Kinetics of the microbial degradation of oil in soil slurry reactors

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of oil in soil slurry reactors

Kinetiek van de microbiële afbraak van olie in grondslurry-reactoren

Proefschrift

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Summary

In recent years biological decontamination of water and soil has become increasingly popular. The main reason for this is its potential of a cheap and relatively simple process. Several processes have been designed using this technique. One of these techniques is the soil slurry process designed at the department of Biochemical Engineering at the Delft University of Technology. The slurry process makes use of the so called DITS (Dual Injected Turbulent Suspension) reactor and one or more air agitated suspension reactors. The need for a more fundamental knowledge of the microbial oil degradation in the reactors was the starting point of this thesis.

The main aim of this research project was to predict required residence times of oil contaminated soils during microbial remediation in a slurry reactor. Further aims were to increase the biodegradation rate of oil components adsorbed to soil, and to reduce the lowest achievable concentration of pollutants in the treated soil.

To determine oil degradation rates in a slurry reactor, the most important variable that should be measured is of course the oil concentration. When using diesel contaminated soil in an aerated reactor, all three phases, air, water and solid, may contain oil. In batch experiments, the oil concentration in the water will decrease due to the (exponential) growth of microorganisms on this substrate. After about 48 hours most of the oil in the water will have disappeared. The concentration in the gas phase will therefore also be negligible. Hence, during most experiments, only the oil concentration on the solids is of importance.

Since a large number of soil samples has to be analyzed during a degradation experiment, a rapid and labour extensive procedure is required. An established analysis procedure consists of a soxhlet extraction, followed by GC analysis, resulting in a total analysis time for a series of eight samples of about 3 days. The Dutch (interim) standard analysis procedure NEN 5733 requires about two days for a series of eight samples. Such long analysis times are not acceptable, especially when dealing with soil residence times in the reactor of just a few days. In addition, both analysis techniques require large amounts of environmentally undesirable chemicals. The soxhlet extraction uses about 50 ml of toluene and 150 ml of methanol per sample, while the standard analysis uses 40 ml of 1,1,2-trichlorotrifluoroethane, a kind of freon.

Therefore a new and fast analysis technique, the so called Thermal Desorption Mass Spectrometry (TD-MS) method, is introduced and compared to the performance of the two techniques mentioned before. The total analysis time for eight samples is about 4 hours. Except

for the pure contaminant (needed for calibration, as in all analyses), no chemicals are needed. Therefore, this method seems very promising as an alternative.

Despite the disadvantages of the standard analysis method, this method was used in most of the experiments described in this thesis for the following reasons. In the first place, TD-MS is still in the experimental stage, and was not available for the time needed to perform all analyses, and secondly, to have the possibility to refer to literature values as well as to regulation (the ABC-values).

The hypothesis on which the rest of this research is based is, that the overall microbial degradation rate of oil in a soil slurry reactor is restricted by one of three limiting conditions:
1) some or all of the components are not (entirely) degradable by the microorganisms present,
2) the diffusion rate of the oil components out of the soil particles is very low,
3) the desorption rate of the (nonpolar) oil components from the soil is very low.

The degradation of individual oil components by pure bacterial strains or by consortia had already been studied extensively. The microbial degradation of complex substrates such as diesel oil by a consortium of microorganisms in water dispersion had been studied, although scarcely, and it had never been related to soil decontamination. Neither had it been determined what part of the diesel oil could be degraded, how fast this degradation could proceed, and what limits the degradation rate of the oil components remaining in the soil.

Previous experiments with the above mentioned DITS reactor used diesel oil as the contaminant. After treatment of the soil, irrespective of the residence time of the soil in the reactor, a constant fraction of the initial oil concentration remained in the soil. To determine how the final oil concentration can be decreased further, first it had to be established which part of the diesel oil can be degraded and how fast this degradation can proceed. The kinetic parameters governing the consumption of oil (in water) as sole source of energy and carbon have been determined and compared to known values for microbial degradation of single components in literature. It is shown that in the absence of soil, diesel oil can be degraded completely within five days.

The effect of the presence of soil on the microbial degradation of diesel oil has been studied in batch and in continuous systems. It was found that after several weeks in a batch reactor, the oil concentration finally decreases to below the detection limit. However, in the continuous system (a DITS reactor followed by an aerated slurry reactor) the concentrations on the suspended fine particles as well as on the fluidized coarse particles remained much too high, irrespective of the residence time in the reactors. Only if a contaminated soil with an initial oil concentration of less than 3.5 g/kg was used could oil concentrations below the Dutch B-level

(1 g/kg) be reached. The fact that only in batch the soil was remediated sufficiently (and this only with a long residence time) posed a serious problem. In practice for a slurry process to be economically feasible, it should be operated in continuous mode with a maximum residence time that is about three times smaller than was necessary in batch.

Next step is to pinpoint which part of the soil exhibits a low ultimate degradation rate, leading to a rest concentration on the soil due to the relatively short residence times used. Therefore the soil is divided into four different fractions, sand, two types of clay, and organic matter. These fractions are used separately to determine the soil fraction(s) exhibiting the highest oil concentration after a short treatment period and the lowest degradation rate during that period. It was found that these soil fractions are organic matter and one type of clay, represented by montmorillonite. Of these two soil constituents, the clay is most retarding the oil components, more than 100 times as much as organic matter.

When it was determined which fraction of the soil is responsible for the rate limitation it was possible to design a model to predict the rest concentration of oil as a function of the residence time in the reactor.

Essentially, the model consists of a partial differential equation describing the oil concentration on the soil. Three different fractions of the soil, i.e. sand, clay and organic matter, are assumed to each participate independently to the oil concentration in the reactor. The degradation of oil on sand (and in the water phase) can be described by Michaelis-Menten kinetics. The oil concentration on the clay and the organic matter are described by the effective diffusion model. The effective diffusion is assumed to consist of a bulk diffusion rate and a sorption coefficient, so desorption and diffusion are both incorporated in the model. The sorption coefficients for hexadecane on the different constituents of the soil are calculated. When for a specific particle size fraction the soil composition is known, as well as the partition of the oil between the three soil fractions, the hexadecane degradation rate for this size fraction can be calculated. It is shown that in this way for well-defined size fractions the residence time in the reactor can be predicted, during the remediation of a contaminated soil.

The best operation mode in which to remediate water contaminated with oil, was found to be batch or plug flow. This operation mode is necessary, because the presence of linear alkanes inhibits degradation of branched alkanes. Residence times should be in the order of two to three days. An additional beneficial effect is that the yield of biomass on a mixture of linear and branched alkanes is low. The water after treatment will therefore contain a relatively low amount of organic matter. This will reduce the costs of release of the treated water into the environment. The residence time of several days could pose serious clogging problems in a true plug flow

reactor due to bacterial adhesion to the reactor wall. Therefore batch operation or continuous operation in a cascade of chemostats seems the best choice. Recirculation of biomass or use of biomass carrier materials may increase the oil degradation rate somewhat due to the increased biomass concentration in the reactor.

For oil contaminated soil, separation into a clean and a polluted fraction is prudent. This reduces the needed treatment capacity and therefore the size, and correspondingly the construction costs, of the reactor. The DITS reactor is very useful in this respect. The soil is separated into a fraction containing the large particles (the sand) and a fraction containing the silt, clay and organic matter. Simultaneously, the soil slurry is inoculated with oil degrading micro-organisms, and part of the contaminant is degraded. This reduces the amount of polluted soil fraction. The clean(ed) soil fraction can be discharged, the polluted soil fraction can be treated in a second stage. The operation mode of this second reactor should be either batch or plug flow. Due to the long treatment time needed, and the slow release of the contaminant into the microbial environment, the energy input should be kept at a minimum. Addition of a unit operation for size reduction of the soil particles prior to the second reactor should be seriously considered, because of a consequential reduction in residence time of the soil in the second reactor. A cheap alternative to the second reactor could be a landfarm. The costs of this type of operation, instead of landfarming the entire soil, will be much lower, due to the small fraction of the soil that needs treatment after the DITS reactor; the soil already contains an increased amount of oil degrading micro-organisms, and the nutrients needed for microbial activity have already been added in the DITS reactor.

Samenvatting

Biologische reiniging van water en grond heeft de laatste paar jaren meer en meer aan populariteit gewonnen. De belangrijkste reden hiervoor is dat biologische reiniging in potentie goedkoop is en de uitvoering ervan betrekkelijk eenvoudig. Op basis hiervan zijn verschillende reinigingstechnieken ontwikkeld. Een van deze technieken is het grondslurry-proces dat is ontwikkeld bij de vakgroep Bioprocestechnologie aan de Technische Universiteit Delft. In het slurry-proces wordt gebruik gemaakt van de zogenaamde DITS (Dual Injected Turbulent Suspension)-reactor, alsmede van één of meer beluchte suspensie-reactoren. De noodzaak van meer fundamenteel inzicht in de microbiële olie-afbraak in de reactoren was de aanzet tot dit proefschrift.

Het voornaamste doel van dit onderzoeksproject was het ontwikkelen van een model voor het voorspellen van de benodigde verblijftijden van met olie verontreinigde grond, tijdens de microbiële afbraak in een slurry-reactor. Secundaire doelstellingen waren het verhogen van de afbraaksnelheid van aan grond geadsorbeerde oliecomponenten, en het verder verlagen van de laagst haalbare olieconcentratie na behandeling in de reactor(en).

Om de olie-afbraaksnelheden in een slurry-reactor te bepalen is natuurlijk de olieconcentratie op de grond de belangrijkste variabele die moet worden gemeten. Wanneer met diesel olie verontreinigde grond in een beluchte slurry-reactor wordt behandeld, kunnen alle drie betrokken fasen, lucht, water en grond, olie bevatten. In batch-experimenten zal de olieconcentratie in de waterfase afnemen door de (exponentiële) groei van micro-organismen op dit substraat. Binnen 48 uur zal de meeste olie uit de waterfase verdwenen zijn. Dientengevolge zal ook de olieconcentratie in de gasfase verwaarloosbaar klein zijn geworden. Daarom is in de meeste experimenten alleen de olieconcentratie op de grond van belang.

Omdat een groot aantal monsters moet worden geanalyseerd gedurende een afbraakexperiment, is een snelle en arbeidsextensieve analyse-methode nodig. Een degelijke analyse-methode voor olie in grond bestaat uit een soxhlet-extractie, gevolgd door een GC-analyse. De benodigde analyse-tijd voor een serie van acht monsters is zo'n drie dagen. De in het (voorlopige) normvoorschrift NEN 5733 beschreven analyse-methode heeft voor dezelfde serie monsters twee dagen nodig. Dit soort van analyse-tijden is onacceptabel lang, zeker wanneer wordt gewerkt met verblijftijden van grond in de reactor van slechts enkele dagen. Bovendien worden in beide analyse-methodes grote hoeveelheden milieu-onvriendelijke chemicaliën gebruikt. In de soxhlet-extractie wordt per monster 150 ml methanol en 50 ml tolueen gebruikt.

Voor een analyse volgens het normvoorschrift is 40 ml 1,1,2-trichloortrifluorethaan, een soort freon, per monster nodig.

Daarom wordt hier een nieuwe en snelle analyse-methode geïntroduceerd, de zogenaamde TD-MS (Thermal Desorption Mass Spectrometry) methode. Deze methode wordt qua analyse-resultaten vergeleken met de resultaten van de voornoemde twee methodes. De benodigde analyse-tijd voor de TD-MS methode voor een serie van acht monsters is zo'n vier uur. Behalve de vervuilende component(en) zelf (in elke analyse-methode nodig voor calibratie), worden bij een analyse volgens deze methode in het geheel geen chemicaliën gebruikt. Al met al lijkt de TD-MS methode een veelbelovend alternatief voor de tot nu toe beschikbare analyse-methodes.

Ondanks de nadelen van het normvoorschrift zijn toch de meeste analyses in de rest van het onderzoek uitgevoerd volgens de NEN. Dit omdat ten eerste, de TD-MS nog steeds in het experimentele stadium is, en ten tweede, om de analyse-resultaten direct te kunnen relateren aan de Nederlandse wetgeving (de ABC-waardes) en aan in de literatuur gegeven waardes.

De hypothese waarop de rest van dit onderzoek is gebaseerd is dat de microbiële olie-afbraak in een slurry-reactor gelimiteerd wordt door één van de volgende drie beperkingen:
1) sommige of geen van de oliecomponenten kunnen worden afgebroken door de aanwezige micro-organismen,
2) de diffusiesnelheid van de olie-componenten uit de gronddeeltjes is zeer laag,
3) de desorptiesnelheid van de (apolaire) olie-componenten vanaf de grond is zeer laag.

De afbraak van enkelvoudige olie-componenten door monocultures of door een consortium van bacteriën is reeds uitgebreid onderzocht. De microbiële afbraak van complexe substraten, zoals diesel olie, door een consortium van micro-organismen in waterdispersie is ook reeds in een enkel artikel beschreven, maar is nooit gerelateerd aan grondreiniging. Evenmin is onderzocht welk deel van de olie afbreekbaar is, hoe snel deze afbraak plaats vindt, en wat de limiterende factor is in de verdere afbraak van de in de grond achtergebleven olie.

In eerdere experimenten met de bovengenoemde DITS-reactor werd diesel olie gebruikt als de vervuiling. Na behandeling van de vervuilde grond bleef een vast percentage van de olie achter op de grond, onafhankelijk van de verblijftijd in de reactor. Teneinde te bepalen hoe de uiteindelijke olieconcentratie verder kan worden verlaagd, is het in de eerste plaats nodig te bepalen welk deel van de olie afgebroken überhaupt kan worden en hoe snel dit gebeurt. De kinetische parameters, die de consumptie van olie (in water) als enige koolstof- en energiebron beschrijven, zijn bepaald en vergeleken met gepubliceerde waardes voor de afbraak van enkelvoudige componenten door monocultures. Het wordt aangetoond dat, in de afwezigheid van grond, diesel olie volledig kan worden afgebroken binnen vijf dagen.

Het effect dat grond heeft op de microbiële afbraak van diesel olie is bestudeerd in batch- en in continu-reactoren. Gevonden werd dat na enkele weken in een batch-reactor de olieconcentratie tot beneden de detectie-limiet is afgenomen. In het continu-systeem (een DITS-reactor gevolgd door een beluchte suspensie-reactor) echter, bleven de olieconcentraties op zowel de gesuspendeerde deeltjes als in het fluïde bed te hoog, onafhankelijk van de verblijftijd. Alleen bij grond met een startconcentratie van minder dan 3,5 g/kg olie was de concentratie op de uitgaande grond beneden de B-waarde (1 g/kg). Dat alleen in batch, en dan ook nog met een zeer lange verblijftijd, de grond voldoende kon worden gereinigd, zorgde voor een probleem. Als een slurry-proces in de praktijk economisch haalbaar wil zijn moet het namelijk in continu-operatie bedreven worden, met een verblijftijd die zo'n drie keer lager is.

De volgende stap op weg naar het model is dan ook het bepalen welk onderdeel van de grond een lage uiteindelijke afbraaksnelheid geeft, welke leidt tot een hoge restconcentratie vanwege de relatief korte verblijftijd. Daarom is de grond verdeeld in vier verschillende fracties, t.w. zand, twee types klei en organisch materiaal. Deze fracties worden apart gebruikt in experimenten om de fractie te vinden die de hoogste olieconcentratie en de laagste afbraaksnelheid geeft na een korte verblijftijd. Het bleek dat het organisch materiaal en een klei-type, vertegenwoordigd door montmorilloniet, het meest aan deze voorwaarden voldeden. Van deze twee vertraagde de klei de olie-afbraak het meest, meer dan honderd keer zoveel als het organisch materiaal.

Toen de limiterende grondfracties waren gevonden was het mogelijk om een model op te stellen, waarmee de olieconcentratie in de grondslurry als functie van de verblijftijd kan worden voorspeld. In feite bestaat het model uit een partiële differentiaalvergelijking die de olieconcentratie in de grond beschrijft. De drie verschillende grondfracties, t.w. zand, klei en organisch materiaal, worden verondersteld alledrie onafhankelijk van elkaar de olieconcentratie in de reactor te beïnvloeden. De afbraak van olie op zand (en ook in de waterfase) kan worden beschreven met Michaelis-Menten kinetiek. De olieconcentratie op de klei en op het organisch materiaal kan worden beschreven met het effectieve diffusie model. De effectieve diffusie wordt beschouwd als zijnde een combinatie van een bulk-diffusiesnelheid en een sorptie-coëfficiënt. Zodoende worden zowel diffusie als desorptie in het model geïncorporeerd. De sorptie-coëfficiënt voor hexadecaan op de verschillende grondfracties werd bepaald. Als voor een bepaalde deeltjesgrootte-klasse de grondsamenstelling bekend is en tevens de verdeling van de olie over de drie grondfracties, dan kan de hexadecaan-afbraaksnelheid voor deze deeltjesgrootte worden uitgerekend. Het wordt getoond dat op deze manier voor goed bepaalde grootte-fracties de verblijftijd in de reactor goed voorspeld kan worden bij de reiniging van vervuilde grond.

De beste bedrijfsvoering om met olie verontreinigd water te behandelen, bleek batch of plug flow. Deze manier van behandelen is nodig, omdat de aanwezigheid van lineaire alkanen de afbraak van vertakte alkanen belemmert. Een verblijftijd in de orde van twee of drie dagen zou voldoende moeten zijn. Bijkomend voordeel is dat de yield van biomassa op een mengsel van lineaire en vertakte alkanen laag is, zodat betrekkelijk weinig organisch materiaal in het effluent zal zitten. Dit zorgt ervoor dat de kosten om dit effluent kwijt te raken laag zijn. Wanneer voor een echte plug flow reactor wordt gekozen kan de benodigde verblijftijd van enkele dagen ernstige verstoppingen veroorzaken, door hechting van biomassa en olie aan de reactorwand. Daarom is uiteindelijk een batch-reactor of een cascade van chemostaten de beste keuze voor de bedrijfsvoering.

Met olie verontreinigde grond kan het beste eerst worden gescheiden in een "schone" en een vervuilde fractie. Dit verlaagt de benodigde reactor-capaciteit en dus de grootte van de reactor, waardoor de investeringskosten lager uitvallen. In dit opzicht is de DITS-reactor zeer veelbelovend. In deze reactor wordt de grond gescheiden in een fractie met zware deeltjes (het zand) en een fractie met lichte deeltjes (slib, klei en organisch materiaal). Tegelijkertijd wordt de grondslurry beënt met actieve olie-afbrekende micro-organismen en wordt een (groot) deel van de olie afgebroken. Het schone zand kan dan worden gelost, de nog vervuilde lichte grondfractie kan verder worden behandeld in een tweede reactor. De bedrijfsvoering van deze tweede reactor kan ofwel batch ofwel plug flow zijn. Vanwege de zeer lange verblijftijden die nodig zijn door het trage vrijkomen van de vervuiling uit de gronddeeltjes, kan de energie-toevoer in de tweede reactor tot een minimum beperkt blijven. Invoegen van een unit operatie voor het verkleinen van de gronddeeltjes voordat deze de tweede reactor ingaan, moet in overweging worden genomen. Hoe kleiner de deeltjesgrootte van de grond, hoe korter de verblijftijd in de tweede reactor kan zijn. Als alternatief voor een tweede reactor kan landfarming worden overwogen. De kosten van deze manier van behandelen van vervuilde grond, i.p.v. de gehele grond op een landfarm te behandelen, zal veel lager zijn, omdat slechts een (klein) gedeelte van de grond nabehandeling behoeft na de DITS-reactor. Bovendien bevat de grond dan al actieve olie-afbrekende biomassa in betrekkelijk hoge concentraties en de voor de olie-afbraak benodigde nutriënten zijn ook al goed gemengd met de grond aanwezig.

Introduction
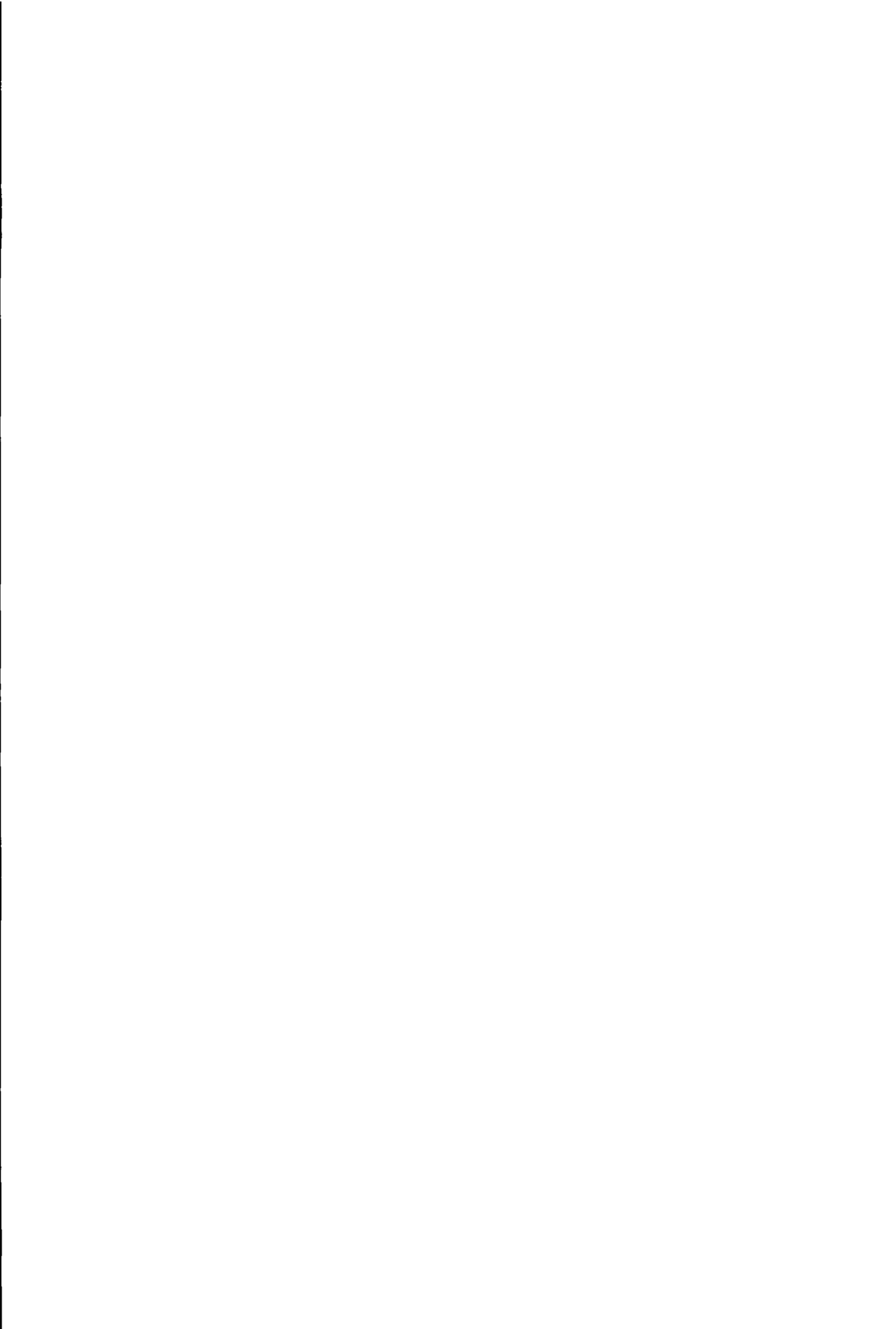
BACKGROUND

From the very start of its existence mankind has been discarding waste into the environment. The more "civilized" the culture, the more waste was produced, and discarded. As long as the population density was not very high, this was not a serious problem. The discarded organic materials were turned over fast enough to prevent accumulation in the environment. However, with the concentration of the population in cities and the resulting centralization of operations, waste production became also concentrated. In many cases the amounts of organics that were dumped largely exceeded the self cleaning capacity of the local area. In order to restore the balance, technologies have been or are being developed to augment the turn over capacity of the ecosystem.

A typical example of water and soil contamination is oil pollution. Oil is a natural product and many microorganisms are capable of degrading oil components. When oil or its refinery products are spilled, accidentally or on purpose, the amount of oil components in the spill area is usually so large that it exceeds the degradation capacity of the microorganisms that are present. The amount of available substrate has suddenly increased manyfold and the microorganisms capable of degrading (specific) oil components start to grow exponentially until a necessary nutrient becomes limiting. In practice it is usually the oxygen or the nitrogen supply that is insufficient to sustain exponential growth. When this happens, soil or water pollution is the observed effect.

Regulation

In most industrialized countries, the concentrations of the most abundant polluting chemicals are not allowed to exceed certain levels, which are designated according to the toxicity of the contaminant. In the Netherlands for each contaminant three concentration levels have been set, the reference level or negligible risk level (verwaarloosbaar risiconiveau, VR), the maximum acceptable risk level (maximaal toelaatbaar risiconiveau, MTR) and the intervention level.

The reference level is the concentration of a component below which the possibility of the occurrence of adversary effects is assumed to be negligible. In principle the VR is set at 1/100 of the MTR. For soils with clay or organic matter deviating from the standard soil, the concentrations of some contaminants must be corrected [1]. The MTR is the concentration above which the risk for the occurrence of adversary effects is unacceptable.

A distinction is made between human toxicity and ecotoxicity. For human toxicity a further distinction can be made between components with and components without a threshold value.

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For components with a threshold value the MTR is set at the acceptable daily intake (ADI) or at the toxicological acceptable daily intake (TDI), both of which are calculated from the no-effect level (NEL). For components without a threshold value, these are the so called genotoxic carcinogens, the MTR for humans is set at 10^{-6} yr^{-1} (the concentration that, for one million exposed humans, results in one extra case of cancer per year). For humans risks the MTR is also the intervention level, because it is perceived that exposure to a component above this concentration is unacceptable.

For ecosystems the MTR is set at 95% protection of all species. the intervention level is set higher, at 50% protection of all species. The concentration at which 50% of all the species present is threatened is perceived as severely threatening to the ecosystem.

Level	Ecotoxicity	Human toxicity	
		components with threshold	components without threshold
Reference (VR)	1/100 MTR	1/100 MTR	1/100 MTR
Maximum acceptable risk (MTR)	95% protection	ADI/TDI	10^{-6} yr^{-1}
Intervention	50% protection	ADI/TDI	10^{-6} yr^{-1}

Table 1. Concentration levels according to the guidelines to soil protection (Leidraad Bodembescherming, adapted from [2]) dated March 1994.

The intervention level is the concentration level above which the soil must be decontaminated as soon as possible. According to Dutch regulation the concentration of contaminants in the soil should not exceed the reference levels after treatment of a contaminated soil. For mineral oil the values for a standard soil are: reference level: 50 mg oil/kg soil, MTR: 1000 mg oil/kg soil, intervention level: 5000 mg oil/kg soil. It should be mentioned explicitly that in contrast to most contaminants, the values for mineral oil are sum parameters. All components that are analyzed and detected using the mandatory protocol of NEN 5733, the interim standard analysis technique, are assumed to constitute mineral oil. No individual component concentrations need to be measured.

Toxicity

Of course it is questionable whether the reduction of the concentration of contaminants in soil after (biological) treatment, to the level required by legislation, is an adequate measure to ensure public health. Probably, the contaminants that are left after biological treatment, are very slowly

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released from the inner volume of the soil particles to the microbial environment [3]. Upon analysis of the soil the components inside the particles are detected as well; (micro)organisms, however, experience only very low concentrations of these components [4]. For example heavy metal ions can be bound so strongly to the soil that only extreme conditions will release them [5, 6]. In the analysis of heavy metals, these extreme conditions are met and a high concentration is found. Under normal conditions however, these ions are released at a extremely low rate, resulting in a very low impact on the environment.

A more realistic approach for setting the allowable concentration levels would be to use a toxicity test [7]. In such a test several organisms, bacteria or higher organisms, are exposed to the soil. Depending on the perceived concentration of contaminant(s), part of the test organisms perish, thus giving a measure for the toxicity. In this way synergistic or antagonistic effects between different components can be detected as well [8, 9].

The toxicity of a contaminant for the test organisms may be very different from that for humans. Even similar species, such as rats and mice, may react differently to certain chemicals [10]. Hence, to assess the toxicity of chemicals towards humans, significant results can only be obtained from tests on humans. However, this is restricted for obvious reasons. For some chemicals the toxicity has been determined through epidemiological research or from accidental exposure. However, for many compounds these figures are not available.

Soil clean up techniques [3, 11, 12, 13]

For the remediation of polluted sites, a large variety of different techniques has been developed. In some cases treatment is performed in-situ and thus the polluted soil is not excavated. This is the preferred operation, because excavation and subsequent treatment of contaminated soil is more expensive.

In-situ remediation is in most cases a variation of the pump-and-treat technique: Aquifer water is pumped to the surface and, after treatment, reinjected into the bottom. Contaminants are removed from the subsurface soil by the washing action of the circulating water. If needed surfactants or other chemicals are added to the water. However, in many cases the treatment time of many years is not acceptable. Moreover, according to an estimate of the EPA (Environmental Protection Agency, USA) 60% of the so called Superfund sites can not be remediated with this kind of technique [14]. Consequently, excavation of the site is necessary. Usually the soil is then treated elsewhere.

These so called ex-situ treatment techniques can be divided into three categories:

1) physical separation of a heavily polluted fraction and a cleaner s.. ..ction, by mechanical techniques like flotation and hydrocyclones, or by physicochemical techniques like extraction by an organic solvent or an emulsifying agent,

2) thermal treatment, in which the contaminants are evaporated and/or oxidized, and

3) biological treatment where the contaminants are mineralized or extracted biologically.

The ex-situ techniques all have their own pro's and con's.

Ad 1) The physical separation processes all split the soil into a clean fraction (usually the large particles, the sand) and a heavily polluted fraction (the fines). Usually an additional extraction step is applied to the polluted fraction. In most cases a surfactant is added and the clean(ed) fraction is separated from the sludge, a suspension in water of the extracting agent, the contaminants and the fine particles [e.g. 15]. The clean soil fraction is reused, in most cases the polluted sludge is removed to special 'temporary' dump areas. The sludge is stored until an economically feasible remediation technique has been developed.

Ad 2) In a thermal soil treatment plant the soil is heated to evaporate the contaminants. The temperature used depends on the contaminants present: For aliphatics 300^0C usually suffices, polycyclic aromatic hydrocarbons (PAH's) and cyanides require 400^0-500^0C, and halogenated components are treated with temperatures sometimes exceeding 600^0C. After evaporation the vapours are burned in a second oven (the post combustor) at much higher temperatures, up to 1200^0C, especially if halogenated components are present. Below 1200^0C, the oxidation products of halogenated components will, in the presence of a proper catalyst such as copper salts [16] (usually present in the fly ash in the gas stream), combine to form dioxines and similar compounds [17, 18]. Therefore, special attention must be given to the cooling stage following the post combustor, to prevent formation of these components.

Ad 3) In a biological treatment process, microorganisms are used to mineralize the organic pollutants. Aliphatics, aromatics, PAH's up to three fused rings, and low halogenated hydrocarbons can be mineralized. High molecular polycyclic aromatic hydrocarbons can be oxidized extracellularly through enzymes, excreted by certain lignin degrading fungi [19, 20]. Further degradation can then proceed intracellularly by the fungi or by other microorganisms. Most highly halogenated hydrocarbons can be dehalogenated

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anaerobically [21], or aerobically through the same extracellular enzymes mentioned before [20], and subsequently mineralized [20, 21].

Heavy metals can be removed through acid production by certain microorganisms and subsequent removal of the metal ions [22], or through accumulation in certain plants [23]. The latter possibility seems very promising: The plants can be harvested and, due to the sometimes very high heavy metal concentrations that are accumulated, they can possibly be used as ore.

In Table 1 the main advantages and disadvantages of the three categories of soil remediation techniques are summarized.

Technique	Advantages	Disadvantages
Physical	Simplicity of operation: No (extremely) high temperatures or complicated process control is needed.	There is always a waste stream, which can be very large, e.g. when organic compounds must be removed from a peaty soil. The fertile part of the soil is removed with the waste stream.
Thermal	All organic contaminants can be removed to below the desired concentration level.	All microorganisms in the soil are killed. Most of the organic matter is destroyed. Clay is petrified when high temperatures are used. No heavy metal removal. High energy costs.
Biological	Minimal changes in soil composition and structure.	Long treatment times are needed to reach the desired low concentration level.

Table 1. The main advantages and disadvantages of the three categories of soil clean-up techniques.

The DITS reactor

Alomst ten years ago the department of Biochemical Engineering at the Delft University of Technology started a soil treatment project. The aim of the project was to develop the technology for the biological clean up of polluted soils in a slurry reactor. The idea behind this was, that, apart from the long treatment times, biological treatment in a landfarm seemed to be a promising strategy for remediation of (oil) polluted soils. In a reactor the reaction conditions can be

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controlled so that optimum conditions for the microbial degradation can be ensured. This will result in a higher degradation rate and consequently in decreased treatment times. Furthermore, treatment of soil in a slurry reactor breaks up the soil clods ensuring good mixing and intensive contact between the soil, the nutrients and oxygen. In Fig. 1 the concentrations for the microbial degradation of α-HCH in end-over-end mixed bottles are shown (+-markers). After a certain
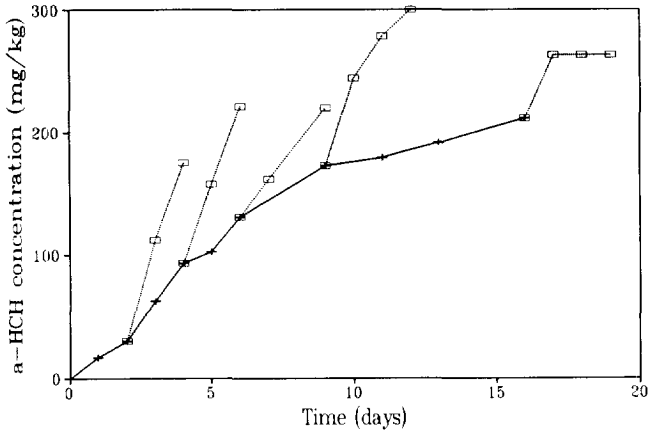

Figure 1. Effect of mixing intensity on the desorption rate. The +-markers represent end-over-end mixing, the open squares represent stirred mixing. (From Rijnaarts et al. [20])

time of end-over-end mixing, some of the bottles were stirred mixed, and the resulting α-HCH concentrations are shown in Fig. 1 as well (open squares). It is clear that more intense mixing results in a higher degradation rate.

Additional advantages of a slurry are that transport and handling of the soil are much facilitated. Also, removal of heavy metals in a later stage seems possible. The soil is already in a slurry, which is needed for this treatment, and can therefore be transferred directly from the last organics treatment reactor.

In 1991 a new type of reactor, the so called Dual Injected Turbulent Separation (DITS) reactor, had been designed for this slurry process (Fig. 2) [25]. The mixing behaviour in the reactor could be described and the scale up rules had been established [26]. It turned out, that the degradation process was quite complex, and was not sufficiently understood. The main problem was that after a residence time of several days, the soil was still not clean enough for the process to be successfully applied in practice. Data from other research groups [27] showed that the process gave satisfactory results for sandy soils. However, the particular aim of the project at the department of Biochemical Engineering was a slurry process for soils with high clay, silt and/or organic matter content, which are hard to clean with any other technique.
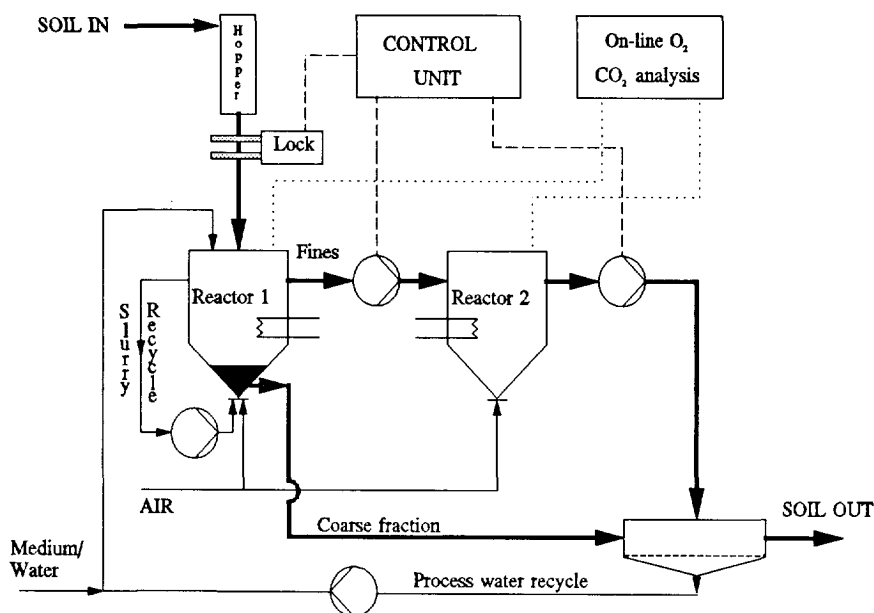
Figure 2. Soil slurry treatment plant.

Therefore the soil used up to that point also contained clay and organic matter. It was assumed, that these soil components were retarding the oil degradation rate. This implied that longer residence times would be needed to reach a lower oil concentration. However, economic feasibility of the process depends on short residence times [28], so a way had to be found to significantly increase the clean up rate. This was the starting point of the project presented in this thesis.

ORGANISATION OF THE THESIS

Microbial degradation of mineral oil in soil is extremely complex due to

1)   the variability of the microbial population with varying environmental conditions and changing composition of the substrate,

2)   the large amount of different components in the substrate,

3)   the complexity of the soil matrix, with varying composition for every type of soil.

In a degradation experiment, be it in batch or in continuous operation, a large amount of soil samples is generated. To keep up with this sample production, a fast reliable analysis method for the determination of oil in soil or sediment is needed. Additionally, it would be preferable if this

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method is as environmentally acceptable as possible. In Chapter 2 such a new analysis method is introduced and validated.

The biodegradation of oil components in a slurry reactor has been studied. The system is kept as simple as possible by first determining the degradation of a single oil component in water by a consortium of microorganisms. The degradation order and the kinetics of degradation of a mixture of a range of slightly differing components, as in diesel oil, can then be related to this single component (Chapter 3). Adding soil to the system can now give insight on the specific effects of the adsorption of diesel oil on the oil biodegradation rate (Chapter 4). A part of the soil probably limits the ultimate oil degradation rate. To investigate this the soil can be divided into several constituent fractions. Each fraction can be used separately as a model matrix (Chapter 5). In the previous chapters the kinetics and order of degradation of the component mixture in diesel oil have already been studied. In Chapter 5 therefore, hexadecane is used as model component for diesel oil.

When the limiting soil fractions are identified, a model can be set up to describe the microbial degradation of oil in a slurry reactor. In Chapter 6 a model, which is based on desorption retarded diffusion in a porous matrix, is proposed. It is demonstrated that it can be used successfully to predict the concentration of diesel oil in a batch reactor, during long term experiments.

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Fast screening of contaminated soil samples using thermal desorption mass spectrometry

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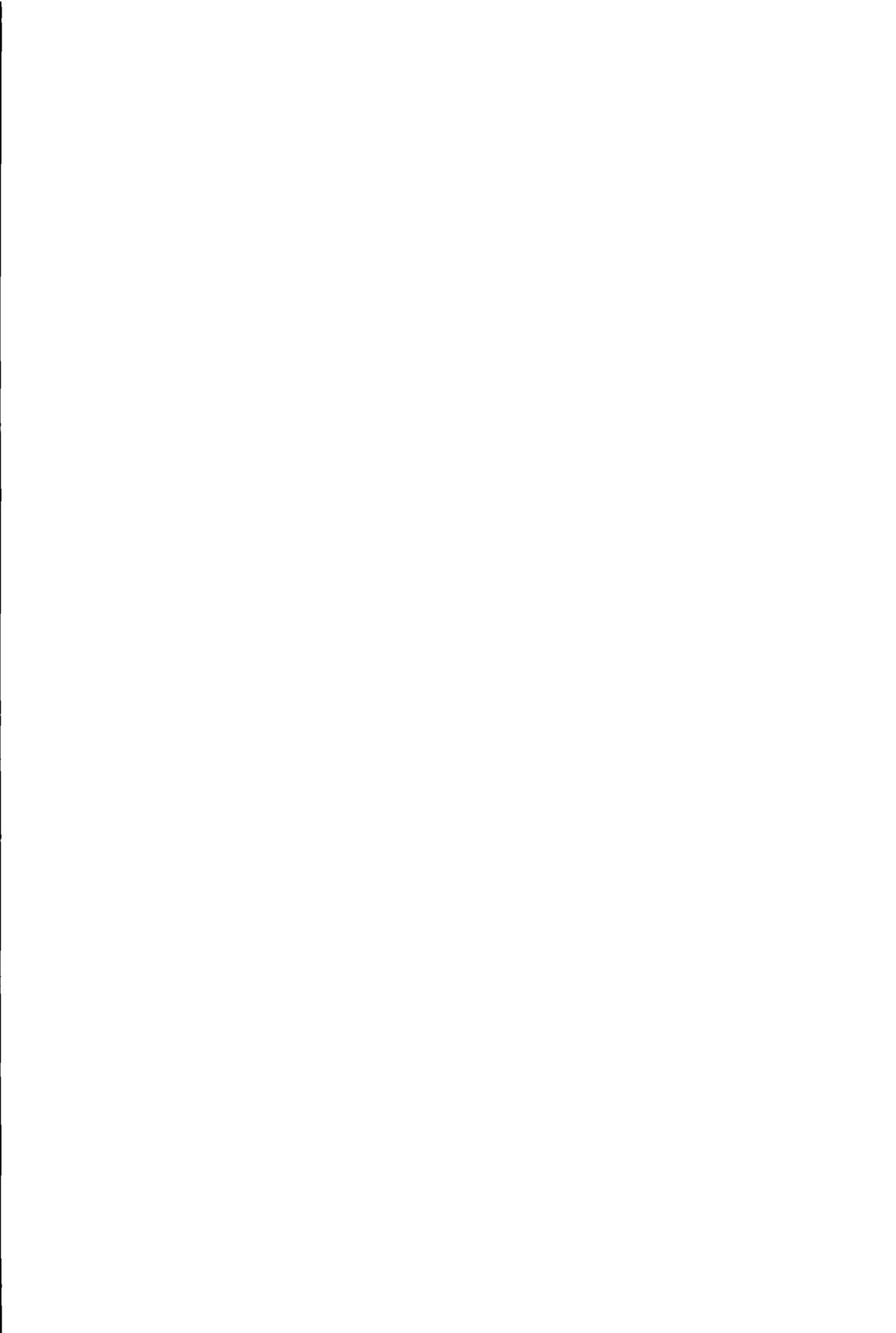
INTRODUCTION

Currently the analysis of contaminated soil requires tedious sample preparation procedures. In the case of contamination with oil, it takes about two days (transport, sample preparation, analysis) until the results are available [1]. This is impractically long and not very cost effective, in particular because many samples are required to get a reliable estimate of the extent of the contaminated site.

Therefore rapid but accurate and relatively inexpensive methods are needed to determine the degree of the contamination, preferably on-site. On-site sampling and analysis would lead to a significant reduction in analysis time and cost: the sampling strategy can be interactively adapted to the results of the analyses and complete mapping of the distribution of the contamination is realized in shorter time. Recently, several fast methods involving in-situ use of a mobile GC-MS combination were described [2, 3, 4, 5]. Although the analysis time is significantly reduced, a sample preparation step, such as solvent extraction or desorption from the soil and concentration in a cold trap or on an adsorbent is still part of the method [3, 4], or the method is only suitable for volatile components [6]. In all cases a gas chromatograph is used to separate the components of interest [2, 3, 4, 5, 6, 7].

In case of a mineral oil pollution the total amount of oil is of interest. The alkanes need not be quantified individually. Therefore a special separation step, e.g. gas chromatography, is not needed. Polycyclic aromatic hydrocarbons (PAH's), which are present in most oil fractions, must be quantified individually. These components evaporate at slightly different temperatures due to the difference in their vapor pressure. In combination with the distinct mass spectra of all PAH's, the separate elution profiles and therefore the concentration of each PAH can be determined individually.

The technique presented here was developed to minimize sample preparation time in the analysis of soil contaminated with oil. Basically, the soil sample is heated in a computer controlled infrared oven and the evaporated contaminants are introduced into the mass spectrometer. There detection of the fragmented components takes place.

MATERIALS AND METHODS

Chemicals

Toluene, methanol, Na_2SO_4 (anh.), talc and hexadec-1-ene were obtained from Merck, Darmstadt, FRG. All these chemicals were analytical grade, except hexadec-1-ene which was synthesis grade. Diesel oil was obtained from a petrol station.

When in the analyses the addition of an internal standard is mentioned, this is always 1.00 ml of a 25.3 g/l solution of hexadec-1-ene in toluene.

Soil samples

Clean soil (sandy loam), obtained from the botanical garden in Delft, was dried to less than 1% water content in an oven at 105^{0}C. The soil was contaminated with 6 g/kg n-hexadecane or 2 g/kg diesel oil. This soil was allowed to thoroughly adsorb the oil component(s) by leaving it in a closed polyethylene container for one week. After that, the samples were submerged in water for one day. In the next step the samples were dewatered by vacuum filtration in cellulose soxhlet thimbles. This procedure was performed to yield samples resembling actual (worst case) contaminated soil.

In addition, soil samples containing unknown amounts of contaminants were obtained from TAUW Infraconsults B.V., the Netherlands.

Sample preparation

The samples from TAUW were cryogenically milled before analysis: 400 g Na_2SO_4 (anh.) and 100 g talc were mixed with 500 g soil sample which was then stored over night at 4^{0}C. After freezing in liquid nitrogen for at least 20 minutes, it was grinded to 1 mm in a hammer crush mill. Then the sample was divided into three portions.

The other samples were analyzed without pretreatment.

TD-MS

To determine the amount of oil on a soil sample, approximately 5 grams of soil was introduced into the sample holder of the IR-oven (Quad Ellipse Chamber, model nr. 5528-0-5 from Radiant Energy Research Inc., Minneapolis, Minnesota, controlled by a Controller/Programmer, type 821 from Eurotherm) (Fig. 1).
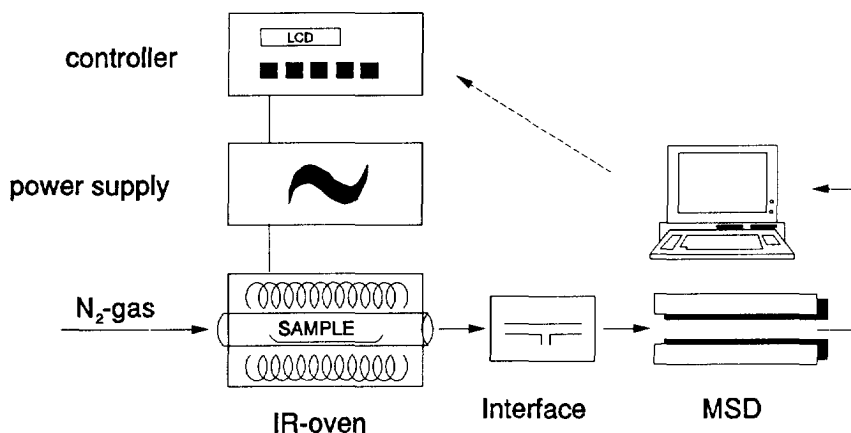

Figure 1. Set up of the infrared oven-mass selective detector combination (TD-MS).

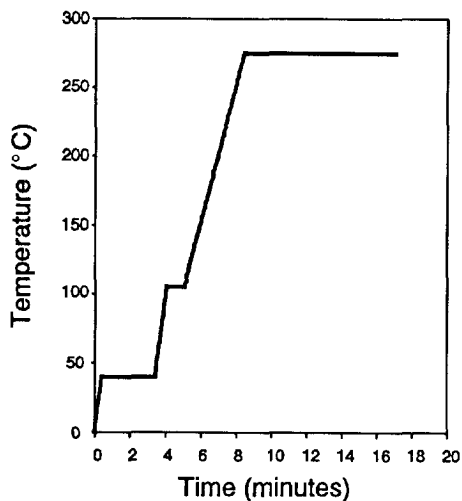Evaporation was performed by heating with a temperature program from 40°C up to 275°C, as shown in Fig. 2:

In the first step the temperature in the evaporation tube is raised to above ambient followed by a three minute stabilization period during which the gas in the tube is replaced by carrier gas. Then the temperature is raised with 100°C/min to 105°C at which temperature the sample is 'dried' during 1 minute. A temperature ramp of 50°C/min up to 275°C is used to evaporate the oil components. To prevent pyrolysis of organic components in the soil the temperature should not exceed this value.

Figure 2. Temperature program for the TD-MS.

The evaporated components are introduced into the mass spectrometer (HP 5970B, Hewlett Packard, Palo Alto, California) through an interface, by a continuous flow of nitrogen gas through the oven. Because the evaporating water causes a dilution of the carrier gas and therefore a decrease of the signal, an external standard (210 ppm xenon) is added to the carrier gas by Air Products Nederland B.V., Waddinxveen, the Netherlands. The area under the resulting Total Ion Chromatogram (TIC) is corrected for water dilution using ion 132 of the external standard, xenon, with the following formula:

$$EI_{corrected}(t) = EI_{measured}(t) \cdot \frac{^{132}Xe(end)}{^{132}Xe(t)} \qquad (1)$$

where $EI_{corrected}(t)$ and $EI_{measured}(t)$ are the actual values of corrected and measured intensities, respectively and $^{132}Xe(t)$ and $^{132}Xe(end)$ the actual and final values of the intensity of mass 132 pertaining to xenon. For $^{132}Xe(end)$ the average of the last 10 scans is taken.

It is assumed that the amount of external standard (xenon) in the carrier gas is constant during an analysis run. The total area under the resulting TIC is a measure for the amount of oil present in the sample. Calibration is performed by evaporating known amounts of hexadecane or diesel oil,

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depending on the contaminant on the soil sample, using the same temperature program as above. Integration is started after 3 minutes, when the sample holder is completely filled with carrier gas, and stopped at 17 minutes.

Soxhlet extraction/GC-FID

A cellulose extraction thimble, with dewatered (filtered) soil, is placed into the soxhlet extraction unit. Extraction is performed with a mixture of 54 ml toluene and 146 ml of methanol to which 1 ml of internal standard is added. After refluxing for 5½ hours and half an hour of cooling, the liquid is poured into a separation funnel and 100 ml of a salt solution (40 g/l NaCl) is added. After 1 minute of shaking, the organic phase is separated from the water phase, and washed with another 100 ml of the salt solution. The organic phase is then poured into a 300 ml conical flask and dried with Na_2SO_4 (anh.), for 4 hours.

After drying, the oil components in the toluene fraction are separated and detected on a GC equipped with a flame ionization detector (FID), by injecting 1 or 2 μl depending on the concentration. The GC was a Varian 3700 gas chromatograph equipped with an autosampler, series 8000, from Varian. The GC-column was a 25 m fused-silica capillary HP-1 column, i.d. 0.22 mm and film thickness 0.33 μm. The temperature program for the oven starts at 60^0C for 5 minutes, followed by a ramp of 15^0C/minute till 300^0C, which temperature is held for 12 minutes.

Calculation of the total area of the resulting chromatogram and of the area of the internal standard was done on a PDP11/03 computer (Datacare, Zeist). Calculation of the total amount of oil present in the sample was performed with a diesel oil calibration curve, obtained by performing the GC-analysis on a series of solutions of diesel oil in 50 ml of toluene, with 1 ml of internal standard added.

The extracted soil sample was dried at 105^0C for 24 hours to determine the dry weight.

Analysis according to the Dutch interim norm NEN 5733

The soil sample is milled cryogenically, as described above. Then 30 g of this sample is extracted two times with 20 ml 1,1,2-trichlorotrifluoroethane by vigorously shaking for 30 minutes, after addition of 1 ml of internal standard. The extract is analyzed by GC as described above. The entire area under the chromatogram is used for the calculation of the amount of oil. Calibration is done by injecting various concentrations of diesel oil in toluene, ranging from 0.2 to 9.8 g/l, with a constant concentration of internal standard.

RESULTS AND DISCUSSION

For TD-MS the total time between two analyses was 25 minutes. For sample preparation an additional 10 minutes is necessary, in which excess water is removed from the sample by centrifugation or filtration.

Linearity of the TD-MS for hexadecane

In Fig. 3 the response of the TD-MS analysis system is shown for 0 to 20 μl of hexadecane. A straight line can be fitted through the data from 0 to 20 μl; $r^2 = 0.995$.
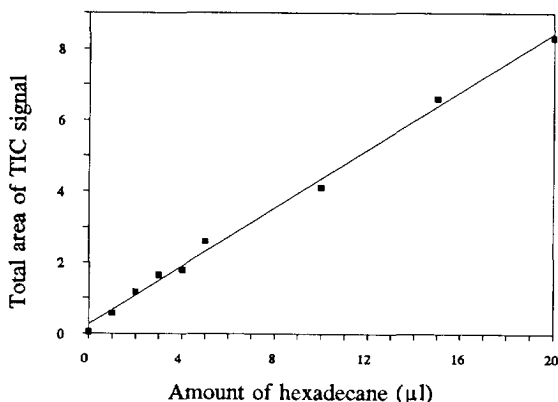

Figure 3. Linearity of the response of the TD-MS analysis system in the range of 1 to 20 µl for n-hexadecane.

Influence of water evaporation for clean soil samples

In Fig. 4 the influence of water on the evaporation profile is shown. Clean soil was analyzed with TD-MS, first dry (Fig. 4a) then wet (Fig. 4b). Due to the evaporation of water the concentrations of the carrier gas and the evaporated components were diluted. This causes the dip in the TIC and in the ^{132}Xe-profile between 5 and 8 minutes. In Fig. 4a it is shown that for a (clean) dry soil sample the correction according to equation 1 shows an elution profile that is nearly identical to the raw TIC. In Fig. 4b a small but significant increase in the signal is seen after correction. This is due to the non-zero average noise level. The photomultiplier (the detector in the MSD) always gives a certain amount of signal, even when no ions are detected. So every ion in each scan has a certain intensity due to this noise. Summation of all ions in a scan yields the TIC-signal, which has a certain off-set due to the noise. When a wet, clean soil sample is analyzed, only the carrier gas (containing
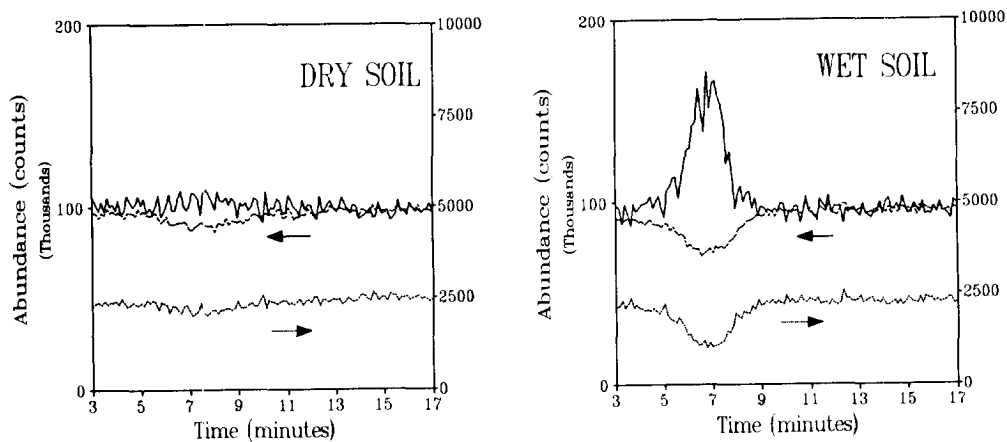
Figure 4. Total Ion Chromatogram Desorption Profile (TIC, dashed curve), ion chromatogram of mass 132 (xenon, dotted curve) and corrected TIC (solid curve) for a dry and a wet clean soil sample.

the xenon) is susceptible to dilution by water evaporation. Just the molecule fragments of xenon will need correction for dilution. All other ions are absent after the ionization of the gas stream. Only the noise level for these ions will be counted by the photomultiplier. When equation 1 is used to correct for this dilution, in effect the noise for the non-xenon ions is increased. This leads to an overcorrection and shows as an increase in the TIC-signal, when the ^{132}Xe-signal is decreased by water evaporation.

Detection limit for TD-MS

When a sample is analyzed the overcorrection limits the lowest detectable concentration. Assuming that an accuracy of $\Gamma=3$ is sufficient, with a standard deviation for the blanco's of $\sigma=0.26$ g/kg and a sensitivity of $S=0.98$ (determined from the calibration samples), the lowest concentration that can be determined accurately with TD-MS is:

$$c_B = \frac{\Gamma \cdot \sigma}{S} = \frac{3 \times 0.26}{0.98} = 0.81 \text{ g/kg} \tag{2}$$

Effect of correction for soil samples with hexadecane and diesel oil

In Fig. 5 two total ion chromatograms (TIC's) are shown, one for a calibration sample (i.e. without soil, Fig. 5a) and one for a soil sample, contaminated with hexadecane, containing water (Fig. 5b).
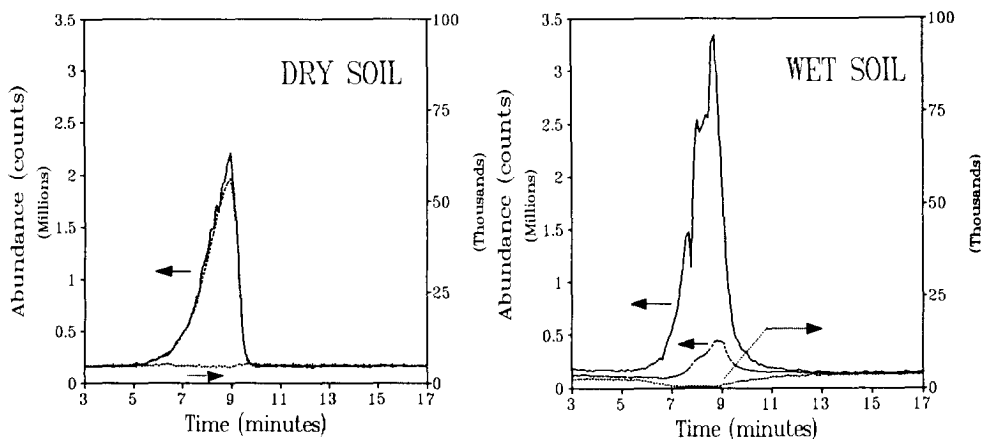
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Figure 5. Total Ion Chromatogram Desorption Profile (TIC, dashed curve), and ion chromatogram of mass 132 (xenon, dotted line) for n-hexadecane on a dry and on a wet soil sample. The solid curve represents the corrected TIC.

For pure hexadecane the evaporation profile is as expected. After correction no significant change is observed. During the evaporation of water part of the hexadecane evaporates (Fig. 5b). A correction must be made to take this into account. Furthermore, the evaporation profile for the soil sample is different from that of the pure component (Fig. 4), due to desorption from or diffusion out of the solid matrix.

In Fig. 6 the elution profiles are shown for diesel contaminated soil, dry and wet. The difference in intensity and total area between Fig. 6a and 6b is due to different amounts of analyzed soil sample.
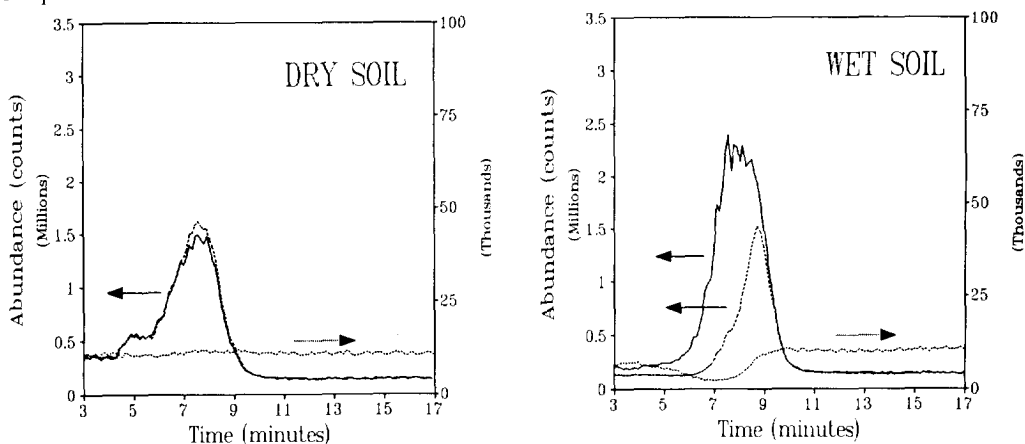

Figure 6. TIC and corrected TIC for a soil sample contaminated with diesel oil, dry (left) and wet (right).

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From Fig. 4a, 5a and 6a it can be seen that correction has no significant effect on dry samples. In Fig. 4b the increase of the intensity after correction for a clean wet soil sample is also small. The corrected elution profiles in Fig. 5b and 6b show a marked increase in the observed concentration, compared to the uncorrected elution profiles. This clearly shows the necessity of correction of the raw TIC's.

Comparison between TD-MS, soxhlet and freon extraction

Fig. 7 shows a comparison of the results between TD-MS and soxhlet extraction for identical soil samples, artificially contaminated with diesel oil. A small but consistent deviation from the ideal line is observed.
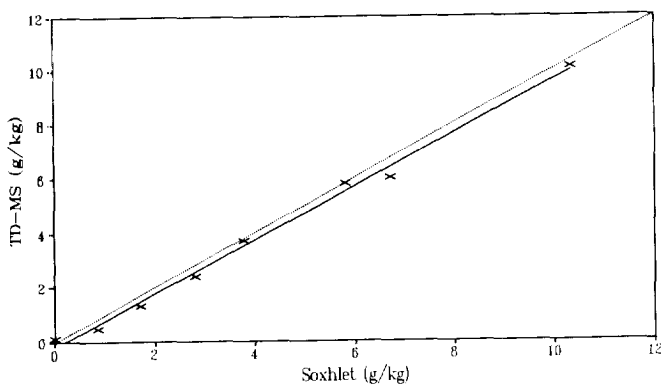

Figure 7. Comparison of the results from TD-MS with those from soxhlet extraction. The drawn line represents the best fit according to linear regression. The dotted line denotes where both methods would give the same results.

Two soil samples, obtained from TAUW Infraconsults, were analyzed with soxhlet extraction/GC-FID, TD-MS and extraction/GC-FID according to the Dutch interim norm NEN 5733. In Table 1 the results of the analyses are shown. As is directly obvious the TD-MS exhibits the lowest relative variance for multiple analyses of the same sample.

In order to determine whether the results are significantly different, and because the true concentration is not known, the so called "distributionfree test for two means", as designed by Wilcoxon, must be applied. The results are given in Table 2.

According to this test two values, S1 and S2, are calculated comprising the agreement of the values of one set to the other. S0 is a value that is solely dependent on the multiplicity of the independent analyses in each set. If for two compared sets S1 is smaller and S2 larger than S0, no significant difference between the sets can be found. It appears that the extraction according to the NEN 5733 is significantly different from both other methods, whereas the results from the soxhlet

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sample nr.	TD-MS	soxhlet	NEN 5733
		0.05	0.22
	0.125	0.13	0.24
I	0.126	0.16	0.25
	0.128	0.21	0.27
	0.141	0.21	0.27
		0.27	0.29
avg±sd	0.130±0.007	0.17±0.08	0.26±0.03
	1.90	1.43	1.30
	1.98	1.54	1.36
II	2.03	1.59	1.38
	2.09	2.29	1.42
	2.14	2.29	1.45
		2.54	1.47
avg±sd	2.03±0.09	1.9±0.5	1.40±0.06

Table 1. Results from three analysis methods executed on two soil samples with unknown oil concentrations. Values are expressed in (g oil/kg soil DW).

sample nr.		TD-MS v. NEN 5733	TD-MS v. soxhlet	soxhlet v. NEN 5733
	S1	11	11	28
I	S0	10	15	25
	S2	33	33	50
	S1	20	20	28
II	S0	45	22	55
	S2	40	40	50

Table 2. Results of the distribution free test for two means, applied on the analysis results in Table 1.
$H_0: \mu_0=\mu_1,\ H_1: \mu_0 \neq \mu_1,\ \alpha=0.05.$

extraction and the TD-MS do not differ significantly. The standard analysis technique yields a significantly higher value for the low concentration than the other two techniques, and a significantly lower value for the high concentration. There could be several explanations for this: 1) Sample II contains a much higher amount of organic matter. It could be that part of this organic matter is detected as oil in the TD-MS and in the soxhlet but not in the standard technique. This would result in an oil concentration that is too high for the first two techniques. 2) Sample I contains less water (6 wt%) than sample II (21 wt%). In the TD-MS water is evaporated before the oil is analyzed. In the soxhlet the oil and the water are extracted from the soil. The water layer is separated afterwards. In the standard analysis water in the sample is bound by an anhydrous salt. It could be that part of the oil is trapped in the salt and cannot be extracted. This would result in an oil concentration, as determined by the standard technique, that is too low. Explanation 1 seems unlikely, because of the entirely different procedure to separate the oil from the soil in the two analysis procedures, yet resulting in the same concentration. Explanation 2 could be correct if it is furthermore assumed that the standard analysis always yields a concentration that is somewhat too high.

CONCLUSIONS

The Thermal Desorption Mass Spectrometry combination (TD-MS) proves to be a very rapid analysis method for mineral oil in soil. When compared to a soxhlet extraction and to a standard analysis, the latter yields significantly different results from the other two methods, while TD-MS and soxhlet do not yield significantly different results from each other. Of the three analysis methods TD-MS yields the lowest variance for multiple analyses.

Further research will be done to determine the applicability of TD-MS for the determination of oil on silt, aquifer material, and soil with high organic matter content. Also the determination of PAH's on soil seems very promising.

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Biodegradability of diesel oil

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INTRODUCTION

Oil contamination of soil and water is a widespread problem nowadays. Microbiological clean-up of this type of contamination can be advantageous, when compared to other remediation techniques. Before deciding to use microbes, it is necessary to determine 1) whether the contaminants can be mineralized (biodegradability), 2) how fast the mineralization can proceed (kinetics), and 3) how the previous two points depend on the type of operation: batch or continuous mode. In this paper we will discuss these three points with regard to dispersions of oil in water.

Oil is a complex mixture of hydrophobic components, such as aliphatic and mono- and polycyclic aromatic hydrocarbons. In general, the degradation rate of a linear alkane is much higher than of e.g. a polycyclic aromatic hydrocarbon [1]. Microbial degradation experiments are often performed with pure cultures growing on one substrate. Especially n-hexadecane has often been used [e.g. 2]. In recent years several papers have been published concerning the degradation of crude oil [3, 4, 5] or a refined fraction thereof. However, in none of these the oil was completely degraded. In this paper the microbial degradability of diesel oil, which is a distillate fraction of crude, is studied. Diesel fuel is one of the major pollutants of soil and ground water near petrol stations.

Diesel oil consists mostly of linear and branched alkanes. We want to compare bacterial growth on this complex substrate with growth on a single substrate to determine the degradation order of the components in the mixture, degradability of the mixture as a whole, and the difference in kinetic parameters between both kinds of substrate. n-Hexadecane was used as a reference for the degradation behaviour of diesel oil. Hexadecane was chosen because of its widespread use in experimental studies [e.g. 2]. Much is already known of its degradation kinetics, and it can be degraded fast in water dispersion. Under these conditions a maximum growth rate has been measured (in batch experiments, at 30^0C) of 0.2 h^{-1} [2], and a corresponding yield Y_{sx} of about 0.5 Cmol biomass/Cmol hexadecane. Rohns [6] has experimented with pentadecane in batch and found a μ_{max} of 0.6 h^{-1} and a corresponding yield of $Y_{sx}=0.2$ Cmol biomass/Cmol pentadecane, at 30^0C. He also experimented with continuous cultures and found the maximum growth rate to be lower than in batch: 0.3 h^{-1}. Several other research groups, used n-hexadecane and a pure culture of a yeast [7, 8]. They also found a significantly lower maximum growth rate in continuous culture, compared to batch.

In this paper the degradation of oil components is studied in batch and continuous cultures. From these experiments conclusions will be drawn concerning 1) the biodegradability of diesel

oil when compared to hexadecane, i.e. are some oil components more easily degraded than others and 2) the best mode of operation when extrapolated to waste water treatment.

MATERIALS AND METHODS

Reactor operation

All degradation experiments were performed in two litres glass reactors. These were equipped with an air sparger at the bottom of the vessel. The amount of air fed to the reactor was controlled by a mass flow controller at 1 l/min. The medium used was composed of: 550 mg/l KH_2PO_4, 275 mg/l $MgSO_4.7H_2O$, 2.75 mg/l $FeSO_4.7H_2O$, 5.5 mg/l $ZnSO_4.7H_2O$, 1.1 mg/l $CaCl_2.2H_2O$, 2.75 mg/l $MnCl_2.4H_2O$, 1.1 mg/l $CuSO_4.5H_2O$, 1.1 mg/l $CoSO_4.7H_2O$, 27.5 mg/l NaCl, 27.5 mg/l KCl, 2200 mg/l NH_4Cl. The reactor contents were stirred with an impeller at 900 rpm. Temperature was kept at 30^0C with a thermostat that circulated water either through the mantle of the reactor, or through a metal heat exchanger inside the reactor. pH was kept between 6.5 and 7.5 with a pH-electrode, coupled to a pH-controller. This controller regulated two peristaltic pumps, one for addition of 1 N sulphuric acid, the other for addition of 2 N NaOH solution.

Batch experiments started with 1.5 l of medium. 5 g of hexadecane (analytical grade) or diesel oil (from a Texaco petrol station) were used as sole carbon and energy source for the microorganisms. The reactor was inoculated with 10 ml (about 10 mg biomass) of an undefined mixed culture of oil degrading micro-organisms.

In continuous mode a CSTR (continuous stirred tank reactor) was used, with hydraulic residence times between 4 and 24 hours. The residence times were set by changing the influent flow of the medium. The oil was added separately as a semi-continuous flow. Several concentration ratios of oil and medium in the influent were used (Table 2). In the continuous experiments the reactor contents of the previous steady-state were used to start the next dilution rate. When needed, an extra inoculum, as described above, was added. To minimize the influence of further adaptation or change in population the experiments were performed in random order.

Analyses

The biomass concentration in the reactor was determined by taking 10 ml of the stirred suspension and determining the mass, after drying for 24 hours at 105^0C. Water and oil were removed during this drying period.

The oil concentration in the water phase was determined by first increasing the pH of the suspension to 13 with NaOH-pellets, to prevent interference from oil metabolites such as fatty

acids during the analysis. 500 ml of the suspension was extracted three times with 50 ml distilled diethylether. 32.050 mg of hexadec-1-ene was added as internal standard (IS). To determine whether any metabolites of oil degradation accumulated in the medium, the extracted water was acidified to pH<1 by addition of concentrated hydrochloric acid [5]. The acidified water was extracted with ether in the same manner as described for the alkaline water. The fatty acids in the "acid" extract were methylated by passing diazomethane through the extract for 5 minutes. Analysis of all extracts was performed on a gas chromatograph (GC), fitted with a 25 m HP-1 column and a flame ionization detector. The temperature profile in the GC was: Start temperature 60^0C, which was kept for 5 minutes, then a temperature ramp of 5^0C/min till 300^0C. This final temperature was kept for 10 minutes. If the oil concentration was found to be low, the extract was concentrated to 1 ml by evaporation of ether at 45^0C. The concentrated extract was subjected to the same analysis procedure as before. The total area of the chromatogram between 5 and 63 minutes and the area of the peak of the IS were used to determine the amount of mineral oil in the extract. This is equal to the amount of oil in the 500 ml of water before extraction. A calibration curve was used to allow for different response factors for oil and IS.

The oil components that were stripped into the gas phase were quantified by adsorption. During the first 48 hours in a batch experiment, or for 48 hours during steady-state in a continuous experiment, 1 l/h of the off-gas was passed through a glass tube, containing an adsorption material (2 g of Tenax TA). The adsorption material was extracted three times with 10 ml distilled diethylether. The extract was analyzed according to the same protocol as used for the water phase extracts.

Nitrate and nitrite concentrations were monitored with Merckoquant 10 020 test strips from Merck, to check whether a significant amount of oxygen was used for nitrification.

Oxygen transfer to the reactor was measured by a paramagnetic O_2-analyzer. Carbon dioxide production was measured with an infrared analyzer. Some of the oil components were stripped into the gas phase and interfered with the carbon dioxide measurements. The carbon dioxide measurements could therefore not be used quantitatively.

RESULTS

In the following calculations it is assumed that both the hexadecane and the diesel oil had a molecular mass, normalized to the amount of carbon atoms, of M=14 g/Cmol. This corresponds to a composition of CH_2. The biomass was assumed to have a normalized molecular mass of M=24.6 g/Cmol, corresponding to an average composition formula of $CH_{1.8}O_{0.5}N_{0.2}$ [9].

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Batch experiments

In Fig. 1 the oxygen uptake rate (OUR) is shown for the degradation of hexadecane and of diesel oil in a batch reactor. It is apparent in both cases that, after a lag phase, the uptake rate
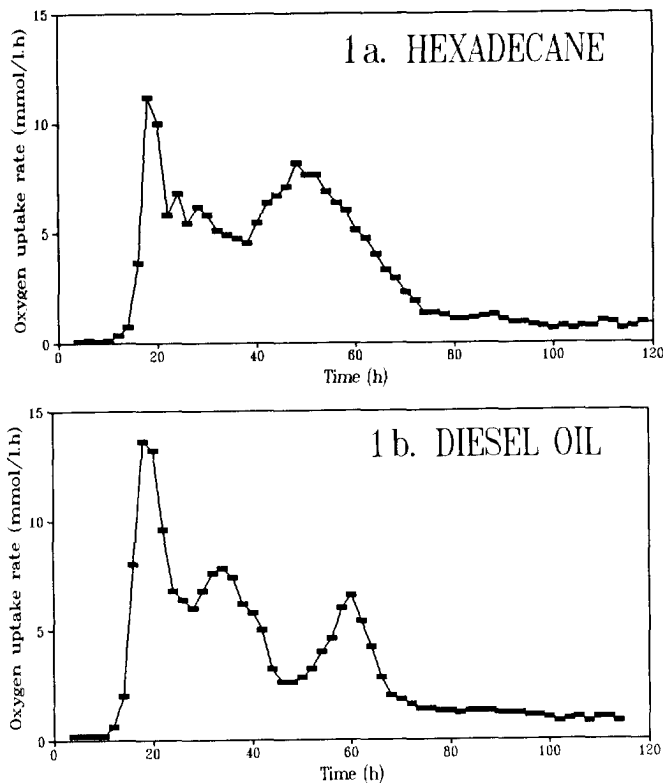

Figure 1. Oxygen uptake rate for the degradation of hexadecane (1a) and diesel oil (1b) in a batch reactor.

shows an exponential increase in the first 18 hours. This corresponds to an exponential growth of the microorganisms. After this period a decrease of the OUR occurs. All the maxima occurred reproducibly in the experiments.

After approximately 40 hours the hexadecane concentration in the water phase was below the detection limit ($<10^{-7}$ Cmol/l). The diesel oil concentration after 22 h was 0.20 Cmol/l (this corresponds to 15% conversion), after 40 h it was 0.013 Cmol/l (95% conversion). Analysis of the water phase after 120 hours showed no detectable amount of diesel oil. The detection limit for diesel oil is approximately 10^{-6} Cmol/l.

Assuming Monod kinetics for the growth in the exponential phase, and assuming furthermore that $C_s >> K_s$ (according to Goldsmith and Balderson [10]: $K_s \approx 0.006$ Cmol/l), integration of the growth equation for a batch reactor yields:

$$\ln(C_{X(t)}) = \ln(C_{X(0)}) + \mu_{max} t \tag{1}$$

If a constant stoichiometry for the degradation in the exponential growth phase can be assumed, then the specific oxygen consumption rate is constant. The oxygen uptake rate is then proportional to the biomass concentration and equation 1 can be changed into:

$$\ln(r_{O(t)}) = \ln(r_{O(0)}) + \mu_{max} t \tag{2}$$

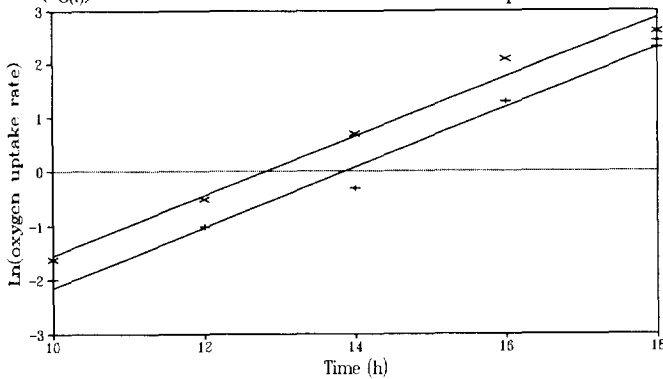Figure 2 shows $\ln(r_{O(t)})$ as a function of time in the batch experiments. From the slope a

Figure 2. Determination of the maximum growth rate from the batch experiments. The +-markers represent the hexadecane, the x-markers the diesel oil.

maximum growth rate of $\mu_{max} = 0.55$ h^{-1} for diesel oil, and of $\mu_{max} = 0.56$ h^{-1} for hexadecane was found.

The yields and oxygen/substrate consumption ratios for the batch experiments are summarized in Table 1.

Time (h)	Hexadecane		Diesel oil	
	Y_{SX} (Cmol/Cmol)	Y_{SO} (mol/Cmol)	Y_{SX} (Cmol/Cmol)	Y_{SO} (mol/Cmol)
40	0.33	0.66	0.10	1.1 ± 0.2
80 (C16) 120 (diesel)	0.4	1.4	0.1	1.6 ± 0.2

Table 1. Yields from the batch experiments.

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With the yields and ratios at the end of the experiments, the stoichiometry of both degradation experiments can be described, within the error margins, by the macroscopic equation:

$$Y_{SN}\ NH_3 + CH_2 + Y_{SO}\ O_2 \rightarrow Y_{SX}\ CH_{1.8}O_{0.5}N_{0.2} + Y_{SC}\ CO_2 + Y_{SW}\ H_2O \qquad (3)$$
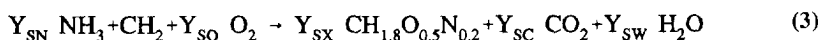
where CH_2 represents hexadecane or oil and $CH_{1.8}O_{0.5}N_{0.2}$ the biomass.

In Fig. 3 extracts of the water phase at the start of the batch experiment (Fig. 3a) and after the first maximum in the OUR (Fig. 3b) are shown, for diesel. In Figure 3a the linear alkanes
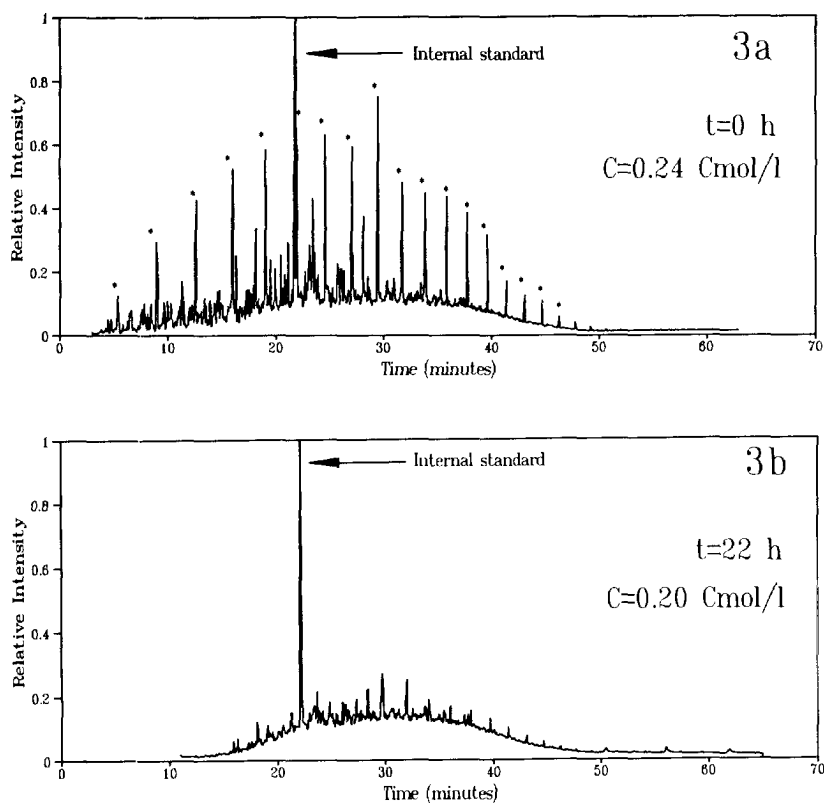

Figure 3. Extract of the water phase at the start of the batch experiment with diesel oil (Fig. 3a), and after 22 h (Fig. 3b).

from undecane (C11) up to octacosane (C28) are indicated with *. The rest of the peaks, as well as the bump, comprise mainly of the branched alkanes. The bump consists of unresolved components. A slower increase of the oven temperature of the GC would resolve more components.

A comparison shows that in the first 22 hours, 90% of the linear and 2% of the branched alkanes are consumed. The chromatogram of the extract of the water phase after 40 h showed no significant peaks, apart from the IS. After concentration a bumpy baseline and several small peaks were observed, some of which could be attributed to the ether or to phtalates from the ABR seal of the vials. After 120 h the diesel concentration was below the detection limit, confirming that diesel oil can be completely mineralized by microorganisms, in batch.

Continuous experiments

Several continuous experiments were performed with dilution rates between 0.04 and 0.25 h^{-1}, corresponding to liquid residence times between 24 and 4 hours. In Table 2 the diesel oil and the biomass concentrations in the continuous experiments are shown.

Dilution rate (h^{-1})	Influent oil concentration (Cmmol/l)	Effluent biomass concentration (Cmmol/l)	Effluent oil concentration (Cmmol/l)	Conversion (%)
0.04	396	35	70	82
0.08	323	44	140	57
0.10	285	37	110	61
0.10	265	44	100	62
0.14	98	12	35	64
0.14	281	36	110	61
0.18	282	47	85	70
0.21	90	6.5	19	79
0.25	90	4.3	25	72

Table 2. Concentrations in the continuous culture experiments.

For a dilution rate of D=0.10 h^{-1} two identical experiments were performed. The effluent biomass and oil concentrations were the same, as expected. For a dilution rate of D=0.14 h^{-1} two experiments with different substrate influent concentrations were performed. According to theory this should have no effect on the effluent oil concentration, or on the specific substrate consumption rate. The specific substrate consumption rates were the same, as expected. However, the measured effluent concentrations in both experiments were different.

The linear Herbert-Pirt relation for substrate conversion was used to evaluate the results of the continuous experiments:

$$q_S = \frac{\mu}{Y_{SX, \, max}} + m_S \tag{4}$$

From the results for low dilution rates (D<0.2 h^{-1}), using equation 4, a $Y_{SX. \, max}$ of 0.3 Cmol biomass/Cmol diesel oil and a maintenance coefficient of m_S=0.2 Cmol oil/Cmol biomass/h was obtained (Fig. 4). In Fig.4 the specific oxygen uptake rate is shown as well.
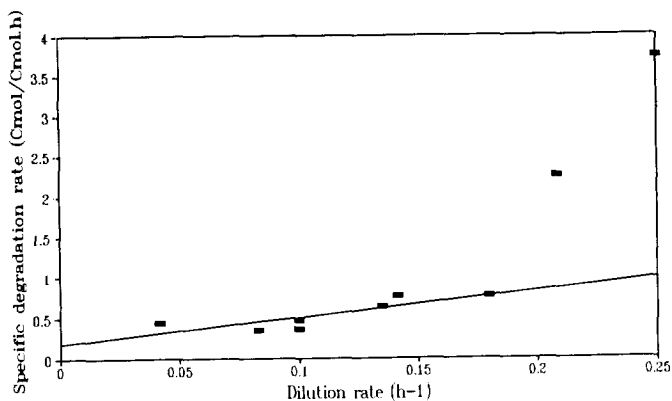

Figure 4. Plot of the specific substrate uptake rate as a function of the dilution rate in continuous cultures of diesel oil. The drawn line represents the Herbert-Pirt relation.

In Fig. 5 a chromatogram of a water phase extract of the experiment with a dilution rate of D=0.18 h^{-1} is shown. When compared to the influent oil (chromatogram similar to Fig. 3a), it is
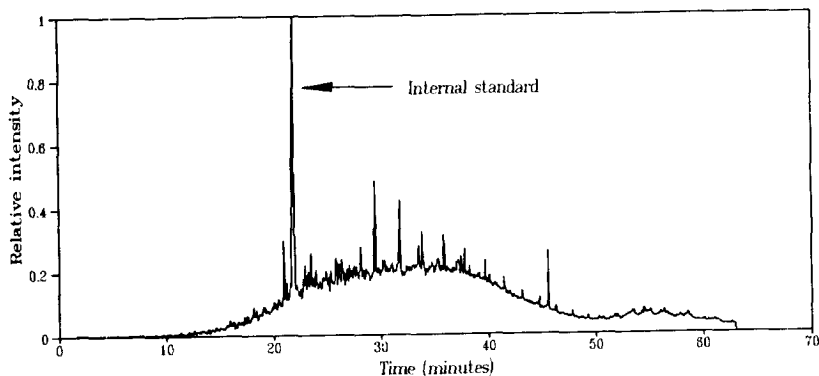

Figure 5. Chromatogram of the extract of the effluent of a continuous experiment (D=0.18 h^{-1})

seen that the linear alkanes are consumed preferentially. The branched alkanes comprising the

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UCM (unresolved complex mixture) [11, 12] are left. Similar chromatograms were found for the other dilution rates.

Strip effect

In order to check that the decrease of the hexadecane or diesel oil concentration in the reactor was due to microbial degradation and not to stripping, an adsorption column was placed in the off gas from the reactor. Fig. 6 shows the chromatogram of the extract of the off-gas adsorption column for a typical continuous experiment, with a dilution rate of $D=0.18$ h^{-1}. It is
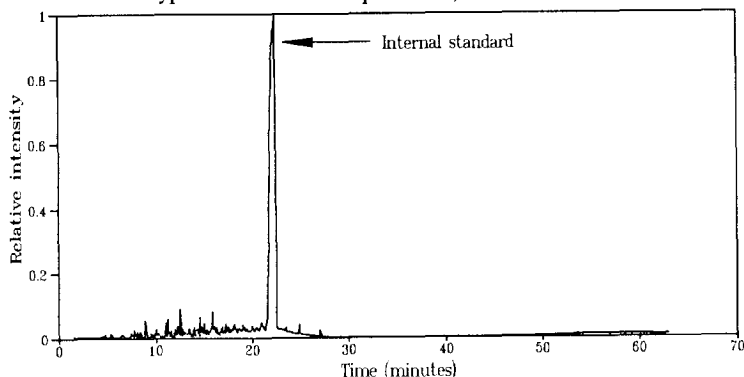

Figure 6. Chromatogram of the extract of the Tenax in the off-gas of a continuous experiment $(D=0.18 \, h^{-1})$.

clear that mainly the lighter hydrocarbons are present. This is as expected because of the higher vapour pressure of these components. The total amount of oil, that is stripped during the experiment, is about 0.01 Cmol/day, or less than 2% of the influent.

In the batch experiments with diesel oil the extracts showed similar chromatograms; mainly lower boiling components were stripped and the total amount of oil components that were stripped was very low. In the batch experiments with hexadecane the total amount of hexadecane that was stripped was negligible, when compared to the initial amount in the reactor.

DISCUSSION

Maxima in the oxygen uptake rates in the batch experiments

Diesel oil exhibits a somewhat different degradation behaviour compared to hexadecane (Fig. 1). However, both show more than one maximum in the OUR. The first maximum represents the oxidation of linear alkanes. In both the hexadecane and the diesel experiments

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linear alkanes were present. After about 22 h, the n-alkanes are mostly degraded, as is clear from Fig. 3a and 3b: Mainly the "grass", comprising largely of linear alkanes, has disappeared. At the start of the diesel experiment, about 14% of the oil mixture consisted of linear alkanes. After 22 h this was reduced to less than 1.5%. The unresolved complex mixture (UCM), the bump in the chromatogram, remained. This UCM consists mainly of branched alkanes [11, 12]. The branched alkanes are oxidized during the second maximum in the OUR of the diesel experiment. Due to the absence of branched alkanes in the hexadecane experiment, no maximum occurs there. After 40 h the extract of the water phase of the diesel experiment showed that almost all oil has disappeared. This is also the case for the hexadecane experiment. Therefore the last maximum in the OUR in both experiments can not be explained by oxidation of oil components.

Metabolites of alkanes, such as alkanoates, could have accumulated in the water phase during the experiments. Therefore, for analysis of the oil concentration in the water phase the pH was raised to above 13. Metabolites like fatty acids will remain in the water phase. After this alkaline extraction, the pH was lowered to below pH 1 [5], and extraction was repeated. In the extracts of the acidified water phase (taken after 40 hours degradation time) no components could be detected. So no metabolites had accumulated during the degradation. Therefore the last maximum cannot be explained by oxidation of metabolites in the water phase.

No significant decrease in the biomass concentration was found between 40 and 120 h. Therefore the maximum in the OUR during this time is not caused by oxidation of biomass. It remains possible that this maximum is the product of oxidation of a storage component [1], formed from excess oil during the first 40 h. This hypothesis is supported by the increasing ratio Y_{so} in the batch experiments, between 40 and 120 h, without a significant decrease in the yield Y_{sx}: Part of the biomass measured after 40 h was storage polymer, which is partly turned over into biomass and partly mineralized.

Another explanation for the last maximum in the OUR for both hexadecane and diesel, is the degradation of emulsifier, formed during the first 40 h to enhance the availability of the oil components. With the extraction procedure followed here, an emulsifier would not have been detected.

Kinetic constants from the continuous experiments

The specific substrate consumption rate for the series of continuous experiments is plotted against the dilution rate (Fig. 4), according to equation 4. From the linear part of the graph the yield, Y_{sx}, and the maintenance coefficient, m_s, are calculated to be: $Y_{sx} = 0.3 \pm 0.05$ Cmol biomass/Cmol oil and $m_s = 0.2 \pm 0.1$ Cmol oil/Cmol biomass/h. This yield compares to the values

found in other studies in continuous culture, $Y_{sx}=0.5$ Cmol/Cmol [2] and $Y_{sx}=0.2$ Cmol/Cmol [6]. The maintenance coefficient is somewhat higher than expected: Up till now, maintenance coefficients between 0.015 and 0.14 Cmol/Cmol/h were found [13] for growth on *n*-alkanes.

Discrepancy between the growth rates from different operations

In the batch experiment the maximum growth rate was calculated to be 0.55 h^{-1} for diesel oil. So it could be expected that the critical dilution rate in a continuous experiment is about 0.55 h^{-1}. In other research [6, 8] however, using pure cultures of microorganisms and one component as substrate, it was found that in continuous culture the maximum growth rate would be lower than calculated from batch experiments. In this study, using diesel oil as substrate and a consortium of microorganisms, this was found too: Table 2 shows that beyond $D=0.18$ h^{-1}, with increasing dilution rate, the biomass concentration is markedly decreasing. This results in a decrease of the yield Y_{sx} with increasing dilution rates, for these high rates. This in turn results in an increase in the specific substrate degradation rate (in agreement with literature [6]), as is shown in Fig. 4.

Rohns [6] explained both phenomena by postulating that for the microorganisms to be able to use the oil components and due to the extreme low solubility of the oil components in water an amount of emulsifier, exceeding the critical micelle concentration (CMC), must be present. The emulsifier will effect a higher concentration of available oil. The emulsifier is produced from the substrate. This means that the amount of substrate that can be used for assimilation is less than without emulsifier production. Therefore the yield (Y_{sx}) will be lower and the specific substrate consumption rate, q_s, will be higher than would be found when no emulsifier is needed. Production of emulsifier by microorganisms is thought to enhance the dispersion of growth substrate into oil-in-water emulsions to increase the interfacial area and thereby enhance the availability of the substrate. According to Hommel [14] the production of emulsifier occurs during the late exponential and during the stationary growth phase of the microorganisms. This would mean that in the determination of the growth rate in the batch experiments this production hasn't started yet. In a CSTR the available substrate concentration is always low (equal to the effluent concentration). Therefore emulsifier production will occur at every dilution rate. Consequently the maximum growth rate determined in the continuous experiments will be lower than for the batch experiments.

In our experiments no analysis for the detection of emulsifier was performed. So the hypothesis of Rohns can not be checked here. However, for many microorganisms it is known that they produce surface active components that enhance the solubilization of nonpolar components [15, 16].

Degradation order of the oil components

Comparison of Fig. 3a and 3b clearly shows that during the first 22 h in batch the linear alkanes in the diesel oil are degraded preferentially: about 90% of the linear and approximately 2% of the other components have disappeared. This is in agreement with Pirnik et al. [5], who found that pristane is not degraded in the presence of hexadecane. Only after almost all the hexadecane had been degraded was pristane degradation observed. According to Pirnik et al. this could be effected by diauxie. Additionally, the rate of degradation of pristane was approximately half as fast as that of hexadecane.

In continuous culture, the chromatogram of the extract of the water phase at $D=0.18$ h^{-1} (Fig. 5) also shows a preferential decrease of the "grass", comprising the n-alkanes, when compared to the chromatogram of the pure oil (cf. Fig. 3a). This is comparable to the first 22 hours in batch. This indicates that the lag phase for degradation of branched alkanes in the presence of linear alkanes is not caused by diauxie, but more probably by a higher affinity of the linear alkanes for one or more of the enzyme systems involved.

A comparison of yields from literature shows that for linear alkanes the yield is higher than for branched alkanes: $Y_{sx}=0.2$-0.5 Cmol/Cmol for linear alkanes [2, 6] and $Y_{sx}=0.05$ for branched alkanes (i.c. pristane) [6]. Comparing these values with the yields for both batch experiments, the yield on hexadecane compares with the yields on linear alkanes: $Y_{sx}=0.3$ Cmol/Cmol, after 40 h. The yield on diesel oil is lower: $Y_{sx}=0.1$ Cmol/Cmol, after 40 h. This could be explained by a combined degradation of the linear alkanes, with a yield of $Y_{sx}=0.3$ Cmol/Cmol, and of the branched alkanes with a yield of $Y_{sx}=0.05$ Cmol/Cmol. The sum of the areas of the linear alkanes is calculated to be between 11 and 18% of the total area of the diesel oil. Assuming 14% of the diesel consists of linear alkanes, an overall yield in the degradation of diesel oil of $Y_{sx}=0.1$ Cmol/Cmol can be calculated. This indicates that the hypothesis might be correct. In the first 22 h of the diesel degradation experiment in batch, 90% of the linear alkanes and 2% of the branched alkanes are degraded. This corresponds to about 14.5% of the initial amount of oil, furthermore confirming the hypothesis.

Operation of a reactor for the degradation of oil sludge

In batch the diesel oil is degraded completely, within 120 h. Therefore it is likely that, in continuous culture, the branched alkanes comprising the unresolved complex mixture can be degraded in a later stadium: The effluent of the continuous stirred tank reactor (CSTR) will be

degraded further in a following reactor. Because the amount of oil in the effluent of the CSTR is still high (Table 2), a possible way to lower the effluent concentration of oil in continuous mode is to use several CSTR's in series or a plug flow reactor (PFR).

In practice this would mean for the use of bioreactors in the remediation of oil pollution that degradation must be performed in a PFR. If needed, inoculation can take place through recirculation of a small amount of effluent. In this way the amount of substrate at the start is high enough for an exponential increase in biomass.

CONCLUSIONS

In batch culture diesel oil is degraded rapidly, with a maximum growth rate (for a consortium of microorganisms) of 0.55 h^{-1}. The corresponding yield Y_{SX} was 0.1 Cmol/Cmol. In a continuous stirred tank reactor the maximum growth rate is about 0.25 h^{-1}, with a yield of 0.3 Cmol/Cmol. With a residence time of 1 day 82% of the influent oil was degraded. In a mixture of linear and branched alkanes the linear alkanes are degraded fastest and with the highest yield. After most of these alkanes have disappeared the branched alkanes are consumed. In a CSTR a large part of the branched alkanes is not degraded. Microbial remediation of oil polluted water should therefore be performed in a batch or in a plug flow reactor. This results in the highest degradation rate and most complete removal of the oil.

ACKNOWLEDGEMENTS

SYMBOLS

D = Dilution rate in a continuous reactor, [h^{-1}]

m_S = Maintenance coefficient, [Cmol oil/Cmol DW/h]

$r_{O(0)}$ = Oxygen uptake rate at time 0, [mol O_2/l/h]

$r_{O(t)}$ = Oxygen uptake rate at time t, [mol O_2/l/h]

q_S = Specific substrate consumption rate, [Cmol oil/Cmol DW/h]

Y_{SC} = Ratio between carbon dioxide production and substrate consumption rates, [mol/Cmol]

Y_{SN} = Ratio between ammonium and substrate consumption rates, [mol/Cmol]

Y_{SO} = Ratio between oxygen and substrate consumption rates, [mol/Cmol]

Y_{SP} = Yield of product on substrate, [Cmol product/Cmol substrate]

Y_{SW} = Ratio of water production and substrate consumption rates, [mol/Cmol]

Y_{SX} = Yield of biomass on substrate, [Cmol DW/Cmol substrate]

$Y_{SX,max}$ = Maximum yield of biomass on substrate, [Cmol DW/Cmol substrate]

μ_{max} = Maximum growth rate, [h^{-1}]

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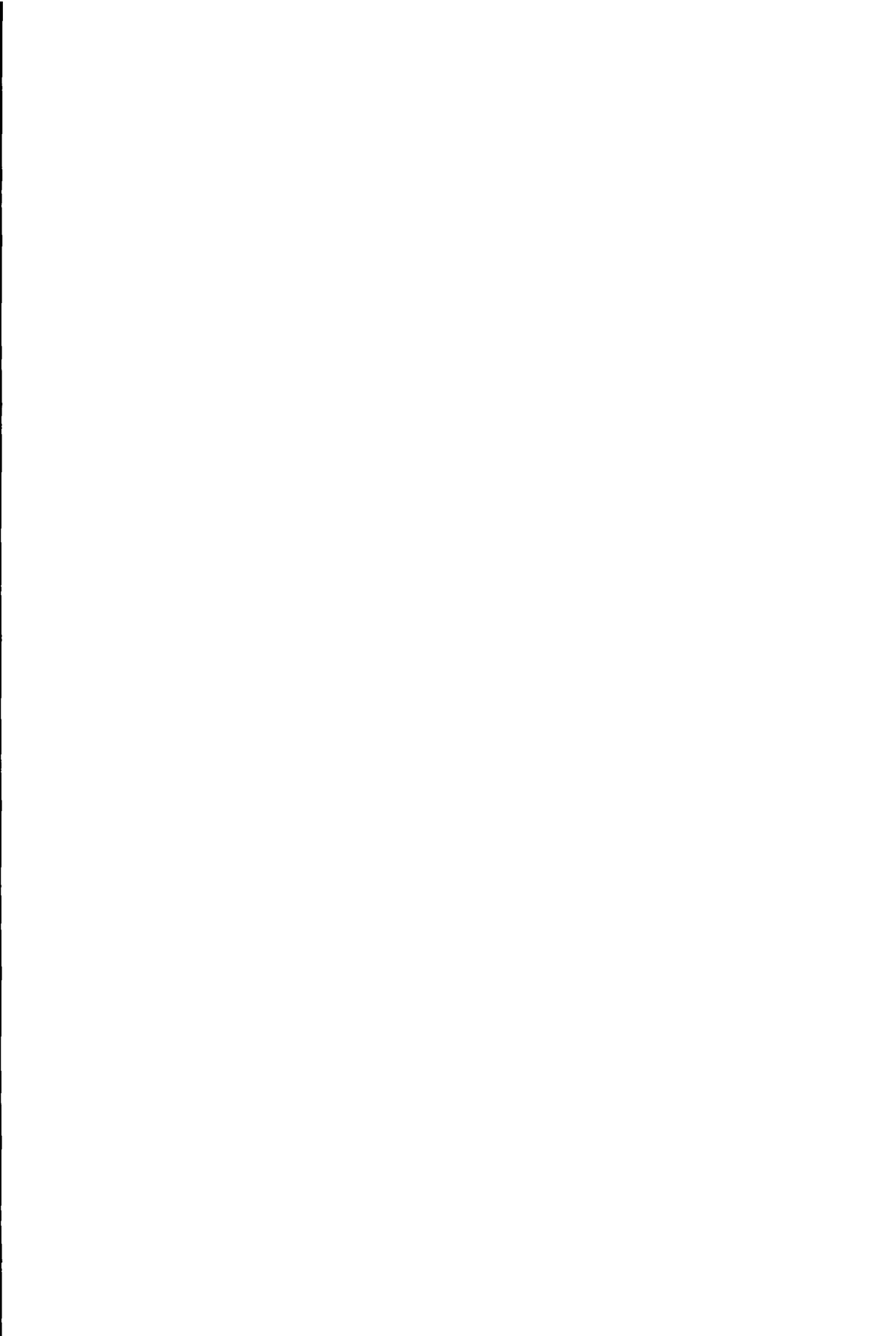
Microbial decontamination of
polluted soil in a slurry process

M.J. Geerdink, R.H. Kleijntjens, M.C.M. van Loosdrecht, K.Ch.A.M. Luyben

INTRODUCTION

Biological treatment of oil contaminated soil in a landfarm has proven to be a good alternative to thermal and extractive reclamation techniques [1]. Drawbacks of landfarming are the requirement for large areas of land and the observed low contaminant degradation rates. Long treatment periods are the result, especially in the colder climates. In order to take more advantage of the benefits of biological soil decontamination, it is desirable to increase the degradation rate. This can be done in a more controlled environment, in which the optimal conditions for microbial activity can be realised. The degradation rate can be further increased by treating the soil in a slurry: Rijnaarts et al. [2] showed a positive correlation between mixing intensity and degradation rate. Based on these and other considerations, a soil slurry process for the decontamination of oil polluted soil has been designed [3].

The process essentially consists of two reactors in series. In the first reactor the soil is mixed with water and divided into two fractions: a fluidized bed of the lightly polluted sand, and a suspension of the heavily polluted fine particles. The oil on the sand is degraded in the first reactor. This sandy soil fraction needs no further treatment and can be discharged from the process. The oil on the suspended fines is usually not degraded as fast. This soil fraction can be further treated in the second reactor.

The economic feasibility of the slurry reactor largely depends on the required residence time of the soil in the reactor [4]. This residence time is governed by the required quality of the treated soil (the maximum allowable oil concentration). Consequently, the oil degradation rate is of prime importance. The degradation rate depends on the type of oil components (degradability) and on the concentration of oil components that are accessible to the degrading microorganisms (availability). In this paper the degradation rate of oil has been studied in relation to the adsorption of oil onto the soil. Degradation of diesel oil in water dispersions was compared to degradation in soil slurries. Data from the degradation of oil in water dispersion (batch and continuous operations) provided the (uninhibited) maximum growth rates, coupled to yield and maintenance requirements. These have been compared with the results from the experiments with soil, to determine the influence of the soil on the oil degradation rate. Finally, the slurry process was tested in the treatment of an excavated soil, which consisted mainly of silt and clay, containing an aged oil pollution. This soil was chosen 1) because this type of soil is considered to be extremely hard to remediate [5], due to the small particle sizes, and 2) because aged pollutions are more persistent than more recent pollutions [5, 6], probably due to a further infiltration of the contaminant into the porous soil particles.

MATERIALS AND METHODS

The bioreactors

For the study of oil degradation in water (E1 and E2, Table 1), a two litres standard glass reactor was used. The contents of the reactor were mixed at 900 rpm with a Rushton turbine. Air was introduced at 1 vvm (1 litre of gas/litre reactor/minute) through a sparger in the bottom of the reactor. pH was maintained between 6.5 and 7.5 through addition of 2 N NaOH solution or 1 N H_2SO_4 solution. In the continuous experiments (E2, see Table 1) the diesel oil was added by a peristaltic pump. Nutrients (see below) were added separately in a C:N molar ratio of the total influent of 10:1. In the batch experiments (E1) this ratio was the same, at the start. The dilution rates used in the continuous experiments ranged between 1 and 4 day^{-1}.

Experiment code	Description
E1	batch 2 litres, diesel dispersed in water, 120 h
E2	continuous 2 litres, diesel dispersed in water, several dilution rates
E3a	batch 2 litres, diesel adsorbed to soil
E3b	batch 2 litres, suspension from E4
E4	continuous 2*20 litres, diesel adsorbed to soil, three dilution rates
E5	continuous 2*20 litres, excavated soil, dilution rate D=0.005 h^{-1}

Table 1. Coding of the various experiments.

For the batch wise degradation of oil in a soil slurry (E3, Table 1), a similar reactor as for the dispersion experiments was used.

For the continuous soil decontamination experiments (E4 and E5, Table 1) a bench scale installation of two reactors in series was used: A tapered 20 litres reactor with a dual injection system (DITS [3]), in series with an air agitated 20 litres reactor (Fig. 1).

The DITS reactor has a combined air-liquid injector in the bottom of the reactor. This creates two zones in the reactor; a fluidized bed of mainly coarse particles, and a suspension zone consisting mainly of the fine soil fraction. The residence times for these two zones can be set independently, so the fluidized bed can have a short residence time, while the suspension zone has a long residence time. The fluidized bed in the DITS reactor was removed manually once a day. The suspended solids from the DITS reactor were transferred to the second reactor. This
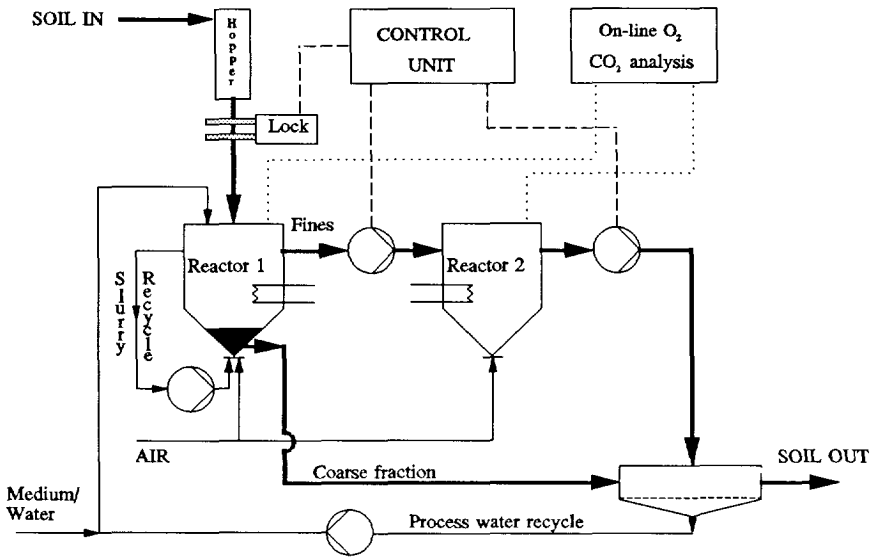
Figure 1. Flowsheet representation of the soil slurry process.

second reactor was used to investigate in a single experiment the effect of a longer residence time for the suspended solids. The two reactors comprised the so called mini-plant. Two different overall (related to the entire mini-plant) soil residence times were investigated: 100 and 200 hours. In both cases, the residence time of the coarse fraction (in the DITS reactor) was about 24 hours.

To keep the coarse particles in the first reactor fluidized, the slurry injection flow at the bottom of the reactor was set at 2 l/min. At higher flow rates, obstructions caused practical problems in the slurry recirculation loop (see Fig. 1), at lower rates the coarse fraction could not be fluidized. Once per hour dry contaminated soil was added from a hopper of 10 litres mounted above the reactor (Fig. 1), through an automated locks system, to the DITS-reactor.

Process conditions

The temperature was kept at 30^0C in all experiments. The oxygen concentration in the water phase was never below 2 mg/l. The batch experiment with diesel oil dispersed in water (E1) lasted 120 h. The batch experiment with soil (E3a) lasted 214 h. Several continuous experiments with diesel oil in dispersion (E2) were performed. Three experiments with the artificially contaminated soil (E4) were performed, followed by one experiment with the excavated soil (E5,

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Table 1). (Residence times and concentrations for these three-phase experiments are given in Table 2.). An additional batch experiment (E3b) was performed using the suspension from a continuous soil experiment. This experiment lasted 1800 h.

Micro-organisms

In all the experiments a mixed microbial consortium capable of degrading oil was used as inoculum. The consortium was enriched from polluted harbour sludge (Rotterdam) obtained from a dredge close to an oil refinery. This consortium was grown on diesel contaminated soil for two years before the start of the first experiment, so it could be expected that this population of microorganisms was already adapted to the reactor conditions that were applied in the experiments.

The batch experiments were inoculated at the start of each experiment. The continuous experiments with dispersed oil (E2) were inoculated once, at the start of the first experiment. Subsequent experiments were started with the reactor contents of the previous dilution rate. During the four consecutive continuous soil decontamination experiments (E4 and E5) the mini-plant was not re-inoculated with microorganisms either. Similar to experiment E2 each experiment started with the reactor contents of the preceding experiment.

The microorganisms were allowed at least 3 residence times to adapt to the new situation, before the actual experiment started. These adaptation times should suffice to establish a new steady state in the reactor.

Medium supply

In batch mode (E1 and E3), nutrients were added at the start of the experiment. This medium was also used for the continuous oil degradation in water dispersion (E2). It consisted of 550 mg/l KH_2PO_4, 275 mg/l $MgSO_4.7H_2O$, 2.75 mg/l $FeSO_4.7H_2O$, 5.5 mg/l $ZnSO_4.7H_2O$, 1.1 mg/l $CuSO_4.5H_2O$, 1.1 mg/l $CoSO_4.7H_2O$, 1.1 mg/l $CaCl_2.2H_2O$, 2.75 mg/l $MnCl_2.4H_2O$, 27.5 mg/l NaCl, 27.5 mg/l KCl, 2200 mg/l NH_4Cl.

In the continuous experiments of contaminated soil in the slurry plant (E4 and E5) the nutrients were added to the reactors in a concentrated form, containing per litre tap water: 20 grams of a standard agricultural fertilizer (NPK fertilizer 12+10+8), corresponding to a concentration of 2.4 g/l nitrogen (75% NH_4^+ and 25% NO_3^-), 2.0 g/l phosphorus (P_2O_5) and 3.6 g/l potassium (K_2O). Also 0.5 gram $MgSO_4$ and 0.25 gram $FeCl_3$ were added. This concentrated

nutrient flow was diluted with tap water in a flow ratio of about 1:10. Trace elements were assumed to be sufficiently available in the soil and in the water. The resulting nutrients supply rate to the first reactor was 0.088 l/h or 0.178 l/h for residence times of 200 and 100 h, respectively. The nutrient supply rate to the second reactor was half that to reactor 1.

Polluted soil

In order to eliminate the influence of soil composition as best as possible, model experiments were performed using a clean soil, which was prepared with different concentrations of the desired contaminant. For the model experiments, a sandy loam was used. According to Ball and Roberts [6] equilibration times for adsorption of nonpolar substances to wet soil can take years. To expedite the adsorption, diesel oil (the contaminant used in this study) was added to dried soil. The soil had been air dried at ambient temperatures for about 2 months, giving a dry weight percentage of 95-97%, and subsequently sieved (1 mm) to remove the larger stones and homogenized [7]. The organic content was 2-4 wt%. The oil was added to the soil two weeks before the soil entered the reactor, to ensure thorough "wetting" of the soil matrix by the oil. The polluted soil was kept in a closed vessel and was frequently mixed during the two weeks "wetting" time. The initial oil concentration for the batch experiment E3a was 11 g/kg. Diesel oil concentrations in the three steady-states of the continuous experiments (E4), as measured at the entrance of the DITS reactor, were 4.8, 8.6 and 5.3 g/kg soil respectively, with a standard deviation of less than 10%.

Besides this model contaminated soil, polluted soil originating from the Amsterdam harbour was used in a continuous experiment (E5). This sandy clay loam (high silt and clay content) had been contaminated with oil from a refinery oil storage tank about 45 years ago. The sludge was dredged up in 1980 and stored on the shore for clean-up. The soil used in this study was excavated from the storage in 1991. The soil was dried (see above) and sieved over a 1.5 mm sieve, before it was added to the hopper. The organic matter content of this soil was about 5 wt%. The average oil concentration was 2.3 g/kg, measured before the reactor entrance.

The batch experiment E3b, with soil from the suspension zone from an experiment in the mini-plant, started with an oil concentration of 17 g/kg.

Analyses

Water phase

Dissolved oxygen concentrations were measured with an oxygen electrode (Electrofact z151, Yokogawa Nederland BV, Maarssen, The Netherlands).

At regular intervals, the concentration of nitrite and nitrate in the water phase (and in the filtrate of the mini-plant) was checked with Merckoquant 10 020 test strips from Merck, Darmstad, FRG, to ensure no significant amount of oxygen was used for nitrification.

The biomass concentration was determined in the solids-free experiments E1 and E2 only, as there are some problems in accurately determining the biomass concentration in a soil slurry. Samples of 10 ml water phase from the batch, or 10 ml of the effluent from the continuous reactor was filtered. The cellulose filter, containing the biomass, was dried at 105^0C for 24 hours, to evaporate water and oil. After drying the weight of the filter was compared to the weight of the dried filter before filtration.

To determine the dissolved oil concentration, soil was removed, if necessary, by centrifugation for 30 minutes at over 1000 m/s^2. The water phase was extracted with a solvent (distilled diethylether, three times 50 ml per 500 ml of water phase) in a shake-flask. Hexadec-1-ene was added as internal standard. Subsequently the solute was analyzed using GC-FID. If no components other than ether were found, the solvent was evaporated at 45^0C until 1 ml was left, and analyzed again by GC-FID.

Gas phase

Since it is possible that dissolved pollutant is stripped by the air flow, the gas flows leaving the reactors were monitored for oil. Part of the off-gas was directed through a glass tube containing solid adsorption material (Tenax TA 60-80 mesh, Chrompack International BV, Middelburg, The Netherlands) at a flow rate of 1 l/h. After several days of being exposed to the off-gas, the adsorption material was extracted with diethylether which subsequently was analyzed by GC-FID.

Oxygen concentrations in the in- and outlet gas were monitored on-line using a (paramagnetic) O_2-analyzer (Taylor Analytics).

Carbon dioxide production was monitored as well. In the solids-free experiments however, adsorption by stripped oil components in the gas phase interfered with the measurement.

Solid phase

In the batch experiments (E3) the oil concentrations were determined by using the Dutch interim standard analysis NEN 5733 (largely similar to the German DIN 38409, teil 18 (1981)).

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In the continuous experiments, the oil concentrations at the different locations in the plant were monitored by frequent sampling. These slurry samples were first dewatered by filtration using cellulose soxhlet extraction thimbles. The oil extraction procedure for these continuous experiments was different from the samples of the batch experiments, because of some doubt concerning the accuracy of the standard analysis: In Chapter 2 of this thesis [8] it was shown that the most reliable analysis method, soxhlet extraction, yielded significantly different results from the standard analysis method. Oil components were extracted with a methanol/toluene mixture (4:1) by soxhlet extraction for five hours [9]. Hexadec-1-ene, which is not dominantly present in oil products, was added to the extracting liquid as an internal standard. The hydrocarbon layer was separated from the water phase and its total oil content was determined by capillary gas chromatography (GC). The GC was equipped with a HP-1 column and a flame ionization detection (FID). Oil concentrations were determined by calculation of the total area of the chromatogram in relation to the internal standard. Using this method, an average extraction yield of $92 \pm 10\%$ was achieved on standards. These standards were prepared in the same way as the model polluted soil, with additionally several oil adsorption equilibration times. Soaking with water and filtration were part of the preparation of the standards. No significant difference was found between extraction of (soaked) soil samples after two weeks equilibration time or longer.

The solids hold-up (volume fraction of the soil in the slurry) and the particle size distribution in the different reactor compartments were monitored during the operation of the plant. The volume fraction of soil in a sample, ε_s, was determined according to equation 1:

$$\varepsilon_s = \frac{V_{solid}}{V_{slurry}} = \frac{\rho_{slurry} - \rho_{water}}{\rho_{solid} - \rho_{water}} \tag{1}$$

in which the density of water is equal to $\rho_{water} = 1000$ kg/m^3. The density of the solids was equal to $\rho_{solid} = 2500$ kg/m^3 for the particles in the fluidized bed, and equal to $\rho_{solid} = 2050$ kg/m^3 for the suspended particles and for the soil used in experiment 4. The density of the slurry, ρ_{slurry}, was determined by measuring weight and volume of the slurry sample. Separate measurement of the dry weight of slurry samples showed a standard deviation of less than 10% in the values of the measured densities. The resulting solids hold-ups had standard deviations never exceeding 8%.

RESULTS

Batch degradation of diesel dispersed in water (E1)

To determine the maximum growth rate of the microbial consortium growing on diesel oil and the stoichiometry of the degradation, several batch experiments, with diesel oil dispersed in water, were performed (coded E1). With an initial oil concentration of 3.3 g/l, the concentration of diesel oil after 22 hours was 2.8 g/l, after 40 hours it was about 0.18 g/l, and after 120 hours the oil concentration had dropped to below the detection limit. The oxygen uptake rate is plotted in Fig. 2.
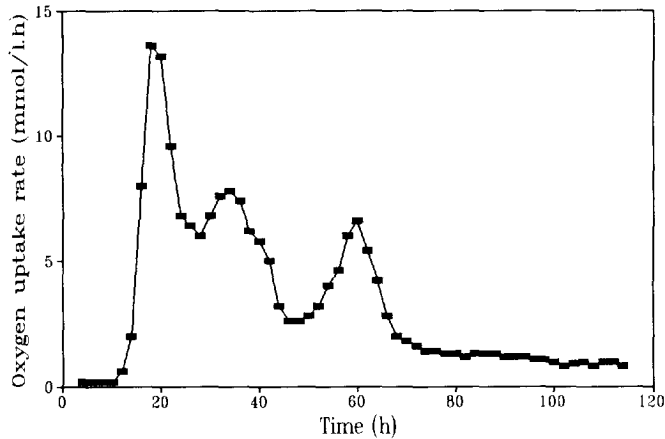

Figure 2. Oxygen uptake rate as a function of time in the batch experiment E1, using diesel oil dispersed in water as the sole carbon and energy source.

From the oxygen consumption rate the maximum growth rate can be determined. It is assumed that in the exponential growth phase the biomass specific oxygen conversion rate is constant, and that Monod kinetics apply. Under these conditions the following linear relation can be derived between the oxygen uptake rate and time:

$$\ln\left(r_{O(t)}\right) = \ln\left(r_{O(0)}\right) + \mu_{max}t \tag{2}$$

This equation is used to calculate the maximum growth rate of the microorganisms from the data in Fig. 2, between 10 and 18 hours. The μ_{max} was found to be 0.55 ± 0.15 h^{-1}.

The yield Y_{sx} after 120 h was found to be 0.10 ± 0.02 Cmol biomass/Cmol oil. The ratio between oxygen and substrate consumption rate was $Y_{so} = 1.6 \pm 0.2$ mol O_2/Cmol oil. The yields

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correspond with each other, within the error margins, according to equation 3:

$$Y_{SN} \; NH_3 + CH_2 + Y_{SO} \; O_2 \rightarrow Y_{SX} \; CH_{1.8}O_{0.5}N_{0.2} + Y_{SW} \; H_2O + Y_{SC} \; CO_2 \tag{3}$$
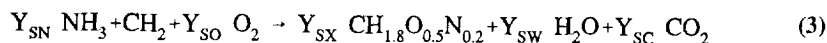
where CH_2 represents oil and $CH_{1.8}O_{0.5}N_{0.2}$ the biomass.

Extracts of the adsorption material in the off-gas of the reactor showed that about 2% of the initial amount of oil had been stripped.

Diesel dispersed in water, in continuous mode (E2)

Several experiments using diesel oil in dispersion in a continuous system (CSTR) have been performed to determine the effect of a change in the operation mode. In the experiments several dilution rates have been applied. The specific substrate consumption rate for each of these experiments, plotted against the dilution rate is given in Fig. 3.
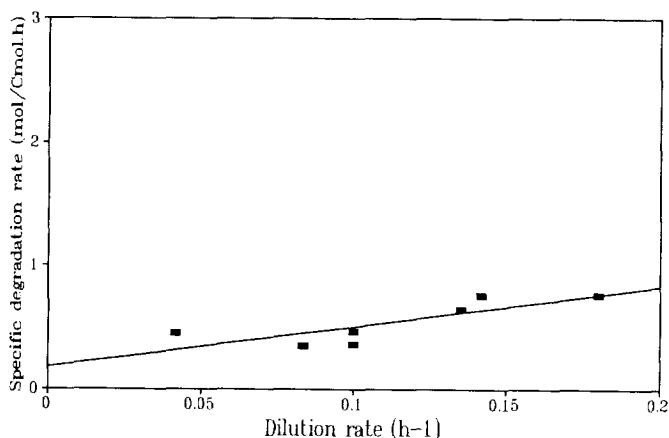

Figure 3. Specific substrate and oxygen consumption rates as a function of the dilution rate for the oil in water degradation in continuous culture (E2).

The specific substrate consumption rate increased linearly. A yield of biomass on substrate of $Y_{SX} = 0.3 \pm 0.1$ Cmol biomass/Cmol substrate and a maintenance coefficient of $m_S = 0.2 \pm 0.1$ Cmol substrate/Cmol biomass/h were calculated from Fig. 3.

In Fig. 4 a chromatogram of the pure diesel oil is shown. The peaks representing the linear alkanes are marked by *. The rest of the peaks and the so called unresolved complex mixture (UCM) [10, 11] (the "bump" in the chromatogram), consist mainly of branched alkanes.

An extract of the water phase of the dispersion experiment E2 with a dilution rate of 0.18 h^{-1} is shown in Fig. 5. The linear alkanes and part of the branched alkanes were preferentially consumed.
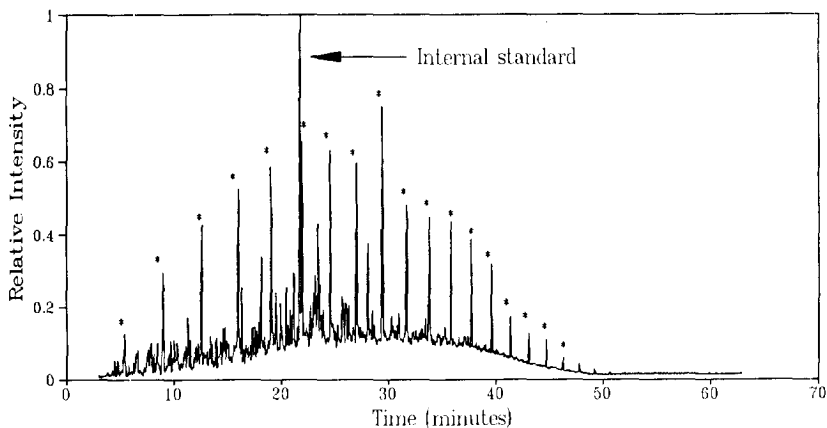

Figure 4. Chromatogram of the pure diesel oil. Intensities are relative to octadecane (the highest peak).
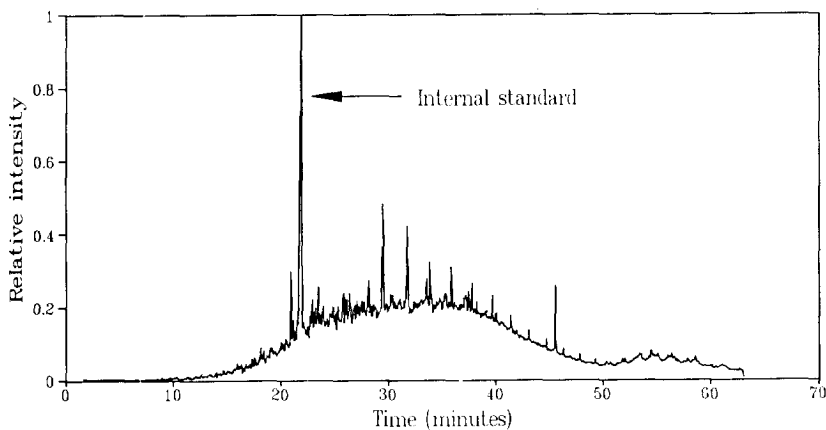

Figure 5. Chromatogram of the extract of the water phase from a continuous experiment (E2) at D=0.18 h^{-1}. Internal standard was tetradec-1-ene. Oil concentration is: 0.085 Cmol/l water.

Measurement of the oil concentration in the off-gas showed that at most 2% of the influent oil was stripped into the gas phase.

Batch wise degradation of diesel in soil slurry (E3)

Batch experiments were performed to determine the influence of soil on the degradation process. In the batch experiment using diesel oil adsorbed to soil (E3a), an oxygen uptake profile (Fig. 6) similar to the dispersion experiment (Fig. 2) was found.
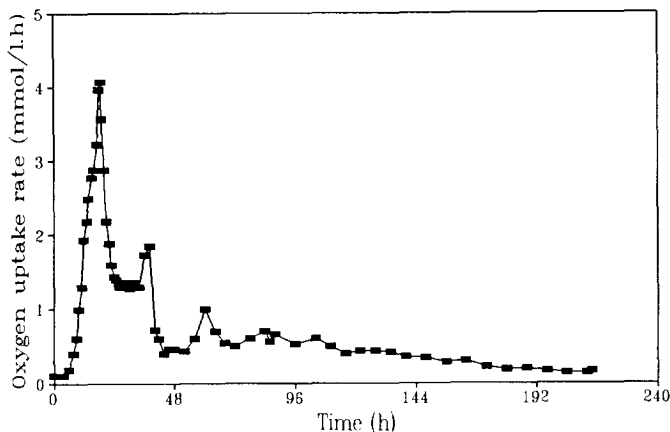

Figure 6. Oxygen uptake rate as a function of time in batch experiment E3a.

From the change in oxygen uptake rate (OUR) the maximum growth rate is calculated to be $\mu_{max}=0.4\pm0.1$ h^{-1} (for t=4 to 12 h). The ratio between oxygen and substrate consumption rate after 214 hours was $Y_{SO}=1.4\pm0.2$ mol O_2/Cmol oil. Contrary to the experiments with diesel in dispersion a relatively high amount of diesel oil remained in the slurry at the end of the experiment (t=214 h: 0.72 g diesel/l). The carbon dioxide production rate matched the OUR. The ratio of carbon dioxide production and oxygen consumption rates after 214 h was found to be $Y_{OC}=Y_{SC}/Y_{SO}=0.90$ mol/mol. This corresponds reasonably well with the Y_{SO}, according to equation 3. With equation 3 a yield of $Y_{SX}=0.14$ Cmol/Cmol is calculated. This corresponds with the yield found in the batch dispersion experiment: $Y_{SX}=0.10$ Cmol/Cmol.

The chromatogram of the extract of the soil (given in Fig. 7) was similar to that of the pure diesel oil (see Fig. 4). No preferential degradation of linear alkanes was observed, in contrast to expectation: It was expected that, as in experiment E1, the linear alkanes would be consumed before the branched alkanes, because these are degraded fastest [12].

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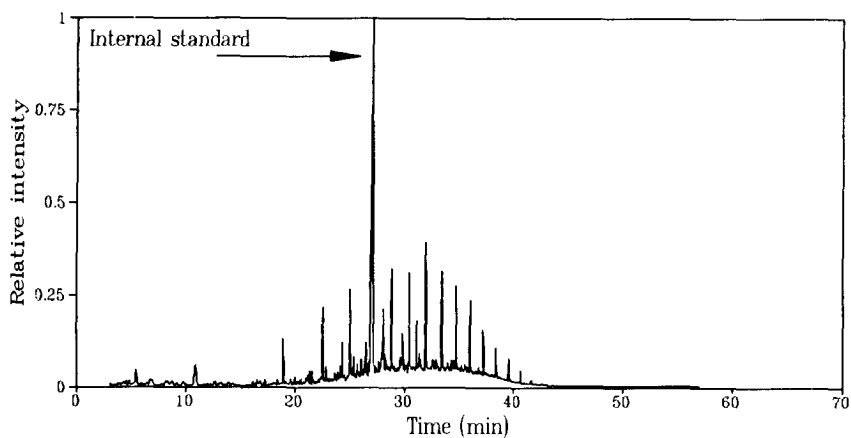
Figure 7. Chromatogram of the extracted soil from the batch experiment (E3a), after microbial degradation for 214 hours. The intensities are relative to the internal standard. Oil concentration is: 3.6 g/kg.

During the experiment measurements were performed to determine the oil concentration in the off-gas and in the water phase. In both phases the oil concentration was below the detection limit (in the water phase <100 $\mu g/l$, in the gas phase <50 $\mu g/m^3$). This means that a maximum of 0.5 mg oil was stripped, which is about 0.01% of the total initial amount of oil.

Continuous degradation of diesel in soil slurry (E4 and E5)

For the continuous degradation of adsorbed oil a bench scale soil slurry plant (Fig. 1) was used. The oil concentration on the influent soil was different for each dilution rate (Fig. 8a, Table 2).
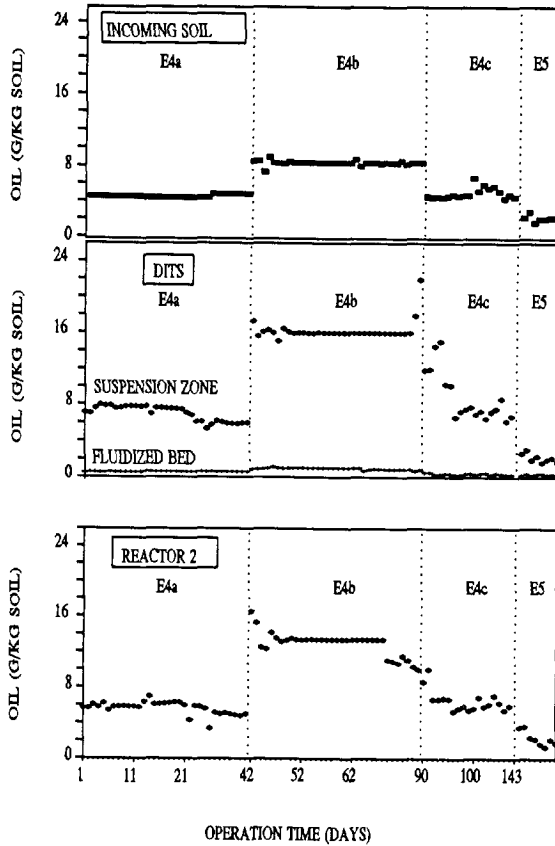

Figure 8. Oil concentrations (g/kg soil DW) in the influent soil flow (8a) and the effluent slurry flows (8b) of the DITS-reactor, and in the effluent slurry flow of the second reactor (8c).

The oil concentration of the influent soil was determined from soil samples taken from the hopper directly before the reactor entrance. In the first (DITS-)reactor the soil is separated into a heavy and a light fraction. The fluidized heavy fraction contained relatively little oil, whereas the suspended light fraction contained the major part of the oil (Fig. 8b). The suspended soil fraction was transferred to the second, air agitated, reactor. The soil in the fluidized bed was discharged from the system once a day. Ultimately, this sand fraction was mixed with the effluent

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from the second reactor. The reconstituted soil slurry was dewatered, after which the final oil concentration was measured (Table 2).

Experiment	E4a	E4b	E4c	E5
Steady state during days:	1-32	42-68	96-108	146-151
Soil residence time (h):	200	100	100	200
Oil concentrations (g/kg soil)				
Soil leaving storage container:	4.8	8.6	5.2	2.26
Reactor 1 (DITS): - suspension:	7.0	16.3	8.9	2.04
- fluidized bed:	0.61	1.00	0.42	0.38
Reactor 2: - suspension:	5.7	13.0	6.5	1.95
Remixed soil:	1.6	2.0	1.1	0.92
Overall reduction (%):	66	77	79	59
Overall conversion rate (mg oil/l reactor/h):	3.5	14	8.5	1.5
Efficiency of reactor 1 (%):	60	73	73	58
Degradation rate in reactor 1 (mg oil/l reactor/h):	5.5	24	14	2.5
Efficiency of reactor 2 (%):	19	20	27	4
Degradation rate in reactor 2 (mg oil/l reactor/h):	1.4	4.6	2.5	0.54

Table 2. Average steady-state oil concentrations in the fine, coarse and remixed soil fractions, relative decontamination levels (in %), and conversion rates for the experiments E4 and E5.

During several of the experiments measurements were performed to determine the oil concentration in the off-gas and in the water phase. In both phases the oil concentration was below the detection limit (in the water phase $<100\,\mu g/l$, in the gas phase $<50\,\mu g/m^3$). This means that at most 1.4 mg oil/day was removed with the gas phase, corresponding to 0.09% of the consumed oil. With the effluent water 0.4 mg/day (less than 0.03% of the conversion rate) could have been removed. The dissolved oxygen concentration in the slurry was 2-3 mg/l.

The steady state oil concentrations and the overall conversion rates are given in Table 2. The data are averaged values measured over a period of several days of steady state in the reactors. From these data it can be seen that, on average, some 70% overall reduction of the oil concentration was reached, independent on the influent oil concentration. The excavated soil with the 45 years old contamination gave the lowest overall reduction: 59%.

In Fig. 9 the chromatograms for the extracted oil from influent and effluent soil are shown.
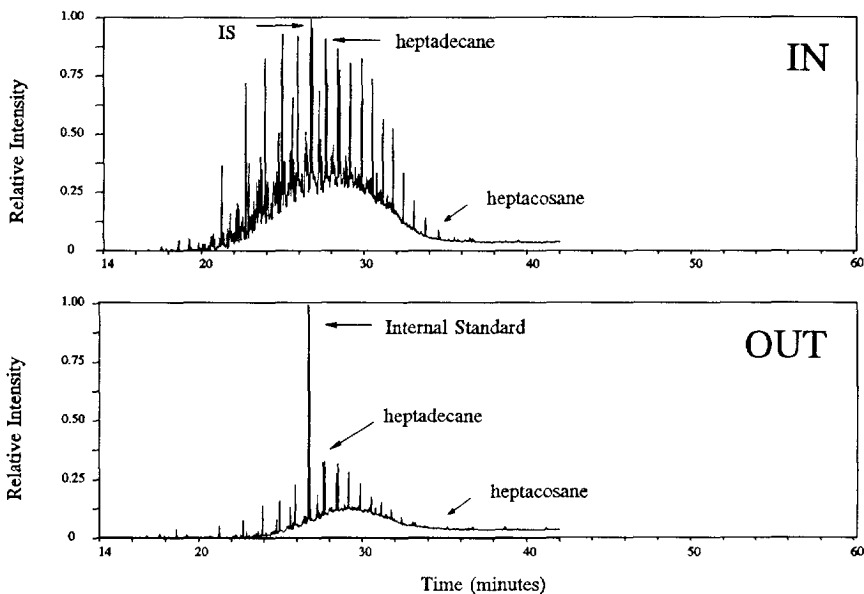
Figures 9a and 9b. Chromatograms of the extracts of the model contaminated soil (experiment E4) before (9a) and after (9b) treatment. Concentrations of oil are 10.1 g/kg (9a) and 2.27 g/kg (9b).
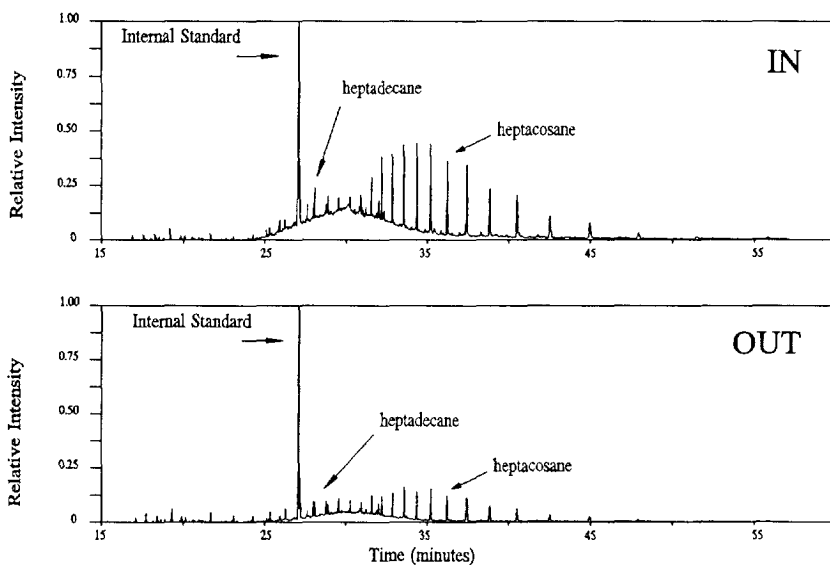
Figures 9c and 9d. Chromatograms of the extracts of the excavated sandy clay loam (experiment E5). Concentrations are 3.8 g/kg (9c) and 0.79 g/kg (9d).

In Fig. 9c it can be seen that the oil in the excavated soil contains more higher boiling

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compounds than the diesel oil (Fig. 9a), which was used in the model experiments. There seems to be no difference in the degradation order of the oil components for the artificial pollution (Fig. 9a and 9b) and the 45 years old contamination (Fig. 9c and 9d). Comparing the chromatograms of the samples before and after treatment, it can be seen that the decrease of the lower molecular compounds such as n-heptadecane is of the same order as of the higher molecular compounds such as n-heptacosane. The branched (and the few aromatic) hydrocarbons are gathered in the UCM [10, 11]. Comparing Fig. 9a with 9b, and 9c with 9d, there seems to be no preference for the degradation of the linear alkanes over the branched alkanes. This is similar to what was observed in the batch experiment E3a.

The diesel oil concentrations in batch experiment E3b using soil from the suspension zone in the mini-plant are shown in Fig. 10. The soil fraction in this experiment consisted mainly of the
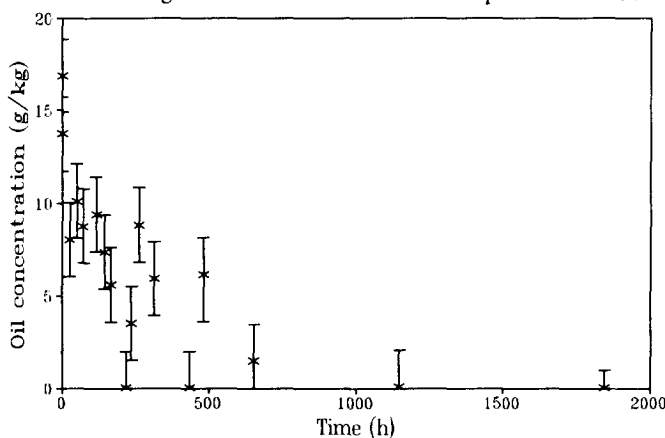
Figure 10. Oil concentrations in the batch experiment E3b using soil from the suspension zone from an experiment in the mini-plant.

fines of the original soil. This soil fraction shows the highest oil concentrations (see Fig. 8b) and exhibits a very low oil degradation rate (see Table 2, reactor 2). In Fig. 10 it is shown that the oil concentration decreased to below the detection level after 1800 h, i.e. after about 11 weeks. The large standard deviation in the measurements of the oil concentrations in this experiment is mainly due to the small volume of the slurry samples. The sample volume had to be quite small so as not to disturb the continuing experiment.

In Table 3 a summary is given of the oil concentrations for all batch experiments, the average concentrations in steady-state for the experiments in the mini-plant, and one typical dilution rate for experiment E2.

Experiment	Solids hold up (l/l)	Reaction time (h)	Oil concentration before the experiment	Oil concentration after the experiment	Units	Efficiency (%)
E1	0	120	3.3	<0.0001	g/l	100
E2	0	12	3	0.42	g/l	86
E3a	0.1	214	11	3.6	g/kg	67
E3b	0.1	1800	17	<0.1	g/kg	100
E4a	0.1	200	4.8	1.6	g/kg	66
E4b	0.1	100	8.6	2.0	g/kg	77
E4c	0.1	100	5.2	1.1	g/kg	79
E5	0.1	200	2.26	0.92	g/kg	59

Table 3. A compilation of the oil concentrations before and after the batch experiments and of the influent and effluent oil concentrations of the continuous experiments.

DISCUSSION

The oxygen uptake rate plots for the batch experiments E1 (Fig. 2) and E3a (Fig. 6) are similar. Both show three maxima. In the batch experiment with diesel in dispersion (E1), the amount of oil remaining after 40 h was 0.18 g/l: After 40 hours virtually all the oil (components) had disappeared. In Chapter 3 it was shown that the first two maxima in the OUR are caused by the oxidation of linear alkanes and branched alkanes, respectively [13]. An explanation for the third maximum was not readily at hand.

In the batch experiment with soil (E3a) the yield after 214 hours is: $Y_{so}=1.4$ mol/Cmol, comparable to the yield in the experiment without soil (E1, after 120 h), $Y_{so}=1.6$ mol/Cmol. Using equation 3, the yield of biomass on substrate in experiment E3a is calculated to be $Y_{sx}=0.14$ Cmol/Cmol. This is comparable to the yield in experiment E1, after 120 h ($Y_{sx}=0.10$ Cmol/Cmol). The composition of the degraded components in experiment E3a was the same as the composition of the pure diesel oil (see Fig. 7 and 5). In experiment E1 all components were

degraded, so the composition of the degraded components was also the same as in the pure diesel oil. This indicates that the soil did not affect the stoichiometry of the oil degradation.

In Fig. 7 it can be seen that all components have been degraded to the same degree; no preferential degradation of linear alkanes is seen. The oil "concentration" in the reactor in experiment E3a, however, is still high: 0.72 g/l. This indicates that there is an availability problem; either the substrate, or the oxygen or nutrients concentrations are too low in the vicinity the microorganisms.

The overall relative reductions of the oil concentrations in the experiments in the mini-plant (E4 and E5) given in Table 2 do not differ significantly from one another. The overall relative reduction was independent (over the range measured) of the average residence time of the solid phase and of the influent oil concentration. It seems that a more or less constant percentage of the oil is bound strongly to the soil. This bound fraction has the same composition as the total oil. It is very slowly released and subsequently degraded. The fact that no decrease in degradation efficiency was observed with shorter residence times, indicates that even higher loading rates to the reactors can be applied.

Degradation rates of up to 24 mg oil/litre reactor/hour, corresponding to 120 mg oil/kg soil/hour, were measured (Table 2). Compared to landfarming, e.g. of an oil sludge with comparable composition and concentration [14], oil degradation rates in this slurry process are some 70 to 260 times higher. In the slurry process a considerable increase in the initial, fast oil degradation rate, i.e. of 70% of the contaminant, can be obtained. Increase of the solids hold-up in the slurry reactor up to 0.3 l/l, without significant decrease of the volumetric degradation rate (in g oil/l reactor/h) is possible. This would result in higher loading rates for the soil, and therefore in increased mass degradation rates (in g oil/kg soil/h). Experiment E4 shows the capability of the process to reduce the oil concentration of highly contaminated soils in a short time. In Fig. 9c it can be seen that the oil on the excavated soil (experiment E5) contains more higher boiling compounds than the diesel oil (Fig. 9a) used in the model experiments. Even for such an aged and persistent contamination on a soil containing a high clay and silt content, the process can be applied successfully.

From Fig. 7 and 9 it is clear that the 'grass', composed mainly of linear alkanes, is not preferentially degraded in the experiments 4 and 5, when compared to the UCM, containing mainly branched alkanes [10, 11]. The same was found for the batch experiment E3a. This is in contrast with observations of degradation order described in the literature [16, 17] and from the

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dispersion experiments: linear alkanes are more readily degraded than branched alkanes. When no soil was present, the linear alkanes were indeed degraded faster (experiment E1, see Chapter 3 [13]), as expected. These observations indicate that, due to the interactions of the oil with the soil, part of the oil (with a composition identical to the initial composition) is unavailable for degradation by the microorganisms during the experiments.

To explain this, the following hypothesis is proposed:

- In contaminated soils, originating from the same area, there will be a rather constant ratio between the amount of oil in micropores (diameter smaller than 1 μm, i.e. too small for microorganisms to enter) and in macropores (>1 μm) of the soil particles. The composition of the oil in the pores will be (almost) the same as the overall composition. The oil in the macropores is easily accessed and is degraded fast. Due to the very high sorption coefficient of the oil components for soil organic matter or some other fraction of the soil [18] the effective diffusion rate [19] of the oil out of the micropores of the particles will be very low to negligible compared to the time scale of the degradation process.

Thus only the oil, which is not in the micropores, will be degraded during the time the soil is in the reactor. The oil in the micropores will continue to "diffuse" slowly out of these pores after treatment. Since in this case the degradation rate is much larger than the desorption rate, there will be less problems with toxicity of the contaminants to other organisms.

In conclusion: It appears that a large part of the contamination in the soil is readily available for microbial breakdown. This is most efficiently handled in continuous mode. Most types of soil exhibit broad particle size distributions. Usually, a large fraction of the soil, mostly the large massive particles, is not or lightly contaminated. It is advisable to split the soil into a clean and a contaminated fraction before continuing, thus reducing the amount of soil to be handled further on. These two operations are combined in the DITS reactor. After the initial degradation of the readily available contaminant and the subsequent separation of the cleaned and the contaminated soil fractions, the latter fraction can be treated further. From the batch experiment E3b it is found that approximately 10 weeks are needed for full remediation of the contaminated fine soil fraction. This treatment can be performed either in a landfarm, or in a plug flow reactor or a cascade of CST reactors with a minimum of energy input.

The main advantages of this type of operation are that:

1) A large fraction of the contaminated soil is cleaned in a relatively short time in the DITS reactor and can be discharged from the process. The contaminated soil fraction is treated further.

2) The contaminated soil fraction is greatly reduced in size, is already supplied with nutrients and contains a relatively high amount of active oil degrading microorganisms.

3) In the contaminated soil fraction all large aggregates are broken up, so further treatment time is reduced, due to increased accessibility of the oil.

CONCLUSIONS

Oil contaminated soil, especially soil with high clay and silt content, can be remediated microbiologically in a slurry reactor. The presence of soil, however, limits the degradation rate of oil. In contrast with oil dispersed in water, the composition of the degraded oil components in a soil slurry is the same as that of the original oil, which is indicative for a physical, rather than a (bio)chemical, process.

It seems that about 70% of the contaminant is readily available and can be degraded in less than 8 days. The DITS reactor is able to combine remediation of part of the contaminated (polydispers) soil with separation of the soil into a heavily and a lightly polluted fraction. In continuous operation lowering the overall soil residence time from 200 to 100 h had no effect on the oil concentration in the effluent soil. Therefore a soil residence time of less than 100 h is feasible. With a residence time of 100 h oil degradation rates were more than 70 times faster than in a comparable landfarm. After treatment in a DITS reactor the oil in the contaminated soil fraction is slowly released from the soil, and from an extrapolation it follows that another 10 weeks are needed to reach the A-level of 50 mg/kg. This can be done in an extensive process, such as a landfarm.

ACKNOWLEDGEMENTS

SYMBOLS

$r_{O(0)}$	Oxygen uptake rate at time 0, [mol O_2/l/h]
$r_{O(t)}$	Oxygen uptake rate at time t, [mol O_2/l/h]

V_{slurry}	Total volume of the slurry, $[m^3]$
V_{solid}	Solid volume of the solids fraction in the slurry, $[m^3]$
t	Time, $[h]$
Y_{OC}	Ratio of carbon dioxide production and oxygen consumption rates, $[mol\ CO_2/mol\ O_2]$
Y_{SC}	Ratio of carbon dioxide production and substrate consumption rates, $[mol\ CO_2/Cmol\ oil]$
Y_{SO}	Ratio of oxygen and substrate consumption rates, $[mol\ O_2/Cmol\ substrate]$
Y_{SX}	Yield of biomass on substrate, $[Cmol\ biomass/Cmol\ oil]$
ε_S	Solids hold-up, $[l\ solids/l\ slurry]$
μ_{max}	Maximum growth rate, $[h^{-1}]$
ρ_{solid}	Solid density of solids, $[kg/m^3]$
ρ_{slurry}	Density of the slurry, $[kg/m^3]$
ρ_{water}	Density of water, $[kg/m^3]$

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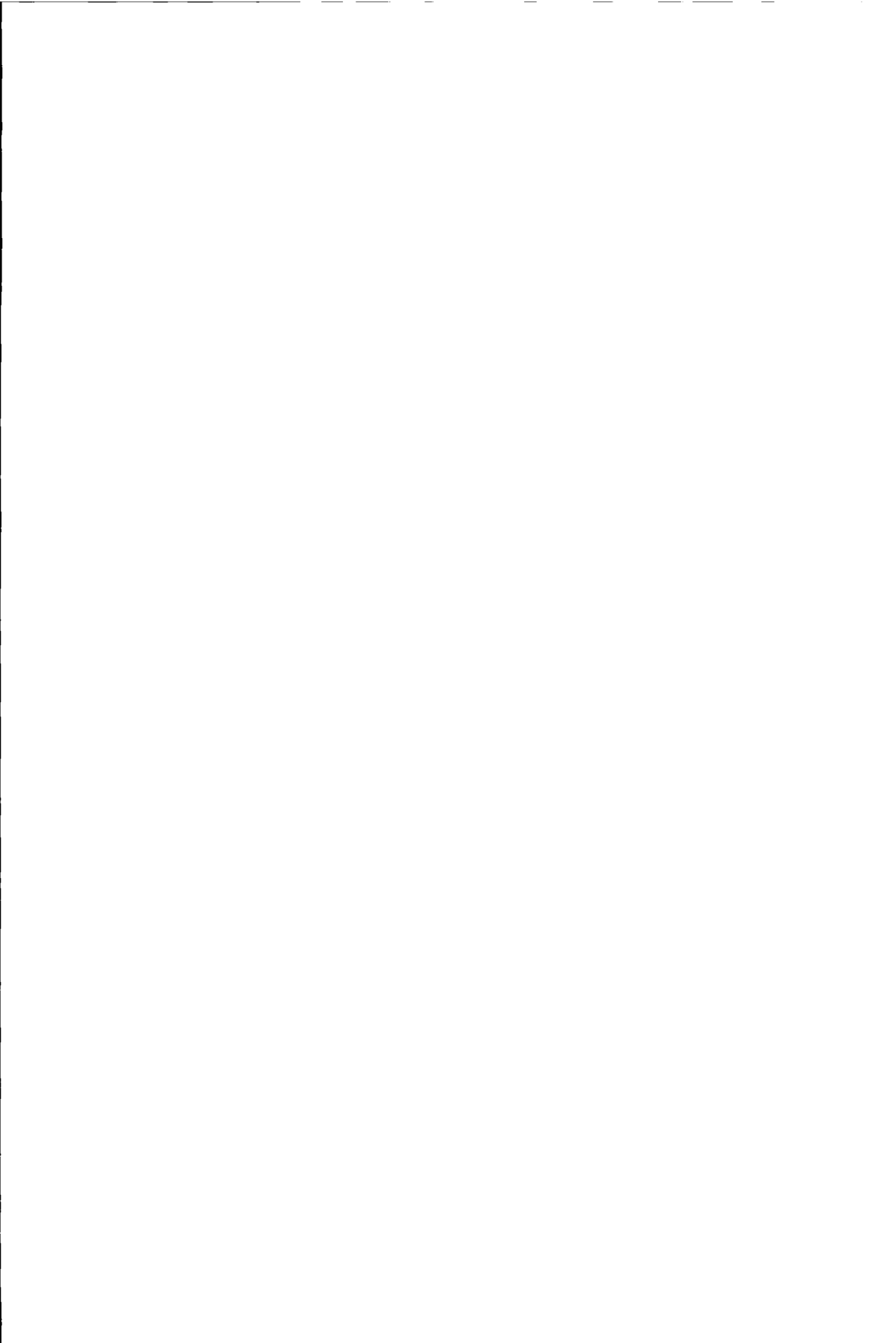
Bioavailability of hexadecane
on several soil constituents

M.J. Geerdink, M.C.M. van Loosdrecht, K.Ch.A.M. Luyben
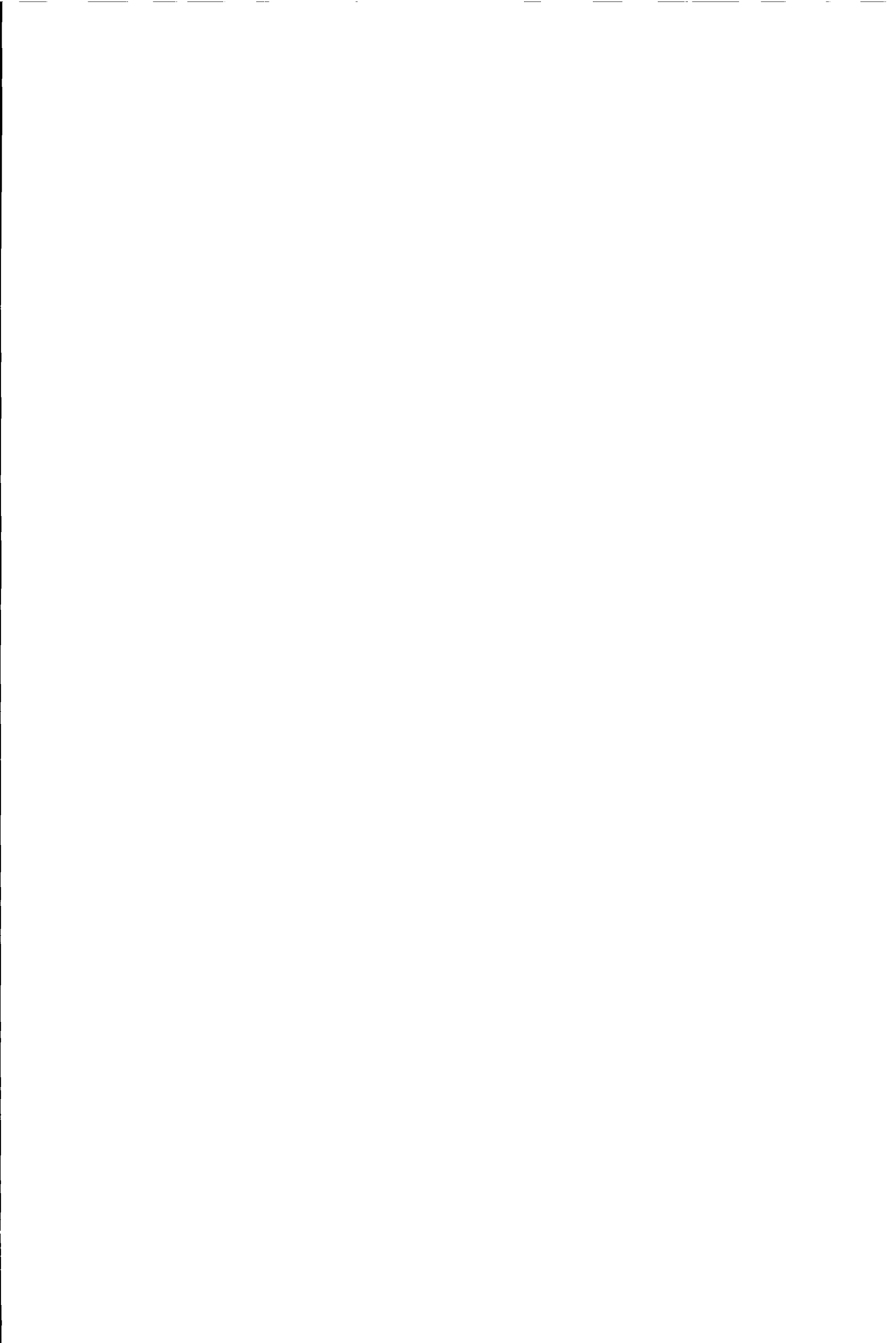
INTRODUCTION

Biological reclamation of soil has many advantages, amongst which are the good quality of the cleaned product. In the case of sandy soils, biological decontamination of polluted soils has been proven to be a viable and competing alternative [1, 2] for already established remediation techniques, such as thermal cleaning. For soils with high clay or organic matter content high rest concentrations are observed. These types of soil are notoriously difficult to clean [3], also with other remediation techniques. Therefore, it is interesting to look at ways to improve the performance of biological techniques for such difficult types of soil.

In previous work [4] it was shown that diesel oil can be completely metabolized when no solid phase is present. The degradation of diesel oil adsorbed to soil, either in batch or in continuous mode, did not proceed as well [5]: In batch a very long residence time, about 10 weeks, was needed to clean up the soil to below the desired level of 50 mg oil/kg soil DW. In continuous mode, using several days residence time, high rest concentrations were observed; typically 30% of the influent concentration. This indicates that (some constituents of) the soil might be limiting the overall degradation rate of diesel oil.

In order to determine the key factor limiting the degradation rate of oil in soil, the problem must be further pinpointed; i.e. which part of the soil shows retardation of the contaminant. Until recently, research on this subject has been focused on the organic matter part of the soil [6, 7]. It was reasoned that the organic matter provides nonpolar adsorption sites and it would therefore be a good adsorption material. In contrast, clay minerals have ionogenic surfaces [8]. This ensures high affinity of the surface of these minerals for water molecules. The water layer around the clay particles would obstruct nonpolar substances to contact. It was assumed that this would prevent the clay minerals from affecting the degradation rate of nonpolar components. Sand (as well as silt) was assumed to have no significant influence on the degradation rate due to its relatively small surface area (no pores), and its nonpolar surface. In the models designed up till now, the fraction of organic matter was the sole parameter with respect to the soil, that would control the degradation rate of a nonpolar component [6, 7]. If this is a correct assumption, soils and sediments with low organic matter content should provide no problem in the biological decontamination. From a recent review it is apparent that this is not the case [3]. Some researchers have started to incorporate the mineral fraction of the soil in the study of bioavailability of contaminants in soil. For example, Farrell and Reinhard [9] found that desorption of TCE from soil proceeds on two distinct time scales. The slower desorption rate proceeds over a time scale of up to several months and could not be correlated to organic matter content. It was assumed to be effected by the mineral fraction of the soil. The faster desorption

rate has a time scale of several minutes and can probably be attributed to organic matter (the sorption coefficient of TCE is about 35 l/kg (calculated from the solubility, according to the relation given by Chiou et al. [10]).

In this study we have investigated the effect of the presence of different soil components on the degradation of hexadecane. These soil components were sand, organic matter and two types of clay minerals. Kaolinite was used as a representative of the 1:1 type of clay, montmorillonite represented the 1:2 type of clay. Kaolinite forms compact agglomerates with a small pore volume, whereas montmorillonite has a relatively open pore structure of its agglomerates.

MATERIALS AND METHODS

Preparation of polluted soil fractions

The soil fractions used in the experiments were sand (particle diameter 260 μm), kaolinite (a 1:1 type of clay, average particle diameter about 0.5 μm) from Janssen Chimica, montmorillonite (a 1:2 type of clay, average particle diameter 4 μm) from Janssen Chimica and peat (organic matter, average particle diameter 10 μm) from Velda. The particle diameters were determined in slurry by an image analyzer. Hexadecane, analytical grade, was added to the dry components in a concentration of 87 g/kg sand, 25 g/kg kaolinite, 10 g/kg montmorillonite and 33 g/kg organic matter. After at least a week contacting time in a polyethylene flask, daily shaking the contents, each soil component was ready to be used. In the reactor only a limited amount of sand could be kept suspended. To obtain a comparable amount of hexadecane in the reactor, less sand is needed when the concentration on the sand is higher. Therefore the hexadecane concentration on the sand was considerably higher compared to the other soil components.

The hexadecane was added to dry soil fractions to reduce the time needed to reach sorption equilibrium. According to Ball and Roberts [11] the equilibration time for sorption of nonpolar components onto wet soil can take years. It was assumed here that this long equilibration time is mainly due to the extreme low solubility of nonpolar components. This effects a very low transport rate of the contaminant into the porous soil particles. In the absence of water this transport is not hindered by interaction with water molecules and can therefore be faster.

Degradation experiments

All experiments were performed in a batch reactor at a temperature of 30^0C. The reactor was a standard 2 litres glass fermenter. It was filled with 1.5 litres of medium. The reactor contents were stirred at 900 rpm with a Rushton turbine. Air was introduced through a sparger in the

bottom of the reactor at a rate, controlled by a mass flow controller, of 1 l/min. The pH was kept between 6.5 and 7.5 using a pH-electrode, which was connected to a pH-controller. If needed 1 N sulphuric acid, or 2 N NaOH solution was added. The medium consisted of 550 mg/l KH_2PO_4, 275 mg/l $MgSO_4.7H_2O$, 2.75 mg/l $FeSO_4.7H_2O$, 5.5 mg/l $ZnSO_4.7H_2O$, 1.1 mg/l $CaCl_2.2H_2O$, 2.75 mg/l $MnCl_2.4H_2O$, 1.1 mg/l $CuSO_4.5H_2O$, 1.1 mg/l $CoSO_4.7H_2O$, 27.5 mg/l NaCl, 27.5 mg/l KCl, 2200 mg/l NH_4Cl in tap water. After setting the pH at 7, 10 ml of inoculation broth (biomass concentration about 1 g/l) was added. The inoculant contained a mixture of oil degrading microorganisms, grown on diesel oil. In the experiments with solid material, 69 g of sand, 200 g of kaolinite, 200 g of montmorillonite or 200 g of organic matter were used respectively. An experiment without a solid phase was performed to observe unhindered degradation behaviour: 6 g of hexadecane was added to 1.5 litres of medium in the reactor. These experiments all lasted 48 hours.

Three long term experiments were done, after it had become clear which soil components were most retarding the hexadecane degradation. For these experiments the hexadecane concentration on the kaolinite was initially 25 g/kg, on the organic matter it was initially 120 g/kg, the initial concentration on the montmorillonite was 24 g/kg. The medium used in these experiments contained about 50 mg of allylthioureum, to inhibit nitrification. The kaolinite experiment was monitored continuously by oxygen consumption and carbon dioxide production and was stopped after 141 hours. The hexadecane concentration on the solid was measured 0.5 h after the start of the experiment, after 48 h and after 141 h. The montmorillonite and organic matter experiments lasted 1000 h and 1500 h respectively. These experiments were monitored by analysing the hexadecane concentration on the solids. The oxygen consumption and carbon dioxide production rates after 48 h were very low and could not be measured accurately enough.

Analyses

The oxygen concentrations in the gas entering and leaving the reactor was measured by a paramagnetic gas analyzer. From these data the oxygen uptake rate (OUR) was calculated.

After 1 hour stirring at the start of each experiment, 50 ml slurry sample was taken and centrifuged for 10 minutes at 2000 m/s^2. The pellet was used for hexadecane analysis. This sample was to represent the start conditions. At the end of the experiment the entire reactor contents were centrifuged for 30 minutes at 1000 m/s^2. 500 ml of the supernatant was used for hexadecane analysis in the water phase. The pellet was used for determination of the hexadecane concentration on the solid phase.

In the long term experiments slurry samples of 20 ml were taken at selected intervals to observe the decrease of the hexadecane concentration on the soil.

Ammonium consumption rates could not be measured due to adsorption of the ammonium on the solid phase. Absence of nitrate and nitrite production was checked through Merckoquant 10 020 test strips.

RESULTS

In Fig. 1 the cumulative oxygen uptake profiles are plotted against time for the respective degradation experiments. The initial amount of hexadecane was slightly different for the various experiments, therefore the oxygen uptake was normalized to 1 Cmol of substrate initially.
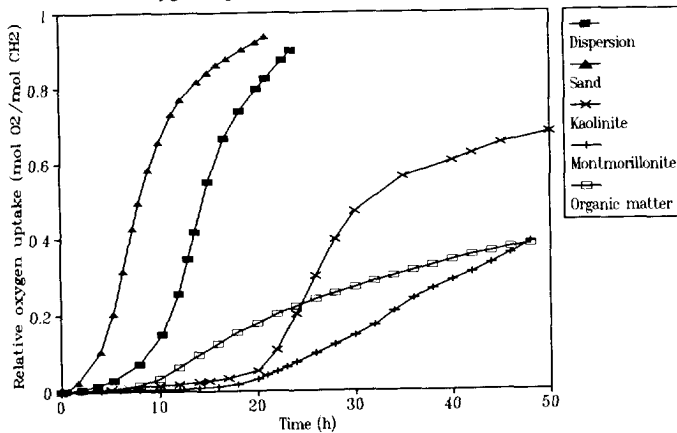

Figure 1. Cumulative oxygen uptake profiles for the different experiments. The amount of oxygen consumed is divided by the amount of hexadecane initially present.

For each experiment a maximum degradation rate can be calculated by assuming Monod kinetics [12] based on exponential growth. It is assumed that the degradation stoichiometry is constant during the exponential growth phase, i.e. the specific oxygen consumption rate is constant. Then the oxygen consumption rate is proportional to the biomass concentration. Furthermore it is assumed that during this phase the saturation constant K_S is far less than the substrate concentration (according to Goldsmith and Balderson: $K_S \approx 0.006$ Cmol/l [13]). After integration this leads to:

$$\ln(r_{O(t)}) = \ln(r_{O(0)}) + \mu_{max} t \qquad (1)$$

In Table 1 the measurements for the various experiments are summarized, as are the maximum growth rates, calculated from equation 1.

Soil component	Hexadecane concentration (Cmol/l)		Conversion (%)	Cumulative oxygen consumption (mol O_2/l)	Maximum growth rate (h^{-1})
	start	end			
water dispersion	0.213	$<10^{-7}$	100	0.19	0.37
sand	0.213	$<10^{-3}$	100	0.20	0.51
kaolinite	0.139	0.053	62	0.15	0.41
montmorillonite	0.064	0.032	51	0.017	0.40
organic matter	0.293	0.098	67	0.075	0.36
				average:	0.41±0.06

Table 1. Measured and calculated data of the experiments lasting 48 h.

The maximum growth rates are not much different from one experiment to another, on average μ_{max}=0.41±0.06 h^{-1}. With an experimental error of 0.1 h^{-1} in the maximum growth rate, all soil components can be considered not to influence the kinetics in the initial degradation stage. In Table 1 it is shown that both types of clay and the organic matter exhibit low conversion, during the first 48 hours. These three components are studied closer in long term experiments.

Fig. 2 shows the cumulative oxygen uptake in the kaolinite experiment, for an extended experiment time. The hexadecane concentration after 141 h was 2.6 g/kg (0.025 Cmol/l).
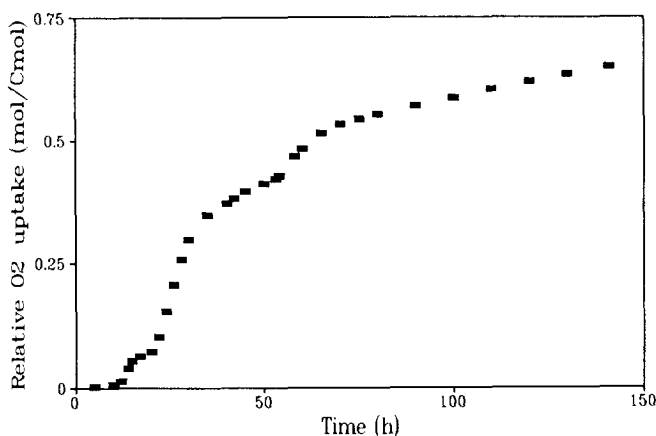

Figure 2. Relative cumulative oxygen consumption for the long term kaolinite experiment.

Fig. 3 shows the hexadecane concentrations for the long term experiments with montmorillonite and organic matter. For the montmorillonite, during the first day the hexadecane concentration
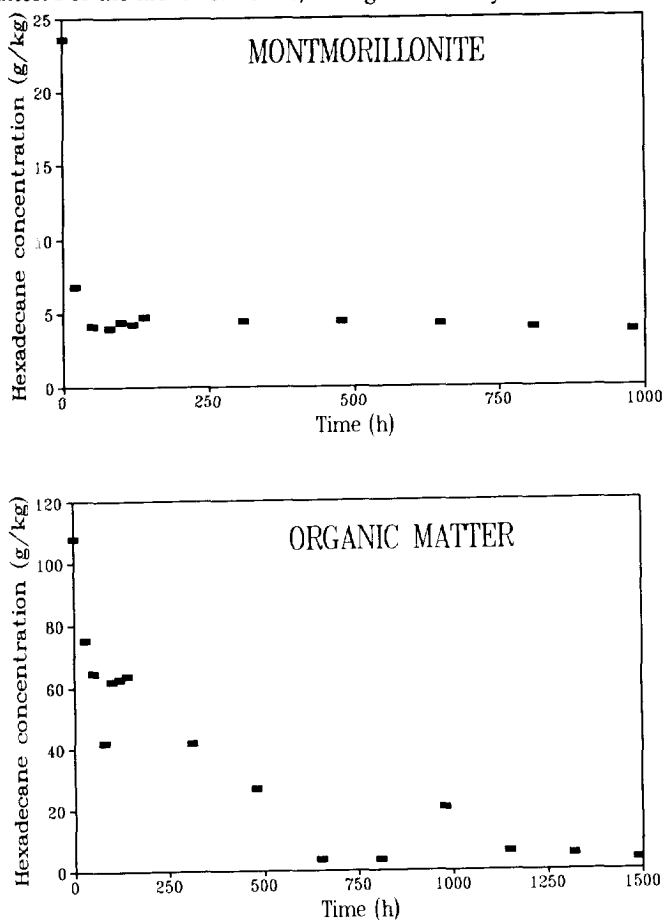

Figure 3. Hexadecane concentrations on montmorillonite and organic matter, during the long term degradation experiments.

decreased rapidly, after which the further decrease was very slow. For the organic matter the decrease was smoother.

Nitrate and nitrite concentrations were very low at the end of each experiment, so no significant amount of oxygen had been used for nitrification.

DISCUSSION

Comparison of growth rates

The values of the maximum growth rates do not differ significantly between the various experiments (Table 1). The maximum growth rates were all calculated in the initial, fast, degradation stage from the change in the OUR. The hexadecane is initially degraded according to maximum growth rate kinetics, independent of the solid phase. The substrate consumption rate is governed by the biomass concentration, and by the maximum growth rate of the biomass in the reactor. This indicates that initially there is no substrate limitation. All hexadecane that is degraded during this period is readily available.

After this first stage the oxygen consumption rate drops. This indicates that the available substrate concentration has been decreased. Measurement of the hexadecane at this moment shows still high concentrations in the reactor except for the experiments with sand and without solid phase. This substrate is concentrated in the soil particles. Probably the hexadecane consumption rate has become limited by the transport rate of the hexadecane from the soil particles to the bulk liquid and consequently to the microorganisms.

Long term experiments

From the cumulative oxygen uptake in the presence of kaolinite (shown in Fig. 2), it is clear that after 48 hours there is still microbial activity in the reactor. This indicates a slow release of the hexadecane from the kaolinite. The hexadecane is degraded as soon as it is released from the micropores (pores with a diameter smaller than 1 μm, i.e. too small for the microorganisms to penetrate). The microorganisms are still active, but their activity is limited by the available substrate concentration.

For the organic matter and the montmorillonite the actual hexadecane concentrations were monitored during long term experiments (Fig. 3). During the first day the hexadecane concentration on the montmorillonite decreased rapidly to about 30% of the initial concentration. The degradation rate for hexadecane on organic matter was lower during the first day. From the second day, the organic matter showed a much steeper decrease in the hexadecane concentration than the 1:2 type clay. The fast decrease of the hexadecane concentration on the montmorillonite in the first day could be caused by a limited storage capacity of the clay. Calculation of the internal storage capacity of montmorillonite (porosity=0.8 l/l, bulk density=1.2×10^3 kg/m^3) yields 0.67 l pore volume/kg montmorillonite. From the liquid density of hexadecane (0.8×10^3 kg/m^3) a total storage capacity for hexadecane of 0.5 kg/kg montmorillonite is calculated. At the initial concentration of 24 g/kg montmorillonite, not more than 5% of the total internal volume will be

occupied, so a limited storage capacity is not the cause of the fast release of hexadecane in the first day.

Several researchers [17, 18] have found that montmorillonite (and illite) platelets adhere to microorganisms. The microorganisms are covered with clay, which might limit the uptake rate of nutrients and sustrate. This could explain the very low hexadecane degradation rate after the first day in the montmorillonite experiment. However, if this is the case, a notably lower maximum growth rate should have been observed. In Table 1 it is shown that the maximum growth rate (determined from the data of the first day) in the montmorillonite experiment is not significantly lower than for the other experiments. Therefore it is unlikely that formation of a layer of montmorillonite covering the microorganisms is the reason for the low degradation rate.

Another explanation is: Initially, part of the hexadecane was available to the microorganisms outside the montmorillonite agglomerates, and was degraded according to Michaelis-Menten kinetics, within the first day. The remaining hexadecane was probably sorbed inside the agglomerates and transport limited degradation occurred, resulting in the very slow decrease of the hexadecane concentration after the first day. With the organic matter little hexadecane is directly available for the microorganisms at the start of the experiment, and no fast decrease of the hexadecane concentration is seen. The transport rate of the sorbed hexadecane to the microorganisms is higher for the organic matter than for the montmorillonite (after the first day), resulting in a faster decrease of the sorbed hexadecane concentration for the organic matter.

Summarizing, in the presence of both soil components part of the hexadecane is adsorbed in the organic matter. This hexadecane is slowly released from the organic matter to the microorganisms and subsequently degraded. The remainder of the hexadecane is partitioned into the montmorillonite. This hexadecane will be so slowly released that, on the time scale of most experiments, it will seem irreversibly adsorbed.

In a mixture of the two soil components the time to reach a certain hexadecane concentration depends on the partition coefficient of hexadecane between organic matter and montmorillonite.

Consequences for biological soil remediation

When first order degradation kinetics are assumed, the residence times in a batch reactor to clean up either sand, kaolinite, organic matter or montmorillonite to below 50 mg/kg, with an initial contaminant concentration of 10 g/kg are: 1, 14, 100 en 1600 days respectively. It is assumed here that the contaminant in the soil components is in the transport limited stage. For practical remediation this means that it is mainly the organic matter that slows down the degradation rate. On the time scale of most remediation processes, the 1:2 types of clay exhibit

degradation rates that are negligible small. From this it is clear that it is important to determine the composition of the soil before deciding which remediation technique is to be used.

The results described here do not rely on any chemical properties of hexadecane. Neither is the shape of the molecule of importance. The solubility of the component in water is the only property that is considered (implicitly). Therefore the results in this paper appear not to be specific for hexadecane and can probably be extrapolated to other nonpolar substances, such as oil or PAH's.

CONCLUSIONS

When degradation of hexadecane on different soil components is compared to degradation of hexadecane in dispersion, the initial degradation rate of hexadecane is the same. Of the soil components sand, organic matter, 1:1 type of clay (kaolinite) and 1:2 type of clay (montmorillonite), the 1:2 type of clay exhibits the lowest ultimate hexadecane degradation rate, followed by organic matter and the 1:1 type of clay. The sand even enhanced the degradation rate slightly. From the experiments discussed here, it follows that it is not only important to know the fraction of organic matter in the soil, but even more so to know the relative amount of 1:2 type of clay (mainly represented by illite and montmorillonite), before deciding to biologically remediate soil from a contaminated site.

ACKNOWLEDGEMENTS

SYMBOLS

$r_{O(0)}$ = Oxygen uptake rate at time 0, in [mol $O_2/l_R/h$]

$r_{O(t)}$ = oxygen uptake rate at time t, in [mol $O_2/l_R/h$]

t = time, in [h]

μ_{max} = Maximum growth rate, in [h^{-1}]

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Model for the microbial degradation of nonpolar organic contaminants in a soil slurry reactor

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INTRODUCTION

Biological decontamination of oil polluted soils and sediments is a good alternative for thermal treatment. Sandy soils can be cleaned to the desired concentration level [1] in an economically feasible process. However, soils with high clay or organic matter content can not be cleaned sufficiently. Typically 20 to 40% of the initial contaminant concentration remains after treatment [2] and is very slowly degraded further. In general it is assumed that one of the following processes is responsible for this very low ultimate degradation rate: 1) degradability of the contaminant by the microorganisms present, 2) desorption of the contaminant from the inner (and outer) surface of the particles, or 3) diffusion of the contaminant out of the particle aggregates. From previous work [3] it was clear that the limiting step in the ultimate degradation rate of diesel oil in a soil slurry plant is not the degradability of the oil. Therefore two possible mechanisms remain; desorption or diffusion limitation of the oil components. If the diffusion of the contaminant out of the soil particle is slowed down by consecutive adsorption and desorption of the oil components to the pore wall, both mechanisms can determine the rate of decontamination. This situation can be described by the radial pore effective diffusion model.

In literature the organic matter content is generally regarded to be the cause of retardation of nonpolar components [e.g. 4, 5]. It has been indicated however, that mineral surfaces possibly contribute to the partitioning of nonpolar components to soil [5, 6, 7, 8]. The study and description of the retarding influence of clay minerals on nonpolar compounds, however, has been rather neglected until recently [4]. At first sight the influence of clay on the ultimate oil degradation rate should not be significant in the presence of organic matter, because of the polar nature of clay platelets. However, if clay poses no problem in the degradation rate of oil, it should be possible to remediate clayey soils and sediments with low organic matter content fast. This is generally not the case [5, 9]. Desorption rates that were much lower than for organic matter were reported. These were possibly caused by the mineral part of the soil. An even stronger argument for incorporation of the mineral part into a desorption model was the finding of two independent desorption stages, one fast and one very slow, by Farrell and Reinhard [4].

In the work described here the influence of clay and organic matter on the oil degradation rate are studied. Each of these soil fractions is assumed to affect the oil degradation rate independently. This is incorporated into an extended effective diffusion model.

A model will be used that was tested on two sets of data, in which the transport of one nonpolar component, γ-hexachlorocyclohexane (lindane) [10] or α-hexachlorocyclohexane [11], out of soil is described. The sorption coefficient for γ-HCH on organic matter was calculated

from the relation given by Chiou et al. [12]. For the sorption coefficient with respect to clay no such relation was known, so this coefficient had to be fitted to the experimental data.

The model has been used to predict the degradation of diesel oil in a sieve fraction of soil. Since diesel consists predominantly of alkanes, the sorption coefficients for the different components in diesel oil were assumed to be equal to those for hexadecane. The coefficients were determined from experiments with hexadecane on pure soil constituents. A sieve fraction of the soil was used instead of a complete soil. According to Cooney et al. [13] more than one (average) particle diameter should be used if the particle size spans more than one decade. Otherwise the model prediction would be too inaccurate.

THEORY

The model

The soil is assumed to consist of three distinct fractions: sand (massive silicate particles, including silt), clay (agglomerates of clay platelets) and organic matter. Each of these three fractions is assumed to have a different sorption coefficient for oil in water. For each particle size the three soil fractions contribute independently to the contaminant concentration.

This results in the following mass balance for a batch reactor, with one particle size:

$$\frac{dC_s(\text{reactor})}{dt} = fo * \frac{dC_s(\text{o.m.})}{dt} + fc * \frac{dC_s(\text{clay})}{dt} + fs * \frac{dC_s(\text{sand}+\text{bulk})}{dt} \tag{1}$$

The last term on the right side of equation 1 describing the oil concentration on the sand and in the water, can be described by a model for unlimited microbial substrate consumption [3]. The other two terms on the right side can each be described with a radial pore effective diffusion model [14]. In this model the soil particle is assumed to be spherical, homogeneous and highly porous. As a result of this last assumption a factor correcting for the tortuosity of the pores is not necessary.

The micro-mass-balance now reads:

$$\frac{\partial C}{\partial t} = D_{eff} \left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r} \frac{\partial C}{\partial r} \right) \tag{2}$$

with:

$$D_{eff} = \frac{D\varepsilon}{K_p(1-\varepsilon)\rho_s} \qquad (3)$$

Equations 2 and 3 are used to describe the concentration in the particle as a function of time. To solve them, one initial and two boundary conditions are needed:

- at time $t=0$, the concentration in the particle is equal to C_0 (in the pore water) and q_0 (on the pore wall), and in equilibrium:

$$q_0 = K_p \cdot C_0 \qquad (4)$$

- at a time $t>0$, the concentration in the bulk outside the particle is $C(r>R)=0$. Only at the start of a batch experiment this will not be true; within 48 hours the oil concentration will have dropped to effectively 0 due to microbial degradation [3].
- In the centre of the particle the concentration gradient is horizontal: $(dC/dr)_{r=0}=0$

A further assumption for the use of this model is that the oil in the pore water is in equilibrium with the oil adsorbed to the pore wall at any time.

The effective diffusion model has been used in several papers, to describe the sorption of organic contaminants in soil [e.g. 7, 10, 14]. Advantages of the model are that it has a fundamental basis and it is able to describe the concentration as a function of time fairly well. Only for long desorption times the model failed and e.g. irreversible adsorption had to be introduced [10, 15]. Two other objections against the model are that it could not explain adsorption-desorption hysteresis [16] nor the sediment/water ratio effect [17].

The purpose of this paper is to demonstrate that it is possible to overcome all these objections and problems by introducing of a second diffusion coefficient and additionally a partition coefficient. It will also be shown that it is indeed relevant to use two effective diffusion coefficients (and a partition coefficient) for the prediction of the oil concentration in soil. In general, these three coefficients can be known a priori, i.e. there are no fitting parameters in the actual prediction stage of the model. Finally we will discuss whether the afore mentioned objections against the model can be discarded.

Partitioning of the contaminant between the three fractions, sand, clay and organic matter, is not necessarily proportional to the sorption coefficients. In the experiments described here this partitioning was fitted. A fraction of the contaminant was thought to be partitioned in the clay, the rest would then be partitioned in the organic matter. The amount of oil on the sand and in the water was assumed to be negligible, unless neither clay nor organic matter were present.

With the effective diffusion model the concentration of oil in a (sieve fraction of the) soil can be predicted, as a function of time, if the following parameters are known:

- The size distribution of the particles in the sieve fraction
- The fraction of organic matter and clay for each sieve fraction
- The oil concentration for each sieve fraction

Clay

It should be noted that there are several types of clay [18] of which the 1:1 and 1:2 type are the most common. The platelets of the 1:1 type, for example kaolinite, have two dissimilar sides, so that two platelets are drawn together with their opposite sides. Very small pores, and a relatively small specific surface area in these aggregates are the result. The platelets of the 1:2 type, for example montmorillonite and illite, have two similar sides. Lose aggregates and consequently a large specific area and a large internal volume are the result.

In the model that will be used here, the distinction between the two types of clay can be expressed. However, it will be shown later that this is not necessary: Both types have the same sorption coefficient and can therefore be described with a single parameter.

Calculation of sorption constants

The measurement of extremely hydrophobic components for which no specific detector is available, e.g. hexadecane, in water is not easy. To our knowledge no measurements of the diffusion coefficients of hexadecane in water have been published. A diffusion coefficient of hexadecane in D_2O has been reported [19]. The diffusion coefficient for hexadecane in water is assumed to be the same as in this "heavy water": $D = 4 \times 10^{-10}$ m^2/s.

According to Franks [20] the solubility of hexadecane in water is 10.9 $\mu g/l$, Sutton and Calder have found a value of 6 $\mu g/l$, and an estimation by UNIFAC [21] yields 15 $\mu g/l$. In this study a solubility of hexadecane in water of 10 $\mu g/l$ was assumed. Several equations have been published that relate the solubility of a component in water with the sorption coefficient for organic matter [e.g. 12, 22]. These equations do not differ much. Chiou et al. [12] proposed the following equation:

$$\log K_p(o.m.) = -0.729 \log S + 0.001 \qquad (5)$$

With this equation the sorption coefficient for hexadecane on organic matter becomes $\log K_p(o.m.) = 5.4$, or $K_p(o.m) = 2.3 \times 10^5$ l/kg.

For the estimation of the sorption coefficient for clay, $K_p(clay)$, no relation was known to us.

MATERIALS AND METHODS

Soil

The pure soil fractions that were used were a 1:1 type clay (kaolinite from Janssen Chimica), a 1:2 type clay (montmorillonite from Janssen Chimica), and organic matter (crushed peat pellets from Velda). Hexadecane (from Janssen Chimica) was used as a model component for oil. The hexadecane was added to the soil fraction, after which it was stored in a polyethylene flask for at least one week, with intermittent shaking. The clays were dry (less than 1% water), the peat had an initial water content of 29%. Since Ball and Roberts [7] reported equilibration times of several years for sorption of nonpolar components to wet soil, the hexadecane was sorbed to the dry soil fractions to prevent equilibration to take a very long time. In each experiment 200 g of soil component was mixed with 1.5 litres of medium. The initial hexadecane concentrations were 25 g/kg for the clays and 120 g/kg for the organic matter.

For the evaluation of the model, clean soil from the Botanical Garden in Delft was used. The soil was classified as sandy loam. The dried soil was contaminated with diesel oil from Texaco (21.4 g/kg), after which it was stored in a polyethylene flask for a week, to ensure good "wetting" of the soil by the oil. Then the soil was mixed with an equal amount (by weight) of water in a stirred reactor, for an hour. The resulting slurry was sieved. The sieve fraction <45 μm was used in the evaluation of the model, because of its high clay (approximately 60%) and organic matter content (Organic carbon content=7.6 wt%, corresponding to an organic matter content of 13.1%, according to Nelson and Sommers [23]), as well as its high oil concentration (43 g/kg) compared to the other sieve fractions.

Porosity was measured by weighing an amount of soil (fraction), adding a known volume of water and, after at least 1 hour equilibration, measuring the resulting volume of the suspension. The porosity is then equal to:

$$\varepsilon = 1 - \frac{V_{solid}}{V_{bulk}} = 1 - \frac{V_{slurry} - V_{water}}{(m_{bulk}/\rho_{bulk})} \tag{6}$$

The bulk densities for the various soil fractions were equal to: ρ_{bulk}=1.6x10³ kg/m³, 1.2x10³ kg/m³, 0.8x10³ kg/m³ and 2.0x10³ kg/m³ for kaolinite, montmorillonite, organic matter and the sieve fraction of the soil, respectively.

Particle size distribution was determined with an image analyzer, using a microscope with a maximum magnification factor of 400. This means that particles down to 2 μm can be measured accurately.

Medium

The medium was a solution of 550 mg/l KH_2PO_4, 275 mg/l $MgSO_4.7H_2O$, 2.75 mg/l $FeSO_4.7H_2O$, 5.5 mg/l $ZnSO_4.7H_2O$, 1.1 mg/l $CaCl_2.2H_2O$, 2.75 mg/l $MnCl_2.4H_2O$, 1.1 mg/l $CuSO_4.5H_2O$, 1.1 mg/l $CoSO_4.7H_2O$, 27.5 mg/l NaCl, 27.5 mg/l KCl, 2200 mg/l NH_4Cl in tap water.

Culture conditions

All experiments were performed in standard 2 litres glass fermenters. Air was introduced through a sparger at the bottom of the reactor. Stirring was done by a Rushton turbine, at 900 rpm. Temperature was kept at 30^0C, pH at 7. The kaolinite experiment lasted 141 h, the montmorillonite experiment 1000 h and the experiment with the organic matter lasted 1500 h. The experiment with the sieve fraction of the polluted soil lasted 920 h.

The inoculum was an undefined consortium of microorganisms adapted to degradation of diesel oil [3].

Analyses

The oxygen consumption rate for the kaolinite experiment was determined by measuring the difference between ingoing air and off-gas with a paramagnetic oxygen analyzer. The carbon dioxide production rate was measured with an infrared analyzer. For the other experiments the hexadecane or diesel oil concentrations on the solid phase were monitored by regularly taking a 20 ml slurry sample and, after centrifuging at over 1000 m/s^2, analysing the sediment. For the kaolinite this procedure was performed too, on samples taken after 27 hours and after 141 hours, at the end of the experiment.

Ammonium concentrations were monitored regularly with Merckoquant 10 024 test strips. Precise measurement of the ammonium consumption rate was deemed not feasible from previous experiments [24]. Nitrate and nitrite production were monitored using Merckoquant 10 020 test strips.

Hexadecane concentrations in the water phase were measured by centrifuging the contents of the fermenter after the experiment at over 1000 m/s^2 and extracting the supernatant [3].

RESULTS

Sorption coefficients for the soil fractions

The oxygen uptake and carbon dioxide production for kaolinite, and the hexadecane concentrations for the organic matter and the montmorillonite are shown in Fig. 1. The effective diffusion coefficients for the respective soil fractions were determined by fitting these results.
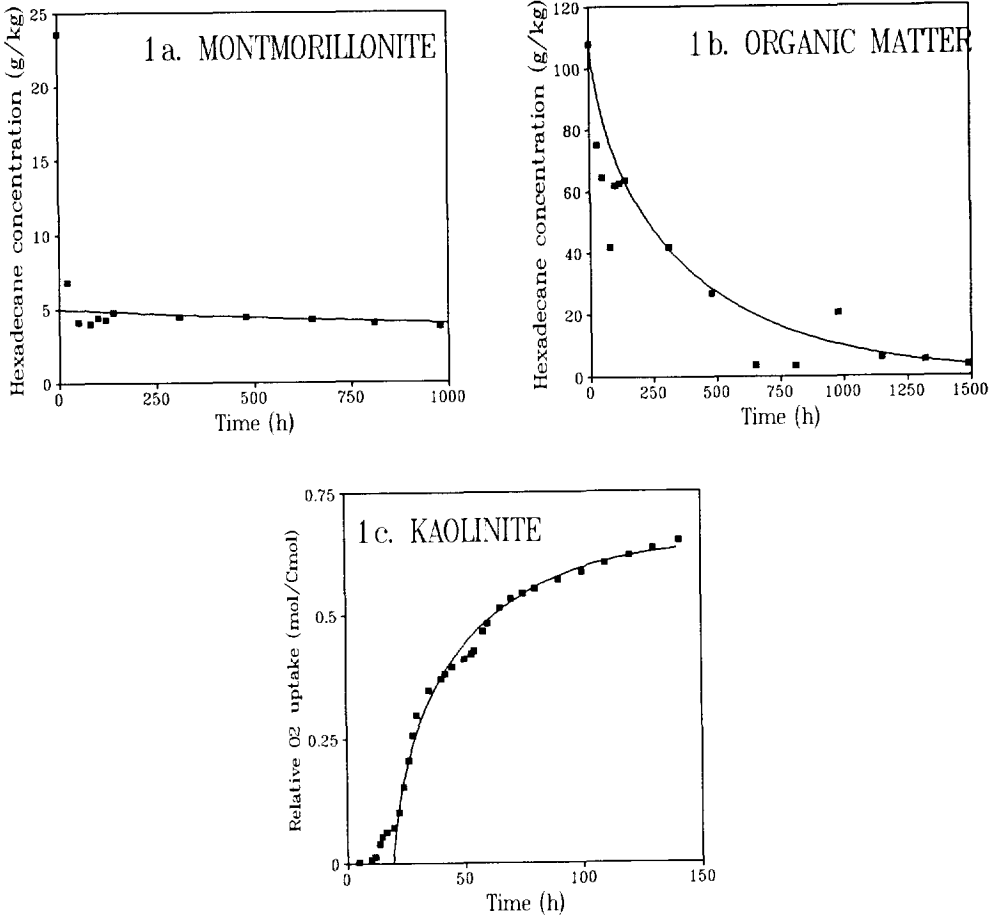

Figure 1. Hexadecane concentrations in case of montmorillonite (Fig. 1a) and organic matter (Fig. 1b) and oxygen uptake and carbon dioxide production rates for the case of kaolinite (Fig. 1c).

The average particle diameter of the soil fractions was: about 0.5 μm for the kaolinite, 4 μm for the montmorillonite and 10 μm for the organic matter respectively. It should be noted, that the determination of the particle size below 2 μm (which is largely the case for kaolinite) is

inaccurate. The porosities for the soil fractions were found to be $\varepsilon=0.65$, 0.80 and 0.90 for kaolinite, montmorillonite and organic matter respectively.

With a diffusion coefficient for hexadecane in water of $D=4\times10^{-10}$ m²/s, the numeric values of the sorption coefficients were calculated with equation 5: K_p(o.m.)$=3.2\times10^9$ l/kg, K_p(montmorillonite)$=1.2\times10^{11}$ l/kg, K_p(kaolinite)$=7.5\times10^{10}$ l/kg, the former two with an estimated error margin of some 25%. The error in the sorption coefficient for kaolinite is not estimated, due to the possible very large inaccuracy in the determination of the average particle size.

The various parameters for the soil fractions are summarized in Table 1.

Soil fraction	Porosity (1 pores/1 bulk)	Average particle diameter (μm)	Partition coefficient (l/kg)	Bulk density (kg/m³)
kaolinite	0.65	0.5	7.5×10^{10}	1.6×10^3
montmorillonite	0.80	4	1.2×10^{11}	1.2×10^3
organic matter	0.90	10	3.2×10^9	0.8×10^3

Table 1. Parameters of the pure soil constituents.

Testing the model

The model was tested on two sets of data of HCH desorption from soil [10, 11]. These two sets were chosen because both used a desorbing component from soils which contained equal amounts of organic matter, whereas the particle size distribution of the soils was quite different.

The solubility of γ-HCH is 2.7×10^{-5} mol/l [25, 26]. According to equation 5 this corresponds to a sorption coefficient of K_p(o.m.)$=2.1\times10^3$ l/kg. The diffusion coefficient of γ-HCH in water is $D=6.4\times10^{-10}$ m²/s [27]. Weber and Miller [10] used soil with a median grain size of 232 μm, having an intraparticle porosity of $\varepsilon=0.38$, and containing 1.14% organic carbon.

The solubility of α-HCH is 6.9×10^{-6} mol/l [26]. According to equation 5 this corresponds to a sorption coefficient of K_p(o.m.)$=5.8\times10^3$ l/kg. The diffusion coefficient of α-HCH in water is assumed to be comparable to that of γ-HCH. Rijnaarts et al. [11] used a sandy soil, amended with 38% of lime. The mean particle diameter was 26 μm and the soil contained 1.1% organic matter. Rijnaarts et al. did not mention the porosity, therefore the porosity is assumed to be 0.1, which is an acceptable value for sandy soil.

In the Figures 2a and 2b the experimental data for both sets are shown together with the model results (drawn lines) obtained by fitting with the effective diffusion model. The effective diffusion coefficients and the partition coefficients that were found are summarized in Table 2.
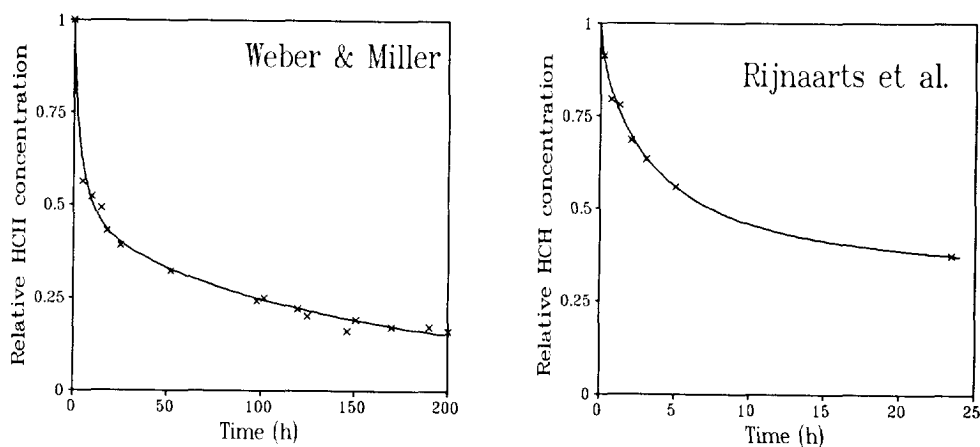
Figure 2. Measured HCH concentrations (markers) on the soil by Weber and Miller [10] (2a) and in the water phase by Rijnaarts [11] (2b) and the fitted concentrations (drawn line).

	D_{eff}(o.m.) (m^2/s)	D_{eff}(clay) (m^2/s)	fo (-)	K_p(o.m.) (l/kg)	K_p(clay) (l/kg)
Weber&Miller	$8.0x10^{-14}$	$1.9x10^{-15}$	0.48	$2.4x10^3$	$1.0x10^5$
Rijnaarts et al.	$3x10^{-15}$	$6x10^{-17}$	0.52	$9.9x10^3$	$4.9x10^5$

Table 2. Fitted effective diffusion and partition coefficients for the HCH data.

Next, an experiment with a sieve fraction of a soil, contaminated with diesel oil, was performed. The particle size distribution of the sieve fraction is shown in Fig. 3, the organic matter content was 13.1%. In Fig. 4 the measured and predicted diesel oil concentrations on the soil are shown. The sorption coefficients for diesel oil on organic matter and on clay were assumed to be the same as for hexadecane. The oil concentration in the water phase was below the detection level. The partition coefficient fo was fitted. The line drawn in Fig. 4 represents the model prediction.

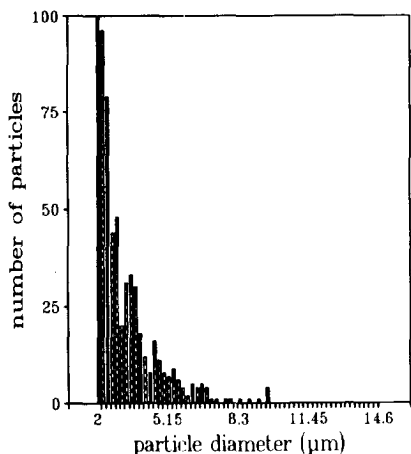The best fit for the partition coefficient was found to be fo=0.71 (and therefore fc=0.29).

Figure 3. Particle size distribution of the sieve fraction used for the testing of the model. Number average: $d_p = 3.4$ μm.

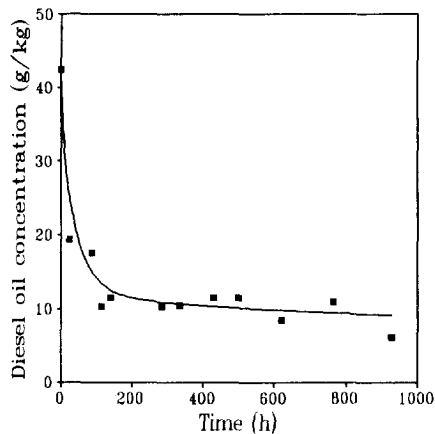*Figure 4. Measured and predicted diesel oil concentrations for the sieve fraction used for the testing of the model.*

DISCUSSION

Pure soil components

From Table 1 it appears that the particle agglomerates of the pure soil fractions are highly porous, as was assumed in the description of the model.

The degradation rate of hexadecane adsorbed to montmorillonite initially is very fast. After 24 h the degradation rate decreases drastically. The drop of the hexadecane concentration in the first hours of the experiment (Fig. 1a) is probably due to expansion of the space between the clay platelets the moment they come into contact with water. This causes an enlargement of the pore diameter. Part of the hexadecane will thus become available to the microorganisms. This indicates that sorption of hexadecane to dry montmorillonite, to forego extremely long sorption equilibration times as mentioned by Ball and Roberts [7], was only partly successfull. A large fraction of the hexadecane is not sorbed to the montmorillonite and is released upon contact with water. After this readily available hexadecane has been degraded the sorbed hexadecane is released with the expected very low rate. For a wet clay that has reached equilibrium during sorption, this means that all hexadecane will be desorbed and degraded according to the low rate that was observed in this experiment after 24 h.

For the organic matter the drop of the hexadecane concentration in the first hours (Fig. 1b) is less markedly than for the montmorillonite. It appears that in the organic matter all the

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hexadecane remains in the matrix. The release rate can be described with one effective diffusion coefficient during the entire experiment.

Testing of the model

The fitted sorption coefficients for HCH on organic matter were 1.7 times (Rijnaarts et al.) and 1.1 times (Weber and Miller) as large as the expected (calculated) coefficients from equation 5. This indicates that the model used here can satisfactorily predict the concentration of components in soil in a desorption/diffusion situation, as long as the sorption coefficients can be estimated or measured accurately. The sorption coefficient for organic matter can be estimated by making use of published correlations [12, 22] between the sorption coefficient and the solubility in water.

For the sieve fraction of the clean soil, artificially contaminated with diesel oil, the measured sorption coefficients for hexadecane to the pure soil constituents were used. In Fig. 4 it can be seen that for a mixture of the soil constituents these same sorption coefficients can be used to predict the overall concentrations of diesel oil.

Evaluation of the fitted sorption coefficients

The calculated value for the sorption coefficient of diesel oil on organic matter (according to equation 5, $K_p(o.m.)=2.3x10^5$ l/kg) is much smaller than the value found in the desorption/degradation experiment (Fig. 1b), $K_p(o.m.)=3.2x10^9$ l/kg. However, the equation to calculate the sorption coefficient from the solubility, had been fitted on components having solubilities up to $3x10^{-4}$ mol/l, and on several estimated solubilities up to $1x10^{-6}$ mol/l [12]. The calculated sorption coefficients do fit well for HCH. Probably extrapolation of the equation to the solubility of hexadecane ($4.4x10^{-8}$ mol/l) is not allowed. This can not completely explain the large discrepancy between both values. Probably, the formation of microscopic droplets or micelles, so called accomodation, by long chain alkanes in water yields too high solubilities for long chain linear alkanes [28, 29]. Formation and transport of small aggregates of hydrocarbons (accomodation) in micropores in the soil could be hindered. This would mean that only truely soluted hexadecane diffuses through the pore water in the micropores to the bulk solution. Consequently a very much smaller value for the solubility must be used to calculate the sorption coefficient of hexadecane on organic matter. A larger sorption coefficient would result, according to equation 5.

The sorption coefficients for hexadecane on both types of clay are nearly the same, well within the error margin. Therefore a lumped coefficient can be used for clay: $K_p(clay)=1x10^{11}$ l/kg.

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Consequently, it is not needed to determine the type of clay for the use of the model. The values for the respective sorption coefficients for hexadecane are larger than found elsewhere [10, 11, 14, 30]. This can be due to the very low solubility of hexadecane, which is related to the low sorption coefficient for organic matter, according to equation 5.

The ratio of the sorption coefficients for HCH is in both cases nearly the same, K_p(o.m.)/K_p(clay)=0.023 for Weber and Miller, and K_p(o.m.)/K_p(clay)=0.020 for Rijnaarts et al.. The partition coefficients for both sets of data are close, fo=0.48 and fo=0.52, respectively, corresponding to the organic matter content which also differs only slightly, 1.14% and 1.1%. These facts and the correspondence between the sorption coefficients for organic matter that were fitted and calculated, indicate that the approach outlined here may be adequate. The ratio for the sorption coefficients for hexadecane is much smaller, K_p(o.m.)/K_p(clay)=4.2x10^{-4}. The partition coefficient that was fitted on data from the desorption and degradation experiment with diesel oil is different from the HCH data, fo=0.72. This is in agreement with the fact that the organic matter content is larger (13% instead of 1%).

A relation similar to equation 5 can probably be found for the sorption coefficients for clay, too. More experimental results of sorption to clays are needed to produce an equation to predict the sorption coefficient of a nonpolar component onto clay. Furthermore an equation for the partitioning between organic matter and clay is needed. When these two are known the model proposed here can predict concentrations of nonpolar components in soil, without any parameters that must be fitted. Oil concentrations can then be estimated without any prior degradation experiments.

Consequences for microbial decontamination of soil in a slurry plant

The most obvious method of increasing the overall oil degradation rate in a soil slurry plant, is to first treat the soil as a whole, to degrade the oil that is readily available (typically 60-70%). After this step, the clean fraction must be separated from the still polluted fraction, which generally consists largely of silt, clay and organic matter. These two operations are combined in a recently developed slurry reactor, called DITS-reactor [31]. When the polluted fraction is to be treated further in the plant, it is expedient to reduce the particle size as much as possible. If the mechanism underlying the model is correct, then the half time for transport out of a particle aggregate with radius R is [14]:

$$t_{0.5} = 0.03 * \frac{R^2}{D_{eff}} \qquad (7)$$

Reduction of the particle size will reduce the diffusion length, and thus the characteristic time. The half time for desorption of oil from clay particles with a diameter of 4 μm is calculated at 167 days. In contrast, for a particle diameter of 1 μm the half time is 'only' 10.4 days. In this way the treatment time for the microbiological decontamination of oil polluted soils will be decreased. This reduction in treatment time should be enough to compensate for the high costs of crushing the fine soil fraction.

Alternatively, the cleaning of the polluted soil can also be performed in a landfarm. Then the use of the slurry reactor as a first treatment step is still attractive, because:

1)   a large part of the contamination has already been removed very fast,

2)   the amount of soil that is to be treated in the landfarm has been reduced considerably,

3)   the nutrients necessary for microbial activity have already been added,

4)   the concentration and activity of oil degrading microorganisms in the soil is high.

Adsorption-desorption hysteresis

Many researchers have found hysteretic isotherms for adsorption and desorption [e.g. 5, 16]. With the sorption coefficients found here this can be easily explained. The fast adsorption takes place between water and organic matter. The sorption to clay is much slower, causing the reported very long equilibration time for nonpolar components to wet soil [7]. When this latter equilibrium has not been reached, and desorption is started, part of the contaminant will continue to adsorb to the clay. Consequently the contaminant concentration in the water phase will be lower than would be expected when only equilibrium with the organic matter is considered. This is seen as hysteresis.

Sediment/water ratio effect

Another effect that was reported, is called the sediment/water ratio effect [17]: With increasing solids concentration, the sorption coefficient K_p decreases. This can be explained by the mistaken assumption of linearity of the sorption isotherm. When the equilibrium water concentration of a component in a water/solids suspension is higher than the maximum soluble concentration, the equilibrium concentration cannot be reached. The water phase concentration will be maximal, the solid phase concentration will be higher than the equilibrium concentration. When the same amount of contaminant is used in an experiment with a solids/water ratio that is lower, then the water phase concentration will again be maximal, while the solid phase concentration will be higher than in the previous experiment. Consequently the measured sorption coefficient will be higher in the second experiment, while the solids/water ratio is lower.

CONCLUSIONS

An extended radial pore effective diffusion model is proposed for the description of the (microbial) decontamination of oil contaminated soil in a slurry. In the model the soil is thought to be split up in three separate fractions, each contributing independently to the oil concentration. The sand is not inhibiting microbial degradation of the oil. The organic matter limits the oil transport rate to the microorganisms. The two types of clay used here showed comparable sorption coefficients. Clay exhibits an extremely low transport rate, about 100 times lower than organic matter.

The model could predict the HCH concentration in the desorption from two different types of soil very well. The diesel oil concentration in a sieve fraction of a contaminated soil, containing high amounts of clay and organic matter, could be predicted well from the sorption coefficients for hexadecane.

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SYMBOLS

C	concentration of component in the water phase, [mol/m^3]
C_s(clay)	substrate concentration in the clay particles, [mol/m^3]
C_s(o.m.)	substrate concentration in the organic matter particles, [mol/m^3]
C_s(reactor)	substrate concentration in the reactor, [mol/m^3]
C_s(sand+bulk)	substrate concentration on the sand particles and in the water phase, [mol/m^3]
D	bulk diffusion coefficient, [m^2/s]
D_{eff}(clay)	effective diffusion coefficient for clay, [m^2/s]
D_{eff}(o.m.)	effective diffusion coefficient for organic matter, [m^2/s]
fc	fraction of the oil on the clay, at t=0, [-]
fo	partition coefficient, fraction of the oil on the organic matter, at t=0, [-]
fs	fraction of the oil on the sand and in the water phase, at t=0, [-]
J	flux, [mol/m^2.s]
K	dynamic equilibrium constant, [-]

k1	desorption rate constant, $[s^{-1}]$
k2	adsorption rate constant, $[s^{-1}]$
K_p(clay)	sorption coefficient for clay, $[l/kg]$
K_p(o.m.)	sorption coefficient for organic matter, $[l/kg]$
m_{bulk}	mass of the soil (fraction), $[kg]$
q	concentration of component adsorbed to the pore wall, $[mol/kg]$
r	distance from centre of the spherical particle, $[m]$
R	radius of the particle, $[m]$
S	solubility, $[mol/l]$
t	time, $[h]$
T	temperature, $[^0C]$
V_{bulk}	bulk volume of the soil (fraction), $[m^3]$
V_{slurry}	volume of the soil slurry, $[m^3]$
V_{solid}	solid volume of the soil (fraction), $[m^3]$
V_{water}	volume of the water, $[m^3]$
ε	intraparticle porosity, $[m^3$ pore volume/m^3 particle]
ρ_{bulk}	bulk density of the solid, $[kg/l]$
ρ_s	density of the solid, $[kg/l]$

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