

READINESS THROUGH RESEARCH

**ACCELERATED BIODEGRADATION OF COAL-TAR
WASTES IN SOIL**

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ACCELERATED BIODEGRADATION OF COAL-TAR
WASTES IN SOIL

LA BIODEGRADATION ACCELERE DES DECHETS
PROVENANT DU CHARBON DANS LE SOL

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ABSTRACT

The Institute of Gas Technology (IGT) has been developing several techniques to improve biodegradation of town gas plant wastes such as multi-ring polynuclear aromatic hydrocarbons (PAH's). These techniques include the use of bioemulsifiers, chemical oxidants or biologically produced chemicals, PAH-degrading cultures developed by IGT through enrichment techniques, and appropriate co-metabolic and/or enzyme-inducing substrates. Depending upon the waste type and concentration, and geological characteristics of the site, a logical combination of any number of these techniques can be used to remediate the site.

This paper discusses several liquid culture and soil treatment experiments conducted at IGT. Results show that PAH's in soils and in waters can be efficiently biodegraded by aerobic and possibly by anaerobic cultures and that chemical treatment and bioemulsifier applications enhance PAH biodegradation. This paper also mentions possible treatment scenarios consisting of various unit operations or remediation techniques.

RESUME

L'Institute of Gas Technology (IGT) développe depuis un certain temps plusieurs techniques pour améliorer la biodégradation des déchets des usines de gaz de ville tels que les hydrocarbures aromatiques polynucléaires (HAP) polycycliques. Ces techniques comprennent l'utilisation de bioémulsifiants; d'oxydants chimiques ou de produits chimiques créés par voie biologique; de cultures, développées par IGT grâce à des techniques d'enrichissement, qui dégradent les HAP; et de substrats cométaboliques ou qui favorisent la production d'enzymes. Selon le type et la concentration des déchets ainsi que les caractéristiques géologiques du site, toute combinaison logiquement cumulée de ces techniques est utilisable pour améliorer la condition des sites.

Ce mémoire traite de plusieurs expériences portant sur les cultures liquides et le traitement du sol effectuées à IGT. Les résultats indiquent que les HAP dans les sols et dans les eaux peuvent être biodégradés par des cultures aérobiques - et peut-être anaérobiques - et que le traitement chimique et les applications de bioémulsifiants améliorent la biodégradation des HAP. Le mémoire mentionne aussi des scénarios possibles de traitement comportant diverses techniques de remédiation ou opérations unitaires.

INTRODUCTION

History and Description of Town Gas Sites

Plants for the manufacture of gas from coal or crude oil, referred to as town gas plants or manufactured gas plants, have existed in many parts of the world including the United States during the nineteenth and twentieth centuries. The first town gas plant was built in England in 1812 by the London and Westminster Charter Gas, Light and Coke Company, although the first record of experimentally manufactured gas production from coal dates back to the seventeenth century in England.¹ North America's first town gas plant was built in 1816 in Baltimore.² It has been estimated that more than 1500 town gas plants were in existence in the United States from 1880 to 1950.

Today, the town gas industry has become extinct because of the inroads made by the current gas industry with its system of interstate natural gas pipelines. Nevertheless, the legacy of town gas plants remains today. While these plants were operational, society and industry were not aware of environmentally safe disposal practices and the potential problems and health concerns caused by on-site disposal. Consequently, many of these plants disposed of process wastes on-site, contaminating the soil with coal tar wastes, oil, spent oxides (from the gas cleaning operation), and ash.

Town Gas Plant Wastes

Although many town gas plant sites have similar types of waste contaminants, the sites may vary considerably in the specific types and quantities of wastes present, depending upon the types of processes used (coal gas, carburetted water-gas, or oil-gas) and the era in which the plant was operational. Polynuclear aromatic hydrocarbons (PAH's) are components of all kinds of tars, and tars were a by-product of all of these plants. The physical and chemical characteristics of these tars varied, however, according to the process employed.^{3,4} Oxide wastes from purifier boxes might contain ferrocyanide and varying amounts of arsenic, chromium, copper, lead, nickel, and zinc (all of which appear on the Environmental Protection Agency priority pollutants list). Lamp black, clinker, cinders, and ash might also be present.

Many of these town gas plants were located near waterways to provide access to the large quantities of coal necessary for their processes. Consequently, aromatic hydrocarbon contamination of streams and rivers has been traced back to some town gas sites.⁴

Town gas plant wastes can be categorized by five major types:

- Free tars, oils, and lamp black
- Hydrocarbon-contaminated soils
- Hydrocarbon-contaminated waters
- Purifier box or spent oxide wastes
- Mixed wastes and fills.

The hydrocarbon- or PAH-contaminated soils represent the largest waste type at most of these sites. Depending on the site location's associated geologic conditions and the available sources for drinking water, problems associated with contaminated waters could also be one of the major problems associated with town gas plant wastes. Therefore, the current focus of the work at IGT is to

develop techniques for efficient treatment of contaminated soils and waters.

The techniques being developed since 1987 at the Institute of Gas Technology (IGT) to accelerate the in situ and/or on-site biological degradation of PAH-laden soils are described below. These techniques include chemical and biological treatments to increase the bioavailability of PAH's to microorganisms for catabolism, the addition of growth substrates to promote the co-metabolism of multi-ring PAH's that cannot be otherwise degraded, and the addition of specially developed, mixed bacterial cultures (both aerobic and anaerobic) to ensure the presence of required types and quantities of PAH-degrading microorganisms when such microorganisms are not present in required mass. Further work is being done to optimize the soil treatment protocols and to determine the effects of non-PAH organic components and heavy metals present in soils and environmental factors on the overall treatability of contaminated soils.

The groundwater can be treated in "above-ground reactors" and the techniques developed for soil treatment can be efficiently used to accelerate the treatment of the groundwater.

BIODEGRADATION STUDIES AT IGT

There are several techniques by which a contaminated site can be cleaned on-site: chemical fixation, soil washing, thermal treatment, and biodegradation. IGT is conducting research primarily in the areas of biodegradation, utilizing microorganisms for degrading contaminants so that the by-products as well as the end products (predominantly water and carbon dioxide) do not pose any environmental concerns. IGT's approach has been very methodical in that we first identified the major rate-limiting steps and then developed or evaluated various approaches to overcome these rate-limiting steps. Some of the techniques under evaluation are not strictly biological; they also include physical or chemical treatments or both that are compatible with biological processes. The strategy of the soil cleanup technology development at IGT is to draw support from every area of science and use that in a manner that would aid biological degradation by accelerating the bioremediation process of multi-ring (2 to 6-ring) PAH's to environmentally acceptable products/levels without adversely affecting the treatment cost.

Major Factors Influencing Soil Cleanup

Factors that influence the rates and the extent of biological degradation of town gas wastes in soil have been identified. These are:

- Mass transfer limitations
 - Low solubility of PAH's in aqueous media
 - Low bioavailability of PAH's to microorganisms
- Toxicity or absence of in situ bacterial growth
- Lack or absence of capable microflora
- Complexity of wastes
- Soil/site characteristics

The following is a discussion of some of the major factors and the IGT approach to overcome these rate-limiting steps. Other

techniques under evaluation have been discussed in previous publications.^{5,6}

ENHANCEMENT TECHNIQUES FOR ACCELERATED BIOREMEDIATION

Improved Mass Transfer

Biodegradation rates appear to be limited by the availability of waste material to microorganisms, i.e. the mass transfer of waste material from the inner pores (micro as well as macro pores) of the soil matrix to the microorganisms. This limitation is caused by many factors including the low aqueous solubility of PAH's, unavailability of PAH's resulting from the formation of soil aggregates trapping components of coal tars, adsorption/absorption of PAH's in soil particles, formation of hydrophobic/hydrophilic layers separating microorganisms from PAH's, and other factors. Two of the techniques being developed at IGT to overcome the above mentioned limitations follow.

Increased Solubility of Wastes. The solubility of PAH's is very low in water and generally decreases as the molecular weight or number of rings increases (Table 1). Benzo(a)pyrene is four orders of magnitude less soluble than naphthalene and three orders of magnitude less soluble than acenaphthylene. Solubility plays an important role in determining the rate of dispersal or migration as well as the effective exposure of these compounds to microorganisms that live and grow in water.

The low solubility of PAH's poses special problems for bioremediation. On one hand, low solubility is beneficial because it limits the migration of PAH's into groundwater. On the other hand, it limits microbial degradation by making these compounds unavailable to microorganisms that are more active when they remain in water or in a moist environment. If the kinetics of degradation of PAH's are the same or better than the kinetics of solubilization, waste reduction can be achieved efficiently without increasing the possibility of further contamination of groundwater. Also, the management of water addition and the maintaining of zero-water-discharge during the treatment to avoid movement into the groundwater are very important factors.

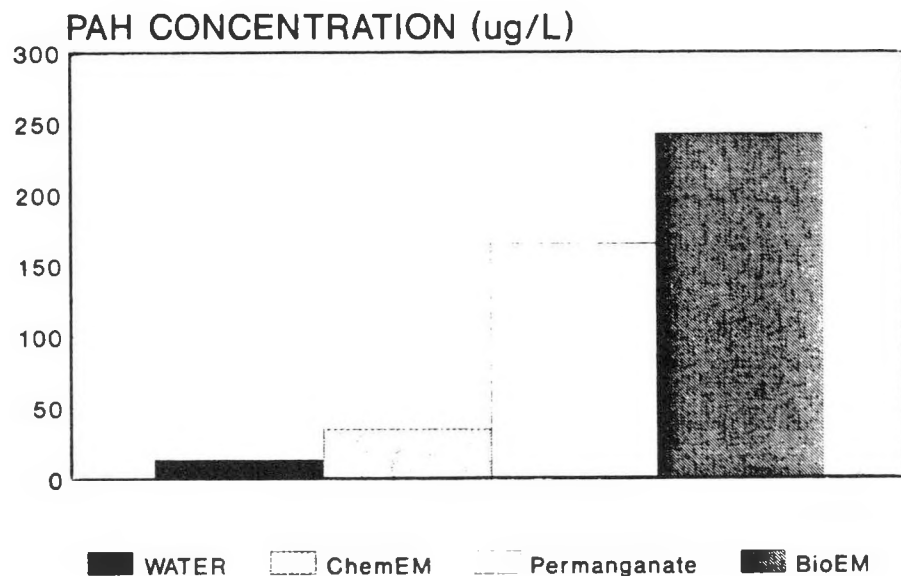
To develop such a strategy, three solubilizing agents -- a chemical emulsifier, a bioemulsifier (biologically produced emulsifier), and potassium permanganate (KMnO_4) -- were evaluated for their effects on increasing the solubility of PAH's. Figure 1 compares the effectiveness of these three solubilizing agents in releasing PAH's bound to the soil. The bioemulsifier solution was the most effective in achieving PAH solubilization. The fact that the bioemulsifier is more effective in liberating PAH's from soil aggregates bodes well for an in situ bioremediation process because biological emulsifiers can also be produced in situ.

Several biological growth experiments on 2- and 3-ring PAH's, as well as 4-ring PAH degradation experiments in liquid and in sand, using pre-grown cells were conducted to determine the effect of bioemulsifiers on PAH use. The growth experiments were conducted in shake flasks with 0.01% of bioemulsifier in the growth medium. The results obtained after subtracting the growth caused by the bioemulsifier itself are presented in Figure 2. In all cases evaluated, greater microbial growth was evident in treatments supplemented with the bioemulsifier. Figure 3 displays the results of another experiment where sand was intentionally contaminated with phenanthrene and then inoculated in one case with PAH-degrading culture and in another case with PAH-degrading culture and biologically produced emulsifier. The rate of degradation of phenanthrene was several times faster in the presence of the bioemulsifier. These data show

Table 1. PHYSICAL-CHEMICAL PROPERTIES OF SOME PAH'S THAT OCCUR IN COAL TAR^{*}

PAH	Rings (No.)	Mol. wt.	mp (°C)	bp (°C)	Solubility (mg/L H ₂ O) 25°C	Vapor Pressure, (torr) 20°C
Naphthalene	2	128	82	218	31.7	4.92 x 10 ⁻²
Acenaphthene	3	154	95	278	3.47	2.0 x 10 ⁻²
Acenaphthylene	3	152	92	265	3.93	2.9 x 10 ⁻²
Anthracene	3	178	218	342	0.073	1.95 x 10 ⁻⁴
Fluorene	3	166	116	295	1.98	1.3 x 10 ⁻²
Phenanthrene	3	178	101	340	1.29	6.80 x 10 ⁻⁴
Fluoranthene	4	202	110	393	0.26	6.0 x 10 ⁻⁶
Pyrene	4	202	156	404	0.135	6.85 x 10 ⁻⁷
Chrysene	4	228	160	400	0.014	5.0 x 10 ⁻⁹
Benz(a)anthracene	4	228	160	400	0.014	5.0 x 10 ⁻⁹
Benzo(a)pyrene	5	252	178	495	0.0038	5.0 x 10 ⁻⁷
Benzo(b)fluoranthene	5	252	167	481	0.0012	5.0 x 10 ⁻⁷
Benzo(j)fluoranthene	5	252	215	480	--	--
Benzo(k)fluoranthene	5	252	215	480	0.00055	5.0 x 10 ⁻⁷
Dibenz(a,h)anthracene	5	278	262	524	0.0005	1.0 x 10 ⁻¹⁰
Indeno(1,2,3-cd)pyrene	6	276	163	--	0.062	1.0 x 10 ⁻¹⁰
Benzo(ghi)perylene	6	276	>220	>500	0.00026	1.0 x 10 ⁻¹⁰
Coronene	7	300	>360	525	--	--

* Data compiled from Simms, 1982; ERT, 1984; Merck Index, 10th Edition, 1983.



The leaching solution was in contact with the soil for 7 days.

Figure 1. EFFECT OF SOLUBILIZING AGENTS ON THE LEACHING OF PFH'S FROM TOWN GAS PLANT SOILS

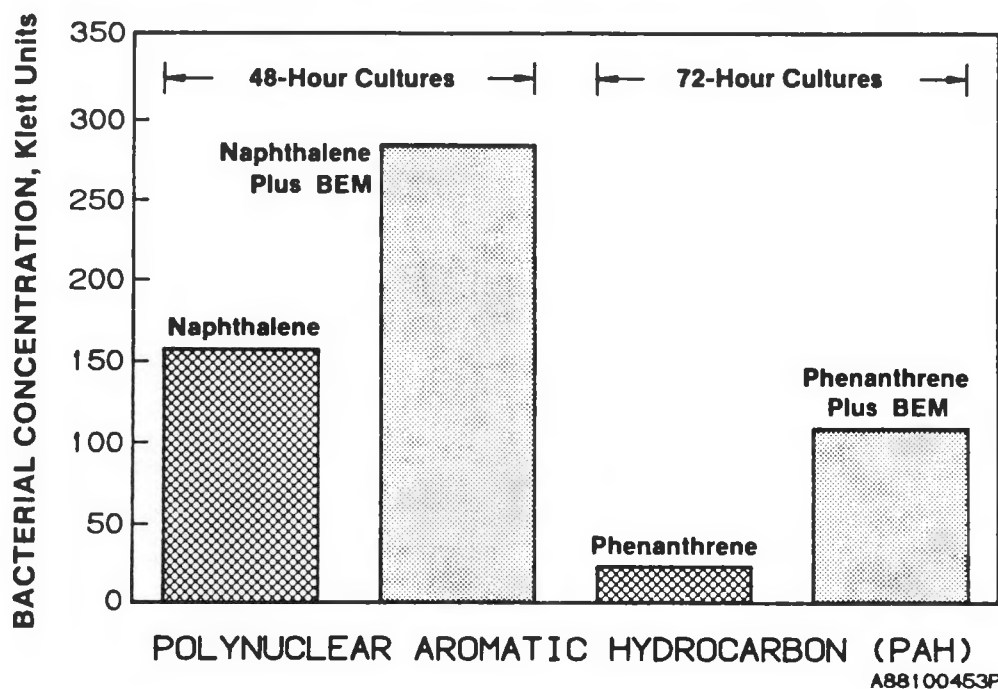


Figure 2. EFFECT OF BIOEMULSIFIER ON PAH UTILIZATION

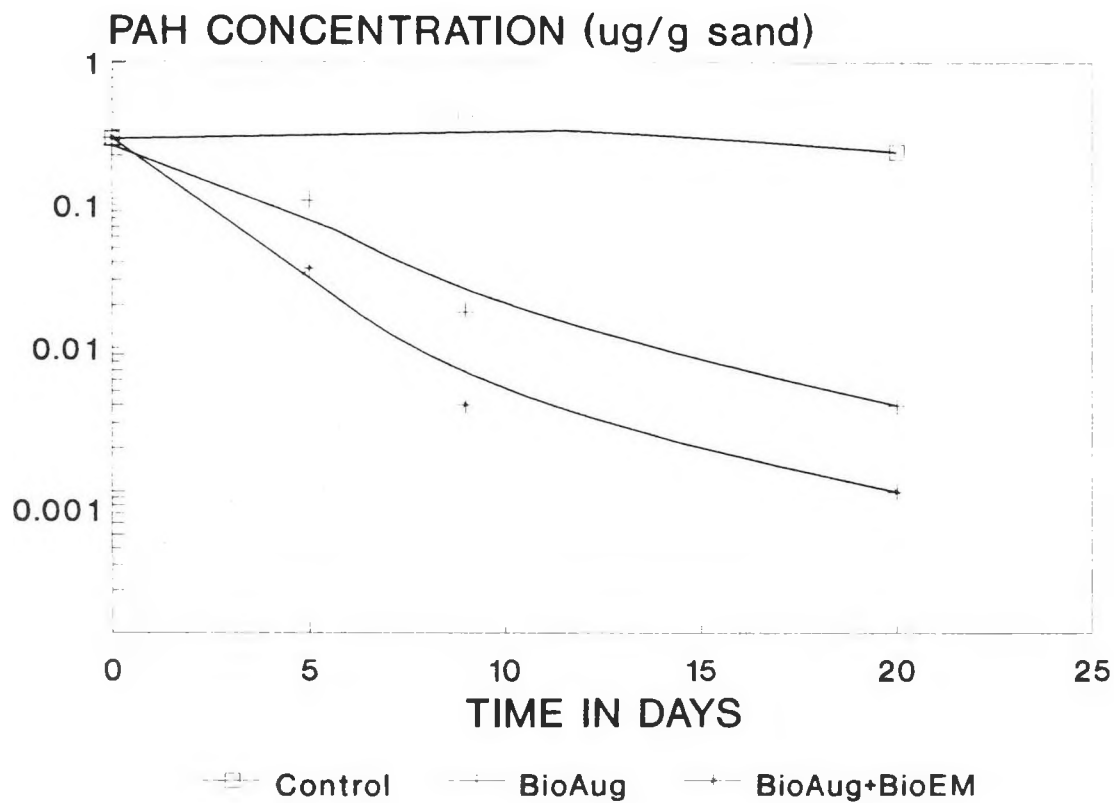


Figure 3. EFFECT OF BIOEMULSIFIER ON THE RATE OF PHENANTHRENE BIODEGRADATION IN SAND

that the bacterial growth rates were higher in the presence of the bioemulsifier; this could be attributed to the increased solubility of PAH's in the presence of the bioemulsifier. Additional experiments are being conducted to determine the effects of bioemulsifiers on the treatment of actual contaminated soil.

Increased Bioavailability of PAH's. Many PAH's present at the town gas sites are higher molecular weight (>250) multi-ring (4, 5, and 6-ring) compounds. These compounds have a tendency to agglomerate. An outer layer of extremely recalcitrant large molecular weight compounds can be absorbed in the pores of soil aggregates, or adsorb to soil particles. Because of this, these compounds are often not available to microorganisms in a form that can be used either for growth (too large a molecule) or for co-metabolism (physically not available, mass transfer limitation). To overcome these limitations and increase the availability of PAH's either by oxidizing the parent molecule, by attaching functional group(s), or by reducing the size of large-ring PAH's through chemical reaction, several chemical oxidants were evaluated for pre-, co-, and/or post-treatment purposes. Of the five chemicals -- potassium permanganate (KMnO_4), hydrogen peroxide (H_2O_2), nitric acid (HNO_3), bleach, and sulfuric acid (H_2SO_4) -- two chemicals, KMnO_4 and H_2O_2 , were most effective in oxidizing PAH's (Figure 4). Additional experiments with H_2O_2 and a mixture of H_2O_2 and ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) showed that the presence of Fe^{++} increases the kinetics of the reaction severalfold (Figure 5). The fact that hydrogen peroxide plus Fe^{++} is effective in oxidizing PAH's, thus making these compounds biologically more available, complements the in situ biological treatment processes because hydrogen peroxide can be biologically produced in the soil matrix by fungi while biodegradation of PAH's is being carried out by another group of microorganisms.

Additional experiments are being conducted to study the dose response of these chemicals on biological degradation. Ultimately, the kinetics of biological degradation of oxidized PAH wastes and cost/benefit analyses will determine the rate and dosage of chemicals most appropriate for site remediation. Also, in some cases chemical treatment can be used as an independent soil treatment technique.

Development and Evaluation of PAH-Degrading Cultures

Low numbers or the absence of pollutant-degrading microorganisms, as well as the toxicity of certain pollutants to microorganisms, present problems for the effective biodegradation of wastes in soil. To reduce growth inhibition, ensure the presence of desired microorganisms, and promote desired microbial growth and activities in the soil, several enrichment culture experiments are being conducted at IGT. The sources of inocula for culture enrichment experiments were several town gas site soils, soils from crude petroleum disposal sites, soils from coal storage areas, or wastewater treatment plants.

Results of several experiments, conducted to determine the kinetics of PAH degradation under aerobic conditions, show that IGT cultures developed using enrichment techniques are well adapted to various PAHs and are capable of degrading multi-ring PAH's. These experiments were conducted in shake flasks and used 2 to 5-ring PAH's present as aqueous solutions. Figure 6 shows the biodegradation kinetics of soluble 2 to 4-ring PAH's by a growing culture. The biodegradation kinetics of all of the 2 and 3-ring compounds were very good. Nevertheless, the kinetics of pyrene biodegradation, a 4-ring compound, for example, was slow. But when a large concentration of pre-grown PAH-degrading culture was incubated in resting cell experiments with PAH concentrations well in excess of

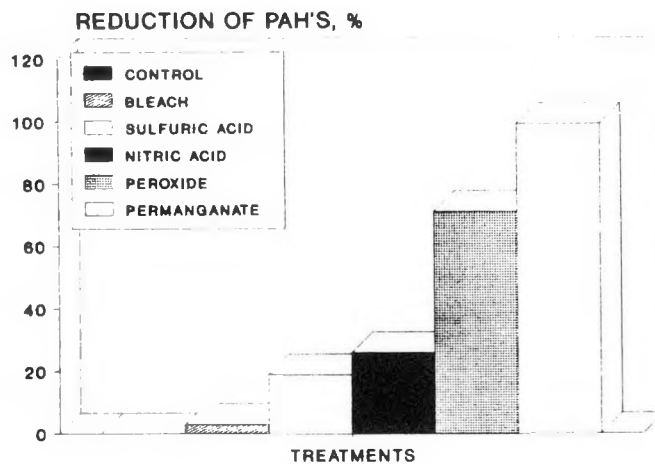


Figure 4. EFFECT OF CHEMICAL TREATMENT ON PAH MODIFICATION

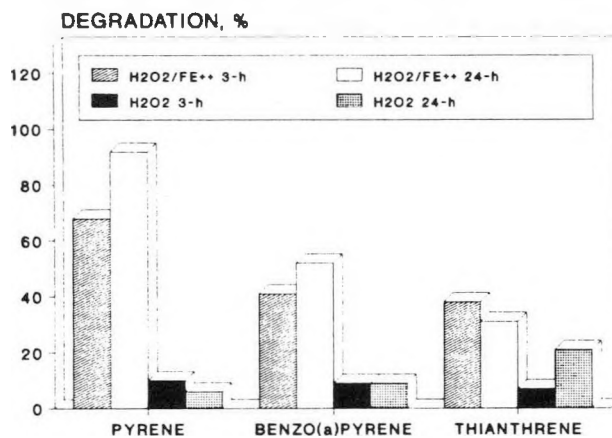


Figure 5. CHEMICAL PRETREATMENT: H_2O_2/FE^{++} EFFECT ON HIGH-MW/HIGH-CONCENTRATION PAH'S

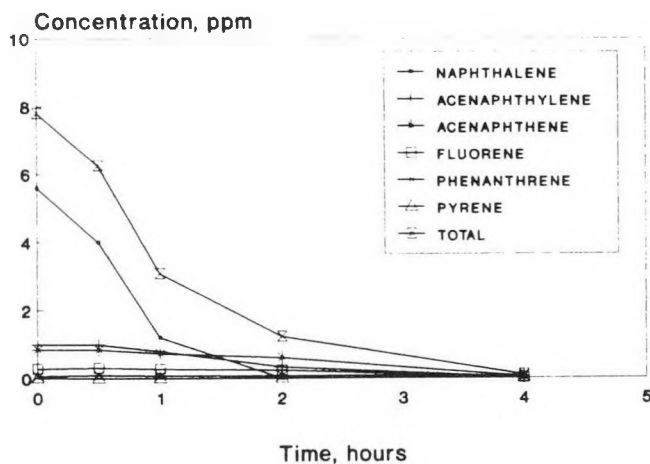


Figure 6. BIODEGRADATION OF PAH'S USING IGT'S MIXED AEROBIC CULTURE

their aqueous solubility, a 4-ring PAH and benzo(a)pyrene, a 5-ring PAH, showed significant amounts of biodegradation (Table 2 and Figure 7).

In addition to aerobic degradation, several anaerobic cultures are also being developed to assess the potential of in situ anaerobic degradation of PAH's in subsurface environments. Figure 8 shows the results of a 72-hour growth experiment under aerobic and anaerobic (denitrifying) conditions. Under aerobic conditions, soluble PAH's almost completely disappeared, as expected. While under anaerobic conditions, the reduction ranged from 40% to 99%, suggesting that anaerobic soil cleanup is quite feasible. Additional experiments with radiolabelled PAH's are underway to better understand both the aerobic as well as anaerobic degradation of PAH's.

These data suggest that PAH's can be degraded under both aerobic and anaerobic environments. Data collected so far also show that lower ring PAH's can support the growth of bacteria. Data collected with pregrown cells show that the presence of PAH-degrading cultures, the appropriate inoculum size, and the absence of any other readily metabolizable carbon source will be conducive to the rapid biodegradation of higher ring PAH's. Further work is under way to delineate the effects of these techniques and observations in soil.

CONTAMINATED SOIL TREATMENT EXPERIMENTS AT IGT

Several soil/solid matrix experiments are under way to determine the effects of bioemulsifier; chemical pre-, co-, and/or post-treatment; addition of pre-grown PAH-degrading cultures; and environmental factors such as presence of air (aerobic) or absence of air (anaerobic), moisture content, mixing, and other factors. The results of a few experiments, although preliminary in some cases, are discussed below.

Moist Soil (Soil Pan) or Landfarming Experiments

Several soil pan experiments have been conducted with contaminated town gas plant soils as well as with an intentionally contaminated soil.^{6,7}

In one experiment, actual contaminated soil was used to determine the effects of a co-metabolism substrate (oleic acid, paraffin or salicylate) and bioaugmentation (addition of pregrown PAH-degrading aerobic culture) on soil decontamination. Biodegradation data are plotted in Figure 9. Although limited in data and number of sample points, the results suggest that the presence of readily metabolizable carbon (co-substrates) interfered with the PAH degradation and that the addition of acclimated cultures was beneficial.

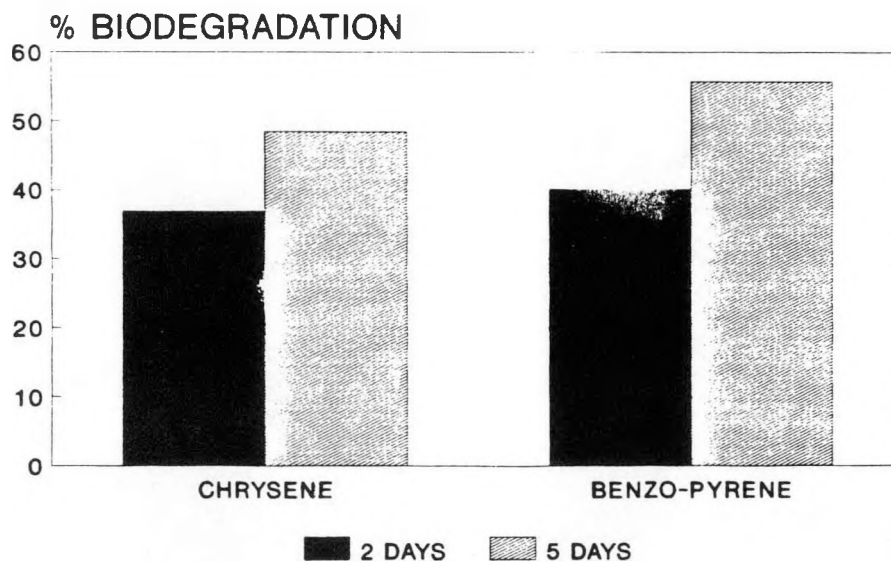
Soil-Water Slurry Experiments

An experiment was conducted with actual town gas soil in a soil-water slurry system using an IGT PAH-acclimated mixed culture. Data obtained after five weeks of incubation are presented in Figure 10 as percent degradation. The results are consistent with the literature which shows that 2- and 3-ring PAH's are degraded more rapidly than 4- to 6-ring PAH's. Nevertheless, it is significant that 4- to 7-ring PAH's, absorbed/adsorbed on soil particles and having gone through an aging process of 50 to 100 years, are degraded in such a short span of time.

Table 2. BIODEGRADATION OF HIGH CONCENTRATIONS OF PAH'S IN LIQUID CULTURE

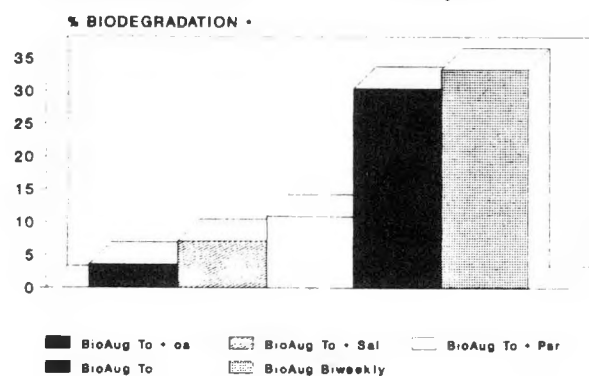
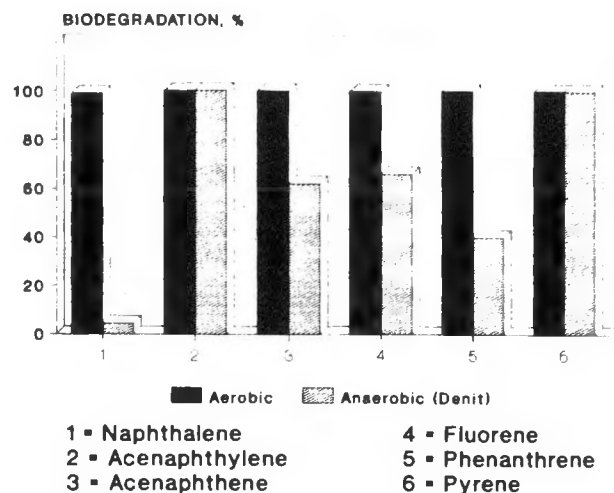
		Chrysene	Benz-pyrene
<u>2 Days</u>	Controls	14.7 15.0 14.9 <u>14.9</u>	1.2 1.2 1.1 <u>1.2</u>
	Avg.		
	Experimentals	9.3 6.0 12.8 <u>9.4</u>	0.76 0.45 1.03 <u>0.75</u>
	Avg.		
	% Biodegradation	36.9	40.1
<u>5 Days</u>	Controls	15.6 16.7 15.3 <u>15.9</u>	1.35 1.42 1.30 <u>1.36</u>
	Avg.		
	Experimentals	6.5 8.9 9.0 <u>8.2</u>	0.46 0.67 0.68 <u>0.6</u>
	Avg.		
	% Biodegradation	48.5	55.8

The washed cell control showed no detectable levels of PAH's.



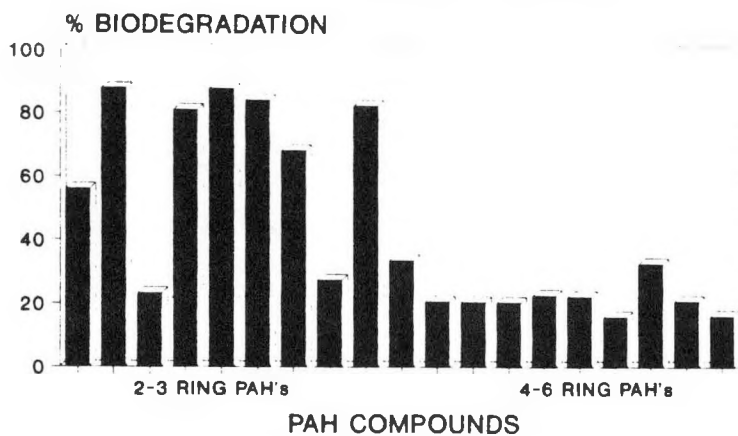
Chrysene and benz-pyrene were present at 150 and 13 ppm respectively.

Figure 7. KINETICS OF 4- AND 5-RING PAH'S IN LIQUID CULTURE EXPERIMENTS BY PREGROWN CELLS



* Average of seven 3-6 ring PAH's after 73 days

Figure 9. PRELIMINARY FINDINGS OF A TOWN GAS SOIL TREATMENT EXPERIMENT IN SOIL PANS



AVERAGES OF FIVE EXPERIMENTALS AND THREE CONTROLS ARE COMPARED

Figure 10. BIODEGRADATION OF PAH'S IN TGS-1 SOIL-WATER SLURRY REACTORS

Soil Treatments Under Anaerobic Conditions

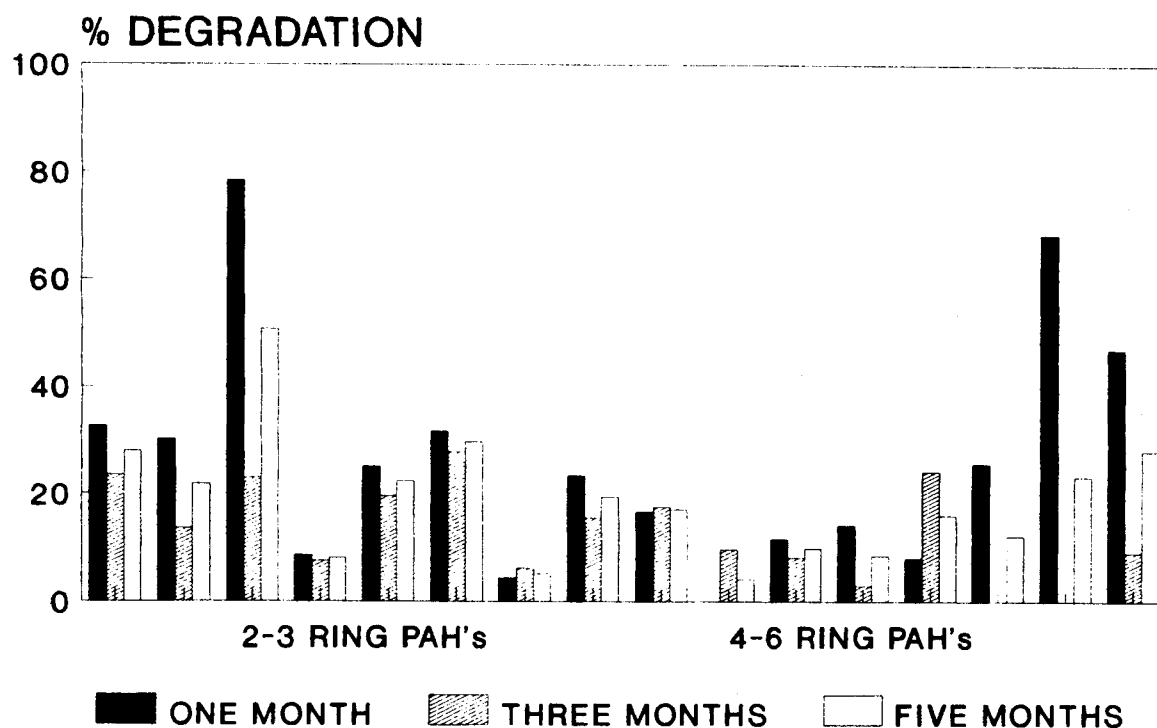
The in situ bioremediation of contaminated soils under anaerobic conditions will be highly desirable as it would require a minimum of intervention at a site which would not only make the treatment less expensive but also widely applicable. The literature suggests that the biodegradation of organopollutants in an anaerobic environment might best be accomplished under denitrifying conditions. To study this treatment scenario, contaminated soil was put under denitrifying conditions using a mineral salts solution and potassium nitrate. Results of soil samples are presented in Figure 11 where the percent biodegradation is plotted for sixteen PAH's. The data seem to indicate that an average of about twenty percent of PAH's were degraded in five months. Although the data are preliminary in nature and indicate a slower rate of degradation, the results are very encouraging. Additional experiments under several different conditions are being initiated to conclusively determine the potential of anaerobic bioremediation.

SUMMARY AND CONCLUSIONS

Research conducted thus far at IGT shows that town gas wastes such as 2- to 6-ring PAH's can be biologically degraded under aerobic and possibly under anaerobic conditions. The rate and extent of biodegradation can be improved by applying any or all of the techniques under development at IGT. These techniques include the use of bioemulsifiers or environmentally acceptable non-aqueous liquids, soil attrition techniques, chemical oxidants, or biologically produced chemicals, PAH-degrading enrichment cultures, and appropriate co-metabolic substrates, and/or enzyme-inducing substrates. Some of these techniques can be used either as a pretreatment or as a co-/post-treatment. Further work is being conducted to develop process schemes with built-in flexibility so they can be used for different town gas plant sites with some modifications.

In summary, we feel that more than one treatment option would be needed to cost efficiently remediate most of the sites. For example, the "hot spots" may be best handled by either thermal treatment (combustion) or by physically removing (excavating) rather than by biological methods. These techniques (thermal or physical) are either well developed or are being developed by other researchers. For most of the contaminated sites, however, as well as most parts of a site, biological treatment with (or without in some cases) enhancement techniques should result in the most desirable treatment scenario. Some of the possible treatment scenarios for contaminated soils are --

- Biological treatment
 - Aerobic alone
 - Anaerobic alone
 - Aerobic for surface and near surface soils and anaerobic for subsurface soils
- Biological treatment followed by chemical treatment (and then biological again)
- Physical or thermal treatment followed by biological treatment
- Chemical treatment followed by biological treatment.



AVERAGES OF TRIPPLICATE SAMPLES
 ARE COMPARED WITH THE
 AVERAGE OF DUPLICATE CONTROLS

Figure 11. BIODEGRADATION OF PAH'S UNDER ANAEROBIC
 (Denitrifying) CONDITIONS

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