Measuring NAPL/Bacteria Interaction Forces using Atomic Force Microscopy

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Coal tar contaminated sites found within the United States are considered to be health hazardous areas that lack a reasonable approach of remediation. Such sites are in dire need of clean-up, but no one satisfactory solution can be found. Chemical clean up has proved inefficient, soil incineration is too expensive, and trying to move it to a more suitable place is unrealistic due to the expansion of the poisonous area. It has been found that certain species of bacterium can survive the toxic environment that coal tar produces. These bacteria break down coal tar using the material as its carbon food source. P*seudomonas* sp. and *Burkhodaria* sp. are two such species of organisms that are found in coal tar soils. While significant progress has been made in identifying organisms responsible for contaminant degradation even at high contaminant concentrations, little is known about key factors that influence NAPL remediation. For example, bacterial adhesion to NAPL interfaces may be advantageous source of knowledge since compounds have to dissolve prior to biodegradation. Therefore, understanding the environmental processes involved, which control bacterial adhesion could have a significant impact in improving current bioremediation processes.

The by-product, coal tar is created by the process of gasification of coal found at Manufactured Gas Plants (MPG's) that predominated the east coat in the late 19th century until the mid 1900's. Coal tar is considered a non-aqueous phase liquid (NAPL) that has polycyclic aromatic hydrocarbons (PAH's) found within its structure. PAH's in high concentrations are found to be carcinogenic and toxic to organisms. An estimated 11 billion gallons of coal tar can be found throughout the United States.[2] The samples that will be used in our experiment came from the Utica Harbor site found in Oneida county New York.

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[2] Eng, R.; Menzies, M. *Survey of town Gas and By-product Production and Location in the United States* (1880-1950); PB-8 J 1738B; National Technical Information Service: Washington, DC, 1985.

It is believed that some bacteria develop high adhesion to coal tar, which works towards enhancing bioavailability. So the two species of bacterium used in this project are *Psudomonus stutzeri* strain P16 and *Burkholderia* RP-037-3. Both organisms are Gram negative, rod-like bacteria (ca. 1µm in length and .5µm in diameter). These two species were chosen because they grow very well in concentrated PAH's such as phenanthrene and naphthalene. We prepare each species by using the following steps: 1) streaking for isolation in R2A agar, 2) Growth in batches of media such as 5% peptone, nutrient broth, and mineral media with either naphthalene or phenanthrene as the carbon source, 3) harvesting bacteria from these batches of media, and then 4) isolation bacteria for visualization and force measurements with the Atomic Force Microscope (AFM). Kanamycin is used in all *Burkholderia* RP-037-3 enoculated samples to reassure that only *Burkholderia sp.* grows.

The Atomic Force Microscopy is a scientific instrument that will be used to take topography images of the two strands of bacterium and make the force measurements. The setup consists of a cantilever with attached sharp pyramidal tip and a laser detection system. Using peizo ceramic positioner, scanner, the sample is moved, scanned in lateral direction, while the tip is gently touching the sample surface. As the tip moves across the sample, the cantilever arm bends resulting in a change of the angle of reflection of the laser beam. This signal is picked up by the computer and is then presented as a digital image. Analysis of the bacteria's force measurements in comparison to digital image will tell where the bacterium is most likely to attach to the NAPL source and at what rate.

Once forces are identified new variables of pH and ionic conductivity can be tested and identified for the processing of an ideal solution that will assist the bacterium in the rapid rate of degradation of coal tar and all of its components. By finding the interacting forces involved between the coal tar and bacterium in different liquid media's, a more favorable form of bioremediation can be established to clean up the MGP contaminated sites.