NOBIS 96-3-04
THE INTRINSIC CAPACITY OF AQUIFERS TO
DEGRADE POLLUTION FROM (OLD) LANDFILLS

Phase 1

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September 1998

Gouda, CUR/NOBIS

Dutch Research Programme In-Situ Bioremediation

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Aantal bladzijden
Rapport: 68
Bijlagen: 32

Uitvoerende organisatie(s) (Consortium)
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Uitgever
CUR/NOBIS, Gouda

Samenvatting
Dit rapport vat de bevindingen van een literatuurstudie naar natuurlijke afbraak (NA) bij voormalige stort-
plaatsen samen. De aandacht in dit rapport gaat voornamelijk uit naar de afbraakcapaciteit van de bodems
en sedimenten rondom de stortplaats. Stortpercolaat heeft een grote reducerende capaciteit. Bij het be-
wegen door de bodem zal dit percolaat de omliggende bodem reduceren. Hierdoor ontstaat een redox-
zonering. Microverontreinigingen, aanwezig in het percolaat, worden afgebroken in specifieke redoxzones.

Recent zijn microbiologische karakterisatietechnieken ontwikkeld om de zones rondom stortplaatsen in
kaart te brengen. Met deze technieken kan enerzijds het metabolisch potentieel (BIOLOG) en anderzijds de
genetische diversiteit (DNA/RNA en DGGE) in kaart worden gebracht. Het combineren van deze nieuwe
methoden met de meer traditionele hydrologische en geochemische methoden lijkt veelbelovend te zijn
voor de toekomst.

Trefwoorden
Gecontroleerde termen:
landfill, literature review, natural degradation

Vrije trefwoorden:

Titel project
The intrinsic capacity of aquifers to degrade
pollution from (old) landfills

Projectleiding
Provincie Zuid-Holland
(F.J. van der Ham, 070-4416611)

Dit rapport is verkrijgbaar bij:
CUR/NOBIS, Postbus 420, 2800 AK Gouda

Report title	CUR/NOBIS report number
The intrinsic capacity of aquifers to degrade pollution from (old) landfills	96-3-04
Phase 1	**Project report number**
	96-3-04 phase 1

Author(s)
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Number of pages
Report: 68
Appendices: 32

Executive organisation(s) (Consortium)
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Publisher
CUR/NOBIS, Gouda

Abstract
This report summarizes the findings from a literature study on natural attenuation (NA) at old landfills. The focus of this study is the attenuation capacity of the soils and aquifers surrounding the landfill. The landfill leachate has a large reduction capacity. As it moves through the soil, it will reduce the surrounding aquifer resulting in sequentially distributed redox zones. The process is catalysed by micro-organisms. Micro-pollutants in the leachate plume tend to be degraded in specific redox zones.

Microbiological techniques to characterize the zones surrounding a landfill have been developed recently. These techniques either characterize the metabolic potential of the population of micro-organisms (BIOLOG) or genetic diversity (DNA/RNA and DGGE). Combining these with the more traditional hydrological and geochemical approaches seems promising.

Keywords
Controlled terms:
landfill, literature review, natural degradation

Uncontrolled terms:

Project title	Projectmanagement
The intrinsic capacity of aquifers to degrade pollution from (old) landfills	Provincie Zuid-Holland (F.J. van der Ham, 070-4416611)

This report can be obtained by: CUR/NOBIS, PO Box 420, 2800 AK Gouda, The Netherlands
Dutch Research Programme In-Situ Bioremediation (NOBIS)

PREFACE

With this report, the first phase in the NOBIS project 'Feasibility project in situ bio restoration of landfills' project number 96-3-04, is closed. The aim of the project is to develop a method for determining the intrinsic degradation capacity of the soil and groundwater in order to assess how it affects environmental risks. This project is carried out by a consortium, consisting of Free University Amsterdam, Province of South-Holland and IWACO B.V.

September 1998

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SAMENVATTING

The intrinsic capacity of aquifers to degrade pollution from (old) landfills

Dit rapport is een samenvatting van de literatuurstudie naar de huidige kennis op het gebied van karakterisatie van stortpercolaat in de bodem en aquifers onder en rond stortplaatsen. De literatuurstudie maakt onderdeel uit van het 'Haalbaarheidsproject in situ biorestauratie bij stortplaatsen. NOBIS-project 96-3-04'. Dit project heeft als doel het ontwikkelen van een methode om de intrinsieke afbraakcapaciteit van de bodem te bepalen en te gebruiken om zo de risico's voor het milieu te minimaliseren. Het betreft bodem verontreinigd bij storts door percolaatuittreding met macro- en microverontreinigingen.

Processen rond stortplaatsen
In de literatuur zijn diverse rapporten aangetroffen waarbij percolaat wordt afgebroken tijdens verspreiding door de aquifer. Met name het instituut Environmental Science and Engineering van de technische universiteit van Denemarken (Lyngby, Denemarken) heeft uitgebreid onderzoek gedaan naar het gedrag van percolaat in de bodem rondom 2 Deense stortplaatsen, Vejen en Grindsted. Natuurlijke afbraak vond in ruime mate plaats nabij deze stortplaatsen. De opzet van onderhavig literatuuronderzoek is gebaseerd op en een vervolg van de inventarisatie gepubliceerd door Christensen et al. [1994]. Dit rapport omvat de meest relevante informatie voor het NOBIS-project.

Om de degradatie van stortpercolaat te kunnen begrijpen, moet men weten wat er gebeurt met het percolaat als het zich door de ondergrond verspreid. Stortpercolaat is een waterige oplossing dat hoge concentraties aan opgelost organisch materiaal (DOC) en een grote verscheidenheid aan andere opgeloste stoffen bevat (o.a. microverontreinigingen). Het percolaat heeft een erg hoge reducerende capaciteit (RDC) ten gevolge van het hoge organische stofpercentage. Zich verplaatsend door de ondergrond reduceert het percolaat het geoxideerde bodemmateriaal waarmee het in aanraking komt. De oxidatie van het percolaat en daarmee samenhangend het reduceren van de ondergrond is primair microbiologisch bepaald.

Andere processen, waaraan percolaat onderhevig is, zijn: verdunning, dichtheiddstroming, sorptie, dispersie, diffusie, microbiologische afbraak, reductie/oxidatieprocessen, oplossing en neerslag enzovoorts. Het resultaat van deze processen is de ontwikkeling van redoxzones stroomafwaarts van de stort. De verdeling van de redoxzones is zo dat de meest gereduceerde zone, de methanogene zone, zich het dichtst bij de stort bevindt. Meer stroomafwaarts bevinden zich achtereenvolgens de minder gereduceerde zones, zoals de sulfaatreducerende zone, de ijzer(III) en mangaan(IV) reducerende zone, de nitraatreducerende zone en ten slotte de aërobe zone.

Microverontreinigingen komen ook voor in stortpercolaat, maar komen in het algemeen in veel lagere hoeveelheden voor dan de macroparameters en het opgelost organische materiaal (DOC). Hierdoor spelen deze verontreinigingen geen belangrijke rol bij het ontstaan en in standhouden van de redoxzones. Wel worden de meeste van deze verontreinigingen afgebroken onder de specifieke redoxcondities die aanwezig zijn onder storts. Momenteel wordt veel onderzoek verricht om het inzicht in dit proces te vergroten.

De redoxchemie van ijzer lijkt erg belangrijk te zijn. De ijzer(III) reducerende zone van de Grindsted- en Vejen-stortplaatsen in Denemarken is relatief groot en Heron [1994] heeft vastgesteld dat ijzer wordt gerecycled in de percolaatpluim. IJzer(III) wordt gereduceerd tot ijzer(II) dat veel beter oplosbaar in water is. IJzer(II) verspreidt zich met het grondwater naar de grens van de

pluim waar het reoxideert in een minder gereduceerd milieu. Vervolgens is het daar weer beschikbaar om het percolaat te oxideren.

Het percolaat zelf heeft een aanzienlijke oxidatiecapaciteit (OXC) ten gevolge van de opgeloste stoffen. Daardoor kan, in situaties waarbij een stort een aquifer verontreinigt met een relatief lage OXC, toch biologische afbraak optreden. De elektronenacceptoren zijn dan van het percolaat zelf afkomstig.

Het verspreidingsgedrag van een percolaatpluim is erg ingewikkeld. Deze complexiteit wordt vergroot door de heterogeniteit van de bodem en de aquifers. De karakterisatie van de stort, de percolaatpluim en de (potentieel) verontreinigde bodem om de biologische afbraakcapaciteit te kunnen bepalen, is hierdoor een moeilijke taak. In dit rapport worden de meest gebruikte en veelbelovende nieuwe technieken en methoden beschreven. Relatief nieuwe moleculair biologische technieken, gebaseerd op de karakterisatie van het genetisch potentieel van de microbiologische populaties in stortpercolaat, worden uitgebreid behandeld. Verder worden waterstofmetingen beschreven, geschikt om het elektronenacceptatieproces af te bakenen, hetgeen een geschikte methode lijkt te zijn voor het afperken van de percolaatpluim bij storts.

Karakterisatie van de natuurlijke afbraakcapaciteit van stortpercolaat
Natuurlijke afbraak van stortpercolaat kan plaatsvinden als de bodem en aquifer een aanzienlijke oxidatiecapaciteit (OXC) hebben. Verder, als er een aanzienlijke OXC of OXC-instroom is, dient er een microbiologisch potentieel beschikbaar te zijn voor de specifieke xenobiotische verontreinigingen. Als de OXC en de microbiologische potentie in voldoende mate aanwezig zijn, worden de processnelheden gemeten om de tijd- en ruimteschaal van de afbraak van de percolaatpluim te bepalen.

Het karakterisatie-onderzoek wordt als volgt samengevat:

1. Bepaal de oxidatiecapaciteit (OXC) van de niet-beïnvloede aquifer (door middel van grond en grondwaterbemonstering).
2. Bepaal de totale reductiecapaciteit (RDC) van het stortpercolaat.
3. Bepaal de afmetingen van de percolaatpluim. Een goede indicator is de elektrische geleidbaarheid (Ec) of het chloridegehalte. De Ec van het percolaat is in het algemeen veel hoger dan van het niet-beïnvloede grondwater. De organisch stofpluim (TOC) zal in het algemeen veel kleiner zijn dan de Ec-pluim ten gevolge van afbraak.
4. Bepaal de algemene geohydrologie in de omgeving van de stort. Modellen kunnen worden gebruikt voor het schatten van de OXC- en RDC-fluxgrootte.
5. Bepaal de locaties waar percolaat naar de ondergrond weglekt (in het algemeen erg moeilijk in verband met de heterogeniteit van storts).
6. Als de aquifer een OXC heeft die voldoende groot is of de OXC wordt voldoende snel aangevuld vanuit de stort om een belangrijk deel van het percolaat af te breken, moet de omvang van de percolaatpluim worden bepaald. Hiervoor kunnen simpele numerieke modellen worden gebruikt. Als de OXC niet groot is, zal de natuurlijke afbraak ook beperkt zijn en zullen andere (sanerende) maatregelen moeten worden genomen.
7. Om de voorspellingen te verbeteren, en om er zeker van te zijn dat toxische verontreinigingen worden afgebroken, moeten laboratoriumproeven gericht op de karakterisatie van de microbiologische afbraakpotentie en de mogelijke afbraaksnelheden worden uitgevoerd. Indien mogelijk, moeten ook in situ experimenten worden uitgevoerd.
8. Als wordt gekozen voor natuurlijke afbraak van stortpercolaat, moet een monitoringssysteem worden geïnstalleerd om de verwachtingen te controleren en de voorspellingen te verbeteren.

Microbiologische karakterisatietechnieken
De detectie van genen, die indicatief zijn voor biodegradzatie, is op het moment niet mogelijk. Wel beschikbaar is de test Random Amplified Polymorphic DNA (RAPD), die geschikt is voor initiële experimenten, om een indicatie te verkrijgen van de biodiversiteit. Verder is de proef Denaturing Gradient Gel Electrophoresis (DGGE) van 16S rRNA beschikbaar; deze werkt goed voor bosbodems en geeft een complex en meer informatief profiel. De DGGE-proef zal verder gaan worden gebruikt in dit project.

Laboratorium microcosms worden gebruikt om de biologische afbraakpotentie te bepalen. Om de biologische afbraakpotentie te kunnen relateren aan de biodiversiteit, lijkt het prepareren van *microcosms*, zoals Johnston et al. [1996] dat heeft gedaan, geschikt. Hiertoe wordt 1 monster verspreid over een groot aantal flesjes en geënt met verontreinigingen. In de tijd worden monsters genomen door iedere keer 2 flesjes te bemonsteren en te analyseren. De concentratie aan verontreinigingen in het bodemvocht wordt bepaald. Het sediment kan worden gebruikt om het DNA en RNA uit te extraheren en een oplossing te maken voor gebruik in BIOLOG-platen om de metabole capaciteit te bepalen.

Door het toevoegen van een kleine hoeveelheid verontreiniging wordt een soort verrijkingscultuur onder redelijk natuurlijke omstandigheden verkregen. Stimulatie resulteert ofwel in groei (meer DNA) of hogere activiteit per cel (meer ribosomes, dat wordt afgeleid uit het rRNA-niveau). Dit soort verrijking verschilt sterk van de traditionele verrijkingsculturen, waarbij monsters worden toegevoegd aan voedingsmedia met relatief hoge concentraties substraten en strengen worden geïsoleerd na diverse cultivatieronden. Door het uitvoeren van DGGE en RNA, of DNA te gebruiken als een indicator, kan een tijdspecifiek profiel worden verkregen, dat wordt gerelateerd aan de verdwijning van verontreinigingen in de batches en voorgaande profielen.

Laboratorium batch microcosms (LBM) en in situ mesocosms (ISM)
Laboratorium batch cosm (LBM) incubatieproeven kunnen volgens de onderzoeksgroep van Christensen worden uitgevoerd. Dit houdt in het aanmaken van 1 grote microcosm waaruit in de tijd grondwatermonsters voor analyse worden genomen. De proeven kunnen ook worden uitgevoerd zoals omschreven door Johnston; dit houdt in het aanmaken van een groot aantal microcosms afkomstig van 1 bodemmonster. In de tijd worden deze cosms bemonsterd. Beide methoden hebben voor- en nadelen ten opzichte van elkaar. Wanneer men echter bodemmonsters wil analyseren gedurende de incubatieperiode (bijvoorbeeld voor het karakteriseren van de microbiologische populatie) heeft de methode van Johnston de voorkeur. Daar staat tegenover dat, indien de microbiologische populatie in de bodem en het grondwater vooraf wordt vergeleken, ook de methode van Christensen kan worden gebruikt.

Tijdens de aanvangsperiode van dit project heerste de gedachte ISM's te gebruiken voor het meten van de afbraak-(snelheden) onder in situ omstandigheden. Deze methode is geoptimaliseerd door de groep van Christensen. Nadat informatie was verkregen van Christensen tijdens het studiebezoek aan Denemarken, werd duidelijk dat ISM's technisch lastig zijn toe te passen, met name de installatie en bemonstering. Afbraaksnelheden gemeten met een LBM of ISM kunnen tot een factor 5 verschillen. ISM's, die dicht bij elkaar in de bodem zijn geplaatst, geven ook soortgelijke verschillen. De experts van de groep van Christensen raden daarom aan om eerst proeven te doen met LBM's. Een meer gedetailleerde studie naar de ISM's wijst verder uit dat deze techniek niet erg geschikt is voor het in situ meten van de biologische afbraak, omdat er geen (fysiek) interactie/contact is tussen de ISM en de omgeving. Alleen de temperatuur is gelijk aan de omgeving. Doorstroming van de ISM vindt niet plaats. Dit kan een belangrijke parameter zijn voor de afbraak: een grondwaterstroming van 10 m/jaar, gemeten nabij de Coupépolder, komt overeen met 0,5 volumeveranderingen per dag in de ISM.

De natuurlijke situatie kan ook worden gesimuleerd door het gebruik van doorstroomkolommen in het laboratorium. Bij deze methode is de kolom gevuld met bodemmateriaal van een bepaald bemonsteringspunt. Grondwater genomen op hetzelfde bemonsteringspunt wordt geënt met verontreinigingen en geleid door de kolom met een snelheid die zoveel mogelijk overeenkomt met de natuurlijke grondwatersnelheid. Omdat de temperatuur in de bodem onder een stort redelijk constant is, kunnen deze experimenten eenvoudig worden uitgevoerd in het laboratorium. In principe kan het effluent van 1 kolom worden gebruikt als influent voor de volgende kolom. Op deze manier kunnen kolommen met bodemmonsters van verschillende redoxzones in serie worden geplaatst voor de simulatie van biologische afbraak onder storts. Effluent kan worden bemonsterd en geanalyseerd om de afname van verontreinigingen te bepalen.

Kolommen leveren vermoedelijk meer informatie op dan ISM's, waardoor in dit project wordt overwogen kolommen te gaan toepassen. Het kortetermijngevolg voor het project is dat gedurende fase 2 ervaring moet worden opgedaan met kolommen in plaats van met in situ mesocosms. Daarnaast is het belangrijk om dit idee verder te onderzoeken, omdat de concentratieveranderingen ten gevolge van natuurlijke afbraak erg klein zijn en onder veldomstandigheden over grote afstanden plaatsvinden (enkele honderden meters). De vraag is of we in staat zullen zijn zulke kleine concentratieverschillen te meten in een laboratorium.

Modellen voor de simulatie van natuurlijke afbraakprocessen
Gedurende de laatste jaren zijn er vele computermodellen ontwikkeld, die de grondwaterstroming inclusief biologische afbraak en andere stoftransportprocessen, zoals sorptie, kunnen simuleren. Deze variëren van indicatieve modellen, zoals BIOSCREEN, tot meer geavanceerde afbraakmodellen, zoals BIOPLUME 3 (in ontwikkeling) die stroombaanberekeningen van de elektronenacceptoren kan uitvoeren.

Modellen die het reactieve transport simuleren, zoals PHREEQC, PHAST en HYDROGEOCHEM kunnen erg nuttig zijn voor het modelleren van de redoxzones stroomafwaarts van storts. De resultaten van deze modellen (ruimtelijke verdeling van de redoxzones) kunnen worden gebruikt als invoer voor modellen die de specifieke afbraak van verontreinigingen kunnen berekenen.

De grondwaterstroming rond de Coupépolder kan worden gemodelleerd met TRIWACO of MICROFEM; het modelleren van sorptie en afbraak langs berekende stroombanen kan worden uitgevoerd met SORWACO. De geochemische reacties kunnen worden gemodelleerd met PHREEQC. Deze stroombaangerichte aanpak wordt ook gebruikt door het model MODFLOW/RT3D; dit model is echter door zijn modulaire opbouw minder geschikt. Een nadeel van de stroombaanaanpak is dat verdunning van de percolaatpluim niet kan worden gesimuleerd; de transversale dispersie wordt immers buiten beschouwing gelaten. Het driedimensionale model HYDROGEOCHEM en het tweedimensionale model FLONET/TRANS kunnen de transversale dispersie wel berekenen.

Aanbevolen wordt als eerste de modellen TRIWACO/MICROFEM, FLONET/TRANS, SORWACO en PHREEQC te gebruiken voor het simuleren van de processen rond de stort Coupépolder.

Voor de integratie van hydrogeochemische processen en microbiologische processen vormt het maken van een koppeling tussen een hydrogeochemisch model en een Metabol Control Analysis (MCA) een uitdaging voor de toekomst.

SUMMARY

The intrinsic capacity of aquifers to degrade pollution from (old) landfills

This report is a summary of a literature review of the present state of knowledge in the field of characterization of landfill leachate in the soil and aquifers below and near landfills. The literature review forms part of the 'Feasibility project in situ bio restoration of landfills', NOBIS (Dutch Research Programme In-Situ Bioremediation) project number 96-3-04. The aim of the project is to develop a method for determining the intrinsic degradation capacity of the soil and ground-water and to use it to minimize environmental risks. This applies to soils and groundwater polluted by landfills from which leachate containing macro- and micropollutants is migrating.

Processes around landfills
In the literature several reports were found which deal with the degradation of leachate during dispersion by an aquifer. The Institute of Environmental Science and Engineering of the Tech-nical University of Denmark (Lyngby, Denmark) in particular has carried out extensive research into the behaviour of leachate in the subsurface around 2 Danish landfills: Vejen and Grindsted. A considerable degree of intrinsic natural degradation was found to occur near these landfills. The objective of the present literature survey is based on, and forms a follow-up to, the review published by Christensen et al. [1994]. This report covers the information most relevant to the NOBIS project.

In order to understand the degradation of landfill leachate, it is necessary to know what is hap-pening to the leachate as it migrates through the subsurface. Landfill leachate is an aqueous solution containing high concentrations of dissolved organic compounds (DOC = Dissolved Or-ganic Carbon) together with wide variety of other dissolved compounds (including micro-pollutants). Because of its high content of organic carbon, the leachate has a very high reduction capacity (RDC). As it migrates through the subsurface the leachate reduces the oxidized material with which it comes into contact. The oxidation of the leachate and the accompanying reduction of the subsurface is primarily controlled by microbiological processes.

Other processes acting on the leachate are: dilution, density flow, sorption, dispersion, diffusion, microbiological degradation, reduction/oxidation processes, solution and precipitation, etc. The result of these processes is the development of a number of redox zones downgradient from the landfill. The redox zones are distributed such that the most reduced one, the methanogenic zone, occurs closest to the landfill. It is followed downgradient by successively less reduced zones, e.g. the sulphate reducing zone, the $Fe(III)$ and $Mn(IV)$ reducing zone, the nitrate reducing zone and finally the aerobic zone.

Micropollutants are also present in landfill leachate but usually in much lower concentrations than the macro parameters and the dissolved organic carbon (DOC). Because of this these pollutants do not play an important role in the creation and preservation of the redox zones. Most of these pollutants are however degraded under the specific redox conditions present below landfills. Much research is being carried out with the aim of gaining a better understanding of this process.

The redox chemistry of iron appears to play a very important role. The $Fe(III)$ reducing zone of the Grindsted and Vejen landfills in Denmark is relatively large and Heron [1994] has established that iron is being recycled in the leachate plume. $Fe(III)$ is reduced to $Fe(II)$ which is much more soluble in water. The $Fe(II)$ then migrates with the groundwater to the edges of the plume where it reoxidizes in a less reducing environment. Subsequently it is again available there to oxidize further leachate.

The leachate itself has a considerable oxidation capacity (OXC) because of the compounds dissolved in it. This means that in situations where a landfill pollutes an aquifer with a relatively low OXC, biological degradation may still occur. In this case the electron acceptors are derived from the leachate itself.

The dispersion behaviour of a leachate plume is very complicated. This complexity is further increased by the heterogeneity of the soil and the aquifers. Characterization of the landfill, the leachate plume and the (potentially) polluted soil and groundwater to determine the biological degradation capacity is therefore a difficult task. In the present report the most commonly used and promising new techniques and methods are described. Relatively new molecular biological techniques based on the characterization of the genetic potential of the microbiological populations in landfill leachate are dealt with in detail. A description is also given of hydrogen measurements suitable for delineating the electron acceptance process, which seems a promising method for demarcating the leachate plume at landfills.

Characterization of the intrinsic degradation capacity of landfill leachate
Intrinsic degradation of landfill leachate can occur when the soil and aquifer have a substantial oxidation capacity (OXC). In addition to a substantial OXC (or OXC inflow) there must also be a microbiological potential available for the specific xenobiotic pollutants. If there is both a sufficient OXC and microbiological potential the process rates are measured to determine the time and spatial scales at which degradation of the leachate plume takes place.

The characterization research can be summarized as follow:

1. Determine the oxidation capacity (OXC) of the unaffected aquifer (by sampling the soil and groundwater).
2. Determine the total reduction capacity (RDC) of the landfill leachate.
3. Determine the dimensions of the leachate plume. A good indicator is the electrical conductivity (Ec) or the chloride content. The Ec of the leachate will as a rule be much higher than that of the unaffected groundwater. The plume of organic compounds (TOC = Total Organic Carbon) will usually be much smaller than the Ec plume because of degradation.
4. Determine the general geohydrology in the immediate vicinity of the landfill. Models can be used for estimating the size of the OXC and RDC fluxes.
5. Locate the spots where the leachate is seeping into the subsurface (usually very difficult because of the heterogeneous nature of landfills).
6. If the aquifer has a sufficiently high OXC, or if the OXC is being replenished from the landfill at a rate sufficient to degrade a significant portion of the leachate, the extent of the leachate plume must be determined. This can be done using simple numerical models. If the OXC is not high, intrinsic degradation will also be limited and other (remediation) measures will have to be used.
7. To improve predictions and to be sure that toxic pollutants are being degraded, laboratory tests aimed at characterizing the microbiological degradation potential and the rates at which degradation (if any) takes place should be performed. If possible in situ experiments should also be carried out.
8. If intrinsic degradation of landfill leachate is to be applied it is necessary to install a monitoring system to check the expected results and to improve future predictions.

Microbiological characterization techniques
At present it is not possible to detect genes that are indicative of biodegradation. There is, how-ever, a Random Amplified Polymorphic DNA (RAPD) test that is suitable for preliminary experi-ments aimed at obtaining an indication of the biodiversity. In addition there is also a Denaturing Gradient Gel Electrophoresis (DGGE) test available for 16S rRNA, which works well for woodland soils and gives a complex and more informative profile. The DGGE test will be used during the continuation of this project.

Laboratory microcosms are used to determine the biological degradation potential. To relate the biological degradation potential to the biodiversity the method of preparing *microcosms* used by Johnston et al. [1996] appears suitable. It involves distributing one sample over a large number of bottles and spiking it with pollutants. Samples are collected as a function of time by sampling and analysing 2 bottles at a time. The concentration of pollutants in the soil moisture is deter-mined. The sediment can be used for extracting the DNA and RNA and for preparing a solution to be used with BIOLOG plates to determine the metabolic capacity.

By adding a small quantity of pollution a sort of enrichment culture is obtained under reasonably natural conditions. Stimulation results in either growth (more DNA) or higher activity per cell (more ribosomes, as deduced from rRNA levels). This type of enrichment culture differs sub-stantially from traditional enrichment cultures in which samples are added to growth media with relatively high concentrations of substrates and where strains are isolated after various rounds of cultivation. By using the DGGE test for RNA or by using DNA as an indicator it is possible to draw up a time-specific profile which can then be related to the disappearance of the pollutants in the batches and previous profiles.

Laboratory batch microcosms (LBM) and in situ mesocosms (ISM)
Laboratory batch cosm (LBM) incubation tests can also be carried out according to Christensen's research group. This involves preparing 1 large microcosm from which groundwater samples are taken for analysis as a function of time. The tests can also be performed as described by Johnston; this involves preparing a large number of microcosms derived from 1 soil sample. The cosms are sampled as a function of time. Both methods have their advantages and disad-vantages. If, however, one wants to analyse soil samples during the incubation period (for characterizing the microbiological population for instance) the Johnston method takes prefer-ence. If, however, the microbiological population in the soil and in the groundwater is compared in advance the Christensen method can also be used.

In the initial stages of the project, the idea was to use ISMs for measuring the degradation (rates) under in situ conditions. This method has been refined by Christensen's group. From information provided by Christensen during a visit to Denmark it became clear that there are practical difficulties in using ISMs, particularly in installing and sampling them. Degradation rates measured by a LBM and an ISM can differ by a factor of 5. ISMs positioned close together in the ground also give similar discrepancies. The experts of Christensen's group therefore recom-mended first doing tests using LBMs. A more detailed study of ISMs further indicated that the technique is not very suited for measuring biological degradation in situ because there is no (physical) interaction/contact between the ISM and its surroundings. Only the temperature is the same as that of the surroundings. There is no flow through the ISM. This could be an important parameter in degradation: a groundwater flow of 10 m/year as measured below the Coupépolder corresponds to 0.5 volume changes per day in the ISM.

The situation in nature can also be simulated by using continuous flow columns in the laboratory. This method involves filling the columns with soil material from a particular sampling point. Groundwater collected at the same sampling point is spiked with pollutants and passed through

the column at a speed as close as possible to the real rate of flow of the groundwater. Because the subsurface temperature below a landfill is reasonably constant these experiments are quite simple to perform in the laboratory. In principle the effluent from a particular column can be used as the influent for the next column. In this way columns with soil samples from a number of redox zones can be placed in series to simulate the biological degradation processes under landfills. Effluent can be sampled and analysed to determine the decrease in pollutants.

Columns will probably yield more information than ISMs so that it is now being considered to use columns for this project. The short-term consequences for the project are that in the course of Phase 2 it will be necessary to gain experience with columns rather than with in situ mesocosms. It is also important to take a closer look into this idea because the changes in concentrations resulting from natural degradation are very small and in the field take place over large distances (several hundreds of metres). The question which arises is whether it will be possible to measure such small concentration differences in a laboratory.

Models for simulating intrinsic degradation processes
In recent years many computer models have been developed that can simulate groundwater flows, including biological degradation and other dissolved-compound transport processes such as sorption. These range from indicative models such as BIOSCREEN to more advanced degradation models such as BIOPLUME 3 (under development) which can perform electron acceptor flow path computations.

Models able to simulate reactive transport, such as PHREEQC, PHAST and HYDROGEOCHEM, can prove very useful for modelling the redox zones downgradient from landfills. The results of these models (spatial distribution of the redox zones) can be used as input for models which can compute the specific degradation of pollutants.

The groundwater flow in the vicinity of the Coupépolder can be modelled using TRIWACO or MICROFEM, modelling sorption and degradation along the computed flow paths can then be done with SORWACO. PHREEQC can be used to model the geochemical reactions. The model MODFLOW/RT3D uses a similar flow-path oriented approach but the modular structure of this model makes it less suitable. A disadvantage of the flow-path approach is that it is not possible to simulate dilution of the leachate plume because transverse dispersion is not taken into account. The 3-dimensional model HYDROGEOCHEM and the 2-dimensional model FLONET/TRANS can compute the transverse dispersion.

It is recommended to start of using the models TRIWACO/MICROFEM, FLONET/TRANS, SORWACO and PHREEQC for simulating the processes in the vicinity of the Coupépolder landfill.

To integrate the hydrogeochemical and microbiological processes it is necessary to establish link a between a hydrogeochemical model and a Metabol Control Analysis (MCA). This remains a challenge for the future.

CHAPTER 1

INTRODUCTION

Contamination of groundwater by landfill leachate is considered to be one of the major environmental concerns related to landfills. In the Netherlands there are about 3000 old (closed) landfills which constitute a major risk to the environment. Although the contents of these landfills consist for 90 % of household and building waste it is well known that illegal dumping of extremely toxic waste has occured. The precise location of this toxic waste is unknown and it is because of this that landfills are considered to be a chemical time bomb.

Because of the immense costs associated with the maintanance of these old landfills so that risks to the environment are reduced to acceptable levels, we have become interested in the possibility of intrinsic (bio) degradation of pollutants in landfill leachate plumes. The basic hypothesis in this project is that aquifers have an intrinsic capacity to degrade pollutants. A result of this intrinsic degradation will be that the spreading of the leachate plume will cease and may eventually be reversed so that the size of the plume may decrease. As a result risks to the environment will decrease too.

Knowledge of the underlying processes allows us to estimate the intrinsic degradation capacity of aquifers. This knowledge may even help us to develop extensive landfill management technology such as biogeochemical degradation screens down stream of the landfill.

Currently a feasibility project is being carried out within the Dutch research program NOBIS (Dutch Research Programme In-situ Bioremediation). The title of the project is 'Feasibility project in situ bio restoration of landfills. NOBIS project 96-3-04'. The goal of this project is to develop a general applicable method for characterizing the intrinsic capacity of the soil and underlying aquifers surrounding landfills to degrade any possible pollution from the landfill.

Within the first phase of the project a literature study has been undertaken. This report is a summary of the findings in the literature and as such can be seen as a state of the art. The approach chosen by us for presenting the results is focused on methods suitable for characterizing the pollution from the landfill and the intrinsic degradation capability of the surrounding soils.

This state of the art takes the review by Christensen et al. [1994] as a starting point. Christensen et al. [1994] presented an excellent overview of the attenuation of landfill leachate pollutants in aquifers. This paper is shortly summarized after which the current report gives an update based on the recent literature. The prime focus of this review are measurement approaches in relation to the processes occuring in aquifers surrounding landfills.

First an outline of the general processes that attenuate landfill leachate is given. Then we focus on several approaches for characterizing the processes that attenuate the leachate. Models used to describe some general features of leachate behaviour in soil and aquifers are presented and finally the report is summarized and conclusions are given on the characterization of the natural attenuation capacity of aquifers.

In order to be complete, we have referred to most references found. Sometimes we chose to included a detailed reference that we found in the literature eventhough we did not consult the reference ourselves. These references are marked with accolades { } instead of brackets [ ].

CHAPTER 2

LANDFILLS ENCOUNTERED IN THE LITERATURE

Research carried out on a number of landfills has been reported in the literature. Table 1 (see appendix B) gives a summary of the landfills discussed in the review by Christensen et al. [1994] supplemented with information from the thesis of Heron [1994] and other literature.

The development of a landfill leachate plume is the result of many interacting physical and bio-geochemical processes. Table 1 (see appendix B) characterizes the plumes reported in literature in terms of landfill age, landfill size, plume size, contaminants measured, etc. Remarkable is that all the plumes are located in sandy aquifers. The lack of reports on leachate plumes in the more complicated geological till deposits and fractured consolidated sediments is probably due to the technical complications and vast demands for economic resources associated with such studies.

Most plumes have lengths of less than 1000 m, probably due to dilution of contaminants. Some plumes have become stationary, i.e. the chloride plume does not develop any further. Most plumes are narrow and undergo little horizontal mixing. An exception is the plume at the Borden landfill, Canada, which is much wider than the landfill. The mixing is supposedly caused by the seasonal changes in flow directions. Mixing and dilution of leachate with groundwater is primarily governed by local variation in hydrogeology.

Figure 1 (see appendix A) gives an example of redox zones in a leachate plume delineated at the Grindsted landfill [Ludvigsen et al., 1995].

GENERAL BACKGROUND ON PROCESSES ATTENUATING LANDFILL LEACHATE

3.1   Leachate composition

Landfill leachate may be characterized as a water-based solution of four groups of pollutants [Christensen et al., 1994]:

1. Dissolved organic matter, expressed as chemical oxygen demand (COD) or total organic carbon (TOC), including methane. The nature of the organic matter in the leachate depends on the phase of the waste stabilization. In the acid phase high concentrations of easily degradable compounds as volatile fatty acids are found, whereas in the methanogenic phase the ratio between the biological oxygen demand and the COD (BOD_5/COD) is much lower indicating that the organic matter in the leachate is much less degradable.
2. Anthropogenic specific organic compounds (ASOCs) from household or industrial chemicals present in the waste. The ASOCs occur in relatively low concentrations (usually less than 1 mg l^{-1}). These compounds include aromatic hydrocarbons, phenols, and chlorinated aliphatics.
3. Inorganic macro components (Ca^{2+}, Mg^{2+}, Na^+, K^+, NH_4^+, Fe^{2+}, Mn^{2+}).
4. Heavy metals (Cd, Cr, Cu, Pb, Ni, Zn).

Of course many other compounds may be found in leachate from landfills (e.g., borate, sulphide, arsenate, selenate, barium, lithium, mercury and cobalt), but, in general these compounds are only of secondary importance as they occur in very low concentrations. Table 2 (see appendix B) is taken from Christensen et al. [1994] and gives an overview of the concentration ranges found in the literature.

3.2   Variation of pollution within landfills; landfill source characterization

The history of the Vejen landfill [Kjeldsen, 1993] and the Grindsted landfill [Kjeldsen et al., 1998a] was investigated using old maps, aerial photographs and by interviews. The waste volume and age of the different areas of the landfill were evaluated using aerial photographs taken 5 times between 1954 - 1980 [Kjeldsen et al., 1998a].

The historical investigation of the Vejen landfill [Kjeldsen, 1993] indicated that the permeability beneath the waste was heterogeneous and that leaching from the landfill could be unevenly distributed. Only a minor part (8 %) of this landfill was a significant source of leachate that polluted the groundwater. The leaching to the groundwater showed significant seasonal and yearly variations. These variations can and could have been of importance in determining the development of redox zonation in the affected aquifer [Lyngkilde and Christensen, 1992a].

The precise location of the pollution in the leachate plume down stream is highly affected by the pronounced spatial variability of the pollution in the landfill [Assmuth, {1992}; Kjeldsen et al., 1998a]. Thus for a cost effective delineation of the downstream pollution plume, information on the location of the main sources is required.

3.3   Processes

Leachate from a landfill is subjected to a large number of processes as it moves through the soil and aquifer downgradient from the landfill. Processes that play an important role are among others, density dependent flow, dilution, dispersion, and biogeochemical reactions which include oxidation-reduction, solution-precipitation, ion exchange, adsorption and degradation. In many

cases all these processes and reactions are interrelated. For example the mobilisation of Fe and Mn is closely related to the decomposition of organic matter. In the following sections some of these processes are explained in more detail.

3.3.1   *Density dependent flow*

Compared to water in pristine aquifers, landfill leachate is a solution with a very high concentration of dissolved compounds. Highly concentrated water solutions have higher densities than water. Christensen et al. [1985] found a good correlation with the specific conductivity and the density of 13 leachates (coefficient of correlation, r^2 = 0.98) with the regression equation given as:

$$\text{Density (in g cm}^{-3}) = 6.87 \cdot 10^{-6} \cdot \text{specific conductivity (in mS m}^{-1}) + 0.9982 \text{ g cm}^{-3}$$

Not much is known about density effects of leachate in the field, but differences in density may have a significant impact on the vertical positioning of the plume. The leachate can sink to deep depths because of this higher density.

3.3.2   *Dilution*

Landfill leachate is subjected to dilution as it moves through the soil. The leachate moves after mixing with the moving soil water. The leachate is diluted due to this mixing. When studying leachate plumes it is essential to account for the dilution. Chloride is thought to be a conservative tracer and as such it can be used to correct for dilution. Bjerg et al. [1995] found that chloride cannot always be used as a tracer. At the Grindsted landfill in Denmark, an additional chloride plume was encountered in the plume that originated from a nearby road. This additional chloride plume was caused by road salt used in the winter.

Local water table gradients just below and around the landfill will most likely differ from the general gradients. This is due to local water table mounds created by the specific hydrogeology of the landfill, giving rise to a higher lateral spreading of the leachate plume. For the formation of the water table mound at the Grindsted landfill three possible reasons are given [Kjeldsen et al., 1998b]:

1. higher infiltration in the domed shaped eastern part of the landfill (not likely);
2. lower hydraulic conductivity in the aquifer underlying this part of the aquifer (likely);
3. higher infiltration in the border regions of the 'domed-shaped' area (likely).

Local water table mounds could also lead to downward-directed hydraulic gradients, causing a vertical flow of leachate. Local water table mounds have been observed at the Borden landfill in Canada {MacFarlene et al., 1983}, the Vejen landfill in Denmark [Kjeldsen, 1993], the Grindsted landfill in Denmark [Kjeldsen et al., 1998b] and the Noordwijk landfill in the Netherlands {Duijvenbooden and Kooper, 1981}.

3.3.3   *Dispersion*

Dispersion is a mathematical term in the solute transport equation and as such it is a controversial term about which there is currently much discussion going on if it is to be considered a process, an artefact or perhaps a completely wrong concept [Lowe and Frenkel, 1996]. Dispersion is used to account for the smearing of the leachate front. This smearing is thought to be caused by processes such as diffusion and mechanical dispersion which is a result of the leachate moving through a porous medium that consists of a wide range of different intersecting pores in a heterogenous soil. It is clear that quantification of dispersion is very difficult and that dispersion depends on the scale of the measurement {Gelhar, 1986}. In addition other processes may result in an apparent dispersion.

As was mentioned before, Kjeldsen et al. [1998b] showed that the streamlines for the water flow showed a seasonal variation implying that the leachate flows through different parts of the aquifer as the seasons change. When taking measurements at a single location this may seem like dispersion.

Perhaps it is because of the difficulties associated with dispersion that most landfills reported in the literature were situated in sandy aquifers. Landfills in more complex systems require more measurements in order to cope with the expected higher heterogeneity in these systems.

3.3.4   Sorption
Sorption covers all surface-related reactions such as surface precipitation, adsorption, absorption, surface complexation, and ion exchange. For reviews on sorption mechanisms see Sposito [1984 and 1989].

The detail of knowledge about sorption differs considerably for different species that can be found in leachate plumes. The detail of information found ranges from simple retardation values for dissolved organic matter [Christensen et al., 1994], to very complex pH dependent adsorption and exchange models for heavy metal adsorption to organic matter [Sposito, 1989]. The following sections follow the approach used by Christensen et al. [1994] in which the sorption phenomena of the different species were discussed separately.

Dissolved organic matter
Sorption of dissolved organic matter seems to be of minor significance according to column experiments [Kjeldsen and Christensen, 1984]. The retardation factor for acid-phase leachate COD was of the order of 0.8 - 1.0. For methane phase leachate it was on the order of 0.7 to 1.0 [Kjeldsen, 1986]. No field observations on the retardation of dissolved organic matter are available.

Anthropogenic specific organic compounds (ASOCs)
Sorption of specific organic compounds to soils is well described in the literature [e.g. Brusseau and Rao, 1989]. Most research concerns non-polar compounds whereas the sorption of polar compounds (e.g. organic acids and bases) is much less researched.

Sorption of non-polar compounds is generally described as a partitioning into the solid organic carbon in the soil. It is a kinetic process that can be described using either a bicontinuum model [Brusseau et al., 1991] or a radial diffusion model [Wu and Gschwend, 1986]. However, sorption of non-polar compounds can also be estimated using a simplified equilibrium approach based on distribution coefficients (Kd). This approach employs empirical regression equations which account for the organic carbon in the soil and the hydrophobicity of the specific organic compound expressed through its octanol/water partitioning coefficient [Christensen et al., 1994]. These regression equations are well established for soil and sediments with organic carbon contents in excess of 0.1 % carbon. These regression equations underestimate the sorption in soils with less than 0.05 % organic carbon. Apparently the other solid components are also active in the sorption process [Larsen et al., 1992a].

Inorganic macrocomponents (cations)
The cation fraction of the inorganic macro components primarily adsorb through ion exchange. Ion exchange is described in nearly all text books on soil and geochemistry [i.e. Sposito, 1989; Fetter 1993; Bolt and Bruggenwert, 1978; Appelo and Postma, 1993].

Although aquifer material usually has a low cation-exchange capacity (CEC is typically in the order of 0.5 to 2 meq/100 g) it is still very significant. In pristine aquifers, cations associated with

the exchange sites typically make up 80 % of the total amount of cations per volume of aquifer [Christensen et al., 1994]. When pristine aquifer material is exposed to leachate having a higher ionic strength and a different relative cationic composition than the natural groundwater, a new equilibrium will be reached. The saturating cations will be expelled from the exchange sites and move with the leachate front in concentrations in excess of the leachate concentrations. This is referred to as the 'hardness halo' and has often been reported in the literature [e.g. Kjeldsen and Christensen, 1984]. Figure 2 (see appendix A) shows an example of a breakthrough curve with a 'hardness halo'.

3.3.5   *Microbiology*

Most uncontaminated aquifers are aerobic oligotrophic. Entry of landfill leachate, reduced and rich in dissolved organic matter, dramatically changes the composition of the orginal microbial population. This causes the number of micro-organisms to increase to numbers relatively high compared with the number of micro-organisms usually found in pristine aquifers (see table 1 in appendix B and [Christensen et al., 1994]. Most of the aquifer bacteria are associated with particle surfaces. Despite high numbers, the abundance of bacteria in aquifer material is too scarce to cause a complete and continuous coverage (biofilm) of the solid particles [Bjerg et al., 1996].

Some attention has been paid to the occurrence of pathogenic micro-organisms in leachate. Fecal coliforms and streptococci can be found in numbers up to 106 colony forming units per ml. Also saphophytic fungi (Aspergillus, Penicillium, Fusarium) are encountered, but no pathogenic virusses and protozoa. Although bacteria can survive and are transported for long distances in aquifers, pathogenics seem to be of minor importance in aquifers affected by landfill leachate [Christensen et al., 1994].

While micro-organisms are responsible for redox zonation and biodegradation, little research has been done on the micro-organisms themselves. Ludvigsen et al. [submitted] examined the distribution of ongoing microbial redox processes (examined via bioassays) and viable populations along a 305 m long transect of the by the Grindsted landfill leachate polluted aquifer. They tried to establish a relation to the geology, distribution of dissolved redox sensitive compounds and sediment geochemistry.

In general, accordance was found between the distribution of the various redox processes in the aquifer and the geochemical features of the leachate plume indicating that the redox processes observed in the bioassays reflected the redox conditions in the plume. The overall distribution of the redox processes reflected increasing redox potentials away from the landfill, but many of the observed redox processes occurred simultaneously (for example sulphate reduction and methane production in the first 80 m).

Laboratory investigations by Christensen et al. [1993] had already shown that most sediments samples contained micro-organisms able to perform several redox processes, for example, denitrification occurred after addition of nitrate to samples from the iron-reducing zone of the Vejen landfill plume. Usually one electron accepting process dominated.

The governing redox process activities were only partly reflected in the distribution and composition of viable biomass, as determined by fatty acid analysis and enumeration techniques. Methanogens were restricted to the most polluted and reduced part of the aquifer, close to the landfill. Sulphate reducers decreased with increase in distance from the landfill. Iron-, manganese- and nitrate-reducers were detected in all samples all over the plume. Microbial activity in terms of iron-, manganese-reduction or denitrification were not reflected in higher numbers. The presence of these bacteria also outside the active zones suggest a great potential

for these microbial processes in the aquifer. Fatty acid analysis revealed the presence of Eukarya close to the landfill. Protazoa could not be detected.

The finding of specific fatty acid biomarkers for sulphate- and iron-reducing bacteria paralleled enumerations and the occurrence of sulfate- and iron-reduction activity. No significant correlation was found between biomass and quantitative measurements of redox processes. Furthermore, fatty acid analysis suggested that the biomass is under stress.

Local heterogeneities in the geology and geochemistry allowed sulfate reduction and iron reduction in the part of the aquifer dominated by manganese reduction and denitrification. These activities occurred at highly microbial active silt/clay layers in the otherwise sandy aquifer. This shows the importance of integrating geology, geochemistry and microbial redox processes, since these processes might also have occurred when the aquifer had not been polluted. Therefore variations in redox activities in a pollution plume may not always be caused by the pollution.

Also other reports in the literature indicate that microbial processes occurring at landfills are complex. At the North Bay landfill, Canada {Acton and Barker, 1992} it was concluded that the presence of high sulfate levels (300 to 850 mg l^{-1} SO_4^{2-}) did not inhibit the methanogenisis. As little as 19 mg l^{-1} SO_4^{2-} was shown to inhibit methanogenesis in other environments. Sulfate reduction in unpolluted aquifers has been shown to be excluded by iron reduction. Iron reduction keeps the concentrations of dissolved hydrogen, formate and acetate lower that the thresholds required by sulfate reducing bacteria [Lovley et al., 1994].

Microbial iron reduction seems to very important in leachate plumes. In situ iron reduction seems to be limited by the availability of Fe(III) [Albrechtsen et al., 1995].

3.3.6   *Redox zonation*
The complex set of reactions taking place in the soils downgradient of the landfill may be described using the concepts of redox and pH buffering. In his Ph.D. Thesis Heron [1994] gives an excellent overview of the concepts and consequences of redox buffering in aquifers containing groundwater that mixes with landfill leachate. In addition Heron [1994] discusses results from other well studied landfills (see table 1 in appendix B) in light of the redox buffering concept.

Key parameters in discussing redox buffering are the reduction capacity (RDC) of the leachate and the oxidation capacity (OXC) of the aquifer. Reduced species produced within the landfill make up the RDC of the landfill leachate. The RDC is a theoretical parameter that equals the amount of electrons generated if all leachate were to be oxidized by a redox titration with an endpoint that equals aerobic conditions:

$$RDC = 4[TOC] + 8[CH_4] + 8[NH_4^+] + 8[S(-II)] + 7[S(-I)] + [Fe(II)] + 2[Mn(II)]$$

The leachate plume is oxidized by the oxidation capacity of the soil downgradient from the landfill. This oxidation (of primarily organic matter) is microbially mediated. The OXC is theoretically defined as the total amount of electrons that can be accepted by an aquifer volume. In a similar fashion as the RDC, the OXC, assuming that organic matter in the aquifer is fully oxidized from oxidation state 0 (e.g. glucose) to the oxidation state +4 (CO_2), is calculated with:

$$OXC = 4[O_2] + 5[NO_3] + [Fe(III)] + 2[Mn(IV)] + 8[SO_4^{2-}] + 4[TOC]$$

As the reduced leachate enters the soil, it tends to reduce the oxidized species in the aquifer. Dissolved organic matter and ammonium contribute most significantly to the RDC of the leachate. Dissolved Fe(II) may contribute during the acid phase of leaching. During plume devel-

opment, retardation of ammonium may occur due to ion-exchange processes. This reduces the contribution of ammonium to the RDC of landfill leachate. Dissolved organic carbon is not significantly retarded by sorption and as it spreads through the aquifer it causes the development of a sequence of redox zones. The OXC is dominated by Fe(III) oxides in most sandy aquifers. Limestone and bedrock aquifers may be poor in Fe(III) and therefore the OXC is primarily made up the dissolved species oxygen, nitrate and sulphate.

The typical sequence of redox zonation which results from the mixing of leachate and groundwater is that the most reducing zone (methanogenic) lies closest to the landfill followed by zones of sulphate reduction, Fe(III)/Mn(IV) reduction and nitrate reduction [Heron, 1994; Baedecker and Back, 1979]. Measurement of this redox zonation is not very easy. Using groundwater analysis alone may very well lead to misinterpretations because many redox sensitive parameters migrate in the plume and may be detected in areas where they have not been formed. Heron [1994], Heron and Christensen [1994] and Albrechtsen et al. [1995] clearly have shown that it is a combination of groundwater and sediment analysis that may provide an insight into the redox buffering processes. For example, using groundwater samples in anaerobic incubation experiments in the laboratory may show extreme reducing conditions (methanogenic) due to depletion of most oxidized species in the groundwater. However an incubation with groundwater and sediment may not reach the methanogenic phase and remain in the Fe(III) reducing phase. In this case the redox chemistry is buffered by the amount of available reducible Fe(III) in the sediment.

Deducing the redox zonation from the distribution of redox sensitive species in the plume (both in the groundwater as well as in the sediment) is also complicated by the historical composition of the leachate plume. For instance large amounts of Fe(II) may be leached from landfills during the early, acid phase. These are deposited in the aquifer, either in the exchange-sites or as reduced precipitates (Siderite, Sulphides) or as readily reducible Fe(III) oxides after oxidation at the plume edges.

Processes during the long lasting methanogenic phase, in which most landfills are studied, then may not be easily distinguished from earlier processes as part of the redox sensitive species have been formed in an earlier stage.

Studies in to the iron geochemistry of the Vejen landfill [Heron, 1994] showed that the cycling of iron between Fe(III) and Fe(II) within the plume may account for the oxidation of organic matter. In short the process is such that oxidized Fe(III) is reduced by organic matter. Reduced Fe(II) is dissolved and moves towards the edges of the plume were it is reoxidized. As such the iron is again available for oxidizing organic matter. The net result of this process after hundreds of years of leaching will be the oxidation of organic matter to carbon dioxide and the reduction of oxygen and nitrate to water and free nitrogen. The redox buffering of iron diminishes the volume of the leachate plume by retarding the movement of the reduced species.

Dilution is also a key factor in controlling the redox processes and subsequently the development of redox zones. Simple spreading due to local water table mounds or plume sinking may dilute the reducing power of the leachate. The leachate is spread over larger areas, but the larger mixing of groundwater also adds dissolved electron acceptors and therefore supports the reoxidation of Fe(III) pool at the plume edges. However, in deep aquifers or aquifers overlain by clay layers, the low infiltration rate may prevent both dilution and the addition of dissolved electron acceptors. In addition such aquifers typically are partly reduced and as a result the oxidation capacity of the aquifer may be very small (lack of oxygen, nitrate and Fe(III)), and the leachate may migrate relatively unretarded far downgradient.

Consequently, the structure and the geochemistry of the aquifer surrounding the landfill controls the development of the leachate plume. Fractured aquifers have very little capacity to attenuate the leachate due to the very high flow velocities and the small transport volumes. Among the porous sandy aquifers, deep, carbonate-rich and homogeneous aquifers may be most vulnerable due to the lack of dilution or addition of oxidized species at the plume edges.

3.3.7   *Degradation of anthropogenic specific organic compounds (ASOCs)*

Anthropogenic specific organic compounds (ASOCs) constitute only a few percent of the total amount of DOC in leachate and leachate contaminated groundwater, and are common in leachate from landfills, which receive chemical and industrial waste (see table 2 in appendix B). Approximately 100 different compounds have been identified in groundwater contaminated by landfills. Of this enormous diversity of compounds only a few compounds occur frequently in higher concentrations (see fig. 3 in appendix A). These are chlorinated aliphatic compounds, BTEX (benzene, toluene, ethylbenzene, and xylene), chlorobenzenes, and vinyl chloride.

Besides dilution, attenuation of ASOCs is due to sorption and degradation (biotic and abiotic). Volatilization is only of importance if the leachate percolates through an unsaturated zone, or if the plume is located at the watertable.

Field and experimental evidence of degradation of ASOCs

In appendix C degradation potentials (No degradation or Degradation) for various ASOCs are presented, and categorized according to the specific redox conditions which were occurring during the compound degradation.

A more precise description of the degradation potential is included in appendix C for the studies of Nielsen et al., [1994a and b]. The degradability of a compound depends for the major part on the governing redox environment (the chemical framework). This table is an extension (with publications 't' to 'M') of Christensen et al. [1994]. Christensen et al. [1994] only included degradation potentials of ASOCs in landfill leachate plumes (publications 'a' to 's') because the environment in landfill leachate polluted aquifers is significantly different from other with ASOCs contaminated sites. Landfill leachate plumes have very high concentrations of DOC, of which the ASOCs constitute only a few percent. The presence of high levels of DOC may either limit the degradation of ASOCs, as the micro-organisms may prefer the dissolved organic matter to ASOCs, or increase the degradation of ASOCs due to the generally high microbial activity caused by dissolved organic matter [Christensen et al., 1994]. The table presented in this review contains results from some investigations which were not conducted at landfill sites.

The occurrence of degradation in the various investigations has been determined by different methodologies. These range from compound disappearance, positive determination of end-products (14C-labeled compounds), to revealing actual degradation pathways, intermediary compounds, and active bacterial species. In detailed field monitoring studies the occurrence of degradation is determined by correcting for dilution by using the chloride-ratio if possible, and by checking the importance of sorption in the attenuation. In this way an indication of degradation potentials can be obtained.

To separate biological degradation from abiotic degradation and loss due to sorption and evaporation, sterile or biologically deactivated control experiments have to be performed. Agents for sterilization are described in the sections discussing the Laboratory Batch Microcosms and the In Situ Microcosms (see chapter 4). Bio-transformation seems to be more important than abiotic transformation, although chlorinated aliphatic compounds have been shown to be reductively dechlorinated under abiotic, strongly reduced conditions in landfill leachate [Christensen et al., 1993].

Abiotic degradation of nitrobenzene was observed at the Vejen landfill [Nielsen et al., 1995b] where it contributed to the total degradation in the more reduced parts of the plume under methanogenic and Fe(III)-reducing conditions, while under nitrate reducing conditions no abiotic transformation was observed.

Generally three strategies have been used to investigate the degradability of ASOCs in leachate polluted aquifers:

1. Laboratory experiments (columns and batches).
2. Field experiments (injection experiments and ISMs (in situ microcosms)).
3. Detailed field monitoring of the distribution of ASOCs along a flow path of the leachate plume.

Until recently, very little effort has been spent on studying on the degradability of ASOCs under Fe(III)-reducing conditions. Investigations at the Vejen landfill and the Grindsted landfill, both in Denmark, revealed that this redox zone is spatially extensive [Lyngkilde and Christensen, 1992a; Bjerg et al., 1995], and that this zone may be important for the degradation of ASOCs]Lyngkilde and Christensen, 1992b; Rügge et al., 1995; Holm et al., 1995].

The variation in the degradability of some compounds, as reported in appendix C, is remarkable. Christensen et al. [1994] explained this variation by the following factors:

1. the investigated concentrations of ASOCs range over three orders of magnitude;
2. single compound experiments versus cocktails;
3. experiments are performed at temperatures ranging from 10 ºC to room temperature;
4. duration of the experiment;
5. the experiments have been performed with different inoculi exposed to different pollution loads and therefore adapted to ASOCs to different degrees;
6. difficulties in assessing the dominating redox environment.

Chlorinated aliphatic hydrocarbons
Most chlorinated aliphatic hydrocarbons are degradable under methanogenic conditions, but appear to be persistent under aerobic conditions (see appendix C [i.e. Nielsen et al., 1996a]). In Laboratory Batch Microcosm (LBM) and In Situ Microcosm (ISM) experiments Nielsen et al. [1995b] found that under methanogenic conditions tetrachloromethane (LBM + ISM), tetra-chloromethane (LBM + ISM) and tetrachloroethene (only ISM) were probably transformed by reductive dechlorination, while trichloroethene was not. Tetrachloroethene degradation did not occur in the LBM experiments. Tetrachloromethane was also transformed under Fe(III)-reducing conditions. Laboratory investigations showed that tetrachloromethane can be transformed abiotically in the presence of sulphide, biotite, vermiculite, Fe(II) and pyrite {Kriegman-King and Reinhard, 1992 and 1994; Doong and Wu, 1992}. Thus part of the transformation of tetra-chloromethane can be abiotic in the presence of Fe(II) and pyrite in this redox zone. The lack of transformation of tetrachloromethane under nitrate-reducing conditions is in good agreement with observations by Semprini et al. {1992} who found that transformation of tetrachloromethane was highest when no NO_3 was present. NO_3 may compete with highly halogenated compounds as electron acceptor as discussed by Murray and Richardson {1993} and may limit the transforma-tion of tetrachloromethane. The compounds were transformed in the sequence: (1) tetra-chloromethane, (2) tetrachloroethane and (3) tetrachloroethene. This sequence can be explained by the preference for reductive dechlorination of compounds with high potential to accept electrons before less electron accepting compounds {Murray and Richardson, 1993}. Tetra-chloroethene was dechlorinated to trichloroethene as indicated by the concentration curves and has been observed in laboratory studies under methanogenic conditions [Vogel and McCarty, 1985].

The observation that strong sorption to the aquifer material hampered the measurements and data interpretation of trichloroethene, tetrachloroethene and 1,1,2,2-tetrachlorethane can be of importance for possible future degradation experiments at the Coupépolder landfill in the Netherlands as these compounds have been detected the landfill leachate.

Aromatic hydrocarbons
Aromatic hydrocarbons are degradable under aerobic conditions (see appendix C). A LBM investigation of the reproducibility and variation in degradability [Nielsen et al., 1994b] showed that the LBM's results were reproducible for some compounds (benzene, toluene, p- and o-dichlorobenzene) but less reproducible for others (naphtalene and especially biphenyl). Significant variation in degradation rates among the 8 localities was found for benzene, toluene, naphtalene and biphenyl, whereas no variation amongst localities was observed for o-xylene, o-dichlorobenzene and p-dichlorobenzene. The overall degradation rates for benzene and toluene and for p- and o-dichlorobenzene, respectively, showed a significant correlation.

Degradation under anaerobic conditions has been observed for some aromatics (nitrobenzene, toluene), but most compounds do not show degradation (cumene, biphenyl, naphtalene) or show different results amongst studies. This indicates the importance of assessing site-specific degradation potentials for these compounds under anaerobic conditions.

Evidence of anaerobic degradation of benzene in landfill leachate plumes has only been observed once. Rügge et al. [1995] concluded, on the basis of detailed monitoring at the Grindsted landfill and correction for dilution using the chloride-ratio, that benzene degraded under Fe(III)-reducing conditions.

The reported benzene degradation under anaerobic conditions [Kazumi et al., 1997; Chapelle et al., 1996a and b] was observed at sites that were not landfills. A possible explanation for the lack of degradation of benzene in aquifer sediments polluted with landfill leachate from Norman, OK, even after three years, was that benzene may not have been an important component of landfill leachate contamination at the site and that the sediments were not enriched with benzene-degrading micro-organisms [Kazumi et al., 1997]. Therefore, the source of sediment inoculum and/or history of contamination may be important for anaerobic microbial benzene degradation to occur. Benzene degradation could be initiated by the addition of Fe(III) chelators such as EDTA and NTA, which make Fe(III) more available for microbial reduction [Kazumi et al., 1997].

Chlorobenzenes
Chlorobenzenes are degradable under aerobic conditions, but under anaerobic conditions [Nielsen et al., 1995b] no degradation was observed. Studies reviewed by Grbíc-Galíc [1990] have shown that chlorobenzenes may be reductively dehalogenated in methanogenic conditions, but the less chlorinated compounds tend to be the most recalcitrant.

Phenols
Phenols investigated, are degradable under aerobic conditions. Clear variation among localities with respect to degradation potential was observed at a LBM study [Nielsen et al., 1994a] for nitrobenzene, o-nitrophenol, 2,6-dichlorophenol and 4,6-o-dichlorocresol, but results between replicates were identical, except in one location for 2,6-dichlorophenol.

Degradation of the eight phenolic compounds was highly sequential: (1) 4,6-o-dichlorocresol, (2) phenol, (3) o-cresol, (4) p-nitrophenol, (5) 2,4-dichlorophenol, (6) 2,6-dichlorophenol, (7/8) o-nitrophenol/nitrobenzene. The highest correlation in this sequence was found for the van der Waals volumes (dissociation constants, pK_a, and K_{ow} also correlated with this sequence). Less

degradation was observed with a increasing van der Waals volume of the compound. Apparently, small molecules with high affinity to water were preferably degradaded.

Pharmaceutical compounds
Pharmaceutical compounds (e.g. sulfonamides and barbiturates) were found to be degraded in the Grindsted landfill [Holm et al., 1995]. A specific compound propyphenazone was expected to show the largest degradation but is found in the highest concentration at 115 m downgradient, so sorption is not significant but degradation is. However no direct proof can be given as no degradation products were identified. At a distance of 150 m downgradient none of the pharmaceutical compounds could be detected in the groundwater. Most of the compounds were degraded under methanogenic/sulfate-reducing and iron-reducing conditions.

Some are not totally degraded in these zones and are found in the transition between the manganese-reducing and nitrate-reducing zone.

CHAPTER 4

METHODS

This chapter gives an overview of the methods which have been applied or could be applied in the characterization of landfills. The methods allow the determination of redox zones in the plume (water and sediment sampling and analysis techniques, dissolved hydrogen (H_2) concentration as an indicator of redox zonation), potential for biodegradation of pollutants (microcosm techniques) and microbial biodiversity (microbial characterization techniques).

4.1   Water and sediment sampling and analysis techniques

Characterizing landfills and landfill leachate plumes requires quantitative information on a large number of parameters. This information is obtained using a combination of laboratory and field techniques. Measurements start by installing wells so that the groundwater can be sampled and by obtaining samples of the sediment in the aquifer. Appendix D gives an overview of the methods used to determine parameters required for landfill characterization.

It is important to realize that when taking samples from aquifers, errors are introduced during sampling handling, transport etc. One the most difficult problems to cope with is to ensure that samples taken from anaerobic spots in the aquifer remain anaerobic. In addition there is the risk that allochtone micro-organisms are introduced that contaminate the sample.

Difficulties that occur amongst many others are the diffusion of oxygen through the tubing used to sample groundwater and degassing of the groundwater due to pressure changes while sampling. Of course careful handling of the samples in the laboratory is also crucial. In addition, samples are relative small and they are taken from heterogeneous systems making things even more complicated. A summary of techniques and methods used for determining the required parameters is given in Appendix D.

4.2   Dissolved hydrogen (H_2) concentration as an indicator of redox zonation

4.2.1   *Background*

Redox environments in the leachate plumes of the Vejen landfill [Lyngkilde and Christensen, 1992a] and the Grindsted landfill [Bjerg et al., 1995] were delineated by assigning a redox status using criteria from the characterization of redox sensitive species in groundwater and sediment samples (see table 3 in appendix B). Although Chapelle et al. [1996b] were able to separate an anaerobic petroleum plume from the aerobic background using Eh measurements, the approach using additional criteria is superior to the measurement of the redox potential with platinum electrodes.

Still complications in the localisation of redox reactions based on redox sensitive species can arise because:

1. reduced products such as methane, Fe(II), and Mn(II) may flow from the zone where they are produced into zones where there is little or no ongoing production of these compounds [Lovley et al., 1994];
2. the detection of sulphate reduction may be difficult. The sulphate concentration does not have to decrease due to replenishment of sulphate from a mineral source or confining bed pore water. The increase in sulphide concentration can be counteracted by precipitation in the presence of metals [Chapelle et al., 1995].

Thus determination of the redox zonation based solely on patterns of electron acceptor depletion or end product accumulation is not always possible [Chapelle et al., 1995].

A very promising method for the delineation of redox zones is based on the identification of the so-called Terminal Electron Accepting Processes (TEAPs) by the measurement of dissolved hydrogen, H_2 [Chapelle et al., 1996a and 1996b; Chapelle et al., 1995; Lovley et al., 1994; Vroblesky and Chapelle, 1994; Chapelle and McMahon, 1991; McMahon and Chapelle, 1991; Lovley and Goodwin, {1988}]. Hydrogen is an important intermediate in the microbial oxidation of organic matter coupled to the reduction of many inorganic electron acceptors.

During the anaerobic decomposition of natural and anthropogenic organic matter, a wide variety of fermentative micro-organisms produce H_2 during metabolism of carbohydrates or aromatic compounds. As rapidly as H_2 is produced, it is consumed by respiratory micro-organisms that use oxidized compounds (nitrate, Fe(III), sulphate, CO_2) as terminal electron acceptors. This rapid turnover between hydrogen producers and consumers has been termed inter species hydrogen transfer. Different anaerobic terminal electron accepting processes exhibit different efficiencies in utilising H_2, resulting in characteristic steady state H_2 concentrations, which are theoretically independent of biomass or overall rates of microbial metabolism {Lovley and Goodwin, 1988}. Therefore the H_2 concentration serves as an indicator of the TEAP that pre-dominates in a given zone. Nitrate reducers are highly efficient H_2 utilisers and maintain very low steady-state H_2 concentrations. Fe(III) are slightly less efficient, sulphate reducers and the methanogenic bacteria are progressively less efficient and therefore maintain higher H_2 concentrations. These characteristic ranges are given in table 4 (see appendix B).

A hierarchical framework for identifying TEAPs proposed by Chapelle et al. [1995] is shown in figure 4 (see appendix A). When no conclusions can be drawn by using solely redox sensitive species, H_2 measurements can aid in the diagnosis. Because H_2 is a continuously cycled inter-mediate product, with a half life in the order of seconds, H_2 concentrations reflect nearly instantaneous conditions at a particular well and a single analysis can, in principle, be a diagnostic of the predominant TEAP [Chapelle et al., 1995]. The use of both redox sensitive parameters (being consumed and produced) and dissolved hydrogen gives a high degree of confidence. Thus the most appropriate protocol for determining the zonation of TEAPs in groundwater systems should include consideration of electron acceptor consumption and final product accumulation in addition to H_2 concentrations [Chapelle et al., 1995].

The measurement of H_2 concentrations was very useful in the study of Chapelle and McMahon [1991], because H_2 measurements suggested that sulphate reduction was taking place, what could not be evaluated from the redox sensitive species. Temporal and spatially changes in TEAPs could be monitored very well with H_2 and are important because of the relation between TEAPs and degradation potential of specific organic compounds [Vroblesky and Chapelle, 1994].

4.2.2   *Sampling and measurement of dissolved H_2 concentrations*
A gas stripping procedure, referred to as the 'bubble strip' method is used to measure dissolved H_2 in groundwater [Vroblesky and Chapelle, 1994; Chapelle and McMahon, 1991]. Figure 5 (see appendix A) shows a schematic of the 'bubble strip' method. Concentrations of H_2 are measured by gas chromatography equipped with a Reduction Gas Detector. The detection limit for H_2 in a gas phase using this method is approximately 0.01 μL/L [Chapelle et al., 1995]. Because H_2 is so dynamic, attempts to preserve H_2 samples in the field for transport to the laboratory were not successful. For this reason, the chromatography equipment was modified to be taken into the field, and all H_2 measurements were made within 30 min of sample collection [Chapelle et al., 1995].

In addition it is important to reduce the contact of the water with metal parts to a minimum because metal parts tend to interfere with the measurements [Wiedemeier et al., 1995]. Of course diffusion through the tubing could also be problem. Because these problems, routine application of this technique is not yet possible and it therefore requires considerable development.

4.3 Methods based on the application of microcosm techniques

4.3.1 *Background*

Microcosm techniques are useful tools when studying degradation kinetics in the field and in the laboratory. The principle behind microcosm studies is that samples from an aquifer are put in to a more or less closed container so that processes can be studied using mass balance approaches. In time samples can be taken from the microcosm which subsequently can be analysed. Generally microcosm experiments start by spiking the water/sediment mixture with a known cocktail of chemicals. Microcosms can be used for both aerobic as well as anaerobic experiments. Results from microcosm experiments can lead to the deduction of degradation potential, redox characterization and degradation kinetics. Two types of microcosms can be distinguished: the Laboratory Batch Microcosm (LBM) and the In Situ Microcosm (ISM) [Nielsen et al., 1996a, 1996b, 1994a and 1994b; Gillham et al. {1990a and 1990b}].

The ISM technique was initially developed at the University of Waterloo (Canada) [Gillham et al., 1990a and 1990b] and further developed at the Technical University of Denmark [Nielssen et al., 1995a, 1995b, 1996a and 1996b]. The ISM technique has been developed to measure degradation potentials and rates under field conditions (in situ) for sandy aquifers. These data are required because there is a lack in understanding how well laboratory-determined degradation rate constants apply to field conditions. Moreover, experiments such as field-injection experiments are in addition to being costly, very difficult to interpret.

Difficulties arise when trying to account for sorptive losses {Brusseau and Rao, 1989}, the length of the microbial adaptation period {Dobbins et al., 1992} and when trying to control the redox conditions {Ghiorse and Wilson, 1988} in the part of the aquifer influenced by the experiment.

Degradation rate constants must be related to aquifer redox conditions to be of any general value; thus it is important to monitor the redox conditions during the experiment.

Both the LBM and the ISM techniques are discussed in more detail below.

4.3.2 *Laboratory batch microcosm (LBM)*
Installation and preparation
LBMs at the Technical University of Denmark [i.e. Nielsen et al., 1996a and b] are 2.5 L glass bottles used to determine degradation potentials of ASOCs under different redox conditions. The bottles are equipped with a glass valve used for sampling. The LBM is loaded with groundwater and sediment.

Groundwater can be collected from drive-point piezometers [Lyngkilde and Christensen, 1992a; Nielsen et al., 1996a]. For anaerobic conditions the water is transferred directly to sterilized glass bottles flushed with N_2 and capped with glass stoppers [Nielsen et al., 1995a and b]. Small amounts of oxygen in the anaerobic experiments were reduced by the addition of an equivalent amount of SO_3^{2-}. Groundwater samples were stored in brown 2.5 L glass bottles with plastic caps. Prior to using the bottles and caps, they were washed in acid and consecutively dry-sterilized and autoclaved [Nielsen and Christensen, 1994a and b).

Aerobic sediment samples were collected with a stainless steel bailer (Eijkelkamp) in a 10 cm cased borehole at the same depth and within 0.5 m from the respective ISMs [Nielsen et al.,

1996a]. Anaerobic sediment was collected with a waterloo piston sampler in aluminium tubes {Starr and Ingleton, 1992} in Nielsen et al. [1995a and b]. Sediment samples were stored at 10 ºC in 2.5 L polyethylene buckets for 1 week [Nielsen and Christensen, 1994a and b].

A suspension of fine fraction sediment (extracted from 1 kg of sediment) and 2 L of groundwater was transferred to the LBM and spiked with the specific organic compounds (~150 μg/l +/- 20 %). The procedure is as follows [Holm et al., 1992; Nielsen and Christensen, 1994a and b]: 1 kg of wet sediment is mixed with 1 L of groundwater in a dry-sterilized 5 L bottle; After 1 min of sedi-mentation, groundwater and suspended clay and silt particles were transferred to the microcosm bottle. Again 1 L of groundwater is mixed with the sediment in the 5 L bottle and the procedure is repeated to yield ~ 5 g of clay and silt and 2 L of groundwater.

This combination of fine fraction of the sediment and groundwater resembles field conditions fairly well [Holm et al., 1992]. The importance of the fine particles is probably related to the fact that the majority of the bacterial biomass in aquifers is associated with the silt and clay fraction of the sediment {Albrechtsen, 1994}.

Atmospheric air was added to the microcosm by bubbling through a diffuser for ~ 1 hour to ensure aerobic conditions (O_2 > 9 mg/L) for the aerobic experiments.

Experimental conditions and sampling
The microcosms were incubated in the dark and under the actual groundwater temperature of 10 ºC in a slowly rotating box for 5 to 6 months [Nielsen et al., 1996a]. For the anaerobic experiments the LBMs were kept submerged under anaerobic water (< 0.1 mg/l O_2) and shaken gently three times a week.

Control experiments were performed by poisoning the LBMs with formaldehyde (250 mg/L) or NaN_3 (2 g/L) depending on the type of ASOCs and the distance from the landfill. Formaldehyde was used for the samples from 2 and 350 m from the landfill, NaN_3 was used for the samples from 135 and 250 m from the landfill.

Aerobic samples were collected for analysis of ASOCs, O_2, NO_3, pH (11 samples in 150 days) [Nielsen et al., 1996a], and DOC [Nielsen and Christensen, 1994a and b] by forcing atmospheric air into the LMB with a syringe [Nielsen and Christensen, 1994a and b]. For the anaerobic experi-ments a water sample was pushed out by an over pressure of N_2 [Nielsen et al., 1995a and b]. Samples were characterized for: NO_3, NO_2, SO_4, NH_4, Fe(II), Mn(II), CH_4, pH, NVOC (non-volatile organic carbon), ATP (adenosine tri-phosphate) and AODC (acridine orange direct counts) [Nielsen et al., 1995a and b]. Concentrations of CH_4 were corrected for volatilisation into the increasing head space. After the anaerobic experiments, sediment from selected LBMs was sampled and characterized for OXC, Fe(II) and Fe(III).

Evaporation of phenols is not of importance (max 0.1 %), since the studied phenols have very low Henry constants (K_H < 3 e-5 atm m^3/mol) [Nielsen et al., 1995a]. For the aromatic and chlorinated aliphatic compounds [Nielsen et al., 1995b] corrections were made. Henry's con-stants were determined at 10 ºC by Ashworth et al. {1988} except for naphthalene and nitro-benzene {Montgomery and Welkom, 1990} and biphenyl {Schwarzenbach et al., 1993}.

Sampling is done about once a week during a period of 80 - 180 days [Nielsen et al., 1995a and b]. In the investigation of Nielsen and Christensen [1994a and b] 24 weekly samples were taken for the aromatic and aliphatic compounds, and 23 samples (5 samples a week in the beginning and 1 a month at the end of the experiment) for the phenols.

Other LBMs from the literature
Johnson et al. [1996] prepared LBMs to examine biodegradation at a landfill differently than the group of Christensen did. The method they use seems to be more commonly used than that of Christensen.

Ambient microcosms are constructed without head space. 20 g of soil is added to a sterile, 10 ml serum bottle and groundwater is added to the serum bottles until nearly full. Next one ml of a spike solution containing xenobiotics, redox indicator (resazurin) and sodium sulphite is added by syringe at the bottom of the bottle to minimize evaporative losses. The bottle is then filled with groundwater and sealed with a Teflon faced butyl rubber stopper. The stopper is secured with aluminum crimp caps. Abiotic microcosms are prepared by autoclaving almost completely filled microcosms for 1 h on two consecutive days. Then a spike solution is added along with ground-water, resazurin and mercuric chloride. Multiple replicates of both live and abiotic microcosms are constructed. Microcosms are destructively sampled in triplicate at each time point. The liquid above the soil-water interface is sampled for xenobiotic and methane analyses by GC. Micro-cosms are incubated in anaerobic incubation jars with oxygen scavenging catalyst envelopes and dry redox indicator strips.

The jars are evacuated and refilled with nitrogen three times, sealed and stored in the dark at 16 ℃. Aquifer sediment for the microcosms is obtained under anaerobic and aseptic conditions. The outer portions of the soil is removed with a sterile spatula. Prior to use, the soil is thoroughly mixed in a sterile aluminum pan in the anaerobic chamber. Groundwater is collected close to the bore hole from which the sediment is obtained by pumping into a collection bottle through a closed system of polyethene tube equipped with a 0.45 µm filter.

Advantages of this method is that due to the destructive sampling, it is possible to use the sedi-ment for nucleic acid based and physiological characterizations. From the LBMs of Christensen it is quite difficult to obtain sediment from analysis. The ratio sediment to groundwater is more realistic than that used by Christensen (who uses a ratio of 1 : 2, while in natural situations the ratio is 2 : 1), as well as the absence of head space. Also, in the method of Christensen some disturbance of the batch occurs every time it is sampled. Furthermore inactivation by boiling is more reliable than by addition of formaldehyde. Disadvantages are that variability can be ob-served due to small scale heterogeneity and that the method, especially the preparation, is more labor intensive.

4.3.3 *In situ microcosm (ISM)*
Installation and preparation
The modified version of the ISM used at the Technical University of Denmark has an inner dia-meter of 6 cm and a length of 65 cm, enclosing about 2 L of the aquifer (see fig. 6 in appendix A). The ISM is hydraulically separated from the aquifer, only diffusion at the open bottom can take place. Tracer tests indicate that this diffusion is insignificant.

In order to collect anaerobic groundwater for determination of the redox conditions and for pro-viding groundwater with which the ISM can be loaded a drive point piezometer is installed prior to installation of the ISM [Lyngkilde and Christensen, 1992a and b]. The redox conditions can be determined with any direct method such as the multi board device (see 4.4).

A sediment core of about 1 m is taken about 0.5 m near the groundwater sampling location to characterize the aquifer material. This can be collected in aluminium tubes with a Waterloo piston sampler [Starr and Ingleton, 1992]. The sediment can be characterized using traditional methods, measurement of oxidation capacities {Heron et al., 1994a}, Fe(II)/Fe(III){Heron et al., 1994b}, sulphur species [Crouzet et al., 1994] and microbiological parameters (AODC and ATP).

Two hours prior to installation, the ISM cylinder and filter body are dry-sterilized at 200 ºC. The installation location of the ISM forms a triangle with the sediment and groundwater sampling spots (0.5 m apart). Installation can be done through a hand-drilled cased borehole (Eijkelkamp) at the bottom of the borehole or through a hollow-stem auger [Gillham et al., 1990a]. For anaerobic experiments the ISM is flushed with N_2 during the installation. About 2 L of groundwater is pumped slowly from the ISM by a peristaltic pump to develop the ISM prior to loading. If no water comes out the ISM must be reinstalled.

From the piezometer 5 L of groundwater is pumped into a TedlarR bag, which has a low O_2 diffusion coefficient, K_p = 5.6 e-15 mol/cm.s.atm. Prior to anaerobic experiments the bag is flushed with N_2.

Tritiated water is added to the water in the bag to a final activity of about 150 Bq m/L and ASOCs to be studied are dissolved in 200 mL of distilled water and transferred to the Tedlar bag.

The 5 L water is pumped slowly through a stainless steel pipe (4 mm I.D.) (anaerobic) or a Teflon tube (aerobic) using a peristaltic pump equipped with a VitonR tube, into the ISM and corresponds to seven to eight pore volumes of the ISM.

For aerobic conditions the loading water is saturated with O_2 (about 10 mg/L), for anaerobic conditions intrusion of O_2 should be avoided. For denitrifying conditions SO_3^{2-} can be added to reduce small amounts of O_2 and low NO_3^- concentrations can be raised by adding NO_3^-. For lower redox levels O_2 is normally not measurable and there are no experiences concerning addition of electron acceptors for these lower redox states.

Biological deactivated control experiments can be carried out using formaldehyde (250 mg/L) or NaN_3 (2 g/L). Formaldehyde is used for aromatic and chlorinated aliphatic compounds and NaN_3 for phenolic. The addition of these control agents resulted in the dilution of several ISMs, probably because of density effects.

Experimental conditions and sampling
A groundwater sample can be collected by lowering a stainless steel syringe to the ISM through the iron pipe. The syringe is attached to the valve of the ISM and a water sample (e.g. 5, 15 and 35 mL) is sucked out of the ISM by pulling the syringe. About 5 mL of water from the ISM is discarded (dead volume of filter body and syringe is less than 1 mL). The advantages of the syringe are: a minimal sampling volume (about a total of 600 mL water in the ISM is available for sampling, 500 mL is used) and dissolved gases are maintained. The first sample is taken immediately after loading, the second 24 h later; this is done to provide information on sorption. The diffusibility through the valve material is still a problem for anaerobic experiments. The iron tube must be flushed with argon during loading and sampling.

Due to the hydraulic separation the electron acceptors cannot be replenished and redox sensitive parameters cannot be controlled during the experiment. So monitoring is necessary to know if redox levels are staying stable, and if different electron acceptors are used either simultaneously or sequentially. Interpretation of ISM degradation results must take changes in redox conditions into account. An overview of the sampling intensity of redox sensitive groundwater and sediment species for particular redox conditions based on experiences from studies reported in Nielsen et al. [1995a, 1995b and 1996a) is given in table 5 (see appendix B). Indicators such as H_2 may also be useful [Lovley et al., 1994]. Methane concentrations cannot be determined because of the suction technique used in sampling of these ISM's [Nielsen et al., 1995a].

High initial O_2 consumption was observed many times and is probably be due to corrosion of the ISM because oxidation of the ASOCs, sediment associated organic carbon or oxidation of reduced inorganic compounds associated with the sediment cannot explain this high O_2 consumption. Therefore ISMs should be made of non-corrodible materials to solve this problem.

It possible that different redox processes occur simultaneously. During aerobic experiments denitrification can occur and during denitrification Fe(III)/Mn(IV) reduction can take place. N_2O production after addition of acetylene is indicative of denitrification. Bioassays can be done on sediment samples to obtain an indication for denitrification and Fe(III)-reduction.

The majority of the microbial biomass in the aquifer is associated with the sediment and only a small fraction is present in the groundwater {Harvey et al., 1984}. This complicates the monitoring of the microbial conditions because with the ISM sediment samples cannot be collected during operation. Therefore it is important to determine microbial conditions on sediment samples before and after the experiment, and on groundwater samples collected during the experiment.

Sediment catchers inside the ISM make it possible to recover sediment inside the ISM for final characterization after completion of the field experiment. For aerobic ISMs wax is put to the bottom, for anaerobic conditions ISM cylinders are kept in N_2 flushed and sealed containers. The containers are stored at 10 °C.

Even in a rather homogeneous aquifer (Vejen) large variations in degradation pattern of ASOCs are found between ISMs installed at short horizontal distances of only a few meters apart. The only solution to this problem is to install sufficient replicates. Loading an ISM several times could be flawed by microbial adaptation, changed sorption properties, or depletion of solid electron acceptors.

Interpretation of the results
The ASOCs studied can be affected by dilution, sorption, abiotic degradation, and microbial degradation.

Tritium ($3H_2O$) is a tracer that is injected into the ISM to see if dilution takes place during the experiment. If the tracer concentration drops or fluctuates dilution occurs and the results cannot be used. Other non-reactive tracers can also be used in stead of tritium. Only a few of the 50 reported ISM experiments suffered from dilution, but 7 out of 10 control experiment showed dilution. This is caused by density effects: the used sterilizing agent increases the density of the water with 2 g/l. The constant level of the tracer also indicates that diffusion into regions with immobile water is of minor importance [Bjerg et al., 1996].

Sorption may limit which compounds can be investigated with an ISM. Even in coarse sandy aquifers (Vejen) with low content of solid organic carbon ($F_{oc} = 0.0001$) the fate of ASOCs in ISMs was affected by sorption which therefore had to be quantified. In theory the seven pore volumes of flushing the ISM should be sufficient to reach equilibrium, assuming instantaneous sorption. But the results of the ISM experiments indicate that a large part of the sorption is kinetic. This kinetic sorption causes an additional decrease in ASOCs after the loading is completed. Extending the time for loading is no solution, because this takes a long time (days) and degradation can occur simultaneously.

In order improve the interpretation of ISM experiments, Bjerg et al. [1996] developed a model that describes the experimental results and that incorporates the most important processes. The model is based on the advection dispersion equation which is supplemented with sorption and biodegradation.

Sorption is assumed to be a bi-continuum process with an instantaneous equilibrium fraction (F) and a kinetic fraction ($1-F$) described by a first order rate constant (α) and the K_d. Biodegradation can either be described using Monod-type kinetics or plain first order degradation. The model distinguishes two flow regimes, convection dominated flow during loading and sampling, diffusion between sampling. The fits obtained with the model were improved considerably when introducing a lag phase before degradation. Batch experiments must be performed to determine the parameters K_d and F since they are confounded. Because of the low concentrations associated with most compounds, the first order degradation approach with lag phase turned out to work best.

For abiotically degrading compounds, it is impossible to determine sorption constants because the compounds can degrade during the deactivated experiments (possible under anaerobic conditions for nitro- and chloro-substituted compounds).

In polluted aquifers with the ASOCs already present, the sorption constants may only be obtainable by means of radio-labelled compounds. This limits the number of simultaneously examined chemicals.

An advantage of studying several compounds simultaneously is that slow sorption can be discerned from degradation by comparing the fate curves with respect to sorptive properties (log K_{ow}) of the chemicals.

With the ISM technique the occurrence of abiotic processes can only be indicated. If a fate curve of a compound differs from the curves of compounds only affected by sorption and if the compound concentration is also decreasing in the biologically active ISM, this chemical is expected to degrade abiotically. Complicating aspects (2 out of four mentioned) are: sterilizing agents influence abiotic processes and the control ISM need not be sterile. Nitrobenzene, nitrophenol and trichloromethane are abiotically transformed under reduced conditions [Nielsen et al., 1995a and b].

A clear deviation between the fate curve of the active ISM and the curve of the control ISM, indicates that biological degradation occurs. Identification of lag phases is subjective and depends on the sampling interval. A lag phase should be supported by model simulations. A long lag phase can be recognised on the large shoulders on the fate curve of the active ISM.

The degradation can be proved by monitoring intermediates or end-products, radio-labelled compounds (e.g. 14C) can be considered.

4.4   Methods based on microbial characterization techniques

While redox zones and for a large part the dissappearance of pollutants are the result of microbial activities, extremely little work has been done on the micro-organisms involved in these processes. Methods reported in the literature on landfills (mentioned in the first part) are rather insensitive. They yield little specific information or even biased information. However, especially molecular techniques could be useful to examine the biodiversity and their relation to redox zones and biodegradation. These methods are discussed in the second part.

4.4.1   *Methods reported in the literature on landfills*
Unamended bioassays
Methanogenesis, sulphate reduction, iron reduction, manganese reduction or denitrification is followed in time to get an indication of the ongoing redox processes. This is done on a sediment and/or groundwater sample which is incubated in the laboratory without (significant) addition of

compounds. Deactivated sediments are used as negative controls. Rates can be determined. For methanogenesis formed methane has been analysed, for sulphate reduction the disappearance of radioactive sulphate (added in small concentration) has been measured while for iron and manganese reduction the formation of Fe(II) and Mn(II) has been determined. For denitrification acetylene is added to stop the last step in denitrification and accumulating N_2O is measured [Ludvigsen et al., 1995 and 1996].

Determination of cell numbers and viability
Total cell numbers are determined by staining with acridine orange staining and counting cells under the microscope, viable biomass has been determined by measuring the total amount of lipids and by ATP content measurements [Albrechtsen et al., 1995; Ludvigsen et al., submitted].

Most probable number (MPN) enumeration for specific redox groups of micro-organisms (sulphate-, nitrate- manganese- or iron reducers, methanogens) has been done by serial diluting samples of sediment or groundwater in tubes containing medium supporting the growth of the specific group of micro-organisms. After incubation for up to 90 days the tubes are judged for growth and the disappearance of electron acceptors or appearance of reduced compounds. Based on the tubes in which still growth/redox reactions are observed, the number can be determined from a table. For landfills no MPN methods have been used for the enumeration of specific degraders [Ludvigsen et al., 1995; Ludvigsen et al., submitted].

MPN is of limited use since only a minor part of the members of a population can be cultured. For example, Fries et al. [1997] observed that reducing toluene and phenol concentrations (as sole source of carbon) from 50 ppm to 5 ppm in MPN increased the population density measured for these degraders by 1.5 and 1 log units at an aquifer where bioremediation was occurring. Lower concentrations could not be reliably prepared and scored, while there were indications that also these concentrations are toxic.

Phospholipid fatty acid analysis (PLFA)
This method does not depend on culturing and has been applied by the group of Christensen [Albrechtsen et al., 1995; Ludvigsen et al., submitted]. Phospholipids are extracted from environmental samples and are analysed via gas chromatography. The composition of the fatty acid profile (presence and amount of certain lipids (so-called biomarkers), ratios between certain lipids) tells which groups of micro-organisms are present and gives information on their physiological state (stress, growth conditions). The use of biomarkers is not without dispute. The method does not yield specific information on species composition, since profiles are very complex due to the fact that each micro-organism contains several fatty acids which all appear in the profile.

4.4.2   *Methods used at the microbial eco-physiology section at the Free University of Amsterdam*
The methods used for microbial characterization described above are either dependent on cultivation or do not yield very specific information on biodiversity and activities. This can be obtained by methods which are used at the microbiology department of the faculty of biology, Free University of Amsterdam. These methods, physiological characterization by the use of Biolog plates and especially nucleic acid based methods can easily be applied for microbial population characterization at landfills.

Biolog microplates
Physiological characterization
During ISM and LBM experiments, microbial populations are in fact characterized for physiological characteristics by determination of degradation potential. However more information might be obtained faster by the use of Biolog microtitre plates. The Biolog system was initially

developed to identify micro-organisms based on differences in their metabolic activities. The core of the system consists of 96-well microplates. Each well contains a different carbon source and a redox dye, 2,3,5-triphenyl tetrazolium chloride (TTC). The chemistry is rehydrated by inoculating a cell suspension. Utilisation of the carbon source results in the formation of a purple color. This color reaction relates to the interaction of TTC with dehydrogenase activity and/or electron transport, by which the colorless TTC is reduced and forms a purple formazan.

Underneath and close to landfills anaerobic conditions prevail. Initially microplates were only used for identification of aerobic gram-negative or gram-positive bacteria. Now, procedures are also available so that the Biolog system can also be used for anaerobic bacteria (Biolog Inc, Technical tips on using the Biolog system with anaerobic bacteria, February 1997).

Community specific profiles with ordinary Biolog plates
As stated above, the microplates (specific for either gram-positive or gram-negative bacteria) were originally developed for the characterization and identification of single microbial isolates. Garland and Mills [1991] were the first to publish the use of ordinary microplates as a functionally based measure for classifying heterotrophic microbial communities. They made suspensions of soil and inoculated these into the plates. They and others found that it is possible to detect site-specific differences in the functional diversity. This has been shown for grassland communities, disturbed soils, rhizosphere communities and wastewater treatment systems [Bossio and Scow, 1995; Victorio et al., 1996; Garland and Mills, 1991; Zak et al., 1994; Garland, 1996a]. This method can also be used to make specific community profiles at landfills, indicative for biodegradation.

A number of groups has done research on how to use Biolog plates for obtaining community specific profiles. Biolog plates can be monitored in two ways:

- for the presence of a negative or positive response to each of the substrates;
- for the amount of reduced TTC formed, or the rate at which this occurs.

It is clear that the latter is far more time consuming. However, it has been observed that community differences in substrate oxidation recorded at a fixed time might simply reflect differences in the total number of micro-organisms in the communities. This can be overcome by either using a certain inoculum density or monitoring the plates at several incubation times [Haack et al., 1995; Garland, 1996b].

For some time it has been disputed whether Biolog plates measure the expressed phenotypes or the phenotypic potential of the community. The facts that relatively short incubation times are used and that some growth in the plates occurs indicate a combination of both. Also, Haack et al. [1995] observed that the growth in wells did not correlate to the extent or rate of substrate oxidation.

There are several limitations to the use of Biolog plates:

- some strains fail to give a response in Biolog plates;
- the degree of oxidation of particular substrates cannot be interpreted with regard to the number or activity of community members carrying out that oxidation [Haack et al., 1995];
- like any culture-based procedure (like MPN), the Biolog method is selective, it only detects activities from bacteria capable of growing or remaining metabolically active in the Biolog plates under conditions that are different from those in the environment. Therefore it cannot be expected to reflect the metabolic capabilities of the entire microbial community.

A discussion group is present on Internet regarding the use of Biolog plates for ecological purposes (www.biolog.com).

Biodegradation specific microtitre plates

More specific information about microbial communities present in landfill leachate plumes and their biodegradation potential can be obtained by using a self-developed microplate containing carbon sources which are encountered underneath and near landfills. Several studies have appeared in which Biolog MT plates are used to assess the biodegradation potential of individual strains [Lee et al., 1995; Fulthorpe and Allen, 1994] or microbial populations [Victorio et al., 1996]. These plates are supplied with the tetrazolium dye chemistry in microplate form without any carbon sources so that users can add carbon sources. The substrates in the ordinary microplates are quite common substrates and therefore the profile generated might not be specific for biodegradation. Candidates are xenobiotics, like BTEX and chlorinated aromatics, and intermediates and end-products of their degradation [CUR/NOBIS, 1996], as well as closely related natural compounds such as (degradation products of) lignins and humic acids and substrates normally encountered at landfills [Barlaz et al., 1989].

The Biolog MT plates have also been applied for the determination of biodegradation potential of microbial populations in waste-water treatment systems, using substrates encountered in these waste-waters [Victorio et al., 1996]. Substrates were added at a concentration of 200 mg/l. Physical extraction methods (homogenisation in the presence of deflocculating agents (Tween 80 and sodium pyrophosphate)) were used to extract cells from flocs. The use of specific compounds produced a more distinctive pattern than the gram-negative specific microplate.

When using xenobiotic substrates, toxicity of these compounds should be addressed. Fulthorpe and Allen [1994] used the Biolog MT plates to screen culture collections of bacteria for degradation of (chloro)aromatic compounds. They found that the substrate concentrations required to allow utilization detection lay between 25 µM and 2500 µM. For several strains, substrates supplied at these concentrations were toxic. The authors recommend that users test a range of substrate concentrations between 0.1 and 10 mM for each test compound, and that before screening is carried out, the toxicity of the test compounds is assessed. Toxicity was assessed by growing strains in a general medium in MT plates supplemented with various levels of the test compounds, and growth inhibition was quantified by reading the tetrazolium dye concentration.

By feeding the wells at 0.5 mM substrate per day toxic effects could be overcome. However, no significant reactions could be induced when substrates were added at lower concentrations. Alternatively, one could use a two-liquid phase system with the toxic compound dissolved in for example undecane. This technique is applied for batch cultures and guarantees continuous and sufficient supply of the compound to the water phase. Also it allows addition of large amounts of a compound to a well, while the concentration in the water phase can be kept below the toxicity level [Holliger and Schumacher, 1994]. It should be noted that Fulthorpe and Allen [1994] used monocultures in their plates and thus toxic intermediates, which might otherwise be removed by other micro-organisms could have accumulated and inhibited further activity.

Lee et al. [1995] observed that strains which failed to grow on a certain substrate were also negative for this substrate in Biolog plates, but that in a few cases a positive growth reaction with a certain substrate did not show a positive reaction in Biolog plates.

Nucleic acid based characterization

Introduction

Every micro-organism contains nucleic acids (DNA and RNA). These stretches of DNA called genes contain the information for every part of the cells metabolism, for example the biodegradation of xenobiotics. Under the right conditions (for example presence of a xenobiotic), a gene is transcribed into messenger RNA (mRNA). Next, the mRNA is translated into protein with the aid of ribosomes and transfer RNA (tRNA). The ribosomes are composed of several

ribosomal RNA (rRNA) molecules and proteins. The ribosome content of a cell is thought to depend on the physiological state of the cell, under non-growing conditions its content is lower than during growth. Thus, analysis of DNA gives an indication for the presence of a gene, the genetic potential, while analysis of RNA gives information on the activity of a certain micro-organism or process.

Methods that analyse nucleic acids extracted from environmental samples have been shown to be of value to bioremediation (reviewed by [Brockman, 1995; Sayler et al., 1995]), but to date have not been used to analyse samples from landfills, and even only in a few cases for bio-remediation in general.

The value of nucleic acid methods relates to the fact that they allow analysis independent of the artefacts that can arise from laboratory biodegradative potential assays and laboratory culture-based enumerations and from the ability to culture only a minor proportion (about 0.1 %) of the micro-organisms in the environment [Brockman, 1995]. Furthermore, samples can be easily stored by freezing immediately after sampling.

All nucleic acid based methods involve chemical extraction and purification of cellular nucleic acids, denaturing the nucleic acid strands, introduction of exogenous nucleic acid sequences (called probes or primers) and controlled reannealing of complementary stretches of nucleic acid to form double-strand hybrids. The term probes is used when one nucleic acid sequence is introduced and used for specific hybridisation. This probe is specifically labelled (radioactive or fluorescent) to allow detection of hybridisation.

The term primers is used for short nucleic acid sequences which are applied in the polymerase chain reaction (PCR). In PCR a so-called forward primer and a reverse primer, a DNA poly-merising enzyme and its substrates, and many cycles of denaturing, primer annealing and synthesis of DNA are used to amplify a target DNA sequence over a million-fold. This method provides far more sensitivity than probe hybridisation for the detection and analysis of genes which are present in low numbers in the sample. Probe sensitivity is generally equivalent to 10^4 - 10^6 gene copies per gram soil or sediment, depending on the size and specific activity of the gene probe and the level of background hybridisation. PCR improves specificity for a particular gene by excluding the amplification of related genes that could be detected by hybridisation analysis. Both DNA and RNA can be used as the nucleic acid template in the analysis. RNA has to be translated into DNA by reverse transcriptase (RT), before it can be used in PCR (RT-PCR).

A good example of the usefulness of nucleic acid techniques in bioremediation is given by Chandler and Brockman [1996]. They wanted to determine whether a lack of genetic potential for bioremediation was responsible for low levels of oxygen utilisation at a jet fuel contaminated site undergoing the first phase of bioremediation. Extracted DNA was amplified with primers specific for genes involved in JP-5 degradation (catechol-2,3 dioxygenase, naphtalene dioxygenase). Results showed that significant aromatic biodegradative potential existed at the site suggesting that physical and/or chemical factors were inhibiting oxygen delivery.

Disadvantages in nucleic acid analysis can be the efficiency of extraction, bias in extraction, spatial variability in community composition and the fact that the presence of a hybridisation signal does not guarantee that the sequence being detected corresponds to the same enzyme. Furthermore, the use of nucleic acid techniques requires knowledge on the nucleotide sequence of the genes one wants to examine. In the case of our project, the targets for amplification might either be functional genes involved in anaerobic biodegradation of xenobiotics or micro-organism specific genes.

Alternatively, populations can be examined independent of sequence information by amplification using randomly chosen primers. These three options are discussed in the next paragraphs.

Functional genes involved in biodegradation
Nucleic acid based studies regarding the detection of genes involved in biodegradation of xenobiotics have so far been limited to (a) gene(s) involved in aerobic degradation of a certain compound or groups of closely related compounds. Nucleic acid based studies addressing anaerobic degradation or the degradation of a large number of different compounds have not been encountered.

Information on chemical intermediates and enzymes involved in aerobic degradation as well as their nucleotide sequences show a very complex picture for aerobic degradation. In many cases a single compound can be degraded by several pathways in different organisms. This applies for both aromatic compounds [CUR/NOBIS, 1996; Van der Meer, 1997], halogenated compounds [Häggblom, 1992; Slater et al., 1997] and nitroaromatic compounds [Marvin-Sikkema et al.,1994]. For example there are at least five toluene oxygenase pathways for the aerobic degradation of toluene.

Also, for aromatic compounds the general thought was that the usual strategy to degrade pollutants was to use a number of so-called peripheral enzymes which convert the substances into a common intermediate, which is channelled into one central pathway. However, gene organisation and the similarities in the different aromatic degradation pathways suggest a much greater variety in the 'central' metabolic pathways, and that the 'peripheral' enzymes are more conservative. It seems that pathways have evolved in a vertical manner by acquisition of genes which would just encode the transformation reactions necessary for a particular step. Expansion in a horizontal manner, which would lead to metabolism of a wider variety of initial substrates in a single organism, that are all channelled in the same 'lower' or 'central' pathway seems to be less frequent [Van der Meer, 1997]. Bacteria acquire novel combinations of previously existing genes; genes for a aromatic ring dioxygenase or dihydrodiol dehydrogenase and genes for a chlorocatechol oxidative pathway. The assembly process may trigger a faster divergence of nearby gene sequences. Also, in a study on biodegradative strains, Pellizari et al. [1996] found that probes based on gene encoding naphthalene or biphenyl dioxygenases are not reliable indicators of catabolic capacity, since a poor relation was observed between hybridisation and substrate on which the strain was isolated.

Furthermore, evaluation of the diversity and distribution of catabolic pathways in nature can be highly distorted by the use of enrichment culture techniques [Dunbar et al., 1997]. It should be realised that the micro-organisms of which biodegradation pathways have been studied have usually be obtained by enrichment culturing with relatively high concentrations of xenobiotics. For example for halogenases often relatively low affinities (millimolar range) are observed [Slater et al., 1997], while only low concentrations are found in the environment (micromolar range). This is another complicating factor if one wants to detect biodegradative genes.

Anaerobic degradation of xenobiotics is far less understood than aerobic degradation [Häggblom, 1992]. While a reasonable amount of information is available on degradation intermediates, far less is known about enzymes involved and only for a few enzymes the genes have been isolated. The limited knowledge so far indicates that anaerobic degradation seems to be less complex.

Benzyl-coenzyme A (Benzyl-CoA) is a central metabolic intermediate during the anaerobic degradation of structurally diverse aromatic compounds. The intermediates in the reductive path-

way of benzoic acid degradation are present as CoA thioesters. Also the degradation of many other aromatic acids is initiated by activation to corresponding CoA thioesters via CoA ligases. Next, the aromatic acid-CoA is via CoA-involving intermediate steps converted to benzyl CoA. CoA ligases are dependent on CoA, Mg^{2+} and ATP, have a narrow substrate specificity and their optimum pH is alkaline These CoA-intermediates remain intracellular. CoA addition facilitates the enzymatic transformations.

Ligases expressed in anaerobically grown bacterial cells are regulated by anaerobic conditions and the presence of aromatic acids. A comparison of the available N-terminal sequences of the aromatic acid-CoA ligases as well as sequenced genes suggests that they are very distinct proteins. However, despite poor homology, sequenced genes all shared a consensus amino acid sequence and conserved glutamine residue which are involved in adenylate binding to the protein. Presently, the limited amount of sequenced genes does not allow to derive CoA ligases specific oligonucleotide probes or primers [Elder and Kelly, 1994; Villemur, 1995].

While aerobic bacteria can dehalogenate chlorinated compounds in four ways, anaerobic bacteria can only reductively dehalogenate aliphatic and aromatic halogenated compounds in a respiratory process (thus independent of availability of electron acceptors). Little is known about the organisms or enzymes involved. In a few cases dehalogenations are unspecifically carried out by certain enzyme systems in a co-metabolic manner [Holliger and Schumacher, 1994].

Concluding, it can be said that for both aerobic and anaerobic conditions it is not yet possible to formulate probes and sets of primers to detect the biodegradation of a wide variety of xenobiotics. This is related to a limited amount of genetic information available and, at least for aerobic conditions, the complexity of degradation pathways.

Denaturing Gradient Gel Analysis of 16S rDNA
Micro-organisms contain ribosomes which are composed of ribosomal proteins and RNA (rRNA). The nucleotide sequences of ribosomal RNA molecules are conserved among micro-organisms and can be used to make phylogenetic trees and evolutionary relationships. Especially the 16S rRNA or rDNA is used for this purpose. Within the 16S rRNA genes regions can be found which are highly conserved among all organisms, as well as variable regions which are group specific (for example sulphate reducers) or species-specific. Due to its phylogenetic importance, several thousands 16S rDNA genes of micro-organisms have been (partially) sequenced and therefore a large database is available. This information can be used to examine a environmental sample by the use of specific probes or primers. Appendix E gives an overview of 16S rDNA probes and primers specific for species which might be encountered near landfills. Specific probes and primers are for example known for sulphate- and iron/manganese reducing bacteria as well as for Archaea [Amann et al., 1992; Fry et al., 1997; Barns et al., 1994].

The amplified product can be separated by denaturing gradient gel electrophoresis (DGGE). In DGGE separation is based on the electrophoretic mobility of a partially melted double stranded DNA molecule in polyacrylamide containing a linear gradient of DNA denaturants (urea and formamid) or temperature (called TGGE: temperature gradient gel electrophoresis). Melting occurs in so-called melting domains. A transition of helical to partially melted molecules occurs when the melting temperature is reached at a certain position in the gel. This transition strongly slows down the migration of the molecule in the gel. Sequence variation in the domains results in different melting temperatures, therefore fragments with different sequences will stop at different positions in the denaturating gradient. This set-up allows 50 % of the sequence variants to be detected in DNA fragments up to 1000 bp. The attachment of a 30 - 40 base pair (bp) long Guanine and Cytosine rich sequence to the fragment increases the percentage to nearly 100 %. In this way a profile of a microbial community is obtained [Muyzer et al., 1993].

More information can be obtained from the DGGE profile by sequencing bands in the profile and establishing phylogenetic relationships to known rRNA sequences or by blotting, denaturating and hybridisation with specific probes, such as species-specific probes or probes indicative for redox environment such as probes for sulphate-reducers. With regard to the latter option it should be realised that the Archaeal domain consists of several kingdoms, for which of only one, the Euryarchaeota, something is known about the physiology. Euryarchaeota do not only consist of methanogens but also sulphate reducers, sulphur reducers, thermophilic heterotrophs and extreme halophiles. No specific 16S rDNA probes are at present known to distinguish between these groups, however it is possible to selectively detect methanogens since the enzyme involved in the final step of methanogenesis, methyl coenzyme M reductase, is conserved in methanogens and specific primers are known [Hales et al., 1996]. Denitrifying bacteria form a very divers phylogenetic group, which does not allow specific detection of denitrifying bacteria via 16S rDNA specific primers. Functional probes based on nitrate reductase, which occurs in two forms, show most promise [Ward, 1996].

A DGGE profile of 16S rDNA molecules by itself does not tell about biodegradation, unlike the use of primers or probes for specific functional genes. It has to be related to other observations, such as degradation of compounds or physiological characteristics or increase in activity or numbers upon spiking with xenobiotics.

Random Amplified DNA Polymorphism (RAPD) PCR
The RAPD technique was originally developed to characterize strains. It implies the use of a single short, about 10 nucleotides long, arbitrary chosen primer in PCR at relatively low annealing temperatures. Under this conditions the primer will initiate synthesis on DNA, even when the match with the DNA template is not perfect. Some of these priming events will occur on opposite strands of the DNA, generating a PCR fragment. This creates a mixture of randomly synthesized fragments of different seizes (0.1 - 1.5 kb). These fragments are separated by size on agarose gels or polyacryl amide gels, resulting in a profile of bands which can be strain-specific (see for example [Röling and Van Verseveld, 1996; Van Rossum et al., 1995]). In the profiles some bands are strain-specific while others are species-specific.

In principle, RAPD can also be used for characterization of biodiversity. If more different strains are present, more different bands will be present in the profile. Since already for one single strain a lot of bands can be obtained, more stringent conditions (meaning higher annealing temperatures) have to be applied. This will lower the amount of bands per strain present in the sample and therefore result in a profile which is interpretable.

MODELS

5.1 Background

In the previous sections of this report a large number of processes have been reviewed that can be of importance in controlling the fate of landfill leachate. When studying the effect of natural attenuation on the leachate, coping with the large time scale on which the processes occur becomes a problem. Due to the complexity of the system, predictions about future behaviour is very difficult. Numerical models can be a useful tool in predicting future behaviour. However, it is essential to realise that models are a gross simplification of reality and as such predictions should be used with necessary care and expert judgement.

There is a wide range of models that can be applied to describe (certain aspects) of leachate behaviour in aquifers. The complexity of the models range from very simple (only one or two processes) to extremely complex (very many interrelated processes). Each of these models have their specific application field. In the following sections we will summarize the main processes in the context of applying a model in order to predict the future behaviour of leachate.

5.1.1 *(Ground) Water flow*

Leachate is a water based solution that is diluted by the groundwater and that moves with the groundwater. Therefore a very important process that has to be accounted for is the flow of water through the landfill and the aquifer. Most models describing water flow in aquifers (and soils) are based on the continuity or mass balance equation. The water flux is mostly calculated using the d'Arcy equation. Often used sink fluxes are water uptake by roots, evaporation at the domain surface and drainage or seepage at the domain boundaries. The most common water source fluxes are precipitation at the surface and seepage at the domain boundaries.

5.1.2 *Production of leachate*

Leachate production in landfills is an extremely complex process influenced by variables such as land filling history, location of sources, precipitation, temperature, permeability, etc. Generally leachate production is modelled using very simple model approaches that consider the landfill to be more or less a black box [Kjeldsen and Christensen, 1997].

5.1.3 *Dissolved solutes (leachate) flow*

In addition to models water flow, models are used for describing the fate of the dissolved leachate. Important processes which can be accounted for are mass conservation, solute flow (using both advection as well as dispersion), sorption, ion exchange, precipitation and dissolution, aqueous complexation, inorganic redox reactions, biologically mediated redox reactions and the growth and decay of organic matter [Brun, 1996].

Many approaches are possible for incorporating all above mentioned processes in the transport differential equations [Yeh and Tripathi, 1989]. One of the most crucial choices to be made is to decide which part of the solutes is taken as the reference. One can choose for the total amount in the soil. Most approaches have the mobile dissolved solute as the reference, from which all other solute related species are calculated.

The sink/source terms can be calculated using different approaches. Fundamental in this respect is if the processes are modelled as being in equilibrium or that kinetic (time dependent) effects play a role. In addition leachate consists of many with each other interacting solute species which calls for a multicomponent transport model.

5.2 Complexity of models

The role of numerical models in evaluating the potential of natural attenuation for controlling leachate from landfills is very diverse. Very simple models, using rough estimations of parameters and only a limited range of processes can be very useful in the development of a first conceptual model. Questions to be answered are:

- the maximum and minimum expected range of the leachate plume based on expert guesses of critical parameters;
- insight into the consequences of degradation (for example based on first order kinetics);
- insight into which reactions play a role when taking the measured chemical environment into account;
- how will the leachate plume develop in the (near) future.

The answers to these questions will mostly be found using a step wise approach, starting with preliminary rough answers which will be improved as more data becomes available. Initially simple models, such as analytical solutions to the flow equations, simple one compartment chemical equilibrium models will be used.

The results obtained in this preliminary phase can be used in determining how and where to take samples and to carry out measurements. As more information becomes available, these can be used to refine the conceptual model. Refining the model can be done by decreasing the margins on the parameters, but also by adding more processes or relations between processes by using more complicated numerical models. In this respect it is crucial to understand that the conceptual model of the landfill consists of the combination of data (obtained from experiments and expert knowledge) and numerical code. Therefore very simple numerical models can be extremely useful with limited but high quality data.

Simulating the development of redox zones as a result of the (bio)degradation of landfill leachate is an extremely complex problem. Currently not much is known about the coupling of biologically mediated redox processes to inorganic redox processes. We know that kinetic processes play an important role, however how these processes should be described is still very uncertain.

5.3 Metabolic control analysis as an alternative approach

Metabolic control Analysis (MCA) is based on establishing the control an enzyme has on a certain flux (for example growth rate or glycolysis) in a micro-organism [Kell and Westerhoff, 1986]. This can done by changing the concentration of the enzyme slightly (for example by the addition of inhibitors or by genetic techniques) and subsequently measuring the change in steady state flux. A control coefficient is calculated by dividing the relative change in flux with the relative change in enzyme. The higher the coefficient the more rate determining the enzyme. This concept is presently only applied to metabolic pathways in a single micro-organism, but can be extended to ecological and non-biological processes. An Ecological Control Analysis (ECA) can be developed and used to determine which functional groups of micro-organisms are important in biodegradation. Only the activity or biomass of these groups then has to be used in mathematical models. With respect to non-biological processes it could be used to establish for instance the importance ('control') of sorption in the disappearance of xenobiotics. Again, only factors with significant control then have to be included in mathematical models.

5.4 Overview of models simulating the processes responsible for natural attenuation of landfill leachate

5.4.1 *Commercial models*

Much of the following information has been taken from Internet. For some models the text found on Internet has been reproduced.

PHREEQC 2.0 (USGS (D.L. Parkhurst) and Free University of Amsterdam (C.A.J. Appelo))
PHREEQC is a 1D flow tube model that calculates advective flow, longitudinal dispersion and diffusion according to the 'mixing cell concept'. Dual porosity media can be simulated with the first order exchange model or with explicit diffusive mixing of stagnant and mobile cells. PHREEQC is currently one of the most sophisticated geochemical reaction models available. PHREEQC version 1 was limited to equilibrium geochemistry: speciation and saturation-index calculations, mixing of solutions, mineral and gas equilibria, surface-complexation reactions, ion-exchange reactions, and inverse modeling.

In the new PHREEQC version 2.0, kinetic rate equations can be defined with BASIC language statements in the input file. The modelling of redox sequences can be easily performed with this novel extension. Transformation (degradation) of organic matter (CH_2O, TOC) to methane, CO_2 or intermediates can be simulated and can be made to depend on the electron acceptors present or any other modelled parameter (temperature, pH). Appelo and Parkhurst (in preparation) give an example in which they simulate the rate of biochemical reactions with the Monod, or Michaelis-Menten equation. It is also possible to simulate the degradation of ASOCs. The rates can be made to depend on electron acceptors present. The modelling of sorption has never been the purpose of PHREEQC, but should not be difficult to include (pers. communication Appelo, 1997). PHREEQC is very capable of modeling redox sequences downstream of landfills, because sorption of DOC is not of significance as observed at the Vejen and Grindsted landfills in Denmark.

PHAST (release in the future; USGS (D.L. Parkhurst))
PHAST is an integration between the 3D transport model HST3D and PHREEQC. This model may be able to model a sequence of redox zones (pers. comm. P. Engesgaard, 1997). In order to obtain more information on PHAST we mailed the developer.

His reply was:

PHAST is a 3D model that is based on PHREEQC version 1. Thus, only equilibrium chemistry can be modeled. I hope to incorporate the kinetics that have been developed for PHREEQC version 2.0, but at this time it has not been done. My priority is to release PHREEQC version 2.0 and then PHAST. I don't know how long this will take. I have had very little time in 1997 so far, but I should be able to get back to work on it soon (mail from David L. Parkhurst, dlpark@usgs.gov).

Currently KIWA (a Dutch company focussing on drinking water quality) is trying to integrate PHREEQC with MODFLOW.

HYDROGEOCHEM (price: $ 1,575)
HYDROGEOCHEM is a coupled model of HYDROlogic transport and GEOCHEMical reactions in saturated-unsaturated media. It is designed to simulate transient and/or steady-state transport of Na, aqueous components and transient and/or steady-state mass balance of Ns adsorbent components and ion-exchange sites. Along the transport path, HYDROGEOCHEM computes the species distribution of N component species, Mx complexed species, My adsorbed species. Mz ion-exchanged species, and Mp potentially precipitated species.

The physical, hydrological and chemical settings are as follows:

Media: Heterogeneous and Anisotropic; Hydrologic Processes: Advection, Dispersion and Diffu-sion; Chemical Processes: Aqueous Complexation, Adsorption/Desorption (Surface Complexa-

tion, Constant Capacitance, and Double Layer Approaches), Ion-Exchange, Precipitation/Dissolution, Redox, and Acid-Base Reactions; Source/Sink: Spatially and Temporally Dependent Element and Point Sources/Sinks; Initial Conditions: Prescribed Initial Condition or the Simulated Steady-State Solution as the Initial Condition; Boundary Conditions: Prescribed Total Analytical Concentrations on Dirichlet Boundaries, Prescribed Fluxes on Flow-In Boundaries, Natural Advective Fluxes on Flow-Out Boundaries - All Boundary Values (Concentrations or Fluxes) are Spatially and Temporally Dependent; Numerical Discretization: Finite-Element Methods with Quadrilateral Elements, Triangular Elements, or the Mixtures of These Two Types; Solvers: Direct Band Matrix Solver, Basic Point Iterations, and 4 PCG Methods (polynomial PCG, Incomplete Cholesky PCG, Modified Incomplete Cholesky PCG, and Symmetric Successive Over-Relaxation PCG); Time Stepping: Implicit Difference, Crank-Nicholson Central Difference, or Mid-Difference; Solution Methods for Geochemical Reactions: Newton-Raphson with Full Pivoting to Solve the Jacobian Matrix Equation and Constraints on Species Concentrations.

HYDROGEOCHEM is the only commercially available model for the simulation of reactive multispecies-multicomponent chemical transport through saturated-unsaturated media. It is not a path model; it is a true transport model coupled with homogeneous and heterogeneous geochemical reactions.

BIOPLUME II (US EPA and Rice University: Rifai et al.)
BIOPLUME II is a two-dimensional solute transport model to compute changes in concentration over time due to advection, dispersion, mixing, and retardation. The model simulates the transport of dissolved hydrocarbons under influence of oxygen-limited biodegradation. It also simulates reaeration and anaerobic biodegradation as a first order decay in hydrocarbon concentrations. BIOPLUME II is based on the USGS 2D solute transport model MOC (Konikow-Bredehoeft). It solves the transport equation twice: once for hydrocarbon and once for oxygen. As a result, two plumes are computed at every time step, and the two plumes are combined using the principle of superposition. The model assumes an instantaneous reaction between oxygen and hydrocarbon. It can simulate natural biodegradation processes, retarded plumes, and in situ bioremediation schemes. The model allows injection wells to be specified as oxygen sources into a contaminated aquifer. It also provides three additional oxygen sources: 1) initial dissolved oxygen in the uncontaminated aquifer; 2) natural recharge of oxygen across the boundaries; 3) vertical exchange of oxygen from the unsaturated zone, and 4) injection wells.

BIOPLUME II comes with a pre-processor for creating data files and a post-processor which generates output that can be read by commercial contouring programs such as SURFER. Grids up to 120 by 120 may be modeled.

BIOSCREEN (version 1.3 - July 1996) (US EPA and US Air Force)
BIOSCREEN is a three-dimensional contaminant transport model for dissolved phase hydrocarbons in the saturated zone under the influences of oxygen, nitrate, iron, sulfate, and methane limited biodegredation. Processes simulated are: advection, dispersion, adsorption, first order decay and instantaneous reactions under aerobic and anaerobic conditions.
With 'instantaneous' reaction is meant that the rate of biodegradation is much faster than the rate of electron acceptor replenishment in the aquifer. This is found valid for aerobic {Wilson et al., 1985} as well as for anaerobic reactions {Newell et al., 1996}. This approach may not apply to sites with low hydraulic residence times. The instantaneous reaction assumption is applicable to almost all petroleum release sites (User's manual BIOSCREEN). This model assumes that all the various aerobic and anaerobic reactions occur over the entire area of the contaminant plume, thus the theoretical zonation of reactions is not simulated in BIOSCREEN.

The theoretical zonation of redox conditions has been observed at the Vejen landfill [Lyngkilde and Christensen, 1992a] and the Grindsted landfill [Bjerg et al., 1995]. A careful inspection of field data of gasoline spill sites shows little or no evidence of this theoretical zonation; in fact all of the reactions appear to occur simultaneously in the source zone (BIOSCREEN User's manual), so the modelling approach is found valid for these cases.

The following conceptual model is used in BIOSCREEN: the upgradient groundwater contains electron acceptors, and flows through a source zone, in which BTEX is released to the groundwater. This zone can be divided into five average concentration parts, and consists of a plain, rectangular to the groundwater flow. Thus the source is only defined by its depth and broadness, but has no length.

Biological reactions occur until the available electron acceptors in groundwater are consumed. The total amount of available electron acceptors can be estimated by the difference in concentrations between upgradient groundwater and the source zone for oxygen, nitrate, and sulfate; in the case of methanogenesis and Fe(III)-reduction, the potential amount is estimated by measuring the production of metabolic by-products (Fe(II) and CO_2).

The amount of electron acceptor depletion is coupled, using stoichiometry, to the amount of hydrocarbon degradation by utilisation factors, which are default for BTEX under different redox reactions {Wiedemeier et al., 1995}.

It is questionable if this conceptual model also applies to landfills. The source zone at a landfill is not a line but a rectangle. A petroleum spill consist for the largest part of BTEX, landfill leachate consists for the largest part of DOC, and contains relative to DOC only traces of BTEX and chlorinated aliphatic compounds. So the the largest part of the electron acceptor decrease is due to oxidation of DOC, and only a small part is due to SOCs degradation.

Modeling a landfill system with BIOSCREEN is probably only warranted using first order degradation kinetics.

BIOPLUME 3 (US EPA and Rice University: Rifai et al.)
The following information about BIOPLUME 3 is gathered from the Internet at the end of May 1997. The site was last updated at March 5, 1997.

BIOPLUME is a new version of the BIOPLUME model (version 3), currently under development at Rice University, Houston, Texas, the developers of BIOPLUME II. BIOPLUME 3 is a numerical model for simulation of two-dimensional saturated flow and transport of biodegradable solutes. The model is an extension of the twodimensional flow and solute transport model MOC/KONBRED of the US Geological Survey. The main improvement in new version of BIOPLUME is that it will be able to simulate up to five electron acceptors instead of one electron acceptor as is the case with BIOPLUME II (oxygen). This makes it possible to use BIOPLUME 3 for anaerobic conditions.

BIOPLUME 3 is being developed for the US Air Force's Center for Environmental Excellence (AFCEE). It is currently in advanced beta testing, which will be followed by an independent review by the US EPA. Its release will follow the testing and EPA review which is expected to take several months.

Upon its release, BIOPLUME 3 will be incorporated in the EIS/GWM modeling system, among others. EIS/GWM is a sophisticated MS Windows based system for modeling of two- and three-

dimensional flow and transport of nonconservative biodegradable constituents and includes various options for simulating (bio-)remediation, including reactive barriers (see EIS/GWM).

BIOPLUME is a more sophisticated biodegradation model than BIOSCREEN. BIOPLUME 3 employs particle tracking of both hydrocarbon and alternate electron acceptors using a numerical solver. The model employs sequential degradation of the biodegradation reactions based on zero order, first order, instantaneous, or Monod kinetics (BIOSCREEN User's manual).

MOC (USGS)
[http://www.epa.gov/cgi-bin/mdb_sw.cgi?modelkey=740]
MOC is a two-dimensional model for the simulation of non-conservative solutetransport in heterogeneous, anisotropic aquifers. MOC is developped by L.F. Konikow and J.D. Bredehoeft (USGS). MOC computes changes in time in the spatial concentration distribution caused by convective transport, hydrodynamic dispersion, mixing or dilution from recharge, and chemical reactions. The chemical reactions include first-order irreversible rate reaction (e.g. radioactive decay), reversible equilibrium-controlled sorption with linear, Freundlich or Langmuir isotherms, and monovalent and/or divalent ion-exchange reactions. The model assumes that fluid density variations, viscosity changes, and temperature gradients do not affect the velocity distribution. MOC allows modeling heterogeneous and anisotropic, confined aquifers.

MOC solves the ground-water flow equation and the non-conservative solute-transport equation in a stepwise (uncoupled) fashion. The computer program uses the Alternating Direct Implicit (ADI) method or the Strongly Implicit Procedure (SIP) to solve the finite-difference approximation of the ground-water flow equation. The SIP procedure for solving the ground-water flow equation is most useful when areal discontinuities in transmissivity exist or when the ADI solution does not converge. MOC uses the method of characteristics of solve the solute transport equation. It uses a particle tracking procedure to represent convective transport and a two-step explicit procedure to solve the finite-difference equation that describes the effects of hydrodynamic dispersion, fluid sources and sinks, and divergence of velocity. The explicit procedure is subject to stability criteria, but the program automatically determines and implements the time step limitations necessary to satisfy the stability criteria. MOC uses a rectangular, block-centered, finite-difference grid for flux and transport calculations. The program allows spatially varying diffuse recharge or discharge, saturated thickness, transmissivity, boundary conditions, initial heads and initial concentrations, and an unlimited number of injection or withdrawal wells.

MT3D/RT3D/MODFLOW (EPA/USGS)
MT3D (WATERLOO HYDROGEOLOGIC, INC.) Price: US $ 500.00
[http://www.flowpath.com/mt3d.html
MT3D is a comprehensive three-dimensional mass transport model for simulating advection, dispersion, and chemical reactions of a single species contaminant in groundwater systems. The model program is based on a modular structure to permit simulation of transport processes independently or jointly. MT3D is capable of simulating advection, dispersion, first-order decay, or production reactions, and linear and non-linear sorption. MT3D is linked to the USGS's three-dimensional groundwater model MODFLOW.

The model input is compatible with standard MODFLOW data files, including three- dimensional transient flow fields with the presence of wells, drains, rivers, recharge, evapotranspiration and a variety of boundary conditions. The model retrieves the heads and various flow and sink/source terms saved by the model and automatically incorporates the specified boundary conditions.

MT3D's modular structure is similar to MODFLOW and allows simulation of sink/source mixing, chemical reactions, advection and dispersion independently without reserving computer memory space for unused options.

Solution options include; the method of characteristics (MOC); the modified method of characteristics (MMOC); and a hybrid of these two methods (HMOC). This approach combines the strength of the MOC method for reducing numerical dispersion and the computational efficiency of MMOC, making MT3D uniquely suitable for a wide range of field problems. In addition, MT3D can also be used in the standard finite-difference mode.

MT3D accommodates the following spatial discretization capabilities and mass transport boundary conditions: confined, unconfined and variably confined/unconfined aquifer layers; inclined model layers and variable cell thicknesses within the same layer; specified concentrations or mass flux boundaries; and the solute transport effects of external sources and sinks.

RT3D
[http://terrassa.pnl.gov:2080/bioprocess/rt3d.htm]
RT3D is a Fortran 90-based software package for simulating three-dimensional, multi-species, reactive transport in groundwater. The code is based on the 1997 version of MT3D (DOD_1.5), but has several extended reaction capabilities. RT3D can accommodate multiple sorbed and aqueous phase species with any reaction framework that the user wishes to define.

RT3D is widely applicable with a variety of pre-programmed reaction packages and the flexibility to insert user-specific kinetics, RT3D can simulate a multitude of scenarios. For example, natural attenuation processes can be evaluated or an active remediation can be simulated. Simulations could potentially be applied to scenarios involving contaminants such as heavy metals, explosives, petroleum hydrocarbons, and/or chlorinated solvents. RT3D is highly flexible. The users can enter their own reaction kinetic expressions or choose from a suite of 6 pre-programmed reaction packages. Pre-programmed packages include:

1. two Species Instantaneous Reaction (Hydrocarbon and Oxygen);
2. instantaneous Hydrocarbon Biodegradation Using Multiple Electron Acceptors (O_2, NO_3^-, Fe^{2+}, SO_4^{2-}, CH_4);
3. kinetically Limited Hydrocarbon Biodegradation Using Multiple Electron Acceptors (O_2, NO_3^-, Fe^{2+}, SO_4^{2-}, CH_4);
4. kinetically Limited Reaction with Bacterial Transport (Hydrocarbon, Oxygen and Bacteria);
5. non-Equilibrium Sorption/Desorption (can also be used for Non-Aqueous Phase Liquid Dissolution);
6. reductive, Anaerobic Biodegradation of PCE/TCE/DCE/VC.

RT3D can be run on either of two of the most widely used graphical user interfaces: the U.S. Department of Defense Groundwater Modeling System (GMS) and Waterloo Hydrogeologic, Inc.'s Visual MODFLOW.

This work was funded by the office of Technology Development, within the Department of Energy's Office of Environmental Management, under the 'Plume Focus Area' and the 'Subsurface Contamination Focus Area.' RT3D was originally developed for the Remediation Technology Development Forum Bioremediation Consortium.

TRIWACO and SORWACO | MICROFEM and CHEMPATH
TRIWACO and MICROFEM are software packages for modelling groundwater flow, based on the finite element method. TRIWACO has been developed by IWACO, and MICROFEM is widely used on the hydrology department of the Free University. A model made in TRIWACO can easily

be translated to a MICROFEM model with a simple program developed by the Dutch drinking water company WZHO. WINDOWS versions are currently being developed for both packages. TRIWACO and MICROFEM can simulate multi aquifer systems, steady and transient flow systems, phreatic conditions, anisotropy and heterogeneous aquifers, and calculate path lines and travel times with 3D particle tracking. Both packages differ in few details. TRIWACO is coupled to ARC/INFO, also simulates unsaturated groundwater flow and can simulate more topsystems.

Solute transport along the calculated flowpaths can be solved with SORWACO or CHEMPATH. SORWACO has been developed by IWACO and calculates the solute transport according to the 'mixed-cell' principle. For each cell parameters can be adopted which describe first order decay rate, and sorption with a non-lineair Freundlich isotherm. Fysical non-equilibrium is modelled with stagnant and mobile zones, and chemical non-equilibrium is modelled with a velocity-dependent Freundlich isotherm.

CHEMPATH (BASELINE Concept Inc. Price: US $ 795) imports output from MICROFEM directly. CHEMPATH simulates zero and first order decay; Linear, Langmuir and Freundlich; equilibrium, kinetic, and two-site sorption. It handles time-variant and constant boundary conditions and can also handle uniform and non-uniform initial conditions for each layer.

FLONET/TRANS (Waterloo Hydrogeologic Software)
FLONET/TRANS is a two-dimensional, finite element model for simulating cross-sectional groundwater flow and contaminant transport in confined, unconfined and leaky aquifers with heterogeneous and anisotropic porous media. It is ideal for applications such as estimating discharge to an excavation for site dewatering design, determining seepage rates from the face of a slope, or predicting contaminant plume migration from a landfill or underground storage tank.

The main features are: a fully-integrated graphical user interface, irregular grid spacing and deformable mesh capabilities, constant head or constant flux boundary conditions (internal nodes and boundary nodes), solves advective-dispersive transport with linear retardation and decay, time-varying concentration or mass flux contaminant source terms, contours equipotentials, streamlines, and concentrations (lines or color shading), plots concentration vs. time at multiple observation points, calculates and displays flux rates along upper model boundaries. Contaminant source terms (constant concentration or mass flux) and observation points can also be specified at any node within the model domain. Solute transport properties include linear retardation, longitudinal dispersivity, diffusion coefficient, transverse dispersivity, source decay rate and solute decay rate. Initial concentrations in the aquifer are also accounted for in the simulation. For time-varying concentrations at the source, a source factor can be applied at each time period to control the concentration entering the system.

FLONET/TRANS solves the steady-state saturated flow equation using the dual formulation of hydraulic potentials and stream functions (E.O. Frind and Mantaga, 1985). The solute transport is solved using the two-dimensional equation for advective-dispersive transport with linear retardation and first-order decay.

FLONET/TRANS displays the simulation results as contour plots of equipotentials, streamlines, and solute concentrations. All contoured results can be represented by lines or gradational color shading. Line contours can be customized by changing the contouring interval, label interval, label location or adding a contour line at any location in the model domain using the mouse to point-and-click on the desired location. Flow velocity vectors can be displayed as either directional or scaled according to the magnitude of the flow velocity. Flux rates across the top boundary can be calculated and graphically displayed as a bar chart along the length of the model

domain, while flow rates anywhere in the model domain can be easily determined by adding the streamlines. For contaminant transport applications, concentration breakthrough curves can be plotted for multiple observation points throughout the model domain. These breakthrough curves can be used to determine potential risks to downstream receptors.

Price: US $ 695.00.

EIS/GWM (US EPA; ZEI/Microengineering, Inc.)
(Environmental Impact System/Geomedia Waste Containment Migration)
EIS/GWM is an integrated environmental modeling platform. EIS/GWM fully supports the USAF/AFCEE Protocol for Intrinsic Remediation (Natural Attenuation). EIS/GWM supports the following simulation capabilities: MODFLOW (USGS) for 3D flow modeling; MIFLOW generalized flow module including slurry walls, liners and faults; CAPTURE for a 3D capture zone delineation including the formation's assimilative capacity; and BIOREM-3D and BioQuick which will be described shortly.

BIOREM-3D
BIOREM-3D is a three-dimensional, multispecies, advective-dispersive migration model that supports simulation of the biodegradation of hydrocarbons dissolved in the saturated zone by interaction with up to six aerobic and anaerobic nutrients providing the system with total electron acceptor capacity. BIOREM-3D includes molecular diffusion, mechanical dispersion (with cross-terms), linear equilibrium sorption (using the retardation factor), nonlinear sorption (Freundlich and Langmuir isotherms), ion exchange using the selectivity coefficient of Goode and Konikow, decay, and aerobic and anaerobic biodegradation. Monod kinetics is used to track the microbial population. BIOREM-3D solves the migration equation in synchronization with the head and associated velocity field generated by the saturated zone flow module. The advective term in the transport equation is solved using one of several methods:

1. fixed-grid upwinding or upwind discrete element method;
2. improved method of characteristics;
3. Euler-Lagrange method or streamline upwinding.

The non-advective terms are solved using an 'operator splitting' procedure with a modified back-ward difference approximation in time. BIOREM-3D can also be used for single dissolved species with first order decay for an initial evaluation.

BioQuick
The current version of EIS/GWM also include BioQuick, an entry-level analytical 3D solution. BioQuick is based on Domenico's three-dimensional solution for the spreading of a solute in an uniform ground-water flow field, combined with a full range of biochemical reactions, including Monod kinetics.

GMS (The Department of Defense Groundwater Modeling System)
[http://ripple.wes.army.mil/software/interfaces/gms/]
The Department of Defense, in partnership with the Department of Energy, the U.S. Environmental Protection Agency, Cray Research, and 20 academic partners, has developed the DoD Groundwater Modeling System. The GMS provides an integrated and comprehensive computational environment for simulating subsurface flow, contaminant fate/transport, and the efficacy and design of remediation systems.

GMS integrates and simplifies the process of groundwater flow and transport modeling by bringing together all of the tools needed to complete a successful study. GMS provides a comprehensive graphical environment for numerical modeling, tools for site characterization, model

conceptualization, mesh and grid generation, geostatistics, and sophisticated tools for graphical visualization.

Several types of models are supported by GMS. The current version of GMS provides a complete interface for the codes FEMWATER/LEWASTE, MODFLOW, MODPATH, and MT3D. Many other models will be supported in the future, such as RT3D, UTCHEM, NUFT3D, ParFlow, and ADH.

FEMWATER is a fully three-dimensional finite-element flow and transport model, utilizing the LEWASTE code for contaminant transport. It provides density driven, coupled flow and contaminant transport in both saturated and unsaturated conditions.

The conceptual model approach involves the use of GIS tools (Arc/Info, ArcView), and has numerous advantages over the traditional cell by cell approach for model input. First of all, the model definition process is much faster and much simpler. Complex models can be defined quickly and easily. Second, once a simulation is performed, changes to the model can be made by changing the conceptual model and regenerating the grid data.

Waterloo Transport Code (WTC) (Waterloo Hydrogeologic, Inc.)
WTC is a finite element 3-D groundwater flow and advective-dispersive mass transport model which computes steady-state or transient hydraulic heads, flow velocities, breakthrough curves, contaminant concentrations or groundwater temperatures for a wide range of hydrogeologic systems. WTC can simulate dilute or dense contaminant plumes, (including seawater intrusion) as well as heat transport in porous media. It has been widely used for regional multi-aquifer flow system characterization, 3D capture zone delineation, landfill impact assessments, and heat storage in confined and unconfined aquifers.

WTC can accommodate a wide range of boundary conditions, variable material properties, injection or withdrawal wells and can incorporate linear retardation and first order decay. Future upgrades will include multi-organic biodegradation and multi-component reactive mass transport.

The finite element grid, material properties and boundary conditions are defined using a graphical, interactive preprocessor called Grid Builder_ (also available through Waterloo Hydrogeologic, Inc.). For visualization of results, simulations at user-specified times are written to files suitable for 2-D or 3-D visual display using existing third-party graphics packages (e.g. TechPlot, SURFER, and SiteView). Future upgrades will include compatibility with GMS.

Two numerical finite element solution techniques are provided; the Galerkin approach or a state-of-the-art collocation method. This software also includes an efficient timeweighting scheme for the transport solution and a preconditioned conjugate gradient solver. The 3-D domain is resolved using triangular prismatic elements and is refined internally using tetrahedra for high accuracy.

Price: US $ 2,000.00.

VISUAL MODFLOW and VISUAL GROUNDWATER
(Waterloo Hydrogeologic, Inc.)
These two packages are especially made for the visualisation of groundwater models. Visual MODFLOW is a standard software package for professional, three-dimensional groundwater flow, pathline and contaminant transport modeling. This seamless package combines the official USGS MODFLOW and MODPATH, and the latest versions of MT3D (and RT3D in the future), with the most complete and easy-to-use graphical interface available. It allows the user to

graphically assign input parameters, run the analysis, calibrate the model, and visualize the results in plan view and full-screen cross-sections.

VISUAL GROUNDWATER is a Windows-based graphical software package for advanced 3-D visualization and animation of site characterization data and modeling results. Highimpact displays and presentations of site characterization data and modeling results can easily be developped and produced. In addition, Visual Groundwater also has many applications as a powerful analytical tool for 3-D interpretation of data. Data can be displayed as 3-D isosurfaces, color shaded slices along any plane, or discrete shapes. Visual Groundwater has been specifically designed for groundwater and environmental applications. By staying focused on this particular market, the development efforts are tailored to the specific needs of groundwater and environmental professionals. This is propably the best groundwater model visualisation program currently on the market.

Price: US $1,995.00.

5.4.2   *Codes developed by the Technical University of Denmark*

Brun [1996] made a 2D reactive transport code including coupling of organic-inorganic processes. The code was used to model a cross-section of the contaminant plume at the Vejen landfill in Denmark, with special attention to the redox processes in the plume, which were influenced by both inorganic reactions (complexation, mineral reactions, cation-exchange and inorganic redox reactions) and biological processes (bacterial degradation of organic substrates). A good example of this interaction is found in the behaviour of Fe. The redox zoning downgradient to the landfill was modelled correctly and several of the simulated plumes showed a reasonable agreement with observations. Differences were related to leachate quality changes and limitations of the code. This is the most elaborate simulation of reactive transport on a field scale.

The reactive transport code consists of three separate codes, a flow and transport code, a geochemical code (PHREEQ: equilibrium processes), which make up the equilibrium part, and a biodegradation code, which is the kinetic part. An iterative solution scheme couples the three codes. This is a pseudo-equilibrium approach (pers. comm. P. Engesgaard). The organic processes are described by a maximum degradation rate that is reduced according to the availability of the species that participate in the process, the actual pH, and the presence of inhibiting species. A drawback of the model is the many unknown biological parameters which must be declared.

The kinetic part is a halfcell reaction which delivers electrons to the other halfcell reaction in the equilibrium part. Between these two parts iterations will follow until there is a pseudo-equilibrium. The rate of degradation depends on which electron acceptor is available (oxygen, nitrate, Mn (IV), Fe (III), SO_4 and CO_2). If a electron acceptor is depleted to a certain threshold, the electron acceptor with the next lower redox state will be used.

The degradation of the bulk organic matter is simulated, degradation of specific organic chemicals is not included.

Modelling of degradation of ASOCs using data from injection experiments

M. Petersen is currently modelling an injection experiment performed by Rügge (pers. communication, in prep. 1997) at the downstream part of the Grindsted landfill. Brun's code [Brun, 1996] will be used to model the redox conditions during the experiment. The simulated redox conditions are used to define spatially fixed degradation rate constants for the various ASOCs, which depend on the dominating redox reaction.

The conservative modelling of bromide has already been performed. The redox zone modelling will probably be excluded, because the experiment has no influence on the assumed stationary redox zoning. Spatial separated degradation rate constants (two sections) for the 18 compounds will be used. Water flow will be transient because of the observed groundwater fluctuations during the experiment.

5.5 Other models and modelling concepts encountered in literature

McNab and Narasimhan [1995] made a 1-D model of an aquifer contaminated with petroleum hydrocarbons. This model dynamically couples equilibrium geochemistry of inorganic constituents, kinetically dominated sequential degradation of organic compounds, and advective-dispersive chemical transport. Organic biodegradation, iron reduction and dissolution, and methanogenesis, was successfully modelled using a partial redox disequilibrium approach.

An expert system approach is used to search for and select favourable degradation pathways for the various organic compounds present. Redox half-reactions are coupled through conservation of solution operational valence. An exceptionally high dispersivity value (20 m) has been adopted, while the front had only moved 25 m during the simulation.

This led to the conclusion that the dispersivity is an very important parameter, because high concentrations of degradable organic constituents easily consume much lower concentrations of electron acceptors (oxygen) far downgradient of the front (70 m additionally), when dispersion moves only small quantities ahead of the front.

The degradation of toluene and benzene is modelled, with a sequence of oxidation-half reactions, yielding CO_2 as an end product via phenol and benzoic acid. It is assumed for modelling purposes that that under favourable conditions each of these reactions may be characterized by pseudo-first-order kinetics with a half life of 100 days each. Thus for the electron acceptors oxygen, nitrate, Fe(III), sulphate, and carbon dioxide, the same half lives are applied.

Kool et al. [1994] made a 3-D model to simulate unsaturated and saturated groundwater flow, advection, dispersion, linear or non-linear equilibrium sorption, and first order biochemical transformation of either a single contaminant species, or a multi-species, straight or branched, decay chain. This model has been applied to a hypothetical landfill problem. This model does not account for electron acceptor specific degradation rates, which are the framework for the degradation of ASOCs.

The impact of various parameters in the behaviour of a hypothetical contaminant plume was investigated [MacQuarrie and Sudicky, 1990] with the use of a organic solute transport model presented by MacQuarrie et al. {1990}. In this model dissolved oxygen was the sole electron acceptor. The effect of the following parameters investigated was found to be of importance: initial concentrations of the organic and background dissolved oxygen, the average linear groundwater velocity, the retardation factor of the organic solute, and the heterogeneity of the porous medium. Of secondary importance were the size of the initial microbial population and the biodegradation kinetic constants for easily degraded organics, such as aromatic hydrocarbons, implying that these parameters need not to be known precisely.

Liu and Narasimhan [1989] developed a multi-species, multidimensional reactive chemical transport model. This model is called DYNAMIX. The model includes advection, diffusion-dispersion, transport of oxygen, oxidation-reduction and, as a consequence, acid base reactions, aqueous complexation, precipitation-dissolution, and kinetic mineral dissolution. Thus also in this model only oxygen mediated degradation is simulated.

SUMMARY AND CONCLUSIONS

This report is a summary of a literature review on the current state of the art on the characterization of landfill leachate in soils and aquifers underlying landfills. The literature review was undertaken as part of the NOBIS project 'Feasability project in situ bio restoration of landfills', project number 96-3-04. This project aims to develop a methodology with which the intrinsic capacity of soils and aquifers, contaminated by landfill leachate to degrade pollution, can be assessed and thereby used to minimize the risks to the environment.

6.1   General background

In the literature several reports have been found where landfill leachate was degraded as it moved through the aquifer. Especially the institute of Environmental Science and Engineering at the Technical University of Denmark (Lyngby, Denmark) has done extensive research on the in situ behaviour of landfill leachate and two Danish landfills (Vejen and Grindsted). Natural degradation was found to be significant at both landfills. The scope of this review is based on, and can be seen as an extension of the review published by Christensen et al. [1994]. This summary and conclusions is an extraction of most relevant information for the NOBIS project.

In order to understand the degradation of landfill leachate one must have an idea about what happens to the leachate as it moves through the soil and underlying aquifers. Landfill leachate is a water based solution that contains a high amount of dissolved organic carbon (DOC) together with a wide variety of dissolved components. The leachate has a very high total reduction capacity (RDC) because of this high DOC content. As it moves through the soil and aquifer it will reduce the oxidized matter it encounters. This oxidation of leachate and corresponding reduction of soil and aquifer matter is primarily microbiologically mediated.

Additional processes to which landfill leachate is subjected are dilution, density flow (due to the very high concentration leachate sometimes has a higher density than the groundwater), sorption (including ion-exchange), dispersion, diffusion, (microbiologically mediated) degradation, reduction/oxidation reactions, dissolution, precipitation etc. The result of these processes is the development of a redox zonation downstream of the landfill. The distribution of redox zones downgradient from landfills is such that the most reduced zone, the methanogenic zone lies closest to the landfill. As we move further downgradient from the landfill we encounter progressively less reduced zones, the sulfate reducing zone, the iron(III) and Mn(IV) reducing zone, the nitrate reducing zone and finally the oxic zone.

Xenobiotic pollutants are also part of the landfill leachate, although they occur in much lower concentrations than the macro components and DOC. As such they do not play an important role in the development of the redox zones. However, most of these xenobiotic pollutants can be degraded under specific redox conditions which do occur close to landfills. Currently much research is being carried out, in order to improve our understanding of this process.

The redox chemistry of iron seems to be extremely important. The iron(III) reducing zone of the Grindsted and the Vejen landfills in Denmark is relatively large and Heron [1994] found that iron was recycled in the leachate plume.

Iron(III) was reduced to iron(II) which is much more soluble. Iron(II) moves with the water to the plumes edge where it is reoxidized in a less reduced environment. There it again is available to oxidize the leachate.

Landfill leachate is a very complex mixture of dissolved compounds. The leachate itself has a considerable oxidation capacity (OXC) resulting from dissolved compounds such as tetrachloro-ethylene. Therefore, in situations were the landfill pollutes aquifers with a relatively low OXC, natural biodegradation of the pollution can still occur. The electron acceptors will then come the leachate itself.

The behavior of a leachate plume from a landifill is extremely complex. This complexity is increased by the natural heterogeneity of the soils and aquifers. The characterization of the landfill, the leachate plume and (potentially) affected soils so that the biodegradation capacity can be evaluated is therefore a very difficult task. The review discusses some of the most used and some new promising techniques and methods that can be applied for this task. Relatively new molecular biological techniques based on characterization of the genetic potential of microbial populations in landfill leachate plumes has been extensively covered. In addition we have also covered the measurement of H_2 concentrations in order to determine the terminating electron accepting process, which seems to be an excellent approach for delineating landfill leachate plumes.

6.2 **A general approach to characterize the natural degradation capacity of landfill leachate**

In order to support natural degradation of landfill leachate, soils and aquifers must have a considerable total oxidation capacity. In addition if there is a considerable OXC or a considerable flux in OXC, microbial potential must be available for the degradation of specific xenobiotic compounds. If both the OXC requirement and the microbial potential are satisfied, then process rates must be estimated to determine the time and spatial scale of the leachate plume degradation.

A general approach to characterize the leachate plume is:

1. Estimate the oxidation capacity of the pristine aquifer (by taking sediment and water samples).
2. Estimate the total reduction capacity of the landfill leachate.
3. Estimate the dimensions of the leachate plume (i.e. what part of the soil and the aquifer is affected by the leachate). A very good indicator is the specific electrical conductivity (Ec) or chloride. The Ec of leachate is much higher than groundwater in the pristine aquifer. Try to estimate the concentration of a number of xenobiotics in the leachate. The total organic carbon (TOC) plume will generally be much smaller than the Ec plume due to degradation.
4. Estimate the general hydrogeology of the soils and aquifer surrounding the landfill. Use these models to exstimate the size of the fluxes in OXC and RDC.
5. Estimate the location of the leachate sources (this is extremely difficult because landfills are very heterogeneous).
6. If the aquifer has an OXC that is large enough or if the OXC is supplied fast enough in order to degrade a considerable amount of leachate we should try to estimate the maximum size of the leachate plume. In order to do this simple numerical approaches can be applied. If the OXC is not very large, natural degradation will not be very large and other measures may have to be undertaken.
7. In order to improve our predictions, and to ensure that essential or extremely hazardous xeno-biotics are degraded, laboratory characterization of microbial degradation potential and possibly rates has to be carried out. If attainable in situ experiments may be carried out too.
8. If we are going to rely on natural degradation of leachate, an extensive monitoring scheme has to be developed so that our expectations can be checked and that our predictions can be improved with time. If we are proven wrong, additional measures must be taken.

6.3 Concluding remarks on nucleic acid based techniques with regard to the project

Detection of biodegradative indicative genes is at present not possible. Random Amplified Polymorphic DNA (RAPD) is suitable for initial experiments, to obtain an indication for biodiversity. However, since denaturing gradient gel electrophoresis (DGGE) of 16S rRNA works well for forest soils and there gives a complex and more informative profile, there seems to be no reason to use RAPD in the project. DGGE seems to be most suitable to apply in the project but has to be related to other parameters, as described below.

Integration of characterization methods and laboratory microcosms
Laboratory microcosms will be used to establish the biodegradation potential. To be able to relate the biodegradation potential to biodiversity, a good way seems to prepare microcosms in a manner also done by Johnston et al. [1996]: a sample was divided over a large amount of bottles/microcosms (10 - 30 ml) and spiked with xenobiotics. In time samples were taken by removing two bottles and destructively sampling these bottles. The liquid then was analysed for the presence of xenobiotics. In our case the sediment can be used to extract DNA and RNA and to make a suspension for use in Biolog plates to determine metabolic capacity.

Due to the addition of a small amount of several xenobiotics a kind of enrichment culture under rather natural conditions is established, especially xenobiotic-degrading micro-organisms are stimulated. Stimulation might either result in growth (results in more DNA) or higher activity per cell (results in more ribosomes, thus can be measured on rRNA level). This kind of enrichment differs much from traditional enrichment cultures where environmental samples are added to nutrient media with relatively high concentrations of substrates and strains are isolated after several rounds of cultivation. By performing DGGE using RNA or DNA as the template a time-specific profile can be obtained, which can be related to the disappearance of xenobiotics in the batches and previously observed profiles. In this way (the position of bands in) the profile will indicate the disappearance of xenobiotics. By hybridization of the blotted DGGE gel with probes specific for a redox group a relation can be established with the redox conditions in the batch.

Biodegradative-specific Biolog plates should be used simultaneously to see whether the measured biodegradation potential can be easier determined with these plates. Also from positive wells in the plates DNA and RNA can be isolated and used in DGGE to get a more specific indication of which micro-organisms are involved in the degradation of a certain compound. This profile can be related to the profile of the sampled batch. In DGGE only the dominant species will be visually, however it is not unlikely that a micro-organism present at a low concentration is of major importance in biodegradation of a certain compound. To make sub-dominant species visual by PCR VUA are presently working on a technique, based on subtractive hybridization [Diatchenko et al., 1996].

6.4 Conclusions regarding the use of laboratory batch microcosms (LBM) and in situ mesocosms (ISM)

Laboratory batch microcosm (LBM) incubation can be performed as done by the group of Christensen (preparation of one large microcosm, from which in time groundwater samples are withdrawn for analysis). It can also be done as described by Johnston (preparation of a large number of microcosms from one sampling point, in time these microcosms are destructively sampled). Both methods have their advantages and disadvantages over the other method. However, if one wants analyse sediment samples during incubation (for example for microbial population characterization, since micro-organisms are mainly associated to the sediment), the method of Johnston is to be preferred. Alternatively, one could first examine whether microbial populations of groundwater are comparable to that of sediment. If so, also the LBMs of Christensen could be used.

In the initial stages of the project it was thought to use ISMs for measurement of degradation (rates) under in situ conditions. This method has been optimised by the group of Christensen, information from them obtained during the visit to Denmark learned that ISMs are technically very demanding, especially installation and sampling. Degradation rates of LBMs and ISMs differ up to five fold, also ISMs installed close to each other can show a fivefold difference in degradation rate. Thus, degradation rates can only be determined in orders of magnitude. Therefore, experts from the group of Christensen advised us first to start with LBMs. Also, a close study of literature on ISM indicates that the ISM technique might not be a suitable method for measuring in situ biodegradation, since there is no (physical) interaction/contact between the ISM and its environment. Only temperature is kept at the ambient conditions. There is no through flow of groundwater in the ISM. This may be an important parameter in degradation, as a flow of 10 m/year as measured at Coupépolder is equal to 0.5 volume changes per day in the ISM.

The natural situation can be simulated by using flow-through columns. In this method a column is filled with sediment collected from a sampling point. Groundwater also collected at this sampling point is spiked with xenobiotics and led through the column at a rate close to the actual groundwater flow. Since temperature underneath landfills is quite constant, these experiments can be executed in the lab. In principle the effluent of one column can be used as the influent of another column. In this way columns with sediment from the different redox zones can be placed in series to simulate biodegradation underneath landfills. Effluents can be collected and analysed for the disappearance of xenobiotics.

Through-flow columns are likely more informative than ISMs and therefore instead of ISMs we intend to use through-flow columns. Short term consequences for the project are that during the second phase experience should be gained with through flow columns in stead of ISMs. It is however essential to explore this idea from a theoretical standpoint because the concentration changes due to natural attenuation (NA) are very small and under field conditions these changes occur over very large distances (i.e. hunderds of meters). Are we capable of measuring such small concentration differences in the laboratory?

6.5 Conclusions and summary on models capable of simulating natural attenuation processes

In the last years many codes have been developed, which can simulate groundwater flow including biodegradation and other attenuating processes such as sorption. These range from simple screening models (BIOSCREEN) to more sophisticated degradation models as BIOPLUME 3 (currently being developed) which include particle-tracking of the electron acceptors.

Models which are the most sophisticated in simulating reactive transport (PHREEQC, PHAST and HYDROGEOCHEM) can be very useful in the modelling of redox sequences found downgradient of landfills. The output of these models (spatially distribution of redox zones) can be the input for models simulating specific fates of ASOCs, as the degradation rate constants for the various SOCs are highly related to the redox environments they flow through.

For the Coupépolder landfill groundwater transport can be modelled with TRIWACO or MICROFEM, the modeling of sorption and degradation can be performed along calculated flowpaths with SORWACO, and geochemical reactions can be modelled with PHREEQC. This pathline oriented approach is also used with MODFLOW/RT3D, but is because of it's modular structure less appropriate for modelling curving structures as meandering rivers. A drawback of this approach is that dilution of the plume cannot be simulated, because transverse dispersion is not modelled. A 3D model capable of doing this is HYDROGEOCHEM, a 2D model FLONET/TRANS. It is best to start with TRIWACO/MICROFEM, FLONET/TRANS, SORWACO, and PHREEQC to model the processes at the landfill. When released PHAST will probably be the best model.

To integrate the hydrogeochemical processes with microbiological processes, a coupling between a hydrogeochemical transport model and a metabolic control analysis (MCA) can be an enterprise for the future.

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APPENDIX A

FIGURES

Fig. 1. Example of a leachate plume near a landfill.
(Longitudinal transect of the leachate plume in the aquifer downgradient of the Grindsted landfill showing the distribution of the different redox zones (from [Ludvigsen et al., 1995]).

Fig. 2. Solute breakthrough curves.
(Solute breakthrough curves illustrating cation migration in a sandy soil column exposed
to landfill leachate (from [Kjeldsen and Christensen, 1984]).

Fig. 3. Most frequently detected ASOCs in groundwater near landfills.
(The 15 most frequently detected ASOCs in groundwater at waste disposal sites in Germany and the U.S. (Arneth et al., 1989 from [Christensen et al., 1994])).

Fig. 4. Hierarchial scheme for diagnosis of terminal electron accepting processes (TEAPs).

Fig. 5. Example of the bubble strip technique.
(Example of the bubble strip technique for measurement of the dissolved H_2 concentration (from [Wiedemeier et al., 1995]).

Fig. 6. Exploded sketch of an in situ microcosm (ISM) (from [Nielsen et al., 1996a]).

Fig. 7. Set up used by Lyngkilde and Christensen [1992a] for collecting redox sensitive ground-water samples. The iron tube povided with a filter tip is rammed into the ground by a motor-driven paviour.

Fig. 8. Principle of the Delft Geotechnics multiground water probe.

APPENDIX B

TABLES

Table 1. Summary of leachate pollution plumes reported in literature.
Table 2. Overview of leachate composition.
Table 3. Criteria for assignment of the redox status in groundwater process.
Table 4. Range of hydrogen concentrations for a terminal electron accepting process.
Table 5. Sampling intensity of redox parameters in groundwater and sediment.

Table 1. Summary of leachate pollution plumes reported in literature (extension of [Christensen et al., 1994]).

name	area of landfill (ha)	landfilling period	type of aquifer	length of plume (m)	width of plume (m)	max. depth of plume (m)	type of tracer	number of samples	components described	reference
Borden, Canada	4.5	1940 - 1976	glaciofluvial sand	~700	~600	~20	chloride, tritium	~600	inorganics, redox pairs, organic matter	MacFarlane et al. {1983} Nicholson et al. {1983}
Vejen, Denmark	6.3	1962 - 1981	glaciofluvial sand	~400	~100	~20	chloride	~160	inorganics, redox pairs, organic matter, ASOCs	Kjeldsen [1993] Lynkilde and Christensen [1992a]
Grindsted, Denmark	~10	1930 - 1977	glaciofluvial sands/gravel	~250	-	~12	chloride (for the first 60~70 m) due to road salting	> 300	inorganics, redox pairs, organic matter, ASOCs	Rügge et al. [1995] Bjerg et al. [1995] Kjeldsen et al. [1995] Holm et al. [1995] Kjeldsen et al. [1998a, 1998b]
North Bay, Canada	28	1962 -	glaciofluvial sands/silts	~700	~300	~20	chloride	~300	inorganics, redox pairs, organic matter, ASOCs	Barker et al. [1986] Rheinhard et al. {1984}
Woolwich, Canada	3.5	1965 -	-	~600	-	-	chloride	~500	chloride, organic matter	Reinhard et al. {1984}
unnamed, Germany	15.3	1954 - 1970	coarse glaciofluvial sands	~3000	~500	-	chloride	~20	inorganics, organic matter	Exler {1972}
KL, US	~30	1960 - 1979	glaciofluvial sands	~700	-	-	chloride	~50	inorganics, redox pairs, organic matter	Kehew and Passero {1990}
Army Creek, US	24	1960 - 1968	sand	~700	-	-	chloride	~20	inorganics, redox pairs, organic matter, ASOCs	Baedecker and Apgar [1984] DeWalle and Chian {1981}
Babylon, US	10.0	1947 - 1975	coarse sand and gravel	3000	600	23	chloride	120	inorganics	Kimmel and Braids {1974} Kimmel and Braids {1980}
Islip, US	6.9	1933 - 1975	coarse sand and gravel	1500	400	50	chloride	75	inorganics	Kimmel and Braids {1974} Kimmel and Braids {1980}
Noordwijk, the Netherlands	~6	1960 - 1973	coarse sands	-	-	40	chloride, tritium	22	inorganics, ASOCs	Duijvenbooden and Kooper {1981}

Table 2. Overview of leachate composition [Christensen et al., 1994].

parameter (values in milligrams per litre unless otherwise stated)	range
pH	4.5 - 9
spec. conductivity. (μS/cm)	2,500 - 25,000
total solids	2,000 - 60,000
organic matter	
total organic carbon (TOC)	30 - 27,700
biological oxygen demand (BOD_5)	20 - 57,000
chemical oxygen demand	140 - 90,000
BOD_5/COD ratio	0.02 - 0.80
organic nitrogen	14 - 2,500
inorganic macro components	
total phosphorous	0.1 - 20
chloride	150 - 4,500
sulphate	8 - 7,750
hydrogen carbonate	610 - 7,320
sodium	70 - 7,700
potassium	50 - 3,700
ammonium-N	50 - 1,800
calcium	10 - 7,200
magnesium	30 - 15,000
iron	3 - 5,500
manganese	0.03 - 1,400
heavy metals	
arsenic	0.01 - 1
cadmium	0.0001 - 0.4
chromium	0.02 - 1.5
cobalt	0.005 - 1.5
copper	0.005 - 10
lead	0.001 - 5
mercury	0.00005 - 0.16
nickel	0.015 - 13
zinc	0.03 - 1000
bacteria	
faecal streptococci (CFU ml^{-1})[a]	0.1 - 3 million
faecal coliform (CFU ml^{-1})	0.1 - 100,000
total coliform (CFU ml^{-1})	0.1 - 100,000
aromatic hydrocarbons (μg/l)	
benzene	1 - 570
toluene	1 - 7,500
xylenes	4 - 3,500
ethylbenzene	1 - 1,100
trimethylbenzenes	4 - 250
naphtalene	0.1 - 260
dietylphthalate	10 - 660
di-n-butylphtalate	5.0 - 15
butylbenzylphtalate	5.1 - 8
halogenated hydrocarbons (μg/l)	

parameter (values in milligrams per litre unless otherwise stated)	range
chlorobenzene	0.1 - 110
1,2-dichlorobenzene	0.1 - 32
1,4-dichlorobenzene	0.1 - 16
1,1-dichloroethane	0.6 - 46
1,2-dichloroethane	< 6
1,1,1-trichloroethane	0.1 - 90
trans-1,2-dichloroethylene	1.6 - 88
cis-1,2-dichlorethylene	1.4 - 470
trichlorethylene	0.7 - 750
tetrachloroethylene	0.1 - 250
methylenechloride	1.0 - 64
chloroform	1.0 - 70
carbontetrachloride	4.0 - 9.0
phenols (μg/l)	
phenol	1 - 1,200
ethylphenols	< 300
cresols	1 - 2,100
miscellaneous (μg/l)	
acetone	6 - 4,400
tetrahydrofuran	9 - 430
methylethylketone	110 - 6,600
tri-n-butylphosphate	1.2 - 360
triethylphosphate	15

Note: All information in this table is from Christensen et al. [1994] tables 1 and 3 and the references therein.

Table 3. Criteria for assignment of the redox status in groundwater process.
(Criteria used for assignment of redox status to groundwater samples of the Vejen landfill (left - 95 % complied [Lyngkilde and Christensen, 1992a] and the Grindsted landfill (right - 90 % complied) [Bjerg et al., 1995] in Denmark[1]).

parameter	aerobic		nitrate reducing		manganogenic		ferrogenic		sulfidogenic		methanogenic	
oxygen	> 1.0		< 1.0		< 1.0		< 1.0		< 1.0		< 1.0	
nitrate	-		-		< 0.2		< 0.2		< 0.2		< 0.2	
	< 0.1		-	> 0.1	< 0.1		< 0.1		< 0.1		< 0.1	
dinitrogen oxide[2]	-		> 1		< 1		< 1		< 1		< 1	
ammonium	< 1.0		-		-		-		-		-	
manganese(II)	< 0.2		< 0.2		> 0.2	> 5	-	< 5	-	< 5	-	< 5
iron(II)	< 1.5		< 1.5	< 10	< 1.5	< 10	> 1.5	> 150	-	< 150	-	< 150
sulfate	-		-		-		-		-		< 40	-
sulfide	< 0.1		< 0.1		< 0.1	-	< 0.1	-	> 0.2	< 0.1	-	
methane	< 1.0		< 1.0	-	< 1.0	-	< 1.0	-	< 1.0	-	> 1.0	> 25

[1] All units are in mg/L, except dinitrogen oxide which is in μg/L, N- and S-compounds are in mg/L N and S.
[2] Only measured for the Grindsted leachate plume investigation.
- = no citerion is applied.

Table 4. Range of hydrogen concentrations for a terminal electron accepting process (Wiedemeier et al., 1995).

terminal electron accepting process	hydrogen (H_2) concentration (nanomoles per litre)
denitrification	< 0.1
Fe(III) reduction	0.1 - 0.8
sulphate reduction	1 - 4
methanogenesis	5 - 20

Table 5. Sampling intensity of redox parameters in groundwater and sediment. (Proposed sampling intensity of redox sensitive parameters in groundwater and sediment for monitoring redox conditions in ISMs installed under various redox conditions).

	aerobic	NO_3^- reducing	Fe(III)/Mn(IV) reducing	SO_4^{2-} reducing	methanogenic
groundwater					
O_2	**	*	*	*	*
NO_3^-/NO_2^-	**	**	t_0	t_0	t_0
NH_4^+	*	**	-	-	-
Fe(II)	*	*	**	*	-
Mn(II)	*	**	**	*	-
SO_4^{2-}	*	*	*	**	t_0
sulphide	-	-	*	**	**
CH_4	-	-	*	*	**
pH	*	*	*	*	*
sediment					
OXC	-	t_0 and t_f	t_0 and t_f	t_0 and t_f	t_0
Fe(II)/Fe(III)	-	t_0 and t_f	t_0 and t_f	t_0 and t_f	t_0
Mn(II)/Mn(IV)	-	t_0 and t_f	t_0 and t_f	t_0 and t_f	t_0
sulphur	-	t_0 and t_f	t_0 and t_f	t_0 and t_f	t_0

The species should be measured:

* Occasionally.
** Frequently.
t_0 At the beginning of the experiment.
t_f At the end of the experiment.
- The species should not necessarily be measured during the experiment. Laboratory incubation of anaerobic sediment showed that the potential of the particular microbial redox processes was not affected by the isolation of the sediment inside the ISM.

APPENDIX C

DEGRADATION POTENTIALS OF ANTHROPOGENIC SPECIFIC ORGANIC COMPOUNDS

Table C1. Degradation potentials observed in degradation studies of anthropogenic specific organic compounds in landfill leachate contaminated aquifers (and other contaminated sites) under different redox conditions (extension of the review by [Christensen et al., 1994], table 11).

compound[*1]	unspecified[*2]		methanogenic		sulfate reducing		iron reducing		nitrate reducing		aerobic	
	N[*3]	D[*4]	N	D	N	D	N	D	N	D	N	D
aromatic hydrocarbons												
benzene	f[*5]	d	t, x, e, C, J, F	I	c, e, y	I, J	C	t, J	e, C, G, I			c, f, m, A, E (3-13)
toluene	f	d	C, J	e, x, F	c	e, y, J		r, z, C (90)	e, b, C	G, H		b, c, f, m, A, E (4-12)
ethylbenzene		d	t, x, e?, F	e?	c, y	e		t	e			c, m
o-xylene	f	d	C, F	e, x	c	e, y	C		e, C, G			c, A, E (1.6)
m-xylene			F	e	c	e			e			c
p-xylene			F		c	y						c
xylenes (sum)				t		t						
1,2,4-trimethylbenzene				e	e	e			e			
nitrobenzene				C				C		C	A	D (2/8)
naphthalene	f		C				C		C			f, m, A, E (3-36)
biphenyl			C				C		C			m, A, E (1-50)
cumene					e				e			
chlorinated aromatic hydrocarbons												
chlorobenzene					e							
1,2-dichlorobenzene												m
1,4-dichlorobenzene												m
o-dichlorobenzene			C				C		C			A, E (1.5?)
p-dichlorobenzene			C				C		C			A, E (1.5)
halogenated aliphatic hydrocarbons												
dichloromethane												
trichloromethane									b		b	
tetrachloromethane		f		C (< 10)				C	C		f, m, A	
1,1-dichloroethane												
1,1-dichloroethene		d										
1,2-dichloroethane												
1,2-dichloroethene		d										
trans 1,2-dichloroethene												
1,2-dibromoethane		d										
trichloroethene	f	d	C, F				C		C		f, m, A	

compound[*1]	unspecified[*2]		methanogenic		sulfate reducing		iron reducing		nitrate reducing		aerobic	
	N[*3]	D[*4]	N	D	N	D	N	D	N	D	N	D
1,1,1-trichloroethane		f		j, C (40)		j	C		j, C		f, j, m, A	
1,1,2-trichloroethane												
tetrachloroethene	f			C (100)			C		C		f, m, A	
cis 1,2-dichloroethene												
phenols												
phenol **(phenol-index)**		g	B	l		l		B, z		B		j, k, m, A, D
o-cresol			q, B		q		B	z	B			m, A, D
m-cresol			l	q		i, q				i		
p-cresol				q		q						
2-chlorophenol		a		l	l							
3-chlorophenol				l	l							
4-chlorophenol				l	l							
2-methyl-4-chlorophenol		a										
4,6-o-dichlorocresol			B				B	B?	B		A	A, D (1/8)
2,4-dichlorophenol				l, B	l		B		B			A, D
2,5-dichlorophenol				l	l							
2,6-dichlorophenol			B	B			B		B		A	A, D (3.5/8)
3,4-dichlorophenol			l		l							
2,4,5-trichlorophenol		a	?l		?l							
pentachlorophenol		?a										
o-nitrophenol				B				B		B	A	A, D (6/8)
p-nitrophenol				B				B		B		A, D
2-nitrophenol		?a										
4-nitrophenol		a										
2,4-dinitrophenol		a										
pesticides												
2-hydroxybiphenyl			n			n				n	n	
2,4,5-trichlorophenoxy acetic acid				o		o						
mecoprop[*3]												s
2,4-D[*4]												

compound[1]	unspecified[2]		methanogenic		sulfate reducing		iron reducing		nitrate reducing		aerobic	
	N[3]	D[4]	N	D	N	D	N	D	N	D	N	D
miscellaneous												
byciclic compounds				t, u		t, u		t, u				
NVOC			t, v		t			t, u, v		v		v
aniline			h			h						
o-, m-, p-toluidine			h		h	h						
o-, m-, p-aminobenzoate				h		h						
benzamide				h		h						
methyl-benzamide			?h			h						
dimethyl-benzamide			h		h							
p-toluamide				h		h						
pyridine				p		p						
furan				p		p						
thiophene			p		p							
pharmaceutical compounds				K		K		K		K		

Explanation:

[1] bold compounds are detected as the principal ASOCs in the leachate of the Coupépolder landfill in the Netherlands;
[2] unspecified: the specific anaerobic redox conditions were not measured;
[3] N = Not degraded; [4] D = Degraded.

References cited in table:

a: Kjeldsen et al {1990}
b: Harrison and Barker {1987}
c: Berwanger and Barker {1988}
d: Wilson et al. {1986}; Lab.
e: Acton and Barker; ISM {1992}
f: Nielsen et al. {1992}
g: Rees and King {1981}
h: Kuhn and Suflita {1989b}
i: Ramanand and Suflita {1991}
j: Kle_ka et al. {1990}

k: Deeley et al. {1985}
l: Gibson and Suflita {1986}; LB
m: Lyngkilde et al. {1993}
n: Liu et al. {1991}
o: Gibson and Suflita {1990}
p: Kuhn and Suflita {1989a}
q: Smolinski and Suflita {1987}
r: Albrechtsen et al. {1993}
s: Heron and Christensen {1992}

*5 Additional references about degradation of ASOCs in a broader context (landfill sites, petroleum spill sites)
t: Rügge et al. [1995]; Detailed field monitoring
u: Lyngkilde and Christensen [1992b]; Detailed field monitoring
v: Barker et al. {1986}
w: Reinhard et al. {1984}
x: Grbíc-Galíc {1990} (cited in: Rügge et al. [1995])
y: Edwards et al. {1992} (cited in: Rügge et al. [1995])
z: Lovley and Lonergan {1990}; LB (cited in: Rügge et al. [1995]; Nielsen et al. [1995a and b])
A: Nielsen et al. [1996a]; ISM and LBM
B: Nielsen et al. [1995a]; ISM and LBM
C: Nielsen et al. [1995b]; ISM and LBM; (General transformation rates (1,2,3) | lag phases in days)
D: Nielsen et al. [1994a]; LBM; (1/8): degraded one out of eight localities
E: Nielsen et al. [1994b]; LBM; (x), degradation rate in $\mu g\ L^{-1}\ d^{-1}$
F: Johnston et al., {1996}
G: Hutchins et al. {1991}; Laboratory methods (cited in: Nielsen et al. [1995b])
H: Barbaro et al. {1992}; Detailed field monitoring
I: Kazumi et al. [1997]; Different methodologies; many localities other than landfill sites; at the only landfill site included no degradation of benzene
     occurred under methanogenic or sulfate reducing conditions
J: Chapelle et al. [1996b]; Field evidence and LB; Petroleum hydrocarbon contaminated aquifer
K: Holm et al. [1995]; Detailed field monitoring

METHODS OF SAMPLING AND ANALYSIS OF VARIOUS WATER AND SOIL PARAMETERS

Table D1. Methods of sampling and analysis of various water and soil parameters.

compound/parameter	in situ measurement	sampling and filtering	conservation	determination
water parameters				
Cl^-		P | +	C5 | LBM	L4
Ca^{2+}		P | +		L3
Na^+		P | +		
K^+		P | +		
Mg^{2+}		P | +		
alkalinity	alk.	P | +		
pH	M-d	P		
Ec	M-d	P		
redox potential	M-d	P		
O_2	M-d | F-d	P		
temperature	M-d			
NO_3	F-d	P | +	C4	L4
NO_2		P | +	C4	L4
N_2O				L13
NH_4^+		P | +	C3 | C1 + LBM	L4
SO_4		P | +	C6 | LBM	L4 | L14
S_2^-	F-d	syringe	C8	L1
Fe^{2+}	F-d	P | +	C1 + LBM	L3
Mn^{2+}		P | +	C1 + LBM	L3
CH_4		syringe	C7	L5
CO_2				
H_2		L15		L15
TOC				L11
DOC as NVOC			C1	L2
metals			C2	L3
aromatics, halogenated aliphatics and bicyclics				L6
pesticides			C9	L7
phenolics				L9
SOCs (general)			C10	L10
SOCs in 10 mL samples				L16
screening of org. matter		L17	L17	L17
$3H_2O$				L8
$^{14}CO_2$				L12
sediment parameters				
OXC	-	WPS	C11	S3
RXC	-	WPS		S4
sediment-bound TOC	-	WPS		S1
Fe-(ox)(hydrox)ides	-	WPS		S2
Fe-mineralogy (chemical extraction techniques)	-	WPS		S5
NVOC	-			S6
kinetic sorption parameters	-			S7

Explanation:

Bold printed parameters are redox sensitive species.

Water parameters

In situ measurement

alk.   Gran titration with sulfuric acid (0.02 M).

M-d   Multiboard-device (see fig. 7 in appendix A) [Lyngkilde and Christensen, 1992a]:
        O_2:   WTW Oxi 196 instrument and a WTW 196-1.5 oxygen electrode;
        redox pot.:   WTW pH 196R instrument and an Ingold Pt-4805R electrode;
        pH: WTW pH 196R instrument and a WTW SenTix 96R pH electrode;
        Ec: WTW Conductometer LF 191R instrument and a WTW LS1/T-1,5R conduc-
                  tivity electrode.

F-d   Field determination of redox conditions [Nielsen et al., 1996b]:
        O_2:   Winkler titration analysis (modified for 12-mL volumes);
        NO_3:   test kits (MerckR);
        Fe: test kits (MerckR);
        sulfide: selective electrode (Radiometer R F1212S).

Sampling and filtering

P   Pressure filtered by nitrogen through a 0.1 μm membrane filter and immediate preserva-
        tion after sampling (for anaerobic conditions).

syringe The sampling of these volatile components took place with a syringe in an unbroken water
        stream before filtering.

+   Filtered with 0.15 μm membrane filter.

Sampling of anaerobic water samples

Drive-in piezometer for collecting anaerobic water samples

1-in (2.54 cm) iron pipe rammed in the ground by a motor-driven hammer. The iron pipe is fitted with a tip supplied with a 10-cm-long iron screen (from an automobile carborator) and a Teflon check valve. This technique has been applied by the Technical University of Denmark in the research of the Vejen and Grindsted landfills in Denmark. Samples are taken every 0.5 meter by pressuring the pipe with nitrogen. The Teflon tube is connected to a *multiboard-device.*

Anaerobic sampling of water at the Coupépolder landfill

The technique used at the Vejen and Grindsted landfills will give problems at the Coupépolder land-fill. Firstly obstructions will be encountered when the piezometer is driven through the landfill body, and a 10 m thick clayey cover layer surrounding the landfill prohibits easy access to the aquifer. Secondly the aquifer underlying this landfill is much deeper (bottom aquifer about 45 m below surface landfill), depths this light equipment has not been constructed for.

Because of these reasons, and a shortage of time the anaerobic sampling has been performed with a technique developped by Delft Geotechnics (GD). This technique is called the 'multiground water sonde' and works on the same principle as the drive-in piezometer (see fig. 8 in appendix A). Because of the heavier equipment, samples can be taken at larger depths. After each sample has been taken, the device is demineralised by nitrogen-gas and cleaned in situ. The device is made of stainless steel. To prohibit permeation of oxygen through the Teflon sampling tube, an surrounding tube has been applied which is flushed with nitrogen. Through flow cells are used to measure pH, Eh, EG, temperature and dissolved oxygen in situ. A final sample is taken when a constant level of these parameters is measured.

Conservation

C1 (NVOC)
1 mL conc. sulphuric acid to 100 mL groundwater [Lyngkilde and Christensen, 1992a]. Pressure filtering 50 mL of groundwater through a 0.15-μm membrane filter into a polyethylene vial containing 0.5 mL conc. sulphuric acid [Lyngkilde and Christensen, 1992b].

C2 (metals)
1 mL conc. nitric acid to 60 mL groundwater [Lyngkilde and Christensen, 1992a].

C3 (ammonium)
50 μL conc. sulphuric acid to 10 mL groundwater.

C4 (nitrate, nitrite)
1 drop of a 0.22 M solution of mercury chloride to 10 mL groundwater.

C5 (chloride)
Kept cool at 0 - 10 degree Celcius.

C6 (sulfate)
As C5. When sulfide is present it is driven out of the sample by nitrogen gas at the site immediately after the sample for sulfate analysis is taken. At pH > 7.5: CO_2 instead of nitrogen is used.

C7 (methane)
6.5 mL samples are transferred from the underbroken waterstream before the pressurised filter containers to an evacuated VenmojectR blood sample vial (13 mL) by use of a syringe. Three drops of conc. sulphuric acid were added to the VenojectR vials for preservation by use of the syringe.

C8 (sulfide)
Adding 10 mL of strong basic, antioxidating solution to the 10 mL groundwater sample.

C9 (pesticides)
Rügge et al. [1995].

C10 (SOCs)
See Lyngkilde and Christensen [1992a].

LBM
Kept at 4 °C until analysis.

Polyethylene bottles are used for conservation of all samples except for those used to determine methane concentrations.

Chemical analysis
L1 (sulfide)
Collected with syringe, mixed with buffer solution (pH = 11, constant ionic strength) to obtain constant pH (for further information: [Bjerg et al., 1995]). Sulfide was quantified by a selective sulfide electrode (Radiometer F1212S). The quantification has to be done close to darkness to get consistent results [Lyngkilde and Christensen, 1992a].

L2 (NVOC)
Dohrmann DC-80R TOC (Total Organic Carbon) analyser. Detection limit: 0.05 mg/LC [Lyngkilde and Christensen, 1992b].

L3 (metals)
Perkin-Elmer 370R atomic adsorption spectrophotometer (Fe, Mn, Ca).

L4 = (NH_4^+, NO_3^-, NO_2^-, SO_4^{2-} and Cl^-)
Standard autoanalyser routine (TechniconR Autoanalyser II).

L5 (methane)
GC with flame ionization detector (see further [Lyngkilde and Christensen, 1992a]).

L6 (aromatics (benzene, toluene, o-xylene, o- and p-dichlorobenzene, naphtalene, biphenyl) bicyclics)
Basic pentane extract + GC + GC/MS in scan mode; detection limit: $1\mu g/l$ [Rügge et al., 1995; Nielsen and Christensen, 1994a and b; Lyngkilde and Christensen, 1992b].

L7 (pesticides)
See Rügge et al. [1995].

L8 ($3H_2O$)
Quantified by liquid scintillation counting on a Liquid Scintillation Analyser on 1 mL of water mixed with 5 mL of Optiphase 'Highsave' 3 scintillation liquid [Nielsen et al., 1996a and 1995a].

L9 (phenolics (phenol, o-cresol, o-nitrophenol, p-nitrophenol, 2,6-dichlorophenol, 4,6-o-dichloro-cresol) + nitrobenzene)
Measured [Nielsen et al., 1996a and 1994a] with a GC equipped with a FID (flame ionization detector) and ECD (electronic capture detector) on organic extracts from 10-ml water samples as described by [Nielsen and Christensen, 1994a].

L10 (ASOCs)
See Lyngkilde and Christensen [1992a].

L11 (TOC)
See Heron et al. [1997].

L12 ($^{14}CO_2$)
See Chapelle et al. [1996a]; Lovley et al. {1995}. Liquid scintillation counting (toluene); proportional counting, GC equipped with thermal detection and gas onal counter.

L13 (N_2O)
Samples (6.5 mL) from syringe to evacuated Venoject blood sample vial (13 mL) + sulfuric acid + GC + electron capture detector and packed column (for more information: [Bjerg et al., 1995]).

L14 (sulfate)
Turbidimetric method.

L15 (hydrogen, H_2)
A gas stripping procedure, referred to as the 'bubble strip' method is used to measure dissolved H_2 in groundwater [Vroblesky and Chapelle, 1994; Chapelle and McMahon, 1991]. Concentrations of H_2 are measured by gas chromatography equipped with a Reduction Gas Detector. The detection

limit for H_2 in a gas phase using this method is approximately 0.01 μL/L [Chapelle et al., 1995]. Because H_2 is so dynamic, attempts to preserve H_2 samples in the field for transport to the laboratory were not successful. For this reason, the chromatography equipment was modified to be taken into the field, and all H_2 measurements were made within 30 min of sample collection [Chapelle et al., 1995].

L16 (SOCs in 10 mL samples)
A method is described in Harrison et al. [1994] for the determination of 22 organic compounds in the water extracted from the ISMs. The samples withdrawn from the ISMs (10 mL) were preserved by being immediately made alkaline with sodium hydroxide, which in some cases resulted in precipitates of metal hydroxides. These precipitates were removed by centrifugation. The sample volume is constrained to 10 ml; thus the volume of pentane used for liquid-liquid extraction had to be very small (100 μl). The extract was analysed by capillary gas chromatography (GC). After this analysis the alkaline aqueous phase was neutralized in order to liberate the phenols which were then selectively derivatised to their acetate esters with acetic anhydride. The esters were extracted into pentane-diethyl ether. The method has proved to be reliable.

L17 (screening of organic matter)
Screening of organic matter in leachate with the GC-MS technique is described in detail from the sampling to the analysis phase [Schultz and Kjeldsen, 1986]. The samples were collected from leachate collection tanks or leachate drains in 2L glass bottles with plastic screw caps. The samples were delivered to the laboratory after preservation with 200 ml dichloromethane.

The samples were extracted using dichloromethane followed by sodium hydroxide solution. In this way the sample is divided into one phase containing the basic and neutral compounds and another phase containing the acidic compounds. These extracts were analysed with GC-MS. Capillary columns were used. These give higher resolution, lower detection limits and a higher possiblity of identification of individual components than packed columns. For analysis of volatile compounds pentane extraction was used, and this extract was analysed by GC-MS immediately afterwards. Prior the analysis the extractions were dissolved in hexane. One sample (acidic fraction) could not be dissolved and dichloromethane was used.

The detection limit depends on the extractability, the GC characteristics and the mass spectra of each compound. Identification of the compounds are based mainly on the INCOS data system s library.

Sediment parameters
Sampling
WPS
Sediments were sampled anaerobically with a Waterloo piston sampler {Starr and Ingleton, 1992}, without contact to the atmosphere and with the preservation of pore water.

Delft Geotechnics
Techniques used for sampling sediment anaerobically underneath the Coupépolder landfill were the 'Begemann boring', and the 'spitsmuis' both developed by Delft Geotechnics (GD). The 'Begemann boring' takes undisturbed samples with a large length (up to 18 m), by pushing the the device through the soil, while a nylon stockinette surrounding the soil sample unwinds. A drawback of the 'Begemann boring' is the contamination of pore water by the heavy support solution. The 'spitsmuis' is the most sophisticated technique developed by GD for collecting soil and pore water samples. It collects samples of the same quality as the WPS does.

Conservation
C11
The cores from the WPS were sealed and stored at 10 °C, for a maximum of 10 days, prior to transfer to an anaerobic glovebox. Storing the fresh sediments inside the anaerobic chamber at 20 °C for up to 4 weeks prior to analysis proved acceptable.

Analyses
S1 (sediment-bound TOC)
Determined as CO_2 evolved during dry combustion in a LECO oven after removal of inorganic carbon by treatment with 6 % H_2SO_3. During the experiment, dissolved O_2 was measured immediately after sampling by the Winkler method modified for 12-mL volumes [Nielsen et al., 1996a and 1995a].

S2 (oxidation capacity related to Fe-oxides and -hydroxides)
A method is described in Nielsen et al [1995a]. This extraction method completely dissolves crystalline iron oxides, siderite and magnetite. Fe(II) and Fe(III) bound to clays and silicates will be partly dissolved, see Heron et al. [1994a and 1994b].

S3 (oxidation capacity (OXC))
A laboratory extraction method (0.008 M Ti^{3+}-EDTA (0.05 M) extraction, pH = 6, Eh = -330 mV) for the determination of the oxidation (electron accepting) capacity related to the oxides and hydroxides of aquifer sediments is developed and described in [Heron et al., 1994b]. This operationally defined oxidation capacity (OXC) is determined as μmoles of electrons accepted per gram of sediment sample. Assuming that the aquifer organic matter is fully reduced from the oxidation state 0 (e.g. glucose) to the oxidation state -4 (methane) - currently it is unknown to what extent the aquifer organic matter can be reduced by methanogenic bacteria - the total oxidation capacity of an aquifer volume can be written as:

$$OXC = 4[O_2] + 5[NO_3^-] + [Fe(III)] + 2[Mn(IV)] + 8[SO_4^{2-}] + 4[TOC]$$

In this case solid sulfur species are neglected, assuming insignificant solid sulfur concentrations for these sediments. However gypsum rich sediment may need special consideration. Only a fraction of the sediment Fe(III), Mn(IV) and organic matter may be reducible under the actual field conditions.

Therefore, the reducible fraction of the oxidized species is in focus, and not the total concentration of the oxidized metals. This is also the purpose of the determination.

Currently the quantification of the organic matter part of the OXC is impossible, because the availability of aquifer organic matter to reduction is very poorly understood. A detection limit of 4 μeq/g was assumed. A single extraction lasting 24 h was selected to be appropriate for the OXC determinations. Storing the fresh sediments inside the anaerobic chamber at 20 °C for up to 4 weeks prior to analysis proved acceptable. The drying of reduced samples leads to an increase in OXC, for aerobic samples the OXC remained constant during drying.

S4 (reduction capacity (RDC))
A laboratory method to measure the reduction capacity (RDC) of an aquifer, e.g. a polluted aquifer is described by Heron and Christensen [1994].

The theoretical reduction capacity can be calculated as:

$$RDC = 2[Mn(II)] + [Fe(II)] + 8[S(-II)] + 7[S(-I)] + 8[NH_4^+] + 4[TOC]$$

assuming that aquifer organic matter is fully oxidized from the oxidation state 0 (e.g. glucose) to the oxidation state +4 (carbon dioxide) and that iron, manganese and sulphur end up in the oxidized forms. A very aggressive method (acid dichromate oxidation) is used for determination of RDC and attacks organic matter as well as inorganic precipitates.

The RDC was determined by a modified COD method {Pedersen et al., 1991}. A dry sediment sample of 0.3 - 3.0 g was extracted by acid potassium dichromate. The remaining $Cr_2O_7^{2-}$ was quantified by redox titration with 0.1 N ferrous ammonium sulphate using ferroin as the redox indicator. This method supposedly attacks all of the expected species contributing to the reduction capacity of sediments and possibly overestimates the reduction capacity actually acting as a buffer against entering oxidising agents.

S5 (iron mineralogy (chemical extraction))
The iron mineralogy is described with chemical extraction techniques in Heron et al. [1994b]. Scanning electron microscopy or X-ray diffraction are only semi-quantitative techniques and are difficult to use for total iron contents smaller than 1 %. Methods are described for measuring ion-exchangeable Fe(II) (anaerobic CaCl extraction); the redox status of a sediment sample character-ized by indications of the content of amorphous Fe(III) and reduced Fe(II) species as done by a 0.5 M HCl extraction; Fe(III)oxide content as part of the OXC by Ti(III)-EDTA extraction; Fe(II) sulphide species are distinguished as AVS (acid volatile sulphide, hot 6 M HCl extraction); pyrite (sequential HI and Cr(II)HCl extraction; total distribution of the major Fe(II) and Fe(III) fraction in aquifer sediments can be assessed by a cold M HCl extraction.

S6 (TOC (NVOC))
A method is described in Heron et al. [1997]. Carbonate is removed by 0.73 M sulphurous acid and subsequently the sample is combusted at 800 °C. Quantification is done by infrared de-tection of CO_2 and was accurate at solid organic carbon contents between 30 and 10,000 $\mu gC/g$. This method is design especially for aquifers low in TOC as the glaciofluvial deposits of the Vejen and Grindsted aquifer. Siderite is not removed by the acid satisfactorily. The method is success-ful for aquifers which are low in siderite, $FeCO_3$ and other hardly soluble carbonates. At high con-centrations of dolomite of siderite alternative carbonate-removing agents are recommended. At low OC concentrations TOC can be underestimated due to the potential loss of humic acid carbon during the carbonate removal by sulphurous acid.

S7 (kinetic sorption parameters)
Laboratory sorption experiments are performed to obtain independently determined kinetic parameters. Batch experiments are done in 1-L glass bottles equipped with a glass valve for sampling. A stock solution of ASOCs (180 $\mu g/l$ after dilution) and a sterilizing agent (NaN_2, 2 g/l) are dissolved in 0.6 L distilled water and are placed together with 1.2 kg of sediment. The bottle is incubated in a slowly rotating box. Several samples are taken from the bottle during a period of one month. Carbon 14 labled benzene was included in the experiment to improve the sensitivity of the sorption experiment for benzene. The compounds were analyzed using a GC and scintillation counting. Concentrations sorbed onto the sediment were estimated by mass-balances and water concentrations of the chemicals. The data obtained were fitted using a bicontinuum sorption model.

APPENDIX E

OVERVIEW OF PRIMERS AND PROBES FOR (GROUPS OF) MICRO-ORGANISMS

Table E1. Overview of primers and probes encountered in literature and specific for (groups of) micro-organisms (which are likely encountered underneath and near landfills).

R = A/G; M = A/C; N = G/T/A/C; W = A/T; S = G/C; H = A/C/T; B = C/G/T; Y = C/T; K = G/T; D = A/G/T; V = A/C/G.

Universal primer and probes.

probe	position E.coli	base sequence (5' to 3')	Td/waSh T (C)	targetted group	nontarget bacteria with exact match to probe sequence	from publ.
1100f 530f		CGG ATC CGA ATT CAA CGA GCG MRA CCC GTG CCA GCM GCC GCG G		Universal f-primer		Ueda et al. [1995] Borneman et al. [1996]
1400r UN 1494r	1423-1402	GAA TTC GGA TCC GAC GGG CGG TGT GTR C ACG GNW ACC TTG TTA CGA GTT GGY TAC CTT GTT ACG ACT T		Universal r-primer		Ueda et al. [1995] McInerney et al. [1995] Borneman et al. [1996]
519r		WA TTA CCG CGG CKG CTG		Universal primer		Ogram et al. [1995]

Archaea-specific primers and probes.

probe	position E.coli	base sequence (5' to 3')	Td/waSh T (C)	targetted group	nontarget bacteria with exact match to probe sequence	from publ.
1Af		TC YGK TTG ATC CYG SCR GAG		Gen. Arch. f-primer		Hales et al. [1996]
Arch-20f		TTC CGG TTG ATC CYG CCR G		Gen. Arch. f-primer		Massana et al. [1997]
Arch-958r	2-20	YCC GGC GTT GAM TCC AAT T		Gen. Arch. r-primer		DeLong [1992]
Arch-927r	bij 21f	CCC GCC AAT TCC TTT AAG TTT C		Gen. Arch r-primer		Jurgens et al. [1997]
ARC915		GTG CTC CCC CGC CAA TTC CT		Archeae (r-primer)		Raskin et al. [1994]
ARC344	934-915	TCG CGC CTG CTG CTC CCC GT	56	Archeae (r-primer)		Raskin et al. [1994]
1100Ar	363-344	TGG GTC TCG CTG TTG (?)	54	Gen. Arch. r-primer		Hales et al. [1996]
236f	236	GAGGCCCCAGGRTGGGACCG		Korarchaeal f-primer		Burggraf et al. [1997]
1135r	1135	GTTTGCCCGGCCAGCCGTAA		r-primer		idem
604r	604	TGTCTTCAGGCGGATTTAAC				idem
546r	546	AGTATGCGTGGGAACCCCTC				idem
Cren499r	515-499	CCAGRCTTGCCCCCCGCT		Crenarchaeota		Burggraf et al. [1994]
GII-554	573-554	TTA GGC CCA ATA AAA KCG AC		Gr.II marine Archeae		Massana et al. [1997]
		CCG AGT ACC GTC TAC		Crenarchae - probe		DeLong [1992-nat]
Eury498r	510-498	CTTGCCCRGCCCTT		Euryarchaeota		Burggraf et al. [1994]
GI-554	573-554	TTA GGC CCA ATA ATC MTC CT				Massana et al. [1997]
MER1	Methyl coM	GGG CAC GGG TCT CGC T		Methanogens probe		Hales et al. [1996]
ME1	reductase (MCR)	GCM ATG CAR ATH GGW ATG TC		MCR a-sub. primer		idem
ME2		TCA TKG CRT AGT TDG GRT AGT		idem		idem
ME3		GGT GGH GTM GGW TTC ACA CA		MCR a-sub. probe		idem
MC1109	\1128-1109	GCA ACA TAG GGC ACG GGT CT	55	Methanococcales		Raskin et al. [1994]
MB310	331-310	CTT GTC TCA GGT CCA TCT CCG	57	Methanobacteriaceae		idem
MB1174	1195-1174	TAC CGT CGT CCA CTC CTT CCT C	62	Methanobacteriaceae		idem
MG1200	1220-1200	CGG ATA ATT CGG GGC ATG CTG	53	Methanomicrobiales Fam. I, II, III		idem
MSMX860	880-860	GGC TCG CTT CAC GGC TTC CCT	60	Methanosarcinaceae		Raskin et al. [1994]
MS1414	1434-1414	CTC ACC CAT ACC TCA CTC GGG	58	idem genus I,II,IV,V		idem
MS821	844-821	CGC CAT GCC TGA CAC CTA GCG AGC	60	idem genus I		idem
		TCG CAC CGT GGC CGA CAC CTA GC				
MX825	847-825		59	idem genus III		idem

Eubacterial primers and probes.

probe	position E.coli	base sequence (5' to 3')	Td/waSh T (C)	targetted group	nontarget bacteria with exact match to probe sequence	from publ.
PA	8-28	AGA GTT TGA TCC TGG CTC AG		Eubact. primer		Edwards et al. [1989]
PB	50-70	TAA CAC ATG CAA GTC GAA CG		Eubact. primer		Edwards et al. [1989]
PC	341-361	CTA CGG GAG GCA GCA GTG GG		Eubact. primer		Edwards et al. [1989]
PD	518-536	CAG CAG CCG CGG TAA TAC		Eubact. primer		Edwards et al. [1989]
PE	908-928	AAA CTC AAA GGA ATT GAC GG		Eubact. primer		Lane et al. [1985]
PF	1053-1073	CAT GGC TGT CGT CAG CTC GT		Eubact. primer		Edwards et al. [1989]
PG	1392-1407	GTA CAC ACC GCC CGT		Eubact. primer		Lane et al. [1985]
PH	1522-1542	TGC GGC TGG ATC ACC TCC TT		Eubact. primer		Edwards et al. [1989]
	1509-1491	GGT TAC CTT GTT ACG ACT T		Eubact. primer		DeLong [1992]
pH'		AAG GAG GTG ATC CAG CCG CA		Eubact. primer		
S-D-bact-1512		ACGGYTACCTTGTTACGACTT		Eubact. primer		Fry et al. [1997]

Eubacteria: kingdom specific probes.

probe	position E.coli	base sequence (5' to 3')	Td/waSh T (C)	targetted group	nontarget bacteria with exact match to probe sequence	from publ.
ALF1b		CGT TCG (C/T)TC TAG CCA G		Bacteria alpha-subclass bacteria, most spirochetes, some gamma-subclassers and Flexistipes sinus-arabicus probe		Manz et al. [1992]
BET42a	23S rRNA	GCC TTC CCA CTT CGT TT		Bacteria beta-subclas probe		Manz et al. [1992]
GAM42a	23S rRNA	GCC TTC CCA CAT CGT TT		Bacteria gamma-subclass probe		Manz et al. [1992]
HGC	23S rRNA	TAT AGT TAC CAC CGC CGT		Gram-pos. Bacteria with high GC content		Manz et al. [1992]
Gram+	16S rRNA	AAGGGGCATGATG		Gram pos		Fry et al. [1997]

Eubacteria: sulphate/iron-reducers specific probes.

probe	position E.coli	base sequence (5' to 3')	Td/waSh T (C)	targetted group	nontarget bacteria with exact match to probe sequence	from publ.
	687-702	TAC GGA TTT CAC TCC T		Desulfovibrio probe		Devereux et al. [1992]
	660-679	GAA TTC CAC TTT CCC CTC TG		Desulfobulbus probe		Devereux et al. [1992]
	221-240	TGC GCG GAC TCA TCT TCA AA		Desulfobacterium prob		Devereux et al. [1992]
	129-146	CAG GCT TGA ATT CAG ATT		Desulfobacter probe		Devereux et al. [1992]
	814-831	ACC TAG TGA TCA ACG TTT		Desulfo-coccus multivorans, D-sarcina variabilis, D-botulus sapovorans		Devereux et al. [1992]
	804-821	CAA CGT TTA CTG CGT GGA		Desulfobacterium, Desulfobacter, D-coccus multivorans, D-sarcina varia-bilis, D-botulus sapovorans		Devereux et al. [1992]
	SRB385-f	CGGCGTCGCTGCGTCAGG		Sulfate-reducing bacteria, f-primer		Amann et al. [1992]
	Univ907r	CCCCGTCAATTCCTTTGAGTTT		Universal r-primer		Amann et al. [1992]
	S-Sc-delta-0401-a-S-20	AASCCTGACGCAGCRACGCC		Delta proteobacteria		Fry et al. [1997]
	S-*-Dsv-0683	TCTACGGATTTCACTCCTACAC		Desulfovibrionaceae/metal reducers		Fry et al. [1997]
SPN3	506-477	CCG GTC CTT CTT CTG TAG GTA ACG TCA CAG		Shewanella putrefaciens probe (Fe-reducer)		DiChristina et al. [1993]
PS1	66-82	GAT TGC TCC TCT ACC GT		Leptothrix, some Sphaerotilus natans (Mn, Fe oxidizers), unknown sheathed filamentous bacteria		Siering et al. [1997]
PSP-6	138-155	GGC TAT CCC CCA CTA CTG		SEE PS-1		Siering et al. [1997]

Functional gene-based primers and probes for denitrification.

probe	position E.coli	base sequence (5' to 3')	Td/waSh T (C)	targetted group	nontarget bacteria with exact match to probe sequence	from publ.
	nitrate reductase (narG)	TTA CTT CAA ACA GAA GGG TGA AAC CTT T TTT CGC TTT ATC GGC GTC TTC AAT GAT		Hyphomicrobium/denitri-fiers, f-primer, r-primer		Kloos et al. [1995]
	cyt c,c nitrite reductase (nirS)	ATC TAC TTC CAA CGC TGC GCC GGT TGC CAT GTG TAC GCG TCC TCG TAG CCC TTG AAC TT		Hyphomicrobium/denitri-fiers, f-primer, r-primer		Kloos et al. [1995]
	Cu-cont. nitrite reductase (nirK)	GGC ATG GT(G/C) CCG TGG CAC GT(G/C) ACC TC(G/C) GGC CAT CAG GTC (A/G)TC GT(C/T) CCA (G/C)TC GCC GGT		Hyphomicrobium/denitri-fiers, f-primer, r-primer		Kloos et al. [1995[
	nitrous oxide reductase (nosZ)	GCA GGA CGA GAA CAG CTA CAC CAT GTN CA(T/C) AGC TTC TTC CAT GTT CCA CTT GAC NAC (C/T)TG		Hyphomicrobium/denitri-fiers, f-primer, r-primer		Kloos et al. [1995]

Primer sets for genes involved in xenobiotic degradation.

probe	position E.coli	base sequence (5' to 3')	Td/waSh T (C)	targetted group	nontarget bacteria with exact match to probe sequence	from publ.
XylE	catachol,2,3-dioxygenase	AGG NGT (A/T)AT GCG ICC IGG CCA (C/T)GT TCG TG(A/G) TA(A/G) AAG AT(G/C) GCC TTG GC		forward pr.catachol-2,3 dioxygenase (P. putida), reverse primer		Chandler et al. [1996]
NahAc	polyaromatic hy-drocarbon oxygenase	GTT TGC AGC TAT CAC GGC TGG GGC TTC GAC AAT GGC GTA GGT CCA GAC		forward primer (see position for target), reverse primer		Chandler et al. [1996]

Eucarya specific primers and probes.

probe	position E.coli	base sequence (5' to 3')	Td/waSh T (C)	targetted group	nontarget bacteria with exact match to probe sequence	from publ.
EukF		AAG CTG GTT GAT CCT GCC AGT		Eucary. primer		DeLong [1992]
EukR		TGA TCC TTC TGC AGG TTC ACC TAC		Eucary. primer		DeLong [1992]
		GGG CAT CAC AGA CCT G		Eucary. probe		DeLong [1992]