Journal of Hazardous Materials 187 (2011) 488–494

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

ETBE (ethyl *tert* butyl ether) and TAME (*tert* amyl methyl ether) affect microbial community structure and function in soils

Johanna Bartling[a,*], Jürgen Esperschütz[b], Berndt-Michael Wilke[a], Michael Schloter[b]

[a] Berlin University of Technology, Institute of Ecology, Franklinstr. 29, 10587 Berlin, Germany
[b] Helmholtz Zentrum München, Department of Terrestrial Ecogenetics, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

ARTICLE INFO

Article history:
Received 21 October 2010
Received in revised form
21 December 2010
Accepted 13 January 2011
Available online 20 January 2011

Keywords:
Oxygenates
Ecotoxicity
Functional redundancy
Soil ecology
PLFA
Gasoline additives

ABSTRACT

Ethyl *tert* butyl ether (ETBE) and *tert* amyl methyl ether (TAME) are oxygenates used in gasoline in order to reduce emissions from vehicles. The present study investigated their impact on a soil microflora that never was exposed to any contamination before. Therefore, soil was artificially contaminated and incubated over 6 weeks. Substrate induced respiration (SIR) measurements and phospholipid fatty acid (PLFA) analysis indicated shifts in both, microbial function and structure during incubation. The results showed an activation of microbial respiration in the presence of ETBE and TAME, suggesting biodegradation by the microflora. Furthermore, PLFA concentrations decreased in the presence of ETBE and TAME and Gram-positive bacteria became more dominant in the microbial community.

1. Introduction

Oxygenates such as ethyl *tert* butyl ether (ETBE), *tert* amyl methyl ether (TAME), and methyl *tert* butyl ether (MTBE) are widely used as gasoline additives in order to reduce emissions of carbon monoxide, ozone, and unburned hydrocarbons [1]. In the last ten years, in European countries ETBE has become more and more important, since it can be synthesized from bioethanol [2]. TAME is primarily used in Finland and Italy [3,4]. Gasoline additives enter the environment during storage, distribution, and handling of the pure chemical, but also as a constituent of oxygenated gasoline. Not surprisingly since the 1990's, oxygenates have been found in the subsoil. They have mainly been detected in groundwater, but also in surface waters in the USA, Denmark, Belgium, Great Britain, in the Netherlands, and in Germany among others [5–12].

Since ETBE and TAME are highly water soluble, a rapid leaching into the saturated zone is generally assumed [5,13–15]. Furthermore ETBE and TAME are highly volatile and may be emitted from contaminated soils to the atmosphere. Consequently, little impact on soil ecology had been expected so far. However, recent studies revealed clear effects of oxygenates on soil fauna and (micro-) flora. An [16] and An and Lee [17] observed restless behaviour of earthworms and significant changes in their morphology, particularly coiling, blooding, swelling and fragmentation into two pieces, after exposition to MTBE. In addition, reduced seed germination, seedling growth, and roots elongation have been reported for wild oats, sweet corn, wheat, and lettuce [18,19]. Also the soil microflora can be negatively influenced by MTBE as shown by Shahidi Bonjar [20] and Bartos et al. [21] in growth inhibition tests with pure cultures using *Fusarium solani*, *Erwinia carotovora*, *Streptomyces* spp. and *Pseudomonas* sp. as model organisms. In a recent study, we investigated the consequences of ETBE and TAME on nitrification rates at least in the first two weeks of soil contamination [22]. However, detailed data on the mode of action of oxygenates on microbial community structure and function in soil is still missing.

Therefore, the aim of the present study was to examine the impact of TAME and ETBE on the microbial community structure and function in soils without any contamination history. For this purpose, a standard soil was artificially contaminated with TAME and ETBE, respectively, and incubated for 42 d. At selected time points, respiration measurements and phospholipid fatty acid (PLFA) analysis were carried out to determine the microbial function and possible shifts in the community structure. According to Bartling et al. [22], we hypothesized a toxic effect on microbial communities linked with a reduction of microbial respiration activity (I). Those were assumed to be short-term effects followed by regression based on the high volatilization rates of oxygenates (II). Since biodegradation of TAME and ETBE by microorganisms that never had contact to any contamination with hydrocarbons

* Corresponding author. Tel.: +49 30 314 73682; fax: +49 30 314 736690.
E-mail address: johanna.bartling@tu-berlin.de (J. Bartling).

0304-3894/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2011.01.058

has rarely been reported [23], we did not expect visible microbial degradation of TAME and ETBE (III).

2. Experimental

2.1. Test soil, contamination procedure and sampling

Experiments were carried out using "standard test soil 2.3" from LUFA (Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer, Germany). The soil has been characterized as follows: 9.4% clay, 31.9% silt; 58.7% sand (w/w); organic C content 0.98% (w/w), pH ($CaCl_2$) 6.4; maximum water holding capacity 34.4% at a bulk density of 1.3 kg l^{-1}. All experiments were performed in 500 ml glass flasks using 200 g m_d (dry mass) test soils, that has been adjusted to 50% of the maximum water holding capacity (WHC_{max}). Soil samples were contaminated with 5 ml (equivalent to 6.76 g) kg^{-1} m_d^{-1} ETBE (Merck, Germany), respectively 5 ml (equivalent to 6.41 g) kg^{-1} m_d^{-1} TAME (Sigma–Aldrich, Germany). These concentrations have been proven to affect soil microflora and fauna [16,22] and additionally, they reflect typical levels of contamination at field sites. Control soil samples received 5 ml water. Flasks were closed with screw taps and incubated at 20 ± 1 °C for 6 weeks. To ensure appropriate mixing, the flasks were shaken at 30 rpm in an overhead shaker during the first three days of incubation.

Soil samples for respiration measurements and PLFA analysis were taken 3, 7, 14, 28, and 42 d after contamination. For all treatments and incubation time points 4 replicates were prepared.

2.2. Respiration measurements

Soil respiration curves were measured according to ISO 17155 [24]. Briefly, soil samples equivalent to 20 g m_d were incubated in a respirometer at 20 ± 1 °C. The respiration rate was measured continuously as CO_2 production before and after addition of the substrate (composed of 80 g glucose, 13 g $(NH_4)_2SO_4$, and 2 g KH_2PO_4). After 24 h of incubation in the respirometer, basal respiration rate was constant in all treatments, so 0.2 g of the substrate mixture were added to each soil sample. In addition to the respiration curve progressions, the respiration parameters basal respiration (R_B) and substrate induced respiration (R_S) have been evaluated (Fig. 1). Respiration parameters were computed by SnoopPlotWin 2.x (PRW Electronics, Berlin, Germany).

2.3. PLFA analysis

PLFA analysis was based on Zelles et al. [25]. Aliquots equivalent to 20 g m_d were extracted with 125 ml methanol, 63 ml chloroform and 50 ml phosphate buffer (0.05 M, pH 7). After 2 h of horizontal shaking, 63 ml water and 63 ml chloroform were added for phase separation. After 24 h the water phase was removed and discarded. The total lipid extract was separated into neutral lipids, glycolipids and phospholipids on a silica-bonded phase column (SPE-SI 2 g/12 ml; Bond Elut, Analytical Chem International, CA, USA). Phospholipids were separated into saturated (SATFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. A derivatisation of MUFA was performed prior to measurement to identify the position of the double bond (see Zelles et al. for details). PLFAs were analyzed as fatty acid methyl esters (FAMEs) on a gaschromatograph / mass spectrometry system (5973MSD GC/MS Agilent Technologies, Palo Alto, USA). FAMEs were separated on a polar column (BPX-70, SGE GmbH, Griesheim, Germany, 60 m × 0.25 mm × 0.25 ml, coated with 70% of cyanopropyl polysilphenylene–siloxane). The mass spectra of the individual FAME were identified by comparison with established fatty acid libraries (Solvit, CH 6500 – Luzern, Switzerland) using MSD Chemstation (Version D.02.00.237). For fatty acid description, standard nomenclature was used [26]. For data evaluation, PLFAs were classified into four major groups, Gram-positive bacteria (incl. actinomycetes), Gram-negative bacteria, fungi and microeukaryotes/protozoa according to the literature (Table 1).

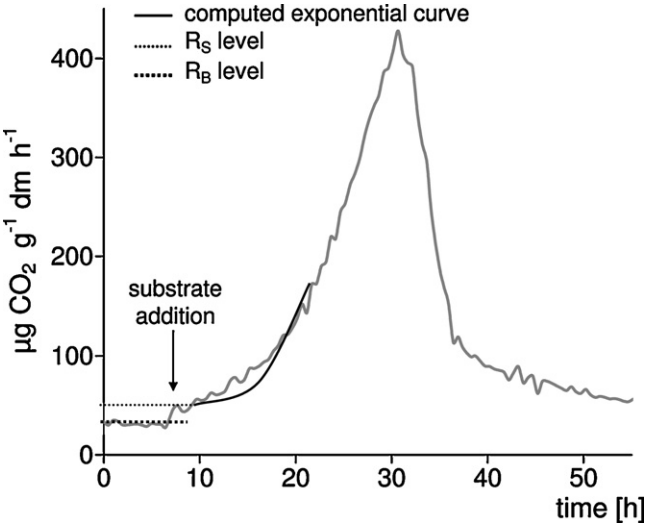

Fig. 1. Typical respiration curve with respiration parameters R_B and R_S.

2.4. Ether concentration measurements

Determination of the bioavailable fraction of ETBE and TAME was carried out after 3, 21, and 42 d of incubation using the method described by Bartling et al. [22]. In brief, soil samples equivalent to 5 g m_d and distilled water (1:2, w/v) were filled into 20 ml glass vials. The vials were closed immediately to prevent exorbitant volatilization. Samples were shaken for 24 h and stored at

Table 1
Classification of FA according to the given references.

Class	Underlying fatty acids	References
Gram-positive bacteria incl. actinomycetes	i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, 10Me17:0, 10Me18:0, br18:0, 10Me19:0, br20:0, br9,17:0	Gattinger [50] Leckie [51] Paterson et al. [52]
Gram-negative bacteria	cy17:0, cy19:0, 16:1ω5, 16:1ω9, 18:1ω7, 15:1?, 17:1ω8	Leckie [51] Paterson et al. [52]
Fungi	18:1ω9, 18:2ω6,9	Vestal and White [53]
Microeukaryotes/protozoa	n22:0, 18:3, 20:4	Gattinger [50] Leckie [51]
Unspecific	a14:0, n15:0, a16:0	Gattinger [50] Zelles [54]