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Microbial Characterization of Internal Active Thermal Control System (IATCS) Hardware Surfaces after Five Years of Operation in the International Space Station

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ABSTRACT

A flex hose assembly containing aqueous coolant from the International Space Station (ISS) Internal Active Thermal Control System (IATCS) consisting of a 2 foot section of Teflon hose and quick disconnects (QDs) and a Special Performance Checkout Unit (SPCU) heat exchanger containing separate channels of IATCS coolant and iodinated water used to cool spacesuits and Extravehicular Mobility Units (EMUs) were returned for destructive analyses on Shuttle return to flight mission STS-114. The original aqueous IATCS coolant used in Node 1, the Laboratory Module, and the Airlock consisted of water, borate (pH buffer), phosphate (corrosion control), and silver sulfate (microbiological control) at a pH of 9.5 ± 0.5. Chemical changes occurred after on-orbit implementation including a decrease to pH 8.4 due to the diffusion of carbon dioxide through the Teflon hoses, an increase in nickel ions due to general corrosion of heat exchanger braze coatings, a decrease in phosphate concentration due to precipitation of nickel phosphate, and the rapid disappearance of silver ions due to deposition on hardware surfaces. Also associated with the coolant chemistry changes was an increase in planktonic microorganisms from less than 100 colony forming units (CFU) per 100 ml to approximately 1 million CFU per 100 ml. Attachment and growth of microorganisms to the system surfaces (biofilm) was suspected due to the levels of planktonic microorganisms in the coolant. Biofilms can reduce coolant flow, reduce heat transfer, amplify degradation of system materials initiated by chemical corrosion, and enhance mineral scale formation.

Enumerations of microorganisms on hardware surfaces were performed by membrane filtration and spread plating on R2A, 1/10-strength tryptic soy agar, and fluid thioglycollate medium agar incubated for 14 days at 25 - 30°C. Enrichments of viable microorganisms from surfaces of the flex hose and SPCU heat exchanger were performed in R2B (R2A without agar), 1/10-strength tryptic soy broth, and fluid thioglycollate medium. Bacterial colonies isolated from these assays were isolated and identified by several methods.

This initial paper reports the results of the membrane filtration analyses performed to assess biofilm on IATCS hardware exposed to coolant and microorganisms during five years of on-orbit operation.

INTRODUCTION

The heat generated by equipment and crew on board the International Space Station (ISS) is removed to maintain a comfortable working and living environment. The Thermal Control System (TCS) in the ISS collects excess heat directly from equipment and indirectly from the atmosphere. The TCS consists of two distinct sections: the Internal Active Thermal Control System (IATCS) which uses an aqueous solution as the working fluid to remove heat from the crew and equipment, and the External Active Thermal Control (EATCS) which uses ammonia as the working fluid to release heat to space via radiation. These two sections interact through liquid-liquid heat exchangers (HX) that transfer heat while maintaining physical separation of the different fluids.

The IATCS consists of two loops that can be independently operated as a low-temperature loop and a moderate-temperature loop. These loops can also be operated in "single-loop mode" using the loop-crossover

assembly, while maintaining their respective temperature ranges. A series of corrugated Teflon hoses and stainless steel tubing connects and transports the IATCS fluid from rack to rack and from the racks to the HX inside the ISS.

The IATCS fluid is also used to cool the spacesuits of the Extravehicular Mobility Units (EMUs). The HX that interfaces with the IACTS fluid and the EMU fluid is called the Special Performance Checkout Unit (SPCU). One side of the SPCU HX contains IATCS fluid; the EMU side contains iodinated water. Hamilton Sundstrand manufactures the IATCS HX and Honeywell manufactures the SPCU HX. The nickel braze material used in these heat exchangers are different. The IATCS HX contains BNi-2 material whereas the SPCU HX contains BNi-3.

MICROBIOLOGICAL ENUMERATION AND ISOLATE IDENTIFICATIONS

Coolant samples were collected aseptically in sterile containers containing 0.3 ml of a 15% w/v solution of ethylenediaminetetraacetic acid (EDTA) per 100 ml to neutralize potential inhibitory effects of nickel on the growth of microorganisms (1). Surface microbial samples were collected onto moistened sterile swabs and placed into 2.5 ml of Neutralizing Buffer containing EDTA. Microorganisms were dispersed from the swab into the neutralizing buffer with sonication on ice 3 times for 30 seconds.

Viable plate counts were performed to enumerate microorganisms by standard membrane filtration (1). Serial dilutions of coolant and swab samples were filtered onto 0.45 μm HAWG membrane filters (Millipore, Bedford, MA) that were 47 mm in diameter. The filters were transferred to 60 mm Petri dishes containing R2A, a growth medium designed to culture heterotrophic bacteria from low nutrient aqueous environments. The cultures were incubated at 30°C for 7 days, then 25°C for an additional 7 days. Colonies were counted at day 7 and day 14.

A minimum of 10% of the colonies for each different morphology were isolated into pure cultures for identification. This approach of identifying representative fractions of all the different colony morphologies on each plate provided increased probability of isolating all species and quantifying species predominance as compared to random sampling.

Identifications were performed with three automated identification systems including the Microbial Identification System (MIDI) fatty acid methyl ester gas chromatography, Biolog substrate utilization identification system, and the RiboPrinter® System that provides an automated genetic snapshot of any bacterial strain. Results from these automated systems were correlated with other published identifying characteristics for colony and cellular morphology, and individual biochemical tests such as nitrate reduction were performed for additional verification.

The MIDI system consists of a HP 6890 gas chromatograph equipped with a flame ionization detector, high resolution fused silica capillary column, split injection, and a robotic arm autosampler for unattended operation. Fatty acid methyl ester chromatographic peaks are automatically named, integrated, and compared to computerized reference libraries of known microorganisms by using the MIDI Sherlock Chromatography Pattern Recognition Software Version 3.1 and library upgrade 5.0. A statistical technique, cluster analysis, is used to generate a similarity index to organisms from the reference libraries (3).

The Biolog system consists of microtiter plates with 96 substrate assimilation tests, a computerized microtiter plate reader, Biolog Microlog 3 release 4.2 software and Gram positive and Gram negative bacterial reference libraries. Utilization of a substrate by bacteria causes the microtiter well to develop a blue color after a 4 or 24 hour incubation period. The reactions are automatically read and compared to the reference libraries. Cluster analysis similar to MIDI is used to generate a similarity index to organisms in the reference library (4).

The RiboPrinter® System is an automated ribosomal deoxyribonucleic acid (DNA) analyzer that lyses the cells and cuts the released bacterial DNA into fragments with a restriction enzyme (EcoRI or PvuII). The fragments are separated by size through gel electrophoresis, transferred to a membrane and hybridized with a DNA probe and mixed with a chemiluminescent agent. A digital camera captures the light emission as an image and the RiboPrinter® software (version 2.1) compares the pattern to a database of known library strains and determines similarity based on cluster analysis (5).

Microbiological enumeration results are reported as colony forming units (CFU) per unit volume for coolant samples or unit area for surface samples since colonies may arise from one or more cells. Coolant removed from the Flex Hose (sample FH002) contained 6.3×10^5 CFU/100 ml of bacteria (7-day incubation). Flex Hose 14-day biofilm counts were 12 CFU/cm^2 on the inlet side (FH024, 1.5-2.5" long), 4 CFU/cm^2 for a section midway along the tubing (FH028, 1.25-2.25" long), and 9 CFU/cm^2 at the outlet end (FH036, 1.5-2.5" long).

The isolate identifications for coolant removed from the flex hose and the inner surfaces of the flex hose are provided in Table 1. The CFU per unit volume or area

are provided for each isolate to highlight species predominance in each of the sampled areas.

Table 1 Isolate Identifications for Flex Hose Coolant and Flex Hose Surfaces

Sample Description	Results	Unit of Measure	Species Identification
FH002 - Fluid	6.10E+05	CFU/ 100 ml	*Ralstonia eutrophalpaucula* (nit +)
FH002 - Fluid	2.00E+04	CFU/ 100 ml	*Acidovorax* species (nit +)
FH024 – Flex hose Inlet	5	CFU/cm^2	*Ralstonia eutrophalpaucula* (nit -)
FH024 – Flex hose Inlet	3	CFU/cm^2	Unidentified non-fermenting Gram negative rod (GNR) (nit -)
FH024 – Flex hose Inlet	2	CFU/cm^2	*Ralstonia eutrophalpaucula* (nit +)
FH024 – Flex hose Inlet	2	CFU/cm^2	*Methylobacterium extorquens*
FH028 – Flex hose Center	2	CFU/cm^2	*Ralstonia eutrophalpaucula* (nit +)
FH028 – Flex hose Center	1	CFU/cm^2	*Methylobacterium extorquens*
FH028 – Flex hose Center	1	CFU/cm^2	*Bradyrhizobium japonicum*
FH036 – Flex hose Outlet	5	CFU/cm^2	Unidentified non-fermenting GNR (nit -)
FH036 – Flex hose Outlet	2	CFU/cm^2	*Ralstonia eutrophalpaucula* (nit +)
FH036 – Flex hose Outlet	2	CFU/cm^2	*Methylobacterium extorquens*

Seven-day enumeration results reported for surfaces of the male quick disconnect (QD) components included the spring poppet interface (FH006) with 32 CFU/swab, webbing of poppet (FH007) with 3 CFU/swab, and the surface of the exposed poppet (FH008) with 107 CFU/swab. The colony forming units per unit area sampled for other male QD surfaces was estimated from engineering drawing measurements including the poppet o-ring (FH009) at 308 CFU/cm^2, forward housing between primary and secondary seals (FH011) at 535 CFU/cm^2, inside aft housing (FH013) at 6 CFU/ cm^2, and inside the hose barb (FH014) at 43 CFU/ cm^2. All of the isolates from these male QD samples were identified as nitrate positive *Ralstonia eutrophalpaucula* with some of the isolates producing nitrogen gas from nitrate.

Seven-day membrane filtration results for female QD surfaces included the hose barb (FH040) with 8 CFU/cm^2, back end of flow path (FH042) with 3 CFU/cm^2, 180° forward part of sleeve stop (FH043) with 49 CFU/cm^2, and outside the spring above the sleeve (FH045) with 7 CFU/cm^2. The 14-day count for the inside housing of the wetted side of the o-ring was 15 CFU/cm^2. The isolate identifications for female QD components are shown in Table 2.

Table 2 Isolate Identifications for Female QD Surfaces

Sample Description	Results CFU/cm^2	Species Identification
FH040 - Barb 1/2" From Couple End	8	*Acidovorax* species (nit +)
FH042 - Back End	2	*Methylobacterium extorquens*
FH042 - Back End	1	*Ralstonia eutrophalpaucula* (nit -)
FH043 - 180° Fwd Part of Sleeve Stop	49	*Ralstonia eutrophalpaucula* (nit -)
FH045 - Spring Above the Sleeve	3	*Bacillus atrophaeus*
FH045 - Spring Above the Sleeve	1	*Ralstonia eutrophalpaucula* (nit -)
FH045 - Spring Above the Sleeve	1	Unidentified non-fermenting GNR (nit -)
FH045 - Spring Above the Sleeve	1	*Microbacterium* species
FH045 - Spring Above the Sleeve	1	*Mycobacterium mucogenicum*
FH047 - Wetted Side of O-Ring	10	*Lampropedia hyalina* (nit-)
FH047 - Wetted Side of O-Ring	5	Unidentified non-fermenting GNR

Coolant removed from the IATCS channel of the SPCU heat exchanger (HX003) contained 4.40E+06 CFU/100 ml of bacteria (7-day incubation). The EMU Channel 1 fluid count at 7-days was 1.15E+04 CFU/100 ml. And

the EMU channel 2 fluid contained 3.50E+03 CFU/100 ml of bacteria (7-day incubation). The isolate identifications for SPCU heat exchanger fluids are provided in Table 3.

Table 3 Isolate Identifications for SPCU Heat Exchanger Fluids

Sample Description	Results CFU/100 ML	Species Identification
HX003 – ITCS Fluid	1.87E+06	*Ralstonia eutrophalpaucula* (nit -)
HX003 – ITCS Fluid	1.78E+06	*Ralstonia eutrophalpaucula* (nit +)
HX003 – ITCS Fluid	7.50E+05	*Acidovorax* species (produces ammonia from nitrate)
HX003 – ITCS Fluid	1.45E+03	*Aspergillus* species (fungi)
HX004 - EMU Channel 1 Fluid	5.75E+03	*Acidovorax* species (nit +)
HX004 - EMU Channel 1 Fluid - 71.73 ml	2.88E+03	*Methylobacterium extorquens*
HX004 - EMU Channel 1 Fluid	2.88E+03	*Sphingomonas parapaucimobilis*
HX004 - EMU Channel 1 Fluid	1.90E+03	*Aspergillus* species (fungi)
HX005 - EMU Channel 2 Fluid	3.50E+03	*Ralstonia eutrophalpaucula* (produces N_2 from nitrate)

The enumeration results for surfaces from the IATCS channel of the SPCU heat exchanger are shown in Table 4.

Table 4 Membrane Filtration Enumeration Results for SPCU IATCS Channel

Sample Description	Incubation Time	Results CFU/cm^2
HX007 - Inlet before Manifold Removal	14-Day	38
HX009 - ITCS Outlet Swab before Manifold Removal	7-Day	3.02E+02
HX015 - Outlet End of Inlet Manifold after Removal	14-Day	11
HX019 - Outer Rim of Inlet Manifold after Removal	14-Day	2.92E+04
HX021 - 1/2 of Inlet Side Face after manifold removal	14-Day	7.28E+02
HX022 - Outer End of Outlet Manifold after Manifold Removal	7-Day	<1
HX025- Outer Rim of Outlet Manifold after Removal	14-Day	4.78E+03
HX027 - 1/2 ITCS Outlet Side Face of Heat Exchanger after Removal	14-Day	88

The isolate identifications from the IATCS channel of SPCU heat exchanger are listed in Table 5. Identifications of a couple of different colony morphologies from a dilution that contained too many colonies to count were also performed and the concentration is reported as not available (NA).

Table 5 Isolate Identifications for SPCU IATCS Channel Surfaces

Sample Description	Results CFU/CM^2	Species Identification
HX007 - Inlet before Manifold Removal	16	*Methylobacterium extorquens*
HX007 - Inlet before Manifold Removal	15	Unid. non-fermenting GNR (nit -)
HX007 - ITCS Inlet before Manifold Removal	7	Unidentified non-fermenting GNR (nit -)

Table 5 Isolate Identifications for SPCU IATCS Channel Surfaces (continued)

Sample Description	Results CFU/CM^2	Species Identification
HX009 - Outlet Swab before Manifold Removal	2.20E+02	*Methylobacterium extorquens*
HX009 - Outlet Swab before Manifold Removal	82	*Lampropedia hyalina* (nit -)
HX015 - Outlet End of Inlet Manifold after Removal	9	Unid. non-fermenting GNR (nit -)
HX015 - Outlet End of Inlet Manifold after Removal	1	*Methylobacterium extorquens*
HX015 - Outlet End of Inlet Manifold after Removal	1	*Bacillus atrophaeus*
HX019 - Outer Rim of Inlet Manifold after Removal	2.03E+04	*Acidovorax* species (nit +)
HX019 - Outer Rim of Inlet Manifold after Removal	5.40E+03	*Methylobacterium extorquens*
HX019 - Outer Rim of Inlet Manifold after Removal	3.50E+03	Unidentified non-fermenting GNR (nit -)
HX019 - Outer Rim of Inlet Manifold after Removal	1.10E+03	*Sphingomonas parapaucimobilis*
HX019 - Outer Rim of Inlet Manifold after Removal	NA	*Acidovorax facilis*

The enumeration results for surfaces from EMU channel 1 and 2 of the SPCU heat exchanger are shown in Table 6.

Table 6 Membrane Filtration Enumeration Results for SPCU EMU Channels

Sample Description	Incubation Time	Results CFU/CM^2
HX013 - EMU 1 Outlet before Manifold Removal	7-Day	1.13E+02
HX014 - EMU 2 Inlet before Manifold Removal	7-Day	1.33E+02
HX030 - EMU 1 Inlet Finstock Area after removal	7-Day	<12
HX032 - EMU 1 Inlet Finstock Upper right Area after Removal	7-Day	26
HX034 - EMU 1 Inlet Finstock Area, Lower Half after Removal	14-Day	68
HX036 - EMU 2 Inlet Manifold Finstock Left Half after Removal	14-Day	1.06E+02
HX038 - EMU 1 Outlet Manifold Finstock after Manifold Removal	14-Day	2.13E+02
HX041 - EMU 2 Outlet Manifold Finstock after Manifold Removal	14-Day	11

The isolate identifications from EMU channel 1 and 2 of the SPCU heat exchanger are provided in Table 7.

Table 7 Isolate Identifications for SPCU EMU Channel Surfaces

Sample Description	Results CFU/CM^2	Species Identification
HX013 - EMU 1 Outlet before Manifold Removal	1.13E+02	*Ralstonia eutrophalpaucula* (produces N_2 from nitrate)
HX013 - EMU 1 Outlet before Manifold Removal	16	*Aspergillus* species (fungi)
HX013 - EMU 1 Outlet before Manifold Removal	5	*Aspergillus* species (fungi, different colony morphology)

Table 7 Isolate Identifications for SPCU EMU Channel Surfaces (continued)

Sample Description	Results CFU/CM^2	Species Identification
HX014 - EMU 2 Inlet before Manifold Removal	1.21E+02	*Methylobacterium extorquens*
HX014 - EMU 2 Inlet before Manifold Removal	12	*Ralstonia eutrophalpaucula*
HX014 - EMU 2 Inlet before Manifold Removal	11	*Aspergillus* species (fungi)
HX032 - EMU 1 Inlet Finstock	13	*Methylobacterium extorquens* (non-pigmented)
HX032 - EMU 1 Inlet Finstock	13	*Bacillus megaterium/ simplex*
HX034 - EMU 1 Inlet Finstock Area	64	Unidentified non-fermenting GNR (nit +)
HX034 - EMU 1 Inlet Finstock Area	64	Unidentified non-fermenting GNR (nit +)
HX034 - EMU 1 Inlet Finstock Area	2	*Bacillus megaterium/ simplex*
HX034 - EMU 1 Inlet Finstock Area	2	*Bacillus filicolonicus*
HX036 - EMU 2 Inlet Manifold Finstock	77	Unidentified non-fermenting GNR (nit +)
HX036 - EMU 2 Inlet Manifold Finstock	27	*Methylobacterium mesophilicum/ radiotolerans*
HX036 - EMU 2 Inlet Manifold Finstock	2	*Bacillus atrophaeus*
HX036 - EMU 2 Inlet Manifold Finstock	2	*Aspergillus* species (fungi)
HX038 - EMU 1 Outlet Manifold Finstock	2.08E+02	Unidentified non-fermenting GNR (nit +)
HX038 - EMU 1 Outlet Manifold Finstock	5	Unidentified non-fermenting GNR (nit +)
HX041 - EMU 2 Outlet Manifold	11	Unidentified non-fermenting GNR (nit
Finstock		+)

SUMMARY AND CONCLUSIONS

The level of planktonic microorganisms in IATCS coolant samples returned from the on-orbit has remained stable between 500,000 to 1 million colony forming units per 100 ml over the past three years verified by analysis of coolant samples returned on the Shuttle and Soyuz. The levels of heterotrophic microorganisms in the fluid removed from the flex hose assembly and the IATCS channel in the SPCU heat exchanger were consistent with the historical data. Microbiological concentrations ranged from 1 to 2 logs lower in the water samples removed from the EMU channels in the SPCU heat exchanger. The lower microbiological levels may reflect a combination of the effect of periodic iodination to control bacterial growth, different concentrations and types of nutrients, and stagnant conditions from infrequent operations required for EVAs.

Historically, microbial growth in water systems has created problems for water circulation equipment and system functionality. Almost all known bacterial species are capable of extended survival in aquatic systems, and are capable of a wide range of metabolic activity even in water with extremely low nutrient content (6, 7). Biofilms readily form on exposed surfaces of almost any material in a flowing water system (Costerton et al., 1985; Willis and Schultz, 1987; Lappin-Scott and Costerton, 1989). These biofilms may actually represent a majority of the microbial biomass in a low nutrient system (8, 9, 10). However, the surface sample microbiological enumerations from the flex hose assembly, associated QDs, and the SPCU heat exchanger were significantly lower than anticipated. The levels are indicative of the presence of individual cells and a few microcolonies on most surfaces. Most surfaces had microbiological counts less than 100 CFU/cm^2 and the counts for the flex hose and IATCS passages in the SPCU heat exchanger were less than 10 CFU/cm^2. Concentrations of bacteria greater than 100 CFU/cm^2 were found on the male QD poppet, o-rings, and between primary and secondary seals, and the SPCU manifolds and welds. The data indicates that microorganisms are not causing any biofouling, significant reductions in flow rates, or reduction of heat transfer in the flex hose assembly and SPCU heat exchanger.

The selection of nickel braze materials as a coating for the stainless steel parting sheets in the IATCS heat exchangers and cold plates may be involved in the inhibition of surface attachment and proliferation of bacterial cells. The passive film that forms on nickel in alkaline borate buffer at ph 8.4 was determined by *in situ* surface enhanced Raman spectroscopy to be a single species consisting of amorphous/microcrystalline $Ni(OH)_2$ (11). The leading hypothesis is that periodic sloughing of the weakly adherent $Ni(OH)_2$ layer prevents permanent attachment of bacteria and significant biofilm

formation. A review of biofouling studies on cupronickel materials have shown that the weakly adherent, porous passive layers formed during secondary corrosion reactions prevents permanent adhesion of microorganisms (12). The same phenomenon may also be preventing biofilm formation on the Teflon flex hose due to the prevalence of loosely associated nickel precipitates.

The predominate bacterial species identified in the IATCS coolant, EMU water, and surfaces on the flex hose, QDs, and SPCU heat exchanger are aerobic, motile, Gram negative, non-fermentative, heterotrophs that grow well on organic acids, amino acids, peptone, and limited carbohydrates. Some of these bacteria are lithotrophs capable of oxidizing the molecular hydrogen produced at a galvanic cathode, thereby depolarizing it and increasing the rate of corrosion. These organisms are also autotrophs and are capable of obtaining all the carbon they need from inorganic sources such as carbon dioxide (13). Most of these organisms including *Ralstonia* species, *Acidovorax* species, *Methylobacterium* species, and *Sphingomonas* species are commonly found in deionized water systems. A few gram positive species including *Bacillus* and *Microbacterium* were also found in low numbers in the QDs and SPCU heat exchanger. These microorganisms may represent laboratory sampling and handling contamination even though good aseptic techniques were utilized due to the fact that these organisms were at low numbers and were found during the later phases of the destructive sampling. An *Aspergillus* species which is a filamentous fungus was isolated from SPCU fluids and surfaces. This may indicate contamination of the SPCU heat exchanger during storage on-board the ISS, presence of fungi in the caps that were placed on the inlets and outlets, or contamination during packing and shipping from the ISS.

In conclusion, surface phenomena associated with nickel hydroxide and nickel phosphate precipitates appear to be preventing significant biofilm formation in the flex hose assembly and SPCU heat exchanger studied. It should be noted that the growth of microorganisms have not resulted in adverse system impacts such as loss of heat transfer or microbiologically influenced corrosion. Nominal IATCS operations have not indicated the requirement for immediate remediation of microorganisms. However, the stable planktonic population of microorganisms in the coolant between 500,000 and 1 million CFU/100 ml may indicate the presence of biofilms on other wetted materials in the system including stainless steel, titanium, ethylene propylene rubber, and epoxy resins. In the long-term, the implementation of a safe, effective, stable and compatible antimicrobial for the IATCS loops currently on-orbit and for future ISS elements and payloads is recommended. The addition of an effective antimicrobial to the IATCS fluid will provide microbial control and minimize microbiological-related problems in the future.

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ACRONYMS

Internal Active Thermal Control System – IATCS
External Active Thermal Control System- EATCS
International Space Station – ISS
Thermal Control System – TCS
Special Performance Checkout Unit – SPCU
Extravehicular Mobility Units – EMUs
Extravehicular Activity – EVA
Heat Exchanger - HX
Colony Forming Units – CFU
Milliliters – ml
Ethylenediaminetetraacetic acid – EDTA
Microbial Identification System, Inc. – MIDI
Deoxyribonucleic Acid – DNA
Quick Disconnect – QD
Nitrate Reduction Test (Nitrate Reduced to Nitrite) – nit