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

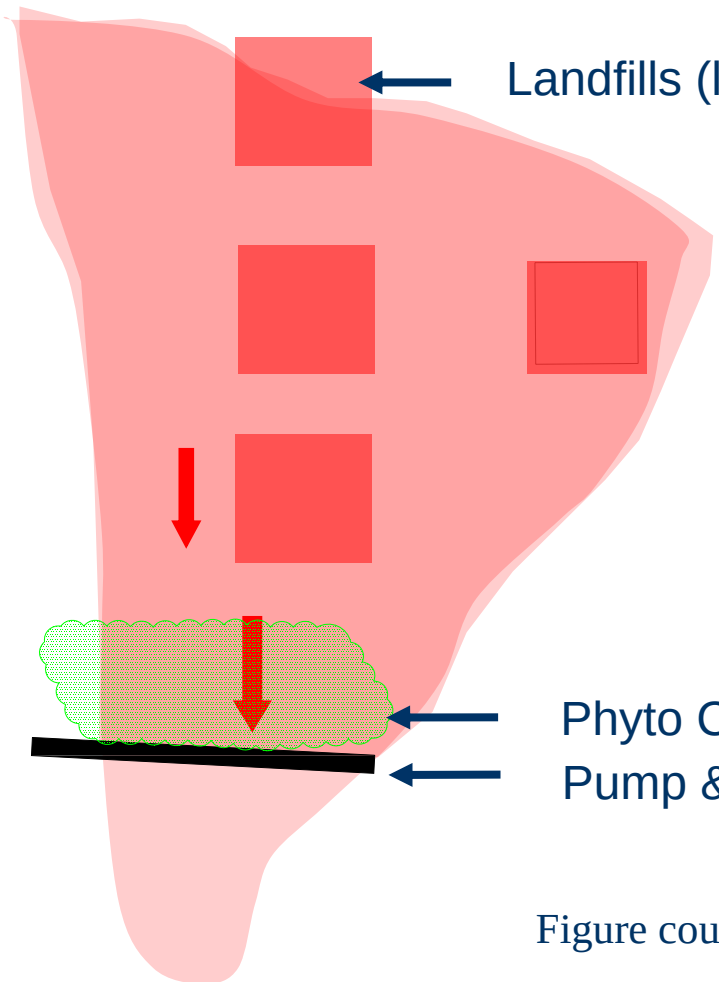
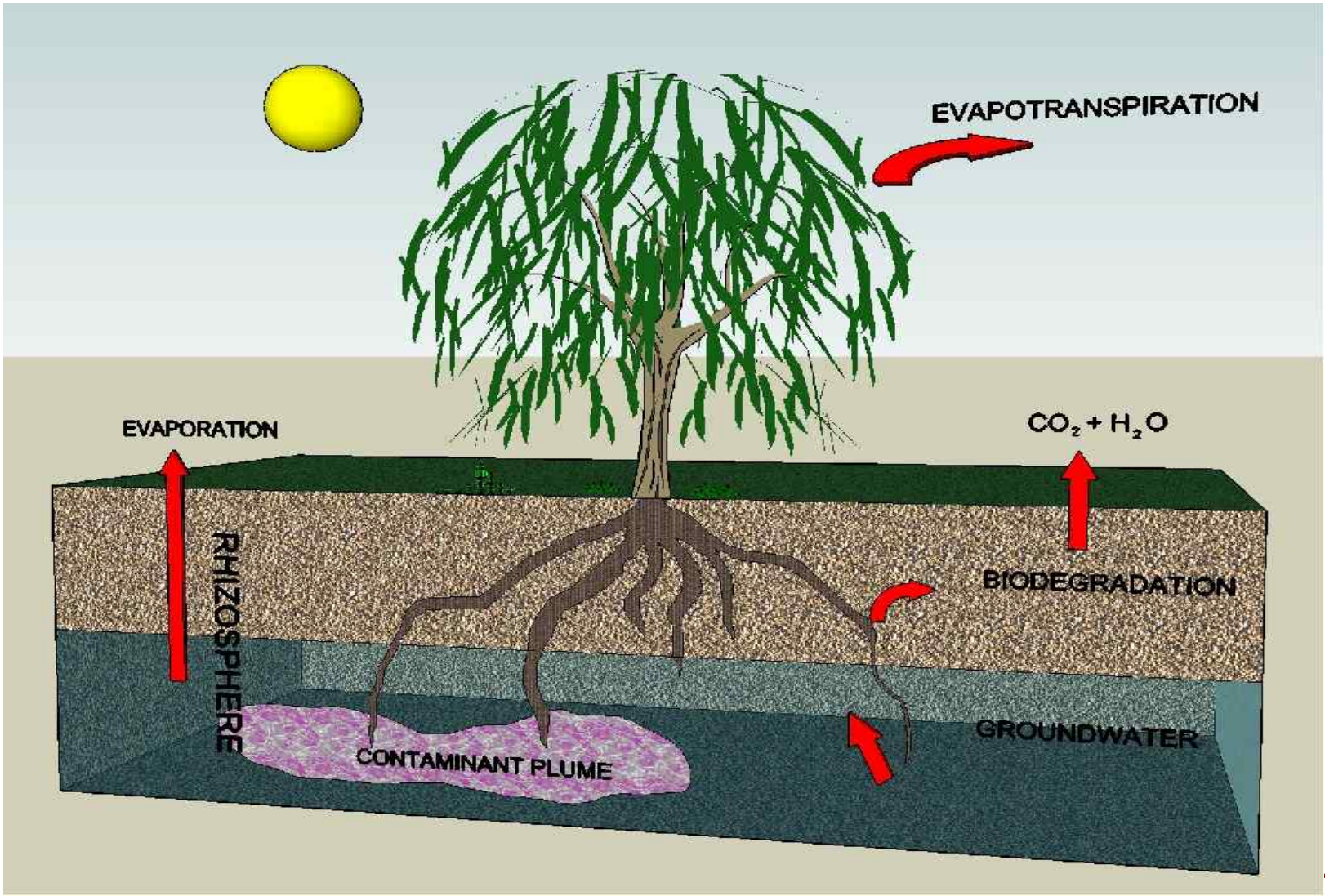


Figure courtesy of Malroz Engineering

Principal of phytoremediation

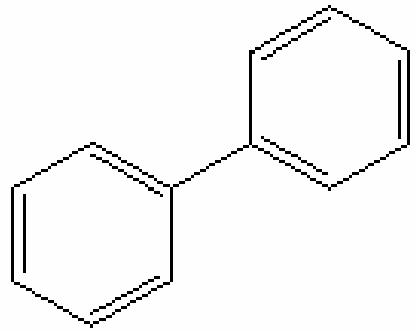


Research objective

- 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



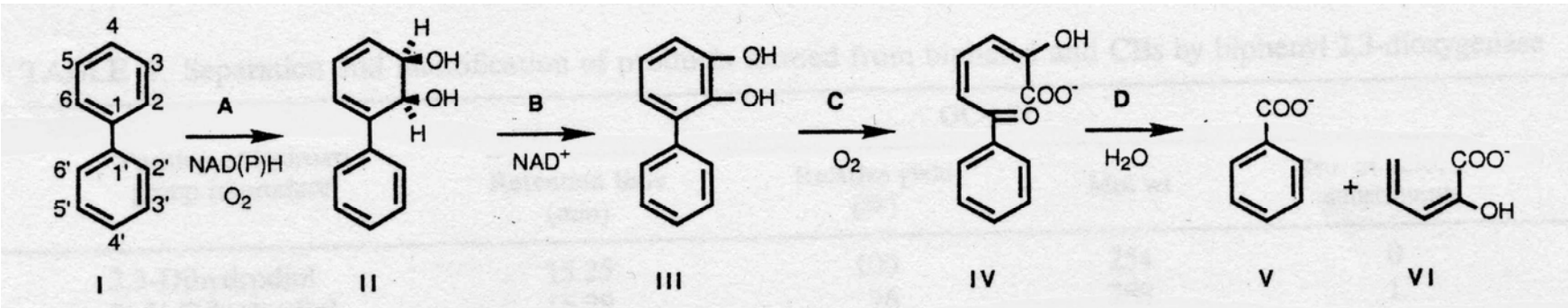
- 885,000 lbs released in USA, 1992
- High environmental hazard rating
- Linked primarily to kidney damage, also to hormonal and CNS disruption

Properties of biphenyl

- Poor water solubility (7.5 mg/L)
- Sorbs strongly to soil (log Kow = 4.09)
- Relatively high volatility (Henry's law constant = 5.94×10^{-4} atm m^3/mol)

Aerobic biphenyl degradation

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

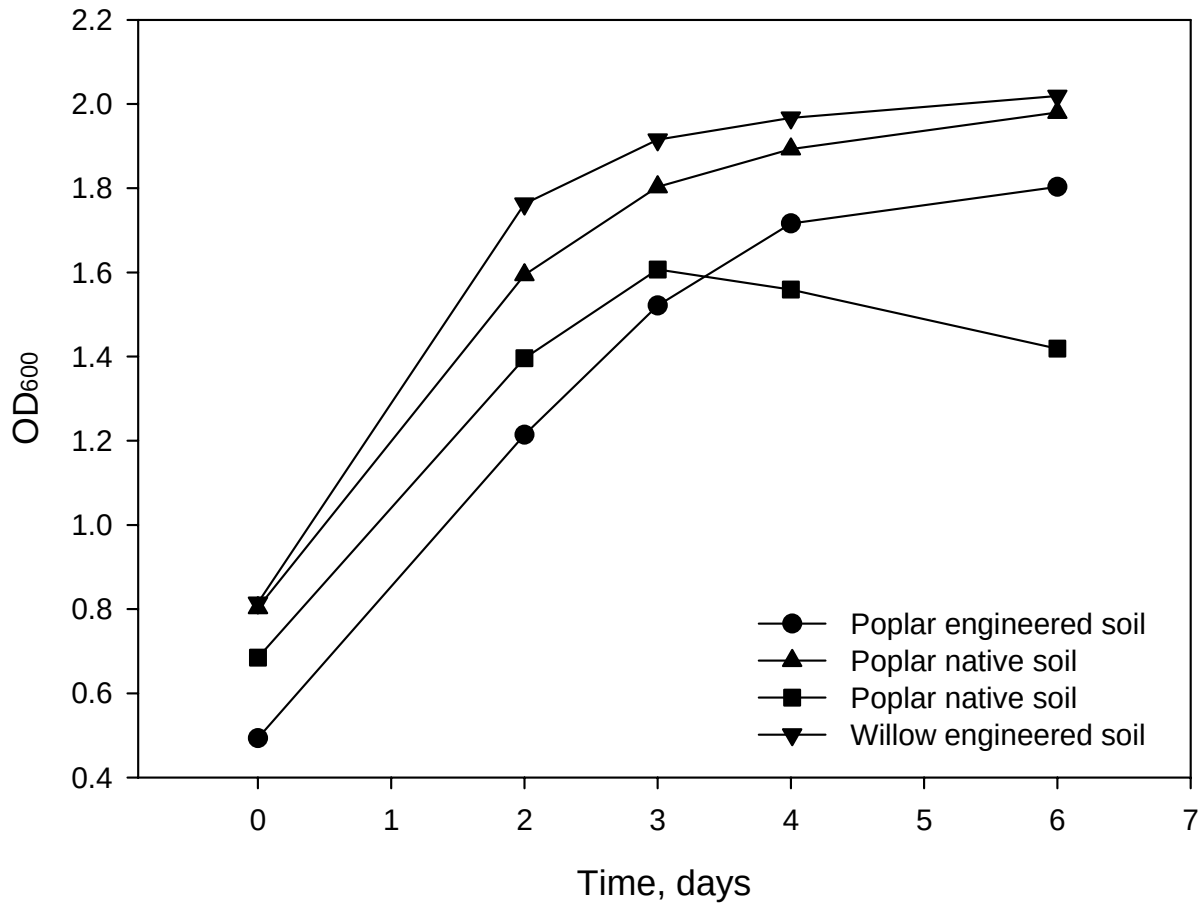


Aerobic biphenyl degradation pathway. Haddock et al 1995

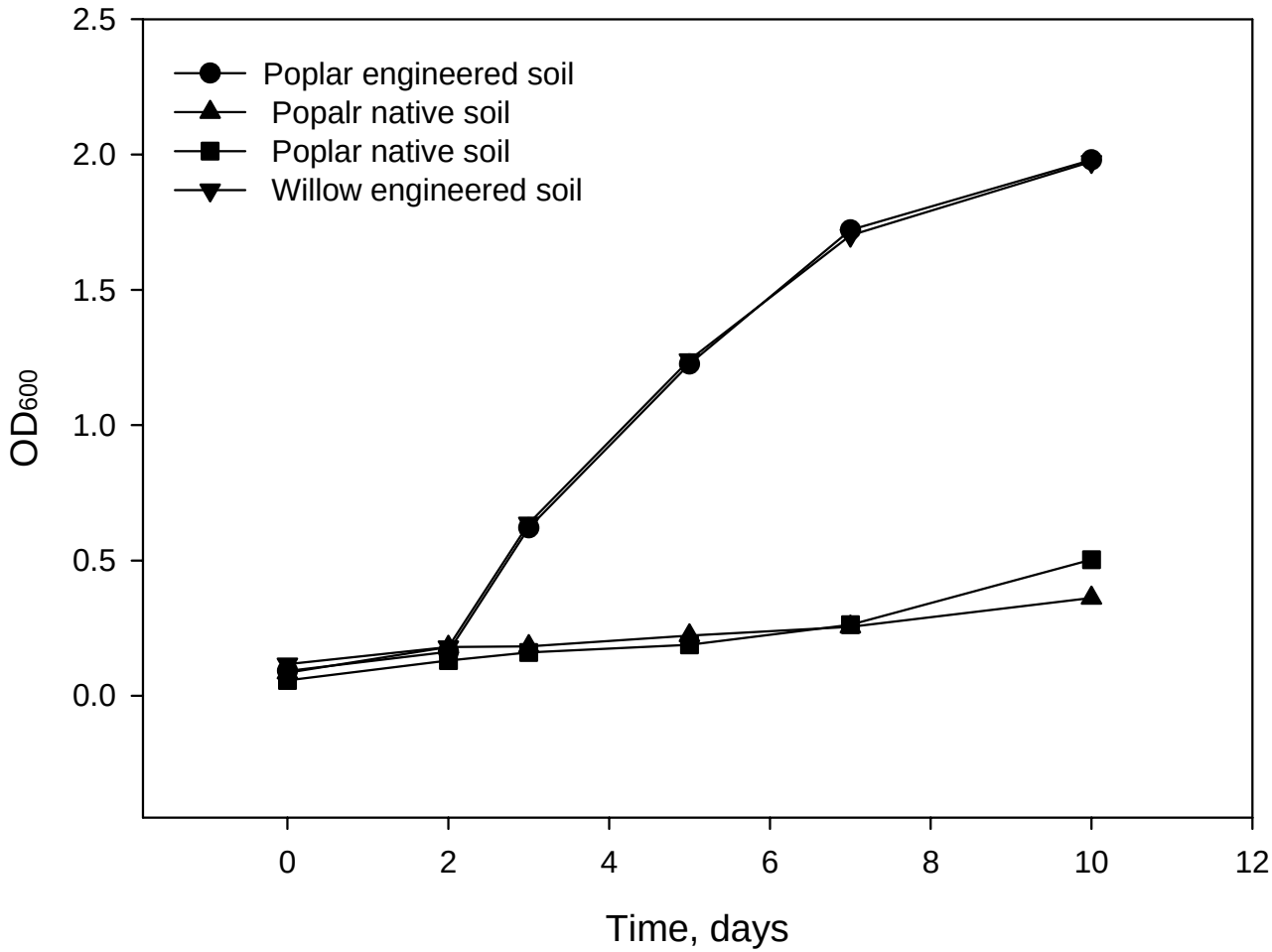
Enrichment of biphenyl degraders from soil

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

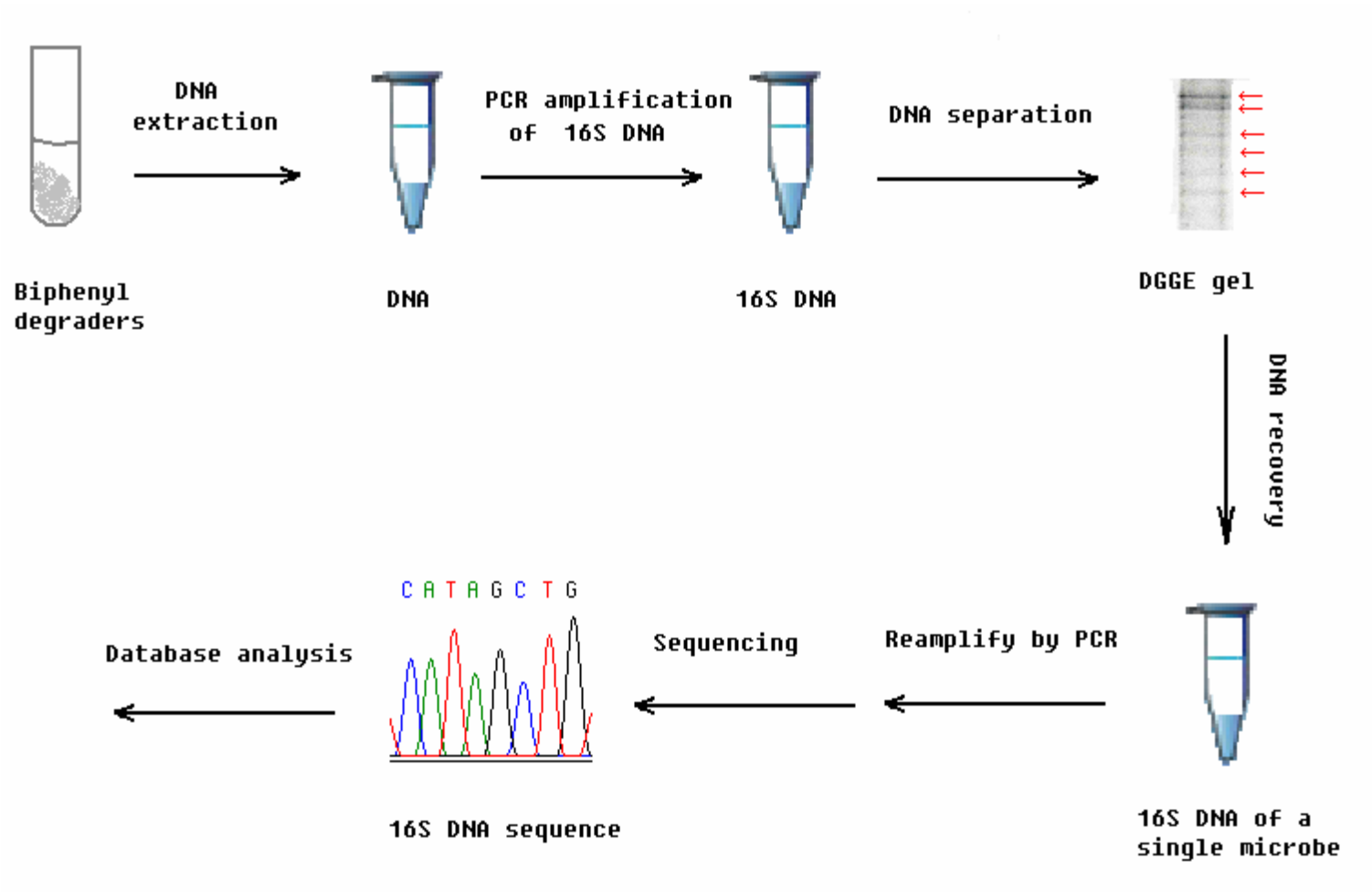
Aerobic growth on biphenyl at 25° C



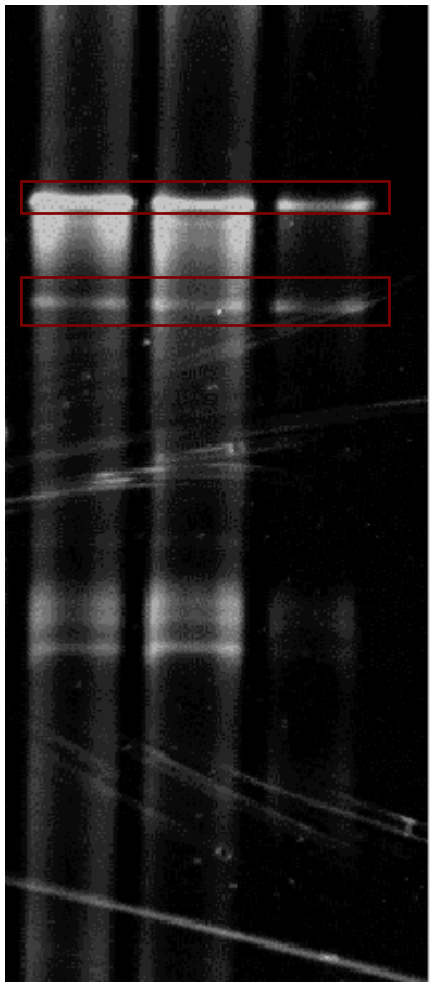
Aerobic growth on biphenyl at 8°C



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 CO_2 (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: Methanogensis

- Natarjan et al. (1999)
  - anaerobic PCB-dechlorinating consortium
  - biphenyl as sole carbon source and as co-metabolite with glucose and methanol
  - 75% (42.2 umol 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 accepters: 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

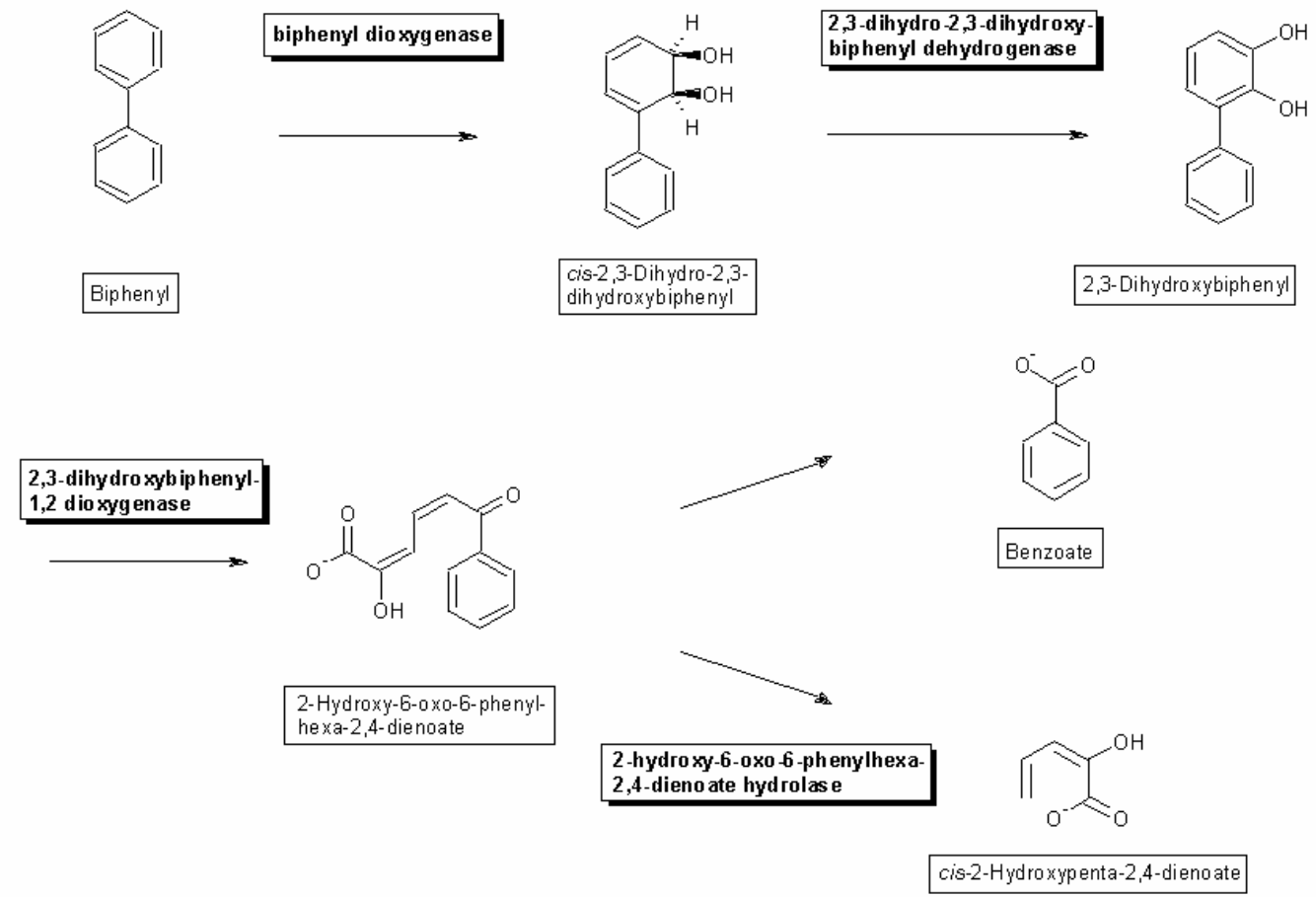
		NO_3^-		SO_4^{2-}		CO_2	
		Deep	Shallow	Deep	Shallow	Deep	Shallow
Control	Sterile soil	--	--	--	--	--	--
Poplar	Engineered soil	--	++	--	+	--	+
	Native soil	++	+++	+	+++	+++	+++
Willow	Engineered soil	--	+++	+++	+++	+++	--
	Native soil	--	+	+	+++	+++	--

(--) 0-25% degradation
(+) 25-50% degradation
(++) 50-75% degradation
(+++) >75% degradation

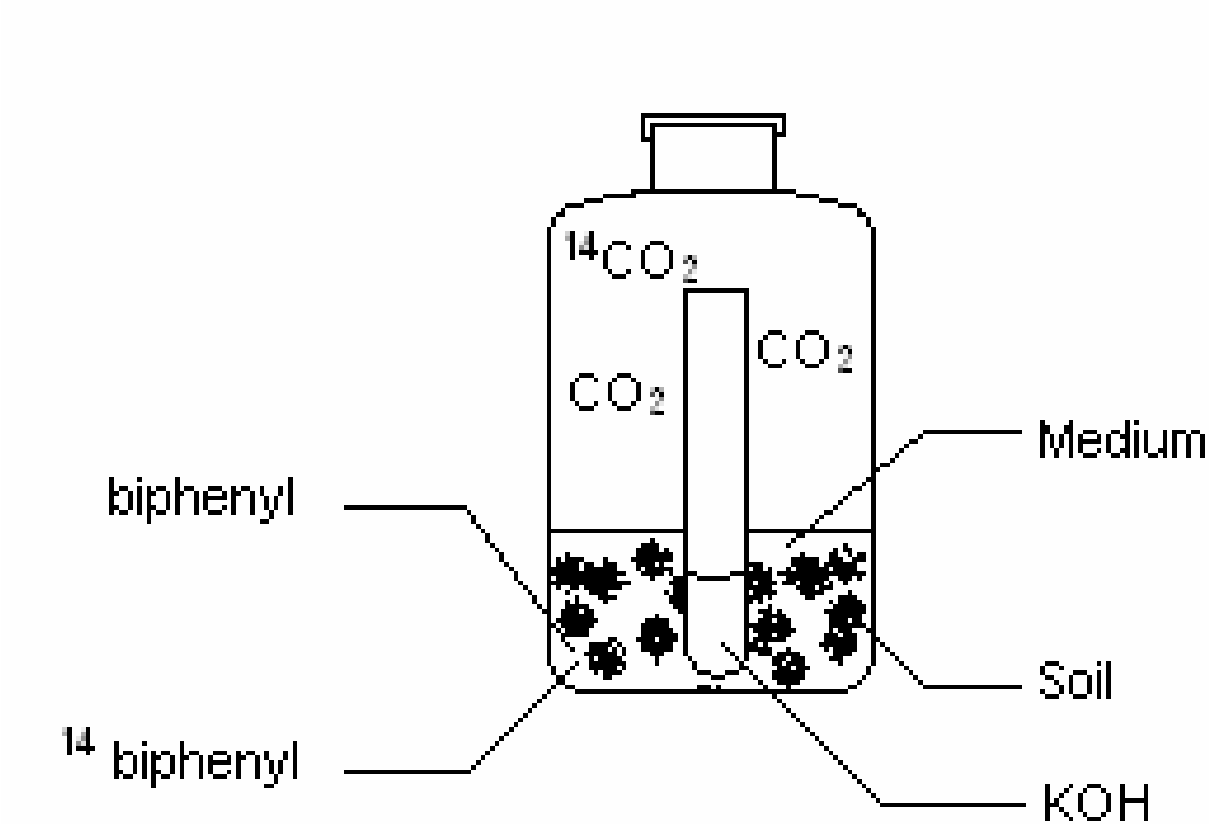
GC/MS analysis of anaerobic biphenyl culture under sulphate-reducing conditions.

Component	Molecular Weight	Concentration (µg/L)
Low MW ester or ether (2-hydroxy propanoic acid)	105	21
Low MW seconday 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-hydroxylbiphenyl	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

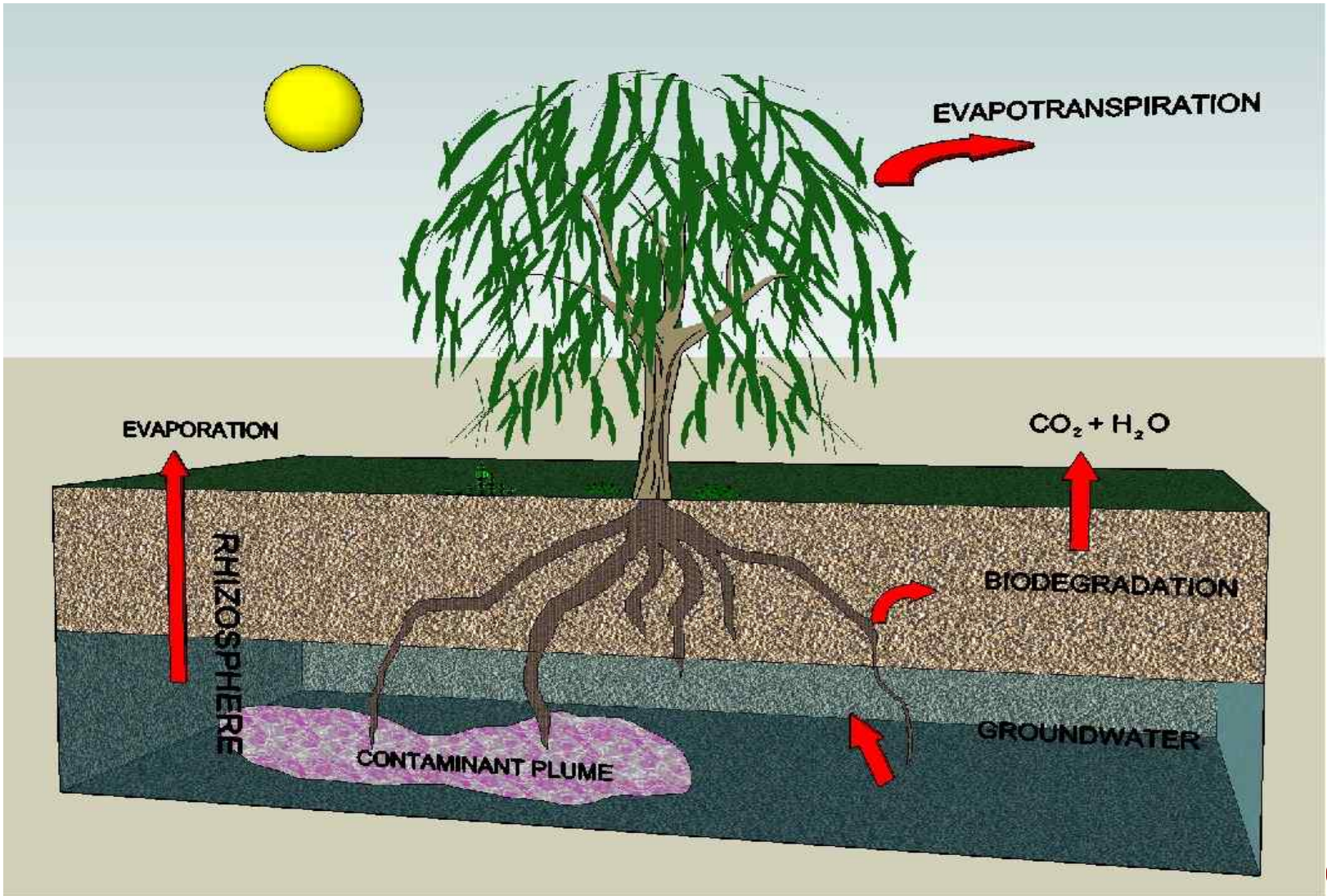
Conclusions 1/2

- 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 2/2

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

Principal of phytoremediation



Acknowledgements

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Questions?