International Biodeterioration & Biodegradation 118 (2017) 45–56

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Anaerobic hydrocarbon biodegradation and biocorrosion of carbon steel in marine environments: The impact of different ultra low sulfur diesels and bioaugmentation

Deniz F. Aktas [a, b], Kathryn R. Sorrell [a, b], Kathleen E. Duncan [a, b], Boris Wawrik [a], Amy V. Callaghan [a], Joseph M. Suflita [a, b, *]

[a] Department of Microbiology and Plant Biology, 770 Van Vleet Oval, Norman, OK, 73019, USA
[b] Institute for Energy and the Environment, University of Oklahoma, 100 E. Boyd Street, Norman, OK, 73019, USA

ARTICLE INFO

Article history:
Received 14 September 2016
Accepted 23 December 2016
Available online 13 January 2017

Keywords:
Biocorrosion
Ultra low sulfur diesel
Fuel biodegradation
Bioaugmentation
Metagenomics
qPCR

ABSTRACT

The anaerobic biodegradation of ultra low sulfur diesels (ULSDs) by marine microbial communities was examined, along with the relationship of this metabolism to carbon steel biocorrosion. Fuel analysis revealed that the ULSDs differed based on the ratio of low to high molecular weight (LMW/HMW) *n*-alkanes. *Desulfoglaeba alkanexedens*, a sulfate-reducing bacterium capable of degrading LMW *n*-alkanes, was used as a positive control and to explore the impact of bioaugmentation. Metagenomic analysis was conducted to determine the genetic potential for anaerobic biodegradation of a range of hydrocarbons. Initial sulfate reduction rates were faster in incubations amended with ULSDs containing a higher ratio of LMW/HMW *n*-alkanes, but total sulfate loss was similar for all fuels. Sulfate removal in bioaugmented incubations exceeded stoichiometric expectations calculated for the mineralization of all paraffins. In combination with metagenomic analysis, these data confirmed that other microorganisms utilized hydrocarbons beyond the range exhibited by *D. alkanexedens*. A positive correlation between coupon weight loss and sulfate loss was observed, and pitting corrosion was more extensive in non-sterile incubations compared to sterile controls. This study demonstrates that while ULSDs vary in their susceptibility to early stage biodegradation, compositional differences were less important for overall fuel deterioration and steel biocorrosion patterns. Further, bioaugmentation stimulated other hydrocarbonclastic marine microorganisms.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Diesel fuel is a global energy resource used primarily for heating and transportation. The U.S. alone is projected to use 600 million liters of diesel per day in 2016 and forecasts indicate that consumption will increase by over 111 million liters/d through 2040 (Anonymous, 2016). Almost all commercial trucks, most farm and construction equipment, and all freight locomotives are powered by diesel engines. Diesel is also important as a marine fuel. Commercial shipping with diesel vessels accounts for approximately 90% of all world trade (Kaluza et al., 2010). Military vessels are powered with marine diesel, and many use seawater-compensated fuel ballast tanks wherein seawater is used to replace the volume and mass loss of fuel as they are consumed in the ship engines (Lyles et al., 2013; Suflita et al., 2014).

Environmentally, diesel tends to be preferred over other fuel options because it is more energy dense and delivers better fuel economy. However, diesel combustion releases particulates and sulfur emissions to the atmosphere, and unintentional spills occur. In 1996, the U.S. EPA mandated that the sulfur content of diesel be lowered to <15 ppm to produce ultra low sulfur diesel (ULSD) (Kilbane, 2006; Song, 2003), and full transition to this fuel for highway and off-road vehicles was completed in 2014. Along the North American coastline, sulfur emission control areas are in place, and more than 90% of maritime ships comply with increasingly stringent sulfur fuel standards (Kattner et al., 2015). While decreased sulfur emission is linked with such regulatory mandates, the level of societal reliance on diesel inevitably results in

* Corresponding author. University of Oklahoma, Department of Microbiology and Plant Biology, 770 Van Vleet Oval, Norman, OK, 73019, USA.
E-mail addresses: deniz.f.aktas@gmail.com (D.F. Aktas), katy.r.brown@gmail.com (K.R. Sorrell), Kathleen.E.Duncan-1@ou.edu (K.E. Duncan), bwawrik@ou.edu (B. Wawrik), acallaghan@ou.edu (A.V. Callaghan), jsuflita@ou.edu (J.M. Suflita).

http://dx.doi.org/10.1016/j.ibiod.2016.12.013
0964-8305/© 2017 Elsevier Ltd. All rights reserved.

accidental releases. For instance, fuel spills in U.S. waterways related to shipping totaled over 32 million liters between 1990 and 2011 (Anonymous, 2013; Ramseur, 2012). Releases to soil and groundwater are well established sources of environmental contamination (Prince and Douglas, 2010). Thus, the environmental consequences associated with widespread diesel reliance are more complex than those associated with atmospheric emissions alone.

In addition, the environmental release of fuels is sometimes associated with the biocorrosion of the carbon steel infrastructure. Fuels that biodegrade too easily serve as carbon and energy sources for the proliferation of microbial communities that ultimately exacerbate biocorrosion through-wall pitting corrosion of pipelines, tanks and other equipment (Aktas et al., 2010; Lenhart et al., 2014; Liang et al., 2016a). Concomitant with the introduction of ULSD were increased reports of infrastructure corrosion (Anonymous, 2012). The essential question is why should diesel reformulated for reduced organosulfur content be inherently more corrosive? The removal of organosulfur compounds from the fuel could conceivably impact its antimicrobial character rendering the resulting formulation more susceptible to microbial attack. It is clear that many organosulfur compounds have important antimicrobial properties (Heldreth and Turos, 2005). However, Lyles et al. (2013) examined the effect of the varying sulfur content on fuel stability in marine systems and found that the sulfur content of diesel sampled during progressive stages of oil refining did not significantly influence the rate of anaerobic hydrocarbon biodegradation or sulfate metabolism.

Gaylarde et al. (1999) report that one of the major issues in the oil industry is the microbial contamination of stored fuels leading to deterioration in fuel quality and compromise of the fueling infrastructure. A study of the microorganisms in several ULSDs (including one fuel used in this study) revealed that they were classified as obligate or facultative aerobes and not taxa that are generally associated with metal biocorrosion (Suflita et al., 2012). In that study, it was concluded that the bacterial load in those fuels was unlikely to substantively contribute to subsequent biocorrosion problems.

Fuel formulations were also changed when environmental regulations in several countries mandated the blending of fatty acid methyl esters (FAME) with petrodiesel in an effort to extend fuel supplies and reduce the net carbon footprint. However, several studies found that these fuel components were labile under both aerobic (Prince et al., 2008) and anaerobic (Aktas et al., 2010) conditions. The latter found that FAME biodegradation exacerbated sulfate loss from marine incubations as well as the deposition of reduced sulfides, fatty acid production and metal biocorrosion. Similarly, Fazal et al. (2010) reported that biodiesel was more corrosive on engine parts made of aluminum, copper, and stainless steel relative to petrodiesel. A subsequent study showed that increasing concentrations of rapeseed methyl ester in an ULSD blend exacerbated the corrosion of aluminum and copper (Norouzi et al., 2012). Still, other investigators suggested that the aerobic bioconversion of contaminating ethanol to acetate was responsible for the increase in corrosion in fueling systems storing and dispensing ULSD (Anonymous, 2012).

It seems clear that the accidental or intentional introduction of labile components into fuels can impact the integrity of the carbon steel infrastructure. However, this study specifically focuses on the ULSDs themselves, rather than the impact of components either added or removed from the fuels. We hypothesized that the susceptibility of ULSDs to biodegradation was likely a function of hydrocarbon compositional differences and that the more labile fuels would differentially impact biocorrosion. To test this prospect, four different ULSDs were added to incubations containing Gulf of Mexico seawater and sediment. When appropriate, a 1018 carbon steel coupon was incorporated in the assay as well as a known hydrocarbon-degrading, sulfate-reducing, positive control bacterium. Our results indicated that ULSDs possessing a greater proportion of low molecular weight *n*-alkanes proved to be more labile in the presence of a positive control bacterium able to metabolize this group of constituents. However, such differences were less significant in longer-term incubations. Remarkably, the bioaugmented bacterium had a stimulatory impact on other hydrocarbonoclastic marine anaerobes that effectively resulted in an increased extent of sulfate utilization and accelerated biocorrosion.

2. Materials and methods

2.1. Marine incubations

Four ULSDs were tested, including one from a refinery (ULSD 1), one from the U.S. Navy (ULSD 2) and two from separate local fueling stations (ULSDs 3 and 4). Coastal seawater and sediment were obtained in January 2013 from the north side of West Ship Island, a barrier island off the Mississippi coast (latitude 30°13′275”; longitude 88°57′243″), and used as both inoculum and growth medium. Sediment was collected by wading into ~0.5 m water depth to scoop sediment into wide mouth high-density polyethylene containers (4 L) until about 75% full. Seawater was used to fill the remainder of the containers to capacity. Environmental samples were cooled, shipped to the laboratory, and refrigerated until use. Anaerobic incubations were prepared in 160-ml serum bottles according to the protocol of Liang and Suflita (2015). The incubations contained 30 ml of seawater, 10 g of sediment, 0.1 ml of a selected ULSD, and a ~1 cm diameter 1018 carbon steel coupon. In addition, a replicate set of incubations contained an inoculum of *Desulfoglaeba alkanexedens* strain ALDC, a model sulfate-reducing bacterium that only mineralizes C_6-C_{12} *n*-alkanes (Davidova et al., 2006). Several attempts to determine whether other hydrocarbons might be metabolized by this organism proved negative (data not shown). The restricted substrate range exhibited by the inoculum has so far been corroborated by a preliminary annotation of the organism's genome (data not shown). The coupons were suspended in the seawater via teflon threads affixed to the serum bottle closures (Liang and Suflita, 2015). The headspace was adjusted to contain 80:20 N_2:CO_2. Negative controls included sterile incubations (autoclaved 20 min; 121 °C, 20 psi) and ULSD-free controls. The initial experiment was designed to monitor terminal electron acceptor depletion as a function of ULSD amendment with and without the *D. alkanexedens* positive control inoculum. It became apparent that the inoculum exhibited a greater-than-expected impact on biodegradation. Therefore, primers for monitoring the fate of *D. alkanexedens* were designed (below) and used in a repeat experiment. Experiments were conducted in triplicate (unless otherwise noted), and all incubations were maintained without shaking in the dark at room temperature. Benzoate (10 mM) and decane (0.02 ml) were used as positive control substrates. Microbial metabolism was routinely monitored by ion chromatography (Liang and Suflita, 2015) as the loss of sulfate relative to negative controls.

2.2. Gas chromatography-mass spectrometry (GC-MS)

The neat ULSDs were dissolved in ethyl acetate and analyzed by GC-MS as a means of comparing their relative compositions. The ULSD content in the incubations was analyzed at the end of the experiment. The aqueous phase of the incubations was acidified to pH $\leq$ 2 with HCl, extracted with an equal volume of ethyl acetate, dried over sodium sulfate, concentrated by rotary evaporation, derivatized with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA),

Download English Version:

https://daneshyari.com/en/article/5740547

Download Persian Version:

https://daneshyari.com/article/5740547

Daneshyari.com