



Selection of autochthonous and allochthonous fungal strains for the treatment of recalcitrant wastewaters



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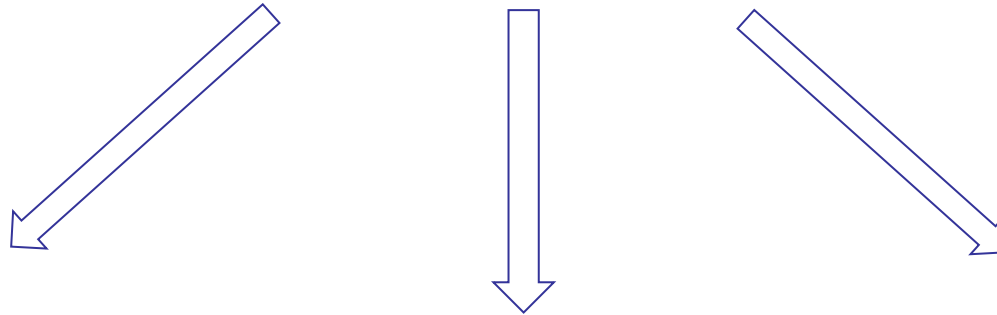
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Lodi 24 September 2015 – FIRB Project (Fondo per gli Investimenti in Ricerca di Base 2014-2016 - MIUR) RBF13V3CH: “In situ bioaugmentation to exploit the combination of fungi and bacteria”

FIRB Project (Fondo per gli Investimenti in Ricerca di Base 2014-2016 - MIUR) RBFR13V3CH: **“In situ bioaugmentation to exploit the combination of fungi and bacteria”**



**Mycotheca Universitatis
Taurinensis (MUT)
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**Department of Biology
University of Pisa**

1 biologist
1 biotechnologist
1 naturalist

2 engineers

1 biologist



Fields of research of MUT

Bioremediation of soil and water:
degradation of different kind of
xenobiotics.

White biotechnology:

flavours, polymers, antioxidants,
anticoagulants and pharmaceuticals.



Marine fungi:

extremozymes,
antibacterial,
antifungal,
antialgal.

Biodeterioration

Taxonomy, Physiology and Biodiversity of Fungi:

database continuously upgraded (www.mut.unito.it).



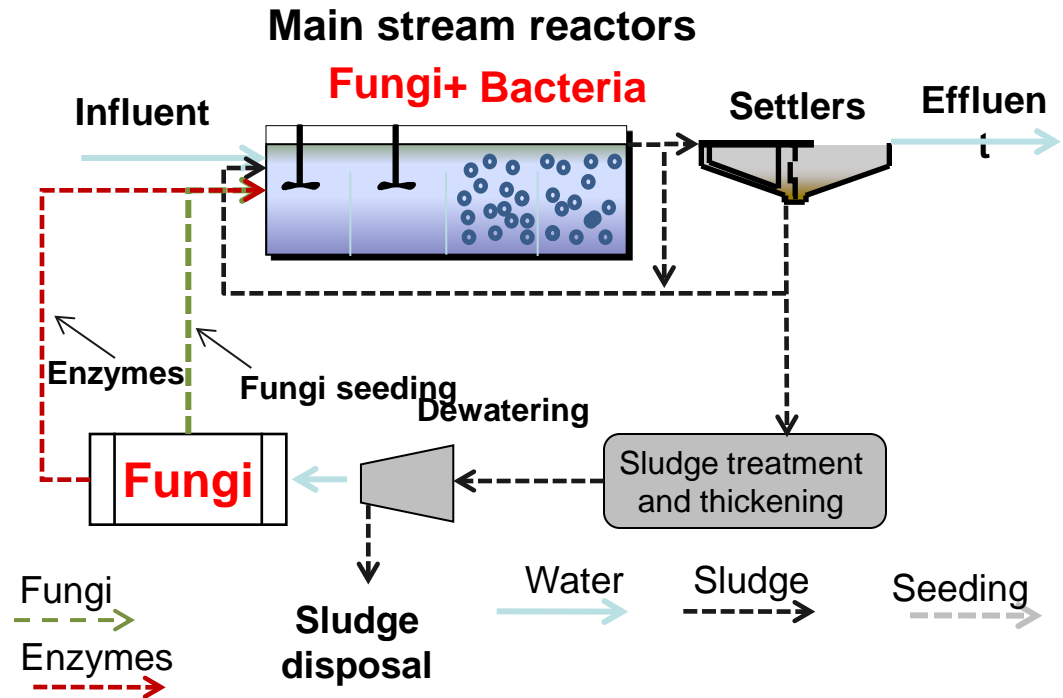
The project idea: in situ bioaugmentation

Previous works demonstrated the cooperation between fungi and bacteria:

Spina et al., 2012.
Environmental Engineering and Management Journal 11(10) 1789-1796.

Strategy: Continuous seeding of fungi from side stream to main stream reactors

INNOVATIVE WASTEWATER TREATMENT PLANT



Advantages of side-stream reactor:

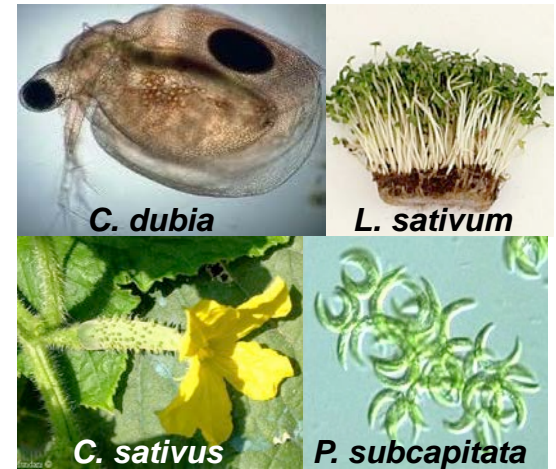
- 1) Scarcity of substrates biodegradable by bacteria
- 2) Presence of recalcitrant compounds and salts
- 3) Possibility of designing and operating the reactors independently

Potentially replicable in all WWTP

Chemical and Ecotoxicity characterisation

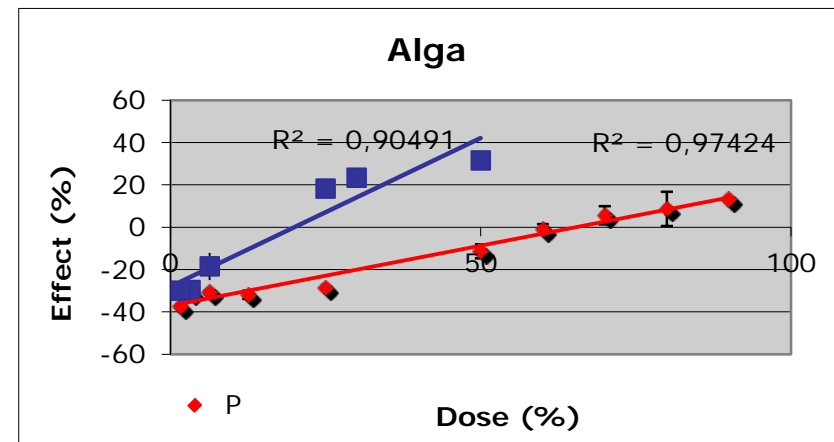
Samples:

- **P** = a landfill leachate after activated sludge (AS) treatment;
- **Co** = a tannery effluent (vegetable tanning process) after nitro-denitro and AS.



	Co	P	Measure unit
pH	7,66	8,48	
Ammonium	1,04	0,05	[mg/L]
Phosphate	5,92	30,57	[mg/L]
Chloride	2.906,90	-	[mg/L]
COD	14.280,00	1603,00	[mg/L]
SCOD	362,00	-	[mg/L]
TSS	1.293,33	3,10	[mg/L]
VSS	1.078,00	-	[mg/L]
Total nitrogen	6,67	-	% dry weight

Co was always more toxic than P.
Alga was selected for further analyses.



Selection of autochthonous fungi

DRBC

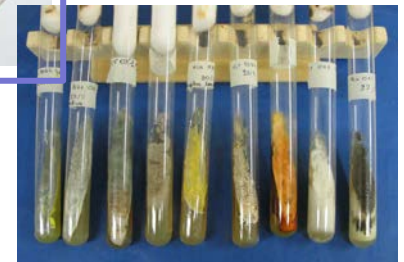
10% Diluted MEA

Agar-Wastewater

15 ° C



25 ° C



Among the 300 isolated strains, 28 were selected for the bioremediation experiments.

Selection of allochthonous fungi from previous studies



MUT 2295 *Bjerkandera adusta*
MUT 2400 *Trametes pubescens*
MUT 1585 *Porostereum spadiceum*
MUT 2976 *Pleurotus ostreatus*

Effective
towards dyes
and landfill
leachate



MUT 990 *Aspergillus tubingenensis*
MUT 918 *Aspergillus niger*
MUT 1125 *Paecilomyces variotii*
MUT 983 *Aspergillus niger*

Effective
towards
tannins

Methods

Sample pH was adjusted to 5-6



Free biomass



Medium was replaced with 100 mL sample



7 days at 110 rpm, T 25° C

Daily analyses ($t_0 - t_{168}$ h):

- Decolourization percentage (DP)
 - enzymes
 - pH
 - glucose
 - COD

Final analyses (t_{168} h):

- biomass growth
 - ecotoxicity
 - phenols

Abiotic controls were set up

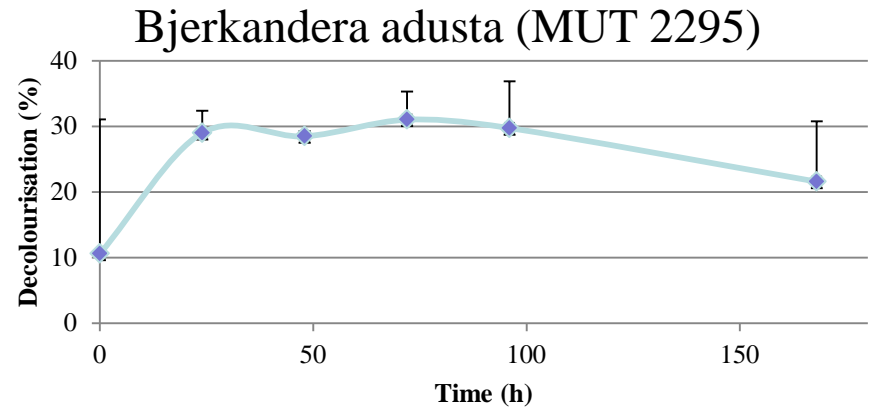
Results

P



Despite the low toxicity of P, only *Bjerkandera adusta* remained active in wastewater.

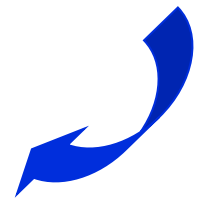
Decolourisation
(up to 30% in 24 h)



Peroxidases were probably the enzyme involved in the biotransformation process.

	MiP	MnP	Tox
MUT 2295	25 U/L	20 U/L	+101%

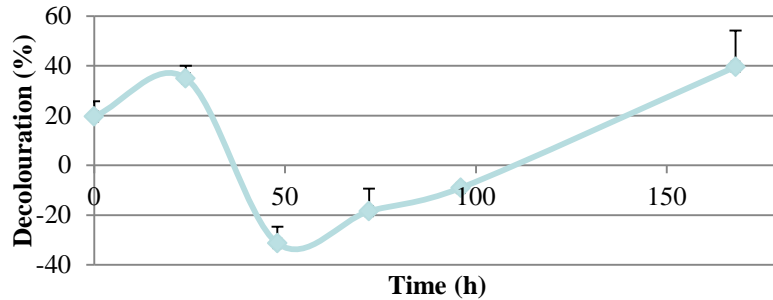
Activated sludge as further treatment to remove toxic but more biodegradable molecules



Results

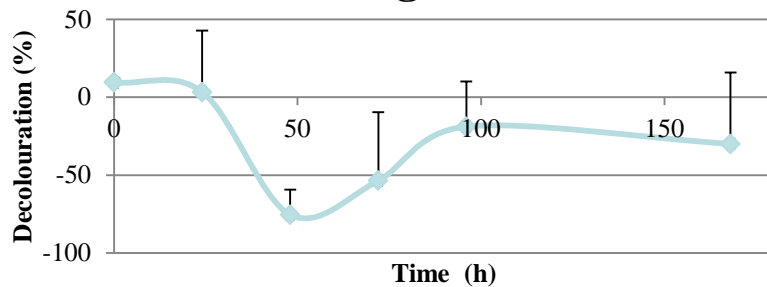
Co

Chaetomium sp. (MUT 5688)



Two different fungi were selected on account of pH control, metabolism activity, toxicity decrease and phenols increase.

A. tubingensis MUT 990



	Lac	Phenols	Tox
MUT 5688	-	+10%	-70%
MUT 990	30 U/L	+38%	-70%

The study of tannase activity is a work in progress

Conclusion and partners research

- Strains well characterised from a fungal collection are precious tools for biotechnology and in particular for bioremediation.
- Several fungal strains were selected for further experiments with immobilised biomass and bioreactors.
- Criticism: evaluation of tannins removal. Partners for tannins characterisation and degradation?



Thanks for your attention



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