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Development of a System to Assess Biofilm Formation in the International Space Station

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ABSTRACT

The design requirements for the water treatment systems aboard the International Space Station (ISS) include and require recycling as much water as possible and to treat the water for intentional contamination (hygiene, urine distillate, condensate, etc.) and unintentional contamination in the form of biofilm and microorganisms. As part of an effort to address the latter issue, a biofilm system was developed by Marshall Space Flight Center (MSFC) to simulate the conditions aboard ISS with respect to materials, flowrates, water conditions, water content, and handling. The tubing, connectors, sensors, and fabricated parts included in the system were chosen for specific attributes as applicable to emulate an orbital water treatment system. This paper addresses the design and development process of the system, as well as the configuration, operation, and system procedures for maintenance to assure that the simulation is valid for the representative data as it applies to water degradation and biofilm/microbial growth. Preliminary biofilm/microbial results are also presented.

BACKGROUND - The testing of the Space Station Environmental Control and Life Support (ECLS) Systems began at NASA/Marshall Space Flight Center (MSFC) in August 1986 with the Phase I subsystem "stand alone" bench tests. During Phase I testing, predevelopment life support systems were operated from facility provided services with no integration to other systems. Since then MSFC has completed a Phase II program, which integrated four Air Revitalization systems and one Urine Processor Assembly, and began a Phase III program, which integrated components of the water recovery system with man-in-the-loop. To date, the Phase III Water Recovery Test (WRT) has evaluated the performance of the Space Station water recovery system throughout nine stages of testing (Table 1). An extensive set of chemical and microbiological analyses were performed during the Phase III testing. Results of those analyses have helped characterize the composition of the waste water and the recycled water generated during the WRT. The analyses include extensive isolation and identification of the population of microorganisms in water samples taken at different stages of processing. However, these analyses are limited to the characterization of planktonic organisms. Limited assessment of the microbial population, accumulated on the surface of the storage tanks and distribution lines, was performed during the WRT. This paper describes the nominal operation requirements for a test that will evaluate biofilm formation in the International Space Station (ISS) water distribution system. The test has two independent setups made of materials currently identified for use in the ISS water distribution system. One setup emulates the conditions (flow rate, and tube material and diameter) in the waste water tank and in the lines upstream of the Water Processor Unibeds. The second system emulates conditions downstream of the polishing beds after the Volatile Removal Assembly (VRA) and in the processed water tanks. Both setups also provide limited simulation of the conditions in the water distribution lines upstream and downstream of the ISS Water Processor.

Assessing biofilm hazards on ISS materials requires a definition of the system materials used in this test. Table 2 contains the list of materials anticipated for use in the ISS Water Recovery System.

The primary constituents evaluated in a biofilm test are:

Biofilm- a layer of microorganisms in an aquatic environment held together in a polymeric matrix attached to a surface. The matrix consists of organic polymers that are produced and excreted by the biofilm microorganisms and are referred to as Extracellular Polymeric Substances. The chemical structure of these varies among different types of organisms and is dependent on environmental conditions. Biofilms can

be continuous, evenly distributed layers but are often quite patchy in appearance. The film also contains organic and inorganic debris from external sources.

Observations of biofilms in water distribution systems indicate that the following are the main parameters that can affect the formation of a biofilm: 1) the structure and chemical composition of the tube wall or surface; 2) water flow rate [and velocity profile]; 3) nutrient level in the water; 4) type and concentration of disinfectant; and 5) other chemical and physical parameters such as pH, hardness (dissolved mineral content), and temperature of the water and/or tubing.

Planktonic microorganisms- microorganisms in an aquatic environment that have little or no resistance to currents and live free-floating and suspended in open or pelagic waters.

OBJECTIVE - The objective of this test is to provide information for use in assessing the extent of microbial growth and biofilm formation in the ISS Water Recovery and Management (WRM) system distribution lines and storage tanks. The test results will be used to identify the areas of concern, and develop countermeasure plans if necessary.

TEST HARDWARE DESCRIPTION

The test uses two very similar system layouts. One is filled with waste water and duplicates the conditions prior to the Water Processor Unibeds and is designated as the "dirty" side. The other system contains clean water and duplicates the conditions in the post processor water distribution lines and is designated as the "clean" side. Both systems are integrated into a single test stand in a horizontal plane. Figure 1 shows a schematic of the system layout and how the components are organized into the biofilm system.

TABLE 1. Stages of the Water Recovery Test

STAGE	DESCRIPTION
1A, 2A, 3A	Comprised of separate hygiene and potable water recovery subsystems (dual-loop) operating in an open-loop "donor" mode.
4 and 5	Same configuration used during previous stages but operating in a closed-loop "recipient" mode.
7A and 7B	"Single-loop" water recovery system with one subsystem processing both hygiene and potable waste streams in "donor" and "recipient" modes; heat exchanger up stream of the Unibeds ("pre-sterilizer").
8	Same as Stage 7 but with the heat exchanger upstream of the Unibeds ("pre-sterilizer") bypassed.
9	Same configuration used during Stage 8, but with higher fidelity hardware and integration.
10	Same as Stage 9, but test incorporated hardware modifications to the WP, UP, and UCS and operated in recipient mode.

TABLE 2. ISS Material Specification for Water Recovery System

LOCATION/APPLICATION	MATERIALS EXPECTED TO BE USED*
Water Distribution Tubing	1/2" Outer Diameter (O.D.) titanium, CP Grade 2
Water Storage Tanks	
Bellows	Inconel 718
Bottom	Inconel 625
Water Processor	
Inlet Tubing (from rack interface to waste ORU)	1/2" O.D. x 0.028" wall w/ 8' runs of titanium as longest lengths
Waste Tubing (from waste tank to Unibed)	1/4" O.D. x 0.028" wall w/ ~15' runs of titanium as the longest lengths
Clean Side Tubing**downstream of Unibeds	1/4" O.D. x 0.035" wall w/ 15' runs of stainless steel 316 as the longest lengths

*Viton and Teflon parts are also expected to be used in the ISS water recovery system.
**Other materials currently being considered include titanium, Inconel 718, 625, or 800 series.

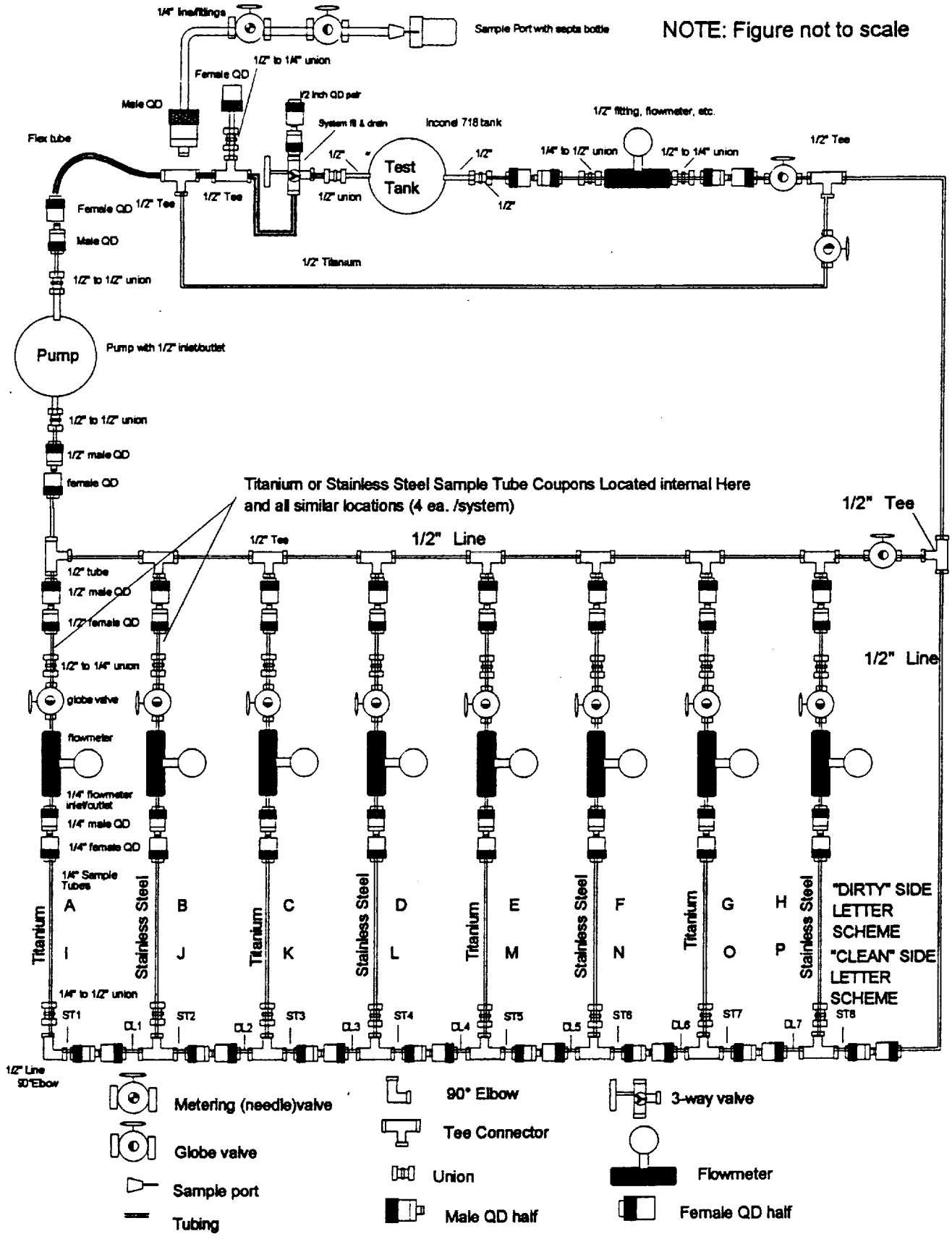

Figure 1. Biofilm Emulator Test System Schematic

SAMPLE TUBES - Four titanium tubes (ASTM B338 Grade 2) and four stainless steel 316 tubes of 1/4 inch O.D. are arranged in parallel as pairs (alternating tubes are stainless steel). Each tube is bent at four equally-spaced intervals to angles of 45° and 90° (Figure 2). The first section of the tube is horizontal, followed by a 45° downward bend. Next is a 90° upward bend, followed by a 90° downward bend and finally another 45° bend to return the tube to horizontal. Each straight section of the tube is 9.6 inches long, and one end of the tube is 6.79 inches higher than the other. Each sample tube is 4 feet long prior to bending, and after bending, each tube has an overall length of 39.48 inches (3.29 feet). The sample tubes are bent to determine if the presence of the bends affects the growth of microbes and to simulate "worst case" conditions in the distribution lines. One end of the sample tube assembly contains a stainless steel quick disconnect (QD) so that the tubes can be removed from the system quickly and easily. The sample tubes are capped when removed from the system and will retain as much internal water as possible. The tubes are provided a safe transportation container to prevent tube damage. Sterile gloves are worn when sample tubes are removed. Since the Water Processor rack will utilize stainless steel, some tubing in the system is stainless steel to determine the long term biofilm effects on this material.

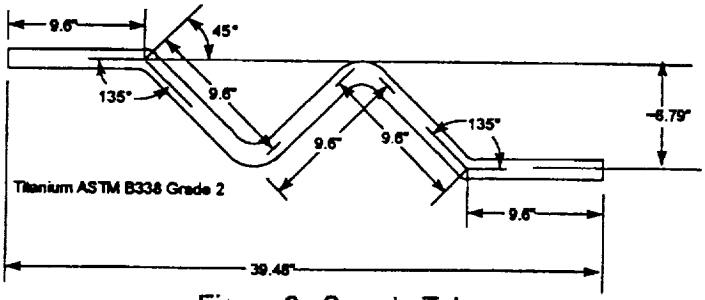

Figure 2. Sample Tubes

TANKS - The storage tank (Figure 3) is 12.75 inches high (from the bottom surface to the top surface of the tank flange) and has an inner diameter of 15.75 inches. The tank is constructed of Inconel 718. The bottom surface of the tank is inclined five degrees to promote drainage into the tank outlet; this inclination raises one end of the tank bottom inner surface 1.875 inches (on the inlet side) while the other is at 0.625 inches (on the outlet side). The inlet and outlet tubes are made from Inconel 625 and are welded to the tank at the inlet/outlet locations. The Inconel 625 inlet and outlet tubes attach to the system by utilizing 1/2 inch Swagelok® weld end unions. These unions are welded to the ends of the tubes and have male connections on the system side for connection to the 1/2 inch stainless steel tubing. The bottom piece of the tank is made from 0.375 inch Inconel 718 sheet material. The inlet to the tank is mounted 2.0 inches from the top of the tank above the highest point of the tank floor. The top flange has thirty-three 0.75 inch holes threaded with 10 threads per inch for coupon/bolt assemblies, and additional holes for temperature, pH, conductivity, and pressurization passthroughs. The pressure transducer is O-ring sealed and connects directly to the tank top in a 7/16 inch threaded hole. The temperature probes utilize a Parker compression fitting with 1/4 inch NPT threads. The pH and conductivity probes are manufactured with a 1 inch NPT fitting that attaches and includes O-ring seals and a globe valve for closing the port when the probes are removed for cleaning and conditioning. The Argon inlet is a 9/16 inch threaded hole and the pressure relief valve will attach to a 3/8 inch national pipe thread (NPT) tapered fitting. The tank top is 19.25 inches in diameter and has 12 additional holes drilled at 30 degree increments around the circumference for attachment to the tank wall flange. The top is sealed with a 1/4 inch Viton O-ring seated in the groove in the lower flange. The flange bolts are 1/2 inch in diameter and utilize lockwashers and hex nuts to draw the flanges tight. These bolts are tightened to a torque of 200 inch-pounds (16.67 foot-pounds). The top is made from 3/4 inch thick Inconel 718 sheet. This arrangement makes it possible to disassemble the tank after the test when the maximum biofilm formation is expected. The lower flange is an annular cylinder of 3/4 inch Inconel 718 plate 19.25 inches in diameter with a 15.75 inch hole in the center as the tank body internal diameter. This flange is welded to the tank wall assembly and then drilled to accept the mating holes on the tank top (upper flange). The coupon holes in the top flange (Figure 4) are pre-drilled and tapped into the tank top; the holes are 0.75 inch in diameter and tapped for a 10 tooth per inch thread (the coupons are attached to standard O-ring sealed bolts and these bolts are screwed into the tank top). After welding, each seam was debeaded, sanded, and polished to prevent the formation of macroscopic pores that might unduly influence biofilm formation at those surfaces. A pressure of 5 psig is maintained in each test tank during testing via argon pressurization by using an adjustable pressure relief check valve. This check valve is attached to a 3/8 inch NPT Swagelok® fitting via 1/4 inch stainless steel tubing. This pressurization is required to maintain positive pressure above ambient to prevent system contamination from external sources. The argon inlet is able to hold pressure whether or not a source of argon is attached to the system. During the tank fill process, the incoming water will force the argon out of the tank through the pressure relief check valve. All fill tank passthroughs perform the same functions as the system tank passthroughs but are stainless steel. Prior to cleaning and sterilization, each tank was hydrostatically tested to 90 psig.

COUPONS - Titanium, stainless steel, and Inconel 718 coupons (Figure 5) are suspended inside the biofilm test tank. The purpose of the coupons is to monitor the growth of biofilm inside the tank. A total of 33 coupons,

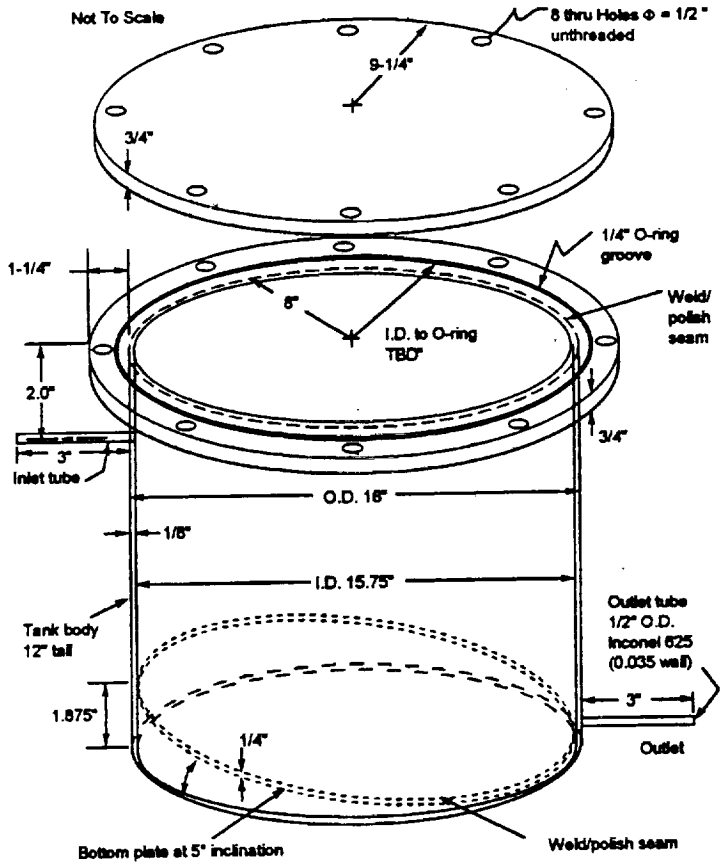

Figure 3. System Tank

eleven of each material, are used in each tank. The coupons attach, via welding or tapped bolts, to 3/4 inch stainless steel, O-ring sealed bolts. The length of the coupon bolt assemblies is 11.0 inches from the lower face of the bolt head to the tip of the coupon, since each coupon is 9 inches and the threaded portion of the bolt is 2 inches. The Inconel and stainless steel coupons are welded directly to the bottom of the bolt while the titanium coupons are attached to the bolt by screws (this is due to the low boiling point of titanium versus stainless steel which prevents welding). The coupon bolts have a Viton O-ring and O-ring groove on the bottom portion of the hex head to provide a gas-tight seal. The width of each coupon is 1/2 inch, and the thickness is 1/8 inch, except the Inconel coupons which are 1/10 inch thick. The coupon bolts mate with threads tapped into the tank top and are sealed to the tank top with Viton compression O-rings. The coupons are manufactured from titanium grade 2, stainless steel 316, and Inconel 718 sheet material. Suitable storage/transport containers for the coupons are used to prevent external contact or contamination during transport to the analysis laboratory. These containers are re-usable, gas-tight, and require sterilization and cleaning prior to and after use. The coupons are transferred "wet", i.e., in water from the test system, or facility provided sterile de-ionized water, to prevent damage or drying of any accumulated biofilm. To

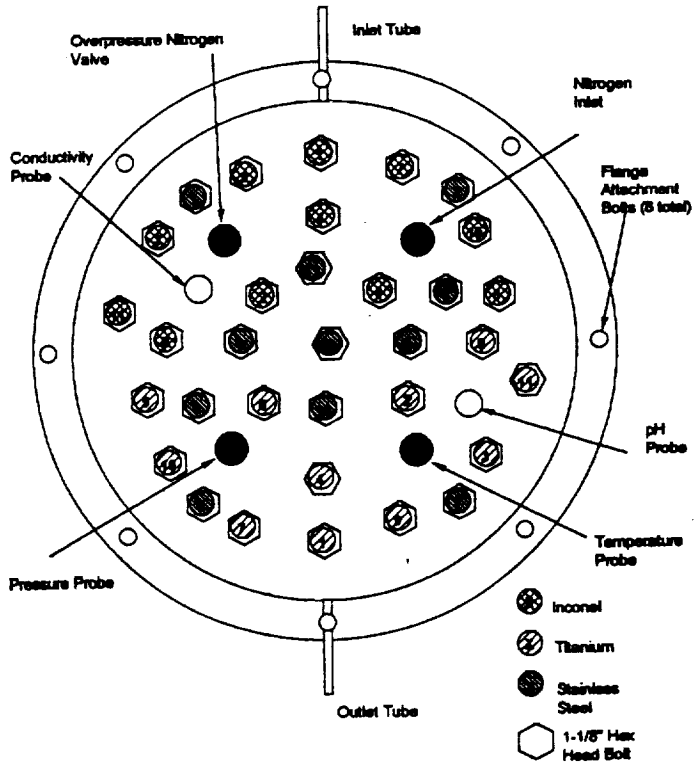

Figure 4. Tank Top with Coupon and Probe Locations

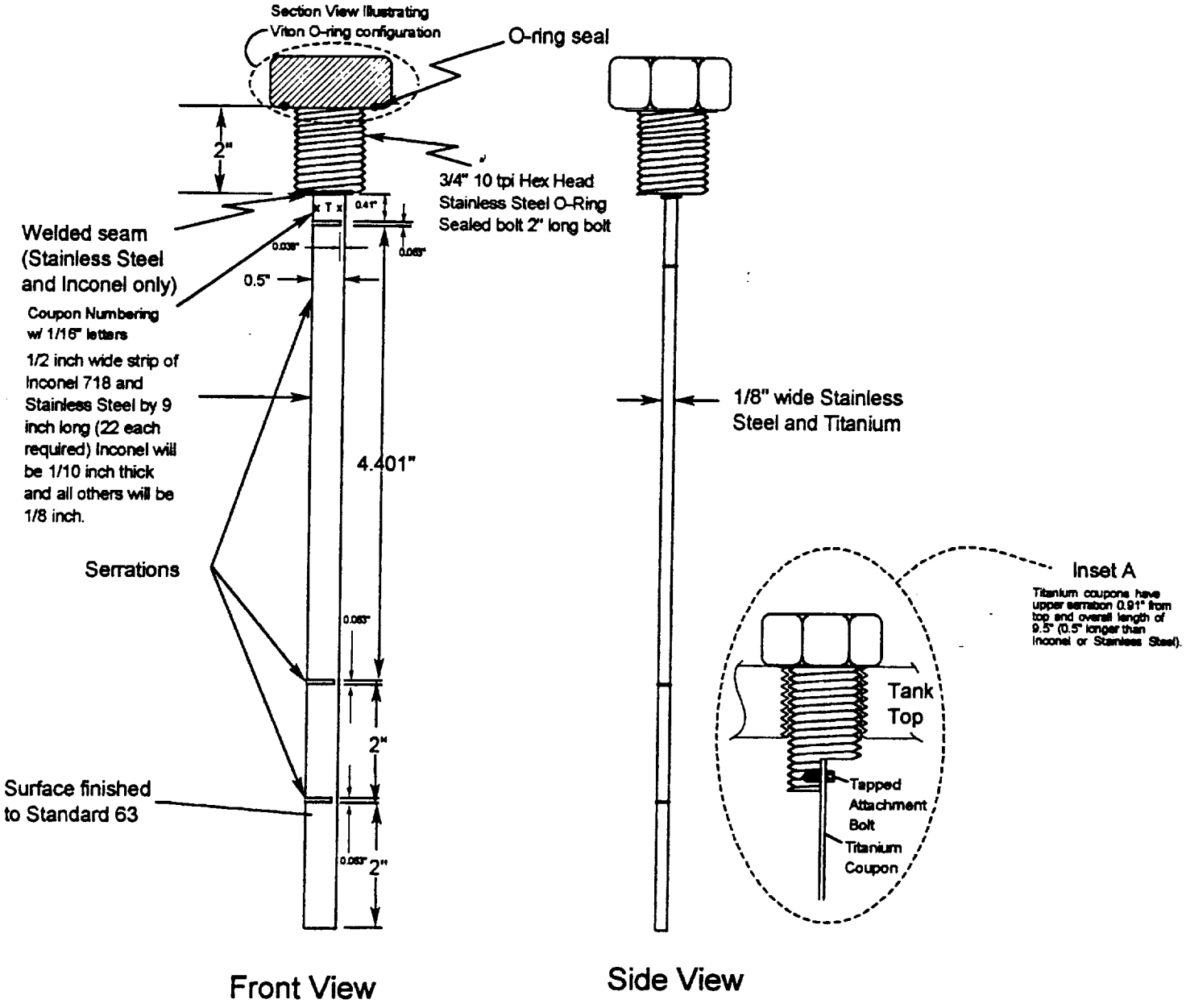

Figure 5. Tank Coupon Schematic

facilitate analysis and sectioning of each coupon after removal from the system, serrations were made in each coupon at 2 inches from the bottom, 4 inches from the bottom, and then at the tank waterline. These serrations provide break points for coupon separation and analysis and provide 2 samples of each coupon that have an overall single side surface area of 1 square inch and an additional sample that extends beyond the tank waterline. The coupons are to be removed in a particular order, as indicated in Figure 4.

The three coupons with the same number are to be removed at the same time, starting with the coupons numbered "1" and counting upward to 11. In addition to the tank coupons, each sample leg contains tube coupons. The tube coupons are 1/4 inch tubing (stainless steel in the stainless steel sample tube legs and titanium in the titanium tube legs) that has been split and separated slightly in a "C" configuration, and inserted into the 1/2 inch tubing upstream of the sample tube flowmeters and valves. Once the desired sample tubes have been removed, these assemblies will be dismantled and the tube coupons removed and examined. Figure 6 shows the tube coupon configuration. These tubes are split along one side and have the other side split except for a small tab of metal to maintain the tube coupon shape during this test. This minimizes the amount of disturbance to the sample during the analysis process.

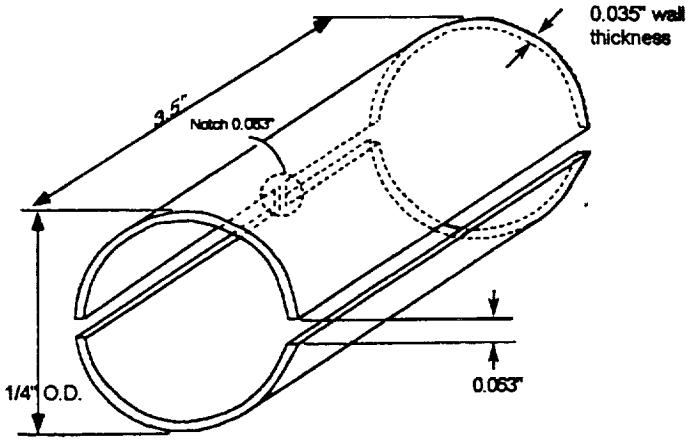

Figure 6. Tube Coupon Inserted into Test System

TEST SUPPORT STANDS - A single test support stand is required to support the experiment apparatus. This stand was manufactured from angle aluminum and welded. This test stand is portable and incorporates casters on each of the support legs such that the system can be moved if necessary.

DATA ACQUISITION - Continuous power to the pump and flow meters will require monitoring. The data acquisition is performed with a data acquisition terminal connected to the System Components Automated Test Systems (SCATS) using 4 alphanumeric descriptions for each line. Since the test runs for 3 years, the SCATS data is broken into more than one file. Data is acquired for each system by the pressure transducers, thermocouples, flow meters, pH meters, and conductivity meters. System performance is checked and monitored daily to prevent loss of data due to extended down periods from system failure, etc. The existing life test database was modified for the biofilm sensors. Calibration curves are supplied with the instrumentation for conversion to the indicated readings (i.e. pressure, pH, flow, etc.).

TEST SETUP - As shown in Figure 1, water enters and leaves the system by way of a three-way, three-port switching valve mounted immediately after the tank outlet. The valve can be set to allow water to flow from the tank out of the system to drain/fill the system and from the tank to the test system. The valve is closed by selecting the center position. Immediately after the valve is a sample port, which is used to take water samples at the same time that the coupons are removed from the tank. After the sample port, the water diverted around the tank rejoins the flow through a TEE (T) connector. The water then passes through a flexible hose and a QD to enter the pump. The desired pump will impart a 25 psia pressure at a maximum flow rate of 120 lb/hr. The water then leaves the pump through another QD and enters the tube manifold. The flow is evenly split among the sample tubes and is controlled by globe valves and monitored with flow meters. Upon leaving the manifold, the water enters the return tube and flows back toward the tank. Just before it reaches the tank, the water is split so that 15 lb/hr flows through the tank and the rest flows around the tank which is also controlled by a pair of globe valves and monitored by a flow meter. In the event that pump flow cannot be matched exactly to the remaining sample tubes, a bypass T connector is located at the end of the inlet manifold and connected to the manifold outlet upstream of the tank. The resistance of this connection is controlled by a globe valve that allows at least 25 lb/hr of flow with minimal resistance at the fully open position. This allows maintenance of the desired flow in the sample tubes while relaxing the flow control requirements on the system pump. The tubing in the system, with the exception of the sample tubes, is 1/2 inch outer diameter stainless and titanium tubing.

The test requires two of these setups, one to mimic the piping system before the water processor, and one to mimic the piping after the processor. From Figure 1, the items labeled DLx are the zero flow portions of the system that remain until the next set of sample tubes are removed. The DLx items are Swagelok® port connectors. The items labeled STx are the stainless steel tubes to be analyzed when the sample tube sets are removed, and they are 6 inches in length. The sample sets removed after the first set will have an attached zero flow region, and the sample port connectors from this location shall be analyzed for biofilm.

MISCELLANEOUS HARDWARE/REQUIREMENTS

PUMPS - Each test system requires a fluid pump. These pumps are removable with minimum effort, and replaceable in the event of failure. The test pumps are required to produce a maximum flow of 120 lb/hr to maintain 15 lb/hr in each of the eight sample tubes in the initial configuration, survive the sterilization temperatures of 195°F, and have variable mass flow output. Variable mass flow is required since sample tubes will be removed and capped, reducing the overall system volume and increasing flow through the remaining tubes. As indicated by the manufacturer these pumps contained "rare Earth" magnets to increase performance and longevity of the pump assembly. These pumps inlets/outlets have 1/2 inch to 1/2 inch Swagelok® unions for system connection.

FLOWMETERS - The flowmeters are specified with a maximum temperature of 300°F, thus, they are sterilized in the system. Four total flowmeters, two in each system, contain a digital readout display thus facilitating visual inspection of system operation. The flowmeters located in the last removed sample leg and at the tank inlet line contain the displays.

CONNECTORS - The QD halves are connected to the appropriate hardware with Swagelok® union fittings which make the halves replaceable and reusable. All connections in the tubing, flow meters, pumps, tank inlets, tank outlets, flex hose, MCV, argon system, etc. are made with the Swagelok® fittings to facilitate system component removal, replacement, and re-use. Certain tank sensors will utilize NPT fittings attached to the appropriate O-ring sealed hardware to facilitate removal. All fittings are stainless steel 316 series, unless otherwise specified.

TRANSDUCERS - Resistance thermocouples or "T-type" thermocouples capable of withstanding 660°F are used in each system to monitor system temperatures. These thermocouples penetrate 5-3/4" into the tank top at the passthrough fitting. The pH and conductivity probes have a 1 inch NPT fitting and globe valve that attaches to the tank and connects to the O-ring sealed probe. The globe valve can be closed when probes are removed from the NPT attachment. This facilitates removal/replacement operations for the pH and conductivity probes and also allows probe cleaning without unduly affecting the system. These probes can withstand 130°F, which means they are removed from the system during sterilization.

SYSTEM PREPARATION

CLEANING - Each part was cleaned on a part by part basis using the following materials/process:

1: Flow through or immerse the part in Amberclean Aqueous Solution.
2: Rinse with De-Ionized water.
3: Dry with warm air.
4: Verify clean with visual inspection.
5. Analyze specified parts (20) for Non-Volatile Residue and Total Hydrocarbon.
6: Double Bag the cleaned parts and combine into sub-assemblies in a clean room.

The pH, conductivity, and pressure probes were chemically washed with Hydrogen Peroxide for cleaning and disinfection.

STERILIZATION - Each test setup was sterilized with a sterilization cart by passing water at 195°F-220°F through the system for 4 hours or more in accordance with the test sterilization procedures. Sterilization access is achieved by a flexible tube mounted upstream of each system pump. One end of this tube has a QD that, when separated, allows connection of the sterilization apparatus. Each free half of the QD is connected to a matching half on the sterilizer. After sterilization, the water is drained from the system by purging with argon. Then, the sterilization apparatus is removed and the flexible tube is reconnected to complete the flow path. The fill tank (one tank used for each system assembly) will also be sterilized (which will require two removable flex hoses attached to each tank inlet and outlet, and 2 pairs of quick disconnects; additional valves may be required to isolate the inlet tube from ambient) according to the sterilization procedure. The pH, conductivity, and pressure probes are removed during sterilization to prevent damage to the sensors. All components of the system that are opened for sterilization are capped with sterile bagging material and taped to exclude atmospheric interaction with the test system.

PRESSURIZATION - Argon tank(s) with two stage constant pressure regulators are required at times of tank filling and pressurization. The argon gas transfer fittings are 1/4 or 3/8 inch O.D. stainless steel tubing with Swagelok® fittings. Pressure relief is used between the Argon gas bottle and the system tank. This pressure relief is required in the event of secondary regulation stage failure and consists of a pressure relief valve. The system is capable of containing the sterilization steam pressure maximum which is quoted as less than 30-40 psig nominal, and the nominal operating pressure of 25 psig for wetted parts and the 5 psig tank headspace pressure for a total maximum of 30 psig. System pressure is relieved using a manual plunger on the Pressure Relief Valve prior to sample section removal or system service.

WATER SAMPLING PORTS - The sample assembly, from which water samples are drawn, is the same as used for the WRT Stage testing and is connected downstream of the tank and upstream of the flexible hose. After each use, the sample port is removed from the system and sterilized. The QD on the system side at the sampling location will be capped with a sterile QD cap when the sampling assembly is not attached to the system.

SYSTEM ACTIVITIES

FLUID INTERFACES - Argon gas is used to pressurize the system and is needed only when sample coupons are removed from the system and when the water in the system is changed. Argon was selected for its inert properties, versus nitrogen which would dissolve in the water at pressure and had the potential to cause anomalies. The clean water side of the biofilm test system initially contained water produced by the ISS water processor that was used during WRT Stage 10 testing. In the case that water from the water processor is not available, sterilized and de-ionized water with a chemical and microbial composition similar to the ISS process water will be used. The dirty water side of the biofilm test setup contains water generated in the End-use Equipment Facility (EEF).

FILLING THE TEST SETUP - The setup is filled through the three-way valve upstream of the test flex tube and

test pump. A secondary tank is connected to the system via a flexible hose and is filled with the water to be placed into the setup. The valve is turned to allow flow into the tank from the outside. The secondary tank is then pressurized to overcome the internal pressure in the test tank, forcing the water through the valve into the test tank. Meanwhile, the pressure relief check valve on the test tank releases the excess argon from the tank to allow the water to enter. Once the tank is filled, the three-way valve is turned so that flow is from the tank to the plumbing. The plumbing is filled from the tank as the argon in the tube is pushed into the tank.

TEST ACTIVITIES - The test began when the first volume of water was put into each system and the system pump activated. The water in the clean system setup was replaced every two weeks with water from the Stage 10 Water Recovery Test (which was then running concurrent to biofilm start). Other water shall be used when WRT 10 water is unavailable. One set (one titanium, one stainless steel, and one Inconel 718) of sample coupons will be removed monthly for the first six months, and every six months thereafter. At the end of the first six months, a set of sample tubes is removed and sent to the lab (a discussion of analyses and results is given at the end of the paper). At this point, the pump flow rate needs to be reduced by 30 lb/hr. Also, the flow rates through each remaining sample tube are balanced so that 15 lb/hr is flowing through each tube. Finally, the valves prior to the test tank are adjusted so that 15 lb/hr flows through the tank and the rest flows through the bypass. Each time a set of sample tubes is removed, the flow rate is reduced and the flow through each sample tube adjusted to maintain 15 lb/hr in each tube. The test is completed when the final set of sample tubes and coupons are removed from each setup. Coupons are removed in sets of 3; 1 Inconel, 1 stainless steel, and 1 titanium coupon from each test tank.

WATER COMPOSITION

WASTE WATER - Waste water for this setup is generated in the EEF on an as needed basis. The waste water is composed of shower water, urine distillate, handwash water, oral hygiene water, and humidity condensate. This water consists of and is representative of the primary ISS system water contaminants as illustrated in Table 3.

Table 3. Waste Water Representative Composition

WASTE WATER STREAM	AMOUNT NEEDED (%)
Shower Water	24
Urine Distillate	10
Handwash	29
Humidity Condensate	23
Oral Hygiene	2
Fuel Cell	12
	total amount 100%

PROCESS WATER - During the WRT Stage 10 test (and possibly after this test), water from the water processor will be used to fill the clean water setup when processed water is available. In the event that processed water is not available, the following alternatives will be used to fill the test setup (in order of preference).

- Processed water frozen shortly after being produced. The water will be handled aseptically and after unfreezing it will be "seeded" with a representative microbial population.
- Sterile de-ionized water seeded with representative microbial population and chemical levels comparable to the ones found in the WRT processed water.

An effort will be made to use water that was processed by the ISS water processor. In the event that this is not possible, clean water processed by the Water Processor will be collected and frozen for use during the periods when the processor is not treating water. When previously frozen water is used for the biofilm test, the water will be "seeded" with a population of microorganisms and total organic carbon (TOC) nutrients comparable to the one observed during the WRT.

TEST REQUIREMENTS

Coupons, water, and tube samples are all to be taken at the times indicated in Table 4. The sample tubes are to be removed in a particular order. After six months, the tubes labeled "A" and "B" (see Figure 1) will be removed. At one year, tubes "C" and "D" will be removed and each subsequent year thereafter another set of tubes will be removed. Similarly, for the "clean" setup, tubes "I" and "J" will be removed first. The water and coupon samples are to be taken at the same time, at least on the same day. During sample tube removal, the pumps are turned off and all valves in the system closed. The flow meters for each sample tube are removed, sterilized, and then stored for future use. Sterile cap nuts are placed over the ends of the T connectors on the removed sample tubes to block the flow path and prevent the chance of leakage/ contamination. Sterile QD caps are placed on the QDs in the sample line legs after the appropriate sample tube assemblies are removed. After sample tube removal and cap placement, the system valves are opened, the system pump turned on, and the flow adjusted in the remaining tube lines. During tank coupon removal, the system tank is de-pressurized to 1 psid by adjusting the pressure relief check valve and positive argon flow will be maintained in the system at 0.5 lb/min from the argon tank system. The argon flowrate is maintained by using a needle valve in-line to the system tanks. Prior to removing the coupon sets the valve upstream of the test

tank is closed, thus bypassing system flow around the tank through the bypass line. Sterilized blank O-ring sealed bolts are placed in the empty coupon holes (there are 66 bolts with coupons and 66 "blank" bolts). The system is then re-pressurized with argon, the valve opened, and the pump turned on.

Table 4. Sampling Timeline

Sample Tubes	Coupon Set	Water	Removal	Duration in Test
(refer to Figure 1)	[1]Clean 1 Dirty 1	Clean Side Dirty Side	Sept. 1, 1996 TBD	31 days
	Clean 2 Dirty 2	Clean Side Dirty Side	Oct. 1, 1996 TBD	61 days
	Clean 3 Dirty 3	Clean Side Dirty Side	Nov. 1, 1996 TBD	92 days
	Clean 4 Dirty 4	Clean Side Dirty Side	Dec. 1, 1996 TBD	122 days
	Clean 5 Dirty 5	Clean Side Dirty Side	Jan. 1, 1997 TBD	153 days
[2]Sample tubes I & J Sample Tubes A & B	Clean 6 Dirty 6	Clean Side Dirty Side	Feb. 1, 1997 TBD	184 days
Sample tubes K & L Sample Tubes C & D	Clean 7 Dirty 7	Clean Side Dirty Side	Aug. 1, 1997 TBD	365 days (1 yr.)
	Clean 8 Dirty 8	Clean Side Dirty Side	Feb. 1, 1998 TBD	549 days
Sample tubes M & N Sample Tubes E & F	Clean 9 Dirty 9	Clean Side Dirty Side	Aug. 1, 1998 TBD	730 days (2 yr.)
	Clean 10 Dirty 10	Clean Side Dirty Side	Feb. 1, 1999 TBD	914 days
Sample tubes O & P Sample Tubes G & H	Clean 11 Dirty 11	Clean Side Dirty Side	Aug. 1, 1999 TBD	1095 days (3 yr.)

[1]The Clean side will start six months earlier than the dirty side system.
[2]Sample tubes assemblies removed will include the tube coupons contained upstream of the valves and flowmeters.

SAMPLE ANALYSIS - The stainless steel tubes and coupons, the Inconel coupons, and the titanium tubes and coupons will be analyzed for biofilm. The sample water is analyzed for microbial content. The analyses performed are shown in Table 5. The composition of the tubing/material used in the test is shown in Tables 6 through 8. Except where noted, this data was taken from analysis sheets shipped with the separate tubing orders. Other components, such as the thermocouples, pH meters, conductivity meters, and miscellaneous fittings could be assessed for biofilm formation at test completion. The accumulation of microorganisms on the surface of the test coupons and tubes is monitored for the duration of the test. Visual assessment of the biofilm accumulation is performed and documented with pictures. Quantification and characterization of the biofilm accumulated on the surface of the tubes and coupons is performed using methods that include viable plate counts, changes in impedance in liquid culture, and phospholipid fatty acid analysis. Direct microscopic examination of the surfaces is performed using epifluorescent staining (acridine orange) and fluorescent labeled monoclonal antibodies to microorganisms of interest such as *Legionella* and *Salmonella*. Additional analyses may be performed if deemed necessary.

Table 5. Analyses To Be Performed on Water Samples

Parameter	Units
*pH	pH units
*Conductivity	µmho/cm
*Turbidity	NTU
*Iodine, Residual	PPM
*Iodide	PPM
*Total Iodine	PPM
*TOC (organic)	mg/L
*TIC (inorganic)	mg/L
*Total Carbon	mg/L
*R2A-7day	CFU/100 mL
†Chromium	mg/L
Iron	mg/L
Nickel	mg/L
Molybdenum	mg/L
Titanium	mg/L
Aluminum	mg/L
Copper	mg/L
Zinc	mg/L

†Interim samples may be deemed necessary during test.
*Fill test set-up and sample 24 hours later.
†All metal measurements made at coupon.

Table 6. Composition of Stainless Steel Alloys Used in Test

Alloy Composition (%)	Ni*	Cu	Cr	Mo	Si	Mn	C	S	P
316 Stainless ½" O.D.	13.08		17.62	2.29	0.53	1.46	0.014	0.008	0.014
316L Stainless ¼" O.D.	12.3	0.1	16.74	2.16	0.38	1.72	0.017	0.003	0.03

*Ni=Nickel, Cu=Copper, Cr=Chromium, Mo=Molybdenum, Si=Silicon, Mn=Manganese, C=Carbon, S=Sulfur, P=Phosphorous

Table 7. Composition of Titanium Alloys Used in Test

Alloy Composition (%)*	°N	Fe	C	Ti	H	O
Titanium ¼" O.D. Tube	0.011	0.035	0.016	Balance	.002	0.101
Titanium ½" O.D. Tube	0.008	0.08	0.013	Balance	<.002	0.14
Titanium Plate	0.01	0.02	0.02	Balance	.002	0.1

*Vendor Supplied Analysis Information.
°N=Nitrogen, Fe=Iron, Ti=Titanium, H=Hydrogen, O=Oxygen

Table 8. Composition of Inconel Alloys and Tubing Used in Test

Alloy	Inconel 625 ½" O.D. Tube	Inconel 718 (%)†
Ni	61.41	70*
C	0.25	0.50
Fe	3.6	5.9
Cr	21.79	15.5
Mo	8.97	
Al	0.34	0.7
Si	0.08	0.7
Mn	0.04	1
C	0.02	0.08
S	<0.001	0.010
P	0.006	
Ti	0.018	2.3
Cb‡	3.56	
Cb+Ta	3.56	

†Nominal Composition for this alloy, used for tank coupons
*Including Cobalt.
‡Columbium (Niobium), Ta=Tantalum

TEST DURATION AND SCHEDULE - The test is scheduled to last three years. The clean side system will start approximately six months prior to the dirty side system; therefore, the sampling cycle for the clean side will start and end six months earlier than the dirty side, unless a continuance option is chosen for the clean side. The continuance is based upon replacing the last sample tubes removed in each system with "blanks" to complete the flow circuit and allow water to continue to flow in the remainder of the system. Future analysis of tubing from the original test startup configuration will allow longer duration testing than is called for in the test plan. This will be important if long duration biofilm formation becomes critical to water processing and water processor operation for the useful life of the Space Station.

SAMPLING ANALYSES AND RESULTS - The biofilm samples were sent to a contractor laboratory for chemical, microbial, and biofilm analyses. Table 9 shows the parameters and water quality specifications that are tested on each sample.

Clean Side – The Biofilm Clean Side Life Test began on January 16, 1997. Several months into testing, the conductivity, total iodine, and iodide levels showed an increase, while the pH values slowly declined. Microbial counts averaged 3 CFU per 100 mL (CFU/100 mL) with *Burkholderia cepacia* and *Methylobacterium radiotolerans* being the predominant organisms isolated.

On June 13, 1997, the test was stopped due to pump failure. The pump operated for approximately 149 days. When the pump was removed, there was a significant amount of fine, black particles in the water downstream of the pump head. Pump wear or MCV resin degradation were two suspects of the particles. The MCV was removed from the system and fine, black particles were observed exiting the MCV when backflushed. On July 3, 1997, the test was restarted. A new MCV was installed, the storage tank was drained, and a manufacturer rebuilt pump head was placed in the system. The pump manufacturer's evaluation found pump failure was due to a mechanical failure. A gear shaft spring failed causing misalignment of the shaft and gears. This resulted in pump degradation.

The first set of bent tube sections was removed for laboratory analysis shortly after test restart. When water was removed from the tubes; fine, black particles were observed. This raised concern that debris was throughout the system possibly clogging the quick disconnects and causing inadequate flow. The MCV was removed and flushed; and fine, black particles were collected from the inlet and outlet sides. The MCV was replaced with a tube assembly; however, the flow was still not within specifications. On August 8, 1997, the test was stopped after 37 days of operation due to inadequate system flow with the pump at maximum load.

After a thorough assessment, it was decided to remove contaminants by draining and flushing the system. Hot water was flushed through the Biofilm Clean Side system using a sterilization cart to remove. Then the tank and tubes were exposed to 225 °F water for 6 hours. Sterile deionized water was placed in the storage tank and circulated through the system. Before draining the system, a microbial water sample was taken to determine a baseline microbial count. The storage tank was filled with processed water and ersatz water, and the test was initiated on September 10, 1997. A straight tube assembly replaced the MCV and a saturated aqueous iodine solution was injected in the water to achieve iodination.

Following test restart, an increase in microbial counts was observed. On October 28, 1997, the test was stopped due to pump failure after 89 days of operation. The pump head was replaced with a spare, and testing continued. The pump manufacturer's evaluation found the pump failure was due to a mechanical problem. The gears were not trimmed properly during assembly.

Microbial counts showed a 3-log increase and variation in microbial identifications during December 1997, raising the concern of contamination. Microbial tests of the iodine solution, processed water, and evaluations of water delivery procedures to the storage tank, were initiated. The iodine solution had a microbial count of 500 CFU/100 mL and appeared to be a single bacterial type. The process water had a microbial count of <10 CFU/100 mL; therefore, not believed to be the source of the system microbial contamination. The iodine solution was determined as the source of contamination. Contamination of iodine solutions has been thoroughly documented by Dr. Favero (2).

On January 26, 1998, the pump was removed due to inadequate flow after 90 days of operation, and replaced with a spare. Water that was collected downstream of the pump contained fine, black particles and a visual inspection of the pump head observed catastrophic wear of the driven magnet assembly. The decision was made to terminate the Clean Side test because mechanical failures of the pump severely compromised test integrity.

The harsh, non-lubricating environment that the Biofilm Clean Side water pump was subjected to during this test could have been a contributing factor in the pump failures. Debris (fine, black particles) believed to originate from the pump driven magnet assembly was found in the water downstream of the pump. Blockage of the quick disconnects due to accumulation of this debris severely impaired flow through the system, possibly contributing to the pump failure.

Table 9. Parameters and Water Quality Specifications

Parameter	Water Quality Specifications	Units
pH	6.0 – 8.5	pH units
Turbidity	1	NTU
Iodine, Residual	1.0 (minimum)	mg/L
Iodine, Residual	4.0 (maximum)	mg/L
Total Iodine	15	mg/L
TOC (organic)	500	mg/L
Chloride	200	mg/L
Sulfate	250	mg/L
Nitrate	10	mg/L
Chromium	0.05	mg/L
Iron	0.3	mg/L
Nickel	0.05	mg/L
Copper	1.0	mg/L
Zinc	5.0	mg/L
Total Count Bacteria	100	CFU/100 mL

Dirty Side – The Biofilm Dirty Side Life Test, began on April 11, 1997. As of February 28, 1998, the test had operated for approximately 324 days. The pH values average 6.8 pH units and the TOC values average 209 mg/L. The conductivity levels have decreased slightly throughout testing. Microbial counts in the water have ranged from 4.24E05 to 2.30E09 CFU/100 mL showing a 4-log fluctuation. *Bacillus*, *Pseudomonas*, *Methylobacterium*, and *Citrobacter* species have been isolated. Microbial counts for the titanium tank coupons show a 4-log fluctuation with counts between 23 and 1.15E05 CFU/100 mL, while the stainless steel and the Inconel 718 tank coupons both show a 3-log fluctuation. The stainless steel coupon counts range from 31 to 1.50E04 CFU/100 mL and the Inconel 718 coupon counts range from <50 to 2.12E04 CFU/100 mL. *Pseudomonas* and *Bacillus* are the predominant isolates found on the coupons. One set of the bent tube sections has been removed and the microbial counts ranged from 4.13E+05 to 5.94E+06 CFU/100 mL with *Pseudomonas* and *Alcaligenes* isolated as the predominant organisms.

On December 17, 1997, the pump failed, after approximately 250 days of operation, and was replaced with a spare pump. The spare pump head began operating at high capacity to achieve acceptable flowrates for test requirements. The manufacturer provided a pump failure evaluation on the failed pump. There was excessive gear wear that may suggest abrasion, high differential pressure, or both.

By implementing lessons-learned from the Biofilm Clean Side Test, modifications were incorporated into the Dirty Side test to ensure test integrity. A cartridge filter was added downstream of the pump to aid in particle removal. After adding the cartridge filter, the pump's flowrate was within specifications. Magnets were externally placed on tubing prior to the pump to attract particles that may have entered the system during pump failure. The test is presently on-going.

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(2) Favero, M. S., *Iodine-Champagne in a Tin Cup*, Infection Control, Vol. 3, No. 1, 1982.

ABBREVIATIONS

Ar	Argon
ASTM	American Society for Testing Materials
CFU	Colony Forming Units
DLx	Sample tube no flow leg
ECLS	Environmental Control and Life Support
EEF	End-use Equipment Facility
°F	Degrees Fahrenheit
ISS	International Space Station
lb/hr	pounds per hour flow
lb/min	pounds per minute flow

MSFC	Marshall Space Flight Center
mg/L	milligrams per liter
NASA	National Aeronautics and Space Administration
NPT	National Pipe Thread (tapered)
NTU	Nephelometric Turbidity Units
O.D.	Outer Diameter
ORU	Orbital Replaceable Unit
ppm	parts per million
psia	lb/square inch absolute pressure
psig	pounds per square inch, gauge
psid	pounds per square inch, delta
QD	Quick Disconnect
SCATS	System Components Automated Test Systems
STx	Stainless Steel system sample part
T	TEE connector (3-way flow fitting)
TOC	Total Organic Carbon
UCS	Urine Collection System
UP	Urine Processor
VRA	Volatile Removal Assembly
WP	Water Processor
WRM	Water Recovery and Management
WRT	Water Recovery Test
µmho/cm	micro-mho per centimeter