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Evaluating *in situ* biodegradation of ^{13}C-labelled naphthenic acids in groundwater near oil sands tailings ponds

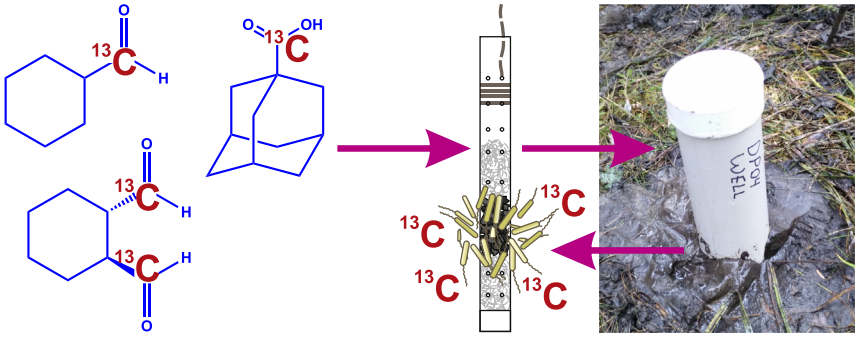
Jason M.E. Ahad [a,*], Hooshang Pakdel [b], Paul R. Gammon [c], Tariq Siddique [d], Alsu Kuznetsova [d], Martine M. Savard [a]

[a] Geological Survey of Canada, Natural Resources Canada, Québec, QC G1K 9A9, Canada
[b] INRS, Centre Eau Terre Environnement, Québec, QC G1K 9A9, Canada
[c] Geological Survey of Canada, Natural Resources Canada, Ottawa, ON K1A 0E8, Canada
[d] Department of Renewable Resources, University of Alberta, Edmonton, AB T6G 2P5, Canada

HIGHLIGHTS

- *In situ* biodegradation of NAs in groundwater assessed using ^{13}C-labelled surrogates
- Highly ^{13}C-enriched PLFAs at one site unambiguously confirm microbial uptake.
- Direct real world proof of NA biodegradation as previously seen in lab experiments
- No evidence for biodegradation of 1-adamantanecarboxylic acid at another site
- Certain types of NAs (tricyclic diamondoid acids) may persist in the subsurface.

GRAPHICAL ABSTRACT



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ABSTRACT

Potential seepage of naphthenic acids (NAs) from tailings ponds into surface water and groundwater is one of the main environmental concerns associated with the Canadian Athabasca oil sands mining operations. Here we report the application of ^{13}C-labelled NA surrogate compounds to evaluate intrinsic biodegradation along groundwater flow-paths originating from oil sands tailings ponds at two different sites: a glacio-fluvial aquifer (Site 1) and a low-lying wetland (Site 2). Microcosms containing the carboxyl group labelled (99%) NA surrogates (cyclohexanecarboxylic acid, CHCA; 1,2-cyclohexanedicarboxylic acid, CHDCA; 1-adamantanecarboxylic acid, ACA) were lowered into monitoring wells for several months to allow sufficient time for substrate degradation and formation of a biofilm in conditions characteristic of the local aquifer. Phospholipid fatty acids (PLFAs), biomarkers for the active microbial population, were extracted from the biofilms for stable carbon isotope (δ^{13}C) analysis. At Site 1, highly ^{13}C-enriched δ^{13}C values (up to ~+7100‰) confirmed the *in situ* microbial breakdown of CHCA and CHDCA. At Site 2, δ^{13}C-PLFA values from −60.6 to −24.5‰ indicated uptake of a ^{13}C-depleted substrate such as biogenic methane and not ^{13}C-labelled ACA. Determination of the microbial community using 16s RNA sequencing confirmed the presence of methane-oxidizing bacteria in the subsurface at Site 2. The *in situ* biodegradation of NAs at Site 1 demonstrates that the indigenous microbial population in the shallow subsurface near tailings ponds can readily break down some of these compounds prior to surface water discharge. The lack of evidence for microbial uptake of ^{13}C-labelled ACA at Site 2 demonstrates that other NAs, in particular tricyclic diamondoid acids, may persist in the environment following seepage from tailings ponds or natural sources.

* Corresponding author.
E-mail address: jason.ahad@canada.ca (J.M.E. Ahad).

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1. Introduction

With around 165 billion barrels, Northern Alberta's oil sands represent the third largest proven oil reserves in the world (Government of Alberta, 2018). The development of this resource, however, has raised awareness regarding its impact on the surrounding environment. One of the main concerns associated with oil sands surface mining, which is responsible for roughly half of the total daily bitumen production, is the fate of the large volumes of oil sands process-affected water (OSPW) stored in surface impoundments (*i.e.*, tailings ponds). OSPW contains high levels of naphthenic acids (NAs), a complex mixture of alkyl-substituted acyclic and cycloaliphatic carboxylic acids with the general chemical formula $C_nH_{2n+Z}O_2$, where n indicates the carbon number and Z is zero or a negative, even integer that specifies the hydrogen deficiency resulting from ring formation (Clemente and Fedorak, 2005). Found naturally in crude oil deposits, NAs become concentrated in OSPW during bitumen extraction due to water recycling. These compounds are harmful to a wide range of aquatic organisms (Bartlett et al., 2017; Debenest et al., 2012; Marentette et al., 2015; Melvin and Trudeau, 2012) and are considered the main source of toxicity in OSPW (Li et al., 2017; Morandi et al., 2015). Recent work has demonstrated that NAs may seep into groundwater and surface water near tailings ponds (Ahad et al., 2013; Frank et al., 2014; Oiffer et al., 2009; Savard et al., 2012). Understanding their behaviour and fate in the shallow subsurface is thus crucial for both current and long-term OSPW management strategies.

The breakdown of NAs by a variety of microorganisms under both aerobic and anaerobic conditions is well documented in controlled laboratory experiments, including for individual surrogate compounds and commercial or OSPW mixtures (Biryukova et al., 2007; Clemente and Fedorak, 2005; Clothier and Gieg, 2016; Del Rio et al., 2006; Demeter et al., 2014; Demeter et al., 2015; Headley et al., 2008; Herman et al., 1993; Herman et al., 1994). Low molecular weight (LMW) NAs, and thus commercial NA mixtures dominated by LMW components, are typically more readily biodegradable than high molecular weight (HMW) NAs (Scott et al., 2005). Due to this preferential degradation of LMW components, OSPW becomes increasingly enriched in HMW NAs over time (Holowenko et al., 2002; Quagraine et al., 2005). Aged OSPW is also thought to be less harmful than fresh OSPW (Anderson et al., 2012; Holowenko et al., 2002), although a recent study reported fresh OSPW to be similarly or less toxic than aged OSPW to aquatic invertebrates (Bartlett et al., 2017). These findings suggest that in addition to changes in OSPW composition and structure that take place in tailings ponds over time, other factors such as potential interactions with other organic and inorganic constituents may influence the overall toxicity of NA mixtures (Li et al., 2017). This highlights the need for techniques that allow for examination of NA biodegradation under conditions that represent as close as possible the actual *in situ* environment.

The use of isotopically labelled surrogate compounds is one approach that has the potential to provide direct evidence of *in situ* biodegradation of organic contaminants in the real shallow subsurface. This technique, referred to as stable isotope probing (SIP), involves tracing the incorporation of heavier stable isotopes (*e.g.*, ^{13}C, ^{2}H) from labelled compounds into biomarkers such as fatty acids, amino acids and nucleic acids (Fischer et al., 2016). Previous work utilising ^{13}C-labelled benzene, toluene and ethyl *tert*-butyl ether demonstrated significant ^{13}C-enrichment in phospholipid fatty acids (PLFAs) – biomarkers for the active microbial population – extracted from *in situ* microcosms (or "biotraps") lowered into groundwater monitoring wells at contaminated sites (Bombach et al., 2009; Bombach et al., 2015; Geyer et al., 2005; Stelzer et al., 2006). The highly ^{13}C-enriched stable carbon isotope ratios ($\delta^{13}C$) of PLFAs far above natural abundance levels provided unequivocal evidence for *in situ* microbial uptake of the labelled substrates in the cited studies. The application of SIP to examine biodegradation of organic contaminants can therefore eliminate the uncertainty associated with extrapolating laboratory experiments to complex groundwater systems.

In this study, we utilised ^{13}C-labelled LMW ($n < 12$) NA surrogate compounds (cyclohexanecarboxylic acid, CHCA; 1,2-cyclohexanedicarboxylic acid, CHDCA; 1-adamantanecarboxylic acid, ACA) to evaluate intrinsic biodegradation of NAs along groundwater flow-paths originating from tailings ponds in the Athabasca oil sands region. The first compound, CHCA ($C_7H_{12}O_2$, MW = 128), is one of the simplest NAs as defined by the general formula $C_nH_{2n+Z}O_2$, where Z = −2, and is a commonly employed NA surrogate in controlled laboratory biodegradation experiments (Del Rio et al., 2006; Herman et al., 1994). The second compound (CHDCA; $C_8H_{12}O_4$, MW = 172), while not following the classical definition of NAs, represents an "O_4 species" diacid – a class of compounds detected at significant levels in the acid extractable organics (AEOs) of OSPW (Ahad et al., 2013; Headley et al., 2011; Lengger et al., 2013). The third compound (ACA, $C_{11}H_{16}O_2$, MW = 180) represents another class of compounds (tricyclic diamondoid acids) recently detected in OSPW (Bowman et al., 2014; Rowland et al., 2011). The main purpose of our study was to determine whether the microbial breakdown of NAs reported in controlled laboratory experiments can be directly verified in the subsurface in areas prone to potential seepage of OSPW. Two different sites that represent typical environments found near tailings ponds were selected: a glacio-fluvial aquifer (Site 1) and a low-lying wetland (Site 2). Stable isotope probing of PLFAs was carried out on *in situ* microcosms lowered into monitoring wells for several months to allow sufficient time for substrate degradation and formation of a biofilm under real-world conditions. In order to characterise the indigenous microbial community potentially involved with the breakdown of NAs, 16s RNA sequencing was performed on microcosms at one of the two study sites. This study is the first to employ SIP to find evidence for *in situ* biodegradation of NAs in the shallow subsurface near oil sands tailings ponds.

2. Material and methods

2.1. Study sites

Both study sites are situated in the active surface mining area of the Athabasca oil sands, north of the city of Fort McMurray, Alberta, Canada. Site 1, ~40 km north of Fort McMurray, was the location of a previous groundwater investigation into sources of AEOs along a groundwater flow-path from an oil sands tailings pond to the Athabasca River (Ahad et al., 2013; Savard et al., 2012). The site has been in operation for several decades; thus, sufficient time has passed to allow AEOs containing NAs to have potentially moved through the subsurface. The two wells chosen for placement of *in situ* microcosms (N-05 and C2-B) were drilled into the Pleistocene glacio-fluvial aquifer located above the Cretaceous Clearwater/McMurray formations and Devonian limestone. Further hydrogeological information on Site 1 can be found in Ahad et al. (2013) and Savard et al. (2012). Site 2, around 55 km north of Fort McMurray, is the location of an ongoing hydrogeological investigation into sources and transport of AEOs along a groundwater flow-path from an oil sands tailings pond to a tributary of the Athabasca River. In contrast to Site 1, Site 2 is situated in a low-lying wetland containing significantly higher levels of organic material in the aquifer matrix. The wells chosen for placement of microcosms at Site 2 (DP-04 and DP-24) were thus shallower than at Site 1 (Table 1.). The main well parameters at the time of microcosm deployment are reported in Table 1.

2.2. In situ microcosms

The microcosms/biotraps (Fig. 1) consisted of perforated, solvent rinsed (methanol, dichloromethane and hexane) polytetrafluoroethylene (PTFE) tubes (15.5 cm length, 1.6 cm o.d., 1.0 cm i.d.) containing 1–2 g of pre-combusted (450 °C for 4 h) granular activated carbon (GAC) (Sigma-Aldrich, Oakville, ON, Canada) previously passed through a 4 mm stainless