

Role of rhizosphere microorganisms in phytoremediation of biphenyl in a contaminated groundwater plume

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Introduction

- ◆ Malroz Engineering Inc. uses Pump and Treat technology to remediate a contaminated groundwater plume
- ◆ Estimated at \$860,000 over 20 years
- ◆ Malroz estimates that phytoremediation would cost \$125,000 over 20 years i.e. significant savings of \$735,000 per site

Site diagram

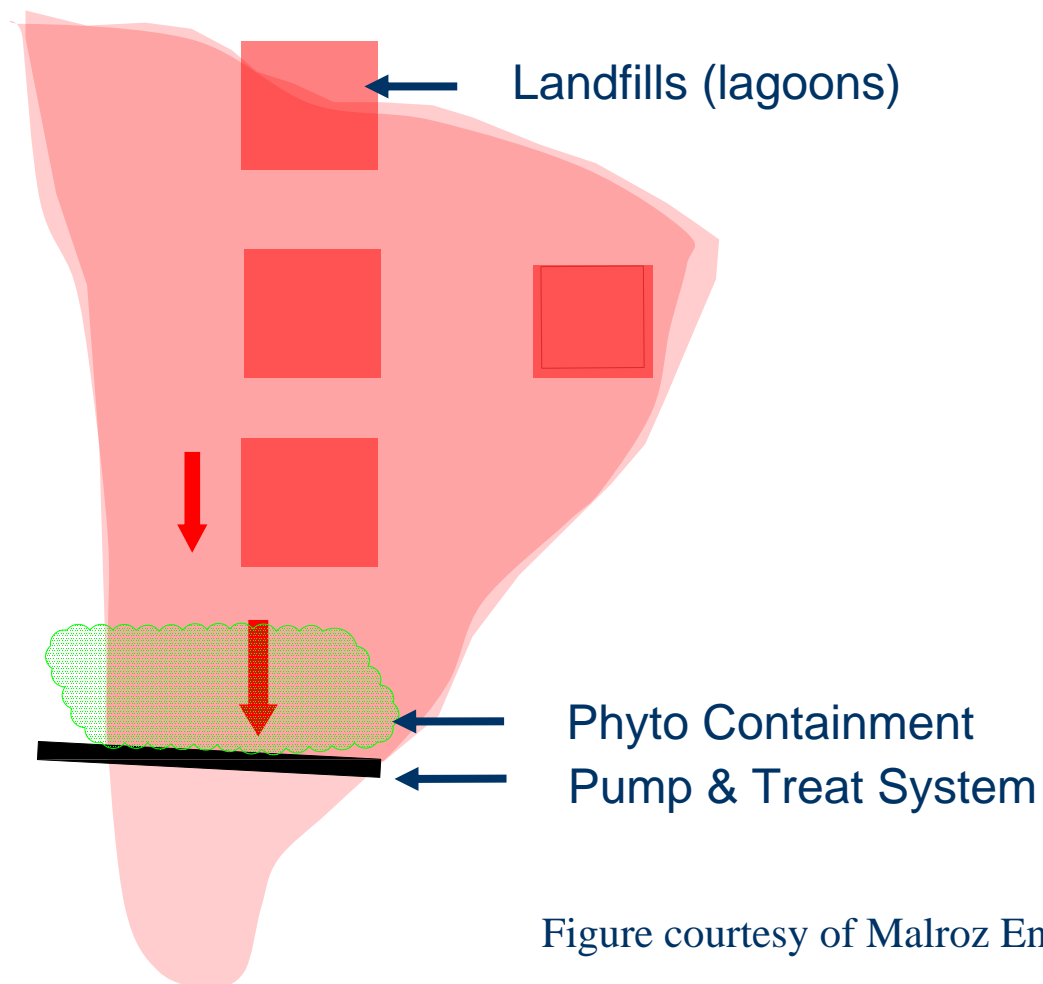
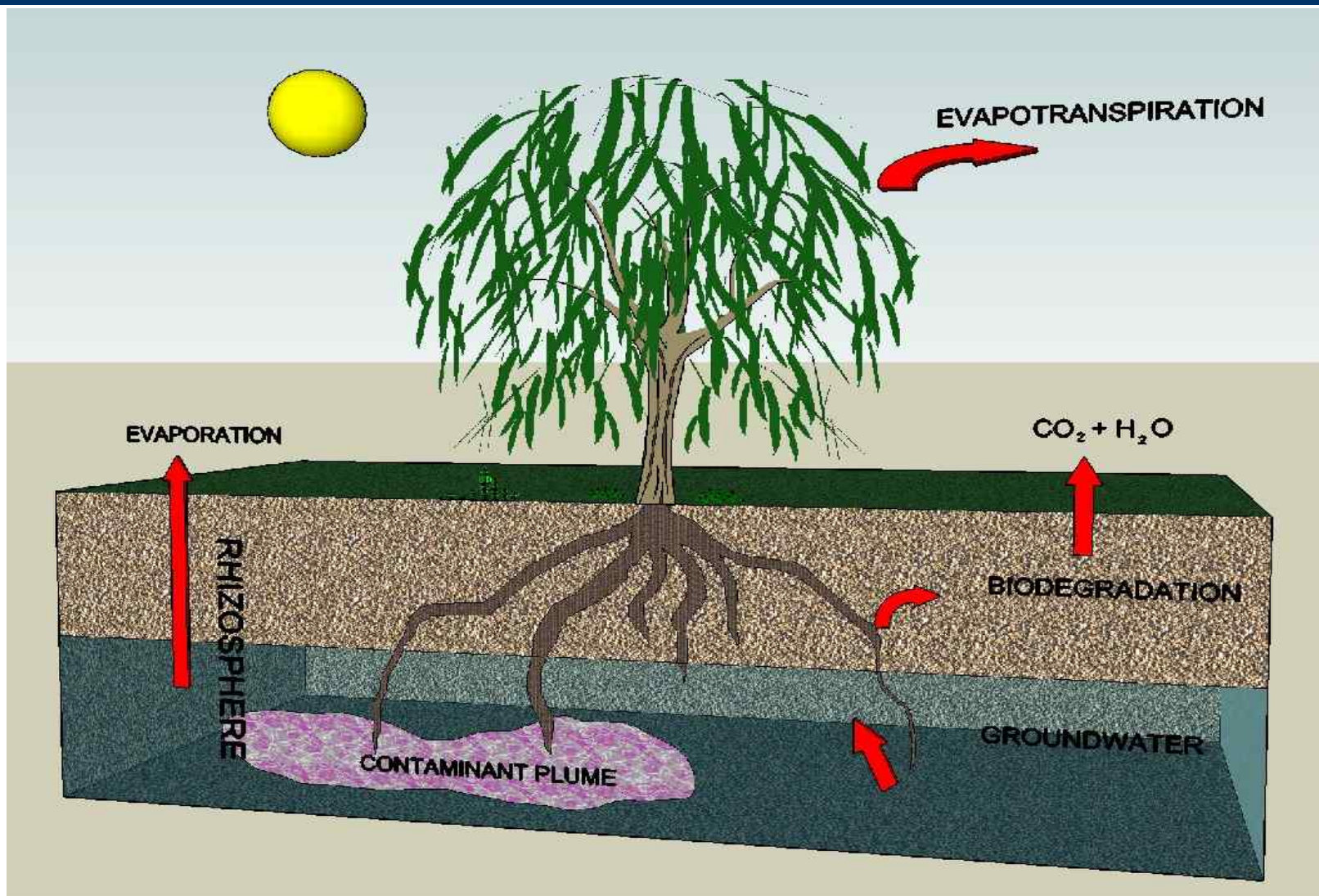


Figure courtesy of Malroz Engineering

Principal of phytoremediation

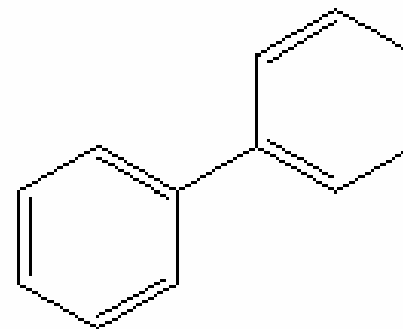


Research objective

- ◆ Evaluate the in situ biodegradation of biphenyl by microorganisms in poplar and willow rhizosphere
- ◆ Investigate methods to enhance biphenyl degradation

Biphenyl

- ◆ Industrial applications
 - Dye carrier
 - Intermediate of PCB
 - Fungicide
 - Heat transfer agent
- ◆ 885,000 lbs released in USA, 1992
- ◆ High environmental hazard rating
- ◆ Linked primarily to kidney damage, also to hormonal and CNS disruption

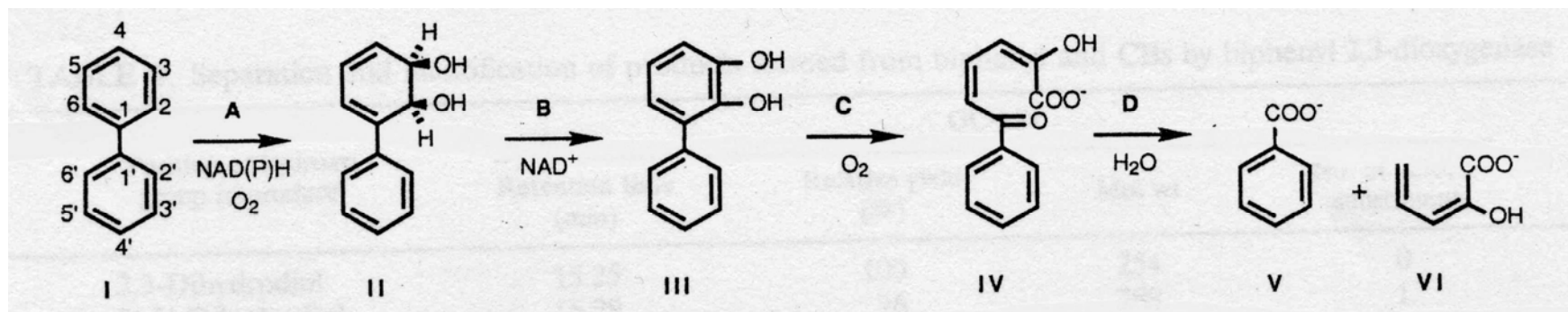


Properties of biphenyl

- ◆ Poor water solubility (7.5 mg/L)
- ◆ Sorbs strongly to soil (log K_{ow} = 4.09)
- ◆ Relatively high volatility (Henry's law constant = 5.94×10^{-4} atm m³/mol)

Aerobic biphenyl degradation

- ◆ Aerobic degradation well documented
- ◆ *bph* genes regulate biphenyl degradation, and induced by biphenyl

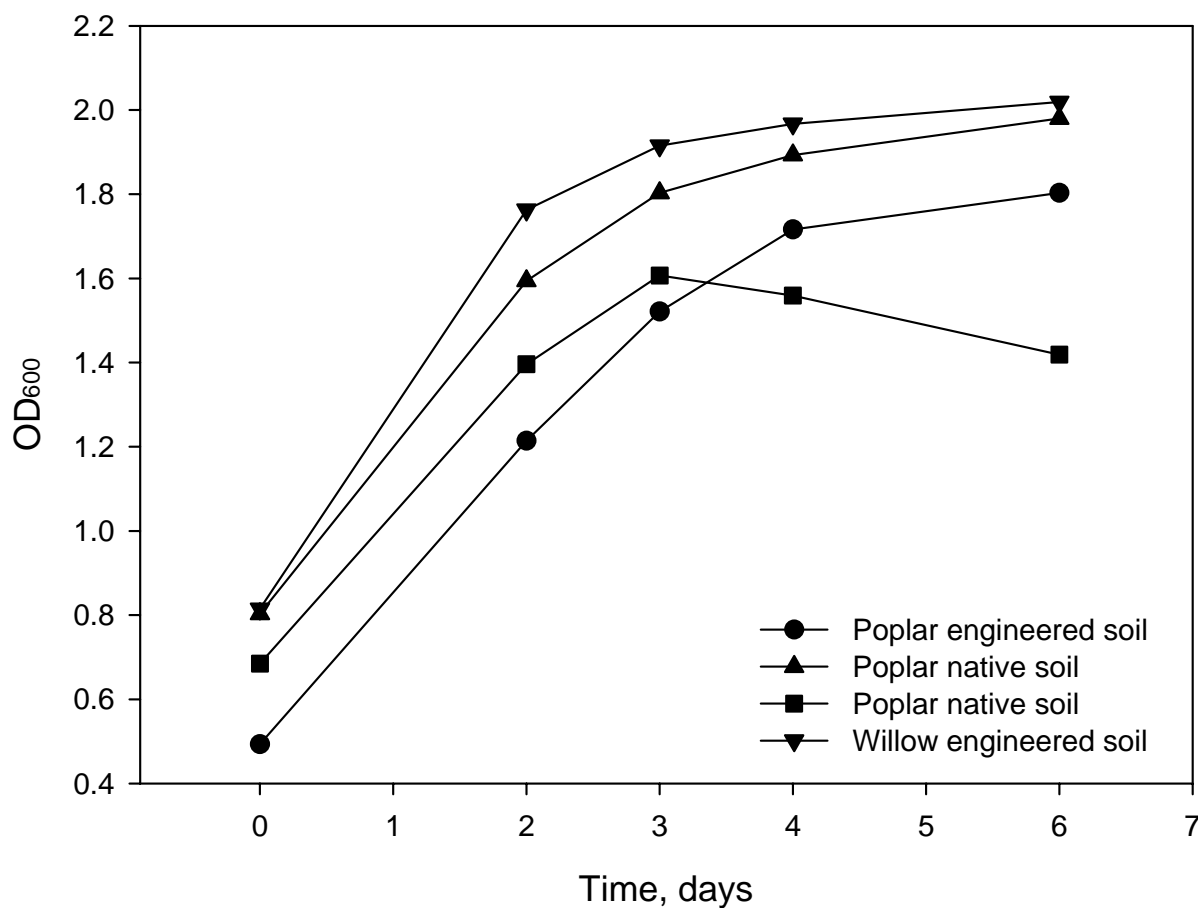


Aerobic biphenyl degradation pathway. Haddock et al 1995

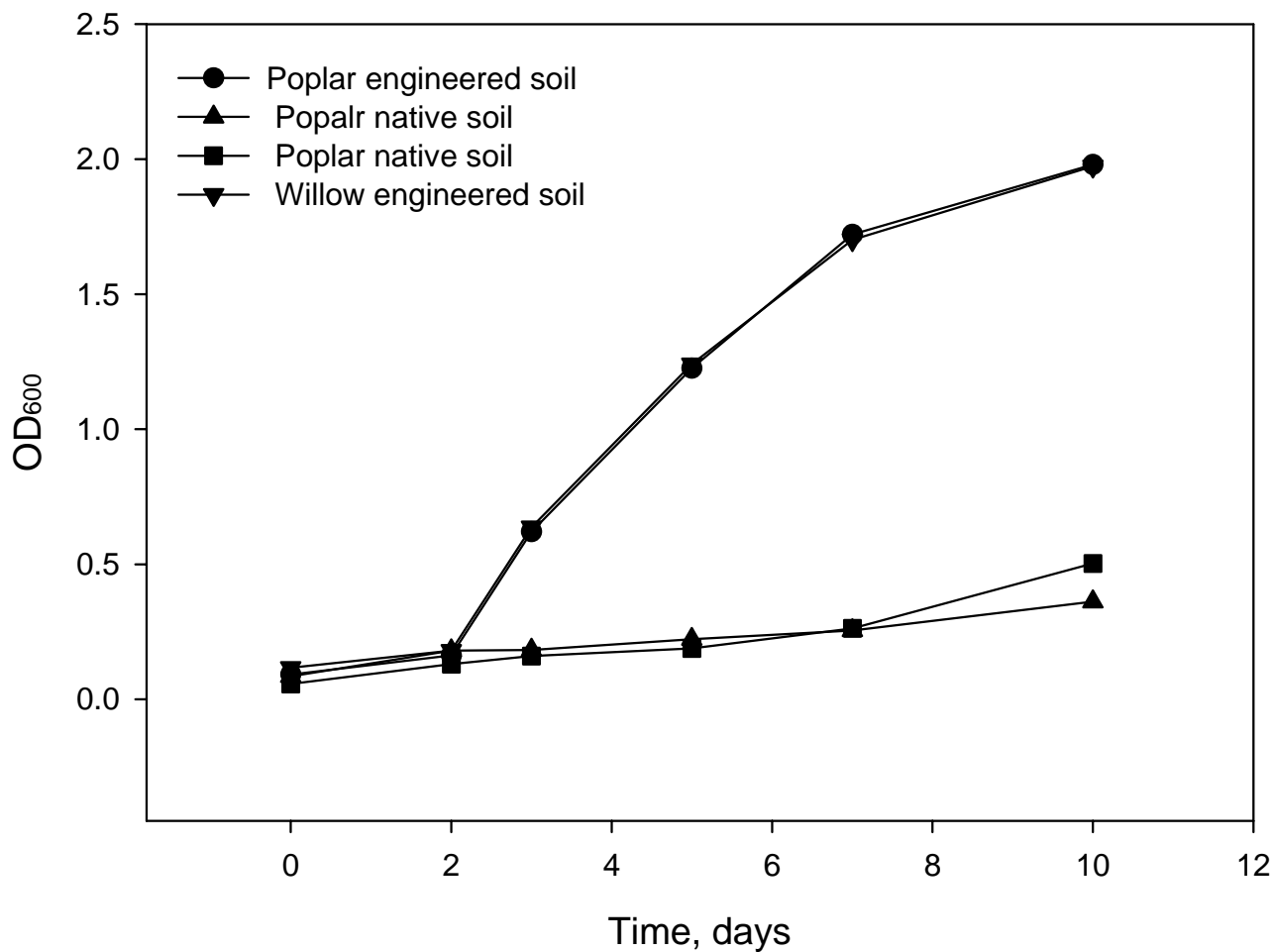
Enrichment of biphenyl degraders from soil

- ◆ Serial enrichment to obtain biphenyl degraders
- ◆ Rhizosphere soil from poplar and willow trees as inocula
- ◆ Basal salts medium (BSM) as culture medium

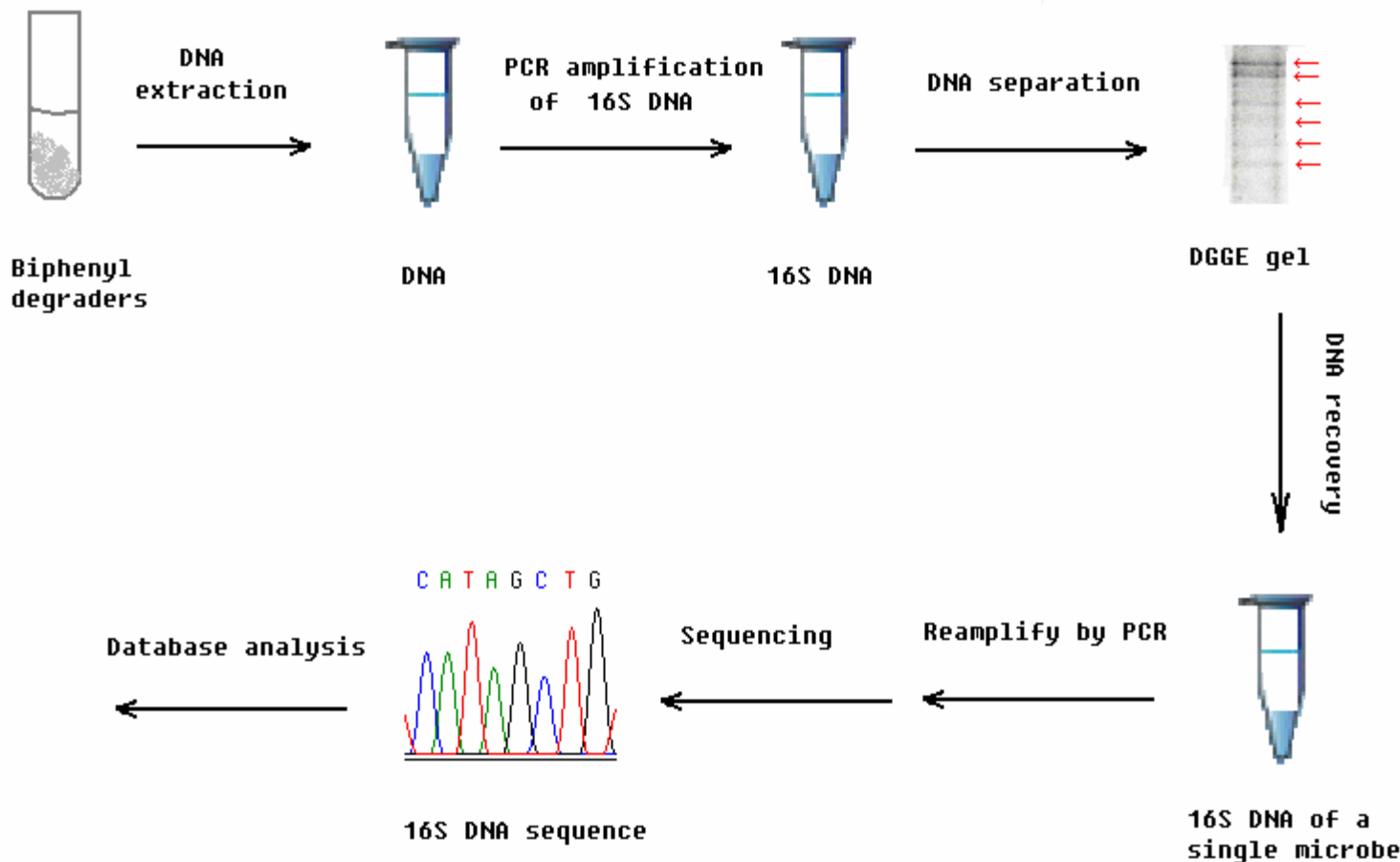
Aerobic growth on biphenyl at 25° C



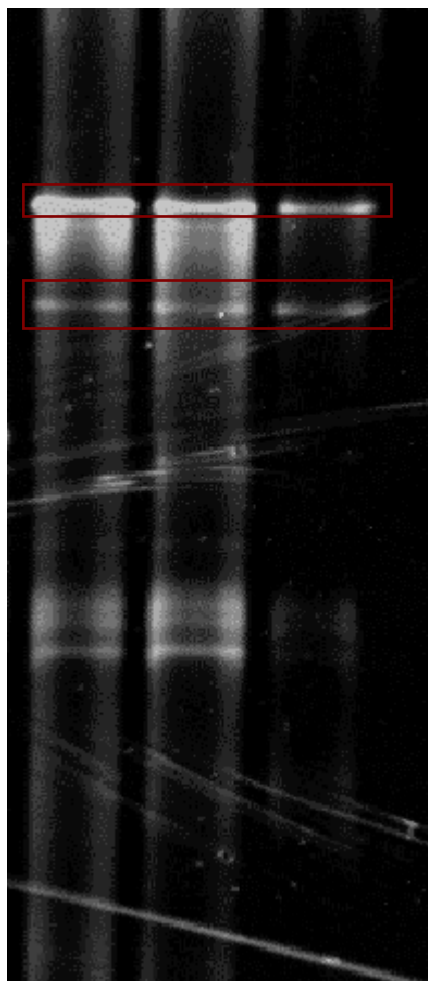
Aerobic growth on biphenyl at 8°C



Identify microbial population



DGGE analysis of biphenyl degraders



- ◆ One consortium with highest biphenyl degradation was analyzed by DGGE
- ◆ Two major members were identified as *Burkholderia xenovorans* LB400 and a strain of *Burkholderia xenovorans*

Anaerobic degradation: nitrate reduction

- ◆ Rockne and Strand (1998)
 - 3.2 ppm biphenyl degraded by enriched culture in ~ 10 days but not coupled to nitrate reduction
- ◆ Rockne and Strand (2001)
 - ¹⁴C-biphenyl degraded to CO₂ (55%) and biomass (23%) after 80 days
- ◆ Grishchenkov et al. (2002)
 - 27% biphenyl (150 ppm) degraded in 3 days, ~ 10% mineralized

Anaerobic degradation: Sulphate-reduction

- ◆ Rockne and Strand (1998)
 - creosote-contaminated marine sediment
 - 5.5 ppm biphenyl within 18 weeks

Anaerobic degradation: Methanogenesis

- ◆ Natarjan et al. (1999)
 - anaerobic PCB-dechlorinating consortium
 - biphenyl as sole carbon source and as co-metabolite with glucose and methanol
 - 75% (42.2 μmol or 6.5 ppm) degraded in 50 days with CH_4 and CO_2 produced at 2:1 ratio

Enrichment of anaerobic biphenyl degraders

- ◆ Anaerobic microcosms were set up
- ◆ Factors considered
 - Tree type: Poplar and Willow
 - Electron acceptors: NO_3^- , SO_4^{2-} , CO_2
 - Depth of soil inocula: shallow and deep
 - Soil: Native soil and “engineered” soil

Anaerobic biphenyl degradation after 90 days

		NO ₃ ⁻		SO ₄ ²⁻		CO ₂	
		Deep	Shallow	Deep	Shallow	Deep	Shallow
Control	Sterile soil	--	--	--	--	--	--
Poplar	Engineered soil	--	++	--	+	--	+
	Native soil	++	+++	+	+++	+++	+++
Willow	Engineered soil	--	+++	+++	+++	+++	--
	Native soil	--	+	+	+++	+++	--

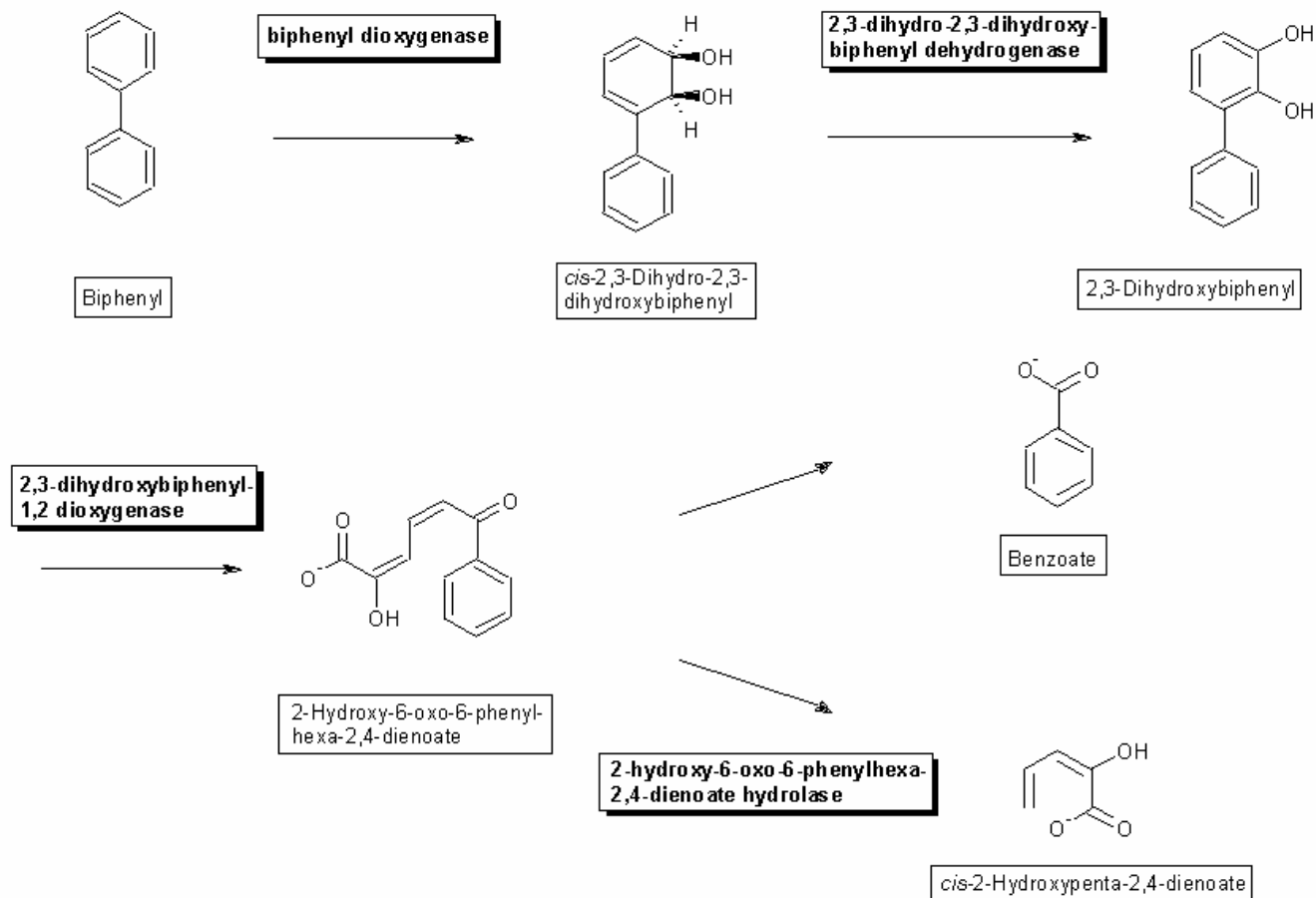
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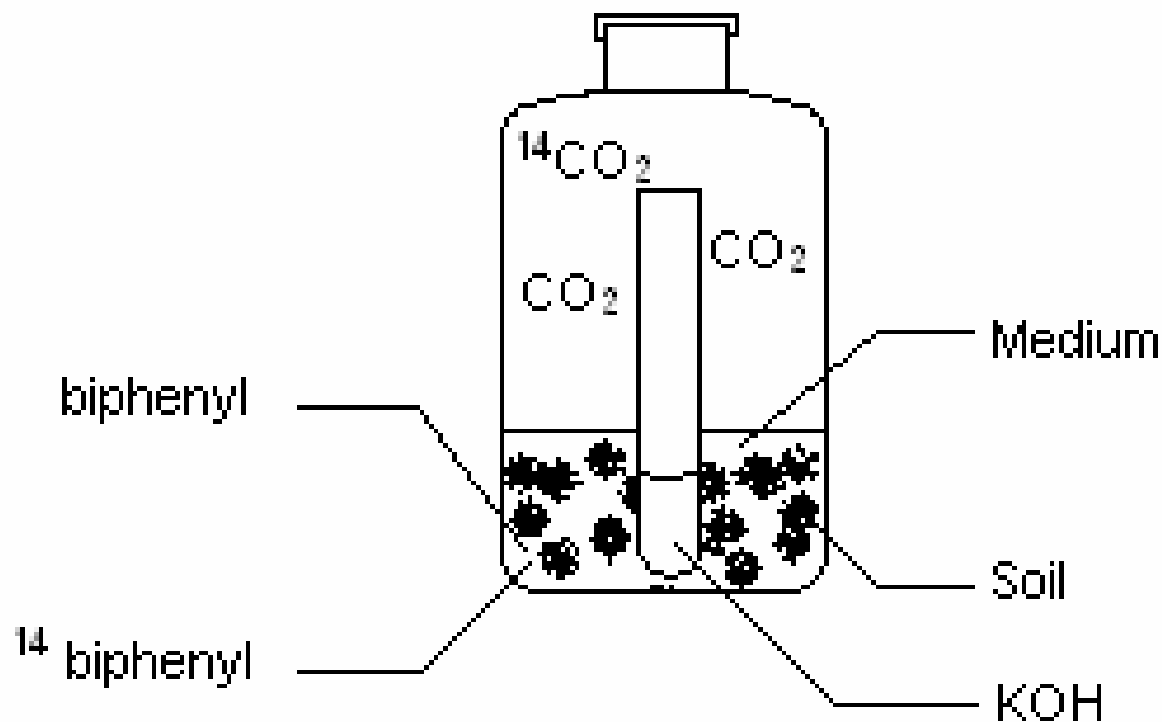
GC/MS analysis of anaerobic biphenyl culture under sulphate-reducing conditions.

Component	Molecular Weight	Concentration (µg/L)
Low MW ester or ether (2-hydroxy propanoic acid)	105	21
Low MW secondary alcohol (C9-C12 alcohol)	154	6
3-(2-methoxyethyl)-1,2-cyclopentanedione)	156	6
Ethoxy ether	135	15
1,1'-oxybis[2-ethoxy-] ethane	128	23
Alkyl ether	228	8
Alkenyl or alkadienyl ether	144	7
Undecyl ether	184	7
1,1'-biphenyl	154	653
Methyl-4,6-decadienyl ether	168	8
Alkyl dienyl methylether	207	7
o-hydroxybiphenyl	170	6
p-hydroxybiphenyl	170	12
Alkyl dienyl methylether	214	5
di-n-butylphthalate		5

Aerobic biphenyl degradation pathway



Anaerobic mineralization experiments



Factors investigated in anaerobic degradation

- ◆ 20 g of soil in 30 mL BSM
- ◆ Factors investigated:
 - Terminal electron acceptor
 - Fertilizer
 - Poplar and willow root exudates

Effects on anaerobic ^{14}C -biphenyl mineralization

	Biphenyl Mineralization
BSM only	1.0
Fertilizer	1.8
SO_4^{2-}	1.3
CO_2	1.5
Poplar root exudates	1.4
Willow root exudates	1.0

Conclusions

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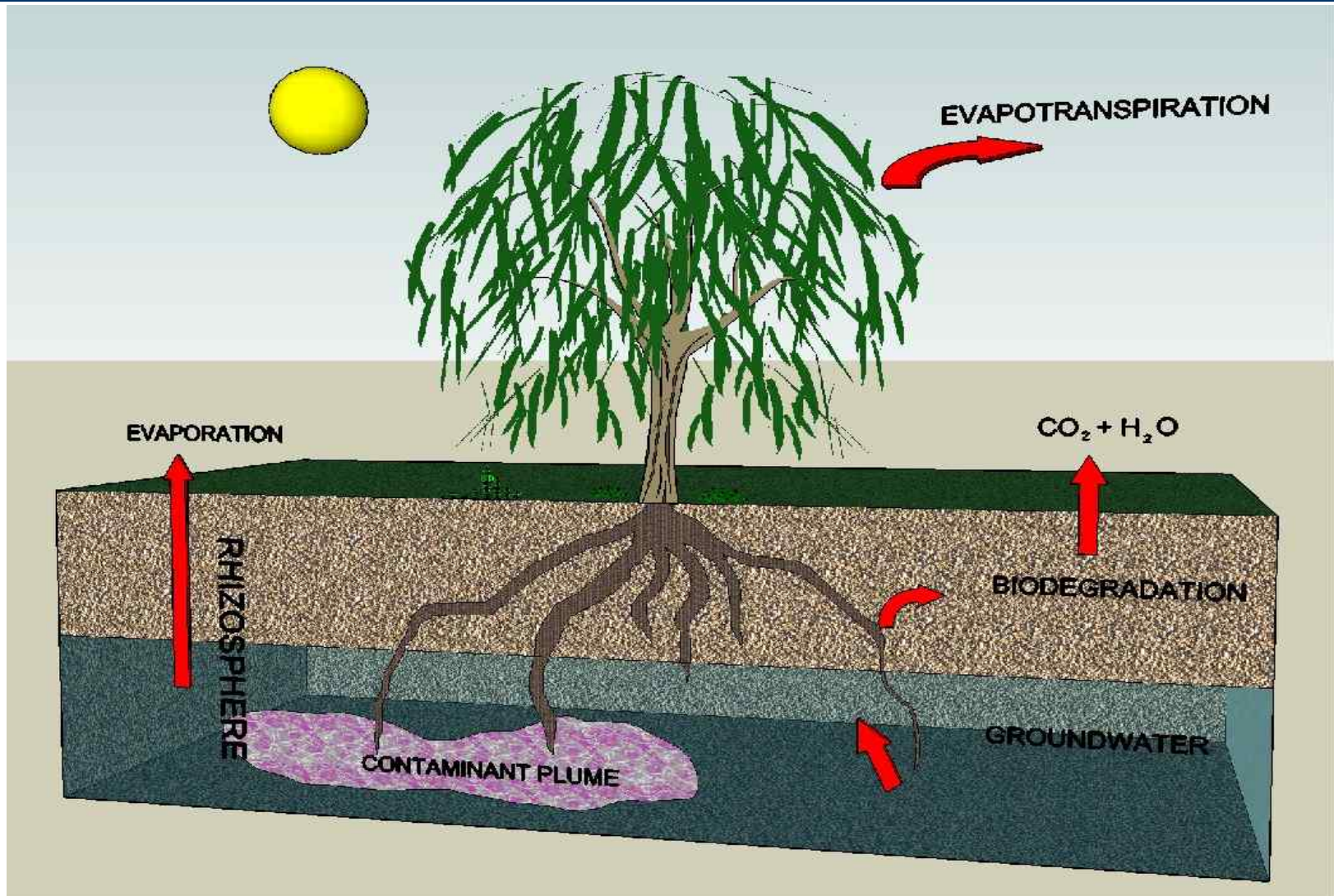
- ◆ Indigenous biphenyl degraders present on site
 - Aerobic and anaerobic degraders were enriched
 - Under aerobic conditions
 - 2000 ppm biphenyl degraded within one week
 - Aerobic biphenyl degraders identified
 - Biphenyl can be degraded at groundwater temperature
 - Under anaerobic conditions
 - Enriched cultures use NO_3^- , SO_4^{2-} , CO_2 as TEA
 - 92.06% and 99.94% biphenyl degradation under nitrate- and sulphate-reducing conditions respectively
 - No significant accumulation of a single intermediate esp benzoic acid

Conclusions

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- Anaerobic biphenyl degradation enhanced
 - By TEA and fertilizer addition
 - Poplar root exudate

Principal of phytoremediation





Acknowledgements

- ◆ Malroz Engineering, Kingston, ON
- ◆ Ontario Centres of Excellence, Centre for Earth and Environmental Technologies
- ◆ NSERC
- ◆ Queen's University

2004



Questions?

2006



2005

