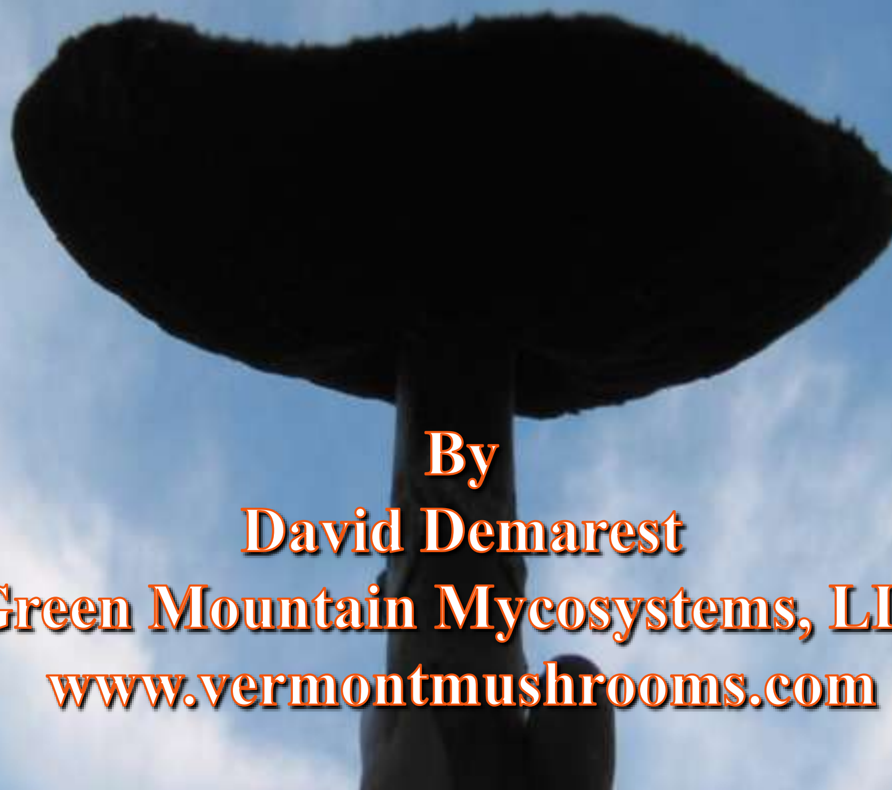
Mycofacilitation:
Increasing Ecosystem Health With Fungi



By
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Who?

~ 1.5 million species of fungi

~140,000 species of mushrooms

What?

"The natural & true ecosystem engineers"

(Lawton and Jones 1995, as cited in Singh 2006)

When?

Fossils of spores/hyphae ~460 million years ago

(Vellinga, 2002)

Where?

Ubiquitous

Why?

Life as we know it would not be possible without them...

Mycoremediation

Phytoremediation

Mycorrhizomal Remediation

Mycoremediation: Separating Fact from Fiction

Fungi can make arsenic disappear.

Fact, sort of…

Fungi can transfer methyl groups as carbonium ions (CH_3^+) by S-adenosyl-methionnine (Tamaki & Frankenberger, 1992, Gadd 1993b as cited in Gadd 2001)

The biomethylation of metalloids frequently results in their volatilization (Gadd 1993b, as cited by Gadd 2001)

But obviously it's still arsenic, and there is no legitimate reason to ever emit arsenic into the atmosphere!

Mycoremediation: Separating Fact from Fiction

Mycoremediation is simpler and cheaper than conventional remediation options.

Fiction!

In fact, mycoremediation *can* be a substantially less expensive remediation option in some situations.

HOWEVER

In many situations this is not the case, especially when the project timeline is considered and the fact that the project is dependent on substantially more variables for success.

All that should be claimed is that mycoremediation has the potential to be cost competitive with existing remediation technologies.

Mycoremediation: Separating Fact from Fiction

Turkey Tail mushrooms have been proven to break down dieldren so I can order some mushroom spawn to mix into my dieldren contaminated soils and end up with clean soil.

Fiction!

In fact, Turkey Tail Mushrooms (Trametes versicolor) have demonstrated the ability to degrade dieldrin in the lab (Morgan et al. 1991 as cited in Gadd 2001)

HOWEVER, successful degradation of a compound in the lab does not directly correlate to success under field conditions!

Mycoremediation: Separating Fact from Fiction

Mycoremediation can save the world.

Fiction!

In fact, mycoremediation *can* play a pivotal role in breaking down numerous toxic substances and mycofacilitation *can* help transform a degraded location into a thriving ecosystem with increased diversity and resilience to environmental stresses.

Saving the world will require a complete paradigm shift.

We, as a society, need to adopt a Traditional Chinese Medicine approach to our vital ecosystems. Preventing the problems in the first place MUST become everyone`s priority.

Potential for Mycoremediation

Petroleum hydrocarbons

Industrial wastewaters

Polychlorinated biphenyls and dioxins

Pesticides, especially persistent pesticides

Phenols, chlorophenols, and pentachlorphenol

Polycyclic aromatic hyrdocarbons (PAHs)

Heavy metal biosorption

Dyes, pulp and paper effluents, brewery waste, etc

Mycoremediation Strategies

Depending on the contaminant compound it may be:

Used as a carbon source

Enzymatically attacked. but not used as a carbon source (cometabolism)

Taken up and concentrated (bioaccumulation) but not metabolized

(Bennett, 2002)

Petroleum Hydrocarbon Remediation

Many fungi are known to be able to utilize hydrocarbons as a carbon source (food), although oxidation by enzymes can also be a significant remediation option.

Fungi can assist in remediation of crude oil and diesel fuel spills, MTBE in groundwater, and more in many ways...

The phytoremediation of arabian medium crude oil contaminated soils using *Lolium multiflorum* (Italian ryegrass) was significantly improved by inoculation with *Glomus intraradices* (arbuscular mycorrhizal fungus), *Sphingomonas paucimobilis* (petroleum degrading bacteria) and *Cunninghamella echinulata* (filamentous fungus).

Petroleum Hydrocarbon Mycoremediation

Tall fescue and meadow fescue (*Festuca arundinacea* and *F. pratensis*) infected with EM (*Neotyphodium coenophialum* and *Neotyphodium uncinatum*) had improved ability to assist in the breakdown of C10-C25 and TPH than uninfected plants (Soleimani et al. 2010)

Practical side note: The addition of compost has proven effective at enhancing degradation of diesel oil and four-ring PAHs (Gandolfi et al. 2010)

NOTE: Significant impact of type of HC (aliphatic hydrocarbons vs aromatics)

Peak Oil,
Global Climate Change & Oil Spills

Convert sustainable sources of energy into useful forms:

Gliocladium roseum growing in *Eucryphia cordifolia* of Northern Patagonia actually converts cellulose to diesel!!!

Arbuscular mycorrhizal fungi are an important tool we can use in carbon sequestration.

While changes in our climate and dwindling supplies of oil are becoming increasingly obvious few things so dramatically demonstrate the impact of our society's oil addiction as the devastation caused by **OIL SPILLS...**

Oil Spill Remediation Steps

1) Prevention, prevention, prevention!

2) Rapid *correct* response in the event of an oil spill!

effective recovery and containment systems

3) Determination of best oil spill treatment options

in situ vs ex situ

identification of key variables to remediation success

4) Realistic analysis of remediation project results and specific variables involved for future use and improvement of existing methods

Demarest Process™

Integrated Oil Spill Remediation and Energy Recovery System

Containment and Recovery of Oil Spill

↓

Rapid Deployment of Methane Digesters in Proximity to Oil Spill

↓

Injection of liquid effluent into stranded oil reserves and/or low performing wells

Demarest Process™

Integrated Oil Spill Remediation and Energy Recovery System

Containment and Recovery of oil spill using currently available and new technologies.

The Demarest process can begin with oil recovered using a wide variety of methods. Even oil recovered from a spill using oil-sorbant materials such as peat moss, hemp, textile fabric waste, human hair, coir, and other natural or synthetic sorbant materials can be converted into useable energy!

Rapid onsite (or near site) deployment of methane digesters to break down oil.

Mixing biological inoculums and local organic waste materials with the recovered hydrocarbons enables degradation of petroleum hydrocarbons into usable methane for use or sale. Emphasis on achieving appropriate digester biology for optimal methane yield and development of appropriate inoculums is essential for success.

Anaerobic transport and injection of liquid methane digester effluent into stranded oil reserves and/or low performing wells.

This cost-effective biostimulation and bioaugmentation approach to recovery of stranded energy can convert oil reserves into easily recoverable methane and/or facilitate well pressurization.

This less environmentally destructive source of inexpensive energy can be used to help transition away from fossil fuel dependence and reduce the desperate implementation of more and more environmentally destructive energy extraction methods.

Demarest Process™ (continued)

Integrated Oil Spill Remediation and Energy Recovery System

Gieg et al. 2008 calculated the amount of methane that could theoretically be recovered from known domestic petroleum systems (375 billion barrels)

1-5 TRILLION CUBIC FEET PER YEAR!!!

(citation from Gray et al, 2010)

But obviously when it's gone, it's gone...

The Demarest Process merely hopes to help provide a less environmentally destructive source of affordable energy to help in a transition away from fossil fuels while reducing the impacts of oil spills and building methane digesters that can later be used with natural organic waste streams as a component of a sustainable energy system.

Chlorinated Compound Mycoremediation

Ligninolytic fungi such as *Phanerochaete chrysosporium* have demonstrated ability to degrade chlorinated compounds such as DDT.

Lestan et al. published methods for inoculation of *P. chrysosporium* into contaminated soils in 1996. However it is worth noting that as far back as 1968 research had already demonstrated the ability of certain fungi to degrade DDT (known species included *Mucor alternans, Fusarium oxysporum,* and *Trichoderma viride*).

P. Chrysosporium, P. Ostreatus, Phellinus weirii and Polyporus versicolor were able to mineralize 5.3-13.5% of added DDT, dicofol, and methoxychlor over 30 days under ligninolytic growth conditions (Kennedy et al. 1990 as cited in Pointing et al. 2001)

NOTE: Biodegradation of DDT can result in toxic and persistent metabolites (Pointing et al. 2001) Degradation of metabolites into nontoxic substances must be factored into the remediation project!

Industrial Wastewater Mycoremediation

Example: Dairy Bioreactor

Why needed?

COD of whey 50000-80000 mg/l Lactic acid bacteria will significantly lower effluent pH and many other organisms cannot effectively utilize it

Airlift (repeated fed batch of whey/dairy effluent) using mixed yeast cultures can accomplish a 96% reduction in COD within 60 hours (Christiana-Urbina et al. 2000 as cited in Singh 2006)

Polychlorinated Biphenyls Mycoremediation

P. Chrysosporium, in addition to Pleurotus ostreatus, Coriolopsis polysona, Trametes versicolor, Bjerkandera adusta, and Lentinula edodes have demonstrated the ability to degrade many PCBs.

Close to 90% degradation of PCD-contaminated soil has been demonstrated using *P. chrysosporium* grown on sugarcane bagasse pith (Fernandez-Sanchez et al. 1999)

Grifola frondosa accumulates dichloromethoxyphenol during degradation of PCB 48 in low-nitrogen medium (Seto et al 1999)

Xenobiotic Degrading Filamentous Fungi

(copied from Bennett et. al. 2002)

TABLE 3 Representative xenobiotic-degrading filamentous fungi

Group	Species	Substrate(s)	Reference(s)
White rot fungi	*Agrocybe aegarita*	Benz[a]anthracene	118
	Agrocybe praecox	Phenanthrene, pyrene	71
	Clitocybula duseni	Lignite	86
	Coriolopsis gallica	Anthracene, phenanthrene, pyrene	137
	Dichomitus squalens	Benz[a]anthracene	118
	Doedoela quercina	Benz[a]anthracene	118
	Ganoderma applanatum	Benz[a]anthracene	118
	Hypholoma fasciculare	Anthracene, fluoranthene, pyrene	71
	Kuehneromyces mutabilis	Anthracene, fluoranthene, phenanthrene, pyrene	71
	Lentinus edodes	Benz[a]anthracene	118
	Lenzites betulina	Anthracene, phenanthrene	71
	Nematoloma frowardii	Dinitrotoluene and trinitrotoluene, lignite coal, pentachlorophenol	85, 86, 148
	Pleurotus dryinus, P. eryngii, P. fusculatus, P. flabellatus, P. pulmonaries, P. sajor-caju	Benz[a]anthracene	118
	Pycnoporus cinnabarinus	Dibenzofuran	97, 118
	Stropharia rugosoannulata	Anthracene, fluoranthene, phenanthrene, pyrene	71, 118
	Trametes hirsuta	Textile dyes	1
Mycorrhizal fungi	*Morchella conica*	Anthracene, fluoranthene, phenanthrene	71
	Tylospora fibrilosa	Fluorobiphenyl	73
Others	*Agaricus bisporus*	Anthracene, fluoranthene, phenanthene, pyrene	71
	Coprinus comatus	Anthracene, fluoranthene, phenanthrene, pyrene	71
	Crinipellis stipitaria	Pyrene	112
	Gloeophyllum striatum	Dichlorophenol	52
	G. trabeum	Pentachlorophenol	149
	Marasmiellus troyanus	Benzo[a]pyrene	171
	M. rotula	Pyrene	112

Polycyclic Aromatic Hydrocarbons Mycoremediation

PAHs (polycyclic aromatic hydrocarbons) are often degraded more rapidly by bacteria; however, in certain circumstances (such as PAHs with more than 4 aromatic rings) fungi play a pivotal role in successful degradation of PAHs.

Remember: The addition of compost has proven effective at enhancing degradation of diesel oil and four-ring PAHs (Gandolfi et al. 2010)

Many fungi have demonstrated ability to degrade two to six ring aromatics include Zygomycete *Cunninghamella elegans*, Ascomycetes *Aspergillus niger* and *Penicillium* sp. and the white-rot Basidiomycetes *Phanerochaete chrysosporium, Trametes versicolor, Pluerotus ostreatus*, and *Bjerkandera* sp. (Singh, 2006)

Generally PAH metabolites are less toxic than parent compounds BUT some quinones or hydrozy derivatives are more toxic and mutagenic.

Heavy Metals and Radionuclitides

Hyperaccumulation and Biosorption are really your only practical options...

Marcellus Shale Hydofracking: JUST SAY NO! Catskillmountainkeeper.org

Filamentous fungi can hyperacculate metals and radionuclitides in mycelium or fruitbody.

Mycorrhizal fungi can be influential in either avoidance or sequestration of pollutant.

Fungi Breaking Down Used Tires, Concrete, & Plastic!

Bredberg et al. demonstrated *Resinicium bicolor* to be effective at detoxifying tire rubber prior to devulcanization (2002)

Species of *Fusarium* contribute to loss of calcium and weight in concrete (Gu et al., 1998) and loss of concrete bonding strength has been attributed to acids produced by *Aspergilis niger* and *Mycelia sterilia* (Perfettini et al., 1991)

Gusse et al. (2006) demonstrated the ability of Phanerochaete chrysosporium to degrade phenolic resin polymers (a common industrial polymer used in plywood, particle board and Formica counter tops and many other products)

Contaminated Water

Benefits of using fungi
Many pollutants can be rapidly broken down by fungi
Biosorption capabilities for heavy metals

Concerns to consider if using fungi
Volume of water creating saturated conditions without effective aeration

High loading rates of:
biological contaminants
or
nitrogen

Some Common Methods

Flow through outdoor beds composed of wood chips, etc

Stirred tank bioreactors

Membrane bioreactors (MBRs)

"Successful use depends on a comprehensive knowledge of fungal physiology, biochemistry, enzymology, ecology, genetics, molecular biology, engineering, and several related disciplines. The field conditions and factors that induce fungal biodegradation are taken into consideration before the final design." (Singh, 2006)

AMRET

Aerobic Microbial Respiration and Evapo-Transpiration discussed in The Composting Toilet Book by Del Porto and Steinfeld

Outdoor ET rates ~ 0.05 to 0.2 gpd/sf

Greenhouse ET rates ~ 0.5 to 3.0 gpd/sf

(Del Porto and Steinfeld, 1999)

or a well designed constructed wetland is generally a more effective long-term option for high nutrient waste steams.

Where fungi really excel is at dealing with complex compounds plants can’t break down...

Contaminated Soils

Benefits of using fungi

- Many pollutants can be rapidly broken down by fungi
- Soil mycoremediation strategies build healthy soil

Concerns if using fungi

- Heavy metal and radionuclide contamination
- Ability of desired species to colonize substrate, and survive long enough, to achieve treatment goals

Soil Mycoremediation Methods

Ex-Situ verse In-Situ

Project must:

- Provide biological needs of fungi (adequate air, water, nutrients, pH, etc)
- Prevent effective biological competitor
- Be monitored until completion

EXCEPTION to first to points: if previously myceliated substrate contains required enzymes for breakdown of toxic substances simple bulk addition of substrate can be an effective option...

Mycorrhizomal Remediation

Benefits

- Enhances breakdown of organic contaminants
- May enhance phytoextraction of heavy metals
- Intended mycorrhizal communities can sustain themselves under field conditions longer than saprophytic fungi

Downsides

- Time-frame until completion
- Potential difficulties establishing optimal mycorrhizal species
- Mixed pollutants may require successional strategy

7 Associations Classified

Arbuscular, Ectomycorrhiza, Ectendomycorrhiza, Ericoid, Arbutoid, Orchid, Monotrophic

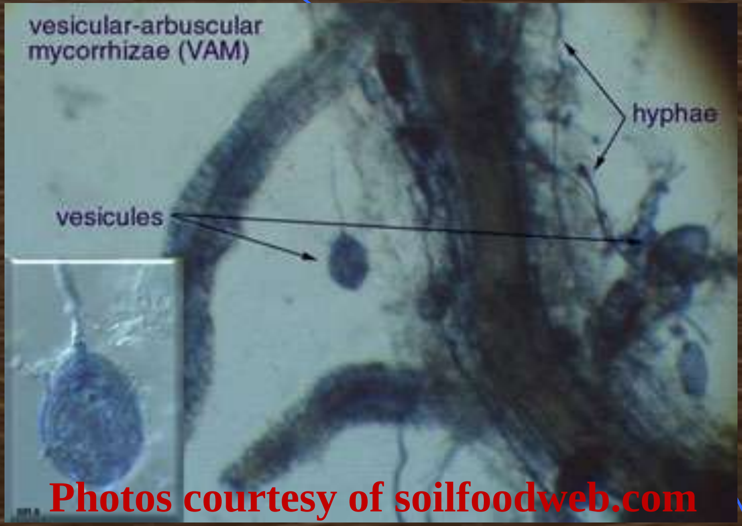
Ectomycorrhiza (ECM)

No host cell penetration

Relatively host specific

Extremophiles

Degrade a range of POP



Vesicular-arbuscular mycorrhiza (VAM)

Host cell penetrated by hyphal network

Occurs in about 2/3 of plants

Endomycorrhizas help considerably with phosphorus uptake

Mycorrhizomal Remediation Potential

PCBs, atrazine, and many more POPs can be degraded by mycorrhizal fungi

(Donnelly and Fletcher, 1994 as cited in Chaudhry, 2005)

(Anderson and Coats, 1995 as cited in Chaudhry, 2005)

A mix of grasses inoculated with mycorrhizal fungi can hyperaccumulate arsenic

Festuca rubra, Festuca eliator, Agropyron repens, and Trifolium repens inoculated Glomus intraradices had a maximum As shoot concentration of 1,713 μg/g growing on soils with an arsenic content of 50 μg/g under optimal conditions (Giasson et. al. 2006)

General Rule of Thumb

(like all rules of thumb there are always exceptions!)

VAM

Very good for Accumulation of Metals

ECM / ERM

Excellent Chemical Metabolization / Enzymes

Substantial research on mycoremediation is done...

The future promises the development of successful field applications for this technology...

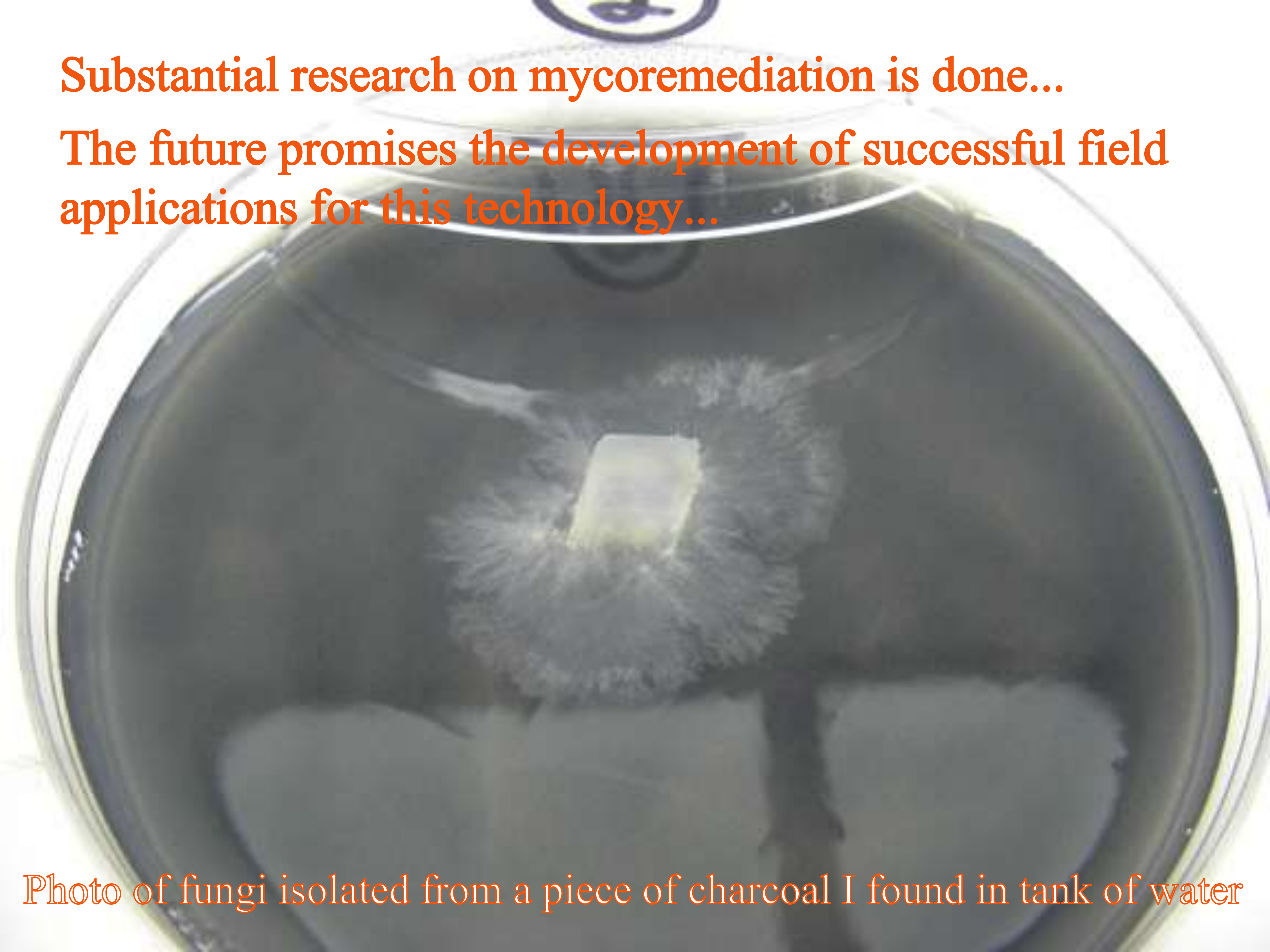


Photo of fungi isolated from a piece of charcoal I found in tank of water

Ramial Woodchips

How to Make and Use Ramial Woodchips

- *Chipping the TOPS of hardwoods (preferably maple/oak/beech, avoid softwoods)*

Use as you would mulch around your plants and any other areas you wish to build healthy vibrant soil

King Stropharia

Soft maple, box elder, poplar and other "softer`` hardwoods ideal, but otherwise standard RWC rules

Main difference between simple RWC and growing King Stropharia is that you add 10 lb of sawdust spawn per 100 sq. ft of beds (3-4" deep)

Oh, and that they taste great...

NOTE: Avoid alcohol when consuming K.S.

Soil Testing Services

University of Vermont Soil Testing Lab

http://www.uvm.edu/pss/ag_testing/?Page=soils.html

Alternative Soil Testing Lab Resource List

http://attra.ncat.org/attra-pub/soil-lab.html

GREAT Laboratory to Better Understand What is Living of Your Soil

http://www.soilfoodweb.com

Woods End Microbiological Testing www.woodsend.org

Endyne, Inc – For a wide range of analytical tests www.endynelabs.com/

Mycofacilitation Resources

Green Mountain Mycosystems website

www.vermontmushrooms.com

A Good Article about "Fungal Curve" by Michael Phillips

www.groworganicapples.com/organic-orcharding-articles/orchard-ecosystem.php

A Quebec Group with Information on Ramial Woodchips

www.hydrogeochem.qc.ca/pages/rcw.html

A searchable database of literature, includes mycoremediation

www.pubmed.com (hint: search for *specific* keywords)