BIOREMEDIATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SOIL AT FORMER MANUFACTURED GAS PLANT SITES

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ABSTRACT

The operation of manufactured gas plants has led to contamination of the soil and groundwater around these sites. The nature of this contamination is organic compounds in the form of long chain, cyclic, and poly-cyclic hydrocarbons. Natural degradation of this contamination has been observed, and studies have been done to determine the organisms involved. Further study has focused on methods of decontamination and how these processes can be optimized. The use of solvents has been shown to improve bioavailability of these pollutants to native and introduced microorganisms. This paper discusses the types of organisms involved in degradation, the biological processes involved, and how natural processes might be enhanced to more quickly and effectively remove this contamination.

KEYWORDS

Manufactured gas plants (MGPs); coal tar; polycyclic aromatic hydrocarbons (PAHs); bioremediation; bacteria; fungi; soil; environment

INTRODUCTION

Manufactured Gas Plants

Manufactured gas plants were used in the late 19th and early 20th century to produce coal gas. This production process heated coal in the absence of air, releasing volatile compounds present in the coal. This gas was then condensed and purified, and distributed as a fuel to provide lighting, heating, and cooking energy in homes and industrial settings prior to the introduction of natural gas (Wikipedia[1], 2006). One of the main waste products of this process was coal tar – a thick oily liquid that condensed as the coal gas was cooled and purified. Although several methods were employed to use or dispose of this waste, the most common method of disposal was to dump the waste into nearby streams or earthen pits, and several former MGP sites remain contaminated with coal tar; specifically the soil surrounding the site (Wikipedia[1], 2006). There are currently seven MGP sites in the state of Iowa that are classified as environmental hazards and that require active monitoring or remediation (Iowa DNR, 2005). Around the United States, there are over 2500 former MGP sites (Allbusiness, 2005)

Hydrocarbons

Coal tar consists of several organic compounds known as hydrocarbons. Hydrocarbons are chemical compounds made primarily of carbon and hydrogen. Hydrocarbons are best known for their role in combustion as kerosene, gasoline, and diesel fuels. These fuels are made primarily of long chain hydrocarbons which have the structure shown in Figure 1.

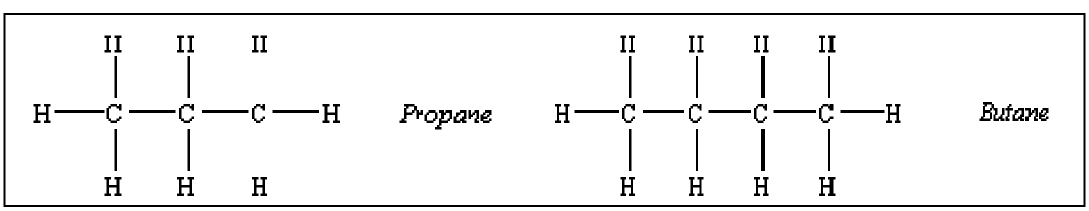

Figure 1 – Chain Hydrocarbons

Source: http://chemed.chem.purdue.edu/genchem/topicreview/bp/1organic/organic.html

Hydrocarbons can also be found in ring, or cyclic structures containing six carbon atoms in the ring. These structures are known as aromatic compounds. These compounds are used primarily as solvents or cleaners. Some examples of aromatic compounds are shown in Figure 2.

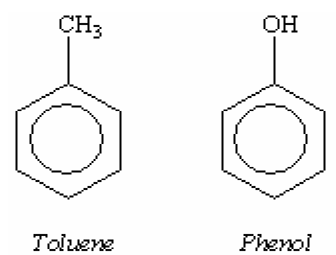

Figure 2 – Aromatic Hydrocarbons

Source: http://chemed.chem.purdue.edu/genchem/topicreview/bp/1organic/hydro.html

When several hydrocarbon rings containing 5 or 6 carbon atoms are joined together, the compounds are known as polycyclic aromatic hydrocarbons, or PAHs. Figure 3 gives some examples of PAHs present in coal tar.

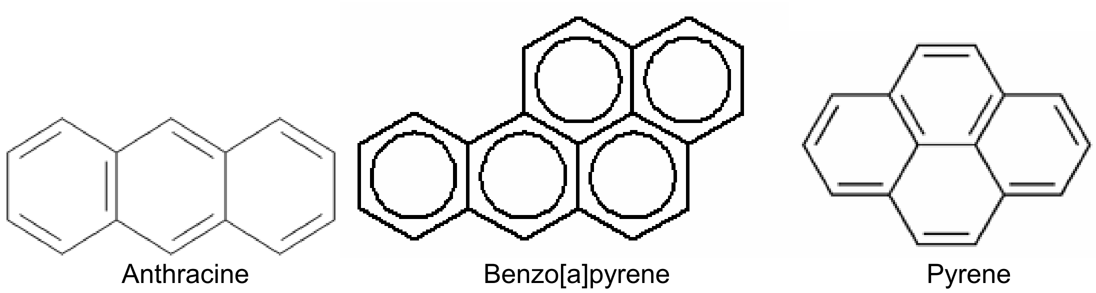

Figure 2 – Polycyclic Aromatic Hydrocarbons

Source: http://en.wikipedia.org/wiki/Polycyclic_aromatic_hydrocarbons

In general, the higher the molecular weight of an organic compound, the less soluble it is in water, the higher its boiling point, and the more viscous it is at low temperatures. These physical properties of the compounds affect their removal from the soil and water they contaminate as well as how long they remain in the water and soil (Cerniglia, 1992). In addition to their physical properties, many of these compounds have dangerous chemical properties that have prompted the EPA to list them as "priority pollutants."

Priority pollutants have been defined by the Environmental Protection Agency (EPA) as a "list of 126 compounds and elements developed by the EPA pursuant to Section 307(a)(1) of the Clean Water Act" (EPA, 1972). These compounds have been shown to have harmful effects to humans, including carcinogenety. These compounds can migrate from contaminated soils into the groundwater and then into water treatment facilities. For this reason, these and other chemicals are monitored regularly to ensure that they are not present in drinking water. The soil and water surrounding MGP sites often contain high concentrations of these pollutants, especially benzo[a]pyrene, which is a known carcinogen (Bamforth and Singleton, 2005). The EPA has set standards regulating the concentration of these compounds in drinking water. These limits are set based on their effects on humans and their potential for treatment (EPA, 1972).

METHODS OF BIOREMEDIATION

Bioremediation is essentially the process of microorganisms consuming compounds as a source of energy and effectively changing the compounds to less harmful or harmless products (Mueller *et al*, 1997). There exist many methods and processes under the definition of bioremediation, but these fall into two broad categories; *in-situ* and *ex-situ* processes. These categories divide bioremediation into processes carried out at the site of contamination, or in another setting by removing contaminated soil from the site and treating it elsewhere.

In-Situ treatment of contaminated soils has the advantage of reducing cost by eliminating the need to transport the soil in which the pollutants reside. This type of treatment often uses microorganisms native to the area to carry out the bioremediation. One study examined whether the rate of pollutant degradation was limited by nutrients required by the microorganisms other than the pollutant compounds, or whether the degradation was limited by the rate of desorption of the pollutants from the soil. It was concluded that when nutrients were added to the soil the rate of degradation increased, but no further increase was observed when the soil was inoculated with additional microorganisms (Li *et al*, 2005).

Ex-Situ treatment involves excavation of contaminated soils and removal for bioremediation at another location. This method of treatment has the advantage of more control over parameters such as moisture content, temperature, and nutrient content. Treatment can be carried out more quickly once actual decontamination has begun, but the quantities of contaminated soil usually present at MGP sites can make *ex-situ* treatment cost prohibitive. Another form of *ex-situ* treatment involves washing contaminated soil with solvents or a combination of surfactants and water, and then removing the water for treatment. This method also has higher cost as the water must be transported, and has the additional problem of successful desorption of the contaminant from the soil. This process is more favorable for the treatment of groundwater, as the apparatus can be set up in such a way that the water is pumped out, treated, and then returned to the aquifer (Guieysse *et al*, 1999).

MICROORGANISMS AND MECHANISMS OF UPTAKE

Types of Microorganisms

Research investigating natural degradation of soil contaminants has included study of naturally present microorganisms, and those introduced to contaminated soils by inoculation. Generally, the types of microorganisms present at a site where the contaminants have been present for a long period of time are able to survive and thrive in the presence of those contaminants. In this case, the native microorganisms are abundant, and need only additional nutrients to increase their degradation of pollutants (Li, *et al*, 2005). In fact, the nutrient deficit observed in long-term pre-existing PAH contaminated soils seems to be the most critical factor slowing the degradation of such contamination (Li *et al*, 2005).

In those sites where contamination has just taken place, there are a few microorganisms present that can naturally metabolize the contaminants, but they have not had time to dominate the flora at that location. In these circumstances, it might be beneficial to inoculate these sites with known species of microorganisms that can degrade the contaminant in question. This would increase the rate of remediation, and much of the contaminant could be removed before adsorbing to the soil at the site.

The bacterial genus *Geobacter* is ubiquitous in many types of soil, and has been used to clean up petroleum spills for many years (Wikipedia[2], 2006). This microorganism's ability to utilize hydrocarbons as a carbon source makes it ideal for remediation of PAH contaminated soils. Other bacterial genus capable of degrading aromatic hydrocarbons include Pseudomonas, *Achromobacter, Bacillus, Arthrobacter*, and *Phanerocheate* (Mellor *et al*, 1996).

Fungi are also capable of degrading aromatic hydrocarbons. They are less numerous than bacteria, and are more sensitive to changes in their environment. Some genus of fungi that have been observed to break down PAH are *Penicillum, Aspergillus,* and *Fusarium* (Mellor *et al*, 1996).

Biological Uptake of PAH

Microorganisms take in various compounds as sources of carbon and energy. Since these MGP pollutants have been present for so long, the microbial communities present in these soils are populated by those organisms that can utilize these compounds to sustain life. In addition to these contaminants, microorganisms need other compounds to maintain themselves; most notably terminal electron acceptors (TEAs). In any microbial metabolic process, the reactions used to break down nutrients result in oxidation of the nutrients and a subsequent reduction of the TEA. If there are no TEAs available to the microorganism, cellular metabolism cannot take place. For this reason, it may be necessary to add TEAs to the contaminated soil to aid these processes.

Microorganisms have been observed to move toward beneficial chemical compounds and away from those that are harmful to them. This action is known as chemotaxis – the movement of an organism in response to a chemical stimulation. A study of chemotaxis in PAH degrading bacteria found evidence of movement rates of up to 1mm/min for some strains of bacteria (Ortega-Calvo *et-al*, 2003). This reveals the potential for microorganisms to remediate lower concentrations than is presently observed.

There are three main mechanisms by which microorganisms metabolize PAHs – bacterial degradation, lignolytic fungal degradation, and non-lignolytic bacterial degradation – but these mechanisms share a common path of "oxidation of the aromatic ring, followed by the systematic breakdown of the compound to PAH metabolites and/or carbon dioxide" (Bamforth and Singleton, 2005). Figure 3 shows the biochemical paths of the three types of metabolism.

ENHACING BIOAVAILABILITY OF CONTAMINANTS

Several methods to make pollutants more available to microorganisms have been researched. The main focus of these processes is to increase not only the ability of microorganisms to access pollutants, but also to increase microorganisms' ability to ingest and metabolize pollutants.

Many chemical compounds such as surfactants and solvents have been tested to determine their effects on increasing bioavailability. Surfactants have a tendency to adsorb to soil particles making them more difficult to remove following treatment (Lee *et al*, 2001). The application of solvents to contaminated soils increases bioavailability by releasing pollutants from the soil particles they are adsorbed to. Solvents are also easier to remove following treatment than surfactants because they tend to be more soluble in water. Acetone and ethanol were compared in one study to determine which solvent improved bioavailability of PAHs to microbial action. These solvents were chosen because of their wide availability and low cost (Lee *et al*, 2001). It was determined that both solvents were well suited to increasing bioavailability, and that the solvents themselves might possibly serve as a nutrient in the microbial metabolic process (Lee *et al*, 2001). Biodiesel has also been shown to improve bioavailability of PAH to microorganisms. The coal tar was observed to be soluble in the diesel fuel, which the desorbed from the soil particles, and this solubility also served to increase the surface area of the contaminant, which increased the rate of microbial degradation (Taylor and Jones, 2001). Another advantage to using biodiesel is that it is renewable resource, making it more desirable for use in bioremediation.

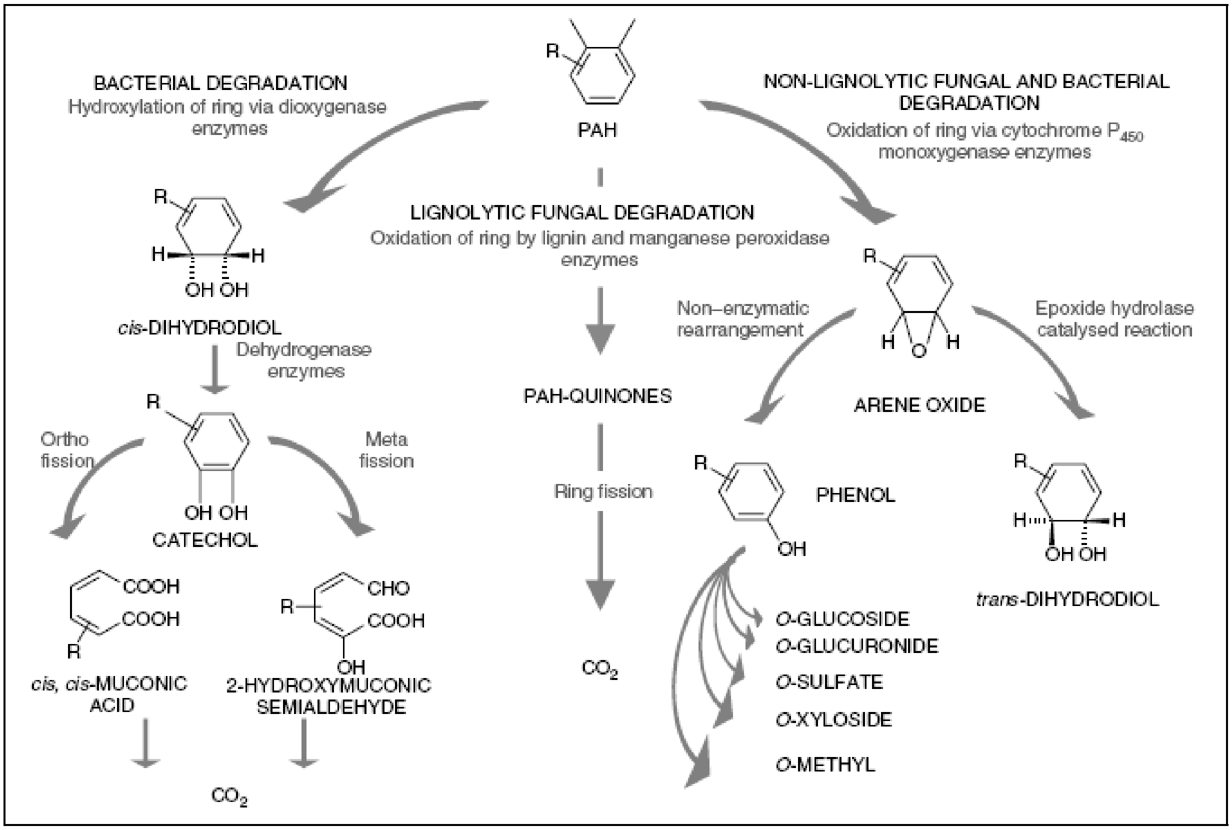

Figure 3 – Paths of Microbial Uptake

Source: Bamforth, S.M., and Singleton, I. (2005) Bioremediation of Polycyclic Aromatic Hydrocarbons: Current Knowledge and Future Directions. *J. Chem. Technol. Biotechnol*. 80 (7): 723-736.

Fenton's reaction is an oxidation reaction carried out by adding hydrogen peroxide to contaminated soil, although this reaction requires a low pH, and therefore inhibits normal biological activity of microorganisms (Nam *et al*, 2001). This limitation is overcome by using a modified Fenton reaction in which hydrogen peroxide and Fe^{2+} are applied to contaminated soil to facilitate oxidation of pollutants (Bogan and Trbovic, 2003). This pretreatment allows more rapid metabolism of the pollutants by the microorganisms because the oxidation step has been done requiring less energy expenditure by the organisms. It was observed that alternating treatment between chemical and biological degradation steps increased the percentage of contaminant removed from the affected soil.

The study of the effects of temperature on microbial metabolism was found to show that increased temperature made PAHs more bioavailable to microorganisms because the increased temperature made the PAHs more soluble (Bamforth and Singleton, 2005). Of course, this increase in solubility is only beneficial if the microorganisms present can withstand the temperatures required to increase the solubility. Often the bacteria present in contaminated sites are mesophilic, and remediation is carried out at temperature suitable for the survival of these

microorganisms (Antizar-Ladislao *et-al*, 2006). Mesophiles can withstand temperatures ranging between 25 and 40 °C (Wikipedia[3], 2006).
The effect of pH on biodegradation of contaminants is less easily determined. Throughout the studies mentioned above, several treatment processes were observed to determine their effect on remediation. Each of these treatment processes is itself affected by pH, and the action of the microorganisms themselves is affected by pH. Generally, once a treatment regimen has been adopted, it is necessary to determine at what pH that process performs best, and how this pH will affect native or inoculated organisms that will be used to carry out the bioremediation. In this way, it is the method and microorganisms used in the remediation that determines the pH, rather then the pH determining the effectiveness of remediation.

EFFECTIVENESS OF BIOREMEDIATION

Bioremediation alone has been shown to reduce PAH contamination by between 60 – 80 percent, depending on the relative age of the contamination (Antizar-Ladislao *et-al*, 1996). The application of solvents to desorb the pollutants from the soil particles also served to raise the percent conversion, as well as the efficiency (Lee *et-al*, 2001). The complexity of the contaminant mixture also plays a role in the effectiveness of bioremediation alone (Guieysse and Mattiasson, 1999). Characterization and adjustment of the bioremediation process can serve to increase the effectiveness of microbial remediation.

Bioremediation with chemical treatment such as application of solvents or Fenton's reaction has been shown to be more effective than microbial degradation alone (Srivastava *et-al*; 1994). In fact, sequential biological-chemical-biological treatment resulted in nearly 98% conversion of PAH levels to innocuous products compared to about 80% after biological treatment alone (Srivastava *et-al*; 1994). This is an encouraging finding with regards to reducing the required monitoring of a contaminated site. A significant reduction in contaminant concentration will result in less need for frequent monitoring, freeing up time and resources for action at other contaminated sites.

CONCLUSIONS

The process of bioremediation of PAH contaminated soils has been underway for almost three decades. This process has been done *in-situ* with the soil left in place, and *ex-situ* where the contaminants are washed off the soil, or the soil itself it hauled away for treatment. Much of the research done so far with regards to PAH bioremediation has been focused on ways to enhance the efficiency of the process. Through the use of pretreatment with solvents or surfactants, researchers have shown that increasing the availability of pollutants to microorganisms results in remediation that is more efficient and complete. Fenton's reaction has been successfully used to hydrolyze compounds to allow microorganisms more efficient uptake and utilization of contaminants.

The future of PAH bioremediation will continue along this same path for the most part. New ways to enhance the effectiveness of known processes will be discovered. New process will be developed utilizing treatments and techniques heretofore unknown. New pollutants will be researched to determine if bioremediation can be used to remove them from contaminated soils. The foundations have been laid for continuing success in bioremediation processes – the work that has been done will be built upon in the years to come.

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