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Journal of Contaminant Hydrology 45 (2000) 165–241

JOURNAL OF Contaminant Hydrology

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Review article

Characterization of redox conditions in groundwater contaminant plumes

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Received 12 July 1999; received in revised form 22 March 2000; accepted 29 May 2000

Abstract

Evaluation of redox conditions in groundwater pollution plumes is often a prerequisite for understanding the behaviour of the pollutants in the plume and for selecting remediation approaches. Measuring of redox conditions in pollution plumes is, however, a fairly recent issue and yet relative few cases have been reported. No standardised or generally accepted approach exists. Slow electrode kinetics and the common lack of internal equilibrium of redox processes in pollution plumes make, with a few exceptions, direct electrochemical measurement and rigorous interpretation of redox potentials dubious, if not erroneous. Several other approaches have been used in addressing redox conditions in pollution plumes: redox-sensitive compounds in groundwater samples, hydrogen concentrations in groundwater, concentrations of volatile fatty acids in groundwater, sediment characteristics and microbial tools, such as MPN counts, PLFA biomarkers and redox bioassays. This paper reviews the principles behind the different approaches, summarizes methods used and evaluates the approaches based on the experience from the reported applications.

Keywords: Redox; Contaminant plumes; Characterisation of plumes; Hydrogen; Microbiology; Sediments

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0169-7722/00/$ - see front matter
PII: S0169-7722(00)00109-1

1. Introduction

Redox conditions of a groundwater contaminant plume from a point source usually differ from the redox condition of the pristine aquifer. When sufficient organic matter and other reduced components leak from a point source into an aquifer, strongly reduced redox conditions will develop close to the source and the plume will develop a redox gradient along as well as transversal to the main groundwater flow direction. In the outskirts of the plume, the redox conditions will approach the redox conditions of the pristine aquifer.

The redox conditions of a contaminant plume constitute an important part of the chemical framework controlling the behaviour of the contaminants in the plume. Knowledge of the actual redox conditions, therefore, is important for interpretation of field observations, evaluation of plume development and risks to downgradient groundwater resources, assessment of natural attenuation as a remediation option, and in engineering of remedial measures. Examples of the importance of understanding redox conditions of plumes are: (1) Elevated concentrations of manganese in groundwater sampled downgradient from the source may not originate from the source, but be a result of redox processes in the plume; (2) the presence of a large reduced plume in an aerobic aquifer suggests that the plume has been there for some time; (3) the presence of strongly reduced redox conditions allowing for methane formation suggests a significant potential for natural reductive dehalogenation of, e.g., tetrachloroethene; (4) the feasibility of remediating a benzene plume by injecting oxygen strongly depends on the current redox condition of the plume as well as the redox buffering capacity of the aquifer sediment in the plume; and (5) laboratory studies of microbial activity and contaminant degradation is of limited value unless the samples are incubated or cultured at a redox state representing the in situ conditions.

The changes in redox conditions in contaminant plumes have been recognised for at least 30 years. Golwer et al. (1969) described a landfill leachate plume in terms of an anaerobic, a transition zone and an aerobic zone and observed elevated dissolved iron concentrations in part of the pollution plume. The redox concept of groundwater contaminant plumes was first introduced by Baedecker and Back (1979a,b) and Champ et al. (1979), but apparently not used rigorously to describe actual plumes in any detail until the 1990s. Actually, two papers (Hostettler, 1984; Lindberg and Runnells, 1984) addressing redox potentials of groundwater, in general, concluded that improved approaches were needed to better understand the redox conditions of groundwater.

Determination of redox conditions in contaminant plumes is still no simple task and no universally accepted procedures exist. However, the need to understand contaminant behaviour in plumes has fostered several approaches and attempts to characterize redox conditions of contaminant plumes. Although several of these approaches are rather pragmatic or restricted in their application, major progress has been made in recent years. Most of the development so far has taken place within the framework of research projects, but there is little doubt that determination of redox conditions will be an important issue in the future also in the context of actual contaminant plumes subject to mapping, risk evaluation and remediation on a routine level.

This paper reviews the different approaches described in the literature for determination of redox conditions in contaminant plumes. After an introductory presentation of the concepts of electron activity, redox potentials, redox conditions and redox buffering, the various approaches are described: electrochemical redox potentials, groundwater sample composition with respect to redox-sensitive parameters, hydrogen concentrations in groundwater, volatile fatty acid concentrations in groundwater, sediment characteristics (iron species, sulphur species, oxidation capacity (OXC), reduction capacity (RDC), extractants), microbial characteristics in terms of biomass composition, biomarkers (PLFA), and redox bioassays.

2. Electron activity, redox potential, redox capacity and redox conditions

2.1. Electron activity ($p\varepsilon$) and redox potential (E_H)

Aqueous systems contain no free electrons, but the relative electron activity, as an intensity parameter, can still be defined (Stumm and Morgan, 1996).

$$p\varepsilon = -\log\{e^-\} \tag{1}$$

$p\varepsilon$ gives the hypothetical electron activity, $\{e^-\}$, and measures the tendency of a system to accept or transfer electrons. In a highly reducing system, the tendency to donate electrons, that is the hypothetical "electron pressure", or electron activity, is relatively large and $p\varepsilon$ is low. In contrast, high $p\varepsilon$ values indicate a relatively low electron activity and a relatively oxidized system.

Any reduction reaction (an oxidized species Ox reacts with n electrons to form a reduced species Red) can be written as:

$$Ox + ne^- \rightarrow Red \tag{2}$$

The Law of Mass Action defines the constant K^* as:

$$\{Red\}/\{Ox\}e^{-n} = K^* \tag{3}$$

This leads to:

$$p\varepsilon = (1/n)\log K^* + (1/n)\log[\{Ox\}/\{Red\}] \tag{4}$$

Since no free electrons are present in the system, the shown reduction reaction must be linked to an oxidation reaction. For reference purposes, the oxidation of hydrogen is used leading to the equation:

$$p\varepsilon = p\varepsilon^0 + (1/n)\log[\{Ox\}/\{Red\}] \tag{5}$$

Here, $p\varepsilon^0$ is the standard electron activity of the actual reduction half reaction when coupled to the oxidation of hydrogen under standard conditions.

The electron activity is, via the Nernst equation, linked to the redox potential, E_H:

$$p\varepsilon = E_H/(2.3RTF^{-1}) \quad (6)$$

or:

$$E_H = E_H^0 + (2.3RT/nF)\log[\{Ox\}/\{Red\}] \quad (7)$$

T is the absolute temperature in Kelvin, R is the gas constant, and F is Faraday's number. At 25°C, $2.3RTF^{-1} = 0.059$ V mol^{-1}.

Another useful relation is

$$\Delta G = -nFE_H \quad (8)$$

where ΔG is Gibb's energy of the reaction.

Eqs. (7) and (8) can be combined yielding an equation for calculating the actual energy yield, ΔG_r, for a given redox reaction at in situ conditions:

$$\Delta G_r = \Delta G^0 + 2.3RT\log[\{Ox\}/\{Red\}] \quad (9)$$

where ΔG^0 is Gibb's energy of the reaction at standard conditions.

As an example, the oxidation of H_2 with the reduction of the crystalline FeOOH, goethite is given:

$$H_2 + 2FeOOH + 4H^+ \rightarrow 2Fe^{2+} + 4H_2O$$

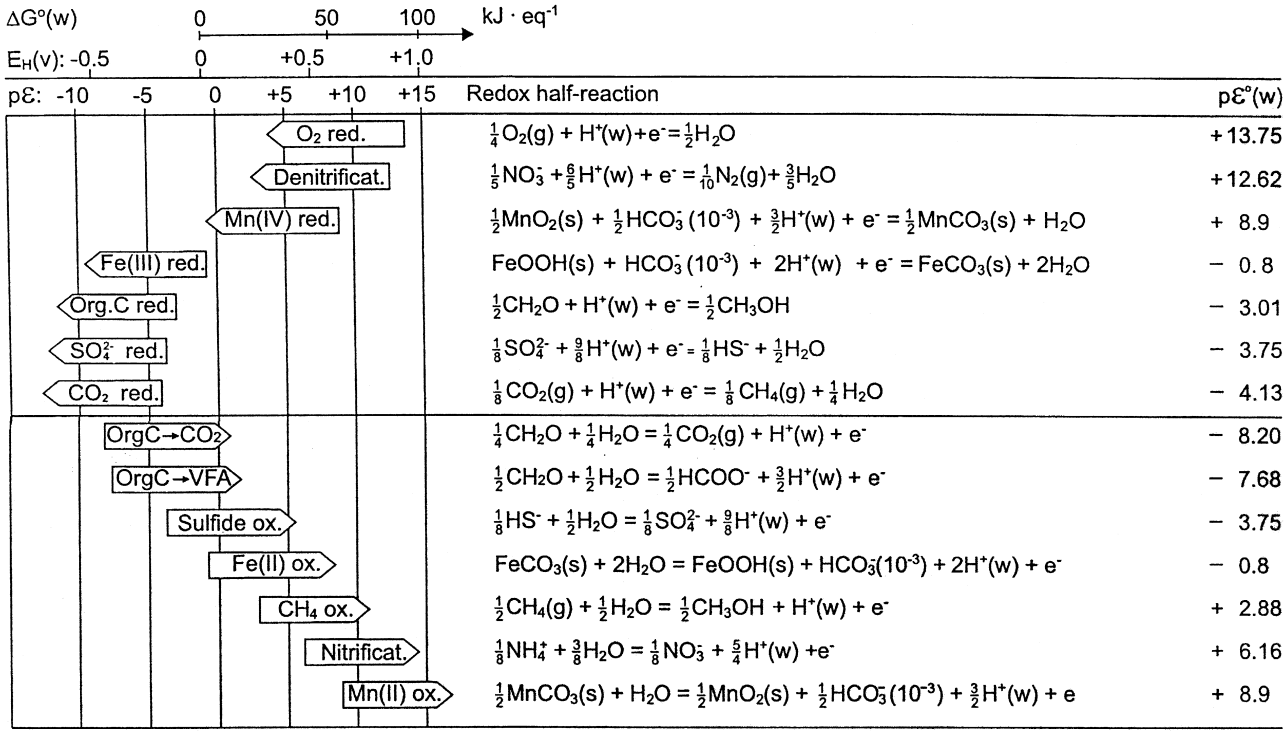
Gibb's energy of this reaction is calculated from:

$$\Delta G_r = \Delta G^0 + 2.3RT\log\left[\{Fe^{2+}\}^2/\left(\{H^+\}^4\{H_2\}\right)\right]$$

If the activities of H_2, H^+, and Fe^{2+} in the system are known, the energy available ($= -\Delta G_r$) for the reaction can be calculated and the feasibility of the reaction being microbiologically mediated can be evaluated if minimum energy requirements are known (see Section 5).

2.2. Aquifer-relevant redox processes

Several redox processes are relevant for contaminant plumes in aquifers. Fig. 1 shows the most relevant half reaction for reduction (electron-accepting) and for oxidation (electron-donating) and their corresponding $p\varepsilon^0(W)$ values (standard electron activity of the half reaction at pH 7), as adapted from Stumm and Morgan (1996). A reduction half reaction (upper part of Fig. 1) can, from a thermodynamical point of view, be combined with any oxidation reaction, if the $p\varepsilon^0(W)$ of the reduction half reaction is higher than the $p\varepsilon^0(W)$ of the oxidation half reaction. Note that the redox system of contaminant plumes may involve gases (O_2, N_2, CH_4, CO_2), dissolved components (NO_3^-, NH_4^+, CH_2O, Fe^{2+}, Mn^{2+}, SO_4^{2-}, HS^-, H^+) as well as solids (FeOOH, MnO_2, $FeCO_3$, $MnCO_3$) and components (Fe^{2+}, Mn^{2+}, NH_4^+) associated with the solids by ion exchange.



a. pE°(w) is the standard electron activity of the halfreaction at pH = 7.

Fig. 1. Representative redox half reactions for contaminant plumes and their standard electron activity at pH = 7 ($p\varepsilon^0(W)$) (after Stumm and Morgan, 1996).

Combining half reactions to create a full redox reaction does not necessarily indicate that the reaction will occur, nor does it suggest how the reaction proceeds. The "redox ladder" only indicates that the reaction is possible from a thermodynamic point of view. It should also be noted that the reactions involving organic compounds and iron and manganese solids are only sample reactions, since the actual organic matter and the composition of the iron and manganese minerals may vary substantially and hence shift the $p\varepsilon^0(W)$ of the reaction. Since most of the significant redox reactions in contaminant plumes are microbially mediated and involve conversion of organic matter, it is important to realise, as illustrated in Fig. 2 (from Lovley and Chapelle, 1995) that the actual pathways may be much more complex than suggested by the half reactions. A redox process is sometimes referred to as a terminal electron-accepting process (TEAP), a chemical reaction picturing the overall process of electrons from a reduced electron-donor, terminally being accepted by an oxidized species without paying attention to the actual electron-transfer chain and the role of intermediates.

The Gibb's free energy of reaction calculated in Table 1 for oxidation of organic matter with various electron acceptors and in Table 2 for oxidation of reduced inorganic species with oxygen (after Stumm and Morgan, 1996) illustrates the order in which the reactions are expected to occur from a thermodynamic point of view, since the reaction with the most negative $\Delta G^0(W)$ is most favourable. The overall reactions listed in Table 1 have all been observed in contaminated groundwater, while the overall reactions listed

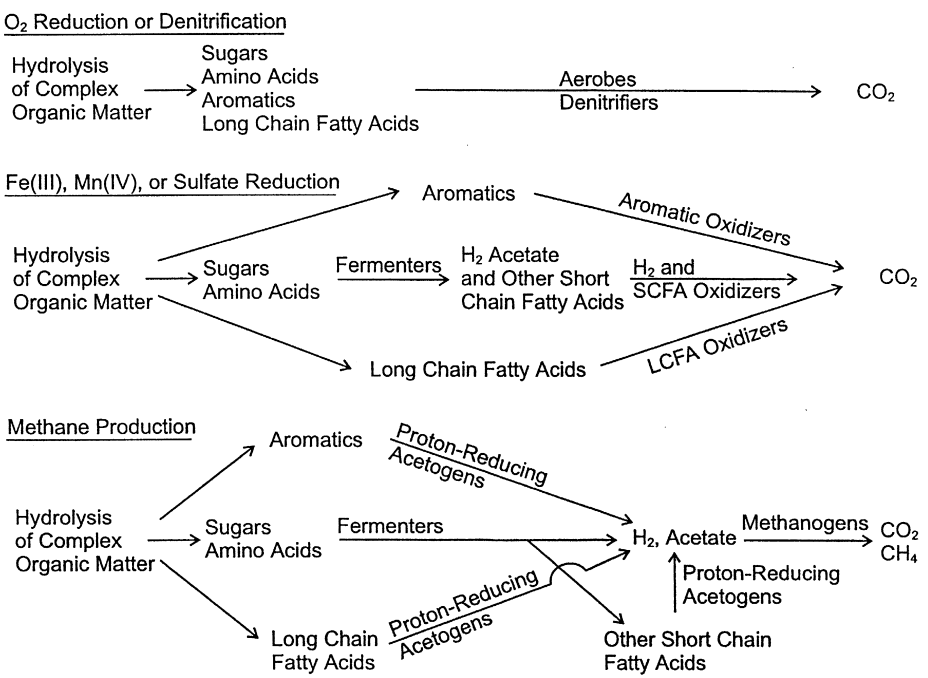


Fig. 2. Pathways of microbial decomposition of organic matter in various redox environments (from Lovley and Chapelle, 1995, with permission).

Table 1
Gibb's energy of reaction calculated for oxidation of organic carbon by various electron acceptors and by methane fermentation of organic carbon

Process	Reactants	Products	$\Delta G^0(W)$ [kJ eq^{-1}]
Aerobic respiration	$1/4\ CH_2O + 1/4\ O_2$	$1/4\ CO_2 + 1/4\ H_2O$	−125
Denitrification	$1/4\ CH_2O + 1/5\ NO_3^- + 1/5\ H^+$	$1/4\ CO_2 + 1/10\ N_2 + 1/2\ H_2O$	−119
Mn reduction	$1/4\ CH_2O + 1/2\ MnO_2 + 1/2\ HCO_3^- + 1/2\ H^+$	$1/4\ CO_2 + 1/2\ MnCO_3 + 3/4\ H_2O$	−98
Fe reduction	$1/4\ CH_2O + FeOOH + HCO_3^- + H^+$	$1/4\ CO_2 + FeCO_3 + 7/4\ H_2O$	−42
SO_4 reduction	$1/4\ CH_2O + 1/8\ SO_4^{2-} + 1/8\ H^+$	$1/4\ CO_2 + 1/8\ HS^- + 1/4\ H_2O$	−25
CO_2 reduction/ methane fermentation	$1/4\ CH_2O$	$1/8\ CO_2 + 1/8\ CH_4$	−23

in Table 2, although likely where the reduced plume encounters oxygen, are not well documented in actual plumes.

2.3. Kinetics of redox reactions

The kinetics of redox reactions often depend strongly on the number of electrons that must be transferred to form stable reaction products. This is because multiple electron transfers are generally associated with complex changes in molecular configuration, while single electron transfers often involve much less change in molecular structure. As an example, the oxidation of the ferrous aquo ion (Fe^{2+}) to ferric aquo ion (Fe^{3+}) can be a relatively rapid and reversible reaction. Both the oxidised and reduced forms of the iron complex have an octahedral structure; i.e., the central Fe atom is bound to four water molecules at the corners of a square plane and two water molecules at either end of an axis oriented perpendicular to the square plane. The activation energy to transform Fe^{2+} to Fe^{3+} is relatively small, and the reaction can proceed rapidly at groundwater temperature and pressure. On the other hand, reductive dissolution of ferric oxyhydrox-

Table 2
Gibb's energy of reaction calculated for oxidation of various reduced species by oxygen

Process	Reactants	Products	$\Delta G^0(W)$ [kJ eq^{-1}]
Aerobic respiration	$1/4\ O_2 + 1/4\ CH_2O$	$1/4\ CO_2 + 1/4\ H_2O$	−125
Sulfide oxidation	$1/4\ O_2 + 1/8\ HS^-$	$1/8\ SO_4^{2-} + 1/8\ H^+$	−100
Fe oxidation	$1/4\ O_2 + FeCO_3 + 3/2\ H_2O$	$FeOOH + HCO_3^- + H^+$	−83
NH_4^+ oxidation	$1/4\ O_2 + 1/8\ NH_4^+$	$1/8\ NO_3^- + 1/4\ H^+ + 1/4\ H_2O$	−43
Mn oxidation	$1/4\ O_2 + 1/2\ MnCO_3 + 1/2\ H_2O$	$1/2\ MnO_2 + 1/2\ HCO_3^- + 1/2\ H^+$	−28

ide minerals requires much greater changes in the coordination environment for iron. Structural Fe(III) at the mineral surface must form chemical bonds with adsorbed reductants, organic compounds, reduced metal complexes or with extracellular iron reductase (Quigley and Banwart, 1999). Subsequent to electron transfer, multiple metal-oxygen bonds in the mineral crystal must then be broken to release structural Fe(II) to solution. As a consequence, the reduction of structural Fe(III) to form Fe^{2+} ions is a much slower reaction than analogous reaction between the aquo ions. As a further example, the configurational changes that are required in oxidizing aromatic organic compounds to carbon dioxide are even more complex. The ring structure of benzene, e.g., is very different from the structure of the CO_2 gas molecule. The reaction mechanism proceeds by multiple electron transfers with substantial activation energies corresponding to the significant structural rearrangements that must take place as electrons are removed from the carbon atoms in the benzene ring.

There are a large number of chemical, mineralogical and microbiological factors that play a role in electron-transfer kinetics of redox processes in groundwater. Slow abiotic kinetics due to the high activation energies for structural rearrangement are an important reason that many redox processes in contaminant plumes require microbial catalysis to proceed at appreciable rates. Enzyme systems act to stabilise intermediates, and facilitate the step-wise transfer of electrons to and from intermediates, with stable reaction products as the end result. Abiotic redox reactions involving dissolved iron often proceed at measurable rates in the presence of suitable oxidants and reductants. Redox processes involving C, S and N, often require microbial catalysis due to the multiple electron transfers required to reach stable products, and the extensive structural rearrangement between reactants and products.

2.4. Redox capacities / redox buffering

In addition to the intensity parameter, the electron activity ($p\varepsilon$) or the theoretical redox potential (E_H), the redox properties of the system also include its capacity to buffer the effects of entrance of reduced or oxidized components (Scott and Morgan, 1990).

In theory, the OXC of a volume of an aquifer can be expressed by the sum of oxidized equivalencies, that potentially could be reduced within a contaminant plume, minus the sum of reduced equivalencies, that could be oxidized in an aquifer system (Scott and Morgan, 1990). This definition would for the redox system be an analogue to alkalinity for acid-base systems. However, since internal redox equilibrium is uncommon it is more practical to define the OXC as the sum of oxidized equivalencies and the RDC as the sum of reduced equivalencies within a volume of the plume:

$$\mathrm{OXC} = \sum(\mathrm{Oxidized\,equiv.}) \tag{10}$$

$$\mathrm{RDC} = \sum(\mathrm{Reduced\,equiv.}) \tag{11}$$

The relevant redox species are defined by their presence as redox species in pristine or strongly contaminated aquifers. Tables 3 and 4 show sample calculations of OXC in two

Table 3
Calculated OXC (milliequivalents per dm^3 (litre) of aquifer) for two aerobic aquifers

Species	Reduction	Vejen (DK)		Sand Ridge (IL, USA)	
		Content	OXC (meq/dm^3)	Content	OXC (meq/dm^3)
O_2 (aq)	$O(0) \rightarrow O(-II)$	10 mg/l	0.44	9 mg/l	0.39
NO_3^- (aq)	$N(V) \rightarrow N(0)$	15 mg/l	1.9	1 mg/l	0.12
Mn(IV) (s)	$Mn(IV) \rightarrow Mn(II)$	0.2 mg/g	12	0.4 mg/g	23
Fe(III) (s)	$Fe(III) \rightarrow Fe(II)$	6 mg/g	175	6.8 mg/g	200
SO_4^{2-} (aq)	$S(VI) \rightarrow S(-II)$	40 mg/l	1.2	36 mg/l	1.1
CH_2O (s)	$C(0) \rightarrow C(-IV)$	0.2 mg/g	110	0.4 mg/g	220

The contents of the oxidized species are per litre of groundwater and grams of sediment, respectively, and converted to dm^3 of aquifer assuming a porosity of 0.35 and a dry bulk density of 1.6 kg/dm^3. Partly based on Barcelona and Holm (1991) and Heron et al. (1994a).

aerobic, uncontaminated aquifers and of RDC in two strongly polluted anaerobic plumes, respectively. The lack of internal equilibrium allows an aquifer volume to have both OXC and RDC at measurable levels, although this is not illustrated by the cases in Tables 3 and 4.

Of the inorganic species, iron and, to some extent, manganese contribute significantly to the OXC in the pristine aquifer, while contributions by dissolved species are very minor. In the contaminant plume, reduced iron and sulphur species as well as ammonium contribute significantly. Organic matter, as the model component CH_2O with a carbon oxidation state of zero, is included in both the OXC and the RDC calculated for

Table 4
Calculated RDC (milliequivalents per dm^3 (litre) of aquifer) for two anaerobic contaminant plumes

Species	Oxidation	Leachate plume (Vejen, DK)		Leachate plume (Grindsted, DK)	
		Content	RDC (meq/dm^3)	Content	RDC (meq/dm^3)
CH_4 (aq)	$C(-IV) \rightarrow C(IV)$	20 mg/l	3.5	15 mg/l	2.6
CH_2O (s)	$C(0) \rightarrow C(IV)$	600 mg/kg	320	200 mg/kg	106
CH_2O (aq)	$C(0) \rightarrow C(IV)$	200 mg/l	23	75 mg/l	8.8
Reduced S (s)	$S(-I) \rightarrow S(VI)$	500 mg/kg	200	100 mg/kg	40
HS^- (aq)	$S(-II) \rightarrow S(VI)$	0.5 mg/l	0.04	0.2 mg/l	0.02
Reduced Fe (s)	$Fe(II) \rightarrow Fe(III)$	1000 mg/kg	29	250 mg/kg	7.3
Fe^{2+} (aq)	$Fe(II) \rightarrow Fe(III)$	50 mg/l	0.3	200 mg/l	1.3
NH_4^+ (s, iec.)[a]	$N(-III) \rightarrow N(V)$	20 mg/kg[b]	14	20 mg/kg[b]	14
NH_4^+ (aq)	$N(-III) \rightarrow N(V)$	40 mg/l	6	25 mg/l	4
Reduced Mn (s)	$Mn(II) \rightarrow Mn(IV)$	50 mg/kg	3	15 mg/kg	0.9
Mn^{2+} (aq)	$Mn(II) \rightarrow Mn(IV)$	10 mg/l	0.1	5 mg/l	0.06

The contents of the reduced species are per litre of groundwater and grams of sediment, respectively, and converted to dm^3 of aquifer assuming a porosity of 0.35 and a dry bulk density of 1.6 kg/dm^3. Data is partly based on Heron et al. (1994b, 1998), Bjerg et al. (1995), and partly assumed by the authors.

[a] iec., Ion exchanged onto aquifer sediment.

[b] The content is assumed by the authors.

the samples. Actually, organic carbon may have an oxidation state varying from C(−IV) in methane to C(III) in formic acids, so the organic model component in the solids represents an average well-matured organic pool. It is, however, questionable to which extent this pool is reactive and significant in the context of a contaminant plume. Griffioen et al. (1999) showed that the bulk organic matter of aquifer sediments had a measurable RDC against oxygen, while Jakobsen and Postma (1994) showed that organic matter in aquifers, in general, had a very low reactivity. Likewise, some uncertainty exists about the availability of the solid iron as well as sulphur components.

To illustrate the development in $p\varepsilon$ and pH as a volume of aerobic aquifer is titrated by dissolved organic matter, Scott and Morgan (1990) carried out a series of equilibrium calculations, as shown in Fig. 3. All the redox buffering was gone after about 500 μm carbon was oxidized, suggesting that the model redox system had very little buffer capacity. For example, compared to the actual sandy aquifers characterised in Table 3, the iron oxide content of the Vejen aquifer solids (6 mg Fe/g of dry sediment) would provide a buffer capacity per litre of aquifer that would match on the order of 120 000 μm carbon corresponding to 1400 mg C/l in the porewater contained in 1 l of the aquifer. However, Fig. 3 still shows how the $p\varepsilon$ of the system develops in a stepwise fashion, assuming the redox reactions establish their equilibria fast. The redox buffering takes place on the horizontal plateaux of the $p\varepsilon$ diagram as $p\varepsilon$ does not change as DOC is being oxidized by a given electron acceptor. When the electron acceptor is depleted, the $p\varepsilon$ drops rapidly until the level of the next redox couple is met. The main drop in $p\varepsilon$ happens between manganese and iron reduction as expected from the difference in $p\varepsilon^0(W)$ values of the two redox couples (see Fig. 1). The redox buffering plateaux for

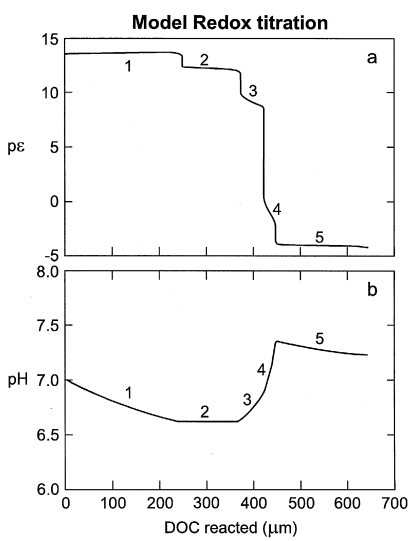


Fig. 3. Model calculated developments in $p\varepsilon$ (a) and pH (b) as a function of dissolved organic matter titrating a fictitious groundwater volume: Numbered segments correspond to sequential reduction of (1) O_2 (aq), (2) NO_3^- (aq), (3) MnO_2 (s), (4) $Fe(OH)_3$ (s) and (5) SO_4^{2-} (aq) (from Scott and Morgan, 1990, with permission).

manganese and iron are not quite horizontal, due to the effect of changes in pH, caused by the redox processes, on the solubility of the electron-accepting minerals.

The redox titration, as calculated by Scott and Morgan (1990), clearly suggests an analogy between pε-titration and pH-titration (pH-titration: pH is the intensity parameter and alkalinity/acidity is the capacity parameter; pε-titration: pε is the intensity parameter and OXC/RDC is the capacity parameter). But as will be learned, the systems are not really analogous, because redox reactions are very slow kinetically, have weak links between different reactions, and the pε-intensity parameter is not as easily measured as in the case of pH.

2.5. Difficulties in applying redox concepts to contaminant plumes

A contaminant plume is likely not to be in internal equilibrium. However, regardless of equilibrium, there is an individual, instantaneous pε for each redox couple in the system, corresponding to the relative activities of the oxidized and reduced species in the redox couple and defined by the Nernst relationship (Scott and Morgan, 1990). Only when the pε is identical for all redox couples is the system at internal or thermodynamic equilibrium, and only in this case can a system pε be defined and applied to estimate reduced and oxidized species of all redox couples.

Since several of the redox processes have very slow kinetics, it is unlikely that a groundwater system is in thermodynamic equilibrium with respect to redox. Lindberg and Runnells (1984) investigated 30 different natural groundwater samples and found none in equilibrium with respect to measurable redox couples. This suggests further that contaminant plumes, where titration with reduced organic carbon may have taken place only within recent years, are less likely to have well-defined system electron activities or redox potentials.

However, while we may abandon the idea of assigning a specific electron activity or redox potential to the environments in contaminant plumes, they often have such strong redox gradients that it is still convenient to identify the redox conditions within the plume. We suggest that the term redox condition be used to characterize the dominating redox processes, instead of trying to assign a specific but meaningless redox potential to the system. Ideally, the evaluated redox condition should be accompanied by an evaluation of associated capacities depending on the purpose of the investigation.

The various approaches for assessing the redox conditions are discussed in detail in the following sections, but as an introduction to the application of the concepts and approaches, first a theoretical framework for the development of redox conditions in a contaminant plume is presented and the expected degree of resolution is discussed.

2.6. The redox dynamics of contaminant plumes

Fig. 4 illustrates the development in redox conditions in a hypothetical contaminant plume from a point source. The scenarios range from the first entrance of reduced leakage into the aquifer, over the maximal development of strongly reduced redox zones

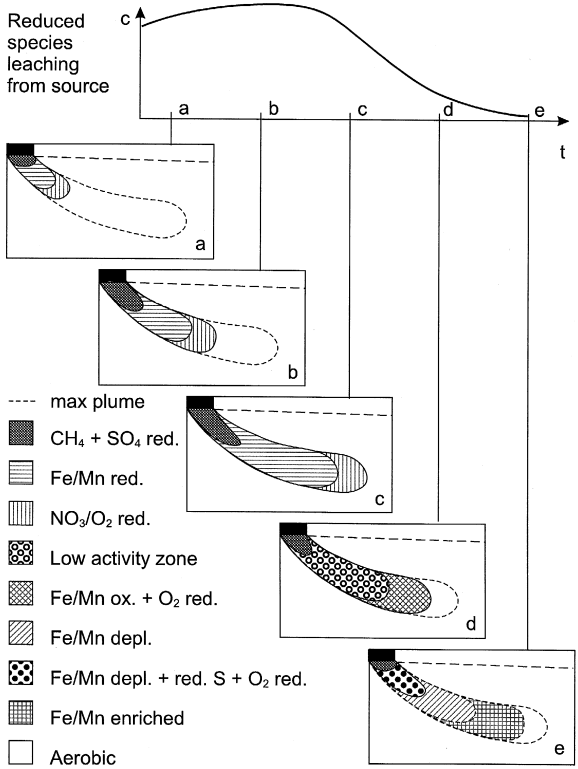


Fig. 4. Illustration of developments with time of redox zones in a plume from a point source.

to the slow recovery of the plume as the strength of the point source declines. The actual length and time scales, related to the plume as well as to the different redox zones (a volume of the plume with somewhat homogeneous redox conditions), depend strongly on the size and strength of the point source, the groundwater flow velocity and the geochemistry of the aquifer. These factors, thus, may also change the importance of the various redox zones.

While the progressive development in redox zones of a contaminant plume is partly supported by experiences from real plumes, the regressive development is still fairly speculative. However, it is likely that after the source term is fully depleted, the aquifer will have permanently changed features where the plume has been located. For example, pyrite produced in the former strongly reduced zones may exist long after the aerobic conditions again have taken over. Furthermore, Fe and Mn may be depleted in the central part of the plume and enriched further out in the plume due to migration of reduced Fe and Mn.

Understanding of the dynamics of the contaminant plume and the purpose of the redox characterization will be useful in selecting the appropriate methods for the redox characterization.

3. Electrochemical measurement

3.1. Background

The definition on electron activity presented in Section 2 and the link to the redox potential, via the Nernst equation, may suggest that immersing two electrical leads in a groundwater sample and connecting them to the input of a voltmeter may well result in a measurable electrical potential that is associated with the redox condition of the sample. Electrochemical redox measurements are easily performed and have been successfully applied in analytical chemistry to aquatic solutions of pure chemicals. However, prior to application of electrochemical redox measurements to assessing complex biological and geochemical processes in a plume of groundwater contamination, a number of difficult questions must be addressed. To what solutes and processes do such measurements respond? Do measured potentials correspond to rigorously defined electrochemical potentials and, thus, free energies of relevant reactions? If so, is the information useful to assessment of groundwater redox conditions?

There is an enormous difference between the thermodynamic concept of an electrochemical potential, and a measured electrochemical potential. The first is a theoretical construct, while the second results from a practical exercise in analytical chemistry. Under certain conditions, the two can be associated in a meaningful way to help assess groundwater redox conditions. Often, electrochemical redox measurements, as referred in the literature, are either considered useless or interpreted beyond their reliability. This probably is due to lack of understanding of the basic concepts and, therefore, of the possibilities and limitations of the measurements. The classic paper by Lindberg and Runnells (1984) abolished meaningful interpretation of measurements of electrochemical redox potentials due to lack of internal equilibrium. Still, redox potentials have been helpful in field monitoring to identify strongly reducing conditions and, when measured with care as discussed in the following sections, can be interpreted quantitatively with respect to the redox speciation of dissolved iron.

3.2. Principles

The electrochemical measurement involves two electrodes, a nonpolarisable reference electrode and a polarisable working electrode. If a small potential is applied, current will flow at the working electrode as redox-active ions undergo oxidation and reduction at the electrode surface. The working electrode will respond to kinetically fast redox couples and the potential between the working electrode and the reference electrode, measured when no current flows, corresponds to electrochemical equilibrium for the redox couple. A review of the basic electrochemical principles behind direct measurements of redox potentials can be found at www.shef.ac.uk/~gprg. The main problem in applying these principles to measuring redox potentials in polluted groundwater is the very slow chemical kinetics of redox reactions involving C, S and N species. Actually, the only redox couple that may respond kinetically fast and have sufficient ion activity is the Fe^{3+}/Fe^{2+} redox couple.

Although a reading of electrochemical redox potential can be obtained for most anaerobic groundwater samples, the lack of internal equilibrium and the slow kinetics of most redox couples, may make the reading meaningless unless it is based on measurable ion activities within the Fe^{3+}/Fe^{2+} redox couple and this redox couple is at equilibrium. Pitfalls and consequences in electrochemical measurements of redox potentials are summarized in Table 5.

3.3. Methods

Redox measurements are not valid in aerobic groundwater due to the presence of O_2 (www.shef.ac.uk/~gprg). Although most contaminant plumes are anaerobic, it is important for measuring reliable redox potentials that the sample is unchanged when pumped from the well to the measuring cell by using gas impermeable pumps and tubing. Specifications for measurement cell and electrode configurations are available in the literature (Walton-Day et al., 1990; Grenthe et al., 1992; Lyngkilde and Christensen, 1992). A standard configuration is a gas-tight cylinder through which groundwater can pass, and into which electrodes are inserted while maintaining a gas-tight seal around openings for flow connections and electrodes. Measurement must take place in a gas impermeable cell since diffusion of oxygen and slow oxidation of ferrous iron species can result in mixed potentials that drift slowly. Since it is essentially impossible for the cell to be completely gas impermeable, insurance against mixed potentials is provided by a continuous flow of anaerobic groundwater past the measuring electrode such that the concentration of dissolved ferrous iron species is representative of the groundwater.

An important precaution against mixed or otherwise unrepresentative potentials is to record measured potentials with time, until a stable potential is obtained. This ensures

Table 5
Pitfalls and their consequences in determination of electrochemical redox potentials in contaminant plumes (after Grenthe et al., 1992)

Pitfalls and consequences
Measurement of non-equilibrium potentials
Incorrect application of measured potential in thermodynamic calculations
Measurement of mixed potentials
Incorrect assignment of measured potential to a particular redox pair
Incorrect application of measured potential in thermodynamic calculations
Measurement in oxic or sub-oxic groundwaters
Drifting or fluctuating potentials
Incorrect assumption that measured potential reflects O_2/H_2O equilibrium
Incorrect assumption that measured potential reflects redox status of groundwater
Measurement below detection limits due to low ion activities
Drifting or fluctuating potentials
Incorrect assumption that measured potential reflects redox status of groundwater
Incorrect assignment of measured potential to a particular redox pair
Incorrect application of measured potential in thermodynamic calculations

that there is no drift due to traces of O_2, and it indicates that the composition of the flowing groundwater is not changing dramatically and the measured potential is representative for the groundwater in the well capture zone. Fig. 5 shows data obtained using a standard flow cell configuration. Data were recorded after a purge time that was suitable to flush the tubing and pumps of any residual air or groundwater. Stable readings in conductivity, pH and E_H were then obtained by the electrodes in the cell within about 30 min. Shorter or longer times are possible, depending on pumping rates, groundwater composition and its variability and the design and performance of the sampling system, measurement cell and electrodes.

An additional refinement for obtaining reliable potentials is to simultaneously record potentials obtained with two different electrodes with time. Fig. 5 compares potentials for simultaneous measurements using platinum and glass carbon electrodes housed in a

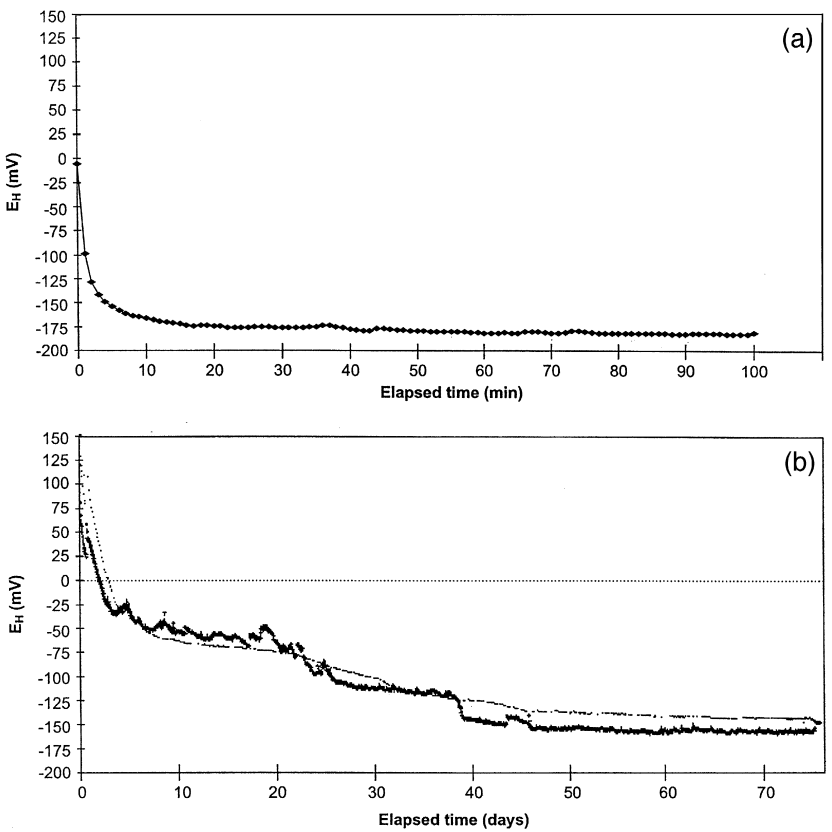


Fig. 5. (a) Establishment of stable redox potential reading with time using a flow through cell receiving pumped discharge from a well within a phenolic contaminant plume in a sandstone aquifer (unpublished data courtesy of S. Thornton, The University of Sheffield, UK). (b) Long-term continuous measurement of redox potentials using comparative measurements from a glassy carbon and a platinum electrode (unpublished data courtesy of Swedish Nuclear Fuels and Waste Management, Stockholm).

flow-through cell. In this case, the flow-through cell was used to monitor a continuous discharge from a well in a shallow granitic aquifer, on a long-term basis. The measurements show that the two electrodes gave measurements within about 10 mV of each other after the first 3 or 4 days. During the next two months, the measured potentials continued to agree well and showed a drop in redox potentials from −50 to near −150 mV. Since the different electrode materials catalyse to different degrees, the redox processes giving rise to the potential of the working electrode, the potentials should only coincide if the aqueous redox species to which the electrodes respond are in chemical equilibrium. Because the potentials for the two electrodes agree, it is inferred that the recorded potentials do reflect electrochemical equilibrium in the groundwater. The data may, therefore, be used for thermodynamic modelling of dissolved iron species.

Calibration of redox electrodes is best carried out using suitable redox buffer solutions in the sealed measurement cell without flow. Single-point calibrations can be used assuming a Nernstian response of the electrode potential to changes in redox species activities in solution. Walton-Day et al. (1990) report a single-point calibration with a standard buffer (Light, 1972). Grenthe et al. (1992) used a two-point calibration where the standard curve is obtained by correcting slope and intercept values to give (stable) millivolt readings that coincide with theoretical redox potential values for standard buffer solutions. Suitable standards are prepared with commercial pH buffer solutions of two different pH values to which a spatula of quinhydrone is added (BDH Technical Services, personal communication, 1999). In these solutions, the equilibrium potential changes with pH; 10°C: $E_H = +487$ mV at pH 4; $E_H = +316$ mV at pH 7.

It has been suggested that electrochemical redox measurements in groundwater might be improved if mediators (active redox couples added at trace levels) were added to the samples. In this case, the mediators help stabilise the measured redox potential by providing a kinetically labile redox couple, presumed to maintain equilibria with other dissolved redox species, to which the working electrode can respond. This may yield a more stable measurement but such additions of mediators do not circumvent the fundamental difficulty that the redox reactions in a contaminated groundwater sample are not at internal equilibrium. The “improved” measurements, therefore, do not improve the interpretation and use of the measurement.

Guidance on performance and interpretation of electrochemical redox measurements are summarized in Table 6.

3.4. Applications

Electrochemical measurements of redox potentials have only been reported in very few cases for contaminant plumes, supposedly due to lack of confidence in their usefulness. Redox potential measurements, however, have often been made in the field as a means to identify strongly anaerobic samples when mapping plumes.

Fig. 6 shows redox potentials measured in 52 sampling points representing a 250-m-long vertical transect of the plume downgradient from the Grindsted Landfill (DK). In the strongly reducing part of the plume, by other techniques characterized as

Table 6
Guidelines for measuring electrochemical redox potentials in groundwater

- The sampled groundwater must be strictly anaerobic.
- Dissolved iron concentrations should be above 1 μM.
- Measured potentials should be stable with time; within a specified rate of drift such as 10 mV h^{-1}.
- Ideally, the measurements should be made with two different working electrodes.
- The difference between measurements with different electrodes should be less than a specified limit such as 30 mV.
- Measurements should be made in a flow-through cell such that the anaerobic groundwater in contact with electrodes is continually being replaced.
- The measured potentials are likely to reflect only the redox equilibrium of the Fe^{3+}/Fe^{2+} couple.

methanogenic, sulphate-reducing and iron-reducing, the electrochemical measurements consistently gave values between −70 and −100 mV. In the aerobic part of the plume, the readings were of the order of 200 to 370 mV. A few measurements in-between these two intervals (−20 to 90 mV) were located in slightly reducing zones of the plume. The electrochemical measurements were used only as a fast field method for locating strongly reduced environments in the plume. However, where reduction of ferric iron or precipitation of ferrous sulphide take place, measurements of redox potentials within the plume might well be controlled by the iron redox couple, and could be applied to thermodynamic modelling of the iron redox system.

Fig. 7 shows redox potentials measured in 20 sampling points in a 50 m wide transversal transect of the plume downgradient the fire-training area at the Wurtsmith site (MI, USA). The core of the plume, identified as methanogenic, sulphate-reducing and iron-reducing according to the distribution of redox-sensitive groundwater species

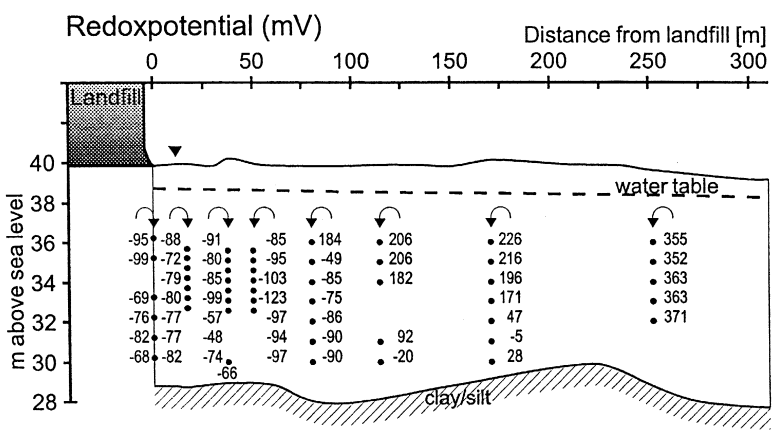


Fig. 6. Redox potentials measured by a platinum electrode (Ag/AgCl reference electrode) in a longitudinal transect of the leachate plume downgradient from Grindsted Landfill (DK) (unpublished data courtesy of P.L. Bjerg, Technical University of Denmark).

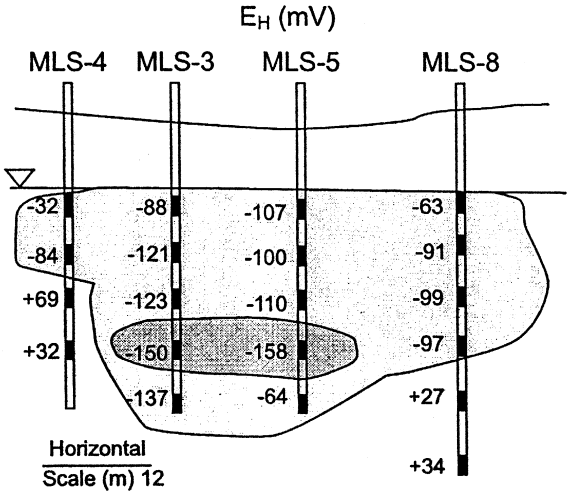


Fig. 7. Redox potentials measured by a platinum electrode in a transversal section of the plume at the Wurtsmith site (MI, USA) (from Chapelle et al., 1996, with permission).

and hydrogen concentrations, showed values in the range −64 to −158 mV (except one value of −32 mV). Four sampling points showing oxygen above 0.5 mg/l revealed positive redox potentials. The authors, Chapelle et al. (1996), found some inconsistencies among the different redox-indicating parameters and warned against interzonal mixing by pumping wells.

Lendvay et al. (1998a,b) combined flow-cell measurement of redox potentials with hydrochemical data for depth profiles at the St. Joseph site (MI, USA) to elucidate redox environments and biological processes within a shallow contaminant plume and their seasonal dynamic behaviour. They observed a sharp drop from positive to negative redox potentials across the dissolved O_2 gradient at the plume fringe. Due to the abundance of iron mineral phases in the aquifer sediment and the existence of significant concentrations of dissolved iron, redox potentials below −40 mV (read from the published illustration) seem to indicate strongly reducing conditions, important for the reduction of chlorinated solvents in the plume.

The data shown in Fig. 5 were applied to model the aqueous iron speciation, and the solubility and redox equilibria of ferrous clays sampled at that site, as a sink for Fe(II) produced during microbial iron reduction by organic carbon species (Banwart, 1999). The measured potentials proved essential to understanding the iron mass balance at the site, which helped quantify the extent and rate of biodegradation processes. Because iron oxide, carbonate, silicate and sulphide mineral phases are potential reactants and products during anaerobic biodegradation processes, understanding and quantifying the iron system can be of tremendous importance when attempting to close electron and mass balances based on field data, and to quantify the associated in situ rates of degradation processes. Jakobsen et al. (1998) recently outlined how the equilibrium redox potential for TEAPs can help quantify conditions where simultaneous TEAP occur

(overlapping iron- and sulphate-reducing and methanogenic zones). In this case, redox potential measurements coupled with hydrochemical data provides the only possibility to assess the mineral solubility and associated redox equilibria of the phases present.

3.5. Evaluation

In the applications described above, redox potentials were used in two different senses. In the first three cases, indicative measurements were used qualitatively to identify strongly reducing zones within a plume. Electrochemical measurements in contaminant plumes are likely to respond primarily to the iron redox couple and, at neutral pH values, measurements below -50 mV seem to suggest that strongly reducing conditions (iron-reducing, sulphate-reducing or methanogenic) prevail in the plume. However, lower values cannot be interpreted as representative of more reducing conditions, such as sulphate-reducing or methanogenic conditions. Depending on the methodology, electrochemical measurements may also yield information on iron speciation in the plume. In the fourth case, considerable effort went into obtaining reliable, long-term and continuously measured redox potentials at a single discharging well. Because the objectives for that research project focused strongly on the thermochemical stability and redox behaviour of iron-bearing mineral phases within the well capture zone, such measurements were essential to interpretation of relevant processes at the site.

It seems clear that redox potential measurements can be routinely carried out at field sites, within a standard sampling protocol, and may help to at least rapidly distinguish between strongly reducing zones (iron-reducing, sulphate-reducing and methanogenic conditions) and zones representing higher redox conditions. In some cases, where measurements have been made carefully and methods documented, a more quantitative interpretation may be possible, although limited to helping interpret iron speciation in the groundwater. Because of the difficulties in obtaining reliable quantitative redox potential data, the cost in time and equipment needs to be weighed against the potential value of the data to a particular field problem.

4. Groundwater composition

4.1. Background

The redox half reactions presented in Fig. 1 and the overall redox reactions exemplified in Tables 1 and 2 involve reactants and products that are present in groundwater as dissolved ions or as dissolved gases. Outside a narrow interval containing the standard redox potential of a given reaction, either the reactants or the products dominate and their presence, therefore, reflects the current redox conditions. The primary redox-sensitive species in groundwater are the dissolved ions SO_4^{2-}, HS^-, Fe^{2+}, Mn^{2+}, NH_4^+, NO_2^-, NO_3^- and the dissolved gasses CH_4, N_2O and O_2, but also other components in groundwater (e.g., DOC and organic N) may reflect the redox levels in

contaminant plumes. Since groundwater sampling is used extensively to characterize the plumes in terms of pollutants (e.g., BTEX and chlorinated aliphatic compounds), it is obvious, also to sample and analyse for redox-sensitive species in the same locations.

4.2. Principles

Assuming that both the oxidized and the reduced species of the important redox processes in groundwater could be quantified analytically with good accuracy and that the solute species were in equilibrium with the solid species, a redox potential could be calculated for each redox couple according to Eq. (7). However, this is usually not possible for various reasons. (1) Analytical methods may have detection limits too high to quantify both species of a redox couple due to a very low concentration of one of them (e.g., Fe(II)/Fe(III)). (2) Analytical methods may not exist for both redox species (e.g., Mn(II)/Mn(IV)). Lack of analytical detection of one species of a redox couple may be circumvented by measuring total concentration (e.g., Fe) and one of the species (e.g., Fe(II)) in the redox couple and then assuming that the other species makes up the difference (Nicholson et al., 1983). However, this introduces significant uncertainty for low concentrations. (3) Several redox-sensitive species are also precipitates and the dissolution/precipitation reactions are not necessarily at equilibrium or the actual precipitate is not known (e.g., Fe^{3+}/FeOOH/Fe_2O_3). Therefore, the quantification of redox-sensitive species usually cannot be used to calculate a redox potential, but the redox levels can still be deduced paying attention to the geochemistry of species, as done below for the hierarchy of electron acceptors.

Issues related to sampling and behaviour of the most commonly used redox-sensitive species are summarized in Table 7. Aerobic conditions require that free O_2 is present and O_2 can in practice (standard wells and sampling equipment) with some caution be measured reliably down to at least 0.5–1 mg/l. Lower concentrations can be measured, but it is hard to tell whether the reading represents the oxygen concentration in the plume or is caused by intrusion of oxygen during pumping and sampling (e.g., by diffusion through plastic tubing, Kjeldsen, 1993). Clearly aerobic conditions are, thus, fairly easy to identify, but microaerophilic (low oxygen concentrations) are hard to document. Denitrifying conditions do not necessarily require that measurable nitrate is present, since the process may limit the nitrate concentration below detection limits. Denitrification may, thus, be determined as decreased nitrate concentrations and/or the presence of reduced intermediates as, e.g., N_2O. Manganese reduction is recognized by the presence of dissolved manganese. Oxidized manganese is present as a solid (e.g., MnO_2) and has such a low solubility at near neutral groundwater pH that all soluble manganese above background concentrations, for all practical purposes, can be assumed to be Mn^{2+}. Presence of dissolved iron may indicate iron reduction as dissolved iron can be assumed to be Fe(II) at neutral pH. Analytical methods do exist for Fe(II) and the validity of this assumption has been proven (Postma and Lyngkilde, 1990). Sulfate reduction may be detected as decreased sulphate concentrations and/or the presence of hydrogen sulphide. However, sulphide has very low solubility if Fe^{2+} is present and analytical detection may be difficult. Methanogenesis results in production of CH_4,

Table 7
Use of redox-sensitive compounds for assignment of redox conditions

Redox-sensitive compound	Indicator	Transport and geochemistry	Sampling precautions
Oxygen, O_2 (dissolved gas)	Electron acceptor: Low concentrations (< 0.5–1 mg O_2 /l) indicate anaerobic conditions.		Field analysis by flow cell is recommended
Nitrate, NO_3^-	Electron acceptor: Presence of nitrate indicates aerobic or nitrate reducing conditions		
Dinitrogen oxide, N_2O (dissolved gas)	Intermediate in the conversion of N-compounds. May indicate nitrate reduction or nitrification	Transport	Volatile
Nitrite, NO_2^-	Intermediate in the conversion of N-compounds. May indicate nitrate reduction	Transport	Instable
Manganese, Mn^{2+}	End product generated by manganese reduction. Presence of Mn^{2+} indicates anaerobic conditions and manganese reduction	Transport, cation exchange, precipitation as carbonates, sulfides and oxidiation by oxygen	Filtration, elevated concentration of dissolved manganese mainly presents Mn (II) at pH 5–8
Iron, Fe^{2+}	End product generated by iron reduction. Presence of Fe^{2+} indicates anaerobic conditions and iron reduction.	Transport, cation exchange, precipitation as carbonates, sulfides and oxidation by oxygen	Filtration, dissolved iron mainly present as Fe(II) at pH 5–8.
Sulfate, SO_4^{2-}	Electron acceptor. Presence but decreased sulfate concentrations under anaerobic conditions indicate sulfate reduction		
Sulfide, S^{2-} (dissolved gas)	End product generated by sulfate reduction. Presence of sulfide indicates anaerobic conditions and sulfate reduction.	Transport, precipitates with dissolved iron and manganese	Volatile, field analysis
Methane, CH_4 (dissolved gas)	Methane is created from reduction of carbon dioxide or degradation/fermentation of organic carbon. Presence of methane indicates anaerobic conditions and methanogenesis	Transport	Volatile

The table also includes processes weakening the interpretation of redox conditions and sampling precautions.

which is easy to analyse. A complicating issue is that some of these redox-sensitive species are subject to transport in the plume. This is, in particular, the problem with respect to CH_4 and N_2O, where the presence of, e.g., methane may indicate production of methane at the sampling point or somewhere upgradient from the sampling point. Also, Fe^{2+} and Mn^{2+} may migrate, but ion exchange and also precipitation may limit this issue. Actually, their retarded movement could maintain elevated concentration in the groundwater after the redox processes no longer are active (e.g., Albrechtsen et al., 1995). Thus, the presence of Fe or Mn in a groundwater sample indicates that manganese/iron reduction is on-going or has happened at the sampling point or upgradient. Hydrogen sulphide may also migrate, but the precipitation of iron sulphide often limits its significance.

In addition to the above-mentioned redox-sensitive species, ammonium and arsenic species have been used as redox indicators. Ammonium is oxidized at high oxygen concentrations (see also Table 2) and its presence may, therefore, indicate reduced conditions, but can also be a result of high dynamics of organic matter degradation. Arsenic exists in the reduced form As(III) or in the oxidized form As(V). Cherry et al. (1979) investigated the behaviour of arsenic downgradient of the Borden Landfill (CND). They found no As(III) in samples with measurable dissolved oxygen presumably due to oxidation to As(V). Closest to the landfill, the highest ratio of As(III)/As(V) was detected. The findings indicate that arsenic may be useful as a redox indicator in a landfill leachate plume; however, low total arsenic concentrations as found in the Borden Landfill plume may limit the use of this indicator.

4.3. Methods

Using redox-sensitive compounds for redox characterization requires proper sampling of redox-sensitive species from representative screens. Screen length is critical, as long screens may cause mixing of groundwater having significantly different composition with respect to groundwater chemistry. This may be specifically critical in layered geological media or shallow contaminant plumes.

Sampling equipment in terms of pumps and tubing must be evaluated for volatilization and diffusion of the compounds in question. Volatile compounds (O_2, H_2S and CH_4) are ideally sampled without the presence of headspace or by nitrogen pressure using a check valve system (e.g., Lyngkilde and Christensen, 1992). Sampling by traditional centrifugal pumps is also possible. Vacuum pumps are widely used for small-diameter wells, but application of vacuum of more than 40% may cause loss of volatile compounds (see Albrechtsen et al., 1999). Diffusion of dissolved gases (H_2S and CH_4) through the sampling tubing can be a problem with soft plastic materials (Kjeldsen, 1993), but also intrusion of oxygen can cause erroneous oxygen measurements or precipitation of Fe^{2+} upon oxidation. This problem is mainly related to sampling with small tubing and low flow rates. Estimates of the importance of diffusion can be made by simple calculations (Kjeldsen, 1993).

When sampling at the ground surface, electrode measurements (e.g., oxygen) should be performed in-line (tubing connected directly to a flow cell) to avoid contact with the

atmosphere. Similarly, samples used for analysis of compounds where solid species may interfere (Fe^{2+}, Fe, Mn) should be properly filtered. Dissolved gases (H_2S and CH_4, and O_2 when measured by the Winkler method) should be sampled in an unbroken streamline and transferred to gas-tight containers without contacting air until analysis is complete. Samples should not be filtered prior to analysis of these compounds as the filtration process may cause significant losses. Preservation of samples to be analysed in the laboratory should be done in the field to avoid precipitation (Fe^{2+}, Mn), oxidation (e.g., H_2S, Fe^{2+}, Mn, NH_4^+) or conversion of the compounds (NO_3^-, CH_4). Preferably, analysis of Fe^{2+} and H_2S should be performed in the field, as storage of such labile compounds may be very difficult.

4.4. Applications

The use of redox-sensitive compounds for deducing redox conditions is, as indicated above, no easy task; however, the advantages (easy analysis and low cost, assuming that the groundwater is sampled anyway) call for use in most cases. Redox-sensitive compounds have been used in at least three different ways:

- Identification of reduced and oxidized conditions,
- Assignment of redox zones,
- Determination of predominant redox reactions.

Identification of reduced and oxidized conditions in a plume follows closely the lines presented in the section on principles (Section 4.2). This has been presented in many different ways for contaminated sites. A comprehensive discussion of redox couples in the Borden Landfill (CND) leachate plume is presented in Nicholson et al. (1983). A recent example presents the redox conditions in a groundwater/surface water interface contaminated by chlorinated ethenes (Lendvay et al., 1998a,b). Amirbahman et al. (1998) described aqueous phase data as indicators of redox processes beneath and downgradient the Winterthur Landfill (CH). Evaluation of dynamic changes is of great interest and here the redox-sensitive compounds may be a good approach, because

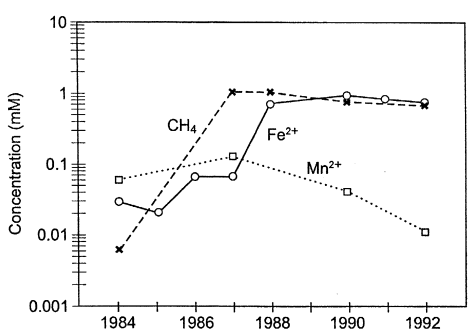


Fig. 8. Concentration (mM) of Fe^{2+}, Mn and CH_4 in groundwater downgradient an oil spill at the Bemidji (MN, USA) site (after Baedecker et al., 1993 and Baedecker and Cozzarelli, 1994, with permission).

repeated sampling in the same point is possible (opposed to sediment samples). Detailed monitoring of temporal or spatial changes has been done during an anaerobic field injection experiments at the Grindsted Landfill site (DK) (Albrechtsen et al., 1995). At the Bemidji site (MN, USA), contaminated by crude oil, the spatial and temporal trends in groundwater chemistry were investigated in the downgradient wells (e.g., Fig. 8, Baedecker et al., 1993). Significant increases in methane and Fe(II) were observed indicating a change in the overall redox conditions from aerobic to anaerobic conditions due to the oil spill. In general, the data presented in the literature support the usefulness of characterizing groundwater samples in terms of redox-sensitive species, but the limitations with respect to species interacting with solid phases are apparent. Generally, evaluation of redox conditions based on measurements of only a few redox-sensitive compounds in a few wells should only be taken as indicative.

Assignment of redox zones to leachate plumes has been done in several cases. The early cases separated leachate plumes, on the basis of nitrogen compounds and oxygen, into an anaerobic zone, a transition zone and an aerobic zone (Golwer et al., 1969; Baedecker and Back, 1979a,b). In the latter case, the ratio between Kjeldahl N and

Table 8
Criteria used for assigning redox conditions to groundwater samples from the Vejen Landfill (DK) leachate plume (Lyngkilde and Christensen, 1992) and Grindsted Landfill (DK) leachate plume (Bjerg et al., 1995)

Parameter	Methanogenic	Sulfate-reducing	Iron-reducing	Manganese-reducing	Nitrate-reducing	Aerobic
Methane	> 1	< 1	< 1	< 1	< 1	< 1
	> 25	–	–	–	–	< 1
Sulfide	–	> 0.2	< 0.1	< 0.1	< 0.1	< 0.1
	–	> 0.1	–	–	< 0.1	< 0.1
Sulfate	< 40	–	–	–	–	–
	–	–	–	–	–	–
Dissolved iron	–	–	> 1.5	< 1.5	< 1.5	< 1.5
	< 150	< 150	> 150	< 10	< 10	< 1.5
Dissolved manganese	–	–	–	> 0.2	< 0.2	< 0.2
	< 5	< 5	< 5	> 5	< 0.2	< 0.2
Ammonium	–	–	–	–	–	< 1
	–	–	–	–	–	< 1
Dinitrogene oxide	NI	NI	NI	NI	NI	NI
	< 1	< 1	< 1	< 1	> 1	–
Nitrite	< 0.1	< 0.1	< 0.1	< 0.1	> 0.1	< 0.1
	< 0.1	< 0.1	< 0.1	< 0.1	> 0.1	< 0.1
Nitrate	< 0.2	< 0.2	< 0.2	< 0.2	–	–
	< 0.2	< 0.2	< 0.2	< 0.2	–	–
Oxygen	< 1	< 1	< 1	< 1	< 1	> 1
	< 1	< 1	< 1	< 1	< 1	> 1

The upper criteria are for the Vejen Landfill and the lower criteria are for the Grindsted Landfill. All concentrations in mg/l, except dinitrogenoxide in μg/l, nitrate, nitrite and ammonium in mg N/l and sulfate in mg S/l.
–, No criterion defined.
NI, Not included in the criteria for the Vejen Landfill.

nitrate was also used for characterising the plume. The concept of assigning redox zones on groundwater redox-sensitive compounds was fully developed by Lyngkilde and Christensen (1992), but Chapelle et al. (1995) also used redox-sensitive compounds for characterization of redox conditions. Lyngkilde and Christensen analysed the data on redox-sensitive compounds from 366 groundwater samples from the Vejen Landfill (DK) leachate plume. Each sample was given a specific redox label based on the criteria given in Table 8. The concept was based on the ideas presented Table 7, but the actual concentration levels were selected from an evaluation of leachate and plume composi-

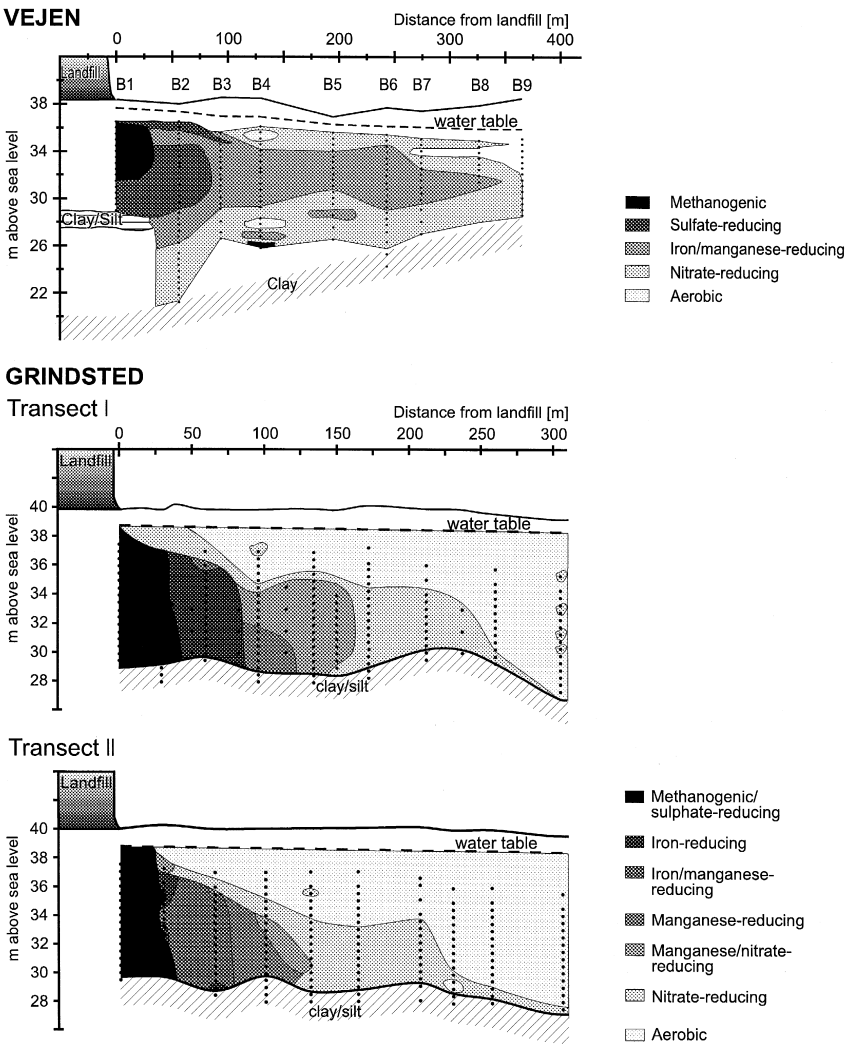


Fig. 9. Identified redox zones at the Vejen Landfill (DK) (Lyngkilde and Christensen, 1992) and the Grindsted Landfill (DK) (from Bjerg et al., 1995, with permission).

tion with respect to redox-sensitive compounds. The criteria values used are, thus, subjective, which does weaken the generality of the approach, as other criteria values may lead to a somewhat different redox assignment (see next paragraph). At the Vejen Landfill leachate plume, 95% of the water samples could be assigned a redox label. This is a high percentage, which indicates the robustness of the procedure, but also the features of the plume. The application of short screens and small sample volumes allowing for depth-specific water samples was a key point. The information from these water samples was subsequently spatially integrated into a redox zone map (Fig. 9). Water samples that did not completely fit the criteria scheme were assigned a redox level based on the overall picture assuming continuity and coherency in the plume.

The concept was further developed at the Grindsted Landfill site (DK), where 285 groundwater samples were characterized with respect to redox-sensitive compounds in two transects (Bjerg et al., 1995). Distributions of redox-sensitive compounds in one of these transects are shown in Fig. 10. Criteria for dinitrogen oxide were added to the redox scheme and turned out to be a sensitive indication of nitrate reduction. The redox criteria at the two sites differ significantly (see Table 8, which contains both criteria). The differences are with respect to methane, iron and manganese, which were found in much higher concentrations in the Grindsted Landfill leachate plume than in the Vejen Landfill plume. Such differences will be common and depend on the redox activity, but also the flow velocities, sediment characteristics and dilution in the plume. This emphasizes that a redox criteria scheme for the assignment of redox status based on concentrations of redox-sensitive compounds in a dynamic system as a contaminant plume is site-specific and that actual conditions at the field site must be taken into account. The investigations in two parallel transect (30 m apart) at the Grindsted site also indicated some differences in the redox chemistry and the resulting redox zonation (see in Fig. 10), again emphasising the impact of local variations on the actual redox zonation. The proposed redox zonation at Grindsted and Vejen Landfills indicates a thermodynamic sound redox sequences; but it also shows some overlaps in the reduced parts of the plume. This may partly be an artifact due to the transport of compounds from upgradient locations (e.g., methane), but may also reflect that more redox processes take place simultaneously within the same zone. This cannot be sorted out solely on basis of the distribution of redox-sensitive compounds in groundwater samples. However, for the assignment of redox zones, a high spatial resolution in the sampling network, short screens, and small sample volumes are crucial in order to avoid mixing of groundwater from different redox zones and to identify the steep redox gradients often observed. This is especially critical in the outskirts of the plume, where horizontal and vertical variations will be significant, and in geological settings with local heterogeneity containing deviating redox environments by nature.

Recently, the protocol for natural attenuation as a remedy for chlorinated solvent plumes has introduced a screening tool for evaluation of the potential for reductive dechlorination of, e.g., PCE and TCE (Wiedemeier et al., 1996). The redox-sensitive compounds are part of this evaluation; however, it should be emphasises that this is not a redox characterization scheme and the numbers given are only indicative for aerobic conditions (no potential for reductive dechlorination) or strongly anarobic conditions (potential for reductive dechlorination).

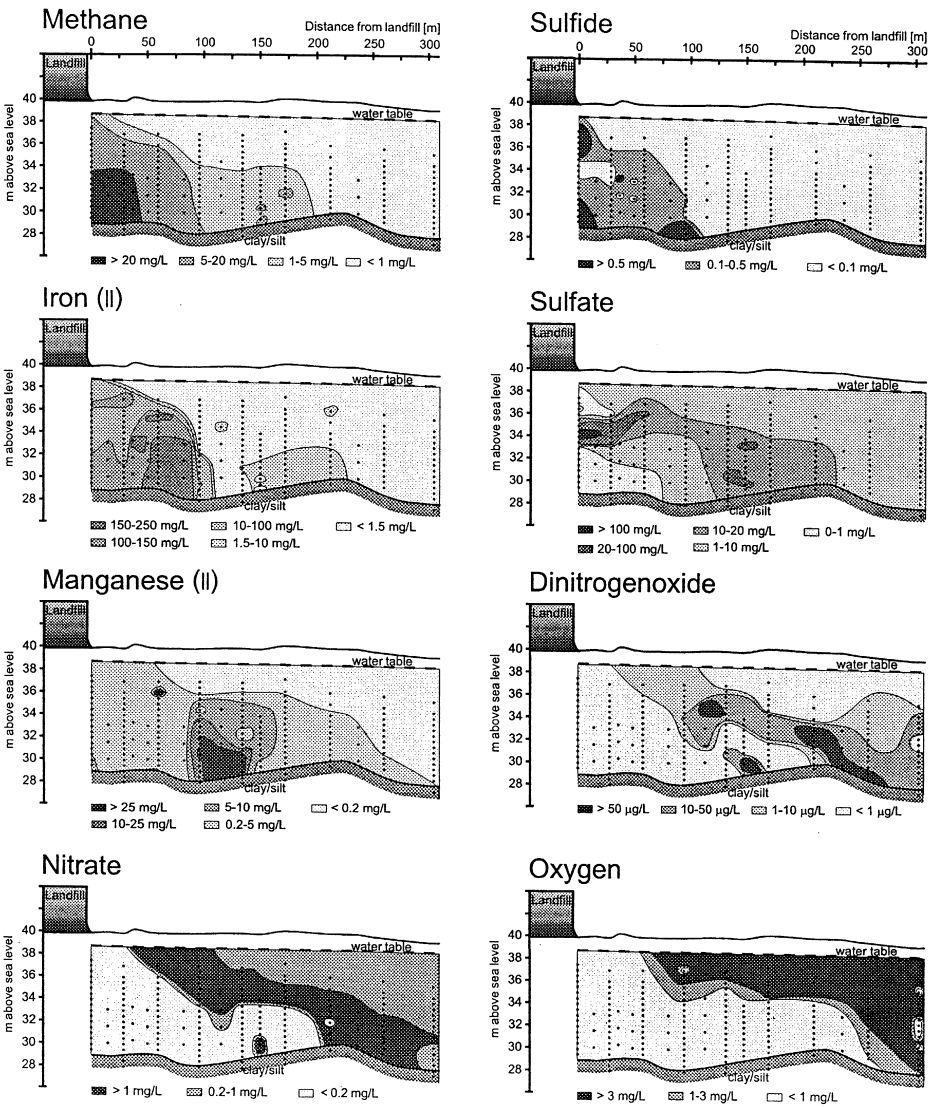


Fig. 10. Longitudinal vertical transect contours of redox sensitive compounds in the plume (Transect I) at Grindsted Landfill (DK) (modified from Bjerg et al., 1995, with permission).

The relative importance of the different redox reactions has also been evaluated using dissolved redox-sensitive species. Typically, concentrations of electron acceptors or generated end products are compared inside and outside the plume (Borden et al., 1995; Ravi et al., 1998). The relative importance of each electron acceptor process is calculated assuming a known stoichiometry of the reaction. This has especially been applied in petroleum hydrocarbon plumes undergoing natural attenuation (Wiedemeier et al., 1995). In some cases, it has been done along a flow line, where the dominating

redox process in a specific section of the plume or the actual rate of the process can be estimated (Amirbahman et al., 1998; Ludvigsen et al., 1998). The major drawback of this method is a clear underestimation of the iron-reduction and manganese-reduction processes as the presence of ferrous iron and manganese in the aqueous phase only represent a minor fraction of the total reduced pools (see discussion in Section 7). Likewise, sulphate reduction can be underestimated if sulphide is used for the calculation (sulphide is likely to precipitate); thus, dissolved sulphate should be the preferred parameter unless there are indications of sulphate sources, such as dissolving barite or gypsum or oxidizing sulphides.

4.5. Evaluation

Quantifying redox-sensitive species in groundwater samples is a simple and useful tool for identifying the redox conditions of a groundwater contaminant plume. The basic principles are thermodynamically sound; but the actual criteria for assigning the redox status depend, to some extent, on local conditions, such as the natural groundwater geochemistry, compounds leaching from the source and quality of sampling and analytical equipment. The major limitation in using the concept is related to migration of redox-sensitive species away from active zones, geochemical processes involving precipitation of compounds and the actual overlap between different redox zones. The transport of reduced redox-sensitive species, e.g., methane and ferrous ion, will smear out the redox zones identified by groundwater sample composition. Presumably, the strongly reduced zones will be slightly overestimated. Precipitation and ion exchange of iron and manganese and precipitation of sulphur species will also affect the identification of the redox state in a given point. For example, iron and sulphate reduction, based on the groundwater composition, may be suggested in areas where the presence of reduced sulphur and ferrous iron primarily is due to dissolution of solid precipitates rather than actually on-going redox processes. However, precipitation of reduced sulphur, iron and manganese may also cause underestimation of significant redox processes if solely quantified in the groundwater. The above limitations cannot be avoided, but a general awareness of the potential problems can limit mistakes and put the interpretation and use of the identified redox zones into the correct perspective.

Redox-sensitive species have been successfully applied for characterization of redox environments in contaminant plumes in sandy aquifers. Identifying a full sequence of redox zones requires many high-resolution samples from short screens. The actual assignment of redox zones will be influenced by the specific criteria set in each case and the zones identified are, therefore, only indicative of the actual redox conditions. However, despite these limitations, the approach seems useful and further applications in different types of contaminant plumes would be interesting.

Changes in concentrations of redox-sensitive species in groundwater have been widely used for calculating the dominant electron acceptor processes. However, a simple calculation based on the groundwater composition alone will often be erroneous, because the solid phases are neglected. This is, in particular, problematic with respect to iron reduction, because high amounts of reduced iron may have precipitated on to the solids. This should be taken into account in the future.

5. Hydrogen

5.1. Background

The use of H_2, as a general indicator of the predominant redox process, was introduced by Lovley and Goodwin (1988). The paper indicated that each anaerobic redox process was characterized by a well-defined range of H_2 concentrations. The proposed theory suggested that in a steady-state system, limited by the availability of organic matter, the H_2 concentration is constant and controlled by parameters related to the physiology of the mediating bacteria, which again is related to the redox process they are mediating. The physiological parameters were also assumed to be related to the energy available at "standard" (pH = 7) conditions. This, in turn, suggested that redox zonation was a result of the competition for the limited substrate, where microorganisms able to use lower H_2 concentrations, e.g., Fe-oxide-reducing bacteria, out-compete microorganisms which demand higher H_2 concentrations, e.g., sulphate-reducing bacteria. This competition would lead to characteristic hydrogen concentrations for different redox zones. The concept was applied to pristine and polluted groundwater by, e.g., Chapelle and McMahon (1991) and Chapelle et al. (1995). However, the concept of characteristic H_2 levels neglects the influence of solute concentrations, the stability of iron oxides and temperature, shown later, and now seems of less general value.

Recent studies have shown that H_2 values reflect the energetics of the system studied much more precisely (e.g., Hoehler et al., 1998; Jakobsen et al., 1998; Jakobsen and Postma, 1999) than assumed by Lovley and Goodwin (1988). As discussed below, this implies that a given measured H_2 concentration does not necessarily indicate a given redox process. Theoretically, two different redox processes may, depending on substrate and product activities, lead to the same H_2 concentration. Still, the measurement of H_2 is an extremely valuable tool in analyzing the energetics of microbiological processes (Hoehler, 1998), and it appears to enable the integration of redox processes in a thermodynamically based description of a given system (Jakobsen and Postma, 1999).

5.2. Principles

As shown in Fig. 2, the anaerobic oxidation of organic matter from complex organic compounds generally goes through a three step process. After an initial hydrolyzation of the organic substances, the evolving substances are fermented into smaller organic molecules, such as lactate, propionate, butyrate, acetate and formate, as well as CO_2 and H_2. These fermentation products are then used as electron donors in the TEAPs by the bacteria mediating the TEAPs. One of the dominant products is H_2. The relative distribution of products is a function of the concentration of these, so at low H_2 concentrations relatively more H_2 is produced (Dolfing, 1988). The described stepwise degradation is not necessary in aerobic systems (Fig. 2); exactly what hydrogen measurements from the aerobic zone relate to is currently not clear.

The initial hydrolyzation has experimentally been shown to be overall rate limiting for the rate of methanogenesis (Gujer and Zehnder, 1983), implying that the supply of

fermentation products is limited. This is also indicated in a more general sense by the extremely low concentrations, in the nM range for H_2 and μM range for acetate measured in groundwater, compared to the mM changes in concentrations observed for the other reactants (Postma and Jakobsen, 1996). This implies that the concentration of the intermediates is under the control of the bacteria carrying out the TEAPs. H_2 is special in this context in several ways. The nM concentrations of H_2 in groundwater makes the pool of H_2 extremely small compared to the rate of production and consumption of this very important intermediate. The residence time is, thus, on the order of seconds at high TEAP rates (> 30 mM/year), to minutes in systems with low TEAP rates (< 0.5 mM/year). Therefore, H_2 concentrations must reflect ongoing processes, as transport is extremely limited within this time frame. Even in a groundwater system with a high flow rate of 1 m/day, a residence time of H_2 of 1 min, the advective transport distance for H_2 is < 1 mm. As described in Section 6.2, there may be short transient phases, when shifting TEA, where the microbiology itself, and not the H_2 concentration, is rate limiting. The low H_2 concentrations otherwise observed confirms that the supply of intermediates is normally rate limiting.

The representativity of measured H_2 concentrations may depend on the system generating the H_2. In systems where particulate organic matter is fermented and the TEA being reduced is dissolved in the porewater (e.g., sulphate), H_2-producing fermenters are presumably associated with the organic matter and are surrounded by TEAP bacteria. In this case, there will be a short gradient in the H_2 concentration from the fermenting to the juxtaposed (closely associated or even directly connected) TEAP bacteria (Conrad et al., 1985; Hoehler et al., 1998). The TEAP bacteria will lose whatever H_2 they do not use to the bulk water by diffusion. This should mean that the bulk water ideally reflects the intracellular concentration of H_2 of the TEAP bacteria (Hoehler et al., 1998). Generally, when the aquifer is sampled by taking water samples, the H_2 concentration measured will reflect the bulk water. If the organic matter being fermented is dissolved, as in most pollution plumes, the fermenting and the TEAP bacteria may be less closely associated. In this case, there would be a gradient in the H_2 concentration from the fermenting to the TEAP bacteria, and sampling of bulk water might give an intermediate concentration. In the case of a solid electron acceptor, such as Fe oxides, the bulk water would also have an intermediate concentration with the detailed distribution depending on the rate of Fe-oxide reduction (Hoehler, 1998). In systems with internal gradients and several TEAPs occurring in different parts of the sediment, a correct interpretation of a measured concentration in a pumped water sample is not straightforward.

The H_2 concentration, controlled by the TEAP mediating bacteria, is related to the minimum energy required in order that the microorganisms can store (through ATP synthesis) the energy released by the TEAP for later use in their life processes (e.g., Westermann, 1994; Hoehler et al., 1998). The energy available, to a microorganism for a given TEAP, is a part of the Gibb's energy of the reaction (ΔG_r). The more negative the ΔG_r the higher the energy available. Table 9 lists estimates of the required energy for the different redox processes. If the H_2 activity is isolated in equations for calculating Gibb's free energy of reaction for a TEAP involving oxidation of H_2 (see Eq. (9) and the example associated), we can calculate the H_2 activity as a function of

Table 9
TEAP's, minimum energy requirements, H_2 ranges, and thermodynamic data, for terminal electron acceptor processes (TEAP's) with H_2

TEAP	Minimum ΔG_r for TEAP (kJ/mol H_2)	"Characteristic" H_2 conc. (nM)	ΔG_r^0 (kJ/mol)	ΔH_r^0 (kJ/mol)
cis-DCE + H_2 → VC + H^+ + Cl^-	−115.5	2[a]	−122.9[a]	–
H_2 + 2FeOOH + $4H^+$ → $2Fe^{2+}$ + $4H_2O$	−25[b]	0.1–0.5[c]	−178.5; −157.5	198.7
$4H_2$ + SO_4^{2-} + H^+ → HS^- + $4H_2O$	−25[d]	1–4[c]	−262.4	−235
HCO_3^- + $4H_2$ + H^+ → CH_4 + $3H_2O$	−18[d]; −7[e]	5–25[c]	−229.8	−237.8

Thermodynamic data on inorganic solutes are from Stumm and Morgan (1996). The ΔG_r^0 values for Fe-oxide reduction corresponds to the reduction of the least ($\Delta G_f^0 = -472.8$ kJ/mol) and most stable ($\Delta G_f^0 = -483.3$ kJ/mol) lepidocrocites (derived from Langmuir, 1997). ΔH_r^0 values was calculated from $\Delta H_{f\,(goethite)}^0 = -559.4$ due to the lack of a value for lepidocrocite. The error is minor because the effect of ΔH is small, the major effect is directly related to T.

[a] Yang and McCarty (1998).

[b] Uncertain value from a preliminary study (R. Jakobsen, Technical University of Denmark).

[c] Chapelle et al. (1995).

[d] Hoehler et al. (1998) (marine).

[e] Schulz and Conrad (1996) (limnic).

temperature and species activities, if ΔG_r is set equal to the required value. This can be compared with observations, as it is done for sulphate reduction and methanogenesis in Fig. 11. The correspondence between data and the theoretical curves must imply that the H_2 concentration in the studied system is extremely closely tied to the thermodynamics of the system. Fig. 12 shows how the combined effect of variations in temperature, as well as Fe-oxide stability and Fe^{2+} activities, theoretically should affect H_2 concentrations for Fe-oxide reduction. Small variations in ΔG_f of the lepidocrocite, in this case 1 kJ/mol FeOOH, compared to a possible 21 kJ/mol variation just for lepidocrocite (Table 9), and a small variation in the Fe^{2+} activity (a factor of 3) could lead to variations in H_2 concentration of more than one order of magnitude for a given temperature. Including the variation due to temperature adds another order of magnitude. If the effects are similar in real systems, the use of a specific H_2 range to indicate Fe reduction seems difficult, especially at high temperatures. It also implies, as has been observed in several cases, that Fe reduction may take place concurrently with sulphate reduction (Canfield et al., 1993; Bjerg et al., 1995; Postma and Jakobsen, 1996; Ludvigsen et al., 1998; Jakobsen and Postma, 1999) and methanogenesis (Bjerg et al., 1995; Ludvigsen et al., 1998; Jakobsen and Postma, 1999).

It is perhaps puzzling why redox zonation develops in the first place if the energy available to any microbial TEAP is always at a minimum for the given bacteria. Hoehler et al. (1998) suggest a slight modification of the concept proposed by Lovley and Goodwin (1988). According to Hoehler et al. (1998), the bacteria trade their potentially higher energy yield for competitive edge. In other words, the TEAP that allows for the

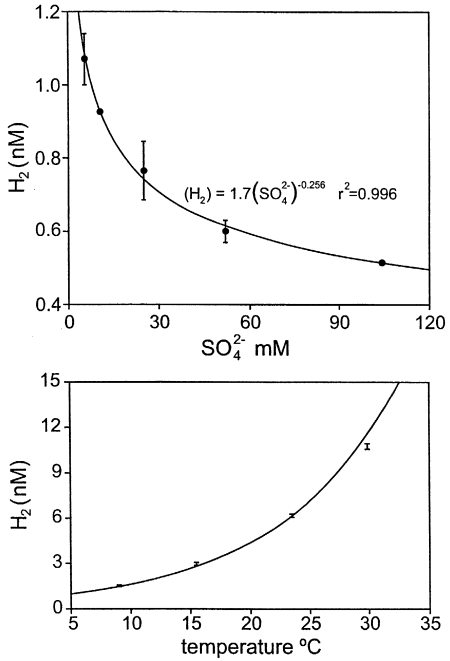


Fig. 11. Effects of SO_4^{2-} concentrations and temperature on the steady-state H_2 concentration during sulfate reduction and methanogenesis (modified from Hoehler et al., 1998, with permission).

lowest H_2 concentration at the given in situ conditions is preferred as this excludes possible competitors not able to use this TEA. The bacteria may or may not be able to succeed in this depending on both the thermodynamical and the physical in situ conditions. In some cases, several TEAPs will be able to proceed concurrently, perhaps with different minimum energy yields related to the specific TEAP, but at the same H_2

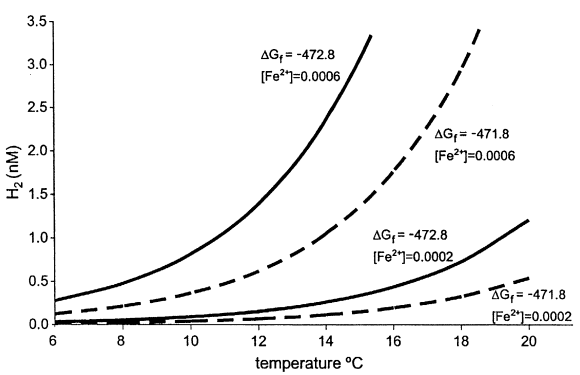


Fig. 12. Theoretical effects of temperature, solute activities, and Fe-oxide stabilities on the steady-state H_2 concentration during Fe-oxide reduction (modified from Jakobsen et al., 1998, with permission).

concentration. An alternative explanation, proposed here, is that when lowering the H_2 concentration as much as possible, by using the TEA allowing the lowest H_2 concentration at the given in situ conditions, the fermentation is shifted towards a more complete oxidation of the organic carbon to CO_2. This would imply a larger supply of H_2 to the H_2 oxidizing bacteria, and perhaps an overall more efficient use of the total energy available in the organic carbon.

The tendency of the bacteria to always minimize the energy of the ongoing TEAPs to a fixed value close to equilibrium suggests that the system can be viewed as being in a state of partial equilibrium (Postma and Jakobsen, 1996; Jakobsen et al., 1998, Jakobsen and Postma, 1999). The term partial equilibrium was originally used by Helgeson et al. (1968) for describing irreversible weathering processes. In terms of redox processes, partial implies that the rate of hydrolyzation and fermentation of the organic carbon controls the overall kinetics of the system, while the TEAPs are described by equilibrium chemistry by taking into account the energy needed for the bacteria. Based on this, the redox state of the system is evaluated by calculating the ΔG_r of the TEAPs using the expressions shown in Table 9. The calculated ΔG_r will indicate the relative potential for the given TEAP, just as calculating the E_H for a given process would. However, by using the ΔG_r it is possible to compare the calculated value with the minimum energy yield for the given TEAP, ideally enabling a judgement of whether a process may or may not occur. With this approach, the interpretation does not rely on the assumption of steady state implied in the Lovley and Goodwin (1988) approach. Especially in contaminant plumes, an assumption of steady state may not be valid (Vroblesky and Chapelle, 1994). Calculating the ΔG_r's, however, has other limitations. Evidently, the relevant parameters must be available to calculate the relative potential (ΔG_r) for the different TEAPs, but to precisely evaluate whether a given process may or may not occur requires a value for the minimum energy yield for that process. The values of Hoehler et al. (1998) are well documented (Fig. 11), but relate to a marine environment where the high Na^+ concentration might have an influence (Hoehler, 1998). It could explain why the minimum energy yield observed by Schulz and Conrad (1996) in limnic Lake Constance (CH) sediments are lower (Table 9). This would imply that H_2 concentrations in pollution plumes might vary, depending on the ionic strength of the plume, related to the plume source. For Fe oxides, it is close to impossible to know exactly which Fe oxides are being reduced. However, the measured H_2 concentration should, under circumstances where there are indications of Fe-oxide reduction, enable an estimate of which Fe oxide apparently controls the H_2 concentration. Currently, the database is too small to address the various limitations and aspects related to the use of ΔG_r values in detail.

An advantage of the ΔG_r approach is that it can be used to directly address the potential for degradation of xenobiotic compounds, if thermodynamic parameters and minimum energy yields are known. Fortunately, these values are starting to appear (Yang and McCarty, 1998). The approach may also be used the other way around. In cases where a downgradient increase in methane indicates methanogenesis, but the calculated ΔG_r is too high for methanogenesis to be feasible, methanogenesis must be occurring in stagnant subdomains of the plume. Knowing the minimum energy yield for methanogenesis enables a calculation of the minimum H_2 concentration in these

subdomains. With this, the degradation potential within the subdomains for a compound for which thermodynamic parameters and minimum energy yields are known may be evaluated.

5.3. Methods

The nanomolar concentrations of H_2 found in groundwater are quantified by gas chromatography with a reduced gas detector (RGD2 or 3, TraceAnalytical), calibrated with standards of H_2 diluted in N_2. The principle is that reduced gases react with a heated bed of HgO producing free Hg vapour, quantified by a UV spectrophotometer.

Measurements of groundwater concentrations are usually made using "bubble-stripping" (Chapelle and McMahon, 1991) where a bubble of N_2 is equilibrated with the groundwater by letting groundwater flow continuously through the bubble. The method can be scaled down as described in Bjerg et al. (1997), and current tests indicate that further downscaling is possible. When the bubble is equilibrated a gas sample is taken from the bubble with a syringe and injected into the gas chromatograph. The equilibration time is 10–30 min depending on bubble size, water flux, agitation intensity, and temperature. Care should be taken to limit flow velocities in the sampled aquifer volume, as high flow rates tend to lower the measured H_2 concentration (Jakobsen and Postma, 1999). The measurement must be made in the field since H_2 is generally not stable in the mixed gas phase, which develops in the bubble, and H_2 is extremely volatile and tends to escape.

An alternative is to let the gas sample equilibrate with the groundwater by diffusion through a gas permeable wall. This can be done by lowering a teflon-tubing spiral into the well (Chapelle et al., 1997), requiring that the water in the well is continuously renewed either naturally or by pumping during the equilibration. In small-scale tracer tests, prolonged continuous pumping can be a problem, but the spiral can be inserted in direct contact with the sediment (Frank, 1996). Removal of water is then not needed, but the teflon tubing must be connected directly to the surface by, e.g., copper tubing, limiting the depth to which the method can be applied. The known air volume of a spiral system is sampled by mixing it with a known volume of N_2 using a syringe. Equilibration times are on the order of 12–24 h, depending on sample volume, tubing surface area, wall thickness, and temperature. Other alternatives are described in Chapelle et al. (1997).

Generally, reactive metal surfaces (Bjerg et al., 1997) and electrical DC pumps should be avoided as they may reduce the protons in water to hydrogen (Chapelle et al., 1997). Wells should not be used for sampling until the elevated H_2 concentrations induced by the well installation is gone, and working close to the well with heavy equipment prior to sampling should be avoided (Bjerg et al., 1997).

As discussed by Hoehler (1998), measurements of concentrations in sediment samples cannot be made by transferring the sediment to an incubation flask, waiting for 1–2 h for equilibrium between the pore water and the gas, and then sampling the overlying headspace. It is necessary to wait until the entire system is reequilibrated. Due to the very dynamic nature of the H_2 pool, the physical pore water/gas equilibration with the

headspace will remove H_2 from the sediment, but H_2 will rapidly be replenished by fermentation in an attempt to reach equilibrium. More H_2 will go into the headspace and so on, until the headspace is finally in equilibrium with the sediment H_2 concentration. It is impossible to calculate the sediment concentration, until this new equilibrium is obtained because the total amount of H_2 in the system is increasing over time at an unknown rate. However, once equilibrium is reached, incubation flasks may give very good results, if great care is taken with regards to keeping the temperature constant and if flasks are not resampled until the system has reequilibrated. Equilibration times of headspace flasks are on the order of days and weeks depending on sediment/headspace ratios and volumes and the H_2 production rates.

5.4. Applications

There is a limited number of studies of H_2 distributions in plumes. Three studies describe specific pollution plumes: Hanahan (SC, USA) dominated by JP-4 jet fuel (Vroblesky and Chapelle, 1994), Kingsbay (GA, USA) dominated by chlorinated solvents (Chapelle and Bradley, 1998), and Wurtsmith (MI, USA) dominated by petroleum products and solvents (Chapelle et al., 1996). Two studies describe mixed landfill plumes: the Grindsted Landfill (DK) leachate plume (Jakobsen et al., 1998), and the Norman Landfill (OK, USA) (Harris et al., 1999). Finally, there is a study from a plume of MAHs and PAHs entering a complicated fill-aquifer in Charleston (SC, USA) (Landmeyer et al., 1998).

Fig. 13 shows the H_2 levels and the concentrations of redox-sensitive parameters from a volume at the Hanahan site (SC, USA). Chapelle et al. (1995) used a combination of geochemical data, in terms of changes in solute concentrations along the flowpath, and the H_2 concentrations in each well to describe zones predominated by methanogenesis or sulphate reduction. The geochemical data, however, suggest that several redox processes may well be occurring concomitantly, at comparable rates, along several paths in this system. A pronounced increase in methane is seen along the proposed sulphate reduction flow path (Well 41B → Well 33B). This increase is 35 times larger on a molar basis, than the decrease in sulphate. The flow path from Well 31A to Well 34A also has a larger increase in methane compared to the sulphate decrease and furthermore shows an increase in the Fe^{2+} concentration. Along the proposed predominantly methanogenic flow path (Well 31B → Well 34B), a pronounced increase in Fe^{2+}, not noted in the paper, is seen. The increase might be due to the reduction of a rather stable Fe oxide, or it could be related to a decrease in pH (Chapelle, personal communication) due to acid-producing reactions, leading to dissolution of FeS phases. The latter is supported by a similar increase in the total sulphide concentration. It is noted that even if Fe-oxide reduction is occurring, there is no doubt that the flow path from Well 31B to Well 34B represents the most reduced conditions within the shown volume of the aquifer.

The Kingsbay (GA, USA) (Chapelle and Bradley, 1998) study is similar in the way that at a closer look several redox processes appear to take place within the same aquifer volume. Though this shows that the H_2 levels alone should not be used for identifying

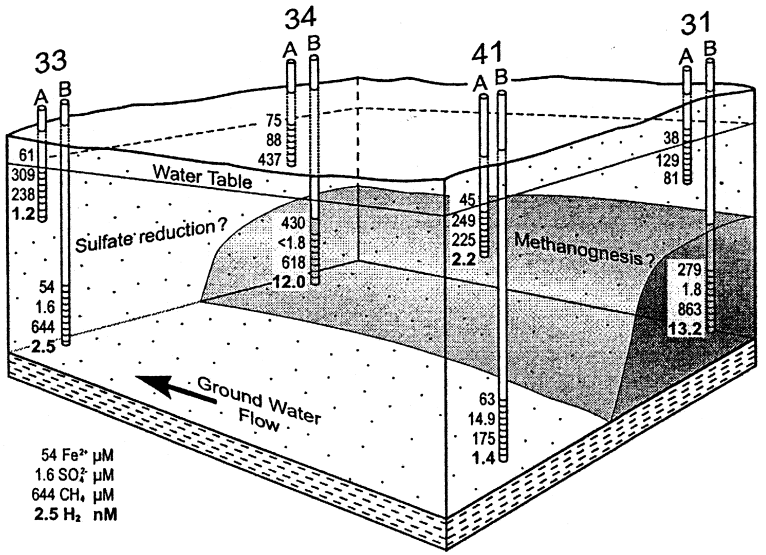


Fig. 13. H_2 and redox sensitive species at the Hanahan jet fuel plume (SC, USA) (modified from Chapelle et al., 1995, with permission).

specific redox processes, it is still clear that the highest H_2 levels ~ 2 nM are related to the most reduced part of the plume, closest to the source, while lower concentrations $\sim 0.2-0.5$ nM are found in the more distal parts. This fits well with disappearance of higher chlorinated compounds PCE and TCE in the most reduced part and subsequent oxidation of *cis*-DCE and VC in the less reduced part of the plume.

The Grindsted Landfill leachate plume (DK) was used to apply the partial equilibrium approach (Jakobsen et al., 1998). The measured H_2 concentrations are shown in Fig. 14. The highest H_2 concentrations are seen close to the landfill where you would also expect the most reduced conditions. This is also where methane, at concentrations around 1 mM, is found. Fe reduction seems to occur in most of the anaerobic volume of the plume. However, as shown in Fig. 12, it is difficult to directly apply calculations of ΔG_r to the reduction of Fe oxides. Still, the deeper part of the plume generally shows higher H_2 concentrations than the upper part, presumably because the underlying and older Miocene sediment is characterized by more stable Fe oxides, than the upper and younger Quaternary sediment. The calculated ΔG_r values for sulphate reduction and methanogenesis are shown in Fig. 14(b,c). Assuming that the minimum required energy is -7 kJ/mol H_2, there seems to be a zone close to the landfill in the upper part of the plume where sulphate reduction could occur. This corresponds to where decreases in the sulphate concentrations are seen. In the lower Miocene part, ΔG_r values also indicate sulphate reduction though this is less clear from the sulphate concentrations. The high ΔG_r values for methanogenesis, indicating no available energy, combined with methane concentrations that increase downgradient, implies that methanogenesis occurs in microniches with adequately low ΔG_r values. The H_2 measured in the aerobic part of the

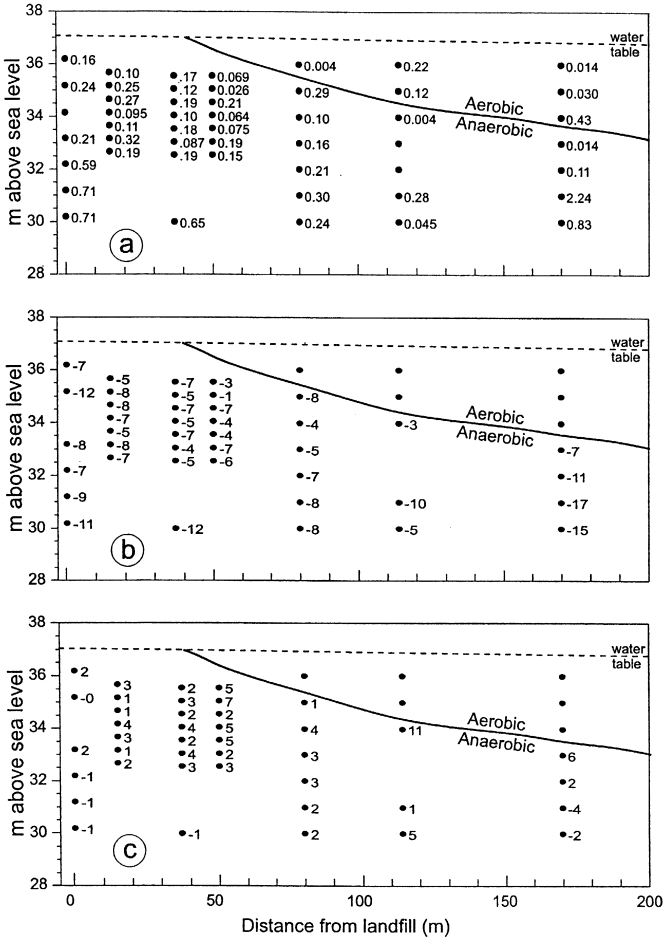


Fig. 14. H_2 profile (a) and calculated energy yields in kJ/mol. H_2 for methanogenesis (c) and sulfate reduction (b) for the Grindsted Landfill leachate plume (DK) (modified from Jakobsen et al., 1998, with permission).

aquifer is perhaps also related to microniches, though as mentioned earlier, the meaning of these values is uncertain.

The Norman Landfill plume (OK, USA) study (Harris et al., 1999) again shows that overlapping redox zones are common in plumes, and that especially Fe-oxide reduction is difficult to pinpoint. Measurements in sediment slurries showed that nitrate reduction led to a relatively low ($\approx$ 0.8 nM) H_2 level. For Fe-oxide and sulphate reduction, and for methanogenesis, H_2 values fell within $\approx$ 6–8 nM, making a separation of these based on H_2 difficult. In a depth profile in the plume, high H_2 concentrations of 1.6–1.9 nM were related to the central part of the plume where sulphate reduction occurred at high rates accompanied by methanogenesis. In the lower part of the plume, values ranged from 0.32 to 0.56 nM, presumably related to Fe-oxide reduction, although this

could not be confirmed by microcosm measurements. So though H_2 concentrations were not clear in diagnosing the redox processes, the highest H_2 values were clearly found in what was expected to be the most reduced part of the plume.

5.5. Evaluation

H_2 measurements are a potentially powerful tool for studying microbial processes, in general, and more specifically TEA degradation processes involving H_2. This is a consequence of the close link, through the thermodynamics of these processes, between H_2 concentrations and the microbiological processes actually occurring in the plume. However, a number of issues need to be addressed before this approach to quantification of redox levels can be fully exploited.

One of the difficulties is to know precisely what a given H_2 sample represents. Depending on aquifer conditions, sampling method, as well as electron donor and acceptor distributions, bulk and stagnant water may be represented differently and the H_2 concentrations in the two might well be different. This has implications for the prediction of degradation potentials. Also needed, is a larger database on what minimum energies are necessary for the different processes to occur under different conditions. There are indications that the minimum energy required is related to environmental factors, such as temperature (Schulz and Conrad, 1996) and Na^+ concentration (Hoehler et al., 1998). Further complications are related to TEA threshold concentrations. This is important for sulphate reduction, which appears to more or less stop when the sulphate concentration is lower than 5–20 μM (Ingvorsen et al., 1984; Lovley and Klug, 1986). Jakobsen and Postma (1999) found sulphate reduction to cease even though ΔG_r was still low enough for the reaction to be feasible. How TEA threshold concentrations affect degradation of TEAs, in general, is not known. Finally, insight is still lacking on precisely how TEAPs, involving the reduction of solid phases, such as Fe oxides and Mn oxides, are related to in situ H_2 concentrations.

Specific steady-state H_2 levels as suggested by Lovley and Goodwin (1988) are not applicable to pollution plumes. The main shortcomings are that fixed H_2 levels do not account for the effects of actual concentrations of dissolved redox species, variability in iron oxides being reduced, actual temperature of the aquifer and the fact that redox processes may be overlapping and not exclusive. Also, the necessity of assuming steady state within the studied system is a limitation. The concept of specific H_2 levels could be viewed as a special case of a more general "partial-equilibrium" approach where the in situ activities of reactants and products controlling the redox processes are taken into account.

The H_2 concentration will, regardless of the chosen approach, indicate the redox level in a relative sense. High H_2 concentrations are found in systems that are more reduced than systems with low H_2 concentrations. In terms of degradation of specific compounds, designating the presence of highly reducing conditions may be more important than knowing the specific inorganic TEAP. Repeated monitoring of H_2 concentrations, may be a sensitive tool in evaluating the stability of plume conditions, which is of importance in relation to in situ bioremediation.

6. Volatile fatty acids (VFAs)

6.1. Background

VFAs are produced as fermentation products through the degradation of organic matter (Fig. 2) and specific levels of volatile fatty acids, such as acetate, have been reported for different redox processes in different types of sediments, e.g., by Lovley and Phillips (1987a) and McMahon and Chapelle (1991). Of the VFAs, acetate and formate are observed in the highest concentrations, whereas propionate and butyrate are measured in lower concentrations. So far, only little data have been published regarding the relation between the VFAs and the redox conditions in aquifers.

6.2. Principles

VFAs are intermediates produced under anaerobic conditions, as H_2 is, and it has been observed that the concentration of VFAs often increase as electron acceptors become depleted. This has raised the hypothesis, that the concentration level of VFA can be used as an indicator of redox processes (Vroblesky et al., 1997). However, the dependency of the available energy for the oxidation of VFA is much lower than it is for H_2, because more electrons are transferred per mole of VFA and, therefore, the use of VFAs as indicators of redox processes is inherently more difficult. Recent results from Hoehler et al. (1999) indicate that transient high peak concentrations of VFAs may be related to a shift in the TEAP, rather than to a substantially different steady state concentration for the new TEAP. Data both from an in situ marine sediment and an incubation experiment (Fig. 15) show how high concentrations of VFA occurred in connection to a shift in the predominant redox process from sulphate reduction to methanogenesis as sulphate was exhausted. In the lag time from the exhaustion of sulphate until full methane production was reached, the buildup of acetate resulted in a pool that lasted for some time — especially in the natural setting. A similar phenomenon was seen for H_2 in the incubation. An intermittently high, but relatively long-lasting concentration peak in the acetate concentration, may have been misinterpreted as plateaus in short term experiments and in noncontinuous studies of various systems. In other words, the higher concentrations are observed when the TEAP changed may have erroneously been attributed to the TEAP taking over, and not to a transient phenomena related to the TEAP shift itself.

6.3. Methods

Groundwater samples collected for analysis of VFAs should be protected from loss of the volatile acids through evaporation during, and after sampling. Since the VFAs are readily degradable under aerobic, as well as anaerobic conditions, the samples have to be preserved immediately. Addition of 0.2% chloroform and immediate freezing in polypropylene vials has been found to be a very simple and yet efficient preservation

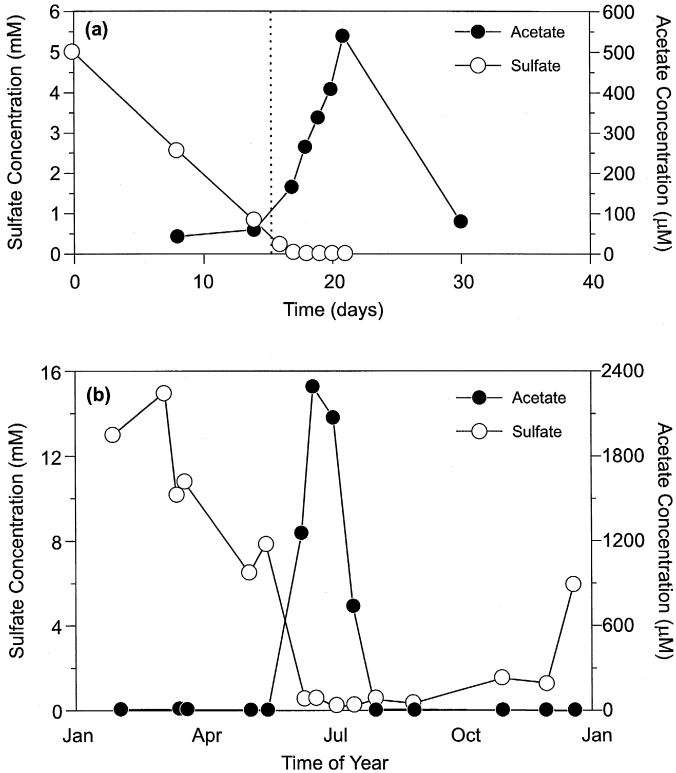


Fig. 15. The transition from sulfate reduction to methanogenesis and the peak in the acetate concentration related to this transition. (a) An incubation experiment, (b) At 13.5 cm depth in Cape Lookout Bight sediments (from Hoehler et al., 1999, with permission).

method (Hansen, 1998; Albrechtsen et al., 1999). This method of preservation also prevents generation of additional VFAs, which may occur in samples containing dissolved organic matter, e.g., humic and fulvic acids. The VFAs are generally analyzed for by Ion Exclusion Chromatography (Bradley et al., 1993). Using a Dionex AS-10 column and suppressed EC detection, Hansen (1998) obtained a detection limit of 0.2 μM formate or acetate.

6.4. Applications

In spite of the inherent difficulties, different levels of VFAs have been reported for different redox conditions. Formate has been observed at 5–60 μM in sulphate-reducing environments (McMahon and Chapelle, 1991; Chapelle and Lovley, 1992) and at 0–6 μM in Fe(III)-reducing environments (Chapelle and Lovley, 1992). Acetate has been observed at 2–50 μM in sulphate-reducing environments (Lovley and Phillips, 1987a; McMahon and Chapelle, 1991; Chapelle and Lovley, 1992) and at 0.5–3 μM in Fe(III)-reducing environments (Lovley and Phillips, 1987a; Chapelle and Lovley, 1992).

In a crude oil-contaminated aquifer at Bemidji (MI, USA), the acetate concentration increased over 3 years at one location from 0.47 to 42.5 μM, probably due to a shift from reduction of Mn(IV) and Fe(III) to methanogenic conditions. At another location in the plume, the acetate concentration decreased during the same period from 1.08 to 0.16 μM in accordance with an increase of Mn(II) and Fe(II) (Baedecker et al., 1993). Since acetate is more readily consumed by Fe-reducing bacteria, than by methanogenic bacteria (Lovley and Phillips, 1987a), the different developments in acetate concentrations may reflect a shift in redox processes.

In the Grindsted Landfill (DK) plume, the volatile fatty acids remained relatively constant throughout the plume (Table 10), which on the basis of results from several redox characterizing methods was predominantly iron-reducing. The concentration range of formate in a given sample was generally higher than the acetate concentration: e.g., 2.4–7.1 μM of formate vs. 1.3–5.4 μM of acetate (Table 10). These concentrations are comparable to those observed in an Fe-reducing zone by Chapelle and Lovley (1992). The propionate concentrations (< 0.1–0.3 μM) were significantly lower than the other two volatile fatty acids (Albrechtsen et al., 1999).

Despite the mentioned observations of different levels of VFAs under different redox conditions in some aquifers, a detailed study of a petroleum-contaminated aquifer at

Table 10
Groundwater and sediment chemistry in samples collected in a vertical longitudinal transect along the centre line of the injected cloud in the Grindsted Landfill (DK) plume

Parameter	Units				
Distance from injection	M	3	18	33	48
Distance from landfill	M	18	33	48	63
Investigated depths[a]	m	33.4–35.4	34.1–35.4	33.5–35.1	33.3–35.1
Groundwater					
pH		6.5–6.6	6.6–6.7	6.6–6.7	6.7–6.8
Temperature	°C	9.2–9.8	8.9–10.8	8.5–9.6	8.8–10.4
Electrical conductivity	mS/cm	1.4–4.5	1.5–1.6	1.4–1.5	1.3–1.5
Cl^-	mg/l	32–45	43–61	39–67	28–39
S^{2-} S	mg/l	< 0.2–1	< 0.2–1	< 0.2–1	< 0.2–1
CH_4	mg/l	13.3–25.5	3.8–25.0	4.9–23.5	6.2–16.4
SO_4^{2-} S	mg/l	0.2–0.5	0.2–0.4	0.2–0.7	0.5–1.4
Fe^{2+}	mg/l	116–130	116–134	141–171	219–269
Mn^{2+}	mg/l	1.7–3.2	1.0–3.4	1.3–2.6	2.5–5.0
NO_3^- N	mg/l	< 0.05	< 0.05	< 0.05	< 0.05
O_2	mg/l	< 0.2	< 0.2	< 0.2	< 0.2
H_2	NM	0.24–0.47	0.20–0.30	0.22–0.30	0.25–0.54
Formate	μM	3.6–5.1	3.7–7.1	2.4–6.1	4.7–5.6
Acetate	μM	2.6–4.2	2.5–4.9	1.3–5.4	3.3–4.4
Propionate	μM	< 0.1–0.2	< 0.1–0.3	< 0.1–0.2	< 0.1–0.2
NVOC	mg/l	51–56	54–60	52–58	41–58

Distance from the border of the landfill is given in parenthesis (modified after Albrechtsen et al., 1999, with permission).

[a] Meters above sea level.

Hanahan (SC, USA) found no correlation between VFAs and hydrogen concentrations (Vroblesky et al., 1997). However, the ongoing redox processes were inferred from H_2-mesurements and were not verified by TEAP bioassays. Furthermore, the detection limits were rather high in the Hanahan study, so possible relations of VFA concentrations to redox processes might be hiding in a lower range of values, while the observed high concentrations could be related to temporal TEAP changes described for the same site (Vroblesky and Chapelle, 1994). This and the fact that VFAs can be transported imply that the use of VFA concentrations for identifying local redox processes should be approached with great caution.

6.5. Evaluation

VFAs do not seem to function well as redox indicators — neither through a "specific level" nor an "available energy" approach. Furthermore, the steady-state VFA pool is normally three orders of magnitude larger than the H_2 pool, and as VFAs are mobile this pool can move correspondingly further downstream. However, this does not make them uninteresting in terms of redox processes, as they could perhaps be used for monitoring plume stability. As described above, shifts in the predominant TEAP can lead to an accumulation of the intermediates VFAs and H_2. A H_2 peak concentration would presumably not last long, making acetate a better candidate for indicating plume stability. The net production of acetate in a plume dominated by advective transport should lead to an intermittent acetate plume within the plume. Evaluating the stability of redox conditions in a plume, by monitoring VFA levels, could be useful in the context of intrinsic bioremediation, though more data is needed to verify that this would work in pollution plumes.

7. Aquifer sediment characterization

7.1. Background

Aquifer sediments contain large pools of redox-sensitive species in terms of minerals, precipitates and ions associated with exchange sites on particle surfaces. Important oxidized solid species are iron oxides, manganese oxides and sulphate present on exchange sites. Sulfate minerals may, in some cases, also constitute a significant pool of potential electron acceptors. Important reduced solid species are organic matter, sulphides, and iron and manganese carbonates. Reduced species, such as Fe^{2+}, Mn^{2+} and NH_4^+, may also be present on exchange sites. The sample calculations presented in Table 3 for two aerobic aquifers suggest that, in terms of OXC per volume of aquifer, solid iron and manganese oxides are predominant. In terms of reducing capacity, Table 4 suggests that solid sulphur species and iron species were predominant in the plumes. The sample calculations also showed that solid organic carbon may have significant capacities, both as electron donor and acceptor, if the pools are reactive. However, little is

known about the reactivity, and usually the reactivity of the sediment-bound organic carbon is considered to be low (see Section 2.4).

Redox potentials and activity relate to on-going processes but do not reflect the history of redox processes in terms of pools of species reacted over time, nor do they reflect possible future changes due to limitations of pools. To learn about plume development with respect to redox and to assess the pools of redox species that have reacted or may react, the sediment pools must be addressed. This may be done by quantifying solid pools of primarily organic carbon, iron, sulphur, and manganese species or as capacities in terms of OXC and RDC as defined in Eqs. (10) and (11).

7.2. Principles

Sediment redox characterisation may involve:

- Identification of the nature of individual sediment minerals;
- Species capacity (quantification of the bulk content of individual species, such as a total iron content);
- Reactive fraction measurements (an operationally defined fraction of a species that is available for a given reaction or the kinetics of a given reaction at a given capacity level);
- Redox capacity measurements (quantification of the available RDC and OXC).

Identification of individual sediment minerals is done by traditional mineral analysis. These methods identify minerals at the grain scale level and are mostly used for direct confirmation of minerals anticipated from other observations, e.g., from capacity measurements. The methods are primarily used qualitatively, since quantitative measurements are very demanding and fairly uncertain.

Species capacity measurements are used to determine bulk contents of species, such as iron, manganese, sulphur, and organic carbon (Ball et al., 1991). These elements, however, are found in a variety of different solid compounds; specific extraction and quantification of individual compounds is cumbersome, uncertain and for some compounds not feasible at all. Table 11 summarizes forms of iron, manganese, sulphur and carbon found in aquifers and possible extractants. Consequently, most bulk methods neglect the individual species and their redox state and determine only the element content. This can be done by a variety of methods, such as strong acid digestion and analysis of the digest, combustion of the sample followed by quantification of the evolved gases, and X-ray diffraction (XRD). Redox reactions in groundwater often are surface-related reactions, and since only a minor fraction of the bulk content of a mineral participates in these reactions (1–10%; Heron and Christensen, 1995; Kennedy et al., 1998), capacity measurements have limited value for identifying temporal changes and spatial gradients in sediment composition caused by redox reactions.

Several wet chemical methods for obtaining a more useful indication of the "reactive fraction" (or the expressed capacity) have been developed (Lovley and Phillips, 1986; Heron et al., 1994b; Kennedy et al., 1998). These relatively mild extractions preferen-

Table 11
Major solid species of Fe, Mn, S and C found in aquifers and contaminant plumes (based on Heron, 1994)

Species	Formula	Oxidation state	Extraction
Iron, Fe			
Ion-exchangeable Fe(II)	Fe^{2+}	+2	Anaerobic neutral salt solution[a]
Siderite	$FeCO_3$	+2	No good method
Trolite, greigite, Mackinawite	FeS, Fe_3S_4	+2	AVS extraction[b]
Pyrite	FeS_2	+2	Sequential extraction[c]
Silicates, clays	Fe(II), Fe(III)	+2, +3	No empirical methods
Ferrihydrite	$Fe(OH)_3$	+3	Reductive dissolution[d]
Goethite	FeOOH	+3	Reductive dissolution[d]
Hematite	Fe_2O_3	+3	Reductive dissolution[d]
Magnetite	Fe_3O_4	+8/3	No good method
Green rust	$Fe_3(OH)_8$	+8/3	No good method
Manganese, Mn			
Ion-exchangeable Mn(II)	Mn^{2+}	+2	Anaerobic neutral salt solution[a]
Pyrosulite	MnO_4	+4	Reductive dissolution[d]
Mixed Mn oxides	MnO_xH_y	+2, +3, +4	Reductive dissolution[d]
Rhodochrosite	$MnCO_3$	+2	No good method
Sulphur, S			
Adsorbed sulfate	SO_4^{2-}	+6	NaH_2PO_4 extraction[b]
Solid sulfate	$CaSO_4$, $FeSO_4$	+6	NaH_2PO_4 extraction[b]
Elemental sulfur	S(0)	0	Chemical extraction
Acid volatile sulfur	FeS, Fe_3S_4	−2	Hot HCl extraction[b]
Pyrite, marcasite	FeS_2	−1	Sequential extraction[c]
Carbon, C			
Organic carbon, TOC	Humic and fulvic acids and humin	? (assumed 0)	Standardized TOC analysis[e]
Inorganic carbon, TIC	$CaCO_3$, $FeCO_3$	+4	Acid extraction[e]

[a] For example, Hodgson (1960).
[b] Landers et al. (1987).
[c] Canfield et al. (1986).
[d] For example, Heron et al. (1994b).
[e] Heron et al. (1997).

tially address surface-related or very reactive minerals and leave the bulk and more stable minerals behind. A standard reaction time is chosen for a given method, but due to differences in sediment composition and the possibility of this interfering, a given method does not necessarily extract the same “reactive fraction” from different sediments. The analysis of extracts and evolved gases is used for determining the composition of the reacted phases. In the kinetic approach described by Postma (1993), the reaction is followed over time, by monitoring the extract concentration, allowing assessment of initial reactivities and a description of how the reactivity will change as a function of how much of the phase has reacted. Results for Fe oxides using this method give continuos curves for the release of Fe, showing that there are no well-defined pools of distinct reactivities, no “reactive fractions”.

Redox capacity measurements have been suggested as an overall quantification of sediment redox properties (Barcelona and Holm, 1991; Pedersen et al., 1991, Heron et al., 1994a). OXC and RDC are lumped variables containing contributions from multiple electron acceptors or donors and determined by the chemistry of the proposed reagent. These capacities fall in a category somewhat in between the bulk capacity measurements and the reactive fraction measurements, since the nature and strength of the chemicals, and the duration of the extraction affects the measured capacities (Barcelona and Holm, 1991; Heron et al., 1994a,b).

7.3. Methods

7.3.1. Sampling

Reliable sediment analysis heavily depends on obtaining a representative solid sample and keeping the reactive species intact during sampling, handling and storage. The current experiences include:

- The outer layers of soil cores should be pared of and discarded in the laboratory in order to avoid interference from the collection sleeve and from contamination.
- Drying of sediment can lead to oxidation of reduced species and loss of volatile components.
- Wet sample splitting is difficult and the reproducibility is usually poor.
- Storage in inert atmosphere is essential. Storage in an oxygen-containing atmosphere leads to oxidation of, e.g., ferrous sulphides, while storage in atmospheres containing hydrogen may result in reduction of electron-acceptors.
- No matter how samples are stored, microbial activity can change the composition; so short holding times and cooling are preferred before solids analyses.

In conclusion, rapid sample handling in the field, flushing with an inert gas, sealing and cold storage in an inert atmosphere, such as nitrogen or argon for only a limited time is recommended (see also Section 8.3.1).

7.3.2. Identification of individual sediment minerals

Direct identification of individual solid phases in reduced contaminant plumes has been done primarily using Scanning Electron Microscopy (SEM) (Baedecker et al., 1992; Heron et al., 1994b, 1998; Tuccillo et al., 1999) and energy-dispersive X-ray analysis (EDS) (Cozzarelli et al., 1999a). For SEM, the sediment must be dried anaerobically (e.g., by freeze-drying) and sieved to remove larger grains (e.g., > 0.2 mm) to allow for coating (e.g., carbon and/or gold) prior to the analysis. Samples should preferably be run the same day as prepared. SEM and EDS analysis allow for qualitative description of the sample with respect to abundance of minerals, the mineral shape and size, and bulk atomic composition. Heron et al. (1994b) identified iron oxides, Fe(II)-rich carbonates, and framboidal pyrite crystals in a landfill leachate-affected aquifer. Baedecker et al. (1992) and Tuccillo et al. (1999) identified iron-oxide coatings on pristine sediments and ferroan calcite crystals in solids from the reducing plume at

the oil spill site at Bemidji (MN, USA). Cozzarelli et al. (1999a) identified iron-sulphide coatings on ilmenite grains from a gasoline-contaminated aquifer (NJ, USA).

Some examples of the SEM pictures obtained and interpretations are given in Fig. 11. Direct identification of these minerals added certainty to associated studies on the overall plume geochemistry, and provided essential input for microbial activity studies by defining both abundant electron acceptors and reduced precipitates formed during microbially mediated redox reactions (Ludvigsen et al., 1998).

7.3.3. Species capacity and reactive fraction measurements

Lovley and Phillips (1987b) proposed a 0.5 N HCl extraction for determining reducible Fe(III) in sediments. A short extraction time was selected (1 h), based on the assumption that reducible iron hydroxides are noncrystalline, amorphous hydroxides, such as ferrihydrite ($Fe(OH)_3$), and that their dissolution kinetics are rapid compared to the dissolution kinetics of the crystalline iron oxides. This method is sensitive to relatively small changes in amorphous Fe(III) contents and, therefore, also useful for studying short-term changes during, e.g., laboratory incubations.

For studies of iron speciation in long-term experiments and in field plumes of several years of age, not only the amorphous iron hydroxides are reactive, and more aggressive methods are warranted. Heron et al. (1994a) developed and compared several iron speciation techniques, including 0.5 N and 5 N HCl, dithionite, ammonium oxalate, acetic acid and $CaCl_2$ extractions of landfill leachate-contaminated sediments. Traditional soil science extraction techniques (dithionite and ammonium oxalate) were rejected for use to determine Fe(III) contents in reduced sediments due to interference from Fe(II). Simple hydrochloric acid (HCl) extractions were suggested for iron speciation, based on the work by Sidhu et al. (1981) and Lovley and Phillips (1987b). Although these extractions yield an operationally defined quantity of Fe(II) and Fe(III), the simplicity of the methods was appealing. Mild (0.5 N for 24 h) and strong (5 N HCl for 21 days) extractions were used to describe the reactive fraction (expressed capacity) and the bulk content, respectively. Both Fe(II) and total Fe were quantified in the nonreductive extracts.

Capacity and reactive fraction measurement techniques for sulphur species were presented by Heron et al. (1994b), using cold and boiling HCl extractions combined with Cr(II) and HI digestions to separate pyrite, acid-volatile sulphides (AVS), chromium-reducible sulphur, elemental sulphur, and solid sulphates in sediments from a landfill leachate-contaminated aquifer. The combination of a hot HCl extraction for determination of AVS and a subsequent reduction by Cr(II) for pyrite determination proved sufficient for speciation of the major reactive sulphur species. The findings were supported by SEM, showing the abundance of framboidal pyrite crystals in samples from the strongly reduced zone near the landfill.

The simple HCl extractions were further developed by Kennedy et al. (1998), who combined the cold iron extraction with the determination of reduced sulphur minerals (AVS and chromium-reducible sulphur). The methods proved useful for studying iron species in three different reduced plumes, and were recommended for use in studies on natural attenuation of contaminants in aquifers. Lyon and Glass (1997) developed a simple protocol for using the 0.5 N HCl extractions to quantify Fe(II) and Fe(III).

The HCl extractions are inappropriate in sediments with high background of Fe(II) carbonate or calcite, due to interferences and neutralization of the acid during extraction (Amirbahman et al., 1998). A milder extraction using ascorbic acid was used for Fe(II) quantification.

Approaches specific to the determination of solid manganese in aquifer sediment have not been reported. Usually, Mn is of less importance than Fe and separate extraction methods have not been given high priority. This is partly due to lack of analytical methods for separation of Mn^{4+} and Mn^{2+}. Instead, total manganese is sometimes measured in extracts obtained for iron extraction.

Quantifying ion-exchangeable Fe(II), Mn(II), SO_4^{2-}, and NH_4^+ is done by extraction with an anaerobic salt matrix. Kota et al. (1999) found that a large fraction of the microbially produced Fe(II) was extracted by a neutral $CaCl_2$ solution.

All of the above-mentioned techniques involve measuring operationally defined quantities of extracted or reacted species. Postma (1993) proposed an ascorbic acid extraction technique for assessing iron oxide reactivity and crystallinity from dissolution rates. The method was tested on several well-known aquifer sediments, and extraction kinetics were compared to those of standard iron minerals.

7.3.4. Redox capacity measurements

Redox-capacity measurement techniques were developed by Barcelona and Holm (1991), Pedersen et al. (1991), and Heron et al. (1994a), and used for sediment analysis in several plumes (Heron and Christensen, 1995; Heron et al., 1994a, 1998; Amirbahman et al., 1998; Pannatier et al., 1999).

Barcelona and Holm (1991) suggested a Cr(II)-reduction step for OXC determination, but this technique was later abandoned due to the inherent instability of Cr(II) and, therefore, the gross overestimation of OXC. Heron et al. (1994a) developed a 8 mM Ti(III)–50 mM EDTA extraction technique based on the work of Ryan and Gschwend (1991) on reductive dissolution of iron oxides using Titanium–Citrate–EDTA–Bicarbonate mixtures. Good results are also obtained by eliminating the citrate and bicarbonate, leading to a simpler reaction chemistry and facilitating quantification of the unreacted Ti(III) by redox titration (Heron et al., 1994a; Pannatier et al., 1999) or later by direct photometric measurement (Amirbahman et al., 1998). The quantification of the amount of Ti(III) reacted yields a direct measure of the OXC.

RDC quantification has been done by a modified Chemical Oxidation Demand determination using dichromate oxidation of organic matter, reduced iron, manganese and sulphur species (Barcelona and Holm, 1991; Pedersen et al., 1991; Heron and Christensen, 1995; Heron et al., 1998; Amirbahman et al., 1998). The RDC is determined by redox titration, yielding the amount of Cr(VI) reacted during extraction. Here, air-tight capping of the reaction vessels is essential, since the acidification of the samples can lead to volatilization of sulphides and losses if the hydrogen sulphide gases are allowed to escape.

7.4. Application

Heron et al. (1994b) and Heron and Christensen (1995) studied the sediments from 25 different locations in the reduced plume downgradient of the Vejen Landfill (DK). A

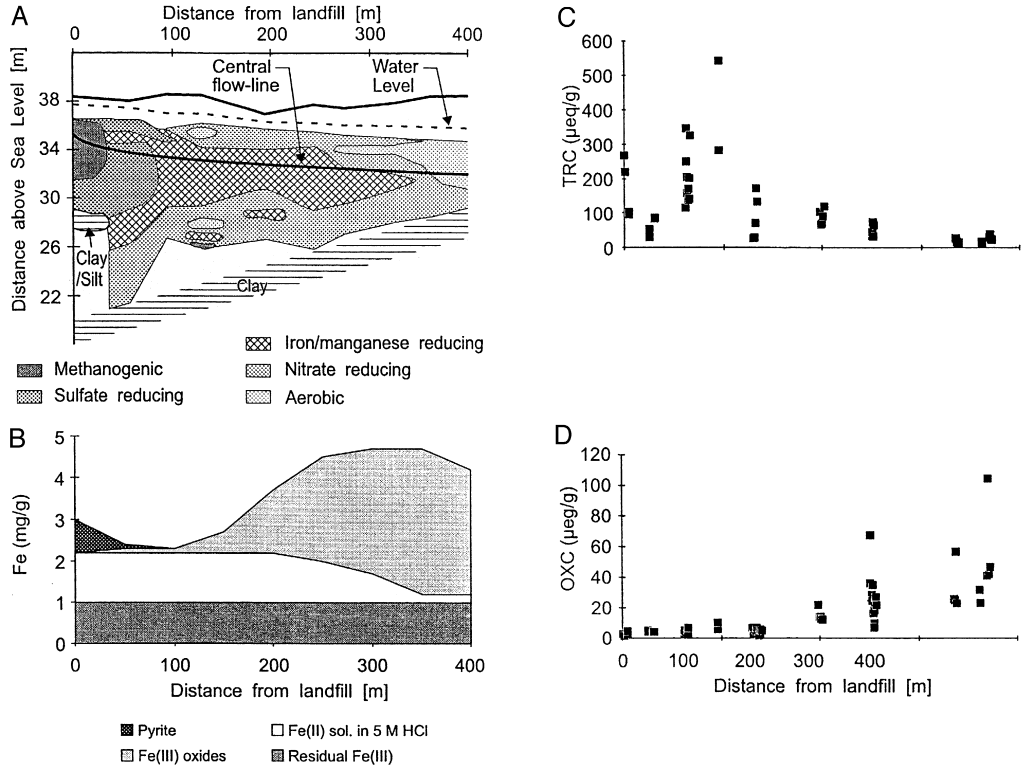


Fig. 16. Iron mineralogy and redox buffer capacity changes along a streamline in the Vejen Landfill (DK) leachate plume. (A) Vertical transect showing the major redox environments inferred from groundwater samples and the central flow-line. (B) The schematic distribution of iron species along the central flow-line inferred from analysis of 45 sediment samples. (C) Reduction capacity (here TRC, equal to RDC) of the sediment determined by a modified chemical oxygen demand method, and (D) Oxidation capacity (OXC) mainly related to iron oxides determined by the Ti(III)-EDTA method (Heron et al., 1994a) (modified from Heron and Christensen, 1995, with permission).

summary of the findings is presented in Fig. 16. It was shown by OXC determination and HCl extractions that Fe(III) oxides, such as goethite, were depleted in the methanogenic and sulphate-reducing zones close to the landfill. The distribution of iron and sulphur species along a central flow line in the plume strongly indicated that reduced precipitates included pyrite (identified positively by SEM; Fig. 17), amorphous sulphides (seen as elevated AVS contents in the reduced zones), and iron carbonates (increased Fe(II) contents extracted by HCl as well as Fe-rich carbonates observed by SEM). The findings of reduced precipitates were supported by elevated RDC in these sediments. Further downgradient, in the transition zone dominated by nitrate reduction and aerobic respiration, elevated Fe(III) contents as well as higher OXC indicated that part of the reduced iron had travelled downstream and then precipitated as ferrihydrite when oxidized (Fig. 16). This was supported by high Fe(III) contents extracted by 0.5 N HCl, indicating a relatively amorphous iron hydroxide (Heron et al., 1994b). Analysis of sediment-bound Fe(II) formed during microbial degradation of organics under Fe(III)-reducing conditions provided evidence of the iron reduction not revealed by groundwater sampling alone (Nielsen et al., 1995).

Baedecker et al. (1992, 1993), and Tuccillo et al. (1999) studied the iron chemistry in sediments in the proximity of the Bemidji oil spill (MN, USA) with a recently developed redox gradient caused by hydrocarbons leaching from an oil body. Close to the source,

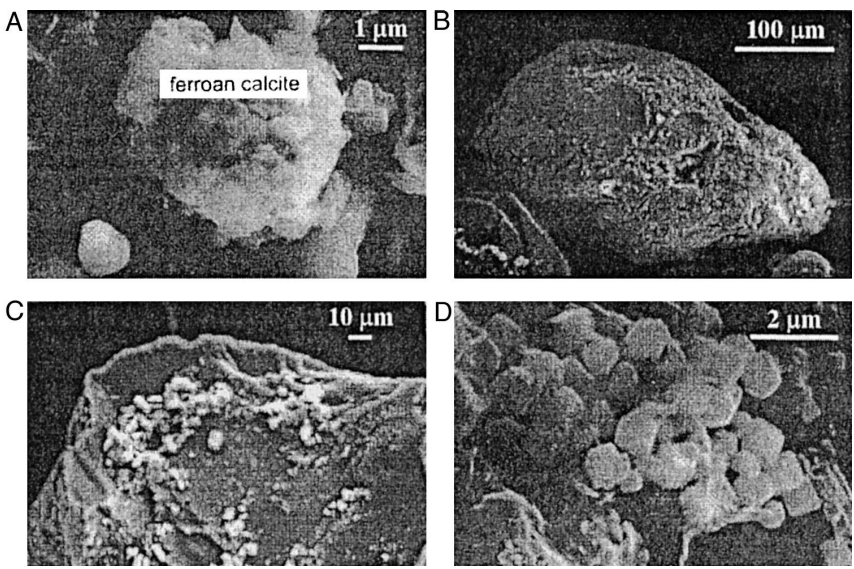


Fig. 17. Scanning Electron Microscopy photos of redox-sensitive iron and sulfur minerals. (A) Ferroan calcite identified in reduced sediments at the Bemidji (MN, USA) oil spill site (Tuccillo et al., 1999). (B) Framboidal pyrite crystals found in the methanogenic zone downgradient of Vejen Landfill (DK) (Heron et al., 1994b). (C) Iron oxide/hydroxides appearing as bright coatings on a quartz grain from the Fe(III)-reducing/precipitating zone at Vejen Landfill (DK) (Heron et al., 1994b). (D) Weathered iron-dominated grain found in the methanogenic/sulfate reducing zone at Grindsted Landfill (DK). This crystalline iron oxide grain constitutes a large capacity of iron, but its reactivity is low (from Heron et al., 1998, with permission).

anoxic conditions prevailed, and a 24–32% reduction in the 0.5 N HCl-extractable Fe(III) was observed. Changing ratios of Fe(II) to Fe(III) along the longitudinal plume axis and a significant increase in the total iron content in the anoxic/oxic transition zone indicated downstream iron transport and reoxidation (Fig. 18). The identification of ferroan calcite in the reduced samples (Fig. 17) and the abundance of Fe(III) oxides on feldspar grains from the transition zone presented a direct evidence of the contrasts in mineralogy along the plume transect (Tuccillo et al., 1999). The finding that iron reduction was a very important redox process in the plume was somewhat surprising, since dissolved iron concentrations were low in the entire plume (Bennet et al., 1993). Precipitation of ferrous iron carbonate (siderite and ferroan calcite, Fig. 17, Tuccillo et al., 1999) was identified as the reason for the low dissolved iron (Baedecker et al.,

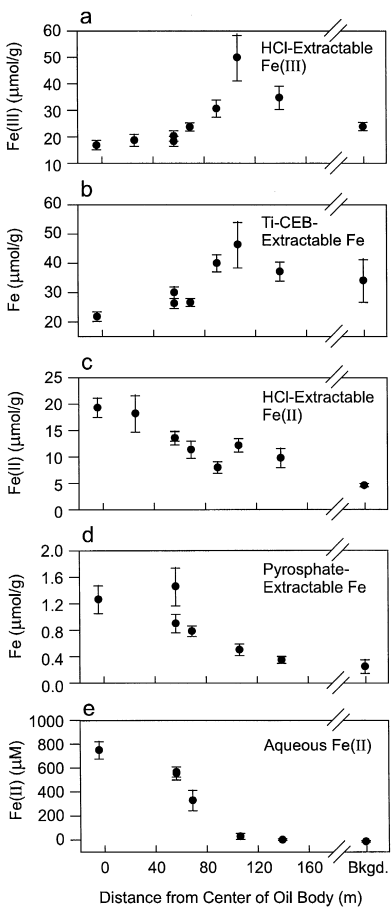


Fig. 18. Distribution of Fe(III) and Fe(II) downgradient from the oil spill at the Bemidji (MN, USA) site. The distance of 160 m corresponds to the toe of the reduced plume, where the aquifer is still under aerobic conditions. Fe(III) is lowered close to the source, and elevated downgradient in the anoxic/oxic transition zone. Fe(II) is elevated in the reducing environments (from Tuccillo et al., 1999, with permission).

1993). Extractions of organically bound iron by pyrophosphate indicated that a minor fraction of the produced Fe(II) was bound to organics (Fig. 18). This study clearly showed the value of an integrated approach to the description of redox conditions.

Kennedy et al. (1998) used vertical depth profiles at three different sites in Oklahoma (USA) contaminated with organics (a landfill, a fuel-spill site, and a methane gas leak site) to evaluate methods for sediment iron and sulphur determination. The combined 0.5 N HCl and 6 N HCl extractions showed that in sediment layers with recent Fe(III) reduction, the fraction of Fe(II) to Fe(total) is higher in 0.5 N HCl extractions than in the stronger 6 N HCl extraction. A limit value of the Fe(II)/Fe(total) ratio was defined by the 6 N HCl extraction, and when the Fe(II)/Fe(total) ratio of the 0.5 N HCl extraction exceeded this limit, the Fe(II) increase was considered significant and used as an indicator of iron reduction. This study documented the occurrence of Fe(III)-reducing zones in plumes in hematite-rich Oklahoman sediments, for plumes with very different electron donors.

Lendvay et al. (1998a) studied a plume of dissolved chlorinated solvents downgradient of an industrial waste water lagoon at St. Joseph (MI, USA). The anaerobic degradation of trichloroethylene (TCE) had resulted in the formation of metabolites in a more than 500-m-long plume. Sediment analysis revealed a lowering of solid Fe(III) in the central part of the reduced plume determined by a 1-h extraction using 0.5 N HCl. Elevated Fe(II) was found in the same extractions, indicating that Fe(III) reduction had led to significant changes in the aquifer iron distribution and speciation. Potentially, the apparent redox buffering by sediment-bound iron hydroxides could explain why the chlorinated solvents persisted as far as 500 m downgradient; since the chlorinated solvents are known to dechlorinate most readily under more strongly reducing, preferably methanogenic conditions.

One possible complexity to interpreting the relatively weak chemical extraction techniques used to determine amorphous and weakly crystalline iron is the fouling of these solids by Fe(II) precipitates (Kota et al., 1999). In systems supersaturated with respect to ferrous carbonate or sulphides, precipitation of these reduced minerals can cover the sediment grains, and render large amounts of Fe(III) unavailable for microbial reactions. Although no direct field evidence of this phenomenon exists, Kota et al. (1999) documented the Fe(II) fouling in controlled laboratory microcosms. It was shown that Fe(III) reduction ceased, and the Fe(II)/Fe(III) remained constant after a period where Fe(II) carbonate was deposited. Fe(III) reduction resumed when part of the coatings was removed. At the grain scale, it is likely that microbial processes will be inhibited where a thin layer of precipitate covers the electron acceptor, e.g., iron oxide. This will effectively block the enzymatic contact involved in the process. Chemical dissolution of the same oxide may not be inhibited, due to chemical dissolution of such precipitates. Such dissolution was documented on several mixed Fe(II)–Fe(III) sediments (Heron et al., 1994b).

Amirbahman et al. (1998) studied the geochemistry downgradient of a municipal landfill in Winterthur (CH). The old landfill presently has very limited leaching of organic matter and, therefore, only mildly reducing conditions exist. However, sediment analysis showed that past processes had led to partial depletion of Fe(III) oxides beneath the landfill and precipitation of iron sulphides, such as ferrous sulphide and pyrite (both

AVS and Cr(II)-reducible sulphur were elevated). The Fe(III) was quantified using both the Ti(III)–EDTA method for OXC determination (yielding total iron and manganese oxide) and an ascorbic acid extraction (milder). Both quantities were reduced below the landfill and immediately downgradient. This study showed how sediment analysis can be used to derive information on previous plume extents, and to provide information on whether the plume is expanding or retracting.

At the Grindsted Landfill (DK), detailed geological and geochemical description of the aquifer sediment showed that the majority of the aquifer consisted of fine sands, low in organic matter, OXC and iron oxides (Heron et al., 1998). Iron and manganese reduction was less important than at the Vejen Landfill located in the same geological setting, even though dissolved Fe^{2+} and Mn^{2+} concentrations were higher (in the 2 mM or 100 mg/l range). Both a lower initial iron oxide content and a different iron mineralogy (presumably solid grains of crystal iron oxides sparsely distributed between Fe(III)–poor quartz grains) indicated lower iron reactivity. These differences were revealed by a detailed geological interpretation (based on 185 samples) identifying an iron-poor, mica-containing Tertiary sand in the bottom part of the aquifer (Heron et al., 1998). In addition, it was demonstrated that geological heterogeneities can lead to unexpected redox activities, exemplified by the high sulphate reduction activity 170 m from the landfill (Ludvigsen et al., 1998). This was caused by local sulphate- and organic matter-rich deposits and was not related to the leaching from the landfill. The Grindsted Landfill plume is host to all of the proposed redox reactions, but also secondary oxidation–reduction reactions involving methane, sulphides, ferrous iron, ammonium and manganese oxides seem important (Bjerg et al., 1995).

7.5. Evaluation

On the basis of an aquifer volume, iron, sulphur and manganese species associated with the solid phase are the most important redox species in a contaminant plume, and several simple and operational chemical extraction techniques have been developed for speciation, especially for iron and sulphur minerals. The studies have been addressing capacities as well as reactivities of aquifer sediments. However, the data base on actual field studies is limited.

Based on data from relatively few aquifers and contaminant plumes, iron oxides seem to be the dominant electron acceptor in most aerobic aquifers, contributing much more to the overall OXC than dissolved species, such as oxygen, nitrate and sulphate. In reduced plumes, precipitates of ferrous iron and sulphides may be abundant products of redox reactions, even when low dissolved concentrations are measured in the groundwater. Reduced iron and sulphur species associated with the sediment, hence, seem to constitute the main RDC of contaminant plumes. Theoretically, solid organic matter also may contribute to both sediment oxidation and RDC. But the reactivity is not well studied and often assumed to be limited. However, solid organic matter in reduced plumes may constitute a measurable RDC.

Not only capacities of solid sediment species, but also reactivity is crucial for understanding the importance of the groundwater–sediment interactions. Reactive fractions have been addressed by mild chemical extractions, leaving most of the bulk iron

and sulphur species intact, and extracting amorphous minerals, such as ferrihydrite and ferrous sulphide. Hydrochloric acid and ascorbic acid extractions are the most promising approaches for reactive fraction measurements.

Direct identification of mineral forms and crystal sizes by SEM has added confidence to geochemical interpretations and helped explain different levels of reactivity for comparable bulk iron and sulphur contents. Key parameters are crystallinity, size, and surface area of the minerals.

Sediment analysis revealing depletion of OXC and Fe(III) as well as increased RDC and reduced Fe(II) and sulphide contents has been shown to describe the importance of past processes in older plumes and may also contribute to identification of currently limiting factors for sediment associated redox processes. Quantification of redox capacities is, in particular, important for remediation approaches considering changes in redox levels, since the dominating capacities are associated with sediments.

8. Microbial measurements

8.1. Background

Most redox processes in contaminant plumes are microbially mediated; and 30 years back, Farkasdi et al. (1969) distinguished between reduction, transition and oxidation zones in leachate plumes at German landfills. This investigation related the observed zones to microbial activity by enumerating sulphate- and nitrate-reducing (NO_3^- to NH_4^+) as well as denitrifying (NO_3^- to N_2) bacteria in the plumes. On this background, it seems obvious that characterization of redox environments in contaminant plumes should include the microorganisms performing the redox processes.

Different approaches are used to identify, enumerate and quantify the different microorganisms carrying out each of the redox processes. This may involve traditional techniques for culturing of the organisms or modern chemical techniques based on direct detection of biomarkers, e.g., ester linked PhosphoLipid Fatty Acids (PLFA) for specific processes or organisms, and the use of molecular DNA or RNA probes.

Instead of focusing on the number of the individual microorganisms or groups of microorganisms, specific types of processes (e.g., sulphate reduction, denitrification, etc.) can be investigated by TEAP bioassays, where each of the processes can be detected and quantified. Furthermore, the TEAP bioassays harbor a unique opportunity to detect ongoing processes. Albeit the TEAP bioassay approach implies incubation of field samples in the laboratory, this approach is currently the only approach used in plume studies providing a direct estimate of field-related redox processes and their rates.

8.2. Principles

The presence of microorganisms able to conduct specific redox processes are traditionally quantified by growth on synthetic media, e.g., agar plate-spreading techniques where each single bacteria is allowed to form colonies, which then can be

counted. This technique is mainly suitable for aerobic conditions, but poor for investigations of contaminated anaerobic aquifers. Instead the most probable number (MPN) enumeration technique is used, since this technique allows to a higher degree for controlled, anaerobic conditions.

The MPN enumeration technique is based on the principle of preparing serial dilutions of a sample, and from each of these dilutions, a set of replicate tubes with defined media are inoculated. The activity in each replicate tube is registered, e.g., in terms of production of reduced end product from the electron acceptor of concern. At a certain dilution, the density of microorganisms becomes so low that activity is detected only in some of the replicates and by means of a probability table, the pattern of positive and negative scores is converted to the MPN of the investigated type of organisms in the original sample.

An alternative to the classical culturing methods is a chemical characterization of microbial communities. A significant advantage of the chemical approaches is that the samples can be preserved immediately after sampling, thus avoiding artificial changes in the microbial population during transport and handling. Furthermore, the samples can be stored for long periods, which reduces the peak in the workload immediately after the sampling. One promising approach is the measurement of PLFAs, which is ubiquitous in cell membranes. The PLFAs can be extracted from an aquifer sample and the content and composition of the PLFAs can be measured by GC-MS, and from the composition of the PLFAs the presence and relative occurrence of different types of microorganisms (White et al., 1983) can be detected, e.g., in an aquifer sample (Ludvigsen et al., 1999). Some PLFAs occur in the lipid membranes of sulphate-reducing bacteria, including 15:1 (*Desulfobulbus* sp., D.C. White, personal communication), i17:0 and i17:1ω7c (*Desulfovibrio* sp., Edlund et al., 1985; Kohring et al., 1994), and 10me16:0 (*Desulfobacter* sp., Dowling et al., 1986), and the presence of some of these biomarkers in a sample may, thus, indicate the presence of sulphate reducers. The monoenoic 16:1ω7c is the most abundant PLFA in cell membrane of *Geobacter metallireducens* and the second most abundant PLFA in *Shewanella* sp. (Coleman et al., 1993), which both are iron reducers. On the other hand, 16:1ω7c is also common in the cell membranes of all Gram-negative bacteria as well as many microeucaryotes (Ludvigsen et al., 1999). Thus, the suggested biomarkers for different specific redox groups may not be that specific, implying a risk of false positive results. The interpretation of PLFA analyses is further complicated by the fact that more than 50 different PLFAs have been detected in aquifer samples (e.g., Ludvigsen et al., 1997)

Other emerging alternatives are the RNA and DNA probes. The principle in these techniques is to establish a fragment or a piece of DNA or RNA (the probe) corresponding to the gene analyzed for, and to label this probe by, e.g., a stain. When added to the sample the probe will bind (hybridize) to the gene, allowing for detection of the gene. Smith and Tiedje (1992) identified specific denitrifying strains in aquifer samples by DNA probes. Sulfate-reducing bacteria have been identified by 16S rRNA probes in other environments, e.g., marine sediments (Devereux et al., 1996; Ramsing et al., 1996; Teske et al., 1996). RNA probes may even be used to estimate the activity of a certain process, such as sulphate reduction. An important strength of the technique is that it is not necessary to grow or culture the investigated organisms, a procedure step that always

causes some selectivity. An important limitation is that probes can only be prepared for known genes and, thus, for organisms that have been isolated. DNA/RNA techniques may also be combined with the MPN approach (MPN-PCR), which gives information about the dominant species cultured in the MPN tubes as done for iron reducers by Anderson et al. (1998). These techniques may prove of interest, but they will not be discussed further because of their limited use in pollution plumes to date.

The above-mentioned methods focus mainly on the presence and enumeration of the microorganisms, but the actual processes are more important in the characterization of the redox environment. The TEAP bioassay is a useful approach to investigate redox processes by enclosing a sample and over time following the consumption of the electron acceptor of concern or the production of intermediates or end products. The assays are set up without any amendments, but sampling and handling of the samples may, in the short term, increase the availability of some sediment associated electron-acceptors, such as Fe(III) and Mn(IV) or of electron donors (organic matter or H_2). This may influence the rates depending on what is the limiting factor in the bioassay. One of the major limitations of this technique is that the change over time in concentration of the measured variable is often very small compared to the background concentration of the variable. This is especially problematic for determining low rates of iron reduction (Ludvigsen et al., 1998). To overcome this, long incubation times are often required before the changes in the concentrations are detectable. Besides indicating if a certain redox process occurs, TEAP bioassays also allow for estimation of redox rates.

8.3. Methods

It is a prerequisite for meaningful microbial investigations that the investigated sediment and groundwater have been collected intact with respect to redox conditions. Microbiological contamination during sampling and handling must also be avoided. Thus, sterile techniques have to be used although the investigation concerns mixed populations.

8.3.1. Sampling and handling

The sampling requirements are similar to the requirements for groundwater and sediment sampling discussed in the previous section; but on top of these requirements, all sampling equipment and containers should ideally be handled by sterile techniques. The samples should be processed as soon as possible, since just a few days of storage even at low temperature may alter the composition and activity of the microbial population (Brockman et al., 1998).

The outer 2–5 mm of sediment cores should be pared off in the laboratory by a paring device to remove potentially contaminated sediment. The use of this procedure reduces the demand for strictly sterile work in the field. Unfortunately, similar approaches are not available for water samples, unless the samples are sterilized by, e.g., filtration, which removes the bacteria to be investigated. The sediment samples are often handled in an anaerobic box to prevent contamination by oxygen in the laboratory. During the setup in the laboratory, the choice of materials should minimize the release of potential substrates to the samples and, therefore, glass and stainless steel are

preferred. For anaerobic samples, all containers and stoppers should be gas-tight. In TEAP bioassays, syringes with needles are convenient for collection of subsamples during the incubation, since the subsample remains enclosed after withdrawal. For this purpose, stoppers of high quality, e.g., butyl rubber, neoprene or viton, are required, allowing penetration of the needle and gas-tight closing afterwards. Finally, for anaerobic TEAP bioassays, the gas in the head space of the bioassay vessels has to be considered. Since aquifer systems often are carbonate buffered, an oxygen-free N_2 with 1–20% CO_2, preferably corresponding to the in situ conditions, is often used to prevent outwash of CO_2. To maintain anaerobic TEAP bioassays, it is crucial to remove even trace amounts of oxygen since even small concentrations of oxygen in the headspace may contribute substantially to the total content of oxygen in the bottle.

Hydrogen gas should be avoided, since hydrogen is a readily used substrate, which may be a limiting factor in TEAP bioassays. Especially, anaerobic boxes using 2–4% hydrogen and a catalyst to remove oxygen may be a serious source of hydrogen contamination of TEAP bioassays. The headspace gas should always be flushed out with a hydrogen free gas if the bottles have been opened in such an anaerobic box.

8.3.2. MPN Measurements

Usually a defined synthetical mineral medium is used for MPN enumeration. Although many different media have been used (e.g., Beeman and Suflita, 1987; Essaid et al., 1995; Kao and Borden, 1997; Ludvigsen et al., 1999), it is important in aquifer studies to use oligotrophic media (Albrechtsen and Christensen, 1994). Different electron donors, such as yeast extract, tryptone acetate, lactate or H_2, as well as the electron acceptor for the process investigated, are added to the mineral medium: sulphate for sulphate reducers; synthetic amorphous iron oxides (ferrihydrite) for iron reducers; MnO_2 for manganese reducers; nitrate for nitrate reducers. The MPN tubes are incubated in the dark for long periods. Ludvigsen et al. (1999) used 2 months for methanogens, sulphate and nitrate reducers and 6 months for iron and manganese reducers. One dilution series per MPN analysis should be autoclaved to serve as a sterile control, allowing for distinguishing between biological and abiotic processes and control for handling errors.

Tubes tested for methanogens can be scored as positive if methane concentrations rise to a level twice that of the sterile controls. The presence of sulphate reducers is indicated by the production of a black ferrous-sulphide precipitate or the detection of sulphide production by lead-acetate paper. Tubes tested for iron and manganese reducers are scored as positive when concentrations of Fe(II) and Mn rise to a level twice that of the sterile controls. Tubes for nitrate reducers are scored positive once they become depleted in NO_3^- or formation of N_2 gas in inverted tubes is observed (e.g., Essaid et al., 1995; Ludvigsen et al., 1999). The MPN method may be combined with PCR methods and molecular probes to investigate the dominant cultured species (Anderson et al., 1998).

8.3.3. PLFA measurements

The samples for PLFA analysis may be preserved by freezing ($-80°C$). The frozen samples are lyophilized and extracted in a chloroform:methanol:phosphate buffer

(1:2:0.8, v:v:v, White et al., 1997). Total extractable lipids are recovered, and separated into three general classes by silicic acid column chromatography. After transesterification into methyl esters, the PLFA are quantified by gas chromatography using flame ionization detection. The presence of individual PLFA may be confirmed by mass spectral analysis (Guckert et al., 1991).

8.3.4. TEAP bioassays

Typically, TEAP bioassays are carried out as batch incubations of subsamples of sediment transferred to serum bottles (50 and 100 ml) in an anaerobic glove box and mixed with groundwater collected at the same location and depth as the sediment, typically in a ratio of 2 g wet weight per 3 ml of water (Albrechtsen et al., 1999). The assays are carried out in the dark to avoid growth of phototrophic organisms, such as algae, and incubated at a temperature similar to the aquifer under investigation. Alternatively, the TEAP bioassays may be performed in intact sediments cores, incubated in a well in the field. This was done for sulphate reduction, where trace amounts of $^{35}SO_4^{2-}$ were injected in the cores (Jakobsen and Postma, 1999). A set of TEAP bioassays should serve as sterilized controls for each of the tested redox processes investigated allowing for distinguishing between biotic and abiotic processes.

The individual redox processes are typically followed by measuring the accumulation in the bottles of dissolved or gaseous products of each process: methane from methane production can be measured by gas chromatography; Fe(II) from iron reduction can be measured spectroscopically by the ferrozine method (Stookey, 1970) or by atomic absorption spectroscopy after filtration. To improve this method, the Fe(II) can be extracted by 0.5 M HCl for 1 h, from the fines in unfiltered samples. Mn(II) from Mn(IV) reduction can be measured by atomic absorption spectroscopy after filtration. For denitrification, the production of N_2 is difficult to measure, because of the high background concentration. By adding acetylene the final step in the reduction of nitrate (NO_3^-) to nitrogen (N_2) is inhibited resulting in accumulation of laughing-gas (N_2O), which is easy to measure by gas chromatography. Furthermore, ^{14}C acetate and ^{14}C bicarbonate can be added in trace amounts to distinguish between autotrophic methane production and acetoclastic methanogenesis (Harris et al., 1999).

The products of some processes react rapidly with other compounds. Sulfide will react with Fe(II) if present and precipitate; it can then not be detected in the water. Sulfate reduction can be determined by measuring production of $H_2{}^{35}S$ after addition of a trace amount of $^{35}SO_4^{2-}$. Replicates of each sediment suspension are incubated and are harvested over time. The acid volatile $^{35}S^{2-}$ solids produced can be extracted by addition of HCl. Dissolved $H_2{}^{35}S$ is collected by purging of the suspension with N_2 and fixation in a Zn-acetate trap, next $Zn^{35}S$ is analyzed by scintillation counting (Jørgensen, 1978). To include ^{35}S associated with pyrite, extraction by, e.g., chromium, should be included. Alternatively, sulphate reduction can be measured by a passive procedure, where the Cr(II)–HCl is added to a closed serum bottle with the sample and a zinc acetate trap for the ^{35}S released (Ulrich et al., 1997). For studies focusing on spatial variability in the rate of sulphate reduction on a very small scale, silver foils have been used as sulphide traps to visualize and detect sulphate reduction in near-continuous core sections (Harris et al., 1999).

An indirect approach for determining the TEAP is to add ^{14}C acetate and follow the production of $^{14}CO_2$ and $^{14}CH_4$. When this is combined with addition of molybdate, which selectively inhibits sulphate reduction, it is possible to distinguish between methane production (by increase of $^{14}CH_4$), sulphate reduction (by increase of $^{14}CO_2$, when molybdate was not added) and iron reduction (by increase of $^{14}CO_2$, when molybdate was added and by absence of $^{14}CH_4$) (Anderson et al., 1998).

8.4. Applications

8.4.1. MPN

The enumeration of redox-specific microorganisms has been used as a characterization and to support that the redox processes observed are biologically mediated. Essaid et al. (1995) counted aerobic heterotrophic, anaerobic fermentative, denitrifying, iron reducing, sulphate reducing and methanogenic bacteria in a crude oil spill site at Bemidji (MN, USA). They found iron reducers in high numbers close to the oil body. Denitrifiers and sulphate reducers were present in lower numbers than all other types of organisms in accordance with the low availability of nitrate and sulphate in the groundwater. These counts were in accordance with the conceptual model of an aerobic, Mn/Fe-reducing, and methanogenic degradation sequence in the plume. However, the MPN numbers of acetate-oxidizing iron reducers were high, and more or less even, in both the iron-reducing part of the plume area and an anaerobic, uncontaminated reference site (Anderson et al., 1998). An investigation by molecular techniques (16S rRNA probes) of the iron reducers in the MNP-tubes revealed that only a sequence closely related to *Geothrix fermentans* was recovered from the uncontaminated site, whereas the sediments from the iron-reducing zone also contained a sequence characteristic of *Geobacter* species. *Geothrix fermentans* are not known to oxidize aromatic compounds with the reduction of Fe(III), in contradiction to *Geobacter*. Their occurrence seemed to be stimulated by the presence of aromatic contaminants, which may indicate benzene degradation by iron reduction. High MPN numbers of iron reducers and *Geothrix fermentans* were also observed at two other oil spill sites: at Hanahan (SC, USA) and at Rocky Point (NC, USA), but no significant anaerobic oxidation of benzene was observed and *Geobacter* species were not found. This suggests that comparative studies on the size of the *Geobacteraceae* community in petroleum-contaminated aquifers might aid in the location of zones in which benzene degradation coupled to iron reduction is taking place (Anderson et al., 1998).

Different physiological groups of sulphate reducers and methanogens using different carbon substrates have been observed in the leachate-contaminated aquifer at Norman Landfill (OK, USA) by the MPN technique (Beeman and Suflita, 1987, 1990). The composition of the microbial population changed along the plume downstream of the landfill with the main occurrence of methanogens and sulphate reducers close to the landfill and decreasing numbers in the more distant part of the plume. Later investigations confirmed the occurrence of relatively high numbers of methanogenic bacteria in the center of the leachate plume where the methane concentration was the highest and the sulphate concentration was relatively low. Higher numbers of sulphate reducers were observed outside the center of the plume in the shallow and the deep portion of the aquifer (Harris et al., 1999).

The MPN technique has also been used in the leachate plume at the Grindsted Landfill (DK) to enumerate nitrate, manganese, iron, and sulphate reducers and methanogens (Fig. 19) (Ludvigsen et al., 1999). The iron, manganese, and nitrate reducers occurred in surprisingly high numbers compared to the total cell numbers and varied only little with distance. The number of sulphate reducers was higher in areas where sulphate reduction occurred and the number of methanogens was higher where methane production was observed. However, the different bacteria groups were present in nearly all the samples investigated without any clear relation to the dominant redox processes. This may reflect that the MPN technique enumerates all the bacteria with a potential for conducting the investigated process, no matter whether this potential is expressed or not. The same type of organisms may also be enumerated in several groups, e.g., as iron, sulphate and manganese reducers.

The density of a specific group of microorganisms has been tested as a measure of the capacity of the specific redox environment, but with little success. Kao and Borden (1997) found no correlation between the number of denitrifiers enumerated by the MPN technique and the degradation rates for toluene, ethylbenzene or xylene under denitrifying conditions in a range of fuel oil-contaminated sites (including Rocky Point, Fort Bragg, and Chapel Hill in NC; Traverse City and Sleeping Bear Dunes in MI).

Finally, quantification of redox-specific bacterial groups in modelling of redox processes in the oil-contaminated aquifer at Bemidji (MN, USA) has been attempted with limited success (Essaid et al., 1995). The MPN technique is useful as a confirmation of the presence of a redox-specific microbial population and a potential for certain redox processes, despite the fact that the results obtained with the MPN technique may be limited by the growth conditions defined by the choice of medium, substrate, etc. However, the presence of certain bacteria does not necessary mean that these processes predominate.

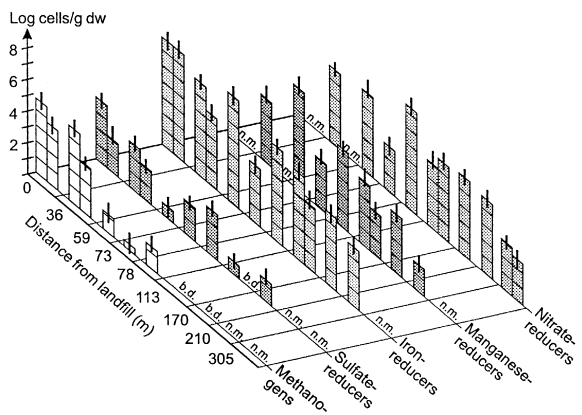


Fig. 19. The (log) number of methanogens (Archaea), sulfate-, iron-, manganese- and nitrate-reducing bacteria measured by MPN enumerations of aquifer sediments at different locations down gradient of the Grindsted Landfill (DK) plume (95% confidence limits shown). Samples were pooled in sets of two or three per distance. When more than one set of samples were collected per distance, 2 bars are shown. 'b.d.' indicates below detection limits, 'n.m.' indicates not measured. (modified from Ludvigsen et al., 1999, with permission).

8.4.2. PLFA

The presence of PLFA biomarkers has been used as indication for the presence of specific bacteria in various environments (e.g., Coleman et al., 1993), including landfill leachate plumes (Albrechtsen et al., 1995; Ludvigsen et al., 1999). Only a few results from analysis of PLFAs are available and PLFAs seem of limited value in identifying specific microbial populations. This was shown in a multivariate statistical analysis of the relation between the PLFA profile and different microbial redox processes in a landfill leachate plume (Ludvigsen et al., 1997). Some specific lipid biomarkers, however, suggested that the proportion of sulphate- and iron-reducing bacteria was increased at the border of the landfill (Ludvigsen et al., 1999). Also, in a jet fuel-contaminated aquifer, elevated concentrations of terminal-branched and cyclopropan fatty acids were coincident with sulphate-reducing conditions (Fang et al., 1997; Fang and Barcelona, 1998).

8.4.3. TEAP bioassays

TEAP bioassays are useful for identification of ongoing microbial redox processes, for verifying redox sequences, for geographical location of the activities resulting in observed distribution of dissolved redox-sensitive parameters, and for estimating rates of the investigated redox processes.

TEAP bioassays with unamended groundwater and sediment samples verified the presence of the following metabolic redox activities in the Grindsted Landfill (DK) plume (Ludvigsen et al., 1998): denitrification, iron, manganese, sulphate reduction, and methane production (Fig. 20). Similar results have been obtained from the Vejen Landfill plume (DK) (Albrechtsen and Christensen, 1994; Christensen et al., 1994). At the Norman Landfill site (OK, USA), the TEAP bioassays demonstrated that sulphate reduction is a dominant electron-accepting process in a narrow zone just below the water table where the sulphate concentrations were the highest, but in this zone also some iron reduction was detected by TEAP bioassays. No iron reduction could be detected in a deeper sulphate-depleted zone, although the Fe(II) concentration was very high, making it unlikely that the dissolved Fe(II) was generated at this location. Maximum methane production was observed in this zone in accordance with high methane concentrations (Cozzarelli et al., 1999b; Harris et al., 1999). When testing for different processes, TEAP bioassays set up with material from the same sediment sample, the Grindsted Landfill (DK) plume showed simultaneous occurrence of several different microbially mediated redox processes (Ludvigsen et al., 1998) (Fig. 20). Thus, the TEAP bioassay has the ability, as no other current redox characterizing approach, to identify not only the dominant redox process, but also less significant redox processes in the same sample. This more differentiated description may have implications for the potential of a given redox zone, as identified by the dominant redox process, to degrade organic chemicals. Since the TEAP bioassays allow for estimating rates for each of the electron-accepting processes, the organic matter (assuming oxidation level zero) mineralized to carbon dioxide can be calculated from the measured rates. The calculations, presented in Table 12 for the Grindsted landfill (DK) plume, showed that one electron-accepting process clearly dominated each sample accounting for more than 70% of the equivalent carbon conversion (Ludvigsen et al., 1998).

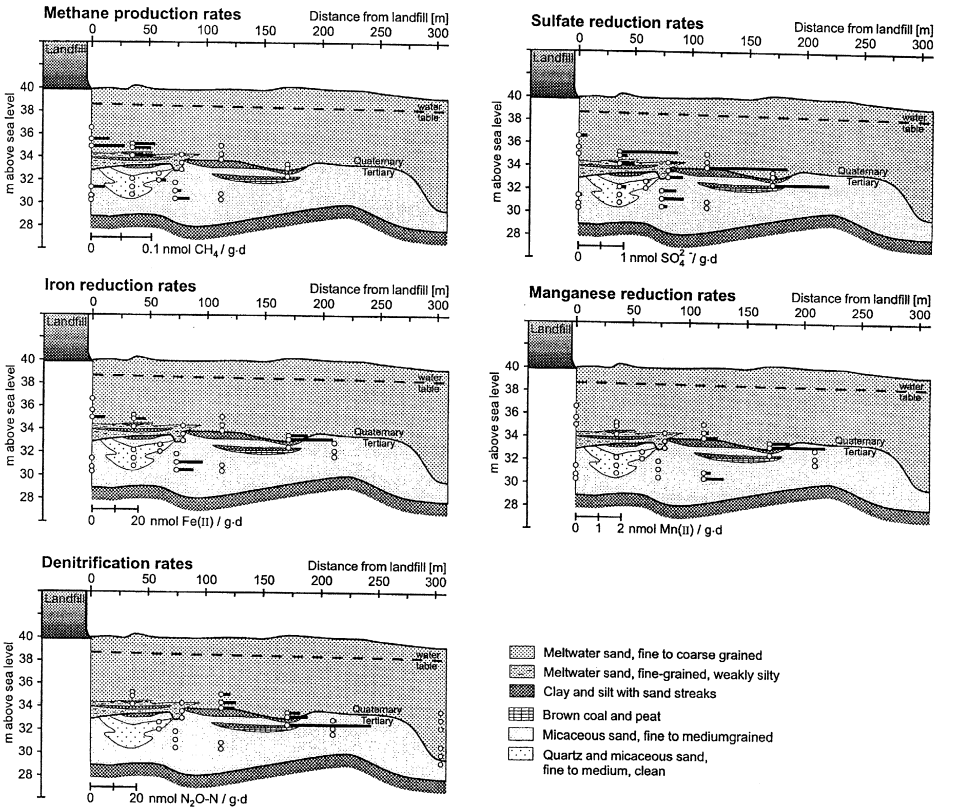


Fig. 20. Rates observed in TEAP-bioassays for individual redox processes at several locations in the Grindsted Landfill (DK) plume. Sampling points are indicated by white dots, and all TEAP-bioassays were conducted for each sampling points, except for denitrification in samples from 0 m and the three lower samples from 36 m, and for methane production and sulfate reduction in samples from 210 m (from Ludvigsen et al., 1998, with permission).

At the crude oil-contaminated aquifer at Bemidji (MN, USA), TEAP bioassays demonstrated reduction of Fe(III), but no methane production. Thus, despite the presence of relatively high concentration of dissolved methane in the groundwater at this site, Fe(III) reduction was the significant TEAP (Lovley et al., 1989).

At the jet fuel (JP-4)-contaminated aquifer at Hanahan (SC, USA), TEAP bioassays revealed that Fe(III) reduction dominated at one sampling site, whereas methane production dominated at the other site (Lovley et al., 1994). The use of TEAP bioassays may, thus, reveal spatial variation in a contamination plume, which may not be discovered by analysis of the overall water chemistry, which may somehow represent an average. Substantial spatial variations in microbial redox processes caused by the geological settings were observed in the leachate plume of the Grindsted Landfill (DK) (Ludvigsen et al., 1998), where layers with silt and clay showed sulphate and iron reduction, which probably was independent of the leachate plume.

Table 12
Estimated carbon oxidation rates (nmol C/g dw/year) calculated from simple stoichiometric reactions and the TEAP-bioassays conducted for one sample from each distance

	Locations				
Distance from the landfill (m)	0	36	73	113	210
Depth (m.b.s.)	4.2	4.9	8.7	9.7	8.4
C-removal rates nmol C / g dw / year					
Methane production	20	30	7	<	n.m.
Sulfate reduction	<	960	355	<	n.m.
Iron reduction	<	<	1055	<	<
Manganese reduction	<	<	<	155	20
Denitrification	n.m.	<	<	<	375
ΣC-removal rate	20	990	1420	155	395

Carbon is assumed converted from redox status 0. End products are CH_4, S(−II), Mn(II), Fe(II) and N_2, respectively (modified from Ludvigsen et al., 1998, with permission).
<, Below detection limit.
n.m., Not measured.

The Vejen Landfill (DK) plume showed locations close to the landfill with high dissolved Fe(II) concentrations, but these locations revealed no or very low iron reduction by TEAP bioassays. Further downstream from the landfill dissolved Fe(II) concentrations were lower, but with higher iron reduction (Albrechtsen et al., 1995). The high concentration of dissolved iron at the location close to the landfill supposedly originated from passed iron-reduction activity.

TEAP bioassays can be manipulated by enrichment by different substrates and electron acceptors and thereby be used for examination of the potentials for different redox processes in a given aquifer, and for identification of limiting or controlling parameters. Such an approach showed that the availability of Fe(III) was a major controlling factor for the microbial iron reduction in parts of the Vejen Landfill (DK) plume. The available Fe(III) was depleted close to the landfill, but a potential for iron reduction was evident after the samples were enriched with acetate and amorphous Fe(III) whereas very little, if any, iron reduction occurred in samples that were stimulated by addition of amorphous Fe(III) alone or acetate alone (Fig. 21). In the samples collected more distant from the landfill, the addition of acetate alone stimulated the iron reduction, but the addition of amorphous Fe(III) alone had no effect. This demonstrated, that close to the landfill the content of organic carbon in the plume was sufficient for the iron-reducing bacteria to reduce all the available Fe(III) in the sediment; but more distant from the landfill, the supply of organic carbon was too small to reduce all the Fe(III) in the sediment (Albrechtsen et al., 1995). Thus, TEAP bioassays may provide insight into the controlling parameters of redox processes.

At the Norman Landfill site (OK, USA), TEAP bioassays were used to investigate the factors controlling shifts between methane production and sulphate reduction by amending the TEAP bioassays with different substrates. The different populations utilized different substrates, since methanol and trimethylamin were utilized by methanogens but

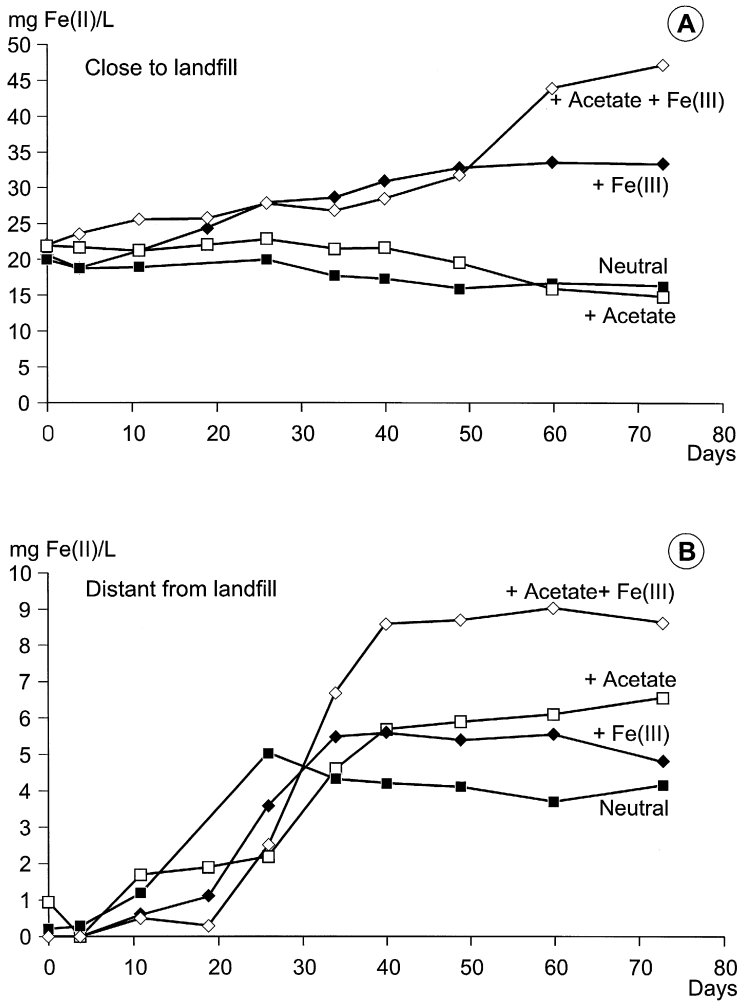


Fig. 21. Unamended and amended bioassays with sediment from the iron reducing zone of the Vejen Landfill (DK) plume illustrating limiting availability of iron and dissolved organic matter (from Albrechtsen et al., 1995, with permission).

not by sulphate reducers. Also, the occurrence of different volatile fatty acids differed between the locations, a fact that might influence the competition between sulphate reduction and methane production (Beeman and Suflita, 1987, 1990). TEAP bioassays conducted with different ^{14}C-labelled substrates were also used to show the dominance of acetoclastic methane production (from acetate) over autotrophic methane production (from bicarbonate) (Harris et al., 1999).

TEAP bioassays can also be used to confirm the actual ongoing microbial redox processes during degradation studies. Nielsen et al. (1995) used TEAP bioassays to evaluate the redox processes (methane production, iron reduction and denitrification)

during degradation of phenolic, aromatic and chlorinated aliphatic compounds investigated by in situ microcosms. The TEAP bioassays showed that the processes changed during the experiment in the in situ microcosms. Baedecker et al. (1993) used TEAP microcosms in connection with degradations studies at a crude-oil aquifer at Bemidji (MN., USA) to verify that iron and manganese reduction were connected with oxidation of toluene and benzene. Another example is the increase of Fe(II) in TEAP bioassays, suggesting that the mineralization of vinyl chloride was coupled to Fe(III) reduction (Bradley and Chapelle, 1996). In a similar way, Hunt et al. (1997) related mineralization of BTEXs with an increase of Fe(II), and Adrian and Suflita (1994) related mineralization of halogenated N-, S-, and O-heterocyclic compounds with an increase in methane.

Finally, investigations by TEAP bioassays in a field injection experiment at Grindsted Landfill (DK) (Rügge et al., 1999) revealed the occurrence of methane production in some locations in the plume dominated by iron-reducing conditions according to the water chemistry (Albrechtsen et al., 1999). The small methane production may reveal the presence of strongly reduced areas explaining the observed reductive dechlorination of some chlorinated aliphatic compounds (Rügge et al., 1999).

8.5. Evaluation

The enumeration of redox-specific bacteria by the MPN technique is resource-demanding and has some flaws since the technique is biased toward culturable organisms. Since some organisms are able to use several electron-acceptors, they may contribute to the number of bacteria in several redox groups. Thus, the technique primarily confirms the presence of a certain microbial potential for specific microbial redox processes.

There is no growth requirement for measuring PLFA and biomarkers, which is a strong advantage. This technique may confirm on-going redox processes, but presently the technique cannot stand alone regarding quantifying or identifying dominant redox-specific populations. Molecular probes (DNA and RNA) also do not require growing or culturing of organisms. On the other hand, a major limitation is that probes can only be prepared for known genes and, thus, for organisms that have been isolated. These techniques may prove of interest but, so far, their use in aquifers has been limited.

Generally, although present or detectable under optimum growth conditions, the different redox-specific bacteria (sulphate reducers, denitrifiers, etc.) are not necessarily active in situ, and significant correlation between the abundance of any of the different redox-specific bacteria measured by MPN or estimated from specific PLFA biomarkers, and quantitative measurements of redox reaction rates has not been observed.

The TEAP bioassays, although tedious and laborious, provide the most valuable information, such as identification of on-going processes, physical location of on-going processes in a redox sequence, identification of spatial variation of redox processes, which cannot be observed from dissolved redox-sensitive parameters, and verification of the occurrence of simultaneous redox processes. Transformation rates can be estimated from the TEAP bioassays and a dominant redox process can be appointed by transforming these rates to carbon conversion. The TEAP bioassays can be manipulated by amendments and, thus, provide insight into the complexity of processes in a contaminated aquifer and identify factors limiting or controlling the redox processes. Further-

more, TEAP bioassays can verify redox conditions during experiments and relate ongoing redox processes to mineralization processes. When assessing TEAP bioassays, however, it is important to bare in mind that the results are limited by the electron acceptors, which were investigated, and consequently by the TEAP bioassay setup. Also, the detection limit is a problem, especially in relation to iron reduction. Iron reduction may be underestimated, since even small rates, which may not be measurable or be considered significant in the iron-reduction bioassay, may convert more carbon than methane production rates, which can be detected with a lower detection limit. A way to lower the detection limits is to increase the incubation time. However, long incubation time is a problem from a practical point of view, and since the TEAP bioassays are set up as batches, limitations or successions in microbial populations may develop over time in the batches. The approach would benefit from further development in terms of improving the sensitivity, especially with regard to iron-reduction, and by including fermentation — which could be important in the context of landfill leachate plumes. However, so far, TEAP bioassays may be considered as the most useful and ultimate approach for characterizing the on-going microbial redox processes in contaminated aquifers.

9. Discussion and conclusion

Redox conditions in a contaminant plume must be addressed to understand the biogeochemistry of the plume, and much progress, as reviewed in the previous sections, have been made in this regard in the last decade. However, it should be emphasised that our current documentation and experience on measuring redox conditions in pollution plumes is still rather limited.

- The number of actual plumes, where assessment of redox has been addressed in any elaborated way (e.g., in more than 20 sampling points and by more than one method), is currently less than ten. Apparently, no investigation has used all the approaches discussed in this review on a plume containing several redox environments.
- Most of our current experiences are related to plumes in relatively shallow, sandy and, prior to contamination, originally aerobic aquifers.
- In nearly all current cases, the plumes have been present for more than a decade suggesting that the central part of the plume and maybe the full plume has reached a pseudo steady state. To our knowledge, only the Bemidji oil spill site (MN, USA) represents a newly formed progressing plume, and only the Winterthur (CH) landfill leachate plume represents a significantly receding plume.
- Most, if not all, current plumes assessed with respect to redox have been addressed within the framework of reduced, dissolved organic carbon being oxidised by inorganic electron acceptors (including carbon dioxide) present naturally in the aquifer. Oxidation of, e.g., methane, sulphides, ferrous iron, and ammonium has been dealt with only rudimentarily in the context of plumes. This is in spite of the fact that recent modelling of redox conditions in plumes (Hunter et al., 1998) suggests that such secondary oxidation reactions may be very important in contaminant plumes.

• Most of the elaborate cases addressing redox deal with natural gradient and non-engineered plumes. Hardly, any significant experience regarding redox is currently available on engineered and redox-manipulated plumes.

Therefore, the obvious recommendation on measurement of redox conditions would be to measure in, as many as possible, different plumes in many spatially distributed sampling points, repeat the measurements over time to learn about temporal variations and trends, and always use several different methods. This way, the database would improve and allow for a qualified evaluation of cost-effective approaches to measuring of redox conditions. However, usually, redox is not the main purpose of a scientific project or plume investigation, but only a supportive framework for addressing the main issues, e.g., degradation of organic pollutants. In addition, the resources may not allow for addressing redox conditions on an elaborate level. However, evaluating current, albeit rudimentary, experience may still contribute to advancement of the field. But it should be kept in mind that redox measurements in pollution plumes are still in their infancy.

9.1. Current understanding of redox environments in plumes

The experiences from measuring redox in pollution plumes, in particular, during the last decade, have improved our understanding of redox environments in plumes significantly. The approaches we take to measure redox conditions should respect this understanding to be useful and cost-effective. The key points in this understanding are as follows:

• Pollution plumes usually contain several redox environments that are physically distributed with the most reduced environments close to the source and the more oxidized environments at the front and in the outskirts of the plume. The diversity of redox environments present depends on the geochemistry of the pollution source, the natural geochemistry of the aquifer and the physics of their interaction, including the temporal and spatial variability of the plume. For example, if no significant nitrogenous compounds are present in the combined system, denitrifying redox environments are unlikely to be important in the plume.

• The overall distribution of redox environments in a pollution plume agrees with the basic thermodynamic theory as presented in Section 2, and where the redox gradients are small, zones having common dominating redox characteristics may be identified. These zones are often referred to as redox zones. However, it has been shown that several different redox processes may take place within the same zone. This has been observed even in aquifer sediment samples as small as 200 ml. It appears, at any practical scale, that concomitant redox processes are a general feature at least in pseudo-stable plumes. Redox zones may be dominated by one redox process, but other redox processes may also take place.

• In pollution plumes, redox changes over depth may be very dramatic. Changes may occur within a few centimetres, suggesting that mixing of samples over depth may be detrimental to accurate assessing and mapping of redox conditions. This applies to groundwater sampling through screens and to sediment sampling by coring. Local, small-scale, low-permeable hydrogeological heterogeneities may have natural redox

conditions not associated with the plume and should not to be mistaken as representing redox conditions in the plume.

· Ferric oxyhydroxides are very common in shallow aquifers and constitute, based on chemical assessment of redox capacity and microbial redox rates, the major chemical redox buffer in many plumes. Assessment of rates of iron reduction in actual plumes is a fairly recent accomplishment. Many previous discussions on important redox processes in pollution plumes have consistently underestimated the importance of iron reduction due to the fact that account has been made only of dissolved reduced iron neglecting the significant pool of reduced iron accumulating in the sediment. An example is the experience from the Bemidij oil spill plume (MN, USA), where little reduced iron was present in the groundwater, but including the sediment in the investigation revealed that iron reduction was the dominant redox process in the plume. It has also been observed that high concentrations of reduced iron in the groundwater as well as on the sediment did not reflect on-going iron reduction. The potential complexity in properly addressing iron reduction should not lead to iron reduction being ignored.

· Microbial processes are believed to be the most important redox processes in pollution plumes. However, at least in pseudo-stable plumes, the actual, general redox processes do not seem to be controlled by limitations in the type of microbial populations present. Most likely, the redox activity is limited by one or more factors, such as mass transfer, availability of electron donors or acceptors, the thermodynamic energetics of the processes, and microbial kinetics (affected by low substrate concentrations, presence of toxic compounds, and end-product inhibition).

9.2. Current needs to address redox conditions in pollution plumes

Redox assessment is usually carried out as a supportive framework for understanding the behaviour of pollution plumes and their pollutants. As the concepts of redox zones in plumes were developing in the late 1960s into the 1980s, redox was used as the framework for explaining the different types of groundwater samples obtained from the plumes and as an indication of the effect of the plume on the aquifer. Emphasis was on inorganic constituents as nitrogen compounds, sulphur compounds and iron. In the 1990s, redox zones were used as the chemical framework for understanding the degradation of organic chemicals in plumes. Focus was originally on aerobic vs. anaerobic redox conditions since many chemicals were considered easily degradable under aerobic conditions but recalcitrant under anaerobic conditions. However, as our understanding of degradation of organic chemicals under anaerobic conditions has increased in recent years, the focus on redox and degradation of organic chemicals has shifted towards compounds that are slowly degrading or incompletely mineralised under various redox conditions. For example, in anaerobic environments, benzene degradation or reductive dehalogenation of chlorinated solvents have been shown to be related to specific redox conditions.

In the view of plume mapping and remediation, by natural attenuation or engineered systems, several issues may relate to redox, and information on redox conditions may be useful in answering questions including the following:

· Has the plume existed for a long time and is it fairly stationary or receding? This question may be crucial in evaluating the environmental risks associated with the plume

and in applying monitored natural attenuation as a remediation strategy. A pseudo-stationary plume often will have changed significantly the redox capacities of the aquifer as measured on the sediment, which seldom will be the case in a recent or temporary plume.

- Is the apparent attenuation of an organic chemical in a plume consistent with our current knowledge on the degradability of that chemical at the redox conditions in the plume? Naturally, this requires that we can determine which redox conditions are present, not necessarily dominating, in the zone of the plume, where the organic chemical apparently is degrading. This may also require that the size of the zone and the retention time of the organic chemical in the zone are estimated to provide apparent degradation rates for comparison with the current database.
- Are the redox conditions in a degradation experiment (laboratory or field) regarding a critical organic chemical comparable to the redox conditions in the plume? If the redox conditions in an experimental system change over time, e.g., due to leaks or intrusion of air during sampling or due to depletion of a critical electron acceptor caused by the spiking of the system with the organic chemical, the degradation patterns and rates observed in the experiment may not apply to the plume. Monitoring of both redox environments may be the only way to assure compatibility.
- How easily can the redox conditions in the plume be altered in order to enhance the degradation of a critical pollutant? In the context of an engineered remediation, it may be of interest, e.g., to reduce the redox conditions to enhance dehalogenation of chlorinated solvents or to provide aerobic conditions to enhance benzene degradation. Evaluation of the feasibility of such approaches must involve assessment of the oxidation and RDC of the plume and the availability of redox buffers at the aimed redox condition beneficial for the enhancement of degradation.
- What is the capacity of the aquifer for buffering against accidental spills of fuels from planned or existing storage tanks located on the land overlaying the aquifer? Aquifers with a high OXC are likely better at limiting the pollution of the groundwater from an accidental spill and, therefore, the land above more suited for hosting the storage tanks.

The above-mentioned issues that redox measurements can help address show large diversity and remind us that the approach to measuring redox conditions must be selected according to the purpose. However, consistently linking approaches and purposes based on the current limited experiences would be premature. But it is important in each case, in view of the purpose, to pay attention to the following issues:

- How detailed should the redox characterization be? The resolution wanted in the redox characterization should of course be balanced with the number of sampling points. However, even if a high resolution is not needed, the sample volume should ideally be smaller than sediment heterogeneities. Otherwise, samples may contain contributions from aquifer volumes of different redox conditions, resulting in averaged, nonrepresentative results. The redox mapping must be sufficiently detailed that hydraulic retention time in a given redox zone can be estimated, if degradation rates are to be linked to redox conditions.
- Is it sufficient to determine dominating redox environments, e.g., in terms of redox zones defined by the dominating redox process, or is the diversity of redox processes in

a given zone also of importance? If the pollutants of concern also are the pollutants that drive the redox processes (e.g., petroleum hydrocarbons as dominant electron donors), it may suffice to know only the dominating redox condition. However, if the pollutant of concern is a trace component and the redox zones are primarily created by the transformation of other secondary pollutants, e.g., nonspecific dissolved organic carbon, it may be important also to know that other redox processes take place.

- Are current redox conditions or processes to be identified only (do they happen) or are estimates of the reaction rates needed (how fast do they happen)? If the pollutants of concern also are the pollutants driving the redox processes, estimates of redox rates may supplement observed rates of pollutant degradation, but with respect to trace pollutants, major redox process rates will not provide much information.
- Are redox capacities important? Measurements of redox capacities may, in some cases, help in identifying limiting factors controlling current redox processes, remaining potentials, or redox equivalents needed to change current redox conditions. The capacity measurements, in contrast to the intensity measurements, are needed in understanding past plume history and predicting future natural or engineered changes.

9.3. Promising and less promising approaches to measuring redox conditions

In view of the current experience, the diversity of purposes for measuring redox conditions, and the variable resources available for the measurements, specific recommendations on how to measure redox conditions in groundwater pollution plumes are currently not warranted. However, some approaches seem more promising than others, as summarised below:

- Electrochemical redox potentials are easy to measure with a combination electrode and a potentiometer, but any elaborate interpretation is not defendable. In strictly anaerobic redox environments, the electrode is likely to respond to the iron redox couple, but the readings may not reflect the iron couple at equilibrium and cannot be used for meaningful general electrochemical calculations. Such simple measurements of redox potential, performed in-line in a flow cell, are primarily useful in the field for quickly assessing whether the groundwater is sampled from strongly reducing conditions (typical readings: $E_H < -50$ mV), i.e., in the central part of the plume, or from the outskirts of the plume at higher redox conditions (showing higher, typically positive values). Experience from unpolluted groundwater environments may suggest, however, that a better performed redox potential measurement (two working electrodes of different material, sufficient stability, etc., as specified in Table 6) in certain redox environments dominated by iron reduction may provide a redox potential useful for electrochemical calculations regarding the iron system.
- Groundwater composition with respect to key pollutants is determined in most pollution plume studies. By ensuring redox intact sampling and handling of the samples, and analysing for redox-sensitive inorganic compounds in the samples, dominant redox conditions can be assessed from the relative distribution of the compounds. The strength of this approach is the use of conventional technology, but the mobility of some of the redox-sensitive species (in particular, methane) and the site-specific empirical criteria used for assigning the redox labels add some uncertainty to the precise physical

positioning of the redox zones. However, it is very likely that the relative groundwater composition with respect to redox-sensitive species will reflect the redox processes dominating in the different zones. We suggest that redox-sensitive species always be measured in groundwater samples and used, together with other approaches, in assessing redox conditions.

- Hydrogen concentrations in groundwater samples can be fairly easily determined, but special precautions in well design, sampling and analysis are needed. Measurement of hydrogen concentrations has great potential for identification of redox processes under strongly reducing conditions. It is uncertain whether the technique is diagnostic at manganese and denitrifying redox conditions. The strength of the approach is that it reflects on-going redox processes precisely at the point of sampling. The direct use of hydrogen concentrations as a diagnostic tool for assigning redox zones is not appropriate. Temperature, concentrations of dissolved species as well as type of solid iron oxides involved in iron reduction influence the energetics of the system and actually may allow both iron reduction and sulphate reduction to take place at the same hydrogen concentration. However, the combination of measured hydrogen concentrations with groundwater composition in thermodynamic calculation of Gibb's energy of reactions may provide valuable insight into which redox processes may occur and, in the future, which chlorinated or other organic compounds may act as electron acceptors. Full exploitation of the potential of hydrogen measurements for determination of redox conditions requires improvements in the current thermodynamic data bases as well as further experience from real plumes. So far, experiences from just six plumes have been reported in scientific journals.
- VFA concentrations in groundwater samples might theoretically, in analogy to the use of hydrogen measurements, reflect ongoing redox processes, but the few attempts to apply this to real plumes have so far provided no useful insight into redox conditions in plumes. Measurement of VFAs, however, may potentially turn out to be useful for evaluating short-term plume stability.
- Aquifer sediment characterization primarily addresses iron and sulphur compounds. Manganese contents may also be determined, but since analytical distinction between Mn(IV) and Mn(II) is not easily accomplished and manganese is usually a less significant redox components, the focus is on iron and sulphur. Aquifer sediment has a far larger redox buffer capacity than the groundwater, and any evaluation of capacities must include sediment analysis. Applications may include, but not be limited to, assessing plume stability, limiting factors or capacities for iron reduction, quantification of the significance of iron reduction, and documentation of a receding plume by identification of pyrite accumulation in previous, sulphate-reducing zones of the plume. The strength of sediment redox characterization is that only the sediment accumulates information about past processes and provides a basis for evaluating future capacities. These aspects are, in particular, important in the context of plume remediation. The limitation of the sediment characterization is the costly sampling, extraction and analysis. Since iron reduction seems to be a very important redox process in many plumes and both Fe(III) and Fe(II) accumulates in the sediment, characterization of sediments is suggested in all plume investigations, where plume stability is a key issue or manipulation of the plume is considered.

· Microbial measurements in terms of MPN countings, PLFA-based biomarkers and emerging molecular probes have shown the diversity of microbial populations in pollution plumes and have confirmed that the microbial potential for various major redox processes is generally present in pollution plumes. However, these approaches have not been effective in identifying actual redox conditions. PLFA-based biomarkers, which have limited life time outside the cell, have in a few cases been used to confirm otherwise identified sulphate reduction. The unamended TEAP bioassays monitored for a variety of redox processes are, on the other hand, a very powerful approach to identifying multiple redox processes and also to obtain estimates of actual rates. TEAP bioassays are, however, very laborious, time consuming and costly and furthermore require careful handling of sediment samples. A potential limitation may be the fairly high detection limit found for iron reduction, in some cases. Systematic application of unamended bioassays has only been done in a few cases and further experience should be gained to fully understand advantages and disadvantages of this approach.

Acknowledgements

The preparation of this review would not have been possible without the valuable and much appreciated support from Ms. Birthe Brejl (illustrations) and Ms. Grete Hansen (bibliography and permissions to print published material), Technical University of Denmark, and Dr. Dale Carlson and Mr. Joseph Greer, the Valle Scandinavian Exchange Program, University of Washington, Seattle (logistics and telecommunication). Thorough reviews by Dr. J. Griffioen (TNO, the Netherlands), Dr. J. Lendvay (University of Michigan, Michigan, USA) and Dr. P. Bradley (USGS, South Carolina, USA) were very much appreciated.

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