BIOREMEDIATION OF CONTAMINATED SOIL: A STRATEGY BASED ON FUNGI AND BACTERIA


Mycotheca Universitatis Taurinensis
Department of Life Sciences and Systems Biology
University of Turin

20 settembre 2018

RemTech Expo 2018 (19, 20, 21 Settembre) FerraraFiere
www.remtechexpo.com
Fidenza, Emilia Romagna, Italy
Site of National Interest (SIN)

almost 150,000 m²

1 km far from the city center
Tar distillery
Lead tetraethyl production
Sulfuric acid and phosphate fertilizers production

Bombarded during Second World War

Starting disposal of industrial installations and soil remediation by Fidenza Municipality using traditional techniques
On-going activities foresee the soil excavation from the polluted area, followed by *in-situ* remediation. An adjacent area is used to built up biopiles where bio-attenuation processes take place.

Degradation yields are low (industrial use of the soil) and the process is time consuming (6-9 months).

There is room for improvement of the process.

**Selection of microorganisms adapted to this peculiar ecological niche**
RESTORE ECOLOGICAL FUNCTIONS OF THE CONTAMINATED LAND
• Which are the microorganisms adapted to this ecological niche?

• Which are the best performing strains?
  • Degradation skills, production of enzymes, biosurfactants, etc.

• Will be the microorganisms effective in real conditions?
  • Degradation assessment: microcosm, mesocosm and *in situ* biopile
Microorganisms isolation

Selective pressure posed by the use of:

- **PAHs**
  - Pyrene
  - Phenanthrene
  - Naphthalene

- **BTEX**
  - Benzene

- **alkanes**
  - Paraffin oil

**aliphatic and aromatic compounds**

Crude oil extracted from the soil
PAHs are good substrates, whereas fewer strains were isolated from the oil and benzene, due probably to the toxicity or the low availability (e.g. evaporation).
Liquid Enrichment

Different fungal communities developed according to pollutants as sole carbon source and the soil depth.

91 isolated fungi, belonging to 19 genera and 31 taxa

Fusarium, Trichoderma and Aspergillus are the most abundant genera
Molecular analyses of microbial community

All samples were dominated by *Fusarium* (more than 80%), followed by *Aspergillus*, *Penicillium*, *Trichoderma* and *Arthrinium*. 
The pollutants significantly affected the fungal community. PAHs (pyrene and phenanthrene) grouped separately from oil or the volatile naphthalene.
Isolation from different cultural techniques...

<table>
<thead>
<tr>
<th></th>
<th><strong>Fungi</strong></th>
<th><strong>Bacteria</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total count</strong></td>
<td>54 isolates</td>
<td>37 isolates</td>
</tr>
<tr>
<td><strong>Solid Screening</strong></td>
<td>164 isolates</td>
<td>125 isolates</td>
</tr>
<tr>
<td><strong>Liquid Enrichment</strong></td>
<td>91 isolates</td>
<td>94 isolates</td>
</tr>
</tbody>
</table>

**565 isolates**

**309 isolates**

**256 isolates**
309 isolates

256 isolates

Total isolates

309 isolates

219 strains

133 strains

De-replicated strains

256 isolates

352 strains

352 strains

Fungi

Bacteria
• Which are the microorganisms adapted to this ecological niche?

• Which are the best performing strains?
  • Degradation skills, production of enzymes, biosurfactants, etc.

• Will be the microorganisms effective in real conditions?
  • Degradation assessment: microcosm, mesocosm and *in situ* biopile
Microplate Screening

Set up of a innovative microplate screening for the quantitative evaluation of the pollutants biotransformation.

spectrophotometric analysis of the fungal and bacterial growth

growth up to 3 weeks
Many fungi (53 %) grew more than the control (glucose) in the presence of at least one substrate. **Alkanes** are easily used as source of nourishment (often better than the control with glucose).
Cladosporium cladosporioides

Fusarium solani
Biosurfactants Screening

70% of strains showed a positive response in at least one test.

Drop collapsing assay

Oil dispersion test

Blue agar test

Emulsification Activity

WORK IN PROGRESS
LIFE-BIOREST Outline

- Which are the microorganisms adapted to this ecological niche?
- Which are the best performing strains?
  - Degradation skills, production of enzymes, biosurfactants, etc.
- Will the microorganisms be effective in real conditions?
  - Degradation assessment: microcosm, mesocosm and in situ biopile
Any waste transported from the site that is required by the regulations is subject to... an alternative to landfarms, especially on sites near sensitive receptors, as emissions can be more easily controlled.
Colonized carriers were inoculated in 500 g soil (3 months): thesis with only bacteria or fungi (as single or mixed consortia) and thesis with both bacteria and fungi.

Fungi and bacteria synergically transformed the target pollutants. Combined consortia resulted faster and more efficient against the most recalcitrant pollutants.
Mesocosms trials

Six microbial consortia were selected and inoculated in 10 kg soil. Experiments last for 3 months. Three replicates were set up.

The total hydrocarbons content was reduced up to 70 %, but the choice of the microbial consortia came to be a critical issue.

Among the six selected consortia, the yields varied from 20 to 70 %.

The most concentrated pollutants (e.g. phenanthrene, fluoranthene and pyrene) were almost completely transformed.
Bioaugmentation with the selected *microbial consortium* guarantees a high and stable degradation.

The control gave variable results due to the heterogeneous qualitative and quantitative distribution of the autochthonous microbiota.

![Bar chart showing degradation percentage of total hydrocarbons between abiotic control and mesocosm A.](chart.png)
Which are the microorganisms adapted to this ecological niche?

Which are the best performing strains?
- Degradation skills, production of enzymes, biosurfactants, etc.

Will the microorganisms be effective in real conditions?
- Degradation assessment: microcosm, mesocosm and in situ biopile
400 m³ of soil has been inoculated with the best performing consortia (fungi and bacteria). Chemical and ecotoxicological analysis are carried out to follow the microbial degradation.
GRAZIE PER L’ATTENZIONE,

Dott. Federica Spina

Università degli Studi di Torino

+39 0116705964

federica.spina@unito.it