Precision Bioremediation: A New Frontier for the Treatment of Environmental Pollutants

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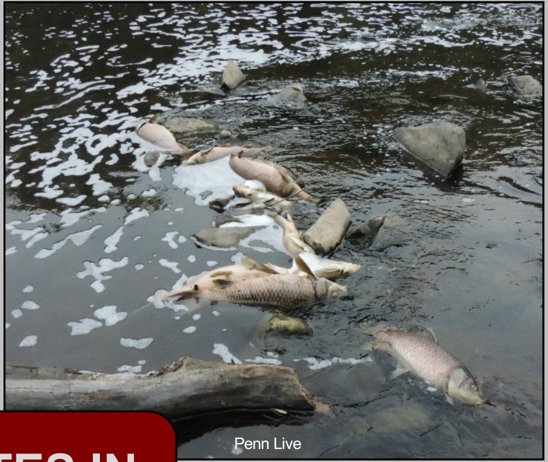
Claudia Gunsch

October 3, 2019

Delta Environmental

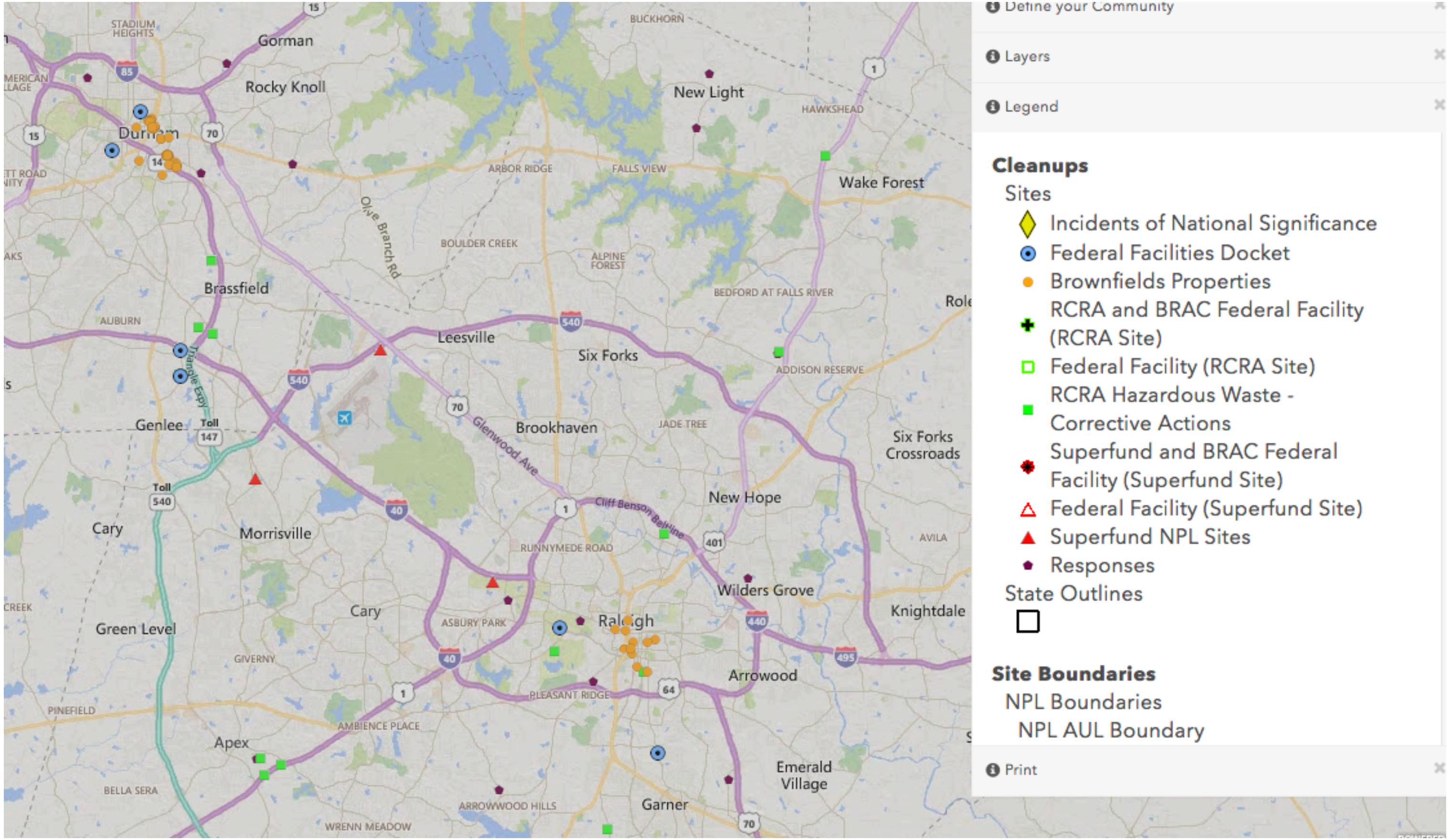


Penn Live

>4,700 WASTE SITES IN THE US

National Geographic

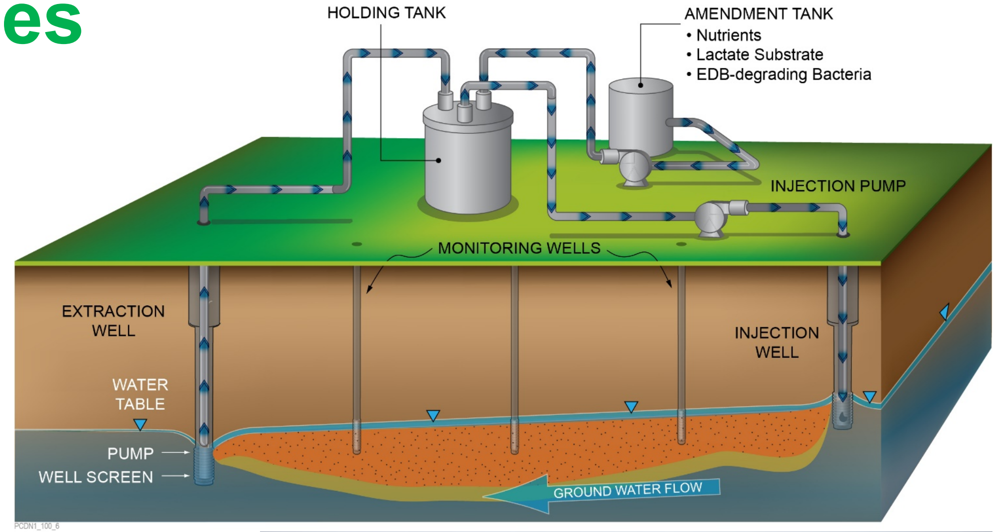
Kuwait photos

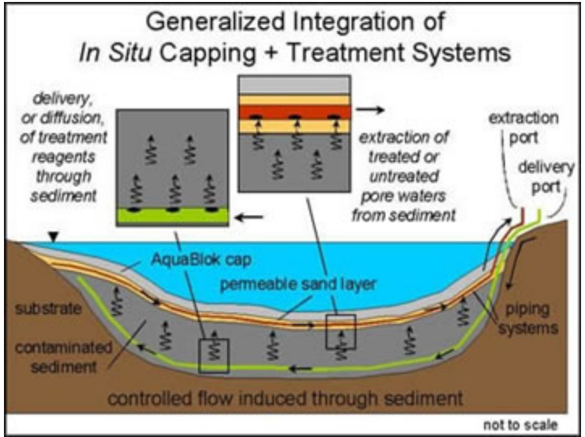


Nearly **53 million** Americans lives within three miles of a major hazardous waste site (EPA, 2014).

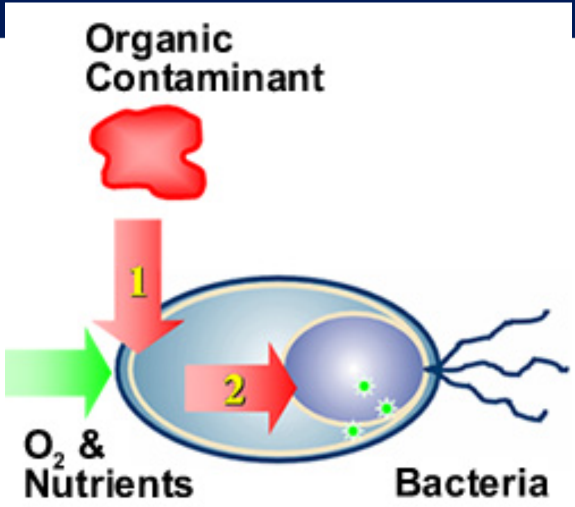
Remediation Technologies

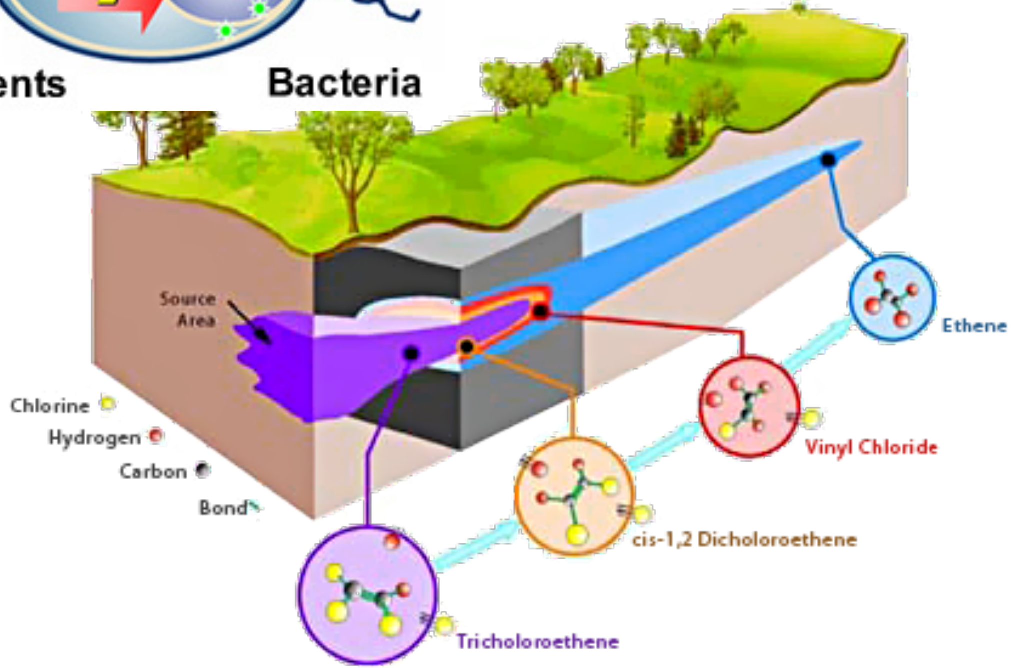
- Pump and Treat
- Soil Vapor Extraction
- Excavation
- Capping
- Vitrification
- …





In Situ Bioremediation



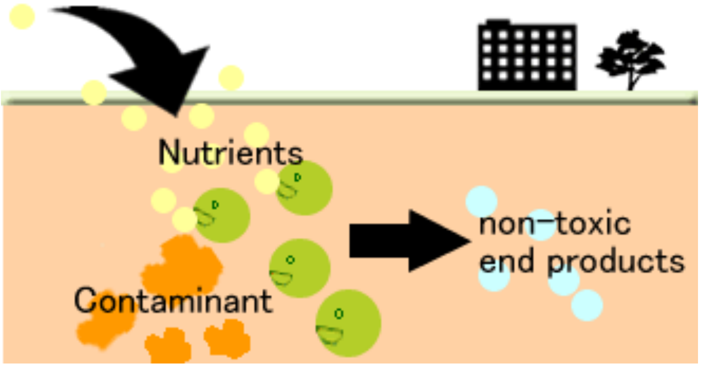


- More sustainable
- Less intrusive
- Cost effective

Challenges

- Slow degradation
- Absence of degradation

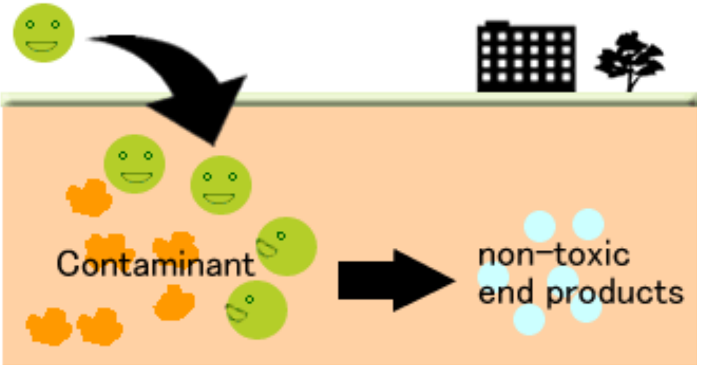
In situ bioremediation can be accelerated by bioaugmentation or biostimulation



Biostimulation

http://www.ecocycle.co.jp

Bioaugmentation



http://www.ecocycle.co.jp

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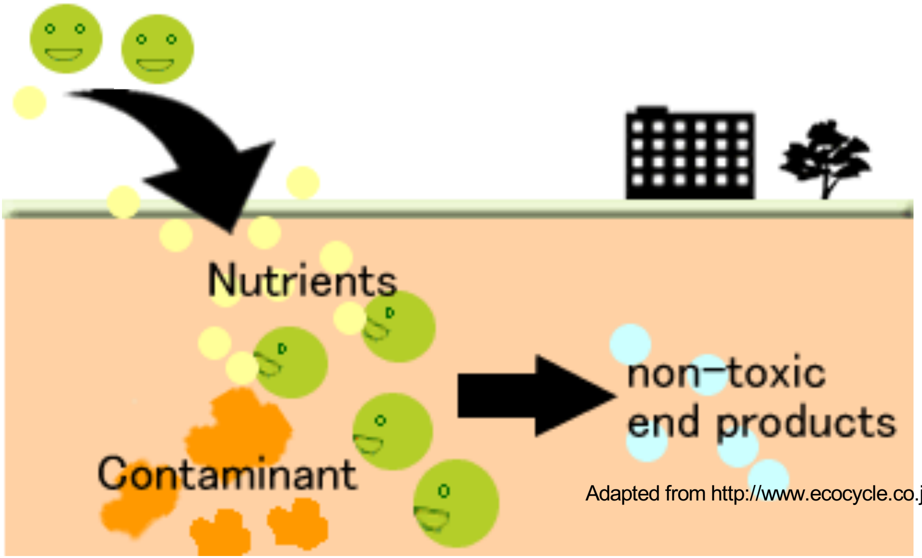
Combined biostimulation & bioaugmentation may result in better biodegradation



Adapted from http://www.ecocycle.co.jp

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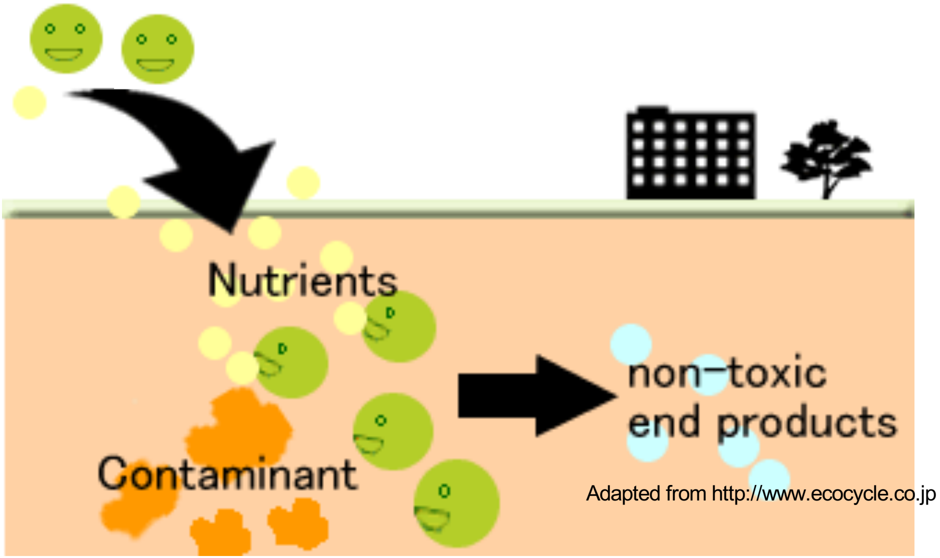
Combined biostimulation & bioaugmentation may result in better biodegradation



BUT amended microbes may not survive in the new environment

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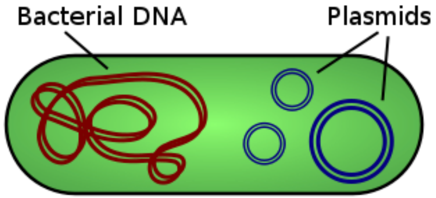
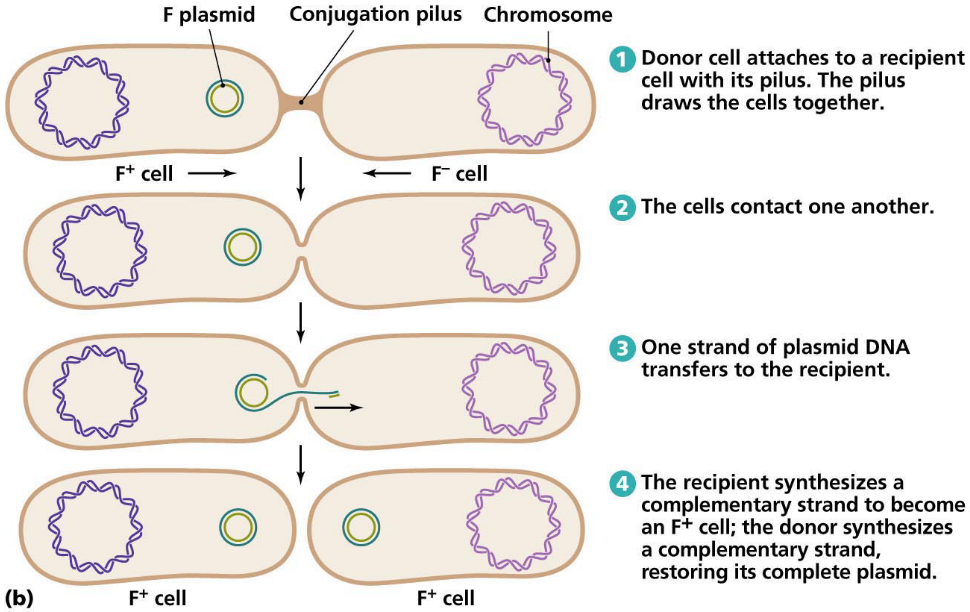
Precision Bioremediation Approach #1



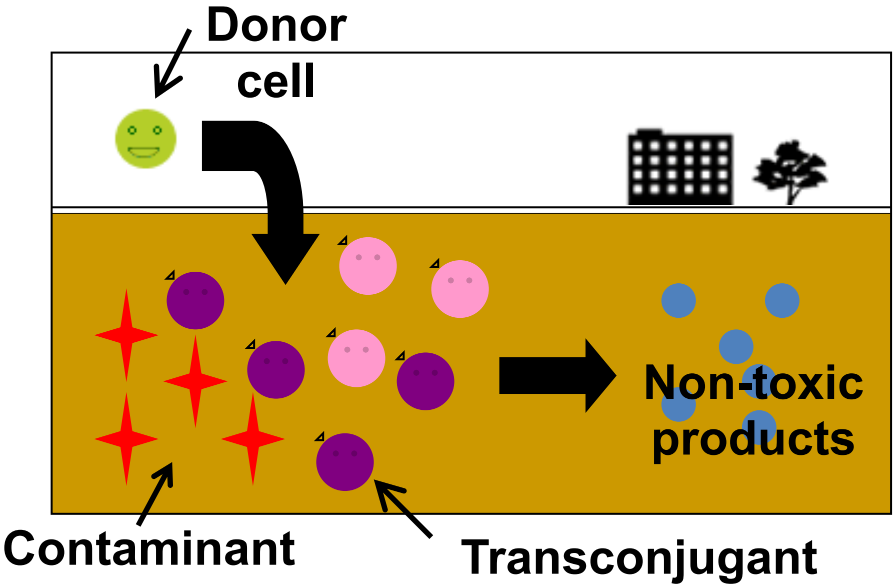
Genes for contaminant degradation are often found on <u>catabolic plasmids</u>!

Plasmid conjugation is a form of horizontal gene transfer



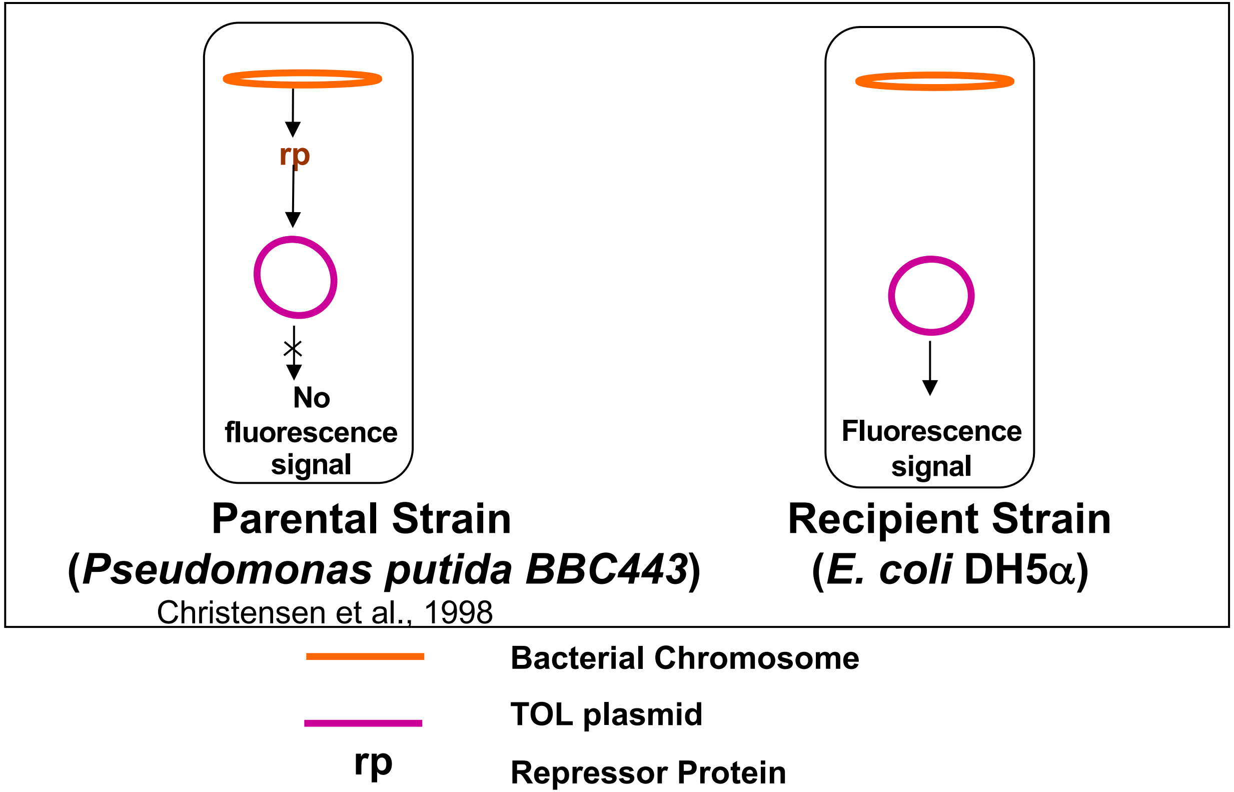
Genetic bioaugmentation using plasmid conjugation as a means for effective bioremediation

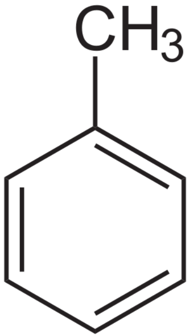
<u>Genetic bioaugmentation</u> = Increasing the amount of microbes capable of degrading certain contaminants by promoting HGT occurrence *in situ*



<u>Advantage</u>: Requires less foreign microbe addition
Donor cells do not need to survive for bioremediation

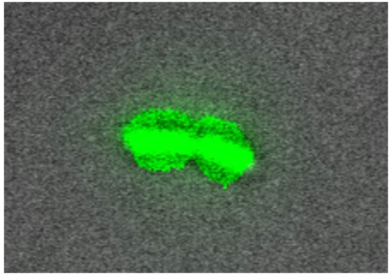
Pseudomonas putida BBC443 harboring a TOL plasmid tagged with GFP and kanamycin resistance used as donors



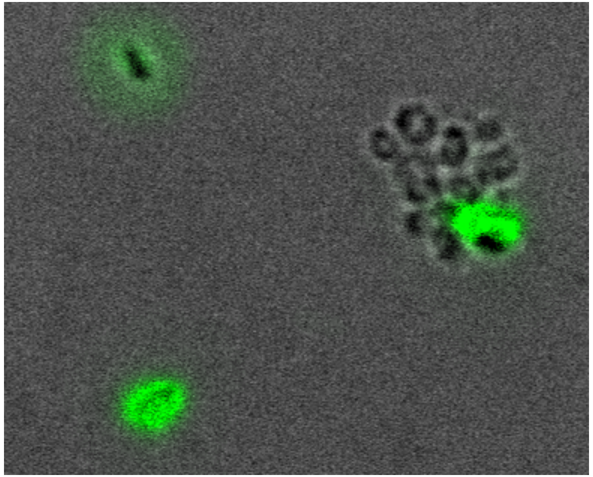


Model Contaminant: Toluene

Transconjugants can be verified through epifluorescence microscopy and flow cytometry

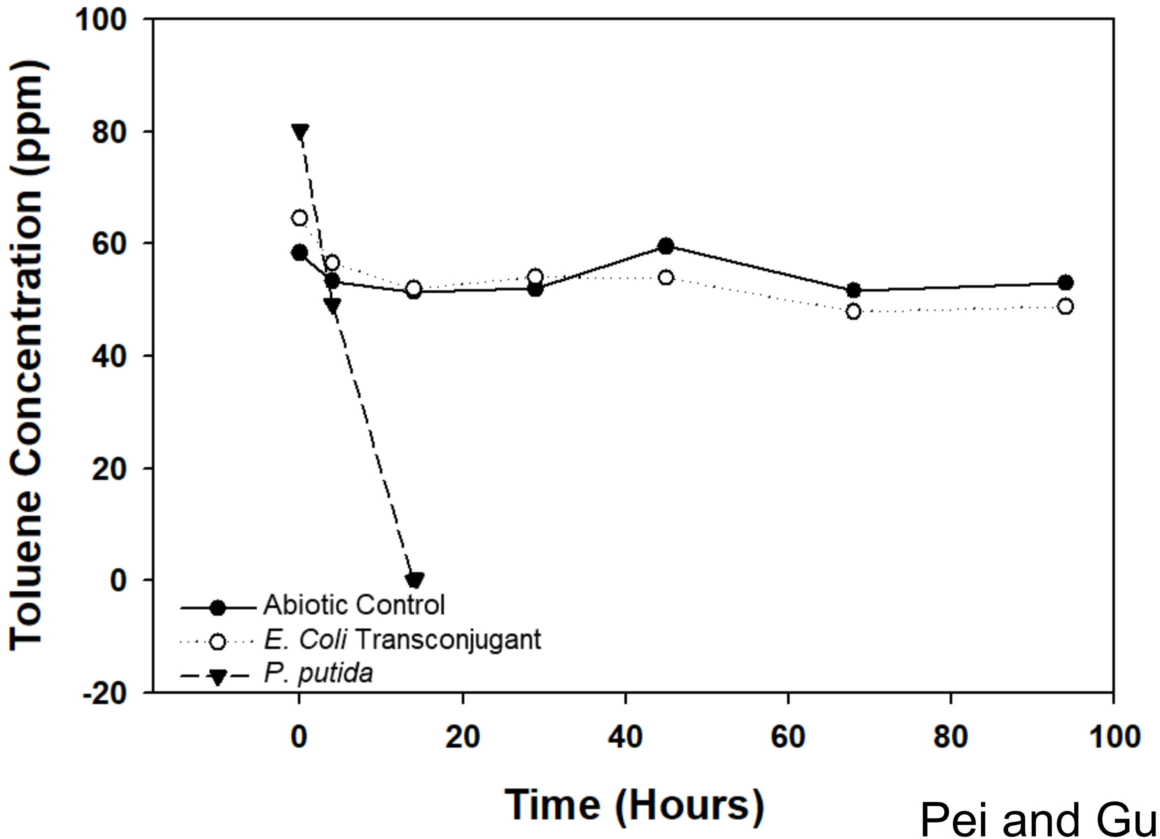


Escherichia coli transconjugant cells



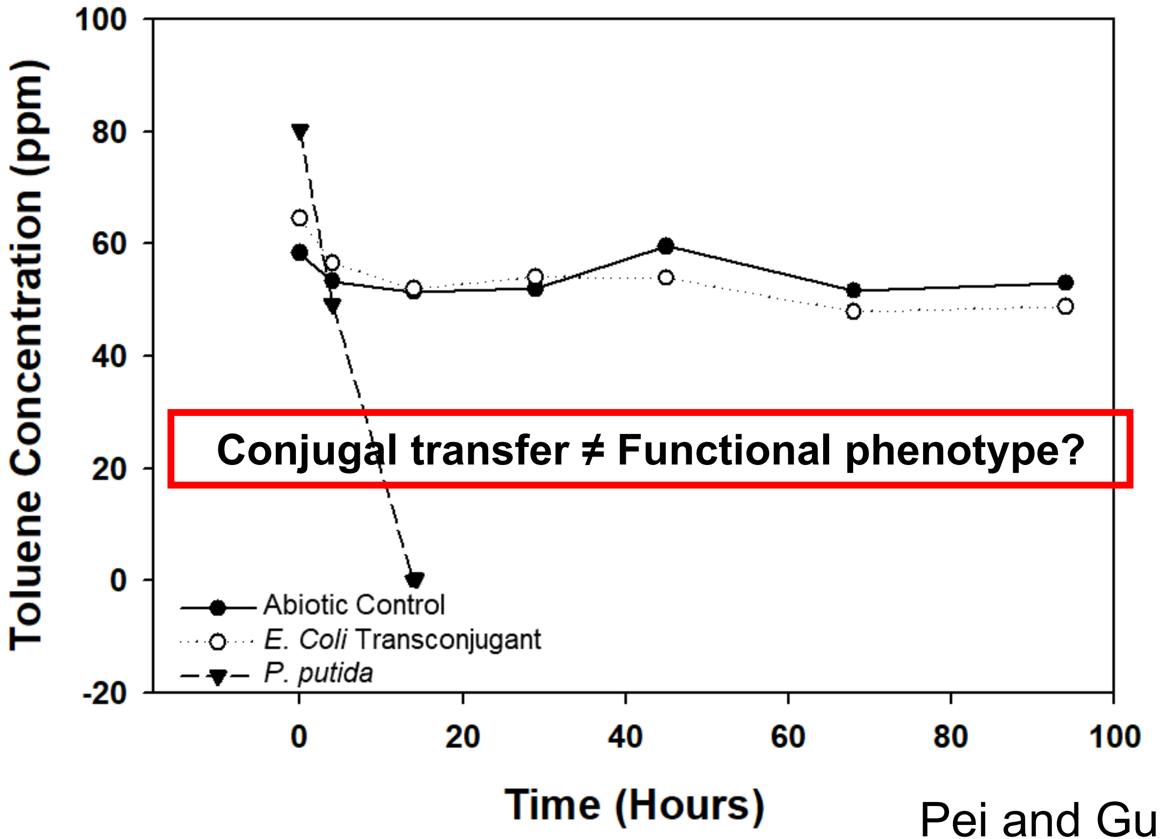
Mixture of *P. putida* cells and *E. coli* transconjugants

E. coli transconjugants harboring the TOL plasmid could not degrade toluene



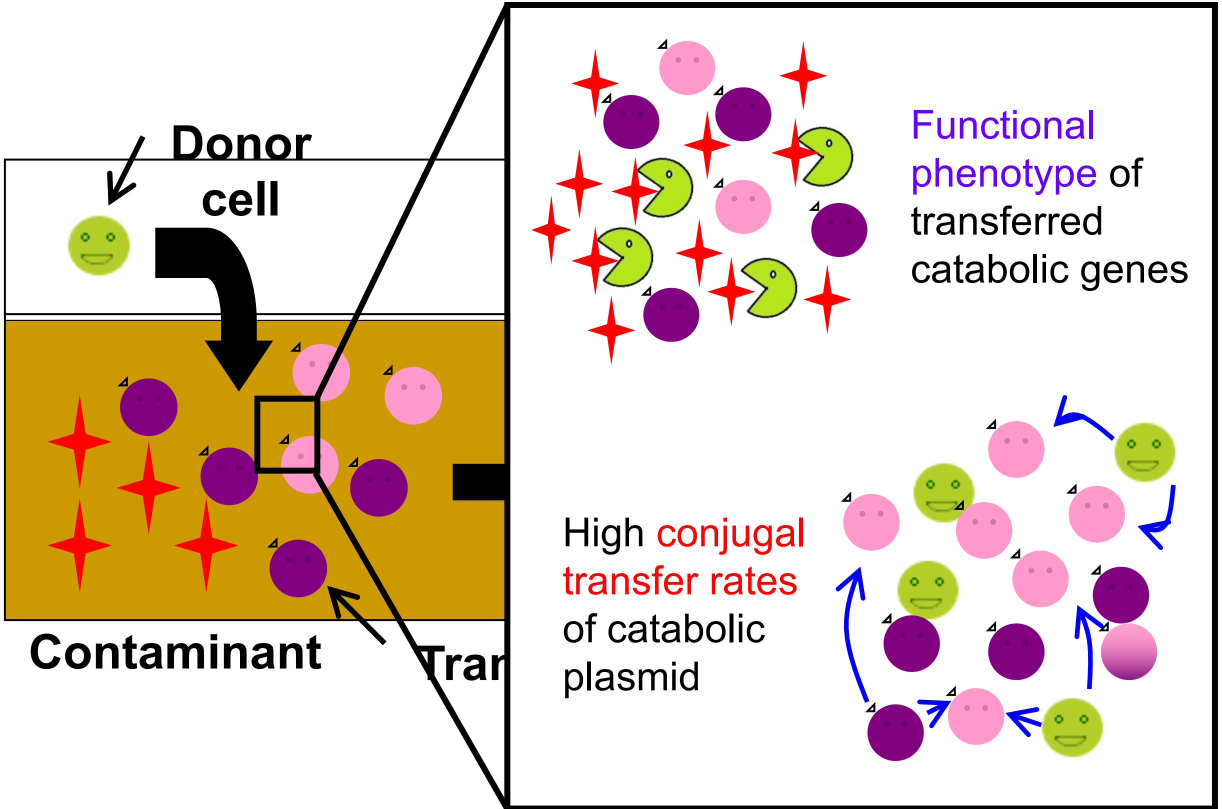
Pei and Gunsch, 2008

E. coli transconjugants harboring the TOL plasmid could not degrade toluene

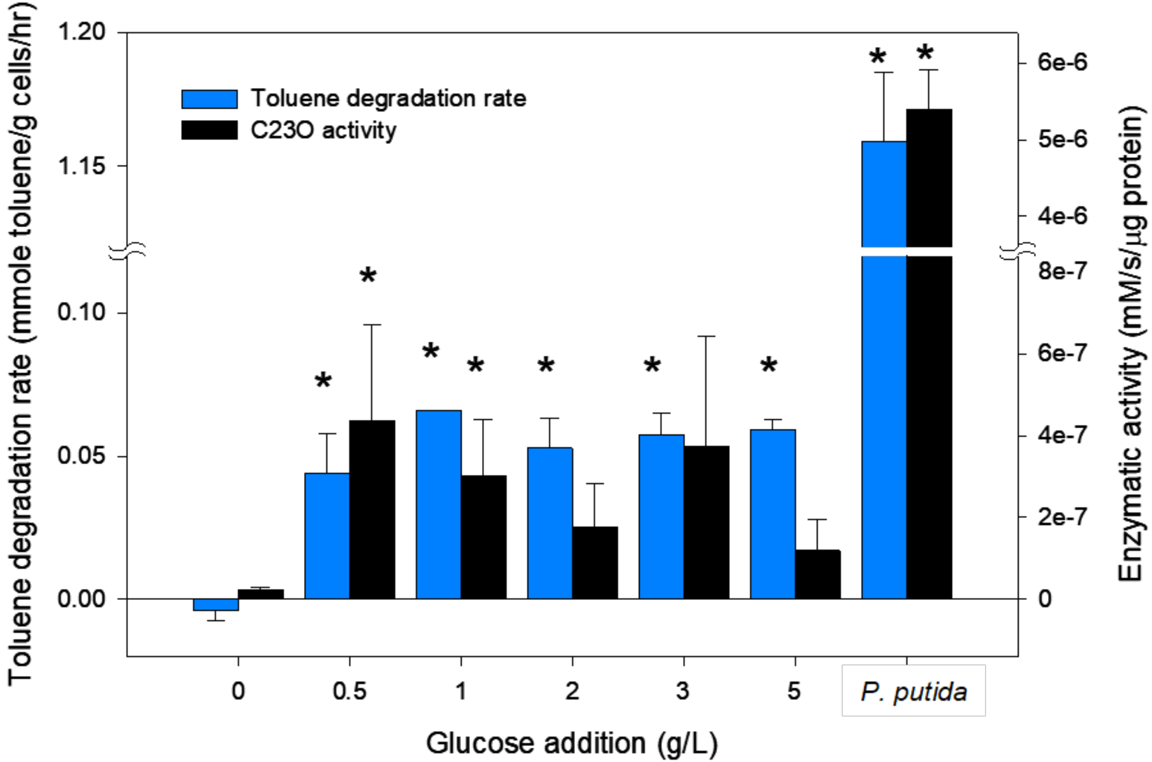


Pei and Gunsch, 2008

High plasmid functionality and transfer rates are necessary in genetic bioaugmentation



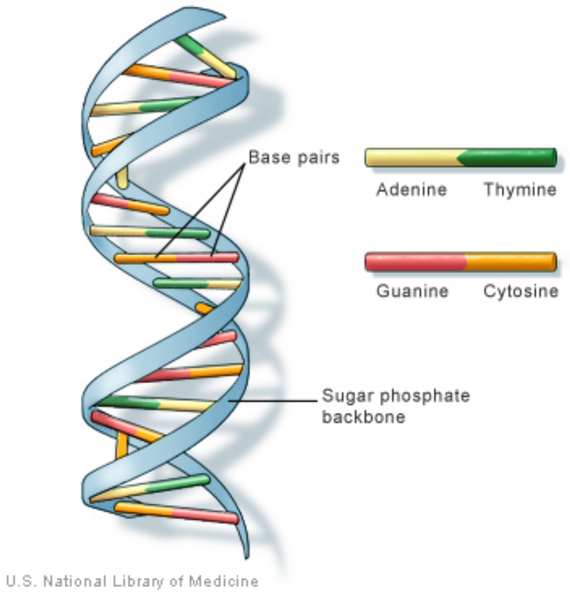
Addition of alternative C source increased TOL plasmid activity in *E. coli* transconjugants



(* indicates statistical significance compared to 0 g/L glucose)

(Ikuma and Gunsch, 2010)

E. coli transconjugants may not have functional phenotypes due to GC content differences



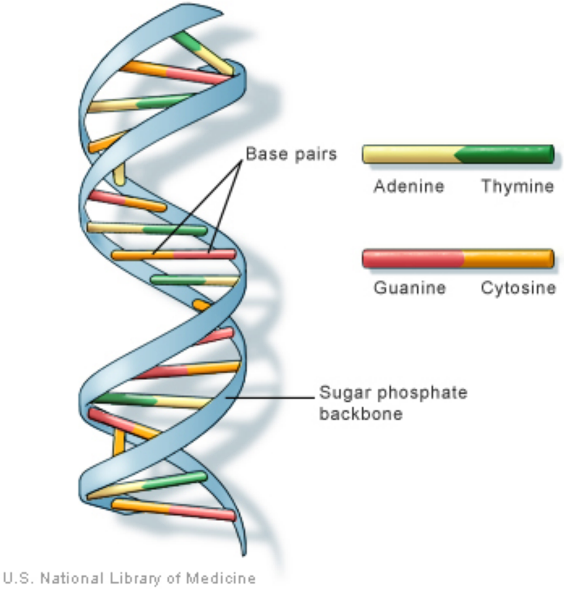

```
ATGGAGCAAA ACCCGCAGTC ACAGCTGAAA
CTTCTTGTCA CCCGTGGTAA GGAGCAAGGC
TATCTGACCT ATGCCGAGGT CAATGACCAT
CTGCCGGAAG ATATCGTCGA TTCAGATCAG
ATCGAAGACA TCATCCAAAT GATCAACGAC
ATGGGCATTC AGGTGATGGA AGAAGCACCG
GATGCCGATG ATCTGATGCT GGCTGAAAAC
ACCGCGGACG AAGATGCTGC CGAAGCCGCC
```

E. coli = 50% GC ⟷ TOL plasmid = 59% GC

~10% difference

P. putida = 60% GC

E. coli transconjugants may not have functional phenotypes due to GC content differences



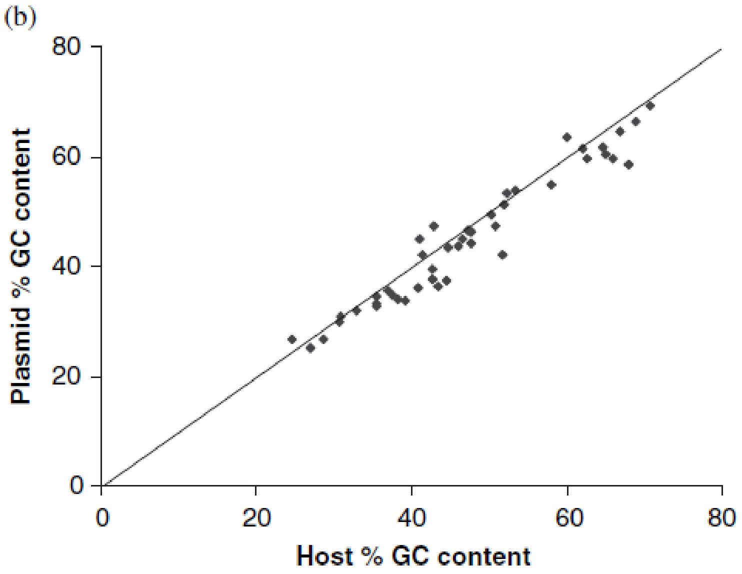
ATGGAGCAAA ACCCGCAGTC ACAGCTGAAA
CTTCTTGTCA CCCGTGGTAA GGAGCAAGGC
TATCTGACCT ATGCCGAGGT CAATGACCAT
CTGCCGGAAG ATATCGTCGA TTCAGATCAG
ATCGAAGACA TCATCCAAAT GATCAACGAC
ATGGGCATTC AGGTGATGGA AGAAGCACCG
GATGCCGATG ATCTGATGCT GGCTGAAAAC
ACCGCGGACG AAGATGCTGC CGAAGCCGCC

E. coli = 50% GC ⟷ TOL plasmid = 59% GC; *P. putida* = 60% GC

~10% difference

Presence of additional carbon source can overcome phenotype functionality issues.

Recipient genomic GC content may play an important role in TOL plasmid functionality



$y = 0.94x + 0.72$
$(n = 43; R^2 = 0.940)$

(Martiny and Field, 2005)

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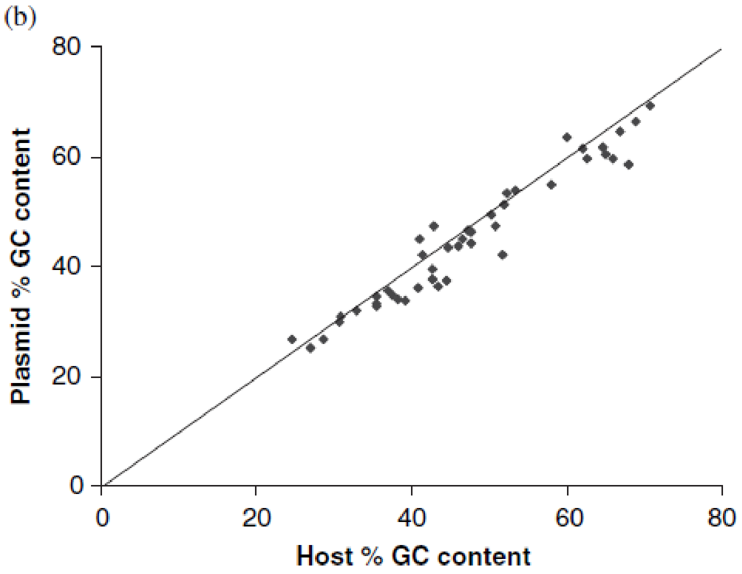
Recipient genomic GC content may play an important role in TOL plasmid functionality

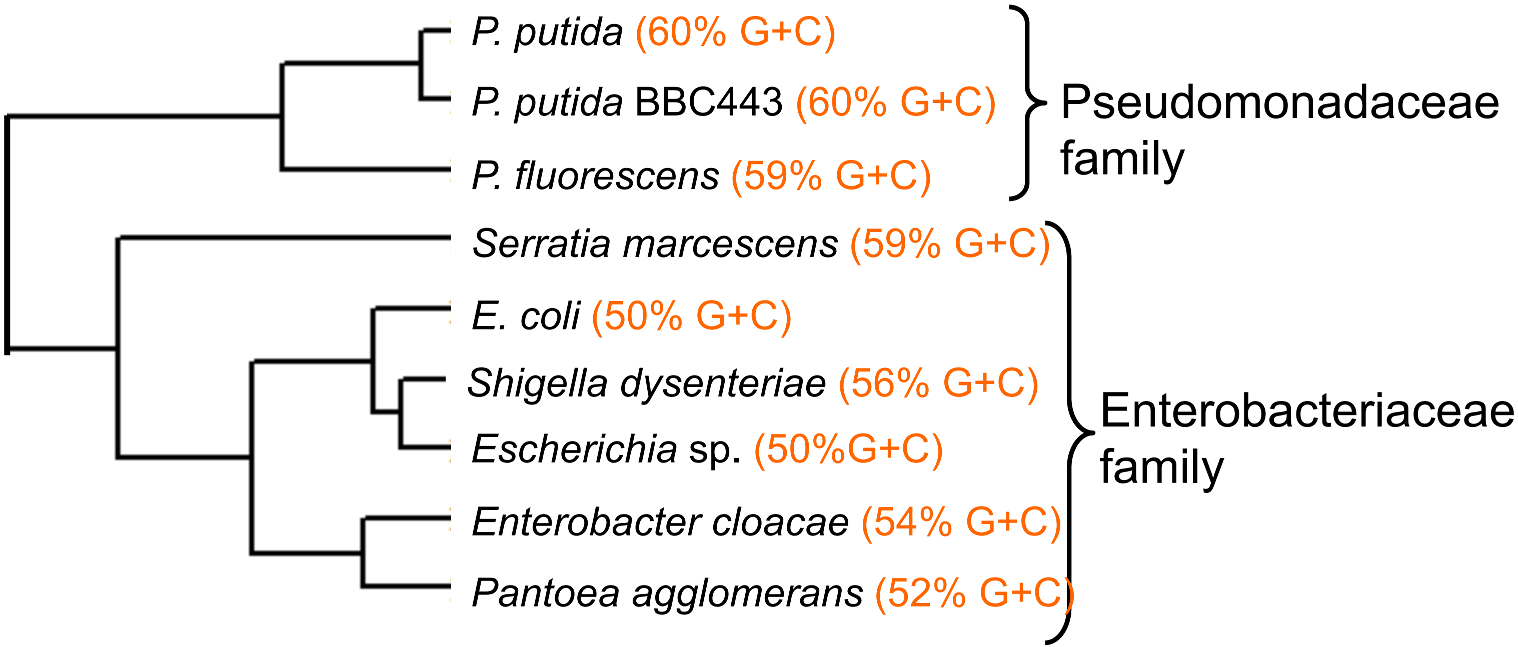


$y = 0.94x + 0.72$
($n = 43$; $R^2 = 0.940$)

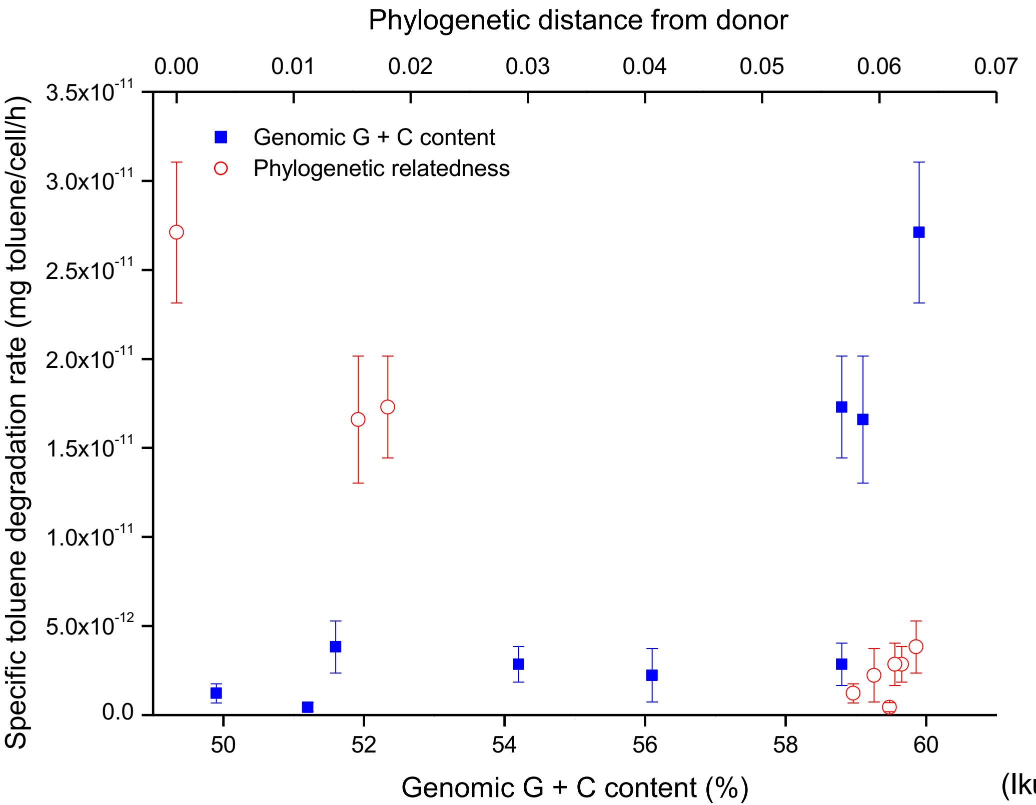
(Martiny and Field, 2005)

Will strains with genomic GC contents similar to that of the TOL plasmid (59%) have functional phenotypes?

Transconjugants obtained and tested belong to the γ-Proteobacteria family



Host cell G+C content and phylogenetics may limit TOL plasmid activities in transconjugants



(Ikuma and Gunsch, *Appl Microbiol and Biotech*, 2013)

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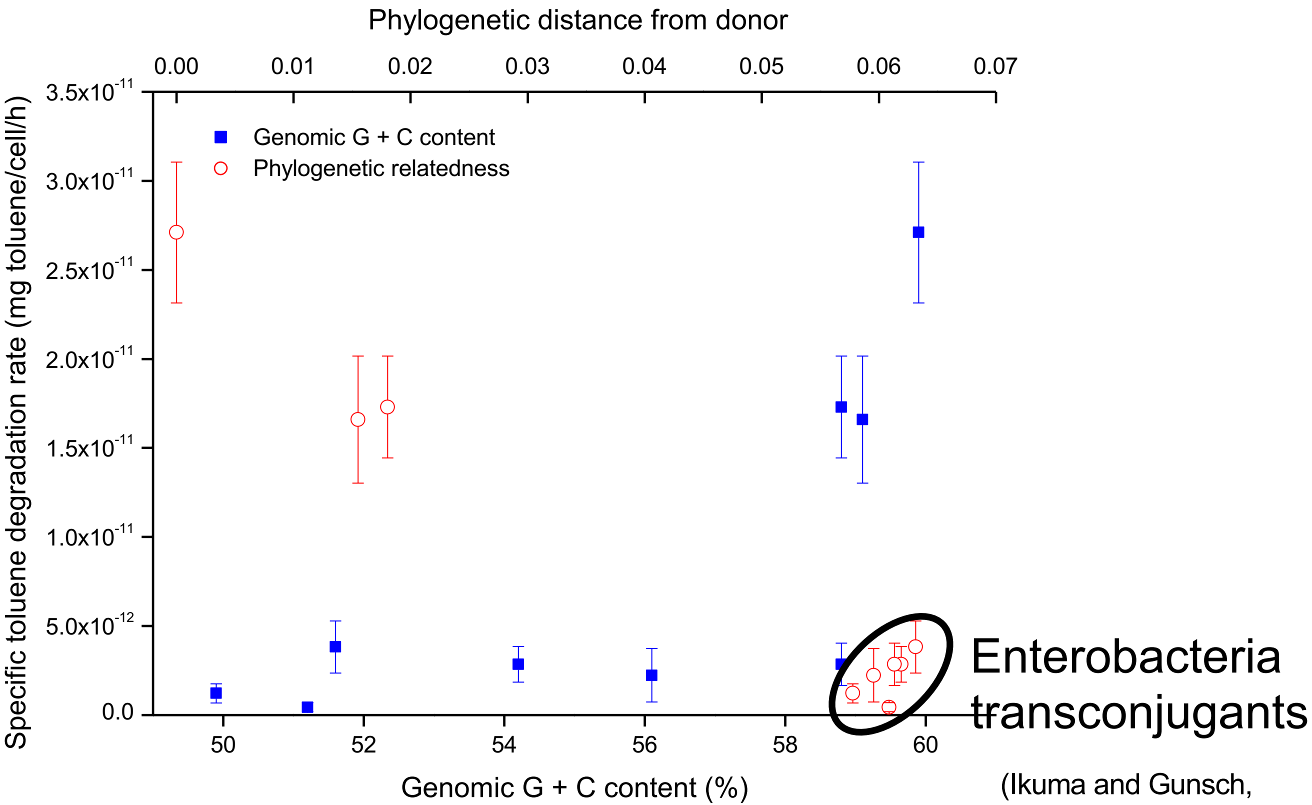
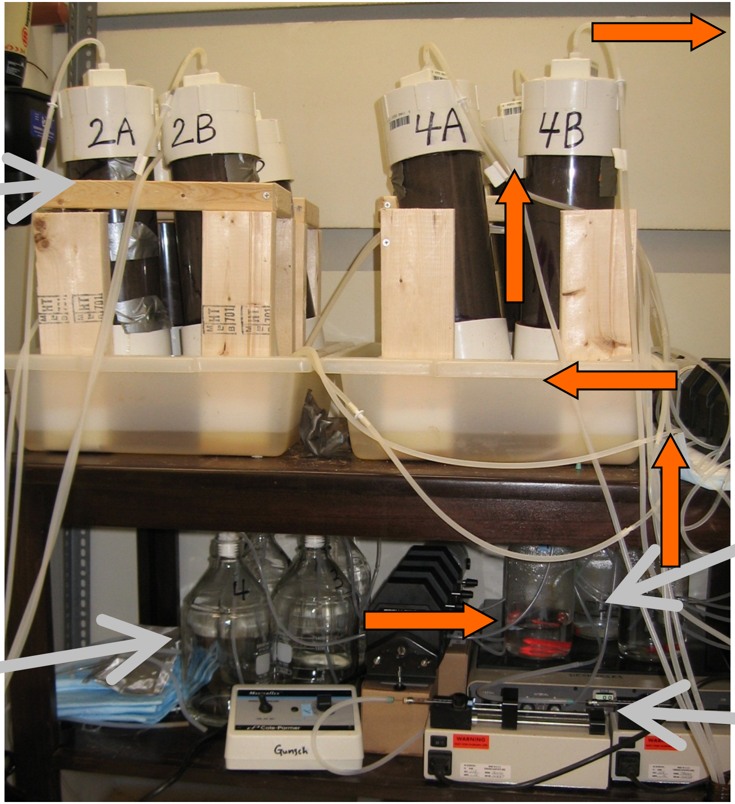


(Ikuma and Gunsch, *Appl Microbiol and Biotech*, 2013)

Soil column experiments

Column (10 cm diameter, 30 cm height)

Influent medium



Flow rate: 33 mL/h
HRT: 30 h
Toluene in the influent

Mixing chamber

Contaminant addition

Soil columns tested various scenarios of genetic bioaugmentation

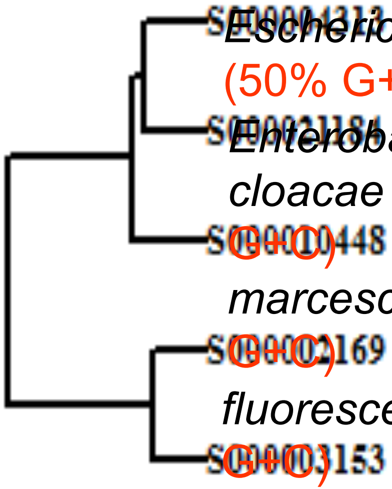
<u>Column conditions</u>

1A/B: Autoclaved soil +
4 recipient strains

2A/B: Autoclaved soil +
4 recipient strains +
continuous glucose input

3A/B: Autoclaved soil +
4 recipient strains +
pulse glucose input

4A/B: Non-sterile soil +
pulse glucose input



Escherichia coli (50% G+C)

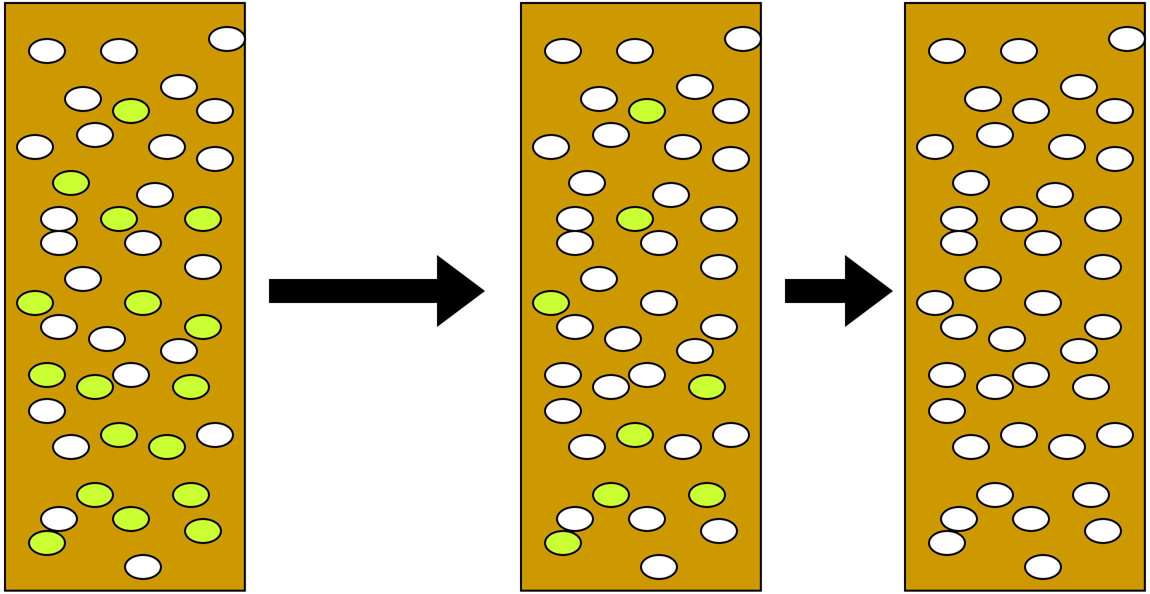
Enterobacter cloacae (54% G+C)

marcescens (59% G+C)

fluorescens (59% G+C)

(plasmid donor) (60% G+C)

Long-term fate of TOL plasmid genetic bioaugmentation was studied over 60 days



Presence of selective pressure: **28 days**

Absence of selective pressure: **32 days**

Total column operation: **60 days**

(Ikuma and Gunsch, *Environmental Chemistry Letters*, 2013)

Lessons learned

- It is possible to induce horizontal gene transfer of catabolic plasmids
- Functional phenotype requires luck or knowledge of host phylogenetic genetics and phylogenetic relatedness to catabolic plasmid

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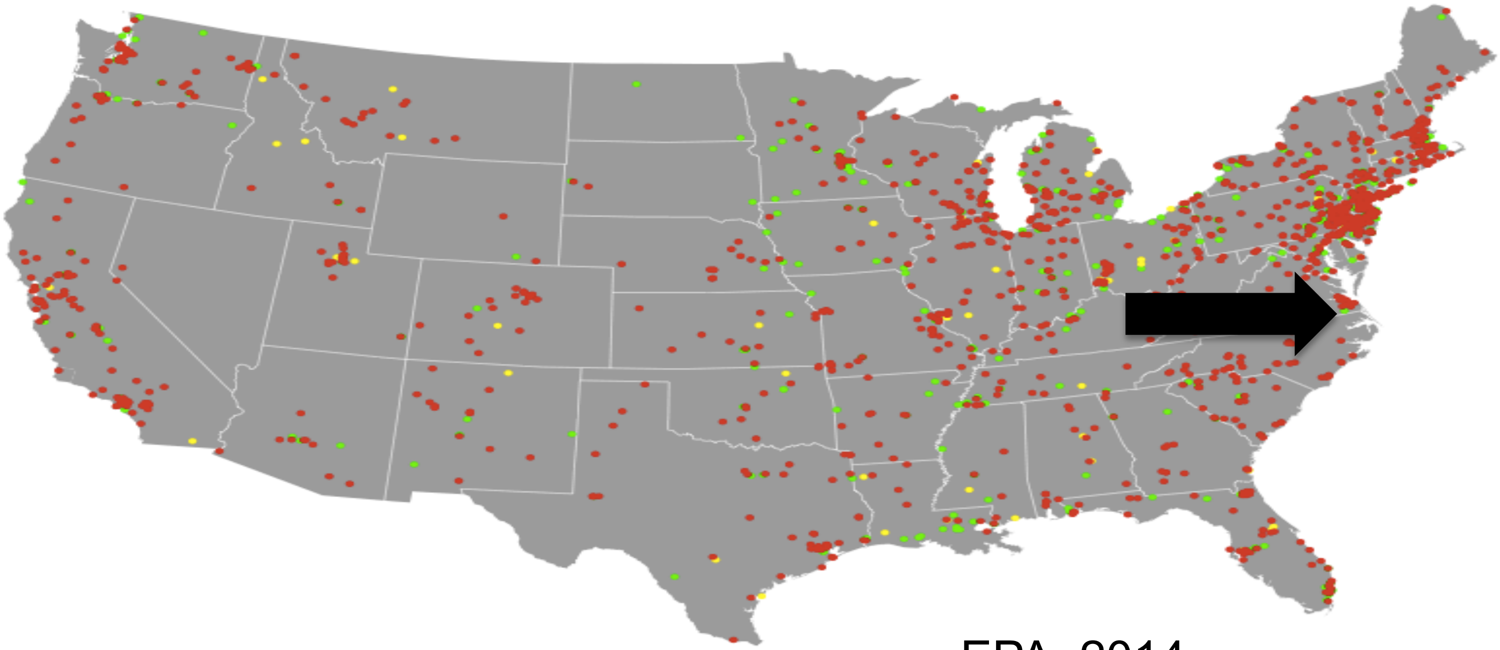
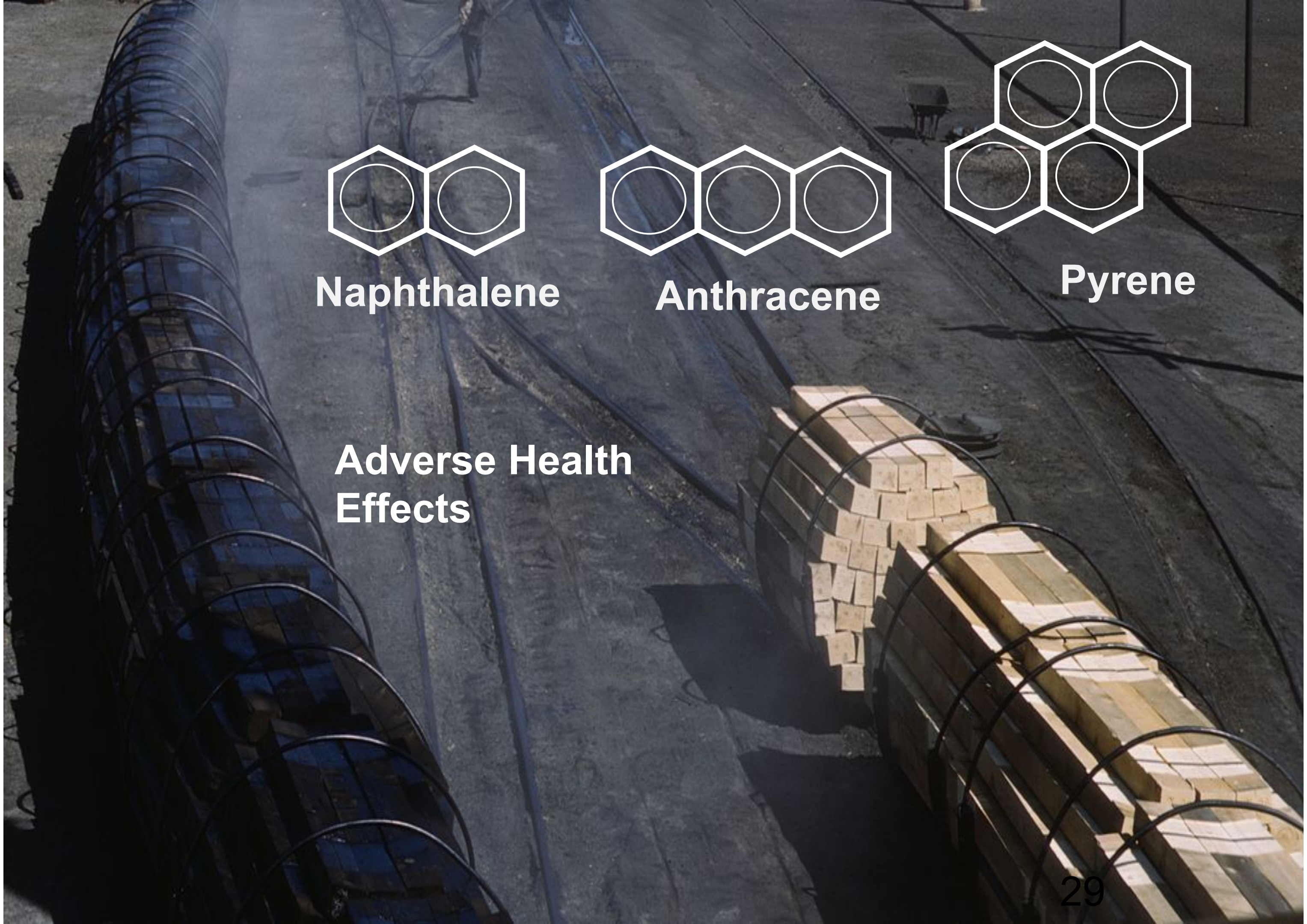
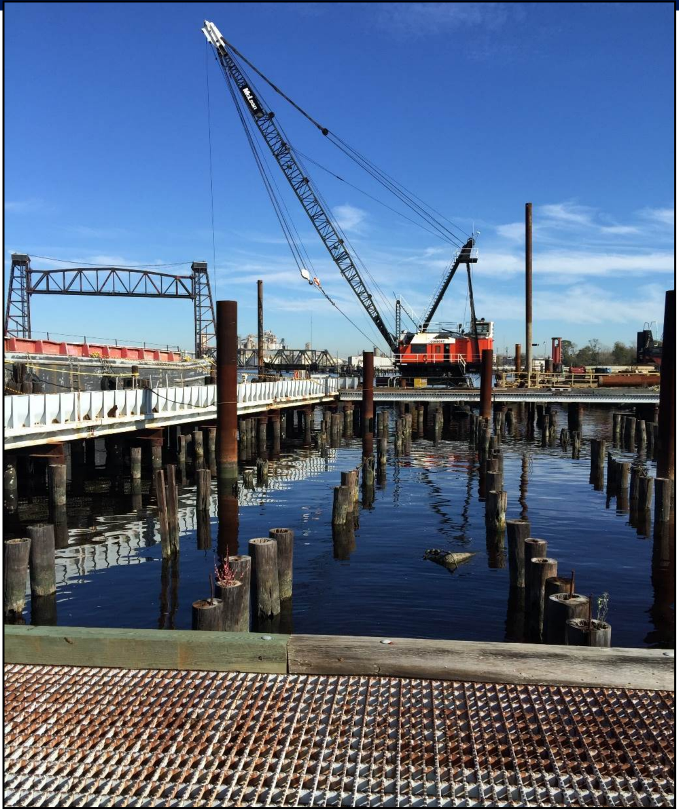
Extrapolating to the Real World

1322 National Priority Superfund Sites



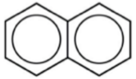
EPA, 2014


Naphthalene
Anthracene
Pyrene
Adverse Health
Effects

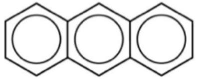


Republic Creosoting, Elizabeth River

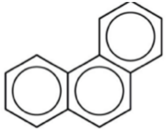
Norfolk, VA



Naphthalene

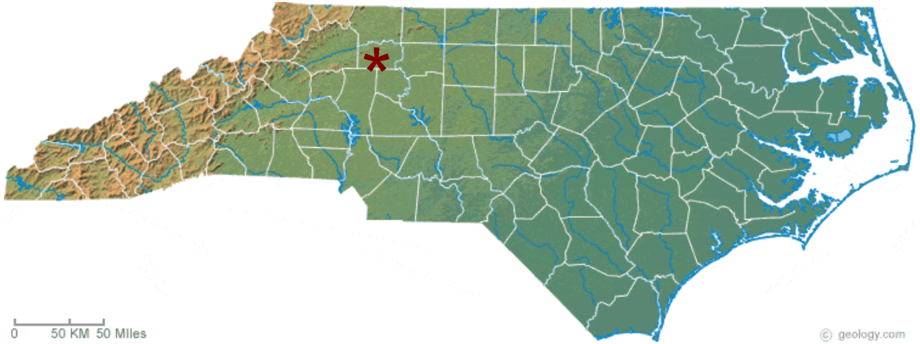


Anthracene



Phenanthrene

Holcomb Creosote



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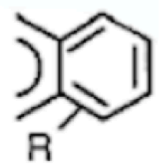
Project Goal: Integrated Microbial Metabolism

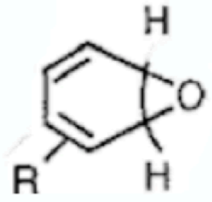
Fungal Metabolism

- Increase bioavailability of HOCs
- Nonspecific extracellular enzymes



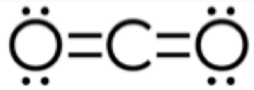
Metabolites

- Increased water solubility
- Increased bioavailability



Bacterial Metabolism

- Complete Mineralization

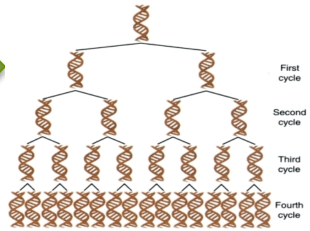


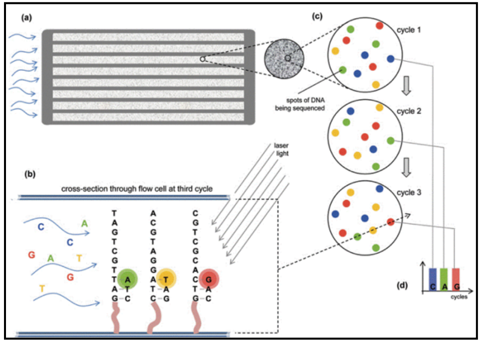
Precision Bioremediation Approach #2: Next-Generation Sequencing

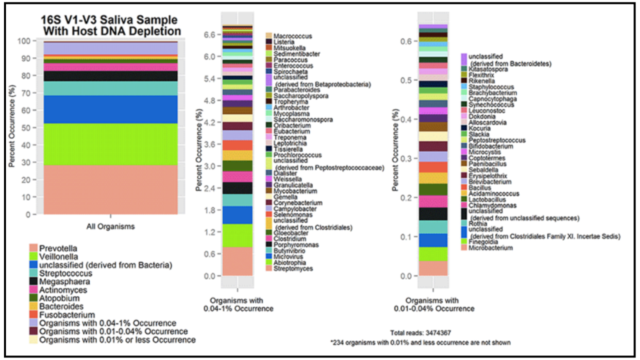
- Universal amplicon sequencing
  - 18S/LSU (fungi)
  - ITS (fungi)
  - 16S (bacteria)
- Helps identify potential target organisms for bioremediation


Republic Creosoting site.

Amplicon Based Metagenomic Community Analysis

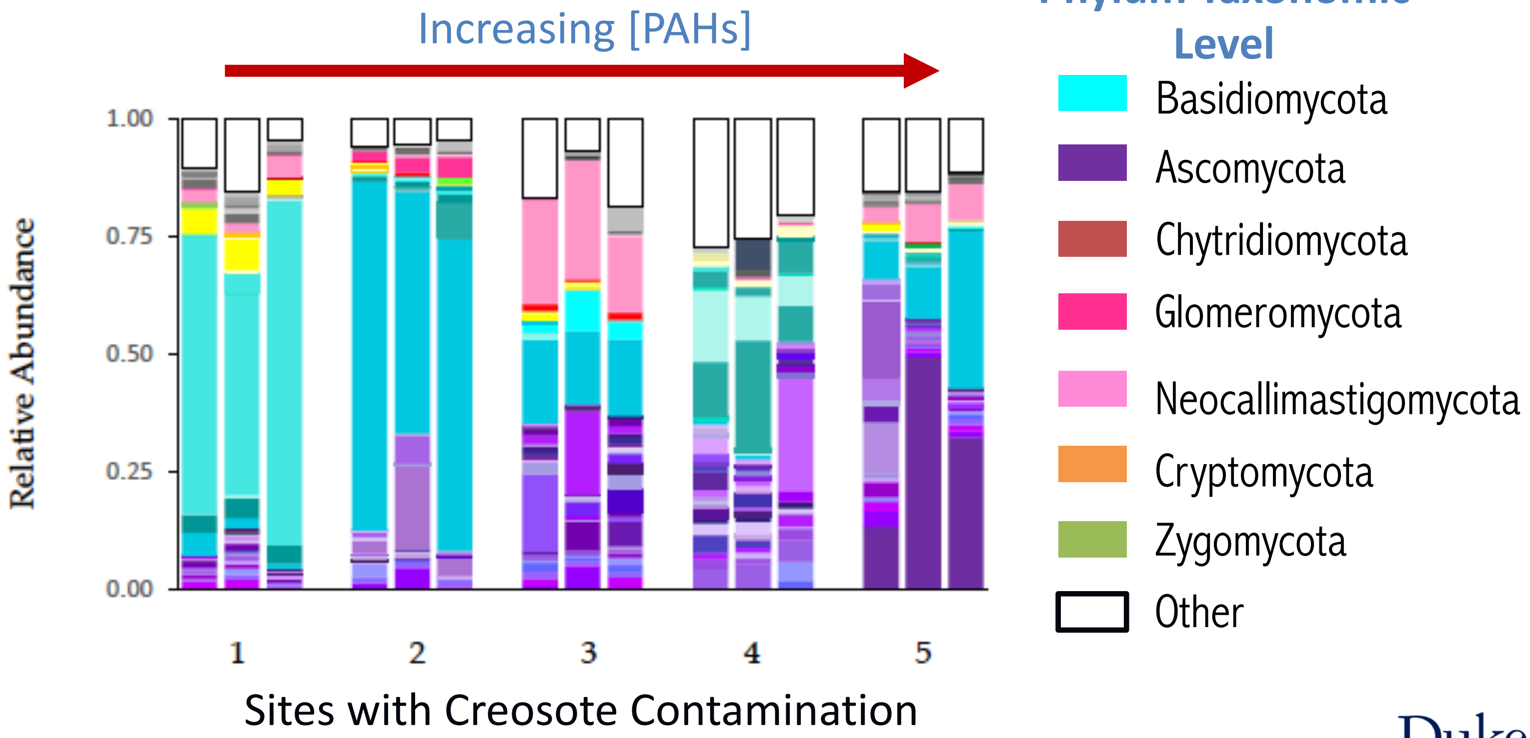






http://tucf-genomics.tufts.edu/images/illumina-large.gif?1378237298

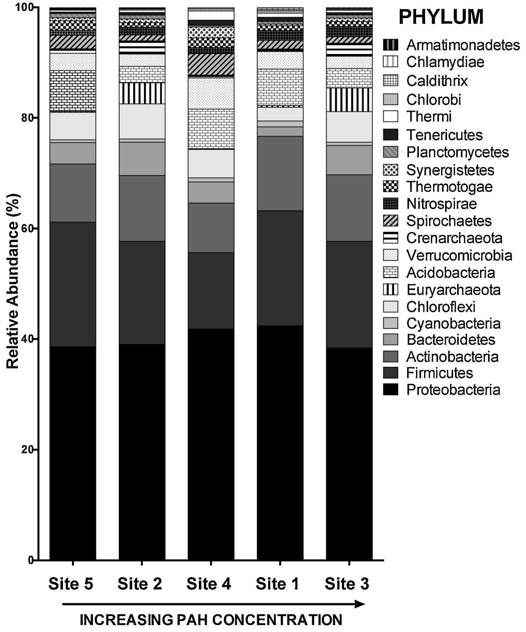
Soils with High [PAHs] Host Ascomycota

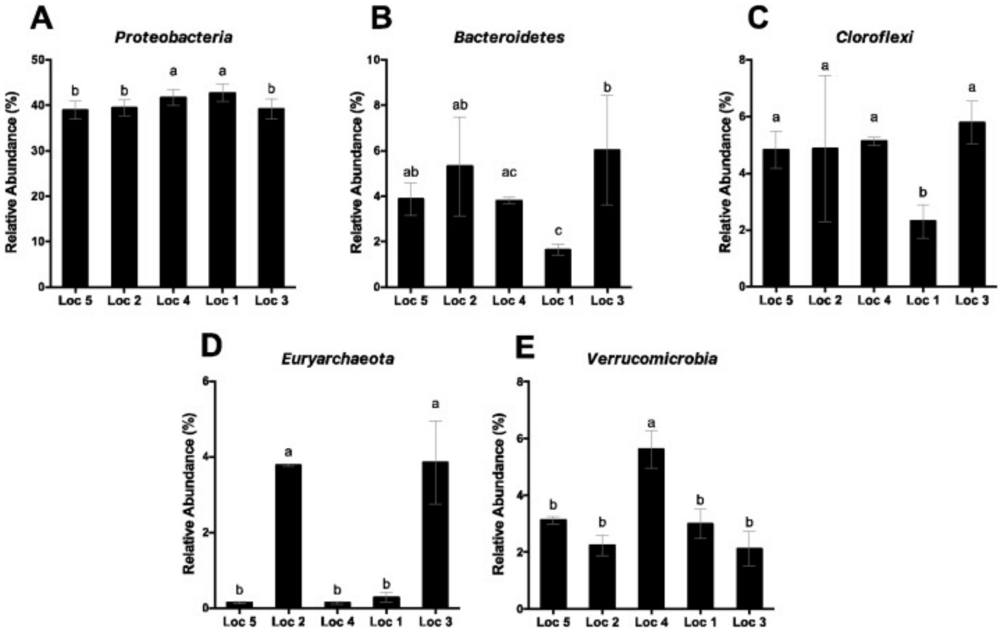


Czaplicki et al., *Remediation*, 2016)

Method: Illumina MiSeq 18S amplicon sequencing

Sediment Bacterial Communities





Method: Illumina MiSeq 16S amplicon sequencing

(Redfern et al., *J. Haz Mat*, 2019)

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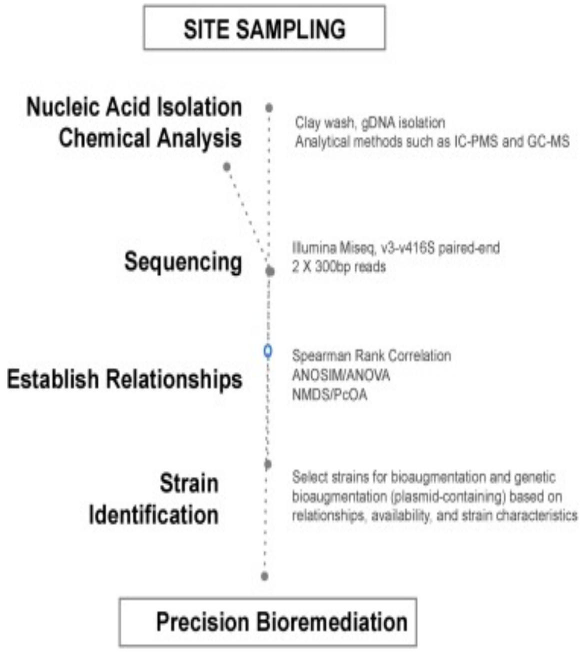
Can Identify Targets for Engineering Microbial Consortia



Genetic Bioaugmentation

1. ***Sphingomonas* sp. strain KS14**
   (PAH-degrading plasmid)
2. ***Sphingomonas aromaticivorans* F199**
   (PAH-degrading plasmid, shown to conjugate)

Bioaugmentation

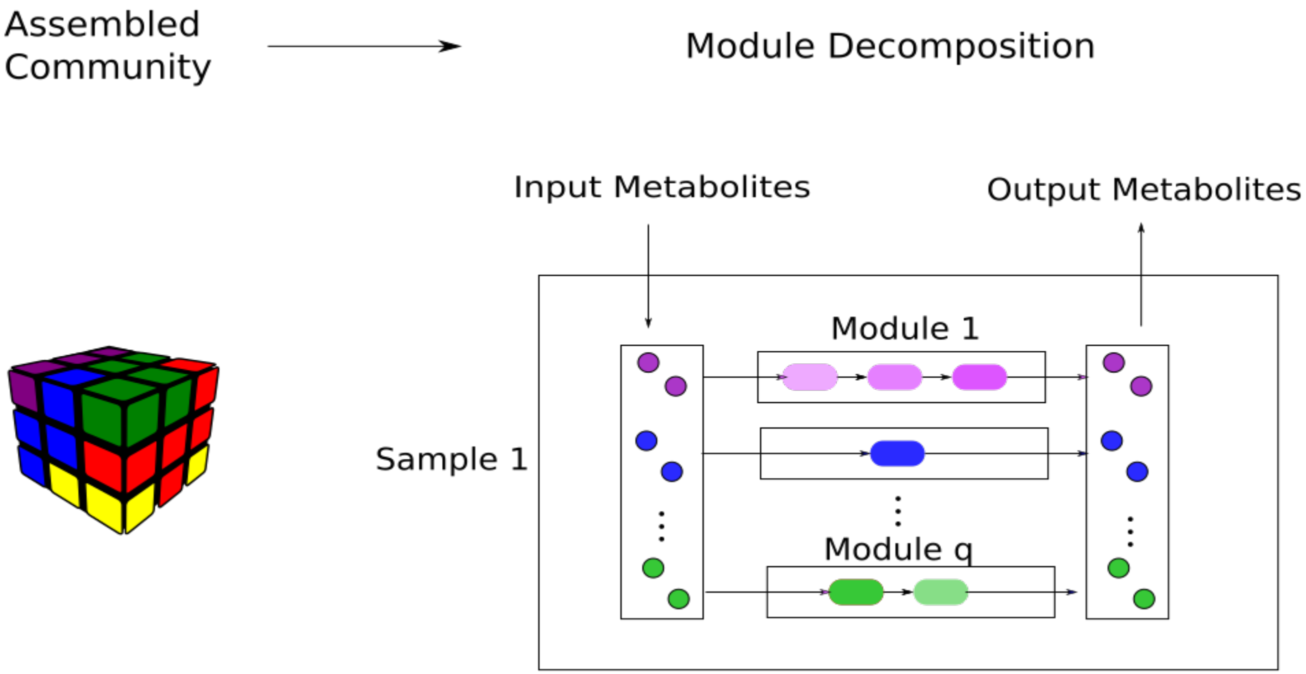
3. ***Paracoccus* sp. strain HPD-2**
   (PAH-degrader, bioaugmentation candidate)
4. ***Pseudomonas xanthomarina*** (4M14) and ***Arthrobacter nitroguajacolicus*** (1B16A)
   (effective at PAH degraders in consortia)
5. ***Bacillus subtilis* BMT4i (MTCC 9447)**
   (BaP degrader)

Biostimulation

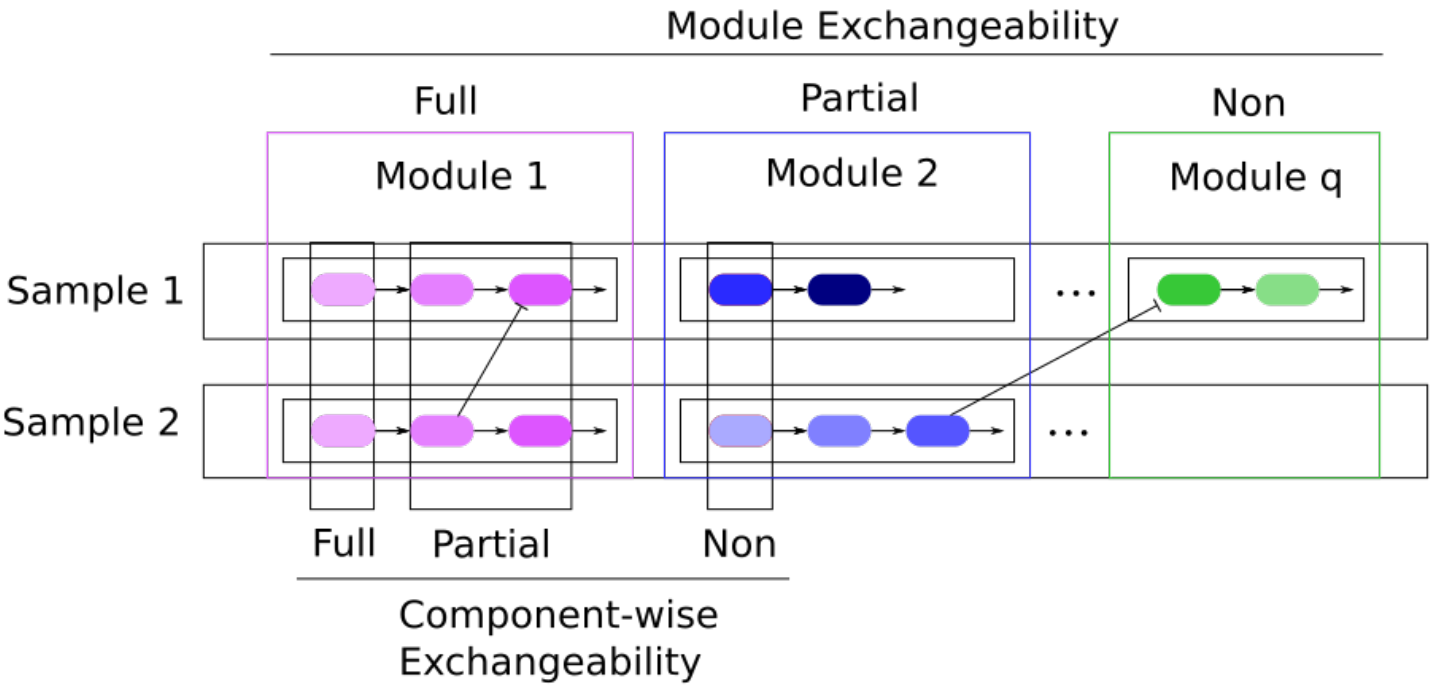
6. **Cysteine (stimulate Geobacter)**
7. **Carbon, Nitrogen, Phosphorus amendments**

(Redfern et al., *J. Haz Mat*, 2019)

Next Steps
Precision Bioremediation Strategy #3



Conditional Exchangeability of Microbes Across Samples



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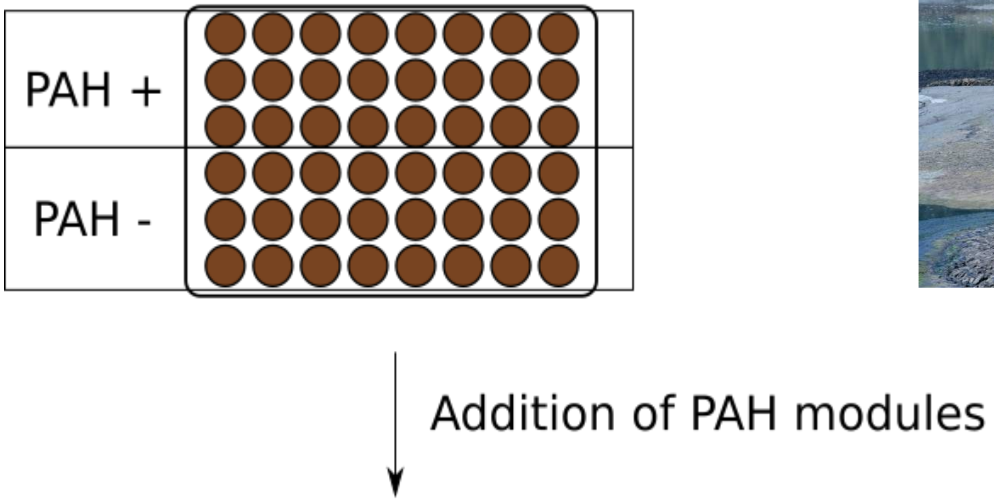
Future Work



High throughput sequencing
Bioaugmentation Stability Analysis

Comparison with
Modeling Results

High throughput Sequencing
Bioaugmentation Stability Analysis
Comparision with
Modeling Results

Acknowledgments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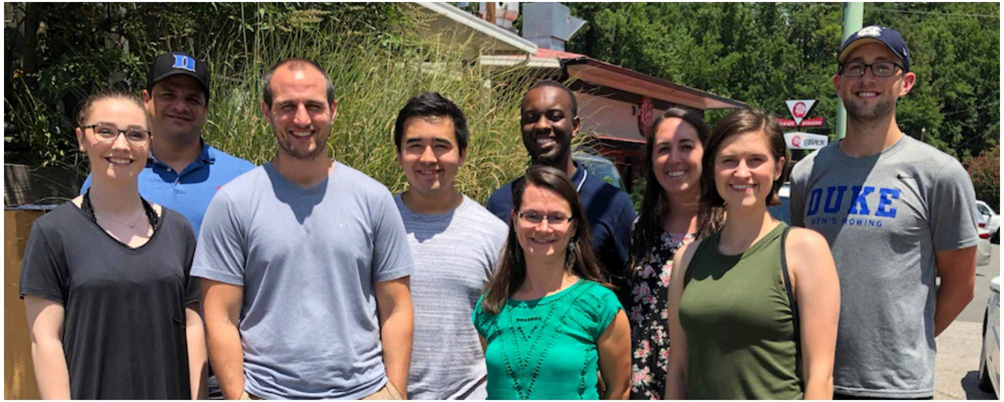
P. Lee Ferguson, PhD




David R. Singleton, PhD

National Science Foundation

National Institute of Environmental Health Sciences
Superfund Research Program