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Oxygenated gasoline biodeterioration and its control in laboratory microcosms

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Abstract

An array of microcosms containing California Air Resources Board (CARB)-compliant, oxygenated 87-octane gasoline over nutrient-amended water was monitored over a 7-month period. The array included triplicate microcosms of each of four conditions: unchallenged control, challenged control and challenged with two different antimicrobial agent treatments. After 7 months, significant fuel chemistry and physical changes occurred in all the microcosms that were challenged with an uncharacterized microbial inoculum drawn from a contaminated fuel system. Most noteworthy was the average 67% loss of oxygenates and the marked shift from isoparaffins and normal paraffins to alkyl isoparaffins, coupled with a shift to higher carbon numbered compounds. Moreover, in the untreated, challenged control microcosms, mild-steel corrosion rates were approximately double, and filter-plugging rates were greater than four times those observed in the unchallenged control microcosms. Both antimicrobial agent treatments attenuated the physical and chemical changes. There were no significant physical or chemical changes in the unchallenged control microcosms, indicating that physical weathering during the test period played only a minor role in the changes. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Microbial growth at the expense of distillate petroleum fuels has been recognized for over a century (Atlas, 1984). At least three monographs address the topic of fuel microbiology (Beerstecher Jr, 1954; Davis, 1967; Atlas, 1884). Although there have been reports of gasoline biodeterioration (Hill and Koenig, 1995) most research has addressed middle distillate fuel biodeterioration (Littmann, 1980; Hill, 1984; Smith, 1988, 1991; Neihoff, 1988).

During the period 1992–1996, one of the authors (Passman) surveyed approximately 400 refinery, terminal and retail outlet storage tanks, ranging in size from 37.8 to 31,800 m³. Approximately 60% of all gasoline tanks surveyed contained significant levels of microbial contamination as evidenced by enzymatic activity, viable recovery methods and the presence of an intermediate zone (rag layer) between the fuel and bottom-water layers. However, the field surveys did not determine whether the

observed levels of microbial contamination affected the commercial value of the fuels in contaminated systems. The present study was designed in order to determine whether microbial contaminants growing primarily in microcosm rag layers and bottom waters altered the chemistry of the overlying fuel significantly. Additionally, the experimental design to evaluate the effect of two antimicrobial pesticides presently approved for use in on-highway fuels in the United States (US EPA, 1994).

2. Materials and methods

2.1. Chemicals

We used California Air Research Board (CARB) Phase 2 compliant, regular unleaded gasoline (RUL) for all microcosms. ASTM D 4814 (ASTM, 1999) defines properties of this fuel. This gasoline was augmented with 12% (w/w) of an oxygenate blend comprised primarily of methyl tertiary-butyl ether (MTBE) and tertiary

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amyl methyl ether (TAME). Sufficient product was obtained from a single production run to ensure that all testing was performed using the same fuel.

The two antimicrobial agents tested were methylenebis-thiocyanate (MBT, Buckman Laboratories, Memphis TN) and a blend of 4-(2-nitrobutyl)morpholine and 4,4'-(2-ethyl-2-nitrotrimethylene) dimorpholine (NMEND; ANGUS Chemical, Buffalo Grove, IL). MBT was tested at 200, 100 and 50 ppm (as supplied; 40, 20 and 10 ppm a.i.). NMEND was tested at 1000, 300 and 135 ppm (as supplied; 850, 255 and 115 ppm a.i.). These doses represent the minimum, maximum and mid-range concentrations recommended by the respective manufacturers.

2.2. Organisms and microcosms

Aliquants (1 ml) from a stock solution mixture containing microorganisms collected and pooled from each of several contaminated gasoline tank bottom-water samples were used to inoculate a French-square bottle. Each bottle contained 300 ml oxygenated, regular unleaded gasoline (RUL) over 100 ml synthetic bottom water (SBU). The SBU was formulated from deionized water augmented with 3 g/l Instant Ocean (Aquarium Systems, Mentor, OH; synthetic bottom-water — SBW) inorganic salts (nitrogen and phosphorous-free) and 100 mg/l sodium sulfate (to support sulfate-reducing bacteria). Within a week, a characteristic third layer developed between the fuel and water phases. Periodically, the bottom water was tested for catalase activity and viable cell recoveries. The HMB IV system (BioTech International, Houston, TX) and Liqui-Cult broths (MCE, Lake Placid, NY) were used to determine catalase activity and most probable numbers (MPN) (Passman et al., 1995). At intervals not exceeding 1-month, 75 ml of the bottom water was replaced with fresh water for the first 3 months of the study (no changes were made between 3 and 7 months). Make-up SBU salt and sulfide concentrations were adjusted to maintain 3 g/l salt and 100 mg/l concentrations. To challenge test microcosms, an aliquant providing the equivalent of 1 ml of 16 psig catalase activity ($\sim 10^6$ – 10^7 MPN/ml) was transferred to the test microcosm.

Twenty-one 1 liter French-square jars were filled with 300 ml RUL and 100 ml SBW at the initiation of the experiment. Triplicate microcosms were prepared for each of seven biocide treatments, including biocide-free controls. An additional microcosm was set up after 30-day biocide performance data indicated that the strongest biocide concentrations were not inhibiting microbial growth completely. This additional microcosm was identical to the other microcosms, except that neither biocide nor microbial challenge population was added. This unchallenged control microcosm, prepared at the 30-day time point, was capped and shaken vigorously, then allowed to stand until sampled at the 7-month time point. Data listed under

“Unchallenged” or “Unchallenged Control” came from this microcosm.

All microcosms, except for the aforementioned unchallenged control, were handled identically throughout the study. At each sampling point, microcosms were vented, 75 ml bottom-water samples were removed and replenished with 75 ml fresh SBW containing fresh inoculum. All microcosms were stored in the dark at room temperature $20 \pm 2^\circ\text{C}$.

2.3. Gross observations

Microcosms were observed and samples were drawn after 1 week and 1, 3 and 7-months. Gross changes were documented photographically. In addition, the formation and appearance of interfacial and bottom-sediment layers, color and turbidity changes were recorded.

2.4. Corrosion testing

To evaluate the impacts of microbial contamination on corrosion rates, standard corrosion coupons were placed into the aqueous phase of each test system. The coupons were completely immersed in the water phase with the maximum surface area (4.5 in^2) parallel to the interface layer maximum surface. At the end of 7 months, the corrosion coupons were harvested and processed to determine the corrosion rate and provide physical examination assessment of corrosion processes.

2.5. Fuel filterability

Samples of fuel were filtered through $0.22 \mu\text{m}$ GS Millipore membrane filters (Millipore Corp., Bedford, MA) under 4.3 kPa vacuum. Sequential 5 ml samples were added to the filtration units until the filter plugged as determined by no further flow through the membrane. The volume of fuel able to be filtered is referred to as the *plugging volume*. Due to limited sample volume, we used only 600 ml to test all treatments except for the unchallenged microcosm. We filtered 1 liter of unchallenged microcosm fuel. Two replicate samples were filtered for each treatment.

2.6. Chemical analysis

Bottom water pH was measured using a Corning pH meter, total dissolved solids (TDS) with a VWR digital conductivity meter, and dissolved oxygen (DO) with an OM-1 oxygen meter and MI730 modified oxygen electrode (Microelectrodes, Inc., Bedford, NH). Hach (Loveland, CO) test kits were used to determine alkalinities. Nitrate and nitrite concentrations were determined using Reflectoquant TM Analysis System (EM Sciences, E. Merck, Darmstadt, Germany). Gasoline hydrocarbon

analyses were performed using a proprietary Chevron detailed hydrocarbon analysis (DHA) method. The method was similar to the Canadian Government Standard Board high-resolution gas chromatographic method (CGSB Method No. 14.3-94) and the method described by Schubert and Johansen (1993). We used either a SE30 or DB1 column with the stationary phase of 100% methyl silicon and a temperature programmed operating mode (-30° – $+250^{\circ}$ C) on a Hewlett Packard 5890 GC.

2.7. Microbiological analyses

As noted above, microbial activity in fuel and bottom water samples was measured using the catalase test. Chemoorganotrophic bacterial (COB) population densities were determined by inoculating Liqui-Cult broths with 1 ml samples, serially diluting (1:10) and observing color reactions after 24, 48 and 72 h incubation at room temperature. Acid-producing bacteria (APB), total anaerobic bacteria (TAB) and sulfate reducing bacterial (SRB) population densities were determined using MIC-KIT (BioIndustrial Technologies, Inc., Georgetown, TX) test vials. Appropriate media were inoculated with 1 ml of sample then observed for up to 1-month for turbidity and/or color change in accordance with the manufacturer's recommendations.

3. Results

As noted above, two of the three concentrations tested for each antimicrobial pesticide reflected the lowest and highest treatment rates approved under their respective US EPA pesticide registrations. The third concentration represented the respective manufacturer's "typical" treatment dose as described in their product literature. Consequently, since the maximum permitted concentration of NMEND is 850 ppm a.i., and the maximum allowable concentration of MBT is 40 ppm a.i., their respective performance is assessed based on the relative efficacy of maximum allowable dosages, rather than equivalent active ingredient concentrations. Only the highest concentration of the two antimicrobials inhibited fuel biodeterioration after 7 months. Consequently, only data for 40 ppm a.i. MBT and 850 ppm NMEND are presented below.

3.1. Gross observations

Except for some fuel-phase darkening, 7-months of storage over water had no gross impact on RUL. In contrast, the gross characteristics of challenged RUL microcosms began changing within a week after inoculation. Fig. 1 demonstrates the gross changes that occurred in the challenged microcosms over time. Within 1-week, 0.1–0.2 mm diameter clumps began to develop at the fuel–water interface. By 1-month, a semi-consolidated 0.1–0.2 mm

interfacial layer (IL) has formed. The IL darkens with time, and by 3-months, the 1–2 mm thick IL is dark-orange to reddish-brown. A bottom sediment layer has also formed at 3-months. By the end of the study, at 7-months, the challenged microcosms have turned black.

Although the fuel-phase ASTM haze rating never exceeded 2 in the unchallenged microcosms, it was >3 in all challenged microcosms by the end of month 3. By the end of the study, haze ratings are >5 for all challenged microcosms.

Although an intermediate layer (IL) eventually formed in all microcosms, both antimicrobials delayed IL development and attenuated terminal IL volume. Moreover, both biocides reduced the extent of fuel and water-phase turbidity and color changes (Fig. 2). Universally soluble NMEND was more effective than water-soluble MBT in inhibiting gross product and bottom-water changes.

Fig. 3 illustrates the effect of microbial contamination on carbon steel coupons corrosion rates (0.2 – 0.4 ± 0.05 mil/yr) in unchallenged control microcosms. There was no gross evidence of corrosion on the coupons after 7-months exposure. Coupons from challenge microcosms had corrosion rates of 0.8 ± 0.06 mil/yr. Although no pits were present, surface granularity has increased substantially.

Neither of the biocides affected bottom-water corrosivity, significantly, relative to the untreated control. In all microbially contaminated microcosms, carbon steel coupons, in the water phase corroded at an average rate of 1.0 ± 0.16 mils/yr. Fig. 4 compares untreated, 850 ppm a.i. NMEND and 40 ppm a.i. MBT treated microcosm corrosion coupon surface appearances. There is no gross evidence of microbially influenced corrosion on any of the coupons. However, the MBT-exposed coupon showed significant pitting.

3.2. Filterability

Table 1 compares fuel filterability among treatments at $T_{7\text{-months}}$. The presence of microbial contamination caused RUL gasoline to plug filters with significantly less volume of gasoline (419 ml + 69 ml) than RUL gasoline from the unchallenged microcosms (>1000 ml). Thus filter plugging occurred at least twice as fast as when no microbial contamination was present. Both antimicrobials protected fuel filterability.

3.3. Bottom-water chemistry

Except for pH, bottom-water chemistry data were obtained for challenged microcosms only during the first 3 months of the study. Table 2 summarizes the initial and 3-month bottom-water chemistry data. The pH did not change significantly during the first 3 months. However, by the end of 7-months, it had fallen to 6.1. NMEND