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Anaerobic Metabolism of Biodiesel and Its Impact on Metal Corrosion

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Biodiesels have gained widespread acceptance because they are domestically produced carbon-neutral fuels that ultimately decrease greenhouse gas emissions and reduce dependence on fossil imports. While they are chemically and physically stable fuels, their susceptibility to biological degradation in the absence of oxygen is underexplored. We incubated five anaerobic inocula with biodiesel. The microorganisms originated from fresh and marine environments with differing histories of exposure to hydrocarbons, biodiesel, and oxygen. All inocula were able to biodegrade biodiesel within 1 month. Biodiesel metabolism accelerated the rate of both sulfate reduction and methanogenesis above biodiesel-unamended controls. Metabolite profiling indicated that the methyl esters of biodiesel were readily hydrolyzed to the corresponding suite of fatty acids, and the latter were also metabolized. Electrochemical/corrosion experiments showed that the anaerobic microbial metabolism of biodiesel in coastal seawater samples accelerated the rate of pitting corrosion in carbon steel. The susceptibility of biodiesel to anaerobic biodegradation and its propensity to stimulate biocorrosion suggest caution when integrating this alternate fuel with the existing infrastructure.

Introduction

Biodiesel is a mixture of monoalkyl esters of long-chain fatty acids derived from plant or animal lipids. In the United States, the term "biodiesel" is standardized as fatty acid methyl ester (FAME). Biodiesel content is reported as the number of carbon atoms in the FAME backbone, e.g., FAME C16. Biodiesel mixes easily with petroleum diesel as a fuel additive for use in blends of up to 20%. Some countries encouraged biodiesel production with policy incentives. For example, in 1998, biodiesel as a 20% blend (B20) with petroleum diesel was designated an "alternative fuel" under the U.S. Energy Policy Act. This designation allows the government fleet services to purchase the B20 blend for operation in normal diesel vehicles and receive credit for those vehicles. In the European Union, the Renewable Transport Fuel Obligation mandates that suppliers include 5% renewable fuel in all transport fuel sold by 2010. The use of biodiesel reduces the societal dependence on imported oil; therefore, it is produced as a major biofuel throughout the world.[1] The worldwide production of biodiesel dramatically increased to about 9.4 million metric tons in 2007.[2] Despite the global acceptance of biodiesel, the impact of integrating this alternate fuel with the existing infrastructure has not been fully explored.

The chemical stability characteristics of biodiesel are well-documented,[3,4] but the susceptibility of this fuel to biodegradation is not well-known. Biodiesel methyl esters are sparingly soluble in seawater, with a saturation concentration of 7 ppm at 17 °C.[5] Several studies showed that aerobic microorganisms readily degrade biodiesel.[6−8] The half-life for the biodegradation of the vegetable methyl esters in agitated San Francisco Bay water was less than 4 days at 17 °C.[9] However, anaerobic conditions prevail whenever heterotrophic microbial respiration consumes oxygen at a rate that exceeds diffusion. This is typically the case in subsurface environments, including oil reservoirs,[10−12] oil-contaminated habitats,[13] refineries, storage vessels, pipelines, oil−water separators, and ballast tanks.

We exposed biodiesel to anaerobic microorganisms from fresh and marine environments with differing histories of exposure to hydrocarbons, biodiesel, and oxygen. Biodegradation of biodiesel was evaluated using gas chromatography−mass spectroscopy (GC−MS), and corrosion of carbon steel was monitored over a 60 day period using electrochemical and imaging techniques. Corrosion product chemistry and localized corrosion morphology were documented using environmental scanning electron

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microscopy (ESEM) and energy-dispersive spectroscopy (EDS) techniques.

Experimental Section

Biodiesel Incubations with Anaerobic Inocula. A soy-based biodiesel was used in the experiments. The major components were FAME C16 and C18. Anaerobic biodegradation of biodiesel was evaluated using (1) an inoculum capable of hydrocarbon metabolism under both sulfate-reducing and methanogenic conditions, obtained from the contaminated aquifer sediments that overlie a natural gas field in the Denver Basin near Ft. Lupton, CO, (2) an alkane-degrading methanogenic bacterial consortium enriched from the same aquifer, (3) a marine oil-degrading sulfate-reducing inoculum obtained from the sunken remains of the USS Arizona in Pearl Harbor, HI, (4) an unenriched sample obtained from a seawater compensated ballast tank aboard the USS Gettysburg, and (5) natural Key West, FL, coastal seawater. The collection, storage, and cultivation of inocula were as described by Gieg et al.[14,15] and Johnson et al.[16]

All incubations were in sterile serum bottles. Strict anaerobic conditions, as described by Widdel and Bak,[17] were maintained throughout the experiment. Brackish mineral[17] and freshwater[14] media were used for marine and freshwater inocula, respectively. The serum bottles were capped with butyl rubber stoppers and crimp sealed. The headspace was adjusted to N_2/CO_2 (8:2). Approximately 10 mM sulfate, in the form of Na_2SO_4, was initially added to sulfate-reducing incubations. Each bottle aseptically received excess biodiesel (0.5−1 g) by syringe. Negative controls included both sterile and biodiesel-free incubations. Sterile controls were autoclaved for 20 min (121 °C, 20 psi) on 3 consecutive days. The experiments were conducted in triplicate. All serum bottles were incubated in the dark at room temperature. Microbial metabolism of the biodiesel was evidenced by sulfate reduction or methanogenesis in excess of substrate-unamended controls. Methane production was monitored by gas chromatography,[15] and sulfate reduction was analyzed by ion chromatography.[18]

Electrochemical/Corrosion Experiments. Biocorrosion experiments were carried out with natural Key West, FL, coastal seawater, collected as described by Lee et al.[19] A sealable plastic chamber was instrumented as previously described[20,21] to monitor electrochemical parameters and dissolved oxygen concentration. The chamber was filled with 6 L of coastal seawater and 4 L of biodiesel and placed in an anaerobic hood (0.01% CO_2, 10% H_2, balance N_2). The CO_2 concentration was chosen to maintain a seawater pH between 7.8 and 8.2.[22] Nine epoxy-mounted carbon steel (UNS C10200) coupons (1.58 cm diameter × 0.16 cm) were arranged vertically, so that triplicate coupons were exposed to three conditions: (1) seawater immersion, (2) seawater/biodiesel interface, and (3) biodiesel immersion, where the lighter biodiesel forms a separate layer on top of the seawater. Polarization resistance (R_p) (Ω-cm^2) was monitored daily for 60 days for coupons exposed at and below the biodiesel/seawater interface using the linear polarization technique.[23,24] The inverse of polarization resistance ($1/R_p$) (Ω-cm^{-2}) is proportional to the instantaneous corrosion rate. Electrochemical corrosion monitoring during the experiment was not possible above the interface because of the low electrical conductivity of the biodiesel. After 60 days, the seawater H_2S concentration was measured as previously described.[22] The carbon steel coupons were removed and imaged using a macro digital camera. Corrosion morphology and corrosion product chemical composition were characterized with ESEM and EDS, respectively.[20,21] Coupons were acid-cleaned[25] to remove corrosion products and re-examined with ESEM.

Metabolite Profiling. The pH of the incubations was raised to 11 with 12 N NaOH and kept at room temperature for 2−3 h. The samples were then acidified with 10 N HCl until the pH was ≤2. After 2 h, the samples were extracted with ethyl acetate [10% (v/v), 4 times] and the extracts were combined, dried over anhydrous Na_2SO_4, concentrated by rotary evaporation, and reduced further under a stream of N_2 to a volume of 50 μL.[26] The extracts were derivatized with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Pierce Chemical Co., Rockford, IL) prior to analyses of the resulting compounds on an Agilent 6890 model gas chromatograph (GC) coupled with a Agilent model 5973 mass spectrometer (MS). Derivatized components were separated on a HP-5 ms capillary column (30 m × 0.25 mm inner diameter × 0.25 μm film, J&W Scientific, Folsom, CA), using the method described by Duncan et al.[27] All fatty acid and methyl ester identifications were made by a comparison of the GC−MS profiles to (1) authentic standards purchased from Sigma-Aldrich (St Louis, MO) and analyzed identically or (2) the National Institute of Standards and Technology (NIST) Mass Spectral Library, version 2.0a.

Results

Biodiesel Incubations with Anaerobic Inocula. The inoculum from a gas-condensate-contaminated aquifer (inoculum 1) metabolized sulfate at a net rate of 255 ± 5 μM/day in biodiesel-amended incubations and depleted 9 mM oxyanion within a month (Figure 1). Continued incubation resulted in net methanogenesis (245 ± 16 μM/day; Figure 1) and the accumulation of more than 20 mM methane after 2 additional months of incubation. Comparable rates of methanogenesis (208 ± 37 μM/day) and methane accumulation were also noted (Figure 1) when a defined alkane-degrading methanogenic enrichment derived from the same aquifer (inoculum 2) was exposed to biodiesel. The oil-degrading bacterial inoculum originally enriched from marine samples taken from the USS Arizona (inoculum 3) was also capable of biodiesel metabolism, as evidenced by net sulfate reduction at 82 ± 7 μM/day (Figure 1). The inoculum from a seawater compensated ballast tank (inoculum 4) reduced sulfate at a net rate of 827 ± 224 μM/day (Figure 1) and used 25 mM sulfate within 1 month.

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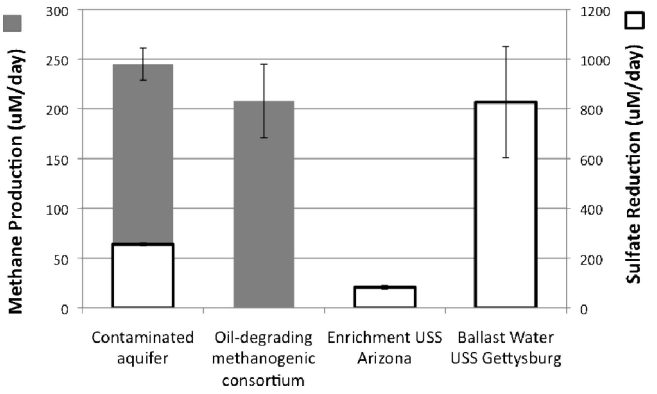

Figure 1. Net rates of sulfate reduction and/or methane production in anaerobic incubations amended with biodiesel. The error bars reflect the standard deviation of triplicate determinations. The rates were corrected for biodiesel-unamended controls (the rates of sterile controls were negligible and not depicted).

Electrochemical/Corrosion Experiments. The seawater (inoculum 5) oxygen concentration fell below 0.2 ppm after 24 h (data not shown). Averaged $1/R_p$ for coupons suspended in seawater and at the biodiesel/seawater interface indicated that corrosion rates decreased (because of oxygen removal) from 5×10^{-4} to 10^{-6} Ω-cm^{-2} over the first 10 days of exposure (Figure 2a). On day 12, $1/R_p$ corrosion rates began to increase, reaching peak values of 10^{-3} Ω-cm^{-2} by day 18, representing an increase of 3 orders of magnitude. Subsequently, $1/R_p$ corrosion rates for both coupon positions remained constant (10^{-3} Ω-cm^{-2}) until the conclusion of the exposure on day 60. In comparison, carbon steel coupons exposed to anaerobic Key West seawater in the absence of biodiesel resulted in $1/R_p$ corrosion rates an order of magnitude lower (10^{-4} Ω-cm^{-2}) after 60 days.[20]

At the conclusion of the exposure, the chamber was opened and a very strong sulfide odor was evident. The seawater H_2S concentration was 6 ppm. Black deposits were obvious on all coupons (all positions) (panels b−d of Figure 2). The intensity of the blackening and distribution of deposits varied with the location within the exposure tank. All coupons exposed to seawater were uniformly black, whereas deposits on coupons exposed in biodiesel alone were localized and coupons at the interface were a combination of the two extremes. Subsequent analysis of all of the coupons by EDS demonstrated the presence of sulfur and chloride (presumed chloride). After acid cleaning, pitting was observed in coupons at all exposure locations (panels e−g of Figure 2).

Metabolite Profiling. Mass spectral analysis of biodiesel showed that linoleic (9,12-octadecadienoic), oleic (9-octadecenoic), stearic (octadecanoic), and palmitic (hexadecanoic) methyl esters were the primary components, typical of soy-based biodiesel.[3] Fatty acids were below detection limits. In non-sterile incubations, a complex suite of even numbered saturated fatty acids ranging from C_6 to C_{24} chain lengths and a few odd-numbered saturated fatty acids were observed (Figure 3), whereas in sterile controls, only C_{16}−C_{24} methyl esters were detected.

Discussion

A common feature of the five diverse anaerobic inocula described in this paper was that they could reduce sulfate or produce methane in ≤1 month in biodiesel-amended incubations, well in excess of biodiesel-free or sterile negative controls. Inocula 1 and 2 were obtained from a contaminated

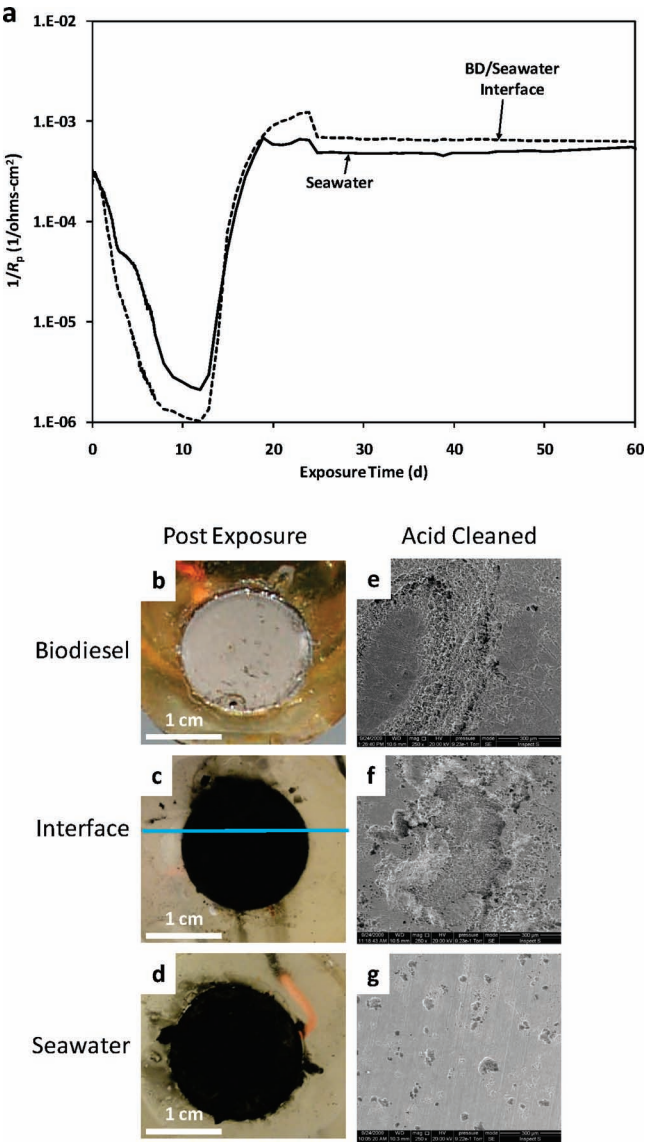

Figure 2. Carbon steel coupons after 60 days of exposure to Key West seawater and biodiesel under anaerobic conditions. (a) Averaged $1/R_p$ instantaneous corrosion for coupons suspended in seawater and at the biodiesel/seawater interface. (b, c, and d) Coupons suspended in biodiesel, at the biodiesel/seawater interface, and in seawater, respectively. Corresponding micrographs (e−g) indicate pitting in each coupon after acid cleaning.

aquifer, whereas inoculum 3 was obtained from the USS Arizona. These three inocula were chronically exposed to hydrocarbons, but none were previously exposed to biodiesel. In fact, the widespread use of biodiesel was almost 30 years after the sinking of the USS Arizona. Inoculum 4 from the ballast tank of a navy ship was likely exposed to biodiesel previously. This presumably explains the higher rate of biodiesel biodegradation for this inoculum. The common feature of these experiments was that all inocula metabolized biodiesel, regardless of their freshwater or marine origins or prior exposure history to biodiesel, over a very short time period, from weeks to 1 month. Over the past 2 decades, it has been recognized that many fuel components are susceptible to anaerobic decay.[28] Because biodiesel is a modern component

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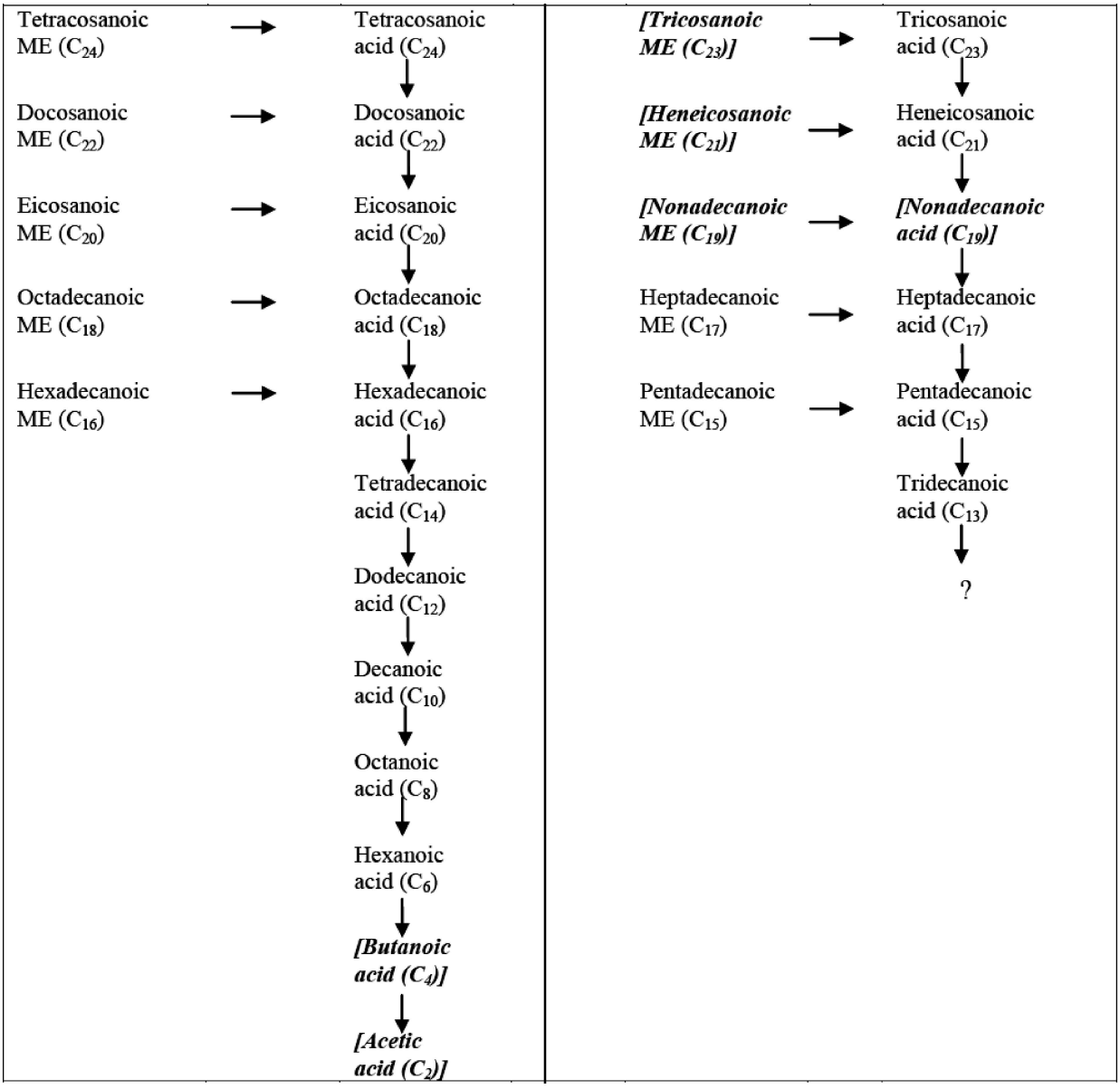

Figure 3. Starting methyl esters in biodiesel and likely corresponding fatty acids detected in incubations from all inocula, except the sample obtained from the USS Gettysburg (not assayed). The left part of the figure shows the even-numbered methyl esters (MEs) and their hydrolysis products. The fatty acids presumably undergo β-oxidation to form a series of smaller molecular-weight counterparts, each two carbon atoms shorter than the precursor metabolite. The right part of the figure shows the odd-numbered MEs and their acid products. The ones that are written in italic and in brackets could not be detected. Unsaturated fatty acids, while detectable, are not illustrated in the figure.

of transportation fuels, it too must be held in similar regard. However, our evidence suggests that this biofuel is far more amenable to biodegradation processes than traditional hydrocarbons.

Indications of the role of microorganisms in fuel decomposition processes can be deduced through the analysis of metabolic intermediates. Identification of the intermediate(s) can often attest to the predominant mode of decay and reveal the particular fuel constituents preferentially degraded.[29,30] This is because diverse life forms exhibit remarkably similar strategies for metabolizing particular substrates. We assayed the metabolites differentially associated with biodiesel biodegradation by GC−MS and found a comparable suite of fatty acids in all incubations. The formation of these acidic intermediates helps explain the accelerated rate of corrosion of metals. This array of fatty acids would be expected if the parent methyl esters were initially hydrolyzed and the resulting intermediates thioesterified and subject to β-oxidation processes. This is the well-known catabolic pathway, whereby fatty acid intermediates are shortened by successive removal of two carbon fragments from the carboxyl end of the molecule as acetyl-CoA residues. The latter components ultimately become mineralized. Presumably, lower molecular-weight fatty acids were also produced and consumed but were not detected by the GC−MS procedures employed. Lapinskiene et al.[31] evaluated the anaerobic degradation of biodiesel in a soil medium and concluded that the

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primary hydrolysis took place between 10 and 20 days (which is consistent with the onset of activity in this study), producing large amounts of butyric acid. We also detected a few odd-numbered saturated fatty acids and their presumed β-oxidation intermediates (Figure 3), but these compounds were quantitatively about 2 orders of magnitude less than even-numbered fatty acids. Presumably, this reflects the relatively low abundance of odd-chain fatty acid methyl esters in the original biodiesel.

It is well-known that many of the methyl esters in biodiesel have some degree of unsaturation.[3] In consistent fashion, we observed several unsaturated fatty acids in our mass spectral profiles (e.g., linoleic and oleic acids) that were presumably formed from the initial hydrolysis of the corresponding methyl esters (data not shown). The presence of unsaturated fatty acids added to the complexity of metabolite profiles observed with various inocula. It is not known if the anaerobic microorganisms initially hydrogenated the unsaturated fatty acids to convert them to saturated compounds before β-oxidation, as previously suggested by Sousa et al.,[32] or merely biodegraded them more rapidly than their corresponding saturated fatty acid counterparts.[33,34]

Prior research suggests that, while the anaerobic metabolism of hydrocarbons in fuels is fairly restricted and requires "specialist organisms",[35] the ability of bacteria, archaea, and even eukaryotes to hydrolyze the component esters of biodiesel is common. Such bioconversions result in the formation of fatty acid intermediates, the metabolism of which has been extensively documented.[32−34] In fact, syntrophic microbial partnerships based on fatty acid metabolism are widespread and obligate for the turnover of many forms of organic matter in anaerobic environments.[32,36]

The analysis of fatty acid components of bacteria, typically detected as methyl esters, has proven to be diagnostic for the identification and differentiation of bacteria at the species level.[37] It is therefore possible that some of the fatty acids detected in our study originated from microbial cells proliferating at the expense of the added organic matter. However, we would not expect nearly the same suite of fatty acids in the diverse array of both freshwater and marine anaerobic incubations if the primary origin of these components was indeed microbial lipids.

To test whether anaerobic methyl ester biodegradation could accelerate the rate of biocorrosion, we immersed carbon steel coupons in biodiesel-amended coastal seawater from Key West, FL (inoculum 5). Experiments demonstrated corrosion of unprotected carbon steel exposed in either phase of a two-phase biodiesel and seawater combination under anaerobic conditions. Corrosion in biodiesel was influenced by the presence of seawater. In previous work[38] with distilled water (simulating water of condensation), alloy exposures to B5, B20, and B100 indicated the lowest corrosion rates for carbon steel. Visible biofouling was observed in all biodiesel/distilled water combinations. In the current work, sulfur and chloride from the seawater were located on surfaces exposed to biodiesel. These elements were detected by EDS on biodiesel-exposed electrodes, suggesting that chloride and sulfur species diffused through the hydrophobic phase and came into contact with the electrodes. These results indicate that seawater in the fuel condensed at localized areas on the electrode surfaces and in combination with chloride and/or sulfide, caused pitting. In general, black corrosion products were associated with an elevated sulfur concentration. On the basis of prior work,[21,22] the black corrosion products were likely iron sulfides.

Conclusion

The increasing world reliance on biodiesel has important environmental and financial consequences. Our studies suggest that biodiesel can be quite easily hydrolyzed and converted to a variety of fatty acid intermediates by anaerobic microorganisms, regardless of their previous hydrocarbon- or biodiesel-exposure history. The acidic nature of these intermediates accelerates the pitting corrosion process of the most common metal alloy used throughout the fuel infrastructure.[39] The corrosion of pipelines, tanks, storage units, and associated equipment increases the risk of the release of hazardous materials to the environment, with concomitant pollution issues. With the widespread use of biodiesel as an additive to fuel supplies, it is at least prudent to consider how best to avoid the negative consequences associated with the microbial metabolism of these labile fuel components.

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