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Hydrogen as indicator for in-situ redox condition and dechlorination

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Samenvatting

Intrinsieke biodegradatie en gestimuleerde natuurlijke afbraak van chloorkool-
waterstoffen (zoals PER en TRI) zijn belangrijk bij *in situ* reiniging van grondwa-
ter vervuild met deze stoffen. Het doel van dit project was de bruikbaarheid van
waterstofmetingen als aanvullende redoxkarakteristiek en als graadmeter voor de *in
situ* dechloreringsconditie in de praktijk te toetsen. Op drie met chloor-oplosmiddel
verontreinigde locaties en in het grondwater nabij een stortplaats zijn waterstofme-
tingen in combinatie met andere bepalingen uitgevoerd. Verschillende methodes
om deze gegevens te interpreteren zijn gebruikt en met elkaar vergeleken.

Uit het onderzoek is naar voren gekomen dat redoxkarakterisering middels het me-
ten van waterstof gekenmerkt wordt door een goede prijs/prestatie verhouding. Met
waterstofmetingen kan een eerste snelle on-site screening van de redoxsituatie
worden verricht. Vervolgens kan een voldoende gedetailleerde redoxkarakterise-
ring worden verkregen door gericht een aantal punten te kiezen en naast waterstof
ook de macrochemische parameters te bepalen. Het weglaten van waterstofmetin-
gen lijkt in een aantal gevallen een onvolledig en niet consistent beeld op te leve-
ren.

Het meten van waterstof in grondwater is waarschijnlijk ook bruikbaar voor het in-
schatten van de *in-situ* dechloreringsconditie. Bij *in-situ* waterstofconcentraties ho-
ger dan 2 nM werd een hoge mate van dechlorering gevonden. In aanwezigheid
van voldoende DOC, BTEX, minerale olie, en 1,2-dichloorethaan, bleek de water-
stofconcentratie verhoogd te zijn en ook een hoge mate van dechlorering op te tre-
den. Enkele kennisleemtes moeten nog aangepakt worden om de toepassing van
een waterstofmeting als indicator voor dechlorering volwaardig te maken.
samples are taken for analyses on other chemical parameters. At some of the sites it
was shown that omitting the hydrogen data results in an inconsistent redox identifi-
cation.

Intrinsieke biodegradatie en gestimuleerde natuurlijke afbraak van chloorkoolwa-
terstoffen (zoals PER en TRI) zijn belangrijk bij de beheersing en extensieve reini-
ging van grondwater vervuild met deze stoffen. De sanering van bodems die ver-
ontreinigd zijn met PER en TRI zijn in de meeste gevallen complex en kostbaar.
Een mogelijkheid om de kosten van deze saneringen te beperken en daarmee de
(financiële) haalbaarheid te vergroten is het bewust gebruik maken en stimuleren
van natuurlijke processen. Hiertoe is het belangrijk dat de bodem de juiste intrin-
sieke dechloringsconditie heeft. Dat wil zeggen dat de juiste redoxpotentiaal voor
reductieve dechlorering moet heersen, de aanwezigheid van voldoende elektronen-
donor en -acceptor en de geschikte microbiële populatie moet aanwezig zijn.

De op locatie gemeten concentratie van waterstof in grondwater kan mogelijk als
een graadmeter worden gebruikt om de intrinsieke dechloringsconditie van een wa-
tervoerend pakket vast te stellen. Daarnaast kan met waterstofmetingen een meer

completere redox karakterisering worden uitgevoerd hetgeen belangrijk is voor een effectief gebruik van natuurlijke of gestimuleerde biologische afbraak.

Het onderzoek is opgedeeld in twee fasen. De eerste fase bestaat uit het opzetten van een meetmethode en het testen van deze methode, het meten van waterstof op vier verschillende locaties en het interpreteren van deze gegevens. De tweede fase, die nog niet is uitgevoerd, bestaat uit een multi-locatie analyse waarbij getracht wordt correlaties tussen waterstof en andere parameters (o.a. DOC, macrochemie) te identificeren.

In dit rapport wordt de eerste fase besproken, deze fase heeft gelopen van mei '98 tot en met maart '99.

Doel
Dit onderzoek had tot doel de bruikbaarheid van waterstofmetingen te onderzoeken. Enerzijds om de intrinsieke dechloreringsconditie van een watervoerend pakket vast te stellen. Anderzijds wordt nagegaan of de waterstofconcentratie gebruikt kan worden als een goede redoxkarakteristiek.

Werkzaamheden fase 1
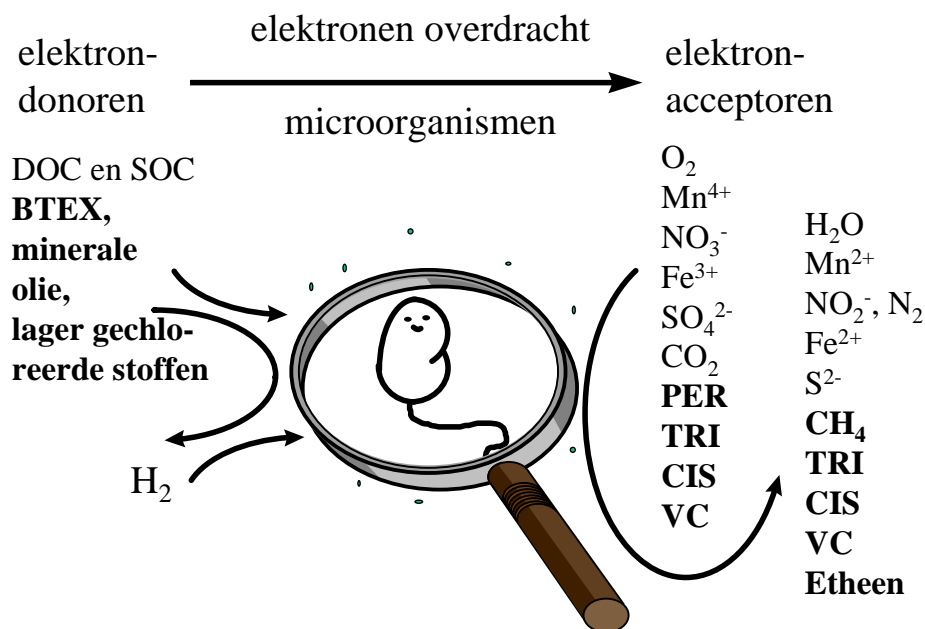In de eerste fase van dit onderzoek zijn de volgende onderdelen uitgevoerd:
i) uitwerken en verhelderen van de grondslagen voor de interpretatie van waterstofmetingen ten behoeve van karakterisering van de redoxsituatie en van het natuurlijke afbraak potentieel,
ii) testen van apparatuur en opstellen van een methode voor on-site bemonstering en analyse van waterstof,
iii) uitvoering van waterstofmetingen op een aantal locaties en interpretatie op basis van geformuleerde grondslagen (i) voor het gebruik van waterstof als indicator voor redoxkarakterisatie en voor natuurlijke biodegradatie van chloorkoolwaterstoffen. Gaande het project zijn uiteindelijk vier locaties in het meetprogramma opgenomen.
  - drie locaties verontreinigd met chloorkoolwaterstoffen en in mindere mate met BTEX, namelijk:
    - de locatie Rademarkt te Groningen,
    - het DAF-terrein te Eindhoven,
    - het NS-revisieterrein te Tilburg
  - één stortplaats, verontreinigd met zware metalen, aromaten en chloorkoolwaterstoffen, namelijk:
    - Banisveld te Boxtel.
De resultaten van de waterstofmetingen zijn per locatie vergeleken met andere redoxparameters en met de dechloreringsindex. Vervolgens zijn de resultaten voor de vier verschillende locaties met elkaar vergeleken en is een inschatting gemaakt van de toegevoegde waarde van waterstofmetingen voor redoxkarakterisering en inschatten van de mate van dechlorering. Een samenvatting van de bevindingen is hieronder weergegeven.

Uitwerken en verhelderen van de grondslagen

In de theoretische hoofdstukken twee en drie is geanalyseerd en beschreven wat de rol van waterstof is bij de in de bodem optredende redoxprocessen die grotendeels door micro-organismen worden gekatalyseerd. Waterstof is een tussenproduct dat gevormd wordt bij de natuurlijke microbiologische fermentatie en vertering van organisch materiaal in de bodem. Het gevormde waterstof reageert met behulp van micro-organismen en vervolgens met andere in de bodem aanwezige componenten. Het waterstof is een elektronendonor en geeft daarbij elektronen af aan die componenten. Dit kunnen natuurlijke elektronenacceptoren zijn zoals zuurstof, nitraat, ijzer(III), sulfaat, en kooldioxide, maar ook verontreinigingen zoals PER, TRI en andere chloorkoolwaterstoffen, die daarbij gedechloreerd worden (Figuur I)


elektron-donoren

elektronen overdracht

microorganismen

elektron-acceptoren

DOC en SOC
**BTEX,
minerale
olie,
lager gechlo-
reerde stoffen**

H_2

O_2
Mn^{4+}
NO_3^-
Fe^{3+}
SO_4^{2-}
CO_2
**PER
TRI
CIS
VC**

H_2O
Mn^{2+}
NO_2^-, N_2
Fe^{2+}
S^{2-}
**CH$_4$
TRI
CIS
VC
Etheen**

Figuur l   *Rol van waterstof en micro-organismen bij de in de bodem optredende processen*

Het concentratieniveau van waterstof in het grondwater wordt aldus bepaald door een complex geheel van processen waarbij enerzijds waterstof geproduceerd wordt uit organische stof en anderzijds waterstof geconsumeerd wordt door reactie met bodem en grondwater bestanddelen. In deze studie is geïnventariseerd welke mechanismen, en welke biologische en chemische parameters relevant kunnen zijn. Voor een gedetailleerde beschrijving wordt verwezen naar de betreffende hoofdstukken in dit rapport. Hier wordt volstaan met het geven van enkele hoofdpunten. In een niet te zeer verstoord bodemsysteem zijn de processen vaak (dicht bij) evenwicht en correspondeert de waterstofconcentratie met het heersende redox milieu.

Een aantal onderzoekers hebben *ranges* van waterstofconcentraties proefondervindelijk (empirisch) gerelateerd aan de heersende (dominante) redoxconditie. Deze empirische benadering kan aldus gebruikt worden voor interpretatie van gemeten waterstofconcentraties ten behoeve van redoxkarakterisatie (Figuur II).

Steady-state hydrogen range
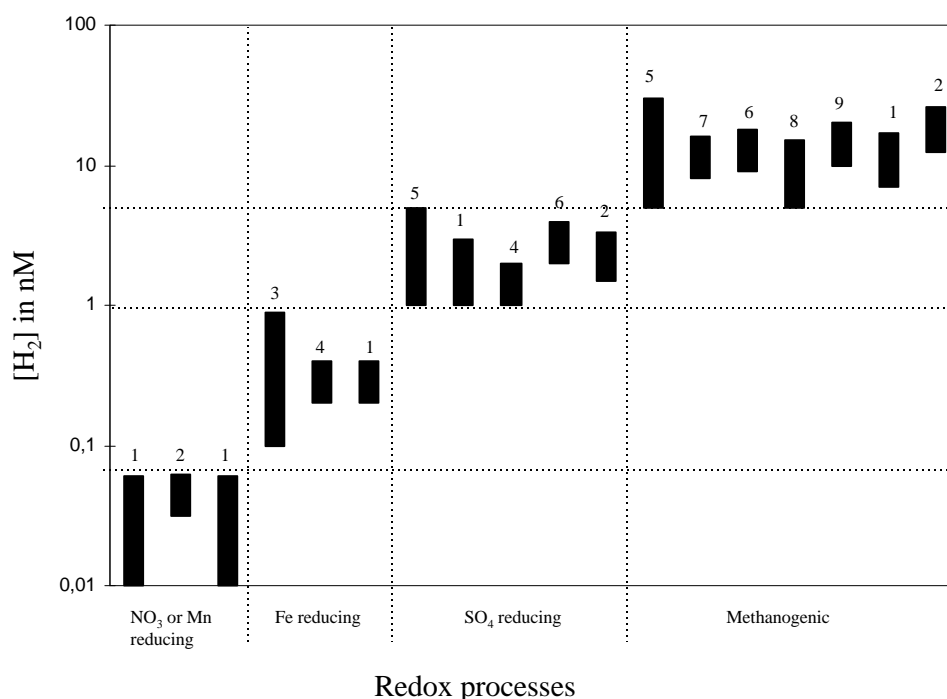
Figuur II   *Overzicht van steady-state H_2-concentraties gerelateerd aan verschillende redoxprocessen bij standaardtemperatuur (25°C). De waarden zijn afkomstig uit de volgende referenties: (1) Lovley and Goodwin, 1988; (2) Hoehler et al., 1998; (3) Chapelle and Lovley, 1992; (4) Lovley and Philips, 1987; (5) Vrobleski and Chapelle, 1994; (6) Lovley et al., 1982; (7) Lovley and Klung, 1982; (8) Goodwin et al., 1988; (9) Conrad et al., 1987.*

Deze **empirische relatie** kan dus gebruikt worden voor de redoxkarakterisering aan de hand van waterstofmetingen. Belangrijk daarbij is dat rekening wordt gehouden met de locaal heersende temperatuur van de bodem. In dit rapport zijn correctiefactoren voor de temperatuur opgenomen.

De meetwaarden van waterstof kunnen ook geïnterpreteerd worden door combinatie met macrochemische gegevens van bodem en grondwater en vervolgens de uitvoering van **thermodynamische berekeningen** (in dit rapport ook wel de partiële evenwichtsbenadering genoemd). Op deze manier kan voor een bepaald monsterpunt worden aangetoond welke redox processen <u>kunnen</u> verlopen. Dit kan voor "normale" redoxprocessen maar ook voor dechloreringsreacties.

Het voordeel van de thermodynamische methode is dat deze locatie onafhankelijk is; er zijn geen locatie specifieke criteria waarmee vastgesteld wordt welke redox processen kunnen op treden. Uit de inventarisatie kwam nog wel een belangrijke kennisleemte naar voren. De minimale energie die micro-organismen uit een redox- en dechloreringsreactie moeten kunnen winnen om daarop te kunnen leven is een invoer parameter in de thermodynamische evaluatie. Er konden slechts zeer algemene waarden voor deze minimale energieën in de literatuur gevonden worden. In dit onderzoek is bij alle thermodynamische berekeningen standaard een waarde voor de minimale energie behoefte van 5 kJ/mol gebruikt. Zeker voor de dechloreringsreacties is het mogelijk dat de werkelijke waarden veel hoger liggen. Voor dechloreringsreacties waarbij waterstof een rol speelt heeft een grote minimale energie behoefte het effect dat er een relatief hoge grensconcentratie voor waterstof bestaat. De waterstofconcentratie moet dan deze waarde overstijgen om dechlorering te kunnen laten plaatsvinden.

Voor een bodem systeem dat ver uit evenwicht is, is de situatie veel complexer. Dit kan voorkomen bij stortplaatsen waar recentelijk grote hoeveelheden vers organische stof in de bodem zijn gekomen en bij toepassing van een gestimuleerde biologische *in-situ* sanering op een chloorkoolwaterstof locaties waar organische hulpstoffen worden geïnjecteerd. De in grondwater voorkomende waterstof concentraties kunnen dan sterk afwijken van de (dicht bij) evenwichtssituatie. Ze worden bepaald door de *in-situ* waterstof productie en consumptie snelheden, die onder andere weer afhangen van het type organische stof, en de snelheid van infiltratie. Een eerste conceptueel model voor een kinetische beschouwing van dit soort situaties is besproken in de hoofdstukken 2 en 3. Deze methode is verder niet uitgewerkt en toegepast in deze fase van het project.

In deze fase 1 zijn zowel de empirische relatie als de methode met thermodynamische berekeningen gebruikt voor interpretatie van gemeten waterstofconcentraties. De thermodynamische berekeningen zijn uitgevoerd voor de waterstofconsumerende processen, d.w.z. de redox- en dechloreringsprocessen, waarbij voorlopig is aangenomen dat voor alle reacties een minimale energie behoefte van 5 kJ/mol geldt. Grenswaarden van waterstof zijn niet berekend; er is volstaan met weergave van een thermodynamische grootheid, de Gibbs Energie. Indien deze meer negatief is dan -5 kJ/mol wordt geconcludeerd dat de condities gunstig zijn om dat proces op dat meetpunt te laten verlopen.

Methode voor on-site bemonstering en analyse van waterstof
Aangezien waterstof een klein molecuul is diffundeert het door vrijwel alle kunststoffen, opslag en transport naar het laboratorium is daarom niet wenselijk. Tevens is waterstof zeer reactief. Waterstof dient daarom op locatie gemeten te worden. Dit is mogelijk met behulp van een gaschromatograaf uitgerust met een reduction gas detector (RGD). Met behulp van de "gas-stripping" methode wordt waterstof uit het grondwater in de gasfase overgebracht. Na het verversen van het grondwater in de peilbuis wordt met een debiet van minimaal 500 ml per minuut, grondwater door een "gas-bulb" van 250 ml gepompt. In de "gasbulb" wordt vervolgens 20 ml

stikstofgas gebracht. Na 25 minuten worden uit de gasfase vier monsters op de GC-RGD geanalyseerd. Na vijf minuten worden wederom vier monsters uit diezelfde gasfase genomen en geanalyseerd op de GC-RGD. Wanneer de waarde minder dan 5% afwijkt van de gemeten waarde bij 25 minuten wordt aangenomen dat er een evenwicht is bereikt. Voor het omrekenen van de waterstofconcentratie in de gasfase naar de concentratie in de waterfase wordt gebruik gemaakt van de Henry coefficient. De kosten van een waterstofanalyse op locatie bedragen, inclusief reis- en materiaalkosten, ongeveer 450 gulden per meetpunt. De kosten zijn exclusief concentratieprofielplots en interpretatie van de gegevens.

Waterstofmetingen op vier locaties: uitvoering en interpretatie
De volgende vier locaties zijn onderzocht:
−   drie locaties verontreinigd met chloorkoolwaterstoffen en in mindere mate met BTEX, namelijk: de locatie Rademarkt te Groningen, het DAF terrein te Eindhoven, het NS revisieterrein te Tilburg.
−   één stortplaats, namelijk Banisveld te Boxtel.

Naast waterstofmetingen zijn op deze locaties op alle meetpunten ook de macrochemie, de redoxpotentiaal, het DOC gehalte en (indien relevant) de concentraties aan chloorkoolwaterstoffen en afbraakproducten (PER, TRI, CIS, VC, en etheen) bepaald. Aldus zijn vier redoxidentificatie methoden toegepast:
−   **redox-potentiaal** meting (de Eh-methode),
−   interpretatie van **macrochemische** parameters (de macrochemische methode),
−   interpretatie van **waterstofmetingen** middels de empirische relatie tussen de redoxconditie en waterstofconcentraties (de empirische methode),
−   en interpretatie van **waterstofmetingen** middels thermodynamische berekeningen op basis van waterstofconcentraties en macrochemische parameters (de thermodynamische methode).

Uit de concentraties van de CKW's en afbraakproducten is de mate van *in-situ* dechlorering afgeleid, in termen van de dechloreringsindex. Ook de mate waarin de dechlorering thermodynamisch gezien gunstig is werd berekend aan de hand van gemeten concentraties. De mate van dechlorering is vergeleken met de redoxkarakteristiek en andere parameters zoals DOC-gehalte en het voorkomen van cocontaminanten zoals BTEX.

Rademarkt te Groningen
Redox-karakterisatie. Op deze locatie werd een grote overeenkomst gevonden tussen redoxkarakterisering middels de macrochemische, de empirische en de thermodynamische methoden. Eh-resultaten gaven geen consistent beeld. Een hoge variatie aan redox-condities (variërend tussen aëroob en methanogeen) werd aangetroffen, overeenkomstig een eerdere karakterisering in het kader van NOBIS-onderzoek. De meerwaarde van de thermodynamische methode bleek uit het feit dat in redox overgangszones kon worden aangetoond dat de condities voor verschillende redoxprocessen gunstig zijn.

In situ dechlorering. Berekeningen toonden aan dat dechlorering onder alle condities thermodynamisch gunstig is. Op de Rademarkt locatie werd geen overall relatie gevonden tussen de dechlorering en de waterstofconcentratie. Wel werd bij de meetpunten met een relatief hoge waterstofconcentratie (> 2-3 nM) ook de meest volledige dechlorering gevonden. Op deze locatie ligt het DOC-gehalte relatief laag (5 - 25 mg DOC/l) en er kon een correlatie tussen waterstofconcentratie en DOC-concentratie worden aangetoond.

DAF terrein te Eindhoven

Redox-karakterisatie. Op deze locatie werd een grote mate van overeenkomst gevonden tussen redoxkarakterisering middels de empirische en de thermodynamische methoden. Er werd een grote discrepantie gevonden tussen dit resultaat en de redoxpotentiaal metingen en de macrochemische analyse, deze laatste leverden geen consistent redoxpatroon op voor de verschillende bodemlagen van de locatie. Een beperkte variatie aan redox-condities (in hoofdzaak variërend tussen ijzerreducerend en methanogeen) werd aangetroffen. De meerwaarde van de twee waterstofmethoden bleek uit dit bovenstaande resultaat: alleen op deze manier konden redoxpatronen worden afgeleid.

In situ dechlorering. Op deze locatie kon geen overall relatie gevonden worden tussen de dechloreringsindex en de waterstofconcentratie. Over de gehele pluim worden afbraakproducten van dechlorering gevonden ongeacht de waterstofconcentratie. Op punten met hoge waterstofconcentraties bleek ook het DOC-gehalte verhoogd, of bleken co-contaminanten zoals BTEX en dichloorethaan aanwezig, dit zijn stoffen die als substraten voor waterstofproductie en/of elektronendonor voor dechlorering kunnen fungeren.

NS revisieterrein te Tilburg

Redox-karakterisatie. Op deze locatie werd een grote mate van overeenkomst gevonden tussen alle methoden voor redoxkarakterisering, ofschoon door de beperkte set aan meetpunten, duidelijke redoxpatronen moeilijk waren af te leiden, en niet alle methoden volledig konden worden toegepast. De empirische waterstof methode gaf hier het meest consistente beeld en correspondeerde met de Eh resultaten. Een variatie aan redox-condities (variërend tussen nitraatreducerende en methanogene condities) werd aangetroffen.

In situ dechlorering. Op deze locatie werden indicaties gevonden dat er een relatie is tussen de dechloreringsindex en de waterstofconcentratie. Wederom werd bij de meetpunten met een relatief hoge waterstofconcentratie (> 2-3 nM) ook de meest volledige dechlorering gevonden. Op die punten bleek het DOC-gehalte sterk verhoogd (tot ca. 100 mg DOC/L) ten gevolge van additionele verontreiniging met minerale olie en BTEX, stoffen die als substraten voor waterstofproductie en/of elektronendonor voor dechlorering kunnen fungeren.

Banisveld te Boxtel

Redox-karakterisatie. Op deze locatie werden grote verschillen gevonden tussen redoxkarakterisering middels de macrochemische, empirische en de thermodynamische methoden. De thermodynamische methode wordt hier gezien als de methode die het meest betrouwbare en volledige redoxpatroon oplevert. De empirische waterstof methode levert hier geen consistent beeld, mogelijk ten gevolge van de hoge in-flux van DOC en andere redox-species waardoor niet-evenwicht situaties kunnen zijn ontstaan. Ook de macrochemische gaven geen consistent beeld. Eh-metingen zijn niet uitgevoerd.

Vergelijking karakterisering middels waterstof voor vier locaties

Redox-karakterisatie. De bruikbaarheid van de waterstofmeting voor redoxkarakterisatie blijkt uit de volgende samenvattende tabel (zie Tabel 1).

Tabel I   Overzicht van de bruikbaarheid van de verschillende redox-identificatiemethoden. De gegevens zijn gebaseerd op de metingen uitgevoerd op de vier locaties.

Locatie	Redox potentiaal	Macro chemie	Empirische methode	Part. evenwichts benadering
Rademarkt	-	+	+	++
DAF	+/-	-	+	++
SBNS	+/-	+	+	(*)
Banisveld	(**)	-	-	++

- geen consistent beeld

+ consistent beeld, identificatie van dominante processen

++ consistent beeld, identificatie van gelijktijdig optredende processen

(*) niet genoeg data

(**) niet gemeten

Zowel de empirische methode als de thermodynamische methode (ook wel partiële evenwichtsbenadering genoemd) leveren in drie van de vier gevallen een consistent redoxpatroon, in tegenstelling tot de meer traditionele redox-karakteriseringmethoden op basis van macrochemische analyse en redox-potentiaalmetingen. De empirische waterstof methode lijkt een goed instrument voor een eerste screening van de redoxsituatie op een locatie. Daarna kan een meer gedetailleerd bemonstering- en analyse programma worden uitgevoerd om zo op basis van macrochemische parameters en waterstofmeetwaarden middels de thermodynamische methode een meer complete en meer accurate redoxkarakteristiek vast te stellen.

In situ dechlorering. Op de locaties werd niet altijd een volledige correlatie gevonden tussen de waterstofconcentratie en de mate van dechlorering (de dechlorerings-index). Wel werd geconstateerd dat bij de meetpunten met een relatief hoge waterstofconcentratie (> 2-3 nM) op alle locaties ook de meest volledige dechlorering werd gevonden. Op die punten bleek het DOC-gehalte verhoogd en/of andere co-contaminanten aanwezig die als substraten voor waterstofproductie en/of elektronendonor voor dechlorering kunnen fungeren. Er werd een relatie gevonden tussen

DOC-gehalte en de waterstofconcentratie, met toenemende DOC-gehaltes nam de H_2-concentratie toe.

Conclusies en aanbevelingen

Het doel van fase 1 van dit project was de bruikbaarheid van waterstofmetingen als aanvullende redoxkarakteristiek en als graadmeter voor de intrinsieke dechloreringsconditie van de bodem, in de praktijk op een aantal locaties te toetsen.

Waterstofmetingen blijken een sterk toegevoegde waarde bij de redoxkarakterisering te hebben. Waterstofmetingen in grondwater kunnen gebruikt worden bij een eerste snelle on-site screening van de redoxsituatie. Hierbij wordt voor de interpretatie gebruik gemaakt van de empirisch vastgestelde relatie tussen de waterstofconcentratie en de redoxtoestand. Voor een meer nauwgezette redoxkarakterisering is naast meten van de waterstofconcentraties ook een bepaling van macrochemische parameters noodzakelijk. Een interpretatie van deze gegevens met thermodynamische berekeningen geeft het meest complete redoxpatroon. Wanneer bij een redoxkarakterisering op basis van macrochemie of redoxpotentiaal de waterstofmetingen worden weggelaten lijkt dit vaak een onvolledige en inconsistent redoxpatroon op te leveren.

Er zijn sterke aanwijzingen dat waterstof een bruikbare parameter kan zijn voor het inschatten van de *in-situ* dechloreringsconditie. De mate waarin dit ook werkelijk het geval is, is nog niet volledig duidelijk. Er werd voor één van de vier locaties een relatie tussen de dechlorering en de waterstofconcentratie gevonden. Op alle locaties werd geconstateerd dat in aanwezigheid van voldoende DOC, BTEX, minerale olie, dichloorethaan, etc., de waterstofconcentraties relatief hoog zijn (> 2-3 nM) en dat in die gevallen ook een hoge mate van dechlorering optreedt. Mogelijk is het noodzakelijk dat de waterstofconcentratie boven een grenswaarde van enkele nM uit moet komen om dechlorering voldoende snel en volledig te laten plaatsvinden.

Aanbevolen wordt in een eventuele vervolgfase van het project, conform het oorspronkelijk projectplan (= fase II), de relatie tussen DOC, waterstofproductiesnelheid, waterstofconcentraties, snelheid en mate van dechlorering en andere parameters nader te onderzoeken. Dit kan middels multiparameter analyse van de data die in fase 1 van dit project gegeneerd zijn. Tevens kunnen data uit aanpalende projecten gebruikt worden. Op korte termijn staan metingen gepland bij Rademarkt (IWACO), Zaltbommel en Amsterdam (BioSoil), CombiRem (SBNS) en 14 stortplaatsen (IWACO).

In aanvulling worden laboratoriumexperimenten met natuurlijke sediment en grondwatermonsters aanbevolen om ook nog bestaande thermodynamische en microbiologische kennishiaten te kunnen ophelderen. Zo zijn grensconcentraties voor productie van waterstof uit natuurlijke organische koolstof en voor dechlorerings processen belangrijke kennisleemtes waar nader onderzoek zich op zou moeten richten. De verwachting is dat na afronding van dat onderzoek de waterstofmeting

als een bewezen en volwassen methode voor vaststelling van redox- en dechlore-
ringscondities kan worden gebruikt.

Leeswijzer
In hoofdstuk twee wordt een theoretische overzicht gegeven over de basis beginse-
len van de redoxreacties in grondwatersystemen en de waterstofproductie en -
consumptie in anaërobe ecosystemen. Het derde hoofdstuk behandeld het dechlore-
ringsproces en de rol van waterstof in deze. Een aantal verschillende redox identi-
ficatiemethoden wordt besproken in hoofdstuk vier. Waarna in hoofdstuk vijf een
methode besproken wordt hoe de gemeten waterstofconcentratie te gebruiken bij de
bepaling van de mate van dechlorering. In hoofdstuk zes wordt een algemeen ka-
rakterisatieschema gegeven en uitgelegd.

Een procedure om waterstof te meten in het veld is weergegeven in hoofdstuk ze-
ven. Tevens wordt stap voor stap uitgelegd hoe in het veld gemeten moet worden,
welke apparatuur hiervoor nodig is en wat de kosten zijn.

Vier locaties zijn in dit onderzoek gemeten en worden één voor één behandeld in
achtereenvolgens hoofdstuk acht, negen, tien en elf. In hoofdstuk twaalf worden
deze verschillende locaties met elkaar vergeleken. In hoofdstuk dertien staan de
conclusies van het onderzoek, inclusief de aanbevelingen.

Preface

With this report, the first phase in the NOBIS project "Hydrogen as indicator for *in-situ* redox condition and dechlorination" project number 96.024, has been delivered. The aim of this project is two-fold and divided into two phases. In the first phase a method was developed to demonstrate the usefulness of hydrogen measurements as an extra redox identification method and as an indicator for *in-situ* dechlorination. This method was tested at four locations.

In the second phase a multiple location analysis is planned in order to determine the correlation of hydrogen and other parameters by specific measurements at the locations, followed by a principal component analysis of relations between hydrogen concentrationsand these parameters.

The first phase of this project was carried out by TNO Institute of Environmental Sciences, Energy Research and Process Innovation, Department of Environmental Biotechnology. During the measurements at the different sites TNO was assisted by various persons and organisations listed below.

Site	Organisation	Person
Rademarkt, Groningen	IWACO	Adri Nipshagen
DAF, Eindhoven	Grontmij	Paul Verhaagen
SBNS, Tilburg	IWACO	Simon Hofstra
Banisveld, Boxtel	IWACO Vrije Universiteit Amsterdam	Timo Heimovaara Boris van Breukelen

Rademarkt, Groningen IWACO, Groningen (Rademarkt), Grontmij Houten (DAF), IWACO Den Bosch (SBNS) and IWACO Rotterdam and VU Amsterdam (Banisveld).

June 1999.

Table of contents

1.   Introduction

World-wide, many sites are contaminated and pose a potential threat to the well-being of man, flora and fauna. Chlorinated hydrocarbons are among the most frequently occurring contaminants in soil and groundwater. The use of intrinsic degradation processes (the most significant process of natural attenuation) is becoming more and more an option for the control and restoration of contaminated sites. The hydrogen concentration in groundwater may be an important parameter in assessing the natural attenuation (NA) potential at contaminated sites, especially those polluted with chlorinated compounds. The feasibility of NA as remedial option of chlorinated solvents can be assessed through the following phases (Wiedemeier et al.,1997; Rijnaarts et al., 1998; Sinke et al., 1998):

1. **Collecting indicative information on NA.** Gathering information on indicators and appropriate conditions for intrinsic degradation are often used at first instance and address the following parameters:

    – **Parent compounds and degradation products**. Concentrations of parent compounds and formed degradation products indicate the degree at which intrinsic dechlorination has occurred.

    – **Redox condition and electron acceptors**. The in situ redox condition and concentrations of chemical species corresponding to electron accepting processes

    – **The in situ electron donor activity.** (organic) electron donors are driving the reductive dechlorination of chlorinated solvents and other chlorinated compounds. Different types of organic carbon sources can serve as intrinsic electron donors, namely naturally present dissolved organic carbon (DOC) or co-contaminants such as BTEX, petroleum hydrocarbons, or other organics.

2. **Quantification of NA** This is determined by a documented loss of mass of contaminant over time and space in a contaminant plume.

This last phase is performed at each site at which monitored NA has been accepted as the most feasible pollution control approach. In many cases (new sites, complex situations) NA cannot yet be completely quantified, and one starts with phase 1). Sinke et al. (1998) have developed (and are further developing) a decision tool to proceed through these steps in an efficient way.

Where the assessment of the parent compounds and degradation products is relatively straightforward, the other two parameters often form a bottleneck, for which measurements of hydrogen concentrations in groundwater may offer a solution:

– **Redox condition and electron acceptors**. Redox conditions as measured with an electrode, have been proven to give often unreliable results in practice. Hydrogen measurements may provide a more reliable method and may be useful for a first on-site quick scan prior to a more detailed characterisation. Interpretation of groundwater chemistry is also hampered by uncertainties. Chemical species consumed and formed by electron accepting processes can disperse through the site after the reactions took place. As a consequence, interpretation in terms of electron accepting processes that occur at a specific monitoring point (and the related condition for intrinsic degradation) remains difficult. Hydrogen concentrations may give a better an more complete picture of the redox condition, of the potentially occurring electron accepting processes, and therefore also of the basic in-situ condition for intrinsic degradation of the organic pollutants present.

– **The in situ electron donor activity**. The amount of in situ electron donor which drive the reductive dechlorination of chlorinated compounds has been found to be important for intrinsic dechlorination. Different types of organic carbon sources can serve as intrinsic electron donors, namely naturally present DOC or co-contaminants as BTEX, petroleum hydrocarbons, or other organics. The in situ activity of these compounds and mixtures with respect to releasing reduction equivalents needed for dechlorination is difficult to assess. The hydrogen concentration in groundwater may be indicative for this activity, and may form a simple lump-sum parameter to qualify the intrinsic dechlorination condition.

Thus measuring hydrogen concentrations may provide a way to improve the assessment of the NA potential at contaminated sites. However, the theoretical fundaments, the practical possibilities and limitations, and the adequate tools for interpretation are either unclear or described fragmented in the international literature. The aims of this project were therefore the following:

– to systematically review the theoretical fundaments and assumptions underlying the role of hydrogen in in-situ redox and dechlorination processes
– to review and further develop the methods of measuring and interpreting hydrogen concentrations
– to test the usefulness of the hydrogen concentration as a redox characterisation and/or intrinsic dechlorination parameter at four different sites in the Netherlands
– to indicate the potential use of the hydrogen concentration as monitoring tool for NA and enhanced natural dechlorination

These aims are addressed in the various chapters of this report. The second chapter in this report gives a theoretical overview of the basic principles of redox reactions in groundwater systems, hydrogen production and consumption in anaerobic ecosystems and gives conceptual models that describe the reactions in soil and groundwater in which hydrogen takes part. The third chapter describes dechlorination processes and the role of hydrogen in it. Chapter four describes some different redox identification methods. Chapter five discusses different ways to use hydrogen as a method to evaluate dechlorination and in chapter six, a general scheme for
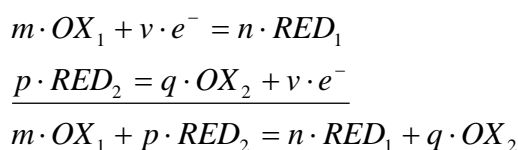
characterisation is explained. A description of the operation procedure for hydrogen measurements in the field is described in chapter seven. Including a step by step guideline and an overview of the equipment needed to measure hydrogen in the field. In chapter eight, nine, ten and eleven, four different case studies are reported according to the general characterisation scheme. In chapter twelve these results are compared to each other. Chapter thirteen gives conclusions about the hydrogen measurements in relation to redox characterisation and *in-situ* reductive dechlorination, including the outlook and recommendations for future application and optimisation.

## 2.	Hydrogen: redox and microbial interactions

### 2.1	Basic principles of redox reactions in groundwater systems

Organic matter in soils and aquifers is subjected to decomposition. Decomposition can be seen as a microbially mediated redox reaction. The organic matter is oxidised and donates electrons through various intermediary compounds to terminal electron acceptors, which subsequently become reduced. Redox reactions can be described as the combination of two reduction half-reactions.

$$m \cdot OX_1 + v \cdot e^- = n \cdot RED_1$$
$$\underline{p \cdot RED_2 = q \cdot OX_2 + v \cdot e^-}$$
$$m \cdot OX_1 + p \cdot RED_2 = n \cdot RED_1 + q \cdot OX_2$$

Where OX_1 is the terminal electron acceptor (oxidator) and RED_2 the electron donor (reductor), e^- is an electron and v, m, n, p and q are constants.

The terminal electron acceptors most often found in natural systems are oxygen, nitrate, Mn(IV), Fe(III), sulphate, and carbon dioxide (table 1). Organic compounds such as quinone moieties of humic acids or other organic acids (e.g acetate, fumarate, caffeate) are also known to be used as electron acceptor by anaerobic micro-organisms. Because the contribution of organic compounds to the electron-flow in anaerobic groundwater systems is still unkown their role will not be further considered in the present report. Among the inorganic electron acceptors there is a well-defined sequence which is related to the amount of energy which is gained from the redox reaction. Micro-organisms tend to oxidise organic matter by using the electron acceptor that provides the most energy [Stumm and Morgan, 1981]. Due to this sequence of terminal electron accepting processes, distinct zones may develop in the subsurface, marking the domination of particular electron acceptors. Under steady state conditions where the supply and demand of electron acceptors and donors is in balance, one microbially mediated redox reaction will predominate within a distinct zone [Ponnamperuma, 1972; Froelich et al., 1979; Reeburgh, 1983]. However, in many cases redox processes are not in steady-state and change within time and in space [Vroblesky and Chapelle, 1994]. Moreover, oftentimes several redox processes take place simultaneously.

Table 1   *Relevant half reactions for electron acceptor utilisation in natural systems.*
             This table is not a complete overview, but rather covers the most common
             reactions in natural systems. Half reactions are normalized to one electron
             (e⁻).

Half reactions		
$1/4\ O_2\ (g) + H^+ + e^-$	=	$1/2\ H_2O$
$1/5\ NO_3^- + 6/5\ H^+ + e^-$	=	$1/10\ N_2(g) + 3/5\ H_2O$
$1/2\ NO_3^- + H^+ + e^-$	=	$1/2\ NO_2^- + 1/2\ H_2O$
$1/8\ NO_3^- + 5/4\ H^+ + e^-$	=	$1/8\ NH_4^+ + 3/8\ H_2O$
$1/2\ MnO_2(s) + 2\ H^+ + e^-$	=	$1/2\ Mn^{2+} + H_2O$
$Fe(OH)_3(s) + 3H^+ + e^-$	=	$Fe^{2+} + 3\ H_2O$
$1/8\ SO_4^{2-} + 5/4\ H^+ + e^-$	=	$1/8\ H_2S(g) + 1/2\ H_2O$
$1/8\ SO_4^{2-} + 9/8\ H^+ + e^-$	=	$1/8\ HS^- + 1/2\ H_2O$
$1/8\ HCO_3^- + 9/8\ H^+ + e^-$	=	$1/8\ CH_4(g) + 3/8\ H_2O$
$1/8\ CO_2(g) + H^+ + e^-$	=	$1/8\ CH_4(g) + 1/4\ H_2O$
$H^+ + e^-$	=	$1/2\ H_2\ (g)$

2.2   Microbiological hydrogen production and consumption

2.2.1   The anaerobic food-web

The decomposition of organic matter in anaerobic ecosystems is brought about by a
complex microbial food web [Conrad 1989, Schink 1997]. The dominant primary
sources of organic material in most natural ecosystems include polysacharides (e.g.
cellulose, hemicellulose, pectin, chitin), lignin, fulvic and humic acids, protein, lip-
ids and nucleic acids, derived from plants and lower animals. These complex
polymers are broken down into simpler molecules in a stepwise process, mediated
by the concerted action of different specialised microbial species. The anaerobic
microbial communities can be arranged in a general scheme comprising (at least
four) specific "trophic" or "functional" groups (figure 1). The metabolism of these
groups is linked through excretion and consumption of degradation products. In a
first step the high-molecular organic compounds are depolymerised by hydrolytic
and fermenting bacteria yielding oligomers, e.g. peptides and disacchrides, and
monomers, e.g. sugars, amino acids, purine and pyrimidine bases, aromates and
long chain fatty acids. The oligo- and monomers can be utilised as direct growth
substrates by respiring bacteria which use nitrate, Fe^{3+}, Mn^{4+}, sulphate or CO_2 as
electron acceptor. Alternatively, the monomers may be fermented to short-chain
fatty acids, aromates, alcohols, CO_2 and hydrogen. The fatty acids, alcohols and
aromates are further oxidised to acetate and CO_2 by obligate proton reducing bacte-
ria which use H^+ as electron acceptor, forming H_2. Because this step is energeti-
cally unfavourable and - for thermodynamic reasons, see below - only proceeds at

low H_2 concentrations, the obligate proton reducing bacteria live in syntrophic association with H_2-consuming anaerobes. Thus the oxidation of the final products of the anaerobic food-chain requires the activity of anaerobic H_2-consuming bacteria, particularly nitrate-reducing, metal-reducing, sulphate-reducing, methanogenic or homo-acetogenic bacteria. These hydrogenotrophic micro-organisms use H_2 as an energy source and catalyse the transfer of electrons from hydrogen to one of the electron acceptors, nitrate, manganese(IV), iron(III), sulphate or bicarbonate (table 1), and maintain the H_2-concentration below the thermodynamic <u>maximum</u> permitted for the activity of the obligate proton reducing bacteria. The turnover of H_2 between hydrogen producers and consumers has been termed interspecies hydrogen transfer [Iannotti et al., 1973; Gottschalk, 1986].

Overall the process of organic matter mineralisation in anaerobic ecosystems thus yields CO_2, water and reduced electron acceptor molecules. Obviously, the specific pathways of carbon and electron flow through the food web, and the related activity of specific microbial communities depends on many factors, including :
–   The prevailing physical and chemical conditions (pH, temperature, groundwater flow, soil matrix structure etc.)
–   The quality of the organic matter. Aged organic matter usually consists of more refractory carbon, such as an increased amount of humic acids. Especially in deeper soils (older groundwater) this may be important because the reactivity (i.e. availability as electron donor for dechlorination) may be limited.
–   The (bio)availability of inorganic electron acceptors, which strongly controls the kinetics of the reduction processes in soil.
–   The presence of organic and inorganic contaminants. These may either act as electron donor or acceptor, or inhibit microbial activity due to toxicity effects.
–   The presence or absence of specific microbial species or communities with unique biodegradation capacities.
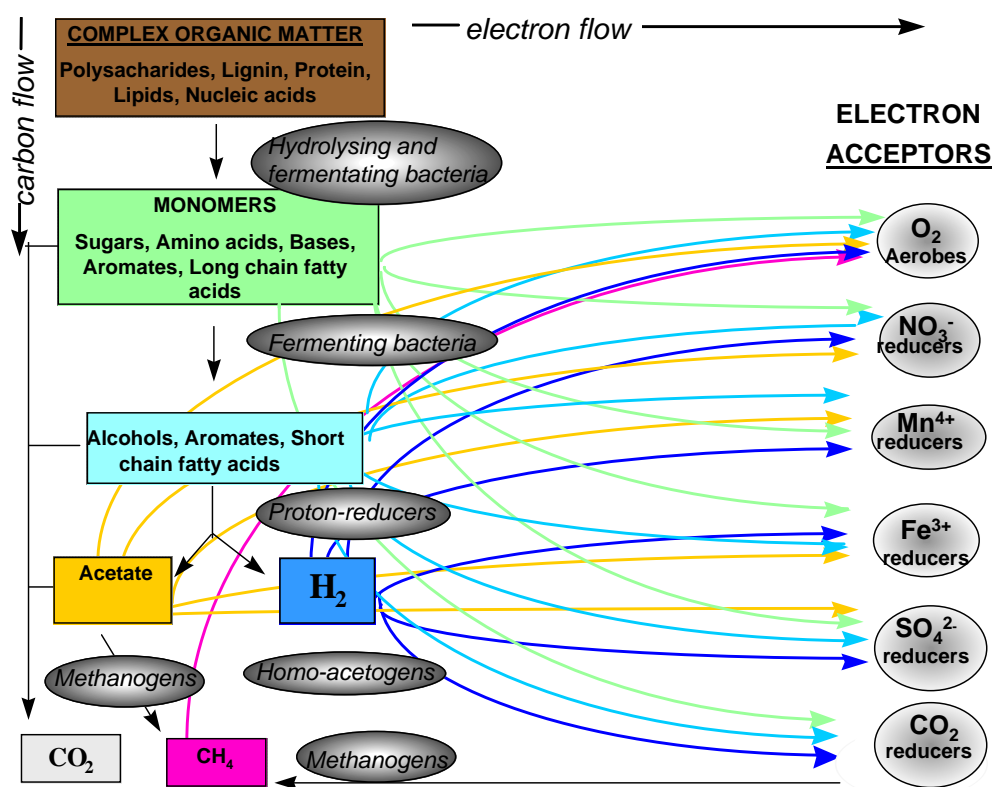
*Figure 1   Microbial food-web for degradation of organic matter, showing a carbon
                flow between the different trophic groups (vertically) and an electron flow
                from the trophic groups to an electron acceptor (horizontally)*

2.2.2   Control of H_2-concentration in groundwater

The concentration of molecular hydrogen in the groundwater reflects the flow of
carbon and electrons from electron donor substrates to electron acceptors through
the anaerobic microbial food-web. Hydrogen is continuously produced and con-
sumed during different stages of the decomposition of organic matter (figure 1).
The most important H_2-producing bacteria are the fermenters and the obligate pro-
ton reducers. Hydrogen production by the fermenting bacteria is relatively inde-
pendent on its prevailing concentration. This is caused by the fact that oxidation of
the substrates used by the fermenters (e.g. glucose, fructose, lactate) is thermody-
namically feasible, even at high pH_2. In contrast, oxidation of the substrates of the
obligate proton reducing bacteria (e.g. alanine, phenol, butyrate, benzoate, propi-
onate) is energetically unfavourable, particularly at high hydrogen concentrations.
Hence, different electron donor substrates each have certain "maximum threshold
H_2-concentrations" associated with their degradation. For example, the maximum
allowed H_2-concentration for oxidation of the following substrates decreases from
saturation to less than 0.1 nM in the order: glucose, fructose > lactate > methanol >
alanine > ethanol > phenol > glycolate > butyrate > benzoate > propionate > ace-
tate. Obviously, the specific maximum hydrogen concentrations depend also on the

prevalent *in situ* conditions - particularly the temperature and pH - surrounding the H_2-producing microbial communities [Schink, 1997].

The hydrogenotrophic bacteria use different terminal electron acceptors that all compete for hydrogen as an energy source for growth. Interestingly, these different H_2-oxidising populations have different affinities for H_2, and in addition, require distinct <u>minimum threshold H_2-concentrations</u> for growth. Generally, micro-organisms mediating an energetically favourable terminal electron accepting process are strong competitors for H_2, because they consume H_2 at high rates, have high growth yields, and are capable of growing at relatively low H_2-concentrations. Consequently they reduce the H_2-concentration to lower levels than the H_2-thresholds required for organisms catalysing energetically using less favourable electron acceptors [Lovley and Goodwin, 1988; Lovley et al., 1982]. Experimental evidence indicates that indeed micro-organisms mediating less favourable reactions are out-competed for H_2 by bacteria mediating energetically more favourable reactions [Conrad, 1996; Cord-Ruwisch et al., 1988; Schink, 1997]. A general order of ranking of bacteria with decreasing affinity for H_2 is as follows: aerobes, nitrate-reducers, manganese-reducers, iron-reducers, sulphate-reducers and methanogens (table 2).

Table 2   Some redox reactions mediated by hydrogen consuming bacteria.

Reaction		
$2H_2 + O_2$	=	$2H_2O$
$5H_2 + 2NO_3^- + 2H^+$	=	$N_2 + 6H_2O$
$H_2 + NO_3^-$	=	$NO_2^- + H_2O$
$4H_2 + NO_3^- + 2H^+$	=	$NH_4^+ + 3H_2O$
$H_2 + MnO_2 + 2H^+$	=	$Mn^{2+} + 2H_2O$
$H_2 + 2FeOOH + 4H^+$	=	$2Fe^{2+} + 4H_2O$
$4H_2 + SO_4^{2-} + H^+$	=	$HS^- + 4H_2O$
$4H_2 + HCO_3^- + H^+$	=	$CH_4 + 3H_2O$

As a result, the syntrophic degradation of organic substrates takes place within a specific range of H_2-concentrations. The upper and lower H_2-threshold concentrations allowing growth of a syntrophic consortium depend on the particular electron donor / electron acceptor substrate combination utilised by the bacteria (figure 2).
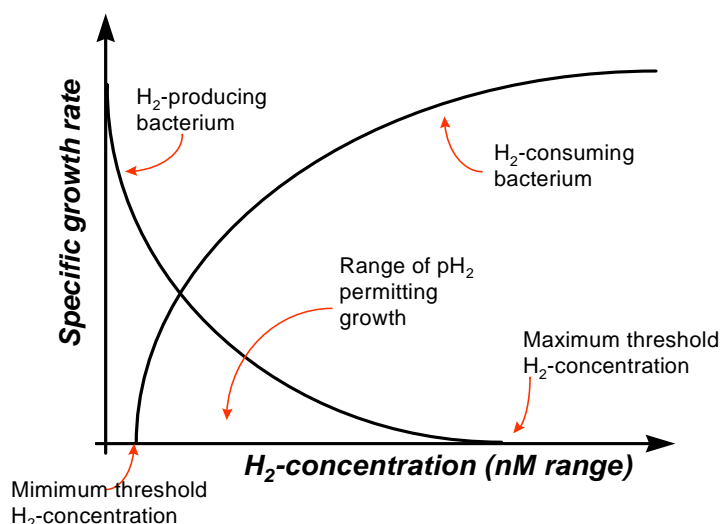
Figure 2   *Influence of the H_2-concentration on the specific growth rate of a H_2-producing and a H_2-consuming bacterium. Obviously, the precise shape and position of the curves depends on the growth properties of the bacteria and the electron donor and acceptor substrates utilised*

Altogether, the H_2-concentration in groundwater systems is controlled by both the kinetic properties of the H_2-producing and the H_2-consuming bacteria, as well as by the thermodynamic constraints of mediated redox reactions. The <u>kinetics</u> are especially important in <u>non-steady-state</u> situations. For example, upon a sudden input of electron donor substrates in an anaerobic system, the H_2 formation will increase up to the maximum rate catalysed by the enzymes of the hydrogen producing bacteria. In case electron acceptor availability is limited this increased H_2-production can not be counteracted by H_2-consumption and the dissolved H_2-concentration will rise. This increased H_2-concentration in its turn will restrain the H_2-production rate until a level is reached where both H_2-production and H_2-consumption are balanced again. <u>Thermodynamics</u> appear to control the hydrogen concentration in <u>steady-state</u> situations. In a groundwater system with a constant input of electron donor substrates the steady-state H_2-concentration is eventually reduced to the lowest possible level allowed by the inorganic electron acceptors available. Under electron donor and acceptor limiting conditions, redox reactions take place at a constant Gibbs energy [Hoehler et al., 1998; Jakobsen et al., 1998]. This Gibbs energy appears to be around the critical Gibbs energy that the micro-organisms need to live and to carry out the reaction. Below the critical Gibbs energy, redox reactions are energetically favourable, whereas above this threshold they are energetically unfavourable. More specifically, the threshold Gibbs energy corresponds to the minimum amount of energy which is required for bacteria to conserve the energy from a particular redox-reaction as ATP [e.g. Schulz and Conrad, 1996]. This threshold value is assumed to be similar for all of the terminal electron accepting processes because ATP-synthesis a the universal energy-conservation process for all bacteria. Nevertheless, the values of the threshold Gibbs energy for ATP-synthesis reported for various micro-organisms and situations range between -5 and -20 kJ/mol H_2: -9/-15 kJ/mol H_2 [Hoehler et al., 1994]; -7 kJ/mol H_2 [Jakobsen et al., 1998]; -14/-18

kJ/mol H_2 [Thauer and Morris, 1984]; -5.6/-8.6 kJ/mol H_2 [Schulz and Conrad, 1996]; -5.8 kJ/mol H_2 [Yang and McCarty, 1998].
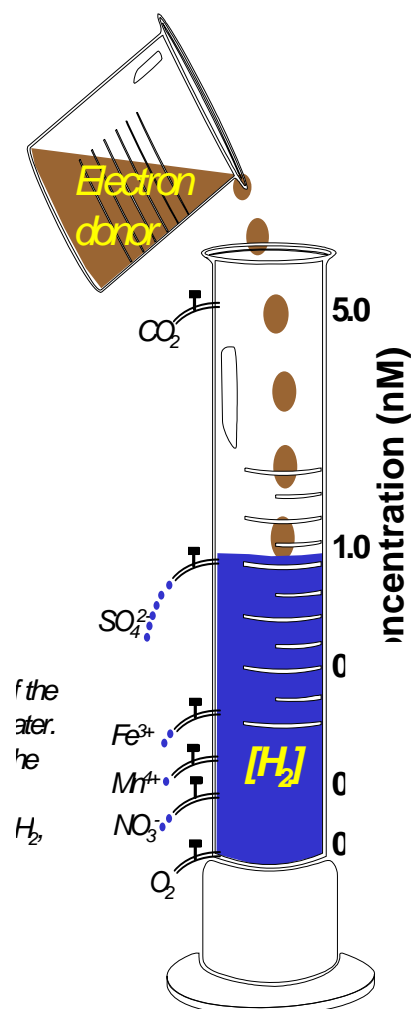
Figure 3   *Cartoon-like impression of processes affecting the H_2-concentration in groundwater. The level of H_2 depends on the supply of electron donor substrates and its conversion to H_2 and the H_2-flow to electron acceptors. In this particular example sulphate reducing conditions prevail, and dominate over iron reducing, manganese reducing, nitrate reducing and oxygen reducing conditions*

The control of the dissolved hydrogen concentration in groundwater systems is further schematically illustrated in figure 3. The level of H_2 in a microbial ecosystem (represented as the cylinder) is elevated by its production which is directly related to the supply- and oxidation-rate of electron donor substrates. The H_2-formation is counterbalanced by the flow of H_2 to the acceptor molecules, each with their specific minimum threshold concentrations (indicated by the height of the taps).

2.2.3 Conceptual model of H_2-production and H_2-consumption

The complex scheme of hydrogen production and consumption within the anaerobic food-web can be translated into a conceptual model (figure 4). The H_2-concentration is lifted by the activity of fermenting and proton-reducing bacteria (V_1 and V_2). The H_2-concentration is pulled down (V_4) by the activity of hydrogen consumers among which are the dechlorinating micro-organisms. The net H_2-level in the groundwater is the resultant of hydrogen production and consumption rates between thermodynamically imposed maximum and minimum concentrations (steady-state hydrogen threshold). Consumption and production rates and threshold values vary among the numerous micro-organisms involved. The availability of inorganic electron acceptors for H_2-oxidation is additionally determined by their reduction mediated by bacteria using other electron donors such as organic matter or its degradation products (V_3, V_5 and V_6).
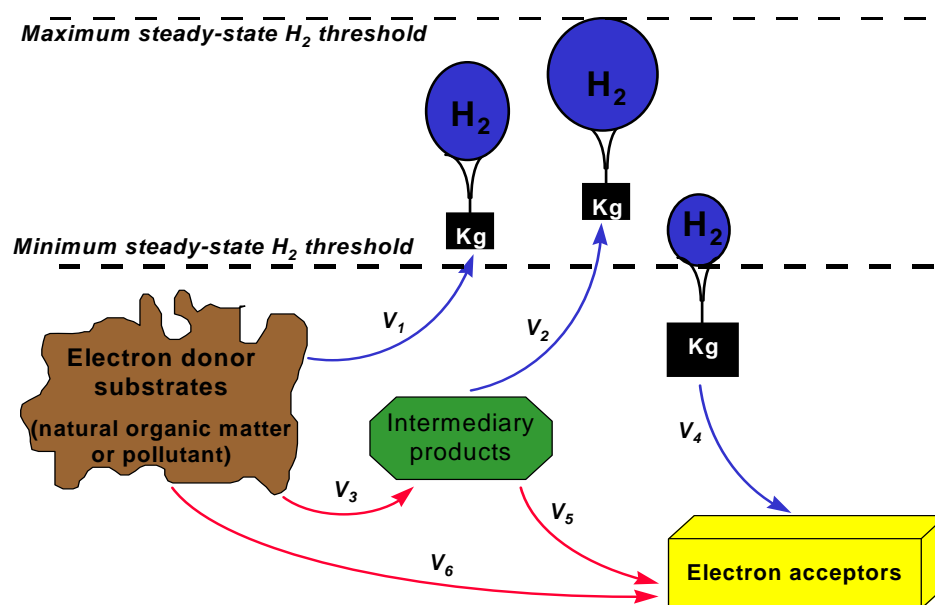

Figure 4 *Conceptual model of H_2-production and H_2-consumption in anaerobic groundwater systems. The arrows indicate the flow of electrons*

The conclusion is, that in a natural groundwater system a multitude of processes, micro-organisms, and thermodynamic constraints determine the hydrogen concentration. No model can be expected to give a complete deterministic description of all these phenomena. Hence, a more empirical evaluation of these relations between hydrogen and electron transfer processes is warranted.

3. Hydrogen: Dechlorination processes

3.1 Biotransformation of organic pollutants

Biodegradation by bacteria is the most important mechanism for the removal of organic pollutants from groundwater systems. Micro-organisms catalyse the transformation of pollutants through two principally different mechanisms: metabolism and co-metabolism [Alexander, 1985, Gerritse et al. 1995, Gerritse et al. 1997, Rijnaarts et al, 1998]. During metabolic biodegradation the organic pollutants are used by the micro-organisms for the generation of energy and/or biomass (figure 5). In contrast, co-metabolic transformations are "by-reactions" of enzymes which are actually intended for microbial growth on natural substrates.
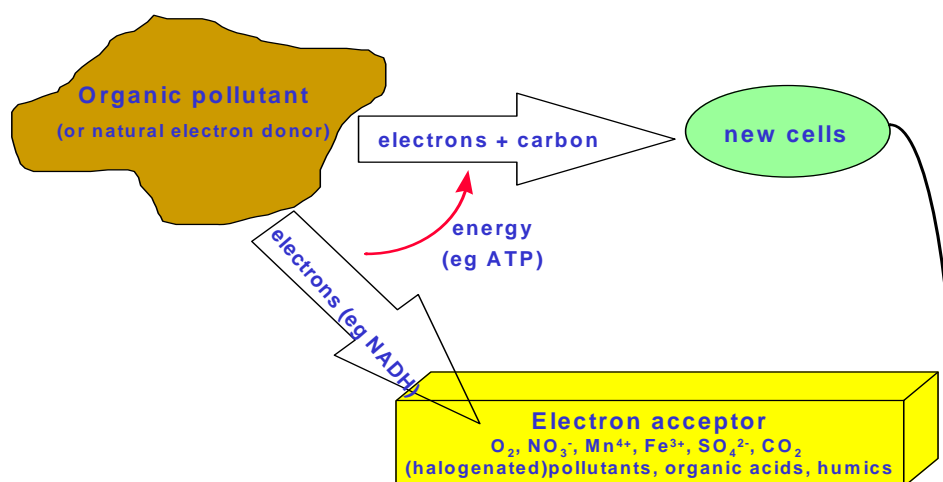
Figure 5   Metabolic degradation of organic pollutants
(after Rittmann et al., 1993)

The organic compounds can be degraded either as an electron donor or as an electron acceptor.

In case the organic pollutant is used as an <u>electron donor</u>, it is oxidised, e.g. to CO_2 or acetate. The discharged electrons are either excreted as H_2, and/or organic fermentation products, or transferred within the organism (e.g. as NAD(P)H or FADH) to an electron acceptor (e.g. O_2, NO_3^-, Fe^{3+}, Mn^{4+}, SO_4^{2-}, or CO_2). Examples of pollutants that can be oxidatively degraded (can function as electron donor) are: BTEX-aromatics, mineral oil constituents, poly-aromatic hydrocarbons (PAHs) some low halogenated aliphatics (e.g. dichloromethane, 1,2-dichloroethene, vinylchloride) and aromatics (e.g chlorobenzene, monochloro- and monofluorobenzoates).

Other pollutants like chlorinated organic compounds can be used as <u>electron acceptors</u>. This reductive dechlorination process is called "halorespiration" in case the

microbes involved conserve the energy released via electron transport and synthesis of ATP (oxidative phosphorylation). To fuel reductive dechlorination the micro-organisms require an electron donor substrate (figure 6). The electron donors known to be used by halorespiring bacteria are molecular hydrogen, short chain fatty acids (e.g. acetate, lactate, butyrate), ethanol, amino acids (e.g serine), or reduced inorganic compounds such as sulphide.
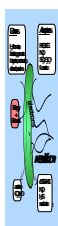

Figure 6   Use of electron donors and acceptors by dehalorespiring Desulfitobacterium species

The presence and availability of the various electron acceptors strongly controls the structure and the activity of the local microbial communities and related pathways, rates and extents of pollutant biodegradation . Generally, in an "oxidised environment" (e.g. aerobic or denitrifying conditions) oxidative degradation of BTEX, oil, PAHs and mono-chlorinated compounds proceeds readily, whereas reductive transformations are relatively slow and incomplete. The opposite is true for a strongly "reduced environment" (e.g. sulphate-reducing or methanogenic conditions) favouring reductive dechlorination of PCE, TCE, and other highly chlorinated pollutants (PCB'S, Chlorobenzenes, HCH, DDT, Dioxins) and restraining oxidative biodegradation. In general, for organic compounds that contain halogen-, sulphoxy-, nitro- or azo-groups, the biotransformation characteristic changes with decreasing substitution number, from "preferentially degraded through reduction" (many groups per molecule) to "preferentially degraded through oxidation" (one or few groups per molecule).

3.2   Reductive dechlorination of chloroethenes

Reductive dechlorination is the most important mechanism for the transformation of a wide spectrum of chlorinated aromatic and aliphatic pollutants. In fact, for poly-chlorinated compounds as tetrachloroethene (PCE), tetrachloromethane, hexachloroethane, hexachlorobenzene and PCBs containing more that six chlorines it is the only biotransformation mechanism known [Mohn and Tiedje 1992, Rijnaarts et al. 1998]. This process occurs under anaerobic conditions and proceeds faster and more complete when the environment becomes more reduced. Reductive dechlorination of PCE involves the stepwise replacement of chloride atoms for protons (figure 7). Thus PCE is subsequently reduced to trichloroethene

(TCE), cis-1,2-dichloroethene (DCE), vinylchloride (VC), and ethene. [Vogel, 1985; Bruin, 1992; DiStefano, 1991; Freedman, 1989].
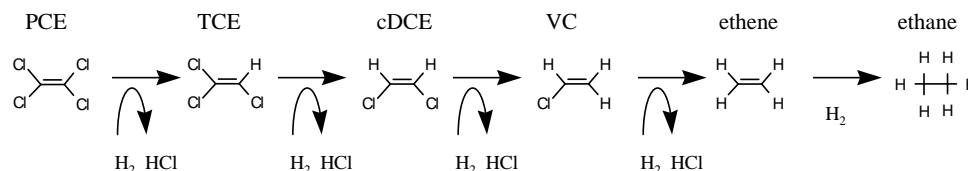

Figure 7   Reductive dechlorination of chloroethenes with H_2 as electron donor

Anaerobic bacteria that use PCE or TCE as terminal electron acceptor have recently been isolated and include: *Dehalospirillum multivorans*, *Dehalobacter restrictus*, *Desulfitobacterium* species, *Dehalococcus ethenogenes* and *Desulfuromonas chloroethenica* [Gerritse et al. 1996, Gerritse et al. Submitted]. These halorespiring bacteria were capable of dechlorination-dependent growth at relatively high rates and indicate the great microbial potential for natural and enhanced remediation of chloroethene-contaminated aquifers [Gerritse et al, 1998]. In reduced (sulfido- or methanogenic) environments reductive dechlorination of PCE and TCE usually proceeds at relatively high rates and has been demonstrated at lower rates under nitrate and iron reducing conditions During reductive dechlorination of PCE and TCE the lower chlorinated ethenes, particularly DCE and VC, often accumulate. Apparently, their reduction is often more restrained. Several explanations have been proposed for this phenomenon: i) reductive dechlorination of DCE and VC is a co-metabolic process, not providing an energy and electron source required for the development of a VC/DCE degrading microbial community; ii) dechlorinating enzymes have less competitive kinetics for DCE/VC than for PCE/TCE; and iii) PCE/TCE dechlorination is thermodynamically more favourable than DCE/VC dechlorination. So, in general, the rate of reductive dechlorination decreases as the degree of chlorination decreases [Vogel, 1985; Wiedemeier, 1997].

For reductive dechlorination of chloroethenes, an appropriate carbon source is required as electron donor. This can be either natural organic matter or an organic contaminant like most fuel hydrocarbons [Wiedemeier, 1997; Rijnaarts et al. 1998, Middeldorp et al. 1998]. During the anaerobic degradation of these organic compounds, products are formed such as molecular hydrogen, short chain fatty acids (e.g. formate, acetate, lactate, butyrate), ethanol, amino acids, or reduced inorganic compounds such as sulphide. These intermediates are the actual fuel for the bacteria that are responsible for the dechlorination process [Gerritse et al. 1996, Gerritse, submitted]. Molecular hydrogen is an important but not the sole electron donor in reductive dechlorination.

Recent experiments with ^{14}C-labelled DCE and VC indicated that these lower chlorinated ethenes may also be mineralised through anaerobic oxidative pathways occurring under iron- and manganese-reducing conditions [Bradly and Chapelle

1996, Bradly and Chapelle 1997, Bradly and Chapelle 1998[a], Bradly and Chapelle 1998[b]].

3.3   The importance of hydrogen for reductive dechlorination

Molecular hydrogen (H_2) plays a key role during the reductive dechlorination process. In this chapter various mechanisms in which hydrogen interacts with dechlorination of chloroethenes are considered.

Hydrogen may be used as a direct electron donor by anaerobic dechlorinating micro-organisms [Distefano, 1992; Fennell, 1997; Maymo-Gatell, 1995; Smatlak, 1996] (figure 6 & 7, table 3). Indeed, several anaerobic bacteria, *Dehalospirillum multivorans*, *Dehalobacter restrictus*, *Desulfitobacterium* species strain TCE1, have been isolated which are able to respire with H_2 as energy source using PCE or TCE as terminal electron acceptor [Gerritse et al., submitted; Holliger et al., 1994; Schulz-Muramatsu et al., 1995]. The availability of H_2 therefore directly influences the kinetics of such reductive dehalogenation. However, it should not be overlooked that reductive dehalogention can also be fueled by other electron donors, and even PCE-dechlorinating bacteria, e.g. *Desulfuromonas chloroethenica* or *Desulfitobacterium* species strain PCE1, are known that are <u>not</u> capable of using H_2 at all [Gerritse et al., 1996].

Table 3   Dechlorination reactions with hydrogen

Reaction		
PCE + H_2	=	TCE + Cl^- + H^+
TCE + H_2	=	DCE + Cl^- + H^+
DCE + H_2	=	VC + Cl^- + H^+
VC + H_2	=	ethene + Cl^- + H^+
ethene + H_2	=	ethane

The presence of natural electron acceptors often inhibit the dechlorination process. Particularly O_2, NO_3^- and Mn^{4+} are known to inhibit dechlorination of chloroethenes. Some studies indicated that PCE dechlorination was retarded and less complete in the presence of iron-oxides or sulphate. However, other investigations do not confirm these observations. Although the influence of electron donors and acceptors on the reductive dechlorination process is complex and not well understood, some mechanisms involved have been identified:
1. Competition for electron donors between dechlorinating and not-dechlorinating bacteria (see below).
2. Reduction of alternative electron acceptors with H_2 as donor. In this way H_2 reduces the redox potential of the environment, and optimises the conditions for reductive dechlorination.
3. Preference of halorespiring bacteria for natural instead of chlorinated electron acceptors. When alternative electron acceptors are available, halorespiring bac-

teria can shift the electron-flow from PCE or TCE to for example nitrate or sulfoxy-anions (figure 6 [Gerritse et al. 1996; Gerritse et al. 1997; Gerritse et al. Submitted]).

In soil and groundwater systems there is a strong competition for electron donor substrates between dechlorinators and <u>non</u>-dechlorinating bacteria that use natural acceptors, especially O_2, NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-}, or CO_2. Particularly use of O_2, NO_3^- and Mn^{4+} as electron acceptor is energetically favourable, and the bacteria respiring with these electron acceptors have a high affinity for electron donors (including H_2) and high growth yield. They appear capable of out-competing other anaerobic including dechlorinating bacteria.

An additional important factor controlling the competition for hydrogen between different terminal electron acceptors utilising bacteria was shown to be the "threshold" for hydrogen uptake (see chapter 2). Bacteria using nitrate, manganese or iron in the terminal electron accepting process can grow at very low hydrogen concentrations (<0.2 nM). Such concentrations are too low for the growth of bacteria mediating less favourable terminal electron accepting processes as sulphate- or carbon-dioxide reduction which require at least 1 nM H_2.

From a thermodynamic point of view, reductive dechlorination is a very favourable process. The Gibbs energies that are involved in dechlorination reactions remain negative even at extremely low hydrogen concentrations (<0.01 nM). This suggests that dechlorinators should in theory be able to compete successfully with methanogens, sulphate-, iron-, manganese- and nitrate-reducing bacteria. Indeed, laboratory studies indicated that dechlorinators can use hydrogen at lower levels than methanogens [Smatlak, 1996; Fennell, 1997; Yang, 1998]. In the presence of chloroethenes the dechlorinators maintained steady-state hydrogen levels around 2 nM and out-competed the methanogens. In methanogenic batch cultures the steady-state hydrogen concentration was significantly higher, around 11 nM, which is in line with other studies. This lower threshold H_2 value of 2 nM observed for dechlorination is in the range of the minimum hydrogen concentration required by sulphate- or iron-reducing bacteria (figure 9, chapter 4). These threshold hydrogen concentrations appeared independent on the type of electron donor used [Yang, 1998].

Obviously the precise value of these threshold H_2-concentrations depends on environmental factors as pH and temperature. Nevertheless, H_2-threshold concentrations observed for dechlorination are considerably higher than expected on the basis of thermodynamic calculations which suggest a similar value as for nitrate reduction i.e. < 0.05 nM. This discrepancy may be explained by the fact that dechlorinators are not as efficient at capturing the energy available from hydrogen oxidation as bacteria mediating "natural" redox reactions and operate at very low Gibbs-energies [Yang, 1998; Smatlak, 1995]. In contrast, thermodynamic calculations indicate that the available energy for methanogenesis that is taking place at the

steady-state (critical) hydrogen concentration is close to the reported threshold Gibbs energy for ATP production.

This is significant because competition effects among different terminal electron accepting processes appear of particular importance for the extent to which dechlorination of PCE takes place. The observation that DCE and VC often appear the bottleneck in the complete reductive dechlorination process can be understood in case PCE- and TCE-dechlorinating bacteria would have a considerably lower threshold for H_2 then DCE and VC dechlorinators. The fact that bacteria respiring with H_2 as electron donor and PCE and TCE as acceptor are frequently isolated, whereas anaerobes capable of respiring with and DCE or VC have not been described seems to approve this possibility. However, detailed research on the H_2-levels during dechlorination of the different chloroethenes is needed to confirm this hypothesis.

Various experiments indicate that reductive dechlorination of chloroethenes is restrained not only at very low but also at relatively high H_2-concentrations. It appears that in cultures in which H_2 levels are relatively high, most of the H_2 is consumed by methanogenic bacteria. This may be explained by competitive exclusion of dechlorinators by methanogens at high hydrogen concentrations. Such a mechanism is likely in case the methanogens would have significantly higher H_2 uptake-capacity or growth-rate on H_2. An alternative explanation may be the direct inhibition of the dehalogenating activity of dechlorinators at high H_2-concentrations. Indeed, such inhibition was observed for 3-chlorobenzoate dehalogenation by *Desulfomonile tiedjei* and in enrichment cultures containing a PCE dechlorinating *Desulfitobacterium* species [Gerritse et al., 1996]. Although the reasons for the apparent selective advantage of methanogens over dechlorinators at high hydrogen concentrations are still unclear, the general picture that has emerged from different studies is that the competitive strength of the dechlorinators resides within their capacity to capture H_2 at lower levels than the methanogens. Therefore, the competition between methanogens and dechlorinators can be controlled by the type supply of electron donor. Based on thermodynamic considerations a selection of primary electron donors can be made beforehand. Different electron donor substrates generate different hydrogen concentrations (see chapter 2). Substrates which have a positive Gibbs energy at high hydrogen concentrations for fermentation are probably optimal primary electron donors to stimulate dechlorination. These substrates are unable to produce high concentrations of hydrogen and will be a long term source of hydrogen. For example, the oxidation of propionate or butyrate to acetate, CO_2 and H_2 by obligate proton reducing bacteria is only energetically favourable at H_2 concentrations just below or close to the threshold for methanogens (<1 nM). Therefore, methanogens can not compete successfully with dechlorinators for the low levels of H_2 generated from propionate or butyrate oxidation. In contrast, substrates as glucose, lactate, benzoate, methanol and ethanol can be fermented at H_2-concentrations well above the levels required for optimal growth of methanogens and the competitive advantage of dechlorinators falls away. Low levels of H_2 can also be created by limiting the supply-rate of electron donor substrates. This can for

example be accomplished by slow release of electron donors for example in con-
tinuous flow systems (soil columns, chemostats, infiltration wells in aquifers) or by
addition of complex or relatively "inert", slowly degradable, compounds such as
poly-lactate esters (hydrogen release compound, HRC), paper- or wood chops,
compost extract, or natural organic matter which deliver hydrogen over long peri-
ods of time [Middeldorp et al. 1998, Wu et al. 1998].

4.   Redox identification methods

4.1   Traditional methods to identify redox conditions

Several attempts have been made to develop a method to determine which redox conditions take place at a given location. The most direct way is to measure the rates of the redox reactions. Since these are very slow and difficult to measure (all phases must be considered), this option is not applicable in practice. Alternatively, redox potential measurements and macro chemical methods are currently used to indicate redox conditions.

4.1.1   Redox potential (Eh)

Redox potential measurements are done with an electrode (e.g. Pt-electrode) against a standard electrode of known potential. Redox potentials are measured in Volts and are directly related to the free electron concentrations. It is assumed that each terminal electron accepting process has a characteristic redox potential range. However, this method is generally found to be of limited value [Lindberg and Runnells, 1984; Berner, 1981; Stumm and Morgan, 1981]: electrodes do not respond to many of the important redox couples, redox couples are not in thermodynamic equilibrium, and the formation of oxide precipitates on the surface of the electrode leads to analytical problems. Consequently, different redox potential ranges associated with redox processes have been reported in several literature sources (summarized by: Lovley and Goodwin, 1988).

4.1.2   Macro chemistry

The term macro chemistry will be used to indicate a redox identification method that is based on the presence of and changes in the concentrations of compounds that participate in natural redox reactions. These compounds will further on be called redox species. Redox species can either be electron acceptors (O_2, NO_3, Mn(IV), Fe (III), SO_4, HCO_3) or end products that are formed by transformation of electron acceptors in redox reactions (NO_2, N_2, NH_4, Mn(II), Fe(II), HS, H_2S, CH_4). In principle, each redox specie that is involved in redox processes can be used, but their performance as indicator is strongly variable. Some redox processes are more suitable under certain conditions than others, but in fact each redox specie has its own specific difficulties which must be taken into account when making interpretations. Redox species may be transported, produced in other processes (e.g. methane), precipitated (i.e. divalent iron and sulphides), and can have analytical problems (e.g. oxygen and iron(hydr)oxides). As a result of these complications the redox species give an indirect image of the redox processes that actually take place.

Redox identification protocols based on macro chemistry have been developed by Berner (1981), Lyngkilde et al. (1991) (figure 8), and for the Dutch situation by Sinke et al. (1998).These protocols are ment to be general, but are not applicable for all locations. At some locations strongly deviating conditions, processes and background concentrations exist, for which the criteria that form the basis of the redox identification protocols cannot be used. In such a case, one has to deduce the criteria from the background concentrations at and around that site, a rather arbitrary method.
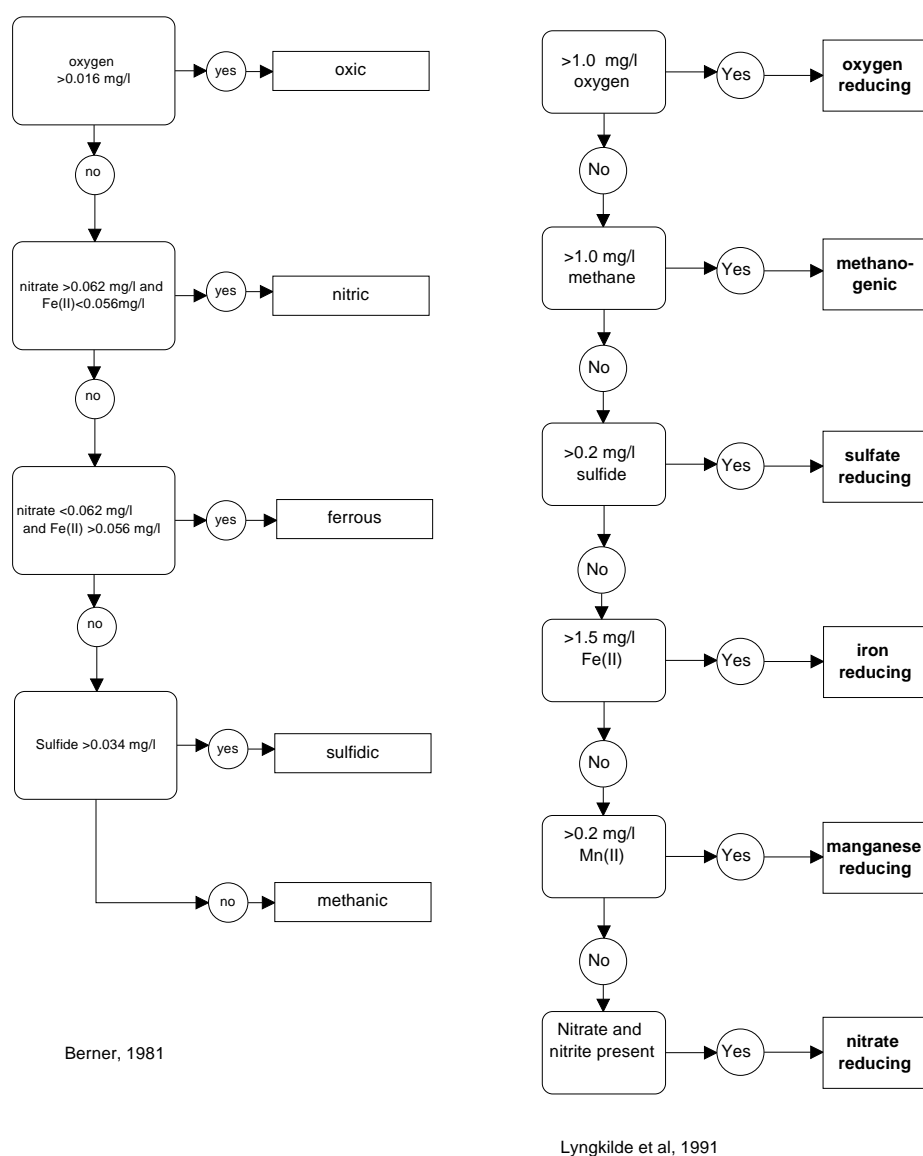
Figure 8   Two different types of redox classifications

4.2   Methods based on hydrogen

There are two ways in which hydrogen can be used to identify redox conditions: Comparing measured hydrogen concentrations with empirical ranges, and the partial equilibrium approach.

4.2.1   Comparison with empirical ranges

In many experiments hydrogen concentrations have been measured under (artificially produced) conditions that where demonstrated to be either nitrate, manganese, iron(III), or sulphate reducing, or methanogenic. It appears that each redox reaction has its own specific hydrogen range, which is severely different from the hydrogen range of the other redox reactions. Therefore H_2 concentrations can be an indicator of the terminal electron accepting process that predominates in a given zone (Lovley and Goodwin, 1988). H_2 is a continuously cycled intermediate product with a half-life in the order of seconds, and therefore reflects nearly instantaneous conditions at a particular well. A single analysis can, under steady-state conditions, be diagnostic of the predominant terminal electron accepting process and concentration changes along a flowpath are not required to be diagnostic of terminal electron accepting processes. Figure 9 summarises steady state concentrations corresponding to the terminal electron accepting processes at a standard temperature of 25^oC. Although, the values are empirically determined by different authors and under different conditions, they show to be rather consistent. Steady-state hydrogen concentrations for nitrate and manganese reducing and dechlorination reactions are less studied and therefore scarce.

Steady-state hydrogen range
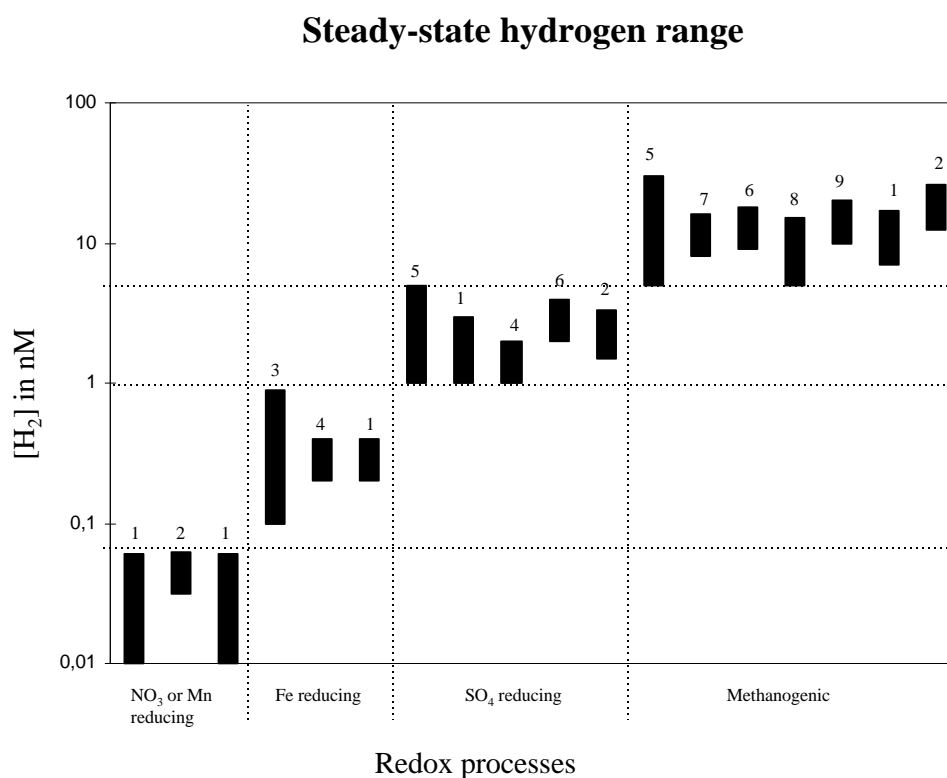
*Figure 9   Overview of ranges of steady-state H_2 concentrations (at the standard tem-
            perature of 25^oC) associated to the different redox processes. The ranges are
            based on the following literature: (1)Lovley and Goodwin, 1988; (2)Hoehler
            et al., 1998; (3)Chapelle and Lovley, 1992; (4)Lovley and Philips, 1987;
            (5)Vrobleski and Chapelle, 1994; (6)Lovley et al., 1982; (7)Lovley and
            Klung, 1982; (8)Goodwin et al., 1988; (9)Conrad et al., 1987.*

Several studies have determined the effect of temperature on the steady state hy-
drogen ranges (Hoehler et al., 1998; Jakobsen et al., 1998; Schulz and Conrad,
1996; Westerman, 1994). They all concluded that the hydrogen level is not just a
function of the given redox process, but is strongly affected by the in situ tempera-
ture. A decrease in temperature leads to a decrease in the steady state hydrogen
concentration. Therefore the hydrogen ranges in figure 9 are not necessarily appli-
cable to situations in which the temperature is significantly different from the stan-
dard temperature. A temperature between 10 and 15^oC is more common in temper-
ate regions like The Netherlands. Table 4 indicates the hydrogen ranges that are as-
sociated with three redox processes for temperate regions. The ranges are based on
data from experiments carried out in this temperature range.

Table 4   *Criteria for determining redox processes by the empirical hydrogen method.*
These criteria are applicable in temperate regions where the groundwater is
approximately 10 to 15°C. These ranges are based on steady-state hydrogen
concentrations that have empirically been determined in experiments indi-
cated by the references.

Redox condition	[H_2] (nM) at 10 to 15°C	Based on
Iron reducing	[H_2] ≤ 0.3	Jakobsen et al. (1998)
Sulphate reducing	0.3 < [H_2] < 1.5	Hoehler et al. (1998), Jakobsen et al. (1998)
Methanogenic	[H_2] ≥ 1.5	Hoehler et al. (1998), Westerman (1994) and Jakobsen et al. (1998)

In non-steady state environments empirical hydrogen concentrations can not be in-
terpreted unambiguously. Terminal electron accepting processes do not always oc-
cur in well separated zones, but to some extent they occur simultaneously, indicat-
ing that competitive exclusion is not always efficient. Terminal electron accepting
processes can co-exist when the more energetically favourable reaction is limited
by electron acceptor availability. Under these conditions, the more favourable reac-
tion can no longer maintain the hydrogen concentration below the minimum
threshold necessary for hydrogen metabolism by the less favourable reaction. Ad-
ditionally, a temporary increase in the rate of hydrogen production can disturb the
steady state situation, and might lead to a misinterpretation of the hydrogen con-
centrations. Furthermore, microzones can form another potential situation where
there could be more than one electron acceptor for organic matter oxidation within
a readily sampled interval. In these microzones different terminal electron accept-
ing processes occur compared to the bulk phase (Lovley and Philips, 1987) (Lovley
and Goodwin, 1988). Thus, the existing approach to using empirical hydrogen
ranges is only valid if the system studied is in a steady state, a prerequisite that is
very difficult to be certain of.

4.2.2   The partial equilibrium approach

The partial equilibrium approach, which was introduced by (Postma and Jakobsen,
1996) and (Jakobsen et al., 1998) implies that fermentative hydrogen production is
overall rate limiting, while terminal electron accepting processes occur at negative
Gibbs energies, close to thermodynamic equilibrium. Measured concentrations of
hydrogen and redox species may be combined, through simple thermodynamic cal-
culations (see appendix A) into an actual potential in situ energy yield, representing
the potential for a given hydrogen oxidising terminal electron accepting process.
This approach is able to indicate the occurrence of concomitant terminal electron
accepting processes and to evaluate where (in the leachate plume) the different
terminal electron accepting processes can take place.

In practical terms, for each separate sampling point Gibbs energies are calculated for all relevant redox processes. The calculated Gibbs energies are subsequently used to delineate zones in which the Gibbs energy is above or below the necessary threshold (see section 2.2.2). Below this threshold (i.e. more negative) the reaction is energetically favourable and is likely to occur. Above the threshold the reaction is less favourable, but is not totally excluded to proceed. The process can still occur in stagnant subdomains if the hydrogen concentration in these is higher due to fermentation of organic matter or if hydrogen is directly transferred from fermenting to hydrogen consuming bacteria (Conrad et al., 1985). Since different values have been reported for this threshold Gibbs, no single value can be considered to be the true threshold Gibbs energy (section 2.2.2). Therefore it is recomended to perform a low and/or a high case scenario.

A precondition to perform this approach is the determination of the appropriate parameters. Depending on the redox reactions of interest, several redox species (both electron acceptors and final products, and of course hydrogen) must be measured in order to calculate the Gibbs energy of those reactions (appendix A). Apart from the concentrations of redox species also the temperature and pH are important parameters that must be measured.

In case of the iron(III) and manganese(IV) reducing processes, not only the concentrations of species in solution are a determining factor, but also the ones that are present as a solid phase (electron acceptors). A variety of different minerals exist in soils, each having a characteristic reactivity. The energy yield of the reaction strongly depends on the reactivity of the particular minerals that are present. The reactivity, in turn, is roughly paralleled by the stability of the minerals (Postma and Jakobsen, 1996). Thus the stability of the minerals present determines whether iron (III) and manganese (IV) reduction are energetically favourable and likely to take place (Postma and Jakobsen, 1996).

Trivalent iron is found in several minerals, but only a few are assumed to be present in soils. Most common are ironhydroxides: α-FeOOH (goethite) and amorphous FeOOH. In some regions (e.g. The Netherlands), lepidocrocite is also present. These minerals are relatively reactive, and will be reduced first. The more stable (hydr)oxides are less reactive and yield less energy upon reduction and will be reduced in a further stage of reduction. Fe mineralogy seems to play an important role and should therefore be determined at each site in order to use the partial equilibrium approach optimally. However, this is not always achievable, and therefore best and worse case estimations can be made by assuming both stable and less stable minerals.

5.   Methods that use hydrogen to evaluate dechlorination

The ultimate goal in this and many other projects is to obtain a tool to evaluate and to predict dechlorination processes. Here, two different methods that are based on hydrogen are discussed. The first is based on thermodynamic calculations of dechlorination reactions and is a variation on the partial equilibrium approach for redox reactions. The second method is making use of steady-state hydrogen concentrations to judge if dechlorination reactions are in a favourable range. Research on both methods is still in a very preliminary stage, but in this project a first start is made.

5.1   Thermodynamic calculations for dechlorination

Similar to the partial equilibrium approach for natural redox reactions (section 4.2.2), thermodynamic calculations can be made to evaluate dechlorination processes. On basis of thermodynamic calculations which have been done in previous research, dechlorination reactions are considered to have very low Gibbs energies under all natural conditions. This implies that these reactions are always favourable under these conditions, when assuming that the same threshold Gibbs energy for ATP synthesis applies as for natural redox reactions (chapter 2). However, indications have been found by Yang and McCarty (1998) that dechlorination reactions would be less efficient than normal redox reactions. This suggests that the threshold Gibbs energy for dechlorination might be lower than for natural redox reactions. Instead of a value between -5 and -20 kJ/mol, a value which is much lower might be used as threshold Gibbs energy. Its exact value, though, has not been studied sufficiently and could therefore not provide a basis for this method. For this moment only thermodynamic calculations will be made, without comparing Gibbs energies to threshold values.

5.2   Steady-state hydrogen range for dechlorination

Dechlorination reactions that use hydrogen as electron donor operate optimally at a certain range of hydrogen concentrations. When hydrogen concentrations become too low, the process will be unfavourable; if they are too high, competition with natural redox processes might occur. Like natural redox processes, dechlorination processes are also expected to be able to maintain hydrogen at a steady-state hydrogen concentration which is in this favourable range. Until now, only little research has taken place on the values of this range. Data published by Yang and McCarty (1998) identify a favourable range between 2 nM and 11 nM.
In order to obtain a method to evaluate dechlorination, measured hydrogen concentrations that fall within this range will be considered favourable for dechlorination processes. In this project, this range will be further validated by comparing measured hydrogen concentrations and observed dechlorination in the field.

6.   General characterisation scheme

The use of hydrogen as tool for the characterisation of redox conditions and its relevance for dechlorination processes is being determined at four different locations. These locations are: Rademarkt (Groningen), DAF (Eindhoven), SBNS (Tilburg) and Banisveld (Boxtel).

Since these four locations are all different types of locations as far as (geo)hydrology, the contaminated situation, and the conditions that exist are concerned, they will be discussed individually in separate chapters. In each chapter the location will be described first. Secondly, redox conditions will be characterized in different ways including traditional methods and methods based on hydrogen. Criteria that form the basis of these methods do not necessarily have to be the same and applicable for all possible locations, but can also be formulated for one particular type of location or in a site-specific way.

The redox potential will not be used as a separate tool to identify the exact redox reactions, but will be used in a comparative way to locate redox trends and to verify the results of the redox characterisation of the other methods.

For the macro chemical method, the criteria that are formulated depend on the type of location, which represents distinct background conditions and processes. In a previous NOBIS report (Sinke et al., 1998) macro chemical criteria have been formulated for the determination of the redox conditions into three distinct groups: aerobic, anaerobic and strongly anaerobic. These criteria are applicable for most types of locations in The Netherlands. In this report, the criteria for the determination of the redox conditions at the Rademarkt and SBNS site will be based on the macro chemical criteria formulated for these typical locations, however, more specific redox conditions are considerd (figure 10). The criteria are based on the concentrations of a limited number of common redox species. Significant methane concentrations indicate that methane is produced during methanogenesis. Sulphate reduction generally occurs after iron reduction has been taken place, leading to increased divalent iron concentrations. Therefore, decreased sulphate concentrations in combination with increased divalent iron concentrations indicate sulphate reduction. The presence of oxygen and nitrate indicate oxygen and nitrate reduction, respectively. Iron reducing conditions apply to the cases that do not fit any of the criteria mentioned above.

CH$_4$ > 1 mg/l ↓	→yes:	methanogenic
SO$_4$ < 1.5 mg/l Fe(II) > 1.5 mg/l ↓	→yes:	sulphate reducing
O$_2$ > 1 mg/l ↓	→yes:	oxygen reducing
NO$_3$ > 0.5 mg/l ↓	→yes:	nitrate reducing
no:		iron reducing

Figure 10   Criteria for the determination of the redox conditions for a typical location using macro chemistry

The DAF and Banisveld location strongly deviate from the typical locations in terms of background concentrations. The formulated macro chemical criteria (figure 10) cannot form a basis for the determination of the redox conditions at these sites. Instead, distinct site-specific criteria have been formulated for these locations (chapter 9 and 11).

Also the steady-state hydrogen concentrations associated with redox processes are expected to be different per location. Hydrogen concentrations are not considered to be a function of redox processes only, but are also affected by other local conditions (chapter 3). For the Dutch situation, the hydrogen range in table 4 (section 4.2.1) can be used for most of the sites. Also for the Rademarkt, DAF, and SBNS site it will provide the criteria for the emperical hydrogen method. However, for the Banisveld location, this range was found to be inadequate for the interpretation of the redox conditions (chapter 11).

The criteria for the partial equilibrium approach on the other hand, are the same for all locations because the variability in conditions is already included in this method. For the four locations a Gibbs energy of -5 kJ/mol has been selected as criterion. This value is the highest of the range of values that has been reported in literature (chapter 3). The reason for doing so is to be sure that no reactions that possibly occur are excluded beforehand, when the Gibbs energy of a reaction is close to the threshold. This reduces the possibility of excluding a favourable reaction. Consequently, reactions might be falsely included. However, a better estimate of the threshold value would bring partial equilibrium interpretations closer to the real situation.

All redox identification methods will be evaluated and the results will be compared in order to come to a reliable redox characterisation.

At all locations, dechlorination processes will be analysed and related to hydrogen. A comparison between dechlorination and the redox conditions according to methods based on hydrogen will be made. More direct comparisons are made between dechlorination and empirical ranges of hydrogen concentrations. Additionally, thermodynamic calculations are made for the dechlorination reactions with hydrogen as electron donor to see if they are energetically favourable. Furthermore, comparisons are made with the type of electron donor which is present.

In order to make comparisons, dechlorination has to be quantified. The chloride number (Cl#) is a useful tool to indicate the stage of dechlorination that exists at a certain part of the location. The Cl# is defined as follow:

$$Cl\# = \frac{4[PCE] + 3[TCE] + 2[DCE] + [VC]}{\sum [chloroethenes] + [ethene] + [ethane]}$$

The Cl# ranges from 4, indicating that only PCE is present and no dechlorination has yet taken place to 0, indicating that no chloroethenes are present (any more) and might have been dechlorinated.

In figure 11 the general characterisation scheme is summarised.
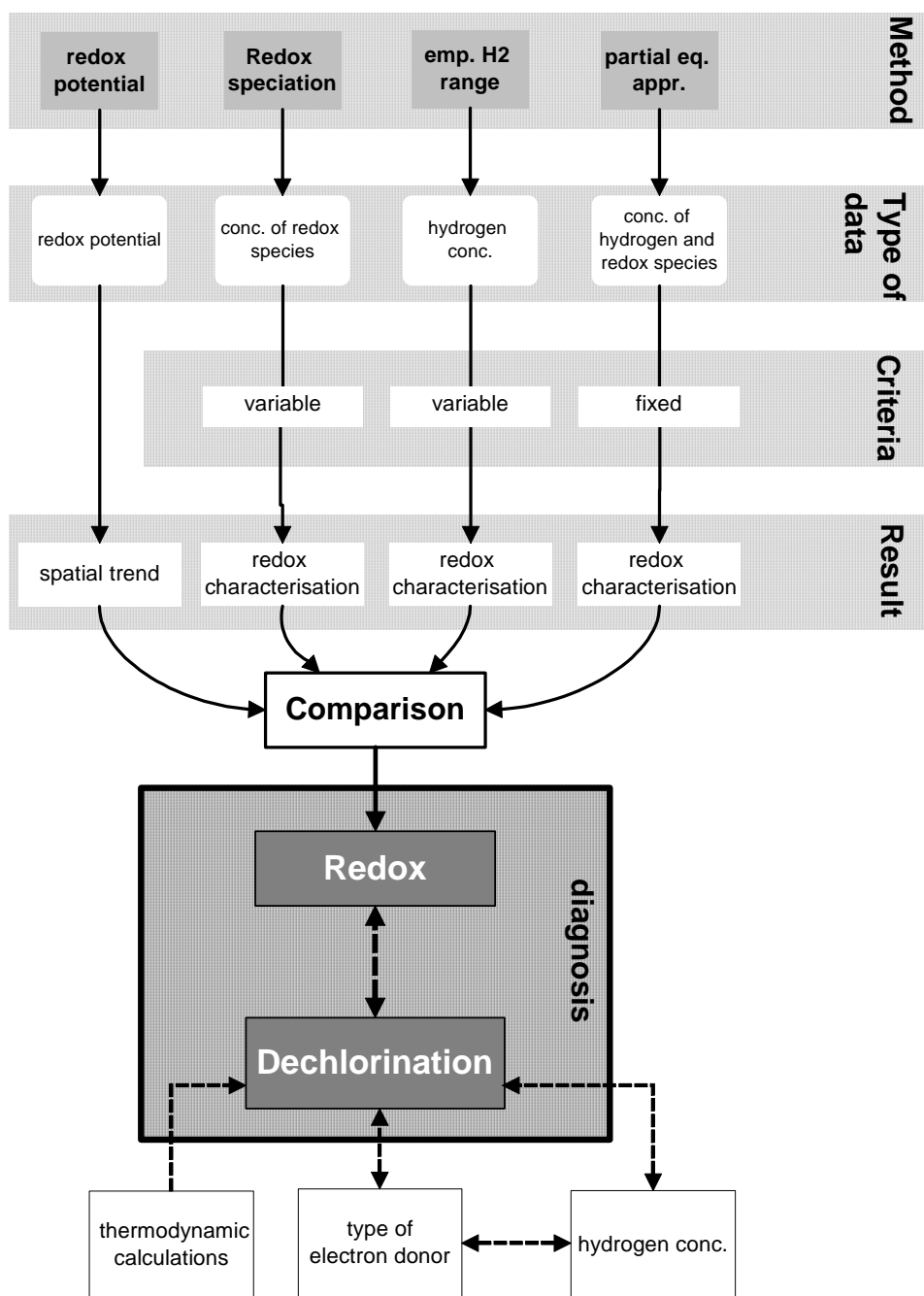
*Figure 11   General characterisation scheme showing the different steps that are taken
                to come to a diagnosis of redox conditions and dechlorination at the four
                case studies. Criteria that form the basis of the redox identification methods
                are either fixed, indicating that for all locations criteria are identical, or
                variable, indicating that they can be general for a particular type of location
                or have to be determined in a site-specific way*

7. Measuring hydrogen in the field

7.1 Introduction

Hydrogen is a very small and reactive molecule, therefore it is necessary to measure hydrogen directly in the field. Transport of H_2 samples to the laboratory is not advisable. In the United States and Denmark a lot of experience has been obtained over the last five years with hydrogen measurements [Chapelle et al, 1997; Jakobsen et al, 1998]. Low concentrations of hydrogen can be measured with a gas chromatograph equipped with a reduction gas detector (RGD). This patented detector responds to reducing compounds that generate mercury from a mercuric oxide bed. The mercury vapour is detected by adsorption in the UV part of the spectrum.

$$H_2 + HgO \text{ (solid)} \rightarrow H_2O + Hg \text{ (gas)}$$
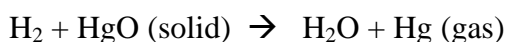
The gas-stripping method is described by Chapelle as the best suited method for measuring hydrogen in the field [Chapelle, 1997]. Groundwater is pumped through a sampling bulb with a H_2-free nitrogen gas bubble. The dissolved hydrogen gas is being transferred from the water to the gas phase and asymptotically reaching equilibrium. The gas-stripping method is a kind of an accumulation technique. The H_2 concentration will increase from ppt levels in the water to ppb in the nitrogen bubble which can conveniently be detected by the reduction gas detector.

7.2 Operation procedure for Hydrogen measurements in groundwater

Samples for hydrogen analysis were taken using the gas-stripping method developed by Chapelle [Chapelle *et al*, 1997]. Water from a well was continuously pumped through a 250 ml gas-sampling bulb with a flow of at least 500 ml/min. In some cases a flow rate of 360 ml/min was used. A 20 ml bubble of nitrogen gas was introduced into the sampling bulb through a septum. The slightly soluble hydrogen gas diffuses from the water to the gas phase. The equilibrium was achieved in about 25 minutes. Samples were taken after 25 and 30 minutes and when the difference was less than 5%, it is assumed that equilibrium has been obtained.

A 2.5 ml sample from the gas bubble is taken with a gas-tight glass syringe and analysed immediately in the field using a gas chromatograph with a reduction gas detector from Trace Analytical (Bester, Amstelveen). The gas chromatograph is equipped with a Carbosieve II column and the loop size on the gas chromatograph is 1 ml. The carrier gas is N_2 with a flow rate of 20 ml/min. The column and detector temperature are 104 °C and 265 °C, respectively.

Hydrogen gas phase concentration values are converted directly into aqueous concentrations using Henry's law.

Figure 12   Measuring hydrogen in the field, with the GC-RGD on a trolley

7.3   Experimental section:

7.3.1   Calibration

Calibration of the GC-RGD is carried out with three standard gases containing 1, 2 and 10 ppm H_2 in nitrogen. A 2.5 ml sample of the calibration gas was injected on to the gas chromatograph using a gas-tight glass syringe. Figure 13 shows a typical calibration curve.
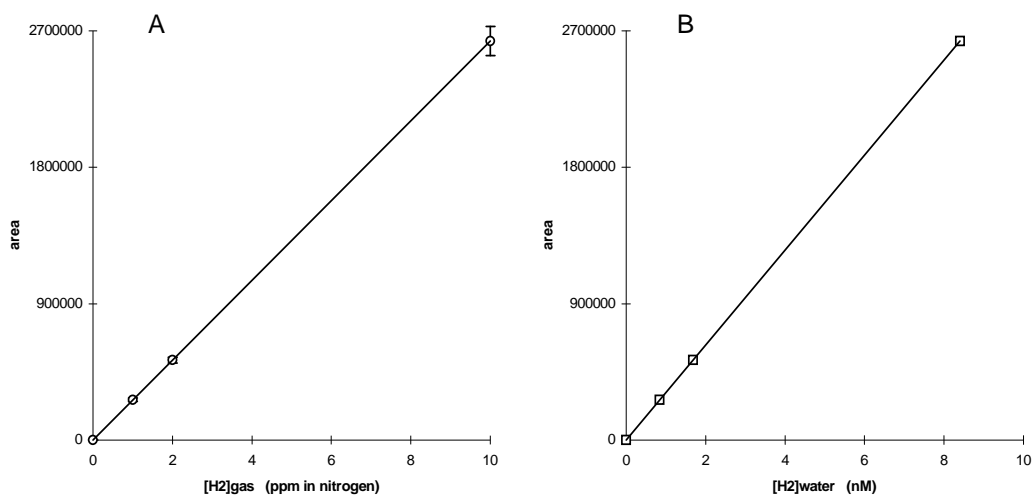
Figure 13   Calibration of the RGD gas chromatograph. Graph A shows the results of the calibration measurements. Graph B shows the same results converted to the water phase. The results are the means of duplicate injections.
$R^2 = 0.9999$

7.3.2   Equilibrium

The developed method (groundwater sampling and analytical procedures) used in this study is similar to that described by Chapelle and Jakobsen [Chapelle *et al*, 1997; Jakobsen *et al*, 1998]. A test in the field was performed to check equilibrium times required and to get experienced with the method.

The first experiment is carried out using a groundwater sampling flow rate of 360 ml/min. Figure 14 shows two other equilibration control experiments.
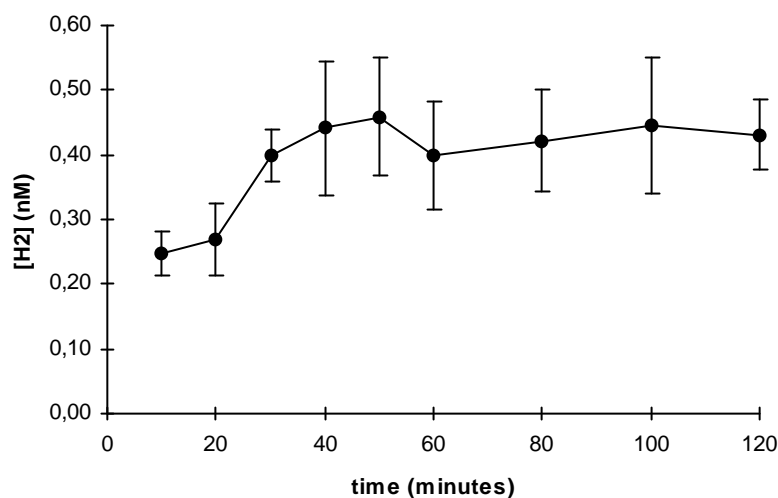
*Figure 14   Hydrogen concentration measured with the bubble-stripping method using a
               peristaltic pump. Flow rate: 360 ml/min. The results are the means of quad-
               ruplicate injections*

In about 30 minutes equilibrium was achieved, with a flow rate of 360 ml/min. In general in this study, a flow rate of 500 ml/min was used with corresponding equilibration times of 24 minutes. These results are consistent with the results of Chapelle [Chapelle *et al*, 1997] and Jakobsen [Jakobsen *et al*, 1998]. They found that an increase of the flow rate from 500 to 1000 ml/min, equilibrium is achieved in proportionally less time (12 minutes).

Figure 15 shows two other equilibration control experiments.
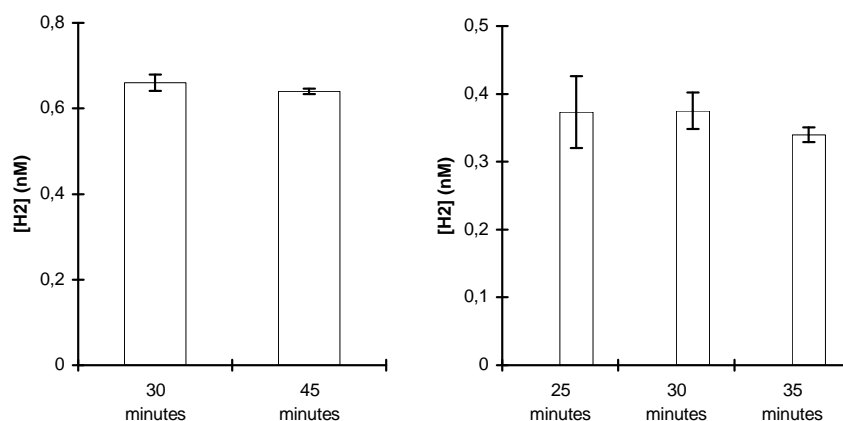
*Figure 15   Hydrogen concentration over time measured in two different wells with the
               bubble-stripping method using a peristaltic pump. Flow rate: 500 ml/min.
               The results are the means of quadruplicate injections*

In both experiments it appears that an equilibration time of 25 or 30 minutes is enough to obtain equilibrium. The difference between the first measurement and second or third one differs less than 5%.

7.3.3   Type of pump

A comparison of different types of pumps was carried out by Chapelle [Chapelle et al, 1997]. Four methods for pumping groundwater were evaluated. These were a peristaltic pump, a stainless piston pump, a bladder pump and a stainless steel submersible pump. The submersible pump is not suitable because hydrogen can be produced through interaction between direct current and water. The three other pumps are suitable for pumping groundwater for hydrogen measurement.

In the experiments carried out in this study a peristaltic pump was used. In some experiments the bladder pump was used to compare results obtained with the peristaltic pump. A peristaltic pump, which draws water under a negative pressure, is not working properly when the water table is at a depth of more than five metres. A bladder pump, which pushes water under positive pressure upwards can be used at water table depths of more than five metres. A disadvantage of the bladder pump is the need to clean the pump before sampling a new well, which is laboriously and costly, to prevent cross contamination between different wells. In The Netherlands water tables deeper than five metres are not common and in most cases a peristaltic pump can be recommended.

Figure 16 describes two equilibrium curves, one is measured using the peristaltic pump and one is measured using the bladder pump. The sample was taken at a depth of 15 to 16 metre and the groundwater table was at a depth of 2.5 metre.
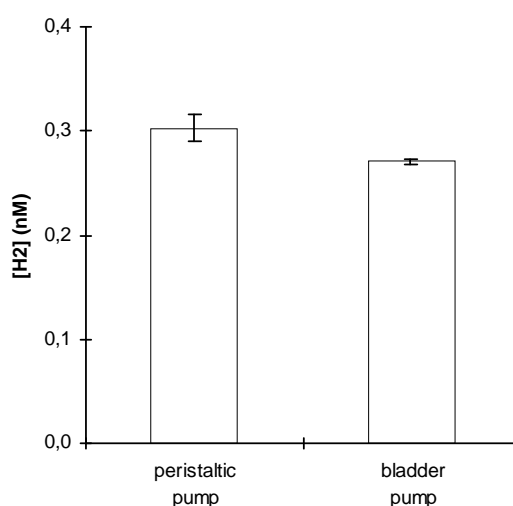
Figure 16   Comparison of H_2 concentrations sampled in the same well sampled, during 30 minutes with a peristaltic pump (flow rate: 500 ml/min) and a bladder pump (flow rate: 680/ml/min). The results are the means of quadruplicate injections

The two pumping methods show similar results which is consistent with the findings of Chapelle. For measuring hydrogen in the field the use of a peristaltic pump is preferred since it has no risk of cross contamination. In some cases, if the water table is more than five metre, the bladder pump should be used. Special care must be taken of decontamination of the bladder pump and tubing material to avoid cross contamination.

7.3.4   Tubing material

Hydrogen is a very small molecule and therefore use of appropriate tubing material is required to minimise the transport of hydrogen through the tubing. According to the literature butyl rubber is the most suitable tubing material. A disadvantage of this material is the high price, one metre costs about 2.50 Euro. It is recommended to use clean tubing material for each new well to avoid cross contamination, therefore butyl rubber is an expensive option. Figure 16 describes the comparison between butyl rubber tubing and the much cheaper tubing material polyethylene (PE). The price for 1 metre PE tubing is about 0.25 Euro.
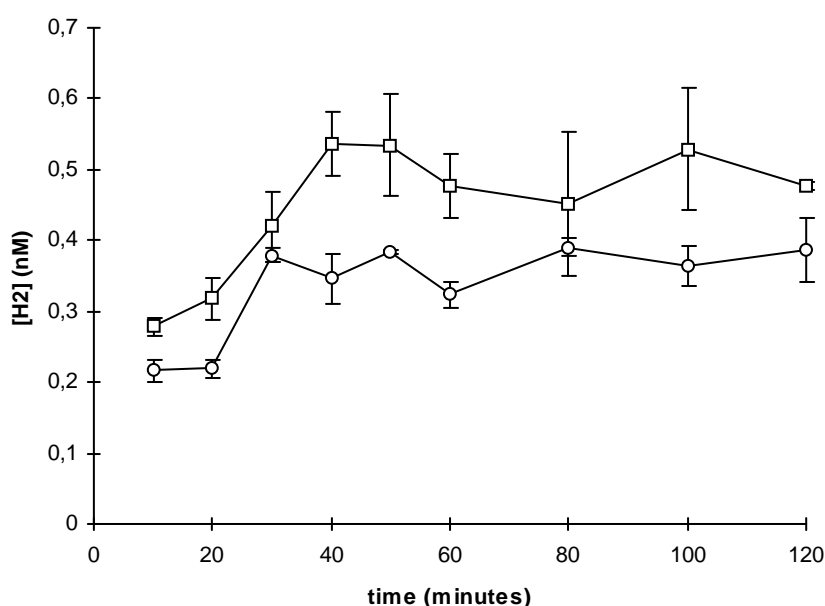
Figure 17   Comparison of equilibration time of the gas-stripping procedure using butyl rubber tubing (◯) and polyethylene tubing (☐). Flow rate: 360 ml/min. The results are the means of duplicate injections.

The time required for equilibrium for both tubing materials is about 30 minutes. A small difference between the hydrogen concentration measured with the two different materials exists, with slightly higher H_2 concentrations for PE. Apparently PE tubing is a suitable material for hydrogen measurements.

7.4   Guideline for H_2 measurements in the field

General instruction:
- Refresh the groundwater in the well with a volume equal to four times the volume of the well.
- Use clean PE tubing for each well
- Clean the bladder pump (when used) carefully before sampling a new well to prevent cross contamination

Instruction for H_2-measurements
- Use a pump with a flow rate of 500 ml/min
    - Use a peristaltic pump when the water table is less than 5 metre.
    - Use a bladder pump when the water table is deeper than 5 metre.
- When the well has a discharge capacity too low to provide 500 ml/min, use lower flow rates and proportionally longer equilibrium times.
- Use a 250 ml gas bulb and connect this to the PE tubing and put on the pump.
    - Fill the bulb with groundwater and remove the air in the gas bulb.
- Inject 20 ml of nitrogen in the gas bulb.
- Take four samples of the headspace for GC-RGD analysis with a glass gas-tight syringe.
- Take after 5 minutes (total time 30 minutes) again four samples and analyse. When the difference in hydrogen concentration is less than 5%, it is assumed that equilibrium has been obtained.
- Inject several times a calibration gas with a hydrogen concentration between 1 and 10 ppm in nitrogen.
- To convert the hydrogen concentration in the gas phase to the concentration in the water phase the Henry coefficient is used. Figure 18 shows the Henry coefficient in relation to the temperature.
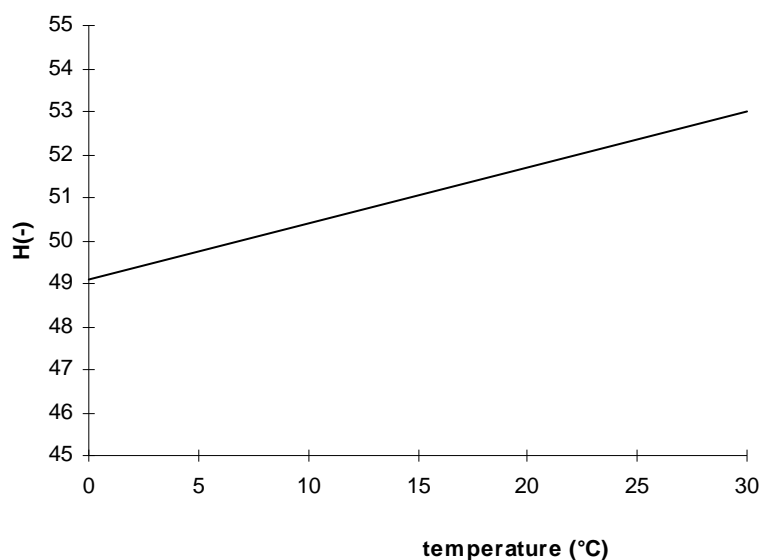
Figure 18   The Henry coefficient in relation to the temperature [Lide and Frederikse, 1995]

Table 5 gives an overview of the main equipment needed for hydrogen measurements in the field.

Table 5   An overview of the main equipment.

gas chromatograph with RGD detector
N_2 carrier gas 10 litre 150 bar
pressure regulator (150 bar to 4 bar)
power supply (220V, ± 350W)
computer + interface
peristaltic pump (500 ml/min)
gas bulb (250 ml)
PE tubing
zero gas (H_2 free N_2)
Cali-5 bond gas sampling bag with calibration gas (50, 10 and 5 ppm H_2)
glass gas-tight syringe with luer lock and valve (2.5 ml)
plastic syringe (20 ml)

7.5   Costs of H_2 measurements

Besides reliability and practicability, the cost determine whether or not a particular measurement will be considered successful. Four different types of cost are taken into account:

- equipment costs
- consumable costs
- mobility costs
- personal cost

The cost of interpretation, data evaluation, and the assessment of the in-situ redox conditions and dechlorination is not incorporated. The cost is purely based on the measurements of hydrogen concentrations in groundwater. The costs mentioned in the rest of the text are mentioned without value added tax (VAT).

Equipment costs involve solely the fixed costs of the portable gas-chromatograph, while consumable costs include all recurring costs such as: maintenance of the GC, tubings and reagents. Mobility costs are made to reach the site and include the vehicle and fuel. The personal costs include the rates of two engineers that have to drive to the site and to do the measurements.

In order to properly estimate the costs of a the hydrogen measurement the following aspects have to be considered:
- number of samples taken (per day and per year)
- measurements of other species
- distance travelled
- working hours per day

The costs are estimated for a typical hydrogen analysis. Since these costs strongly depend on the aspects mentioned above, the cost dependency is shown in two different figures. In the first one, the hydrogen measurement costs are shown to be dependent on both the amount of samples taken per day and the amount of samples that are taken per year. The costs decrease with the amount of samples taken per year and per day, but not linearly. For a relatively small amount of samples taken per day costs decrease very sharply, but after about 10 to 12 samples per day (the number of samples that can be taken during one working day) the costs do not decrease substantially when increasing the amount of samples per day. The same applies to the amount of samples per year. Initially, for a relatively small amount of samples per year, the costs change significantly, but above 400 S/y the cost do not decrease drastically (figure 19).
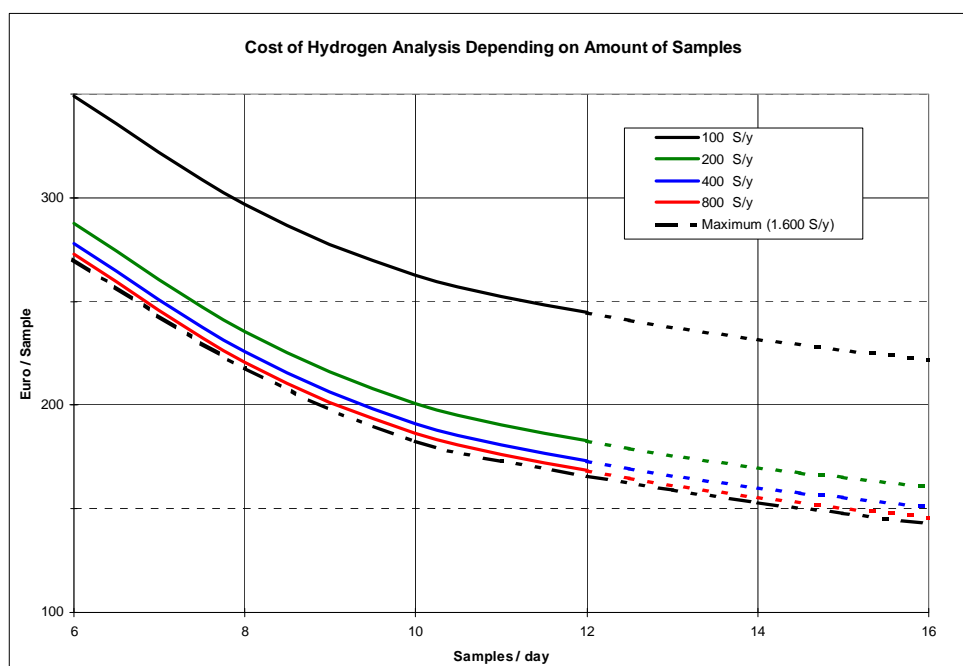
Figure 19   The cost of the hydrogen measurement per sample and its dependency on the
*   number of samples taken per day and per year*

In figure 20 the specification of the costs is shown assuming that ten samples are
taken per day and 200 per year. The personal cost for the engineers forms the major
part of the total costs (41 %). The equipment cost form 31 % of the total costs.
Mobility costs strongly depend on the situation of the location but only form a
small part of the total costs (6 %). Costs for consumables is estimated to form 22 %
of the total costs.
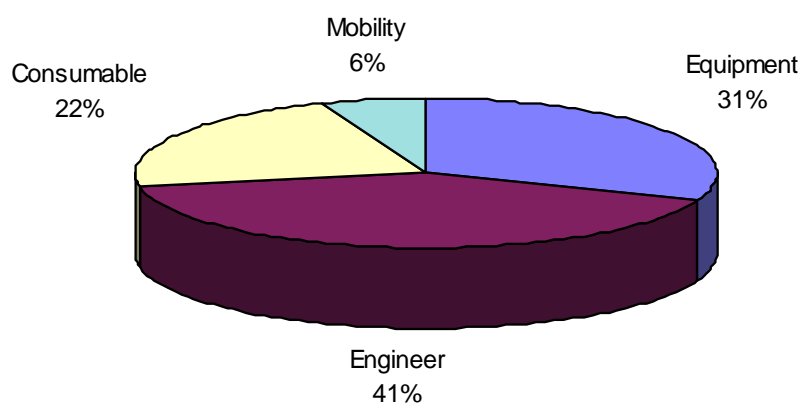
Figure 20   The specification of the costs for a hydrogen measurement, assuming that
*   200 samples per year and 10 samples per day*

8.   The Rademarkt location

8.1   Describing the location

The Rademarkt location is situated in the centre of the city Groningen (The Nether-lands). From 1969 to 1990 a chemical laundry was situated in a former building at Rademarkt 25. Due to spillage to the sewer system chloroethenes have been trans-ported and contaminated the soil and groundwater. In the direction of the ground-water flow a contaminant plume has developed. Additionally, the same site was po-luted with BTEX by a garage which was established at Rademakt 25 from 1955 to 1969. Both plumes partly overlap as is shown in figure 21.

Plume dimensions for BTEX and chloroethenes
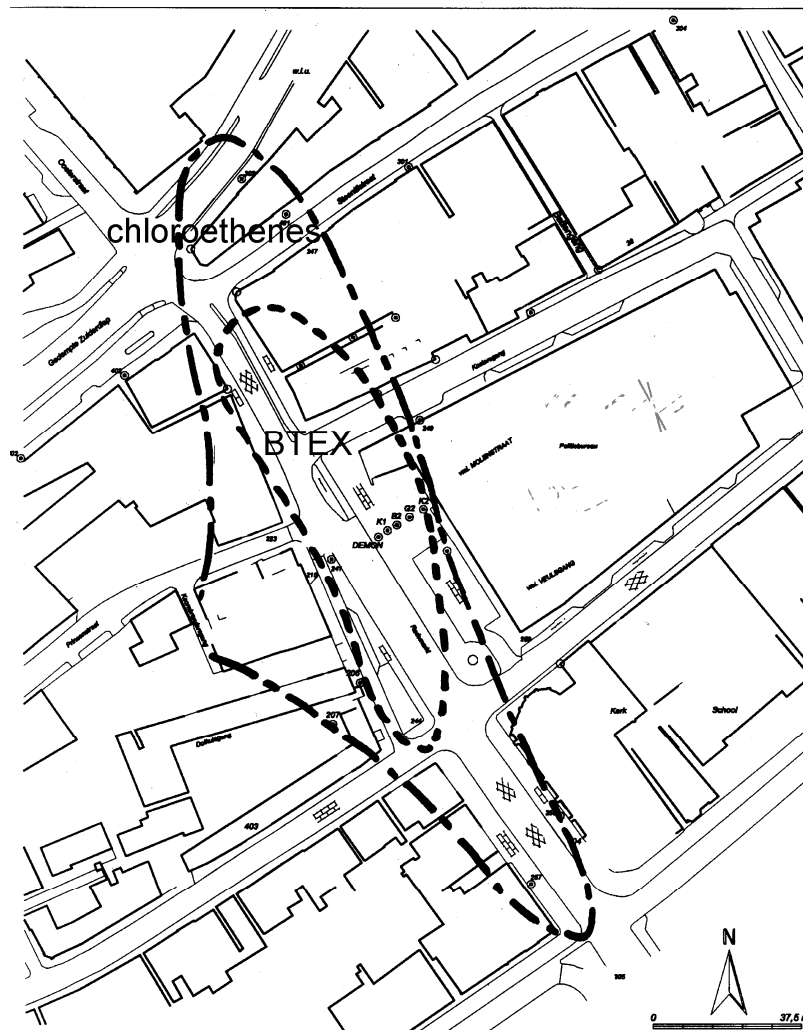
Figure 21   Rough schematic representation of the dimensions of the chloroethene and BTEX plume at the Rademarkt site. Contours are based on previous meas-urements and represent concentrations above 1 μg/l

The contaminants are primarily present in a sandy loam top layer of 8 metres thick. At a depth of 8 metres to 12 metres a loam layer is present below which a sandy aquifer is located. The phreactic groundwater flow is slow and in a variable direction ranging from north-east to south-east. The groundwater flow in the first aquifer is directed mainly to the east.

The Rademarkt site has been studied in various projects. In a former NOBIS project (number 95-1-41) the redox conditions have been identified. It appears that stronger reduced redox conditions are found in the highest contaminated zones. The correspondence between low redox conditions and the source zone of the plume has been explained by the presence of BTEX compounds and DOC leaking from the sewer. These compounds act as electron donors that reduce electron acceptors, and cause a decrease in redox conditions. At the edges of the plume, such influence is not present and the redox conditions approach the natural background, i.e. oxygen, nitrate or iron reducing. These sequential redox conditions between the different redox zones favour the degradation process of the chloroethenes since the higher chlorinated ethenes dechlorinate under strongly reduced conditions in the core of the plume, and the lower chlorinated ethenes can be oxidatively degraded under less reduced conditions in the periphery of the plume.

8.2   Redox characterisation

In a previous project redox conditions have been identified at the Rademarkt location (NOBIS 95-1-41). This characterisation has been done on basis of macro-chemical analysis of the following species in groundwater: oxygen, nitrate, nitrite, total iron, divalent iron, sulphate, sulphides and bicarbonate. By doing so, a rough interpretation could be made in which transitional redox zones could be indicated but not defined in detail. In the additional characterisation reported here the redox conditions are more thoroughly identified by using several different methods: the traditional methods involving redox potential measurements (Eh) and macrochemistry. Hydrogen measurements will be interpreted in two different ways; by comparing measured hydrogen concentrations to an empirically determined range and by the partial equilibrium approach. In the following paragraphs the exact criteria for the different approaches are formulated and applied to the Rademarkt location. Sampling points distributed over the highly contaminated parts of the plume and in the perephery of the plume were selected. All the wells had a filter depth between 4 to 9 metres. For each redox identification method, redox conditions are indicated on a map of the location.

8.3   Redox potential

The redox potential could not be used directly to identify redox conditions. However, the redox potential might be used to observe spatial redox trends, in which

zones can be distinguished as either more or less reduced, without identifying the actual redox condition. In further chapters the results of the redox potential are compared to the results of the other methods.

In figure 22. the redox potential is shown for several wells. The pattern does not display a clear and logical trend, since a large variation has been found on relatively small scale. It is therefore not considered to be a basis for an interpretation of the redox situation at this site.

8.3.1   Macro chemistry

For the Rademarkt location the criteria for a typical location (chapter 6) can be used as a basis for the macro chemical method (figure 26).

$CH_4 > 1$ mg/l $\rightarrow$yes: methanogenic
$\downarrow$
$SO_4 < 1.5$ mg/l $\rightarrow$yes: sulphate reducing
$Fe(II) > 1.5$ mg/l
$\downarrow$
$O_2 > 1$ mg/l $\rightarrow$yes: oxygen reducing
$\downarrow$
$NO_3 > 0.5$ mg/l $\rightarrow$yes: nitrate reducing
$\downarrow$
no: iron reducing

Figure 26   Criteria for the macrochemical method

These criteria have been used to characterise the redox conditions at several different sampling points. High methane concentrations, which form the criteria for methanogenesis, are found at two sampling points (404 and 304). Sulphate reducing conditions, indicated by decreased sulphate concentrations in combination with increased iron concentrations, are found only at sampling point 247. Oxygen is below 1 mg/l throughout the site, except for one sample (305). Nitrate concentrations seem to be strongly related to divalent iron concentrations. For samples where nitrate concentrations were below the detection limit, the divalent iron concentrations were higher than the detection limit, and vice versa (except for sample 401 where both nitrate and divalent iron occur simultaneously). Increased nitrate concentrations indicate nitrate reducing conditions at the following sampling points: G2, 244, 403, 401, 303, 215 and 223. At three sampling points iron reducing conditions take place: DEMO, B2 and 259.

So, redox conditions have been found to vary from oxygen reducing to methano-genic (figure 23). The most reduced conditions (sulphate reducing and methano-genic) occur in a zone which is following the dimensions of the contaminated plume. However, possibly due to the influx of nitrate rich groundwater this strongly reduced zone is more narrow than would be expected on basis of the plume dimensions. Nevertheless, the redox conditions seems to be related to the contaminated plume since nitrate reducing redox conditions dominate outside the plume. At sampling point 304 methanogenic conditions have been identified, al-though it is not located in the core of the plume.

8.3.2 Comparing hydrogen concentrations with empirically determined hy-drogen ranges

Hydrogen concentrations have been measured at several sampling points through-out the Rademarkt location (table 6).

Table 6   Measured hydrogen concentrations in nM.

sampling point	[H_2] in nM	sampling point	[H_2] in nM
demo	0.36	304	0.17
B2	0.29	401	0.12
G2	0.18	247	7.29
259	0.49	303	0.09
404	0.37	215	0.14
244	0.17	305	-
403	0.11	223	-

The steady-state hydrogen concentrations that are associated with redox processes are temperature dependent. Therefore, measured hydrogen concentrations must be compared to empirical determined ranges for the local temperature. At the Rade-markt location the temperature ranges between 10°C and 15°C and the typical em-pirical hydrogen range for Dutch situations can be used (table 7, table 4 section 4.2.1).

Table 7   Criteria for the empirical hydrogen ranges for typical Dutch situations.

Hydrogen concentration		redox condition
H_2<0.2	→	nitrate reducing
0.2<H_2<0.3	→	iron reducing
0.3<H_2<1.5	→	sulphate reducing
H_2>1.5	→	methanogenic

These criteria have been used for the redox identification as depicted in figure 24. Redox zones similar to the macro chemical method have been identified with this method. The distribution of zones with sulphate reducing and methanogenic redox conditions seems to be related to the presence of the contaminated plume, although there is not a perfect fit. Nitrate reducing conditions do not only exist outside the

plume, but also enter the plume from the western and eastern side. Therefore the strongly reduced zone appears to be relatively narrow.

In some parts there is a transitional zone in which redox conditions change from more to less reduced. This redox transition at some parts occurs very abruptly, i.e. from methanogenic to nitrate reducing on a very short distance. In other parts a more gradual transition is observed, where the conditions change from sulphate reducing to iron reducing and then to nitrate reducing, over large distances.

8.3.3   The partial equilibrium approach

Based on the many parameters that have been measured for several different reactions, Gibbs energies can be calculated. Some reactions have not been covered for several reasons. Manganese reduction has not been covered since manganese (hydr)oxides are generally not present in high amounts in Dutch soils to be an important electron acceptor. Sulphides have not been measured because they where assumed to precipitate to iron sulphides as soon as they where produced. Therefore the reaction from sulphate to sulphide has not been included. Instead, a reaction is covered in which sulphate reacts directly to iron sulphide. For the nitrate reducing reaction only the one in which nitrate reduces to nitrogen has been taken into account. The nitrogen concentration has not been measured but assumed to be at maximum solubility ($5.15*10^{-4}$ M). Nitrite an ammonium have not been measured and the reactions in which nitrate reduces to these products have not been taken into account. Concentrations from species which were below the detection limit and concentrations which where unreliable when low (e.g. oxygen below 1 mg/l) where discarded and consequently the involving reaction was assumed not to take place. For the iron reducing reaction goethite has been selected to be the predominant iron(III) mineral.

For relevant reactions, Gibbs energies have been calculated. Based on this energy, reactions have been determined as being favourable if Gibbs energies are more negative than threshold, or as unfavourable if it exceeds the threshold. For reasons discussed in chapter 6, the value of the threshold has been chosen to be -5 kJ/mol. From the Gibbs energies that have been calculated for many reactions at this site, it appears that Gibbs energy values are clustered slightly below -5 kJ/mol. This clustering implies that these values might indicate the actual threshold Gibbs energy, since bacteria tend to operate at situations as close as possible to this threshold.

For several sampling points the redox conditions have been identified by using the partial equilibrium approach (see figure 25). Also these results show that more reduced conditions correspond to the source zone of the contaminated plume. Outside the plume nitrate reducing conditions dominate, at the edges intermediate conditions exist and inside the plume more reduced conditions. In the western and eastern part of the plume nitrate reducing conditions take place, narrowing the zone of highly reduced conditions.

Eh (mV)
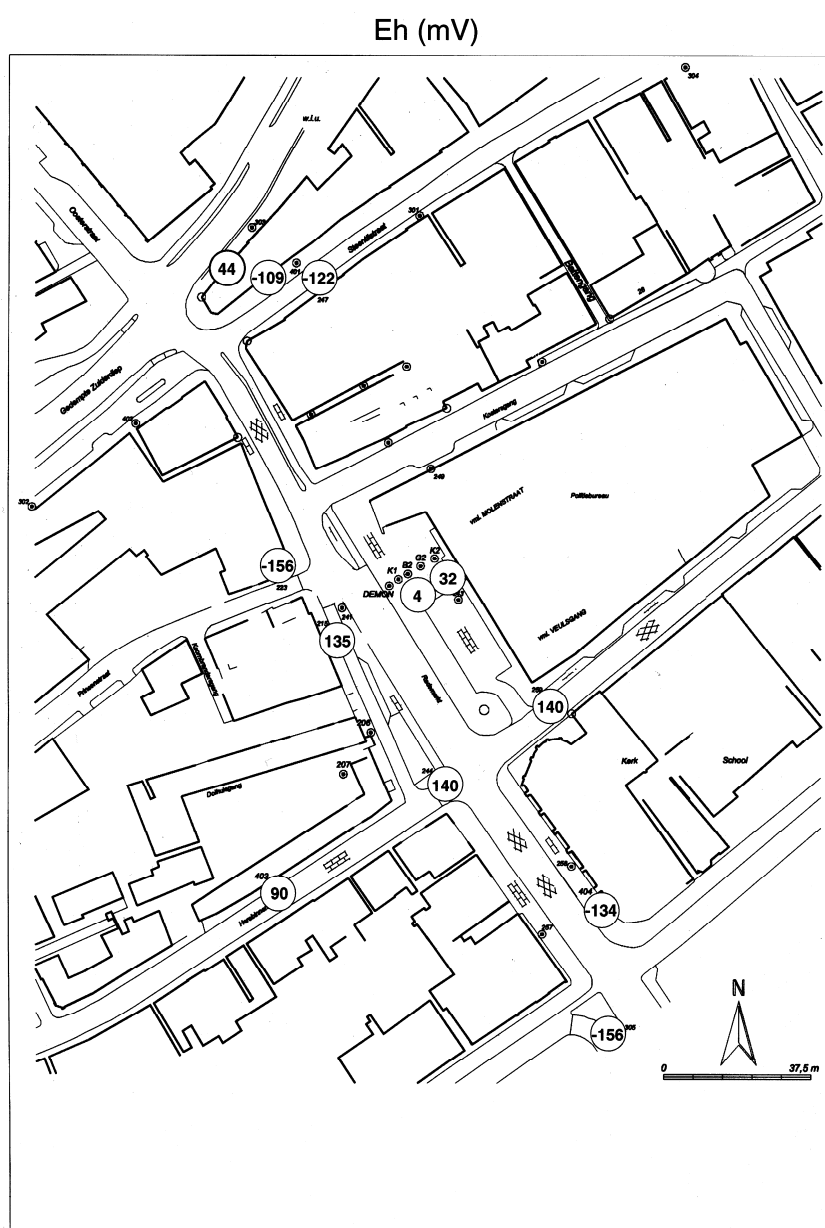
Figure 22   Patern of redox potential.

Figure 23   Redox characterisation according to the macro
                   chemical method

Figure 24   Redox characterisation based on comparison with
                   empirical hydrogen range.

Figure 25   Redox characterisation according to the partial equilibrium
                   approach.

Some regions can be considered as transition zones in which more redox reactions occur simultaneously. Very clearly, a gradual change in redox conditions can be observed in these zones. In the northern part of the location conditions change from methanogenic/ sulphate reducing inside the plume to sulphate/ nitrate reducing conditions and nitrate reducing conditions outside the plume. Furthermore in the centre of the plume redox conditions change from iron/ sulphate reducing to nitrate reducing.

8.3.4   Comparing the methods

Except for the redox potential the different methods all give results that are consistent with each other. It appeared that a sulphate reducing and methanogenic zone exist in the vicinity of the contaminated plume. A complete fit has not been found since nitrate reducing conditions from outside the plume appear to penetrate the plume on both the western and eastern side.
Only small differences exist between the three different methods, especially the distinction between nitrate and more reduced conditions could be made perfectly in each method. Only small differences between the methods have been found for iron reducing, sulphate reducing and methanogenic conditions, but a relatively large difference has been found at sampling point 304 where according to the macro chemical method methanogenic and according to the empirical hydrogen method nitrate reducing conditions exist. Unfortunately, the partial equilibrium approach could not give a decisive answer at this point due to a lack of macro chemical data.

The partial equilibrium approach has been shown to be a useful method because it was able to account for the discrepancy between the two other methods. At samples for which the macro chemical and empirical hydrogen method indicated distinct dominant redox processes, the partial equilibrium approach often appeared to cover the redox processes according to both other methods. These sample locations are often found in a redox transition zone, an area where more redox processes are likely to take place simultaneously. The partial equilibrium approach identified simultaneous favourable conditions for various redox processes, i.e. iron and sulphate reducing conditions and simultaneous sulphate reducing and methanogenic conditions, and even simultaneous nitrate reducing and sulphate reducing conditions. All these combined redox processes were identified in redox transition zones and were consistent with the rest of the redox pattern.

8.3.5 Conclusion for redox characterisation

Thus, the partial equilibrium approach has been shown to give very the most detailed and consistent redox interpretation at this location, since it is capable to identify zones where different redox processes simultaneously can occur (redox transition zones). However this method requires an extensive set of measurements including both redox parameters and hydrogen measurements. The macro chemical method and the empirical hydrogen range, which are less extensive, can be used to get an indication of the redox processes, but are incapable to indicate the transition zones. The redox potential has not been found to match with any of the other methods and seems to be an inappropriate tool for redox characterisation of this site. Measuring hydrogen has proved to be beneficial since it provides a stronger based redox characterisation than by macro chemical methods alone.

8.4 Hydrogen and dechlorination

The ultimate goal is to find a tool to quantify and control dechlorination processes. In this section the usefulness of hydrogen is tested by finding relations with dechlorination. These relationships could be either direct, when hydrogen concentrations are directly coupled to dechlorination processes, or indirect, when relationships exist between redox identification methods based on hydrogen and dechlorination. Also relationships between hydrogen concentrations and different carbon sources are being investigated.

Similar to other sites, also at the Rademarkt site dechlorination reactions with hydrogen as electron donor have been found to be energetically highly favourable throughout. Gibbsenergies could not be calculated for all dechlorination reactions, the ones that have been calculated were all in a range between -122 kJ/mol and -158 kJ/mol (table 8), well below the threshold for ATP synthesis. Thus from a thermodynamic point of view dechlorination should not be hindered in any part of the site. The lowest Gibbs energies have been found for PCE dechlorination to TCE, showing to be a very favourable dechlorination reaction. The highest Gibbs energies have been found for the less, but still highly favourable reactions of the lower chlorinated ethenes. Furthermore, the hydrogen concentrations are possitively coupled to the energetics of the reaction. The lowest Gibbs energies are calculated at points where high hydrogen concentrations a present, and these points have been determined to be the most reduced (see section 8.2).

Table 8   *Calculated gibbsenergies (in kJ/mol) of dechlorination reactions with hydrogen.*

	PCE dechl	TCE dechl	DCE dechl	VC dechl	Ethene red
pb demo	-148	-141	-125	*	*
pb B2	-148	-140	-125	*	*
pb G2	*-152*	*-140*	*-127*	*	*
pb 259	-152	*	*	*	*
pb 404	-153	*	*	*	-63
pb 244	-154	-142	-124	-122	-69
pb 403	-148	-137	*	*	*
pb 304	*-148*	*	*	*	*
pb 401	-146	-137	*	*	*
pb 247	-157	*	*	*	-77
pb 303	-145	-134	*	*	*
pb 215	-149	-140	-126	-128	-61

*   the required chloroethenes for this reaction have not been detected

italics pH has been extrapolated

Thus thermodynamics indicate that dechlorination is more favourable at zones where high hydrogen concentrations exist, but the actual relationship between hydrogen and dechlorination has not been proven. The dechlorination stage of the Rademarkt can be quantified by the chloride number (Cl#, chapter 6).

The redox characterisation that has been made shows that the most reduced conditions exist in the core of the contaminated plume. Those conditions are sulphate reducing and methanogenic and are thus considered to be favourable for dechlorination processes. In these zones the Cl# is expected to be low, since the lower chlorinated ethenes will be present in relatively high concentrations. In the periphery of the plume where nitrate reducing conditions exist, reductive dechlorination will not play an important role and high concentrations of higher chlorinated ethenes will result in high chloride numbers. A strong correlation between dechlorination and redox conditions, however, has not been observed at the Rademarkt site (figure 27). The bulk of the samples have Cl# between 2 and 4. At two points, however, Cl# numbers are significantly lower than that. At sampling point 247 low Cl# suggest that dechlorination is in far stage, which could be in accordance with the redox conditions that are determined as methanogenic. Unfortuanately, at sampling point 304 where also a lowered Cl# has been observed no relation could be made between the dechlorination process and redox conditions, since no consistent redox characterisation could be made. According to the empirical hydrogen method the conditions are nitrate reducing, while the macrochemical method indicated methanogenic conditions.
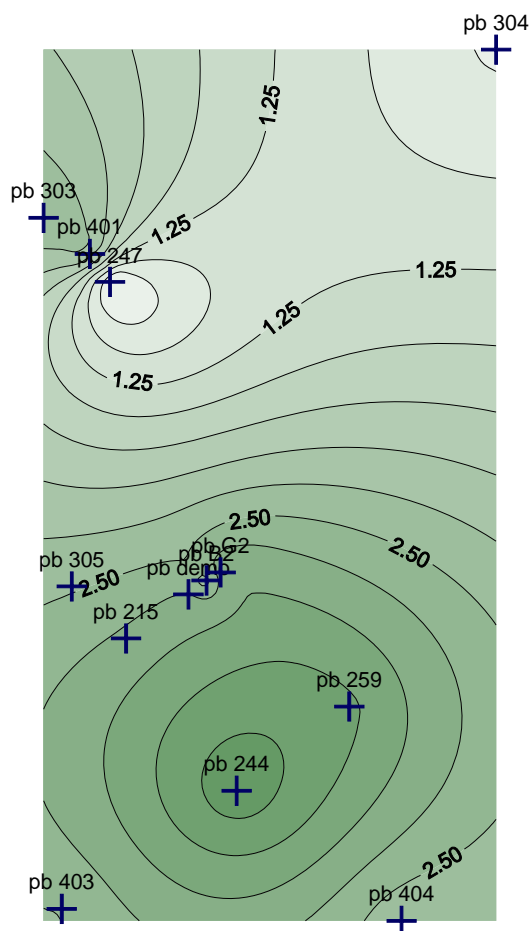
Figure 27   Contour plot of Cl#, based on Kriging method

A direct correlation between dechlorination and hydrogen concentrations has not been found. In figure 28 the hydrogen concentration is plotted against the Cl#. The points with the lowered Cl# correspond to the same points that have been discussed above.

H2 vs. Cl#
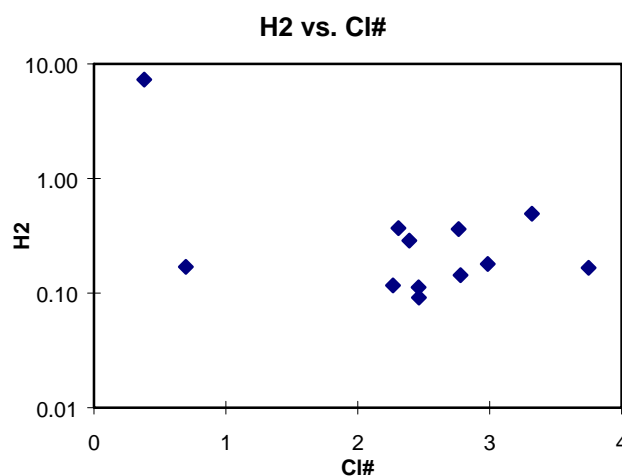
Figure 28   Plot of hydrogen against chloride number

Although no clear correlations have been found between hydrogen and dechlorina-tion, hydrogen appears to be related to the DOC content in the groundwater (figure 29). It seems that high DOC contents cause high hydrogen concentrations. DOC is the sum of all carbon compounds that are dissolved in the groundwater including many compounds that could be fermented to hydrogen.

H2 vs. DOC
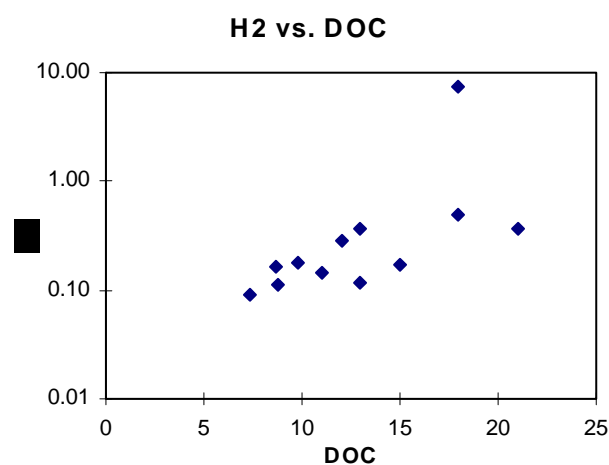
Figure 29   Plot of hydrogen against DOC

8.4.1   Conclusion of hydrogen and dechlorination

Although from a thermodynamic point of view dechlorination is considered more favourable at high hydrogen concentrations, a proof that an enhancement of dechlorination processes takes place at high hydrogen levels has not been found for the "natural" situation at the Rademarkt location. But, for the point with the lowest Cl# the highest hydrogen concentration and methanogenic conditions have been

determined, suggesting that there is a relation between hydrogen concentrations and redox conditions on one hand and dechlorination on the other.

Data analysis showed a significant correlation between DOC (a potential electron donor for dechlorination) and hydrogen. More research is needed to support these observations.

9.   The DAF location

9.1   Describing the DAF location

9.1.1   Situation

The location is about 17 acres (7 hectare) and forms a part of the DAF industrial terrain which is about 220 acres (90 hectare). In the central part of the location DAF started its activities in 1949. This part has been used and is still being used for production and maintenance activities and is considered the most contaminated part of the entire terrain. The main source of this contamination is located on the north-wester side of the building, where until 1975 metals have been cleaned and chemicals have been stored.

9.1.2   (Geo)hydrology

The surface layer of the DAF location forms a part of the Nuenen formation. locally this formation consists of a sequence of loamy sand and loam. Additionally, in the upper seven meters of the profile thin peat layers are present. The Nuenen formation reaches to 25-30 metres depth. Below this layer there is an aquifer consisting of coarse sand and gravel.
The average groundwater level is at 2.5 metres below ground level. The direction of the groundwater flow is variable with depth:
–   on 3-4 metre depth the groundwater is south to southwesterly directed. On the western part of the location the groundwater turns off to the west;
–   from 6-8 metre depth the groundwater is directed to the southwest;
–   around 14-15 metre depth, the groundwater is directed northwesterly. Also the water in the aquifer is flowing in this direction.

9.1.3   Description of the contamination

The DAF location has been contaminated with both volatile aromatics and chlorinated solvents. The source of both contaminants is located northwest of building E55 around well C40. In the direction of the groundwater flow a plume has been developed. In preliminary investigations TRI has been found to be the major contaminant. However, in more recent research the contamination consisted mainly of CIS and VC, indicating that dechlorination has taken place. Also a limited ground contamination has been found for TRI and is closely coupled to the presence of the groundwater contamination. Additional contaminated sources have been identified at two spots. Around C40 a BTEX contamination partly overlaps the chloroethene plume and around C46 a DCA source has been located.

9.1.4   Arrangement of the wells

Around twenty wells throughout the site have been selected and sampled for all
relevant parameters that are needed to characterise redox conditions by four differ-
ent methods, and to analyse dechlorination processes. Most wells have been sam-
pled at different depths. Two approaches have been followed in this chapter. For
the first approach layers have been selected at three different depths, at 6-8 m, 15-
28 m, and 29-30 m below the ground level. In the second approach a depth profile
has been made along the groundwater flow path (see figure 30). The usefulness of
both approaches will be discussed further on.

Figure 30   Site map including the selected flowpath. The flowpath curve is a result of in-
*   filtrating canal water into the upper "Nuenen" formation. The regional*
*   groundwater flow direction in the underlying aquifer is in the direction from*
*   NOB2 to NOB3.*

9.2   Redox characterisation

In order to understand the biodegradation process of chloroethenes at the DAF lo-
cation it is important to identify the redox conditions that exist. Four different
methods will be used and tested for their applicability to identify the redox condi-
tions. The results will be presented in two different ways, one in three layers of
depth and the other in a depth profile along the groundwater flow.

9.2.1   Redox potential

Similar to the locations which have been described in other chapters, the redox potential has only been used to identify trends in redox conditions on this location. A statement of the exact redox condition that exists is not considered to be reliable (see chapter 4).

In contrast to the other redox identification methods, the redox potential is only presented in a depth profile and not in layers. In figure 31 is shown that the redox potentials range between -31 and -260 mV. Roughly, three zones with different redox potentials could be distinguished in the chloroethene/BTEX plume. In the source a redox potential of -100 to -200 mV has been found. Even lower redox potentials of -200 to -250 mV are present in a zone halfway downstream the plume. In the deepest parts far away from the source, redox potentials are higher again and range from -50 to -100 mV. Outside the chloroethene plume a less consistent pattern was present. Low redox potentials are found in the vicinity of the canal, and higher values for most other parts. Also relatively high redox potentials have been found for the zone associated with the DCA contamination, which is not in accordance to expectations since these contaminants can act as electron donor and consequently cause a drop in redox conditions.

Figure 31   Soil profile with redox potentials

9.2.2   Macro chemistry

On basis of the macro chemical criteria formulated for typical locations (chapter 6), redox conditions could not be defined properly. Probably the background concen-

trations and the processes that take place at the DAF location are very distinct from the typical situation for which the redox identification protocol was developed. This resulted in an ambiguous redox interpretation in which methanogenic conditions are identified for samples where divalent iron is not even increased, and sulphate reducing conditions could not be identified at all, since the sulphate concentrations were higher than the criteria at all sampling points probably due to increased background concentrations. It is unlikely that methanogenic conditions take place together with iron reducing conditions, without the occurrence of sulphate reducing conditions is not very likely. Therefore the standard protocol appears to be inappropriate and macro chemical criteria have to be formulated site-specifically, in order to be able to identify the redox conditions at the DAF site.

The presence of oxygen and nitrate is indicative for oxygen and nitrate reducing conditions. Although at the DAF location oxygen has been encountered in the groundwater in considerable concentrations, the measurements are not considered to be reliable. It is suspected that the sampling method or an erroneous oxygen detection caused these strange results. Nitrate which is commonly present under oxygen reducing conditions, is in fact absent throughout the site. Redox conditions are therefore considered to be more reduced. Iron reducing, sulphate reducing and methanogenic conditions seem to play an important role. Manganese reducing conditions, on the other hand, are not expected to be important, because in the Dutch subsurface manganese(hydr)oxides are only present in small amounts.

Iron reducing conditions can be demonstrated by the loss of trivalent iron and the production of divalent iron. Trivalent iron is always present as mineral phase and has not been measured. Divalent iron is dissolved in the groundwater and has been measured thoroughly. Increased concentrations of divalent iron might indicate that iron reduction has taken place, but it could also have been transported from elsewhere. The absence of divalent iron might indicate that iron reduction has not (yet) taken place. In this case it is also possible that iron reduction has taken place but the produced divalent iron directly precipitated as ironcarbonate or ironsulfide. This could explain that certain samples at the DAF location did not contain divalent iron, although both oxygen and nitrate where absent. As criterion for the characterisation of iron reducing conditions therefore not the presence of divalent iron but the absence of oxygen and nitrate will be used.

Sulphate reduction can be demonstrated by the disappearance of sulphate and the production of sulfides. In this project only sulphate has been selected, since sulphide concentrations are disturbed by the precipitations with divalent iron to form ironsulfides. Decreased sulphate concentrations suggest that sulphate reduction has taken place. However, sulphate concentrations are not always reliable as redox parameter since sulphate might have been trasported from elsewhere.
A difficulty which is encountered in this method is the distinction which must be made between high and low sulphate concentrations. For the DAF location values of 10 mg/l and 20 mg/l have been selected as criteria. The selection of these values is based on the (statistical) distribution of sulphate concentrations. The group with

sulphate concentrations below 10 mg/l is considered as sulphate reducing (or methanogenic). Another group could be identified above a value of 20 mg/l. In this group the high sulphate concentrations indicate that sulphate reduction has not taken place and iron reduction is more likely. For the group between the two values it is uncertain if sulphate has been reduced, and therefore both processes possibly take place.

During methanogenesis bicarbonate is being reduced to methane. Especially methane might be a good indicator for the occurrence of methanogenesis. However its use is limited if methane is being transported from elsewhere or if methane is locally produced by processes other than methanogenesis (e.g. dechlorination of chloromethanes). Also for methane the distinction between high and low concentrations forms a obstacle. Based on the distribution of the data, two groups have been identified. The group having methane concentrations above 5 mg/l is identified as methanogenic, provided that sulphate concentrations were below 10 mg/l and oxygen and nitrate were absent. The other group has methane concentrations below 5 mg/l and is considered as sulphate reducing.

Table 9   *Overview of the site-specific criteria on which the redox conditions have been identified following the macro chemical method.*

Redox characterisation	criteria		
Iron reducing	$O_2 + NO_3$ absent	SO_4^{2-} >20 mg/l	
Iron/sulphate reducing	$O_2 + NO_3$ absent	$10 < SO_4^{2-} < 20$ mg/l	
Sulphate reducing	$O_2 + NO_3$ absent	$SO_4^{2-} < 10$ mg/l	
Methanogenic	$O_2 + NO_3$ absent	$SO_4^{2-} < 10$ mg/l	$CH_4 > 5$ mg/l

In figure 32 the redox conditions are shown at three different layers. In the upper layer in most parts the redox conditions have been identified as iron reducing. At two other parts a combination of iron and sulphate reducing conditions exist. Those part are found at the eastern part of the location. The same pattern could be found in the second layer except that the more reduced area has been moved in a northern and western direction. In the deepest layer a more heterogeneous pattern is shown. Sulphate and iron reducing conditions alternate at a small scale.
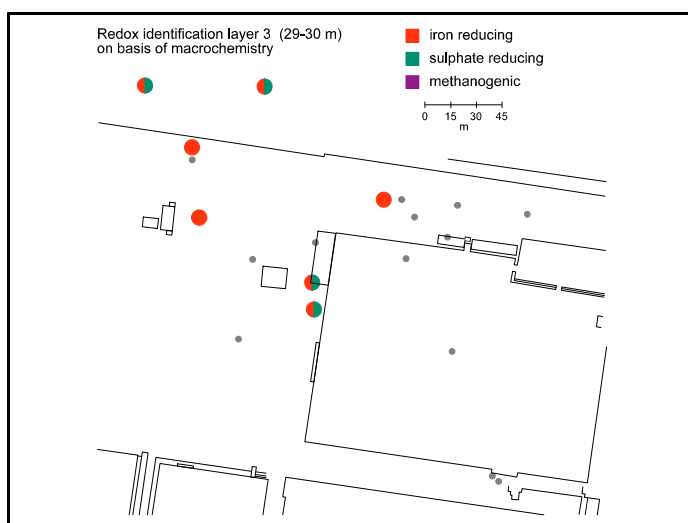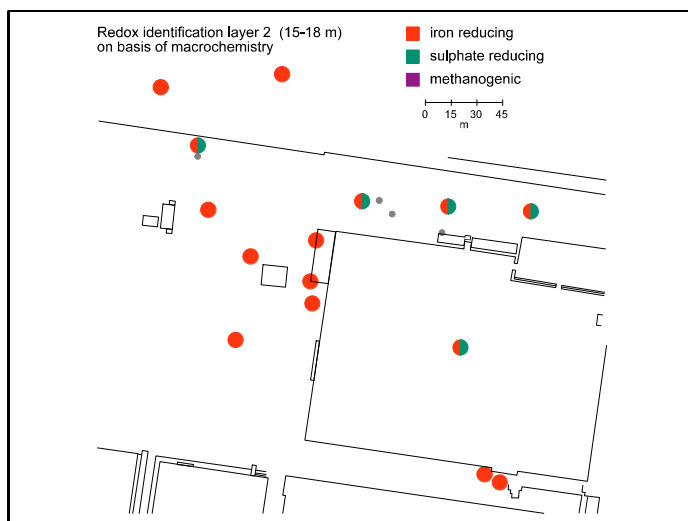
Figure 32   Redox characterisation in layers according to macro chemical method.

From the profile (figure 33) it does not becomes clear that the redox conditions are associated with the contaminated plume.

9.2.3 Comparing hydrogen concentrations with empirically determined hydrogen ranges

The following hydrogen concentrations have been measured at the DAF location (table 10)

Table 10   Hydrogen analysis.

sampling point	[H_2] in nM	sampling point	[H_2] in nM
NOB5-1	0.46	C39-2	0.23
C4-2	0.23	C41-1	0.11
C40-1	4.77	NOB4-2	0.53
C40-2	1.09	C4-3	0.63
C47-2	0.49	C32-2	0.25
NOB2-1	3.07	C48-3	0.44
NOB3-1	0.30	B12	0.13
C33-1	0.20	C51-3	1.01
NOB1-1	0.80	C47-4	0.54
C32-1	0.18	NOB2-3	1.15
B12	0.40	NOB3-3	2.49
C47-3	0.50	C27-3	0.27
C46-2	0.29	NOB1-3	0.40
NOB2-2	4.92	C41-2	0.25
NOB3-2	0.29	C48-4	4.25
C45-3	0.46	NOB4-3	0.66
C27-2	0.13	C61	1.30
C33-2	0.10	C61	0.58

At the low temperatures of about 14°C that exist at the DAF location, the empirical hydrogen range which is based on results of experiments at standard temperature, could not be used. Instead, the empirical range for temperatures between 10 and 15°C is also applied to the DAF location (table 11, table 4 section 4.2.1).

Table 11   Criteria for the empirical hydrogen method.

Hydrogen concentration		redox condition
0.2<H_2<0.3	→	iron reducing
0.3<H_2<1.5	→	sulfate reducing
H_2>1.5	→	methanogenic

For each layer of depth the hydrogen concentrations have been compared to the empirical range in order to determine the redox conditions. According to this method a combination of iron reducing, sulphate reducing and methanogenic conditions is found in all layers. On a relatively small scale, the redox conditions show a large variation.

In the upper layer two spots have been identified as methanogenic, four as sulphate reducing and three as iron reducing. These spots appear to be randomly distributed, however in the second layer the same spots have been found to be in a highly reduced stage. At this depth it becomes more clear that the sulphate reduced zone extends towards the south. North from this zone an iron reducing zone and a zone with variable conditions have been observed. In the deepest layer the stronger reduced zone seemed to be moved northwesterly, in the direction of the groundwater flow.
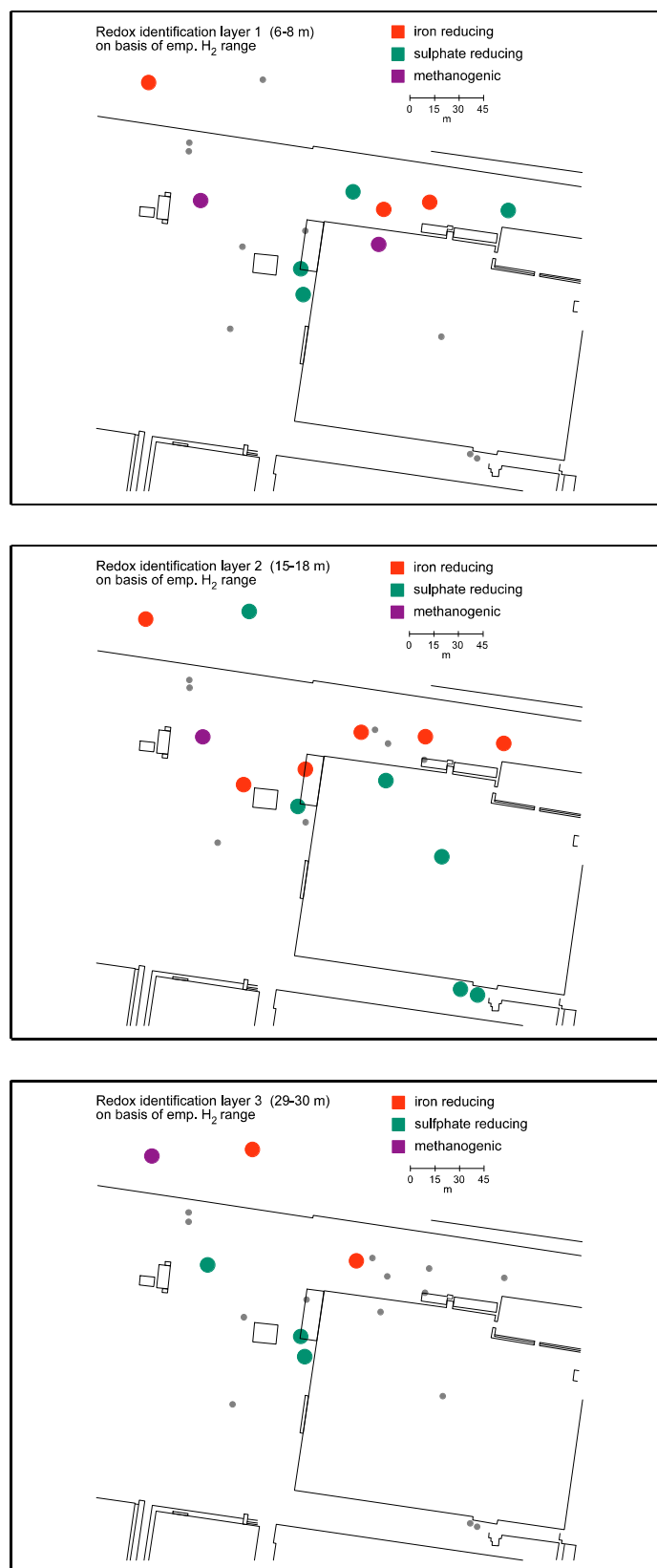
Figure 34   Redox characterisation in layers according to the empirical hydrogen range

In the depth profile shown in figure 33 the spatial distribution with depth becomes more clear, and redox zones seem to be related to the contaminated zones. Basically, two major sources of contaminants can possibly account for more reduced conditions. A BTEX source at sampling point C40, and a DCA source at sampling point NOB2. Both type of contaminants can act as electron donor and are expected to reduce natural electron acceptors and consequently cause a drop in redox conditions. At both spots methanogenic conditions exist according to the emperical hydrogen method, while sulphate reducing and iron reducing conditions exist outside these zones. The deepest sampling point of NOB3 is also determined as methanogenic, and could be associated with the DCA contamination.

9.2.4   The partial equilibrium approach

The partial equilibrium approach is useful to determine for each sample which redox processes are favourable and might take place.

The groundwater at the DAF location has been analysed thoroughly for several different redox parameters and enough information is available to be able to use the partial equilibrium approach. The absence of oxygen and nitrate in the groundwater indicates that redox conditions are strongly reduced. Therefore only iron reduction, sulphate reduction and methanogenesis will be covered in the partial equilibrium approach. Manganese reduction is not considered to be an important process for reasons that are mentioned earlier, and is therefore not covered in this method.
For several sampling points Gibbs energies have been calculated for iron reducing, sulphate reducing and methanogenic reactions. Those calculations are based on the concentrations of hydrogen and redox species and also on pH and temperature. The calculations of the sulphate reducing reactions are partly based on sulfide concentrations. On this location sulfide has not been analysed, but extrapolating is justified in this case. It appears that a large change in the sulfide concentration leads only to small change in the Gibbs energy of the reaction. At a few sampling points also bicarbonate concentrations had to be extrapolated, which has also been shown to be justified.
The Gibbs energies that have been calculated for individual redox reactions form the basis for the identification of redox processes. Depending on whether this calculated Gibbs energy is below or above the threshold Gibbs energy, the reaction is considered as either favourable or unfavourable, respectively. Similar to the other locations, also at the DAF location a threshold Gibbs energy of -5 kJ/mol, has been chosen as critical value.

The partial equilibrium approach resulted in the redox characterisation shown in figure 35. For each layer of depth is indicated which redox processes are favourable. For some spots more processes might take place simultaneously.
In the first layer various different redox conditions exist. In the centre a zone is present in which the redox conditions seem to be stronger reduced than in the surrounding area. In this zone sulphate reduction and methanogenesis might take place

in addition to iron reduction. In the surrounding area sulphate reduction and methanogenesis are unfavourable and only iron reducing conditions are expected. In the second layer a similar pattern has been found. In the same two wells where methanogenesis was favourable in the first layer, also in the second layer stronger reduced conditions exist. In the western situated well three redox processes are favourable and might occur simultaneously. On the other well only sulphate reducing conditions are expected. In the south sulphate reduction and iron reduction are favourable and a combination of both processes might take place. In the northern part only iron reducing conditions are favourable.

At the deepest layer, a sulphate reducing zone has been developed in the northeast. Possibly this zone has been moved from the central part of the location in the direction of the groundwater flow. Due to the limited amount of sampling points at this depth the redox conditions in the other parts could not be determined.
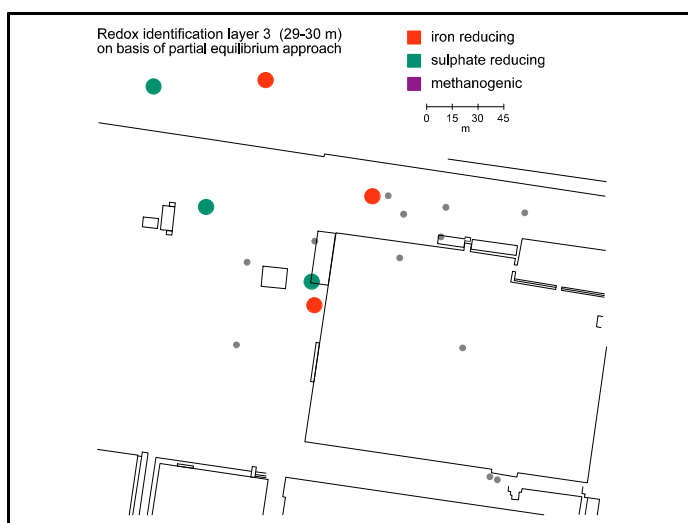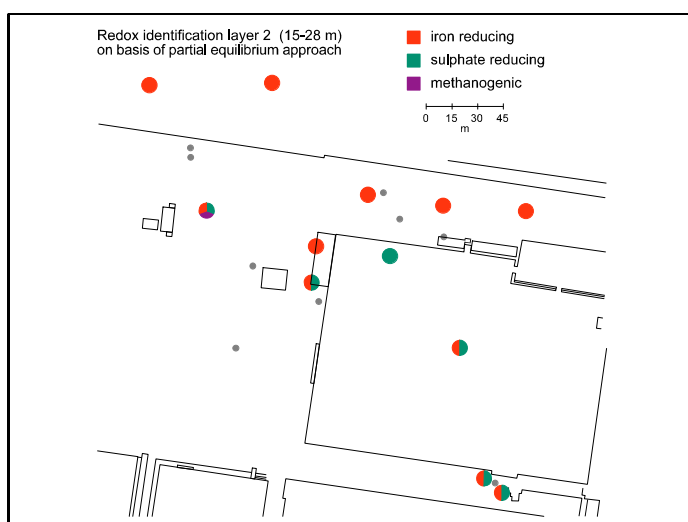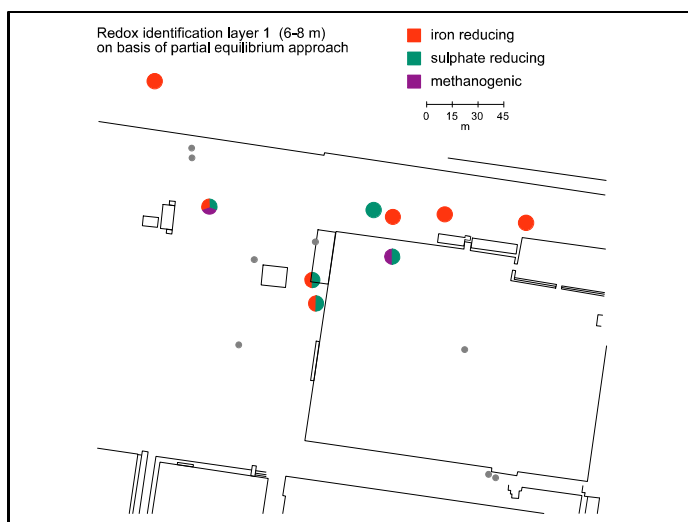
Figure 35   Redox characterisation in depth layers according to the partial equilibrium
             approach

DAF location Eindhoven
Soil profile with redox conditions

according to the macro chemical method
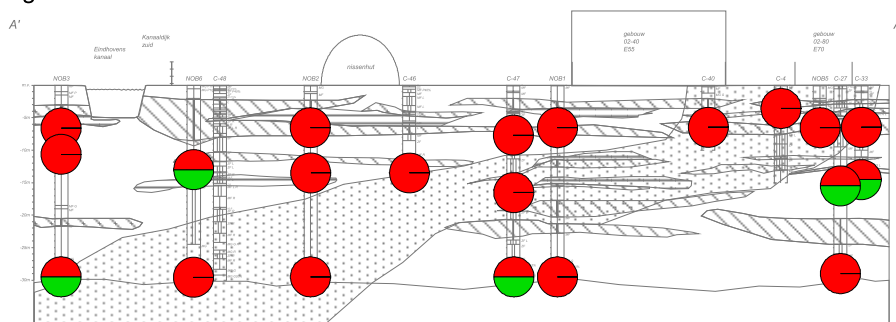
according to empirical hydrogen range
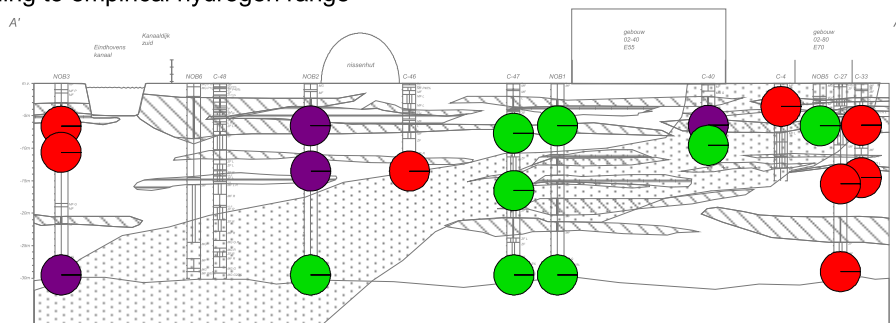
according to partial equilibrium aporach
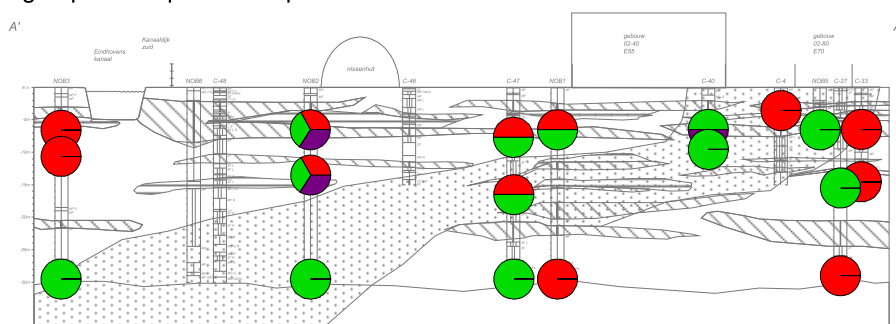
Soil profile

▭ peat
▨ loam, containing moderate to strong amounts of sand
▭ sandy, containing moderate to strong amounts of loam
▭ sandy, containing moderate amounts of loam

Pollutants

▦ cloroethene plume

Redox conditions

🟥 iron reducing
🟩 sulphate reducing
🟪 methanogenic

0   50   100   150   200 Meters

*Figure 33   Redox characterisation in the depth profile according to the partial equilib-
rium approach*

In the depth profile the same two stronger reduced spots become visible (figure 33). Apparently, more redox processes are favourable simultaneously at these spots. A local supply of electron donor could have raised the hydrogen concentration temporarily and thus enabling sulphate reduction and methanogenesis to take place, while trivalent iron is still available as electron acceptor. As soon as the electron donor supply ceases, the hydrogen concentration decreases and these reactions will become unfavourable again leaving iron reduction as the only process. It seems that the background redox conditions are iron reducing, but sulphate reducing conditions, or a combination of both occurs in the vicinity of the plume.

9.2.5   Comparison of the methods

Not all methods give similar results for the redox characterisation. Especially the macro chemical method is strongly different from the methods which are based on hydrogen concentrations. Overall, the redox conditions according to the macro chemical method are less reduced than for the other methods.
The macro chemical method is not considered to give reliable results for this location. The redox zones that have been identified do not match well to the pattern which is expected on basis of the plume dimensions of contaminants that are likely to act as electron donor (BTEX and DCA). In contrast to the macro chemical method, both the results of the empirical hydrogen range and the partial equilibrium approach follow the contours of the plume. A second argument is the agreement with other field observations. The macro chemical method fails in this respect, since it indicates iron reducing conditions at sampling points where a sulphide smell has been observed. The same points have been identified as sulphate reducing and methanogenic by the two methods that are based on hydrogen. The pattern of redox potentials is only partly in accordance with the redox characterisation of the methods based on hydrogen. In the core of the chloroethene/BTEX plume, where conditions have been determined as methanogenic and sulphate reducing, low redox potentials have been found. However, deeper in the plume the methods do not fit completely. Also the values of the redox potential are not reflected in the redox conditions that have been characterised inside the DCA spot.

Compared to the other methods the partial equilibrium approach gives a consistent pattern. The redox identification made by this method is the most specific in the sense that more redox processes can be indicated simultaneously. At this location where in some parts the supply and consumption of electron donors and acceptors is not expected to be in steady-state, redox transition zones form in which more redox processes occur simultaneously. These zones could not be identified by the other methods. This method thus provides additional information about the processes that take place. The results of the emperical hydrogen range lack the high specificity of the partial equilibrium approach but nevertheless are consistent and useful to get an indication of the redox conditions.

9.2.6   Conclusions for redox characterisation

For redox characterisations hydrogen has been shown to be a very useful tool. In fact, without hydrogen a consistent redox identification could be made neither with the macro chemical method and only partly with the redox potential. Although the results of the empirical hydrogen range and the partial equilibrium approach are comparable, the partial equilibrium approach is more specific. For a complete redox characterisation this method is advised, while for a preliminary characterisation the less extensive and cheaper empirical hydrogen range could be used. Depth profiles are preferred over a presentation in layers, because the former gives better spatial insight.

9.3   Hydrogen and dechlorination

In this section comparisons are made between dechlorination and hydrogen on one hand, and the redox conditions derived from hydrogen on the other. Also the relation between the type of electron donor and dechlorination processes is investigated. Similar to the other locations, also for the DAF location the Cl# is used to quantify the stage of dechlorination.

Thermodynamic calculations indicate that dechlorination reactions are energetically favourable throughout the location. All values are well below the reported threshold Gibbs energy for ATP synthesis. For the different dechlorination reactions, a range is indicated with the minimum and maximum value of all samples (table 12).
According to expectations, dechlorination of the higher chlorinated ethenes is energetically more favourable than dechlorination of lower chlorinated ethenes. The energy which is involved in DCE and VC dechlorination is in the same range, but slightly lower for VC dechlorination. Ethene reduction to ethane is far less favourable than the dechlorination reactions.

Table 12   The ranges of the calculated Gibbsenergies for different dechlorination reactions with hydrogen.

dechlorination process	minimum observed Gibbs energy (kJ/mol)	maximum observed Gibbs energy (kJ/mol)
PCE dechlorination	-162	-135
TCE dechlorination	-152	-127
DCE dechlorination	-141	-110
VC dechlorination	-143	-122
ethene reduction	-77	-54

In principle, dechlorination reactions with hydrogen are considered favourable, but if dechlorination actually takes place still has to be proven. One of the ways to quantify dechlorination processes is the Cl# (chapter 6). At the DAF location Cl# range between 1 and 2.5 which is considered to be very low. Dechlorination seems

to be a very important process. In figure 36 Cl# have been plotted in the depth pro-
file. A spatial trend of Cl# has been observed along the flowpath. In the core of the
chloroethene plume the highest Cl# are found, but along the flowpath Cl# decrease,
indicating that dechlorination takes place. According to the pattern of Cl#, the
dechlorination process has taken place in all zones of the plume. Apparently, the
redox conditions are favourable for dechlorination processes throughout the entire
plume. Strong relationships between the Cl# and redox conditions have therefore
not been found. Also a direct relationship between hydrogen concentrations and
dechlorination has not been found (see figure 37). The samples with the highest
hydrogen levels (above 2 nM) do not have the lowest Cl#. So, the hydrogen con-
centrations found at the DAF location are all considered to be in a range where
dechlorination processes take place, but no optimal range for dechlorination was
detected. These results are in conflict with the results of Yang and McCarty (1998)
which indicate an optimal hydrogen range for dechlorination between 2 nM and 10
nM. Most hydrogen concentrations found here are well below this range and imply
that the conditions would be unfavourable.
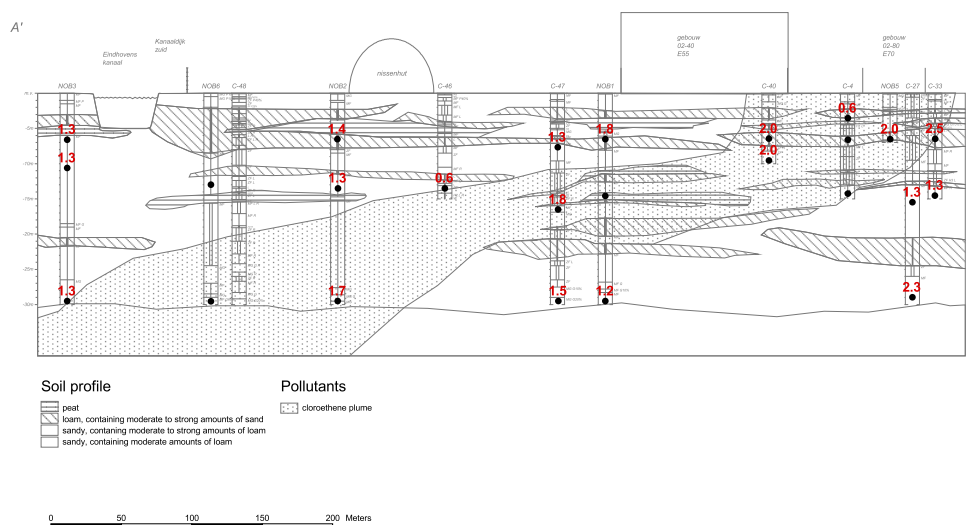
*Figure 36   Spatial distribution of Cl#, indicating zones that are in different stages of
               dechlorination*

Since BTEX and DCA both might act as electron donors in dechlorination proc-
esses, the zones in which these compounds are present in large amounts are ex-
pected to stimulate dechlorination processes. This, however, does not seem to be
the case at this location, since no association between BTEX and DCA sources and
decreased Cl# have been found.

H2 vs. Cl#
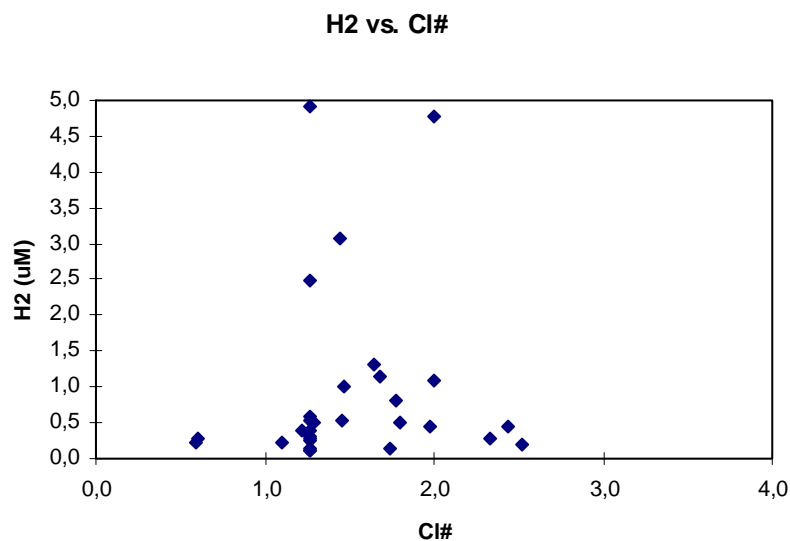
Figure 37   Plot of hydrogen against Cl#

From figure 38 can be concluded that no clear relationship is exists between hy-
drogen concentrations and the concentrations of dissolved organic carbon (DOC).
It is expected that higher DOC levels cause an increase in the hydrogen concentra-
tions, but only a very weak association can be found.

H2 vs. DOC
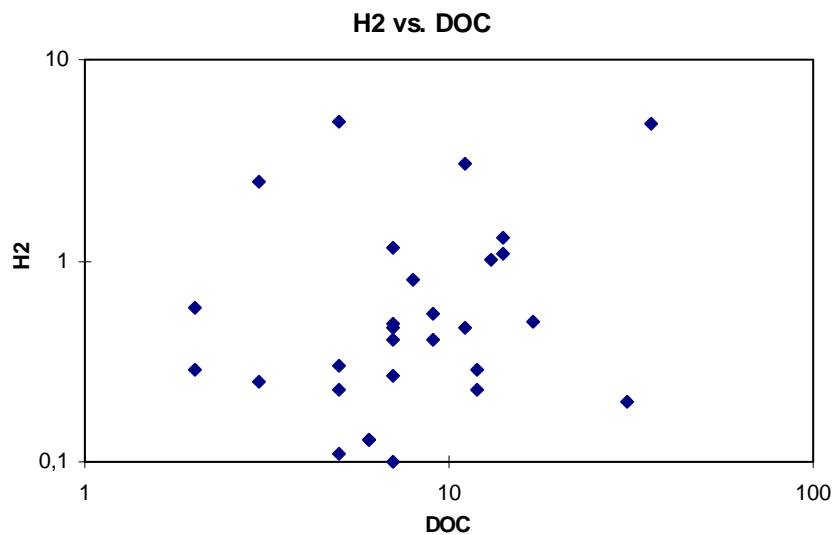
Figure 38   Plot of hydrogen against DOC

10.   The SBNS location

10.1   Description of the SBNS site

10.1.1   Situation

The site discussed in this chapter has been owned by the Dutch railroad company (Nederlandse Spoorwegen) for more than a century. The site covers an area of approximately 12 acres, and is located in the centre of the city Tilburg. The site has been used for maintenance activities of railroad material including cleaning, surface treatment, metal casting and paint spraying. Nowadays, the central part is still being used for the cleaning of engines and pipes. As a result of these activities the site has become contaminated with several different compounds, including chloroethenes, BTEX compounds, mineral oil, polycyclic aromatic hydrocarbons and heavy metals.

10.1.2   Geohydrology

The top layer of the site is part of the Nuenen formation and is variable in thickness from 5 till 12 meters. The first aquifer, which is laying beneath the top layer, is the formation of Sterksel an Veghel. This formation reaches to a depth of 57 metres below ground level. The boundary between the upper layer and the first aquifer cannot be defined sharply because both layers strongly resemble in composition. The phreatic groundwater would normally flow in a northerly to north-easterly direction but due to a groundwater sanitation nearby, the groundwater flow is north-westerly directed.

10.1.3   Description of the contamination

High levels of BTEX and Chlorinated Solvents have been found out around sampling points 907 and 103. These points might be considered as the source and the core of a plume. A second BTEX plume is located north-westernly from the previous source, but the exact dimensions of this plume are not sufficiently known.
In the direction of the groundwater flowpath, 11 sampling points have been arranged. The point labelled 251-b is located upgradient in respect to the SBNS site but could not be considered to represent the background conditions, since considerable amounts of PCE, TCE and BTEX have been found here. From this point of view the sampling point 156-2 is more reliable in defining background conditions, since at this point all contaminants remain below the instrumental detection limit.

10.2   Redox Characterisation

10.2.1   Redox Potential

Similarly to the other locations, also at the SBNS location the redox potential will
be used only for a spatial comparison to distinguish between reductive zones. Re-
dox potentials have been measured for all sampling points and range between -75
and 312 mV (figure 39). The redox potential of 156 point, which might represent
the background conditions, is 40 mV. In the core of the principal plume the lowest
redox potentials have been found (between -75 and -50 mV), which is in accor-
dance with the expectations. Due to the presence of contaminants that might act as
electron donor (e.g. BTEX), natural electron acceptors become reduced, which
leads to a drop in the redox conditions. For the other sampling points (251b; 107;
902-1; 902-2; 905 A13-2) the redox values range between 145 and 312 mV. As ex-
pected, redox potentials, that have been determined in a simple standard way, can
be considered inefficient to describe sharply the exact redox conditions that take
place.

Pattern of redox potentials (Eh)
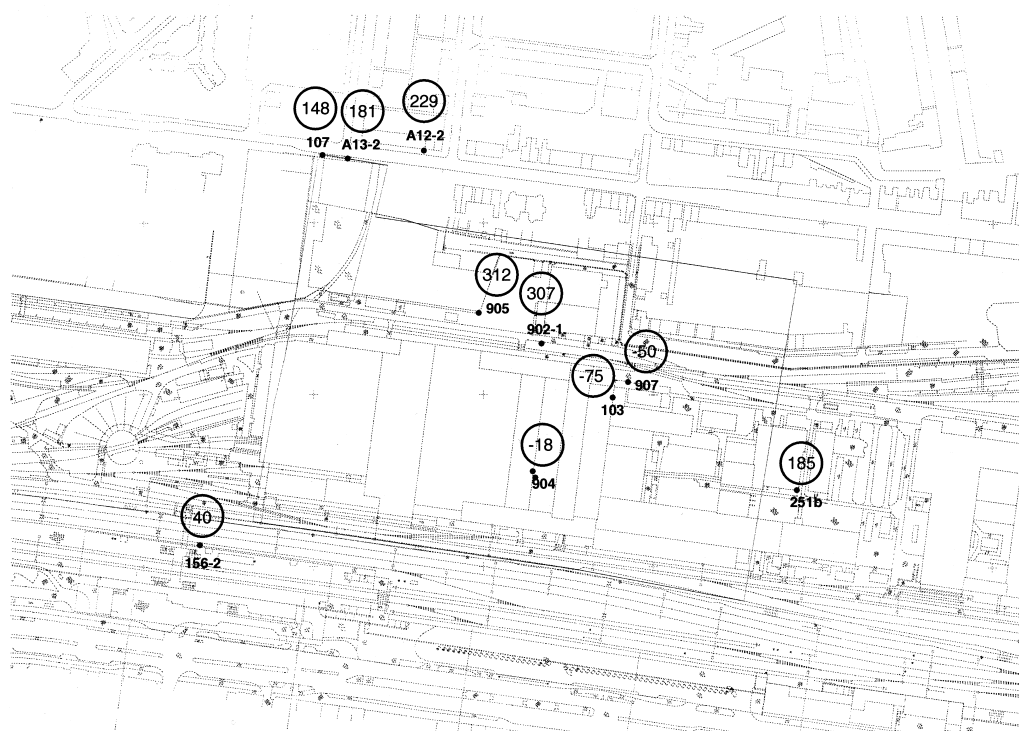
Figure 39   Spatial distribution of redox potentials at the SBNS site Tilburg

10.2.2   Macro Chemistry

Several relevant species have been measured in order to identify the redox conditions but not all the species are useful for that aim. According the criteria for a typical situation as used in the redox identification protocol (chapter 6), the species considered are the following: O_2, NO_3-, Fe_2+ , SO_4^{2-} and CH_4 (figure 40). The dataset is complete for most of the sampling points and sufficient to make a redox characterisation, except for sampling point A12-2 which has been neglected.

$CH_4 > 1$ mg/l	→yes:	methanogenic
↓		
$SO_4 < 1.5$ mg/l	→yes:	sulphate reducing
Fe(II) > 1.5 mg/l		
↓		
$O_2 > 1$ mg/l	→yes:	oxygen reducing
↓		
$NO_3 > 0.5$ mg/l	→yes:	nitrate reducing
↓		
no: iron reducing		

Figure 40   Criteria for macro chemical redox characterisation

At the points labelled 907 and 103 the methane concentration is clearly higher than the critical value, indicating methanogenic conditions. The methane concentrations found were 2.6 mg/l and 1.4 mg/l respectively. At non-methanogenic areas, a decreased sulphate concentration and an increased divalent iron concentration indicated sulphate reducing conditions. Although divalent iron has only been measured three sampling points, it is safe to state that sulphate reducing conditions have not been encountered throughout the site since sulphate concentrations are nowhere decreased (except at the methanogenic sampling points). For the sampling points where oxygen has been determined it is not encountered in significant amounts. Nitrate, on the other hand is found at sampling points: 904, 905, 251b, 902-1 and 902-2, and are therefore considered to be nitrate reducing. According to the formulated criteria, the other sampling points (156-2, 107 and a13-2) are considered iron reducing.

According the macro-chemical approach, it could be concluded that the core of principal plume, i.e. the most contaminated sampling points (i.e. 103; 907), is characterised as methanogenic and the periphery as nitrate reducing (figure 41). In the downgradient plume, including the wells 107, A13-2, and 156-2 iron reducing conditions prevail . The point 156-2 has been considered to be under iron reducing conditions. Comparing the assumed background conditions with the conditions in the periphery of the principal plume, it is expected that an influx of nitrate has

taken place. According the available data is not possible to assess if the influx is temporary, e.g. through rain infiltration or a sewer leak.
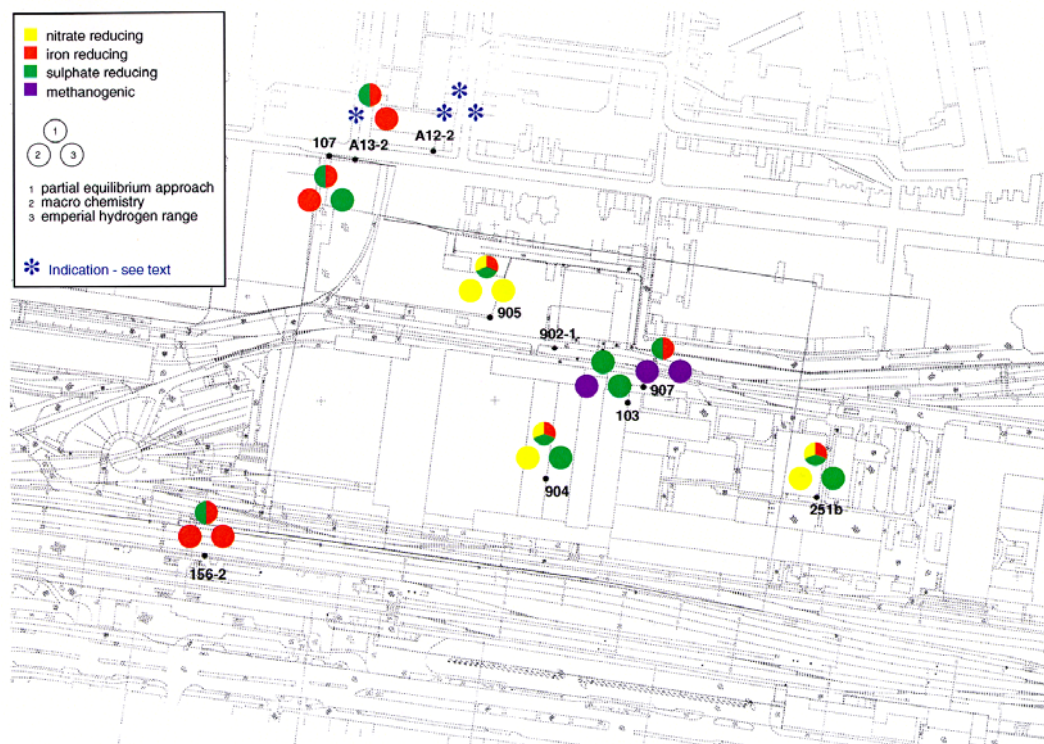
*Figure 41   Overview of the redox characterisations according to three different redox identification methods: macrochemical, empirical hydrogen and partial equilibrium approach (see legenda). * means that data were incomplete*

10.2.3   Comparison with empirical hydrogen range

The hydrogen concentration and temperature have been measured throughout the entire site. The H_2 concentrations range between 0.16 and 2.14 nM (table 13) and the temperature between 11.5 and 17.1 °C. This temperature fits with the temperature of the hydrogen range that applies to most situations between 10 and 15 °C. This range is shown in table 14 and explained in chapter 4.2.1.

Table 13   Hydrogen analysis for different sampling points.

Sampling point	[H_2] in nM	Sampling point	[H_2] in nM
156-2	0.28	905	0.16
251-b	0.42	107	0.59
907	2.14	902-1	0.15
103	0.31	A12-2	-
904	0.37	A13-2	0.25
902-2	0.31		

Table 14   Criteria for the empirical hydrogen method.

Hydrogen Concentration		Redox Condition
$H_2 \leq 0.2$	$\rightarrow$	nitrate reducing
$0.2 < H_2 \leq 0.3$	$\rightarrow$	iron reducing
$0.3 < H_2 \leq 1.5$	$\rightarrow$	sulphate reducing
$H_2 > 1.5$	$\rightarrow$	Methanogenic

In this way two sampling points have been classified as nitrate reducing, two as iron reducing, five as sulphate reducing and only one as methanogenic (figure 41). The most reduced spot is found at sampling point 907, at which methanogenic conditions appeared to exist. Around this point a sulphate reducing area has been identified (point 103, 904 and 902-2) and a little further, downgradient, a nitrate reducing zone (point 905, 902-1). At sampling points 156-2, 107 and A13-2, iron reducing, sulphate reducing and iron reducing conditions have been found, respectively.

10.2.4   Partial equilibrium approach

Gibbs energies have been calculated for several relevant redox reactions. Some reactions have not been covered. Manganese reduction has not been considered since manganese hydroxides are not sufficiently present in Dutch soils to be an important electron acceptor. Since sulphide concentrations were always below the instrumental detection limit, it has been assumed that sulphides precipitated to iron sulphides as soon as they where produced. No reaction in which sulphate reduces to sulfide has therefore been included. Instead, the reaction in which sulphate reacts directly to iron sulphide is considered.
In order to estimate the nitrate reducing reactions, the nitrogen concentration has been assumed to be at maximum solubility ($5.15*10^{-4}$ M). Bicarbonate concentrations have not been measured, but extrapolations of this compound have been made and have been justified since large concentration differences have only a minor effect on the calculated Gibbs energy. For the iron reducing reaction, goethite has been considered as the predominant iron mineral. Also for iron reducing reactions extrapolations had to be made, since divalent iron concentrations have only been measured at a few sampling points. For some points extrapolations were not justified and no characterisation could be made.

The Gibbs energies which have been calculated for the individual redox reactions are compared to the threshold Gibbs energy of -5 kJ/mol in order to determine if the reaction is favourable or unfavourable. Arguments for the selection of this value have been given in chapter 6.

For all sampling points where nitrate is detected, the nitrate reducing reaction can be considered as energetically favourable (figure 41). The calculated Gibbs energies are around -180 kJ/mole, which is well below the threshold value. So nitrate reducing conditions have been shown to be favourable for the sampling points 904,

902-1, 902-2 and 905. According to the partial equilibrium approach at most parts, more processes are favourable simultaneously. Methanogenesis is not considered to be favourable in any part of the site. In the core of the combined chloroethene and BTEX plume, sulphate reducing conditions exist. West from this zone nitrate reducing conditions are favourable as well as iron and sulphate reducing conditions. Apparently, an influx of nitrate is not capable to suppress the hydrogen concentration to a level that excludes the other reactions. Either it is a temporary influx or the supply is too small. In both cases a situation of non steady-state is developed. In the other parts of the site a combination of iron and sulphate reducing processes is favourable.

10.2.5   Comparison of the methods

Although small differences exist between the redox identification methods, they all show the same sequence of redox zones. All methods have identified a sulphate or methanogenic zone around 103 and 907, which is associated with the BTEX contamination. West from this area, wells 905 and 902 mark a zone where an influx of nitrate has caused nitrate reducing conditions. At 904 and 251b less consistent results have been found for the macro chemical and empirical hydrogen methods. In this situation the partial equilibrium approach has shown to be very useful, since it provides results that are consistent with the those of the other two methods. At 904 and 251b both the macro chemical method and empirical hydrogen method indicate conditions that have been determined as favourable by the partial equilibrium approach. In the western zone with sulphate and iron reducing conditions all methods are consistent with each other.

In contrast to the other redox identification methods the partial equilibrium approach was able to identify multiple redox conditions typical for redox transition zones. Since this appears to be the case in many parts of this site, the partial equilibrium approach was not very discriminative with respect to identifying the dominant redox process.

10.2.6   Conclusion redox characterisation

In general, the four redox identification methods give different redox results, at the level of individual sampling points. In contrast, all the methods produce the same pattern, a similar sequence of redox zones. Comparing the macro chemical and empirical hydrogen method, both producedures characterise the periphery of the plume and the more reduced core zone similarly. Both methods could not account for simultaneously favourable redox conditions as often found in redox transition zones For such areas the partial equilibrium approach appears to be a better method On the one hand the partial equilibrium approach gives a more detailed picture of the prevailing redox conditions, on the other hand it gives a less discriminative result.

Due to a lack of data, several assumptions and extrapolations had to be made in order to apply the partial equilibrium approach. An improved performance of this method is expected if more wells are included and more parameters are measured. According to the results of this case study, the empirical hydrogen method appears to be a valid detection method at least for a first screening investigation. The partial equilibrium approach can be used for improved interpretations, especially in redox transition zones.

10.3   Hydrogen and dechlorination

The aim of this paragraph in not to assess the distribution of contaminants at SBNS site, but to understand if there is any correlation between the hydrogen concentration and lines of evidence of dechlorination. Also the relationship between hydrogen and the type of carbon source as principal electron donor is being investigated. Comparisons between dechlorination and hydrogen and electron donor source are being made in location maps.

Dechlorination processes are expected to be more favourable under reduced redox conditions than under less reduced conditions. So based on this consideration it is expected that at the SBNS site most dechlorination takes place in the core of the contaminated plume (907 and 103), where the concentration of both the total chlorethenes and BTEX are highest and where sulphate reducing and methanogenic conditions exist. Patterns of the Cl# show that this is indeed the case.

In figure 42 the hydrogen concentration is plotted. In the core or the plume the concentrations of hydrogen are around 2 nM while at the periphery much lower values have been observed. When comparing this hydrogen pattern with the Cl# (figure 43), it becomes clear that in zones where high hydrogen concentrations are found, Cl# remain low (lower than 2). This suggests that high hydrogen concentrations favour the dechlorination process. It seems that these observations are in accordance with the reported range of optimal dechlorination between 2 and 11 nM (Yang and McCarty, 1998).

SBNS Tilburg
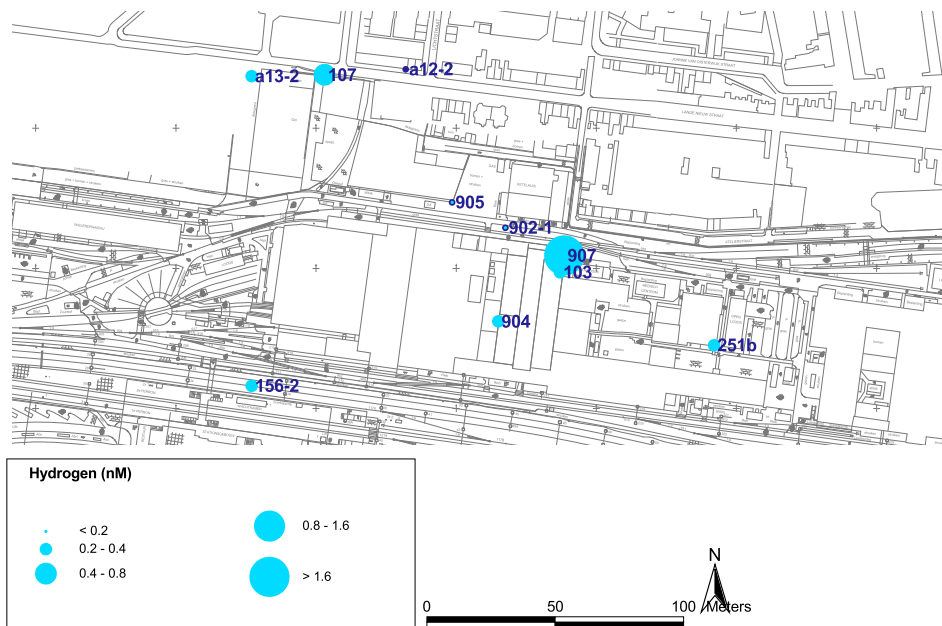
Figure 42   Spatial distribution of hydrogen concentrations at the SBNS site

SBNS Tilburg

Figure 43   Spatial distribution of chloride number at the SBNS site

Figure 44   Spatial distribution of BTEX concentrations at the SBNS site

Figure 45   Spatial distribution of total chloroethene concentrations at the SBNS site

Further spatial comparisons have been made with the plume dimensions of BTEX and chloroethenes. In figure 44 and 45 is seen that the chloroethene plume and the BTEX plume partly overlap. Since BTEX compounds might act as primary carbon

source, they can be fermented to produce hydrogen. It is therefore expected that in zones where BTEX compounds are found, hydrogen concentrations are also increased. For well 107, however, increased BTEX concentrations have only led to a small increase of hydrogen concentrations.

10.3.1   Conclusion dechlorination

In conclusion it appears that there is a very close correlation between hydrogen and BTEX on one hand and dechlorination on the other. High hydrogen concentrations (around 2 nM) which might have been caused by the presence of BTEX, seems to stimulate dechlorination processes.
This case study could be considered as a good starting point, but more analytical data are required to support a statistical interpretation and a validation of hydrogen as a tool to evaluate dechlorination.

11.   The Banisveld location

11.1   Describing the Banisveld location

11.1.1   Situation

The banisveld location is a former landfill southwestern of Boxtel (The Netherlands). From 1965 to 1977 and in 1990 and 1991 several types of waste have been dumped, including household, building, industrial and possibly chemical waste. As a result a variety of different pollutants have contaminated the soil and groundwater. In the groundwater some of these compounds are very mobile and form a potential threat for a nearby nature reserve.

11.1.2   (Geo)hydrology

The Banisveld location is situated in a region where the Nuenen formation and the formation of sterksel form the upper parts of the subsurface. The Nuenen formation is geologically complex and consists of alternating layers of fine sands, loam, and peat. The formation of Sterksel consists of gravels and sands and forms the first aquifer. At the Banisveld location the Nuenen formation forms a toplayer of 15 to 25 metres thickness. To a depth of 10 metres below groundlevel fine sands are locally alternated by a peat or loam layer. From 10 to 16 metres below groundlevel a loam layer is present together with layers of sand. Sand is found below 16 metres. The groundwater flow is strongly influenced by both the complexity of this formation and seasonal variations. During the winter the groundwater level is high and groundwater from the toplayer drains in surface waters like the Heiloop. During the summer when the groundwater level is low the Heiloop infiltrates its water to the deeper layers mainly in the direction of Smalbroeken. Additionally, a water flow is observed from the first aquifer to the Nuenen formation.

11.1.3   Contaminated situation

In previous research increased concentrations of macro chemical contaminants (chloride, chemical oxygen consumption, nitrigen, ammonium etc.), heavy metals, aromatics and chlorinated solvents have been found in the groundwater. The spreading of these contaminants is determined by the groundwater flow over years, which is a combination of winter and summer variations. In a recent project the dimension of the contaminated plume have been characterised (Boris van Breukelen, in preparation). Also the potential for natural attenuation has been determined (Nobis 95-2-09).

11.1.4   Arrangement of the wells

Eleven wells have been placed in a transect along the groundwater flowpath (see figure 46). Well p10 and p9 are considered to represent the background conditions and the source of the contaminated zone, respectively. At different depths until 10 meters, samples have been taken from these wells. In this way it was possible to make a cross-section which has been used for the redox characterisation.
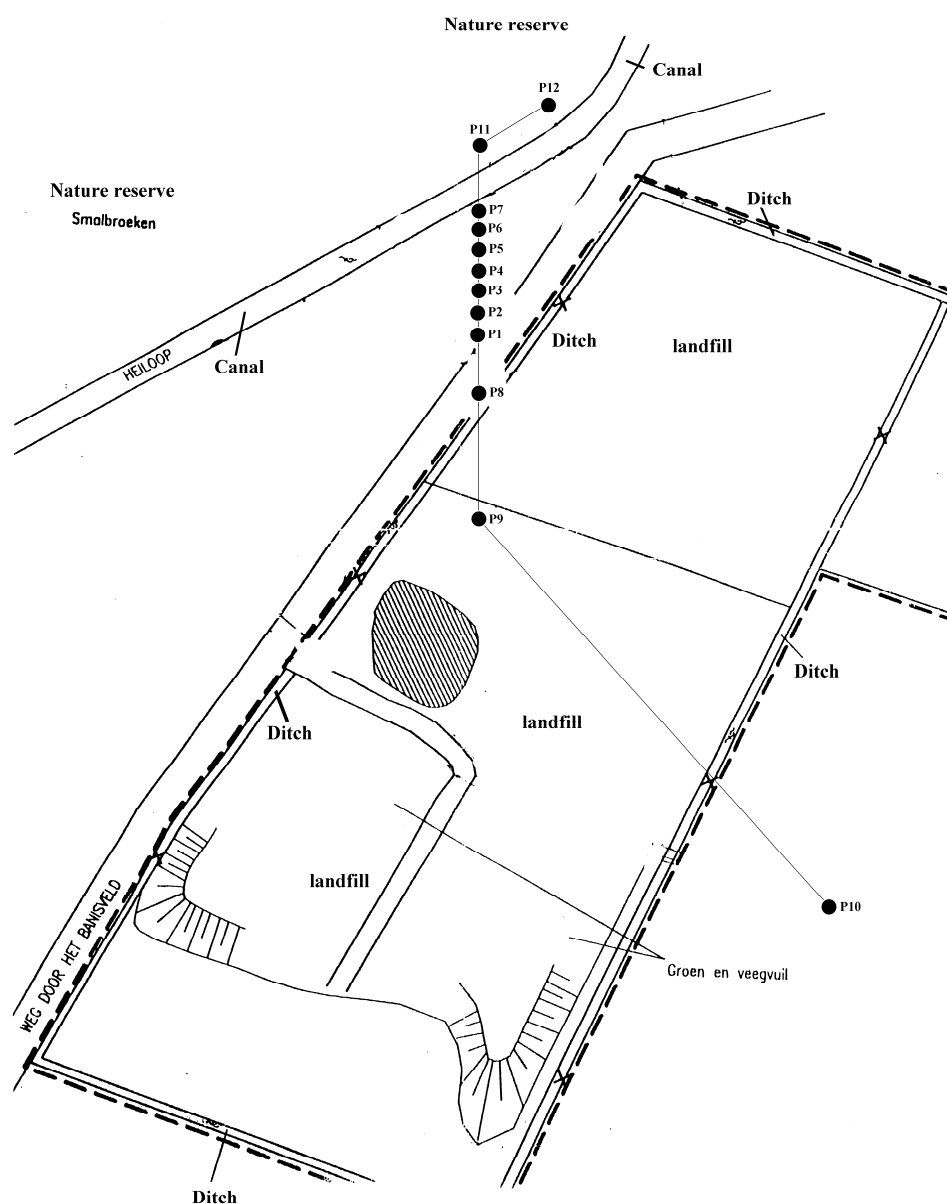
Figure 46   Map of the location including the arrangement of the wells and the transect

11.2   Identification of the redox condations at the Banisveld location

In the Banisveld case the use of hydrogen will be considered as a method to identify the local redox conditions. Two different approaches that use hydrogen will be applied on this site. The first method is based on hydrogen concentration ranges, which have been determined empirically. The second is called the partial equilibrium approach and is based on both hydrogen concentrations and concentrations of several redox species. These approaches will be validated by comparing the result to the macro chemical method and the processes in the contaminated plume. Redox potentials have not been measured at this site.

11.2.1   Macro chemistry

On basis of the various species that have been measured, redox conditions could be identified at the Banisveld location. The criteria that have been used for this location are shown in figure 47. These criteria have been determined in a site-specific way instead of the generalistic criteria for a typical location using the standard redox identification protocol (chapter 6). For setting the criteria not all species were useful, in fact, only nitrate, sulphate and methane have been used. Since the Banisveld site is a landfill, also several other contaminants are present that could have initiated several different processes and could have caused increased background concentrations of redox species like methane, sulphate, divalent iron. In this site-specific approach these increased concentrations are taken into account, by increasing the critical values and by eliminating divalent iron as criterion.

$NO_3 > 1.5$ mg/l $\downarrow$	$\rightarrow$	Nitrate reducing
$SO_4 > 50$ mg/l $\downarrow$	$\rightarrow$	Iron reducing
$SO_4 < 50$ mg/l $CH_4 < 1$ mg/l $\downarrow$	$\rightarrow$	Sulphate reducing
$SO_4 < 50$ mg/l $CH_4 > 1$ mg/l	$\rightarrow$	Methanogenic

Figure 47   Criterial for the macro chemical redox identification method

No significant amounts of oxygen have been found at the Banisveld location, indicating that oxygen reducing conditions do not take place. The presence of significant amounts of nitrate is characteristic for nitrate reducing conditions. Two groups can be identified: a group in which nitrate was absent or present in very small amounts (0-0.4 mg/l), and a group in which nitrate is present in relatively large amounts (above 17 mg/l). Conveniently, a certain critical value has been used

which in this case is 1.5 mg/l. If the conditions are not nitrate reducing, sulphate and methane can be used to make a further distinction between iron reducing, sulphate reducing, or methanogenic conditions. High sulphate concentrations indicate that sulphate reduction has not (yet) taken place and low concentrations that sulphate reduction might have taken place. Similarly, high methane concentrations are associated with methanogenesis. The determination of critical values for sulphate and methane is less straightforward as for nitrate. No clear distinction between low and high concentrations could be found, possibly due to local heterogeneity. Although there is a high probability of misinterpretation, critical values had to be determined and implemented in order to use this method. Based on the distribution of these parameters, a reasonable critical value for sulphate is 50 mg/l and for methane is 10 mg/l.

In Figure 49 redox characterisations according to the three redox identification methods are shown.
For all sampling points redox conditions have been identified using these criteria. Different redox zones can be identified: a strongly reduced zone is located inside the contaminant plume. Although methanogenic conditions seem to dominate this zone a serious probability of misinterpretation exists due to the transport of methane. Possibly, methane has been produced in the core of the landfill where truly methanogenic conditions exist and has subsequently been transported along the groundwater flow to zones in which sulphate reducing conditions exist. Therefore these zones could have been falsely characterised as methanogenic. A more oxidised zone dominated by nitrate reducing conditions has been located outside the plume at shallow depth. Sulphate and iron reducing conditions are found in deeper zones and possibly mark the background redox conditions.

11.2.2   Empirical hydrogen ranges

At the Banisveld location hydrogen has been measured throughout the entire site. Solely by comparing the hydrogen concentration to an empirically determined range, redox conditions could be identified. The following critical values have been chosen as criteria:

Table 13   Criteria for the empirical hydrogen method.

Hydrogen concentration	Redox condition
$H_2 < 0.7$ nM	iron or sulphate reducing
0.7 nM$< H_2 < 5$ nM	sulphate reducing
$H_2 > 5$ nM	methanogenic

These criteria are distinct from the standard criteria used for situations between 10 and 15°C (table 4, section 4.2.1). When applying these standard criteria to the hydrogen concentrations at the Banisveld site, no discriminative results were obtained. The majority of the sampling points would in this way be identified as methanogenic or sulphate reducing, while also nitrate and iron reducing conditions are expected (section 11.2.1). A reason to deviate from the standard criteria could

not be found, since the temperatures at the location seem to fit the indicated range. Surprisingly, the criteria do perfectly fit to empirical ranges that are based on experiments done at standard temperatures (25°C). Possibly, the high organic carbon loads percolating from the landfill into the subsurface have created non-steady-state conditions. Hydrogen levels may not be associated with dominant redox processes. Instead of using the standard criteria, criteria were based on the distribution of measured hydrogen concentrations. In figure 48 is shown that the hydrogen concentration is distributed into three distinct intervals, representing different redox processes. Values between these zones have been used as the critical hydrogen concentrations.

Distribution of the hydrogen concentration
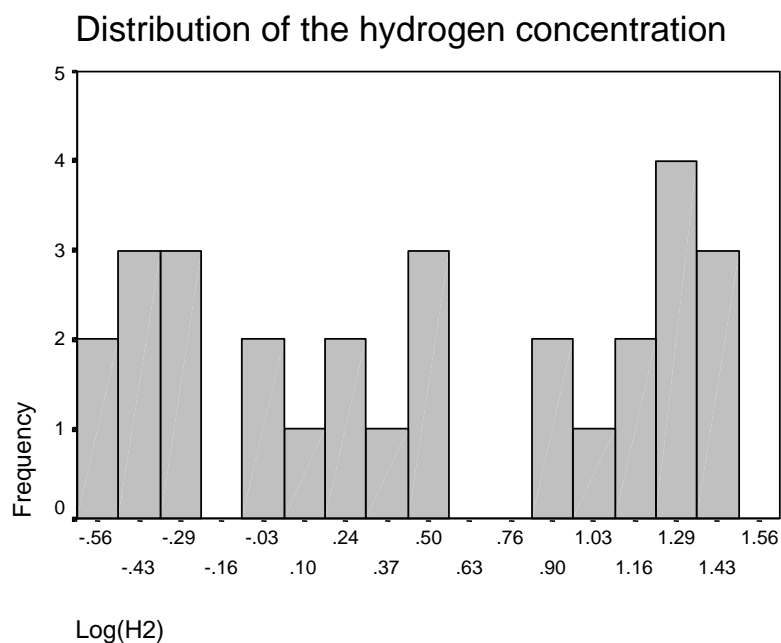
Figure 48   Distribution of hydrogen concentration into three distinct groups

Redox identification on basis of Macrochemistry

Redox identification on basis of emperical hydrogen range

Redox identification on basis of the partial equilibrium approach

Legend: ■ nitrate reducing   ■ iron reducing   ■ sulphate reducing   ■ methanogenic
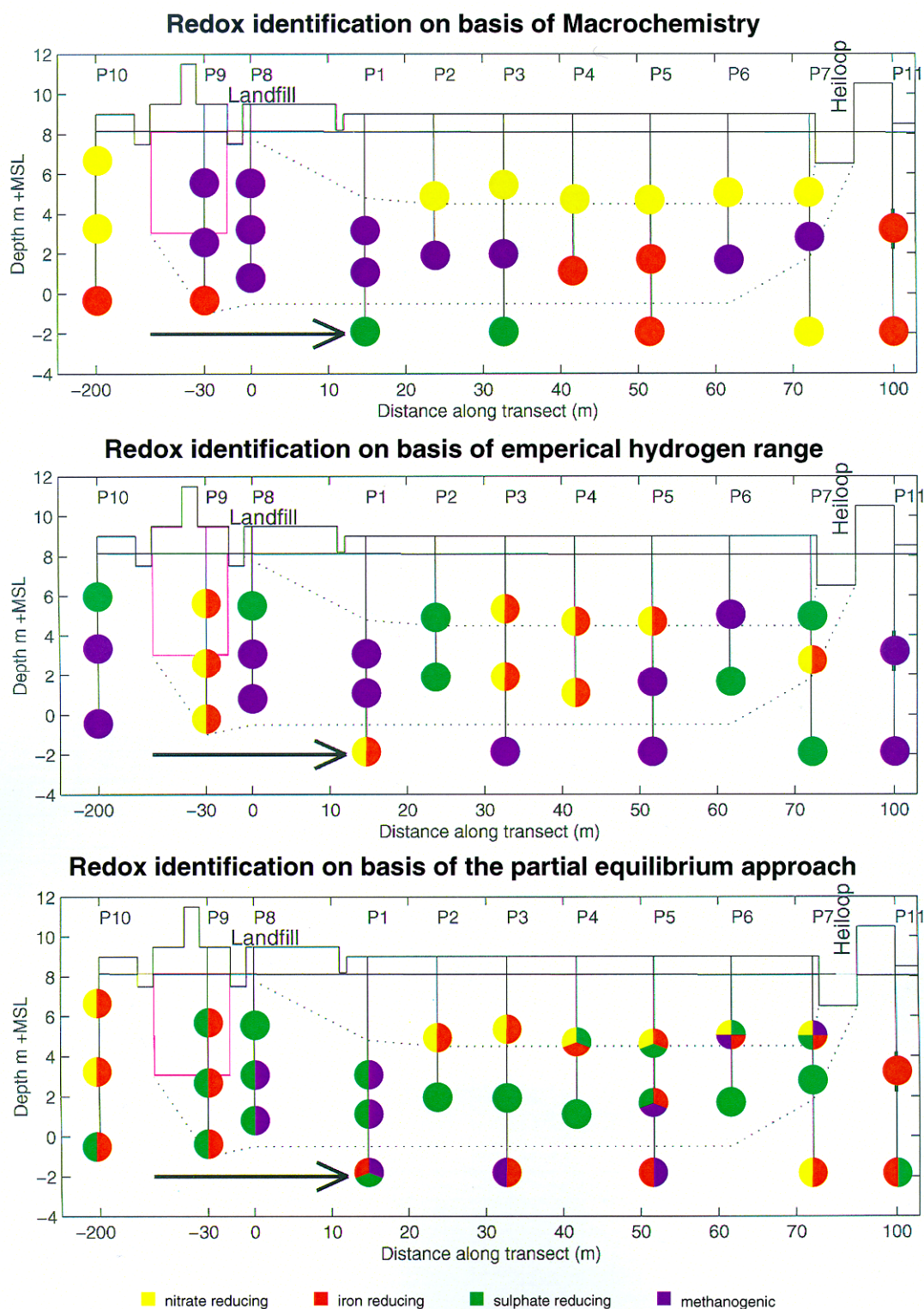
Figure 49   Spatial distribution of redox conditions according to the three methods

The group with the highest hydrogen concentrations is considered methanogenic, the one with intermediate hydrogen concentrations sulphate reducing, and the one with the lowest hydrogen concentrations corresponds to iron reducing or nitrate reducing conditions. A further discrimination between nitrate reducing and iron reducing conditions could not been made within this group, because not enough variation has been found between the values. This automatically leads to a reduction of specificity and performance of this method.

The redox zones with similar conditions that have been identified are shown in figure 49. Although there is a strong variation, redox conditions can be recognised. No logical explanation could be found for the position of the different redox zones. A less reduced zone involving nitrate and iron reducing conditions is present in the core of the landfill and between p3 and p5 close to the surface, indicating a possible influx of nitrate rich water (i.e. through rain or other infiltration). More reduced conditions i.e. sulphate reducing and methanogenic conditions, exist in other parts of the site.

11.2.3   Partial equilibrium approach

Because of the numerous measurements that have been done, a redox identification could be made following the partial equilibrium approach. Based on the concentration of hydrogen and redox species in combination with temperature and pH, Gibbs energies have been calculated for several relevant redox reactions. Depending on whether the calculated Gibbs energy is below or above the threshold value, the reaction can be considered favourable or unfavourable, respectively. Similarly to the other locations a value of -5 kJ/mol has also been selected as threshold for the Banisveld location.

The results of the partial equilibrium approach have led to a pattern in which all reactions are indicated that are considered favourable (see figure 49). Apparently, on several parts of the site, for example in the shallow parts, different redox processes are favourable and can take place simultaneously. The possibility that more reactions take place simultaneously at this location is very likely. Redox transition zones are expected when variations in the supply of electron acceptors and donors occur. A temporal influx of nitrate together with infiltrating rainwater in the upper part of the soil, might account for a combination of different processes when nitrate reducing bacteria are unable to lower the hydrogen concentration. Similarly, a temporary increase in the amount of electron donor due to a local influx of an anthropogenic carbon source can lead to temporary increased hydrogen concentrations. Due to such an increase even the less favourable reactions might become favourable. As soon as the hydrogen level drops, these reactions become less favourable again and stop.
More uniform redox conditions have been found in the contaminant plume where sulphate reducing conditions are favourable, and sometimes in combination with iron reducing or methanogenic conditions.

11.3 Comparison of the different redox identification methods

The results of the different methods that have been used to identify redox conditions do not closely match. A very large difference between the macro chemical method and the empirical hydrogen range has been found. Although it does not indicate the relative importance and dominance of redox processes, the partial equilibrium approach is more specific than the other methods since it indicates all processes that are favourable and might occur. Moreover, the partial equilibrium approach is less dependent on arbitrary criteria used for discriminating redox conditions. It is therefore a more generic method not dependent on site specific conditions. By comparing the macro chemical and emperical hydrogen redox interpretation to the redox processes that are indicated as favourable according to the partial equilibrium method, interpretations that are not very likely from a thermodynamic point of view will become clear. It appears that for the macrochemical method several interpretations are not supported by thermodynamics, since the identified processes were shown to be thermodynamically unfavourable and therefore not likely to take place. Especially for the more reduced conditions the macro chemical method fails. Nitrate reducing conditions have been determined correctly, which is logical since nitrate reducing conditions will always be determined as being favourable as long as nitrate is present.
Many interpretations have been made using the empirical hydrogen method, which are also inconsistent with the results of the partial equilibrium approach. Especially the characterisation as nitrate reducing is often not supported by thermodynamics, indicating an insensitivity of hydrogen for nitrate reducing conditions. Samples that have been identified as sulphate reducing or methanogenic with the empirical hydrogen method seems to be unfavourable according to the partial equilibrium approach. And vice versa, at points that have been identified as nitrate or iron reducing, only sulphate reducing conditions are considered favourable. The expected correlation between reduced redox conditions and the contaminated plume does not exist for the redox characterisation according to the empirical hydrogen method.

11.4 Relation between hydrogen concentration and type of carbon source

This section will focus on the association between hydrogen and the type of carbon source. Since many carbon compounds might be fermented to hydrogen and produce different hydrogen levels, an correlation between the carbon source and hydrogen concentration is expected. At the Banisveld site this might be of particular interest since many different carbon compounds are present here.
However, from the two plots (figure 50 and figure 51) it does not become clear that there is a relationship between hydrogen concentrations and DOC level or BTEX concentrations, respectively. Possibly, the actual hydrogen concentration is not only determined by the production of hydrogen by carbon sources, but also by the consumption by electron accepting processes.
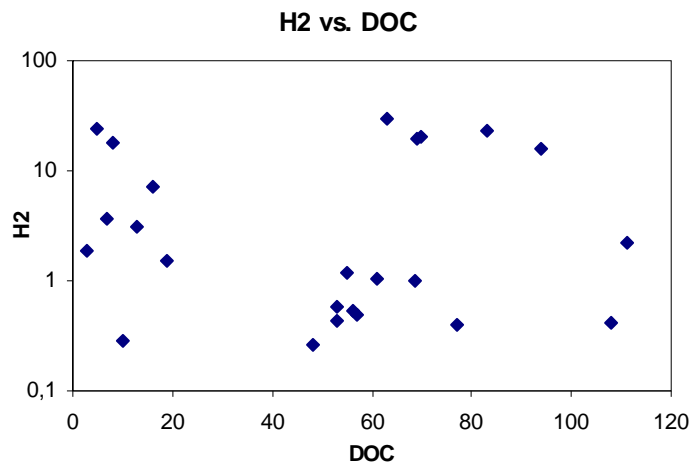
Figure 50   Plot of hydrogen against DOC
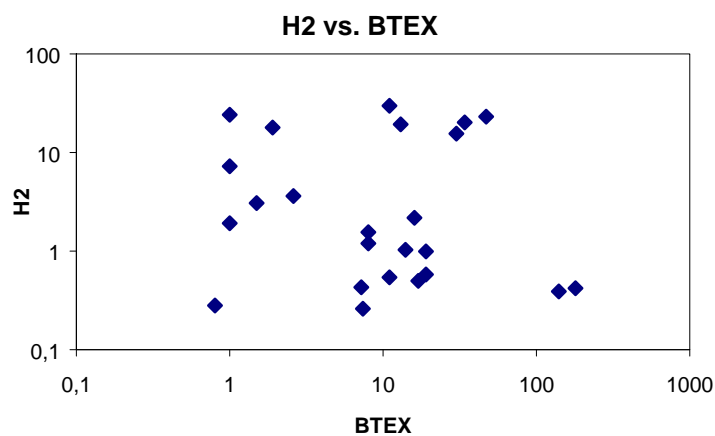
Figure 51   Plot of hydrogen against BTEX

11.5   Conclusions

The partial equilibrium approach has been proven to be a relatively site independent redox characterisation method. The macro chemical and empirical hydrogen method dependent on site-specific criteria and provided inconsistent results with the partial equilibrium approach. Both methods have been shown to determine redox conditions that from a thermodynamic point of view are unfavourable and not likely to take place. These methods are therefore considered as less useful for identifying the redox conditions at the Banisveld site. The redox identification made by

the partial equilibrium approach appears to be most consistent. Additionally, the partial equilibrium approach is able to identify zones where more processes might take place simultaneously. These zones are very likely to be formed at this location, since a non steady-state situation is expected as a result of a changing supply of electron donors and acceptors. The redox potential is partly supporting the partial equilibrium approach.

Summarising it may be concluded that the redox conditions are generally more reduced inside the contaminated plume than outside. The (temporary) influx of nitrate in the shallow regions close to the surface seems to influence the local redox conditions strongly. Furthermore the conditions inside the plume seem to be in a steady-state situation, since in many cases only one reaction is favourable at the same time. This is in contrast to transition zones in which more redox conditions are favourable simultaneously. For the Banisveld location the partial equilibrium approach has been proven to be to only method to produce a consistent redox identification.

No clear relation has been found between different carbon sources and the hydrogen concentration.

12.   Site comparison

The applicability of the different redox identification methods strongly depends on the location. For all locations, the partial equilibrium approach has been shown to be a very useful tool to identify the redox processes that can take place and to provide a less site specific dependent reference for the other methods that have been used. In contrast to the other methods, the partial equilibrium approach was able to indicate redox transition zones where more redox processes take place simultaneously. In other words it is applicable for all types of locations and gives the most specific results. An additional advantage is that the same criteria can be used for all locations and do not have to be formulated for each location separately. Therefore, the method is more objective than the other methods and less affected by the scope of the researcher. A major disadvantage is that the partial equilibrium approach is not always discriminative. It does not pin point one single dominant redox process.This was most pronounced at many parts of the Banisveld location and the SBNS location.

The performance of the other redox identification methods was more strongly depending on the type of location. The Rademarkt location is the location where the most redox identification methods were consistent with each other. Except for Eh, all other methods gave a correct redox characterisation. Apparently, the conditions at the Rademarkt are at least close to steady-state, implying that the supply and consumption of electron donors and electron acceptors are in balance. Low DOC values indicate that electron donor limitation exist and redox processes take place in sequence. This is directly reflected in the presence of redox species and hydrogen levels which validates the macro chemical method and the empirical hydrogen method at this location.

At the DAF location the redox identification according to the empirical hydrogen method was in accordance with the partial equilibrium approach. The macro chemical method gave less satisfying results, since difficulties were encountered by the formulation of the criteria. Apparently, steady-state redox zones have not been developed sufficiently to affect the concentrations of the redox species. Also highly fluctuating background concentrations of these species could be the reason for the failure of this method. Hydrogen concentrations, which adept more easily to a change of redox conditions, have been shown to be less influenced by non steady-state situations, possibly present in parts of the site. In these situations, however, the specificity is not as high as for the partial equilibrium approach. This is most pronounced in the contaminated zones where a large supply of electron donor causes a temporary situation in which more redox processes take place simultaneously, but this could not be indicated by the emperical hydrogen method.

At the SBNS location all redox identification methods gave a more or less similar pattern, with strongly reduced conditions in the core of the contaminated plume and less reduced conditions outside and at the edges of the plume. However, a

complete fit between the results of the various methods has not been found. The results of the macro chemical and the emperical hydrogen method are not always consistent, and the partial equilibrium approach was not always discriminative in these cases. The fact that the different redox identification methods indicated comparable general trends, could be an indication that the redox species, the steady-state hydrogen concentration and the redox potential responded reasonably well to changing conditions at the site.

The Banisveld location is an example of a location where both the macro chemical method and the emperical hydrogen method do not give a correct redox characterisation. The partial equilibrium approach indicates that at many parts more redox processes occur simultaneously and the site can therefore be considered to be in a non-steady state situation where the supply and consumption of electron donors and acceptors is not in balance. As a result of the various carbon sources that exist in the landfill the electron donor supply is expected to be highly fluctuating. In a same way an influx of nitrate could influence the electron acceptor supply and cause a non steady-state situation. The presence of redox species on which the macro chemical method is based might therefore not reflect the occurrence of the actual redox processes, but the ones that have taken place. Also transportation of species like methane seriously limits this method. The non steady-state situation affected the hydrogen concentration in such a way that hydrogen concentrations did not correspond to the redox processes that are taking place, but are the result of temporal fluctuations.

Summarising it can be stated that the performance of the various redox identification methods is (partly) determined by the extent to which a location is in steady-state. At locations where steady-state conditions are approached (Rademarkt), the macro chemical and empirical hydrogen methods are considered to be validated redox identification methods. At locations that are far from steady-state (Banisveld), the redox conditions could not be identified using these methods and only the partial equilibrium approach gave the consistent results. For locations that are somewhere in between those extreme situations, the validation of the macro chemical and emperical hydrogen method is less straightforward and both methods might characterise the redox conditions partly in a correct way (DAF and SBNS).

Table 14   Overview of the applicability of different redox identification methods on the four locations that have been studied.

Location	Redox potential	Macro chemistry	Emp. H_2 range	Part. equil. Appr.
Rademarkt	-	+	+	++
DAF	+/-	-	+	++
SBNS	+/-	+	+	(*)
Banisveld	not measured	-	-	++

- No consistent pattern

+ Consistent pattern; indication of dominant process

++ Consistent pattern; indication of simultaneous occurring processes

(*) Not enough data

Dechlorination processes have been studied at three locations: Rademarkt, DAF and SBNS. According to the Cl#, dechlorination takes place at these three locations, but in some parts of the location more than in others.

For the SBNS site a close association exists between dechlorination processes, redox conditions, hydrogen concentration and the type of carbon source. In some parts of the site due to the presence of BTEX compounds that acted as electron donor, decreased redox conditions have developed and resulted in increased hydrogen concentrations. This situation has been favourable for dechlorination processes. In other parts where this situation has not been present, less dechlorination has been taken place.

At the other two sites no such strong relations have been found for dechlorination processes. At the Rademarkt site only indications have been found that at relatively high hydrogen concentrations as well as under methanogenic conditions, dechlorination plays an important role. A significant relation between hydrogen levels and DOC has been found.

Also at the DAF location hydrogen concentrations are related to the presence of electron donor sources like 1,2-DCA and BTEX. In the plumes of these compounds increased hydrogen concentrations and reduced redox conditions have been observed. A relation with dechlorination could, however, not be found, probably because the conditions are favourable for dechlorination processes throughout the entire location. This is supported by the fact that no nitrate reducing conditions have been observed here. At the other two locations, these conditions did occur and might have suppressed the dechlorination process.

13.   Conclusion and outlook

As stated in the introduction, measuring hydrogen concentrations may be important in the assessment of natural attenuation potential at contaminated sites. The hydrogen concentration in groundwater may form a convenient and accurate redox parameter and may give insight into the in situ electron-donor activity, which is important for intrinsic dechlorination.

Hydrogen as convenient and accurate redox parameter

Hydrogen measurements in groundwater have proven to provide a more consistent insight into the in-situ redox conditions at contaminated sites as compared to traditional methods as redox potential (Eh) measurements with electrodes and the evaluation of the concentrations of macro chemical species in groundwater. Hydrogen measurements can be interpreted in two ways: through empirical relations between hydrogen concentration and redox conditions (the empirical method) and by combining with macro chemical data and thermodynamic calculations (the so called partial equilibrium approach).

The partial equilibrium approach is the most elaborate method and gives the most complete insight into the redox processes that potentially can occur at the site. This method also does not depend on site specific criteria. Hence, it can serve as a reference to which the results of other methods can be compared. In this project, this was done for the four sites investigated, three chlorinated solvent sites and a landfill site. The redox potential (Eh) measurements were demonstrated to give inconsistent results at three sites. The macro chemical method was inconsistent at two out of four sites tested, including one chlorinated solvent site. Hence, these two traditional methods appear to include a significant risk of erroneous interpretation. The empirical hydrogen method, a relatively simple and straightforward procedure, was demonstrated to give consistent redox results at all three sites contaminated with chlorinated solvents. For the landfill site, the results were inconsistent. The hypothesis is that this deviation was a result of the non-steady state conditions probably present in the landfill plume.

Hydrogen measurements can become an important new tool in the redox characterisation as part of the stepwise assessment of the natural attenuation potential at contaminated sites. A quick on site scan using the empirical method followed by a more detailed characterisation using the partial equilibrium method appears to be the most appropriate way. The empirical hydrogen method yields consistent redox results at the chlorinated solvent sites tested. Hence, it can serve as a method for an on site quick scan of the redox situation. The costs of this method (200 - 300 EURO per measurement, depending on sample numbers taken per day and per year) are competitive with currently used methods, i.e. standard macro chemical characterisation. In addition, the empirical method has the advantage to yield an immediate impression of the redox situation at the site. The more elaborate method

of the partial equilibrium approach, i.e. extending hydrogen measurements with macro chemical analysis and thermodynamic interpretation, can be used to further characterise in more detail. Especially in redox transition zones, this extensive method gives a more complete picture.

Additional research could further improve the hydrogen based redox characterisation. The results of the partial equilibrium approach seem to be very promising, but knowledge gaps have to be addressed in order to use the method optimally. Most important is to study the variations in and the parameter-dependency of the threshold Gibbs energy for biologically mediated electron-accepting reactions. Furthermore the energetics of different iron minerals have to be studied more thoroughly.

On most locations, the comparison of hydrogen concentrations to empirically determined ranges can be used as a single method to obtain a first impression of the redox conditions that exist. More research is needed to achieve a range which is applicable to the Dutch situation where temperatures of the groundwater range between 10 and 15°C. For the landfill site, the results of the empirical hydrogen method were inconsistent with the partial equilibrium results. The hypothesis was that this deviation was a result of the non-steady state conditions probably present in the landfill plume. Thus, redox interpretations in non-steady state situations (landfills, recently formed contaminated situations) cannot be made. On the other hand, discrepancies between the results of this method and those of the partial equilibrium approach may be indicative for such non-steady state conditions. In order to test the validity of this assumption additional research is required.

Hydrogen as indicator for the intrinsic dechlorination and in-situ electron-donor activity

Measuring hydrogen concentrations may also be valuable for evaluating intrinsic dechlorination processes. This can be done in three different ways:
−   applying thermodynamic calculations to evaluate *in situ* dechlorination reactions. In this project, most Gibbs energies for the dechlorination reactions were found to be strongly negative, which indicates that dechlorination should occur under almost any condition. This finding is inconsistent with the observations in the field. For an appropriate interpretation determination of threshold Gibbs energies that are valid for the biological dechlorination reactions is needed.
−   comparison of the degree of dechlorination with the hydrogen concentration, in order to determine a range of hydrogen concentrations for which dechlorination is considered optimally. In this project, indications were found that a significant intrinsic dechlorination occurred at sample points where the hydrogen concentrations exceeded a value of 2 nM.
−   Correlation between hydrogen concentration and diluted organic carbon concentration (DOC) or co-contaminant concentrations (BTEX, DCA) were found in several data-sets obtained at the various test sites. Hence, hydrogen may also

be used as an indicator for the in situ electron donor activity. These relations and their relevance for *in situ* dechlorination should be further explored

From the above it becomes clear that the three methods could not be fully applied in this project, since several knowledge gaps first have to be resolved. Future research should be focused on:

−   Determination of <u>threshold</u> Gibbs energies that are valid for the biological dechlorination reactions
−   Identification of optimal hydrogen range for dechlorination processes for conditions typical for the Dutch subsurface and other situations.
−   Determination of the relation between types of natural carbon sources and their hydrogen release characteristics
−

Hydrogen and enhanced natural remediation

The project performed here primarily addressed hydrogen measurements in the context of assessment of natural attenuation. Hydrogen can be considered to be also an important parameter in monitoring enhanced attenuation. The major advantage of such a monitoring is that hydrogen is a very sensitive lump-sum parameter responding immediately to changes in redox conditions, making it an effective on site monitoring tool. Introducing more reducing conditions (i.e. when infiltrating electron donor into a chlorinated solvent site) leads to an elevation of hydrogen concentration, and hydrogen can be used to monitor the effect and penetration of the electron donor in the subsurface. On the other hand, introducing more oxidised conditions (i.e. by adding nitrate or oxygen at a BTEX or mineral oil contaminated site), will result in a decrease in the hydrogen concentration. Hence, hydrogen can be used to monitor the performance of an oxidation zone. For the enhanced reductive dechlorination, hydrogen controlled supply of electron donor is probably crucial for an effective remediation. Hydrogen can be controlled by using the appropriate substrate and by using the suitable supply method. Which natural and man-made organics are most suitable as electron donors for intrinsic and enhanced bioremediation is still insufficiently clear. Laboratory and field experiments, some of which are already performed or in preparation, will bring hydrogen based in situ bioremediation again important steps further.

14.   References

Alexander, M. (1985). "Biodegradation of organic chemicals." Environmental Science Technology **18**(2): 106.

Berner, R.A. (1981). "A new geochemical classification of sedimentary environments". Journal of sedimentary petrology, **51**: 359-365.

Bradley, P. M. and F. H. Chapelle (1996). "Anaerobic mineralization of vinyl chloride in fe(III)-reducing, aquifer sediments." Environmental Science & Technology **30**(6): 2084-2086.

Bradley, P. M. and F. H. Chapelle (1997). "Kinetics of DCE and VC mineralization under methanogenic and Fe(III) reducing conditions." Environ. Sci. Technol. **31**(9): 2692-2696.

Bradley, P. M. and F. H. Chapelle (1998[a]). "Microbial mineralization of VC and DCE under different terminal electron accepting conditions." Anaerobe **4**(2): 81-87.

Bradley, P. M., F. H. Chapelle, et al. (1998[b]). "Field and laboratory evidence for intrinsic biodegradation of vinyl chloride contamination in a Fe(III)-reducing aquifer." Journal Of Contaminant Hydrology **31**(1-2): 111-127.

Bruin, W. P. d., M. J. J. Kotterman, et al. (1992). "Complete biological reductive transformation of tetrachloroethene to ethane." Appl. Environ. Microbiol. **58**: 1996-2000.

Chapelle, F.H. and Lovley, D.R. (1992). "Competitive exclusion of sulfate reduction by Fe(III)-reducing bacteria: a mechanism for producing discrete zones of high-iron groundwater". Groundwater, **30**: 29-36.

Chapelle, F.H., D.A. Vroblesky, J.C. Woodward and D.R. Lovley (1997); Practical considerations for measuring hydrogen concentrations in groundwater; Environmental Science and Technology, **31**, 2873-2877.

Conrad, R., B. Schink, et al. (1986). "Thermodynamics of H2-consuming and H2-producing metabolic reactions in diverse methanogenic environments under in situ conditions." FEMS Microbiology Ecology **38**: 353-360.

Conrad, R., Lupton, F.S. and Zeikus, J.G. (1985). Applications of environmental microbiology, **50**: 595-601.

Conrad, R., Lupton, F.S. and Zeikus, J.G. (1987). "Hydrogen metabolsm and sulfate-dependent inhibition of methanogenesis in a eutrophic lake sediment (Lake Mendota)". FEMS microbiological ecology, **45**: 107-115.

Cord-Ruwisch, R., H.-J. Seitz, et al. (1988). "The capacity of hydrogenotrophic anaerobic bacteria to compete for traces of hydrogen depends on the redox potential of the terminal electron acceptor." Arch. Microbiol. **149**: 350-357.

DiStefano, T. D., J. M. Gossett, et al. (1991). "Reductive dechlorination of high concentrations of tetrachloroethene to ethene by an anaerobic enrichment culture in the absence of methanogenesis." Appl. Environ. Microbiol. **57**(8): 2287-2292.

DiStefano, T. D., J. M. Gossett, et al. (1992). "Hydrogen as an electron donor for dechlorination of tetrachloroethene by an anaerobic mixed culture." Appl. Environ. Microbiol. **58**(11): 3622-3629.

Fennell, D. E., J. M. Gossett, et al. (1997). "Comparison of butyric acid, ethanol, lactic acid, and propionic acid as hydrogen donors for the reductive dechlorination of tetrachloroethene." Environmental Science & Technology **31**(3): 918-926.

Freedman, D. L. and J. M. Gossett (1989). "Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions." Appl. Environ. Microbiol. **55**: 2144-2151.

Froelich, P. N., G. P. Klinkhammer, et al. (1979). "Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. " Geochimica et cosmochimica acta **43**: 1075-1090.

Gerritse, J., G. Kloetstra, et al. (1997). "Complete degradation of tetrachloroethene in coupled anoxic and oxic chemostats." Appl. Microbiol. Biotechnol. **48**(4): 553-562.

Gerritse, J., V. Renard, et al. (1995). "Complete degradation of tetrachloroethene by combining anaerobic dechlorinating and aerobic methanotrophic enrichment cultures." Applied Microbiology and Biotechnology **43**(5): 920-928.

Gerritse, J., V. Renard, et al. (1996). "*Desulfitobacterium* sp. strain PCE1, an anaerobic bacterium that can grow by reductive dechlorination of tetrachloroethene or ortho-chlorinated phenols." Arch. Microbiol. **165**: 132-140.

Goodwin, S., Conrad, R. and Zeikus, J.G. (1988). "Relation of pH and microbial hydrogen metabolism in diverse sedimentary eco-systems". Applications of environmental microbiology, **54**: 590-593.

Gottschalk, G. (1986). Bacterial methabolism, second edition, Springer-Verlag.

Hoehler, T. M., M. J. Alperin, et al. (1994). "Field and laboratory studies of methane oxidation in an anoxic marine sediment: evidence for methanogen-sulfate reducer consortium." Global biogeochemical cycles **8**(4): 451-463.

Hoehler, T. M., M. J. Alperin, et al. (1998). "Thermodynamic control on hydrogen concentrations in anoxic sediments." Geochimica et cosmochimica acta **62**(10): 1745-1756.

Holliger, C. and W. Schumacher (1994). "Reductive dehalogenation as a respiratory process." Antonie van Leeuwenhoek **66**: 239-246.

Iannotti, E. L., D. Katkewite, et al. (1973). "Glucose fermentation products of Ruminococcus albus grown in continous culture with Vibrio succinogenes:changes caused by interspecies transfer of H2." Journal of bacteriology **114**: 1231-1240.

Jakobsen, R., H-J. Albrechtsen, M. Rasmussen, H. Bay, P.L. Bjerg and T.H. Christensen (1998); H_2-concentrations in a landfill leachate plume (Grindsted, Denmark): *In situ* energetics of terminal electron acceptor processes; Environmental Science and Technology, **32**, 2142-2148.

Lide, D. R. and H. P. R. Frederikse, Eds. (1995). CRC Handbook of Chemistry and Physics. Boca Raton, Ann Arbor, London, Tokyo, CRC Press.

Linberg, R.D. and Runnells, D.D. (1984). "Groundwater redox reactions: an analysis of equilibrium state applied to Eh measurements and geochemical modelling". Science, **225**: 925-927.

Lovley, D. R., D. F. Dwyer, et al. (1982). "Kinetic analysis of competition between sulphate reducers and methanogens for hydrogen in sediments." Applications of environmental microbiology **43**: 1373-1379.

Lovley, D.R. and Klung, M.J. (1982). "Intermediary metabolism of organic matter in the sediments of a eutrophic lake". Applications of environmental microbiology, **43**: 552-560.

Lovley, D. R. and S. Goodwin (1988). "Hydrogen concentrations as an indicator of the predominant terminal electron-accepting reactions in aquatic sediments." Geochimica et cosmochimica acta **52**: 2993-3003.

Lovley, D.R. and Philips, E.J.P. (1987). "Competitive mechanisms for the inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments". Applications of environmental microbiology, **53**: 2636-2641.

Lyngkilde, J., Christensen, T.H., Skov, B. and Foverskov, A. (1991). "Redox zones downgradient of a landfill and implications for biodegradations of organic

compounds". In: R.E. Hinchee and R.F. Olfenbuttel (Editors), In situ bioreclamation applications and investigations for hydrocarbon and contaminated site remediation. Butterworth-Heineman, Stoneham, MA.

Maymogatell, X., V. Tandoi, et al. (1995). "Characterization of an H-2-utilizing enrichment culture that reductively dechlorinates tetrachloroethene to vinyl chloride and ethene in the absence of methanogenesis and acetogenesis." Appl. Environ. Microbiol. **61**(11): 3928-3933.

Middeldorp, P. J. M., M. A. van Aalst, et al. (1998). "Stimulation of reductive dechlorination for in situ bioremediation of a soil contaminated with chlorinated ethenes." Water Sci. Technol. **37**(8): 105-110.

Mohn, W. W. and J. M. Tiedje (1992). "Microbial reductive dehalogenation. " Microbiol. Rev. **56**: 482-507.

Ponnamperuma, F. N. (1972). "The chemistry of submerged soils." Advances in agronomy **24**: 29-96.

Postma, D. and Jakobsen, R. (1996). "Redox zonation: equilibrium constraints on the Fe(III)/SO4-reduction interface". Geochimica et cosmochimica acta, **60**(17): 3169-3175.

Reeburgh, W. S. (1983). "Rates of biogeochemical processes in anoxic sediments." Ann. rev. Earth Planetary Science letters **11**: 269-298.

Rijnaarts, H.H.M., J.H. de Best, H.C. van Liere and T.N.P. Bosma (1998); Intrinsic biodegradation of chlorinated solvents: from thermodynamics to field. Report number 96004; CUR/NOBIS, P.O.Box 420, 2800 AK Gouda.

Schink, B. (1997). "Energetics of syntrophic cooperation in methanogenic degradation." Microbiology and Molecular Biology Reviews **61**(2): 262-280.

Scholz Muramatsu, H., A. Neumann, et al. (1995). "Isolation and characterization of *Dehalospirillum multivorans* gen. nov., sp. nov., a tetrachloroethene-utilizing, strictly anaerobic bacterium." Arch. Microbiol. **163**(1): 48-56.

Schulz, S. and R. Conrad (1996). "Influence of temperature on pathways to methane production in the permanently cold profundal dediment of lake Constance." FEMS microbiology Ecology **20**: 1-14.

Sinke, A.J.C., Heimovaara, T.J., Tonnaer, H. and Veen, H.J.v., 1998. "Beslissings-sondersteunend model ten behoeve van de acceptatie van natuurlijke afbraak als saneringsvariant". Report number 97102; CUR/NOBIS, P.O. Box 420, 2800 AK Gouda.

Smatlak, C. R., J. M. Gossett, et al. (1996). "Comparative kinetics of hydrogen utilization for reductive dechlorination of tetrachloroethene and methanogenesis in an anaerobic enrichment culture." Environmental Science & Technology **30**(9): 2850-2858.

Stumm, W. and J. J. Morgan (1981). Aquatic chemistry. New York, J. Wiley &Sons.

Thauer, R. K. and G. Morris (1984). Methabolism of chemotrophic anaerobes: old views and new aspects. The microbe. procaryotes and eukaryotes. M. Florkin and H. S. Mason, Cambroidge university press.

Vogel, T. M. and P. L. McCarty (1985). "Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinylchloride, and carbondioxide under methanogenic conditions." Appl. Environ. Microbiol. **49**: 1080-1083.

Vroblesky, D. A. and F. H. Chapelle (1994). "Temporal and spatial changes of terminal electron-accepting processes in a petroleum hydrocarbon-contaminated aquifer and the significance for contaminant biodegradation." Water resources research **30**: 1561-1570.

Westerman, P. (1994). "The effect of incubation temperature on steady-state concentrations of hydrogen and volatile fatty acids during anaerobic degradation in slurries from wetland sediments". FEMS Microbiology Ecology, **13**: 295-302.

Wiedemeier, T.H. et al. (1997). "Overview of the technical protocol for natural attenuation of chlorinated alifatic hydrocarbons in groundwater under development for the U.S. airforce center for environmental excellence".

Wu, W. M., J. Nye, et al. (1998). "Anaerobic dechlorination of trichloroethylene (TCE) to ethylene using complex organic materials." Water Research **32**(5): 1445-1454.

Yang, Y. R. and P. L. McCarty (1998). "Competition for hydrogen within a chlorinated solvent dehalogenating anaerobic mixed culture." Environmental science and technology **32**(22): 3591-3597.

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Appendix A

Thermodynamic calculations

The energy micro-organisms gain from dechlorination and redox reactions can be calculated thermodynamically as Gibbs (free) energies. By calculating the Gibbs energy for each reaction, terminal electron accepting processes can be classified as energetically favourable or as energetically unfavourable.

Recall the general form of the redox or dechlorination reaction from section 2.1:

$$m \cdot OX_1 + p \cdot RED_2 = n \cdot RED_1 + q \cdot OX_2$$
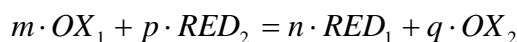
The Gibbs energy which is associated with this reaction is calculated in the following way:

$$\Delta G_r = \Delta G^0 + RT \ln \frac{[RED_1]^n \cdot [OX_2]^q}{[OX_1]^m \cdot [RED_2]^p}$$

Where ΔG_r is the Gibbs energy of the reaction, ΔG^o the Gibbs term at standard temperature, R the gas constant, T the temperature, and between [ ] the concentrations of the species. For natural redox reactions, such Gibbs equations are listed in the next table. ΔG^o is calculated from the Gibbs energies of formation of the individual species, which are listed for relevant species (see thermodynamic facts):

$$\Delta G^0 = \sum \Delta G_f^0 (products) - \sum \Delta G_f^0 (substrates)$$

Since ΔG^o is temperature dependent, a correction must be made. This is done by the Van't Hoff equation in the form:

$$\Delta G^{0T_2} = \frac{\Delta H^0 (T_1 - T_2) + T_2 \Delta G^{0T_1}}{T_1}$$

Where, ΔH^o is the enthalpy of the reaction, and T_1 and T_2 the standard and actual temperature, respectively. Since hydrogen is the electron donor of primary interest, the Gibbs energies of reactions have to be calculated per mol hydrogen. Apart from the concentrations of reactants and products, also the in situ conditions (pH and temperature) influence the Gibbs energy of the reactions. The calculated Gibbs energy is thus not a constant value for the entire system, but rather a parameter that applies to one single reaction under specific conditions. Of particular concern in Gibbs energy calculations for iron and manganese reducing reactions is the existence of several different types of iron and manganese oxides. Each of these oxides have a different reactivity and Gibbs energy of formation. It is therefore essential to consider differences in mineralogy and if possible, make a entire characterisation of the oxides involved.

On basis of the calculated Gibbs energy, terminal electron accepting processes are classified as favourable if the calculated Gibbs energy is lower than a threshold value and considered unfavourable if exceeding this value. For redox and dechlorination reactions, this value is between -5 and -20 kJ/mol. The origin of this value is discussed in section 2.2.2.

A second application of thermodynamics is to calculate the minimum hydrogen concentrations, which are required for dechlorination and redox reactions to occur. For each terminal electron process this minimum hydrogen concentration can be calculated with use of the Gibbs energy equation. The minimum hydrogen concentration corresponds to the calculated hydrogen concentration when the Gibbs energy is at the critical value. In the general form of the Gibbs energy equation, the term RED_2 corresponds to the hydrogen concentration.

$$\Delta G_r = \Delta G^0 + RT \ln \frac{[RED_1]^n \cdot [OX_2]^q}{[OX_1]^m \cdot [H_2]^p}$$

In another form it becomes:

$$\Delta G_r = \Delta G^0 + RT \ln \frac{[RED_1]^n \cdot [OX_2]^q}{[OX_1]^m} + RT \ln \frac{1}{[H_2]^p}$$

Where, ΔG_r is between -5 kJ/mol and -20 kJ/mol and ΔG^o, R, T, RED_1, OX_1 and OX_2 are known parameters. The minimum hydrogen concentration is the only remaining variable and can thus be calculated. This extrapolation of hydrogen ranges is only validated in situations where hydrogen concentrations are negligible compared to the concentrations of the other components. In these situations the second term in the Gibbs equation is expected to remain constant. Commonly, the hydrogen concentrations in natural environments are in the nM range and the concentrations of other species several factors higher. Therefore it is expected that such low hydrogen concentrations do not affect the concentrations of the other species, and that the second term remains constant. The concentration H^+, though will be in the same range as hydrogen, but is expected to be buffered. It is important to realise that the calculated minimum hydrogen concentration, associated with a terminal electron accepting process, apply to one particular situation with unique concentrations and conditions. The calculated minimum hydrogen concentrations must therefore not be generalised.

Gibbs equations for important redox and dechlorination reactions

Oxygen reduction	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{1}{\left[O_2\right]\left[H_2\right]^2}$
Nitrate reduction (I)	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{1}{\left[NO_3^-\right]^2\left[H_2\right]^5\left[H^+\right]^2}$
Nitrate reduction (II)	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{\left[NO_2^-\right]}{\left[NO_3^-\right]\left[H_2\right]}$
Nitrate reduction (III)	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{\left[NH_4^+\right]}{\left[NO_3^-\right]\left[H_2\right]^4\left[H^+\right]^2}$
Manganese oxide reduction	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{\left[Mn^{2+}\right]}{\left[H_2\right]\left[H^+\right]^2}$
Iron hydroxide reduction	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{\left[Fe^{2+}\right]^2}{\left[H_2\right]\left[H^+\right]^4}$
Sulfate reduction	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{\left[HS^-\right]}{\left[H_2\right]^4\left[SO_4^{2-}\right]\left[H^+\right]}$
Methanogenesis	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{\left[CH_4\right]}{\left[HCO_3^-\right]\left[H_2\right]^4\left[H^+\right]}$
PCE dechlorination	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{\left[TCE\right]\left[Cl^-\right]\left[H^+\right]}{\left[PCE\right]\left[H_2\right]}$
TCE dechlorination	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{\left[DCE\right]\left[Cl^-\right]\left[H^+\right]}{\left[TCE\right]\left[H_2\right]}$
DCE dechlorination	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{\left[VC\right]\left[Cl^-\right]\left[H^+\right]}{\left[DCE\right]\left[H_2\right]}$
VC dechlorination	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{\left[ETHENE\right]\left[Cl^-\right]\left[H^+\right]}{\left[VC\right]\left[H_2\right]}$
ethene reduction	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{\left[ETHANE\right]}{\left[ETHENE\right]\left[H_2\right]}$
Iron hydroxide reduction and formation of siderite	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{1}{\left[HCO_3^-\right]^2\left[H_2\right]\left[H^+\right]^2}$
Iron hydroxide reduction and formation of pyrrhotite	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{1}{\left[HS^-\right]^2\left[H_2\right]\left[H^+\right]^2}$
Sulfate reduction and formation of pyrrhotite	$\Delta G_r = \Delta G^0 + RT \ln \dfrac{1}{\left[Fe^{2+}\right]\left[H_2\right]^4\left[SO_4^{2-}\right]}$

Thermodynamic facts

Thermodynamic calculations are based on the Gibbs energy of formation and enthalpy of formation. These data are shown for the relevant species. Values are in kJ/mol and apply to the specie in solution (unless noted else).

	Specie	ΔG_f^0	ΔH_f^0
	O_2	16.32	-11.71
	H_2	17.57	-4.18
	H+	0	0
liquid	H_2O	-237.18	-285.83
	NO^{3-}	-111.3	-207.3
gas	N_2	0	0
	NH^{4+}	-79.37	-132.5
	NO^{2-}	-37.2	-104.6
Pyrolusite	MnO_2	-465.1	-520
	Mn^{2+}	-228	-220.7
Goethite	FeOOH	-489	-559.3
Amorphous	FeOOH	-452	
Siderite	$FeCO_3$	-666.7	-737
Pyrrhotite	FeS	-100.4	-100
	Fe^{2+}	-78.87	-89.1
	SO_4^{2-}	-744.6	-909.2
	H_2S	-27.87	-39.75
	HS^-	12.05	-17.6
	HCO^{3-}	-586.8	-692
	CH_4	-34.39	-89.04
	CO_2	-394.37	-393.5
	Cl^-	-131.3	-167.2
	H_2	17.57	-4.18
	PCE	*27.59*	**-12.43**
	TCE	*25.41*	**-19.1**
	cDCE	*27.8*	**4.27**
	VC	*59.65*	**29**
	ethene	*81.43*	**52.47**
	ethane	*-17.43*	**-83.85**

Source: Stumm and Morgan, 1981
             Dolfing and Janssen, 1994
             http://webbook.nist.gov

Example of a thermodynamic calculation

The redox reaction in which PCE is dechlorinated with H_2 as electron donor becomes:

$$
\begin{array}{l}
PCE + H^+ + 2e^- = TCE + Cl^- \\
H_2 = 2H^+ + 2e^- \\
\hline
PCE + H_2 = TCE + H^+ + Cl^-
\end{array}
$$
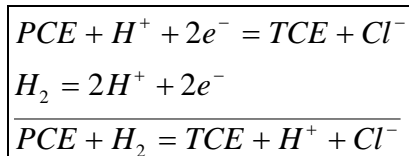
In this example the following specie concentrations are assumed:
- [PCE] = $2 \cdot 10^{-4}$ M
- [TCE] = 10^{-4} M
- [H_2] = 10^{-9} M
- [Cl$^-$] = 10^{-3} M

And the following conditions:
- pH = 7   ([H^+] = 10^{-7} M)
- T = 298.15 K (25° C)

The following constants and values were used:
- R = 8.31451 J/mol
- ΔG^o_f (PCE) = 27.6 kJ/mol
- ΔG^o_f (TCE) = 25.5 kJ/mol
- ΔG^o_f (H_2) = 17.8 kJ/mol
- ΔG^o_f (H^+) = 0   kJ/mol
- ΔG^o_f (Cl$^-$) = -131.3   kJ/mol

(The ΔG^o_f values all apply to the aqueous phase at 298.15 K)

From this information it is now possible to calculate the energy which is involved in the redox reaction mentioned above.

$$
\Delta G_r = \Delta G^0 + RT \ln \frac{[TCE] \cdot [H^+] \cdot [Cl^-]}{[PCE] \cdot [H_2]}
$$

$$
\Delta G_r = \left[\Delta G^0_f(TCE) + \Delta G^0_f(H^+) + \Delta G^0_f(Cl^-) \right] - \left[\Delta G^0_f(PCE) + \Delta G^0_f(H_2) \right] +
$$

$$
RT \ln \frac{[TCE] \cdot [H^+] \cdot [Cl^-]}{[PCE] \cdot [H_2]}
$$

$$
\Delta G_r = (25.5 + 0 - 131.3) - (27.6 + 17.8) + RT \ln \frac{10^{-4} \cdot 10^{-7} \cdot 10^{-3}}{2 \cdot 10^{-4} \cdot 10^{-9}} = -158.6 \quad kJ/mol
$$

Appendix B

Analytical results

Chapter 8:   Rademarkt, Groningen.

well	depth (m)	T (°C)	pH	EC (µS/cm)	Eh (mV)	O₂ (mg/l)	H₂ (nM)
Demo	6,00-7,00	12,3	6,70	2060	4	0,28	0,36
B2	5,65-6,65	13,0	6,70	1525	32	0,88	0,29
G2	5,95-6,95	13,9					0,18
259	4,60-5,60	12,5	7,00	1800	140	0,25	0,49
404	5,00-7,00	12,3	6,90	1820	-134		0,37
244	8,10-9,10	11,8	7,00	1777	140	0,25	0,17
403	5,00-7,00	11,9	7,10	1802	90	0,29	0,11
304	4,00-5,00	12,7					0,17
401	5,00-7,00	13,6	6,90	2080	-109	0,27	0,12
247	4,90-5,90	13,7	7,10	1718	-122	0,14	7,29
303	5,00-7,00	13,7	6,80	3130	44	0,18	0,09
215	7,50-8,50	13,0	7,00	1920	135	0,11	0,14

well	NO_3^-N (mg/l)	SO_4^{2-} (mg/l)	Cl^- (mg/l)	TOT-S (mg/l)	DOC (mg/l)	H_2CO_3 (mg/l)	Fe(II) (mg/l)
Demo	< 0,04	140	300		13	640	0,21
B2	< 0,04	75	170		12	660	0,1
G2	4,8	73	160		9,8	640	< 0,05
259	< 0,04	170	180		18	720	10
404	< 0,04	0,39	150	< 0,10	21	1200	11
244	6,7	58	290	< 0,10	8,7	490	< 0,05
403	8,3	41	370		8,8	400	< 0,05
304	< 0,04	7,5	260		15	860	4,7
401	1,65	180	250		13	910	3,8
247	< 0,04	1,3	160	< 0,10	18	1000	7,4
303	6,3	150	520		7,3	910	< 0,05
215	0,76	89	280		11	610	< 0,05

Chapter 8: Rademarkt, Groningen.

well	methane (µg/l)	eth-ane(µg/l)	ethene (µg/l)	VC (µg/l)	cis-DCE (µg/l)	TCE (µg/l)	PCE (µg/l)
Demo	61,1	1,3	0,0	17,1	2655,6	1999,3	2414,8
B2	18,6	2,1	0,0	5,5	520,5	167,3	166,2
G2	3,8	0,2	0,0	0,9	161,6	57,5	276,4
259	150,4	0,1	0,0	0,0	0,0	5,5	6,8
404	5989,9	1,5	0,6	0,0	0,0	4,8	13,2
244	4,4	0,1	1,0	1,1	109,8	146,9	1990,4
403	65,2	0,0	0,0	0,0	23,6	5,1	9,8
304	1239,1	6,8	0,0	0,0	0,0	3,3	5,0
401	276,0	0,3	0,0	0,0	19,6	4,7	4,8
247	14,4	1,2	9,6	0,0	0,0	4,3	2,7
303	124,8	0,0	0,0	0,0	37,2	8,7	15,4
215	107,1	0,8	0,2	4,3	1226,1	681,2	1213,7

Chapter 9:   DAF, Eindhoven.

well	depth (m)	T (°C)	pH	EC (µS/cm)	Eh (mV)	O$_2$ (mg/l)	H$_2$ (nM)
NOB5-1	6 - 7	14	7		66	2.8	0.46
C4-2	6.2 - 7.2	14	6		-60	2.8	0.23
C40-1	5.8 - 6.8	14	8		164	0	4.77
C40-2	9 - 10	15	7.8		-177	0	1.09
C47-2	7 - 8	14	6.4		-107	1	0.49
NOB2-1	6 - 7	14	3.8		-94	0	3.07
NOB3-1	6 - 7	13	6.7		0.2	2.5	0.3
C33-1	6 - 7	14	6.5		-150	0	0.2
NOB1-1	6 - 7	14	6.9		-167	1.3	0.8
C32-1	6 - 7	14	6.7		-130	1.3	0.18
B12	6.4 - 7.4	14	6.8		-133	2.8	0.4
C47-3	16 - 17	14	6.4		-170	6.4	0.5
C46-2	13 - 14	15	7.2				0.29
NOB2-2	14 - 15	15	6.4		-132	1.5	4.92
NOB3-2	14 - 15	13	6.9		-183	4.5	0.29
C45-3	14 - 15	14	4.4		-141	0.3	0.46
C27-2	15 - 16	14	6.9		-99	3.4	0.13
C33-2	14 - 15	14	7		-155	2.5	0.1
C39-2	15 - 16	14	6.4		-121	2.6	0.23
C41-1	14 - 15	14	6.9		-167	4.4	0.11
NOB4-2	14 - 15	13	6.3		-100	0.2	0.53
C4-3	14.2 - 15.2	14	7		-80	4.1	0.63
C32-2	14 - 15	14	6.9		-80	0	0.25
C48-3	14 - 15		7.3		-105	0	0.44
B12	14 - 15	14	6.7		-139	1.5	0.13
C51-3	14 - 15	14	6.5		-50	1.3	1.01
C47-4	29 - 30	14	6.7		-166	1.2	0.54
NOB2-3	29 - 30	15	6.6		-120	0.2	1.15
NOB3-3	29 - 30	13	7.3		-198	3.8	2.49
C27-3	28 - 30	14	6.7		-130	1.5	0.27
NOB1-3	29 - 30	16	6.8		-165	1.3	0.4
C41-2	29 - 30	14	7.1		-176	4	0.25
C48-4	29 - 30		6.5				4.25
NOB4-3	29 - 30	13	6.6				0.66
C61	44 - 45	15	6.2		-45	0	1.3
C61	63.5 - 64.5	15	6.9		-120	0	0.58

Chapter 9:   DAF, Eindhoven.

well	NO$_3^-$N (mg/l)	SO$_4^{2-}$ (mg/l)	Cl$^-$ (mg/l)	NO$_2^-$ (mg/l)	DOC (mg/l)	HCO$_3$ (mg/l)	Fe(II) (mg/l)
NOB5-1	0.2	350	56	0.2		5	0.3
C4-2	0.2	22	14	0.2			1.8
C40-1	0.2	98	220	0.2		390	28
C40-2	0.2	97	230	0.2		210	14
C47-2	0.2	120	56	0.2		59	0.5
NOB2-1	0.3	52	490	0.2		190	19
NOB3-1	0.2	33	60	0.2			0.3
C33-1	0.2	81	210	0.2		69	0.3
NOB1-1	0.2	140	10	0.2		46	0.3
C32-1							
B12	0.2	14	38	0.2		23	0.3
C47-3	0.2	21	190	0.2		22	2
C46-2	0.2	86	250	0.2		180	23
NOB2-2	0.2	40	48	0.2		45	0.3
NOB3-2	0.2	21	36	0.2			0.6
C45-3	0.2	10	15	0.2		34	1.2
C27-2	0.31	10	40	0.2		22	0.3
C33-2	0.2	16	5.2	0.2		5	0.4
C39-2	0.2	53	170	0.2		65	0.3
C41-1	0.2	27	56	0.2			0.4
NOB4-2	0.2	22	14	0.2			1.8
C4-3							
C32-2							
C48-3							
B12	0.2	13	44	0.2		13	0.3
C51-3	0.2	28	14	0.2			1.4
C47-4	0.2	11	23	0.2		5	0.7
NOB2-3	0.2	80	180	0.2		110	7.5
NOB3-3	0.2	13	21	0.2			0.3
C27-3	0.3	34	32	0.2		61	0.7
NOB1-3	0.2	18	21	0.2		5	1.9
C41-2	0.2	13	47	0.2			0.3
C48-4							
NOB4-3							
C61	0.2	660	16	0.2			1
C61	0.2	30	10	0.2			0.3

Chapter 9:   DAF, Eindhoven.

well	methane (µg/l)	ethane (µg/l)	ethene (µg/l)	VC (µg/l)	cis-DCE (µg/l)	TCE (µg/l)	PCE (µg/l)
NOB5-1	25	0.25	0.25	0.5	2.5	0.6	0.2
C4-2	100	0.46	1.2	0.5	1	0.2	0.2
C40-1	89	6.3	70	1200	340000	2900	33
C40-2	610	1.3	1.9	310	67000	540	25
C47-2	38	0.25	0.59	4.4	5.6	0.2	0.2
NOB2-1	320	800	0.79	19	29	0.7	0.2
NOB3-1	25	0.25	0.25	0.5	1	0.2	0.2
C33-1	420	3.8	10	52	3500	5700	90
NOB1-1	25	0.25	0.47	2.6	7.9	4.3	0.2
C32-1	250	22	1000				
B12	410	0.25	0.25	0.5	1	0.2	0.2
C47-3	3300	27	420	5200	43000	82	4.7
C46-2	340	0.85	68	56	76	0.5	0.2
NOB2-2	25	0.25	0.25	0.5	1	0.2	0.2
NOB3-2	1600	0.25	0.25	0.5	1	0.2	0.2
C45-3	13000	5.8	0.25	9.2	63	110	5.6
C27-2	25	0.25	0.25	0.5	1	0.2	0.2
C33-2	450	0.25	0.25	0.5	1	0.2	0.2
C39-2	520	0.44	81	780	460	1.8	0.2
C41-1	25	0.25	0.25	0.5	1	0.2	0.2
NOB4-2	16000	0.28	0.25	0.5	1	0.2	0.2
C4-3	< 25	0.25	0.25				
C32-2	45	0.25	15				
C48-3							
B12	210	0.25	0.25	0.5	2.3	1.3	0.2
C51-3	8400	0.25	0.25	0.5	1	0.7	0.2
C47-4	860	0.25	0.25	10	14	0.2	0.2
NOB2-3	540	1.9	93	1200	5500	81	0.2
NOB3-3	780	0.25	0.25	0.5	1	0.2	0.2
C27-3	25	0.25	2.8	55	100	70	140
NOB1-3	2400	0.25	0.6	5.2	4.6	0.2	0.2
C41-2	2500	0.25	0.25	0.5	1	0.2	0.2
C48-4							
NOB4-3	11000	0.25	0.25				
C61	12000	0.96	0.25	2.3	9.2	0.2	0.2
C61	25	0.25	0.25	0.5	1	0.2	0.2

Chapter 10:   SBNS, Tilburg.

well	depth (m)	T (°C)	pH	EC (µS/cm)	Eh (mV)	O_2 (mg/l)	H_2 (nM)
156-2	5.5	11.5	5.8		40		0.28
251b	6	12.4	6.2		185		0.42
907	6	12.2	6.7		- 50	0.2	2.14
103	6	15.0	6.8		- 75	0.1	0.31
904	6,5	17.1	5.8		- 18	0.1	0.37
902-2	5 - 6	13.5	6.4		310		0.31
905	5 - 6	12.1	6.3		312		0.16
107	5 - 6	12.5	6.4		148		0.59
902-1	10 - 11	14.3	6.6		307		0.15
A12-2	11	14.0	6.0		229		
A13-2	9 - 11	13.1	6.1		181		0.25

well	NO_3^- (mg/l)	SO_4^{2-} (mg/l)	Cl^- (mg/l)	Mn-tot (mg/l)	DOC (mg/l)	HCO_3^- (mg/l)	Fe(II) (mg/l)
156-2	0	1.6	34	0.16			0.25
251b	5.6	5.6					
907	0.08	0.17	75	2			
103	0	0.1	160	1.1			17
904	11.6	6.7	47	0.32			0.85
902-2	7.2	3.7	27.3				
905	1.8	9.3	34.2				
107	0	3.2					
902-1	1.1	1.1					
A12-2							
A13-2	0	0					

well	methane (mg/l)	ethane (µg/l)	ethene (µg/l)	VC (µg/l)	cis-DCE (µg/l)	TCE (µg/l)	PCE (µg/l)
156-2	0.00						0
251b	0.09	0.8	146	50	420	270	540
907	2.60	33	1450	31000	45000		
103	1.46	7.8	11000	34000	12000		
904	0.10	1.2	0.4				780
902-2	0.08	2.3	1.1				20
905	0.06	0.6	0.1				
107	0.13	1.9					
902-1	0.11	2.1	3.7		230	980	4600
A12-2	0.16		4.2	88	2100	1900	
A13-2	0.20	1.2	1.3		400		

Chapter 11:   Banisveld, Boxtel

well	depth (m)	T (°C)	pH	EC (µS/cm)	Eh (mV)	O$_2$ (mg/l)	H$_2$ (nM)
BP10F1		9	5.93				2.9
BP10F2		9	5.15				8.3
BP10F3		9	5.58				12.3
BP9F1		9	6.51				0.4
BP9F2		9	6.56				0.4
BP9F3		9	6.09				1.6
BP8F1		9	6.25				2.2
BP8F2		9	6.25				15.6
BP8F3		9	6.25				19.2
BP1F1		9	6.28				23.2
BP1F2		9	6.23				20.2
BP1F3		9	6.10				3.6
BP2F1		9	5.92				1.0
BP2F2		9	6.13				1.0
BP3F1		9	5.78				0.5
BP3F2		9	6.04				0.6
BP3F3		9	6.00				17.9
BP4F1		9	5.20				0.4
BP4F2		9	6.03				0.5
BP5F1		9	5.07				0.3
BP5F2		9	5.96				29.9
BP5F3		9	6.43				24.1
BP6F1		9	5.50				7.2
BP6F2		9	6.15				1.2
BP7F1		9	4.65				3.1
BP7F2		9	5.85				0.3
BP7F3		9	6.20				1.9
BP10F1		9	5.55				21.3
BP10F2		9	6.02				16.1

Chapter 11: Banisveld, Boxtel.

well	NO$_3$$^-$N (mg/l)	SO$_4$$^{2-}$ (mg/l)	Mn (mg/l)	NO$_2$$^-$ (mg/l)	NH$_4$ (mg/l)	Alk-lab (mmol)	Fe(II) (mg/l)
BP10F1	370.5	43	0	0.1	0.1	0.9	0.3
BP10F2	46.2	108	0.4	0.2	0.6	0.3	15.5
BP10F3	0	138	0.4	0	0.3	0.3	8.9
BP9F1	0	7	0.5	0.2	323.2	48.9	0.3
BP9F2	0	7	0.4	0.3	346.3	56.2	0.1
BP9F3	0	92	0.8	0.1	3	12.2	26.2
BP8F1	0	13	0.8	0.2	410	52.2	50.1
BP8F2	0	32	1.2	0.2	308.3	44.7	55.9
BP8F3	0	22	1.4	0.1	138.3	29.1	76.7
BP1F1	0	6	1	0.2	253.6	36.2	51.4
BP1F2	0	11	1.2	0.1	197.5	33	73.3
BP1F3	0	7	0.5	0	0.9	3.8	6.6
BP2F1	19.7	58	0.4	0.2	73.7	11.5	14.7
BP2F2	0	16	1.1	0.1	206.4	30.1	83.1
BP3F1	17	45	0.4	0.2	77.5	10.1	4.3
BP3F2	0	25	1.3	0.1	124.4	22.1	90.2
BP3F3	0	23	0.7	0	0.7	5.5	16.2
BP4F1	0.1	66	0.3	0.1	1.7	0.7	0.2
BP4F2	0.1	53	1.8	0.1	30.6	19.4	116.3
BP5F1	0.2	157	0.4	0.2	4.4	0.7	1.9
BP5F2	0.1	77	0.6	0.1	111.7	16	61.3
BP5F3	0.1	58	0.3	0.1	0.8	2.6	5.5
BP6F1	0.1	119	0.3	0.1	0.3	0.7	0.1
BP6F2	0.1	9	1	0.1	114.1	19.7	77
BP7F1	0.1	62	0.1	0.1	0.9	0.4	0.2
BP7F2	0.1	7	1.1	0.1	41.2	14.1	76.5
BP7F3	0.1	65	0.2	0.1	0.3	0.5	2.6
BP10F1	0	134	0.6	0	0.5	0.1	26.4
BP10F2	0.1	53	0.3	0.1	0.4	1.2	7.5

Chapter 11:   Banisveld, Boxtel.

well	methane (mg/l)	ethane (µg/l)	ethene (µg/l)	VC (µg/l)	cis-DCE (µg/l)	TCE (µg/l)	PCE (µg/l)
BP10F1							
BP10F2							
BP10F3							
BP9F1	21.36						
BP9F2	23.64						
BP9F3	8.50						
BP8F1	17.81						
BP8F2	23.4						
BP8F3	20.16						
BP1F1	23.38						
BP1F2	19.14						
BP1F3	1.82						
BP2F1	10.61						
BP2F2	20.88						
BP3F1	8.69						
BP3F2	15.35						
BP3F3	0.02						
BP4F1	5.84						
BP4F2	12.53						
BP5F1	0.42						
BP5F2	12.34						
BP5F3	0.53						
BP6F1	0.74						
BP6F2	17.38						
BP7F1	3.58						
BP7F2	12.27						
BP7F3	0.53						
BP10F1							
BP10F2							