ABSTRACT
As an alternative to conventional remediation technologies, the engineered use of natural attenuation (NA) processes for site remediation coined Monitored Natural Attenuation (MNA) is becoming more and more accepted in a growing number of industrialized countries. The analytical “cookbook” of methods to investigate NA processes matured during the 1990’s and has amalgamated in a wealth of guidance documents. Practical experience with MNA has shown site conditions to be more complex than in theory requiring a more profound understanding of the governing NA processes. Case studies are presented focusing on the application of novel tools such as compound-specific isotope analysis on $^{13}$C/$^{12}$C (CSIA), signature metabolites analysis (SMA) and redox-sensitive tapes (RST), which are aimed at providing answers as to whether the contaminants of concern are actually (bio)degraded and to what extent. These tools have the potential to provide more reliable field quantification of degradation, and improved investigation and assessment of small-scale biogeochemical processes.

RÉSUMÉ
Les phénomènes d’atténuation naturelle (“Natural Attenuation” — NA), communément appelés “Monitored Natural Attenuation” (MNA), utilisé pour réhabiliter des sites pollués, propose une alternative aux techniques conventionnelles de dépollution, qui est de mieux en mieux acceptée dans un nombre croissant de pays industrialisés. Les méthodes analytique utilisées pour comprendre les procédés de NA a mûri aux cours des années 90 de manière à constituer une somme de documents de référence. L’application des techniques de MNA a montré que les conditions des sites traités étaient plus complexes que la théorie ne le supposait, ce qui exige une bien meilleure compréhension des principaux phénomènes de NA. Les cas pratiques qui sont présentés mettent l’accent sur l’utilisation de nouveaux outils tels que l’analyse du composé spécifique des isotopes stables $^{13}$C/$^{12}$C (CSIA), l’analyse de la signature des métabolites et les bandes sensibles au redox, dont le but est montrer si les agents polluants recherchés sont réellement biodégradables et jusqu’à quel point. Ces outils offrent la possibilité de fournir des données plus fiables pour quantifier le degré de dégradation, et proposent des moyens améliorés d’investigation et d’évaluation des processus bio géochimiques intervenant à petite échelle.

1. INTRODUCTION

Monitored Natural Attenuation (MNA) represents a groundwater monitoring scheme to characterize the groundwater chemical processes for natural attenuation (NA) to confirm they are sufficient to achieve site remedial objectives. For common contaminants such as petroleum hydrocarbons (PHCs) and chlorinated hydrocarbons (CHCs), NA processes result in characteristic changes of biogeochemical patterns in groundwater. Typically, NA occurs at the majority of contaminated sites. However, the effectiveness of these processes varies depending on the nature and extent of contaminants present and the physical, chemical, and biological site characteristics. NRC (2000) proposed that NA would be appropriate only when a “cause-and-effect relationship” is established between naturally occurring destruction and/or immobilization processes and the loss of contaminant. For NA assessment a three-part strategy is proposed (NRC 2000):

1. Develop a Conceptual Site Model (CSM) aimed at identifying the site-specific NA processes;
2. Obtain site-specific information that documents the cause-and-effect relationship between the NA processes identified and the observed contaminant loss; and
3. Implement a long-term data acquisition and evaluation program that documents (monitoring) that these NA processes are sustained and consistent with remedial objectives.

When appropriately employed, NA can represent an “effective knowledge-based remedy” protective of human health and the environment when a thorough analysis provides for the understanding, quantitative documentation, prediction, and monitoring of the NA processes (US EPA 2001). Practical experience with MNA has shown site-specific conditions to be more complex than in theory requiring a more profound understanding of the governing NA processes. Since biodegradation of organic contaminants is the only process resulting in actual contaminant and/or mass elimination, it is preferred over other NA processes such as sorption and dispersion. Unfortunately, to date the direct proof of contaminant degradation and a practical and more reliable quantification of the active biodegradation is generally the most challenging to
achieve. However, compound-specific isotope analysis (CSIA) on $^{13}$C/$^{12}$C, signature metabolites analysis (SMA) in combination with redox-sensitive tapes (RST) are emerging innovative tools which have the potential to provide a more reliable assessment of NA processes on the field-scale. Two case studies are presented focusing on the scope of NA data acquisition required for two different sites and contaminant conditions to demonstrate that MNA is a viable approach.

2. DATA ACQUISITION STRATEGY

Depending on the complexity of site conditions, different tools for the NA assessment can be selected. Our data acquisition strategy (DAS) extends the lines-of-evidence approach of NRC (1993) insofar as it incorporates these novel tools into the standard NA assessment procedures (Figure 1):

1. Of high significance are the contaminant data, which are used to assess contaminant trends in groundwater in space and time.
2. Hydrochemical data are collected which are indicative of biodegradative processes in groundwater, since NA depends upon the contaminant reactivity, the site-specific geologic and chemical conditions. Assessment of the spatial and/or temporal changes in hydrochemical conditions constitutes a secondary line of evidence.
3. In addition, SMA, CSIA and RST investigations are carried out alone or in combination aimed at acquiring an improved picture in support of MNA by gathering direct and quantified evidence of contaminant degradation supported by the assessment of the actual redox zone distribution.

![Figure 1. Illustration of the data acquisition strategy.](image)

In the following, the methodology of these tools is described.

2.1 Compound-Specific Isotope Analysis (CSIA)

Stable isotope fractionation occurs in many biochemical reactions and is therefore a process which occurs in various environments. For example, the carbon fixation of plants is a well studied field. Lebedew et al. (1969) were the first who recognized a stable isotope effect caused by the degradation of contaminants. In recent years several studies showed that the compound-specific isotope analysis (CSIA) can be used to prove and quantify the biodegradation of various organic compounds with a focus on benzene, toluene, ethylbenzene, and xylenes (BTEx) and chlorinated solvents (e.g. Meckenstock et al. 1999; Hunkeler et al. 1999), as well as several polycyclic aromatic hydrocarbons (PAHs) (Griebler et al. 2004). The Rayleigh (1896) equation is used to describe and quantify the isotope fractionation:

$$C_t = C_0 \left( \frac{R_t}{R_0} \right)^{\alpha_{\text{C}} - 1}$$  \[1\]

where $C_t$ is the theoretical remaining substrate fraction, $C_0$ is the initial substrate concentration, $R_t$ is the $^{13}$C/$^{12}$C isotope signature at time $t$, $R_0$ is the initial isotope signature and $\alpha_C$ is the isotope fractionation factor, which can be derived from laboratory experiments and is often expressed as the isotope enrichment factor $\varepsilon = (\alpha_C - 1) \times 1,000$. A comprehensive summary on available isotope enrichment factors is given by Meckenstock et al. (2004).

Further, the percentage of biodegradation ($B$) of the residual substrate fraction can be calculated with the following equation:

$$B = \left(1 - \frac{C_t}{C_0}\right) \times 100$$  \[2\]

It should be noted that the determined percentage of biodegradation is based on the kinetic isotope fractionation factor, which is a constant for a given reaction at experimental conditions. Hence, the determined percentage of biodegradation only describes a certain fraction of the entire biodegradation occurring in or at the plume fringes. Nevertheless, a site- and process-specific degree of biodegradation dependent on the isotope fractionation factor can be estimated. In summary, the CSIA allows for a qualitative and semi-quantitative proof of biodegradation.

2.2 Signature Metabolite Analysis (SMA)

The acceptance of MNA is closely related to demonstrating that decreasing contaminant concentrations are unambiguously linked to biodegradation rather than physical processes such as dilution, sorption and volatilisation. This objective can be accomplished by acquiring direct evidence of biodegradation through the detection of metabolites consisting of aromatic acids generated through the initial activation of the contaminant molecule (e.g. Beller 2000; Griebler 2004).
Subsurface microbes have been shown to be able to degrade compounds such as BTEX under both aerobic and anaerobic conditions to obtain energy. Primarily under anaerobic conditions, but also under aerobic conditions, metabolites are being formed. These signature metabolites are considered biogeochemical indicators for in-situ degradation of aromatic hydrocarbons, because these compounds do not occur in pristine aquifers nor are they constituents of the original mineral oil (Beller 2000). According to Cozzarelli et al. (1990), Beller et al. (1996) and Beller (2000), the metabolites of aromatic compounds structurally correspond to their parent compounds. Moreover, aromatic acids do exhibit a transient behaviour (i.e. likely, they are continuously being formed and degraded, resulting in a good potential for their detection under field conditions).

In broad terms, the degradation sequence for BTEX compounds is as follows:

\[
\text{BTEX} \rightarrow \text{benzylsuccinic acids} \rightarrow \text{methylated benzoic acids} \rightarrow \text{benzoic acid} \rightarrow \text{CO}_2 + \text{H}_2\text{O}.
\]

Moreover, there are indications that higher alkylated benzenes up to C\textsubscript{7} yield in similar structurally corresponding aromatic acids (Martus & Puettmann 2003).

Analogous to this, PAHs may be subject to the formation of polyaromatic acids. For example, the pathway for anaerobic naphthalene degradation is:

\[
\text{Naphthalene} \rightarrow \text{naphthoic acid} \rightarrow \text{CO}_2 + \text{H}_2\text{O}.
\]

In summary, signature metabolites may be indicative of in-situ biodegradation processes, but will not allow a quantification of biodegradation due to their transient behaviour.

### 2.3 Redox-Sensitive Tapes (RST)

The vertical and lateral distribution of redox zones within a dissolved contaminant plume can be investigated by the use of redox-sensitive tapes (RST). The tapes consist of a 2 cm wide synthetic textile coated with reactive manganese dioxide minerals. The tapes are submerged into existing monitoring wells for approximately four weeks. Given adequate reaction time, site microbiological communities readily occupy the tape utilizing the manganese dioxide minerals through redox reactions. These reactions lead to characteristic colour changes of the tape surface, indicative of a distinctive redox process. This allows for an investigation of the actual redox zonation on the centimetre-scale for manganese, iron and sulfate reduction.

The redox-environment specific colors are as follows:

- **Drab** = manganese(II)-oxidizing redox zone;
- **Ochre** = manganese(IV)-reducing and iron(II)-oxidizing redox zone;
- **Light ochre to white** = iron(III)-reducing and sulfide-oxidizing redox zone; and
- **Light grey to dark grey** = sulfate-reducing redox zone.

### 3. CASE STUDIES

#### 3.1 Case Study 1 – Creosote-contaminated Site

##### 3.1.1 Site Description

The investigated site is located in northern Germany approximately 40 km south of Hamburg. The subsurface conditions at the site are characterized by Pleistocene glacial deposits, mainly consisting of fine to coarse sands. Between 1904 and 1986, coal tar creosote was used to impregnate wooden railroad cross ties. Substantial coal tar contamination of both the unsaturated and the saturated zones has occurred. The extent of the contaminant plume in groundwater is around 450 m.

In the 1990’s, a conventional pump & treat remediation of the groundwater contamination had been in operation downstream of the main contaminant source. In 2003, URS commenced the assessment of the feasibility of MNA as an alternative remediation strategy. Hence, a DAS was developed to obtain all the required data to prove and quantify the biological activity in the contaminant plume.

##### 3.1.2 Site-specific Data Acquisition Strategy

The site-specific conditions showed significant contaminant mass to be present in the source area. Moreover, the groundwater contaminant plume extended to approximately 48 m below ground surface (bgs). These conditions made the site a candidate for the use of the full array of DAS tools. Moreover, the location was considered a reference site, aimed at facilitating transfer of DAS experience gained to other NA projects. The site-specific DAS pursued was based on the general description in Section 2. Therefore, by using CSIA, SMA and RST in combination, alongside with hydrochemical and contaminant data, reliable evidence for biodegradation was obtained.

In the following, the application of these tools is briefly described.

In October 2004 and November 2005, groundwater samples for the CSIA were obtained from 12 groundwater monitoring wells located mainly along the plume centre line. The isotope analysis was carried out on the individual BTEX constituents, as well as on naphthalene, 1- and 2-methylnaphthalene, acenaphthylene, acenaphthene and fluorene. Groundwater samples for the SMA were collected from 7 monitoring wells located along the plume centre line and at a control plane (CP) close to the contaminant source. The CP consisted of a transect of monitoring wells established perpendicular to groundwater flow direction across the plume. Moreover, a threefold RST investigation (April and November 2004, May 2005) was conducted at 8 groundwater monitoring wells located along the plume centre line and at a CP aimed at delineating the redox zone distribution in space.
and time. Finally, four comprehensive site-wide NA screenings were performed to delineate the distribution of both contaminants and NA indicators such as \( \text{SO}_4^{2-} \), \( \text{Fe}^{2+} \), and \( \text{O}_2 \) in groundwater.

### 3.1.3 Results

The observed contaminant trends from previous groundwater samplings (1994-2002) and four specific NA screenings (2003-2005) indicated that the contaminant plume is currently at steady-state conditions.

Strong aromatic acid signals were obtained from wells located within the contaminant plume. Direct evidence of active contaminant biodegradation was found for toluene, the isomers of xylene, as well as naphthalene and the isomers of methylnaphthalene (2). For benzene, ethylbenzene and the trimethylbenzene isomers, general transformation pathways suggest metabolism through the central intermediate benzoic acid. Peak naphthoic acid as well as benzoic/methylbenzoic acid concentrations in the upper \( \mu \text{g/L} \) range were detected at the near-source monitoring wells. Metabolite concentrations mimic the decrease in contaminant concentrations along the groundwater flow path of both PAH and BTEX compounds very well. Based on the current scientific understanding on metabolite transformation, all aromatic acids eventually are transformed to \( \text{CO}_2 \) and water.

Figure 3 ideally illustrates the CSIA results for o-xylene. The o-xylene concentrations significantly decreased with increasing distance from the contaminant source from 90.2 \( \mu \text{g/L} \) at G10m (near source) to 1.2 \( \mu \text{g/L} \) at the leading edge of the contaminant plume (G24u). In contrast, the \(^{13}\text{C}/^{12}\text{C} \) stable isotope ratios of o-xylene increased with increasing distance from the source. This indicates an enrichment of \(^{12}\text{C} \) in the residual dissolved contaminant, which demonstrates the effectiveness of the in-situ biological activity. Assuming an isotope fractionation factor \( \alpha \) for sulfate-reducing bacteria of 0.9972 (Griebler et al. 2004), the mean calculated percentage of biodegradation in the considered aquifer is 66 % ± 31 % with a maximum of 92 % observed at the leading edge of the contaminant plume. Similar CSIA results were obtained for the other compounds studied, however the isotopic effects for the BTEX compounds were stronger than for PAH compounds. This is considered a common observation, which is due to the lower biodegradation of PAH (e.g. Zamfirescu & Grathwohl 2001). A quantative analysis and determination of the percentage of biodegradation was achieved for toluene, ethylbenzene, o/m/p-xylene and naphthalene. Moreover, the CSIA enabled a qualitative analysis proving the biological activity for toluene, ethylbenzene, 1- and 2-methylnaphthalene and acenaphthene.

Figure 2. Simplified anaerobic degradation pathways of dominant contaminants at the Buchholz site: (1) naphthalene, (2) naphthoic acid, (3) methylnaphthalene isomers, (4) benzene, (5) toluene, (6) xylene isomers, (7) ethylbenzene, (8) trimethylbenzene isomers, (9) methylbenzoic acid isomers, (10) benzoic acid. Solid arrows indicate proven pathways, whereas suggested transformation steps are dashed.
3.2 Case Study 2 – Gas Station Site

3.2.1 Site Description

The site, which is located on Vancouver Island, is used as a parking lot for a shopping mall (Figure 4). Environmental investigations identified free phase gasoline product migrating to the site from two adjacent gasoline retail sites. The product plume initially covered 700 m$^2$ area at the site boundary and downgradient area. The resulting dissolved BTEX plume extended approximately 90 m downgradient. In 2002, the BC Ministry of Environment approved a phased remediation plan combining active and passive approaches. Remedial objectives were the removal of free-phase product to permit risk management of residual soil and dissolved phase contamination. In summer 2002, remediation works were initiated with the commissioning of a dual phase extraction system. Extension of the remedial system to the neighbouring off-site source site was undertaken in July 2005 to permit initiation of the risk assessment.

A total hydrocarbon mass removal of approximately 100,000 L (liquid product equivalent) was achieved, with all mobile free-phase product removed from the mall site by January 2006.

While the remedial system reduced concentrations of dissolved phase contaminants, post-remediation concentrations exceeded standards for groundwater quality in some locations on the site. Confirmatory drilling indicated residual soil contamination remains in the area of the former product plume. This residual contamination has been risk-managed. A thorough NA assessment was conducted in support of the risk assessment problem formulation.

A network of 41 groundwater monitoring wells is present across the site to facilitate the delineation of the hydrocarbon impacts.

3.2.2 Site-specific Data Acquisition Strategy

In February 2006, URS conducted a state-of-the-art assessment of both NA processes in groundwater and dissolved contaminant plume stability. The assessment included contaminant mass flux calculations utilizing two CP (Figure 2), plume statistical tests (Mann-Kendall, t-test), geochemical sampling, and investigation of plume redox zone distribution using RST. The DAS rationale was based on:

- the availability of a multi-year record of environmental data dating back to 1994, indicating a shrinking plume related to the effects of the remediation system and/or NA processes;
- the overall influence of the remediation system on the dissolved contaminant plume resulting in a presumed widespread alteration of NA conditions;
- the constraints imposed by the operational requirements of the remediation system (i.e. the minimum time lag after temporary system shut-down to create undisturbed NA groundwater conditions within the radius-of-influence of the remediation system).

Consideration of these site-specific factors led to the conclusion that due to the presumed impact of the remediation system on hydrochemical conditions, the use of SMA and CSIA would likely be equivocal. Moreover, strong signals indicating a receding plume due to the combined effects of the remediation system and NA processes downstream were evident. Therefore, the data acquisition strategy was rather to assess the distribution of redox conditions inside and outside of the influence of the remediation system after temporary system shutdown. This was aimed at assessing the response of redox conditions on a future permanent shutdown of the remediation system.

To ascertain the minimum response lag time between shutting off the system and the geochemical assessment, URS monitored dissolved oxygen (DO) in groundwater and well vacuum response. The measured parameters reached steady-state within about seven days of shutting down the system.
3.3 Results

The data collected demonstrated significant ongoing NA processes and indicated a shrinking dissolved plume in response to ongoing source remediation. An apparent shift of minimum and maximum distribution patterns of NA parameters into the downstream direction outside the influence of the remediation system (i.e. NNW of MW37 and MW 38 (Figure 2)) indicated undisturbed NA ground-water conditions downstream of the remediation system were not fully established when the NA screening was conducted.

Evidence of the actual small-scale distribution of redox processes within the vertical water column of the contaminant plume was successfully gathered (Figure 7). There was a good correlation of RST data with hydrochemical data at the wells investigated. In the majority of plume wells across CP1 located 50 m downstream of the source area and beyond, manganese reduction appeared to be prevailing. Moreover, the results of the redox tape investigation attest for a widespread absence of actual iron and sulfate reduction along CP1 at the time of investigation. Therefore, the decreased sulfate and elevated dissolved iron concentrations detected at CP1 point to ongoing iron and sulfate reduction in the upstream direction of CP1, and provide evidence for the transport of transformation products with groundwater flow.

Following successful demonstration of NA processes to be active, the RST can be used to assess the spatial and temporal distribution of biodegradation processes within the contaminant plume. A reliable demonstration of plume stability is an important common feature in support of MNA under most environmental legislative regimes. The RST allows for a high-resolution confirmation of the plume stability, potentially using this tool as a surrogate in favour over more expensive laboratory chemical investigations. We deem that the costs for the DAS presented herein have the potential to be offset by the benefits through reduction of uncertainty with respect to the applicability of MNA at a given site. Hence, the DAS for MNA should be considered on a regular basis during NA investigations where an improved understanding of biodegradation conditions is required.

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