CONTRACT NUMBER: FA8532-17-C-0008 (CDRL A002)

Evaluation of the Aircraft Ground Equipment (AGE) at Pacific Air Force (PACAF) Locations

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Date: 16 January 2018



This Battelle Task report 'Evaluation of the Aircraft Ground Equipment (AGE) at Pacific Air Force (PACAF) Locations' is CDRL A002 of the project funded by AFLCMC/WNZE under contract FA8532-17-C-0008.

Battelle expresses its sincere thanks to all members of the following organizations responsible for the maintenance and corrosion repair of the AGE for their excellent support during our onsite visits from 1 - 10 November. Without their cooperation and support this report would not have been possible.

- Kadena Air Base, Okinawa 18th Equipment Maintenance Squadron (EMS)
- Kunsan Air Base, Gunsan 8th Maintenance Squadron (MXS)
- Andersen Air Force Base, Guam 36th MXS

Special thanks to the leadership of the following for personnel during our onsite inspection visits;

- MSgt Mauricio Lopez 18 EMS/MXMFD and MSgt Devin Williams 18 EMS/MXMFS
- MSgt Jesse Crosse, 8 MXS/MXMG and MSgt Easterling 8 MXS/MXMG
- TSgt Andre s. McNeil, Andersen AFB 36 MXS/MXMFS

The visit would not have been possible without the prompt coordination activities led by the following:

- Mr. Douglas Dennis, SMSgt Matthew Hertel, PACAF/A4MYE
- SMSgt Johnny Barton 18 EMS/MXM
- MSgt Thomas Flammger 8 MXS/MXMF
- MSgt Robert B Madsen Jr. 36 MXS/MXMF

Finally, support and guidance from Mr. Jason McDuffie, AFLCMC/WNZ and the Air Force Project Manager for this project was critical to the coordination and data collection activities on this Task.



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List of Acronyms

AB	Air Base
AFB	Air Force Base
AGE	Aircraft Ground Equipment
BCO	Battelle Columbus, OH Operations
BLAST	Basic Local Alignment Search Tool
CDRL	Contractor Data Requirements List
CFU	Colony Forming Units
CONUS	Continental United States
COTS	Commercial-Off-The-Shelf
EDS	Energy-dispersive X-ray Spectroscopy
EMS	Equipment Maintenance Squadron
EPS	Extracellular Polymeric Substances
HALS	Hindered Amine Class)
IOB	Iron Oxidizing Bacteria
ITS	Internal Transcribed Spacer (gene)
LTPC	Low Temperature Powder Coating
MIC	Microbially Influenced Corrosion
MXG	Maintenance Group
MXS	Maintenance Squadron
NCBI	National Center for Biotechnology Information
PACAF	Pacific Air Force
PBS	Phosphate Buffered Saline
PDA	Potato Dextrose Agar
PTMLB	Portable Trailer Mounted Load Bank
PU	Polyurethane
PWS	Performance Work Statement
SRB	Sulfate Reducing Bacteria
TSA	Tryptic Soy Agar
USAF	United States Air Force
UV	Ultraviolet

Executive Summary

The United States Air Force (USAF) equipment and assets that are stationed in Pacific Air Force (PACAF) locations are exposed to highly corrosive environments resulting in damage of the equipment and adding costs for its upkeep. Battelle is working with Hentzen to modify their Commercial-Off-The-Shelf (COTS) Low Temperature Powder Coating (LTPC) 6191-61003. The objective is to tailor the COTS LTPC formulation to include suitable ultraviolet (UV) stabilizers to improve weathering performance and add relevant biocides to mitigate Microbially Influenced Corrosion (MIC).

This report is the deliverable for CDRL A002 of Phase II (PWS paragraph 4.3). The objectives were for Battelle to directly inspect, review and document aircraft ground equipment (AGE) conditions and environmental conditions at the following PACAF installations:

- Kadena Air Base (AB), Japan
- Kunsan AB, Korea
- Andersen Air Force Base (AFB), Guam

At each installation Battelle inspected five AGE assets for degradation of coatings from UV light and effects of corrosion in general and specifically, MIC. The selected AGE had coatings with varying levels of maturity ranging from 1 month to several years. Since MIC cannot be characterized by visual inspection, gauze samples were collected from corroded and non-corroded (i.e. background) areas of AGE using standard sample collection techniques, microbes were extracted, and were then characterized for potentially MIC causing bacteria and fungi species.

<u>Coatings Degradation from Exposure to UV light.</u> Color, gloss, and coatings thickness measurements were taken from the AGE inspected at the three PACAF locations. Results as seen in Figure ES- 1 show

significant loss of gloss across the inspected AGE as quickly as 3 months after being painted. Gloss readings on the MIL-PRF-85285 coating were below the minimum gloss specification of 15 units for semi-gloss in the 60° angle of incidence. Readings for color showed significant change over time from UV exposure. The two parameters indicate high levels of UV degradation of the polyurethane backbone, which in turn reduces the binding between the PU and the pigments. This can cause chalking and roughening of the coating surface which reduces the gloss of the coating. The inspection of AGE at each PACAF locations and the respective readings to determine



Figure ES- 1. Overview of UV Degradation

level of coatings degradation from exposure to UV and, the general modes of corrosion are discussed in details in section 3.0 and evaluation of the PACAF AGE results are discussed in sections 4.1 - 4.3 of this report.

<u>Characterization of Corrosion Modes and MIC at PACAF Locations.</u> Several types of corrosion were observed in the inspection of AGE in the PACAF region and as stated earlier, all of the corrosion products

cannot be attributed to MIC. For example, most corrosion emanating from fasteners and metallic joints is likely crevice or galvanic corrosion or a combination of the two modes. Preferential weld corrosion was observed on stands and similar AGE where the metal used to make the weld is slightly anodic compared to the parent metal. Therefore, the weld metal corrodes at a higher rate than the parent. Corrosion appearing through paint at corners and edges is likely a result of edge effects and the coating naturally being thinner there, so it is the first spot to form pores and leads to corrosion propagation along the edge. Corrosion observed with paint bubbling up is likely filiform corrosion but could be MIC as well. Therefore, the analysis of the microbial samples was critical for determination and for characterizing the MIC. The microbial samples from corroded areas of the inspected AGE showed no major fluctuations in the bacterial species / activity among the three PACAF locations. Kadena AB had the largest sample to sample variability, while Andersen AFB had the least. Equivalent levels of bacterial species were characterized on several samples from the non-corroded areas of the AGE, although the microbial levels observed on the non-corroded AGE samples at Andersen AFB was found to be approximately 2 logs lower than those found from Kadena AB and Kunsan AB. This is expected as bacteria and fungi can be found essentially everywhere and in equivalent amounts. Sphingomonas were the most prevalent across all of the PACAF locations, followed by Massilia. Pseudomonas/Brevundimonas was guite prevalent across all PACAF locations and is also known to contribute to MIC, likely due to its ability to form biofilms. Acidovorax/Variovorax, a known iron oxidizing bacteria (IOB) as well as a biofilm producer and Methylobacterium, known to cause corrosion in copper pipes and therefore likely contributors to MIC were not nearly as prevalent as the other two on the PACAF samples. A summary of results is presented in Table ES-1.

Prevalent Bacteria Across the Three PACAF Locations	Mechanism Influencing MIC	Cell Wall Type	
Sphingomonas	Degrades alkane, Copper-degrading, Nitrate- reducing	Gram Negative	
Acidovorax / Variovorax	Biofilm formation (Variovorax), Copper- degrading (Variovorax), Iron-oxidizing (Acidovorax), Nitrate-reducing (Acidovorax)	Gram Negative	
Pseudomonas / Brevundimonas ₉	Forms biofilms, Stainless steel corrosion, Copper-degrading, Nitrate-reducing, Carbon Steel degrading	Gram Negative	
Massilia	Found in MIC-causing consortia, Copper- degrading	Gram Negative	
Methylobacterium	Copper corrosion	Gram Negative	

The *Exophiala* genus was by far the most abundant fungal species across the three PACAF locations. The results for other species were more scattered across the locations. *Dothideomycetes* were found on the AGE samples from Kadena, but were not quite as prevalent on the AGE in Kunsan and Andersen. *Toxicocladosporium/Cladosporium* and *Aureobasidium* were also found across all the PACAF sites, but not on all the AGE at each site. Less prevalent yet were the *Aspergillus* (Kadena and Andersen only), *Nigrospora* (Kaden and Kunsan only), and *Penicillium* (all three sites) genuses; however, species within these genuses are known to be involved with MIC. A Summary of fungal results is presented in Table ES-2.

Prevalent MIC-causing Fungi across three PACAF Locations	Mechanism Influencing MIC	Substrates Affected by MIC
Exophiala	Degrades wood and organic materials; forms biofilms	Water and Metal
Dothideomycetes	Often found as pathogens, endophytes, or epiphytes of living plants; also degrades cellulose and other complex carbohydrates. Several species are lichens, while others occur as parasites.	Unknown
Aureobasidium ₆	Forms biofilms by extracellular polymeric substances (EPS)	Aluminum alloy
Toxicocladosporium / Cladosporium ₇	Metal-ion binding via fungal mycelia	Carbon steel and aluminum alloy
Aspergillus ₈	Oxalic acid metabolite	Magnesium alloy
Penicillium ₈	Forms biofilms by EPS	Aluminum alloy
Nigrospora	Found on decayed wood	Unknown

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Characterization of the PACAF samples collected for this task showed presence of wide varieties of bacteria and fungi with the potential for causing MIC. Therefore, it is important to control the microbe's formation on the coatings substrate as these microbes can contribute to the corrosion of the AGE assets. In-depth discussion on the approach for characterization of MIC and the evaluation results are presented in sections 2.3 and 4.4 of this report.

Commercially available UV stabilizers and anti-microbial additives typically added to coatings to improve weathering performance and reduce or eliminate microbial growth are discussed in section 4.5 of this report. The results were discussed with Hentzen coatings and are in consideration for the modification of the LTPC 6191-61003 for subsequent tasks of this project.

The organization of this report is as follows; Introduction of the Project and Objectives of this task are summarized in Section 1. Details on Battelle's coordination activities to schedule the inspection visits and the approach to data collection and achieving the task objectives are discussed in Section 2. Section 3 summarizes the findings from inspections at each of the three locations and documents the samples collected. Section 4 presents the evaluation results of the samples collected during the inspection visits, and the task conclusions are summarized in Section 5. Multiple Appendices are included along with the report for additional raw data.

1.0 Introduction

1.1 Background

The United States Air Force (USAF) equipment and assets stationed at the Pacific Air Force (PACAF) locations are exposed to highly corrosive environments resulting in excessive damage of the equipment and costs for its sustainment and upkeep. For the USAF, Battelle is working with Hentzen to modify their Commercial-Off-The-Shelf (COTS) Hentzen (Crosslink) Low Temperature Powder Coatings (LTPC) No. 6191-61003 to improve its resistance to Ultraviolet (UV) light and microbiologically influenced Corrosion (MIC). The intent is to transition to this powder coating initially on the Aircraft Ground Equipment (AGE). As part of the project, Battelle inspected and evaluated the AGE at three PACAF locations for the level of degradation from exposure to UV light and MIC.

1.2 Scope and Objectives

This technical report is the deliverable for CDRL A002 of the Phase II (PWS paragraph 4.3). It presents Battelle's findings from inspection and evaluation of the AGE at the three PACAF locations for degradation of the coatings from exposure to UV light and potentially from MIC.

The objectives of the task are to inspect the AGE for coatings degradation, corrosion, and evidence of MIC and to understand the similarities as well as differences in the level of UV and MIC induced degradation of coatings on the AGE at different PACAF locations and finally to report these findings to support subsequent project steps. To achieve this, select samples were taken from the AGE and surrounding areas for the purposes of identifying, characterizing and classifying field-prevalent microorganisms and MIC-related bacteria and fungi. The information was used to identify the UV additives and biocides for modification of the LTPC.

2.0 Approach for PACAF AGE Inspection & Evaluation

The following three PACAF locations were identified in the performance work statement (PWS) for the inspection of AGE to meet the objectives of the task;

- Kadena Air Base, Okinawa 18th Equipment Maintenance Squadron (EMS)
- Kunsan Air Base, Gunsan 8th Maintenance Squadron (MXS)
- Andersen Air Force Base, Guam 36th MXS

With the Air Force Program Manager's (AFPM) assistance, Battelle coordinated the visits directly with the maintenance squadrons at the three PACAF locations for the inspection and evaluation of the AGE. Twoday inspection visits were planned for each location and the following visit objectives were communicated in advance with the PACAF locations to effectively use the onsite time:

- Inspect, review, and document the condition of a minimum of five (5) AGE assets degraded by microbially induced corrosion (MIC) and from exposure to UV. Collect delaminated coatings, and soil or swab samples to characterize the prevalent microbes in the region
- Discuss the local environment, concerns relative to corrosion, maintenance challenges and the resulting challenges of the AGE groups/squadrons at each location, and
- To explore potential sites at Kadena and Andersen for installation of at least two (2) test racks for the 18-month beachfront testing.

2.1 AGE Inspection and Sample Collection

Battelle inspected the AGE for corrosion and weathering performance of the coatings at all the PACAF locations, and photos were taken of each AGE unit being sampled. MIL-PRF-85285E for polyurethane topcoats for aircraft and AGE states in section 3.7.5 that the specular gloss of the coating should be a minimum of 15 and a maximum of 45 at a 60° angle of incidence. Several gloss readings were taken from

AGE assets at the three PACAF locations with a Gardner micro-TRI-gloss meter set for 60° angle of incidence.

Color measurements and specular gloss measurements were taken from the inspected AGE to determine the level of coatings degradation from environmental effects. The color and gloss readings were analyzed at Battelle for the deleterious effects that the environment has on the CIELAB color coordinates of Fed-STD-595 color 26173 as well as the 60° specular gloss for MIL-PRF- 85285 coating. Coating stack-up film thickness measurements were also collected from these assets and are reported in the results section. Where the coating stack-up was delaminated from the inspected AGE, samples of the coating were removed and will be characterized optically to determine primer and topcoat thicknesses within the stack-up.

Samples were collected from the corroded areas on the inspected AGE assets to characterize the observed corrosion. Prior to taking the sample, gloves were disinfected with isopropyl alcohol wipes and a sterile piece of gauze was moistened with 1X phosphate buffered saline (PBS). The coated surfaces of ferrous and non-ferrous substrates of the AGE were wiped thoroughly either by swabbing back and forth three times or by wiping in an "S" shape both horizontally and vertically during sample collection. The gauze was then placed in a sterile 50 mL conical tube containing 5 mL of PBS, sealed with parafilm, and packed into coolers with ice packs to keep temperatures at around 2-8°C, and shipped to Battelle Columbus, Ohio (BCO). Five (5) samples were taken at each base, and three (3) background samples were taken at each base. Four soil (environmental) samples were taken at Kadena, 2 were taken at Kunsan, and 3 were taken at Andersen.

2.2 Evaluation of AGE for Degradation from UV

The harsh weather conditions at these locations can cause a high aesthetic degradation, due to the significant development of chalking. The severe environment can cause light induced degradation of the binder along the pigment interfaces, followed by the dissolution of the polymeric breakdown products in water that propagate the loosening of the pigment. These UV-induced and hydrolytic changes in the surface of the coating film can result in a significant loss of gloss.

The Battelle and Hentzen team is experienced in development of coatings as well as their test and assessment in different environment and on substrates and has a good understanding of the material structure. The MIL-PRF 85285E topcoat in the coatings stackup applied on AGE is a polyurethanes (PU) based acrylic polyol and is prone to degradation from exposure to outdoor environments. Although temperature, moisture, and other weathering elements may contribute to the degradation of the polymeric coatings, the primary cause of PU degradation during outdoor exposure is ultraviolet (UV) light. For that reason, the photodegradation mechanism of PU and its model compounds have been studied extensively. Both aromatic and aliphatic isocyanate-based polyurethanes undergo chain scission by photo-oxidation process. Photo-oxidation is a chemical change that reduces the polymer's molecular weight. As a consequence of this change, the material becomes more brittle, with a reduction in its tensile, impact and elongation strength. Discoloration and loss of surface smoothness accompany photo-oxidation.

To determine degradation from UV exposure, color Lab coordinates were collected from the evaluated AGE equipment at three PACAF locations using a X-Rite model SP64 hand held spectrophotometer. The source used was D65/10°. The baseline L*a*b* coordinates are taken from a color standard for FED-STD-595 26173 measured with the same X-Rite hand held color spectrophotometer. Specular gloss readings were taken with a BYK Gardner micro-TRI- gloss meter. Several gloss readings were taken from various locations on the AGE.

Weather and shortwave radiation which includes visible light and ultraviolet radiation historical data were gathered from meteorological web site weatherspark.com for the three PACAF locations included for inspection of the AGE. This data will be used to interpret results in determining the average monthly

temperature, precipitation and incident shortwave solar energy the panels will experience during the beachfront testing of the candidate coatings. This data is included in section 3 of this report for each PACAF location visited.

2.3 Microbiology

Samples received at Battelle were unpacked, checked for damage, and images captured. See Table 1 for receipt inventory and Figure 1 for an example of sample receipt. Samples were then placed into a 2 - 8 °C refrigeration unit until ready for extraction. Battelle developed, proprietary work instructions and test protocols were used for extraction and analysis of the sample and are described briefly in the subsequent sections. After extraction via the method listed in Section 2.3.1.1, content was analyzed via 16S and ITS sequencing and for culturable microbes via standard spread plating.

Site	Corrosion Samples	Background Samples	Environmental Samples (soil)	Control Samples	Condition
Kadena AB, Japan	6	5	5	1	Good
Kunsan AB, South Korea	5	6	2	1	Good
Andersen AFB, Guam	5	5	4	1	Good

Table	1.	Sample	Recei	nt [·]	Inven	torv
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Figure 1. Example Sample Receipt

2.3.1 Extraction

All samples collected for microbial testing from corroded and non-corroded (*i.e.* background) areas were extracted by vortexing the vials containing the sample and 1X PBS buffer and using a serological pipette or pipettor to press the gauze against the side of vial to manually extract liquid from the gauze. The liquid was removed from the vial and placed into a new vial labeled with the appropriate sample name. Next, 5 mL of 1X PBS was added into the vial containing the gauze, capped, and vortexed for 1 minute. The manual extraction described previously was repeated to remove the extraction buffer from the gauze a second time, and was pooled with the first extraction buffer aliquot. The final volume of buffer from each extraction was recorded. For background samples, after individual extraction, 1 mL aliquots of each background sample were removed and pooled into a new vial for each site. Furthermore, some equipment in the field were swabbed at multiple locations; these samples were combined into one composite sample after each swab was extracted individually.

2.3.2 Enumeration

Serial 1:10 dilutions of sample extracts were performed using 1X PBS. Neat, 10^{-1} , and 10^{-2} dilutions were spread plated on Tryptic Soy Agar (TSA) for aerobic bacteria and Potato Dextrose Agar (PDA) for yeast and mold and incubated at 25 ± 2 °C. Plates were checked daily for growth and observations were recorded. After 24 hours, some plates were overgrown and were replated using 10^{-3} , 10^{-4} and 10^{-5} dilutions. Other plates had slower growth and were incubated for up to 72 hours. Colony counts were recorded, and CFU/mL calculated. Colonies with similar morphologies were observed, and data compiled; all colonies with similar morphologies that were grown on TSA were further isolated onto TSA to examine if those samples were indeed the same species. TSA plates were removed after isolation and stored at 2-8 °C. PDA plates were placed back into the incubator, right-side up to promote fungal growth. These plates were checked weekly thereafter and were removed from incubation after two weeks.

2.3.3 Sequencing and Bioinformatics

2.3.3.1 Sample Extraction

Samples suspended in 1X PBS were extracted using the Qiagen DNeasy PowerLyzer Microbial Kit. Samples went through a series of centrifugation steps and were washed with various buffers to lyse open cells and extract the DNA. An ethanol buffer was used to wash away any extracellular debris, and the purified DNA was suspended in an elution buffer prior to library preparation.

2.3.3.2 Library Preparation and Metagenomics Sequencing

After extraction, library preparation was performed on the sample DNA which included a series of PCR reactions to add adapters, primers, and barcodes to amplify DNA prior to sequencing. These steps allowed 16S primers to bind to the DNA and specifically target the 16S rRNA gene sequence present in bacteria. The same steps were followed a second time, only this time, Internal Transcribed Spacer (ITS) primers were used to amplify a region of the rRNA fungal genome. Once library preparation was complete, samples were analyzed using the Agilent Bioanalyzer and Qubit to ensure concentration requirements were met for sequencing. Samples were sequenced using the Illumina MiSeq and per the developed test protocols.

2.3.3.3 Bioinformatics Methods: 16S and ITS

Sequencing data was received on an external hard drive from the laboratory. The hard drive contained the raw fastq files in gzipped format. These files were transferred to the Battelle High Performance Computing System for analysis.

Raw files were unzipped and then merged using the bioinformatic command line paired read merging tool, FLASH₁ v.1.2.11. Before running the reads through the Basic Local Alignment Search Tool (BLAST), a filter was applied to the merged reads to remove low quality reads using the fastq quality filter of the FASTX Toolkit₂. All reads that did not pass the fastq quality filter were excluded from further analysis. A plot of the quality for each sample was created using the fastq quality boxplot graph shell script, also part of the FASTX Toolkit. These plots allowed a visual inspection of the average quality for each sample after merging and filtering. All samples were of acceptable quality to move on to Blast analysis.

The merged and quality filtered reads were sent through BLAST v.2.6.0, to identify the organisms in each sample. The 16S samples were analyzed through BLAST using the NCBI 16S database v. June 11, 2017. The ITS samples were analyzed through BLAST using the UNITE₃ fungal ITS database v. January 12, 2017. Post BLAST, additional filtering was used to remove false identifications and low-quality identifications. BLAST results were filtered to remove all results with less than 97% identity over less than 80% of the read length. Following this filter, a filter was applied that is designed to remove false positive identifications are defined as hits that comprise less than 0.01 percent of the

identifications in the results. False positive hits have too few reads identified as belonging to a given organism to be considered strong identifications.

Two scripts from KronaTools₄ were used to get both the TaxID of all filtered BLAST results (reported in an .xlsx spreadsheet) and to generate an interactive Krona plot for relative abundance visualization of the results. The ktClassifyBLAST script gathers the TaxID for all results when the -s flag (summary) is used. The ktImportBLAST script organizes all the results into a single, interactive plot for visualization and exploration of results.

For each organism identified at greater than 1% of the sample, a quick search through the scientific literature was performed to determine origin or bio-activity for the identified organisms. The R programming and statistical analysis language was used to create heat maps to compare sample community composition in a plot.

2.4 Industry Available UV Inhibition Additives and Biocides

The performance of an additive is directly related to its chemical structure and exposure conditions. Therefore, it is important to know the chemical functionality of the additives and their activity during manufacturing process and weathering conditions (UV or microorganisms). Battelle conducted a literature search to identify commercially available additives (biocides and UV inhibitors) using the following databases

- a) SciFinder® (Service provided by American Chemical Society),
- b) United States Patent and Trademark Office (USPTO)
- c) Peer reviewed articles from journals focused on paints and coatings namely Coatings Journal, Progress in Organic Coatings and J. Coatings & Technology
- d) Technical and Safety data sheets from selected commercial sources.
- e) Websites reporting about environmental and safety regulations such as California Pro 65, EPA

We used the information gathered from the literature along with other guiding factors such as LTPC manufacturing process, Environmental regulations, field evaluation of microorganisms and weatherability to down select potential additives. The details are reported in section 4.5

3.0 PACAF Site Inspections & Findings

3.1 AGE Inspection at Kadena Air Base

The 18th EMS at Kadena is split between two teams operating on the north and the south side of the flight

line. The north team maintains the AGE for large Cargo, Recon, and re-fueling aircraft while the south side team primarily maintains AGE for Fighters. At Kadena AB Battelle evaluated five AGE assets, three from the south side of the flight line and two from the north side of the flight line. According to the 18th EMS team, the AGE on the north side is exposed to a more severe environment due to its proximity to the ocean and more frequent precipitation than that on the south side.

According to weatherspark.com at "Kadena Air Base, the summers are hot, oppressive, wet, and overcast; the winters are cool and mostly clear; and it is windy year-round. Over the course of the



Figure 2. Kadena AB (Source: google maps)

year, the temperature typically varies from 57°F to 89°F and is rarely below 49°F or above 92°F. The total daily incident shortwave solar energy reaching the surface of the ground over a wide area, taking full account of seasonal variations in the length of the day, the elevation of the Sun above the horizon, and absorption by clouds and other atmospheric constituents. Shortwave radiation includes visible light and ultraviolet radiation. The average daily incident shortwave solar energy experiences some seasonal variation over the course of the year. The darker period of the year lasts for 2.4 months, from November 20 to February 2, with an average daily incident shortwave energy per square meter below 3.6 kWh. The brighter period of the year lasts for 3.3 months, from June 17 to September 27, with an average daily incident shortwave 5.1 kWh".



Figure 3 Average annual weather at Kadena AB (Source: https://weatherspark.com)

3.1.1 Inspection Findings of AGE

Battelle started inspection of the AGE at Kadena on the South side primarily supporting the fighter aircraft. The 18th EMS is supported by a contractor operated corrosion repair facility capable of scuff sanding and painting off aircraft components as well as AGE. Because the AGE is subjected to excessive use in harsh open environment and is seldom sent to depots at the CONUS locations for maintenance, this facility is a critical capability for sustainment of the AGE at Kadena. At an interval of about 60 months, corrosion maintenance is performed at this facility on all AGE / assets in accordance with the Tech Order 35-1-3 and coatings are reapplied.

AGE was staged in the parking lot for inspection. To get a representative effect of UV exposure and corrosion effects, Battelle selected five (5) AGE with varying durations from corrosion maintenance ranging from 1 month to close to 60 months.

The following five (5) AGE were inspected at Kadena AB

- C-10 Aircart AU21: 1 month from corrosion maintenance
- Portable Trailer Mounted Load Bank (PTMLB) LB21: 3 months from corrosion maintenance
- Generator 60 GT 28: 60 months from corrosion maintenance
- B-1 stand B1 64 about 48 months from corrosion maintenance
- Air conditioning and Heating Unit: AA05. This AGE is on the north side supporting large aircraft. Because there are only two of these units, this asset was not scheduled for corrosion maintenance in over 7 years

Many of the corrosion products found on the AGE equipment at Kadena AB were in areas of bolts, leaf springs, under carriage, welds, and crevices between dissimilar metals. These are areas where moisture can collect and in the case of dissimilar metals an electrochemical process can occur in which one metal corrodes preferentially when it is in electrical contact with another, in the presence of an electrolyte. In the areas of welds, the problem arises from the fact that weld metal compositions (which are normally optimized for mechanical properties) tend to be slightly anodic to the parent steel. Therefore, the weld metal corrodes at a higher rate than the parent.

Significant loss of gloss can be seen between these two assets evaluated at Kadena. The AU-21 aircart was painted 1 month prior to this photo, while the GT28 generator was painted 60 months



Figure 4. Photos of AGE in Kadena to show the loss of gloss over time



Photos of corrosion products found on AGE at Kadena AB. These are most likely the result of galvanic, crevice or preferential weld corrosion

Figure 5. Corrosion observed on Inspected AGE at Kadena

3.1.2 AGE Inspected at Kadena

Swabs were taken from both corroded and non-corroded areas of the AGE units. Table 2 provides an overview of the sample name, collection location and collection technique used.

AGE Identification	Corrosion Sample Location	Sample Technique	Non-corrosion Sample Location
J-AU21	Leaf Spring of aircart	Sample was taken by wiping PBS soaked gauze back and forth on three leaflets.	Non-corroded area of aircart
J-LB25	Leaf Spring and left side of generator	Sample was taken by wiping PBS soaked gauze back and forth on three leaflets and	Non-corroded area on left side of AGE

Table 2. AGE Inspected at Kadena

AGE Identification	Corrosion Sample Location	Sample Technique	Non-corrosion Sample Location
		wiping up and down 3 times left side where CPC was sprayed	
J-GT28	Left side of break linkage and leaf frame on front of generator	Samples taken by swabbing PBS soaked gauze back and forth three times.	Non-corroded area of generator
J-B164	Front step and bar on the back end of B1 stand	Samples taken by swabbing PBS soaked gauze back and forth three times.	Sample taken from non-corroded area of AGE
J-AA05 & J- AA05.2	Lower back right corner and from door on the right side. (J-AA05.2) piece of corroded material.	Samples taken by swabbing PBS soaked gauze back and forth on three leaflets. back and forth three times.	Sample taken from non-corroded area of AGE
Note: All sampl soaked gauze	es from the non-corro	ded areas were taken by allv in "S" format	swabbing PBS

3.1.3 Beachfront Testing Site

While at Kadena, Battelle also worked with the 18th EMS to explore location for the 18-month beachfront testing site. Three potential locations were evaluated for southern exposure, proximity to the oceanfront, contractor access to the test site, safety during extreme weather conditions, and other factors of relevance to the location.

Battelle plans to install two exposure racks to mount test coupons for the beachfront testing task. These racks will require about $350 \text{ ft}^2 - 400 \text{ ft}^2$ in area. During this task, Battelle's subcontractors will periodically evaluate the test coupons each quarter and send a set of coupons back to Battelle for evaluation.

3.2 AGE Inspection at Kunsan AB



According to weatherspark.com "in Gunsan, the summers are warm, muggy, and wet; the winters are very cold, dry, and windy; and it is partly cloudy year-round. Over the course of the year, the temperature typically varies from 24°F to 87°F and is rarely below 15°F or above 92°F. The average daily incident shortwave solar energy experiences significant seasonal variation over the course of the year. The brighter period of the year lasts for 3.9 months, from April 15 to August 12, with an average daily incident shortwave energy per square meter above 5.7 kWh. The darker period of the year lasts for 2.8 months, from November 10 to February 5, with an average daily incident shortwave energy per square meter below 3.1 kWh".

Figure 6. Kunsan AB (Source: google maps)



Figure 7 Average annual weather at Kunsan AB (Source: https://weatherspark.com)

3.2.1 Inspection Findings of AGE

The AGE at Kunsan is maintained by the 8th MXS. The assets were similar to those at Kadena. A B4 stand at Kunsan has been with the 8th MXS since 1989. Kunsan also has a corrosion repair facility equipped with abrasive blast media and painting capabilities. Records showed that the B4 stand was painted in 2007 and then again in 2014. While several AGE / assets were inspected, samples for characterization of corrosion effects were collected from the following five (5) AGE selected based on the duration from the previous corrosion maintenance/painting.

- Dash 60 Turbine Generator: TG-27; Fresh received from the corrosion repair
- Dash 95: TC-21; Old DRMO, arrived at Kunsan 2001
- 7000 lb Bomb lift; MH10 Arrived at Kunsan 2011; CAT3 and never been painted
- C1-12 stand; No additional information available
- Load Bank LB-04 Arrived at Kunsan 2015 CAT 1



Example of probable crevice corrosion or corrosion due to compromised coating seen on the undercarriage of AGE at Kunsan AB

Figure 8. AGE Inspection Findings at Kunsan AB



(a) Example of Possible example of MIC from hydraulic oil. (b) Edge effects seen on the fairing of a Dash 95 TC 21 at Kunsan AB. Corrosion peeking through paint at corners is likely a result of edge effects and the coating naturally being thinner there, so it is the first spot to form pores and thus lead to corrosion

Figure 9. Additional AGE Inspections at Kunsan

3.2.2 AGE Inspected at Kunsan

Swabs were taken from both corroded and non-corroded areas of the AGE units. Table 3 provides an overview of the sample name, collection location and collection technique used.

AGE Identification	Corrosion Sample Location	Sample Technique	Non-corrosion Sample Location	Sample Technique
K-TG27	Top of turbine generator	Sample taken in "S" format by swabbing PBS soaked gauze horizontally and vertically	Non-corroded area of turbine generator	Sample taken in "S" format by swabbing PBS soaked gauze horizontally and vertically
K-TC21	Inside of AGE and on tow bar	Sample taken by swabbing PBS soaked gauze back and forth three times	Non-corroded area of aircart, on both steel and aluminum	Sample taken by swabbing PBS soaked gauze back and forth three times
K-MH10	Bomb lift and front of AGE	Sample taken by swabbing PBS soaked gauze back and forth three times	Non-corroded area of bomb lift	Sample taken by swabbing PBS soaked gauze back and forth three times
K-CC12	Under steps of bomb lift	Sample taken by swabbing PBS soaked gauze back and forth three times	Non-corroded area of steps of bomb lift	Sample taken by swabbing PBS soaked gauze back and forth three times
K-LB04	Side of load bank	Sample taken by swabbing PBS soaked gauze back and forth three times	Non-corroded side of load bank	Sample taken by swabbing PBS soaked gauze back and forth three times

Table 3. AGE Inspected at Kunsan

3.3 AGE Inspection at Andersen Air Force Base



According to weather spark.com at "USAF Andersen Air Force Base. the wet season is hot and overcast, the dry season is warm and partly cloudy, and it is oppressive and windy year round. Over the course of the year, the temperature typically varies from 77°F to 87°F and is rarely below 75°F or above 89°F. The average daily incident shortwave solar energy experiences significant seasonal variation over the course of the year. The brighter period of the year lasts for 2.5 months, from February 28 to May 10, with an average daily incident shortwave energy per square meter above 6.4 kWh. The darker period of the year lasts for 4.0 months, from July 5 to November 6, with an average daily incident shortwave energy per square meter below 4.5 kWh".

Figure 10. Andersen AFB (Source: google maps)



Figure 11 Average annual weather at Andersen AFB (Source: https://weatherspark.com)

3.3.1 Inspection Findings of AGE

The AGE at Andersen is maintained by the 36 MXG/MXMG and was similar in type and function as the AGE found at Kadena and Kunsan. AGE at Andersen is refurbished just off base by Milspray. This facility has the capability to blast and paint. The AGE at Andersen selected varied in time from last refurbishment from 1 month to 60 months with one asset that was still green in color. Three of the assets were staged outside and two of the assets the Dash 86 power generator PC70 and the 809 generator B8W4 were in the maintenance bay. Evaluations of corrosion and samples were taken from the following.

- MJ-1C bomb lift: MJW6 painted 1 month previously
- Dash 86 generator: PC03 refurbished 1 year ago 9/29/16
- 809 generator: B8W4 green in color, never painted
- Dash 86 generator: PC70 5 years since last painted (Nov 2012)
- B5 stand: B597 no additional information available

Figure 12 shows the striking difference in color and gloss from UV degradation on the bomb lift painted a month ago and the Dash 86 generators painted five year ago and Figure 13 shows corrosion damage on the inspected AGE at Andersen AFB.



Figure 12. UV degradation of coatings at Andersen AFB resulting in loss of gloss



Figure 13 Corrosion types observed on the AGE at Andersen AFB

3.3.2 AGE Inspected at Andersen

Swabs were taken from both corroded and non-corroded areas of the AGE units. Table 4 provides an overview of the sample name, collection location and collection technique used.

AGE Identification	Corrosion Sample Location	Sample Technique	Non-corrosion Sample Location	Sample Technique
A-MJW6	Front and side of bomb lift	Sample was taken by swabbing PBS soaked gauze back and forth three times	Non-corroded area on front of bomb lift	Sample taken in "S" format by swabbing PBS soaked gauze horizontally and vertically
A-PC03	Side of power generator	Sample was taken by swabbing PBS soaked gauze back and forth three times	Non-corroded steel area on front of power generator	Sample taken in "S" format by swabbing PBS soaked gauze horizontally and vertically
A-B8W4	Side, back, and top of generator	Sample was taken by swabbing PBS soaked gauze back and forth three times	Non-corroded area on generator	Sample taken in "S" format by swabbing PBS soaked gauze horizontally and vertically
A-PC70	Side of power generator	Sample was taken by swabbing PBS soaked gauze back and forth three times	Non-corroded steel area on power generator	Sample taken in "S" format by swabbing PBS soaked gauze horizontally and vertically
A-B597	Bottom, side and top of B5 stand	Sample was taken by swabbing PBS soaked gauze back and forth three times	Non-corroded area on B5 stand	Sample taken by swabbing PBS soaked gauze back and forth three times

Table 4. AGE Ins	pected at Anderse
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4.0 PACAF AGE Evaluation Results

4.1 Impact of UV on AGE Coatings Gloss

Loss of surface gloss and fading is a typical response of a coating due to degradation via UV radiation absorption. Subjectively there is a general reduction in shine, and the surface starts to look chalky. This commonly accepted phenomenon can be observed with any exterior coating. It may be more pronounced in some locations versus others, but the overall trend should be the same.

Figure 14 presents the trend observed on the inspected AGE in gloss reduction through pictures of the AGE at the three PACAF locations.



AU21 Kadena 1 month Gloss = 30.13





TC21 Kunsan 192 months Gloss = 1.53



LB25 Kadena 3 months Gloss = 9.47



GT28 Kadena 60 months Gloss = 2.37



Figure 14 UV degradation over time of AGE coatings at the three PACAF Locations

The gloss readings recorded on AGE at all the three PACAF locations are presented in Table 5.

Location	Equipment Nomenclature	Unit Serial #	Avg. 60° gloss	Months since last
Kadena South	C10 aircart	AU 21	30.13	1
Kadena South	generator	LB 25	9.47	3
Kadena South	generator	GT 28	2.37	60
Kadena North	B1 stand	B1 64	7.33	unknown
Kadena North	AC + Heat unit	AA 05	1.55	unknown
Kunsan	Dash 60	TG 27	17.63	0*
Kunsan	Dash 95	TC 21	1.53	192
Kunsan	Bomb lift	MH 10	4.23	72
Kunsan	C1 stand	C1 12	28.42	unknown
Kunsan	Load bank	LB 04	4.06	unknown
Andersen	bomblift	MJW6	22.17	1
Andersen	Dash 86	PC-03	23.17	12
Andersen	809 generator	B8W4	14.77	unknown**
Andersen	Dash 86	PC-70	4.47	60
Andersen	B5 stand	B5-97	1.80	unknown
*Unit still tacky,	not completely cui	red		
**Unit was gree	n in color			

Table 5 Average gloss readings taken from PACAF weathered AGE

Assets in which the maturity of the coating could be verified through records were plotted for the 60° gloss readings versus time. This plot included three assets from each of the three PACAF locations. In Figure 16 below we see a dramatic drop in gloss, falling below the minimum specification as soon as three months.



Figure 15. Plot of 60° gloss readings vs. time

4.2 Impact of UV on AGE Coatings Color

The Air Force's technical order 35-1-3 states in section 3.5 that the standard color for all flight line support equipment is gray 26173 FED-STD-595, MIL-PRF-85285.

Several color readings were taken from PACAF AGE assets with a X-Rite model SP64 hand held spectrophotometer. The source used was D65/10°. The CIELAB coordinates were evaluated to see the effects on the topcoat color of AGE assets located in the PACAF region. The ΔL , Δa , Δb and ΔE were calculated for each of the readings and an average delta is shown in table 6. If you refer to the diagram of the CIELAB color space in Figure 17 you will see that positive (+ve) delta (Δ) corelates to a color reading becoming lighter while a negative (-ve) Δ would mean the color is darkening. A positive Δa would mean the color is moving to the red spectrum while a negative Δa shows a move toward the green. A positive move in the Δb shows the color is vellowing and a negative Δb shows the color moving toward the blue spectrum. The ΔE deals with how the human eye perceives changes and a general rule of thumb is that the untrained human eye cannot perceive color changes that are less than 1 ΔE . A look at the ΔL ,



Figure 16 CIELAB color space

 Δa and Δb shows a general lightening, reddening and yellowing of the polyurethane topcoat on assets that have been sitting in the PACAF environment for any extended period. This is observed by the ΔL number moving in a positive direction as well as the Δa and Δb becoming positive in the Lab color space in the Figure 17. Table 6 presents the recorded color coordinates from the AGE inspected at the three PACAF locations.

Location	Equipment normenclature	Unit Serial #	ΔL	∆a	Δb	ΔE	Months since last recorded painting
Kadena S	C10 aircart	Au 21	0.47	0.51	0.44	0.88	1
Kadena S	generator	LB 25	-2.40	0.57	0.76	2.60	3
Kadena S	generator	GT 28	5.42	0.46	1.61	7.52	60
Kadena N	B1 stand	B1 64	-1.07	0.66	0.66	2.33	unknown
Kadena N	AC + Heat unit	AA 05	9.32	0.33	0.42	9.33	unknown
Kunsan	Dash 60	TG-27	-1.95	0.71	0.33	2.11	0*
Kunsan	Dash 95	TC 21	2.44	0.63	2.11	4.95	192
Kunsan	Bomb lift	MH 10	7.56	-0.14	-0.16	7.59	72
Kunsan	C1 stand	C1 12	-2.11	0.59	2.50	3.50	unknown
Kunsan	Load bank	LB 04	6.74	-0.27	-0.85	6.84	unknown
Andersen	Bomb lift	MJW6	0.00	0.36	-0.72	0.81	1
Andersen	Dash 86	PC 03	0.72	0.02	0.11	1.04	12
Andersen	generator	B8W4	-19.12	0.28	8.55	20.96	Unknown**
Andersen	Dash 86	PC 70	5.06	0.25	1.18	5.21	60
Andersen	B1 stand	B1 97	6.63	0.43	1.26	6.81	unknown
*Unit still tae **Unit greer	cky, not completely n in color	y cured					

Table 6 Average ΔL , Δa , Δb and ΔE color coordinates from AGE in PACAF locations

4.3 Evaluation of Coating Stack-up and Film Thicknesses

Many film thickness measurements were taken on the various AGE assets at various locations with a DeFelsko Positector 6000 coating thickness gage. The results of these film thickness readings can be found in Appendix B. The film thicknesses varied significantly because during the corrosion repair process the AGE is only sanded down to the base metal in areas showing corrosion, and over time the film build can be quite significant. Where the coating stack-up was peeling away from the assets substrate, samples of the coating were removed and were characterized optically to determine primer and topcoat thicknesses within the stack-up. Energy-dispersive X-ray spectroscopy (EDS), is an analytical technique used for the elemental analysis or chemical characterization of a sample. EDS was used to determine the elements in each film layer so it could be identified. Figure 17, Figure 18, and Figure 19 present the evaluation of the corroded samples from the three PACAF locations.



Figure 17. Evaluation of Corroded Coatings Sample from Kadena AB



Figure 18. Evaluation of Corroded Coatings Sample from Kunsan AB



Figure 19. Evaluation of Corroded Coatings Sample from Andersen AFB

4.4 Microbiology Results

The first 24 hours of growth on TSA and PDA plates resulted in overgrown plates for some samples, which led to further dilutions and re-plating (10⁻³, 10⁻⁴ and 10⁻⁵). Other plates had organisms that grew slower, requiring incubation for up to 72 hours. Counts were similar on TSA and PDA, with the enumeration results from samples plated on both PDA and TSA shown in Table 7. Overall, there were no major fluctuations in the enumerations from site to site, with an approximate range of 10² CFU/mL to 10⁶ CFU/mL for Kadena AB, 10² CFU/mL to 10⁴ CFU/mL for Kunsan AB, and 10³ CFU/mL to 10⁴ CFU/mL for Andersen AFB. This range shows that Kadena AB had the largest sample to sample variability, while Andersen AFB had the least. Enumerations from non-corroded areas of the AGE were also equivalent in number to the AGE enumerations, although the background taken at Andersen AFB was ~2 logs lower than the backgrounds from Kadena and Kunsan AB. This is expected as bacteria and fungi can be found essentially everywhere and in equivalent amounts.

Site	Sample ID	Avg. CFU/mL (PDA)	Avg. CFU/mL (TSA)
	J-AU21	6.60E+02	6.07E+02
	J-LB25	3.07E+05	2.87E+05
	J-GT28	3.06E+04	1.41E+04
Kadena AB, Japan	J-B164	5.87E+05	6.17E+05
	J-AA05	1.21E+06	1.42E+06
	*J-AA05_C	1.02E+04	1.40E+04
	J-BKGRD	1.25E+05	1.12E+05
	K-TG27	4.07E+04	9.73E+04
	K-TC21	1.53E+04	1.55E+04
Kunsan AB, South	K-MH10	3.77E+02	8.27E+02
Korea	K-LB04	9.10E+02	1.01E+03
	K-C112	**TNTC	1.30E+03
	K-BKGRD	5.93E+04	6.13E+05
	G-MJW6	1.81E+04	7.31E+04
	G-PC03	3.74E+04	5.59E+04
Anderson AER Quem	G-B8W4	2.07E+04	2.37E+04
Andersen AFD, Guam	G-PC70	1.60E+04	6.50E+04
	G-B597	4.69E+04	7.69E+04
	G-BKGRD	6.70E+03	6.17E+03

Table 7. Bacterial and Fungal Enumeration Results

*Sample= Piece of chipped coating, not gauze.

**TNTC= Too numerous to count; plate overgrew after the 24-hour checkpoint

Table 8 describes the bacterial morphologies observed on the TSA plates prior to colony isolation. Figure 18 and Figure 21 show two samples plated on TSA after incubation for 24 hours and 48 hours, respectively. All colonies with similar morphologies on TSA were further isolated onto TSA. Further examination of bacterial morphology on the isolated TSA plates was undertaken (see Figure 22 for an example) and Table 8 updated where possible. No further action past that was undertaken as the

microbiology portion at this point was for informational purposes and the sequencing data provided the details needed for identification (Section 4.4.1).

Table o	Table 8. TSA FTC-Isolation Morphology Observations					
Morphology Label		Samples Displaying				
Morphology A	Pinpoint/small, white/off- white, transparent, round/raised/convex, entire	All Plates at All Sites				
Morphology B	small, round, raised, entire, shiny, wet	J-B164, J-AA05_C, J- BKGRD, K-TC21, K-NH10, K-LB04, K-C112, K-BKGRD, G-MJW6, G-B8W4, G-PC70, G-B597, G-BKGRD, J-AA05, K-TG27				
Morphology C	small-medium, yellow, raised shiny, round	J-AU21, J-LB25, J-GT28, J- AA05_C, K-TC21, K-MH10, K-LB04, K-C112, G-MJW6, G-B8W4, G-PC70, G-B597, J-AA05				
Morphology D	white, rhizoid	J-AU21, J-GT28				
Morphology E	filamentous, green, raised	J-AU21, K-C112				
Morphology F	small, pink, raised round	G-BKGRD, K-LB04, K-TC21, G-B597				
Morphology G	yellow/white/filamentous, raised	J-GT28, G-MJW6, G-PC03				
Morphology H	yellow/white, wrinkle, craterform	J-AA05_C, G-PC70, G-B597				
Morphology I	flat, irregular, medium, clear, yellow	K-TC21				
Morphology J	medium, dark orange, raised, round	K-TC21				
Morphology K	white, umbonate	G-PC70				
Morphology L	yellow, flat, shiny	G-PC70				
Morphology M	Small - medium, yellow center, cone, shiny, edge clear/off-white	J-AA05				

Table 8, TSA Pre-Isolation Morphology Observations



Figure 20. K-TG27 on TSA after 24 hours at 25 \pm 2 $^{\circ}\mathrm{C}$



Figure 21. J-GT28 on TSA after 48 hours at 25 ± 2 °C



Figure 22. Morphology A, J-LB25 Colony Selection for Before (a) and After (b) Isolation

Table 9 describes the fungal morphologies observed on the plates. No isolations were performed on PDA since sequencing was performed also (Section 4.4.1). See Figure 23 for an example of fungal growth observed. Fungal growth was especially prevalent on sample J-AA05_C, the sample from Kadena AB that was a piece of chipped corroded coating (Figure 24) and was presented in Figure 17 under section 4.3.

Samples collected showed abundant bacterial and fungal growth. Most of the bacterial growth had foul odors, indicating sulfate reducing bacteria (SRB) may be present, which can lead to MIC; as well as other bacteria and fungi. See Section 4.4.1 for the discussion on sequencing results and a more in-depth data analysis as to which organisms are prevalent across all sites.

Fungal Morphology Description	Samples Displaying Organism	Possible Microbe
Large, white, rhizoid,	G-B8W4, J-GT28, G-PC03,	Microsporum,
Cloud-like	K-1G27, J-AA05_C, J-A021	Cunningnamella
	G-B8W4, J-GT28, G-PC70,	
Black medium to large	K-TC21, J-AA05, J-AA05_C,	
shiny, wet	J-AU21, J-LB25, K-MH10, K-	Aspergillus
	C112, K-LB04, G-BKGRD,	
	K-BKGRD, J-BKGRD	
Yellow, medium, rhizoid	G-B8W4	Microsporum
Large, white rhizoid, red		Microsporum,
wet under white rhizoid	G-D0114	Trichophyton, Fusarium
grov modium volvot	G-B8W4, G-B597, G-	Cladophialophora,
gray, medium, vervet-	BKGRD, J-AA05_C, J-B164,	Chromoblastomyosis,
like	J-LB25	Aspergillus
Small – medium, white,	C REOT C MIWE	Fusarium,
filamentous	G-6597, G-105000	Cladosporium

Table 9. PDA Morphology Descriptions



Figure 24. G-PC03 on PDA Before and After Fungal Growth Promotion



Figure 23. J-AA05_C PDA plates

4.4.1 16S and ITS Metagenomics Sequencing Results

Sequencing data showed a number of microbial communities present in each sample. Due to the large amount of data produced, approximately the top 90% of organisms present in each sample were analyzed. These organisms are presented in Table 10 - Table 12. The tables show bacterial DNA detected on each AGE, and bacterial genuses common across AGE are color coded (e.g. all Sphingomonas are highlighted in orange). These color codes are carried across tables so that it can be determined which bacterial genuses were also found on AGE across sites. Common soil microbes found on the AGE, not considered to be contributors to MIC, are highlighted in grey. In one case, in Table 10 (Sample J-B164), some common (and uncommon) pathogenic organisms were observed during sequence analysis (i.e. Salmonella enterica, Trabulsiella guamensis, Brevundimonas vesicularis, and Salmonella bongori) and were omitted from further analyses. These organisms are highlighted in grey. After thorough analyses of Table 10 - Table 12, the top set of five bacterial genuses most likely contributing to the MIC across all PACAF locations was compiled. Sphingomonas were the most prevalent across all of the PACAF locations, followed by Massilia. Pseudomonas/Brevundimonas was also selected as it was quite prevalent across all PACAF locations and is also known to contribute to MIC, likely due to its ability to form biofilms. While not nearly as prevalent, Acidovorax/Variovorax was also selected as it is known to be an acid producer as well as a biofilm producer, and could very likely be a contributor to MIC. Finally, another potential contributor, again while not as prevalent, is Methylobacterium. This genus has been shown to be involved in copper pipe corrosion. Table 13 lists the bacterial genuses of most interest.

ITS sequencing data resulted in the same type of data for fungus. These data are shown in Table 15 - Table 17, and again the tables show fungal DNA detected on each AGE, and fungal genuses common across AGE are color coded (*e.g.* all *Exophiala* are highlighted in orange). These color codes are carried across tables so that it can be determined which fungal genuses were also found on AGE across sites. After thorough analyses of Table 15 - Table 17, the top set of 7 fungal genuses most likely contributing to the MIC across <u>all</u> PACAF locations was compiled (Table 18). The *Exophiala* genus was by far the most abundant within a PACAF site as well as across sites; however, after that the results were more scattered. *Dothideomycetes* were found across the AGE at the Kadena AB, but were not quite as prevalent on the AGE in Kunsan AB and Andersen AFB. *Toxicocladosporium/Cladosporium* and *Aureobasidium* were also found across all the PACAF sites, but not on all the AGE at each site. Less prevalent yet were the *Aspergillus* (Andersen AFB and Kadena AB only), *Nigrospora* (Kadena AB and Kunsan AB only), and *Penicillium* (all three sites) genuses; however, species within these genuses are known to be involved with MIC so they were included in Table 18.

J-AU21	J-AA05	J-AA05.2	J-LB25	J-GT28	J-B164
Alkanindiges hongkongensis	Stenotrophomonas maltophilia	Alkanindiges hongkongensis	Pseudomonas soli	Sphingomonas hankookensis	Trabulsiella guamensis
Pseudoxantho- monas spadix	Stenotrophomonas pavanii	Acidovorax delafieldii	Sphingomonas hankookensis	Brevundimonas vesicularis	Brevundimonas vesicularis
Sphingomonas paucimobilis	Brevundimonas vesicularis	Noviherbaspirillum psychrotolerans	Pseudomonas punonensis	Pseudomonas stutzeri	Salmonella enterica subsp. enterica serovar Typhimurium
Rhizobium pakistanensis	Sphingomonas kyungheensis	Pseudoxanthomonas mexicana	Sphingomonas dokdonensis	Sphingomonas dokdonensis	Agrobacterium fabrum
	Sphingomonas adhaesiva	Blastocatella fastidiosa	Massilia namucuonensis	Microbacterium chocolatum	Janthinobacterium agaricidamnosum
	Massilia suwonensis	Brevundimonas lenta	Novosphingobiu m barchaimii LL02	Microbacterium aurantiacum	Pseudomonas oryzihabitans
	Sphingomonas hankookensis	Noviherbaspirillum suwonense	Sphingomonas kyungheensis	Sphingomonas kyungheensis	Sphingobium abikonense
	Noviherbaspirillum suwonense	Sphingomonas roseiflava	Roseomonas gilardii	Microbacterium oleivorans	Sphingomonas pituitosa
	Paracoccus carotinifaciens	Xenophilus aerolatus	Enterobacter kobei	Bosea robiniae	Acidovorax wautersii
	Noviherbaspirillum canariense	Methylobacterium tarhaniae	Pseudoxanthom onas spadix	Spirosoma rigui	Massilia consociata
	Sphingomonas paucimobilis	Sphingomonas adhaesiva	Leclercia adecarboxylata	Pseudoxanthom onas mexicana	Salmonella bongori
	Sphingomonas gimensis	Tetrasphaera remsis	Massilia oculi	Massilia arvi	Sphingomonas hankookensis
	Blastocatella fastidiosa	Sphingomonas kyungheensis	Curtobacterium oceanosediment um	Sphingomonas aquatilis	Bordetella sp. R- 39474
	Sphingomonas desiccabilis	Chryseobacterium profundimaris	Rhizobium qilianshanense	Rhizobium cellulosilyticum	Massilia arvi
	Methylobacterium phyllostachyos	Sphingomonas ginsengisoli	Rhizobium grahamii	Agrobacterium larrymoorei	Curtobacterium oceanosedimentum
	Spirosoma rigui	Acetobacteraceae bacterium WS10	Agrobacterium fabrum		

Table 10. To	n ~90% of Bacteria	Found on A	GE at Kadena AB
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J-AU21	J-AA05	J-AA05.2	J-LB25	J-GT28	J-B164
	Sphingomonas	Methylobacterium			
	dokdonensis	phyllostachyos			
	Mucilaginibacter litoreus	Methylocapsa sp. NE2			
	Spirosoma sp. MSd3	Microvirga guangxiensis			
	Tersicoccus phoenicis	Flavisolibacter ginsenosidimutans			
	Brevundimonas viscosa	Noviherbaspirillum canariense			
	Methylobacterium tarhaniae	Variovorax defluvii			
	Massilia brevitalea	Methylobacterium soli			
	Microbacterium aerolatum	Alsobacter metallidurans			
	Novosphingobium naphthalenivorans	Sphingomonas dokdonensis			
	Microbacterium assamensis	Azospirillum oryzae			
	Variovorax defluvii	Sphingomonas desiccabilis			
	Rhizobium cellulosilyticum		_		
	Rhizobium				
	endophyticum CCGE 2052				
	Mesorhizobium mediterraneum				
	Pseudarthrobacter equi				

K-TG27	K-TC21	K-MH10	K-LB04	K-C112
Massilia timonae	Sphingomonas sp. DCY91	Brevundimonas vesicularis	Massilia jejuensis	Pseudochrobactrum Iubricantis
Pseudomonas abietaniphila	Pseudomonas punonensis	Noviherbaspirillum suwonense	Sphingomonas sp. JM-791	Luteibacter rhizovicinus
Sphingomonas paucimobilis	Noviherbaspirillum suwonense	Rhodococcus corynebacterioides	Pseudomonas oryzihabitans	Variovorax boronicumulans (NBRC 103145)
Brevundimonas vesicularis	Sphingomonas kyungheensis	Sphingomonas hankookensis	Sphingomonas paucimobilis	Brevundimonas vesicularis
Sphingobium rhizovicinum	Ochrobactrum pseudogrignonense	Pseudorhodoferax caeni	Massilia oculi	Bacillus aquimaris
Novosphingobium lindaniclasticum LE124	Sphingobium rhizovicinum	Pigmentiphaga litoralis	Sphingomonas dokdonensis	Okibacterium fritillariae
Noviherbaspirillum suwonense	Exiguobacterium acetylicum	Sphingomonas kyungheensis	Brevundimonas vesicularis	Sphingomonas kyungheensis
Sphingomonas sp. JM- 791	Brevundimonas vesicularis	Luteibacter rhizovicinus	Pigmentiphaga litoralis	Aureimonas ureilytica (DSM 18598 = NBRC 106430)
Massilia suwonensis	Sphingomonas kyeonggiensis	Massilia consociata	Massilia tieshanensis	Curtobacterium oceanosedimentum
Roseomonas aerophila	Sphingomonas hankookensis	Massilia putida	Sphingomonas kyungheensis	Rhizobium pakistanensis
Massilia namucuonensis	Paenibacillus taohuashanense	Sphingomonas roseiflava	Massilia varians	Curtobacterium flaccumfaciens
Microbacterium assamensis	Pseudomonas oryzihabitans	Microbacterium ginsengiterrae	Stenotrophomonas pavanii	Curtobacterium albidum
Agrobacterium Iarrymoorei	Exiguobacterium indicum	Sphingomonas yunnanensis	Noviherbaspirillum suwonense	
Rhizobium soli DS-42	Bacillus vireti LMG 21834	Achromobacter animicus	Microbacterium ginsengiterrae	
Rhizobium skierniewicense	Sphingomonas roseiflava	Agrobacterium Iarrymoorei	Sphingomonas roseiflava	
Curtobacterium oceanosedimentum	Sphingomonas dokdonensis	Curtobacterium oceanosedimentum	Paenibacillus pabuli	
Agrobacterium rubi	Methylobacterium phyllostachyos	Rhizobium cellulosilyticum	Massilia suwonensis	

K-TG27	K-TC21	К-МН10	K-LB04	K-C112
	Rhizobium soli DS-42	Pseudarthrobacter equi	Rhizobium cellulosilyticum	
	Pseudarthrobacter phenanthrenivorans Sphe3	Pseudarthrobacter phenanthrenivorans Sphe3	Rhizobium soli DS- 42	
	Pseudarthrobacter equi	Curtobacterium flaccumfaciens	Rhizobium grahamii	
	Curtobacterium oceanosedimentum Agrobacterium		Curtobacterium oceanosedimentum	

Table 12. Top ~90% of Bacteria Found on AGE at Andersen AFB

G-B8W4	G-PC70	G-B597	G-MJW6	G-PC03
Pseudomonas stutzeri	Pseudomonas stutzeri	Alkanindiges hongkongensis	Sphingomonas kyungheensis	Noviherbaspirillum canariense
Chryseobacterium hagamense	Sphingomonas dokdonensis	Pseudomonas stutzeri	Sphingomonas paucimobilis	Sphingomonas hankookensis
Pedobacter sp. THG- G12	Pseudomonas sp. KMM 9500	Sphingomonas hankookensis	Mycoplana ramosa	Sphingomonas kyungheensis
Massilia consociata	Sphingomonas hankookensis	Pseudarthrobacter equi	Aureimonas phyllosphaerae	Noviherbaspirillum soli
Brevundimonas vesicularis	Sphingomonas kyungheensis	Agrobacterium Iarrymoorei	Microbacterium assamensis	Sphingomonas adhaesiva
Sphingobacterium kyonggiense	Brevundimonas vesicularis	Sphingomonas kyungheensis	Methylobacterium komagatae (DSM 19563)	Oxalicibacterium solurbis
Devosia lucknowensis	Cellulomonas pakistanensis	Oxalicibacterium solurbis	Methylobacterium pseudosasae	Novosphingobium subterraneum
Brevundimonas olei	Curtobacterium albidum	Noviherbaspirillum canariense	Sphingomonas hankookensis	Sphingomonas kyeonggiensis
Sphingobacterium daejeonense		Rhodococcus cerastii	Aureimonas ureilytica (DSM 18598 = NBRC 106430)	Methylobacterium hispanicum
Massilia suwonensis		Noviherbaspirillum soli	Microbacterium oleivorans	Novosphingobium naphthalenivorans

Evaluation of the COTS LTPC (CDRL A002)

G-B8W4	G-PC70	G-B597	G-MJW6	G-PC03
Pseudomonas sp. KMM 9500		Sphingomonas adhaesiva	Microbacterium marinum	Microbacterium mitrae
Parapusillimonas granuli		Tersicoccus phoenicis	Massilia timonae	Agrobacterium larrymoorei
Sphingobacterium lactis		Sphingomonas dokdonensis	Brevundimonas vesicularis	
Sphingobium rhizovicinum		Novosphingobium naphthalenivorans	Curtobacterium pusillum	
Shinella zoogloeoides		Curtobacterium oceanosedimentum	Curtobacterium oceanosedimentum	
Chryseobacterium arachidiradicis				
Achromobacter				
Agrobacterium				
larrymoorei Agrobacterium				
tumefaciens				

Prevalent Bacteria Across the Three PACAF Locations	Mechanism Influencing MIC	Cell Wall Type
Sphingomonas ^{1,2,}	Degrades alkane, Copper-degrading, Nitrate-reducing	Gram Negative
Acidovorax ^{3,4} /Variovorax ⁵	Biofilm formation (Variovorax), Copper-degrading (Variovorax), Iron- oxidizing (Acidovorax), Nitrate-reducing (Acidovorax)	Gram Negative
Pseudomonas ^{6,7,8,9} /Brevundimonas ₉	Forms biofilms, Stainless steel corrosion, Copper-degrading, Nitrate- reducing, Carbon Steel degrading	Gram Negative
Massilia ¹⁰	Found in MIC-causing consortia, Copper-degrading	Gram Negative
Methylobacterium	Copper corrosion	Gram Negative

Table 13. Potential MIC-causing Bacteria Prevalent Across All PACAF Locations

Table 14. Top ~90% of Fungi Found on AGE at Kadena AB

J-AU21	J-AA05	J-AA05.2	J-LB25	J-GT28	J-B164
Cystobasidium minutum	Exophiala xenobiotica	Dothideomycetes sp.; Didymellaceae sp. ZLY- 2010	Aureobasidium namibiae	Exophiala xenobiotica	Exophiala oligosperma
Nigrospora oryzae	Dothideomycetes sp.; Didymellaceae sp. ZLY-2010	Exophiala xenobiotica	Cystobasidium benthicum	Aureobasidium namibiae	Exophiala xenobiotica

¹ Pavissich *et al.*, Culture dependent and independent analyses of bacterial communities involved in copper plumbing corrosion. 2010. Journal of Applied Microbiology 109; 771–782.

² Leigh *et al.*, Microbial communities biostimulated by ethanol during uranium (VI) bioremediation in contaminated sediment as shown by stable isotope probing, 2015. Front. Environ. Sci. Eng. 9(3): 453–464.

³ Wang et al., Effects of disinfectant and biofilm on the corrosion of cast iron pipes in a reclaimed water distribution system. 2012. Water Research 46 (2012) 1070 e1078.

⁴ Leigh *et al.*, Microbial communities biostimulated by ethanol during uranium (VI) bioremediation in contaminated sediment as shown by stable isotope probing, 2015. Front. Environ. Sci. Eng. 9(3): 453–464.

⁵ Pavissich et al., Culture dependent and independent analyses of bacterial communities involved in copper plumbing corrosion. 2010. Journal of Applied Microbiology 109; 771–782.

⁶ Jia R. *et al.*, Anaerobic Corrosion of 304 Stainless Steel Caused by the *Pseudomonas aeruginosa* Biofilm. 2017. Front. Microbiol. 8:2335.

⁷ Aruliah and Ting, Characterization of Corrosive Bacterial Consortia Isolated from Water in a Cooling Tower. 2014. ISRN Corrosion Volume 2014, Article ID 803219.

⁸ Pavissich et al., Culture dependent and independent analyses of bacterial communities involved in copper plumbing corrosion. 2010. Journal of Applied Microbiology 109; 771–782.

⁹ Rajasekar *et al.*, Characterization of corrosive bacterial consortia isolated from petroleum-product-transporting pipelines. 2010. Appl Microbiol Biotechnol. 85:1175–1188

¹⁰ Aruliah and Ting, Characterization of Corrosive Bacterial Consortia Isolated from Water in a Cooling Tower. 2014. ISRN Corrosion Volume 2014, Article ID 803219.

J-AU21	J-AA05	J-AA05.2	J-LB25	J-GT28	J-B164
Toxicocladosporium irritans	Exophiala phaeomuriformis	Resinicium saccharicola	Toxicocladosporium irritans	Toxicocladosporiu m irritans	Nigrospora oryzae; Nigrospora spaerica
Dothideomycetes_s p.; Didymellaceae sp. ZLY-2010	Phialophora chinensis	Marasmius tenuissimus	Dothideomycetes sp.; Didymellaceae sp. ZLY-2010	Nigrospora oryzae; Nigrospora spaerica	Cyphellophora europaea
Cordyceps bassiana; Beauveria bassiana	Exophiala dermatitidis	Gymnopilus dilepis	Chaetothyriales sp	Cystobasidium benthicum	Dothideomycete s sp.; Didymellaceae sp. ZLY-2010
Exophiala xenobiotica		Coniosporium uncinatum	Nigrospora oryzae; Nigrospora spaerica	Dothideomycetes sp.; Didymellaceae sp. ZLY-2010	
Penicillium oxalicum		Diatrypaceae sp	Pestalotiopsis coffeae-arabicae		
Moesziomyces parantarcticus		Ascomycota sp	Exophiala xenobiotica		
· ·	-	Aplosporella javeedii		•	
		Eutypella citricola			
		Toxicocladosporium irritans			
		Exophiala oligosperma			
		Ochroconis musae			
		Knufia epidermidis			
		Nigrospora oryzae;			
		Exonhiala dermatitidis			
		Ochroconis globalis			
		Aureobasidium namibiae			
		Entolomataceae sp			
		Ascomycota sp.; fungal sp. E08			
		Hydnochaete japonica			

J-AU21	J-AA05	J-AA05.2	J-LB25	J-GT28	J-B164
		Agaricus rotalis			
		Aspergillus pulvericola			
		Hymenochaete muroiana			
		Ustilaginaceae sp			

Table 15. Top ~90% of Fungi Found on AGE at Kunsan AB

K-TG27	K-TC21	K-MH10	K-LB04	K-C112
Hypocreales sp	Calyptrozyma sp	Exophiala sp	Chaetothyriales sp	Cystobasidium lysinophilum
Nigrospora oryzae; Nigrospora spaerica	Aureobasidium namibiae	Exophiala heteromorpha	Aureobasidium namibiae	Aureobasidium namibiae
Montagnulaceae sp.; Dothediomycetes sp.	Sarocladium bactrocephalum	Aureobasidium namibiae	Exophiala sp	Pseudozyma pruni
Sarocladium bactrocephalum	Hypocreales sp	Pleosporales sp	Cryptococcus saitoi	Nigrospora oryzae; Nigrospora spaerica
Penicillium cinerascens	Sporobolomyces carnicolor	Exophiala xenobiotica	Hypocreales sp	Montagnulaceae sp.; Dothediomycetes sp.
Ustilaginaceae sp	Exophiala xenobiotica		Trichomeriaceae sp	Ascomycota sp
Cladosporium delicatulum	Chaetothyriales sp		Pleosporales sp	Dioszegia zsoltii var. zsoltii
Agaricomycetes sp	Sporobolomyces phaffii		Exophiala xenobiotica	Pleosporales sp

Table 16. Top ~90% of Fungi Found on AGE at Andersen AFB

G-B8W4	G-PC70	G-B597	G-MJW6	G-PC03
Hortaea werneckii	Aureobasidium namibiae	Toxicocladosporium irritans	Dothideomycetes sp	Exophiala xenobiotica
Aspergillus penicillioides	lschnoderma resinosum	Exophiala xenobiotica	Pseudozyma hubeiensis	Dothideomycetes sp
Gymnoascaceae sp		Sympoventuriaceae sp	Curvularia pseudorobusta	Claviceps pusilla
Aspergillus tamarii		Aureobasidium namibiae	Penicillium multicolor	
Dothideomycetes sp.; Didymellaceae sp. ZLY- 2010		Ochroconis musae	Pseudozyma sp	
Aspergillus salwaensis				

Prevalent MIC-causing Fungi across three PACAF Locations	Mechanism Influencing MIC	Substrates Affected by MIC
Exophiala ₁₁	Degrades wood and organic materials; forms biofilms	Water and Metal
Dothideomycetes	Often found as pathogens, endophytes, or epiphytes of living plants; also degrades cellulose and other complex carbohydrates. Several species are lichens, while others occur as parasites.	Unknown
Aureobasidium ₆	Forms biofilms by extracellular polymeric substances (EPS)	Aluminum alloy
Toxicocladosporium/Cladosporium7	Metal-ion binding via fungal mycelia	Carbon steel and aluminum alloy
Aspergillus ₈	Oxalic acid metabolite	Magnesium alloy
Penicillium ₈	Forms biofilms by EPS	Aluminum alloy
Nigrospora	Found on decayed wood	Unknown

 Table 17. Potential MIC-causing Fungi Across all PACAF Locations

4.5 Analysis of Literature for Available UV Inhibiting Additives and Biocides

4.5.1 List of UV Inhibiting Additives to Improve Weathering Performance

The results obtained from weathering studies (section 4.1 and 4.2) were discussed with the manufacturer and it is mutually agreed to add UV stabilizer to improve the weathering of the current LTPC composition. Based on the literature search and performance requirements (thermally stable at LTPC process conditions, no adverse interaction with the ingredients present in the LTPC composition), Battelle down selected potential UV inhibition additives and presented in Table 18.

Class	Chemical Name	CAS	Melting range (°C)
Hindered amine class (HALS)	Bis (1, 2, 2, 6, 6-pentamethyl-4- piperidinyl)-[[3, 5-bis (1, 1- dimethylethyl)-4- hydroxyphenyl]methyl]butylmalonate	63843-89-0	146 - 150
	Polymer of 2,2,4,4-tetramethyl-7- oxa-3,20-diaza-dispiro [5.1.11.2]- heneico-san-21-on and epichlorohydrin	202483-55-4	>148°C (softening point)
	Tetramethyl-7-oxa3,20- diazadispro[5.1.11.2]-henicosan-21- one	64338-16-5	>225°C
UV Absorber	2-Ethyl,2'ethoxy-oxalanilide	23949-66-8	126- 128°C
	2-(2H-benzotriazol-2-yl)-4, 6-bis (1- methyl-1-phenylethyl)phenol	70321-86-7	137 – 141°C

Table 18: Typ	oical UV absorbe	rs and HALS use	ed in powder coating	25 ^{1'}
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The potential UV inhibition additives will be provided to LTPC manufacturer and assist them to select the top additive for the formulation.

4.5.2 List of Microbicides to Improve MIC Performance

Microbicides are increasingly regulated due to their adverse effects on health and environment. Therefore, formulators should be aware of the safety and environmental concerns for specified microbicides used in the formulation.

Table 13 and Table 17 summarize bacteria and fungi, respectively, potentially causing MIC on the AGE. It is apparent wide varieties of organisms are present on the AGE and are possibly the cause for the corrosion. Therefore, it is critical to control these microbes' formation on the coating substrates as these

¹¹ George Wpych, e. (2015). Handbook of UV Degradation and Stabilization 2nd edition. Toronto: Chemtech publishing.

microbes are potentially contributing to the corrosion of the coatings. Battelle identified a preliminary list of microbicides (Table 19) that are typically added to coatings to reduce or eliminate microbial growth.

Name	CAS	Melting Point	Boiling Point	Vapor Pressure	Chemistry, Mode of Action
3-lodo-2- propynylbutylcarbamate (IPBC)	55406-53-6	~65- 67°C		2.4- 4.5x10 ⁻³ (25°C)	Carbamate; Mode of Action: Disturbs the cell membrane permeability and affects the fatty acids metabolism
Thiabendazole (TBZ)	148-79-8	~297- 298°C		4.6- 5.3x10 ⁻⁷ (25°C)	Benzimidazole; Mode of Action: Contact and systemic fungicide; interferes with the assembly of microtubules (components of fungal exoskeleton)
Carbendazim (BCM)	10605-21-7	~302- 307°C		1.5x10 ⁻⁴ (25°C)	Benzimidazole, Carbamate; Mode of Action: Interferes with DNA synthesis, mitosis, cytokinesis or related processes
Zinc Pyrithione (ZnP)	13463-41-7	≥240°C		1x10 ⁻⁶ (25°C)	Pyridine-N-oxide; Mode of Action: Membrane- active substance with chelating properties; influence on ATP levels, protein synthesis and nutrient transport
2-n-octyl-4-isothiazolin- 3-one (OIT)	26530-20-1		120°C	4.9x10 ⁻³ (25°C)	Isothiazolinone; Mode of Action: Electrophilic active agent. Reacts with nucleophiles (e.g. amines or thiols); enzyme
4 5-dichloro-2-n-octyl-4- isothiazolin-3-one (DCOIT)	64359-81-5	~41- 42°C		9.8x10 ⁻² (25°C)	Isothiazolinone; Mode of Action: Electrophilic active agent with activated N-S bond and vinyl activated halogens; reacts with nucleophilic elements of cell proteins resulting in interruption of

Table 19. Microbicides used in coating formulations

Name	CAS	Melting Point	Boiling Point	Vapor Pressure	Chemistry, Mode of Action
					important metabolic processes
2-Butyl-1,2- benzisothiazolin-3-one (BBIT)	4299-07-4		Decom. before boiling	1.5x10 ⁻² (25°C)	Isothiazolinone; Mode of Action: Electrophilic active agent
Tebuonazole	107534-96-3	105°C		1.7x10 ⁻⁶ (20°C)	Azole; Mode of Action: Inhibition of ergosterol biosynthesis in fungal membranes; at lower azole concentration, retardation of fungal growth, at higher azole conc. Disruption of fungal membranes
Propiconazole	60207-90-1		>250°C	5.6X10 ⁻⁵ (25°C)	Azole; Mode of Action: Inhibition of ergosterol biosynthesis in fungal membranes; at lower azole concentration, retardation of fungal growth, at higher azole conc. Disruption of fungal membranes
Silver nanoparticles	Multiple CAS# based on the chemistry		Not Applicable	Not Applicable	Multiple mode of action and well documented additive for inhibiting bacterial, algal and fungal growth
Quat Salts	Multiple CAS# based on the chemistry		Not Applicable	Not Applicable	Disturbs the cell membrane permeability and affects the fatty acids

Considering the broad spectrum of microorganisms present in the environment, a combination of microbial additives is necessary to improve the MIC performance. Furthermore, the additives should be compatible with the LTPC process condition and should not have any adverse environmental and regulatory aspects of the microbicides. Based on the above discussion, we suggest inclusion of silver nanoparticles, Quat Salts, and IPBC in the LTPC formulation.

4.5.3 Applicable Environmental Regulations to Additives and Biocides

It is important to consider the addition of a biocide to provide a long-lasting coating must be balanced with their health effects and potential damage to the environment. There has been a history of additives used in years past like mercury salts, Carbendazim (BCM), and chlorothalonil (CTL) that have been linked to

toxicity, genetic defects, and carcinogenicity. (Shauer, 2017) A few of the microbicides listed are skin sensitizers and care must be taken especially during the coating application whereupon it is airborne. In regard to general structure toxicity, these particular chemistries were not listed by CAS number in California's Proposition 65 (updated December 2017). As with the chemistry, and with specific state regulations, their individual toxological review may be incomplete and may not be fully representative of actual effects on humans. The Globally Harmonized System of hazard communication details environmental and specific health hazards. It has been adopted to some degree in all major countries, including United States, Japan, and Korea. It may be projected as to what concentration would trigger the requirement of a health hazard pictogram.

Name	CAS	GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)
IPBC	55406-53-6	Acute toxicity, Oral (Category 4), H302
		Serious eye damage (Category 1), H318
		Skin sensitisation (Sub-category 1B), H317
		Specific target organ toxicity - repeated exposure (Category 1),
		H372
		Acute aquatic toxicity (Category 1), H400
		Chronic aquatic toxicity (Category 1), H410
TBZ	148-79-8	Acute aquatic toxicity (Category 1), H400
		Chronic aquatic toxicity (Category 1), H410
BCM	10605-21-7	Germ cell mutagenicity (Category 1B), H340
		Reproductive toxicity (Category 1B), H360
		Acute aquatic toxicity (Category 1), H400
		Chronic aquatic toxicity (Category 1), H410
ZnP	13463-41-7	Acute toxicity, Oral (Category 3), H301
		Acute toxicity, Inhalation (Category 3), H331
		Serious eye damage (Category 1), H318
		Acute aquatic toxicity (Category 1), H400
		Chronic aquatic toxicity (Category 1), H410
OIT	26530-20-1	Acute toxicity, Oral (Category 4), H302
		Acute toxicity, Inhalation (Category 3), H331
		Acute toxicity, Dermal (Category 3), H311
		Skin corrosion (Category 1B), H314
		Serious eye damage (Category 1), H318
		Skin sensitisation (Category 1), H317
		Acute aquatic toxicity (Category 1), H400
		Chronic aquatic toxicity (Category 1), H410
DCOIT	64359-81-5	Acute toxicity, oral(Category 4), H302
		Skin corrosion/irritation(Category 2)H315
		Serious eye damage/eye irritation(Category 2A) H319
		Acute toxicity, inhalation(Category 4)H332
		Specific target organ toxicity, single exposure(Category 3)H335
BBIT	4299-07-4	Acute toxicity, oral(Category 4), H302
		Skin corrosion/irritation(Category 2), H315
		Serious eye damage/eye irritation(Category 2A), H319
		Specific target organ toxicity, single exposure(Category 3), H335
Tebuonazole	107534-96-3	Acute toxicity, Oral (Category 4), H302
		Reproductive toxicity (Category 2), H361
		Acute aquatic toxicity (Category 2), H401
		Chronic aquatic toxicity (Category 2). H411
Propiconazole	60207-90-1	Acute toxicity, Oral (Category 4), H302
		Acute toxicity, Inhalation (Category 2), H330

Name	CAS	GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)
		Skin sensitisation (Category 1), H317 Acute aquatic toxicity (Category 1), H400 Chronic aquatic toxicity (Category 1), H410

GHS labeling pulled from safety data sheets from concentrated ingredients.

4.6 LTPC Manufacturer's Statement on Compatibility of Additives

This task required that the Contractor provide a written and signed statement from the manufacturer ot the LTC indicating that the additive and inhibitors presented in this report to AFLCMC/WNZE and those in considerations for modification of formulations for the subsequent laboratory testing tasks are compatible with the LTPC system, and appropriate for PACAF AGE environmental applications. Accordingly, Battelle has received a letter from Hentzen Coatings and it is included in Appendix A.

5.0 Conclusions

In terms of UV degradation of the coatings we see a significant loss of gloss across AGE inspected at all the three PACAF locations. We measured the AGE in this region falling below the minimum gloss specification of 15 units for semi-gloss in the 60° angle of incidence as quickly as 3 months on AGE in the PACAF region. This is a sign of UV degradation as the chain scission takes place in the backbone of the polyurethane the bonding between the pigment and resin in reduced. This can cause a chalking and roughening of the coating surface which reduces the gloss of the coating.

We also notice a change in the color of the coating as it sets in the harsh environmental conditions of this region. We see a lightening of the topcoat color as well as the color moving toward the red and yellow color spectrum. This is a sign of UV degradation as polyurethanes will tend to amber with exposure to UV radiation.

Several modes of corrosion were observed in the inspection of the AGE in the PACAF region. It is difficult to determine the exact cause of the corrosion product and impossible to associate it to MIC during visible inspection. Most of the corrosion emanating from fasteners and metallic joints is likely crevice or galvanic corrosion or a combination of the two modes. It appeared there is preferential weld corrosion in couple of cases as the metal used to make the weld is slightly anodic compared to the parent metal. Therefore, the weld metal corrodes at a higher rate than the parent. Corrosion appearing through paint at corners and edges is likely a result of edge effects and the coating naturally being thinner there, so it is the first spot to form pores and thus lead to corrosion propagation along the edge (and out). The corrosion where the paint is bubbling up is likely filiform corrosion but could be MIC.

As for the potential organisms that may contribute to the corrosion, there have been numerous studies demonstrating that microorganisms may be involved in corrosion. For example, bacteria belonging to the genus *Sphingomonas* were detected in 2011 in an underground pyrite mine.¹² The bacteria were detected in acid streamer samples at pH 3, and resulted in better growth under microaerobic and anaerobic conditions than when grown aerobically. Colonies of the sample were gelatinous, and liquid cultures were highly viscous, suggesting the sample produces exopolymeric materials, which is a common characteristic of *Sphingomonas spp*. These characteristics are all indicative of a type of bacteria that can produce a type of biofilm, grow anaerobically (*i.e.* without oxygen), and live in a highly acidic environment. These are all essential characteristics of an organism that helps to promote MIC. As was shown in Table 10-Table 12, bacteria from the *Sphingomonas* genus were found most frequently across

¹² Kimura *et al.*, Biodiversity and geochemistry of an extremely acidic, low-temperature subterranean environment sustained by chemolithotrophy. 2011. Environmental Microbiology. 13(8), 2092–2104.

all three sites and across all AGE samples. Ultimately, as listed in Table 13, *Sphingomonas*, along with four other organisms, was selected for testing to observe whether it contributes to corrosion. *Acidovorax* is known to be an IOB as well as a nitrate reducer, and *Variovax* is known to produce biofilms and degrade copper. The bacteria that belong to the genus *Pseudomonas* are another strong candidate to contribute to MIC, as they are known to form biofilms and contribute to stainless steel, carbon steel, and copper corrosion. They can also reduce nitrate. All of these characteristics make it very likely to be a contributor to MIC. Finally, bacteria belonging to *Massilia* and *Methylobacterium* genuses were selected as candidates. They are found in MIC-causing consortia, and are both known to degrade copper.

Next, the fungi that potentially contribute to corrosion were considered. They are listed in Table 14-Table 16, and the downselected group is listed in Table 17. All fungi found in these genuses are found across the sites as well as across the AGE samples. These fungi are known to form biofilms and/or degrade wood or other organic materials. These candidates are the most promising in terms of potentially contributing to corrosion.

6.0 References

- 1. Magoc T. and Salzberg S.L. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27(21) 2957-2963.
- 2. FASTX-Toolkit http://hannonlab.cshl.edu/fastx_toolkit/ Accessed December 19, 2017.
- 3. UNITE (User-friendly Nordic ITS Ectomycorrhiza database). <u>https://unite.ut.ee/</u> Accessed December 5, 2017.
- 4. Ondov B.D., Bergman N.H. and Philippy A.M. 2011. Interactive metagenomic visualization in a web browser. *BMC bioinformatics* 12(1), 385.
- 5. Critchley, MM. Pasetto, R. O'Halloran, RJ. Microbial influences in 'blue water' copper corrosion. Journal of Applied Microbiology. 2004;97(3):590-7.
- McNamara CJ¹, Perry TD, Leard R, Bearce K, Dante J, Mitchell R. Corrosion of aluminum alloy 2024 by microorganisms isolated from aircraft fuel tanks. PubMed. Biofouling. 2005;21(5-6):257-65
- 7. Little, Brenda. Lee, S, Jason. 2007. Microbiologically Induced Corrosion. Page 200.
- T. A. Alekhova. A. A. Aleksandrova. T. Yu. Novozhilova. L. V. Lysak. N. A. Zagustina.
 A. M. Bezborodov. Monitoring of Microbial Degraders in Manned Space Stations. 2005. Applied Biochemistry and Microbiology. Volume 41, Issue 4, pp 382-389.
- S.J. Yuan. S.O. Pehkonen. Microbiologically influenced corrosion of 304 stainless steel by aerobic *Pseudomonas* NCIMB 2021 bacteria: AFM and XPS study. Volume 59, Issue 1. 2007, Pages 87-99.
- Hu, Jun. Qiang, Zhimin. Impacts of water quality on the corrosion of cast iron pipes for water distribution and proposed source water switch strategy. Water Research. 2017. Volume 129, Pages 428-435.
- 11. Biedunkiewicz, Anna. Schulz, Lukas. Fungi of the genus Exophiala in tap water-potential etiological factors of phaeohyphomycoses.
- 12. Altimira, Fabiola, Yanez, Carolia. *et al.* Characterization of copper-resistant bacteria and bacterial communities from copper-polluted agricultural soils of central Chile. 2012. BMC Microbiology. 2012; 12: 193.
- 13. Donlan, RM. Costerton, JW. Biofilms: survival mechanisms of clinically relevant microorganisms. 2002 Apr;15(2):167-93.
- Tinh Nguyen, Jonathan W. Martin, Eric Byrd and Edward Embree, National Institutes of Standards and Technology, Effects of Spectral UV on Degradation of Acrylic-Urethane Coatings. 80th Annual Meeting of the Program of the Federation of Societies for Coatings Technology, 2002.

Appendix A: Hentzen Coatings Letter per the AF PWS requirements



Vinay V. Gadkari, PMP Battelle Program Manager Office: 614.424.5751 Email: gadkariv@battelle.org 505 King Ave. Columbus, OH 43201 January 12, 2018

SUBJECT: Additive Statement per PWS 4.3.1 for Phase II PR Number: FD2060-17-30718

Dear Vinay,

Hentzen Coatings, Inc. has researched and down-selected commercially available UV and MIC additives compatible with Commercial Off The Shelf Low Temperature Powder Coating (COTS LTPC) formulations.

A Hindered Amine Light Stabilizer (HALS) will be used to improve weatherability from UV degradation.

3 down selected additives for inhibiting Microbial Induced Corrosion (MIC) will be approved by Battelle microbiologists for testing in the following test systems in Air Force Color 26173 Semi-Gloss Gray:

- 1) Control COTS LTPC (No UV or MIC Additives)
- COTS LTPC + HALS
- 3) COTS LTPC + MIC Inhibitor #1 + HALS
- COTS LTPC + MIC Inhibitor #2 + HALS
- 5) COTS LTPC + MIC Inhibitor #3 + HALS
- 6) COTS LTPC (Anti-Microbial resin modification to reduce MIC)

If you have any additional questions, call me or Andy Daly at (414) 353-4232 adaly@hentzen.com.

Best Regards,

R. J. Pourghil

Rick Pomykala – Defense & Aerospace Product Mgr. Mobile: (630) 776-4748 Office: (630) 761-4310 roomykala/Dhentzen.com

6937 West Mill Road Milwaukee, WI 53218-1225 (414) 353-4200 • www.hentzen.com ISO 9001:2008 AS9100 REV.C

Appendix B: Coatings thickness measurements recorded on the inspected AGE at the three PACAF locations

	Equipme	ent	Coating Thickness Measurement (mils)				5)
Location	Nomenclature	Serial #	Sampling Location	#1	#2	#3	AVG
Kadena	C10 aireart	AU 21	side	7.2	8		7.6
South	oroandare	10 21	axle	6.1	6.4	7.2	6.6
Kadena			bumper	4.3			4.3
South	Generator	LB 25	tow bar	6	4.8		5.4
			axle	9.4	5.1	6	6.8
			tow bar	4.2	3.4	2.3	3.3
Kadena	Generator	GT 28	frame	2.7	2.4	2.5	2.5
South			fuel tank	7.8	0.1		
			suap	7.0	0.1	5.1	0.5
			Frame				
Kadena North	B1 Stand	B1 64	around				
			standing area	5.1	3.9	5.9	5.0
			axle	17.9	9	13.8	13.6
Kadena North	AC + Heat Veh	AA 05	Back Bumper	3.8	7.6	6.8	6.1
			frame	25	25.8	21.1	24.0
	Dash 60		Left side				
			frame	5.8	4.8	10.2	6.9
Kupcap		TC 37					
Kunsan		IG 27	front tow bar	15	12.3	12.8	13.4
			tank strap	5.3	2.3	2.4	3.3
			tow bar	2.9	3.6	4.7	3.7
Kupsan	Dach 05	TC - 21	right side				
Kunsan	0451195	10-21	frame	9.9	9	7	8.6
			front	6.5	8	6.1	6.9
			Back side				
			above				
Kunsan	7000# bomb lift	MH-10	exhaust	7	5.6	6.3	6.3
			top	3	3.8	7.6	4.8
			top frame	6.4	6.7	4.3	5.8
			left side	5	2.2	1.5	2.9
Kunsan	C1 stand	C1-12	right side	1.9	1.1	1.6	1.5
			front step	1.2	1.1	2.5	1.6
			Front frame				
			low	1.7	1.7	1.4	1.6
Kunsan	Load Bank	LB-04	back colling				
			grate	1.9	2.7	2.4	2.3
			top	2.2	3	2.6	2.6

	Equipm	ent	Coating Thickness Measurement (mils)					
Location	Nomenclature	Serial #	Sampling Location	#1	#2	#3	AVG	
			top	15.2	11.7	9.3	12.1	
Andersen	MJ-1C bomblift	MJW6	right side	14.4	16.4	21.7	17.5	
			left side	9.6	6.6	8.2	8.1	
			tow bar	5.5	6	6.4	6.0	
Andersen	Dash 86	PC-03	left side	8.2	9.6	9.9	9.2	
			right side	7.2	8	8	7.7	
			left side	3.5	4.9	4.9	4.4	
Andersen	809 Generator	B8W4	top	5.3	5.9	4.3	5.2	
			right side	5.3	4.6	5.1	5.0	
			tow bar	4.1	3.9	4.2	4.1	
Andersen	Dash 86	PC-70	top	8.5	8.5	11	9.3	
			back side	6.2	6.2	6.5	6.3	
			Left side	4.2	4.3	3.1	3.9	
Andersen	B5 stand	B5-97	right side	3.3	3.6	3.5	3.5	
			front side	3.5	5.4	4.6	4.5	
			-					