Cold Regions Science and Technology 86 (2013) 55–68

Contents lists available at SciVerse ScienceDirect

Cold Regions Science and Technology

journal homepage: www.elsevier.com/locate/coldregions

Bacterial community evidence for anaerobic degradation of petroleum hydrocarbons in cold climate groundwater

C. William Yeung [a], Dale R. Van Stempvoort [b], John Spoelstra [b], Greg Bickerton [b], John Voralek [b], Charles W. Greer [a,]*

[a] National Research Council Canada, 6100 Royalmount Ave., Montreal, Quebec, Canada H4P 2R2

[b] Water Science & Technology Directorate, Environment Canada, 867 Lakeshore Road, Burlington, Ontario, Canada L7R 4A6

ARTICLE INFO

Article history:
Received 31 July 2012
Accepted 26 October 2012

Keywords:
Groundwater
Hydrocarbon contamination
Anaerobic hydrocarbon degradation
Microbial diversity
DGGE
Nitrate reduction

ABSTRACT

There is currently limited scientific data to assess whether groundwater bacterial communities in fractured rock environments can degrade petroleum hydrocarbon plumes in cold regions. The former Colomac Mine is located in the Canadian Shield in the Northwest Territories (mean annual air temperature of −5 °C) and is currently listed on the Federal Contaminated Sites inventory. Groundwater in fractured rock beneath the former fuel tank-farm at the mine site is contaminated by petroleum hydrocarbons. The objectives of this study were to investigate the bacterial community structure in the groundwater associated with hydrocarbon contamination, and to probe for potential anaerobic microbial processes involved in intrinsic bioremediation. Groundwater monitoring wells previously installed at the site were used to collect samples for chemical, isotopic and microbial analyses. Denaturing gradient gel electrophoresis (DGGE) analysis identified a relatively high bacterial diversity in the least contaminated locations, but as the hydrocarbon contamination increased, bacterial diversity decreased. Sequence analysis of the 16S rRNA gene demonstrated that the bacteria belonged to a wide range of genera, such as *Pseudomonas*, *Thiobacillus*, and *Geobacter*, all of which have been associated with the anaerobic degradation of hydrocarbons. Two putative nitrate-reducing genes were detected in samples with high nitrate reducing activity. Both chemical and microbiological results indicated the presence of microbial anaerobic processes by using nitrate, manganese(IV), ferric iron and sulfate as electron acceptors and suggest that these anaerobic processes play an important role in the biodegradation of dissolved petroleum hydrocarbons in the groundwater at the Colomac site. Our results also revealed that more than one biogeochemical process linked to hydrocarbon degradation could be present in a single borehole and these processes could vary spatially at this site.

1. Introduction

In general, bioremediation is a common choice for the clean-up of petroleum hydrocarbon-contaminated sites in warm or temperate regions because of the relatively low-cost (Domenico and Schwartz, 1990; Suthersan, 2002). However, cold temperatures and remote locations pose challenges for bioremediation. Current bioremediation methods for petroleum hydrocarbons have been developed and employed largely at temperate sites, whereas relatively little is known about microbial processes in groundwater under cold-climate conditions (Van Stempvoort and Biggar, 2007). Due to the large number of petroleum hydrocarbon contaminated sites in cold-climate regions (e.g., Arctic regions: Robin des Bois, Non Governmental Organization for Protection of Man and the Environment, 2009) there is a need for more information on the effectiveness of natural bioremediation for groundwater in cold climates.

Aerobic degradation is the most rapid and complete method for the removal of aromatic hydrocarbon pollutants in the environment and is an important bioremediation process. The initial intracellular attack of aromatic hydrocarbons is an oxidative process that incorporates oxygen by key enzymes such as oxygenases and peroxidases. Under aerobic conditions, oxygenases initiate attack on the benzene ring and prepare it for subsequent ring cleavage.

Under anaerobic conditions, microbes have been shown to use various electron acceptors, such as nitrate, manganese(IV), ferric iron, and sulfate to replace molecular oxygen for respiration and to oxidize hydrocarbons in petroleum plumes in groundwater (Azadpour-Keeley et al., 2001). These natural processes have been shown to significantly reduce the environmental impacts of petroleum contamination in warm or temperate climates.

One of the main objectives of this study was to gather the first detailed information about the occurrence and structure of microbial communities present in hydrocarbon-contaminated groundwater in a fractured rock environment at a cold climate site. A second main objective was to determine if hydrocarbon degradation was occurring

* Corresponding author. Tel.: +1 514 496 6182; fax: +1 514 496 6265.
E-mail address: charles.greer@cnrc-nrc.gc.ca (C.W. Greer).

0165-232X/$ – see front matter. Crown Copyright © 2012 Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.coldregions.2012.10.013

and, if so, to use microbiological, geochemical and isotopic techniques to provide information on the potential anaerobic pathways that might be used. Although the focus of this study was microbial analyses, it also involved gathering relevant information about physical conditions at the site (temperature, water table fluctuations), and groundwater chemistry.

This study examined the relationship between redox-sensitive species in the groundwater, specifically nitrate, manganese, iron, sulfate, and the bacterial community. In addition to standard analyses of petroleum hydrocarbons and a range of dissolved inorganic species, the stable isotope composition of dissolved sulfate ($\delta^{34}S–SO_4^{2-}$, $\delta^{18}O–SO_4^{2-}$) in groundwater was determined. Sulfate isotope ratios were used to obtain evidence about the source(s) of the sulfate, and the potential role of microbial sulfate reduction as an electron accepting process within the contaminated groundwater at the site (cf. Van Stempvoort et al., 2009). The stable isotope composition of the groundwater ($\delta^{18}O–H_2O$, $\delta^2H–H_2O$) was also analyzed, to provide information on the hydrology at the site.

2. Materials and methods

2.1. Study site location

The Colomac Mine (latitude 64.38°, longitude 115.11°) is located 220 km northwest of Yellowknife, Northwest Territories (Fig. 1), in the Canadian Shield region, which comprises nearly half of the area of Canada. Operated as an open-pit gold mine from 1989 to 1997, the site is currently in the custody of Aboriginal Affairs and Northern Development Canada (AANDC) after responsibility for the property reverted to the Government of Canada in 1999 when the mine's owner went into receivership.

There were at least twenty-four releases of petroleum-hydrocarbons (primarily diesel) documented in the vicinity of the mine's main fuel tank-farm (Iwakun et al., 2008). The fuel releases infiltrated the overburden and contaminated the groundwater within the underlying fractured rock in the area of the former fuel tank farm and machine shop (Fig. 1). To investigate the groundwater contamination, a series of monitoring wells was installed at the site (Bickerton et al., 2007; EBA Engineering Consultants Ltd., 2001). These monitoring wells are cased through the overburden and open boreholes below the casing, ranging in total depths up to 27.5 m below ground surface (EBA Engineering Consultants Ltd., 2001). Some boreholes (BH-103, -104, -106, -108) were drilled at approximately 50° to 55° from the horizontal to improve the likelihood of intercepting vertical fractures (Bickerton et al., 2007).

Based on the Environment Canada climate data from the nearest weather stations (Yellowknife data in Table 1; incomplete data from 1993 to 2008 at Snare Rapids, about 150 km southwest of the study site as provided by Environment Canada indicated an annual average temperature of −3.6 °C), the Colomac Mine site has a mean annual air temperature around −4 to −5 °C. Based on the Climate Normals record at Yellowknife, Northwest Territories (Table 1), the annual precipitation is ~280 mm, of which about 120 mm is snowfall. There is an average of 175 days per year with maximum daily temperatures of ≤0 °C (Yellowknife Climate Normals data). The natural topography of the site is rugged with large areas of outcropping bedrock at the surface.

2.1.1. Groundwater conditions at the study site

By the monitoring of water levels within boreholes in the fractured rock Bickerton et al. (2007) determined that the regional groundwater flow from the tank farm and machine shop areas is generally towards Steeves Lake (Fig. 1), consistent with earlier interpretations by consultants. Therefore groundwater flow at the site has the potential to transport dissolved fuel plumes towards the lake, where the hydrocarbons could impact aquatic organisms. In 2004, AANDC contracted the construction of an engineered, subsurface "barrier wall" between the former fuel tank farm and Steeves Lake (Fig. 1). The barrier wall was intended to produce a shallow zone of permafrost that would limit seepage of contaminated groundwater from the tank farm area towards Steeves Lake.

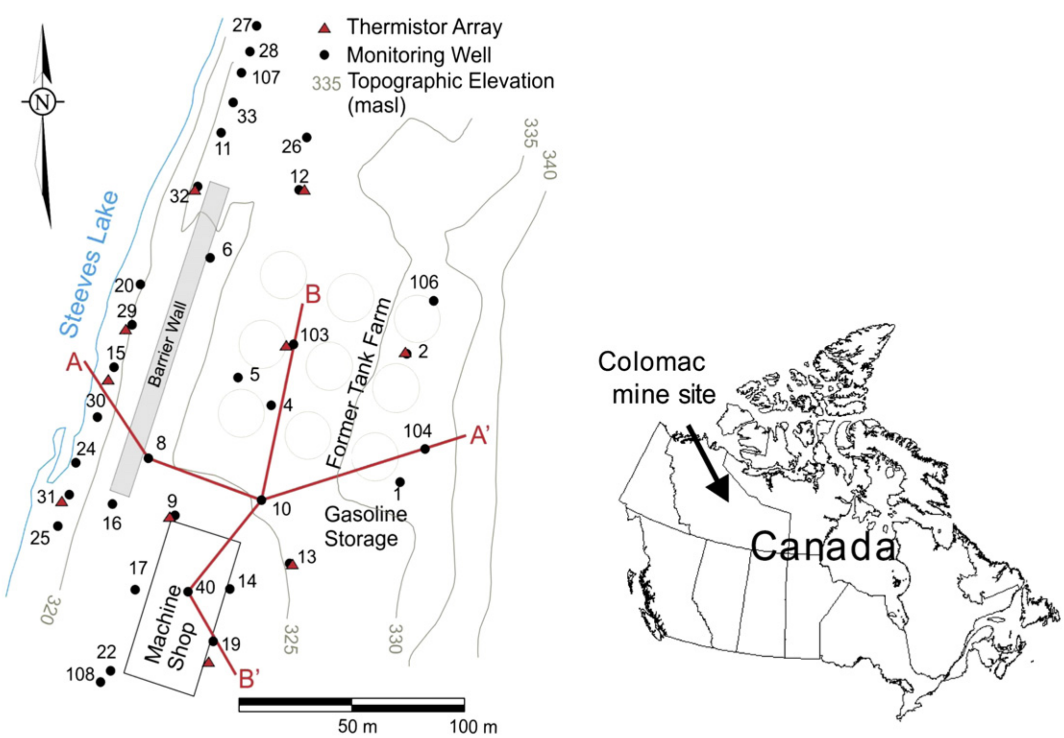

Fig. 1. Colomac study site.
Modified from Bickerton et al., 2007.