mechanisms involved in BaP degradation



Benzo(a)pyrene

→ Identification of produced metabolites → CO_2

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

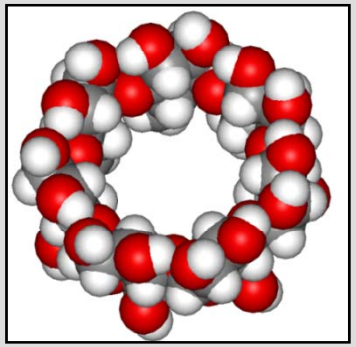
Conclusion

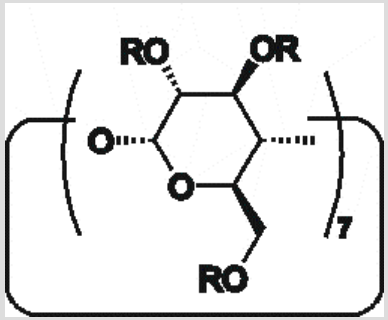
- **Diversity of fungi isolated from polluted soil and able to degrade high molecular weight PAHs**
- **Fungi different from ligninolytic, wood-decaying fungi which are well known and extensively investigated (e.g. *Phanerochaete chrysosporium*)**
- **The use of ROS producing fungi, well adapted to contaminated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

II. Research objectives : Chemical and Biological oxidation

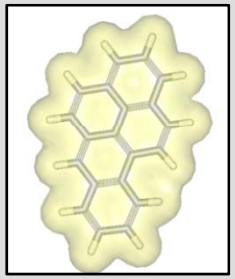
- **Use of cyclodextrins to enhance BaP bioavailability**
- **Fenton's reaction (generating ROS)**
- **Evaluation of the efficiency of Fenton's reaction on BaP degradation**
- **Interest of combining simultaneously chemical oxidation with biological treatment for BaP degradation**

Use of cyclodextrins to enhance BaP bioavailability





BaP



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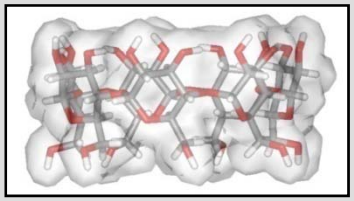
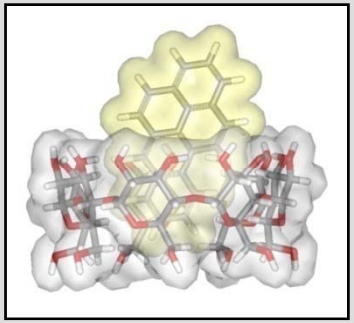


CD

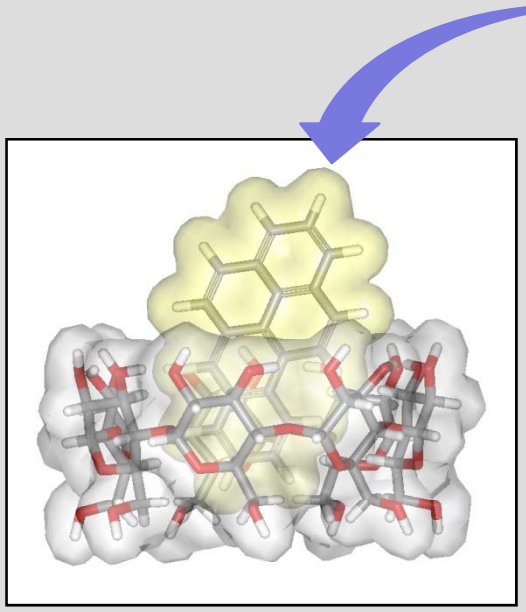


CD/BaP

BCD: R=H
RAMEB: R=H or CH_3 (mean degree = 13)
HPBCD: R=H or $CH_2CHOHCH_3$ (mean degree = 5)

Veignie *et al.* 2009 Journal of Hazardous Materials

Fenton's reaction on BaP degradation



CD/BaP

Fenton reaction

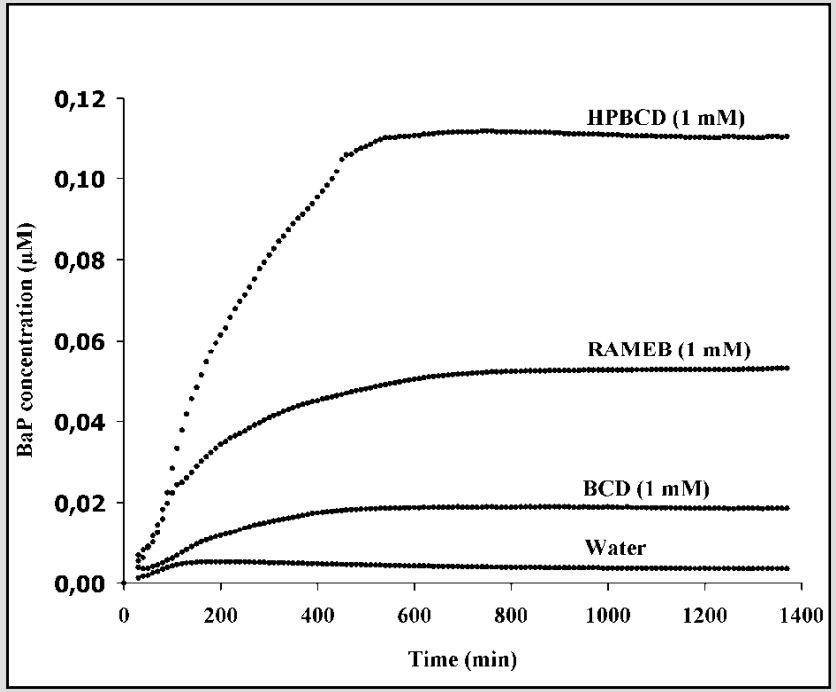
$$H_2O_2 + Fe^{2+} \rightarrow OH^{\bullet} + OH^- + Fe^{3+}$$

$$H_2O_2 / Fe = 5$$

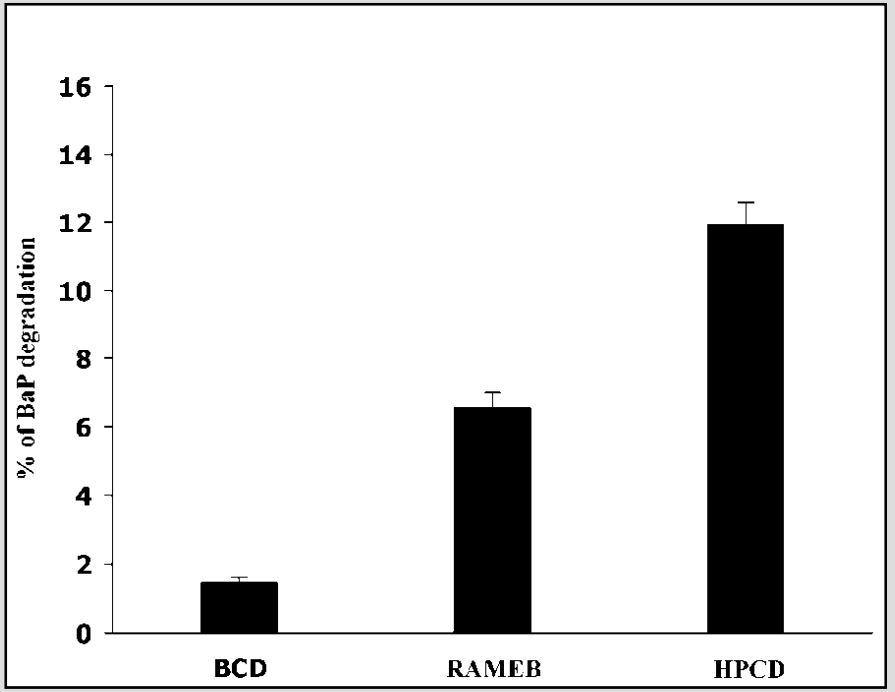
$$[H_2O_2] = 10^{-2}\ M$$

II. Chemical and Biological oxidation

Kinetics of BaP solubilisation



BaP Fenton degradation

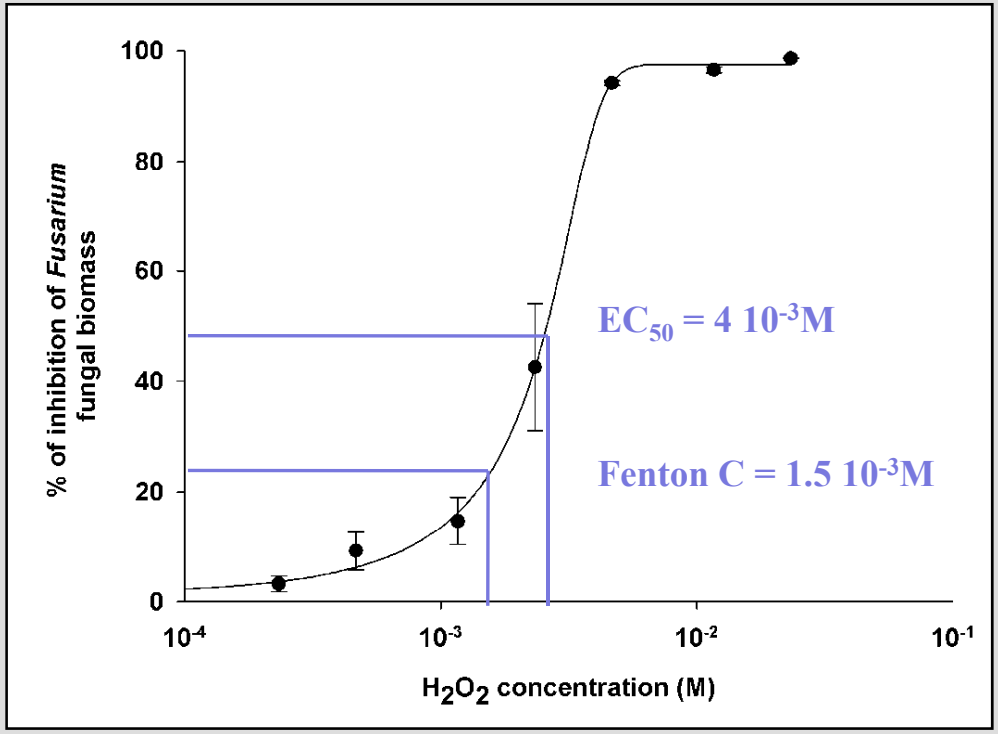


Incubation overnight in Mineral Medium

H_2O_2	10^{-2} M
$FeSO_4$	2.10^{-3} M
Cyclodextrine	5.10^{-3} M

Veignie *et al.* 2009 Journal of Hazardous Materials

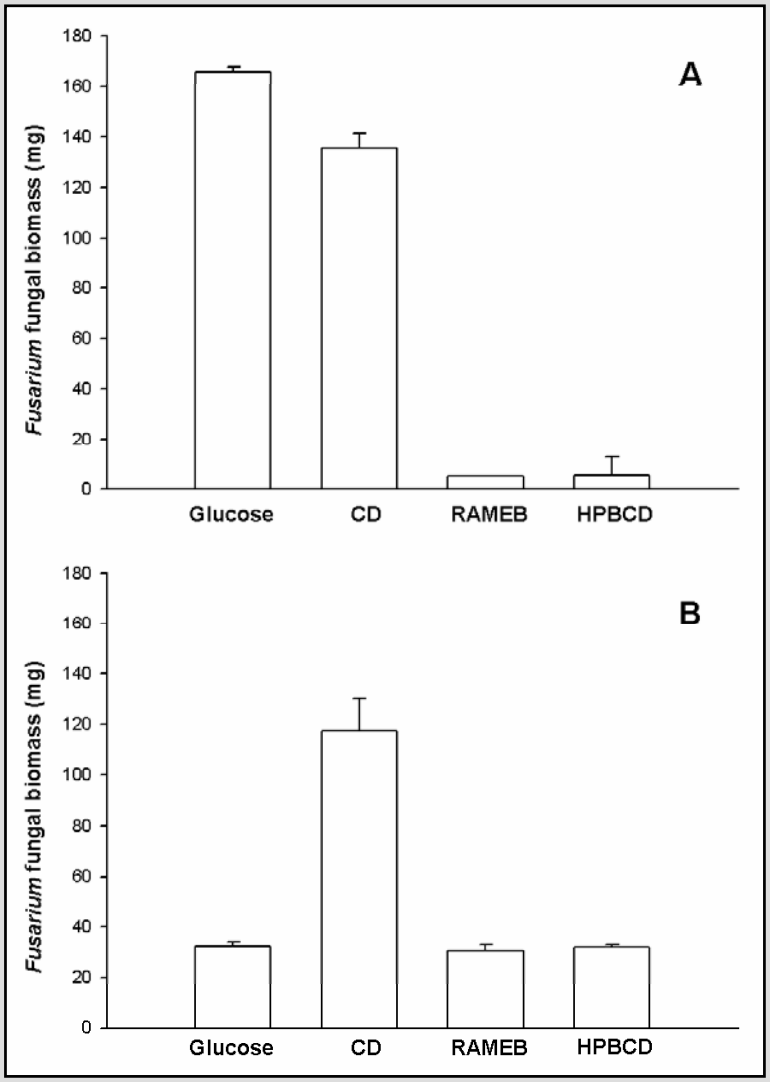
Inhibition percentage of *Fusarium solani* biomass in presence of increased H_2O_2 concentrations



5 days of incubation in Mineral Medium

Rafin *et al.* 2009 Bioresource Technology

Biodegradation of cyclodextrins by *F. solani*



5 days of incubation in Mineral Medium

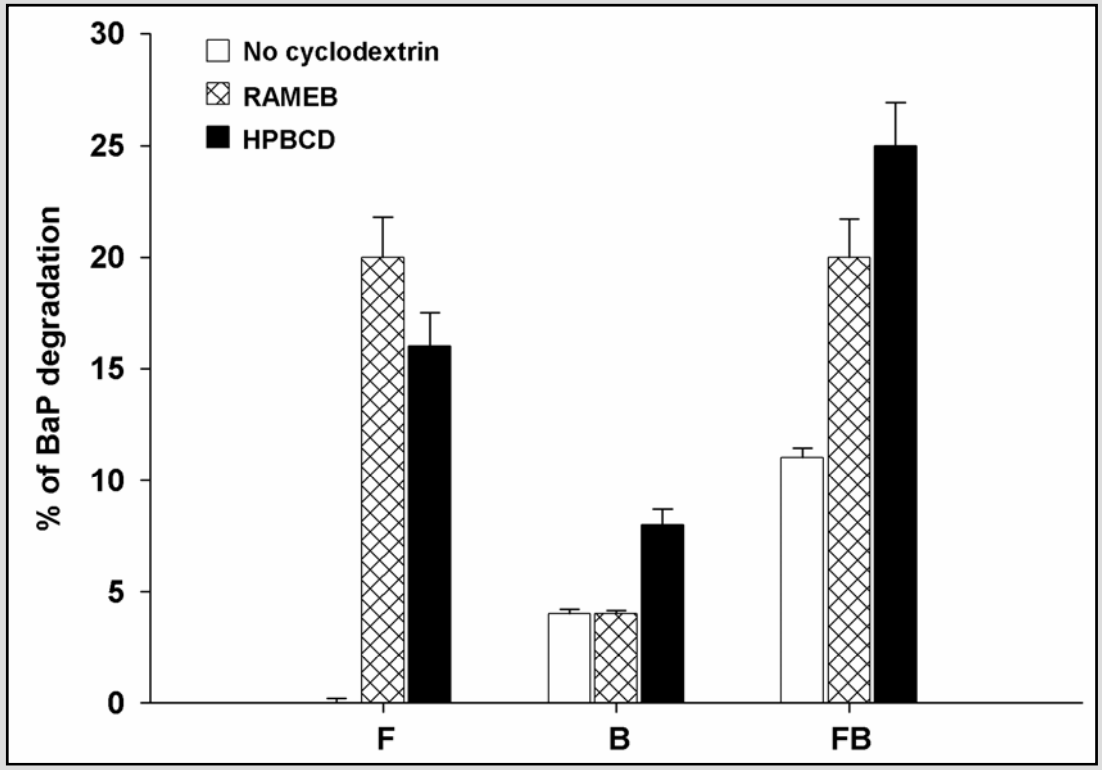
A. In presence of glucose or cyclodextrin as sole carbon source (5.82 carbon eq g l^{-1})

B. Glucose was added (1.42 carbon eq g l^{-1}) in each cyclodextrin treatment to induce fungal growth

Rafin *et al.* 2009 Bioresource Technology

II. Chemical and Biological oxidation

Degradation of benzo[a]pyrene



12 days of incubation in Mineral Medium

H_2O_2 1.5 10^{-3} M, $FeSO_4$ 5.10^{-4} M, Cyclodextrine 5.10^{-3} M

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

Rafin *et al.* 2009 Bioresource Technology

Conclusion

- **High efficiency of HPBCD to solubilise BaP**
- **Fenton degradation conducted at low H_2O_2 concentrations compatible with fungal growth**
- **Degradation study conducted for 12 days**
- **Strategy based on two simultaneous complementary remediation approaches**

References

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Keywords

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

Ecology and Biotechnology of Fungi (EBF Team)

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)**

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