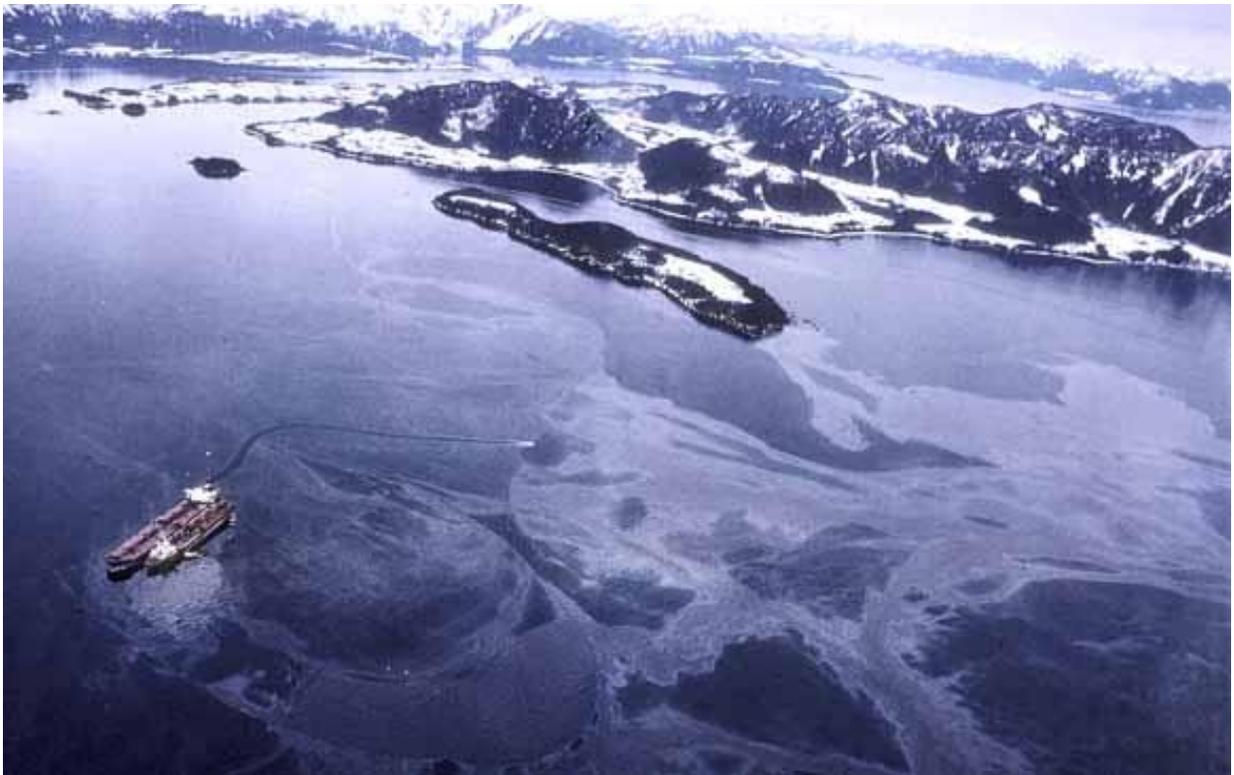


Environmental Degradation of petroleum hydrocarbons



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Front: Petroleum spill in Prince William Sound, Alaska, after the tanker accident with the Exxon Valdez (2)

Abstract

Petroleum hydrocarbons are causing widespread pollution in both the aquatic and the terrestrial environment. Petroleum consist of alkanes, cycloalkanes, aromatics, polyaromatics (PAHs) and other compounds. All of these hydrocarbons groups are toxic for the environment, but especially the PAHs and their degradation products are known for their carcinogenic properties.

The partitioning of hydrocarbons in the environments depends on volatility and hydrophobicity, influencing the partitioning between soil, water and sediment. The risk and degradation rates of hydrocarbons are besides this partitioning strongly dependent on bioavailability. Presence of other reactive or absorbing substances and environmental conditions have shown to influence the fate and degradation rates of hydrocarbons in the environment. Sediments, and more specific the carbon compounds in sediment, are the most important absorbing substances.

The most important degradation pathways in the environments are performed by light and organisms, bacteria, fungi and algae. Where bacterial degradation results in total degradation of nearly all hydrocarbons light is only able to make some photochemical modifications in the hydrocarbons. The degradation mechanisms have shown enormous influence not only on degradation but also on physical and chemical properties of hydrocarbons, resulting in change of partitioning in the various compartments in the environment. Environmental condition influencing degradation are pH, temperature, oxygen, nitrogen compounds, humic acids and salinity.

Due to the widespread use of hydrocarbons and subsequent pollution several methods for soil and water remediation have been developed. These methods include physical based methods as evaporation of hydrocarbons and biodegradation methods by stimulation of bacteria able to degrade hydrocarbons. For water treatment absorption and biodegradation are the most used methods.

The concequences of soil and sediment remediation for society in the Netherlands are enormous, which is illustrated by two examples; PAH pollution in the river delta in the Netherlands and soil pollution of former gas factories in the area of Utrecht.



Figure 1 Soil remediation in the Griftpark, Utrecht 1994 (1)

Contents

Abstract	3
Contents.....	5
1. Introduction.....	7
1.1. Composition of oil	7
1.2. Oil and toxicity for the environment	8
1.2.1. Toxicity of Polyaromatic hydrocarbons (PAHs)	9
1.3. Fate of petroleum in the environment.....	10
1.3.1. Hydrocarbons dissolved in water	11
1.3.2. Bioaccumulation	12
1.3.3. Sorption to Sediments and soil	12
1.3.4. Formation of NAPLs	15
1.3.5. Petroleum degradation.....	16
2. Petroleum degradation and light	17
2.1. Mechanisms of oil degradation by light	17
2.1.1. Direct mechanism, direct photolysis	17
2.1.2. Indirect photolysis, radical oxidation and electron transfer	18
2.2. Influence of the environment on photodegradation	18
2.3. Effect of oil degradation by light	19
3. Biodegradation of Petroleum by bacteria	22
3.1. Mechanisms for petroleum biodegradation	22
3.1.1. Aerobic degradation	22
3.1.2. Anaerobic degradation	23
3.2. Factors influencing biodegradation	25
3.3. Effect of biodegradation	27
4. Other degrading organisms	29
4.1. Fungi	29
4.2. Algae.....	30
4.3. Plants	30
5. Remediation of petroleum-contaminated soils	32
5.1. Physical en chemical methods.....	32
5.2. Biodegradation methods.....	34
6. Stimulating degradation of hydrocarbons in the aquatic environment.....	36
6.1. Prevention of hydrocarbon toxicity by use of activated carbon	37
6.2. Wastewater treatment.....	37
7. Examples of degradation of hydrocarbon pollution in the Netherlands	39
7.1. Example: PAH pollution in the river delta of the lower part of the Netherlands.....	39
7.2. Example: soil pollution by gas factories in the area of Utrecht	41
8. Conclusions and remarks.....	44
8.1. Further research on petroleum degradation	45
References	48

1. Introduction

During the past 50 years there has been a lot of oil pollution in the aquatic and terrestrial environment due to human activity. This has an enormous effect on the quality of the water and soil in some parts of the world. Petroleum is also a source of environmental pollution on beaches and near oil dump sites. Most notably the aquatic animals have to suffer from this oil pollution(3), but also the plants and animals in the terrestrial system are influenced. There has been a lot of research on oil degradation in the past.

In a lot of tanker accidents it was tried to break down or remove parts of the oil with special separator boats or use of detergents. Removal of parts of the oil in most cases has some positive effect on the environment, but the use of detergents sometimes only worsen the effect of the pollution. Sometimes even detergent was used without knowing what the effect of the addition would be. To make better approaches for the future available several studies were performed on the degradation mechanisms of oil in the aquatic environment and the factors that influence it.

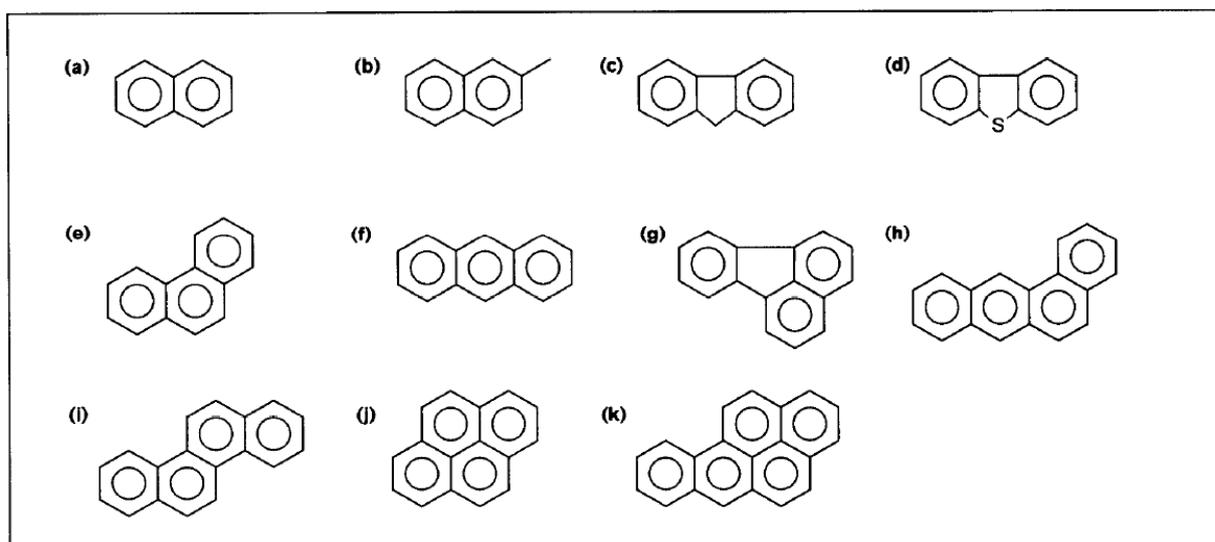
In the past 20 years there has been an increase in research on cleaning soil. This has to do with the change of industry near cities. There were a lot of gas producing companies in cities who closed during the past 40 years, but they left heavy organic polluted ground in the city. To make reuse of this ground possible, there is a need for effective cleaning measures to remove the organic compounds. There are several methods available these days, based on various mechanisms discussed further in these thesis.

There are several mechanisms to degrade and remove petroleum from the environment, both biological and chemical. The most well known are evaporation and degradation by bacteria and fungi. Where the pollution take place in the world does also matter, because the abiotic factors including light intensity and temperature of water and soil have also an effect on degradation. Most of these processes are related or influence each other, degradation by light for example stimulates biodegradation and evaporation is both beneficial and harmful for degrading bacteria. The aim of this thesis is to clarify most of the oil degrading mechanisms and the relation between these mechanisms. With this information it perhaps will be possible to prevent unnecessary pollution in the environment.

1.1. Composition of oil

Before considering the degradation of oil it is first essential to focus on the composition of oil. It is well known that the composition of oil varies both between sources and in source itself during years. Therefore it is not possible to give an exact composition of oil in general. One can say that every oil contains at least the following groups of chemicals: alkenes, cycloalkanes, aromatics and polyaromatics(4). Further it contains some additional nitrogen en sulfur containing compounds. The different components of oil present in the environment can be determined by various techniques including silica gel chromatography(5) and solid phase microextraction (SPME)(6). GC/MS is also used

although it is very time-consuming to find the exact composition of the petroleum mixture(7). Compound of special interest due to their carcinogenic properties are the PAHs. The most common PAHs are shown in figure 2(8).



Structures of representative PAHs. (a) Naphthalene. (b) 2-methylnaphthalene. (c) Fluorene. (d) Dibenzothiophene. (e) Phenanthrene. (f) Anthracene. (g) Fluoranthene. (h) Benz[a]anthracene. (i) Chrysene. (j) Pyrene. (k) Benzo[a]pyrene.

Figure 2 PAHs most considered in degradation and toxicological research

1.2. Oil and toxicity for the environment

The toxicity of petroleum hydrocarbons depend on the solubility and the bioavailability of the hydrocarbons. It is assumed that the water soluble fraction is the most environmental harmful fraction because it is direct available for uptake by organisms. The partitioning of a hydrocarbon in organisms is depending on the hydrophobicity of the compound which is in models expressed by K_{ow} , the partitioning of the organic compound between an octanol and water phase. The height the biological available concentration in organisms can reach is depending on the K_{ow} and the time in contact with the hydrocarbon(4).

In the past it was assumed that the water soluble fractions of the aromatics and polyaromatics were the most harmful and thus these compounds were the molecules for considering in toxicological studies. They are assumed to be mutagenic and carcinogenic(9). Especially the polyaromatic hydrocarbons with 4 or 5 rings are known carcinogens(10). The non aromatic substances in the petroleum were not considered very harmful. This is in fact not thru, and alkanes and cycloalkanes are now also taken into account(4). Hydrophobic hydrocarbons are toxic for microorganisms by accumulation in the membrane, which causes the loss of membrane integrity(11). As toxicology depends on concentration, biodegradation is an important topic in petroleum toxicology because it changes both the nature and concentration of the chemical compounds.

Another uptake route for hydrocarbons is via sediment. The most hydrophobic hydrocarbons will absorb in the sediment and are toxic to the animals living there. For these animals the bioavailability and bioaccumulation of these absorbed hydrocarbons is assumed to be higher than for the water soluble hydrocarbons, because these hydrocarbons are more concentrated and also have higher K_{ow} values. High K_{ow} values in general predict bioaccumulation when the compounds can be taken up.

1.2.1. Toxicity of Polyaromatic hydrocarbons (PAHs)

Although more fractions of petroleum can lead to toxic effects the toxic properties of PAHs are most studied. PAHs are assumed to be carcinogens. The most dangerous PAHs are benzo[a]pyrene, benz[a]anthracene and dibenz[a,h]anthracene. These compounds also appear to be the less bioavailable PAHs. The solubility of only 3% results in low bioavailability. This is something that should be included in a risk assessment(12).

As usual with carcinogens the risk of a chemical is expressed in a measure of added risk due to that compound. In a study benzo[a]pyrene was estimated to be responsible for 48 to 52% of the added risk of a sediment and for 44 to 54% in another sediment. When also concentrations benz[a]anthracene and dibenz[a,h]anthracene were included 90% of the added risk could be estimated. This proves that concentrations of these compounds, in combination with other environmental factors discussed in further chapters, are useful for risk estimation of PAHs(12).

There are several attempts for classifying PAHs in so called Toxicological Equivalent factors (TEF). The result of one of them is shown in figure 3. Benzo[a]pyrene was not considered the most toxic compound in this study. Benzo[a]pyrene is often assumed to be the most harmful PAH(13).

PROPOSED TOXICITY EQUIVALENCY FACTORS (TEFS) FOR INDIVIDUAL PAHS	
Compound	TEF
Dibenzo[a,h]anthracene	5 ^a
Benzo[a]pyrene	1
Benzo[a]anthracene	0.1
Benzo[b]fluoranthene	0.1
Benzo[k]fluoranthene	0.1
Indeno[123-c,d]pyrene	0.1
Anthracene	0.01
Benzo[g,h,i]perylene	0.01
Chrysene	0.01
Acenaphthene	0.001
Acenaphthylene	0.001
Fluoranthene	0.001
Fluorene	0.001
2-Methylnaphthalene	0.001
Naphthalene	0.001
Phenanthrene	0.001
Pyrene	0.001

^a A TEF of 1 appears to be appropriate for high doses of DBA but the TEF of 5 is considered more likely to be applicable to environmental exposures (chemical-related tumor incidence rate of less than about 25%).

Figure 3 Toxic equivalent factors for PAHs

In toxicological studies it is also important to consider the possible toxic metabolites of PAHs. The first step in the degradation of aromatic hydrocarbons in eukaryotes is the formation of trans-dihydrodiol, which will be explained in detail later. These trans-dihydrodiols are in equilibrium with trans-diol epoxides(14). The dihydrodiols are converted to catechols, which autooxidize to quinones(15). The toxic metabolites form adducts on the DNA which leads to DNA damage(16). In animals these adducts showed a very slow turnover, leading to a accumulation of these adducts. The reactive species formed, quinones and trans-diol epoxides, can also cause DNA scission. The metabolic pathways are illustrated in figure 4 for benzo[a]pyrene(14).

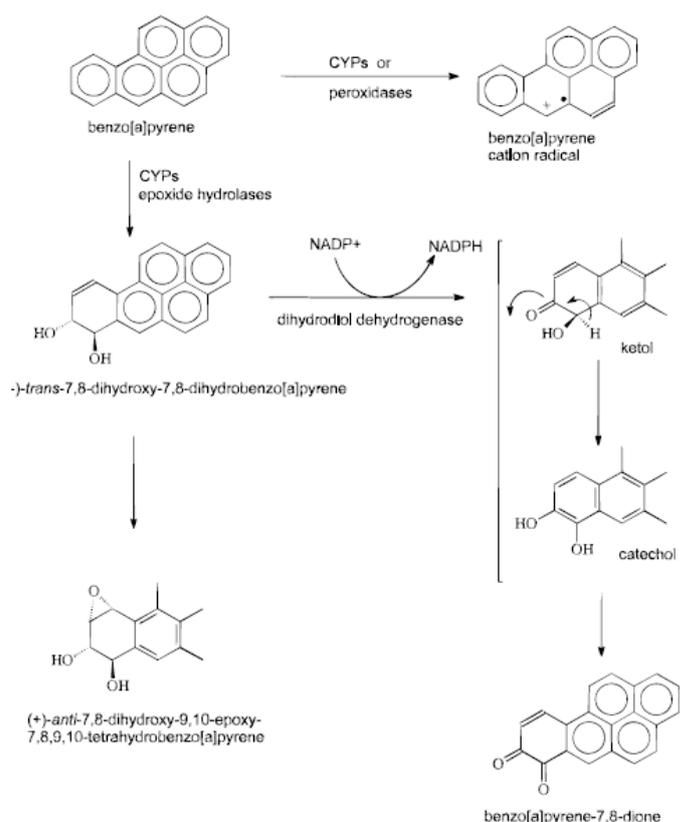


Figure 4 Formation of reactive metabolites from benzo[a]pyrene

A factor that influences the risk associated with PAHs is bioaccumulation in plants and animals(17). This will be explained in the next chapter.

1.3. Fate of petroleum in the environment

When petroleum comes in water a very fast partitioning between the water, air and sediment part of the environment take place(18). The insoluble fraction forms a layer of 0.01 to 3.0 mm thickness on the water layer(19). During the first few hours some parts evaporate and other parts are absorbed in the sediment. When the hydrocarbons are concentrated enough non-aqueous phase liquids (NAPLs) can be formed. The remaining hydrocarbons are present in the aqueous layer or as a film on the water surface. The lighter fractions are removed within twenty-four hours by evaporation. Studies show that the amount of oil that evaporates strongly depends on the oil. The evaporation of

alkanes is possible until an 18 carbon chain(18). The mass loss due to evaporation can range for 0,1% for heavier oils to 17,3% for lighter oils(20). Evaporation of lighter fractions stimulates biodegradation, because the lighter fractions are more toxic to degrading bacteria(21). After the partitioning the degradation starts in the different compartments. In this thesis the degradation in water, sediment and soil will be considered.

1.3.1. Hydrocarbons dissolved in water

For toxicology studies the part of the hydrocarbons that comes in contact with organisms or is accumulative in the environment is the most important. On the other hand the molecules that are absorbed in the sediment will remain longer in the environment because they are less available for degradation. Often a water in oil emulsion is formed in the aquatic environment, due to the increased viscosity of the oil after evaporation of volatile compounds. This makes degradation less favorable(22). In fact bacteria are only able to degrade hydrocarbons solved in water. This explains the persistence of larger PAHs(23). Bacteria have developed a mechanism to circumvent this problem; production of surfactants. This subject will be discussed in the chapter 'effect of biodegradation'.

Only some fractions are dissolved in water after petroleum spill in the environment, and this can be as low as only 2%(22). Other parts are absorbed in the sediment or soil. Lighter 3 or 4 ring aromatic molecules are soluble in water (31.7 mg/L), but the PAHs consisting of 5 or more aromatic rings are not soluble in water (0.003 mg/L) and will become associated with the sediment (10; 12). This makes them more persistent. Recent research revealed that also the presence of humic acids can be very important for solvability of PAHs insoluble in absence of humic acids. The results are shown in figure 5(24).

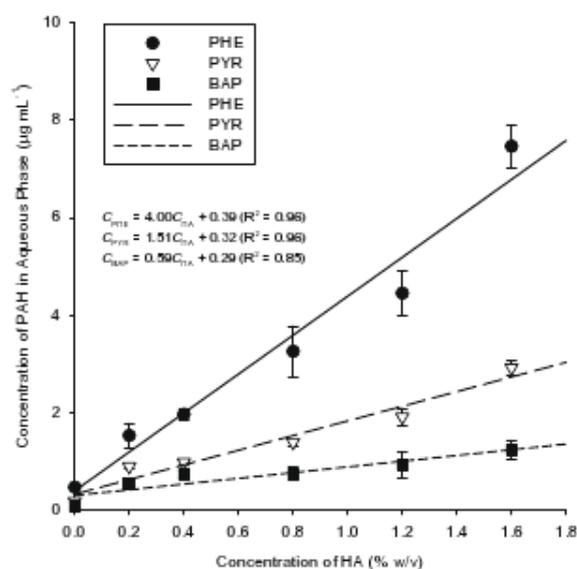


Figure 5 Effect of humic acids on PAH solvability

An important part of oil degradation in the aquatic environment is emulsification, because this leads to hydrated particles with properties differing from the molecular oil layer. This particle formation cause spreading of oil through particles which are too polar for sediment absorption and have a long degradation period in the water. It is mainly the polyaromatic or asphaltic fraction which is associated with emulsion formation. In the following part the degradation mechanisms are explained in more detail.

1.3.2. Bioaccumulation

PAHs are known for their bioaccumulation. PAHs accumulate in the lipid rich tissues of animals. This is especially seen in the liver of fish and in the pancreas of invertebrates. The more hydrophilic PAHs are taken up by aquatic organisms from the ventilated water. The other uptake route for the more hydrophobic PAHs is via food and sediment. The food uptake route implies a accumulation of PAHs in the found chain, which is of interest because humans are frequently the last part of the found chain. For animals the uptake of PAHs shows a seasonal variation(17). The uptake of PAHs by plants also has been investigated, and it was shown that the PAH concentration is the result of a partitioning between the absorbed phase and the gas phase. Due to a large dependence of this partitioning on the temperature also here a seasonal variation was seen(25).

1.3.3. Sorption to Sediments and soil

Sediment absorption is important for degradation of hydrocarbons because it makes the hydrocarbons in general less available for degradation. Uptake of hydrocarbons by microbes was shown to be much slower from the sediment than when the hydrocarbons are in a solved state(26). The absorption of organic compounds depends on a lot of factors. First the composition of the sediment is an important factor. Further the presence of other organic substances in the soil can have an influence. At last also the environmental condition as for example pH, salinity and water temperature play a role in absorption. The pH was found to have a minor influence, with a 6-9% decrease in absorption with an increase of one pH unit in the pH 6.5-8.5 domain(27). The effect of salinity on sediment absorption turned out to be much bigger in an experiment with fatty acids, shown in figure 6. From 0 ‰ to 4 ‰ there is an increase in sediment absorption. With salt concentration higher than 4 ‰ the absorption continues to increase, but much slower(27).

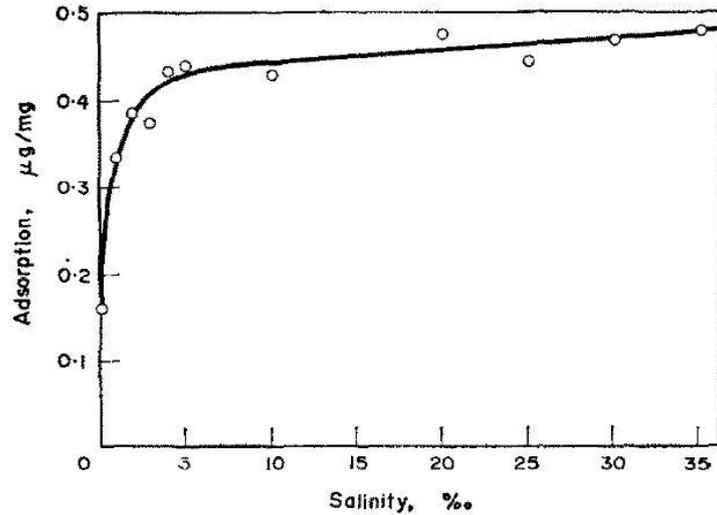


Figure 6 Effect of salinity on sediment adsorption of fatty acids

All of these absorptions are measured in the so called steady state, the partitioning of hydrocarbons is not changing any more. But for practical purposes, for example to estimate sediment pollution during an oil tanker accident, temperature is also important. Temperature has an effect on the absorption rate, and therefore determines how the hydrocarbons which will absorb in the sediment are spread out in a certain area. In a steady state experiment it was found that there was a inverse relation between temperature en sediment absorption, shown in figure 7(27).

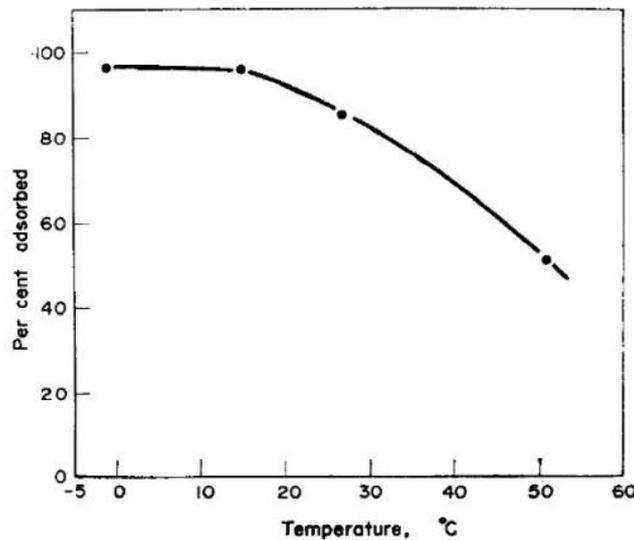


Figure 7 Temperature effect on sediment adsorption

It was expected that the composition of the soil had a great effect on the partitioning of hydrocarbon in soil, but this effect was not as important as expected(28). Except the composition of the sediment itself the presence of other organic substances as for example coal and soot can be important for hydrocarbon absorption(29). Organic substances can also originate from a natural source as shown in figure 8(30).

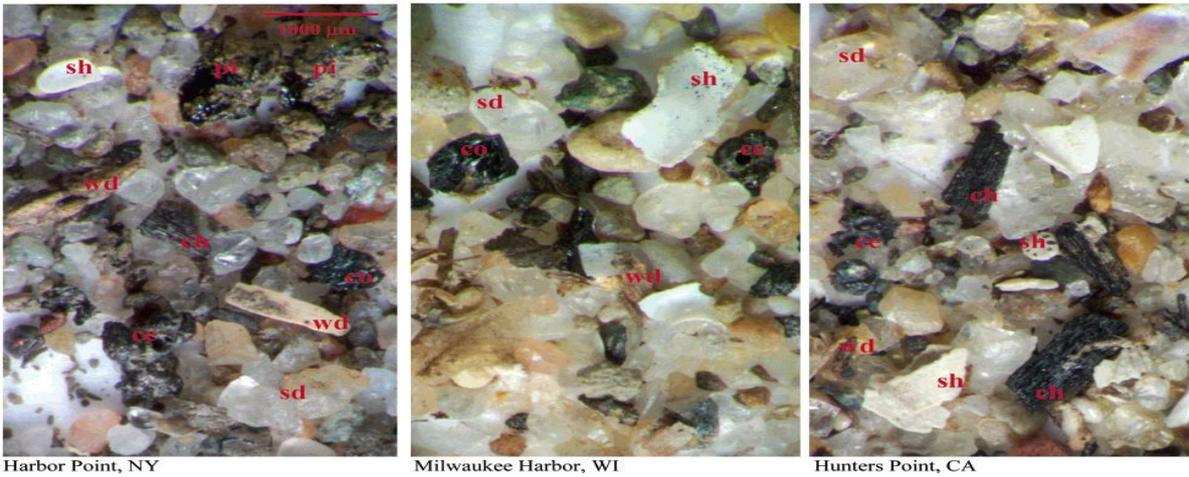


Figure 8 Sediments from 3 cities in the United States, showing their composition, SD sand, SH shell, CO coal, CH Charcoal, PI coal tar pitch, CE cenosphere and WD wood, made by electron microscopy

Partition of hydrocarbons between sediment and water is determined by the partition coefficient K_p of the sediment. This K_p is dependent on the concentration of organic carbon in the sediment. The general formula is;

$$K_p = K_{oc} * f_{oc}$$

in which K_{oc} is the partition constant K_p corrected for the fraction of organic material present in the sediment, and f_{oc} is the fraction of organic material in the sediment. K_{oc} is specific for an organic substance in the sediment. This can be seen in figure 9(30).

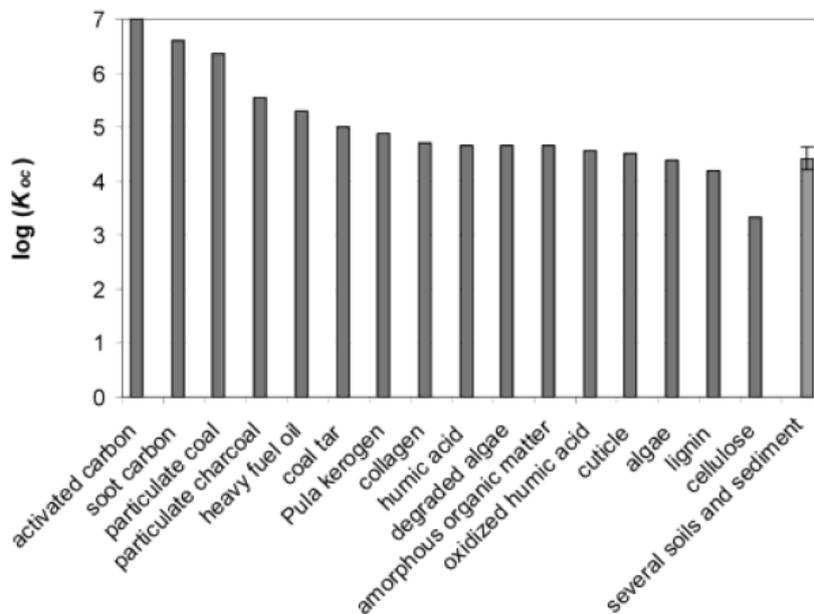


Figure 9 K_{oc} for some organic substances

It is shown that soot and coal have a K_{oc} around 100 times higher than other compounds. It has been shown that this partitioning is independent on PAH concentration(28). The presence of coal and soot in sediments of harbors and in soils of

factory sites is of enormous importance for toxicity of hydrocarbons. The presence of only a few percent of coal or soot can, due to the high K_{oc} values of these substances, have an enormous effect on k_p and therefore on bioavailability and toxicity. In fact this was found for PAHs and other harmful organic molecules(31). Because soils and sediments are complex mixtures it is very difficult to draw conclusions regarding partitioning of organic molecules bases on sediment or soil composition(28).

In the previous part the petroleum hydrocarbon absorption was considered as a partitioning equilibrium between the absorbed en solved phase. At least as important as this equilibrium is the role of kinetic processes in sediment during of this sediment sorption. The effect of declining availability when hydrocarbons are longer absorbed in the sediment or soil is called aging. Soil and sediment sorption appears to occur by a multi step process, and this is in fact what aging is. The effect of aging was being determined by extraction experiments. This effect is being caused by the entering of hydrocarbons in inaccessible parts of the soil matrix. This slows down both biotic and abiotic degradation. It was found that the aging effect increases with hydrocarbon molecular weight, and with K_{ow} and K_{oc} of the sediment(32). Aging is a process that takes week to moths and slows down biodegradation. Aging takes place by diffusion in both organic parts of the soil and through intraparticle nanopores(26).

1.3.4. Formation of NAPLs

Hydrocarbons absorbed in the soil can also be present in the form of NAPLs. This is the case when the hydrocarbons are concentrated and are able to form their own insoluble phase in the soil. This phase contains a mix of a lot of hydrocarbons. Research on soils with GC/MS revealed that the concentration of harmful PAHs is high in these NAPLs(7). NAPLs are important for bioavailability because they make just as sediment sorption and aging the hydrocarbons less available for biodegradation(33).

1.3.5. Petroleum degradation

There are a lot of mechanisms known for petroleum degradation. The most studied ones are without doubt the bacterial pathways which are able to degrade several petroleum compounds. Light is also able to degrade a lot of petroleum hydrocarbons. Some volatile parts of the oil will simple desorb or evaporate short after the pollution occurs, but this is not always advantages because this slows the degradation of the remaining part of the oil. A overview of the possible degradation mechanism is given in figure 10(34).

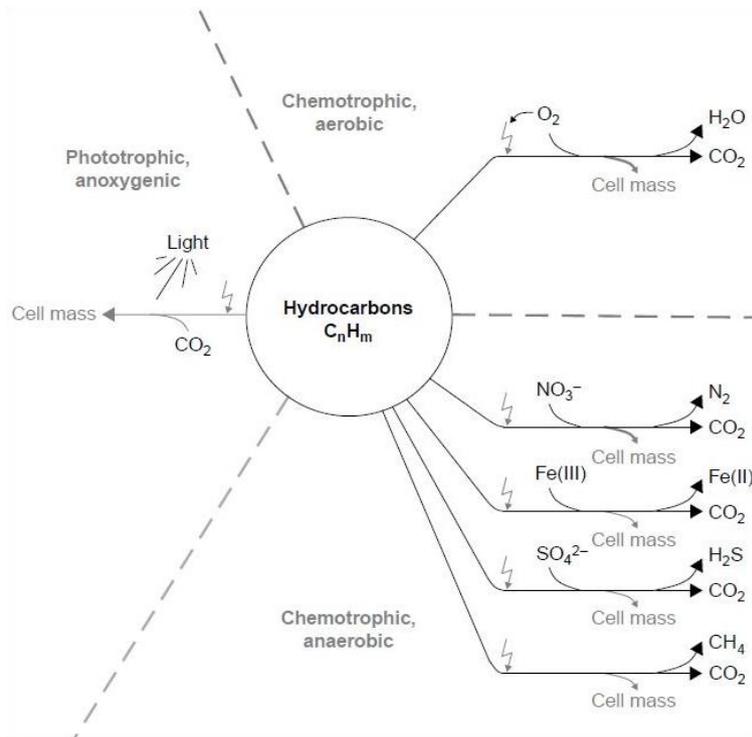


Figure 10 Possible degradation mechanisms for hydrocarbons

2. Petroleum degradation and light

A major cause of petroleum degradation is light. Until recent years the effect of light on the degradation of petroleum was estimated to be small. Recent research reveals that it can be as important as biological degradation, most notably in tropical regions(22). First the presence of light has a positive influence on degradation of some hydrocarbons by biodegradation in presence of algae(35), but it also has the possibility to degrade petroleum components in a direct photochemical way. The photochemical reactions caused by light are mainly able to effect the physical properties of some of the oil fractions. They are able to alter for example the emulsion formation and the solubility of the petroleum fractions. This is done by inducing reaction between oil components and other molecules, which makes the molecules more polar and water soluble, so creating new compounds with other physical en toxicological properties(22). The change in the original hydrocarbon concentration has also effect on toxicity.

2.1. Mechanisms of oil degradation by light

Petroleum is most notably able to absorb light in de visible and UV part of the light spectrum. This is done by aromatic structures. Because light has an influence on both photochemical and biodegradation it is very difficult to prove a mechanism. It seems that there are 3 major mechanism involved in photo degradation of oil(22). These mechanisms can be classified as either direct or indirect photolysis(36). Direct photolysis take place when the molecule of interest absorbs energy from light and further degrades. Degradation is called indirect photolysis when another molecules absorbs the energy from light and reacts to degrade the molecule of interest. Indirect photolysis can be divided in singlet oxygenation and radical oxidation(19). An overview of the reactions is given in figure 11 (37). In this figure P is the PAH molecule.

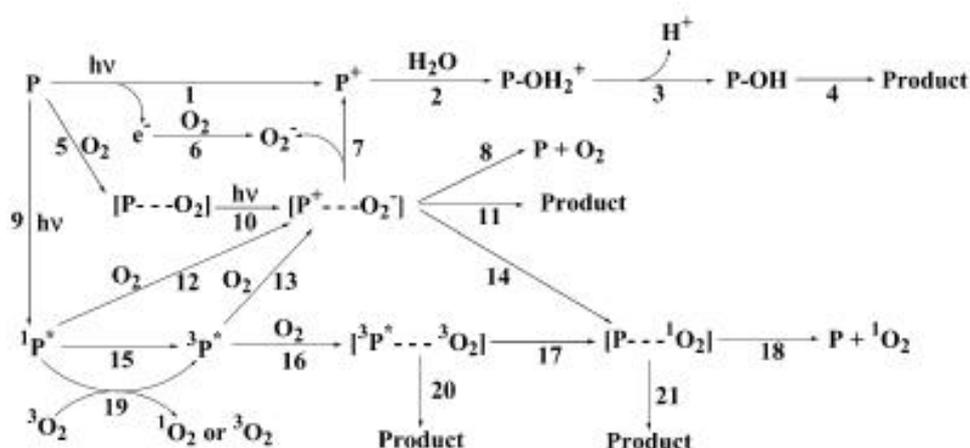


Figure 11 Mechanisms for photodegradation of polycyclic hydrocarbons

2.1.1. Direct mechanism, direct photolysis

The first mechanism starts with excited hydrocarbons (aromatics) or other polar molecules which react with an oxygen molecule(37). The aromatics are for this reaction excited to their triplet state (reaction 9 + 15). The absorbed energy is then transferred to

an oxygen molecule (reaction 17) in a complex formed between the PAH and oxygen (reaction 16). This results in the formation (reaction 18) of a singlet oxygen molecule and the original PAH molecule (19). This singlet oxygen molecule is able to react with aromatic and sulfur containing cyclic molecules. To test for the involvement of singlet oxygen in photochemical degradation of some petroleum hydrocarbons some tests were performed with β -carotene, which is known as a singlet oxygen quencher. The test showed that some mechanisms do have a singlet oxygen involved, while other reactions work by another mechanism (22).

2.1.2 Indirect photolysis, radical oxidation and electron transfer

The second mechanism that is thought of influencing the degradation of oil compounds is radical formation. Photo ionization causes the removal of an electron from the PAH (reaction 1) which results in a PAH radical. This PAH radical can react further with water or hydroxide ions to form secondary radicals (reaction 2), also called radical oxidation. These radicals can react further to form products (reactions 3,4) (37). There is a lot of uncertainty about this mechanism, and the most likely explanation is that it is caused by a photochemical reaction which involves both singlet oxygen formation and free radicals (22). Measurements on the quantum yield of the PAH degradation support the assumption that direct formation of PAH radicals is not possible, because not the right oxidation and ionization potentials were found. The influence of oxygen on the quantum yield in presence of low concentration of electron donors showed the dependence of the PAH radical on oxygen. This can be seen as a proof that reaction 1 is at least not the major reaction pathway (37). The role of electron donors will be further discussed in the next chapter. This singlet oxygen reacts further with other molecules in the oil solution to yield ketones and quinones which are able to form the radicals by hydrogen abstraction and so induce further radical chain reactions (22).

The third mechanism is the least understood. It is oxygenation by electron transfer. In this reaction light causes the transfer of an electron from the PAH molecule to oxygen (reaction 5, 10). This electron transfer is thought to take place at the air-oil surface and creates anion and cation radicals which undergo solvent separation (37). This can lead via reaction 7 to the same situation as during photo oxygenation (22; 37). One of the experiments in which this mechanism was tested was the formation of hexadecanoic acid from an oil solution which at the beginning of the experiment only contained hexadecane (22).

2.2. Influence of the environment on photodegradation

The photochemical reactions that take place in the environment are strongly dependent on environmental factors as for example the hydrophobicity of the solvent and the concentration of oxygen present (36).

The effect of oxygen was measured by determining the quantum yield for different oxygen concentrations. Quantum yield is the number of times a reaction occurred divided by the number of photons absorbed, and is therefore a measure of efficiency. In

an experiment the quantum yield for 9 PAHs was measured under different oxygen concentrations and it was shown that the concentration of oxygen had an almost linear relation with the quantum yield, which shows the importance of oxygen for the photochemical PAH degradation(37).

Another effect that was found was the effect of electron donors. In figure 11 the formation of the PAH radical was assumed to be important for indirect photolysis and can in these mechanisms be formed by reaction 1 and 7. The PAH radicals (positive) are in equilibrium with the PAH molecules. All PAH radicals can in the presence of electron donors as NaI and $K_4Fe(CN)_6$ in high enough concentrations be reduced to PAH molecules. This is in theory a method to prevent PAHs from being degraded. The quantum yield was also measured in the presence of these donors to determine if this has an influence on the quantum yield. The quantum yield was unaffected for low oxygen concentrations, but for higher concentrations there was a decrease in quantum yield. This can be seen as a prove of oxygen being a limiting factor if the concentration of the PAH radical has chance to reach a higher concentration(37).

The major reaction mechanism for PAH degradation appears to depend on the hydrophobicity of the solvent. In hydrophilic solvent the direct reaction mechanism (reaction 16) is the most dominant reaction mechanism, because the polarity of the solvent has a negative influence on the creation and stability of reactive oxygen molecules. In hydrophobic solvents the reaction mechanisms depending on oxygen molecules become more dominant because their lifetime increases 10 times, thus favoring the indirect mechanisms. This proves that the direct reaction mechanism is less important than the oxygen depending pathway in the hydrophobic oil layer, and reaction of other photochemically activated molecules with petroleum hydrocarbons is therefore the most important mechanism for oil degradation in oil layers on beaches in the environment(36).

2.3. Effect of oil degradation by light

The reactions occurring as a result of oil irradiation by light mainly leads to bigger and more polar molecules. These molecules are both aliphatic and aromatic molecules in which polar oxygen containing groups are inserted. In an 30 day experiment 51 to 93% of the saturated hydrocarbons was converted, showing some selectivity for branched alkanes(38). In one of the experiments with saturated hydrocarbons it was found that it were the bigger alkanes which have the greatest degradation rates(39).

Aromatics were degraded from 86 to 99% with also a preference for the branched molecules. The faster degradation of aromatics compared to saturated hydrocarbons can be explained by the fact that aromatics are able to absorb light directly because of their aromaticity. If degradation occurs by light or by biodegradation can be proven by aromatic molecules like phenanthrene, shown in figure 12(40). When an methylgroup is added to this molecule there are several conformation structure isomers possible, C₁, C₂, C₃ and C₉. This was used to distinguish between photodegradation and biodegradation.

For photodegradation the degradation rate is $C_2 < C_1 < C_3 < C_9$. Biodegradation show a totally different pattern with C_2 and C_3 degraded faster(38).

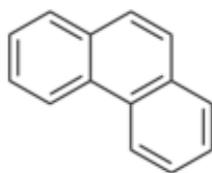


Figure 12 Phenanthrene

Fresh oil is very insoluble. The only fraction that is soluble is the smaller aromatic fraction, and this fraction is lost very soon from the oil layer. The remaining organic substances are nearly insoluble in the water layer. Light causes the insertion of polar groups like ketones, aldehydes, hydroperoxides, sulfoxides, phenol and carboxylic acids. Among the more water soluble fractions of the aromatics, the more substituted fractions are converted faster than the less substituted ones, as has been proven by UV spectrophotometry(41).

These more polar molecules create the possibility to form an emulsion of oil molecules. Also addition reactions occur and lead to other more soluble molecules. These bigger and more polar molecules are able to form a so called mousse, which is a mix of particles. This happens also in absence of light, but light increases mousse formation. During irradiation most notably the asphaltene concentration becomes higher, and this results in higher mousse formation(42). The solubility of hydrocarbons also increases. Photodegradation leads to a tenfold increase in solubility(22).

After photodegradation has made hydrocarbons more soluble the degradation continues in the aqueous phase, so called water column photodegradation. Since photodegradation is depending on the amount of light available, it is expected that the amount of light penetrating in the aqueous layer has an influence. This depend on the thickness of the oil film above the water column. Although little is known about the aquatic degradation processes they are expected to be faster than reactions in the oil layer, and this has been proven with model compounds(22). The hydrocarbons made soluble by photodegradation stimulate also biodegradation(43).

Light can also have influence on the toxicity of hydrocarbons, as is shown for PAHs. In experiments done in tanks with oil pollution the amount of soluble aromatic fractions was measured during the year. In the summer there was a considerable increase in the soluble aromatic fraction of the used oil. This was, except the minor influence of temperature on the solubility, the most important influence on the concentration of the soluble aromatics(44). This higher aromatic concentration leads to higher toxicity among various aquatic species. This proves that light can be an important factor in oil degradation and toxicity. A property of light is that it can lead to lowering of the biodegradation rate of bacteria, because it seems to be toxic to degrading bacteria in the upper water layer(21). It was shown that some amphipods are able to absorb PAHs

which can cause toxicity after irradiation by UV light. The toxicity only occurred when the amphipods have been in contact with the PAHs, and this is not a result of UV irradiation alone. The toxicity is assumed to be caused by oxidative stress from singlet oxygen, formed by triplet state PAHs(45). This toxicity has also been seen for algae, which is due to the formation of quinones under influence of light(46).

3. Biodegradation of Petroleum by bacteria

Bacteria are able to degrade most petroleum compounds. Degrading bacteria are present in both the water and the soil environment. The degradation speed of hydrocarbons depends on the compound, the bacteria present and on the environmental condition(47). Different steps in a degradation pathway can be performed by different bacteria. Except bacteria also petroleum degrading fungi are known. They are able to help bacteria in degradation of some hydrocarbons. In general the degradation mechanism works by introducing a more polar reactive group in the molecule. This more reactive group is used for further degradation of the carbon chain. At the end of the pathway the petroleum is totally degraded to CO₂(48).

3.1. Mechanisms for petroleum biodegradation

Bacteria are during evolution selected for the possibility to degrade hydrocarbons. Hydrocarbons are in smaller concentration made by plants in the environment since they are advantages for them. Protection against animals is an example. Bacteria developed during evolution various mechanisms to degrade these molecules(34). There are a lot of degradation pathways known for bacteria, of which the pathways including oxygen are known for a long time and understood the best. Bacteria need an electron acceptor for degradation of organic molecules. This is in most pathways oxygen, but can also be another molecule.

For aromatics it can be proven that they are degraded by biodegradation instead of chemical or physical degradation. This is done by comparing degradation rates between differently alkyl-substituted PAHs. The degradation of these compounds appears more favorable for some of the isomers compared to other isomers(20).

If oxygen is used during degradation the pathway is called aerobic, otherwise it is called anaerobic. The molecules involved in anaerobic degradation pathways are nitrate, sulfate or iron(34). Anaerobic degrading bacteria can be divided in two classes of bacteria, the so called strict and facultative anaerobes. Strict anaerobes are bacteria which can only degrade hydrocarbons in an anaerobic environment. Most of these bacteria are sulfate reducing bacteria. Facultative anaerobic bacteria have a choice between degradation pathways with or without oxygen. They are in general nitrate or iron using bacteria(49). Anaerobic processes take place in anaerobic environments which can be sediment layers, but also deeper layers in soil or aquatic environments.

3.1.1. Aerobic degradation

Aerobic degradation is characterized by the involvement of oxygen in the pathway. In the following part the degradation possibilities of most of the petroleum components will be discussed. Most of the reactions on aliphatic and aromatic hydrocarbons are performed by monooxygenases, which add one oxygen to the hydrocarbon. Dioxygenases are able to add one or two oxygen atoms to aromatic molecules(34).

Unbranched alkanes are degraded readily in the aquatic environment. This degradation works for small as well as for larger carbon chains, but the shorter chains are degraded faster. Bacteria convert most alkanes first to alcohols. These alcohols can be catalyzed to aldehydes or carboxylic acids. In some rare cases the alcohol is secondary and the next step produces a ketone instead of a carboxylic acid. The carboxylic acids can be further degraded via β -oxidation, which produces a carbon chain with two carbon atoms less than the starting molecule and acetyl coenzyme A. Another possibility is omega oxidation(20).

More branched alkenes are more resistant to bacterial degradation. This is because the carbon sidegroup on the carbon chain make most of the degradation mechanisms of the bacteria impossible. This is also the reason why branched alkanes are degraded by other mechanism. An example is omega degradation which introduces two carboxylic acids. A lot of studies have been done on the effect of introducing methyl groups in the molecule. Some places on the main carbon chain inhibit some degradation mechanisms and thus resulting in slower degradation by other more complex mechanisms, for example α -degradation instead of β -degradation(20).

Cycloalkanes are more resistant to degradation than alkanes. For unsubstituted cycloalkanes the first degradation step is the addition of an alcohol or a ketone group. This group can in a second step being used for ring cleavage. If the cycloalkane is substituted the degradation is faster, and an acidic group is likely to be inserted near the side group(20).

For aromatics the degradation speed depends on the number of rings present in the molecule. The presence of more aromatic groups results in more resistance to degradation. In most aromatic compounds a diol group is inserted in the first degradation step. In a second step the diol group is cleaved and carboxylic acid groups are formed. For bacterial degradation the diol formed is a cis-cis diol(20). The most important bacterial enzymes used to degrade polyaromatics are oxygenase and dehydrogenase enzymes. Fungi use another method for inserting a diol in the aromatic ring, which results in a cis-trans diol. This is a prove that the mechanism involves first addition of one oxygen in the aromatic ring followed by cleavage. Fungi use lignolytic enzymes for these reactions, which will be explained in further chapters. These reactions work by catalyzing oxidation of the aromatic molecule which results in a radical reaction. The smaller aromatic hydrocarbons are because their partially solubility degraded in the water phase. The bigger polyaromatics are degraded in the solid phase, which makes de degradation rates lower(50).

3.1.2. Anaerobic degradation

Anarobic reaction pathways have not been studied for a long time. The first interest in this pathway started with the undesired reduction of sulfate in oil production. Sulfate was reduced by sulfur reducing bacteria. The alkanes in petroleum mix were taught to donate some electrons to make these reactions possible(34). It became clear that

alkanes can be reduced in an anaerobic environment if other oxidators are present. Further research has been done to better understand the mechanisms behind this bacteria. Anaerobic degradation turned out to be able to degrade less compounds than aerobic degradation can. The last 10 years the interest in this kind of reactions has increased due to possible use for bioremediation(51; 52).

Anaerobic biodegradation is degradation with sulfur or nitrogen using bacteria. Alkanes and a lot of aromatics are degraded by an anaerobic pathway to form fat acids in bacteria(34). These reactions start with the addition of a small carbon fragment to the main carbon chain, and this initiates a radical mechanism for further reaction(53; 54). This activated molecule reacts with fumarate. The result is a substituted succinate molecule which can react further in mechanisms presumed to be similar to fat acid degradation. The mechanism of this reactions is still unclear(34).

The degradation of toluene and some related aromatics is better understood and starts also with the formation of benzylsuccinate molecule. This molecule reacts with coenzyme A to form Benzoyl-CoA and acetyl-CoA(55). Benzoyl-CoA is further degraded by β -oxidation. Toluene is one of the minor organic molecules for with the degradation pathway is known. It is show in figure 13 as an example(54).

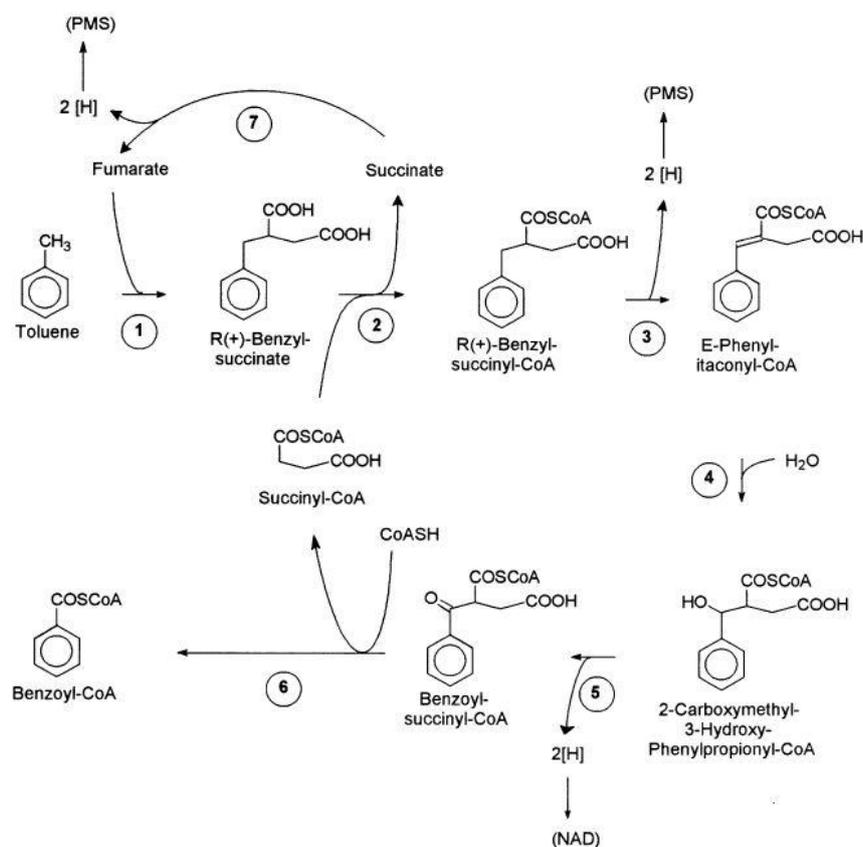


Figure 13 Anaerobic degradation of toluene

3.2. Factors influencing biodegradation

There are a number of factors that influence the biodegradation of petroleum hydrocarbons. The first factor is temperature, because the biological enzymes involved in the degradation pathway have an optimum and will not have the same metabolic turnover for every temperature. Other factors are more environmental, like presence or absence of oxygen and nitrogen. Humic acids can also play a role in biodegradation of petroleum and in degradation in general.

Bacteria use the petroleum hydrocarbons as a source of carbon for assimilation and for energy production. To make degradation of hydrocarbons possible the presence of other compounds is sometimes necessary. That this is true has been proven by Wang et al. They used GC-MS to determine the change in oil composition in different oils after incubation for 28 days at 10°C with six both aliphatic and aromatic petroleum degrading bacteria. They added to some cultures a mix of nitrate, ammonium and phosphate. This results in a clear difference in oil degradation. The degradation was 5-47% for the total aliphatic hydrocarbons in the cultures with the mix and 0-13% in cultures without the mix. For the bigger alkanes there was an even bigger difference, addition of the nitrogen/phosphate mix results in a change in degradation from 16-28% to more than 90%(20).

For the aromatic hydrocarbons this influence of nitrogen addition was not discovered(20). In the natural environment it was shown that presence of nitrogen even slows down aromatic degradation, probably because bacteria switch to another carbon source for degradation(56). In other experiments there was a decrease in nitrate degradation for only the first 2 weeks of the 5 week experiment, which suggests that after a certain period the role of nitrate as an oxidator was taken over by the fermentation process(49).

Oxygen is also an important factor. As discussed above, oxygen determines if the reaction pathway is aerobic or anaerobic. Aerobic degradation is much faster than anaerobic degradation, as have been shown in experiments. Some experiments were done with three strains of bacteria isolated from polluted soils. The bacteria were facultative anaerobe nitrate reducing bacteria. The two experiments were a ten day aerobic and a fifty day anaerobic experiment. The bacteria in the aerobic experiment were able to degrade 20-25% of the organic material and 90-95% of the alkanes. In the 50 day anaerobic experiment 15-18% of the organic material and only 20-25% of the alkenes was degraded. This shows that anaerobic degradation is far more slower than degradation in the presence of oxygen. It was also shown that the degradation of alkanes of intermediate length was slower than degradation of shorter and longer alkanes(49).

Another well understood factor influencing biodegradation is temperature. This effect is due to influence on the metabolic rate of the bacteria. Temperature also has an influence on the composition of the oil and on the bacteria culture. At lower

temperature the toxic low carbon chains do not evaporate and cause toxicity to degrading bacteria. The enzymes of the metabolizing bacteria have an optimum around 30 to 40 degrees Celsius. When the temperature becomes higher the hydrocarbons become toxic to the bacterial membrane. Lower temperatures cause lower metabolic rates. In aquatic environments with year round lower temperatures there is a selection for bacteria with a lower optimal temperature(57).

Humic acids are big biomolecules naturally occurring in the aquatic environment. They are able to bind a lot of organic compounds. This can influence the possibilities for degradation. Some research has been done with humic acids in relation to PAH degradation. Except the higher solubility of PAHs in presence of humic acids it has also been shown that they can have a stimulating effect on the biodegradation of PAHs. In this experiment a concentration of 0.1 and 1.0 g/L⁻¹ humic acids was added to a solution of phenanthrene, a PAH who already creates a maximal possible soluble fraction in water in the absence of humic acids. Increases in biodegradation of 3.1 and 4.8 times were found for the natural occurring concentration of 0.1 g/L⁻¹ and for the artificial concentration of 1.0 g/L⁻¹(24; 58).

Because a lot of oil pollutions occur in oceans it is also worth to consider the effect that salinity can have on biodegradation. There are several experiments done on salinity and biodegradation, and the general trend was that higher salt concentration results in lower degradation rates. Variation in salinity results in lower degradation rates compared to a solution with a stable concentration, which suggest a kind of adaptation of the degrading bacteria(59). In one of the experiments the degradation of hydrocarbons was measured in two soil samples. The reason for this experiment was the fact that the conductivity of petroleum was four times higher than of seawater. In polluted sites both salt and hydrocarbon concentration are higher, and so salt concentration should be considered in degradation processes. This is shown in figure 14.

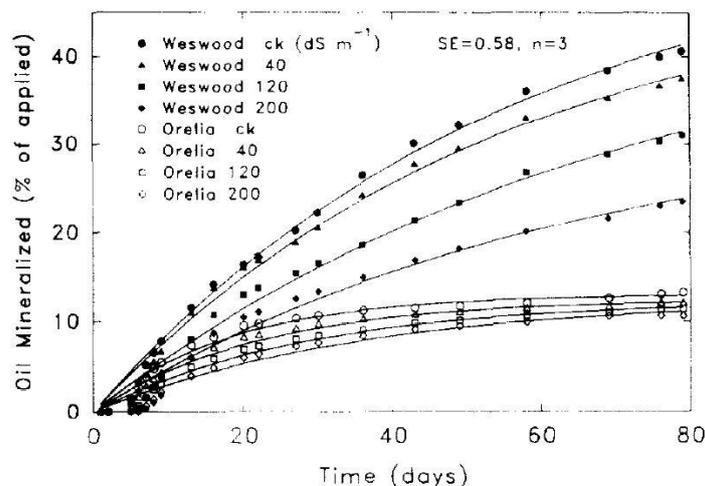


Figure 14 Effect of salinity on hydrocarbon degradation

The concentration were 0, 40, 120 and 200 dS m⁻¹, which is a measure of conductivity. 40 dS m⁻¹ is the normal conductivity of seawater, 200 dS m⁻¹ is the conductivity of petroleum. Westwood is a sample of clay ground and Orelia is a sample of clay and sand. The reason for Orelia soil degrading faster is the presence of a higher number of degrading bacteria(60). Immobilized bacteria on fibers seems to be more resistant to salinity, but this is more important for waste water treatment in industry(61).

3.3. Effect of biodegradation

The effect of biodegradation is a change in petroleum composition. Some part are readily degraded by bacteria, and other compounds are degraded only very slowly due to leak of degrading enzymes/mechanisms by bacteria or because the hydrocarbons were toxic to the bacteria. This causes a difference in degradation for different oils, and thereby creates a different in toxicity between oils(20). The degradation of aliphatic hydrocarbons is faster than degradation of other compounds. A degradation of 50 to 65% was shown for hexadecane(62). That bacteria have a strong preference for compounds can be seen in an experiment with biodegradation of PAHs in a soil sample. The PAH with less rings are degraded faster and the bigger PAH molecules are only slowly degraded as can be seen in figure 15 (63).

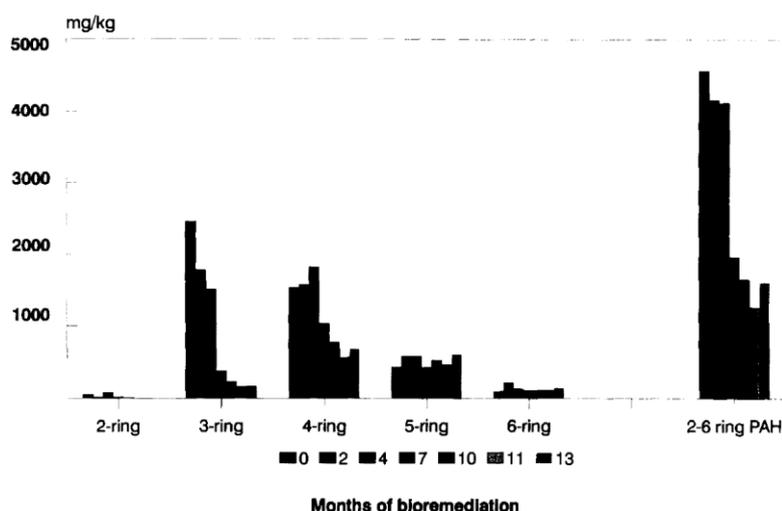


Figure 15 Effect of biodegradation on PAHs

There is also a clear difference in composition change between weathering of petroleum by biodegradation or by chemical or physical degradation. This difference in degradation is that degradation by bacteria is isomer specific. This can be used to estimate the amount of degradation due to degradation by light or by biodegradation(56).

Another property of oil that can be changed by biodegradation is the solubility of oil. Some bacteria are able to make so called biosurfactants. These biosurfactant are able to decrease the surface tension of the solution more than two times, and are therefore more effective than synthetic detergents. The concentration of PAHs was found to be higher in the aqueous phase. This effect was bigger for more substituted PAHs. Despite

the better solubility in the aqueous phase when biode detergent were used, the PAHs still have higher EC50 levels compared to synthetic detergents. This shows the importance of these detergent for bacteria in the degradation on hydrocarbons(64). Another experiment shows that the effect of biosurfactants also increases with concentration of petroleum pollution(65). Enhanced solubility can also have an effect on further biodegradation, because it has been shown that solubility enhances uptake of hydrocarbons by bacteria. This is because the oxygenases are membrane bound and are only available by soluble organic molecules(66).

4. Other degrading organisms

Except the well known hydrocarbon degrading bacteria there are other organisms who are also able to degrade hydrocarbons. Fungi, algae and plants are now known to have degrading possibilities as well. They use enzymes, for example peroxidase and dioxygenase from cytochrome P450 to degrade a lot of hydrocarbons. Due to their persistent character often the PAHs are the subject of this degradation mechanisms.

4.1. Fungi

Fungi are also known to degrade hydrocarbons. They use another mechanism for degradation of hydrocarbons than bacteria do, and they are therefore maybe able to degrade the hydrocarbon compound left by the normally faster degrading bacteria. This can be useful for the five-ring PAHs, which are only poorly degraded by bacteria.

Fungi secrete extracellular oxidizing enzymes for degradation of lignin(67). These enzymes are able to make reactive peroxide from oxygen(68). Especially white rot fungi are able to degrade lignin. Lignin is a complex random molecule containing a lot of aromatic groups. That fungi are able to degrade lignin makes them also possible candidates for PAH degradation. The degrading enzymes lignin peroxidase and manganese peroxidase have shown to be able to degrade some model lignin compounds. Peroxidase has showed to be involved in degradation of PAH to quinones(69).

Fungi do not degrade the hydrocarbons completely to CO₂ as bacteria often do. In the highest conversion of hydrocarbons shown only 19% was converted(67). Instead they form a range of degradation products which are solved in the aqueous phase or become bound to organic fraction in the soil. For benzo[a]pyrene it was found that nearly all degradation product was bound to the compost fraction used in that experiment. The degradation rate of benzo[a]pyrene was found to be double of the degradation rate in the a culture without the fungi. After a month the degradation stopped, which is suggested to be due to nutrient limitation(70).

The presence of fungi and bacteria in one culture leads to an increase in degradation. The culture showed a degradation rate which was higher than the total amount of degradation in a bacterial and a fungi culture separately. Bacteria which have shown no growth on benzo[a]pyrene seems in presence of fungi able to grow. A degradation rate of 27% in 56 days was found for benzo[a]pyrene and 19% for dibenz[a,h]anthracene in the combined culture. The influence of the lignolytic enzymes on the degradation product can be seen in figure 16 (71). Degradation mechanisms of bacteria and fungi show a remarkable difference with cis-trans hydroxylation for fungi and cis-cis hydroxylation for bacteria(10).

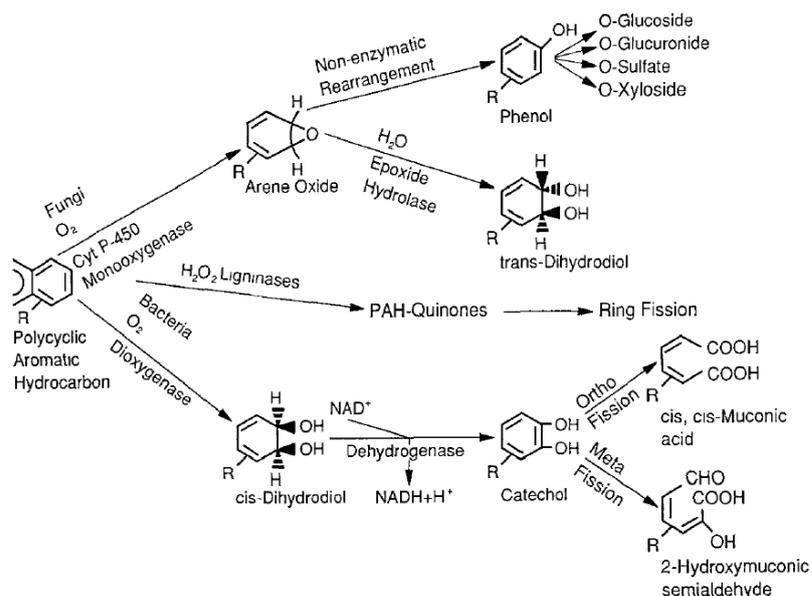


Figure 16 Degradation mechanism for bacteria and fungi

4.2. Algae

Algae have also the possibility to degrade hydrocarbons, and more specific PAHs. They use the eukaryotic mechanism similar to fungi. This mechanism make use of a dioxygenase enzyme which leads to cis-trans hydroxyl groups. Algae are dependent on light for being able to degrade PAHs. The metabolites are depend on the kind of light radiation the algae are irradiated with. The production of quinones was related to the intensity of light and, and therefore the toxicity of PAHs to algae was also related to the light intensity. This toxicity was not seen for al PAHs(46).

Some green algae were very effective, they degraded all PAHs in 5 of 6 days for low concentrations, but other green, yellow and blue-green algae were less effective. For higher concentrations the decrease was smaller(46). It was shown that the difference in degradation rate was enormous for different algae, but also that the fate of the PAHs was different. PAHs can be degraded or accumulated in the algae as biomass. Some algae only accumulate, while other degrade nearly all PAHs, as was shown in an experiment with benzo[a]pyrene. It was found that for a brown algae over 90% of the benzo[a]pyrene was found in biomass(35).

4.3. Plants

Plants are another example of eukaryotic organisms who are able to degrade PAHs. They use the same peroxidase P450 enzyme used by fungi. These enzymes have the same concentration or a higher concentration in presence of PAHs. Plants with lower peroxidase activity also have lower metabolizing rates. It was shown that also oxidative enzymes were important, and both oxidative and peroxidative enzymes were needed for PAH degradation in plants(72).

It was shown that although plants have the possibility to degrade PAHs, it does not often occur due to problems with distribution and uptake from the soil. This is due to the respiration stream which is optimal for moderate hydrophobic molecules, but the PAHs are too hydrophobic for being taken up and transported. That plants are still able to stimulate biodegradation of PAHs has to do with stimulation of bacteria associated with the root system. This is because plants create a for bacteria useful solution around the roots, also known as exudates. This solution contains amino acids, sugars and organic acids and some enzymes. A lot of degrading bacteria need this solution for survival, because they degrade PAHs only to prevent toxic effects and this process uses energy(73).

5. Remediation of petroleum-contaminated soils

Due to widely use of petroleum hydrocarbon in factories there is a lot of pollution caused by these production processes. This pollution happened both in de aquatic environment in for example harbors, but also in the terrestrial environment, mostly near factories or tank stations. That soil pollution is not considered as a big environmental problem is due to the restriction of the pollution to a small local area. But the local areas polluted with petroleum are quite numerous as has been shown by a research on petroleum tanks in de VS(74), and therefore it is important to develop techniques to efficiently clean these areas.

In the terrestrial environment the contaminating organic compounds first migrate downward in the soil due to gravity. While doing this there are some organic compounds left in the pores of the soil it migrates trough. When it reaches the soil layer containing the ground water there is a separation. The lighter compounds go further spreading in that layer above the ground water, while the heavier parts continue their way downwards trough the water layer. Some compounds can be soluble in the water layer, and are also further transported horizontally. This is of interest for soil pollution, because is causes spreading of the pollution and creates the possible entering of organic compound in the ground water used for a variety of purposes. During the past 40 years a lot of mathematical models have been developed to explain the behavior of pollutants in the soil(75).

A lot of methods for cleaning soil polluted with hydrocarbon have been developed during past thirty years. Most of these methods can be classified as physical and chemical or as biodegradation based methods. A lot of these methods are bases on making the hydrocarbons more available for separation and degradation. This has to do with the effect of aging and NAPLs being formed. Application of heat, a low pH and biological and chemical agents seems to decrease the desorption time. This also has a positive effect on what sometimes is the literature is called pollution tailing, the effect that one has to wait much longer than necessary for the last 10 or 20% of the pollution which is deeper absorbed in the soil than the other part of the pollution(26).

5.1. Physical en chemical methods

There are various methods to clean the soil from these hydrocarbons. The first method is removing the upper soil layer when the pollution is concentrated in the upper soil layer. The pollution can be removed from that soil by chemical or biodegradation in the presence of oxygen. Evaporation of the volatile organic compounds is another possibility. This process can be made faster by heating of the soil above a required temperature, depending on the pollution. This removes only some parts of the organic compounds, and it is currently being investigated if the ground with the remaining organic compounds can be reused since the remaining compounds are immobilized in the ground. Removing ground and evaporation by heating is a very expensive method and requires a lot of energy. It is also unsuitable for pollution in deeper ground layers.

There is a lot of research going on to develop better method for degrading these polluting hydrocarbon(76).

In most situations it is better to clean the soil on the location where the pollution occurred. In case of spreading of the pollution there is no other choice than local treatment. These methods make use of both biological degradation and physical methods. Soil washing, soil venting, low temperature treatment and stimulation of biodegradation are methods to clean soil(74).

Soil washing is a method which consist of addition of solvent or detergent to the polluted area to make the organic pollutants more soluble in the aqueous phase. This method is still subject of discussion because the detergents used to decrease the surface tension can be pollutant themselves. Further the aim of the washing process is to increase the bioavailability of the organic compounds, but this effect is not shown in both laboratory and field studies yet(77). For other organic compounds is does result in enhanced biodegradation of liquids, so it may be still possible with other conditions or surfactants. Biosurfactants have shown to lead to increase in biodegradation. The installation used for soil washing with surfactants is shown in figure 17 (78).

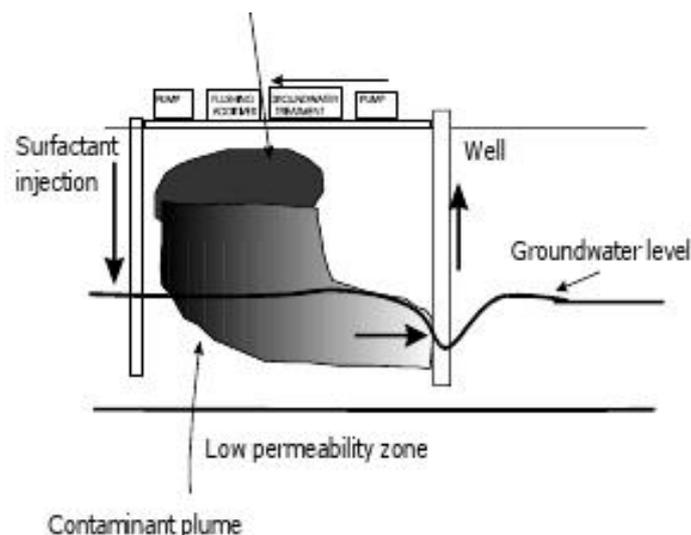


Figure 17 System for soil washing

Although the discussion about the influence of the surfactants on the environment the surfactants have shown to be very effective in removing organic pollution from the soil. The effectiveness of this method is strongly depended on soil characteristics as for example permeability, pH, particle size and the presence of other contaminants(78).

Soil venting is one of the most effective methods for volatile organic compounds. This method use extraction wells from which water is extracted. This creates a vacuum which causes extraction of volatile organic compounds from the soil. The strength of this method is that the extraction of gasses just below the ground water layer is faster that extraction of the nearly insoluble organic compounds. Low temperature soil heating was also tried as an alternative, but this was not shown to be useful(74).

5.2. Biodegradation methods

Most other methods make use of the stimulation of the initially present biodegrading bacteria. Strongly related with the usefulness of these methods is the adaptation to hydrocarbon degradation of bacteria at the pollution site. In most cases it is not useful to import selected bacteria from other polluted sites because the bacteria present are adapted to the local environment, the hydrocarbon mix present and the presence of other (sometimes also degrading) bacteria(74). This adaptation is caused by induction of metabolizing enzymes and genetic changes. Experiments show that this results in much faster metabolism. The microbiological composition of the soil is most times enriched in these bacteria, which has been shown in field experiments by an increase in degrading bacteria of 4 or 5 times(79).

The degrading bacteria are stimulated by addition of oxygen or nutrients. This technique is in contrast to soil venting able to degrade nearly all organic compounds. The stimulating chemicals are added to the ground layers by pumping in water with the chemicals dissolved in it. Oxygen addition results in increase of aerobic degradation instead of the much slower anaerobic degradation. Injection of a combination of oxygen and nitrogen in sandy soil seems to work best. This results in a faster degradation than without the stimulation(74).

One of the difficulties with application of this method is bringing the right oxygen concentration in the deeper ground layers. There are several methods for this. The problem is that oxygen is only poorly soluble in water, which causes the addition of air to water to result in a very low concentration of around 8-9 ml/L. This can be made around 5 times higher by replacing the addition of air by addition of oxygen gas. With this method there is still 75 000 kg water necessary for degradation of 1 kg hydrocarbons. Another method with hydrogen peroxide has a better efficiency, only 12 000 kg is needed. The disadvantage of this method is that the hydrogen peroxide can be harmful for the degrading bacteria(74).

Another method for increasing the concentration of oxygen and nutrients in the soil is the use of plants. They excrete oxygen and nutrients from their root system, and this stimulates bacterial growth around the root system. An advantage of this method is that it is very cost effective for slow and long term soil degradation. It is most useful in ground polluted with lower hydrocarbon concentrations because higher concentrations have shown to be toxic for the root system of plants. The effectiveness of plants differs between plant species, and the species emitting a low oxygen concentration around the roots are most successful(73).

There is also a possibility to stimulate the anaerobic degradation pathway. Techniques are now developed to introduce nitrogen or sulfur containing compounds in the deeper layers of the soil via injection. These compounds are much more soluble in the injected water than oxygen, and so lead to petroleum degradation via an anaerobic pathway(34). The problem with this method is the uncertainty about the completeness of the

degradation, because it is not sure if bacteria can degrade all organic compounds as for example longer alkanes by an anaerobic pathway(57).

A possibility to make biodegradation for the most hydrophobic hydrocarbon in soil better is by adding some detergents. The detergents of choice should be a biosurfactant, because they have shown to lead to greater bioavailability combined with lower toxicity to microorganisms(64; 66). The synthetic detergent sodium dodecyl sulfate (SDS) was also often used in experiments. The presence of SDS showed to decrease degradation rate, because SDS itself was first degraded instead of the PAHs added. SDS was however able to increase the solubility of the PAHs and this lead to an increase in degradation in a linear way. Non-ionic surfactants of the alkylethoxylate type were also used, and some of these detergents have shown to be toxic. Toxicity seems to correlate with hydrophobicity of the detergent, the more hydrophobic surfactant were more toxic. A longer ethoxylate chain makes the detergent more hydrophobic and less toxic. The average ethoxylate of 9 to 12 monomers appears to be toxic for the bacteria(80).

6. Stimulating degradation of hydrocarbons in the aquatic environment

The most well known aquatic pollution caused by petroleum is without doubt pollution due to tanker accidents. This creates a lot of environmental problems for water life, both in the sea and on the beaches. Therefore a lot of methods are developed to clean the water from these oil fractions. Another source of pollution in the aquatic environment is hydrocarbon polluted wastewater from industry. Because this is a much more controlled environment more possibilities for degradation are available. An important factor influencing the degradation of hydrocarbons is their availability for chemical and biodegradation. When oil is able to come in contact with sediment it is far less available for biodegradation and becomes persistent. This also happens on the beaches where the oil absorbs in the sand. This is one of the reasons for cleaning up the oil as fast as possible(48).

The first methods used after a tanker accident are mostly based on physical methods. Most oils have a lower density compared to water, so they will form a layer on the water surface. If possible special boats are used to remove the upper water layer for filtration to remove the most hydrophobic parts of the oil. This is in oceans very difficult and one is only able to remove some parts of the oil. Dispersants and agents to catalyze photo-oxidation are also being used to fasten degradation. Photo-oxidation as explained in one of the previous chapters, is able to cause dispersion by itself, and sometimes this is enough to clean the ocean from a huge amount of petroleum if the accident happens far from the shore. This has been seen in several accidents, for example the tanker incident with the Argo Merchant (1976) and the Khark 5 (1989)(48).

One of the best ways of cleaning petroleum pollution is use of bioremediation. For degradation there has to be contact between bacteria and the hydrocarbons. Normally they are only able to contact the dissolved parts of the petroleum. Therefore sometimes detergents are added to a site of a tanker accident. Some bacteria can achieve this themselves by production of biosurfactants(64). There are also bacteria who are able to perform reactions with very small beads of dissolved hydrocarbons(48).

Bacteria are able to degrade nearly all components of petroleum, but as previously discussed, it strongly depends on the components how fast it degrades. The degradation of petroleum is also strongly depending on temperature. A difference of 10°C turned out to have an effect of a factor 2.7 on hydrocarbon metabolism(81).

The presence of petroleum after a tanker accident is not toxic for aquatic bacteria. In fact the presence of hydrocarbons causes an enormous population growth. This population growth comes to an end when other nutrients as for example nitrogen and iron are limiting. In contrast to degradation in the terrestrial environment this limitation in oceans does not exist, and degradation can therefore be faster. Except nitrogen there are several other agents which can stimulate biodegradation. Twenty years ago some experiments on beaches were performed. This results in a visual cleaner

effect, but a significant degradation effect was not shown. This has to do with the preference of bacteria to degrade only some hydrocarbons. Unbranched alkanes and small aromatics were degraded fastest. To be able to measure degradation due to biodegradation the degradation of unbranched alkanes was in the past compared with that of branched alkanes. Because it turned out that branched alkanes are also readily degraded, other petroleum compounds are now chosen as a reference(48).

Other research on the degradation in the ocean reveals that bacteria have no preference for some organic compounds, and that the composition of the remaining part of the oil did not change during degradation. In this research a shift in bacterial composition of the aquatic environment was seen. The bacteria who were able to use hydrocarbon became more dominant(82).

6.1. Prevention of hydrocarbon toxicity by use of activated carbon

A relatively new method for preventing the aquatic environment from hydrocarbon pollution in sediment is the use of activated carbon. With application of this method the hydrocarbons are not removed, but are made unavailable for organisms. This method is based on the observation that carbon particles are able to reduce bioavailability of hydrocarbons. It was shown that biodegradation was able to reduce PAH degradation in the silt and clay layer of sediment, but in the lighter carbon containing layer there was no reduction in PAH concentration(83). The earthworms used in this experiment showed that the bioaccumulation in presence of carbon is 75% less than without carbon, showing that absorption by carbon makes PAH less available for uptake. That carbon is indeed able to effectively absorb PAHs was shown in a shipyard in San Francisco where addition of 3.4 wt% of activated carbon shows an 84-92% or 77-83% reduction of the PCBs and PAHs in equilibrium with the aqueous phase, depending on the measurement method(84). For this method 1 kg activated carbon was enough for absorption of 10 g of hydrocarbon pollution in soil, which in practice means that an amount of 100-200 kg activated carbon has to be added to one ton of soil or sediment(85).

6.2. Wastewater treatment

A lot of methods have been developed to clear wastewater from petroleum hydrocarbons. One of the methods is sorption with activated carbon. The general method is treatment of the polluted water in a tank with a diffuser to bring oxygen in the solution. Wastewater treatment also make use of bacteria and fungi, which grow under stimulating conditions. This method is popular because the cost are low and the process can be continuous. There is a lot of research on degradation of petroleum hydrocarbons, and the primary aim of these studies is to degrade the persistent hydrocarbons in addition to the aliphatic hydrocarbons. Some processes make use a combination of absorption of pollutants by activated carbon and degrading bacteria. The bacteria are able to degrade the easily degradable hydrocarbons and the more persistent hydrocarbons are absorbed in the activated carbon. This method is effective for PAH removal within 24 hour, with a temperature around 24 °C and a moderate oxygen concentration of 6/7 mg*L⁻¹(86).

Fungi can also have an important role in hydrocarbon degradation in wastewater. This is illustrated by an artificial wetland used for wastewater treatment. Fluoranthene was degraded by 33 fungi species. Anthracene was only degraded by two species for at least 70%(87). Fungi can also work in an vapor of hydrocarbons when they are immobilized on wood chips(88).

Besides bacteria algae are also an option for degrading PAHs. Algal degradation is happening in the oceans by nature, but it can under stimulated conditions also being used in wastewater treatment in industry. In one experiment algae were used in combination with bacteria. The function of the algae was here merely to support the bacteria. The photosynthesis of the algae produces oxygen which stimulates growth and aerobic degradation by bacteria. Further the algae produces surfactants which also stimulated PAH degradation. The PAHs were during this experiment added to an organic phase which was in equilibrium with the aqueous phase, resulting in only a low concentration of phanthrene in the water phase. This reduces toxicity for the algae(35).

7. Examples of degradation of hydrocarbon pollution in the Netherlands

In this final chapter some sites polluted with hydrocarbons will be discussed. This is to give an overview of the pollution in the Netherlands and to illustrate the topics of the past chapters. Although most of the pollution by hydrocarbons in the Netherlands is due to combustion processes, it is still worth to consider because the degradation mechanisms are the same. The Netherlands has a high density of harbors and motorways, as well as a lot of factory sites suspected of causing hydrocarbon pollution. It is therefore worth to look at some cases to see what hydrocarbon pollution does mean in practice and how pollution was being treated. Sometimes it is also possible to give a prediction of the fate and future of the pollution.

In the Netherlands there is a focus on pollution in the aquatic environment. This is because at the other pollution sites, mostly factory terrains, the pollution is far less spread out. In rivers there has been a lot of sediment pollution being caused by wastewater from industry. This has been spread out down the river depending on the sorption characteristics of the hydrocarbon and the ease of degradation. The most studied compounds are without doubt the PAHs, which are known to be very persistent and have a tendency to absorb in the sediment.

7.1. Example: PAH pollution in the river delta of the lower part of the Netherlands

The first river part in the Netherlands who has been extensively studied is the Scheldt (Schelde) estuary. This river is known to have the highest concentration of PAHs in the North Sea area. In this research from 1988 it was found that around 10% of the PAHs present in the river is being transported to the North Sea, the other 90% is absorbed in the sediment. The concentration of PAHs drops when the water becomes more saline, which can be explained by an increase in sediment absorption of the PAHs. The concentration of PAHs is very homogeneous over a large area, which suggests that most of the PAHs comes from coal combustion. There is a seasonal variation in PAH concentration. River discharge is inversely related to PAH concentration. Despite some uncertainty about the measurement it turned out that the PAH concentration in the Scheldt is considerably higher than in other rivers, for example Rhine (Rijn)(89).

There is a general trend towards lower concentrations of PAHs. Despite the high concentration of PAHs in water from the Scheldt river sediment analysis revealed that the concentrations are now 2 or 3 times lower than they were in the 1960s and 1970s. This is shown in figure 18, where the concentrations are in $\text{ng}\cdot\text{g}^{-1}$ and the numbers on the right are the depth of the sediments layers in cm(90).

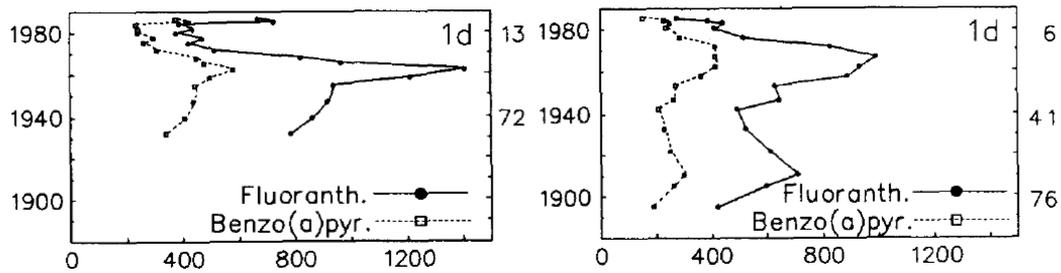


Figure 18 PAH concentration in sediment of the Scheldt river

This trend toward cleaner sediment has also been shown in measurements on other places in the Netherlands. In the Biesbosch a trend was shown with a reduction for all PAHs, although there was a slight increase in sediment pollution after the Delta works, shown in figure 19. This is because the Delta works limit the flow in the Biesbosch, resulting in polluted sediment deposition(91).

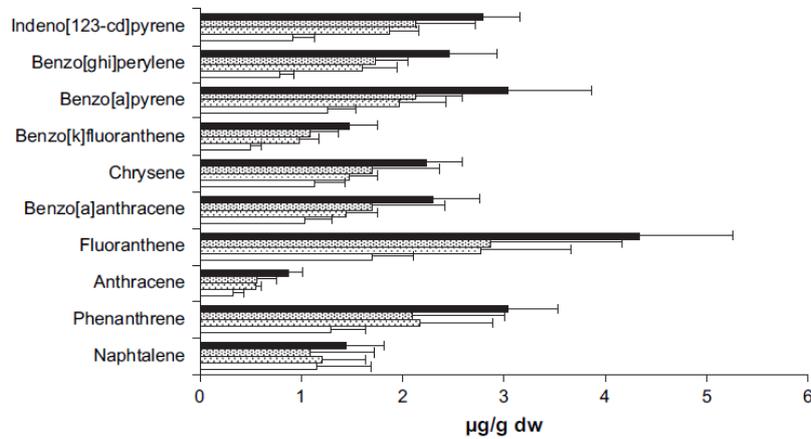


Figure 19 PAH concentration in the Biesbosch, Black to white is from 0 to 45 cm depth

The concentrations of PAH have also been monitored by concentration measurements in organisms, for example in mussels in the Ems Dollard and the Western Scheldt. The result is shown in figure 20, expressed in mg/kg fat weight. There was a decrease in concentration for increase in size, and that was the reason for making a correction for fat weight. The conclusion was the concentration does not exceed the safe consumption limit(92).

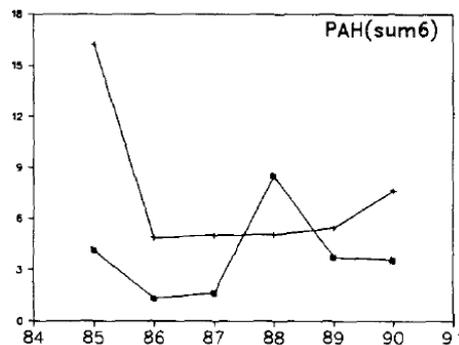


Figure 20 PAH concentration in Mussels in Western Scheldt (upper line) and Ems Dollard (lower line)

The lower hydrocarbon concentration in the upper sediment layer does not always imply a solution for the problem because the PAHs remain in the layer, and need special treatment when removed. This is regularly done in harbors and cities. One of the cities where sediment pollution is a problem is the city of Delft, where sediments in the city center were classified in the highly polluted class 3 and 4 partly because of the PAH sediment concentrations. This led to the need for disposal under strict conditions(93).

Delft is not the only city having these problems. A lot of cities, especially cities which are harbor or former harbors have this problems. The petrol harbor of Amsterdam and the harbor of Rotterdam are other examples(94). In 2010 total around 425 cubic meters of polluted sediment have to be removed from rivers, lakes and harbors in the Netherlands(94).

This disposal is problematic because degradation requires an enormous area for a long time. In the past some polluted harbor sediment has been used for other purposes as happened in The Steendijkpolder in 1983. Although this does not result in toxic concentration for people living on the sediment, it is no longer the method of choice(95). This is the reason for a lot of attention for other methods, for example PAH removing by purging air in the soil. This method has shown to be effective enough in experiments with sediment from Dutch harbors(94).

7.2. Example: soil pollution by gas factories in the area of Utrecht

Before the discovery of a huge amount of natural gas in Slochteren most of the gas in cities in the Netherlands was made by gas factories for more than hundred years. In the factories mainly coal, cokes and sometimes oil were combusted. This was a very polluting production process which was harmful for the environment. Pollution by air gasses spread out over a large area. Therefore the area around the factories had a chance to be cleaned by microorganisms. The concentration levels dropped rapidly in these areas in the past 50 years. What remains is the factory terrain itself with is nearly always heavily polluted by petroleum or petroleum like hydrocarbon side products. The pollution consist of tars and residues which are mostly rich in hydrocarbons as for example PAHs and toluene(96). These tars are examples of the NAPLs commonly found at former gas manufacturing plants(7). Most of the terrains are situated near city centers and the pollution makes redevelopment of the terrains impossible.

Because the hydrocarbons pollution on the factory terrains is very concentrated it has to be degraded or removed before the ground can be used for other purposes. This was for example done near the former gas factory near the Griftpark in Utrecht(97). That soil pollution by former gas factories is a big problem can be seen by the Province of Utrecht, which had 15 of these former factories. Of these factories terrains 8 have already been cleaned. Most soil remediation is made possible by investments in the ground after remediation. This is the most important reasons why some sites are being remediated and other sites not(98).

Especially tars form a problem, because these product were sometimes not used due to the poor quality and insufficient demand for tar during some periods. This causes deposition of the tars on the factory terrain and sometimes even in pits or streams. The resulting concentrated pollution is considered very ecotoxic(96). For better understanding of the risk it is important to use the calculated bioavailability including the properties of the gas factory soil, for example the organic carbon concentration. This approach has been shown to be importance for risk assessment(99).

Except the direct risk for the organisms on the factory terrain also groundwater pollution is a possible risk. The tar which is heavier than water infiltrates in the ground. This is why the Province of Utrecht decided to take faster action in the Utrechtse Heuvelrug, where the sandy ground can be easily infiltrated by the pollution. Another reason is the use of this groundwater for drinking water production.

For estimation of the risk associated with pollution it is necessary to know the ground layer composition under the gas factories. In figure 21 a typical underground of a gas factory is shown. It consist of a layer of soil and ash, underlain by a layer of gravel and sand, which is underlain by a layer of clay. The height of the groundwater is also shown(96).

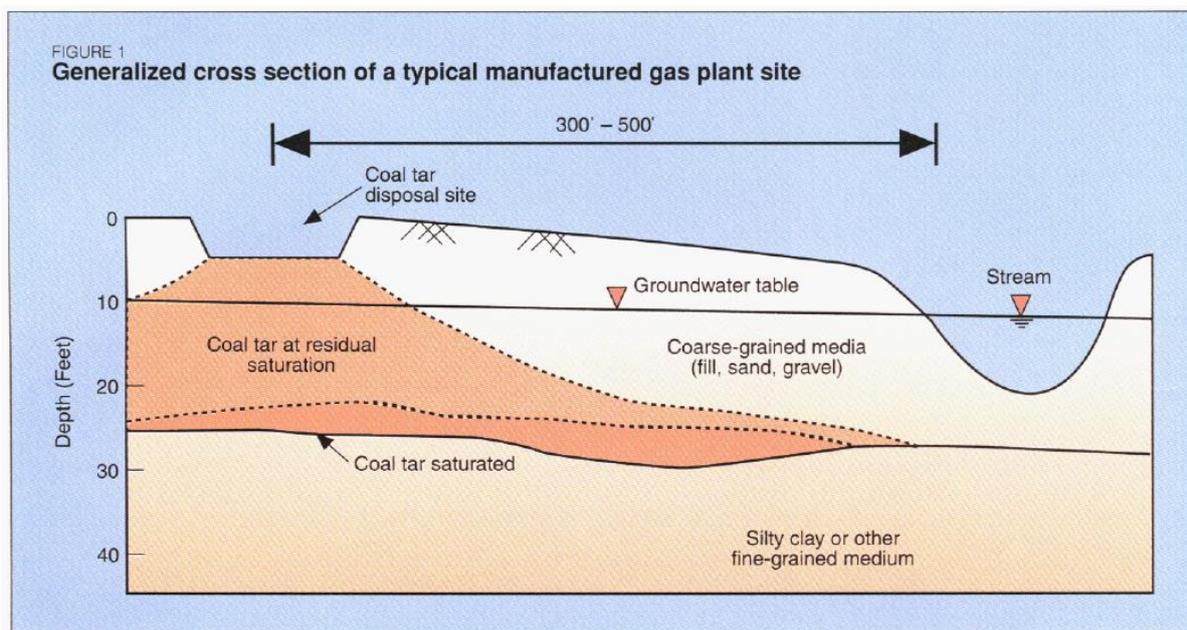


Figure 21 Underground of a former gas factory

In most parts of the Netherlands the sand layer is nearly absent and the clay layer starts directly after the soil layer. The pollution travels downwards until it encounters a layer which it cannot penetrate. The pollution remains in that layer until some parts are solved in the ground water and horizontal spreading starts. Because the high clay layer the pollution in the Nederland is expected more on the surface than in other nations.

This limits both horizontal and vertical spreading. On the other side the level of the groundwater in the Netherlands is in general high, so it is still able to cause spreading. There has also been deposition of petroleum and tart in streams. The former gas factory in Woerden is an example of a place where deposition in a stream took place. The enormous area of the Singel who had to be remediated is shown in figure 22(100).

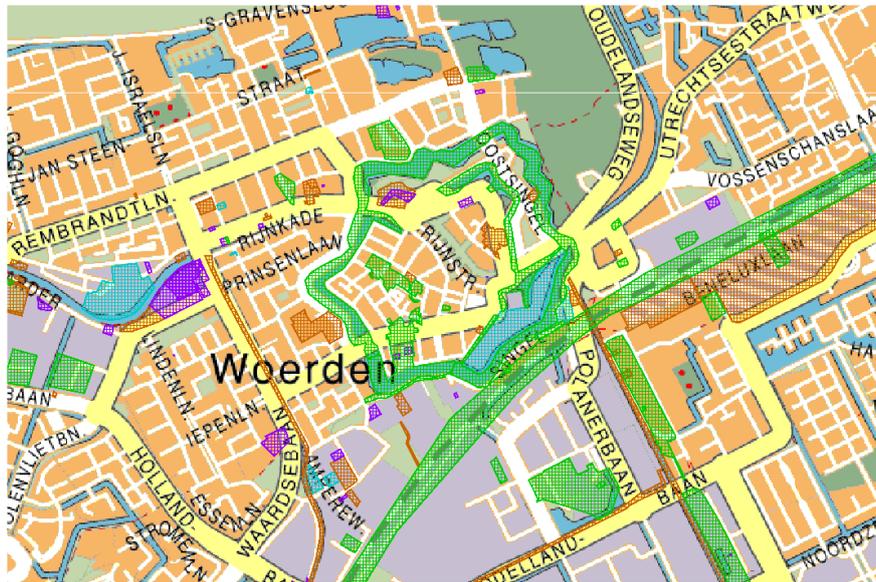


Figure 22 Area remediated around the Singel, shown in green

There are a lot of methods being used for cleaning ground under former gas factories. The problem of this pollution is that it consists of a very dense non aqueous soluble layer. Soil washing is frequently used for remediation of the soil, but often only the lighter soluble fractions are extracted. Sometimes the remaining layer is very insoluble and is left in the soil because without the soluble fraction it is no longer harmful for the environment(96). There were some laboratory experiments done to establish the effectiveness of various techniques. The conclusion was that lighter PAHs are removed by evaporation and that biodegradation and nutrition addition works for heavier PAHs. The added bacteria in this experiment were bacteria selected for metabolizing PAHs. With a combination of these methods it seems to be possible to clean the soil within 50 days(101). A pilot experiment was done at the Griftpark in Utrecht. There were 4 methods tested, 2 physical and chemical based and 2 based on biological methods. The biological methods show much better results than the other methods(97). These methods can further be developed for standard remediation methods for gas factories.

8. Conclusions and remarks

There has been research on petroleum degradation for a long time. This increases during the 1960s and 1970s due to some large oil spills. There was a need for further understanding of the risks for humans and the environment and strategies were developed for remediation of petroleum spills. In the 1980s and 1990s the large scale problem of soil pollution only adds to this demand.

That bacteria are able to aerobically degrade petroleum hydrocarbons is already known for more than a century, but after the Second World War petroleum degradation became a popular topic in microbiology. Huge list of all degrading bacteria and the hydrocarbons they degrade were populated. For the knowledge about the environmental problem of petroleum pollution this is despite only of minor influence. During further research it became more and more clear that degrading bacteria in soil and sediment are nearly everywhere present, and that they only become dominant when they have the genetic advantage over other bacteria to be able to degrade hydrocarbons.

The influence of light was estimated to be small for a long time. In tropical regions it turned out that it is besides biodegradation also an important degrading factor. Degradation by light lead to a totally different pattern of hydrocarbons degradation, with degradation rates differing from biodegradation. Based on the ratios of the degradation products it is possible to distinguish degradation by light from biodegradation.

The toxicology of hydrocarbons is important for assessing the risk that hydrocarbon form for humans and ecosystems. There is not much known about aliphatic hydrocarbons. They do not seem to create a lot of damage to organisms due to their character in the environment. They are in general volatile and the non volatile aliphatic are easily degraded by bacteria. The cycloalkanes are degraded slower and are not very often used in toxicological studies for risks for humans. Finally they are degraded in the environment. The PAHs in comparison have carcinogenic properties and are the compound of interest for toxicological studies and for concentration limits for governments.

The topics bioavailability and degradation are strongly related with toxicity. The distribution of hydrocarbons in the environment is important for risk assessment. The most hydrophobic hydrocarbons absorb in soil and sediment, where their availability is totally different than in the aqueous phase. The composition of the sediment has been shown to be very important. The composition of absorbing compounds in the soil has an influence on the partitioning between the aqueous and the sediment phase. Especially organic compounds are able to absorb hydrocarbons and this is important to include in estimations of the risk for the environment.

Biodegradation is able to degrade nearly all hydrocarbons in the environment. The bigger aromatic hydrocarbons are the only molecules which are poorly degraded by bacteria. Fungi are however able to degrade them with specific enzymes, followed by normal degradation by bacteria. Biodegradation is instead of degradation by light influenced by a lot of factors. For example temperature, salinity, pH and humic acids have shown to have a large influence on toxicity and biodegradation. Presence of oxygen makes aerobic degradation possible, which is faster than anaerobic degradation.

Photochemical degradation in general does not lead to total degradation of the hydrocarbons, but only introduces some chemical groups. These polar groups lead to mousse formation and increase in solubility. For photochemical degradation light intensity and temperature are most important. The increase in solubility in water fastens degradation by photochemical and biodegradation. The importance of water column photodegradation in nature is unsure, because the availability of light in the water layer is not known. Experiments however suggest that this form of degradation is of major influence on the fate of hydrocarbons in water. One side of light is that it is able to degrade toxic hydrocarbons, but on the other side light seems to create compounds which are more toxic for degrading organisms.

There are several methods known for remediation of polluted soils. Many of them have been proven to be successful. Soil remediation can be done by physical methods for volatile compounds, and for less volatile compounds the biodegradation methods are more appropriate. Sometimes a combination of these two methods is used.

For remediation of hydrocarbon pollution in the aquatic environment there has been a huge evolution. In the 1960s dispersants were used during tanker accidents. This led to visual improvement, but the effects on the environment are only poorly understood. Nowadays dispersants are still used, but now also addition of nutrients is important for stimulating biodegradation.

In the Netherlands there are various places where the environment is polluted with hydrocarbons. This is seen near harbors and rivers. The hydrocarbon concentrations do not exceed the current safety limits and the sediment layer, which absorbs the most dangerous compounds, is becoming cleaner since the 1980s. The presence of much higher concentrations in deeper layers sometimes pose a problem when there is a need for sediment removing. The pollution in soil is more concentrated and is therefore less problematic than sediment pollution. On the other side there are a lot of polluted places, a lot of them being caused by former gas factories. Efficient techniques have been developed and are still being developed on a lot of locations in the Netherlands. Methods based on biodegradation method sometimes combined with physical removal of the pollution have shown to work best.

8.1. Further research on petroleum degradation

Most of the degradation studies on hydrocarbons are descriptive. They focus on the effect of the change of one parameter on degradation. In contrast to this one parameter

experiments fate of petroleum in the environment is quite complex, being determined by an almost infinite number of parameters. The most important parameters are now known, although not always well understood. Photodegradation for example is an important process that is only poorly understood. The influence on the environment of each parameter alone is known, but only rarely the influence of these parameters is measured in relation to influence of other parameters. This complexity should be the central thought for developing further research. This is important because these experiments are being used to predict the behavior of hydrocarbons in the environment, and this is always a multi parameter process.

This thesis focus mainly on the distribution and degradation of hydrocarbons in the environment. The final goal however is not distribution or degradation but the harmfulness of hydrocarbons for all different kinds of environments. For risk assessments of hydrocarbons it is important to know the concentration that is taken up by organisms, and even more important the concentration that is able to cause toxic effects. This only add more parameters to the already complex process of distribution and degradation. The difficulty of this problem is already shown in the process of modeling bioavailability.

That toxicity is caused by a lot of parameters is central to toxicology. I think that continuing doing multi parameter experiment combined with collection of already known effects in for example computer programs is useful. This kind of programs can being used for risk estimation of hydrocarbons in a specific environment and provide better information for example for NOAELs.

Except the estimation of risk also the risk diminishing side needs some attention. Although methods for soil, sediment and water cleaning have shown to be very successful it is worth to develop better method for soil and sediment treatment. In most nations soil treatment is planned as soon as pollution forms a threat for groundwater. In most cases this is not what happens and the soil is not remediated. A lot of former industry terrains all over the world are useless because there is a need for remediation of the soil. The ground will only be remediated if the ground is sellable for at least the price of the treatment. Developing faster and more cost effective methods will lead to more terrains being treated, resulting in useful ground for industry and houses. Another reason for being able to develop low cost soil remediation is a possibility to create for example parks at former industry terrains in city centers as has been done in the Griffpark in Utrecht, creating improvement for the local population.

Also in cleaning of sediment there are some improvements expected. In the aquatic environment the use of activated carbon has shown to be a promising solution for sediment pollution. Before wide spread use of this technique it is however important to know that this technique is safe for the environment, because the carbon remains in the soil and maybe has the possibility to cause adverse effects. The use of gasses to clean

sediment showed promising results on lab scale, but it depends on the possibility for upscaling if this can become a useful technique for in the field.

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