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Microbial activities in hydrocarbon-laden wastewaters: Impact on diesel fuel stability and the biocorrosion of carbon steel

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ABSTRACT

Anaerobic hydrocarbon biodegradation not only diminishes fuel quality, but also exacerbates the biocorrosion of the metallic infrastructure. While successional events in marine microbial ecosystems impacted by petroleum are well documented, far less is known about the response of communities chronically exposed to hydrocarbons. Shipboard oily wastewater was used to assess the biotransformation of different diesel fuels and their propensity to impact carbon steel corrosion. When amended with sulfate and an F76 military diesel fuel, the sulfate removal rate in the assay mixtures was elevated (26.8 μM/d) relative to incubations receiving a hydroprocessed biofuel (16.1 μM/d) or a fuel-unamended control (17.8 μM/d). Microbial community analysis revealed the predominance of *Anaerolineae* and *Deltaproteobacteria* in F76-amended incubations, in contrast to the *Beta-* and *Gammaproteobacteria* in the original wastewater. The dominant *Smithella*-like sequences suggested the potential for syntrophic hydrocarbon metabolism. The general corrosion rate was relatively low ($0.83 - 1.29 \pm 0.12$ mpy) and independent of the particular fuel, but pitting corrosion was more pronounced in F76-amended incubations. *Desulfovibrionaceae* constituted 50–77% of the sessile organisms on carbon steel coupons. Thus, chronically exposed microflora in oily wastewater were differentially acclimated to the syntrophic metabolism of traditional hydrocarbons but tended to resist isoalkane-laden biofuels.

1. Introduction

Microorganisms residing in fuel storage and distribution systems can lead to costly problems, including fuel biodeterioration and corrosion of the carbon-steel infrastructure (Suflita et al., 2014). Moreover, the release of fuels due to internal corrosion of steel tanks and pipelines frequently results in severe environmental damage (Vigneron et al., 2016). While corrosion of the energy infrastructure has been extensively examined, a complete understanding of the interplay between biocorroding microbiomes and fuel biodeterioration is still lacking. This is particularly true for environments that are chronically exposed to petroleum hydrocarbons. Similarly, the rapid integration of alternative biofuels into the existing infrastructure raises new concerns about the ultimate compatibility of such products (Lee et al., 2014; Liang et al., 2016). Therefore, it is important to understand the baseline role of chronically petroleum-exposed microorganisms in the biocorrosion of carbon steel and how their metabolism might be influenced by the presence of biofuels in order to help maintain the integrity of the fueling infrastructure.

Petroleum-based F76 (F76) is a marine diesel used mainly to power military ships (Lee et al., 2014). Alternative fuels from renewable biomass resources have been considered as suitable drop-in supplements for F76 (Bartis and Van Bibber, 2011). Marine diesels regularly come into contact with oxygenated seawater, particularly in seawater-compensated fuel ballast tanks common on many warships (Lyles et al., 2013). Several studies have focused on the biodegradation of fuels and its impact on the steel corrosion under transiently aerobic conditions (Aktas et al., 2013; Lee et al., 2014). However, oxygen consumption by microbial heterotrophic respiration and abiotic reactions with iron cause the development of anaerobic conditions in naval ballast tanks and other storage facilities (Aktas et al., 2013; Lee et al., 2014). As a consequence, the proliferation of sulfate-reducing bacteria capable of further anaero-

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bic fuel biodegradation can reasonably be expected. This will in turn contribute to biogenic sulfide induced metal corrosion. Indeed, the microbial community from seawater compensated ballast tank was able to degrade various diesels at the expense of sulfate (Lyles et al., 2013). A subsequent study confirmed that sulfate reduction linked to the anaerobic metabolism of fuel components (F76 and a Fischer Tropsch-F76 – FT-F76) exacerbated corrosion of carbon steel in seawater incubations amended with a model alkane-degrading sulfate reducing bacterium (Liang et al., 2016). However, seawater is most often an aerobic environment that is hardly conducive for the proliferation of anaerobes and indigenous marine microflora are usually not chronically exposed to hydrocarbons. Therefore, long incubation times and long succession patterns can be expected as the norm (Liang et al., 2016; Lyles et al., 2014). This study was initiated to examine the ability of marine microorganisms chronically exposed to petroleum hydrocarbons to degrade F76 or FT-F76 fuels at the expense of sulfate and the potential impact of these fuels on carbon steel corrosion.

Fuel storage and distribution systems usually harbor a wide variety of microorganisms (Lyles et al., 2013) that are primarily responsible for fuel biodegradation and biocorrosion. To date, many pure cultures, particularly aerobic hydrocarbon-degrading bacteria, have been isolated and investigated for their roles in fuel biodegradation and the corrosion of carbon steel (Ching et al., 2016; Muthukumar et al., 2003; Striebich et al., 2014). However, microorganisms typically function as a complex community with a great variety of metabolic interactions between component species. In fact, a thermodynamic basis for syntrophic interactions in anoxic environments (McInerney et al., 2007) is typical. A recent study found that syntrophic fatty acid metabolism by defined strains of *Syntrophus* and *Desulfovibrio* could catalyze the pitting of carbon steel under sulfate-reducing conditions (Lyles et al., 2014). Another study also demonstrated the involvement of syntrophic metabolism in biocorrosion when a natural microbial community was grown with iron as the sole electron donor (Usher et al., 2015). Clearly, inherent complexity is associated with biocorroding microbiota in fuel-degrading environments. Molecular characterization of corrosive biofilms from offshore oil pipelines revealed that similar metabolic processes occurred among diverse microorganisms involved in metal corrosion (Vigneron et al., 2016). Herein, we examined the ability of a microbial community chronically exposed to an aviation diesel (Jet A-1) to biodegrade other petro- and biodiesel (F76 and FT-F76), and its impact on the corrosion of carbon steel. While Jet A-1 and the two marine diesels in the study share common petroleum hydrocarbon classes (*n*-alkanes and isoalkanes, etc.) (Dagaut, 2002; Liang et al., 2016), the FT-F76 contains relatively more isoalkanes and almost no aromatic hydrocarbons relative to the petroleum counterpart (Liang et al., 2016). Therefore, this study addressed how differences in fuel composition impact their susceptibility to anaerobic biodegradation and metal biocorrosion by a chronically fuel-exposed microflora.

Biodegradation and biocorrosion in hydrocarbon-laden systems involve complex interactions between fuel, inocula and metal surfaces (Suflita et al., 2014). Sessile and planktonic microorganisms metabolize the available electron donors to produce chemical agents such as sulfide to accelerate electrochemical corrosion reactions (Chemical Microbially Influenced Corrosion; CMIC) (Enning and Garrelfs, 2014). Recently, Electrical Microbially Influenced Corrosion (EMIC) has been proposed as certain species of bacteria and archaea were capable of corroding iron via direct electron extraction from the metal surfaces (Enning and Garrelfs, 2014; Enning et al., 2012; Venzlaff et al., 2013). However, it is unknown whether EMIC also plays an important role in iron corrosion in hydrocarbon-laden environments where a plethora of organic electron donors are available to the resident microflora. While the proportional contribution of EMIC and CMIC to corrosion can hardly be pinpointed, the comparison of planktonic microbial community and the sessile biofilm on corroded carbon steel in fuel-degrading systems can shed some light on their respective roles in biocorrosion processes.

The overall goal of this study was to characterize the microbial community in an oily wastewater and their role in the both biodegradation of marine diesels (F76 and FT-F76) and the corrosion of carbon steel under anaerobic conditions. We also compared the microbial communities in the aqueous phase and on corroding metal surfaces to elucidate the dominant taxa involved in the biodeterioration processes. Our results indicate that the indigenous microflora chronically exposed to hydrocarbons are likely adapted to the biodegradation of petroleum-based F76 and catalyze corrosion of carbon steel via syntrophic metabolism under sulfate-reducing conditions. However, the biodiesel (FT-F76) tends to resist biodegradation by the same microbial community likely due to the predominance of isoalkane in the Fischer-Tropsch processed fuel.

2. Materials and methods

2.1. Sample collection and fuels

Oily wastewater was collected from a floating reservoir moored in the industrial port of Augusta (Sicily, Italy) as previously described (Mancini et al., 2012). The wastewater was generated using high-pressure hydraulic water to rinse oil tanker compartments that transported jet fuel (Jet A-1). The fresh wastewater was combined in the floating reservoir with residual Mediterranean seawater. The oily wastewater thus contained a mixture of Mediterranean seawater and freshwater that had previously been exposed to a jet fuel (Jet A-1) inside the tanker for at least a month. The wastewater was originally collected in a jerrycan (30 L), mixed thoroughly, and then subdivided into Schott bottles (Schott, Mainz, Germany) and shipped to the laboratory. A 1 Liter Schott bottle containing the wastewater was stored at 4 °C upon arrival (<48 h) prior to DNA extraction and experimental incubation construction. The wastewater was used directly as both medium and inoculum source to assess the biological stability of marine diesel fuels and the potential impact on the corrosion of carbon steel. The test fuels included a biomass-derived marine diesel produced via Fischer Tropsch processes (FT-F76) and a petroleum-based counterpart (F76). The test fuels were obtained from Naval Air Systems Command (NAVAIR) as described previously (Liang et al., 2016).

2.2. Incubations

Oxygen was removed from wastewater samples by flushing with N_2 according to a previously published protocol (Liang and Suflita, 2015). The assembly of the incubations was completed inside an anaerobic glove bag. The anaerobic oily wastewater (30 mL) was then distributed to 70 mL sterile serum bottles. A carbon-steel coupon was suspended in the incubations with a quartz thread as previously described (Liang and Suflita, 2015). The initial concentration of sulfate in the aqueous phase was adjusted to ~10 mM by the amendment of a sterile, anoxic stock solution. The desired filter-sterilized fuel (0.1 mL) was amended to the incubations. Fuel-unamended incubations were included to assess background microbial activity associated with the oily wastewater. Sterile negative control incubations were prepared with autoclaved oily wastewater. Incubation bottles were closed with black butyl rubber stoppers (20 mm diameter × 16 mm thickness, Geo-Microbial Technologies, Inc., Ochelata, OK) and secured with aluminum crimps. The initial headspace was O_2-free N_2:CO_2 (4:1). Incubations were in triplicate and maintained in the dark at ambient temperatures (~21 °C).