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
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 methylene-bis-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

Aliquots (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 (≈10^8–10^9 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 ± 2°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 µm 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 M730 modified oxygen electrode (Microelectrodes, Inc., Bedford, NH). Hach (Loveland, CO) test kits were used to determine alkalinitities. 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°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 (Biindustrial 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 was 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 T7 months. The presence of microbial contamination caused RUL gasoline to plug filters with significantly less volume of gasoline (419ml+69ml) than RUL gasoline from the unchallenged microcosms (>1000ml). Thus filter plugging occurs occurred at least twice as fast as when no microbial contamination is 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
Fig. 1. Fuel deterioration in challenged, untreated simulated gasoline storage tank microcosms.

Fig. 2. Fuel deterioration inhibition, effects of two antimicrobial pesticides.

Fig. 3. Carbon steel corrosion in challenged, untreated microcosms.
Treatment contributed significantly to bottom-water alkalinity. Water-bottom pH increased over time in challenged control microcosms, remained stable in NMEND-treated microcosms, and decreased in MBT-treated microcosms. Bottom-water alkalinity increased from $85 \pm 4.8$ mg CaCO$_3$/l at $T_0$ to $5.973 \pm 500$ mg CaCO$_3$/l at $T_{1\text{-month}}$. The alkalinity increase may have reflected partitioning of weak-base RUL molecules. After $T_{1\text{-month}}$, alkalinity decreased to $2887 \pm 90$ mg CaCO$_3$/l. Bottom-water total dissolved solids (TDS) concentration increased from $3.3 \pm 0.25$ g/l to $3.6 \pm 0.02$ g/l during the first month, then plateaued between $T_{1\text{-month}}$ and $T_{3\text{-months}}$. Dissolved oxygen remained at 1% saturation ($0.1$ mg O$_2$/l) throughout the study. Neither nitrite nor nitrate were detectable at $T_0$. However, at $T_{1\text{-week}}$, 5.7 mg NO$_3$ and 7 mg NO$_2$ were detected. By $T_{1\text{-month}}$ and for the remainder of the study, neither nitrate nor nitrite were detected in bottom-water samples. Neither biocide affected TDS increases in microcosm bottom water. Nitrate and nitrite appearing in NMEND-treated bottom water probably reflect the partitioning of some NMEND into the water-phase.

### 3.4. Gasoline chemistry

At $T_{3\text{-months}}$, RUL chemistry from both challenged and unchallenged microcosms did not differ significantly from that of fresh RUL. However, RUL composition changed significantly between 3 and 7 months. Fig. 5 depicts fuel mass changes due that occurred over the course of 7 months. All fuel component changes presented in this paper have been corrected for the total mass loss. This was done by using the C8+ aromatics as an internal marker, since these molecules are not biodegraded.
The oxygenate blend originally contained 75% (w/w) MTBE (8.6% of total RUL) and 24% (m/m) TAME (2.8% of total RUL), for a total oxygenate content of approximately 12% of total RUL. There was no significant change in either the total oxygenate concentration or the component ratios in the unchallenged controls (Fig. 6). In the challenged controls, final MTBE and TAME concentrations were 1.3% (m/m) and 2.8% (m/m), respectively. Approximately 85% of the MTBE had been removed from the bulk RUL.

Fig. 7 illustrates the significant shift from C5, C6, and C7 molecules (21, 23, and 21% of total RUL weight, respectively, at 0-months; 1.3, 9.3, and 26% at 7-months) to C5, C6, and C7 molecules (21, 18, and 10% at 0-months; 26, 30, and 21% at 7-months) in challenged microcosms. Carbon number distribution in unchallenged RUL was indistinguishable from that in fresh RUL. Both antimicrobials inhibited C5 to C7 losses. At 850 ppm a.i., NMEND inhibited C5 and C6 losses by 40%. MBT inhibited these losses by 20%. Since bottom-water samples were not analyzed for oxygenates, we could not determine whether microbial activity mediated MTBE extraction into the water-phase or actual MTBE conversion. Although volatilization and partitioning were not quantified, the correction for total mass loss in the unchallenged control indicates that these losses were small, at least between months 3 and 7.

Fig. 8 summarizes the relative losses of major RUL constituents. The percentage values above the T7-month bars represent the fraction of original concentration. Biodeterioration resulted in 70% losses for normal and iso-paraffins and cyclopentanes. Aromatics were depleted by approximately 14%. Both antimicrobials inhibited RUL component loss (Fig. 9). At 850 ppm a.i. NMEND showed greater loss inhibition than 40 ppm a.i. MBT for each of the six hydrocarbon groups. NMEND efficacy ranged from 40% normal paraffin loss inhibition to 70% cyclohexane loss inhibition.

3.5. Microbiology

Microbiological data are presented in Fig. 10. Untreated microcosm, water phase, COB recoveries remained at 10^5 MPN/ml from 0 to T90,25-month then decreased to 10^4 MPN/ml for the balance of the study (Fig. 10a). Aerobic bacteria were not recovered from fuel-phase samples until T7-months (Fig. 10b). By the end of the study, fuel-phase, aerobe viable recoveries reached 10^1-10^2 MPN/ml. Water-phase, acid producer recoveries at T7-months were a log greater than at 0 (Fig. 10c). From the end of the first week through the balance of the 7 month period, acid producer recoveries remained stable at 10^3 MPN/ml (Fig. 10d). Fig. 10e shows the variability of the water-phase SRB data. Recoveries ranged from < 10 to 10^5 MPN/ml, with no apparent relationship to microcosm age. Fuel-phase SRB recoveries remained between 10^4 and 10^6 MPN/ml throughout the study (Fig. 10f).

Catalase activity provides a rough estimate of the general intensity of metabolic activity in liquid samples. The
catalase activity trend (Fig. 10g) in the water phase roughly followed the fuel-phase total aerobe, acid producer and SRB trends. By the end of the first month, it had fallen from an initial 15–2 psig. Catalase activity increased between $T_{1\text{-month}}$ and $T_{3\text{-months}}$, and continued to increase though the end of the study. Fuel-phase catalase activity began increasing after the third month (Fig. 10h).

After 7 months, the lowest doses of each biocide provide >50% log inhibition of acid-producer viable recoveries (data not shown). At maximum dosing, both NMEND and MBT inhibit water-phase acid producer recoveries (Fig. 10c) for the full 7 months. In the fuel phase, NMEND loses its efficacy after 3 months, and MBT fails sometime between 3 and 7 months.

As noted above, SRB recoveries appear to be more a function of the sampling date than any antimicrobial treatment (Fig. 10e and f). At 1-week and 7-months >100 MPN SRB/ml were recovered from all treated microcosms, but <1 MPN SRB/ml (detection limit) were recovered from untreated control microcosms. Given the variability of the data, no assessment of antimicrobial performance against SRB in either the water or fuel phases was possible.

Catalase activity decreased, over the first week, in all treated microcosms (Fig. 10g). At 1 and 3-months, catalase activity was relatively low in all microcosms, including the untreated controls. At 7-months, both 850 ppm (a.i.) NMEND, and 40 ppm (a.i.) MBT still inhibited catalase activity in both fuel and water phases (Figs. 10g and h). Until $T_{7\text{-months}}$, fuel-phase catalase activity in treated microcosms was indistinguishable from the low activity levels observed in the untreated controls (Fig. 10h). At $T_{7\text{-months}}$, 850 ppm (a.i.) NMEND provided >90% catalase activity inhibition, relative to challenged, untreated controls. 40 ppm (a.i.) MBT inhibited catalase activity by >75%.

4. Discussion

Biodeterioration of gasoline and other petroleum products is well documented (Davis, 1967; Hill, 1984; Watsonson, 1987). However, in most previous reports, the focus has been on incidental biodeterioration processes. These include, primarily biomass accumulation; biosurfactant production, organic acid production and sulfate reduction (Smith, 1988, 1991). The principal symptoms of these activities are filter plugging, increased water partitioning into the fuel-phase, increased fuel and bottom-water corrosivity, and fuel souring. Actual petroleum product bioconversion is generally considered only in the context of bioremediation (Borden et al., 1997).

Nearly a decade ago, Shennan (1988) listed refinery process, additive and distribution channel changes as causes for increased concern about fuel biodeterioration. In January 1995, the US Environmental Protection Agency’s new fuel and fuel additive regulations (U.S. EPA, 1994) went into effect. The reduced aromatic content and increased use of non-hydrocarbon additives, including oxygenates has made fuels more susceptible to biodeterioration. Industry distribution consolidation coupled with increased product demand has resulted in a net annual throughput rate increase of 7–11% over the past several years (Anonymous, 1997a,b,c). This means that product
at increased biodeterioration risk is increasingly likely to reach engine-operating customers.

In this context, we undertook to evaluate the potential effect of uncharacterized microbial populations on, post-1995, RUL product integrity. Our microcosm design reflected both fiscal and physical constrains on the experimental design. With the possible exception of sea water ballasted fuel tanks, a 3:1 fuel to water ratio is unlikely to be seen in any petroleum distribution tankage. However, since the data were intended primarily to provide direction for routine condition monitoring, the limitations of the microcosm design were deemed acceptable. In a larger microcosm, or in-service tank, fuel stratification would be expected. The types of physical and chemical changes reported in the current investigation might only be detected in the near-interface fuel. This stratification reflects the fluid dynamics of most fuel service tanks. Typically, the top layer, representing > 95% of the total tank volume is exchange at frequent intervals. Product turnover rates at distribution facilities are on the order of 1–2 weeks per volume exchange. Retail service tanks may turnover product within 24–96 h. Generally, the bottom 1% tank volume is quiescent. Bottom-water, sludge and sediment settle in this layer. Normally, the fuel-water interface lies within this quiescent zone. Between the quiescent zone and the rapid exchange zone there is a transition zone. This zone’s dimensions depend on tank geometry, fitting location and design, fluid fill and suction flow velocities and other variables that define the tank’s fluid dynamics. Biodeteriogens produced within the fuel–water interface biofilm are subject to both diffusion and turbulent mixing. The relative importance of these phenomena changes as biodeteriogens move closer to the high-turnover zone. The microcosms used in this investigation most closely simulated quiescent-zone conditions.

Typical of findings from bioremediation studies (Krumholz et al., 1996) the challenge population had little effect on fuel chemistry for the first 3-months of exposure. The gross changes we observed were typical of those previously reported by others (Rogers, 1940; DeGray and Killian, 1952; Park, 1975).

Dramatic fuel property changes did occur after the third month. The microbial community mediated the removal of nearly 80% of the oxygenate. This is one of few investigations that report microbially mediated MTBE removal from fuel, although others (Salanitro et al., 1994; Mo et al., 1997; Eweil et al., 1997) have reported MBTE biodegradation in aqueous systems or soil microcosms. In addition, the community mediated significant shifts in hydrocarbon chain-length and chemical class distributions. These changes affected the fuel’s gross properties, including API gravity, average molecular weight, heat of combustion and vapor pressure. Once the changes began to occur, the rates of change were comparable to the fastest bioconversion rates reported in the bioremediation literature (Borden et al., 1997). At present, 68 biocides are approved for use in fuel applications under Federal Insecticide, Fungicide and Rodenticide regulations (Anonymous, 1996). However, only two are also approved for use in on-highway fuels under the 1994 revisions to the Clean Air Act (CAA) Fuel and Fuel Additives Regulations (US Environmental Protection Agency, 1994). Both
Fig. 8. Major RUL constituent losses in challenged, untreated microcosms.

Fig. 9. Effect of antimicrobial pesticides on major RUL constituent losses.
antimicrobials meet 40 CFR 79 baseline chemistry requirements (contain only carbon, hydrogen, oxygen, nitrogen, or sulfur - CHONS). Manufacturers of both have registered their products under both CAA and FIFRA regulations. No other fuel treatment biocides meet these criteria. There may well be alternative antimicrobial pesticides that would be more efficacious than either of the two products included in this investigation, but are either not approved as industrial pesticides in the United States, do not meet the US EPA's substantially similar chemistry criteria or both.

As reported previously (Passman and Pohlman, 1992), NMEND is both fuel and water-soluble. Its fuel to water partition coefficient is 26:1, with approximately 1.5% solubility in water. The commercial product is nominally 85 to 95% a.i. Its pH ranges from 9.5 to 10.0. In contrast, MBT is only marginally soluble in non-polar solvents like fuels. Commercial, aqueous and glycol-based formulations containing 2.5–20% MBT a.i. are available. The pH range for the glycol-based, 20% a.i. MBT formulation used in this study was 5 to 7. Its oil to water partition coefficient has not been tested.

Since no attempt was made to obtain a material balance, and the effects of volatilization and partitioning were not quantified, we reported the results from biocide treated microcosms relative to those in the challenged, untreated controls. This approach should normalize the data for any of the variables that we did not measure directly. The authors contend that it is reasonable to assume that for identically handled microcosms, any non-biological factors contributing to the observed changes would have essentially the same effect in each test system. Consequently we are confident that the changes we have reported are the result of microbial activity, not physical–chemical processes.

Although this report focuses on biocide performance, another objective of the research was to evaluate the predictive value of relatively simple, field-adaptable test
methods. The methods selected for use in this study offered the most promise, based on extensive preliminary testing (not reported). However, as the data reveal, none of the methods is without its limitations. The relationship between water-phase microbial and chemical changes, and fuel property changes was less clear-cut than the authors had anticipated. However, in the fuel-phase there was a direct relationship between both catalase activity and viable microbe recoveries and biodeterioration.

Reflecting their relative water-solubility characteristics, MBT generally was more effective than NMEND against water-phase, microbial growth. In the fuel-phase, NMEND generally provided better growth and activity inhibition. However, only 850 ppm (a.i.) NMEND inhibited catalase activity at T_{1/2months} in both fuel and water phases.

Defining sub-populations as viable recoveries in each of the different growth media, neither biocide appeared to be equally effective against all sub-populations. Further research is needed to determine whether the RUL biodeterioration we observed was mediated primarily by one or two species present in our uncharacterized, mixed inoculum, or, if a true consortium mediated it. Microbial communities are often capable of mediating processes that individual species cannot (Hill, 1984).

Shennan (1988) has reviewed microbial contamination control strategies. Recognizing that biocide use could be effective only if it was incorporated into a comprehensive contamination control practice, she listed 10 criteria defining the "ideal" fuel biocide. Several of Dr. Shennan's criteria were open-ended, reflecting her recognition that definitions of such terms as cost-effectiveness, storage-stability and wide anti-microbial spectrum are situational.

5. Conclusions

This investigation demonstrates unequivocally that uncontrolled microbial growth can affect bulk fuel chemistry and performance properties. However, it also raises a number of critical questions. Product turnover rates in most market distribution tanks, from the refinery to the retail outlet, are measured in days, not months. In most systems, there is a quiescent zone into which water and particulates settle (1–5% of the total volume). Between the quiescent zone and the dynamic product turnover zone there is a transition zone. Within this zone, sheer forces contribute to co-mingling between fresh and aged product. This co-mingling is a significant potential mechanism for transporting biodeteriogens downstream. Further research is needed to determine the effect of adding fresh product to systems in which mature (older than 3-months) biodeterioration processes are active. What are the biodeterioration rates for the freshly exposed product? Is there sufficient biodeteriogen carry-over to support continued product degradation after exposed fuel has been transferred from a microbiologically contaminated tank to the next stage in the petroleum distribution system?

In this investigation, we evaluated two products for their performance under conditions simulating RUL near-bottom zones. Although neither inhibited biodeterioration completely, for 7-months, both products reduced biodeterioration substantially. Of the two products, NMEND was more effective than MBT in inhibiting RUL biodeterioration. Moreover, combustion, emission, and fuel and additive compatibility data have been reported only for NMEND (Passman and Pohlman, 1992), not for MBT.

More research is needed to define cost-effective treatment strategies, and to better understand the dynamics of fuel biodeterioration.

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