Fuel 116 (2014) 321–327

Contents lists available at ScienceDirect

Fuel

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

Biodegradation of diesel/biodiesel blends in saturated sand microcosms

Piotr Lisiecki [a], Łukasz Chrzanowski [a,*], Alicja Szulc [a], Łukasz Ławniczak [a], Wojciech Białas [b], Mariusz Dziadas [c], Mikołaj Owsianiak [d], Jacek Staniewski [a], Paweł Cyplik [b], Roman Marecik [b], Henryk Jeleń [c], Hermann J. Heipieper [e]

[a] Institute of Chemical Technology and Engineering, Poznan University of Technology, Pl. M. Skłodowskiej-Curie 2, 60-965 Poznań, Poland
[b] Department of Biotechnology and Food Microbiology, Poznan University of Life Sciences, Wojska Polskiego 48, 60-627 Poznań, Poland
[c] Institute of Food Technology of Plant Origin, Poznan University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland
[d] Division for Quantitative Sustainability Assessment, Department of Management Engineering, Technical University of Denmark, Produktionstorvet, Building 426, DK-2800 Kgs. Lyngby, Denmark
[e] Helmholtz Centre for Environmental Research – UFZ, Department of Environmental Biotechnology, Permoserstraße 15, D-04318 Leipzig, Germany

HIGHLIGHTS

- Biodegradation of diesel/biodiesel blends was studied for 578 days.
- Procedure for monitoring the aliphatic and aromatic fractions was developed.
- Additive effect of biodiesel on mineralization of fuel blends was confirmed.
- Biodiesel did not impact the biodegradation of aliphatic and aromatic fractions.
- Biodiesel can affect the growth of microbial consortium members.

ARTICLE INFO

Article history:
Received 4 April 2013
Received in revised form 26 June 2013
Accepted 5 August 2013
Available online 20 August 2013

Keywords:
Biodegradation
Biodiesel
Diesel fuel
Hydrocarbon fractions
Community dynamics

ABSTRACT

The aim of the study was to evaluate the biodegradation extent of both aromatic and aliphatic hydrocarbon fractions in saturated sandy microcosm spiked with diesel/biodiesel blends (D, B10, B20, B30, B40, B50, B60, B70, B80, B90 and B100, where D is commercial petroleum diesel fuel and B is commercial biodiesel blend) augmented with a bacterial consortium of petroleum degraders. The biodegradation kinetics for blends were evaluated based on measuring the amount of emitted CO_2 after 578 days. Subsequently, the residual aromatic and aliphatic fractions were separated and determined by employing GC-FID and GC × GC–TOF-MS. Additionally, the influence of biodiesel-amendment on the community dynamics was assessed based on the results of real-time PCR analyzes. Our results suggest that the biodegradation extents of both aliphatic and aromatic hydrocarbon were uninfluenced by the addition of biodiesel, regardless of the concentration used. This observation leads to the conclusion that blending with biodiesel does not impact the long-term biodegradation of specific diesel oil fractions.

1. Introduction

Biodegradation is a major removal process of petroleum hydrocarbons in the environment, with degradation half-lives ranging from days to weeks, depending on the type of hydrocarbon and the environmental compartment [1]. Both biodegradation rates and extent of hydrocarbon mixtures containing biodiesel in form of fatty acid methyl esters (FAME), such as diesel/biodiesel blends, are usually higher compared to those of non-blended petroleum fuels [2]. Accelerated biodegradation of fuels containing biodiesel can be attributed to the fact that FAME are a better carbon source compared to petroleum hydrocarbons to support microbial growth [3]. Prince et al. [4] have demonstrated that FAME in a B20 blend (containing 20% v/v of FAME) were degraded at about the same rates as n-alkanes, but at lower rates than other petroleum hydrocarbons by unacclimated freshwater inocula.

Little is known about the role of biodiesel in long-term biodegradation of petroleum hydrocarbons. DeMello et al. [5] have shown that biodegradation of aliphatic hydrocarbons in the presence of biodiesel (B8 and B25) in seawater microcosms was accelerated at time scales of days, but no effects on compositions of the residual post-biodegradation mixture were observed after 53 days. Cyplik et al. [6] speculated that due to structural similarities between FAME and intermediate metabolites of alkane biotransformation, the presence of biodiesel could stimulate the growth of alkane-degraders in aqueous media, but the fate of specific hydrocarbons was not investigated. In porous media, biodiesel can mobilize aged,

* Corresponding author. Tel.: +48 61 6653716; fax: +48 61 6653649.
E-mail address: lucaschrz@gmx.de (Ł. Chrzanowski).

0016-2361/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fuel.2013.08.009

strongly sorbed hydrocarbons as it acts like a biosurfactant [7–9]. Miller and Mudge [7] already showed how biodiesel stimulates the biodegradation of petroleum hydrocarbons in sediments, whereas Taylor and Jones [10] showed how biodiesel enhanced biodegradation of desorbed polycyclic hydrocarbons in soils. Recently, Yassine et al. [11], showed how addition of biodiesel accelerates 7-day biodegradation of aliphatic and some aromatic hydrocarbons in aqueous systems through its control of hydrocarbon solubilization. However, long-term effect of biodiesel on biotransformation of aliphatic and aromatic biofuel fractions in porous matrices, has not been investigated up to now.

The aim of our study was to investigate the effects of biodiesel on the biodegradation of aromatic and aliphatic hydrocarbons within diesel/biodiesel blends. We chose aerobic, water saturated sand microcosms spiked with diesel/biodiesel blends as a model experimental system to isolate the effects of biodiesel on biotransformation, from dissolution and mobilization potentially occurring if aged hydrocarbons were considered. The response of hydrocarbon-degraders used to augment the microcosms to the presence of biodiesel was studied employing real-time PCR and the ddCt method for relative quantification.

2. Materials and methods

2.1. Fuels

Eleven types of fuels were used. Petroleum diesel fuel (assigned as letter D) was produced according to EN 590:2004 was purchased from a petrol station (PKN Orlen, Poland). Biodiesel (assigned B100) produced from rapeseed oil according to EN 14214 was purchased from a local supplier in Germany. Nine diesel/biodiesel blends with biodiesel content ranging from 10 to 90% (v/v) (assigned B10, B20, B30, B40, B50, B50, B60 B70, B80, and B90) were prepared by batching and mixing volumetric portions of pure fuels. Prior to experiments, all the fuels had been sterilized by filtration (Millex, pore size of 0.2 μm, Millipore). Elemental composition of diesel and biodiesel carried out using Elementar Vario EL III instrument, is shown in Table 1. The fatty acid profile of biodiesel is shown in Table 2, while a detailed characterization is given in the supplementary materials.

2.2. Microorganisms

Bacterial consortium had been isolated by selective enrichment using 100% pure diesel fuel as a sole source of carbon and energy from soil contaminated with crude oil [12]. The consortium contains strains belonging to the following bacterial taxa: *Achromobacter*, *Alcaligenes*, *Citrobacter*, *Comamonadaceae*, *Sphingobacterium*, *Pseudomonas*, and *Variovorax* [6].

The consortium was stored in 30% (v/v) glycerol stocks at −80 °C. To prepare an inoculum, stock suspension (1 mL) was transferred to a 300-mL Erlenmeyer flask containing 50 mL of mineral medium (composition in [13]) and diesel fuel (0.5%, v/v), and cultivated for 24 h at 25 °C on an orbital shaker (120 rpm). Later, 1 mL aliquot of the cell suspension was transferred to a new enrichment flask and the culture was grown for 3 days in the same conditions. This step was repeated three times and cells from the last enrichment were centrifuged at 10,000 g, washed twice with 40 mL of mineral medium, resuspended in the medium, and used as inoculum.

Table 1
Elemental composition of diesel (D) and biodiesel (B100) fuels.

Fuel	C (%)	H (%)	O (%)	N (%)
D	85.22 ± 0.47	14.67 ± 0.49	0.00 ± 0.01	0.11 ± 0.02
B100	74.13 ± 0.12	14.64 ± 0.34	10.92 ± 0.58	0.31 ± 0.13

Table 2
Fatty acid methyl ester profile of biodiesel.

Fatty acid methyl ester type		Content (%)
C16:0	Hexadecanoic ME	6
C18:0	Octadecanoic ME	2
C18:1	Octadec-9-enoic ME	68
C18:2	Octadeca-9,12-dienoic ME	21
C20:0	Eicosanoic ME	1
C20:1	Eicos-11-enoic ME	2

2.3. Microcosms and mineralization measurements

To prepare the microcosms, 50 g of dry sand was placed in sealed 1-L glass bottles. The sand was rinsed with deionized water, autoclaved, and dried. The microcosms were spiked with fuels applied on a sand surface, yielding a total concentration of about 16 g/kg dry sand. Then, the microcosms were inoculated with the consortium by applying a dense cell suspension (1 mL; $OD600_{nm}$ 3 ± 0.1) on the sand surface. Afterwards 14 mL of the mineral medium with additional 2 g/L of NH_4Cl was added to obtain full saturation. Undisturbed microcosms were maintained at 20 °C

Mineralization of fuels was determined by measuring CO_2 content in a base trap (10 mL of 0.75 M NaOH in a 20-mL vial) placed in microcosms. Titration of the diluted NaOH and Na_2CO_3 solution from the trap with 0.1 M HCl was done using an automatic titrator (Metrohm titroprocessor 686). The content of base traps was replaced with fresh NaOH solution after each measurement. The base trap was also a mean to maintain full saturation in the microcosms, as it provided equilibrium between the headspace phase and the sand.

2.4. Analyses of hydrocarbon fractions

At the end of the mineralization experiment (day 578) the microcosms were sacrificed to determine the content of residual hydrocarbons. First, CO_2 traps were removed from the bottle and 10 mL of acetone were added. This solvent is commonly used for extraction of wet solid samples because of its miscibility with water that allows penetration of solids' pores without need for drying, which may result in loss of volatile analytes [14]. The samples were then vortexed for 1 min. Afterwards, 15 mL of hexane was added and the sample was vortexed for another 1 min. Subsequently, the bottle was placed into an ultrasonic bath and exposed to ultrasound radiation to enhance desorption of the analytes from the solid matrix and improve their transfer to the extractant. Application of ultrasounds has been widely employed for extraction from solid samples, and has been proven to give similar results to those obtained via Soxhlet extraction [14,15]. After 10 min the samples were shaken vigorously to mix the sample matrix sticking on the bottom of the flask and sonicated for another 10 min, after which the bottle was shaken for 15 min at 250 rpm on a horizontal shaker. A 1-mL aliquot of the obtained extract was shaken with 3 mL of 0.1 M NaOH to remove acetone and potentially co-extracted acidic interferences. The upper phase was collected and subjected to the fractionation procedure, as described below.

2.4.1. Separation of aliphatic and aromatic hydrocarbon fractions

Fractionation into aliphatic and aromatic fractions was done by employing adsorptive open column chromatography (OCC), using silica gel impregnated with $AgNO_3$, as described by Bennett and Larter [16]. Briefly, the adsorbent was prepared by mixing 0.2 g