Impact of Hydrogen Peroxide on Horizontal Transfer of Naphthalene-Degrading Genes

Group 1

Anastasia Fox and Kayleigh Millerick
Civil, Environmental and Construction Engineering, Texas Tech University, Lubbock, TX

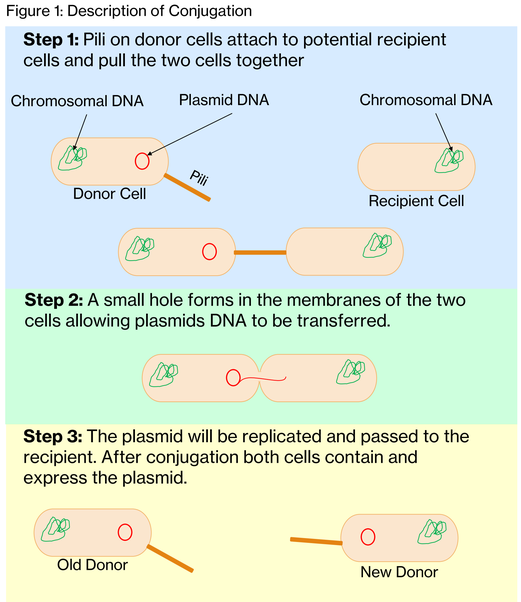
Potential Use of Plasmids in Remediation

- Plasmids are small segments of DNA that can be transferred between cells. They encode genes for:
  - Antibiotic resistance
  - Degradation of xenobiotic compounds. These are known as catabolic plasmids.
- Plasmids are needed for populations of bacteria that degrade:
  - Camphor
  - 2,4-D
  - PCBs and PBBs
  - Nitrotoluene
  - Phenanthrene

Catabolic Plasmids and Conjugation

- Horizontal gene transfer allows plasmids to be replicated independent of cell division
- Conjugation is the most frequent method of horizontal gene transfer

Figure 1: Description of Conjugation



- Recipient cells that express and maintain the plasmid are called *transconjugates*.
- Environmental conditions greatly impact transfer frequency

Environmental Condition	Transconjugate Frequency
Bulk Soil or Water	10^{-5} transconjugates per donor cell [8]
Biofilm	0.29 transconjugates per donor cell [3]

- Although plasmids have to potential to be useful in remeditaion they are often only contained in a subset of the bacterial population. This population may not be dominant at a site.
- **Plasmid conjugation has to potential to increase the number of bacteria that can degrade a contaminant and are well adapted to site conditions.**

Current Knowledge and Gaps

Current Knowledge:

- Current research has focused on optimizing conditions for transfer by varying [1][2][4][9]:

pH
Donor to recipient ratios
Temperature
Bacterial Strains
Carbon additions (glucose and LB)
Soil type and depth

- Most of these parameters are difficult or costly to alter on a large scale, like at a contaminated site.
- Carbon source addition had inconsistent impacts on transfer frequency The addition of an easier to degrade substrate can increase bacterial populations but reduce expression of degradation genes [2][9].
- Field scale experiments or experiments with plants have many complex interactions so it is difficult to determine what had the greatest impact on transfer frequency.

Knowledge Gaps:

- Results are difficult to apply to field scale remediation because the experimental conditions are often very different from conditions seen at sites.
  - Experiments on homogeneous surfaces, like filter mating experiments, have donor and recipient cells in close proximity to each other increasing transfer frequency
  - Additions of antibiotic resistance genes and the use of antibiotics as a selective pressure increase transfer frequency but cannot be replicated in situ.
- There are only limited studies that investigate the impact of stress on conjugation frequency of catabolic plasmids.
  - Exposing only recipient bacteria to stress does not reflect field conditions where all bacteria would be stressed [4].
  - Reactive oxygen species (ROS) is known to increase the transfer of antibiotic resistance plasmids [5][10].

Goals for research:

- ROS are a group of chemicals containing partially reduced, redox-active oxygen molecule.
- ROS can increase the transfer of antibiotic plasmids between 5 and 100 fold [5][10].
- **Can ROS increase the transfer of catabolic plasmids by several folds?**

Determine the effect of hydrogen peroxide on transfer of a naphthalene degrading plasmid, NAH7 from *Pseudomonas putida* donor to *Escherichia coli* recipient

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Determine the impact of other ROS on plasmid transfer

Experimental Setup

Figure 2: Mating Experiment Setup

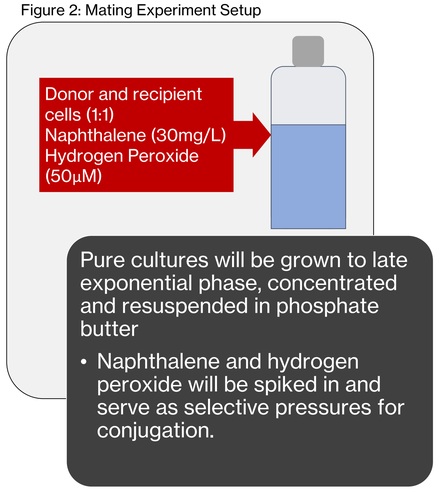
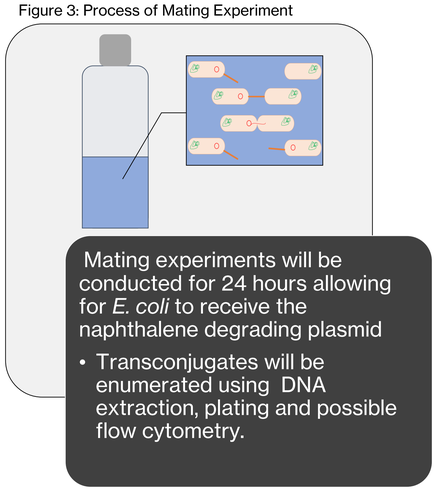
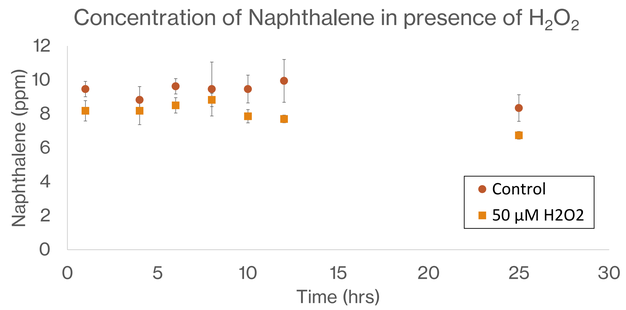


Figure 3: Process of Mating Experiment



Current work

- Methods to get replicable plate counting for NAH7 harboring cells are in the process of being developed.
- Experiments were conducted to determine the impact of H_2O_2 on naphthalene concentration. The concentration of naphthalene was initially reduced due to hydrogen peroxide, but there was still naphthalene in the aqueous phase.



Environmental Sources of ROS

Understanding how ROS impacts gene transfer is important because ROS is ubiquitous and can be easily stimulated. Below are 3 potential sources of ROS to encourage gene transfer.

Oxidizing Chemicals

Figure 4: ROS production from oxidizing chemicals

$$S_2O_8^{2-} + 2e^- \rightarrow SO_4^{2-} + SO_4^{\cdot -}$$

$$SO_4^{\cdot -} + OH^- \rightarrow SO_4^{2-} + OH^{\cdot}$$

Lower concentrations of ROS will stress the cells, but will not cause mass cell death.

Aerobic Respiration

Figure 5: ROS production from aerobic respiration

$$OrgC + H_2O_2 \rightarrow CO_2 + H^+ + e^-$$

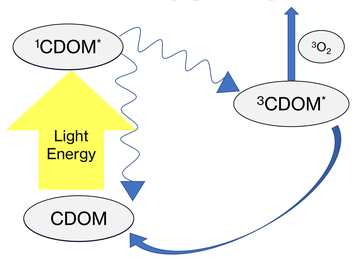
$$O_2 \quad O_2^{\cdot -} \rightarrow H_2O_2 \rightarrow OH^{\cdot -}$$

Increased chance of ROS production with higher O_2 concentration or with specific cultures [6].

Photoexcitation of CDOM

Figure 5: ROS production from photoexcitation of CDOM

H_2O_2, $OH^{\cdot}$, $^1O_2$, $RO^{\cdot}$, $ROO^{\cdot}$, $O_2^{\cdot -}$, ...



References

1. Ikuma, K., & Gunsch, C. (2010). Effect of carbon source addition on toluene biodegradation by an Escherichia coli DH5α transconjugant harboring the TOL plasmid. *Biotechnology and Bioengineering*, *107*(2), 269–277. https://doi.org/10.1002/bit.22808
2. Ikuma, K., Holzem, R. M., & Gunsch, C. K. (2012). Impacts of organic carbon availability and recipient bacteria characteristics on the potential for TOL plasmid genetic bioaugmentation in soil slurries. *Chemosphere*, *89*(2), 158–163. https://doi.org/10.1016/j.chemosphere.2012.05.086
3. Mohan, V. S., Falkentoft, C., Venkata Nancharaiah, Y., Sturm, B. S. M., Wattiau, P., Wilderer, P. A., ... Hausner, M. (2009). Bioaugmentation of microbial communities in laboratory and pilot scale sequencing batch biofilm reactors using the TOL plasmid. *Bioresource Technology*, *100*(5), 1746–1753. https://doi.org/10.1016/j.biortech.2008.09.048
4. Pinedo, C. A., & Smets, B. F. (2005). Conjugal TOL transfer from Pseudomonas putida to Pseudomonas aeruginosa: Effects of restriction proficiency, toxicant exposure, cell density ratios, and conjugation detection method on observed transfer efficiencies. *Applied and Environmental Microbiology*, *71*(1), 51–57. https://doi.org/10.1128/AEM.71.1.51-57.2005
5. Qiu, Z., Yu, Y., Chen, Z., Jin, M., Yang, D., Zhao, Z., ... Li, J.-W. (2012). Nanoalumina promotes the horizontal transfer of multiresistance genes mediated by plasmids across genera. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(13), 4944–9. https://doi.org/10.1073/pnas.1107254109
6. Sekar, R., & Dichristina, T. J. (2014). Microbially Driven Fenton Reaction for Degradation of the Widespread Environmental Contaminant 1,4-Dioxane.
7. Sigler, K., Chaloupka, J., Brozmanová, J., Stadler, N., & Höfer, M. (1999). Oxidative stress in microorganisms I. Microbial vs. higher cells - Damage and defenses in relation to cell aging and death. *Folia Microbiologica*, *44*(6), 587–624. https://doi.org/10.1007/BF02825650
8. Sørensen, S. J., Bailey, M., Hansen, L. H., Kroer, N., & Wuertz, S. (2005). Studying plasmid horizontal transfer in situ: A critical review. *Nature Reviews Microbiology*, *3*(9), 700–710. https://doi.org/10.1038/nrmicro1232
9. Wang, Y., Kou, S., Jiang, Q., Xu, B., Liu, X., Xiao, J., ... Xiao, M. (2014). Factors affecting transfer of degradative plasmids between bacteria in soils. *Applied Soil Ecology*, *84*, 254–261. https://doi.org/10.1016/j.apsoil.2014.07.009
10. Zhang, Y., Gu, A. Z., He, M., Li, D., & Chen, J. (2016). Subinhibitory Concentrations of Disinfectants Promote the Horizontal Transfer of Multidrug Resistance Genes within and across Genera. *Environmental Science & Technology*, acs.est.6b03132. https://doi.org/10.1021/acs.est.6b03132