

Overview of Natural Source Zone Depletion: Processes, Controlling Factors, and Composition Change

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Abstract

Natural source zone depletion (NSZD) has emerged as a practical alternative for restoration of light non-aqueous phase liquid (LNAPL) sites that are in the later stages of their remediation lifecycle. Due to significant research, the NSZD conceptual model has evolved dramatically in recent years, and methanogenesis is now accepted as a dominant attenuation process (e.g., Lundegard and Johnson 2006; Ng et al. 2015). Most of the methane is generated within the pore space adjacent to LNAPL (Ng et al. 2015) from where it migrates through the unsaturated zone (e.g., Amos and Mayer 2006), where it is oxidized. While great progress has been made, there are still some important gaps in our understanding of NSZD. NSZD measurements provide little insight on which constituents are actually degrading; it is unclear which rate-limiting factors that can be manipulated to increase NSZD rates; and how longevity of the bulk LNAPL and its key constituents can be predicted. Various threads of literature were pursued to shed light on some of the questions listed above. Several processes that may influence NSZD or its measurement were identified: temperature, inhibition from acetate buildup, protozoa predation, presence of electron acceptors, inhibition from volatile hydrocarbons, alkalinity/pH, and the availability of nutrients can all affect methanogenesis rates, while factors such as moisture content and soil type can influence its measurement. The methanogenic process appears to have a sequenced utilization of the constituents or chemical classes present in the LNAPL due to varying thermodynamic feasibility, biodegradability, and effects of inhibition, but the bulk NSZD rate appears to remain quasi-zero order. A simplified version of the reactive transport model presented by Ng et al. 2015 has the potential to be a useful tool for predicting the longevity of key LNAPL constituents or chemical fractions, and of bulk LNAPL, but more work is needed to obtain key input parameters such as chemical classes and their biodegradation rates and any potential inhibitions.

Introduction

Natural source zone depletion (NSZD) is emerging as an important remediation approach for petroleum hydrocarbon sites. In recent years, rapid advances have been made with NSZD as documented in a diverse multidisciplinary body of knowledge, indicative of a dynamic and expansive field of research. Unfortunately, the knowledge is spread through a diverse body of literature (e.g., see Figure 1 and Table 1) and has not been synthesized into a coherent state-of-knowledge overview of the topic, which is limiting the potential of NSZD to be effectively applied as a remediation option.

The objectives of this paper are to summarize current knowledge and identify key research questions that need further attention.

First, we present the origins of the NSZD conceptual model and the emergence of methanogenesis and vertical gas transport as key processes underlying NSZD; review knowledge from other methanogenic systems; and evaluate critical issues and data gaps. Then the current NSZD conceptual model is presented with a detailed discussion of potential rate-controlling processes. Finally one key issue, how light non-aqueous phase (LNAPL) composition changes during NSZD, is discussed based on knowledge from hydrocarbon biodegradation literature, and on a model developed by Ng et al. (2015).

Historical View

The widespread acceptance of natural processes being important in hydrocarbon attenuation started in the early 1990s (Chapelle 1999), where monitored natural attenuation (MNA) was recognized as playing a key role in dissolved plume stability. These hydrocarbon source zone mass balance models incorrectly assumed that hydrocarbon mass removal fully accounted for the mass discharge of aqueous phase electron acceptors entering, and soluble byproducts (ferrous iron and dissolved methane) leaving saturated LNAPL source zones (Newell et al. 1996; Wiedemeier et al. 1999). This focus on the horizontal flux of reaction constituents in

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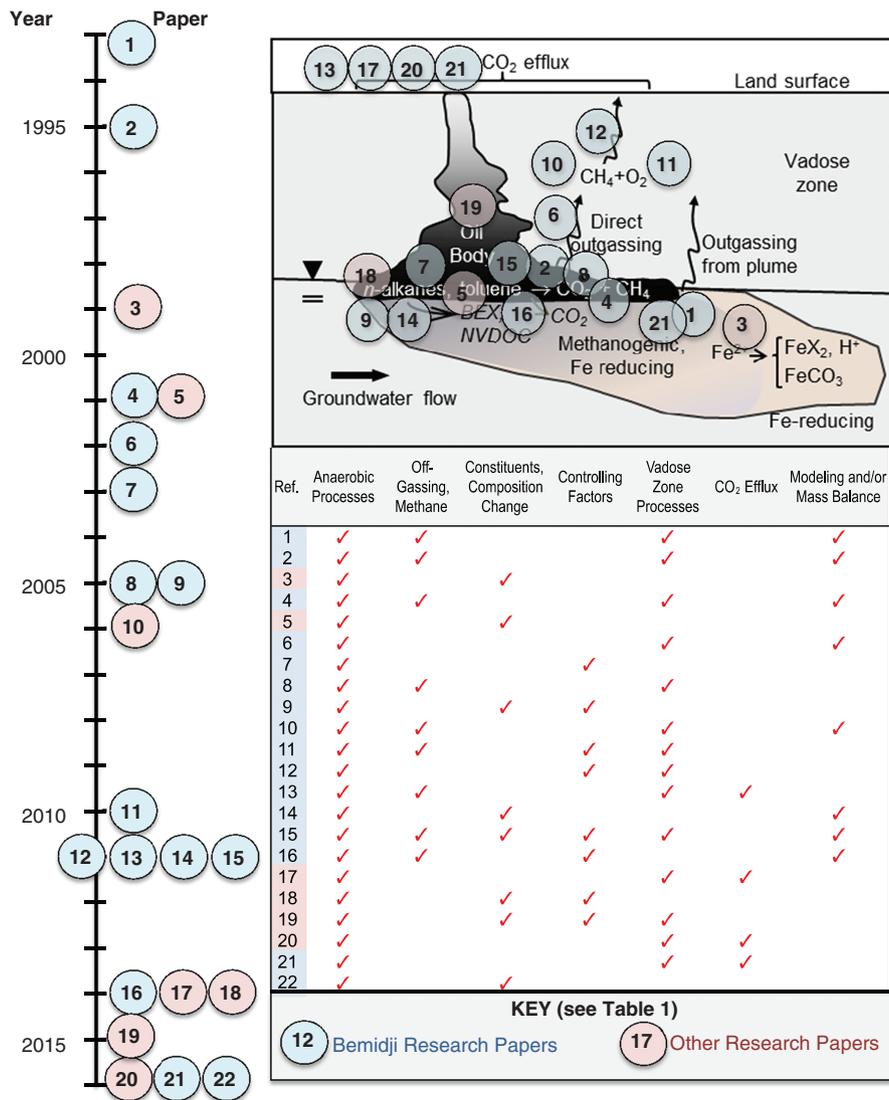


Figure 1. Overview of selected Bemidji and other research focusing on methanogenic degradation of LNAPL sources since 1995. Left panel: Timeline of key papers; numbers are linked to Table 1. Top panel: Conceptual description of research targets for the 22 papers. Each circle is located to represent the physical location of the research, or is located by the key words shown in the figure that is representative of the papers. Figure is adapted from Ng et al. 2015. Bottom panel: Chart identifying the key topics addressed by the 22 research papers.

the saturated zone led to the conclusion that LNAPL source zones biodegraded at a rate of only a few gallons of hydrocarbon/acre/year (see “NSZD Measurement and Observed Rates” and Appendix S1, Supporting Information).

Paradigm Shift

In 1983, a long-term research program was initiated at the Bemidji Site in Minnesota by the U.S. Geologic Survey (USGS) and their collaborators, where about 1.7×10^6 L (10,700 barrels) of crude oil were released due to a pipeline rupture in 1979 (Essaid et al. 2011). Key research was also performed in a series of studies by Arizona State University and Colorado State University. These research papers and other contributions are summarized in Figure 1 and Table 1, showing the timeline and the nature of each research paper. Taken together, the research highlighted in Table 1 represents a paradigm shift from an electron acceptor, dissolved-phase model geared toward assessing plume stability to an

NSZD model where methanogenesis coupled with transport of gases—methane, carbon dioxide, and volatile organic compounds (VOCs)—through the vadose zone is recognized as the primary mass-loss mechanism (Table 2). By 2015, authors such as Suthersan et al. were able to conclude: “It is now apparent that the rate of LNAPL depletion in the subsurface was greatly underestimated. The key piece missing in traditional electron acceptor-driven mass balance models was a complete understanding of LNAPL depletion in the vadose zone and the role of methanogenesis.”

Key Concepts

A brief discussion of some key concepts is instructive before presenting the NSZD conceptual model:

- Methanogenesis is part of a complicated, multiple-step, syntrophic process. Syntrophy is a metabolic phenomenon where two groups of organisms degrade complex organics

Table 1

Selected Research Papers from 1993 to 2016 Focusing on Methanogenic Processes in LNAPL Source Zones

No.	Reference	Key Excerpts
1	Baedecker et al. 1993	<i>“Mass transfer calculations on a 40-m flowpath in the anoxic zone, downgradient from the oil body, indicated that the primary reactions in the anoxic zone are oxidation of organic compounds, precipitation of siderite and a ferroan calcite, dissolution of iron oxide and outgassing of CH₄ and CO₂.”</i>
2	Revesz et al. 1995	<i>“...gas exsolution is removing dissolved CH₄ (along with other dissolved gases) from the ground water.” “Calculations of C (carbon) fluxes in the saturated and unsaturated zones originating from the degradation of the oil plume lead to a minimum estimated life expectancy of 110 years.”</i>
3	Suarez and Rifai 1999	<i>“Toluene exhibited a median (degradation rate) of 0.03 day⁻¹, which is the highest anaerobic value among the BTEX compounds.” “Toluene “almost always” degraded under methanogenic conditions.”</i>
4	Cozzarelli et al. 2001	<i>“The spreading of the dissolved methane and iron plumes between 1993 and 1997 indicate that iron reduction and methanogenesis continue to be important processes in the aquifer close to the oil body, consistent with the previous observations of Baedecker et al. (1993, 1996).”</i>
5	Salanitro 2001	<i>Many alkanes, monoaromatics are biodegradable; some isoalkanes, polyaromatics, and hetrocyclic compounds are biodegradable; and few cycloalkanes are biodegradable. B, T, and X are degraded in methanogenic cultures via benzoate as an intermediate.</i>
6	Chaplin et al. 2002	<i>“Reaction stoichiometry was assumed in converting O₂ and CO₂ gas-flux estimates to rates of aerobic biodegradation and CH₄ gas-flux estimates to rates of methanogenesis.” “It appears that volatilization is the primary mechanism for attenuation in early stages of plume evolution, while biodegradation dominates in later stages.” Rates of hydrocarbon biodegradation above the center of the floating oil were relatively stable from 1985 to 1997, as the primary metabolic pathway shifted from aerobic to methanogenic biodegradation.</i>
7	Warren et al. 2004	<i>“In laboratory toxicity assay, crude oil collected from the site inhibited methane production from acetate but not from formate or hydrogen.” “The ratio of acetate to DOC in a plume may provide an in-situ measure of the cumulative effect of these mixtures of toxic contaminants on methanogenic degradation.”</i>
8	Amos et al. 2005; Amos and Mayer 2006	<i>“If degassing is neglected, and historical rates of methanogenesis are determined based only on measured (dissolved phase) CH₄ concentrations, both methanogenesis and contaminant degradation is underpredicted. Underprediction may be even more pronounced if ebullition of gas bubbles is occurring.” “...the process of bubble formation and partitioning of gases into the bubble as described in this paper is referred to as ‘degassing.’ This is distinguished from the term ‘ebullition’ which refers to the transport of gas bubbles to the unsaturated zone”</i>
9	Bekins et al. 2005	<i>“...methanogenic conditions prevail over all but the top 2 m (6.6ft) of this (oil body) area. Substantial degradation of the n-alkane fraction has occurred under methanogenic conditions. The methanogenic degradation of n-alkanes first depletes the C₁₈ and higher fraction, which is the reverse of the aerobic degradation progression.” “It has only recently been established in the laboratory that n-alkanes biodegrade under methanogenic conditions (Zengler et al. 1999; Anderson and Lovley 2000). We have documented that this process can occur rapidly in the subsurface.” “The most likely explanation for the variation in degradation rates is recharge facilitated transport of microbial growth nutrients from the land surface”</i>
10	Johnson et al. 2006; Lundegard and Johnson 2006	<i>“...the mass loss rate per unit surface area for those (vadose zone gas) processes varies from approximately 0.1 to 1.0kg total petroleum hydrocarbons per m² /year. In comparison, mass losses from the submerged part of the source zone and involving ground water transport processes (i.e., dissolution and biodegradation) were estimated to be about approximately 2 orders of magnitude lower.”</i>
11	Molins et al. 2010	<i>“Simulation results confirm that as of 2007, the main degradation pathway can be attributed to methanogenic degradation of organic compounds in the smear zone and the vadose zone resulting in a contaminant plume dominated by high CH₄ concentrations.”</i>
12	Amos et al. 2011	<i>“Near the source zone, direct-push sampling revealed a sharp aerobic–anaerobic transition at the water table where O₂ concentrations decrease from near atmospheric above the water table to non-detect below the water table and CH₄ concentrations show the opposite trend.” “Model calibration strongly suggests that transfer of biogenically generated gases from the smear zone provides a major control on vadose zone gas distributions and vadose zone carbon balance.”</i>
13	Sihota et al. 2011; Sihota and Mayer 2012	<i>“Results confirm that, in the source zone at the Bemidji site, the majority of CO₂ originates from degradation of the oil body. In addition, radiocarbon in CO₂ proves particularly useful in determining the contribution of contaminant degradation to the measured CO₂ efflux.”</i>

Table 1 (Continued)

No.	Reference	Key Excerpts
14	Baedecker et al. 2011	“Over a 21-year period oil at the Bemidji site, the n-alkanes (C ₆ –C ₁₂), toluene, benzene, and o-xylene were significantly depleted, and the remaining hydrocarbons were slightly depleted or, in some cases, showed no depletion.” “These results have implications for modeling of contaminant plumes because the LNAPL source composition changes and is not uniform within an oil pool over time.”
15	Essaid et al. 2011	“Studies at Bemidji were among the first to document the importance of anaerobic biodegradation processes for hydrocarbon removal and remediation by natural attenuation.” “Model results also have also shown that compounds with high effective solubilities (such as benzene) and/or large biodegradation rates (such as toluene) were depleted in the oil body more than other hydrocarbon compounds.” “The degree of depletion of the insoluble alkane fraction in the oil body (degradation state) did not depend on oil saturation, indicating that it was not caused by dissolution but instead was a result of methanogenic oil biodegradation (Bekins et al. 2005).” “Vapor-phase data indicate that methanogenic biodegradation was occurring in the oil body by 1987 and is the dominant degradation process today” “Geochemical mass-balance modeling (Baedecker et al. 1993) supported the hypothesis of anaerobic biodegradation of hydrocarbons in conjunction with dissolution of manganese and iron oxides, and outgassing of CH ₄ and CO ₂ .”
16	Ng et al. 2014, 2015	“The results show that oil constituents other than BTEX (benzene, toluene, ethylbenzene, o-, m- and p-xylenes), including n-alkanes and other aromatic compounds, play significant roles in plume evolution and secondary water quality impacts” “Model results demonstrate that most of the carbon loss from the oil (70%) occurs through direct outgassing from the oil source zone, greatly limiting the amount of CH ₄ cycled down-gradient” “Our proposed conceptual model includes direct outgassing of CO ₂ and CH ₄ from the oil body source zone in order to match surface efflux measurements from Sihota (2014).” “We attribute 60% of the surface efflux to the modeled oil body at and below the water table.”
17	McCoy et al. 2015	“Results from the deployment of 23 CO ₂ traps at a former refinery indicate natural loss rates of LNAPL (measured in the fall, likely concurrent with high soil temperatures and consequently high degradation rates) ranging from 13,400 to 130,000 liters per hectare per year (L/Ha/year).”
18	Zeman et al. 2014	Collectively, results demonstrated that anaerobic biodegradation processes can be enhanced by increasing the temperature of LNAPL-containing soils, but biodegradation does not simply increase as temperature increases likely due to a lack of microorganisms that thrive at temperatures well above the historical high temperatures for a site. Rather, optimal degradation is achieved by holding soils at the high end of, or slightly higher than, their natural range.
19	Irianni-Renno et al. 2016	“Four distinct zones were identified based on microbial community structure and geochemical data: (i) an aerobic, low-contaminant mass zone at the top of the vadose zone; (ii) a moderate to high-contaminant mass, low-oxygen to anaerobic transition zone in the middle of the vadose zone; (iii) an anaerobic, high-contaminant mass zone spanning the bottom of the vadose zone and saturated zone; and (iv) an anaerobic, low-contaminant mass zone below the LNAPL body.”
20	Palaia 2016	“Rates were generally observed within an order-of-magnitude ranging between 300 to 5600 gal/ac/yr. The lowest rate was measured at a site in Alberta with natural gas liquid LNAPL in sedimentary bedrock. The highest rate was measured at a semi-arid site in Colorado with weathered diesel in a sand and gravel formation. The median, sitewide-averaged NSZD rate for the 11 sites in the data set was estimated at 700 gal/ac/yr.”
21	Sihota et al. 2016	“Overall, results indicated that source zone natural attenuation (SZNA) rates and gas transport processes varied seasonally and that the average annual SZNA rate estimated from periodic surface efflux measurements is 60% lower than rates determined from measurements during the summer.”
22	Bekins et al. 2016	“Two groundwater plumes in north central Minnesota with residual crude oil sources have 20 to 50 mg/L of nonvolatile dissolved organic carbon (NVDOC). These values are over 10 times higher than benzene and two to three times higher than Diesel Range Organics in the same wells. On the basis of previous work, most of the NVDOC consists of partial transformation products from the crude oil.” “These plumes of metabolites are not covered by regulatory monitoring and reporting requirements in Minnesota and other states. Yet, a review of toxicology studies indicates that polar metabolites of crude oil may pose a risk to aquatic and mammalian species. Together the results suggest that at sites where residual sources are present, monitoring of NVDOC may be warranted to evaluate the fates of plumes of hydrocarbon transformation products.”

Note: Each number corresponds to the paper number in Figure 1.

cooperatively; one group degrades the byproducts of another group and ensures that the overall reaction is thermodynamically favorable. In anaerobic LNAPL zones, fermenters biodegrade hydrocarbons and form dissolved hydrogen and/or acetate; and then methanogens utilize the

hydrogen and/or acetate to form methane. (For a comprehensive overview of these reactions see Gieg et al. 2014.)

- While the original conceptual model in references such as Wiedemeier et al. (1999) presented a sequenced or zoned relationship between different reactions where all

Table 2

Paradigm Shift from Monitored Natural Attenuation to Natural Source Zone Depletion

	Hydrocarbon Attenuation in the 1990s–2000s	Hydrocarbon Attenuation Now
Nomenclature	Monitored natural attenuation (MNA) of dissolved plume	Natural source zone depletion (NSZD) of LNAPL body
Management focus	How far plume will migrate	How long will sources last
Key constituents	Dissolved BTEX ¹	All LNAPL constituents
Key biodegradation process	Electron acceptor mediated biodegradation	Methanogenesis
Key unsaturated zone biodegradation process	Volatilization of LNAPL followed by aerobic biodegradation of hydrocarbon vapors	Anaerobic biodegradation (methanogenesis) of LNAPL followed by aerobic methane oxidation
Key saturated zone biodegradation process	Anaerobic biodegradation of dissolved BTEX	Anaerobic biodegradation of LNAPL by methanogenesis with off-gassing and ebullition
Key metric	“Biodegradation capacity” (BIOSCREEN mass balance)	“NSZD rate”
Key measurement	Upgradient vs. downgradient electron acceptors and byproducts	Carbon dioxide efflux; gradient of oxygen consumption in unsaturated zone; thermal flux
Representative attenuation rates	BTEX half-life of 2 to 4 years	NSZD rate of 100s to 1000s gallons/acre/year

¹Benzene, Toluene, Ethylbenzene, and the Xylenes.

electron acceptors would need to be removed before methanogenesis would occur, field observations and microbial process research suggest that some syntrophic behavior and methane generation can occur in the presence of alternative electron acceptors under some circumstances (Gieg et al. 2014); see electron acceptor as a control discussion below.

- “*Direct Outgassing*” is production and release of methane and carbon dioxide from biodegradation of oil that occurs within the pore space adjacent to the entrapped oil (Ng et al. 2015). “Direct outgassing” differs from “degassing” described by Amos et al. (2005) in that the dissolution of biogenic gases is not included as an intermediate step in this conceptualization. This can occur in both the unsaturated zone and saturated zone. They noted that Meckenstock et al. (2014) supported their concept by observing high rates of anaerobic biodegradation within

oil droplets that contain tiny water droplets without any transport from oil to the bulk groundwater phase. Atekwana and Atekwana (2010) discuss how LNAPL biodegradation at the pore level can produce biogenic gases that can change the geophysical properties of an aquifer, for example, changes in bulk electrical conductivity, GPR velocity, and acoustic wave amplitude can be used as indicators of bioactivity at LNAPL-contaminated sites.

- “*Ebullition*” is the transport of gas bubbles generated in the saturated to the unsaturated zone (Amos et al. 2005; Amos and Mayer, 2006). Because almost all significant LNAPL releases in the saturated zone turn methanogenic, and biodegradation represented by outgassing is much larger than the assimilative capacity of groundwater to transport or degrade dissolved methane (e.g., see Ng et al. 2015), our belief is that ebullition is likely common at sites with LNAPL present in the saturated zone.
- Aerobic degradation of hydrocarbons in the vadose zone may be important at new sites, particularly sites with recent releases, but the body of evidence suggests that older releases have anaerobic cores around vadose zone LNAPL. At the well-studied Bemidji site, Essaid et al. (2011) concluded “*Thus, the unsaturated zone vapor plume mirrored the saturated zone groundwater plume, suggesting a similar core of anaerobic degradation near the NAPL oil source*” and Amos et al. 2005 concluded the influence of hydrocarbon vapors on total vapor pressure was “insignificant” and that the main sink of oxygen in the unsaturated zone was microbial oxidation of methane. At the California oil field site, Lundegard and Johnson concluded that the upward diffusion rate of methane and degradation rate inferred from the oxygen gradient were in close agreement. Finally, Irianni-Renno et al. (2016) concluded “*aerobic processes likely only played a major role above 1.4 m bgs,*” which was above almost all of the high-concentration contaminant mass at the site.

NSZD Conceptual Model

A NSZD conceptual model, based on Irianni-Renno et al. (2016), and the combined body of work referenced in Table 1 and Figure 1, is shown in Figure 2. Three distinct zones are defined here for their key role in the overall NSZD process. Our conceptual model is illustrative and reflects only one-dimensional flux of gases; there can be scenarios where heterogeneity can result in lateral movement of gases.

Methane Generation Zone

Significant methanogenesis occurs in the saturated zone, which results in direct outgassing and ebullition of methane and carbon dioxide (Figure 2). Direct outgassing of LNAPL also occurs in the bottom of the unsaturated zone. The combined gas fluxes migrate vertically upwards. For simplicity, the LNAPL smear zone and the capillary fringe are not explicitly shown in the processes depicted in the conceptual model.

The fluxes moving through an *anaerobic transport zone* may also include VOCs, especially if the source contains fuels with lighter-end constituents (e.g., recent release of

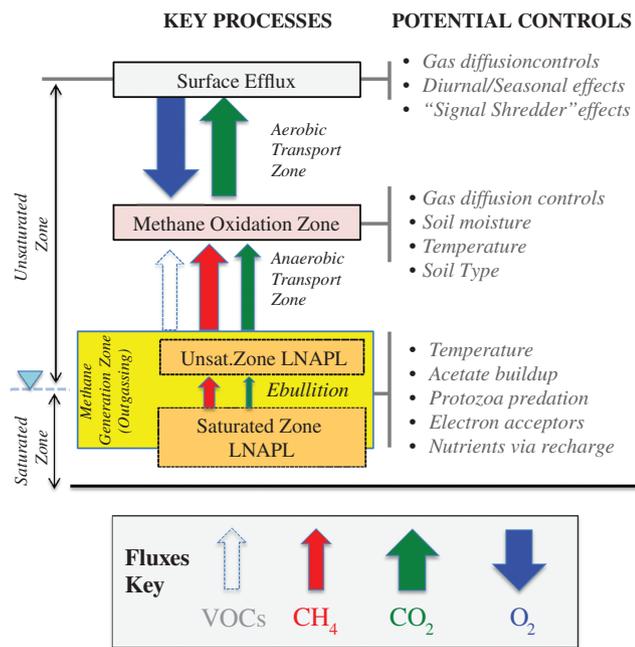


Figure 2. Conceptual model of NSZD processes, gas fluxes, and controls in an LNAPL source zone. Adapted from multiple references shown in Table 1 and Figures 1 and 3. For simplicity, the LNAPL smear zone and the capillary fringe are not explicitly shown in the processes depicted in the conceptual model.

gasoline). As NSZD is typically applied to sites that are at a later stage of their lifecycle, direct volatilization of VOCs may be a minor process at many sites and therefore VOC flux is shown as a dashed arrow in Figure 2.

Methane Oxidation Zone

Methane and carbon dioxide migrate upward through the vadose zone to a relatively thin reaction zone where most or all of the methane and VOCs (if present) are oxidized to carbon dioxide. The importance of oxidation reactions in the vadose zone has been recognized for some time by the vapor intrusion literature to explain VOC attenuation in the vadose zone (e.g., DeVaul et al. 1997; Hers et al. 2000).

Aerobic Transport Zone

Oxygen diffuses toward the methane oxidation zone, and the produced carbon dioxide diffuses upward through the unsaturated zone and can be measured as a surface efflux of carbon dioxide.

The thickness of the aerobic transport zone (or the depth to methane oxidation zone) will depend on the flux of methane and VOCs emanating from the methane generation zone relative to the flux of oxygen into the subsurface (Roggemans et al. 2001). Where methane and VOC flux is relatively low, the aerobic transport zone can extend to the unsaturated zone LNAPL allowing aerobic degradation in this zone. If the shallow vadose zone is also contaminated (e.g., near the point of release), then aerobic biodegradation of the shallow impacts can be a sink for the oxygen and limit its amount diffusing to the methane oxidation zone. If these impacts utilize more oxygen than that necessary to fully oxidize the methane from the methane production

zone, methane efflux could occur. As this process is relevant only when shallow contamination exists, it is not shown in Figure 2.

Figure 2 also lists potential controls that may be affecting NSZD processes in each of the above zones. These controls are discussed later in the paper.

NSZD Measurement and Observed Rates

A variety of methods have been used to measure the carbon dioxide efflux and thereby obtain NSZD rates (e.g., see papers 10, 13, 17, 20, 21 in Table 1). American Petroleum Institute (API) (2017) presents a detailed discussion of the various methods used for NSZD measurement. McCoy et al. (2015) proposed describing these rates in units of LNAPL volume degraded per large unit area per year, e.g., U.S. gallons/acre/year or liters/hectare/year (1 gallon/acre/year=9.4 liters/hectare/year) to facilitate comparisons of NSZD rate to depletions achieved with active remedies including hydraulic recovery of LNAPL. Table 3 summarizes rates from several published NSZD studies. While individual sampling locations show orders of magnitude temporal and spatial differences, the overall site-wide averages are much narrower, with the middle 50% of sites falling between 700 and 2800 gallons/acre/year for the 25 sites, giving a representative median NSZD rate of about 1700 gallons/acre/year. The reasons for this will be explored in a later section of this paper.

To support the contention that methanogenesis is a significant process at NSZD sites, the biodegradation capacity of the electron acceptors in the saturated zone at nine LNAPL sites, calculated using the mass balance methods in the BIOSCREEN model (Newell et al. 1996), and ignoring methanogenesis, is also shown in Table 3. The median value is only 9 gallons/acre/year, or about 0.5% of the typical 1700 gallons/acre/year measured by surface efflux and other NSZD methods (Table 4).

Critical Issues Needing Resolution

While there has been an intense ramp-up in the collection of NSZD rates at hydrocarbon sites (e.g., Table 3), there are still some gaps, and many practitioners and regulators are not sure how to utilize the rates from a site-management perspective, for example:

1. Why is there significant variability in NSZD measurements?
2. What are the differences between vadose, saturated and smear zone NSZD rates, and processes?
3. Does the NSZD rate change over time? How does LNAPL composition change over long time periods as NSZD progresses?
4. Is NSZD measuring the biodegradation of only the soluble compounds, for example, BTEX?
5. What are the rate-limiting factors in NSZD, and how can NSZD rates be enhanced?
6. When will the groundwater and soil meet respective chemical-specific target concentrations?

To answer some of these questions, we conducted a literature review and attempt to integrate several threads of

Table 3
Examples of Site-Wide Average NSZD Rate Measurements at Field Sites

NSZD Study	Number of Sites	Site-Wide NSZD Rate (All Sites)	Site-Wide NSZD Rate (Middle 50%)	Reference
		(Gallons/Acre/Year)		
Refinery terminal sites	6	2100–7700	2400–3700	McCoy 2012
1979 crude oil spill	1	1600	—	Sihota et al. 2011
Seasonal range		310–1100	—	Sihota et al. 2016
Refinery/terminal sites	2	1100–1700	1250–1550	Workgroup, L.A. LNAPL 2015
Fuel/diesel/gasoline	5	300–3100	1050–2700	Piontek et al. 2014
Diverse petroleum sites	11	300–5600	600–800	Palaia 2016
All studies	25	300–7700	700–2800	
Saturated zone electron acceptor biodegradation capacity	9	0.4–53	1.7–19	This paper (see Appendix S1)

Notes: Middle 50% column shows the 25th and 75th percentile values. To demonstrate the significance of methanogenesis, NSZD rates calculated from the biodegradation capacity of electron acceptors in the saturated zone, ignoring methanogenesis, are shown in the last row.

Table 4
Representative Degradation Rates from Different Methanogenic Systems

Methanogenic System	Equivalent LNAPL Degradation Rate (Gal/Acre/Year)	Original Measurement	Reference
Anaerobic digesters	500,000	Methane generation ¹	Gerardi 2003
Ethanol release sites	20,000	Carbon dioxide, methane efflux ¹	Sihota et al. 2013
Landfills	10,000	Methane generation ^{1,2}	Spokas et al. 2006
NSZD at LNAPL sites	2000 ³	Carbon dioxide efflux	Table 3 ³
Wetlands	200	Methane flux to atmosphere ¹	Le Mer and Roger 2001
Peat	4	Methane ebullition ¹	Stamp et al. 2013

Note: All values rounded to one significant figure.

¹Adjusted to account for CO₂ production (see Appendix S1).

²Adjusted to account for oxidized methane in landfill covers (see Appendix S1).

³Representative median NSZD rate of about 1700 gallons/acre/year rounded to one significant figure.

research and engineering practice into a unified framework for understanding and implementing NSZD at LNAPL sites.

Other Methanogenic Systems

With NSZD research pointing to methanogenesis as a key process for bulk attenuation of LNAPL bodies, there is a benefit to reviewing other methanogenic systems in nature and in other fields to compare rates and determine if any lessons can be learned from these fields. Key information

about these related systems is highlighted in Figure 3, and general observations are discussed below.

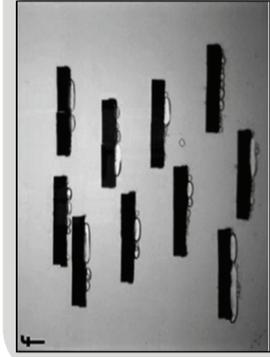
Comparison of Representative Degradation Rates

An approximate comparison of representative anaerobic biodegradation rates for several of these systems was performed by converting the representative methane and/or carbon dioxide flux from each system to an equivalent volume of LNAPL biodegraded per area per time. Table 4 shows the results, rounded to one significant figure, for anaerobic digesters, landfills, peatland, and wetlands. Note that this analysis is approximate, as it requires making assumptions about the volumes of the different reactors (e.g., the volume of water in a typical LNAPL source zone and the typical thickness of a municipal landfill) and assumptions about converting the particular measurement to an equivalent LNAPL degradation rate (see Appendix S1 for details about the conversions from the original data to the values in Table 4). Sihota et al. (2013) also developed a variation of the comparison shown in Table 4 with fluxes of carbon dioxide and methane from several natural and anthropogenic systems. With these caveats, the following observations are made from Table 4:

Anaerobic digesters have extremely high methanogenic degradation rates (Agency for Toxic Substances and Disease Registry (ATSDR) 2001; Gerardi 2003) with an equivalent LNAPL degradation rate on the order of one-half million gallons/acre/year. This value reflects the likely upper limit of methanogenic processes due to the carefully engineered and controlled process within the anaerobic digesters (i.e., temperature, volatile fatty acids, and pH are constantly controlled so there is limited buildup of intermediates such as acetate or hydrogen), the reactors being carefully shaped to reduce dead zones, and the configuration that allows the entire volume to be available for reaction (porosity of close to 100% compared with porosity of porous media reactors like soil of ~30%).

Municipal landfills generate methane over a wide range of methane emission rates; for this paper, a representative

Methane Ebullition in Wetlands and Peat
 In wetlands and peat, anaerobic conditions lead to the generation of methane in the subsurface that comes out of solution via the process of ebullition (Rosenberry et al., 2006). Ebullition is the movement of methane gas bubbles or gas channels by buoyancy forces. In one study of peat systems, the ebullition process was marked by slow accumulation of gas with the subsurface and then sudden release to the surface, making the ebullition process a "signal shredder" that obscured the actual methane production rate in the subsurface. (Ramirez et al., 2015a, 2015b, 2016; figure from 2015a).



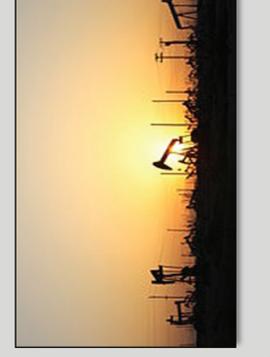
Methane Generation in Wetlands
 Organic matter is anaerobically reduced to methane in saturated wetland and rice paddy soils. Though much of this methane is oxidized before entering the atmosphere, these wetland emissions are the largest source (20-30%) of atmospheric methane. Methane production rates in wetlands are controlled largely by the availability of an organic matter substrate, temperature, and the absence of electron acceptors such as nitrate. Wetlands methane emissions are commonly on the scale of 10^3 mg/m²-sec. (Walter and Heimann, 2000, Le Mer and Roger, 2001).
 Photo: Davaalgn)



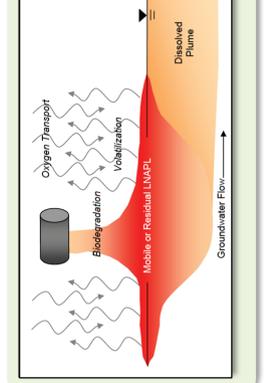
Methane Oxidation in Natural Soils
 Methane oxidation by aerobic methanotrophs is a ubiquitous phenomenon occurring in natural soils, especially wetlands and peatlands. There is broad research interest in methane oxidation not only to the end of better understanding those systems, but also to better understand the role that methane oxidation plays in the global carbon cycle, since methane is a greenhouse gas. The figure to the left shows a methane release/oxidation field experiment (de Sleyes et al., 2016). (Photo: D. Mackay)



Biodegradation in Petroleum Reservoirs
 Biodegradation in petroleum reservoirs is a negative process, as it converts light oil to heavy oil which is more difficult and costly to remove. Researchers have identified that most of the degradation occurs under methanogenic conditions, with preferential removal of n-alkanes. In many systems, fermentation of the substrate forms both dissolved hydrogen and acetate, which are then used by methanogens to produce methane. In crude oil reservoirs, the toxicity effects of oil inhibit the utilization of acetate, so that most of the degradation is by hydrogen-utilizing methanogens (Jones et al., 2008; Gleg et al., 2014; Jimenez et al., 2016). (Photo: A. Huckelheim)

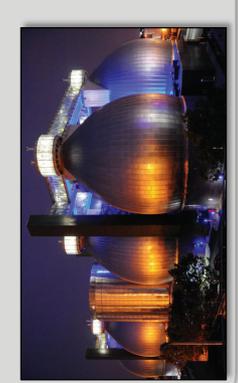


Natural Source Zone Depletion (NSZD)
 Once thought to be sterile or dominated by aerobic reactions, anaerobic biodegradation, in particular methanogenesis, is now known to be the dominant attenuation pathway at mature hydrocarbon release sites in the saturated zone, smear zone and typically the vadose zone.
 Representative rates from NSZD studies are ~6600 liters/hectare/year (~1700 gallons/acre/year; see Table 4). (Graphic: ITRC, 2009)



SYSTEMS WITH RELATED PROCESS KNOWLEDGE

Anaerobic Digesters
 A highly controlled anaerobic process that is used to treat sludge from municipal waste water treatment. Key process variables include pH, volatile fatty acids, residence time, and temperature (typical temperatures are maintained at about 30-35°C) (Gerardi, 2003). Degradation rate in digesters is hundreds of times the NSZD rate at hydrocarbon source zones (Table 4). (Photo: NY D. Env. Prot.)



Methane Generation in Landfills
 Municipal waste is converted to methane via a relatively slow, extended subsurface anaerobic degradation process. After anaerobic conditions are established, methane is emitted at a relatively stable rate for ~20 years and continues to be produced for ~50 years or longer. Controlling variables include the fraction of organics in the waste; waste age; presence of oxygen, moisture content (more moisture promotes more gas production), and temperature (higher temperatures lead to more gas) (ATSDR, 2001).



Methane Oxidation in Landfill Cover Soils
 In landfills without methane capture, methane leaves the waste and migrates to the surface via advection and diffusion. In the cover soils, aerobic bacteria (methanotrophs) consume some of the methane and convert it to carbon dioxide. For climate change studies, it is often assumed that less than 10% of the methane is oxidized; other studies recommend using 22% for clay soils and 55% for sandy soils (Chanton et al., 2009). Moisture content and temperature are two key factors controlling the efficiency of methanotrophs in converting methane to CO₂ before it reaches the atmosphere (Del Grosso et al., 2000; Spokas and Bogner, 2011) (Figure: Cheimchaisri et al., 2012; Tantachoon et al., 2008)

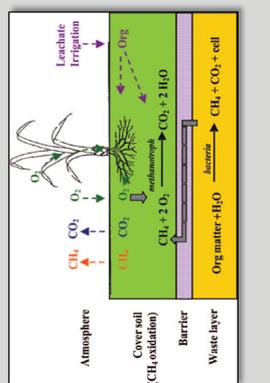


Figure 3. Methanogenic systems with related process knowledge.

rate equivalent to an LNAPL degradation rate of 10,000 gallons/acre/year was estimated (see Appendix S1). Methane generation typical peaks after 5 to 7 years after the wastes are emplaced (ATSDR 2001), but unlike anaerobic digesters, rates decline over time likely due to the fact that the waste source is not replenished and because of mass transfer constraints caused by lack of mixing.

The representative anaerobic rates found in natural porous media systems in the subsurface (hydrocarbon NSZD, wetlands, and peatland) are between 4 and 2000 equivalent gallons/acre/year. These rates are orders of magnitude lower than anaerobic digesters and landfills, suggesting some common controlling process(es) may be acting on all these natural porous media systems. One exception is spills of denatured fuel ethanol (e.g., Spalding et al. 2011; Sihota et al. 2013), which have higher NSZD rates likely because of the high biodegradability of ethanol.

Potential Controlling Factors

Various controlling factors can play a role in each of the different zones identified earlier in the conceptual model (Figure 2). Some of these factors directly affect the rate of methanogenesis, while others may only influence its measurement. For some of the potential controls, the scientific evidence is limited or contradictory.

Potential Controlling Factors in Methane Generating Zone

In the methane generation zone, the methanogenic processes may be controlled by:

Temperature

It is well-known via the Arrhenius equation and operational experience in landfills and anaerobic digesters that methanogenic processes are sensitive to temperature, with maximum rates in the 35 ° to 40 ° C range (e.g., ATSDR 2001; Gerardi 2003). Kulkarni et al. (2017) performed a large data mining study of over 2000 hydrocarbon sites and found that source attenuation rates for benzene and toluene showed a statistically significant correlation to temperature, with an increase in the rate observed for an increase in temperature. However, the first direct study of temperature effects on LNAPL attenuation by Zeman et al. (2014) provided a more detailed conceptual model where they observe that increased temperatures can cause shifts in microbial communities and, correspondingly, higher rates of biogas production, in excess of what is anticipated by the Arrhenius equation.

Acetate Buildup

Acetate-utilizing methanogens are inhibited in crude oil systems, suggesting that hydrogenotrophic methanogenesis, that is, hydrogen oxidization coupled with CO₂ reduction, is the dominant pathway for the methane being generated and that the buildup of acetate may reduce overall hydrocarbon degradation rates (e.g., Warren et al. 2004; Jones et al. 2008; Ma et al. 2015; Stasik et al. 2015). Using data from a crude oil release site, Warren et al. (2004) suggested that the acetate-to-dissolved organic carbon ratio could be a use-

ful metric to evaluate the effect of this acetate-utilization inhibition. One study of a release of ethanol-blended gasoline suggested that acetate concentrations above 64 mg/L might inhibit benzene degradation by decreasing the thermodynamic feasibility of individual reactions (Corseuil et al. 2011). Wilson et al. (2016) used microcosm data and a thermodynamic analysis to conclude that biodegradation of ethanol in fuels would generate hydrogen, acetate, and dissolved organic carbon in high enough concentrations to inhibit anaerobic biodegradation of benzene and toluene. While Dolfing et al. (2007) highlighted that the acetoclastic pathway can be thermodynamically favored in many conditions, biological factors such as inhibition may dictate the actual methanogenic degradation pathway during crude oil degradation rather than thermodynamics. Finally, one study of methanogenesis at a refined product (gas condensate) site indicated that acetate production and oxidation was the primary methanogenic pathway compared with hydrogenotrophic methanogenesis (Struchtemeyer et al. 2005), suggesting that crude oil sites may have different methanogenic pathways than refined product sites.

Alkalinity and pH

A properly operating anaerobic digester maintains pH between about 6.8 and 7.2. The pH is in part controlled by the carbon dioxide content of the biogas that leaves the system (Gerardi 2003) and in part by acetate, propionate, formate, and lactate that are produced during the fermentation stage of methanogenesis. Ripley et al. (1986) developed the concept of comparing intermediate alkalinity (IA) (titration from pH 5.75 to pH of 4.3, a proxy for bicarbonate alkalinity) vs. partial alkalinity (PA) (titration from original pH to pH of 5.75, a proxy for volatile acid alkalinity) to assess the health of an anaerobic digester. They proposed that the ratio IA:PA should be kept below 0.3 for successful anaerobic digestion.

Protozoa Predation

Wilson et al. (2016) suggested that predation may control the hydrocarbon-consuming biomass and be the ultimate control for methanogenesis. Sinclair et al. (1993) measured protozoa at a fuel release site and concluded “*The abundance of protozoa in the biotreatment area was high enough that it would be expected to significantly reduce the bacterial community that was degrading the fuel.*” Another paper observed: “*The co-occurrence of bacteria and protozoa in association with high concentrations of monoaromatic hydrocarbons suggests the involvement of trophic interactions in the process of biodegradation*” (Zarda et al. 1998).

Electron Acceptors

The presence of oxygen at even low concentrations is toxic to methanogens, and the presence of other electron acceptors (manganese, nitrate, ferric iron, and sulfate) serves as an alternative control to the biodegradation pathway (Wiedemeier et al. 1999). They compete for dissolved hydrogen and acetate produced during the fermentation step in the syntrophic biodegradation reactions. But recent research indi-

cates that syntrophic metabolism and methanogenesis can occur in more diverse geochemical environments than first thought. In their opinion article, Gieg et al. (2014) observe that syntrophic metabolism can sometimes occur even in the presence of electron acceptors, while Irianni-Renno et al. (2016) describes concurrent sulfate reduction and methane production in the same zone based on data from multiple-level sampling systems in an LNAPL area.

Volatile Hydrocarbons

Sherry et al. (2014) presented the results of laboratory experiments that suggested that volatile hydrocarbons (nC5–nC10, methylcyclohexane, benzene, toluene, and xylenes) can inhibit, but not stop, methanogenic alkane biodegradation. Weathered crude oil had about twice the methanogenic rate for alkanes than nonweathered oil. As many NSZD sites are likely to be late in their lifecycle and contain weathered LNAPL, inhibition by volatile hydrocarbons may be of less significance.

Nutrients

At the Bemidji site, the amount of weathering of the crude oil was correlated spatially to zones with higher vs. lower recharge from the surface (Bekins et al. 2005). Therefore, this research team hypothesized that the transport of microbial growth nutrients, most likely phosphorus, was a potential factor affecting biodegradation rates of the crude oil. Gray et al. (2010) also hypothesized that adding inorganic nutrients to oil reservoirs could stimulate methanogenesis and convert liquid hydrocarbon to natural gas.

Water Table Fluctuations

Some researchers have posited that degradation in LNAPL near and above the water table is controlled by raising and lowering water tables that exposes the LNAPL to oxygen (e.g., Suthersan et al. 2015), but the lack of oxygen below the methane oxidation zone and above the LNAPL source zone might preclude this process at many sites. An alternate mechanism is fluctuating water tables might flush accumulated reaction byproducts out of the LNAPL zone and/or introduce micronutrients such as some metals (Nyer 2001).

Potential Controlling Factors in Methane Oxidation Zone

The aerobic oxidation of the methane is likely controlled by these factors:

Soil Moisture and Soil Type

Soil moisture is reported to be a key factor that controls methane oxidation rates in most soils (Del Grosso et al. 2000). In dry soils, the lack of moisture limits microbial activity, but in very moist soils, water is a barrier to gas diffusivity and prevents gaseous oxygen from reaching methane (Le Mer and Roger 2001). Increased methane oxidation has been observed in desert soils after rainfall events (McLain and Martens 2004). Del Grosso et al. (2000) found that methane oxidation rates were at a maximum when volumetric moisture content was between 8% and 10% for coarse grained soils, and between 10% and 15% for fine-

grained soils. Spokas and Bogner (2011) developed predictive equations for methane oxidation in earthen landfill covers based on soil moisture and temperature, and determined “*the wilting point is the lower moisture threshold for CH₄ oxidation activity and optimum moisture potential is close to field capacity.*” Overall, more methane oxidation is observed in coarse-grained soils than fine-grained soils (Spokas and Bogner 2011).

Temperature

In general, methane oxidation has been found to increase with temperature (Del Grosso et al. 2000; Luo et al. 2013) though it may decrease at temperatures beyond a threshold of 30 °C (Spokas and Bogner 2011). Some have reported this relationship to be negligible (Bender and Conrad 1995; Borken et al. 2006), compared to the effect of water moisture and diffusivity (Del Grosso et al. 2000). At sites with particularly high methane concentrations, such as composting heaps and landfill cover soils, this temperature effect has been found to be more pronounced (Kallistova et al. 2005; Serrano-Silva et al. 2014).

Potential Controlling Factors for Surface Efflux

The following are factors that may be occurring in any of the zones shown in Figure 2, but overall may control the surface efflux of carbon dioxide and in some cases, methane:

Gas Diffusion Controls

The diffusion rate of carbon dioxide and methane toward the surface and oxygen into the subsurface is controlled by their vertical concentration gradients and more importantly, the effective diffusion coefficients, which in turn are very sensitive to the soil moisture content. At heterogeneous sites, the effective diffusion coefficients can vary by several orders of magnitude making this a difficult parameter to estimate for quantitative calculations of NSZD rates by measuring the oxygen gradient (referred to as the gradient method for measuring NSZD) (Johnson et al. 2006). Using the effective diffusion and stoichiometric relationships from Eqn. 3b in ITRC (2009), and assuming atmospheric oxygen at the surface and no oxygen at a prescribed depth, the NSZD rate was calculated as a function of water saturation in the aerobic zone for four aerobic-zone thicknesses (1, 5, 10, and 20 m). Typical water saturations for soils ranging from sand to sandy clay were used to bracket the calculation. Results plotted in Figure 4 indicate that if the oxygen diffusion rate into the subsurface is constrained by the water saturation, the resulting range of expected NSZD values (20 to 10,000 gallons/acre/year) is close to the range of site-wide NSZD measurements shown in Table 3 (300 to 7700 gallons/acre/year). At sites with significant heterogeneity and fine-grained layers, the conceptual model can be complex due to complicated distributions of moisture content and, correspondingly, effective diffusion coefficients and resulting gas transport.

Diurnal/Seasonal Effects

Carbon dioxide efflux has been observed to vary both diurnally and seasonally, potentially due to temperature

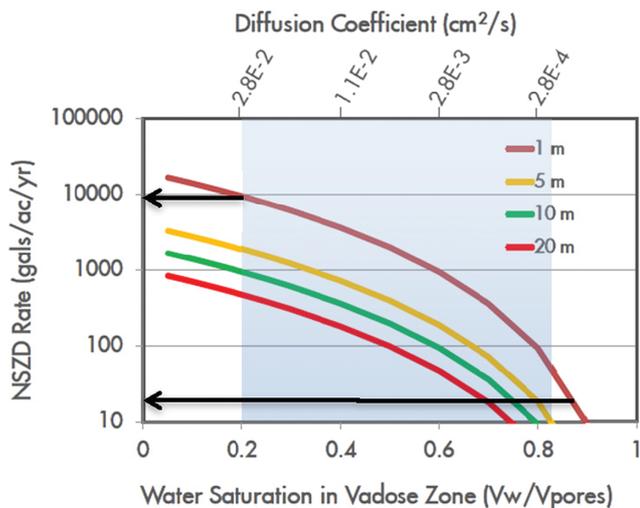


Figure 4. Maximum NSZD rate that can be measured without oxygen diffusion limitation, as function of soil moisture. Note that typical water saturations for seven soil types ranging from sand to sandy clay are shown in light blue (0.2 to 0.82), and the resulting maximum NSZD rate shown by the black arrows (ranging from 20 to 10,000 gallons/acre/year).

and soil moisture effects. Time series analysis showed that the temporal dynamics of CO₂ flux relationships at a primary forest and an active pasture site were closely related to air temperature (Davidson et al. 2000). Sihota et al. (2016) (Table 4) observed a strong seasonal component to NSZD rates at the Bemidji site: “Overall, results indicated that source zone natural attenuation (SZNA) rates and gas transport processes varied seasonally and that the average annual SZNA rate estimated from periodic surface efflux measurements is 60% lower than rates determined from measurements during the summer.” They observed a correlation between contaminant respiration and temperature with a time lag of 5 to 7 months between peak subsurface carbon dioxide concentrations and peak surface efflux, with periods of low carbon dioxide efflux corresponding to periods where frozen soils inhibited gas transport.

Meteorological Factors

Other factors that can affect surface efflux measurements include changing wind and barometric conditions during the measurement period (e.g., Takle et al. 2004; ITRC 2014). For example, Xu et al. (2014) observed a 35-fold variation in day-to-day methane emissions due to barometric pressure alone.

Signal Shredding

At NSZD sites, carbon efflux measurements exhibit significant spatial and temporal variability, sometimes an order of magnitude or more, as illustrated in Figure 5. A similar pattern has been observed in methane efflux from peatlands, giving rise to the term “Signal Shredding” where a presumably steady production signal in the peat is transformed into a highly variable efflux signal at the surface. Rosenberry et al. (2006) reported that “ebullitive gas releases are relatively frequent in some peatlands and

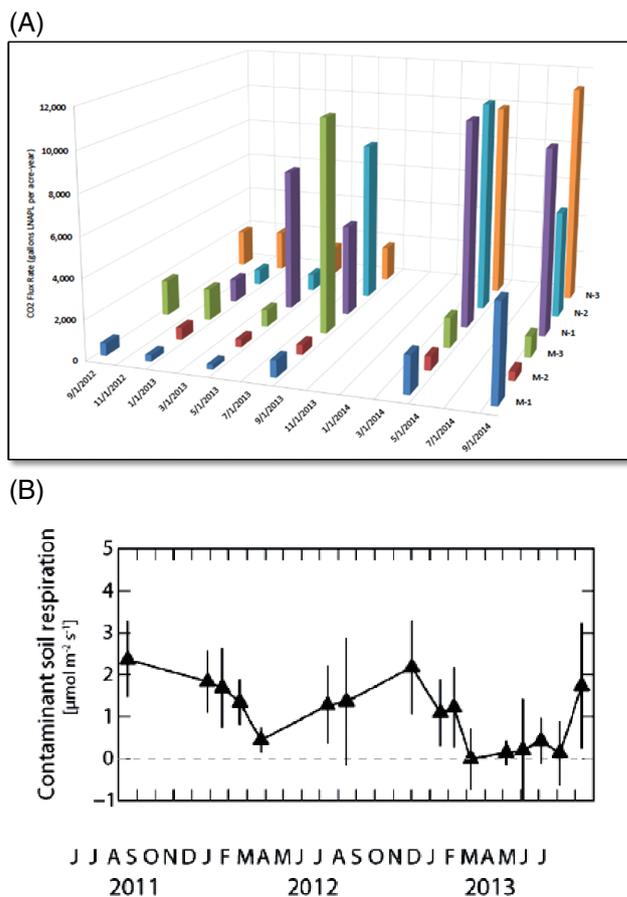


Figure 5. Variability in surface efflux measurements. Top panel: carbon dioxide flux (converted to gallons LNAPL/acre/year) vary both spatially (each row is a different sampling location) and temporally (each column is a different sampling data over a 2-year period. For example, Location M-3 (green bars) varies from 1000 gallons/acre/year to nearly 10,000 gallons/acre/year over the 2-year sampling period. In July 2013, carbon flux measurements varied by a similar amount. (Pontek et al. 2014). Bottom panel: site-wide contaminant respiration rates measured by a dynamic closed chamber with the vertical bars represent the 95 confidence limit of values used to construct site-wide averages; these side wide averages varied from ~0 to ~2.2 µmol/m²/s over the course of the 2-year study (Sihota et al. 2016).

time-averaged rates may be significantly greater than diffusive releases” and “episodic ebullition events occur when an accumulation of gas at some depth in the peat column is released suddenly to the peat surface.” These episodic ebullition events could not be explained by environmental factors such as temperature or barometric pressure. Ramirez et al. (2015a, 2015b) coined the term “signal shredder” and investigated how a relatively constant methane generation signal in the peat is “shredded” by episodic ebullition processes to form a widely varying signal over time and space at the surface. They developed MEGA (model of ebullition and gas storage), an enhanced avalanche-type computer model based on peat structure that could help explain the variable methane efflux signal commonly observed from peatlands. Although unconfirmed, we hypothesize that a similar accumulation/release process may be occurring at some sites and may be contributing to the observed spatial

and temporal variability in carbon efflux measurements at NSZD sites (see Figure 5).

Observations About Controls

Two observations can be made regarding the controls discussed above. First, if the methane production in the methane generation zone is greater than the methane oxidation in the methane oxidation zone, there will be methane efflux to the atmosphere. This occurs commonly in municipal landfills without methane capture systems, where some fraction of the methane generated is not oxidized (Spokas and Bogner 2011); but based on limited data, methane breakthrough to the surface appears to be less common at LNAPL sites.

Second, the controls provide insight into why NSZD rates fall within the range that is being observed at sites and also into ways to potentially enhance these rates. The controls in the methane generation zone affect the rate of NSZD itself, whereas the controls affecting the methane oxidation and aerobic transport zones only *directly* affect measurement of NSZD. If the rate of methane generation exceeds the capacity of methane oxidation, then methane will escape to the surface and the measured NSZD rate will be underestimated if methane emissions are incorrectly assumed to be zero. It is also hypothesized that methane oxidation may have an *indirect* control on methanogenic processes in the methane generation zone via heat transport, where heat generated from methane oxidation warms the methane generation zone and therefore potentially increases the rate of methanogenic NSZD processes.

LNAPL Composition Change During NSZD

NSZD is a bulk measurement of LNAPL loss rate and does not provide information on depletion of specific constituents. Hence, it does not fit neatly into common regulatory metrics, most of which are constituent based (e.g., maximum contamination levels (MCLs) and total petroleum hydrocarbon (TPH) classes). There is a rich body of scientific literature from NAPL forensics, MNA, and hydrocarbon biodegradation that show that biodegradation affects different hydrocarbon constituents in different ways. For example, in his review paper, Salanitro (2001) summarized the state of knowledge about biodegradation and composition change this way: “.....microbes (bacteria and fungi) isolated from soil, sediments, and biosolids can readily metabolize compounds of chain lengths up to C_{30} – C_{44} including *n*-alkanes, branched alkanes with few alkyl groups, and 1- to 3-ring alkylated or nonalkylated aromatics. In general, highly branched alkanes, cycloalkanes, 4- to 6-ring condensed aromatics, and alkylated thiophenes and dibenzothiophenes are partially metabolized or are completely recalcitrant.”

The field of environmental forensics relies heavily on the differences between the biodegradability of petroleum compounds, and looks for specific biomarker compounds that are more degradation resistant in the environment than most other compounds (Wang et al. 2006; Murphy and Morrison 2014). In some cases, forensic tools such as ratios of biodegradable vs. recalcitrant hydrocarbons are applied

(e.g., nC_{17} /pristane, and nC_{18} /phytane ratios) to determine the degree of weathering.

Other researchers have compiled biodegradation rate data from the literature, and show that both laboratory and field anaerobic biodegradation rates are different for the different BTEX compounds, with toluene having the fastest rate compared with BEX (e.g., Aronson and Howard 1997; Suarez and Rifai 1999; Bruce et al. 2010).

Several researchers working at the Bemidji Site have evaluated the change in composition of the crude oil released in 1979. Bekins et al. (2005) reported on the progression of methanogenic degradation as “*The methanogenic degradation of n-alkanes first depletes the C_{18} and higher fraction, which is the reverse of the aerobic degradation progression.*” Baedecker et al. (2011) quantified the percent removal of 25 different hydrocarbon compounds between 1979 and 2008. These data were then used by Ng et al. (2014, 2015) to develop a mass balance and a reactive transport model of the petroleum release undergoing NSZD in the saturated zone.

A data mining effort of the California Geotracker database performed by McHugh et al. (2014) that focused on benzene, MTBE, and TBA at approximately 4800 hydrocarbon sites in California was expanded to include toluene, ethylbenzene, and total xylenes (Figure 6). Source attenuation rates were calculated using the median of the 6-month maximum site concentrations vs. time as described in McHugh et al. (2014). As shown in the legend in Figure 6, toluene degraded significantly faster than BEX over the 10-year time period, while ethylbenzene appears to have a slightly slower biodegradation rate than benzene. Note these first-order attenuation rates are concentration vs. time attenuation rates that reflect source attenuation processes as described by periodic data from monitoring wells (Newell et al. 2002).

The mechanistic reasons for the differences in biodegradation rates between compounds is complex, and includes factors such as thermodynamics, how chemical structure makes it easier or more difficult for enzymes to break hydrocarbon bonds, and how easily different constituents are transported into the cell. Brown et al. (2017)

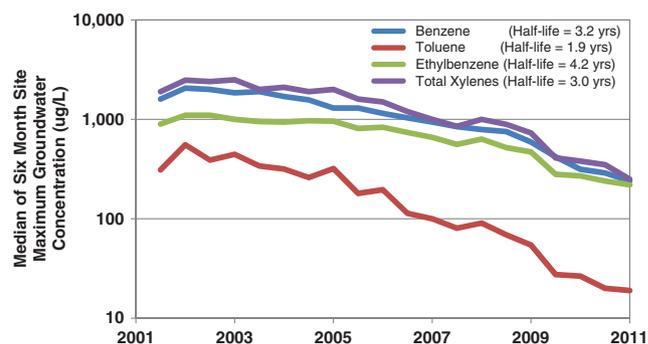


Figure 6. Median aqueous phase groundwater concentration vs. time graphs for BTEX compounds at 1130 California retail gasoline service station sites from 2001 to 2011. Degradation half-lives are shown in the legend. Toluene degraded significantly faster than BEX over the 10-year time period; ethylbenzene appears to have a slightly slower biodegradation rate than benzene or total xylenes.

Table 5**Key Concepts Used in Ng et al. (2014, 2015) Model of Bemidji Crude Oil Release with Example References**

Key Concept	Example Reference
Some hydrocarbons show little or no biodegradation	Salanitro 2001
Biodegradation of petroleum constituents can be modeled as first-order decay process	Suarez and Rifai 1999
Biodegradation of oil constituents can occur without solubilization into bulk groundwater	Hua and Wang 2014; Meckenstock et al. 2014
Some hydrocarbon constituents inhibit the biodegradation of other hydrocarbons	Sherry et al. 2014
Petroleum hydrocarbons can generate polar metabolites	Zemo et al. 2013; Bekins et al. 2016
Zero vs. first-order reaction rate order for bulk petroleum biodegradation	Siddique et al. 2008

compiled Gibbs free energy data calculated by eight different researchers and noted that the electron acceptor used for the reaction is more important than the type of hydrocarbon degraded. For aliphatic hydrocarbons, the size of the molecule had little effect because they are repeating the same carbon-hydrocarbon functional unit that reacts with the electron acceptor. For aromatics, the reduction in saturation (i.e., fewer hydrogen atoms per carbon) with increase in aromaticity and molecular weight reduces the Gibbs free energy and the biodegradation potential. Hua and Wang (2014) report on three different mechanisms for uptake of hydrocarbon molecules into microorganisms: (1) uptake of hydrocarbons dissolved in aqueous phase, which applies to soluble aromatics and aliphatics but not compounds with solubility in the 10^{-10} to 10^{-5} g/L range; (2) uptake of hydrocarbon microdroplets that have been pseudosolubilized by a biosurfactant from the organism; and (3) microorganisms that grow in fatty acids not in the water phase that are in direct contact with large hydrocarbon drops. When combined, these factors can result in different biodegradation rates and different biodegradation kinetics (e.g., first-order decay vs. Monod type) for different hydrocarbon compounds.

However, the connection between the controls on methanogenic processes that drive NSZD and the nature of the composition change in the underlying LNAPL has not fully been explored. We hypothesize:

1. The substrates at NSZD sites are complex mixtures of chemicals that have different propensity to biodegrade anaerobically;
2. The rate of the methanogenic degradation of petroleum hydrocarbons is controlled by one or more nonsubstrate factors (e.g., temperature, acetate, alkalinity, inhibition, protozoa, etc.);
3. NSZD is largely a process where methanogenic microorganisms consume different LNAPL constituents/chemical classes as a first-order process in a semi-sequential basis due to factors such as thermodynamics, inhibition, and solubility, which may cause a quasi-zero-order bulk NSZD rate over long time periods (see Figure 9 for an illustration).
4. Overall, the bulk hydrocarbon methanogenesis rate mimics a Monod-type relation, where for most of the initial part of the source lifetime, the overall rate is zero-order. For example, Siddique et al. (2008) reports that tar sands degradation in oil sands holding ponds appear to be first order when the naphtha concentration is under 5000 mg/Kg and zero order above this concentration. Other authors (e.g., DeVaul et al. [1997] and Hers et al. [2000]) have also discussed the appropriateness of Monod-type kinetics for biodegradation of hydrocarbons.

Modeling LNAPL Composition Change During NSZD

Ng et al. (2014, 2015) synthesized the extensive research that emerged from the Bemidji site (e.g., see Figure 1 and Table 1) and integrated a number of key concepts into a comprehensive mass-balance/reactive-transport

model of the saturated zone at the Bemidji site over a 27-year period. Table 5 shows example references for these underlying concepts. From an NSZD perspective, the key variables tracked in the model (informally called the “Ng Model”) were:

- Five main constituent “buckets”: BEX, toluene, short-chained alkanes (C_6 to C_{12}); long-chained alkanes (C_{13} to C_{18}), and “pre-NVDOC” (non-volatile dissolved organic carbon were simulated as contaminants coming from the oil). In an earlier publication by the authors (Ng et al. 2014), the pre-NVDOC fraction was identified as aromatics, resins, and asphaltenes in the oil (denoted as the ARA fraction), for which they determined a representative stoichiometry of $C_{19}H_{24}O_6$. The pre-NVDOC was biodegraded to NVDOC in groundwater, before its ultimate mineralization. The remaining fraction not already included in the five buckets was described as branched alkanes that were assumed to be recalcitrant. The toxicity and behavior of NVDOC, also referred to as polar metabolites, is an area of focused research at this time (Bekins et al. 2016; Zemo et al. 2016).
- Most existing hydrocarbon source attenuation models are dissolution based where biodegradation occurs only in the dissolved phase (e.g., Huntley and Beckett 2002; Molson 2011). However, the Ng et al. (2015) model accounts for direct outgassing of short- and long-chained alkanes without the need for an intermediate aqueous phase step.
- Dissolved gas phases for CO_2 , CH_4 , O_2 , and inert N_2 were modeled to simulate outgassing. One of the key elements of this mass balance was that “most of the carbon loss from the oil (70%) occurs through direct outgassing from the oil source zone.” Ng et al. (2015) stated, “Our proposed conceptual model includes direct outgassing of CO_2 and CH_4 from the oil body source zone in order to match surface efflux measurements from Sihota [2014].”

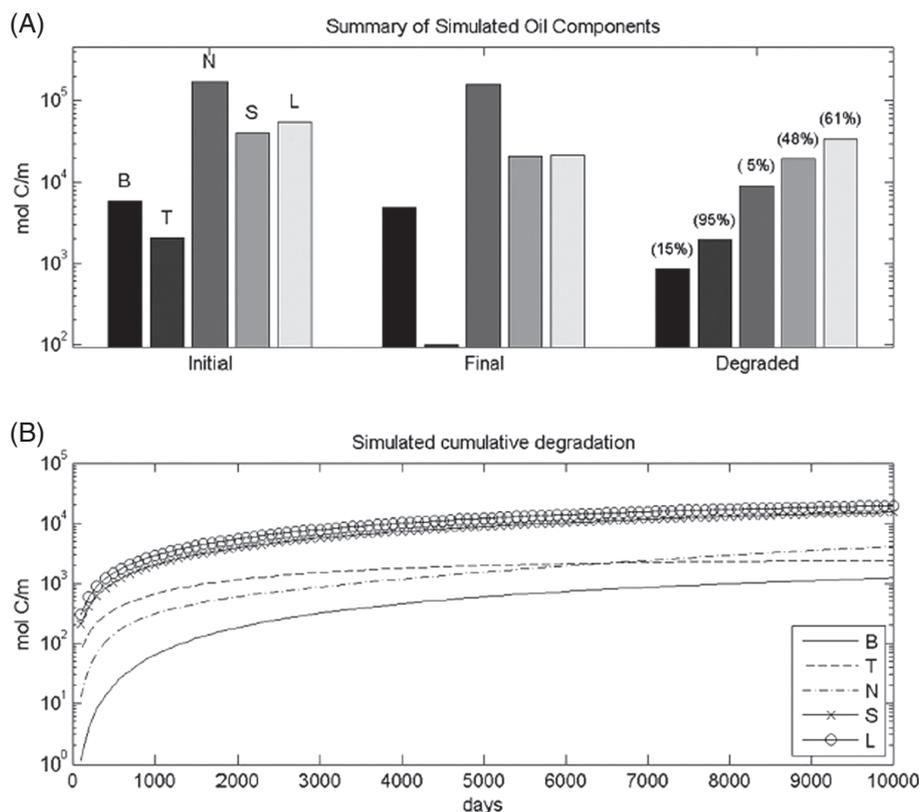


Figure 7. Simulated degradation of B (BEX, combined benzene, ethyl benzene, xylenes), T (toluene), N (pre-non-volatile dissolved organic carbon); S (short-chained alkanes, C_6 to C_{12}); and long-chained alkanes (C_{13} to C_{18}) from the saturated zone at Bemidji (Ng et al. 2015). Units are in moles of constituent in the model domain per meter of model width. The model domain was 260 meters long and 7 meters deep and included both the LNAPL source zone and the downgradient plume.

In the model, we attribute this mechanism to poorly soluble short and long-chain n-alkanes that degrade in pore spaces within the oil body.”

The Ng Model simulated the degradation of the five oil constituents from 1979 to 2008 (Figure 7), which was calibrated to the observed change in oil composition from Baedecker et al. (2011). The source attenuation component of the Ng Model and key input parameters are shown in Figure 8. A spreadsheet model based on most of the Ng model’s processes shown in Figure 8 was used to simulate the mass remaining over time and predict the composition of LNAPL at Bemidji. Figure 9 shows a 100-year simulation of the five key constituent buckets with three different scales for the Y-axis. Figure 9 suggests the overall NSZD rate is approximately constant over time (pseudo zero order), because the main contributors to NSZD, the short-chained and long-chained alkanes, are represented as a first-order decay rate where the biodegradation rate for these two constituent “buckets” gets smaller over time. As they do, the inhibition effect on the pre-NVDOC bucket defined by Ng et al. (2014, 2015) diminishes and the pre-NVDOC starts to biodegrade and “take up the slack” for the declining alkane degradation rate to produce a relatively constant biodegradation rate for much of the site history. The biodegradation rates and inter-bucket inhibitions elucidated by Ng et al. (2014, 2015) are for a crude oil release, and should be extrapolated with caution to other sites or products, such as refined hydrocarbons.

Key Learnings

When the solid foundation of extensive NSZD research summarized in this paper is combined with the modeling framework developed by Ng et al. (2015) and applied in this paper as a simplified model, one can address several questions that practitioners and regulators face when considering NSZD at LNAPL sites.

1. Why is there significant variability in NSZD measurements?

There is significant variability when comparing intrasite data over short time periods (1 to 2 years) (Figure 5), but the variability is reduced when site-wide NSZD averages are used (Table 3). Many of the other controlling factors listed in Figure 2 can contribute to this variability, for example, acetate buildup, soil moisture, temperature, “signal shredding,” etc. NSZD rates may also depend on the type, distribution, and amount of LNAPL in the source zone but the correlation, if any, is not well understood at this time.

2. What are the differences between vadose, saturated and smear zone NSZD rates, and processes?

Methanogenic biodegradation of LNAPL is occurring at a much higher rate than thought a few years ago. Methanogenesis is a dominant process for LNAPL in the saturated and smear zones. Most of the NSZD in these zones occurs via direct outgassing within the pore space adjacent to the entrapped oil (Ng et al. 2014). In the saturated zone, this

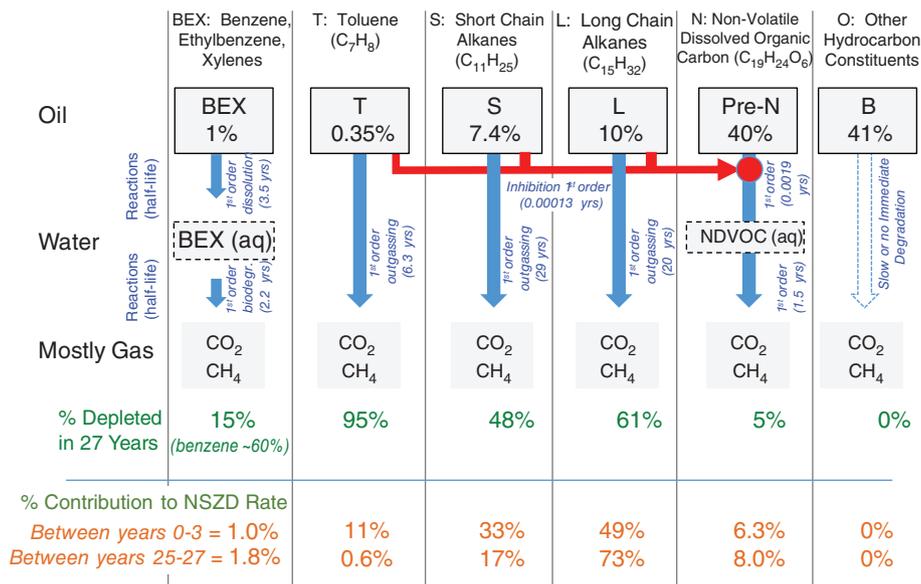


Figure 8. Simplified structure of Ng Model, showing five model constituent buckets (BEX: lumped benzene, ethylbenzene, xylenes; T: toluene; S: Shorted chained alkanes (represented by C11); L: long-chained alkanes (represented by C15); and Pre-N (pre-non-volatile dissolved organic carbon, representative molecular weight of 341 g/mole). BEX and NDVOC go through a dissolution step (modeled as a first-order process), then first-order decay in the aqueous phase; all other compounds are assumed to off from pores with LNAPL gas directly with the first-order decay constants shown in the blue text. These parameters were determined by Ng et al. (2015) from calibration to match observed composition change over a 27-year natural source zone depletion period. The percent degraded over the 27-year period, shown in green, is taken from Baedecker et al. (2011). Using data from the bottom panel of Figure 7, the approximate contribution to the total NSZD rate is shown in orange. Other hydrocarbon constituents included branched hydrocarbons, asphaltenes, and resins.

direct outgassing may be accompanied by ebullition. These processes bring the resulting carbon dioxide and methane to the vadose zone. One method to support that high rates of methanogenesis is occurring in the saturated zone is to collect IsoFlask samples (Isotech Laboratories, Champaign, Illinois) that provide measurements of gas concentrations in the saturated zone (Molofsky et al. 2016). Jones et al. (2008) showed a theoretical molar ratio of methane:carbon dioxide for crude oil methanogenesis of about 3:1; this ratio may be useful for identifying biodegradation processes occurring in hydrocarbon source zones. Aerobic degradation can be an important contributor to NSZD where shallow vadose zone impacts are present (e.g., near the point of release). If the oxygen demand of the vadose zone impacts is greater than the amount that can diffuse to it, methanogenesis and outgassing can occur there as well.

3. Does the NSZD rate change over time? How does LNAPL composition change over long time periods as NSZD progresses?

While there is considerable temporal variability in measured NSZD rates possibly due to factors such as “signal shredding,” soil moisture, etc., there are several lines of evidence that suggest that over decades, the NSZD rate is generally zero order: (1) the presence of controls such as acetate, predation, and so on that may provide a feedback mechanism on the long-term NSZD rate; (2) long-term laboratory studies (e.g., Siddique et al. 2008) showing zero-order behavior at higher hydrocarbon concentrations); (3) the semi-sequenced biodegradation of different buckets observed at Bemidji, add up to a fairly constant bulk

NSZD rate (Ng et al. 2015) (Figure 9); and (4) the hydrocarbon composition literature that shows the potential for sequenced biodegradation of different hydrocarbon chemical classes. Therefore, current knowledge suggests that a zero-order depletion rate can be assumed for much of the life of the LNAPL until a low saturation of LNAPL or a relatively recalcitrant fraction is left, but research is needed to determine if this fraction should be considered important for site management, for example, its magnitude and any persisting secondary water quality effects.

4. Is NSZD measuring the biodegradation of only the soluble compounds, for example, BTEX?

At most hydrocarbon release sites where NSZD is being considered, the LNAPL is weathered and the NSZD rate measured by carbon efflux and other methods originates from the methanogenesis and subsequent outgassing of short- and long-chained alkanes. At the Bemidji site, BTEX constitutes approximately 10% of the measured NSZD rate (see Figure 7 of Ng et al. 2015). Several researchers have noted that the less soluble components of the LNAPL (e.g., alkanes) biodegrade under both aerobic and anaerobic conditions (e.g., Salanitro 2001; Brown et al. 2017), and some have shown that alkanes undergo methanogenesis at rates faster than those for aromatic compounds (Jones et al. 2008; Sherry et al. 2014).

5. What are the rate-limiting factors in NSZD? How can NSZD rates be enhanced?

Figure 2 summarizes potential rate-limiting factors for NSZD. Overall, this research suggests several potential ways to enhance NSZD:

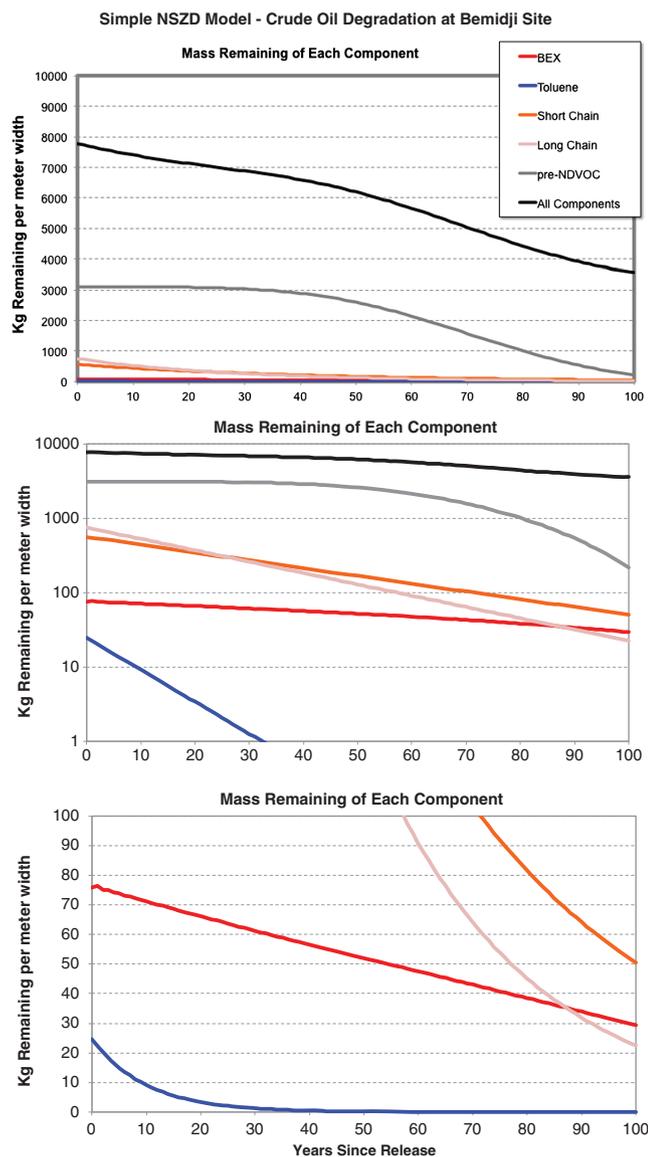


Figure 9. Mass remaining curves for five oil constituents using spreadsheet version of Ng Model for NSZD at Bemidji Site developed by the authors. “Short-Chain” and “Long Chain” indicates short- and long-chained alkanes respectively. All three graphs show same data but with different y-axes. An overall quasi-zero-order rate can be elucidated from the black line (all components) in the top panel, which represents the sum of first-order reactions of different rates for the various “buckets,” and the effect of inhibition on pre-NVDOC biodegradation.

- Adding inorganic nutrients to the methane generation zone might increase the rate of methanogenesis.
- Recirculating groundwater from source zone to the upgradient or cross-gradient zones with no treatment to: (1) remove acetate and other byproducts, which may be restricting the biodegradation rate; (2) help adjust pH to optimal levels for methanogenesis; and (3) “mine” iron in aquifer materials surrounding the site for degradation of the injected plume water; and (4) increase the contact between oil and water and potentially increase the direct outgassing rate. This technique would require the ability to inject contaminated groundwater in clean zones immediately adjacent to the source zone to be cost-effective.

- Heat addition increased methanogenesis significantly in lab columns containing hydrocarbons (Zeman et al. 2014), suggesting that adding low-level heat to the methane generation zone would also increase the overall NSZD rate. Bioventing or soil vapor extraction in the unsaturated zone may also increase the temperature of the methane generation zone, but the overall magnitude of this indirect effect is not known.

6. When will the groundwater and soil meet respective chemical-specific target concentrations?

Using the Ng model as a foundation, a planning-level NSZD model can be used to address questions about how long NSZD will take to remove a certain fraction of the LNAPL that was released, and how long it will take to remove a particular constituent down to a particular target level. The inputs to the model would be: (1) current NSZD rate; (2) type of hydrocarbon that was released and the current mass fraction of the various constituent buckets; (3) degradation rates for the lower solubility buckets, as a starting point, can be obtained from Ng et al. (2015), and for soluble components from site-specific source-zone groundwater concentration vs. time data (e.g., k_{source} for each BTEX compound [McHugh et al. 2014]); and (4) percentage removal of the bulk LNAPL or of a constituent bucket that would be needed to achieve specific remediation targets (such as a groundwater concentration goal or a LNAPL saturation reduction goal). More work is needed to better understand how to measure and process the proper input data for use in this planning-level LNAPL remediation timeframe model. Given the present level of understanding of NSZD, stakeholders should exercise caution when predicting and using long-term projections of source behavior for site management.

Conclusions

NSZD is an emerging option that can play an important role in the restoration of LNAPL-impacted sites once short-term site objectives are met. Methanogenesis is now increasingly accepted as a dominant process in NSZD. Methanogens degrade the LNAPL and generate methane and carbon dioxide that outgas, and subsequently as these gases travel toward the surface, methane oxidizers convert the methane to carbon dioxide. Two ancillary processes, direct outgassing of methane and carbon dioxide from pores containing LNAPL and ebullition of these gases from the saturated zone are also important to the NSZD conceptual model. A review of several strands of literature was conducted to bridge some gaps in the current practice of NSZD, for example, which constituents of LNAPL are actually degrading during NSZD; which rate-limiting factors can be manipulated to increase NSZD rates; and how can longevity of the bulk LNAPL and its key LNAPL constituents be predicted.

Other systems that are methanogenic such as anaerobic digesters, municipal landfills, peatlands, and wetlands were studied to provide insights on the controls that might be influencing NSZD methanogenic processes. Taken together, several factors may control the overall NSZD rate including temperature, inhibition from acetate buildup, protozoa predation, presence of electron acceptors, inhibition from

volatile hydrocarbons, alkalinity/pH, and the availability of nutrients such as nitrogen and phosphorus. Factors that may affect the quality of NSZD measurements include “signal shredding,” soil moisture, soil type, and temperature. Understanding the controls on NSZD processes can provide important insights on how to enhance NSZD rates and make better NSZD measurements.

While NSZD is a bulk measurement, many regulatory programs are based on concentrations of individual constituents. The hydrocarbon composition and biodegradation literature show that different hydrocarbons have different biodegradation potentials, but while this knowledge has been a mainstay for the field of environmental forensics work, it has been difficult to apply directly to predict composition change. A detailed reactive transport model developed by Ng et al. (2014, 2015) was adapted to explore LNAPL composition change as a result of NSZD. This model suggests that methanogenic microorganisms consume different LNAPL constituents/chemical classes in a semi-sequential basis due to inhibition and other effects, which then can produce quasi-zero-order bulk NSZD rates over long time periods. Overall, this body of knowledge can serve as a foundation for a planning-level composition-based LNAPL remediation timeframe tool, but more research is needed to identify representative chemical classes for a particular LNAPL, obtain methanogenesis rates for each of the classes, and test the applicability of the model to sites with varying characteristics.

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Disclosure

GSI Environmental and Colorado State University are collaborating on developing a new technology to measure NSZD rates using temperature (Thermal NSZD).

Supporting Information

The following supporting information is available for this article:

Appendix S1. Calculation of equivalent LNAPL degradation rates.

Figure S1. NAPL depletion not due to methanogenesis.

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