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## Recent developments for fungal bioremediation technologies of soil

NORDROCS  
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## WHY BIOREMEDIATION WITH FUNGI

- Fungi often tolerate high concentrations of pollutants and heavy metals
- Lignin degradation system is
  - non-specific
  - extracellular enzymes
  - secondary metabolism

## WHAT MAKES COMPOUNDS RECALCITRANT FOR MICROBES?

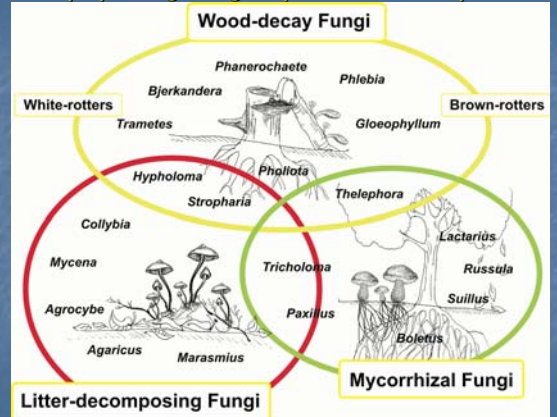
### GENERAL

1. Molecular structure (e.g. substitution with Cl)
2. Molecule is too big and cannot enter the cell
3. The compound is not soluble or it is adsorbed
4. The compound is toxic
5. The concentration of the compound is too low

### FUNGI

1. are able to degrade chlorinated compounds
2. degrade pollutants with extracellular enzymes
3. may degrade even non-soluble or humic bound compounds
4. often tolerate higher concentrations of pollutants and heavy metals than bacteria
5. see 2.

## Ecophysiological groups of basidiomycetes





## ROT TYPES OF WOOD

**White-rot** = All components of wood are degraded, but often lignin selectively, residue is mainly cellulose



White-rot

**Brown-rot** = Cellulose and hemicellulose are degraded, residue is modified lignin



White-rotted wood and *Bjerkandera adusta*

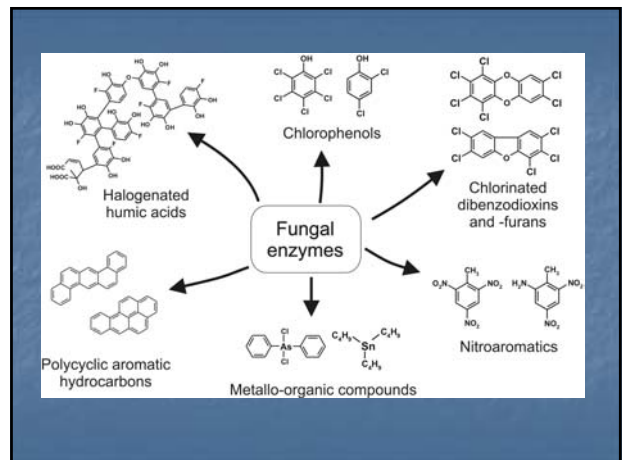
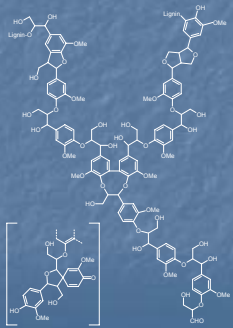


Brown-rot

The degradation capability of white-rot and litter-decomposing fungi is based on their lignin degradation system

### Ligninolytic enzymes

- Manganese peroxidase (MnP)
- Laccase
- Lignin peroxidase (LiP)
- Other peroxidases and oxidases



## Contamination types suitable for fungal remediation

- Filling stations (PAH)
- Saw mills (chlorinated phenols, dioxins)
- Chemical industry
- Oil and petrochemical industry
- Military areas

## CONTROL OF ENVIRONMENTAL CONDITIONS

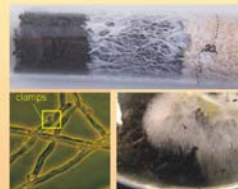
- Temperature
- pH
- O<sub>2</sub>
- Moisture
- Nutrients: **Carbon source** (N, P)

## Challenges of the fungal bioremediation technologies

- Survival of fungus in soil
  - Competition with other organisms
  - Environmental factors
  - Inhibition of soil humic substances (WRF)
- Growth is slower than bacterial growth

## Isolation and Screening of Lignolytic Basidiomycetes

Isolation of fungal strains from fruiting bodies



Microscopic examination



Liquid cultures (culture flasks, bioreactors)

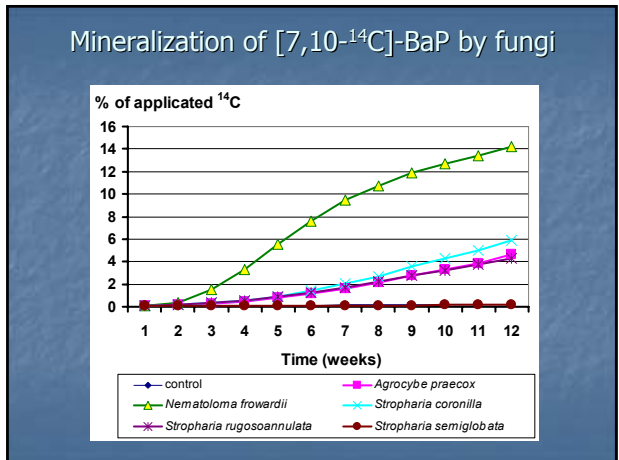
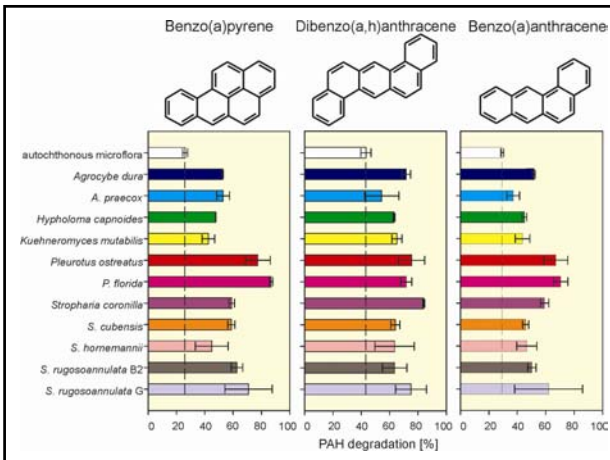


Selective agar-plate tests



### EXAMPLES OF DEGRADATION CAPABILITY OF FUNGI

1. Four- and five-ring PAH-compounds
2. Pentachlorophenol (PCP)



## Over 6000 saw mills in Finland used KY-5

### Commercial product against blue-staining fungi

#### Mixture of:

##### Chlorophenols (60%)

- 2,3,4,6-TeCP 75-85%
- PCP 5-15%
- 2,3,6-TCP 5-15%

##### NaOH (30%), H<sub>2</sub>O (10%)

##### Impurities:

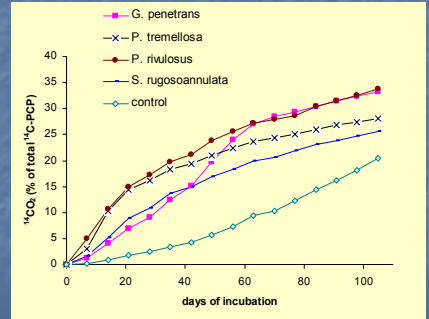
- Polychlorinated dibenzo-*p*-dioxins (PCDD)
- Polychlorinated dibenzofurans (PCDF)
- Polychlorinated phenoxyphenols (PCPP)



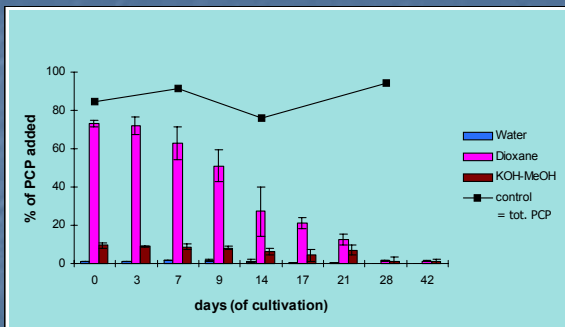
## Mineralization of <sup>14</sup>C-PCP by fungi

Fungi were pregrown eleven days in autoclaved bark and inoculated to non-sterile contaminated soil

control = non-sterile soil

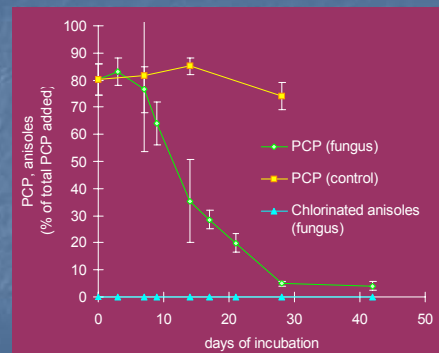


## Degradation of PCP by *Trametes versicolor*



*T. versicolor* degraded both PCP (pink) and humic bound PCP (brown)

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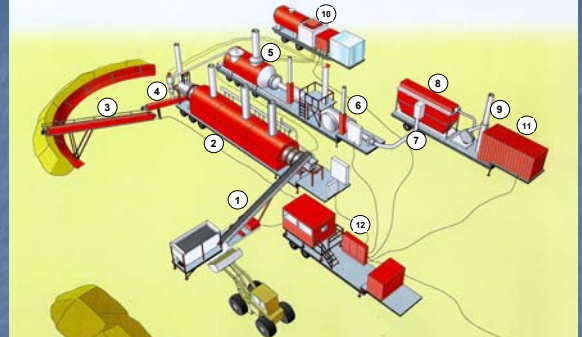
## NEW APPROACH AND PROJECT

- Dioxin contaminated soil from saw mill
- Excavated soil will be combusted
- **Problem:** soil contains  
up to 860 g organic matter / kg dry mass
- **Possible solution:**  
pretreatment with wood-degrading fungus

Started 1<sup>st</sup> August 2006

Together with Niska & Nyyssönen Oy,  
Helsinki University of Technology, Mycocenter Oy

## "Combustion unit" – Thermal desorption unit



1. Belt conveyor
2. Drum furnace
3. Screw
4. Cyclone
5. Secondary burner/Oxidizing unit
6. Heat exchanger
7. Reagent supply
8. Filter
9. Funnel
10. Water/Oil tanks
11. Compressor
12. Electric centre/Control cabin

## COMBUSTION

### High organic matter

- substantial amount of  $\text{CO}_2$  is evolved
- volume of the exhaust gas is increased
- may block up gas treatment unit, i.e. filter



## FUNGAL PRETREATMENT

### FUNGUS should

- degrade organic matter (wood) efficiently and **non-selectively** = **degrade all components of wood**
- grow fast
- be able to compete with native microbes
- tolerate pollutants in soil

### PILES have possibility for

- aeration
- moisture regulation



## SCREENING FUNGI

- Growth speed
- Competition capability
  - non-sterile conditions
- Toleration (and degradation) of toxic compounds
  - tests in contaminated soil
- Degradation capability
  - mass loss of wood
  - production of CO<sub>2</sub>
  - reduction of total organic carbon (TOC)

## FUNGI SELECTED FOR SCREENING

- Earlier experiments
  - Wood block test
  - PCP degradation experiment
- Fungi known to be efficient degraders in nature
- Fungi that degrade wooden building materials

## PLATE TESTS



*Piptoporus betulinus*



*Gloeophyllum trabeum*

Fungi are grown on agar plate with non-sterile and dioxin contaminated piece of wood

## FUTURE RESEARCH

### TOTAL MASS REDUCTION

- Plate tests with contaminated soil
- Laboratory scale experiment with contaminated soil
  - production of CO<sub>2</sub>
  - reduction of total organic carbon (TOC)

### DEGRADATION OF CONTAMINANTS

- Degradation of dioxins and aged chlorophenols
- Mineralization of <sup>14</sup>C-PCP
- Enzymes involved in degradation

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