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Final Report

**Corrosion in Systems Storing and Dispensing Ultra Low Sulfur Diesel (ULSD), Hypotheses Investigation**

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To

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August 13, 2012

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ACRONYMS AND ABBREVIATIONS

ATG automatic tank gauging bp base pair

CDFA Clean Diesel Fuel Alliance

DNA deoxyribonucleic acid

EPA U.S. Environmental Protection Agency

GC-MS gas chromatography-mass spectrometry

IC ion chromatography

ICP-MS inductively-coupled plasma mass spectrometry

LSD low sulfur diesel

MIC microbial influenced corrosion NCH Nationwide Children’s Hospital PCR polymerase chain reaction

PEI Petroleum Equipment Institute ppm parts per million

QA quality assurance

QAPP Quality Assurance Project Plan

QC quality control

SOP standard operating procedure

STI Steel Tank Institute

STP submersible turbine pump

TAN total acid number

taxID taxonomic identification

ULSD ultra low sulfur diesel UST underground storage tank UV ultraviolet

WGA whole genome amplification

XRD x-ray diffraction

XRF x-ray fluorescence

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**Corrosion in Systems Storing and Dispensing Ultra Low**

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Executive Summary

Severe and rapid corrosion has been observed in systems storing and dispensing ultra low sulfur diesel (ULSD) since 2007. In addition, the corrosion is coating the majority of metallic equipment in both the wetted and unwetted portions of ULSD underground storage tanks (USTs). To investigate the problem in an objective manner, multiple stakeholders in the diesel industry, through the Clean Diesel Fuel Alliance, funded this research project. The design included the identification of retail fueling sites and the development of an inspection and sampling protocol to ensure uniform and thorough inspections of USTs. Fuel, water bottoms, vapor, bottom sediments, and scrape samples were taken from six sites: one that was not supposed to have symptoms (but did to a much lesser degree) and five that were to have the severe corrosion. Then, samples from the inspections were analyzed for genetic material and chemical characteristics. These data, in combination with information on additives, have allowed Battelle to draw conclusions with respect to three working hypotheses.

Specifically, the hypotheses are:

1) Aerobic and anaerobic microbes are producing byproducts that are establishing a corrosive environment in ULSD systems;

2) Aggressive chemical specie(s) (e.g., acetic acid) present in ULSD systems is(are)

facilitating aggressive corrosion; and

3) Additives in the fuel are contributing to the corrosive environment in ULSD systems.

All of the sites inspected contained microbes, although at different abundances. The dominant organism identified from three of the sites, *Acetobacter*, has characteristics pertinent to the corrosion observed in all of the sites, such as acetic acid production, ethanol utilization, low pH requirements, and oxygen. Although geographically on opposite sides of the country, with different fuel supplies, and from relatively new construction materials, the presence of the organisms was relatively uniform. The traditionally expected organisms, hydrocarbon degrading organism were found in insignificant abundances. This indicates that the inspected ULSD USTs are selective environments for these specialized, acetic acid producing organisms.

Of note from the chemical analyses is that acetic acid was found to be ubiquitous (water bottoms, fuel, vapor, and scrapings) in all of the sites inspected. In addition, components necessary for the organisms identified to proliferate were analytically determined to be present: trace amounts of ethanol, low pH, oxygen, and water were present in the diesel USTs inspected.

Although additives could play a role in the corrosive environment, it has been determined that they are not a primary cause of the observed corrosion. Acetic acid is believed to be a prime

contributor of the corrosion. The main circumstances around the production of the acid could be thriving *Acetobacter* microorganisms. Finally, there needed to be a mechanism to disperse the acetic acid into the vapor portion of the USTs. Acetic acid has a higher vapor pressure than diesel fuel (0.5 psi compared to 0.1 psi). As a result of this and due to turbulent mixing during fuel deliveries, the acetic acid can be dispersed throughout the upper portions of the tank. Through the wetting and drying cycles (between deliveries), the acid concentrates on the USTs equipment causing severe and rapid corrosion.

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1. Introduction and Background

To protect public health and the environment, the United States Environmental Protection Agency (EPA) Clean Air Highway Diesel final rule stipulated a 97% reduction in sulfur content of highway diesel fuel beginning in June 20061. Accordingly, diesel fuel was altered so that the sulfur content was reduced from 500 parts-per-million (ppm) in low sulfur diesel (LSD) to 15 ppm normally referred to as ultra low sulfur diesel (ULSD). This rule was implemented with a

phased approach where 80% of the change over occurred in 2006 and the remaining 20% occurred by 2010. It was anticipated that the change to ULSD would impact lubricity, energy content, materials compatibility, and microbial growth2. However, accelerated and increased corrosion was not foreseen as a likely outcome.

Almost simultaneously, the Renewable Fuel Standard established by the Energy Policy Act of

2005 and amended by the Energy Independence and Security Act of 2007 mandated significant increase in the volume of biofuels production. Subsequently, there was an increase in retail stations storing and dispensing ethanol blends and biodiesel. Since then, over 90% of all gasoline is being sold with up to 10% ethanol content.

From as early as 2007, the Petroleum Equipment Institute (PEI) started receiving reports of unusually severe and accelerated corrosion of metal parts associated with storage tanks and equipment dispensing ULSD. Reports include observations of a metallic coffee ground type substance clogging the dispenser filters and of corrosion and/or malfunctioning of seals, gaskets, tanks, meters, leak detectors, solenoid valves and riser pipes. These observations were reported to be occurring in as little as 6 months. The corrosion was reported on the unwetted, or ullage, portions of the tanks and equipment in addition to the wetted portions of USTs. Figure 1 shows representative pictures of ULSD system components with rust-colored deposits as reported from industry stakeholders and as found at retail sites inspected for this study.



**Figure 1. Corroded ULSD equipment: Corroded carbon steel submersible turbine pump (STP) shaft removed from pump housing (left), brass ball float extractor cage plug (middle), aluminum drop tube (right).**

By 2009, the Steel Tank Institute (STI) had collected reports and presented the problem to a diverse group of industry stakeholders which included refining, fuel retailing, end-user, petroleum equipment, biodiesel, and fuel additives representatives as well as ASTM and the EPA. As a result of this presentation the need for more information and further investigation was identified. Having many stakeholders with a wide range of interests made developing an objective and inclusive solution imperative to this time sensitive and potentially costly issue.

As an initial step, the Clean Diesel Fuel Alliance (CDFA) developed a simple, five-question survey and members distributed it to various parties in the diesel fuel industry and to regulators to screen for issues with systems storing and dispensing ULSD. The survey results showed that problems were reported from all regions of the country (not in refineries, pipelines, and not associated with any individual supplier), the problems were not related to the age of the equipment, corrosion appeared the same in liquid and vapor areas, and there was an undetermined relationship between tank volume, throughput and tank maintenance. After the surveys were returned, the CDFA met and a taskforce was formed which subsequently funded this research project to begin an in-depth investigation into corrosion issues in systems storing and dispensing ULSD. The CDFA Task Force included the Association of American Railroads, American Petroleum Institute, Ford Motor Company, National Association of Convenience

Stores, National Association of Truck Stop Operators, Petroleum Equipment Institute, Petroleum

Marketing Association of America and Steel Tank Institute.

2. Objective

The objective of the research project was to establish an understanding of factors leading to corrosion of ULSD storage and dispensing systems. For the purpose of this project, the underground storage tanks (USTs), dispensing systems and diesel fuel constitute a “system”. The research was designed to better understand the interconnectedness of the diesel fuel, additives, water (e.g., water bottoms, water emulsion, etc.), polymers and metals as they relate to the material corrosion and degradation issues.

The first phase of this project was a gathering of the anecdotal reports and limited data points (some cultured sample results and chemical analyses) to investigate the feasibility of the approximately 15 hypotheses proposed by the CDFA Task Force. Appendix A presents the (unsubstantiated) information gathered on all of the hypotheses and organizes them in a prioritization decided upon between the CDFA Task Force and Battelle. The output of the first phase was the down-selection to three working hypotheses, based on the discussion of Appendix A. The objective of this second phase was to gather data specific to the chosen three working hypotheses and conclude with a final hypothesis for the problem.

3. Working Hypotheses

Specifically, Phase 2 of the project was designed to investigate the following three working hypotheses.

i. Aerobic and/or anaerobic microbes are producing byproducts that are establishing a corrosive environment in ULSD systems;

ii. Aggressive chemical specie(s) (e.g., acetic acid) present in ULSD systems is(are)

facilitating aggressive corrosion; and

iii. Additives in the fuel are contributing to the corrosive environment in ULSD systems.

The *first working hypothesis* focused on microbial influenced corrosion (MIC), where microbes are producing metabolites that are corrosive to metals found in fuel storage or dispensing systems (i.e., mild carbon steel). To test this hypothesis, genetic sequencing was used to definitively determine whether microbes are present, which microbes are in the samples from

inspected sites, and whether the microbes have metabolites that could contribute to the corrosion.

The *second working hypothesis* focused on chemical corrosion, where specie(s) present in the ULSD are corrosive to the materials found in the fuel dispensing and storage systems. Testing this hypothesis involved analysis of the chemical constituents present in the fuel, water, and headspace vapor within the USTs. These chemical constituents may be corrosive in nature or may contribute to the production of corrosive species, more specifically, acetic acid.

The *third working hypothesis* postulated that additives are contributing to the corrosive environment directly or indirectly as a source of nutrients to microbes that result in corrosive metabolites. The approach for testing this hypothesis focused on gathering information from additives manufacturers, refineries, terminals, stations, and published literature to understand the potential effect of additives on the overall chemical characteristics of the fuel and headspace vapor within USTs.

4. Experimental Methods

The approaches to validate or disprove two of the working hypotheses required knowledge of the contents of the affected UST systems. The research design included the identification of inspection sites to investigate and the development of an inspection and sampling protocol to ensure uniform and thorough inspections of the sites. Samples from the inspections were then analyzed for genetic material and chemical characteristics. These data, in combination with information on additives taken from literature and discussions with suppliers, have allowed Battelle to draw conclusions with respect to the three working hypotheses.

The study allowed for six sites in total to be inspected — one non-symptomatic site and five sites with severe symptoms. The intent was to compare and contrast the characteristics of the sites

that have been effected to the characteristics of a site that has not been effected. This was adjusted to an analysis of all six sites to each other, since severe corrosion was identified at all of

the sites. The following sections describe the experimental methods used to collect data for this research project.

**4.1 Inspection Site Identification**

The purpose of this task was to identify, recruit, and coordinate with the inspection sites for this investigation. For all of the sites, it also included phone discussions with the on-site point of contacts, gathering general site information, and coordination of the inspections.

To identify the inspection sites, a communication asking for sites to be volunteered along with a questionnaire regarding general site information was developed by Battelle. The CDFA Task Force approached potential inspection site owners/operators through their networks of association members, and six sites were volunteered. Then the site owner/operators were contacted for follow-up conversations pertaining to the sites volunteered. In doing this, the site owners offered other potential sites to the list. The total number of volunteered sites rose to 12. A subcommittee of the CDFA Task Force was formed to discuss and evaluate the volunteered sites. As a result, the group decided that there would be six (6) site inspections - one (1) site that was not showing symptoms of corrosion and five (5) sites with a history of severe, rapidly induced corrosive symptoms located across the continental United States. Of the 12, two sites were reported as non-symptomatic, one with a fiberglass tank and one with a steel tank. The material of the tanks inspected was also a factor that could be controlled and, therefore it was decided that all sites inspected should be fiberglass tanks. Six tanks were chosen because had similar tank size, material, and monthly throughput. They were also chosen for a large range of installation years and for them to be spread across the country geographically, meaning different ages, climates and different supplies of fuel by different routes. It was intended that one of the corroded sites would be replaced with a site from the middle of the country for more geographic diversity. After more searching through known networks of industry representatives, it was decided to move forward with six chosen sites.

Three months after the site recruitment and just before deployment to the site for the inspection, the non-symptomatic site was inspected by the owner/operator and determined to have corrosion problems. Therefore, another site through one of the already-engaged site owners was identified to be the non-symptomatic site for the study. Once the research team was on site at the non- symptomatic site, it was clear that the site was, in fact, experiencing effects of the problem, just not as severely as the other five sites.

The site inspections entailed documenting the extent of corrosion in the UST systems and the fuel circumstances (inventory volume, water bottom height, temperature, etc.). The specific names and identifying information of the six inspection sites were stripped from the results. The sites inspected were identified by their state and numerically as designated in parentheses below. There was:

 One site from North Carolina (NC-1);

 Two sites from New York (NY-1 the non-symptomatic site and NY-2); and

 Three sites from California (CA-1, CA-2, and CA-3).

**4.2 Inspection Procedure and Sample Handling**

An inspection procedure and sample handling plan, called the Quality Assurance Project Plan (QAPP), was prepared to ensure the site inspections were conducted in a uniform manner. Battelle and subcontractor field technicians from Tanknology Inc. followed the QAPP to inspect and sample the fuel, water bottom, and vapor from USTs at the inspection sites. One fuel, one water, and two vapor samples from each site were collected, along with scraping or scale samples from various equipment. The inspection steps were followed as described in the QAPP (Appendix B) and briefly described here.

1. Gather printout data from the Automatic Tank Gauging (ATG) system inside retail station.

2. Open and inspect the fill riser pipe and remove the drop tube.

3. Collect vapor samples.

4. Open all other riser pipes (ATG, ball float, etc.), remove equipment where possible, inspect and sample.

5. Collect the fuel sample, consolidate, and split for chemical laboratory analyses. Filter the fuel for biological analysis.

6. Collect the water bottom (and bottom sediment) samples, consolidate, and split for laboratory analyses. Filter the water for biological analysis.

7. Inspect the inside of the UST with a video camera.

8. Inspect the dispensing systems.

9. Reassemble the system and bring the ATG back on line.

10. Ship the samples to respective laboratories.

After collection, the samples were shipped to the appropriate laboratories, and all analysis data were sent to Battelle.

**4.2.1 Sample Handling**

Samples were collected according to ASTM International protocols ASTM D6469-113 and D7464-084. Filtered samples, scrape samples, and bottom sediment samples were shipped overnight in coolers to Battelle (Columbus, OH) and placed into storage at -80°C in a continuously temperature monitored freezer until use. Liquid samples were shipped by ground to the analytical laboratories directly from the inspection sites. Scrape samples and bottom sediment samples were split and shipped to analytical laboratories once all six inspections were complete. All chain of custody forms were retained by Battelle and are available upon request. It is important to note that the sampling equipment was decontaminated at the end of each

inspection day and allowed to air dry. The ethanol evaporated before the next use; therefore, it is unlikely that the decontamination process contaminated the collected samples with ethanol.

Table 1 summarizes the types of samples acquired during the inspections at each site. A

complete list of samples obtained during the inspections is listed in Appendix C.

**Table 1. Sample Collection and Handling**

|  |  |  |
| --- | --- | --- |
| **Sample Type (Number per site)** | **Tank Location** | **Sample Collection and Handling** |
| Vapor (2a) | Headspace |  100 L vapor sample on sorbent cartridges for carboxylic acid and formic acid analyses |
| Vapor (1) | Headspace |  3 L Tedlar bag for sulfur speciation |
| Corrosion scrapings (multiple) | Equipment with excessive corrosion |  Sterile 50 mL conical tubes placed in plastic sample bags for fouling analyses |
| Fuel (1) | Middle of fuel column; Representative sample from multiple risersb |  Amber glass bottles for chemical analyses (~4 L  total split to multiple bottles)   ~700 to 1000 milliliters (mL) of fuel pulled under vacuum through 0.45 µm filter for biological analysis |
| Water (1) | Bottom; Consolidated sample from multiple risers and multiple deployments of the thief sampler |  Amber glass bottle for chemical analyses (~1 L  total split into multiple bottles)   ~50 to 150 mL of water pulled under vacuum through 0.45 µm filter for biological analysis |
| Sediment (1- if thief sampler clogged while sampling) | Bottom |  Sterile 50 mL conical tubes placed in plastic sample bags for fouling analyses and biological analysis |

a Deviation from QAPP. The GC-MS method used for the vapor samples required two sorbent tubes instead of one.

b Deviation from QAPP. The fuel volumes were not large enough to collect multiple samples from different

horizontal sections of the fuel column.

**4.3 Biological Analysis Method**

The purpose of the biological sampling and analysis was to determine the types of microbes present, the conditions under which they would be expected to thrive, and their potential to produce metabolites that could lead to the observed corrosion.

**4.3.1 DNA Extraction**

Frozen samples were thawed and the entire sample was collected in separate 15-mL sterile conical tubes. For solid mass samples (i.e., sediment) Deoxyribonucleic acid (DNA) was extracted via the Ultraclean® Mega Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) using the manufacturer’s recommended protocol with modifications for sediment extraction (Battelle Standard Operating Procedure [SOP]). For filtered fuel and water samples,

the Meta-G-Nome™ DNA Isolation Kit (Epicentre, Madison, WI ) was used according to manufacturer’s protocols for direct extraction from biomass captured on nitrocellulose filters. Post-extraction cleanup for all samples was performed using OneStep™ polymerase chain reaction (PCR) Inhibitor Removal Kit (Zymo Research Corp., Irvine, CA). Purified DNA samples were analyzed with an ultraviolet (UV) absorbance (NanoDrop™ 200 spectrophotometer, Thermo Scientific, Waltham, MA), Qubit® dsDNA HS Assay Kit, and SYBR® Gold Nucleic Acid Gel Stain according to manufacturer’s protocols (Invitrogen/LifeTechnologies, Grand Island, NY).

**4.3.2 Sequencing**

Numerically coded aliquots of approximately 0.5 to 1 µg DNA per sample were used to create sequencing libraries. First, genomic DNA was fragmented using a Covaris™ S220 Sonicator (Covaris, Inc., Woburn, MA) to approximately 300 base pairs (bps). Fragmented DNA was used to synthesize indexed sequencing libraries using the TruSeq DNA Sample Prep Kit V2 (Illumina, Inc., San Diego, CA), according to the manufacturer’s recommended protocol. Cluster

generation was performed on the cBOT using the TruSeq PE Cluster Kit v3 – cBot – HS (Illumina). Libraries were sequenced with an Illumina HiSeq 2000 at Nationwide Children’s Hospital (NCH) Biomedical Genomics Core (Columbus, OH) using the TruSeq SBS Kit v3 reagents (Illumina) for paired end sequencing with read lengths of 100 bps (200 cycles). Primary analysis (image analysis and basecalling) was performed using HiSeq Control Software version

1.5.15.1 and Real Time Analysis version 1.13.48. Secondary Analysis (demultiplexing) was performed using Illumina CASAVA Software v1.6 on the NCH compute cluster. Sequence data (.fastq files) and quality control (QC) reports for library construction were delivered to Battelle via an external hard drive.

**4.3.2.1 Whole Genome Amplification**

DNA extracts with less than suitable yields of DNA for sequencing were subjected to whole genome amplification (WGA) using the Repli-g UltraFast Mini kit (Qiagen, Valencia, CA) according to manufacturer’s recommended protocols. For samples with less than the required 10 ng of DNA input, 1 µL of DNA extract was added. Products were evaluated by UV-absorbance measurements and agarose-gel electrophoresis.

**4.3.2.2 16S rRNA Gene Analysis**

DNA extracts with less than suitable yields of material for sequencing were also subjected to

PCR amplification to detect bacterial DNA. Primers 27F (5,-AGAGTTTGATCMTGGCTCAG -

3’) and 1492R (5,- GGTTACCTTGTTACGACTT-3’) were used to amplify the 16s ribosomal ribonucleic acid (16s rRNA) gene of bacteria using Phusion High fidelity DNA polymerase (New England BioLabs, Ipswich, MA) with parameters of 98°C for 30s, 35 cycles of 98°C for

10s, 56°C for 30s and 72°C for 60s, followed by 72°C for 5 minutes in a PTC-200 thermalcycler

(Bio-Rad, Hercules, CA). Products were visualized by agarose-gel electrophoresis.

**4.3.3 Bioinformatics**

In order to remove poor quality sequencing data (~1% on the Illumina HiSeq), sequence data were quality filtered such that 80% of the bases had a quality of ≥17 (i.e., the probability of a correct base call was ~98%). Following quality filtering, read files were processed using the Battelle Galileo high performance compute cluster and the Basic Local Alignment Search Tool (BLAST®) (National Library of Medicine, Bethesda, MD). Sequences were searched against the

entire genomic DNA sequences reported in the *RefSeq* database v. 12/04/2011 (NCBI, Bethesda, MD), which contained entries for 2,059,236 sequences. Search results were filtered for sequences with ≥97% identity and sequence length of ≥80 bps. The output from this search resulted in a list of taxonomic identifications (taxIDs), associated organism names, and number of sequences per taxID for each sample. Krona5 v. 2.1 was used to create an interactive comparative chart for viewing the relative abundance of organisms in each sample. A final filtering of the results was performed to include only taxa (species) identified by numbers of hits greater than 0.1% (1:1000) of the total representation per sample.

**4.3.4 Diversity Analysis**

To measure the microbial diversity, the Shannon-Weaver Diversity Index, *H*6, was calculated using Equation 1:

*Equation 1: Shannon Diversity Index*

where *pi* is the proportion of identified genetic sequences for each species in the sample and S is the total number of species identified in each sample. In addition, the relative evenness of the identified organisms was measured by Shannon’s Equitability (*EH*)6 using Equation 2:

*Equation 2: Shannon’s Equitability*

As H approaches zero, a microbial ecosystem is dominated by fewer species. EH values range between 0 and 1, with 1 being complete evenness/diversity.

**4.4 Chemical Analysis Methods**

The purpose of the chemical analysis was to determine the chemical characteristics of the sampled matrices and evaluate the relationships between the chemical analysis results with the biological analysis results for a better understanding of the UST environment that is causing the observed corrosion. Table 2 includes what was measured, the standard method number (if applicable), and matrices associated with the samples taken in this project. The standard methods are very detailed and will not be reiterated in this document.

Elemental and crystallographic structural analysis was performed on a number of scraping, deposit and particulate specimens taken from filters, water samples, and other areas of the system. The objective of these analyses was to determine what the elemental composition and crystalline structures were in different areas of the system and to correlate them with observed corrosion and materials used USTs. The primary modes of analysis used were x-ray diffraction (XRD), x-ray fluorescence (XRF), inductively coupled plasma mass spectrometry (ICP-MS) and ion chromatography (IC). Each technique provides slightly different and complementary information which can be used to piece together the sample components. As such, these

methods are designed to analyze for as many elements and chemicals as possible.

The chemical analyses were performed by three members of the CDFA Task Force who have laboratories that regularly perform these analyses and one laboratory that was contracted for vapor analysis. Some methods were performed by more than one lab, resulting in duplicate or triplicate analyses on the liquid samples. Marathon performed analyses on the Tedlar bag vapor, fuel, water, and scrape samples. Chevron analyzed the water and scrape samples. Ford Motor Company analyzed the fuel and water samples. Finally, the contracted laboratory, Columbia Analytical Services, analyzed vapor samples.

**Table 2. Analysis Methods by Sample Type**

|  |  |  |
| --- | --- | --- |
| **Determination of:** | **Method Identification Numbera** | **Sample Type** |
| Biodiesel by Mid Infrared Spectroscopy | Modified ASTM D7371-07 | Fuel |
| Carbon and Hydrogen | ASTM D5291-10 | Fuel |
| Electrical Conductivity | ASTM D2624-09 | Fuel |
| EPA 120.2 | Water |
| Density, Relative Density, and API Gravity of Liquids by Digital Density Meter | ASTM D4052-09 | Fuel |
| Sulfur Compounds and Sulfur Selective Detection (hydrogen sulfide, sulfur content, sulfur speciation) | ASTM D5623-94 | Headspace vapor |
| Dissolved Inorganic Anions by Capillary  Electrophoresis | Modified ASTM D6508 | Water |
| Corrosive Properties | NACE TM-0172 | Fuel |
| Trace Nitrogen in Liquid Petroleum Hydrocarbons by Boat-Inlet Chemiluminescence | ASTM D5762-10 | Fuel |
| Carboxylic Acids and Formic Acid by Gas  Chromatography-Mass Spectrometry | Columbia Method 102 | Headspace vapor |
| Oxygen Concentration by Calculation | Calculation | NA |
| Particulate Contamination by Laboratory  Filtration | ASTM D6217-98 | Fuel |
| Total Acid Number (TAN) | ASTM D664-09a | Fuel |
| pH by Potentiometric Titration | EPA 150.1 | Water |
| Total Sulfur | ASTM D5453-09 | Fuel |
| Water Content by Coulometric Karl Fischer  Titration | ASTM D6304-07 | Fuel |
| Water Content and Temperature | Hygrometer on site | Headspace vapor |
| Flash Point | ASTM D93 | Fuel |
| Analysis of Solid Corrosive Substrate by  XRD, XRF, ICP-MS and IC | Laboratory Fouling Investigation  Methods | Scrapings |
| Determination of Acetate and Formate by  Capillary Electrophoresis | Ford Method - SOP CL029-02 | Fuel and Water |

a References for analytical methods are in the QAPP, Appendix B.

**4.5 Additives Hypothesis Investigation Approach**

The approach for testing this hypothesis focused on gathering information from additives manufacturers and literature to understand the potential effect of additives on the overall chemical characteristics of the fuel and headspace vapor within USTs. Battelle performed literature and internet searches of fuel additives in general and additives important to ULSD service. Also, discussions were held with technical representatives from multiple additive manufacturers. Some discussions were directly related to understanding the data set produced from this research and others were discussing ULSD additives in general.

5. Results

**5.1 Inspection Site Descriptions**

Site inspections took place from February 8–23, 2012. Four people were at each site to conduct the inspections: the Battelle Project Manager, the Tanknology Vice President of Engineering and Research and Development, a Tanknology Quality Assurance (QA) Manager, and a Tanknology Field Technician. NY-1 was intended to be used as a baseline site that would not have symptoms. However, it did have symptoms but they were much less severe than the other sites; therefore, it could not be considered a truly clean site but is identified as “clean” in the

following results tables. Table 3 summarizes some of the site characteristics recorded during the inspections. The complete inspection form data are included in Appendix C.

**Table 3. Inspection Site Characteristics**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Site ID** | **NC-1** | **NY-1**  **“Clean”a** | **NY-2** | **CA-1** | **CA-2** | **CA-3** |
| Inspection  Date (2012) | 8-Feb | 15-Feb | 16-Feb | 21-Feb | 22-Feb | 23-Feb |
| Tank Year of  Installation | unknown | 2008 | 1988 | 1990 | 1991 | 1991 |
| Tank Capacity  (gallons) | 17,265 | 12,000 | 6,000 | 10,000 | 12,000 | 6,000 |
| Tank Diameter  (inches) | 120 | 120 | 92 | 92 | 120 | 92 |
| Tank Material | Fiberglass | Fiberglass | Fiberglass | Fiberglass | Fiberglass | Fiberglass |
| Single/Double  Wall | Double | Double | Single | Double | Double | Double |
| Monthly Throughput (gal/month) | unknown | 18,000 | 6,500 | 26,000 | 20,000 | 25,000 |
| Filter Date  Replaced | 24-Jan-12 | unknown | Filter not identified | 2-Feb-12 | 13-Jan-12 | 9-Jan-12 |
| Biocide Treatment History | Fuel Right™ in Dec 2011 | unknown | BioBor®  2 times in past year | unknown | none | unknown |

a Site was affected by corrosion. It was intended to be the non-symptomatic site; therefore clean is in quotations.

**5.2 Biological Sample Results**

**5.2.1 DNA Yield and Amplification Results**

Sixteen sediment, filtered fuel, or filtered water samples from five geographically distributed sites were subjected to DNA extraction. Nine samples provided DNA measurable by a high- sensitivity dsDNA method (Table 4). In most cases, the filtered fuel provided little to no measurable DNA, while sediment and filtered water samples had measurable amounts of DNA. All sites yielded DNA, suggesting biomass within the systems, with NC-1 providing the least amount of DNA. Survey reports also showed that NC-1 had received a biocide treatment (December 2011) which could be responsible for the low recovery of DNA.

The sequencing method employed in this study requires at least 400 nanograms of high quality DNA. As seen in Table 4, only four samples met this criterion. WGA was attempted in the samples with lower yield to increase the DNA to quantities suitable for sequencing. A common commercial kit that is based on multiple displacement amplification was used, as discussed in the methods. The products of this procedure were measured for quantity and quality. The results showed that the only samples to yield measurable amounts of product from WGA were the same four samples with high DNA yield (Table 4). Thus, the low DNA samples did not achieve high DNA yields following this method.

A confirmatory test for presence of bacteria was also performed on the DNA extract samples to determine if bacterial DNA was present when total DNA was not measurable by the methods used. PCR amplification of the ubiquitous 16s rRNA gene from bacteria was performed. All but two samples yielded 16s amplification in varying amounts (Table 4 and Figure D5 [Appendix

D]) including samples that had less than measurable amounts of DNA following extraction. One sample, 53609-06-09e, had measurable DNA, but gave no 16s rRNA PCR product. This could

be due to interferants in the sample that prohibited the PCR reaction. In conclusion, all sites tested displayed presence of bacterial DNA, although at different abundances.

**Table 4. DNA Yield, Whole Genome Amplification and 16s rRNA Amplification**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Site**  **ID** | **Sample ID** | **Description** | **Purity (Abs**  **260/280 nm)** | **Total DNA (ng)** | **Whole Genome**  **Amplification**  **(WGA)** | **16s**  **Amplification** |
| NC-1 | 8Feb12\_07c | Filtered Fuel | 1.32 | Too Low | - | + |
| NC-1 | 8Feb12\_09 | Filtered Water  Bottom | 1.32 | 123.9 | - | ++ |
| NY-1 | 53609-06-08c | Filtered Fuel | 1.24 | Too Low | - | ++ |
| NY-1 | 53609-06-09d | Filtered Water  Bottom | 1.70 | 463.6 | +++ | ND |
| NY-2 | 53609-08-09e | Bottom Sediment | 1.09 | 75.24 | - | - |
| NY-2 | 53609-08-08c | Filtered Fuel | 1.36 | Too Low | - | + |
| NY-2 | 53609-08-09d | Filtered Water  Bottom | 1.50 | 1353 | +++ | ND |
| CA-1 | 53609-11-11e | Bottom Sediment | 1.11 | 27.36 | - | + |
| CA-1 | 53609-11-08c | Filtered Fuel | 1.18 | Too Low | - | - |
| CA-1 | 53609-11-11d | Filtered Water  Bottom | 1.48 | Too Low | - | -/+ |
| CA-2 | 53609-14-09 | Bottom Sediment | 1.13 | 7714 | +++ | ND |
| CA-2 | 53609-14-07c | Filtered Fuel | 1.56 | 76.00 | - | + |
| CA-2 | 53609-14-08d | Filtered Water  Bottom | 1.72 | 2584 | +++ | ND |



**Table 4. DNA Yield, Whole Genome Amplification and 16s rRNA Amplification (Continued)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Site**  **ID** | **Sample ID** | **Description** | **Purity (Abs**  **260/280 nm)** | **Total DNA (ng)** | **Whole Genome**  **Amplification**  **(WGA)** | **16s**  **Amplification** |
| CA-3 | 53609-17-11 | Bottom Sediment | 1.12 | 340.1 | - | +++ |
| CA-3 | 53609-17-10c | Filtered Fuel | 1.35 | Too Low | - | ++ |
| CA-3 | 53609-17-12d | Filtered Water  Bottom | 1.15 | 94.24 | - | ++ |

Shading indicates samples analyzed by whole metagenome sequencing

ND = not done

- = no product

+, ++, +++ = product and relative amount

**5.2.2 Dominant Organisms by Site**

Sequencing and bioinformatic analysis was performed on four samples (Table 4). The full results of the analysis are listed in Tables D2-D5 and Figures D1-D4 (Appendix D). Table 5 shows the dominant or most prevalent organisms by site, and Table 6 shows a breakdown of the identified organisms by oxygen requirements. *In general, bacteria of the acetic acid producing family (Acetobacteraceae) were prevalent in all four samples*. These are organisms that characteristically require oxygen and utilize ethanol as an energy source. They do not historically utilize hydrocarbons, such as the components of diesel fuel, for energy. In general, the most abundant organisms identified from the four samples have characteristics that can lead to corrosion of metallic equipment, such as acetic acid production, ethanol utilization, low pH requirements, environmental presence, and oxygen. An expanded list of attributes for the organisms in Table 5 is provided in Appendix E.

Some differences were observed between sites. For example, CA-2 had predominantly

*Gluconacetobacer sp*. Over 50% of the DNA identified belonged to this genus. NY-2 had

higher levels of *Lactobacillus sp.* compared to NY-1 and CA-2. NY-1 showed higher levels of a fungus, *Zygosaccharomyces*, and bacteriophage (viruses that infect bacteria) compared to the other samples. Very little difference was observed between the filter water and sediment

samples in CA-2, suggesting that the same organisms reside in these two sample types within this system. It is interesting to note that although the three geographically separate sites had some observable differences in abundance of select organisms, the presence of organisms was relatively uniform. This suggests that the ULSD system is very selective for specialized organisms capable of thriving in these environments, rather than a site specific or environmental effect driving the composition of microbial population.

**Table 5. Dominant Organisms by Site**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Genera*** | **NY-1 (06-09d)** | **NY-2 (08-09d)** | **CA-2 (14-08d)** | **CA-2 (14-09)** |
| *Gluconacetobacter sp.* | 35% | 44% | 53% | 55% |
| *Acetobacter pastuerianus* | 33% | 23% | 24% | 19% |
| *Gluconabacter oxydans* | 4.0% | 3.0% | 20% | 19% |
| *Lactobacillus sp.* | 1.0% | 34% | 0.1% | 4.0% |
| *Fungi (e.g. Zygosaccharomyces sp)* | 9.0% | 0.3% | 0.1% | 0.2% |
| *Bacteriophage (virus)* | 7.0% | 2.0% | 0.8% | 0.7% |

Underlined results highlight which samples had the highest percentages of the different genera.

**Table 6. Identified Organisms According to Oxygen Needs**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Category** | **NY-1 (06-09d)** | **NY-2 (08-09d)** | **CA-2 (14-08d)** | **CA-2 (14-09)** |
| Strictly aerobic | 23% | 63% | 30% | 28% |
| Strictly anaerobic | 1% | 6% | 0% | 0% |
| Facultativea | 33% | 7% | 0% | 2% |
| Viruses and Unknowns | 43% | 24% | 70% | 70% |

aOrganisms that survive in both aerobic and anaerobic conditions.

**5.2.3 Hydrocarbon Degrading Bacteria**

Some species of bacteria contain biochemical pathways to utilize and break down petroleum hydrocarbons of various chemical forms.7 Although these species identified to date are distributed within several bacterial orders, a majority of hydrocarbon degrading bacteria originate from marine environments and are typically of the class *Gammaproteobacteria*. Common hydrocarbon degrading genera include *Alcanivorax, Marinobacter, Pseudomonas,*

*Shawanella* and *Acinetobacter* species. To evaluate if the bacteria are present with the potential of using diesel fuel as a carbon source, bacteria and genes involved in hydrocarbon utilization were evaluated. Table 7 shows the percentage of positive hits for selected groups of bacteria

with the potential to utilize hydrocarbons for each site sampled by metagenomics. In general, the class of bacteria *Gammaproteobacteria* was only a small percentage of the total consortia, ranging from 0.3 to 5% of the identified DNA (Table 7). NY-2 showed that *Pseudomonas sp*.

were the major *Gammaproteobacteria* present, while NY-1 showed *Enterobacteriaceae*, an order of non-hydrocarbon utilizers, were the dominant *Gammaproteobacteria*. The two samples from CA-2 showed near limit of detection levels of total *Gammaproteobacteria*. Further, a search of the alkane hydroxylase (alkane-1-monoxygenase) gene, an essential enzyme involved in

degradation of n-alkanes (C10-C13), was performed using data for sample 06-09d (NY-1). No positive gene hits were discovered for homologues of the alkane hydroxylase gene in NY-1 (data not shown), suggesting that the pathway to utilize n-alkanes is not present for the species of bacteria sampled at this site. *Thus, based on the current library for metagenomics comparison available, the evaluation of hydrocarbon degradation suggests that the hydrocarbons contained within the diesel fuel may not be the primary carbon source for the consortium of bacteria present*.

**Table 7. Percent of DNA Identified for Selected Hydrocarbon Degrading Bacteria**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Category** | **NY-1 (06-09d)** | **NY-2 (08-09d)** | **CA-2 (14-08d)** | **CA-2 (14-09)** |
| ***Gammaproteobacteria*** | 4% | 5% | 0.3% | 0.3% |
| ***Pseudomonas sp.*** | 1% | 4% | <LOD | 0.1% |
| ***Marinobacter sp.*** | <LOD | <LOD | <LOD | <LOD |
| ***Acinetobacter sp.*** | 0.7% | <LOD | <LOD | <LOD |
| ***Shawanella sp.*** | <LOD | <LOD | <LOD | <LOD |

< LOD = below threshold for genetic identification, <1:1000 of total data set.

**5.2.4 Diversity Assessment**

A measurement of microbial diversity was performed to further evaluate the community profiles identified by DNA sequencing. Table 8 shows that overall all four samples have low diversity, as measured by the Shannon Index, compared to environmental sediment samples. This finding

suggests that there are both less overall unique organisms present in the community, and of those present, there are limited species that dominate the community within the USTs surveyed. This

is further evidence that the conditions of the ULSD USTs are conducive to growth of limited, specialized organisms. Lastly, the NY-1 site had the most diverse microbial community, while the CA-2 site was the least diverse (Table 8).

**Table 8. Diversity Assessment**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Shannon Index** | **NY-1 (06-09d)** | **NY-2 (08-09d)** | **CA-2 (14-08d)** | **CA-2 (14-09)** | **Historical sediment samplesc** |
| *Shannon’s diversity (H)a* | 2.6 | 2.7 | 1.5 | 1.7 | 4.8 - 5.3 |
| *b*  *Shannon’s equitability (EH)* | 0.23 | 0.22 | 0.13 | 0.14 | 0.74 - 0.80 |

a As *H* approaches zero, an ecosystem (microbial) is dominated by very few species.

b Equitability assumes a value between 0 and 1, with 1 being complete evenness/diversity.

c Previous data from marine sediments (natural environmental samples) from research studies at Battelle using the same genomics methods.

**5.3 Chemical Analyses Results**

Many analysis methods were performed on the matrices sampled during the inspections. Some of the results that are more relevant to the hypotheses under investigation are presented below in Tables 9 through 11 and all of the results are presented in Appendix F. Table 9 shows results from the analyses performed on the fuel samples taken at each inspection site. Acetate (a form

of acetic acid) is not expected in diesel fuel but was measureable in four of the six sampled fuels. Ethanol was also unexpectedly identified; therefore, in a separate analysis was conducted to estimate the ethanol concentrations of both fuel and water bottoms. This was accomplished by comparing the instrument response to the responses of fuel spiked with ethanol. These results indicate that ethanol could be contaminating ULSD as four of the six fuels contained it. An acceptable NACE analysis result is a requirement for fuel to be transported via pipeline and is

not traditionally performed for fuel transportation via barge, truck, or directly dispensed from a terminal. In this case, three of the six samples failed this test, indicating that the corrosion inhibitor that may have been added at the refinery was consumed by the time the fuel reached the retail sites. Although not the only lubricant, in accordance with the Federal Trade Commission requirements and ASTM D975, biodiesel is allowed to be added to ULSD at up to 5% of the composition. These results indicate that two samples had detectable levels of biodiesel, and only one was close to the 5% at 3.55%. This sample was also the only one that contained Formate

and had the highest composition of water, both of which are related to the presence biodiesel. This could be due to the degradation of biodiesel. Finally, since the corrosion started to be reported after the lowering of sulfur content, the sulfur results for these sites ranged from 5.9 to

7.7 ppmv, which is well below the 15 ppm maximum.

**Table 9. Summary of Fuel Sample Results**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Site ID** | **NC-1** | **NY-1** | **NY-2** | **CA-1** | **CA-2** | **CA-3** | **Standard+** |
| Acetate mg/kg (ppm) | <0.3 | 7.7 | 2.8 | 2.7 | <0.3 | 5.9 | NA |
| Biodiesel (vol%) | <0.3 | 3.55 | 0.40 | <0.3 | <0.3 | <0.3 | NA |
| Conductivity (ps/m @  ambient) | 125 | 1,200 | 183 | 30 | 70 | 64 | minimum  251 |
| Ethanol (vol%)\* | 0.04 | 0.01 | 0.17 | 0.06 | ND | ND | NA |
| Formate mg/kg (ppm) | <0.3 | 5.6 | <0.3 | <0.3 | <0.3 | <0.3 | NA |
| Fouling GC-MS Scan | trace  Ethanol | trace  Ethanol | trace  Ethanol | trace  Ethanol | NTR | NTR | NA |
| NACE TM-172 Rating | A | A | A | D | C | C | B+++1 |
| Particulate (mg/L) | 54.5 | 87.4 | 91.4 | 114.8 | 69 | 122.2 | 122 |
| Sulfur (ppmw) | 7.2 | 7.7 | 7.3 | 5.9 | 6.4 | 6.2 | 153 |
| TAN (mg KOH/L) | 0.01 | 0.04 | 0.02 | 0.002 | 0.005 | 0.006 | 5.04 |
| Water (ppmw) | 39 | 65 | 46 | 44 | 41 | 29 | 501 |

\*Fouling GC-MS Scan results compared to fuel spiked with ethanol for estimated quantification.

NTR = Nothing to report outside of expected hydrocarbons

Table 10 presents chemical analyses results on the water bottom samples collected at each inspection site. Acetate was measured in all six water samples at high levels from 9,000 ppm to

22,500 ppm. Glycolate, a related compound to acetic acid, was detected in appreciable amounts at four of the six sites. In addition, ethanol was identified in five of the six water bottoms. Neither acetate nor ethanol were expected to be in these systems and are considered corrosive agents. Other characteristics of the water that are connected to corrosivity are the conductivity,

pH, and chloride concentration. The conductivity of the water was quite high, ranging from

4,000 µS/cm to 21,000 µS/cm, and the pH of the waters were acidic, ranging from 3.6 to 5.3. Chloride and sodium results were especially high for the three east coast sites, possibly indicating the use of road salts during the winter season although another potential source would be refinery salt driers. Chloride is known to adversely affect corrosion resistance of many metallic materials. The GC-MS fouling scans indicated the presence of a variety of compounds, including alcohols, acids, and amines. Although the exact resins that make up the fiberglass tanks are unknown, methyl vinyl ketone has been identified as a chemical that could have leached from the tank shell.

**Table 10. Summary of Water Bottom Sample Results**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ***Site ID*** | ***NC-1*** | ***NY-1*** | ***NY-2*** | ***CA-1*** | ***CA-2*** | ***CA-3*** | ***Water+*** |
| Acetate  (ppm)a | 16,500 | 9,000 | 21,000 | 22,500 | 17,500 | 20,000 | 2951 |
| Ammonium  (ppmw) | 871 | <1 | 452 | 30 | 37 | 5.2 | < 2002 |
| Calcium  (ppmw) | <1 | <1 | <1 | 732 | 586 | 242 | 6.53 |
| Chloride  (ppmw)b | 6,791 | 3,890 | 1,978 | 785 | 888 | 394 | 173 |
| Carbonate  (ppmw) | 12 | 57 | 19 | 65 | 72 | 41 | 773  bicarbonate |
| Conductivity  (µS/cm) | 21,000 | 17,000 | 12,000 | 4,000 | 7,500 | 8,000 | 3313 |
| Ethanol\* (vol%) | 3.17 | 0.66 | 0.45 | 0.40 | ND | 0.04 | NA |
| Fluoride  (ppmw) | 1,074 | 1,205 | 1,796 | 4,653 | 4,372 | 3,595 | 1.54 |
| Formate  (ppm) | 78 | 1,400 | 69 | 350 | 300 | 280 | data not  found |
| Glycolate  (ppmw) | <100 | 4,000 | <100 | 11,000 | 11,000 | 5,000 | data not  found |
| Magnesium  (ppmw) | <1 | <1 | 112 | 63 | 614 | 25 | 1.13 |
| Nitrate  (ppmw)a | 39 | 514 | 60 | 26 | 308 | 27 | 105 |
| pHb | 5.3 | 4.6 | 4.1 | 3.6 | 3.8 | 3.6 | 6.73 |
| Potassium  (ppmw) | 370 | 639 | 278 | <1 | 45 | 51 | 33 |
| Sodium  (ppmw) | 6,124 | 2,291 | 1,886 | 581 | 158 | 182 | 373 |
| Sulfate  (ppmw)a | 440 | 470 | 312 | 598 | 273 | 376 | 153 |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ***Site ID*** | ***NC-1*** | ***NY-1*** | ***NY-2*** | ***CA-1*** | ***CA-2*** | ***CA-3*** | ***Water+*** |
| Fouling GC- MS Scan (identified peaks) | methyl  vinyl ketone, acetic acid, ethanol,  1,2- ethane diol propylen e glycol, N-butyl-  1- butanami ne,  N-ethyl- cyclohex ylamine | methanol  ethanol, acetic acid,  1,2- ehthane diol, propylene glycol,  N,N- dimethyl formamide, significant N,N- dimethylbe nzenemeth anamine, unidentified phthalate | ethanol, acetic acid,  2- hexanon e | acetic acid, glycol, ethanol | acetic acid,  1,1'- oxybis-2- propanol,  traces of glycol and  dioxane | acetic acid, traces of dioxane, glycol, and  2,5- dimethyl-  1,4-  dioxane, very faint trace of ethanol | NA |

NA = Not applicable

\*Fouling GC-MS Scan results compared to fuel spiked with ethanol for estimated quantification. Typical concentrations seen in groundwater or surface waters according to source.

1. In surface soil solutions: The Influence of Acetate and Oxalate as Simple Organic Ligands on the Behavior of Palladium in Surface Environments, Wood, S. A and Middlesworth, J. V. The Canadian Mineralogist, Vol 42, pp. 411-421.

2. Guidelines for Drinking-water Quality, 3rd Edition, Volume 1, World Health Organization, 2008. Pp 303-

304.

3. Groundwater from volcanic rocks: Natural Variations in the Composition of Groundwater, Nelson, D., Oregon Department of Human Service, November 2002. pp. 3.

4. Water Quality Fact Sheet: Fluoride. British Geological Survey

5. U.S. EPA drinking water MCL

The vapor results are presented in Table 11. The relative humidity of the vapor was high, ranging from 72% to 95%. Given that the acetate was found in the fuel and water, and there were little other organic acids present in the samples (analyzed for 17 other acids, see Appendix F), the determination of acetic acid in the vapor space makes this the suspected corrosive agent corroding ULSD USTs.

**Table 11. Summary of Vapor Sample Results**

a Average of two readings

b Average of three readings c Average of four readings ND – not detected

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Site ID*** | ***NC-1*** | ***NY-1*** | ***NY-2*** | ***CA-1*** | ***CA-2*** | ***CA-3*** |
| Average relative humidity (RH%) | 90.9b | 83.3b | 95.5c | 73.7a | 71.8a | 95.2b |
| Average In tank temp (°F) | 57.1b | 46.8b | 44.7c | 61.8a | 66.4a | 58.2b |
| Acetic acid (ppbv) | 570 | 1,800 | 3,600 | 7,800 | 9,500 | 16,000 |
| Formic acid (ppbv) | 18 | 48 | 110 | 190 | 88 | 72 |
| Propionic acid (Propanoic) (ppbv) | 1.6 | 15 | 2.3 | 1.8 | 1.7 | 2.0 |
| 2-Methylpropanoic acid  (Isobutyric) (ppbv) | ND | 0.79 | ND | ND | ND | ND |
| Butanoic acid (Butyric) (ppbv) | ND | 0.85 | ND | ND | ND | ND |
| Carbonyl sulfide (COS) (ppmw) (Tedlar bag) | 0.14 | Bag ruptured | 0.29 | Lost in transport | 0.12 | 0.22 |
|  | | | | | | 0.14 (duplicate) |

**5.4 Corrosion Sample Results**

The sites inspected were chosen because of the corrosion observed by the owners/operators of the sites. The corrosion is severe and according to the owners happened over a relatively short period of time. During the inspections in this project, severe corrosion was observed on a large number of components *within* the USTs and specifically *not* on the outside of the components in the sump pits and the dispensing systems. The corrosion scrape samples taken from the internal components were coated with corrosion. *The scrape sample results support the conclusion that the internal components made up of all different metals are deteriorating in ULSD USTs.*

Since acetic acid and ethanol have been identified as the most likely corrosive agents from the chemical results, it was not surprising that *acetic acid was identified in 75% of the scrape samples.* Ethanol was not measured in the scrape samples as it is believed to be used as a carbon (food) source for the biological activity in the USTs. Appendix G includes a detailed discussion and tables of all of the results from the scrape sample analyses performed.

**5.5 Additives**

Additives for fuel handling, specifically for de-icing or water removal/encapsulation, may contain various concentrations of alcohols and/or glycols. In the fouling gas chromatography- mass spectrometry (GC-MS) scans of water bottom samples, alcohols (specifically methanol, ethanol, and 1,1’-oxybis-2-propanol) were found in all samples. Glycols were found in five of

the six samples (not seen in NY-2). Additives for de-icing are not generally added at the refinery or terminal points, and are seasonally and infrequently added by retailers.

Fuel stability additives composed of strongly basic amines are added to react with and eliminate weak acids such as acetic acid and formic acid. Amine compounds were found in two of the sites’ water bottoms, NC-1 and NY-1. Although the specific amines used are unknown, amine compounds are also components in biocide additives, which had been added to the NC-1 site in December 2011. Additives containing amines are generally added to eliminate microbes that can

cause corrosion, or as corrosion inhibitors binding corrosive acids. They would unlikely be a factor in the corrosion seen in the USTs inspected.

The sulfur compounds in LSD contribute to increased lubricity when compared to ULSD. In general, ULSD requires a lubricity additive in order to meet lubricity specifications. Mono- and di-acids or biodiesel are commonly added to ULSD to increase the lubricity. Biodiesel composition of the tested fuel samples showed results below detection (< 0.3 % by volume) for four of the six samples. The NY-1 and NY-2 sites showed 3.55% and 0.40% biodiesel, respectively. Chemical breakdown of these small chain acids or biodiesel generally occurs slowly over time and could potentially produce acetic or formic acids. The rate of this chemical breakdown could be increased by presence of a microbiological component that metabolizes these mono- and di-acids, forming corrosive products. As discussed in Section 5.2.3, these microbes were not found in significant amounts in the sampled matrices from the inspected USTs.

Gaylarde, Bento, and Kelley report that trace nutrients in fuel may be limiting factors for

bacterial growth.9 The authors mention phosphorus as a likely limiting nutrient. In this respect it is noted that some additives may contain phosphorus. Trace elements were determined in the sampled fuels as presented in Appendix F.

**5.6 Quality Assurance/Quality Control**

Steps were taken to maintain the quality of data collected during this research effort. 100% of the acquired data were reviewed by the Battelle project manager, and a Battelle QA Manager audited at least 25% of the data acquired in this research effort. Finally, a second review performed by the Battelle QA Manager or designee traced the data from initial receipt from the laboratories, through reduction and comparisons, to final presentation in the report. Battelle did not receive or review the QC data from the laboratories (with the exception of the carboxylic acid, formic acid, and genomics data). The laboratories stated that the ASTM methods were followed and the criteria were met for the chemical analyses.

6. Discussion

**6.1 Corrosion Inducing Factors**

In order to understand why corrosion is occurring, an understanding of the relationships of the factors in the diesel UST environment is needed. Specifically, corrosion inducing factors are: a substrate that corrodes (UST equipment), a corrosive electrolyte, and a mechanism for the electrolyte to be disseminated onto the substrate surface, in addition to being influenced by microbiological activity.

**6.1.1 UST Equipment Materials**

Fuel storage and dispensing equipment is composed of a combination of materials including a variety of different carbon steels, austenitic stainless steels, ferritic stainless steels, cast irons, brasses, and cast aluminum alloys.8 The storage tanks are commonly fiberglass – as were all the tanks evaluated in this study – or steel with a small portion being fiberglass coated steel. This study examined fiberglass USTs. Each of these metals has its own distinct electrochemistry and corrosion susceptibility depending on the specifics of the environment. Additionally, some

components may receive nickel coatings for corrosion resistance, while others may be coated with various epoxy, enamels, varnish rust inhibitors or lacquer topcoats. However, generally, increased acidity leads to increased corrosion damage accumulation and promotes depassivation of most of the materials used in USTs.

**6.1.2 Ingredients for an aggressive corrosive electrolyte**

The ingredients for an aggressive electrolyte exist within the USTs inspected for this study. Namely, available water, oxygen, acids, and aggressive species create an environment that would be expected to attack most of the materials used in USTs. In addition, these environmental characteristics are specific to microbiological organisms that also contribute to the corrosive

cycle in ULSD USTs.

*Water Content and Availability* - In the presence of an aqueous electrolyte, a susceptible metal may corrode. In this instance, water can be present either in solution with the diesel or as “free” water which exists as its own phase.9 The water existing in solution with the diesel has little impact on corrosion or increasing the chances of MIC12 - with measured concentrations in this study ranging from 29 to 65 ppm as shown in Table 9. However, the water accumulated at the bottom of storage tanks or in the vapor spaces can have catastrophic effects7. At the time of measurement, water existed in significant enough quantities on the tank bottoms to be sampled and the relative humidity of the vapor spaces were found to range from 72% to 95%. The high measured humidity is consistent with the observed corrosion in the vapor regions. It has been found that the time of wetness on a surface can increase significantly, leading to increased corrosion of steel in particular, when the relative humidity is above 80%.13 Water accumulation and high relative humidity in tanks are common to the UST environment; however, in this case, it enable the corrosive agent to sustain contact with the equipment for a longer periods.

Water accumulation has been attributed broadly to three different sources: infiltration, temperature affected solubility, and condensation.11 Infiltration refers to the ingress of water from the outside environment through obvious physical routes11, for example rain water entering through an opening to the system, the spill containment bucket being dumped into the tank, or with the fuel load being delivered from the tanker . Temperature and aromatic content are directly related to the amount of water a diesel can hold in solution with warmer, more aromatic rich fuel being capable of holding higher concentrations of water 11. When the fuel is cooled, water in excess of the solubility limit will drop out of solution. Finally, condensation is noted to be a primary source of moisture in fuel storage tanks, which are vented to the atmosphere with condensate forming any time the temperature falls below the dew point.11 Although temperature

fluctuations are relatively mild for USTs and the frequency of this happening would be related to

the climate and season, the possibility for condensate still exists. The introduction of water into the system can occur any time warm fuel is added to a cooler tank – i.e., the transfer of fuel from a truck on a warm day to a UST.

The water samples were highly conductive ranging from 4,000 to 21,000 µS/cm, which is close to brackish water at approximately ~27,000 µS/cm. To cause corrosion, conductivity is needed to complete the circuit with aqueous electrolytes; however, measurements in line with ground water would have enough conductivity to do so (~300 µS/cm). Therefore, it is possible to have the observed corrosion in the ULSD USTs without having the high levels of conductivity found in the inspected USTs.

*Oxygen* - Based on actual oxygen solubility data for some model hydrocarbons, the solubility of oxygen gas in diesel fuel is estimated to be 200 to 300 mg/L.14 This could be significant for supplying oxygen to aerobic bacteria. The dissolved oxygen could be, at least partially, replenished in the new loads of fuel. Each delivery requires the tank to be opened and fuel to be added. This churns the fuel in the storage tank and introduces air into the tank. The added fuel

can bring with it a fresh supply of dissolved oxygen and dissolved water.

*Acid Content* – The presence of acids can accelerate corrosion and depassivate normally passive materials (in this case, the primary acids of concern were acetic and formic acids). Acetic acid appears to be the dominant acid species present among those species which were analyzed for and will be the focus of subsequent discussions. However, the concentration of acetic acid varied widely depending on whether considering the diesel fuel, water bottom, or vapor phase. In the fuel phase, acetate was detected to be between 2.7 and 7.7 ppm among the four sites in

which acetate was present in detectable quantities – two locations were below the detection limit. Acetate/acetic acid values are summarized in Table 12. Additionally, although not a required

test for ULSD specification, a gauge of diesel acidity was measured via TAN values, which were found to vary from 0.006 to 0.04 mg KOH/L.

Significantly larger concentrations of acetate were found to exist in the water bottoms as compared to the diesel fuel as summarized in Table 12. Depending on the site, acetate was found to exist in the water bottoms in concentrations ranging from approximately 9,000 to

22,500 ppm. pH values were also determined for each site and found to range from approximately 3.5 to 5.3.

**Table 12. Summary of Acetic Acid/Acetate and Ethanol Concentrations in UST Systems Inspected**

a average of 2 results from different laboratories

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Acetic Acid/Acetate** | **NC-1** | **NY-1** | **NY-2** | **CA-1** | **CA-2** | **CA-3** |
| Fuel Ethanol (vol%) | 0.04 | 0.01 | 0.17 | 0.06 | ND | ND |
| Water Ethanol (vol%) | 3.17 | 0.66 | 0.45 | 0.40 | ND | 0.04 |
| Vapor Acetic Acid (ppmv) | 0.57 | 1.8 | 3.6 | 7.8 | 9.5 | 16 |
| Fuel Acetate (ppm) | <0.3 | 7.7 | 2.8 | 2.7 | <0.3 | 5.9 |
| Water Average Acetate (ppm)a | 16,500 | 9,000 | 21,000 | 22,500 | 17,500 | 20,000 |

ND = Not detected

*Aggressive Species* – The presence of aggressive anionic species such as chlorides is also detrimental from a corrosion perspective. These species not only increase the conductivity of the solution but can act directly in breaking down passivity and passive films. In

microenvironments, free hydrogen protons (H+) can combine with available anions (Cl-, NO3-

2,SO4-2) to form strong acids that are also corrosive. High conductivity values and appreciable quantities of fluoride, chloride, and sulfate were observed at all locations in the water bottom samples and are summarized in Table 10. Additionally, nitrates, phosphates, and ammonium were observed at some but not all locations.

**6.1.3 Electrolyte Distribution**

The distribution of the electrolyte and the mode of contact between a metal and its environment have direct bearing on corrosion. Generically, within this case, three distinct regions exist in the storage tanks and along the STP. Depending on the region of the tank, materials could be constantly exposed to a bulk aqueous electrolyte, thin electrolyte layers, experience wetting and

drying cycles, or periodic “washing” as the tank is emptied and refilled. The vapor space at the top of the tank theoretically does not see liquid fuel but exists at relatively high humidity. The tank bottom is constantly submerged unless drained off and would contain any water that drops out of the fuel. Finally, an intermediate region, which depending on tank fuel level, can either be submerged or exist in the vapor space.

Each region will experience a different set of conditions that will directly influence the type and extent of attack. In the vapor space corrosion could occur under thin electrolyte layers from a high relative ambient humidity or the condensate, readily available oxygen, and the presence of acetic acid in the vapor space. If these regions experience wetting and drying cycles, there is the potential to significantly concentrate aggressive anions and acidic species leading to much more corrosive conditions than experienced or measured in the bulk water bottoms. The intermediate region would effectively experience “washing” during tank filling. As the diesel level drops, these regions become exposed in a similar fashion as the vapor phase and it is plausible that residue and contaminants are left behind.

**6.1.4 Microbial Presence**

Microbial contamination of hydrocarbon-based fuels has been a well known problem for nearly half a century.10 MIC is not in itself a distinct kind of corrosion, but rather a change to physical or chemical conditions that often accelerate other types of corrosion brought about by local environmental changes induced through microbial activity often associated with bacteria, algae, and fungi.12,15 Microorganisms can produce and consume species involved in corrosion as well as produce a physical bio-film barrier which either directly or indirectly results in the formation of a bio-film composed of extracellular polymeric material, which causes heterogeneities on the surface and can lead to differential aeration and oxygen depleted zones, differences in diffusion, and concentration gradients of other chemical species.12,15

Generically, differences in aeration, diffusion, pH, or concentration gradients of other types can lead to a separation of anodic and cathodic reactions on a surface, which leads to aggressive localized pitting at the anode; this kind of pitting attack is classically associated with MIC on iron (Fe)-based alloys. Once the pit is established and if chlorides are present, the pit will grow independent of microbial activity through autocatalytic processes.12 In these cases, insoluble Fe(OH)2 corrosion products can combine with the bio-film to form a tubercule which itself can trap electrolytes and subsequently can become highly acidic or combine with chloride from the surrounding environment to form an aggressive ferric chloride solution.15

In this case, *Acetobactor* has been identified in the samples and need water, oxygen, and a energy source (ethanol) to thrive and to consequently produce acetic acid. The results from the chemical analyses show that all three components are present in the UST environment. In addition, even though *Acetobacter* are commonly found in the environment, the ULSD UST environment is selective for them. The amount of water needed for microbial proliferation is small and generally the growth of aerobic bacteria and fungi which are likely at play in this

instance grow at the interface between the fuel and water.10 The final component is if ethanol is readily contaminating diesel fuel and whether there is enough ethanol to produce the abundant acetic acid to cause the severe and rapid corrosion.

*Ethanol contamination* – Fuel infrastructure supplies and handles other fuels in addition to diesel, such as gasoline, jet fuel, and ethanol. Diesel fuel is often shipped in the same pipelines

as gasoline and jet fuel. More importantly, ethanol is specifically kept separate from gasoline until splash blended in the tanker trucks. Since the trucks transport and switch between all fuels from 0 to 100 percent ethanol, it is possible that there be some cross contamination from the

fuels and vapors. Because nearly all gasoline sold in the U.S. now contains 10 percent ethanol, it is not surprising that small amounts of ethanol were found in most of the diesel fuel and subsequent water bottom samples as shown in Table 12. However, further study is required to establish this causal link.

Another source of potential ethanol contamination is through manifolded systems. At times, gasoline USTs are converted to diesel service with ventilation systems still connected to other gasoline USTs on site. As ethanol vapors collect in the ullage of the gasoline tanks, it can be forced back into the ULSD tank contaminating the system.

As mentioned earlier, ethanol was used to decontaminate the sampling equipment at the end of each inspection. It was rinsed and allowed to dry before the next inspection. Throughout the six inspections, the fuel sample was taken from the center of the fuel column then the water sample was taken from different riser pipes of the tank. The concentrations indicate that there was a higher percentage of ethanol in the water samples than in the respective fuel samples. The likelihood of ethanol contamination from the sampling process is low.

Ethanol has an affinity to water; therefore, if the fuel is being dropped with some contamination, the ethanol will migrate into the water bottom. This is also indicated by the ethanol concentrations measured in this study. Site NC-1 received a biocide treatment soon before the inspection. The DNA yields for the genomic analysis were low, as expected. In addition, the ethanol concentration in the water bottom was much higher than the others (~3%). Presumably, the ethanol is collecting in the water bottom to be metabolized if the tank becomes contaminated with *Acetobacter* again. Inversely, the CA-2 had the most measureable amounts of *Acetobacter* and ethanol was not detected in the fuel or water. Understanding how ethanol contamination in happening and what levels are occurring in the USTs of ULSD is a topic for further research.

*Feasibility*-The presence of acetic acid in high concentration in the vapor sampled from the tanks, as well as the concentration of acetate in the water bottoms, suggest that acetic acid may be reacting with the iron to produce the scale and corrosion. This section will examine whether it is possible for the corrosion product to have resulted from the reaction of steel or iron with the acetic acid in the tank. This requires determining whether it is possible to create enough acetic

acid to cause the corrosion, and whether this amount of acetic acid can be created in a timeframe consistent with the observations.

Analysis of the scale sampled from the tanks showed the likely presence of multiple compounds. One compound that may be present in the scale is iron acetate, which is formed by the reaction

of acetic acid with iron, although iron acetate is not the only product resulting from the reaction of acetic acid with iron or steel. To set an upper bound on the amount of acetic acid required, it is assumed there is 1 kg of scale or corrosion in the tank and that the scale is composed solely of iron(III) acetate, [Fe3O(OAc)6(H2O)3]+ OAc-. The molecular weight of iron(III) acetate is 650

g/mol, so 1 kg would equal 1.54 mole. Formation of 1.54 mole of iron(III) acetate would require

10.8 mol or 650 g of acetic acid (MW=60 g/mol).

The presence of *Acetobacter* in the tank samples suggests that ethanol is being converted to acetic acid. One of the most common reaction pathways for acetic acid production requires one

mole of ethanol per mole of acetic acid. The 10.8 mol of ethanol equates to about 500 g

(46 g/mol), and at a density of 0.789 g/cm3, this is about 0.63 liter of ethanol. Assuming a 5000 gallon diesel tank (18950 liter), this is equivalent to an ethanol concentration of 0.0033% by volume or 33 ppmv. This is significantly below the miscibility limit of ethanol in diesel.16

The above discussion shows it is feasible for the corrosion to be formed from acetic acid reacting with the iron or steel surface, if the acetic acid can be generated rapidly enough by the *Acetobacter*. A previously published paper measured the acetic acid production by *Acetobacter* in the presence of ethanol.17 The acetic acid production rate was controlled by the O2 concentration in the reactor, as the primary mechanism is the reaction of ethanol and oxygen to produce acetic acid and water.

C2H5OH + O2 → CH3COOH + H2O

The paper reported the acetic acid production was steady at 4.55 g/Lh for 27 g (dry weight) of *Acetobacter* in a 1-L reactor. Based on this performance, producing 600 g of acetic in the course of one week (144 hours) would require less than 27 g dry weight of *Acetobacter*, if there is sufficient O2 and ethanol. *Therefore, with the low levels of ethanol contamination, it is feasible given enough oxygen for the equipment to be corroding as severely and rapidly as observed and reported.*

**6.2 Hypotheses Evaluations**

**6.2.1 Additive Hypothesis Evaluation**

One of the three working hypotheses stated that additives in the diesel fuel were causing the corrosion observed in UST systems. The analysis of the fuel and water bottoms showed the presence of some of the classes of chemicals associated with the additives present in ULSD.

The analysis of the fuel, water bottoms, and vapor phase also showed the presence of acetic acid in large quantities. From the literature search and discussions with additive manufacturers, there is no reason for acetic acid to purposely be added to diesel fuel. To be present in the tank at the concentrations measured, the acetic acid would have to be a significant component of the fuel additive. There is minimal use of ethanol in additives and they are not widely or consistently used. For these reasons, the additives hypothesis is not believed to cause the severe and rapid corrosion occurring in UST systems storing and dispensing ULSD (Figure 2).

**Banetie**

*V.e* Buaine11a/Innovation

HYPOTHESIS: Additives in the fuel are contributing to the

corrosive environment.



Do additives contain corrosive species?

**No.**

- **Acetic acid Is widely-known to be a corrosive species.and Is not used**

**In additives.**

- In **general. only additives used for de-Icing would contain ethanol. which**

**Is not recommended, would not be used In all geographic areas, and would only be used during winter months.**

Have additives changed since introduction of ULSD?

Yes.

- Additive composit on has generally remained consistent, however,removal

of sulfur compunds has altered the lubricity properties when compared to LSD.

- Removal of sulfur from diesel lowered the lubricity of the ULSD, often requiring lubricity additives that were not necessary with LSD. These additives consist primarily of mono- and di-acids, amines, synthetic esters, or biodiesel.

- Detergent additives that maintain clean fuelinjectors are commonly composed of polymers which redissolve deposits.

Could detergent or lubricity additives be responsible for the corrosive species?

**No.**

- **Degradation of these mono- and dl- acids, blodlesel, and polymers occurs slowly over time and would not be responsible for the large concentrations of acetic acid found In the samples.**

- **Blodleselwas found In only one of the six fuel samples.**

- **Further. stabilizers In many fuels would neutralize degradation products.**

**X**

HYPOTHESIS **DISPROVEN**

Additives are not responsible for the levels of corrosive species found in the inspected USTs.

Figure 2. Additives Hypothesis Evaluation



**6.2.2 Chemical Species Hypothesis Evaluation**

Ethanol and acetic acid were the two potential agents identified in the samples from the site inspections as possibly being responsible for the severe and rapid corrosion.

Ethanol is known to influence corrosion to many of the materials used in fuel delivery infrastructure especially in the presence of water, oxygen and aggressive ions.11 For this reason, it is blended with other fuels downstream to prevent transport through pipelines. Detectable quantities of ethanol were determined in the majority of the liquid samples from this study, and may contribute to the corrosion; however, in this case, any contribution is believed to be minimal for two reasons. In gasoline systems, ethanol is present in significant quantities of 5, 10, or even

85 percent total volume as compared to being available in ppm-type concentrations in the diesel

USTs. Second, the pKa of ethanol is ~15.5 and is significantly smaller than the pKa of some of the other aggressive species such as acetic acid (discussed below) with a pKa of ~4.75, which are more likely reasons for the corrosion.18 Although not the only factor in terms of acidifying the solution and corrosion, other species play a much larger role than ethanol potentially does.

As previously discussed in Section 6.1.2, acetic acid was found in the majority of the samples at all of the inspection sites. With the low pKa, the disassociation of the acid is at a rate that could account for the aggressive corrosion. For these reasons, the chemical hypothesis is accepted with respect to acetic acid and not accepted with respect to ethanol (Figure 3).

**Banetie**

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HYPOTHESIS: Aggressive chemical species present in ULSD systems are facilitating aggressive corrosion.

Corrosion

Identified corrosive agents

Acetic acid



Were corrosive agents present in UST systems inspected?

Yes.

Analysis showed trace levels of ethanol in water bottom and fuel samples.

Yes.

Analysis showed significant amounts of acetic acid in the water bottom,headspace vapor, and fuel.

Can the corrosive agent be responsible for the aggressive corrosion?

No.

- EthanolpKa = 15.5,similar pKa to water

• Reaction rate Is too slow to account for the

observed aggressive corrosion.

• Trace amounts of ethanolmeasured would not be responsible for the aggressive corrosion observed.

X

Yes.

• Acetic acid pKa = 4.75

• Reaction rate is appropriate to account

for observed aggressive corrosion.

• Substantialconcentrations of acetic acid measured in the vapor, fueL and water bottom samples correlates to the aggressive corrosion.

HYPOTHESIS **VERIFIED**

Acetic acid may be responsible for the aggressive corrosion.

Figure 3. Aggressive Chemical Species Hypothesis Evaluation

**6.2.3 Microbial Hypothesis Evaluation**

All of the sites inspected in this research project contained microbes, although at different abundances. The dominant organisms identified from three of the sites have characteristics pertinent to the corrosion observed in all of the sites, such as acetic acid production, ethanol utilization, low pH requirements, environmental presence, and oxygen. Although geographically on opposite sides of the country, with different fuel supplies and from relatively new

construction materials, the presence of the organisms was relatively uniform. The traditionally expected organisms were found in insignificant abundances. Anaerobic organisms ranged from

0% to 6% and hydrocarbon degrading organisms from the *Gammaproteobacteria* class ranged from 0.3% to 5% in the samples analyzed. This indicates that ULSD USTs are selective environments for these specialized, acetic acid producing organisms. Therefore, as shown in Figure 4, the microbial hypothesis is accepted with respect to aerobic microbes but rejected with respect to anaerobic microbes.

HYPOTHESIS:

Aerobic and anaerobic microbes are producing by-products that are establishing a corrosive environment in ULSD systems.

Could these microbes be present in the USTs inspected?

Yes.

Water present. food (hydrocarbon) present, slightly acidic pH conditions,

and localized oxygen-deficient areas could

allow anaerobic microbe growth.

Yes.

Water present,food (alcohol) present. slightly acidic pH conditions,

andlocalized oxygenated areas could allow aerobic microbe growth.

Were these microbes found in the USTs inspected?

Yes.

Analysis of boHom water and sediment indicated presence of anaerobic bacteria.

Yes.

Analysi s of boHom water and sediment Indi cated presence of aerobes,specifically from the family Acetobacteraceae.

Could these microbes be responsible for corrosive species?

*r* No.

Anaerobic hydrocarbon-metabolizing bacteria were an lnslgnllcant portion of the populationIn tested samples.

*I*

X

Yes.

Bacteria in the family *Acetobacteraceae*

metabolize ethanolInto acetic acid In the presence of oxygen and water in slightly acidic pH conditions.

HYPOTHESIS **VERIFIED**

Aerobic bacteria ( family *Acetobacteraceae* could be responsible for the production of the acetic acid.

Figure 4. MIC Hypothesis Evaluation

7. Conclusions

From this hypotheses evaluation, the following has been concluded:

 Bacteria of the acetic acid producing family (*Acetobacteraceae*) were prevalent at three inspection sites. These are organisms that characteristically require oxygen and utilize ethanol as an energy source. Oxygen and ethanol were identified in the USTs inspected.

 The evaluation of hydrocarbon degradation suggests that the hydrocarbons contained within the diesel fuel may not be the primary carbon source for the consortium of bacteria present (0.3% to 5%).

 There are both less overall unique organisms present in the community and, of those present, there are limited species that dominate the community within the USTs surveyed. This is further evidence that the conditions of the ULSD tanks are conducive to growth of limited, specialized organisms.

 Geographically separate sites had some observable differences in abundance of select organisms and the presence of organisms was relatively uniform. This suggests that the ULSD system is selective for specialized organisms capable of thriving in these environments, rather than a site specific or environmental effect driving the composition of microbial population.

 Acetic acid appears to be the dominant acid species. It was measured in all vapor samples. Acetate was measured in all water samples and four of six fuel samples.

 The scrape sample results support the conclusion that the internal components made up of all different metals are deteriorating in ULSD USTs. In general, the most abundant identified organisms typed from the four samples have characteristics pertinent to the corrosion, such as acetic acid production, ethanol utilization, low pH requirements, environmental presence, and oxygen.

 Ethanol was measured in four of the six fuel samples and five of the six water samples, suggestion ethanol is contaminating the fuel. The source is unknown; however, diesel

fuel is often delivered in the same trucks as ethanol-blended gasoline. Also, ULSD USTs that have been converted from a gasoline tank could have manifolded ventilation system with gasoline tanks. Thus, it is possible that there be some cross contamination of

ethanol into ULSD.

 It is feasible for the scale or corrosion to be formed from acetic acid reacting with the iron or steel surface, if the acetic acid can be generated rapidly enough by the *Acetobacter*.

 Materials could be constantly exposed to a bulk aqueous electrolyte, thin electrolyte layers, experience wetting and drying cycles, or periodic “washing” as the tank is emptied and refilled.

This project was designed to objectively investigate multiple hypotheses as to why ULSD USTs have been experiencing severe and rapid corrosion. The in-depth site inspections were performed on a limited number of sites and therefore may not be representative all of systems experiencing this phenomenon. Although it cannot be stated with statistical significance,

ingredients necessary for the observed and chemically determined corrosion in this environment were present at the inspected sites. The above conclusions and Figure 5 summarize the supporting evidence and the final hypothesis for this project. The most obvious issues causing this problem were the focus of this research and the development of corrosion at different sites could also be influenced by other factors (environmental, geographical, seasonal, etc.) not discussed in this report.

Battelle recommends continued research into this issue. The hypothesis derived in this study should be investigated with a larger and more diverse sample set and should use a longitudinal design (where sites would be sampled multiple times over a period of time). In particular, steel USTs and tanks without issue should be investigated. This study could not compare the findings to a non-symptomatic site due to the difficulty finding one. Also, the source and magnitude of ethanol contamination should be determined.

In conclusion, the project final hypothesis is that corrosion in systems storing and dispensing ULSD is likely due to the dispersal of acetic acid throughout USTs. It is likely produced by *Acetobacter* bacteria feeding on low levels of ethanol contamination. Dispersed into the humid vapor space by the higher vapor pressure and by disturbances during fuel deliveries, acetic acid is deposited throughout the system. This results in a cycle of wetting and drying of the equipment concentrating the acetic acid on the metallic equipment and corroding it quite severely and rapidly.

**Banetie**

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FINAL HYPOTHESIS:

Aerobic microbes are producing acetic acid which is being dispersed into the humid vapor space coating and re-coating the UST equipment. This process concentrates the acetic acid on

the equipment, resulting in severe and rapid corrosion.

Acetic acid is a corrosive species found in appreciable concentrations in all vapor,fuel, and water bottom samples trom the inspected USTs.

Further,it was found in the chemicalanalysis of the corrosion scraping samples.

Did conditions exist for aerobic bacteria that produce acetic acid?

Yes.

Oxygenated areas,water, and slightly acidic pH conditions were in all USTs inspected.

Ethanol(food) was found in 5 of *6* USTs inspected.

Were these microbes found in the USTs inspected?

Yes.

Analysis of water bottoms with sufficient extractable DNA indicated presence of aerobes, specifically from the family Acetobacteraceae.

Could these microbes be responsible for corrosive species?

Yes.

Research conducted by Ghommidh,Navarro, and Durand indicates that with sufficient ethanol and oxygen, approximately 27 grams of Acetobacter can produce approximately 600 grams of acetic acid in one week. This quantity of acetic acid is sufficient to form approximately 920 grams of iron {Ill) acetate.

Are USTs suitable environments for these microbes?

Yes.

The low diversity of microbes seen in the collected samples indicates the UST diesel environment is selective for the microbes given the food,oxygen, and water.

- *Water* can enter the UST environment via condensation from air displacing fuelremoved during dispensing, dissolved in the fuel, or from surface water entering the UST.

- *Oxygen* enters the UST environment during fuel deliveries and as ambient air displaces fuel during fuel dispensing. Solubility of oxygen in water at 20°C is approximately 9.0 mg/l

and in diesel fuelis estimated to be 200-300 mg/l.

- *Ethanol* could enter the UST in diesel fuelthat has come in contact with fuels containing ethanol. Shipping tankers routinely carry ethanolfuels, jet fuels, and diesel fuels,where cross-contamination is inevitable. Ethanolis highly miscible in diesel fuel.

Is there a mechanism to disperse the acetic acid to the vapor phase?

Yes.

- Turbulence in the UST liquids (fuel and water bottom) during fueldelivery can mix and splash water containing acetic acid to the ullage portion of the UST.

- Acetic acid produced in the water layer has a higher vapor pressure than dieselfuel and can migrate through the fuelinto the vapor phase.

Figure 5. Final Hypothesis

8. References

1. EPA. Fuels and Fuel Additives. 2010 [cited 2011 March]; Available from:

<<http://www.epa.gov/otaq/fuels/dieselfuels/index.htm>>.

2. NRC, New Ultra-low-sulfur Diesel Fuel Oil Could Adversely Impact Diesel Engine

Performance 2006, Office of Nuclear Reactor Regulation Washington, D.C.

3. ASTM D6469-11 Standard Guide for Microbial Contamination in Fuels and Fuel

Systems

4. ASTM D7464 - 08 Standard Practice for Manual Sampling of Liquid Fuels, Associated

Materials and Fuel System Components for Microbiological Testing

5. Ondov B.D., Bergman N.H., and Phillippy A.M. Interactive Metagenomic Visualization

In A Web browser. BMC Bioinformatics 2011. 12:385.

6. John C. Wooley, Adam Godzik, and Iddo Friedberg, A Primer on Metagenomics. PLoS Comput Biol. 2010 February; 6(2):

7. Leahy J.G. and Colwell R.R., Microbial Degradation of Hydrocarbons in the

Environment. Micro Rev. pp 305-315. 1990.

8. Markus, A. Diesel Fuel Basics – What is Diesel Fuel, And Where Does It Come From?

PassageMaker, pp 2-6, 1999.

9. Microbial Contamination of Stored Hydrocarbon Fuels and Its Control, Christine C.

Gaylarde, Fatima M. Bento, and Joan Kelley, Rev. Microbiol., 30, 1 (1999).

10. Lee, J. S., Ray, R. I., and Little, B. J. An Assessment of Alternative Diesel Fuels: Microbiological Contamination and Corrosion Under Storage Conditions. Biofouling, Vol. 26, No. 6, pp. 623-635, August 2010.

11. GE Water and Process Technologies. Moisture Control of Diesel Fuel. Fact Sheet

FS1677EN Jul-07. General Electric Company, 2007.

12. Stott, J. F. D., Corrosion: Fundamentals, Testing, and Protection. ASM Handbook

Volume 13A., pp 644-649. 2003.

13. Christofer Legraf and Thomas Graedel, “Atmospheric Corrosion,” Chapter 7, John

Wiley & Sons, Inc. 2000).

14. Determination of Oxygen Solubility in Refinery Streams with a Membrane-Covered Polarographic Sensor, Xin A. Wu and Keng H. Chung, Ind. Eng. Chem. Res., 45, 3707, (2006).

15. Dexter, S. C., Corrosion: Fundamentals, Testing, and Protection. ASM Handbook

Volume 13A., pp 398-416. 2003.

16. Gerdes, K. R. and Suppes, G. J. Miscibility of Ethanol in Diesel Fuels, Ind. Eng. Chem.

Res., Vol. 40, No. 3, pp 949-956, 2001.

17. Ghommidh, C., Navarro, J. M., and Durand, G. A Study of Acetic Acid Production by Immobilized Acetobacter Cells: Oxygen Transfer. Biotechnology and Bioengineering, Vol XXIV, pp 605-617, 1991-1999 (1982).

18. Handbook of Chemistry and Physics, 82nd Edition, David R. Lide, Editor. P. 8-46.