ELSEVIER

Chemosphere 59 (2005) 983–996

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

Characterization and microbial utilization of dissolved organic carbon in groundwater contaminated with chlorophenols

J.H. Langwaldt *, U. Münster, J.A. Puhakka

Institute of Environmental Engineering and Biotechnology, Tampere University of Technology, P.O. Box 541, 33101 Tampere, Finland

Received 12 February 2004; received in revised form 22 October 2004; accepted 16 November 2004

Abstract

The aim of this study was to characterize the labile part of dissolved organic carbon (DOC) present in groundwater by identification of natural organic carbon substrates and to assess their microbial utilization during aeration of the groundwater. The studied chlorophenol (CP) contaminated groundwater contained 60–2650 $\mu mol l^{-1}$ of DOC of which up to 98.0% were CPs; 1.7% were low-molecular weight organic acids and 0.2% were dissolved free amino acids. Traces of following natural organic carbon substrates were identified: L-alanine, L-isoleucine, L-leucine, L-serine, L-threonine, L-tyrosine, L-valine, L-aspartic, acetic, citric, formic, lactic, malic and oxalic acid. Dissolved oxygen concentration inside the CP-plume was lower (mean 25 $\mu mol l^{-1}$) than outside of the plume (mean 102 $\mu mol l^{-1}$). Over a monitoring period of four years the concentrations of CPs, Fe(II) and NH_4^+ were higher inside than outside of the CP-plume. Oxygen availability within the CP-plume limits in situ biological oxidation of CPs, DOC, NH_4^+ and Fe(II). The microbial enzymatic hydrolysis rates of 4-methylumbelliferyl and 7-amino-4-methylcoumarin-linked substrates varied from 0.01 to 52 $\mu mol l^{-1} h^{-1}$ and was slightly higher inside than outside the plume. Microbial uptake rates of ^{14}C-acetate, ^{14}C-glucose and ^{14}C-leucine were on average 28, 4 and 4 $pmol l^{-1} h^{-1}$ outside and 17, 25 and 8 $pmol l^{-1} h^{-1}$ inside the plume, respectively. The indigenous microorganisms were shown able of hydrolysis of dissolved organic matter, uptake and utilization of natural organic carbon substrates. Therefore, the labile part of DOC serves as a pool of secondary substrates beside the CP-contaminants in the groundwater and possibly help in sustaining the growth of CP-degrading bacteria.

Keywords: Amino acids; Contaminated groundwater; Extracellular enzyme; Microbial degradation; Natural organic matter; Nutrients; Organic acids

1. Introduction

Bacteria play a key role in mineralizing and cycling of the organic carbon in the subsurface. Natural organic matter (NOM) originating from the topsoil, buried organic matter or decaying microorganisms (Thurman,

* Corresponding author. Present address: Finnish Forest Research Institute, Rovaniemi Research Station, P.O. Box 16, 96301 Rovaniemi, Finland. Fax: +358 10 211 4401.
E-mail address: jorg.langwaldt@metla.fi (J.H. Langwaldt).

0045-6535/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2004.11.036

1985) is the dominant carbon source (Morita, 1997) in pristine groundwater, where carbon is the main growth-limiting factor (Madsen and Ghiorse, 1993). The partial biodegradability of dissolved organic matter (DOM), measured as dissolved organic carbon (DOC), has been shown during groundwater treatment (Langwaldt and Puhakka, 2002) and artificial groundwater recharge (Hendel et al., 2001). Thus, the microbial growth in groundwater can be carbon limited, despite elevated concentrations of organic matter as is the case for boreal groundwater (Pönkkä, 1981). Studies on groundwater NOM have mainly focused on high-molecular weight (HMW) organic compounds such as humic substances (Thurman, 1985, 1986). The availability and microbial utilization of natural organic carbon substrates, such as amino acids and organic acids, in groundwater remain to be studied in detail. Extracellular enzymes are excreted by microorganisms to hydrolyze low-molecular weight (LMW) organic compounds from NOM (Münster et al., 1999). The determination of the activity of microbial extracellular enzyme (MEE), such as phosphomonoesterhydrolase (phosphatase), acetate-esterase and leucine-aminopeptidase, has been used to describe microbial activity in saltwater (Hoppe, 1983), microbial utilization of DOM in freshwater (Münster et al., 1999) and artificially recharged groundwater (Miettinen et al., 1996; Hendel et al., 2001). The MEE activity in contaminated groundwater has not been studied in detail.

For this work, a long-term chlorophenol (CP)-contaminated aquifer in Southern Finland was studied (Männistö et al., 2001a). The diversity and aerobic CP-degrading capacity of the microbial community in the aquifer has been demonstrated (Langwaldt et al., 1998; Langwaldt and Puhakka, 1999; Männistö et al., 1999, 2001a,b; Männistö and Puhakka, 2002). Thus, the microbial activity was studied under aerobic conditions as anticipated for in situ bioremediation of the CP-contaminated groundwater. The aims of this work were to characterize the labile part of DOC by identification of natural organic carbon substrates present in groundwater and to determine the microbial utilization of these substrates under aerobic conditions.

2. Material and methods

2.1. Studied aquifer

The studied Quaternary aquifer is less than 10000 years old (Kukkonen, 1988) and is located in the municipality of Kärkölä in Southern Finland (Nystén, 1994). The shallow clay covered aquifer is within a bedrock depression (Fig. 1a and c) (Nystén, 1994) filled with medium to fine glacial deposits, with some coarse material present on top of the bedrock in the center of the depression (Fig. 1b). The esker and bedrock outcrops are not covered with clay. The aquifer was contaminated by use of a wood-preservative Ky-5, containing 2,4,6-tri-, 2,3,4,6-tetra- and pentachlorophenol (Fig. 2), in a sawmill located upstream (Fig. 1a) (Nystén, 1994).

2.2. Groundwater sampling

Groundwater was sampled at 10 points from the studied aquifer and from one reference point (MV5) receiving mainly pristine groundwater and partially CP-contaminated groundwater. The following parameters were measured during sampling of groundwater: temperature, dissolved oxygen (DO) (WTW DO-electrode CellOx 325, calibrated against wet air prior to measurement) and pH (WTW pH-electrode SenTix 41, calibrated with reference pH-solutions). Groundwater samples were stored at 4 °C or frozen. Groundwater samples were filtered (Whatman Puradisc, 0.45 μm pore size) for all analyses performed with the following 17 analyzers: ion-chromatograph, flow injection analyzer, high performance liquid chromatograph and total organic carbon analyzer for DOC-determination.

2.3. Analyses

2.3.1. Groundwater chemistry

Ferrous and total iron were measured following standard protocols, APHA (1992) and SFS (1976), respectively, as described by Langwaldt and Puhakka (2002). The detection limits (DL) (precision, P) were DL 0.5 $\mu mol l^{-1}$ P ±6% and DL 0.5 $\mu mol l^{-1}$ P ±8% for ferrous and total iron, respectively. Samples were diluted with MQ-water prior to analysis if required and MQ-water was used as reference in the spectrophotometer (Shimadzu UV-1601). Blanks were prepared with MQ-water and ran prior to sample determination.

Two flow injection analyzers (AKEA Autoanalyzer in 1998 and Lachat QuikChem 8000 in 2002) were used to measure NH_4^+ (DL 0.2 $\mu mol l^{-1}$) phenolate method (Solórzano, 1969), $NO_2^- + NO_3^-$ (DL 0.4 $\mu mol l^{-1}$) cadmium reduction method (Wood et al., 1967) and PO_4^{3-} (DL 0.01 $\mu mol l^{-1}$) molybdenum blue method (Murphy and Riley, 1962). After alkaline persulfate digestion (Koroleff, 1983) N_{total} (DL 1 $\mu mol l^{-1}$) and P_{total} (DL 1 $\mu mol l^{-1}$) were determined as $NO_2^- + NO_3^-$ and PO_4^{3-}, respectively. The precision was 15% for all inorganic nutrients measured with the flow injection analyzers. The content of CP (DL 5×10^{-3} $\mu mol l^{-1}$, P ±5%) and DOC (DL 20 $\mu mol l^{-1}$, P ±11%) in the groundwater were analyzed as described by Langwaldt et al. (1998). Blanks containing MQ-water were measured prior to and at the end of each DOC run. Blanks with MQ-water were analyzed prior to CP-calibration curve run. The measured CP-concentrations were converted into organic