Final Report

DEVELOPMENT OF AN ELECTROLYTIC SILVER-ION GENERATOR FOR WATER STERILIZATION IN APOLLO SPACECRAFT WATER SYSTEMS

Apollo Applications Program

67-2158
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Manned Spacecraft Center
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SUMMARY

An electrolytic water sterilizer has been developed for control of microbial contamination in the Apollo spacecraft. Individual units are self-contained and require no external power or control. The small size (2.5-in. diameter by 4 in. long), light weight (0.6 lb), and absence of interface requirements make it possible to incorporate such sterilizers at various desirable locations in the potable water system or the waste water system.

The sterilizer produces silver ions in concentrations of 50 ppb to more than 200 ppb in the water flow system, the desired concentration being adjusted to the average water flow rate. After installation, no maintenance is required. The unit can be neglected with no damage to the cell or the system, since it becomes self-limiting if water flow is shut down. An external shunt is provided for on-off functions and monitoring of current flow. Probable life expectancy is 9000 hr without a change of batteries.

Laboratory tests under simulated conditions have demonstrated essentially complete kill of Staphylococcus aureus and Escherichia coli within 8 hr, using initial bacterial concentrations greater than 5×10^5 organisms per ml.

Methods for passivation of aluminum piping systems to minimize losses of silver ions by reduction have been developed. Elimination of system losses enhances bactericidal effectiveness, decreases the required current, and permits closer control over silver-ion concentrations in the water systems.

CONCLUSIONS

Adequate bacteriological control can be obtained within the water systems of the Apollo spacecraft by electrolytic generation of silver ions at concentrations of 50 to 100 ppb.

SECTION I

INTRODUCTION

The silver-ion generator was developed under Task 34 of the Phase II Program Plan for sterilization of water systems in the Apollo Applications Program. The necessity of controlling microbial contamination and the requirements for control were discussed in a previous report (Reference 1).

Silver ions in concentrations of 50 to 100 ppb, although nontoxic when ingested, are an effective bactericide. The oligodynamic effects of silver have been known for a number of years (Reference 2). Since a sterilization unit for spacecraft water systems must operate in zero g, expend little power, and require no elaborate controls, or expendables, the use of silver has many advantages over other possible sterilization techniques.

It has previously been found (Reference 1) that silver plated on stainless steel provided the requisite concentrations, but silver-plated aluminum was not effective because the ionic activity of silver is suppressed by the much greater electrolytic solution pressure of aluminum. Exposed aluminum surfaces (anodic) and silver-plated aluminum (cathodic) created electrolytic cells which effectively plated silver out of solution.

This report covers the development of an electrolytic silver-ion generator. Electrolytic generation at the desired concentration levels (50 to 100 ppb) is advantageous for the following reasons:

Electrolytic silver provides effective bactericidal control.

The silver-ion generator is self-contained, requiring no power from the spacecraft and no control system.

The weight and volume of a single unit are such that several can be distributed throughout the water systems where required.

The generator precisely meters silver ions with the water flow stream, the desired concentration being adjusted to the average flow.

The generator is self-limiting if water ceases to flow.

No maintenance is required, the generator being replaced if necessary.

Life expectancy of 9000 hr is limited by battery life.

No damage, aside from failure to sterilize the water, can occur to other spacecraft components if the generator should malfunction.

SECTION 2

ANALYTICAL METHODS AND PROCEDURES

COLORIMETRIC DETERMINATIONS

The analytical procedure for the detection of silver ions in quantities as low as 5 μgm has been previously described (Reference 1). The procedure used throughout this investigation was essentially the same. Additional information can be found in Reference 3.

The colorimetric method is extremely sensitive to impurities. The necessity for use of distilled water from the plant system rather than bottled distilled water has been previously pointed out (Reference 1). Throughout the present investigation, plant distilled water was used when possible.

The procedure is a refinement of that employed in Reference 3, utilized for examination of waste water, which is relatively complex because it necessitates eliminating ions other than silver ions from the solution. In the present investigation, when silver was the only ion present in distilled water, accuracy could be maintained and a larger number of samples could be analyzed by means of the refined procedure (Reference 1). In those instances where other known ions (chromate) or compounds (thiuram) were found to cause interference, additional steps were employed which were found effective in eliminating the interference while preserving the rapidity of analysis.

An initial estimate must be made of the concentration of silver in the sample so that the sample size can be chosen to yield 5 to 15 μgm of silver. Other metallic ions or organic materials which can be extracted by carbon tetrachloride and contribute to unwanted adsorption must also be absent. The sample is first acidified to a pH of approximately 0.8 by the addition of 1 cc of 5.5N H_2SO_4 solution to each 10 cc of sample. It is then shaken vigorously with a 5-ml solution of 10 μg per ml dithizone in carbon tetrachloride for one minute and the sample is set aside in a dark location until separation occurs. The water layer is decanted and discarded and the quantity of silver in the dithizone is determined by measuring the percentage of yellow light (620 mμ) transmitted using a Bausch and Lomb Spectronic 20 Colorimeter (Figure 2-1). Percentage transmittance is related to the weight of silver in the dithizone using a calibration curve previously derived from standard silver nitrate solutions.

Great care was required to reduce experimental error. The sample size was selected so as to avoid more than one dithizone extraction for each sample, if possible. A new prescription bottle (4 to 16 oz) and cap was used for each sample to avoid reuse of glassware that had previously contained silver. Special precautions were taken with other glassware such as pipettes, burettes, and graduated cylinders to avoid unwanted contamination. Standard silver nitrate solutions were stored in dark bottles that had been preequilibrated with the standard solutions for some weeks prior to storage. Since glass adsorbs silver, such solutions were freshly made up if the solutions made earlier had been stored more than two or three months. Similarly, samples were analyzed within minutes after acquisition to avoid losses of silver by adsorption on glassware.

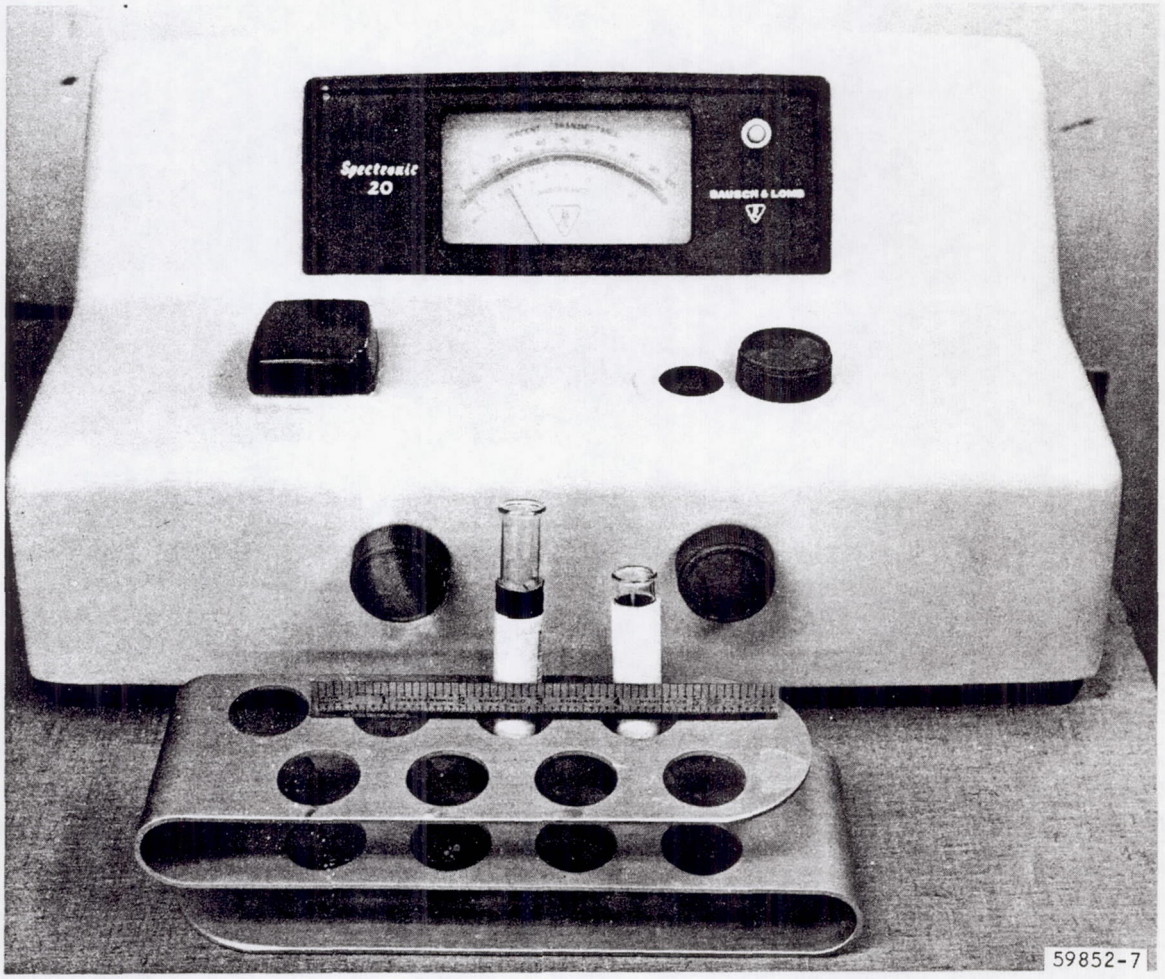


Figure 2-1. Spectronic 20 Used for Analysis of Silver-Ion Concentrations

A fresh sample of the green dithizone solution was used each day to zero the colorimeter. Before analysis of each sample, the colorimeter was calibrated with dithizone and pure carbon tetrachloride solutions. The dithizone solution undergoes color changes with light, so that it is necessary to store such solutions in the dark, to utilize them only as needed in a burette, and store the calibrating solution in the dark when it is not in use. Since the light in the colorimeter also affects the dithizone solution, it was necessary to discard and use fresh calibrating solution more than once a day when numerous analyses were made. Fresh dithizone solutions were made up when required by initially weighing 50 mg of dithizone dry crystal (Baker analyzed reagent) followed by appropriate dilutions with carbon tetrachloride. Minor errors (±1 mg) in weighing might introduce considerable error in successive batches and cause a shift in the calibration curve.

The curve used for converting transmittance to weight of silver in the sample is shown in Figure 2-2. Estimated error based on amount of silver in the sample is shown in Figure 2-3. Where possible the sample size was chosen so that it contained nearly 10 μg of silver. This point on the graph could be calibrated with standard silver nitrate solution (100 ppb) when necessary to improve accuracy. The nature of the investigation, however, made it necessary to analyze various samples of different volumes containing unknown quantities of silver, many of which could not be duplicated. Final results, reported either as efficiencies or concentration, do not indicate the amount of error in the analysis, which is a function of sample size used for the determination.

When solutions other than distilled water--i.e., sweat condensate, fuel cell water, or solutions containing bacteria--were analyzed, it was necessary to check the effects of such solutions on dithizone in the absence of silver to ensure that unwanted adsorption of light had not occurred. Bacteria, in particular, seemed to have some effect on analysis of silver, so that kill rates are reported as a function of theoretical silver ion concentration as well as measured concentrations which cannot be considered as accurate as those for distilled water.

Hexavalent chromium, used for coating aluminum tubes, will cause a yellow shift in the dithizone solution. It was found that hexavalent chromium could be reduced to chromate ion by shaking the acidified water sample with a few drops of a 5-percent hydrogen peroxide prior to analysis. Chromate ion does not complex with dithizone. Tests of water from aluminum tubing treated with hexavalent chromium (Alumigold) indicated little or no chromium content in the effluent. Analyses of samples with and without peroxide treatment generally showed a slight increase in silver after the peroxide treatment, which is the reverse of what is predicted if chromium interference occurred. This slight increase is apparently due to oxidation of collodial silver in the effluent, which had previously been reduced in the aluminum tubing.

Considerable trouble was encountered in using the colorimeter to analyze effluent from the Apollo potable water or waste water tanks. This was believed to be due to the presence of thiuram (dipentamethylene thiuram tetrasulfide) in the water that was leached from the bladder (polyisoprene) used for pressurization of the tank. A carbon tetrachloride (10-ml) extract of the

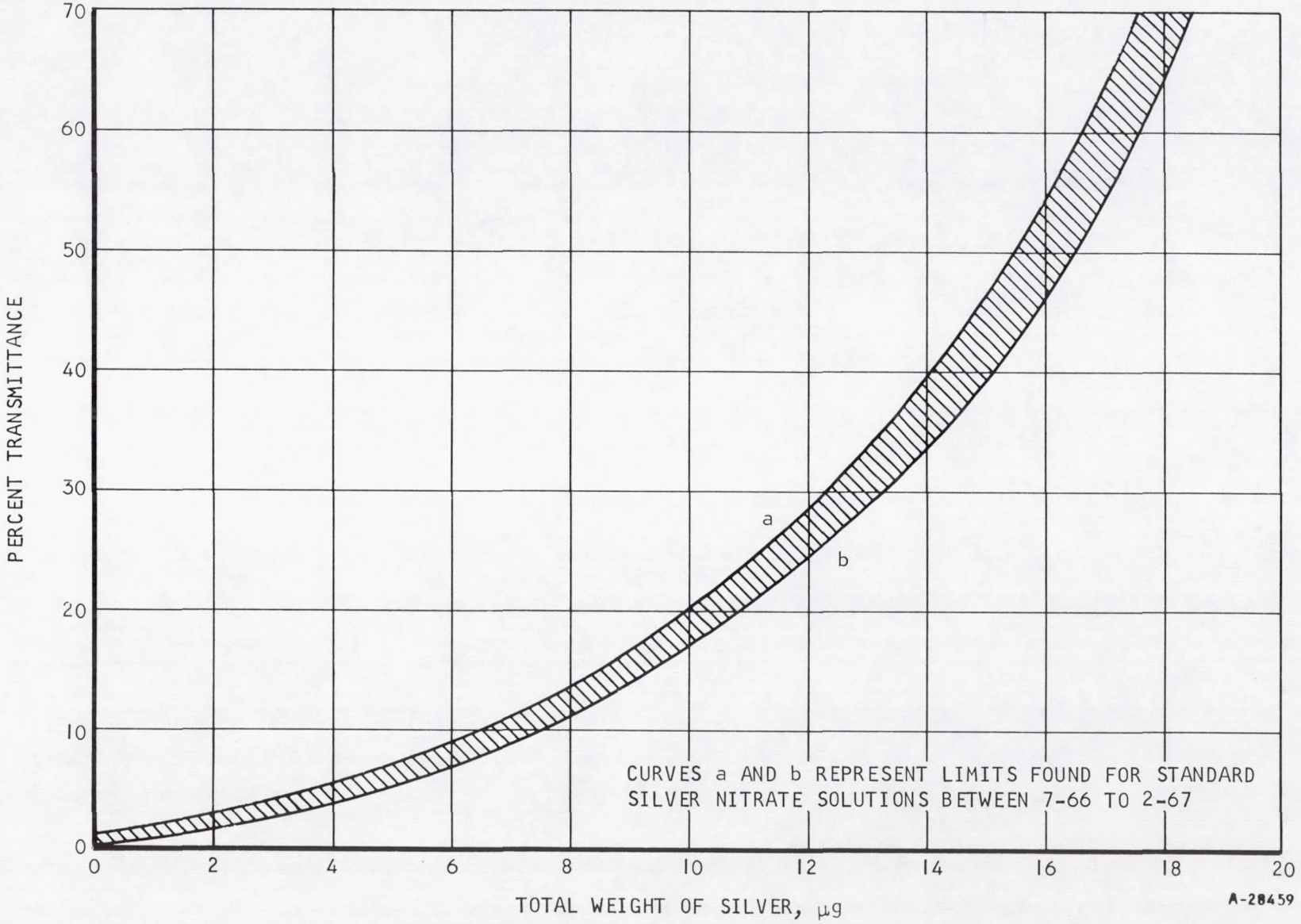


Figure 2-2. Calibration Curve for Analysis of Silver-Ion Solution

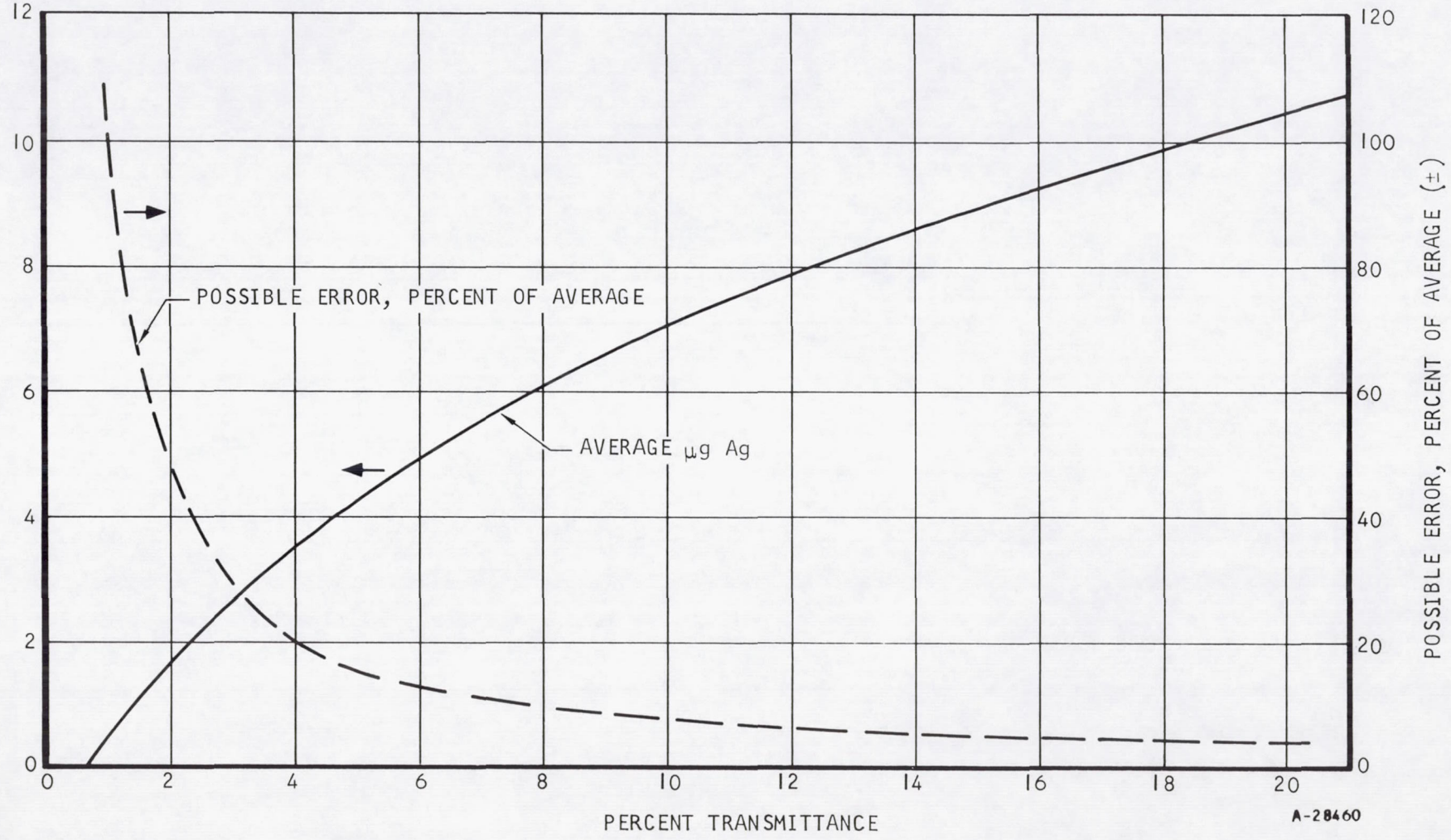


Figure 2-3. Influence of Total Silver Content of Sample on Possible Error Obtained in Analysis

tank effluent (150 ml) will turn yellow, indicating that such impurities exist. This yellow color will disappear if the carbon tetrachloride is exposed to light overnight or will last indefinitely if it is kept in the dark. Since this yellow color is extracted from a silver sample into the dithizone solution, it prevents accurate analysis of such solutions by colorimetric methods.

Atomic adsorption was found to be effective for analysis of silver in the presence of thiuram and is further discussed. A partial colorimetric procedure was worked out, however, by which thiuram interference could be eliminated. It was found that adding a few drops of a potassium permanganate solution (1 percent) to the acidified sample, shaking, and allowing about one minute for complete reaction, followed by destruction of residual potassium permanganate with a few drops of hydrogen peroxide, results in complete oxidizing of the thiuram, and good analytical results can be obtained. The permanganate is a sufficiently strong oxidant to convert the sulfur in thiuram to sulfoxy compounds. Accurate silver analysis could be made using thiuram solutions obtained by soaking a portion of the bladder in distilled water. Effluent from the waste water hold tank, however, analyzed high, indicating contamination by the presence of other metallic ions.

It is possible that such metallic ions could be eliminated by extracting the first dithizone solution with an aqueous ammonium thiocyanate solution (specific for silver), followed by destruction of the thiocyanate with permanganate. The resulting water solution containing only silver could then be extracted with fresh dithizone using the normal analytical procedure. Complete details for this modified procedure were not worked out, but the method described above would preserve the rapidity of analysis while extending the colormetric techniques to contaminated solutions.

POTENTIOMETRIC MEASUREMENTS

Attempts were made to set up an analytical procedure for silver ions in these low concentrations (50 to 100 ppb) using a sulfide electrode (Orion Research, Inc., Cambridge, Massachusetts) reported to be useful for the detection of silver-ion concentrations down to 10 ppb. Measurements against a standard calomel electrode, however, indicated that the small shifts in potential difference encountered with concentrations below the parts per million level could not be measured accurately without exerting extreme care in control of pH, temperature, and aeration. Such techniques would not be practical for average analytical work.

ATOMIC ADSORPTION

Atomic adsorption was used to determine the silver ion concentration in bladder water effluent from the waste water tank. Silver adsorbed on the polyisoprene bladder was also analyzed by this technique. These analyses were performed by an independent analytical laboratory (Truesdail Laboratories, Inc., Los Angeles). Results of such analyses are subsequently reported in Tables 4-5 and 4-6. These analyses are believed to be accurate. A control sample (standard silver nitrate solution) containing 100 ppb of silver, sent for analysis at the same time, was correctly analyzed.

SECTION 3

ENGINEERING AND DESIGN

BASIC DESIGN CONSIDERATIONS

System Requirements

The potable water in the Apollo spacecraft is obtained from the fuel cell. The water flow rate is dependent on vehicle power requirements, but essentially averages 7 cc per min. This water can be considered distilled but saturated with hydrogen. The waste water consists of excess potable water and condensate from the suit heat exchangers. The condensate flow rate averages between 2 and 4 cc per min and varies with work load.

Desired silver-ion concentrations are 50 to 100 ppb. These levels ensure that ingestion of silver will not be injurious to human health (Reference 5). Previous tests have indicated such low concentrations are effective as bactericides. However, health standards are based on a minimum 25-year period, so that it is safe to assume that higher concentrations up to 200 ppb would not be injurious for short intervals and could be utilized for more complete sterilization (Reference 6).

Electrode Reactions

When the electrolytic silver-ion generator operates in a flowing water stream, the silver ions are removed before deposition can occur at the cathode. The electrode reactions can be written as

Anode	Ag	$\rightarrow$	$Ag^{+} + e$
Cathode	$H_2O + e$	$\rightarrow$	$0.5H_2 + OH^{-}$

Current requirements are low (15 μa or less), so that the internal resistance of the cell is not excessive, although distilled water is relatively nonconductive. Furthermore, the amount of hydrogen produced is negligible, so that the cathodic overvoltage is limited. The concentration of ionic silver (50 to 100 ppb) is within the solubility of silver oxide so that little or no precipitation occurs.

Silver is more noble than other metals, excluding gold and metals of the platinum series. An anode of high purity is therefore necessary to prevent introducing other metallic ions into the potable water stream.

In the absence of an imposed potential, silver is cathodic to most other metals. If the cathode were other than silver or a more noble metal, a bucking potential would exist between the silver anode and the cathode. Such bucking potentials can be eliminated by using a silver cathode. Since no metal is being removed, a composite cathode, in which aluminum is plated with silver, has been found effective for weight and cost reduction. When silver is used as a cathode, the internal resistance is reduced, a more uniform

current density results, the plating of unwanted silver under static conditions is facilitated, and stable current levels can be maintained.

Amperage Requirements

If no losses occur by cathodic deposition or other means within the cell, the concentration in the effluent is a function of the silver ion generation rate and the water flow rate, and the three are interrelated by the equation

$$C = \frac{G}{F}$$

where C = Concentration (μg/liter = ppb)

G = Generation rate (μg/min)

F = Water flow rate (liters/min)

From Faraday's Law, the generation rate can be expressed as a function of current: $G = 0.067\ i$, where i = current (μa).

The current and, consequently, the generation rate, are constant, so the concentration in the effluent varies inversely with the flow rate. At an average flow of 10 cc per min and a desired concentration of 100 ppb, the required amperage can be readily calculated as 14.9 μa. Theoretical concentration as a function of flow rate and current is shown in Figure 3-1. Under no-flow or static conditions, a limiting concentration will be reached in the cell where the deposition (or plating) rate equals the generating rate, so that the total quantity of silver in the water reaches an equilibrium level. Uniform concentrations and effective sterilization cannot be achieved in locations where rapid water surges exist if the surge volume exceeds the cell volume and no mixing or diffusion occurs downstream.

Actual amperage requirements will be greater than theoretical, to overcome losses of silver ions within the cell by deposition on the cathode and within the system downstream of the cells by reduction or adsorption.

The rate of anodic loss of silver as a function of operating current is shown in Figure 3-2. The volumetric loss of silver from the anode during extended missions can be calculated from the density (10.5 gm per cc). For missions to 90 days, and at current levels up to 20 μa, the dimensional changes for anodes (1/2-in. diameter by 1/2 in. long) are negligible, representing about 0.5 percent of the original diameter.

Internal Cell Construction

An electrolytic silver ion generator must be designed so as to ensure an even flow of water past the anode to remove all ionic silver. Any "dead" spots in the cell will accumulate silver ions. The higher concentration in this region will increase the solution conductivity and direct the current and ionic flow away from the main water stream. This condition becomes progressively worse as the silver-ion concentration increases. Operating efficiency is then reduced by preferred cathodic deposition.

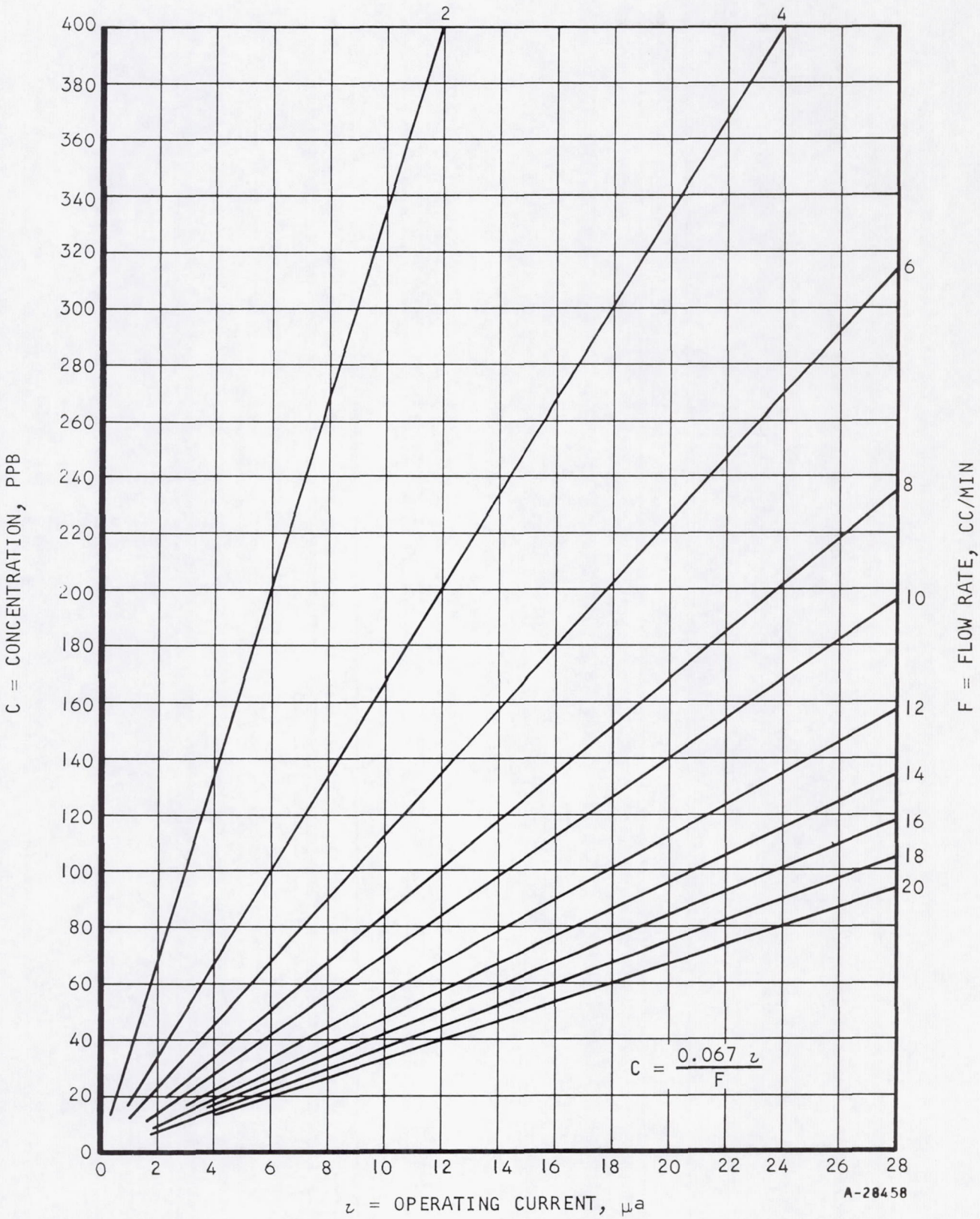


Figure 3-1. Theoretical Silver-Ion Concentration as a Function of Current and Flow Rate

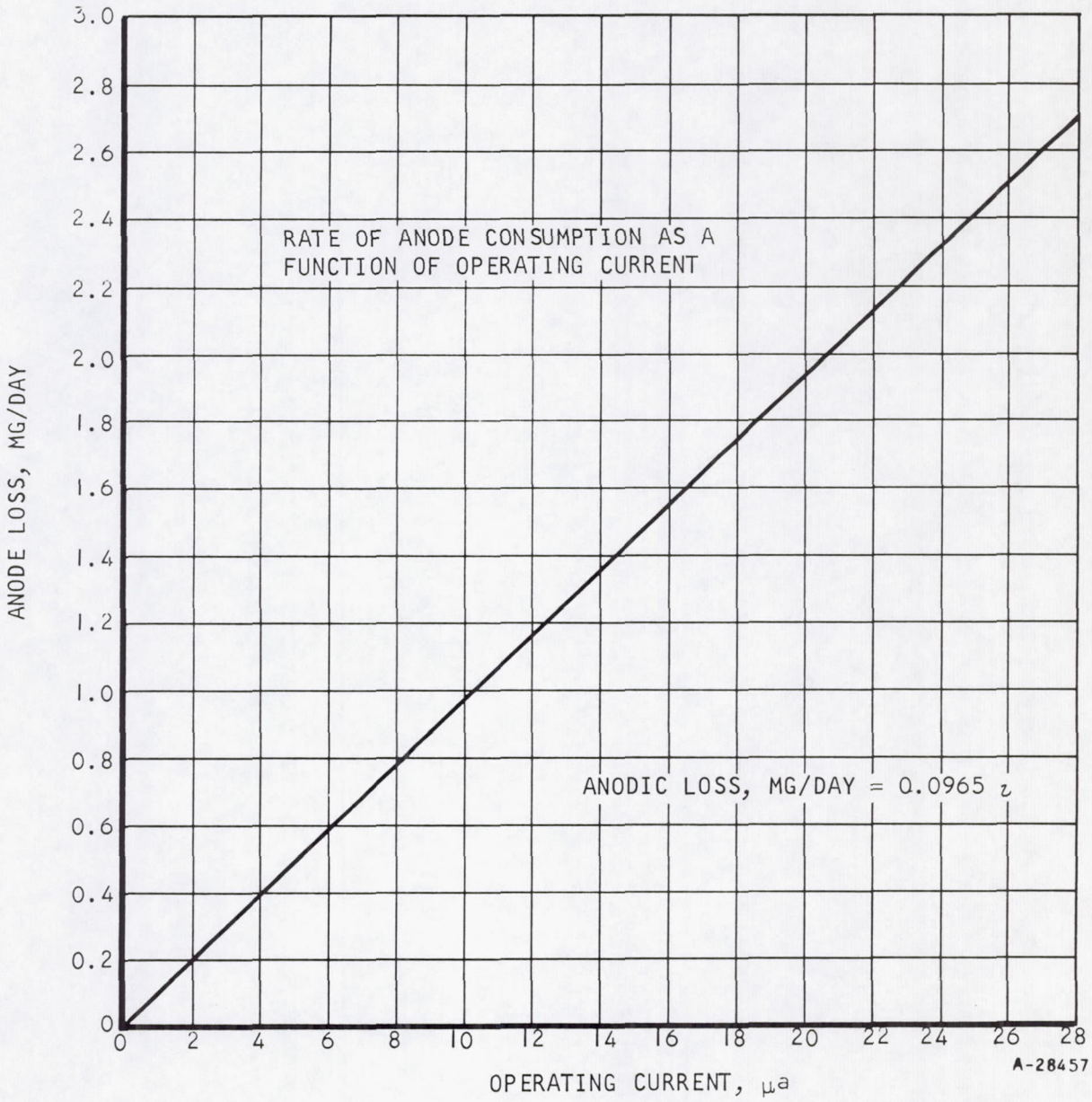


Figure 3-2. Rate of Anodic Consumption as a Function of Operating Current

A similar situation can exist if concentration gradients form in the flowing water. Water flow is considered to be laminar so that no turbulence is available to dispense a gradient once formed. If the residence time in the cell is excessive, such gradients can result from changes in conductivity of water flowing past the anode, imperfections in the anode or cathode, or from improper positioning of the anode in the true center of the cathode. Since current densities are very small, the current seeks the path of least resistance and follows the concentration gradient down the anode until the water leaves the cell. This nonuniform current density heightens losses by cathodic deposition and causes the concentration in the effluent to fluctuate.

The silver-ion concentration is greatest at the cell exit, and maximum losses due to cathodic plating can be expected in this region. Cathodic plating at the exit is minimized by keeping the cathode length less than the anode length and by drawing the water away from the cathode toward the anode as it leaves the cell. Overall cell losses are reduced by minimizing the cathodic area and the cell residence time.

CONTROL CIRCUIT

Basic Circuit

Minor changes in silver-ion concentration are not detrimental and occur with fluctuations in water flow. Minor amperage variations will normally occur with changes in water conductivity within the cell.

The maximum variation in current that can occur at any given current level can be limited by determining the internal cell resistance at the desired current level, using water of both high and low conductivity, and making the variation in resistance a fixed fraction of the overall resistance. This determines the required external resistance from which the battery voltage can be calculated. Fluctuations in output concentrations, which can occur with changes in flow rate or pH, are then fixed.

The required circuitry reduces to a fixed control resistance of such magnitude that changes in internal resistance represent only a small fraction of the overall resistance, with a battery of sufficient voltage to produce the required current.

If the anode and cathode are shorted internally by a stray metallic path, the cell becomes inoperative. The amperage then rises to the maximum value fixed by the control resistance and battery voltage.

Interactions of Dissimilar Metals

Aluminum and silver form an electrolytic cell in which the silver becomes the positive electrode (anode). Both silver electrodes, i.e., anode and cathode, must be electrically isolated from the aluminum system which is considered to be the ground, to prevent undesirable electrical currents, corrosion, and plating out of silver ions. The use of silver-plated aluminum for a cathode is not detrimental if the silver coating is sufficiently thick so that no aluminum is exposed to the water (electrolyte).

To avoid complete electrical isolation of the cell, the silver anode is grounded through a high resistance (22 megohm). The current which would normally flow from the silver anode to the aluminum ground is then effectively nulled by current flowing from the battery to the anode. Corrosion of the aluminum portions of the cell is inhibited, but the aluminum is maintained slightly anodic and tends to repel deposition or reduction of the ionic silver produced at the anode. By placing another resistor (22 megohm) in parallel with ammeter corrections and/or a switch, a trickle current (about 0.1 μa) always flows between the anode and cathode and the condition of zero current flow between anode and ground is maintained even if the cell is essentially in an off status. The cathode is completely insulated to avoid any contact with the ground. Cell design minimizes any interaction between the aluminum ground and the silver cathode.

Battery Requirements

A regulated small current drain is required for extended periods up to six months. This dictates the use of a battery with a very stable voltage output. Mercury batteries (Malloy Duracell, 4.2 v, 1000 mAH) were used during laboratory testing. Flight units were equipped with a more stable silver oxide battery (Union Carbide, Eveready No. 301, 100 mAH, 4.5 v). These batteries, rated at 100 mAH, will have a service life of more than one year under a continuous current drain of 10 μa.

Final Circuit

A diagram for the overall circuitry is shown in Figure 3-3. With the exception that a variable resistor (0 to 1 megohm) was used to control amperage during developmental testing, this circuit was employed throughout for performance evaluation, and is the one used in the final flight type units.

After initial performance data were obtained (see Cell Performance), and possible variations in internal cell resistance were evaluated, a battery potential of 4.2 v (three 1.4-v Mercury batteries) was judged sufficient for practical purposes. For the flight units, 4.5 v (three 1.5-v silver oxide batteries) were used. At current levels of 10 μa, the amperage would vary between 10 and 12 μa so that variations in current due to conductivity changes would not exceed 20 percent.

The final control resistors were selected on the basis of nominal flow rates of 3 cc per min for the condensate and 7 cc per min for the fuel cell water. This gives values of 1 megohm and 390 K ohms, respectively.

ELECTROLYTIC CELL DESIGN

Electrode Construction

Cathodes, fabricated from aluminum, were silver-plated to a depth of 0.001 in. In general, electrical isolation and attachment of leads did not present any problem.

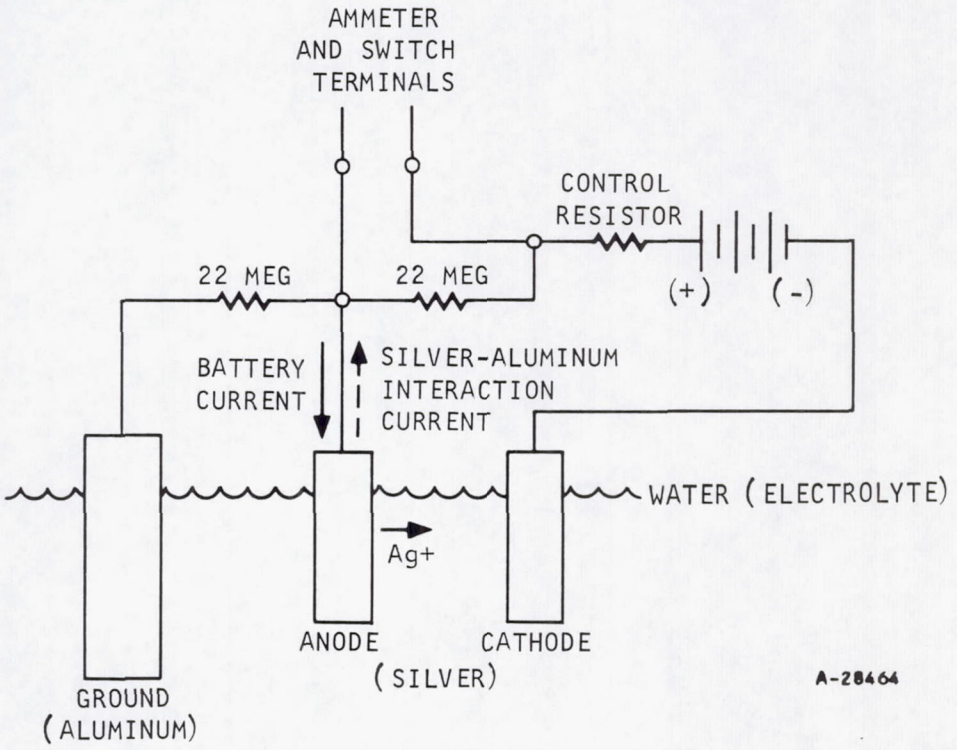


Figure 3-3. Circuit for Control of Current in Electrolytic Silver-Ion Generator

Anodes were fashioned from high-purity silver (99.999 percent or better, Electronic Space Products Inc., Los Angeles) mounted in teflon supports designed both to center the anode and to provide the required water distribution. Wire connections to the anode, which are also within the water system, were similarly fabricated from silver. Such connections were positioned upstream of the cathode so as to avoid any current flow between the thin electrical leads and the cathode, which would result in deterioration of the leads and eventual cell failure. For test cells, a jeweler's grade of silver wire was used and the connection to the anode was made by melting the wire to the anode. The wire was insulated with teflon tubing. For flight cells, a pure (99.999 percent) silver wire was used and the connection was made by crimping the wire to the anode. No insulation was used except where the wire passed through the container walls.

Prototype Cell Design

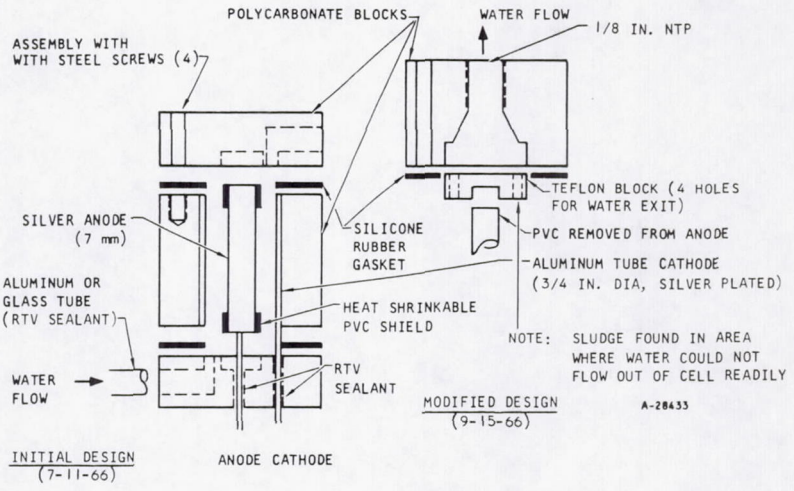
Three different test cell designs were evaluated, the cells differing mainly in geometric configurations and volumetric capacity. Preliminary design drawings and photographs for each of the three different cell designs can be seen in Figures 3-4 and 3-5. Design data are compared in Table 3-I.

The first test cell, designated the polycarbonate cell, was fabricated with a polycarbonate body and used to obtain preliminary performance data. It was later used with modifications in a continuous flow system. This cell did not include all of the design criteria previously set forth, which were observed in later models.

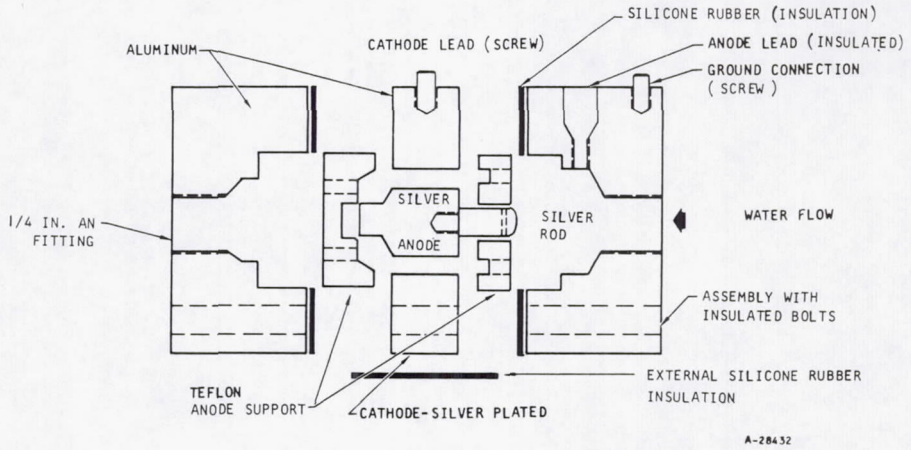
The second group of test cells, designated as Prototype Cells A and B, were built with aluminum bodies. The interior geometry, later used for fabrication of flight cells, differed from the polycarbonate cell in that a shorter anode of larger diameter was employed. The anodic area was virtually unchanged, but the cathodic area, cell volume, and, consequently, the residence time were reduced. Cell performance and efficiency should be enhanced, but actual differences were difficult to detect. The improved design eliminated dead spots in the cell, particularly at the entrance and the exit, and provided for removal of water by drawing it away from the cathode.

A third test cell, designated as Prototype Cell 150, was designed with an internal volume of 150 ml. The large cell volume provided the surge capacity and residence time necessary to obtain the requisite silver-ion concentration before discharge into the hold tank, when located downstream of the cyclic accumulators.

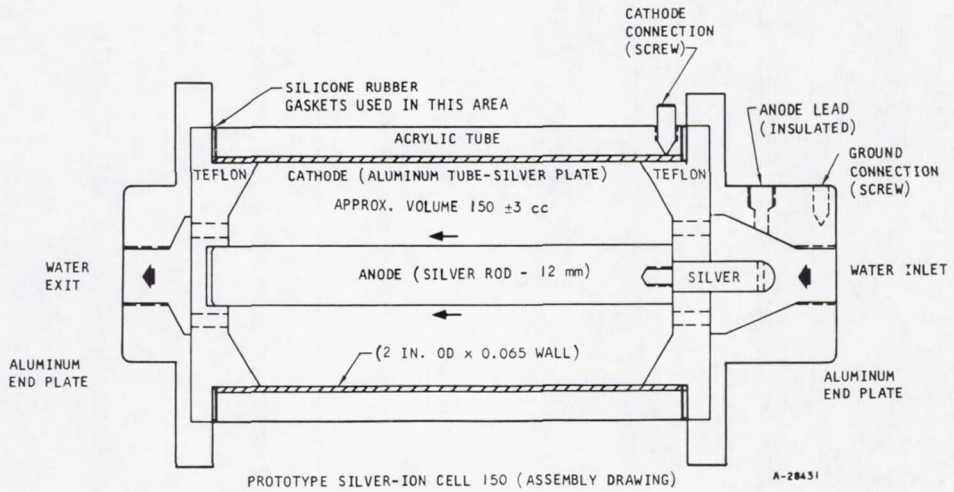
This cell proved useful for obtaining deposition rate data. However, silver-ion concentrations within the cell and exit concentrations were found to fluctuate considerably. Variations in effluent concentration could be traced to concentration gradients formed in the cell due to the large cell volume and small current density. Such gradients have been previously discussed. Redesign of the anode to provide several anodes in parallel was indicated, but was not undertaken. If a water sterilization unit were positioned at the inlet to the cyclic accumulator, where a more uniform water flow prevailed,



POLYCARBONATE SILVER-ION CELL (ASSEMBLY DRAWING)

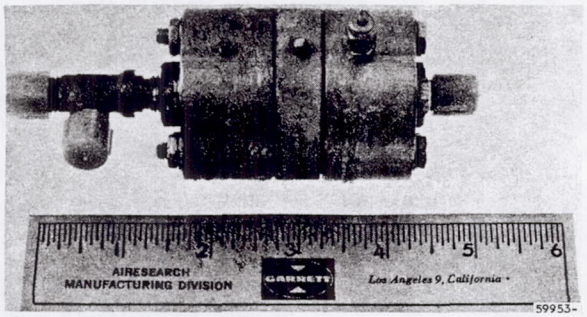


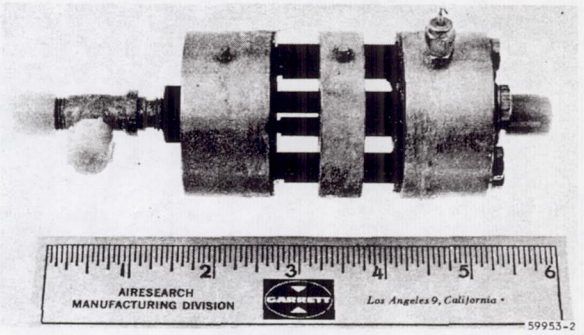
PROTOTYPE SILVER ION CELL A, B (ASSEMBLY DRAWING)

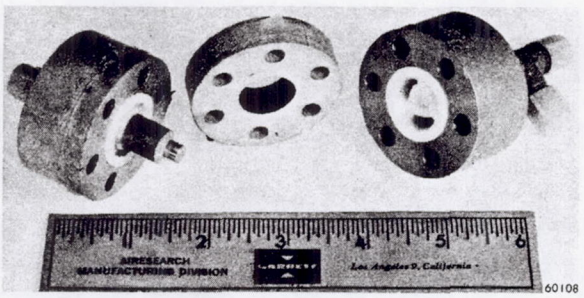


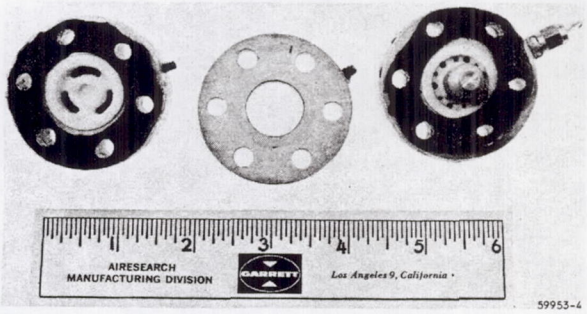
PROTOTYPE SILVER-ION CELL 150 (ASSEMBLY DRAWING)

Figure 3-4. Assembly Drawings for Laboratory Silver-Ion Generators Used to Obtain Development Data

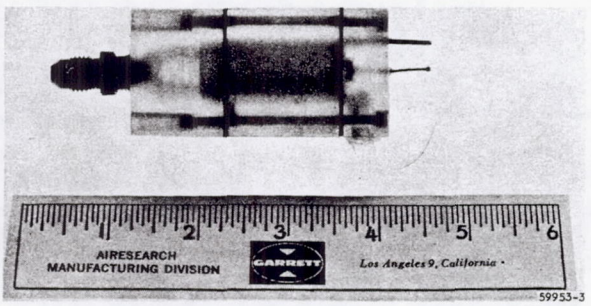




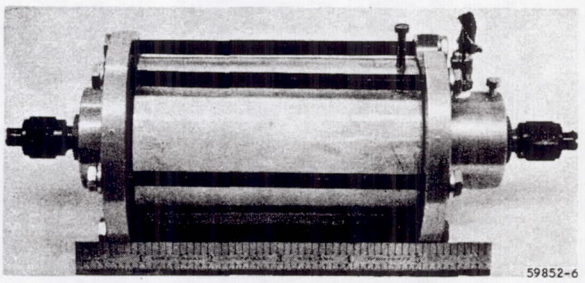




PROTOTYPE CELL A OR B



POLYCARBONATE CELL



PROTOTYPE CELL 150

F-7889

Figure 3-5. Prototype Silver-Ion Generators

TABLE 3-I

DESIGN DATA FOR SILVER-ION GENERATORS

Dimension		Polycarbonate Cell	Prototype Cell, A,B	Prototype Cell 150
Anode				
Diameter	(cm)	0.70	1.20	1.20
Length	(cm)	2.54	1.27	9.53
Effective area	(cm^2)	5.59	4.79	36.0
Cathode				
Diameter	(cm)	1.65	1.905	4.75
Length	(cm)	3.18	1.27	8.26
Effective area	(cm^2)	16.5	7.60	1.23
Electrode spacing	(cm)	0.475	0.353	1.775
Volume between electrodes	(cm^3)	5.57	2.18*	150

*Overall volume assumed 3 cc due to other design factors.

the original prototype unit (3-cc volume) would function more effectively. This arrangement was the one chosen. It was further advantageous in that a single cell design could be used for both the potable water system and the waste water system.

Design and Construction of Anode Supports

Anode support design is crucial for fabrication of a cell that will operate unattended for periods longer than six months at high efficiencies. Water flow paths through the cell, the position (centering) of the anode with respect to cathode, and the possible formation of silver crystals within the cell are influenced by the design and materials of construction of supports.

In the polycarbonate cell, shorting of the anode and cathode was found after about three months of operation. This was caused by a metallic silver sludge which had accumulated in a "dead" area at the exit. In the prototype cells, such sludge at the exit was not noted, but some small silver crystals were found on the teflon support at the entrance, where maximum channeling of the water flow could be expected. Such crystal growth could not readily short the cell, but does point out the necessity for initial water distribution prior to flow across the anode.

Teflon is utilized for a support material, since it is nonconductive and can be readily machined. However, in the machining of the teflon, metallic particles, particularly aluminum, must not be permitted to become embedded in the teflon. Small, electrically isolated, virgin metal particles can serve as secondary cathodic sites, act as a nucleus for silver deposition, and support the eventual growth of large single crystals of silver, which reduces operating efficiency.

Design of Flight-Rated Water Sterilization Units

Three water sterilization units (Figures 3-6 through 3-10, Outline Drawing 133448) were constructed using essentially the same internal design as that used for Prototype Cells A and B. The control circuit was the same as that noted previously (see Final Circuit). The battery (Union Carbide, Eveready, No. 301) and the three required resistors (1/4 w ±5 percent) were incorporated into a receptacle on the unit. The battery connection and circuit board will be potted. Terminals, in series with the current flow, provide for on-off functions or for measuring the current. These terminals are normally shorted with the shorting plug provided when the cell is operating.

Each cell is built as an integral unit, which simplifies installation or replacement. Values for the control resistors were selected to yield 85 to 90 ppb of silver at the cell exit at water flows of either 7 cc per min (potable water system) or 3 cc per min (waste water system). Some differences in current can be expected between various units having the same value for the control resistor, due to the tolerance of the resistor (±5 percent), but these are not critical. These flight units will undergo additional testing in the complete AAP system.

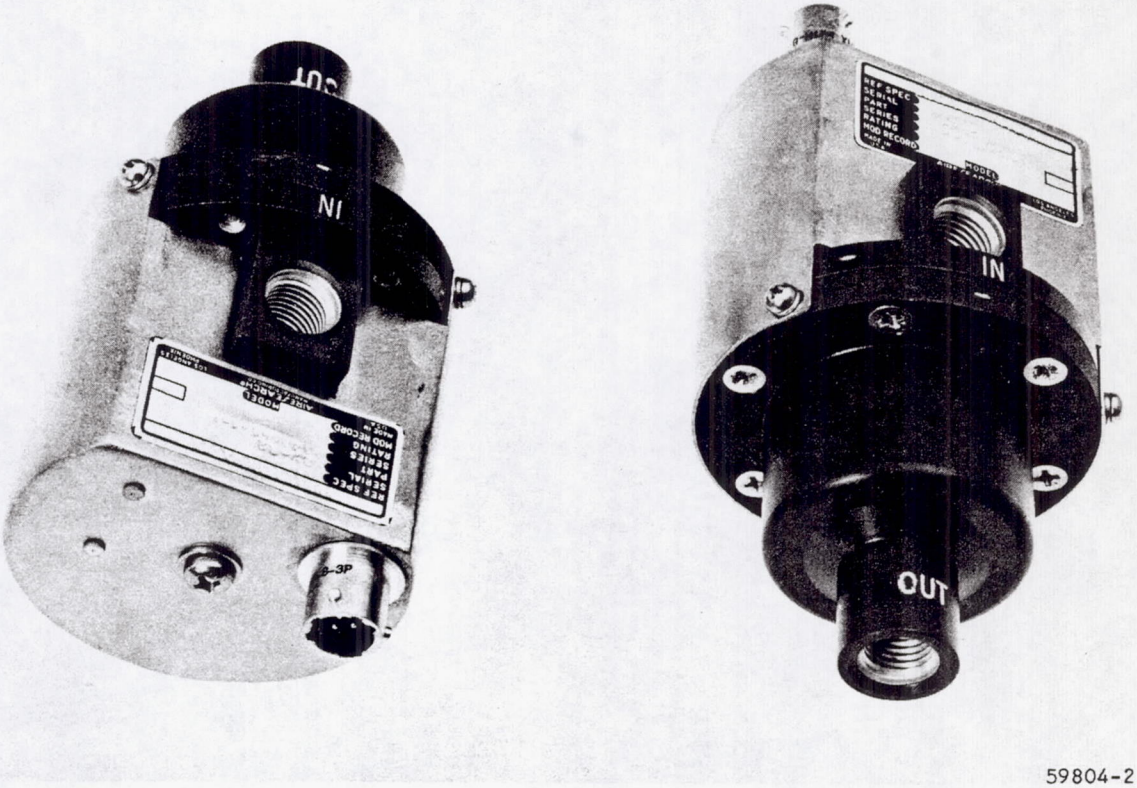


Figure 3-6. Flight-Rated Water Sterilization Cell

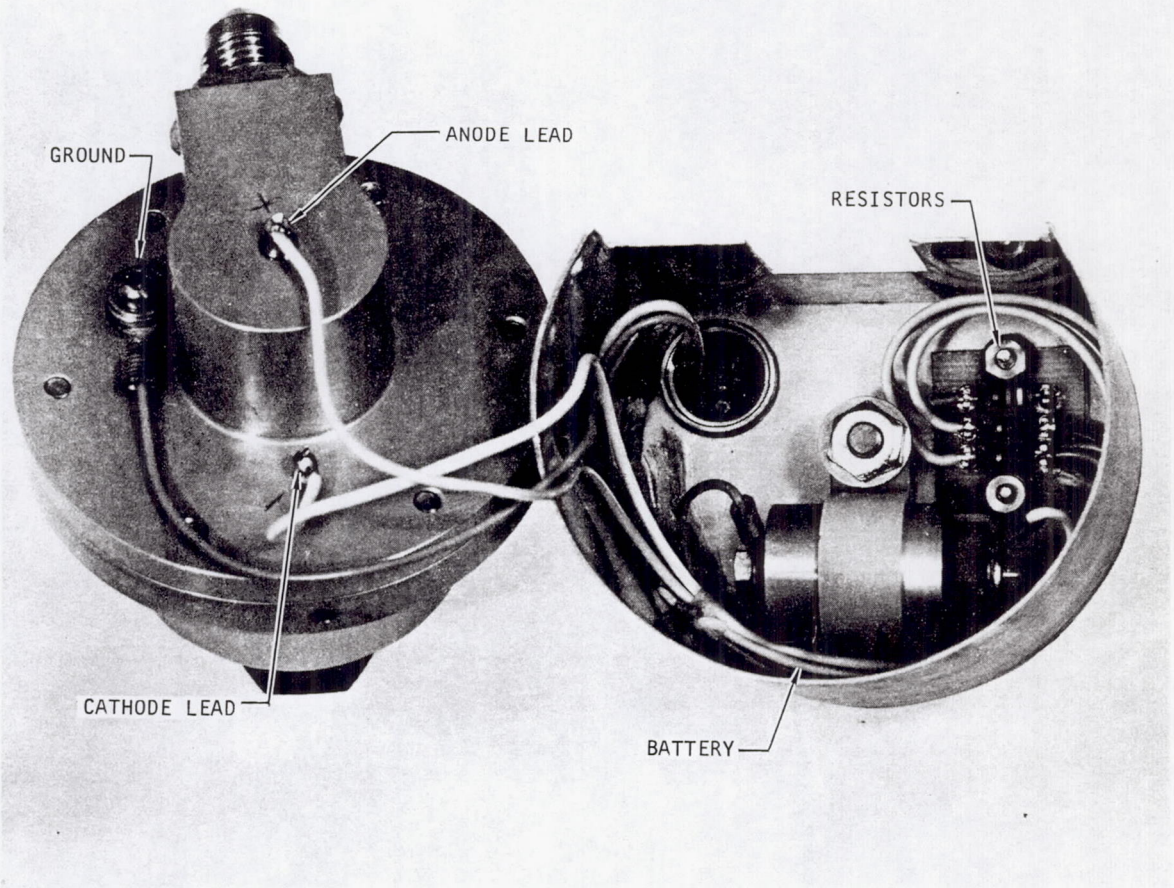


Figure 3-7. Power Supply System, Flight-Rated Water Sterilization Cell

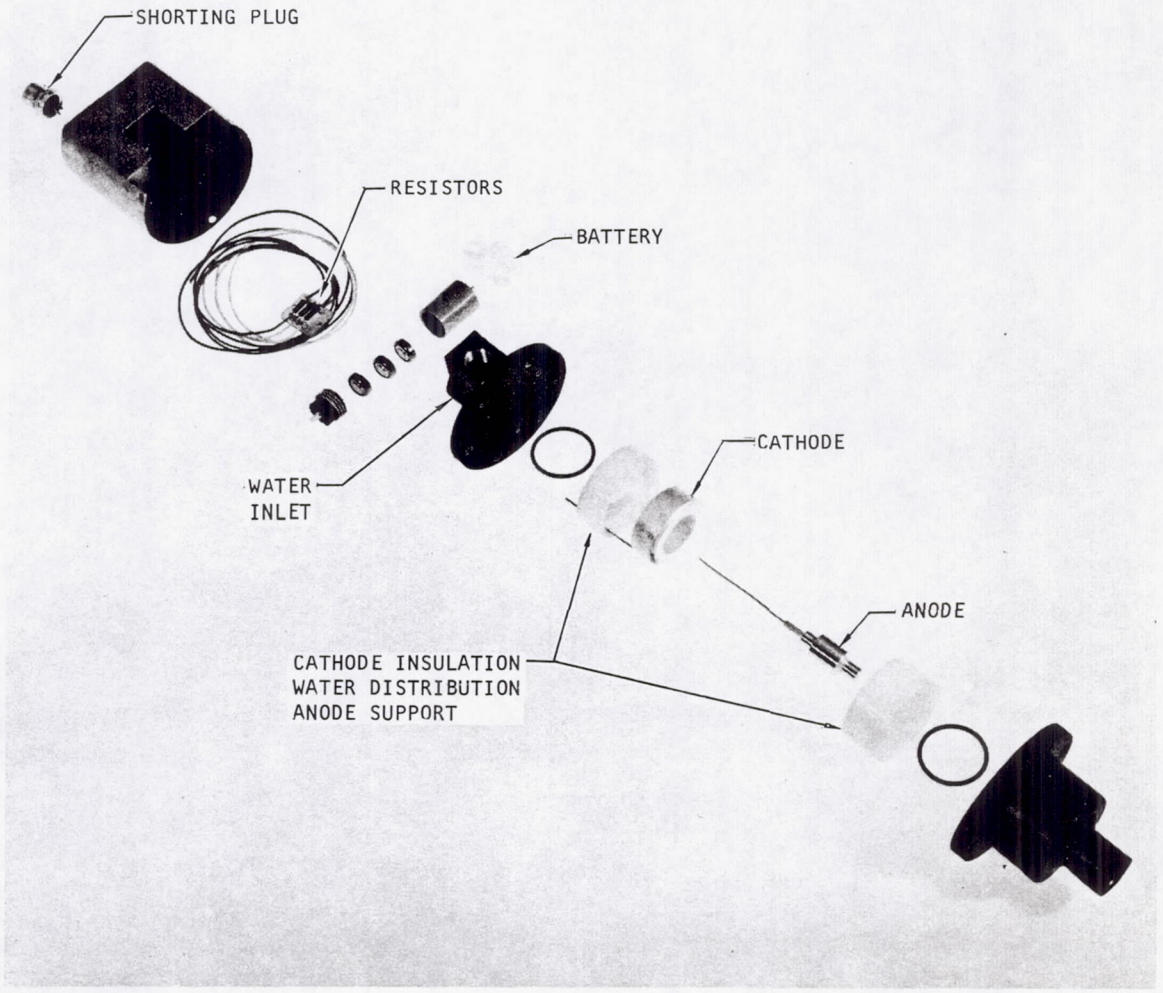


Figure 3-8. Exploded View, Flight-Rated Water Sterilization Cell

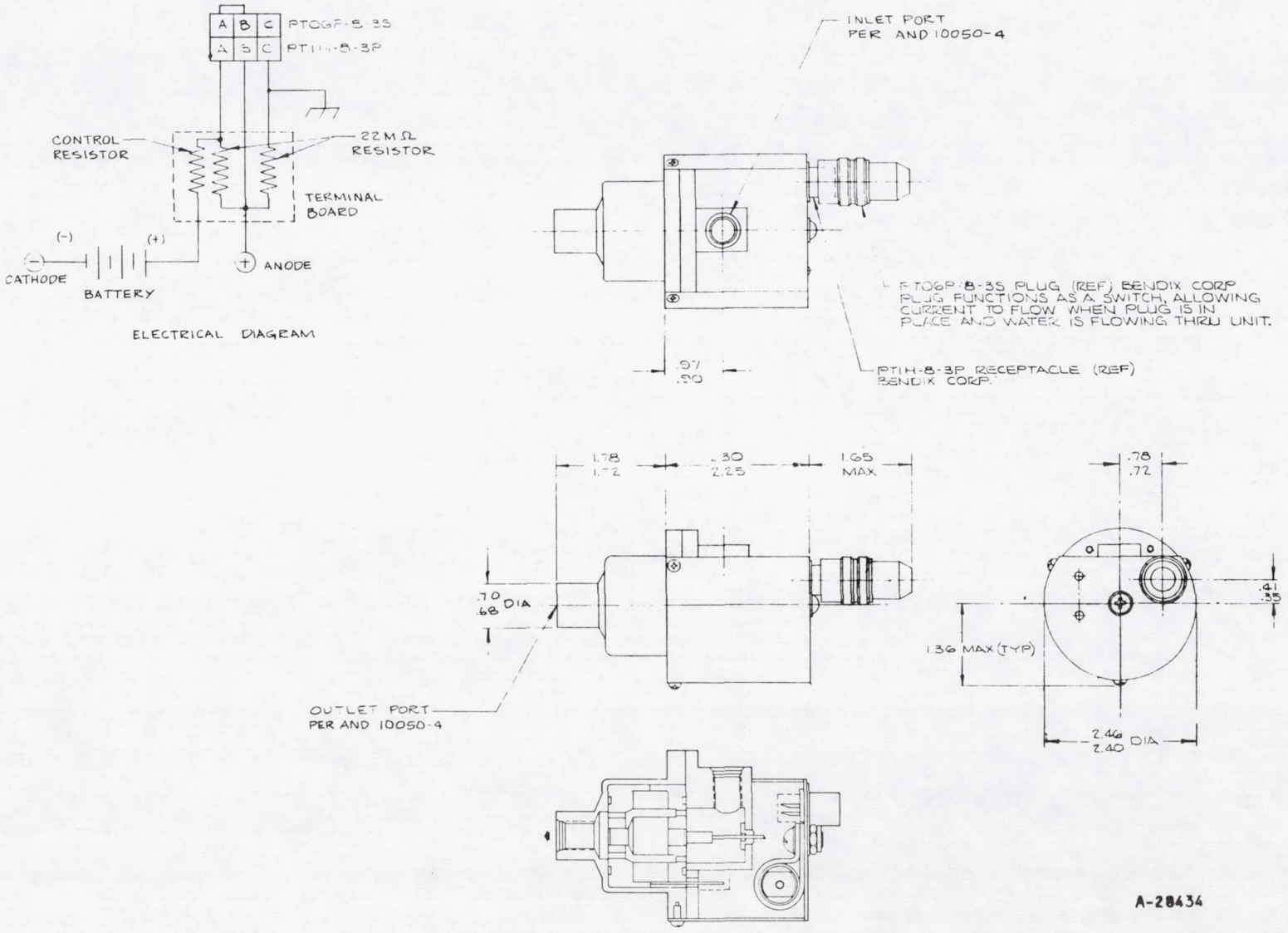


Figure 3-9. Outline Drawing, Part No. 133448

SECTION 4

PERFORMANCE CHARACTERISTICS

CELL PERFORMANCE

Internal Cell Resistance

The internal resistance of each of the three types of cells (polycarbonate, Prototype A and B, and Prototype 150) was obtained at different current levels by measuring the external resistance required to establish the current using a battery of known voltage (4.27 v) and a flow rate of distilled water (15 cc per min) sufficiently high to prevent a change in water conductivity in the cell during the measurements. Resistance was determined with a decade resistance box (General Radio Co., Concord, Massachusetts, Type 1432-B). The original polycarbonate cell was also tested with tap water (assumed to be maximum conductivity) to observe the current variation with change in conductivity of the water. Data are shown in Figures 4-1 and 4-2. These curves directly establish the external control resistance required to obtain the desired current levels in flight units if the power supply is of similar voltage.

Output Efficiencies

Samples of effluent were obtained at constant flow rates during measured time intervals and analyzed for total silver. Analyses were made by colorimetric techniques using dithizone as a complexing agent for silver (see Analytical Methods). The ratio of weight of silver so measured to theoretical weight as established by Faraday's law constituted the cell efficiency.

The concentration, which is independent of the time interval, was calculated from the ratio of total silver to measured sample volume.

$$C = \frac{W}{V}$$

C = concentration (ppb = μg/liter)

W = Weight of silver (μg)

V = Volume of effluent (liter)

For flow systems, either intermittent or continuous, the theoretical output can be established from the average flow and current by the equation given previously

$$C = \frac{G}{F}$$

and compared with concentration obtained by analysis.

Within the limits of experimental error when using distilled water, cell output efficiencies were usually 90 percent or better of theoretical except at very low flow rates (2 cc per min or less). A number of different solutions

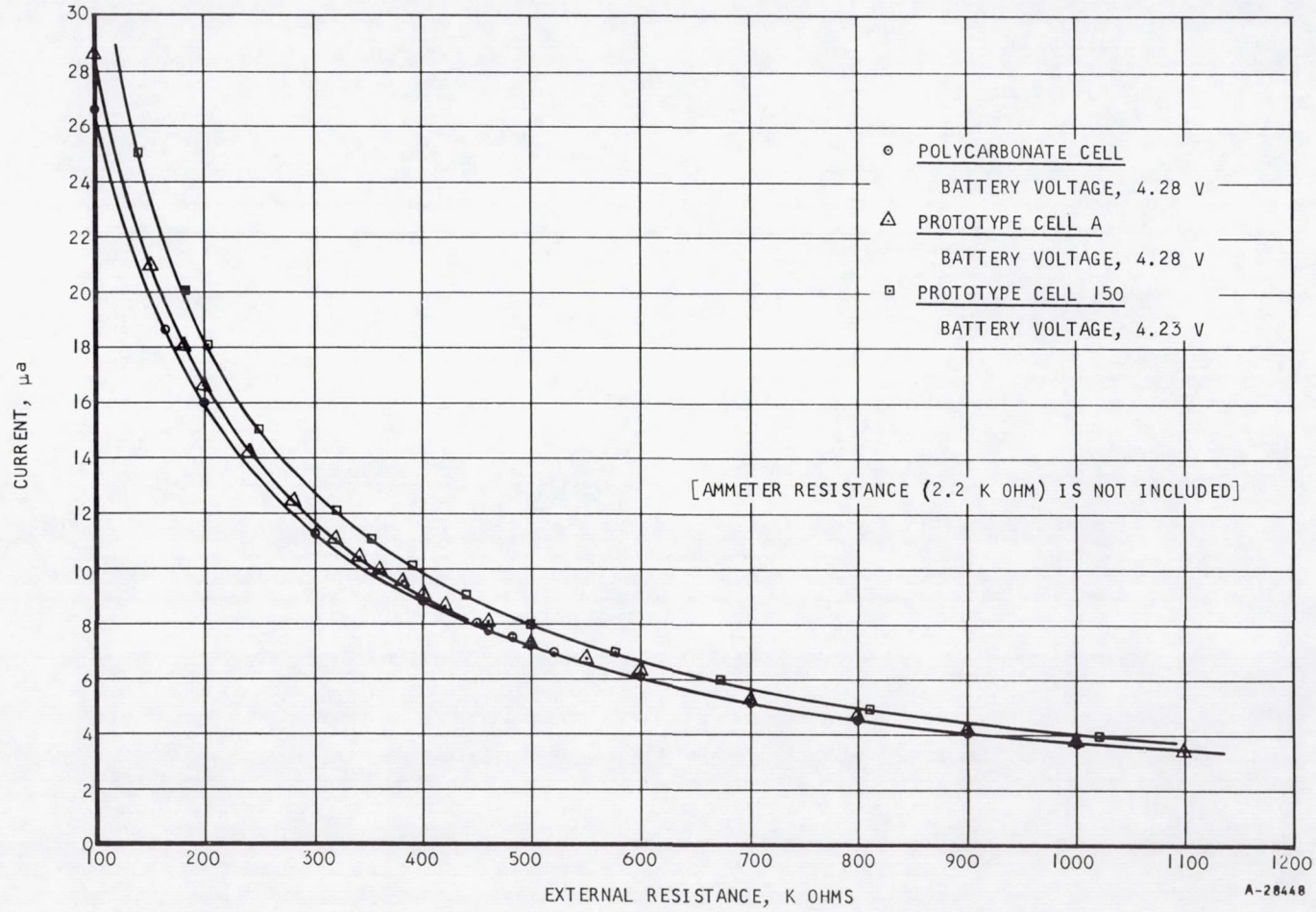


Figure 4-1. Amperage as a Function of External Control Resistance

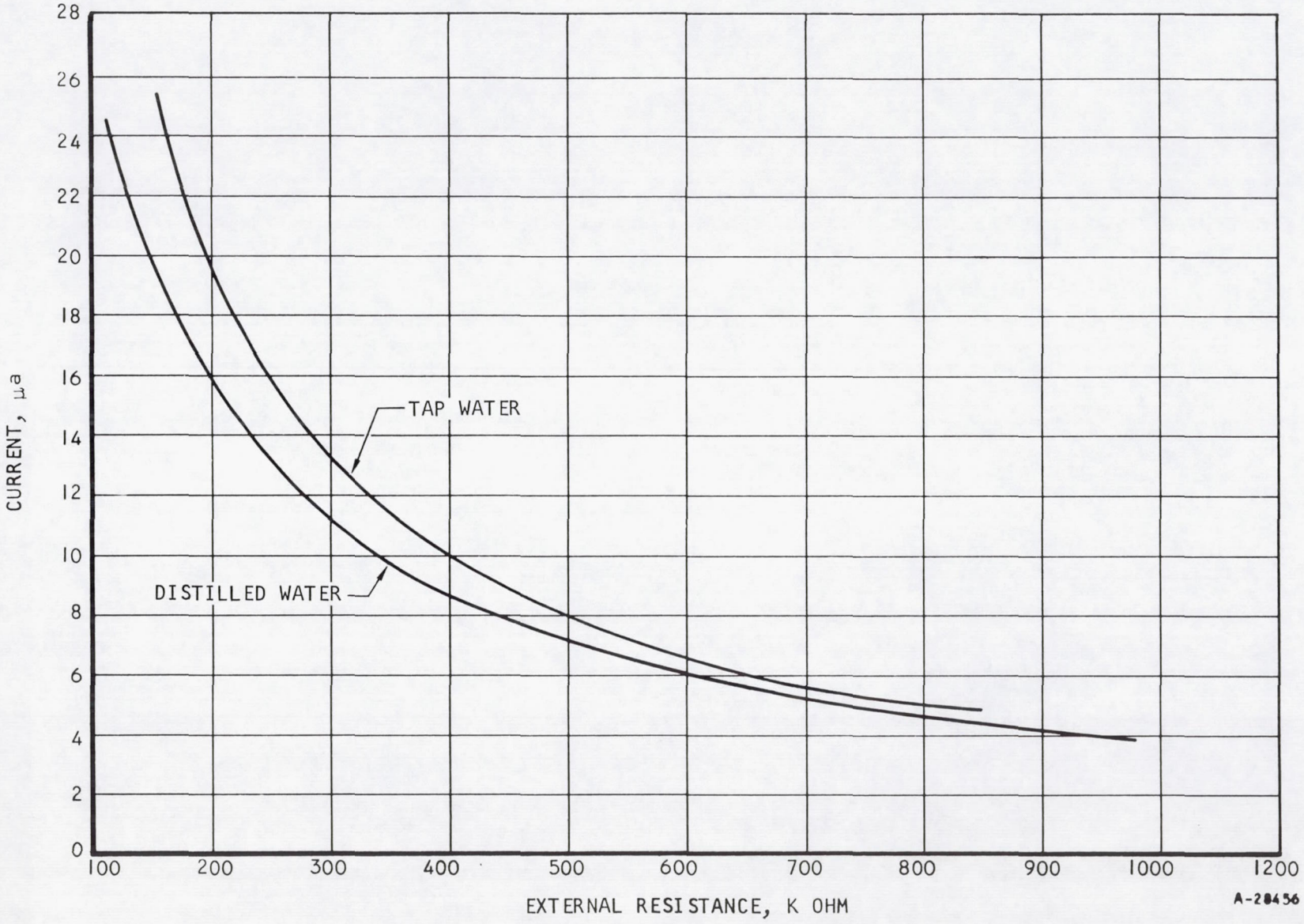


Figure 4-2. Variation in Current with Conductivity of Water

were tested (recognizing the limitations of the analytical procedure) including distilled water, sweat condensate, fuel cell water, hydrogenated water, and a sodium carbonate solution (pH = 9.0), representative of a high pH. Typical values are tabulated in Table 4-1. Variations in efficiency were observed, but are possibly due to analytical error (see Analyses).

The short residence time in the small prototype cells precluded determination of any minor fluctuations in concentration that could be present in the effluent. The sample required for analysis represented essentially an average concentration. Gross variations in concentration were found under flow conditions with the large cell (Prototype Cell 150), since residence times on the order of 15 min or longer occurred, and sample size could be less than the internal cell volume. Such variations are shown in Figure 4-3. These fluctuations in output concentration indicate that the current density in the cell was not diffuse but localized in regions of high silver concentration. These regions then moved through the cell under the low flow conditions, becoming more concentrated before eventually being discharged at the exit. When the cell was vibrated, more uniform values for the effluent concentration were obtained, which approached predicted values (see cathodic deposition rates).

Major losses within the silver ion cells could be attributed to plating on the cathode (more prevalent at lower flow rate), adsorption at the aluminum outlet, and possibly deposition of insoluble silver oxide at the anode.

Although the silver ion concentrations are within the solubility limits of silver oxide (20.5 ppm) and silver chloride (1.13 ppm) (Reference 2), so that precipitation should not occur, the electrode potential can apparently cause deposition of such compounds on the anode due to the higher mobility of the hydroxide and chloride ion which, if present, preferentially carry the current. Oxides were theorized to have formed by the dull appearance of the anode after use, although pH changes to 9.0 or better did not appear to have an appreciable affect on output efficiency.

The presence of dissolved and gaseous hydrogen in the water had no effect on the generation of ionized silver in the tests performed. Theoretically the ionization of hydrogen at the anode would proceed at a lower potential than silver, but apparently the electrode overvoltage on smooth silver is sufficiently high to prevent loss in output efficiency by this means.

Efficiencies of Flight-Rated Water Sterilization Cells

Output concentrations of a flight prototype unit and three flight units were evaluated at different flow rates. These data are tabulated in Table 4-2 and shown graphically in Figures 4-4 and 4-5. Initial tests on the flight prototype unit were made at two different current levels by using an external resistance to augment the internal control resistance of the cell. The battery potential in this unit was determined to be 4.72 v.

Tests on the three flight type units were made only at the current level as determined by the internal control resistances. When an ammeter was utilized in place of the shorting plug a theoretical concentration could be established

TABLE 4-I

TYPICAL ANALYSES OF SILVER-ION GENERATOR EFFICIENCIES UNDER VARIOUS CONDITIONS

Date	Silver-Ion Cell	Solution	Flow Rate, cc/min	Current, μa	Cell Efficiency, Percent	Remarks
7-21-66	Polycarbonate	Distilled water	4.54	15.35	92.2	
7-21-66	Polycarbonate	Distilled water	4.60	15.30	86.9	
7-22-66	Polycarbonate	Distilled water	1.72	15.55	79.5	
7-22-66	Polycarbonate	Distilled water	1.66	15.55	79.9	
7-25-66	Polycarbonate	Distilled water	10.3	15.40	101.0	
7-25-66	Polycarbonate	Distilled water	10.3	15.55	99.7	
7-25-66	Polycarbonate	Distilled water	15.7	15.70	103.6	
7-25-66	Polycarbonate	Distilled water	16.1	15.70	98.6	
7-26-66	Polycarbonate	Distilled water	16.8	15.05	91.4	
7-26-66	Polycarbonate	Distilled water	16.5	15.05	88.8	
8-4-66	Prototype A	Distilled water	14.05	5.2	107.0	Cell is fresh--excessive silver being obtained
8-4-66	Prototype A	Distilled water	13.8	5.25	114.0	
8-4-66	Prototype A	Distilled water	13.7	5.25	116.0	
8-4-66	Prototype A	Distilled water	9.1	5.25	120.0	
8-4-66	Prototype A	Distilled water	9.4	5.25	105.0	
8-4-66	Prototype A	Distilled water	9.4	5.25	125.0	
8-5-66	Prototype A	Distilled water	4.8	5.25	104.0	
8-5-66	Prototype A	Distilled water	4.8	5.30	100.0	
8-5-66	Prototype A	Distilled water	4.73	10.0	112.0	
8-5-66	Prototype A	Distilled water	4.67	10.1	110.0	
8-5-66	Prototype A	Distilled water	9.73	10.05	107.0	
8-5-66	Prototype A	Distilled water	9.73	10.05	100.0	
8-5-66	Prototype A	Distilled water	9.73	10.05	99.0	
8-5-66	Prototype A	Distilled water	15.0	10.00	112.0	
8-5-66	Prototype A	Distilled water	14.8	10.0	116.0	
8-5-66	Prototype A	Distilled water	14.7	10.0	105.0	
8-8-66	Prototype A	Distilled water	10.0	16.2	89.0	
8-8-66	Prototype A	Distilled water	10.0	16.2	102.0	
8-8-66	Prototype A	Distilled water	10.0	16.2	125.0	
8-18-66	Prototype A	Distilled water	6.7	9.7	91.0	
8-18-66	Prototype A	Distilled water	9.6	9.65	91.0	
8-19-66	Prototype A	Distilled water	5.1	20.4	97.0	
9-27-66	Prototype A	Distilled water	11.5	12.5	99.0	
1-30-67	Prototype A	Distilled water	2.86	4.2	86.0	No appreciable change after several months of use in testing cell throughput efficiencies.
1-31-67	Prototype A	Distilled water	2.61	4.2	82.0	
2-2-67	Prototype A	Distilled water	2.86	4.4	94.0	
2-2-67	Prototype A	Distilled water	2.86	4.4	94.0	
2-2-67	Prototype A	Distilled water	2.86	4.4	105.0	
2-2-67	Prototype A	Distilled water	2.63	4.4	98.0	
1-6-67	Prototype A	Na_2CO_3-pH = 9.7	9.4	20.0	97.0	High currents used to ensure presence of sufficient silver for analysis. Tests and solutions were limited.
1-6-67	Prototype A	Na_2CO_3-pH = 9.7	9.2	20.0	71.0	
1-6-67	Prototype A	Distilled water	4.67	16.8	103.0	
1-6-67	Prototype A	Distilled water	4.60	16.8	67.0	
1-6-67	Prototype A	Sweat condensate	2.6	20.1	88.0	
1-6-67	Prototype A	Sweat condensate	4.2	20.0	96.0	
1-6-67	Prototype A	Sweat condensate	2.9	20.0	68.0	
1-6-67	Prototype A	Sweat condensate	7.17	10.2	92.0	
1-9-67	Prototype A	Fuel cell water*	9.9	9.9	93.0	
2-6-67	Prototype A	Bladder water**	(Initial analysis indicated 21 ppb - Analyses below not corrected)			These analyses subject to considerable error due to thiuram but indicate cell output in the presence of bladder water
2-6-67	Prototype A	Bladder water	10.3	10.15	87.0	
2-7-67	Prototype A	Bladder water	10.3	10.15	98.0	
2-8-67	Prototype A	Bladder water	10.2	10.1	105.0	
	Tests of hydrogenated water made under static conditions. Silver anode (12 mm rod) and cathode (7 mm rod) immersed in beaker containing agitated distilled water. Test gas bubbled through dispersion tube. Comparative tests made with nitrogen and the absence of any gas flow. Results reported in sequence obtained. Initial analyses indicated free ionic silver on immersed electrodes.					
			(Static)			
4-18-67	Water only - not agitated		15 min test	10.0	115.0	No appreciable effect noted on generation of silver using different gases or the absence of gas under these test conditions. Probable losses by adsorption on glass and/or cathodic deposition.
4-18-67	Water only - agitated		15 min test	10.0	109.0	
4-18-67	Water only - agitated		15 min test	10.0	90.0	
4-18-67	Hydrogenated water - agitated		15 min test	10.0	74.0	
4-18-67	Hydrogenated water - agitated		15 min test	10.0	84.0	
4-18-67	Nitrogenated water - agitated		15 min test	10.0	84.0	
4-18-67	Nitrogenated water - agitated		15 min test	10.0	75.0	
4-19-67	Water only - agitated		15 min test	10.0	73.0	
4-19-67	Water only - agitated		15 min test	10.0	73.0	

*Allis-Chalmers Test No. 8, June 13, 1966.

**Water collected from waste water tank of simulated Apollo system.

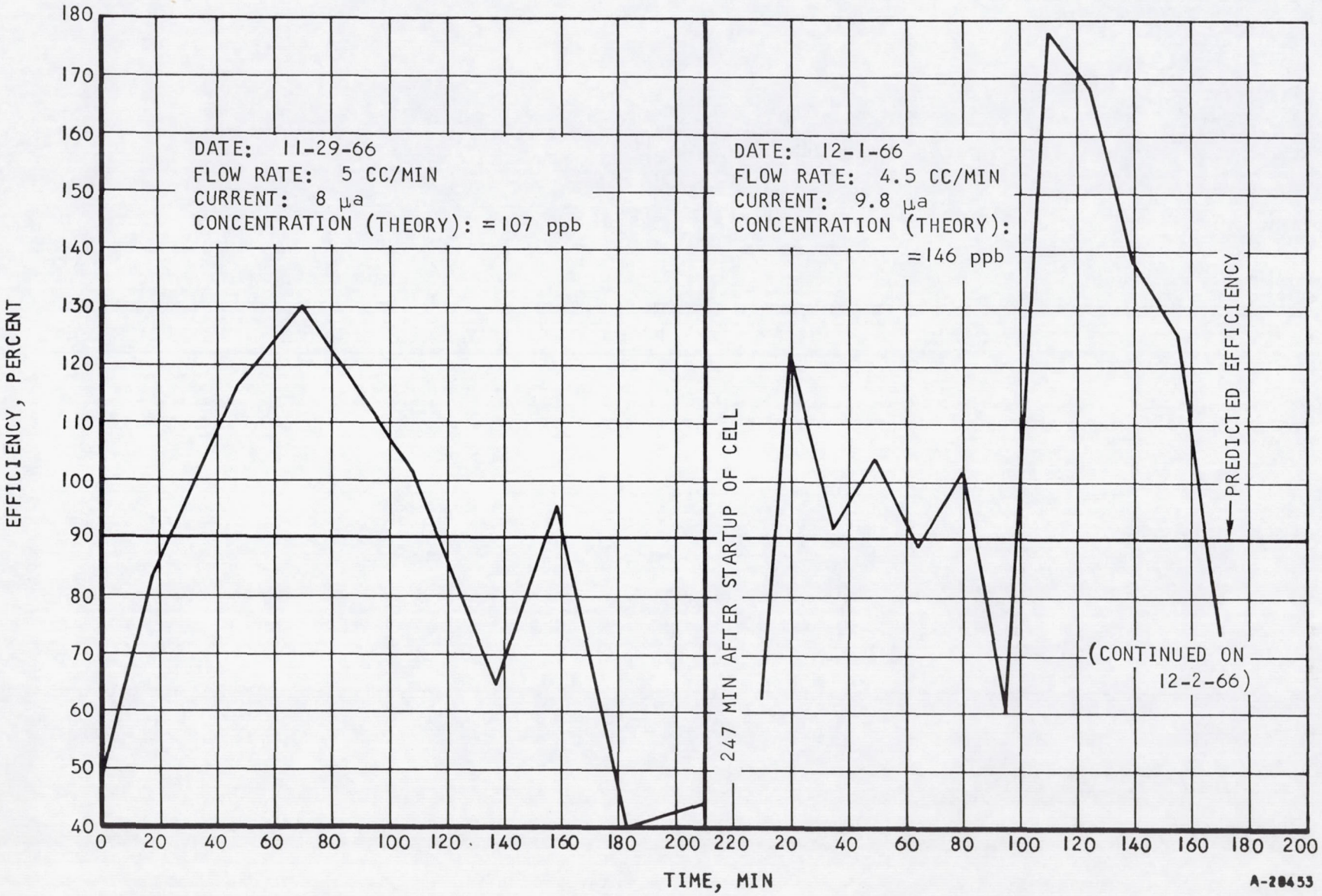


Figure 4-3. Fluctuations in Effluent Concentration for the Large (150 cc) Silver-Ion Generator

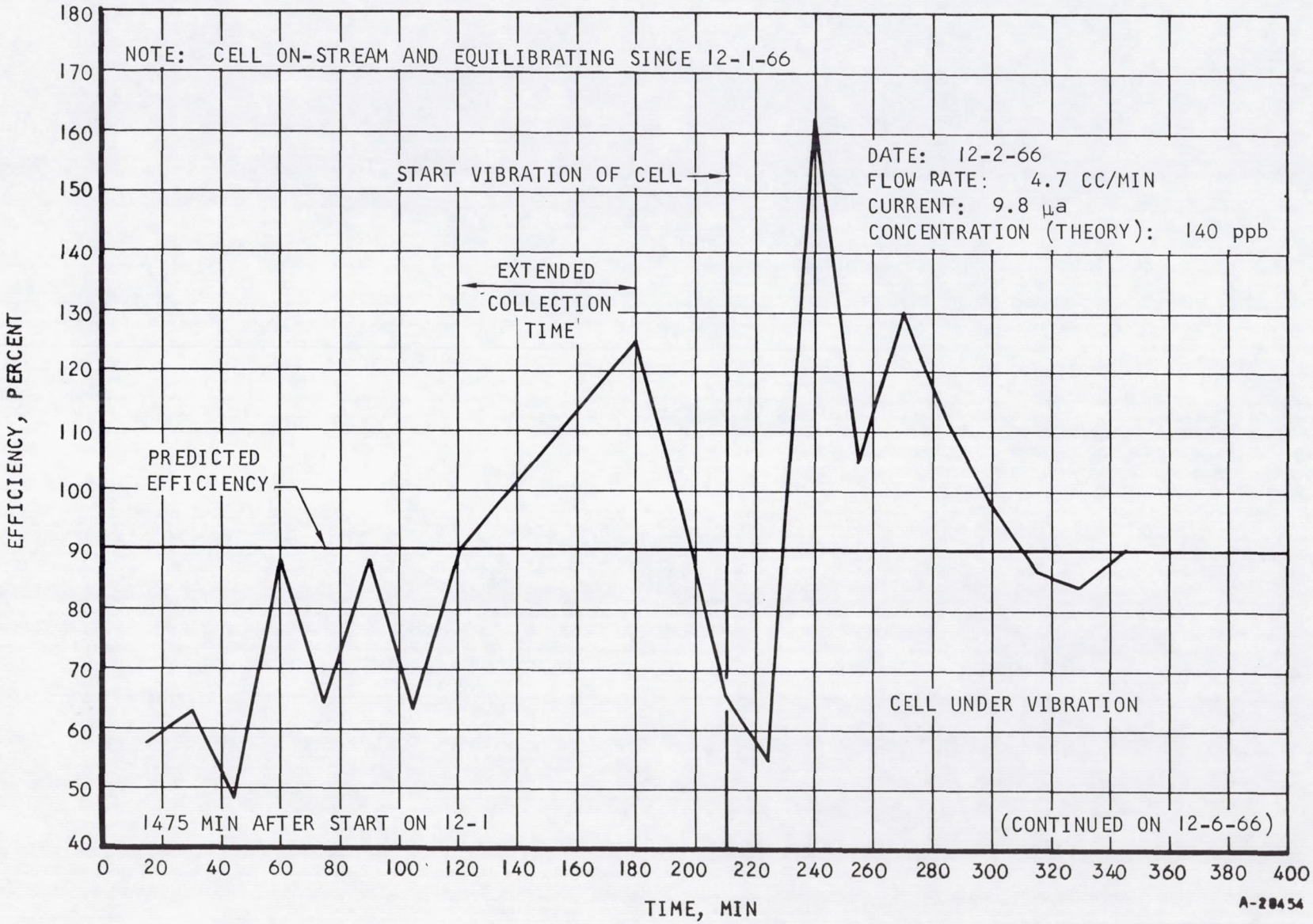


Figure 4-3 (Continued)

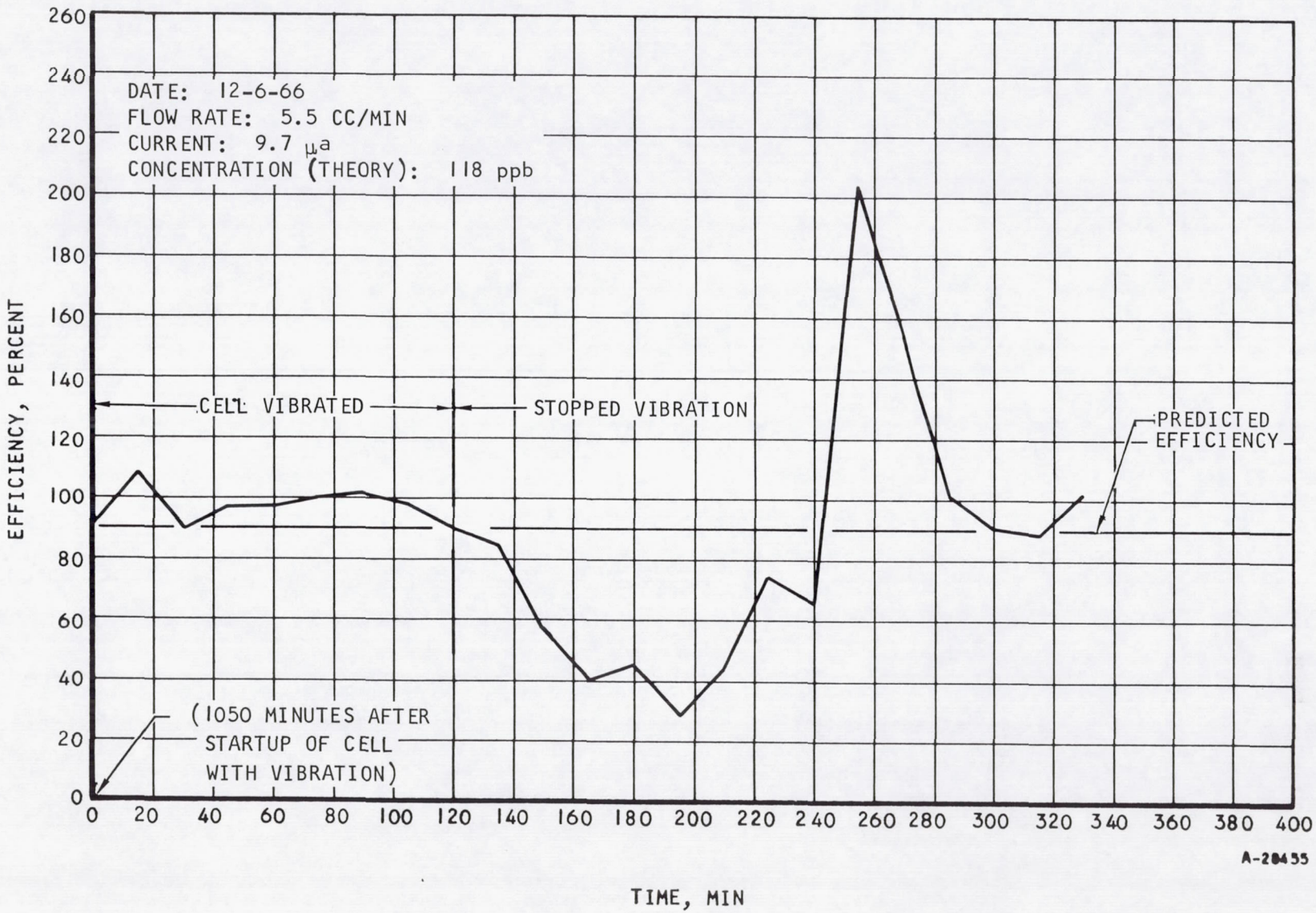


Figure 4-3 (Continued)

TABLE 4-2

OPERATING TESTS FOR FLIGHT RATED
WATER STERILIZATION (SILVER ION) CELLS

Sample No.	Average Flow (cc/min)	Average Current (μa)	Concentration (ppb)			Remarks (2)
			Theory (3)	Measured		
				Ammeter(1)	Short Plug	
No Part No. - Designated as Flight Rated Cell No. 1 (FRC-1) Battery = 4.72v (Tested at two current levels using external resistance to supplement control resistance)						
	At 9-10 μa range					
1-31-FRC1-1	6.57	9.55	97.5	92.9	-	
1-31-FRC1-2	6.67	9.55	96.0	93.6	-	
1-31-FRC1-3	6.56	9.55	97.5	94.0	-	
1-31-FRC1-4	12.60	9.60	51.0	50.8	-	
1-31-FRC1-5	12.53	9.60	51.3	49.8	-	
2-2-FRC1-1	2.90	9.70	224	223	-	
2-2-FRC1-2	2.80	9.80	234	222	-	
2-2-FRC1-3	2.82	9.80	233	220	-	

(1) Ammeter adds 2.2 K ohm to overall resistance. (3) Calculated using average current value.
(2) All samples of approximately 10 μg silver unless otherwise noted.

TABLE 4-2 (Continued)

Sample No.	Average Flow (cc/min)	Average Current (μa)	Concentration (ppb)			Remarks (2)
			Theory (3)	Measured		
				Ammeter (1)	Short Plug	
FRC-1 (Continued)						
	At 4 μa range					
2-3-FRC1-1	3.03	4.1	90.7	85.4	-	
2-3-FRC1-2	3.01	4.1	91.3	86.2	-	
2-3-FRC1-3	7.14	4.1	38.5	36.0	-	
2-3-FRC1-4	7.26	4.1	37.8	36.6	-	
2-6-FRC1-1	13.2	4.0	20.3	18.5	-	5 μg silver sample
2-6-FRC1-2	13.2	4.0	20.3	19.7	-	5 μg silver sample
2-6-FRC1-3	13.2	4.0	20.3	19.7	-	5 μg silver sample
2-6-FRC1-4	1.41	4.0	190	174	-	
2-7-FRC1-1	1.27	4.0	211	210	-	
2-7-FRC1-2	1.23	4.0	218	215	-	

(1) Ammeter adds 2.2 K ohm to overall resistance (3) Calculated using average current value.
(2) All samples of approximately 10 μg silver unless otherwise noted

TABLE 4-2 (Continued)

Sample No.	Average Flow (cc/min)	Average Current (μa)	Concentration (ppb)			Remarks (2)
			Theory (3)	Measured		
				Ammeter (1)	Short Plug	
Part No. 133448-1-1 Serial No. 37-R1 Weight = 273 g						
4-4-37R1(-1)-1	2.93	9.90	226	220	-	
4-4-37R1(-1)-2	2.93	9.90	226	213	-	
4-4-37R1(-1)-3	2.93	9.85	225	218	-	
4-4-37R1(-1)-4	2.93	-	-	-	222	
4-4-37R1(-1)-5	7.07	9.85	93.3	89.0	-	
4-5-37R1(-1)-1	6.80	9.85	97.1	92.4	-	
4-5-37R1(-1)-2	6.73	9.85	98.1	92.0	-	
4-5-37R1(-1)-3	6.67	-	-	-	92.5	
4-5-37R1(-1)-4	11.93	9.50	53.4	51.0	-	
4-5-37R1(-1)-5	11.87	9.50	53.4	52.6	-	
4-5-37R1(-1)-6	11.87	9.60	54.2	49.4	-	
4-5-37R1(-1)-7	11.80	-	-	-	49.5	

(1) Ammeter adds 2.2 K ohm to overall resistance (3) Calculated using average current value.
(2) All samples of approximately 10 μg silver unless otherwise noted

TABLE 4-2 (Continued)

Sample No.	Average Flow (cc/min)	Average Current (μa)	Concentration (ppb)			Remarks (2)
			Theory (3)	Measured		
				Ammeter (1)	Short Plug	
Part N o. 133448-1-1 Serial No. 37-R2 Weight = 271.3 g (with shorting plug = 11.3 g)						
4-4-37R2(-1)-1	3.07	10.20	223	203	-	
4-4-37R2(-1)-2	3.07	10.20	223	209	-	
4-5-37R2(-1)-1	3.00	10.20	228	208	-	
4-5-37R2(-1)-2	2.93	-	-	-	214	
4-5-37R2(-1)-3	7.40	10.00	90.5	84.3	-	
4-5-37R2(-1)-4	7.40	10.00	90.5	88.1	-	
4-5-37R2(-1)-5	7.30	10.00	91.8	83.6	-	
4-5-37R2(-1)-6	7.26	-	-	-	86.2	
4-6-37R2(-1)-1	12.13	9.90	54.7	49.4	-	
4-6-37R2(-1)-2	12.13	9.95	55.0	50.5	-	
4-6-37R2(-1)-3	12.07	9.95	55.2	51.9	-	
4-6-37R2(-1)-4	12.03	-	-	-	51.9	

(1) Ammeter adds 2.2 K ohm to overall resistance (3) Calculated using average current value.
(2) All samples of approximately 10 μg silver unless otherwise noted

TABLE 4-2 (Continued)

Sample No.	Average Flow (cc/min)	Average Current (μa)	Concentration (ppb)			Remarks (2)
			Theory (3)	Measured		
				Ammeter (1)	Short Plug	
Part No. 133448-2-1 Serial No. 37-R1 Weight = 271.6 g (with shorting plug = 11.6 g)						
4-6-37R1(-2)-1	2.86	4.30	100.7	92.2	-	
4-6-37R1(-2)-2	2.84	4.30	101.4	95.5	-	
4-6-37R1(-2)-3	2.77	4.30	104.0	92.8	-	
4-6-37R1(-2)-4	2.74	-	-	-	91.4	
4-6-37R1(-2)-5	6.89	4.25	41.3	37.3	-	
4-6-37R1(-2)-6	6.86	4.25	41.5	40.8	-	
4-6-37R1(-2)-7	6.86	4.25	41.5	37.3	-	
4-6-37R1(-2)-8	6.80	-	-	-	36.9	
4-7-37R1(-2)-1	1.21	4.25	235	200	-	
4-7-37R1(-2)-2	1.19	4.25	239	206	-	
4-7-37R1(-2)-3	1.16	4.25	245	206	-	
4-7-37R1(-2)-4	1.13	-	-	-	216	

(1) Ammeter adds 2.2 K ohm to overall resistance (3) Calculated using average current value.
(2) All samples of approximate 10 μg silver unless otherwise noted

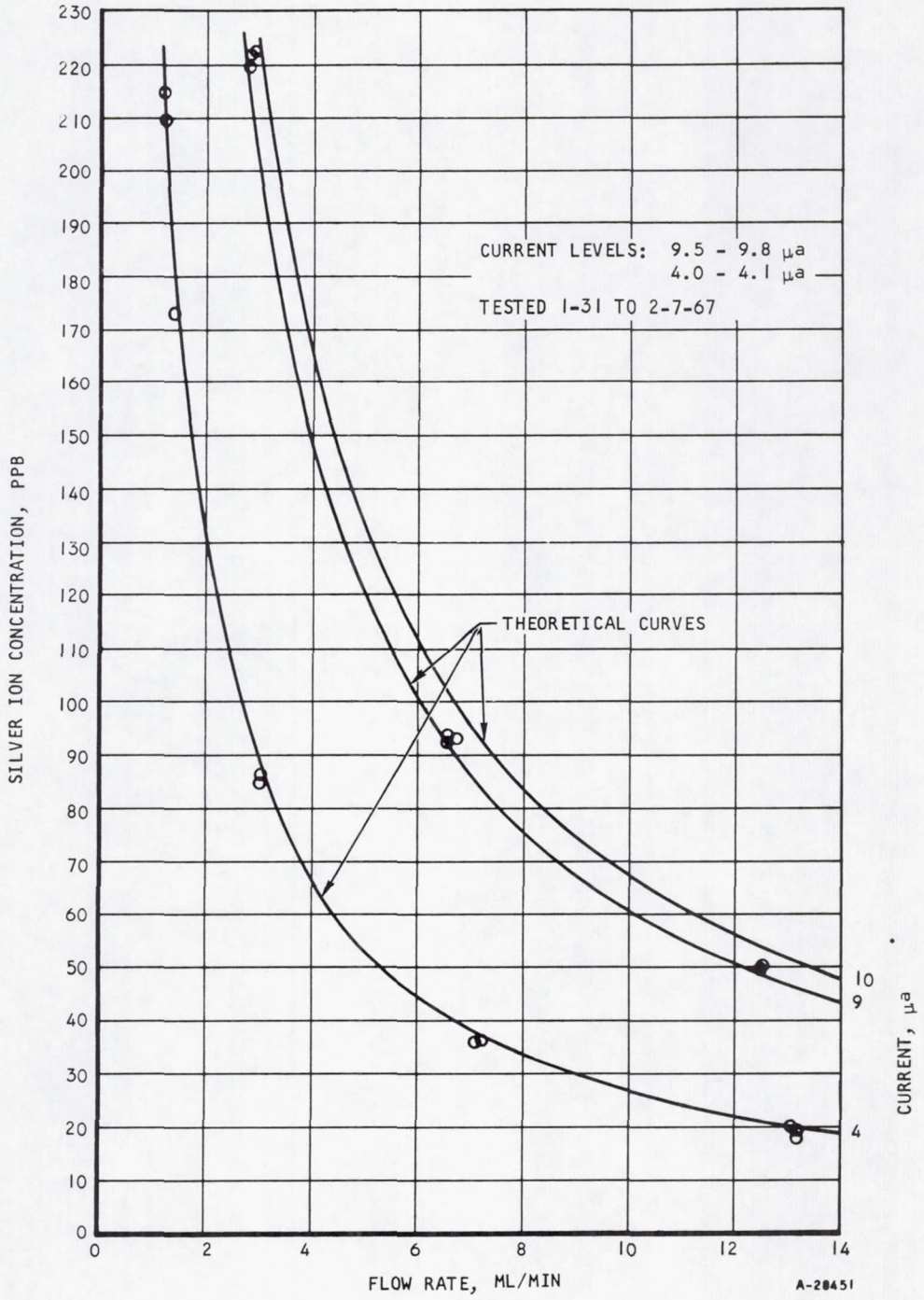


Figure 4-4. Output Concentration as a Function of Flow Rate for Flight-Rated Silver-Ion Cell No.1 (Flight Prototype Unit)

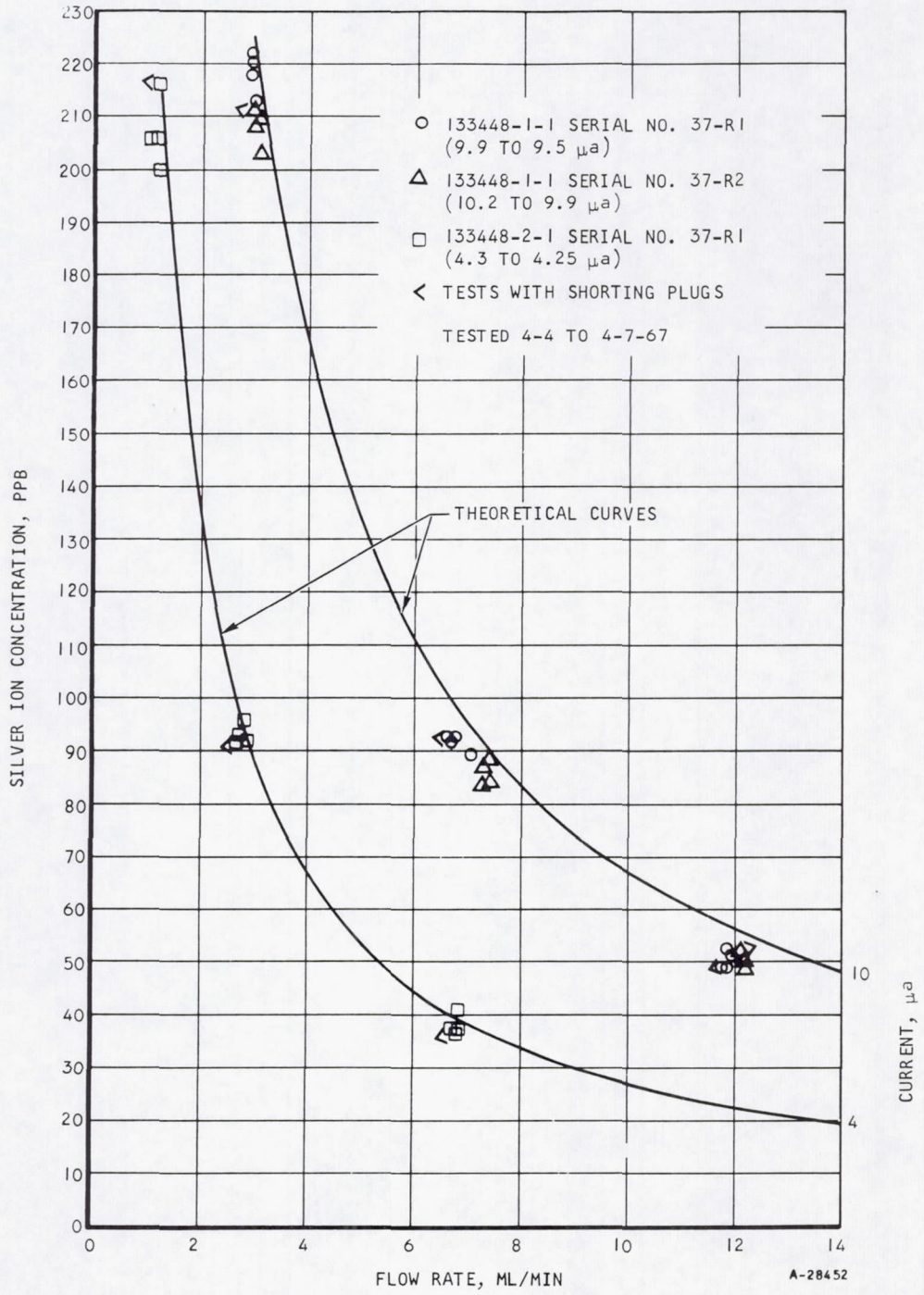


Figure 4-5. Output Concentration as a Function of Flow Rate for Three Flight-Rated Water Sterilization Cells

from the known flow rate and amperage. However, the resistance of the ammeter (2.2 k ohm) reduced the current flow slightly. When only the shorting plug was used, the output concentration could be measured but a theoretical concentration could not be established, since no measurement of current was provided. Output concentrations in Table 4-2 reflect the use of both an ammeter and a shorting plug. Sample size was selected, except as noted, to minimize analytical error. These data are in good agreement with data obtained for the prototype cells and indicate essentially 95 to 100 percent output efficiency.

Cathodic Deposition Rates

In a well-constructed cell, only silver ions can be produced at the anode, and the current is a measure of the weight rate of production. This silver must either be present in the effluent or redeposited on the cathode. Cathodic deposition rates can be obtained by analysis of the concentration of silver in the cell at various time intervals under no-flow (static) conditions. This deposition rate is a function of the instantaneous concentration and the generation rate, which can be considered constant.

It was not practical to measure deposition rates with the original prototype cells because of the small internal volume. Limiting concentrations are reached relatively rapidly, the total weight of silver in the available sample precludes accurate analyses, and, since the overall water volume required for test purposes is more than double the true internal cell volume, diffusion of silver introduces considerable error.

Such rates were measured with the large cell (Prototype Cell 150) in which the overall volume of water analyzed did not differ appreciably from the true internal cell volume. The cell was operated under static conditions for various periods of time at different current levels, and the water in the cell was analyzed for total silver.

Some changes in current were observed as the concentration increased, but these changes were considered negligible for practical purposes. At high operating currents (30 to 40 μa), it was necessary to break up concentration gradients and disperse the silver ions by vibrating the cell.

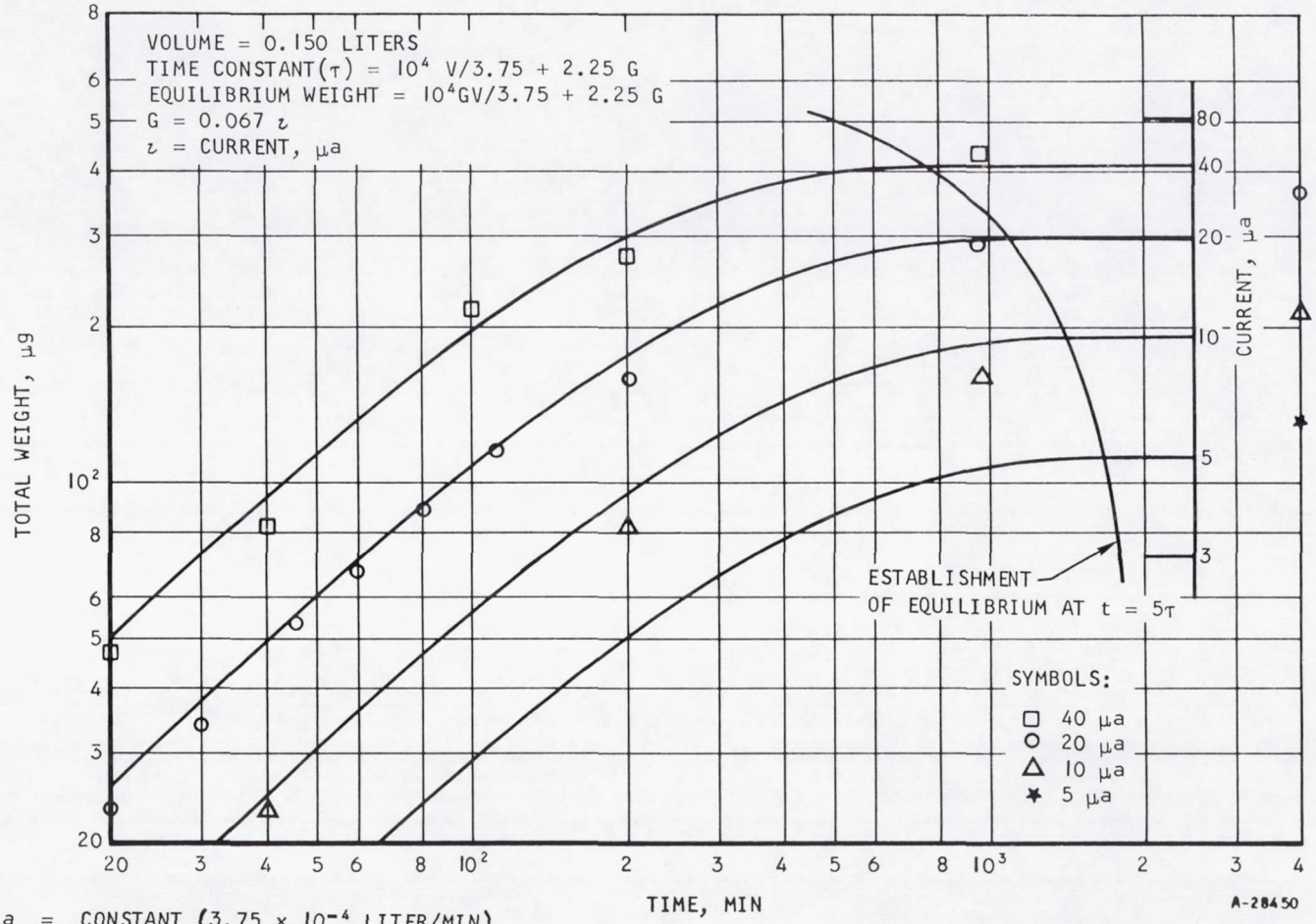
Data obtained are plotted in Figure 4-6.* These data were correlated by determining the constants in the equation

$$\frac{dC}{dt} = \frac{I}{V}\left[G - FC - C(a + bG)\right]$$

which yields upon integration,

$$C = \frac{G}{F + a + bG}\left\{1 - \exp\left[-\left(\frac{F + a + bG}{V}\right)t\right\}\right.$$

*Nomenclature also is presented in Figure 4-6.



a = CONSTANT (3.75×10^{-4} LITER/MIN)
b = CONSTANT (2.25×10^{-4} LITER/μGM)
C = CONCENTRATION (μG/LITER = PPB)
F = FLOW RATE (LITERS/MIN)
G = GENERATION RATE (μGM/MIN)
i = CURRENT FLOW (μa)
t = TIME (MINUTES)
V = VOLUME (LITER)
τ = TIME CONSTANT (TO ESTABLISH EQUILIBRIUM)

Figure 4-6. Establishment of Equilibrium Between Generation and Deposition Rates Under Static (No-Flow) Conditions

The time constant

$$\tau = \frac{V}{F + a + bG}$$

is a function of the cell volume. Final equilibrium can be assumed to be established after five time constants. Note that in the absence of cathodic deposition the time constant reduces to the cell residence time. The concentration at equilibrium ($\tau = 5$) is independent of the cell volume

$$C_{eq} = \frac{G}{F + a + bG}$$

In the above equations, the constant a is not defined if the generation rate is zero or if the initial concentration is other than zero in the absence of an electric potential.

The curves in Figure 4-6 represent the establishment of equilibrium at various current levels if the constants a and b are defined as

$$a = 3.75 \times 10^{-4} \text{ liter/min}$$

$$b = 2.25 \times 10^{-4} \text{ liter/}\mu g$$

The curves so obtained fit the experimental data relatively closely being somewhat raised by the final equilibrium values. It is probable that the constants could be more precisely defined by reducing experimental error but this additional refinement is not considered necessary.

Assuming variations in cell geometry have no appreciable influence, the equations are applicable to the small (3-ml) silver-ion generators. The length of time required to establish equilibrium is directly proportional to the volume, but the final concentration is dependent only on the flow rate and generation rate.

Attempts were made to verify predicted concentrations in the small cell under static conditions so that the validity of the constants for a different geometry could be established. Results (see Table 4-3) indicated either that diffusion into the surrounding (nongenerating) regions was sufficiently rapid to preclude accurate measurements or else that the constants are a function of the anode-to-cathode spacing.

It is possible to estimate, however, the total weight of silver which could accrue in the small cell, assuming the water flow were shut down while the cell continued to generate silver ions. At 10 μa, a final concentration of 1267 ppb will be obtained. This is readily obtained from Figure 4-6 by dividing the equilibrium weight (190 μg) by the volume (0.150 liter) and noting that the final concentration is not a function of cell volume. If the cell volume were 3 ml, representative of the small cells, only 75 ml of water would be required to dilute this concentration to 50 ppb. Even if the final concentration or the volume of solution were three to five times this value,

TABLE 4-3

SILVER ION CONCENTRATIONS OBTAINED IN SMALL PROTOTYPE CELLS UNDER STATIC CONDITIONS

Date	Static Generation Time (min)	Current (μa)	Total Volume Obtained for Analysis (ml)	Concentration (ppb)		
				By Analysis of Total Volume	(1) Adjusted to Cell Volume (3 ml)	Predicted From Deposition Rates
10-31-66	30	22.3	11.6	1322	5100	2100
1-6-67	30	10.2	7.5	547	1370	1290
1-10-67	30	10.0	7.6	1546	3910	1270

(1) Values adjusted by the ratio of actual liquid volume obtained to internal cell volume (3 ml)

the total weight of silver ions would still be insufficient to cause any health hazard on consumption. It is not necessary, therefore, to shut off the silver-ion generators during temporary no-flow conditions, since downstream dilution will occur on start-up.

It is also possible to calculate theoretical efficiencies at various flow rates and current levels from these equations. It is assumed that equilibrium is established in the cell at all times under flow conditions and that no concentration gradients exist. Such theoretical efficiencies are shown in Figure 4-7. Actual efficiencies are in good agreement with the theoretical predictions. For practical engineering purposes, the predicted values, which represent the correlation of a large number of analyses, can be considered more precise than scattered analyses obtained for individual cells operated at various currents and flow rates.

SYSTEM PERFORMANCE

System Losses

1. Reduction in Aluminum Tubing

When installed, the water sterilization cells become part of an aluminum (1/4-in. OD) tubing system. The tubes are treated by an Alumigold process, essentially a chromate treatment. Such tubing has a very high area-to-volume ratio (8.3 cm^2 per cm^3 for 0.030 wall) and losses by adsorption or by reduction on aluminum surfaces in a few feet of tube length can become excessive unless proper precautions are observed.

Losses through tubing of various lengths and treatment were examined using the experimental apparatus shown in Figures 4-8 and 4-9. Initial evaluation indicated that losses could exceed 90 percent within 30 ft of tubing length. The chromate treatment did not appear to enhance throughput efficiency. Such losses would severely limit the application of the water sterilization units and necessitate the precise location of the cell in the system. Furthermore, the current level would have to be increased to offset losses, and no control could be maintained on the silver ion concentration in the system. At low flow rates, all the silver could be lost before it would be of any value as a bactericide.

Various passivation methods for pretreatment of the tubes were examined. To be of any value, a pretreatment procedure had to be applicable to the assembled system and not injurious to metals, gaskets, or polymeric materials in the system. Initially, various weak oxidants such as nitric acid, phosphoric acid, and sodium peroxide solutions were employed, but appeared to have little effect. Inadvertently, it was discovered that if the tubes were filled with water and then sealed off, the water soak provided an excellent method of passivation. Throughput efficiencies increased to 85 to 95 percent and silver-ion losses were negligible. The procedure appears equally applicable to aluminum tubes in an as-received condition or those treated with chromate solutions such as Iridite or Alumigold.

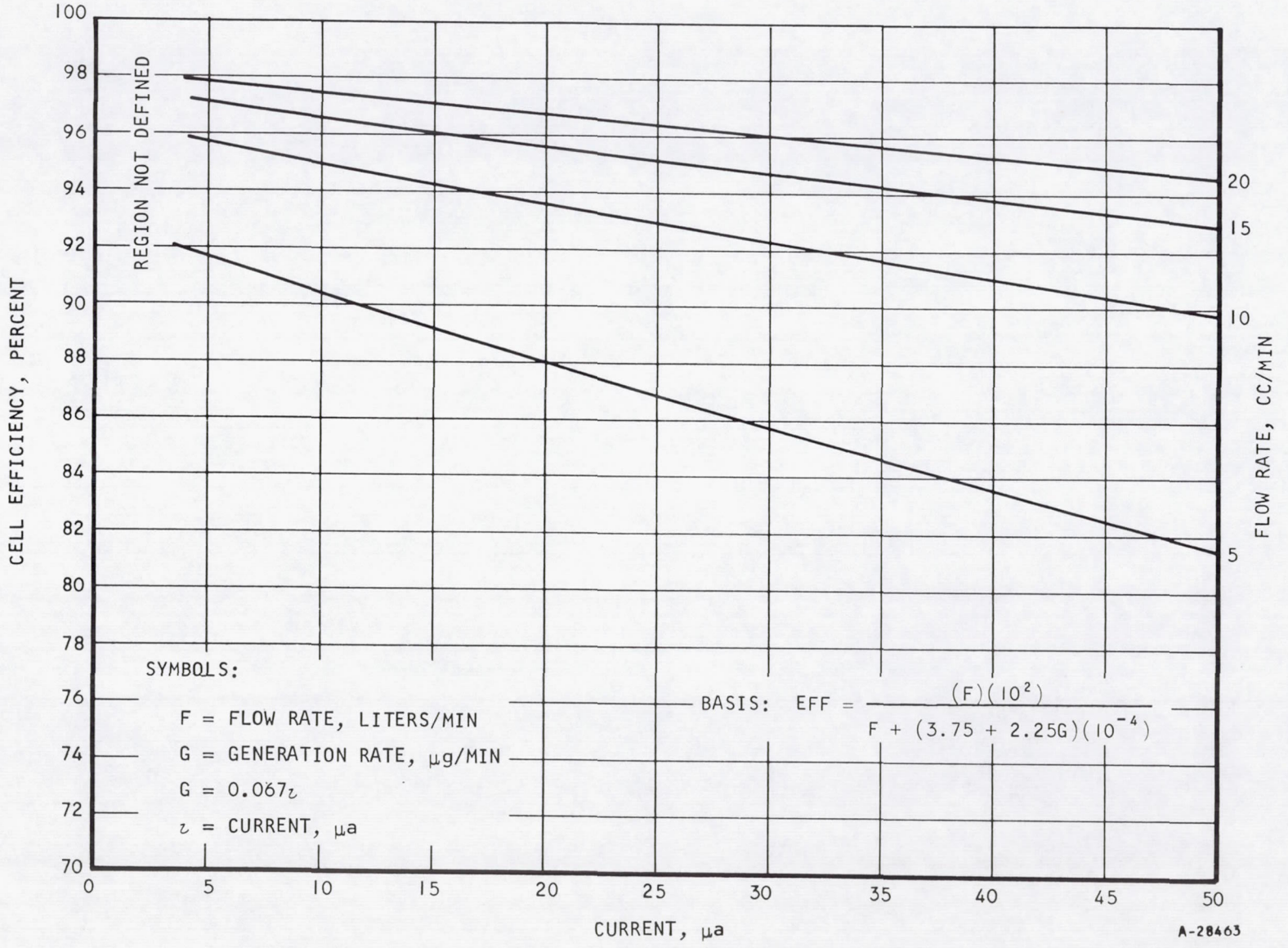


Figure 4-7. Predicted Efficiencies Under Flow Conditions

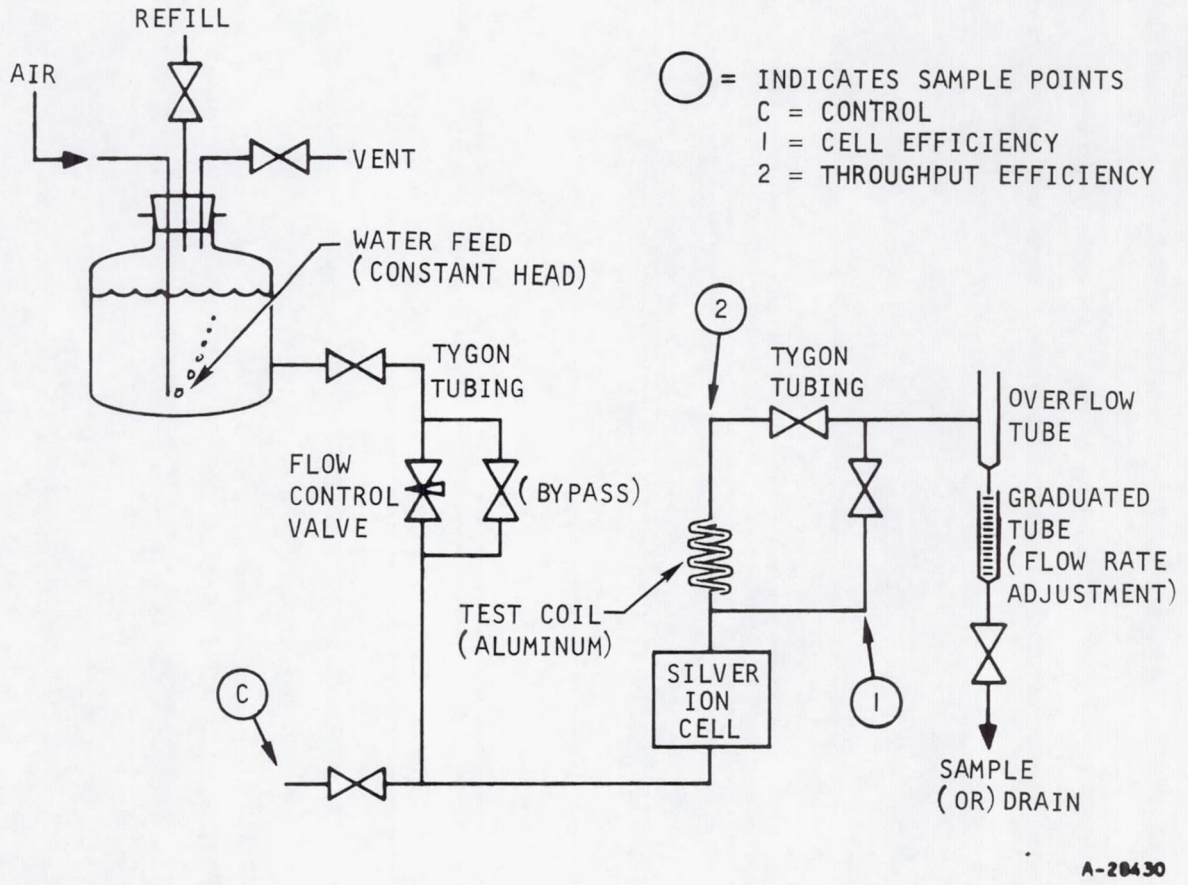


Figure 4-8. Schematic of Test Apparatus for Determination of Cell and Throughput Efficiencies

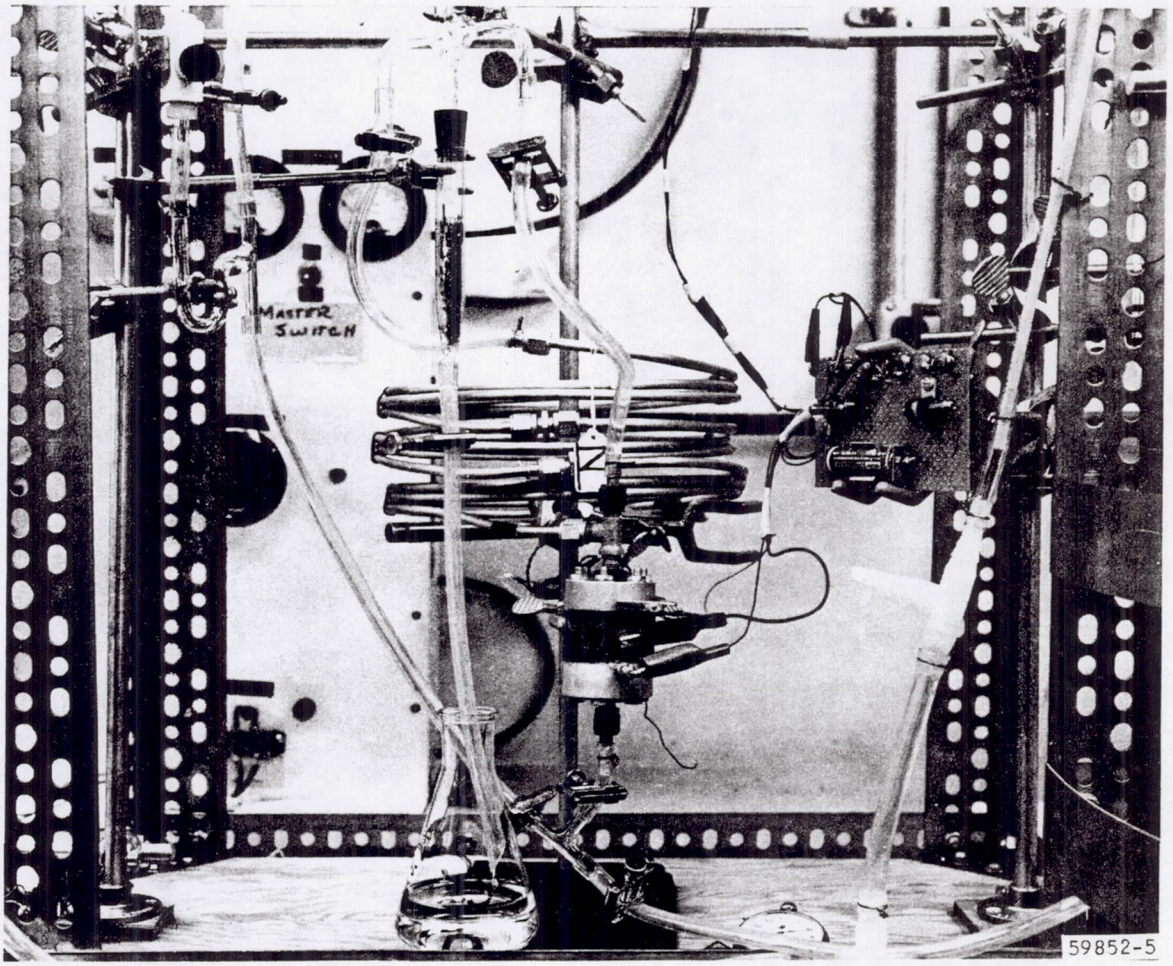


Figure 4-9. View of Test Apparatus Used to Evaluate Cell and Throughput Efficiencies

If the system is filled with water and sealed off for four to seven days, a pressure buildup will occur. This pressure rise occurs from the formation of hydrogen, probably from the reaction of aluminum with water. Once this reaction has occurred, the aluminum apparently loses the ability to reduce silver. In essence, the active sites are oxidized. More than one treatment may be necessary before hydrogen is observed. Initial pressurization may be beneficial, but has not as yet been thoroughly evaluated.

Excessive oxidation of the aluminum surface (anodizing) could lead to adsorption of silver ions rather than reduction. Aluminum oxides are excellent adsorbents and very little area would be required for the small amounts of silver in solution. The effects of the above water treatment on anodized surfaces was not tested, but preliminary tests indicated that such adsorption may take place. Additional investigations of the reactions involved were beyond the scope of this task and were not conducted.

Throughput efficiencies for various tubes and treatment methods are given in Table 4-4. The increase in throughput efficiency by water treatment is shown in Figure 4-10. It should be pointed out that the original tubing used in an as-received condition underwent passivation during initial evaluation of the performance characteristics of the Polycarbonate cell. The tubing underwent several days of testing, during which period, although not sealed, it was left filled with stagnant water when not in use. The formation of bubbles in the water during these stagnant periods was noted, as was the gradual increase in throughput efficiency. The increased efficiency, however, was thought to be due to other factors that were being evaluated at the time.

2. Adsorption on Polyisoprene Bladder (Water Tanks)

The polyisoprene bladder contains sulfur compounds and was found to adsorb silver from solution in minute quantities. Analyses of the bladder material and water solutions in contact with the bladder were made by atomic adsorption techniques (see Table 4-5 and Analytical Methods and Procedures). Analyses of effluent from the waste water tank of the simulated Apollo system were similarly made after equilibrium had been obtained. Results of these analyses are subsequently reported (Table 4-6). Loss of silver within the Apollo water tanks by adsorption on the bladder will be a function of the rate at which such tanks are used and the stagnation periods between use. Effective sterilization was achieved during continuous testing, however, (see Bacteriological Tests) and such adsorption should not be detrimental.

Continuous System

Shortly after the first cells were fabricated, a continuous system was put on stream to obtain reliability data. (Figures 4-11 and 4-12.) Initially, Prototype Cell B was used to supply silver ions, but the modified polycarbonate cell was installed after two months. The effluent of the cell was fed through 4 ft of anodized aluminum tube after which it passed through a 50-ft coil of aluminum tube (Tube Coil A) before entering an aluminum hold tank (20-l volume, area-to-volume ratio = 0.19). At a flow rate of 7 cc per min, the residence time in the hold tank was about 50 hr. A current of 10 μa was used for the first four months, but this was changed to 20 μa after initial evaluation of microbial contamination of the tank. (See Section 5.)

TABLE 4-4

SILVER-ION THROUGHPUT EFFICIENCIES FOR ALUMINUM TUBING SUBJECTED TO VARIOUS TREATMENTS

Coil Designation	Material Type	Length Tested, ft	Treatment and Remarks	Test Date, 66-67	Flow Rate, cc/min	Current, μa	Average Throughput Efficiency, percent
A	Mixture 6061 plus 5052	50	As received (acetone rinse)	7-15 to 7-26	5 to 15	10 to 15	13 → 85
			Continuous system (permanent installation)	8-23 to 2-24	7	10 to 20	70-85
B	5050	50	As received - Tested on continuous system	8-2	7	10	0
			Iridited - (Not rotated)	8-9	7	10	0
			Treated with $AgNO_3$-steamed-desorption noted	8-11	7	10	>100
			Stripped and anodized	9-1	10	12	40
C	6061-0	30	Anodized and steamed	8-18	7 and 10	10 and 20	50 → 25
			Steamed (2nd time)	8-19	10	20	20
			Phosphoric acid treatment (pH = 3.8)	8-23	10	12	15
D	6061-0	30	As received (acetone rinse)	8-25	10	12	25 → 8
			Stagnant water (set inactive in system for 7 days)	9-2	10	12	90
E	6061-0	30	Alumigold (not rotated)	9-9	10	12	10
		16	Tested at midpoint of coil	9-12	10	12	25
		30	Effluent of continuous system (Input = 50 ppb)	9-14	7	10	0
F-1	6061-0	7.5	Alumigold (not rotated)	9-15	10	12	55
			Stagnant water (capped off for 7 days - gas noted)	10-7	10	12	80
			(Used in simulated system as connection between pump and silver ion cell)				
F-2	6061-0	7.5	Alumigold (not rotated) - Nitric acid treatment (pH = 3.2)	9-19	10	12	60
F-3	6061-0	7.5	Alumigold (rotated) - Not otherwise treated (see L below)	9-20	10	12	35 → 55
			Stagnant water (capped off for 5 days - No gas)	10-3	10	12	60 → 80
			Stagnant 0.01M Na_2SO_4 (capped off for 2 days)	10-6	10	12	65
			Stagnant water (capped off for 42 days)		Not Tested		
F-4	6061-0	7.5	Stripped - Not otherwise treated	9-26	10	12	60
			Stagnant water (capped off for 6 days - No gas noted)	10-6	10	12	92-100
G	6061-0 (1/2 in. dia)	10.5	Not tested - 1/2 in. tube	---	---	---	---
H (Used in simulated system between cyclic accumulator and coil 3)	6061-0	7	Alumigold (rotated) - Capped off for 4 days - Gas noted	9-26	10	12	90 to 95
			(Initial flush for 2 hr with distilled water)	NOTE: First direct evidence that stagnant water treatment was beneficial			
			(2nd gas build-up noted when tube recapped with stagnant water for additional 13 days)				

TABLE 4-4 (Continued)

Coil Designation	Material Type	Length Tested, ft	Treatment and Remarks	Test Date, 66-67	Flow Rate, cc/min	Current, μa	Average Throughput Efficiency, percent
J	6061-0	7	Alumigold (rotated) - Capped off for 4 days - Gas noted (Treated with 0.001 m $Na_2 O_2$ after treatment above) (Used in simulated Apollo system as connection to waste tank outlet)	9-28	10	12	90 to 95
K	6061-0	42	Alumigold (rotated) - Treated with 0.001 m $Na_2 O_2$	9-28	10	12	8 to 20
L	6061-0	7	Alumigold (rotated) - Not otherwise treated (see F-3)	9-28	10	12	80 → 60
			Capped off with distilled H_2O for 7 days - No gas noted	10-7	10	12	86 to 89
M	6061-0	7	Alumigold (rotated) - Capped off with H_2O under 25 psig N_2 pressure - Pressure rise to 43 psig	10-3	10	12	87 to 95
N	6061-0	21	Alumigold (rotated) - Capped off with water - Gas noted	10-4	10	12	50
			Capped off with water for 4 months - Gas noted	2-27	6	10	85 → 100
P	6061-0	8	Alumigold (rotated) - Flushed with H_2O- dried with N_2 (Capped off 4 days without water)	10-15	10	12	63
R	6061-0	8	Alumigold (rotated) - Flushed with H_2O- Capped off 4 days with distilled water - No gas noted	10-15	10	12	67
S	6061-0	8	Alumigold (rotated) - Flushed with H_2O- Remained open with H_2O and flushed every 24 hr for 4 days (no gas bubbles noted)	10-15	10	12	64
T	6061-0	8	Alumigold (rotated) - Flushed with H_2O - Purged with continuous water flow of 2 cc/min for 4 days	10-15	10	12	63

NOTE: Aluminum tubes fashioned in coil form required rotation during treatment to ensure the inside of the coil was thoroughly treated with chromate solution.

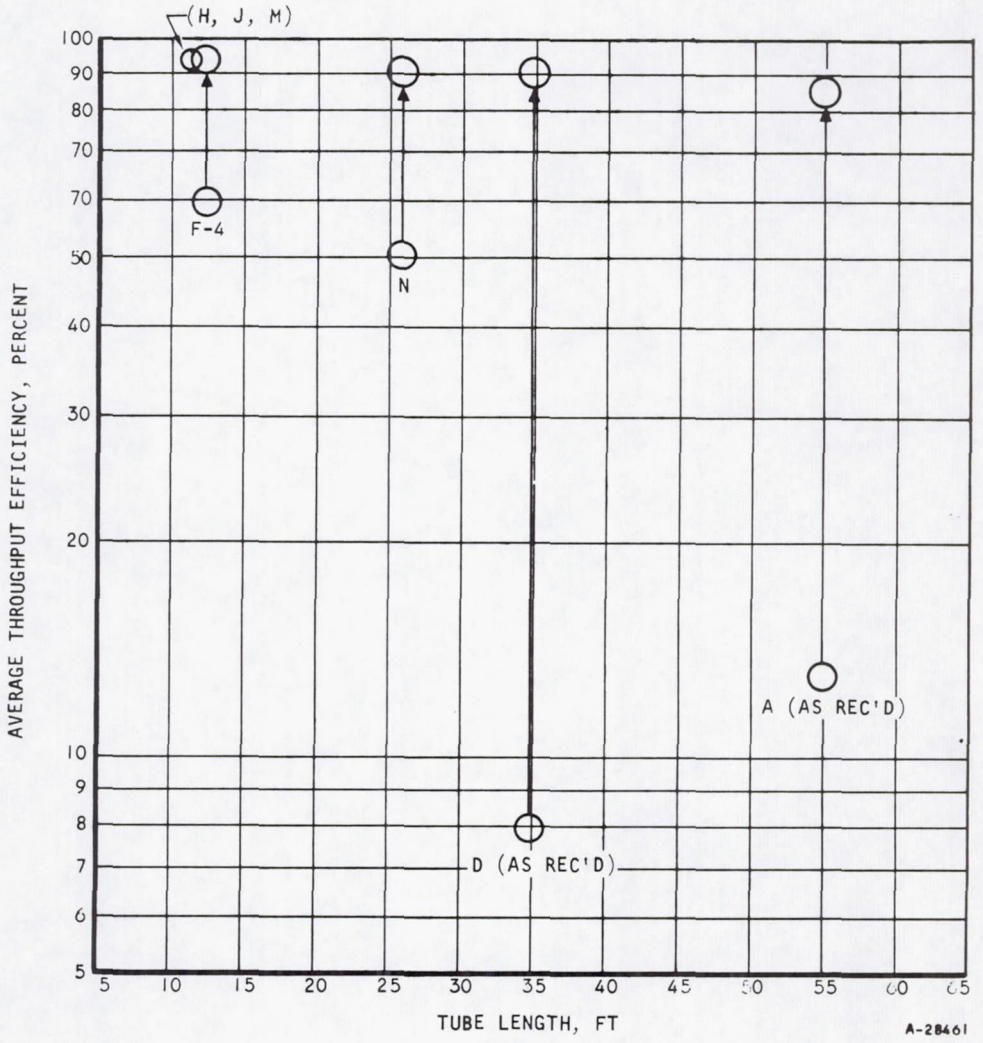


Figure 4-10. Effect of Stagnant Water Treatment on Throughput Efficiency of Aluminum Tubing

TABLE 4-5

ADSORPTION OF SILVER BY POLYISOPRENE BLADDER
(ANALYSIS BY ATOMIC ADSORPTION)

Date	Silver Solution				Polyisoprene Bladder			
	Initial Concentration (ppb)	Amount Used (liter)	Weight of Silver (μg) Initial	Weight of Silver (μg) Final (By analysis)	Exposed Area (cm^2)	Exposure Time (hour)	Adsorbed Silver Total (μg)	Adsorbed Silver μg/cm^2
10-17-66	1×10^5	0.9	9×10^4	-	400	53	7.6×10^3	19
10-19-66	100	0.9	90	36.4	400	21	60	0.15

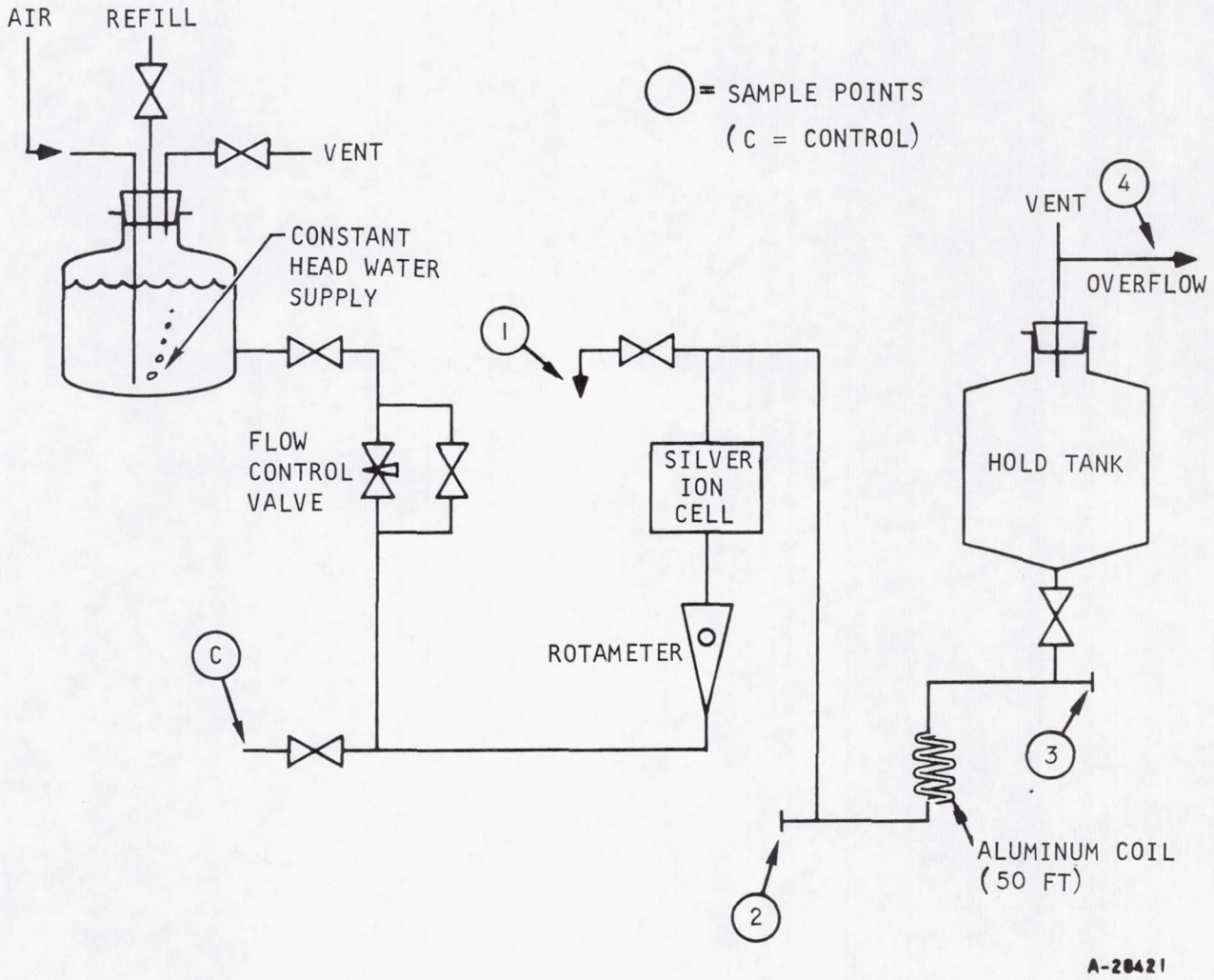
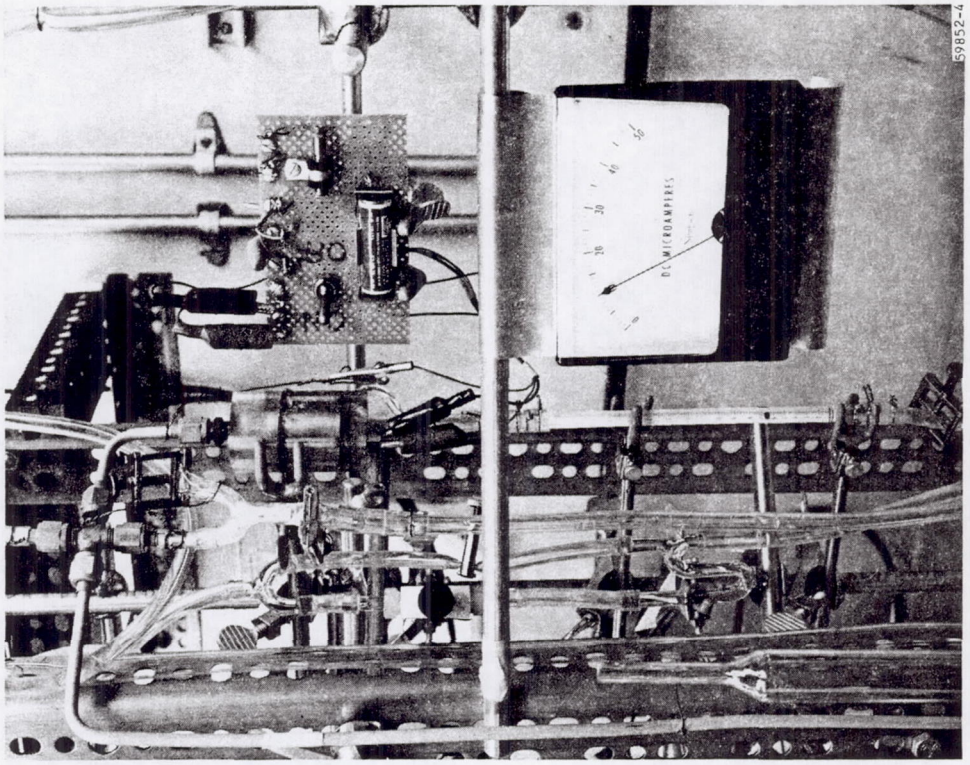


Figure 4-11. Schematic of Continuous Flow System for Reliability Tests



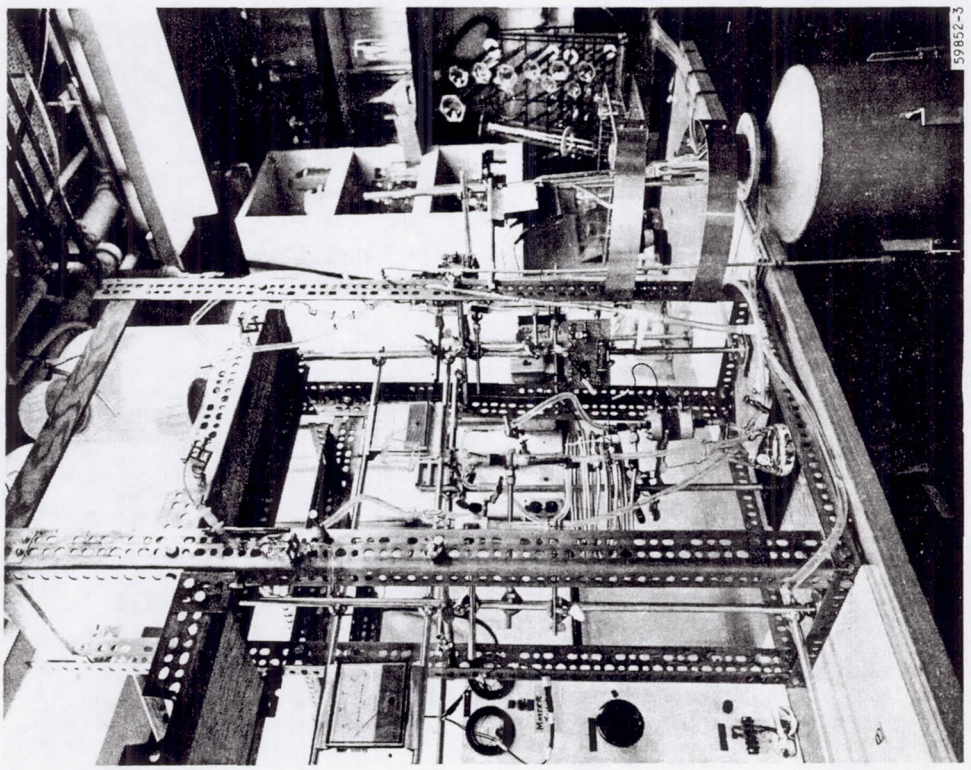


Figure 4-12. Views of Continuous-Flow System

Overall throughput efficiency was about 50 to 60 percent of the theoretical. A concentration of 50 ppb could be obtained at the tank outlet with a theoretical input of 90 to 100 ppb. Flow rates could not be precisely maintained, and, since the time required to insure equilibration in the tank was in excess of a week (10 days at 7 cc per min), the concentration in the tank effluent could not be directly correlated to the theoretical concentration at the time the sample was taken.

The bulk of the silver ion losses appeared to be obtained initially in the cell (about 84 to 90 percent efficient) or through the tubing (75- to 85-percent efficient). Losses through the hold tank having a smaller area-to-volume ratio were about 10 percent of the input.

This system was not used for bacteriological testing, its value being restricted solely to long-term reliability tests. The polycarbonate cell shorted after about three months of operation (five months of testing). This was traced to cell design and has previously been discussed. Such shorting will not occur in the prototype cells.

The system operated overall for a total of eight months. The high current (20 μa) used for the last four months of operation would not be necessary for flight units, but it provided an additional measure of cell reliability under extreme operating conditions.

Simulated Apollo Waste Water System

A more complete set-up was built to simulate conditions that could be expected within the Apollo waste water system. This system is the more complicated and is subject to interrupted and discontinuous water flows. No problems would be expected in the potable water system that are not also present in the waste water system.

In the waste water system, a known source of bacterial contamination and growth is the suit heat exchanger and cyclic accumulators. Condensed moisture (sweat) is withdrawn from the heat exchanger by the cyclic accumulator, from which it passes into the rest of the waste water system, including the glycol evaporators and the waste water tank. Flow rates vary with physical activity, but are generally low, averaging approximately 3 ml per min. The cyclic accumulator (135 cc capacity) discharges at 10-min intervals with approximately 2 min required for a pressure drop after discharge before suction resumes.

By interposing a silver-ion cell between the heat exchanger and the cyclic accumulator, effective sterilization of bacteria cultures withdrawn from the heat exchanger can be achieved at the most uniform flow rate. The residence time in the cyclic accumulator also assures maximum kill before the water is moved into the remainder of the system. If the sterilization unit were located downstream of the accumulator, a uniform silver-ion concentration could never be obtained with a small cell, because of the rapid discharge of a large liquid volume.

The simulated system (Figures 4-13 and 4-14) used an Apollo waste water tank and cyclic accumulator connected by two sections of aluminum tubing (Alumigold treated, 14 ft total length) which had previously been subjected to stagnant water treatment and would effectively pass 90 percent or more of the silver ions. Metals such as stainless steel were incorporated so that any adverse effect of various metals that might be encountered in actual systems would be present in the simulated system. The heat exchanger output flow was simulated by a diaphragm pump (Lapp Pulsafeeder, Microflo Model LS-5). The pump flow could be adjusted between 1 and 65 ml per min and precisely maintained at the desired rate. Distilled water was fed to the pump from a polyethylene carboy (13-gal), which was agitated when inoculated with bacteria.

The cyclic accumulator and the waste water tank require a gas pressure for operation of diaphragms and bladders. Tank nitrogen was used as a pressure source. The tank bladder was operated at a pressure of 16 to 17 psig and the cyclic accumulator at 50 psig. Appropriate relief valves were incorporated to avoid over-pressurization from either the pump or the nitrogen supply. Since only one water opening is available in the waste water tank, the inlet and the exit are identical. The tank was mounted on a scale, so that the weight of water in it at any time could be read. Overflow from the tank during discharge was regulated by a control valve and timer and could be discharged into a carboy or drain as required. The assembly could operate continuously with no maintenance except for water and nitrogen make-up.

A control panel incorporating two timers (Flexopulse, Eagle Signal Corporation 20-min and 20-hr dials) and related switches could be programmed in various ways so as to control the fill and dump cycles. A second set of instantaneous reset time delay timers (Industrial Timer Corp, Series MTD, 15 min and 3 min) was used to control the delay period between discharge of the cyclic accumulator and restart of the pump cycle. During discharge of the waste water tank, other operations were discontinued. A schematic of the control panel circuit is given as Figure 4-15.

Tank input, as determined by pump settings and on-off periods, was balanced against discharge, as determined by the control valve setting and time for discharge, so that the waste water tank was normally maintained between 1/4 and 3/4 full. The time at which the tank discharged could be controlled manually--i.e., at 24-hr intervals, if required, but during normal automatic operation discharge occurred at 16-hr intervals. Using these extended periods, better equilibrium could be obtained in the tank. Since tank capacity was 58 lb of water, it was necessary to feed at a rate of 8 cc per min to ensure a sufficient supply of water to the tank in the 16-hr interval.

A normal cycle (switch arrangement No. I) consisted of the following:

a. The pump operated for 8 min (Valves S1, S2, S3 closed).

b. The pump shut off. Valves S1 and S3 opened for 10 sec, pressurizing the cyclic accumulator to 50 psig and dumping the contents to the waste water tank.

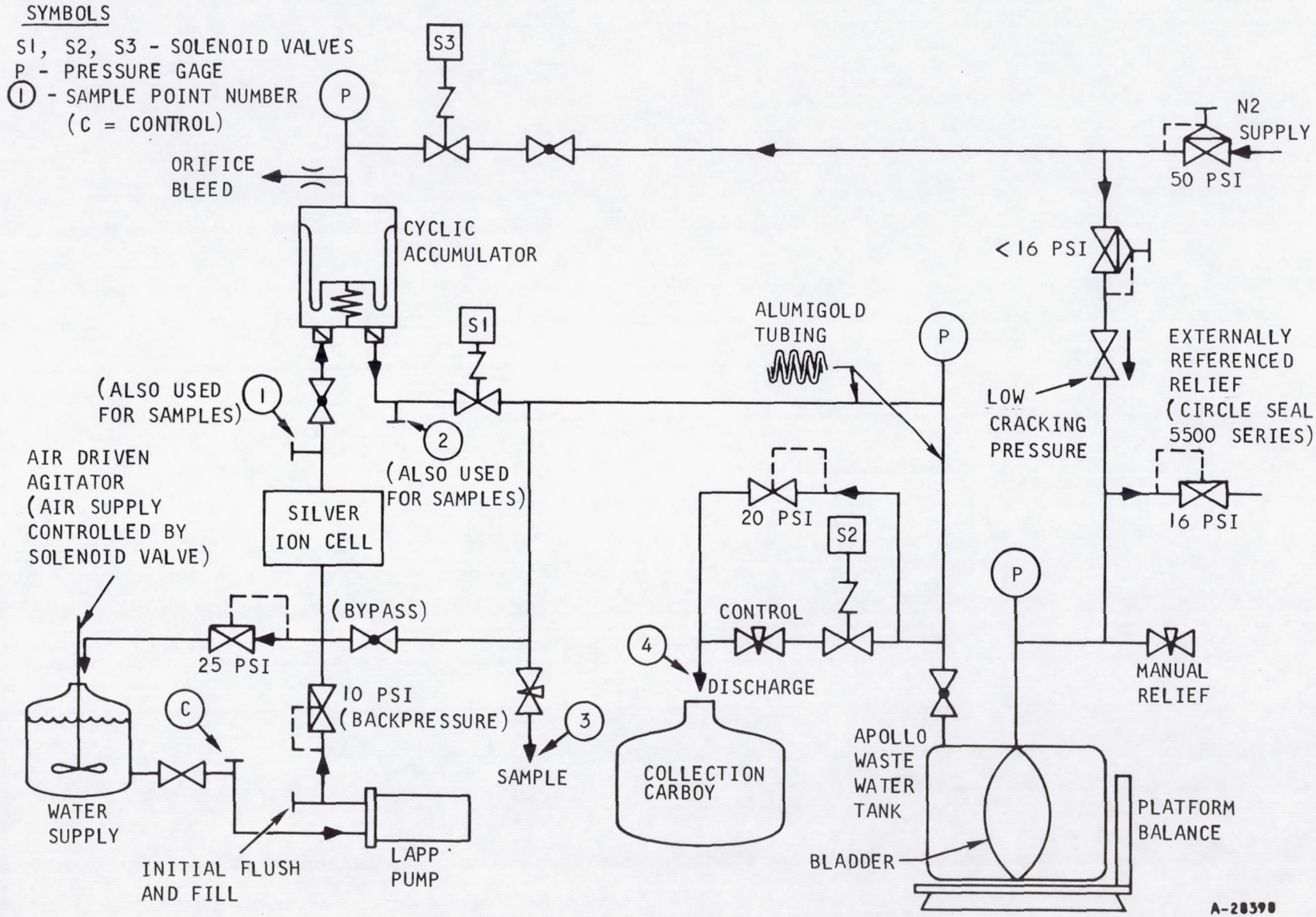
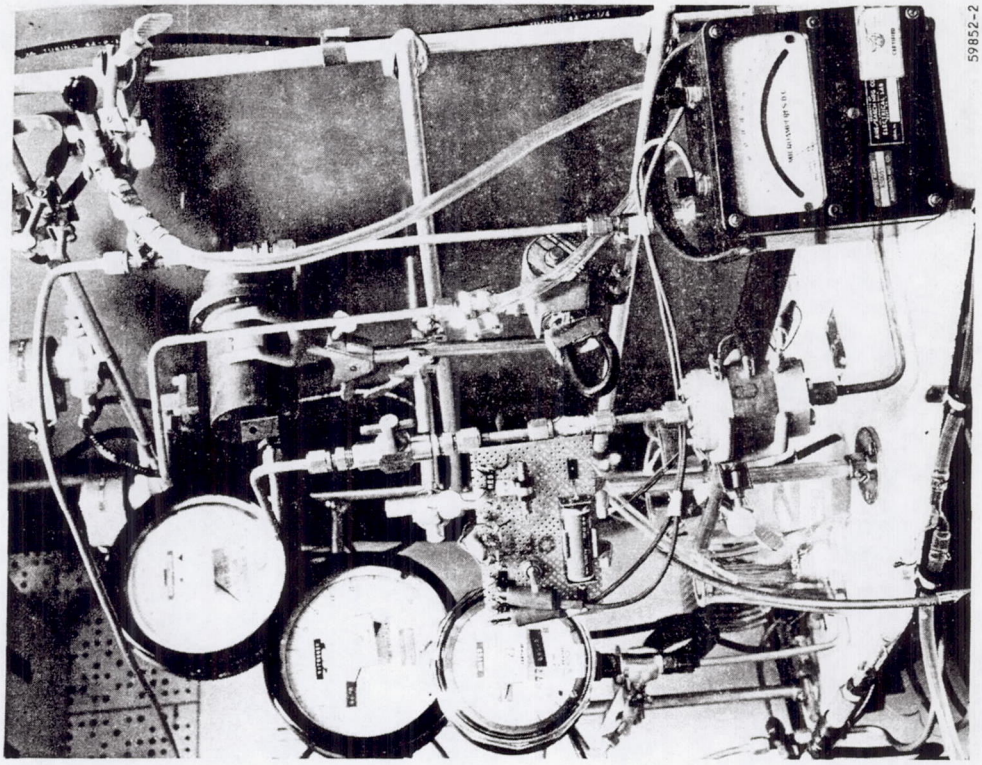


Figure 4-13. Schematic of Simulated Apollo Waste Water System



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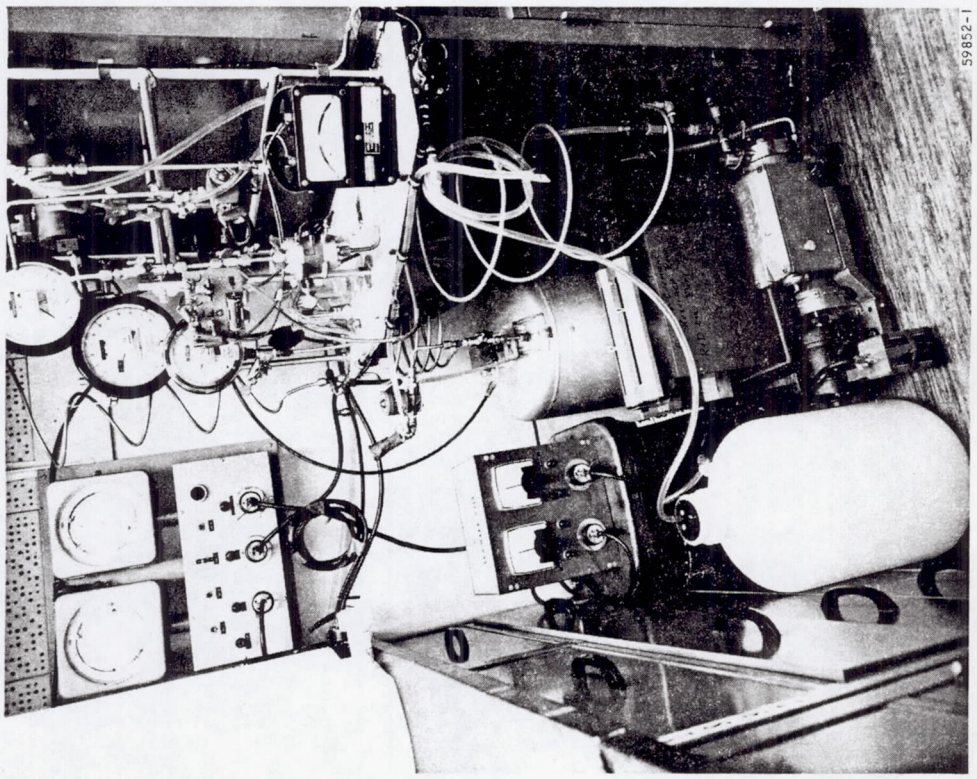

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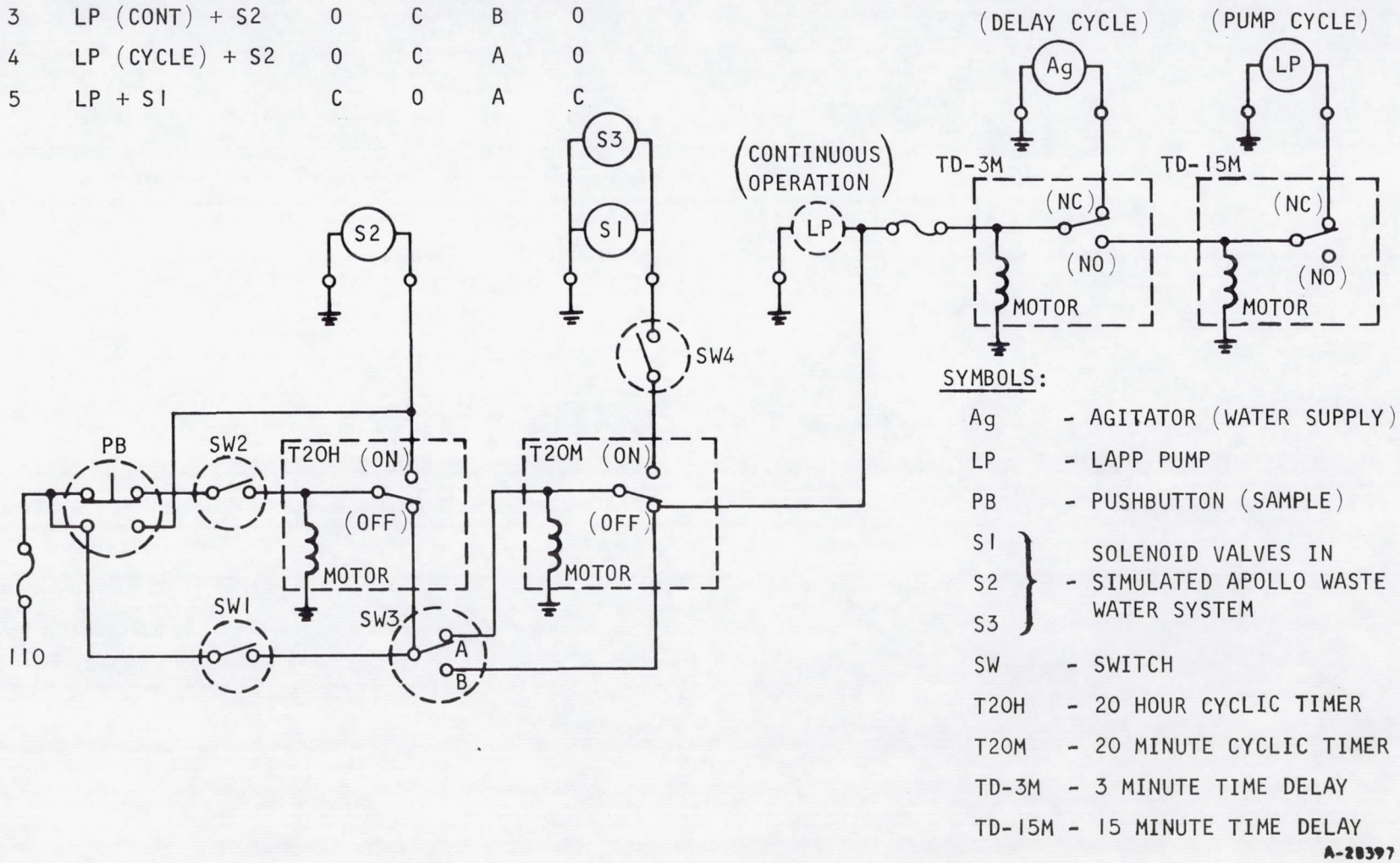
Figure 4-14. Simulated Apollo Waste Water System

SWITCH ARRANGEMENTS

NO.	CONTROL	SW1	SW2	SW3	SW4
1	LP + S2 + S1	O	C	A	C
2	LP (CONTINUOUS)	C	O	B	O
3	LP (CONT) + S2	O	C	B	O
4	LP (CYCLE) + S2	O	C	A	O
5	LP + S1	C	O	A	C



SYMBOLS:

Ag	- AGITATOR (WATER SUPPLY)
LP	- LAPP PUMP
PB	- PUSHBUTTON (SAMPLE)
S1, S2, S3	- SOLENOID VALVES IN SIMULATED APOLLO WASTE WATER SYSTEM
SW	- SWITCH
T20H	- 20 HOUR CYCLIC TIMER
T20M	- 20 MINUTE CYCLIC TIMER
TD-3M	- 3 MINUTE TIME DELAY
TD-15M	- 15 MINUTE TIME DELAY

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Figure 4-15. Wiring Schematic for Operational Control of the Simulated Apollo Waste Water System

c. Valves S1 and S3 closed. Gas bled out of the cyclic accumulator. The time delay relay started to function to delay pump action for an additional 110 seconds. Supply tank agitator was actuated during this period if necessary.

d. The pump started. Supply tank agitator shut off. The pump could be operated for less than 8 min if necessary without upsetting the 10 minute overall cycle.

e. Upon waste water tank discharge solenoid valve S2 opened. Nitrogen pressure was available to the tank bladder at all times and forced water through the discharge control valve. Discharge occurred for 16 minutes. Power to the timers (other than T20H) was off so the remainder of the system shut down.

f. Upon completion of discharge, power was restored. The 20 minute Flexopluse Timer (T20M) commenced operation at the point of interruption. The time delay timer (TD3M) had reset so pump action was delayed an additional 110 seconds on the first start cycle after discharge.

g. A tank sample could be obtained at any time using the pushbutton provided. This pushbutton was also used to set the discharge control valve.

Performance Analysis of Simulated Apollo System

The system was initially operated with silver ion generation at various current levels so as to determine losses within the system. Samples were withdrawn at various sample points (See Figure 4-13). Results are shown in Table 4-6. Analyses of effluent from the tank could not be made using the colorimetric method, since the bladder releases a sulfur compound (thiuram) into the water in trace amounts which affects the color change (See Analyses). Such analyses were obtained by atomic adsorption from an independent laboratory (Truesdail Laboratories, Inc., Los Angeles).

Recognizing the cyclic accumulator introduces rapid flow rates followed by stagnation periods of ten minutes in the aluminum tubing (14 ft) downstream of the accumulator before discharging to the tank the overall losses did not appear excessive.

The system was primarily used to test the bactericidal effects of the silver on cultures of E coli and S aureus. Initially bacteriological tests were made using only the cyclic accumulator without passing the bacteria into the hold tank. Continuous tests were then made with both types of bacteria by passing inoculated water through the water sterilization cell, and cyclic accumulator and then into the hold tank. These tests lasted six days during which time the hold tank was discharged at 24-hour intervals and the effluent was sampled to determine residual contamination. Results of such tests are further presented (See Bacteriological Tests) but essentially complete kill was observed

Bacteriological tests of inoculated sweat condensate were also made using only the cyclic accumulator since insufficient sweat condensate was available to fill the tank.

TABLE 4-6

TYPICAL SILVER-ION ANALYSES AT VARIOUS SAMPLE POINTS
IN THE SIMULATED APOLLO WASTE WATER SYSTEM

Date	Sample Point	Pump Cycle			Average Flow (cc/min)	Current (μa)	Analyses			Remarks
		Off (min)	On (min)	Total (min)			Measured (ppb)	Theory (ppb)	Eff (%)	
1-4	1	0.17	9.83	10	9.4	17.5	100	124	81	
1-19	1	2	8	10	8.2	10.9	68	89	76	Inoculated with E. coli
1-19	1	2	8	10	8.2	11.0	72	90	80	Inoculated with E. coli
1-20	1	2	8	10	8.2	6.7	34	55	62	Inoculated with E. coli
1-4	2	0.17	9.83	10	9.3	17.5	89	127	70	
1-13	2	2	8	10	7.9	18.1	131	154	85	Inoculated with E. coli
1-19	2	2	8	10	8.2	10.9	58	89	65	
1-19	2	2	8	10	8.2	11.0	70	90	78	Inoculated with E. coli
1-20	2	2	8	10	8.2	6.7	35	55	64	Inoculated with E. coli
12-30	3	1	9	10	14.3	17.5	49	82	60	
12-30	3	1	9	10	14.2	17.5	51	82	62	
1-3	3	0.17	9.83	10	9.5	17.5	82	123	67	
1-3	3	0.17	9.83	10	9.5	17.5	84	123	68	
1-4	3	0.17	9.83	10	9.7	17.5	88	121	73	
1-4	3	0.17	9.83	10	9.6	17.5	85	122	70	
1-4	3	0.17	9.83	10	5.0	17.5	143	234	61	
1-4	3	0.17	9.83	10	4.3	17.5	155	272	57	
1-5	3	0.17	9.83	10	4.3	17.5	191	272	70	
1-10	3	2	8	10	7.3	17.0	115	156	74	
1-18	3	2	8	10	8.1	17.1	116	141	82	
1-10	4	2	8	10	7.0	17.0	85	163	52	Before entering tank
1-18	4	2	8	10	8.0	17.1	110	143	77	Concentration in tank by
1-30	4	2	8	10	8.2	6.6	17	54	32	atomic adsorption after equilibrium

SECTION 5

BACTERIOLOGICAL TESTS

INTRODUCTION

The second phase of this program, to determine the efficiency of silver ions in decontaminating the Apollo water system has been completed. During this phase, two microorganisms selected in the Phase I screening program, Escherichia coli and Staphylococcus aureus, were tested under additional simulated conditions. E. coli was chosen because of its relative sensitivity to silver and S. aureus because it is relatively resistant. E. coli, being very sensitive, served as an excellent test organism to examine silver under a variety of conditions, since brief periods of time would suffice for experiments. As data accumulated for E. coli, the more resistant S. aureus was tested, and thus presented anticipated extremes of sensitivity to the various conditions under which the silver-ion generators were studied. As shown in the Phase I report, a far more resistant organism is a sporulated bacillus--e.g., Bacillus subtilis var. niger. We have not included spores in this testing phase because anticipated levels are low and because the conversion of the spores into vegetative forms will result in an increased sensitivity to silver.

MATERIALS AND METHODS

The procedures for preparing test suspensions of the organisms and quantitation techniques are outlined in the screening report (Appendix). Two differential solid media were used: Mac Conkey agar (Difco) for E. coli and Tellurite-Glycine agar (Difco) for S. aureus.

Artificial sweat was made up in double strength solutions, then diluted with either sterile distilled water or silver solution. The resultant pH was usually 6.45. Oxygenation was performed as indicated in the screening report.

Sweat condensate was generated by circulating air at approximately 90°F around a test subject in an enclosed suit as he walked 2 to 4 mph on a treadmill. The moisture-laden air was then passed through a glycol heat exchanger, and the condensate was formed by cooling the air to a dew point of 35°F. Condensate was collected in sterile flasks and refrigerated until needed. Several samples of condensate were initially cooled in a dry ice chest until it was observed that carbon dioxide absorption reduced the pH to 5.3. When silver nitrate was added, small quantities of a concentrated solution were used.

When static bacteriological tests using silver nitrate were undertaken, the requisite amount of silver could be precisely measured into the sample by dilution of standard silver nitrate solutions. Electrolytically produced silver-ion concentrations in this report are given as both (1) theoretical silver concentrations based on the flow rate and amperage and (2) measured results using the dithizone colorimetric analytical procedure. Such analytical methods in the presence of bacteria are considered less accurate than analyses made for silver in distilled water only (see Analytical Methods and Procedures).

Therefore, kill-rate data for electrolytically produced silver are best compared on a theoretical basis.

RESULTS AND DISCUSSION

Effect of Artificial Sweat on Efficacy of Ionized Silver as a Bactericidal Agent

This phase of the program was to use a human sweat simulant as suggested by NASA, and to determine experimentally its effect, if any, on the killing properties of ionized silver ($AgNO_3$). For the purpose of this study, an artificial sweat was put together on the formula obtained from NASA report N66-19642. Table 5-I gives the compounds and the amounts used to make the artificial sweat.

TABLE 5-I

ARTIFICIAL SWEAT FORMULA

Compound	mm/l
Sodium chloride	2.106
Sodium lactate	1.568 (1.2 cc)
Potassium chloride	0.448
Ammonium chloride	0.161
Urea	0.060
Distilled water	1000 cc

Escherichia coli B was used as a test organism throughout these studies, since it is extremely sensitive to ionized silver and is therefore an excellent indicator for any untoward effects of the artificial sweat on the efficacy of the ionized silver.

Various concentrations of $AgNO_3$ were used, ranging from 50 ppb to 800 ppb. The methodology and procedures used for the actual testing, plating, quantitation, and cultural parameters are discussed in the screening report (Appendix). All tests were run at room temperature. The artificial sweat was made up in double-strength solutions and then diluted with either sterile distilled water or silver solution. Sterilization of the distilled water used to make up the artificial sweat solution assured sterility of the preparation, even when the components added were not previously sterilized.

Table 5-2 shows that silver concentrations above 100 ppb were not any more effective than 100 ppb, probably due to binding of the silver by various components of the artificial sweat. Comparison of the percent kill in artificial sweat with the percent kill obtained at similar pH values in distilled water shows that the latter diluent is considerably more effective as a suspending medium for the bactericidal properties of ionized silver.

TABLE 5-2

SILVER SENSITIVITY OF E. COLI IN ARTIFICIAL SWEAT
(STATIC TESTS WITH SILVER NITRATE)

Date (1966-67)	Conditions of Test	Viable Count Reduction					
		With Silver			Control		
		Time (Min)	Viable Count (org/cc)	Kill (%)	Time (Min)	Viable Count (org/cc)	Kill (%)
---	50 ppb pH = 6.14	0 120 240	7.9×10^5 6.8×10^5 4.4×10^5	--- 14 44	0 120 240	9.6×10^5 7.7×10^5 7.3×10^5	--- 20 24
---	Oxygenated Solution 50 ppb pH = 6.6	0 120 240	7.6×10^5 7.4×10^5 2.5×10^5	--- 2.6 67	0 120 240	8.4×10^5 9.6×10^5 1.05×10^6	--- --- ---
10-14	50 ppb pH = 6.5	0 120 240 360	9.1×10^5 8.5×10^5 4.7×10^5 2.04×10^5	--- 6.7 48.3 77.6	0 120 240 360	1.41×10^6 9.9×10^5 8.8×10^5 9.3×10^5	--- 29.8 44.7 34.2
10-19	100 ppb pH = 6.4	0 60 120 240	5.9×10^5 2.4×10^5 2.17×10^5 5.4×10^3	--- 59.3 63.2 99.084	0 60 120 240	6.9×10^5 5.0×10^5 5.8×10^5 5.0×10^5	--- 27.6 9.4 27.6
10-21	200 ppb pH = 6.4	0 60 240	5.9×10^5 4.4×10^5 1.5×10^3	--- 25.4 99.746	0 60 240	6.7×10^5 6.8×10^5 6.6×10^5	--- --- ---
10-22	400 ppb pH = 6.5	0 60 120 240	6.8×10^5 3.1×10^5 1.76×10^5 $>1 \times 10^3$	--- 54.4 74.1 <99	0 60 120 240	6.9×10^5 6.3×10^5 6.6×10^5 6.1×10^5	--- 8.7 4.3 11.6
10-29	400 ppb pH = 6.5 (Repeat of 10-22)	0 60 120 240	6.9×10^5 5.0×10^5 2.81×10^5 4.98×10^4	--- 27.5 59.3 92.77	0 60 120 240	7.6×10^5 6.2×10^5 8.8×10^5 7.6×10^5	--- --- --- ---

TABLE 5-2 (Continued)

Date (1966-67)	Conditions of Test	Viable Count Reduction					
		With Silver			Control		
		Time (Min)	Viable Count (org/cc)	Kill (%)	Time (Min)	Viable Count (org/cc)	Kill (%)
11-2	600 ppb pH = 6.4	0 60 120 240	6.7×10^5 4.0×10^5 1.61×10^5 1.39×10^3	--- 40.3 75.9 99.793	0 60 120 240	7.9×10^5 7.5×10^5 8.1×10^5 6.6×10^5	--- --- --- 16.5
11-2	800 ppb pH = 6.4	0 60 120 240	7.3×10^5 3.5×10^5 1.83×10^5 4.5×10^4	--- 52.1 74.9 93.83	0 60 120 240	7.0×10^5 7.0×10^5 7.3×10^5 7.4×10^5	--- --- --- ---
10-28	259 ppg pH = 6.3 Electrolytic silver from Prototype 150 cell	0 60 120 240	5.4×10^5 3.6×10^5 1.72×10^5 2.9×10^3	--- 33.3 68.1 99.463	0 60 120 240	8.2×10^5 7.2×10^5 7.6×10^5 6.9×10^5	--- 18 16 21.6

It should be realized that the sweat preparation used for the present study is artificial and is not a true example of human sweat. Many of the trace elements normally found in human sweat and the residue of dead and lysed bacteria are not represented. Also, a comparison of the chemical breakdown of artificial sweat and human sweat condensate as it comes out of the Apollo heat exchanger shows sweat condensate to be almost devoid of the compounds used to make up the artificial sweat.

Sweat condensate, representative of the fluid obtained from the Apollo heat exchanger, cannot therefore be considered to be actual human sweat and cannot be simulated by the artificial sweat formula used. Tests with these artificial sweat solutions were discontinued with NASA concurrence.

Human Sweat Condensate as a Suspending Medium for Ionized Silver (Static Tests)

The results obtained using artificial sweat indicated a need for actual human sweat condensate to determine if such sweat condensate would be as detrimental to the efficiency of the silver ions. From Table 5-3 it is evident

TABLE 5-3

COMPARISON OF THE CHEMICAL COMPOSITION OF SYNTHETIC SWEAT AND SWEAT CONDENSATE

Concentration in mM/L

PARAMETER	SYNTHETIC SWEAT[1]	SWEAT CONDENSATE[2]
pH	6.1 - 6.6	7.08
Na	50	0.028
K	6	0.003
NH_4	3	0.212
Cl	45	0.020
Lactate	14	0.005
Urea	10	____[3]
Bicarbonate	0	0.672

1. Formulation based on report by Johnson, Phil, and Sargent, N66 19642.
2. Analyses performed at NASA, MSC, Houston
3. Analysis for urea not performed at time of report.

that the condensate is much more dilute. Three tests were run with E. coli in condensate (Table 5-4, Figure 5-1) at 50 ppb, excellent kills being obtained. S. aureus showed a reduction of only 77.8 percent after three hours of contact time (Table 5-5, Figure 5-2). Essentially complete kill was obtained within 24 hours. Further tests employing a silver cell in conjunction with a cyclic accumulator with slight increases in pH of the sweat condensate showed increased kill. Increasing the silver concentration to 146 ppb (theoretical) gave effective kill in 120 minutes. These experiments will be discussed in another section.

From the results obtained in Tables 5-2 and 5-4, it is evident that human sweat condensate is an excellent suspending medium for the ionized silver as opposed to the artificial sweat. Inhibition of the bactericidal effects of the silver was not observed in the sweat condensate; in the experiments using E. coli as a test organism, the percent kill and the kill rate were enhanced over the results obtained in distilled water with 50-ppb silver. S. aureus, which is more resistant to silver, also showed increased sensitivity to silver in sweat condensate. These data are encouraging, since the sweat condensate, being the solution most likely to contain microorganisms in large numbers, will require the greatest bacterial control.

Silver-Ion Generator Tests Using Distilled Water

The silver-ion generator systems have been previously discussed. Sketches of the three systems are included for comparison and to indicate the sample points employed for bacteriological tests (Figure 5-3). Special sterilization techniques and equipment capable of sterilization were employed during such bacteriological tests to avoid contamination from external sources and to facilitate clean-up.

Initial bacteriological tests using an electrolytically produced silver were made using System 1, Figure 5-3, and either Prototype Cell A or Prototype Cell 150. Inoculated water was passed through the cell and a sample of the effluent was cultured at various time intervals after the sample was removed, to obtain the kill rate as a function of time. A control sample was removed at the same time.

The continuous system (System 3, Figure 5-3), was not used for bacteriogical purposes, but samples of the effluent were cultured occasionally to determine residual contamination in the hold tank.

The simulated Apollo waste water system (System 2, Figure 5-3), incorporated Prototype Test Cell B. This system was used both for short-term tests and for continuous bacteriological tests. In the short-term tests, inoculated water from a separate vessel was pumped through the cell and cyclic accumulator only, and samples were cultured as above at periodic intervals after removal. In continuous tests, the large water supply tank (13-gal carboy) was inoculated on a daily basis. The supply tank was automatically agitated at 10-min intervals to ensure dispersion of the bateria. The inoculated water was then pumped through the cell and cyclic accumulator, where it discharged to the waste water tank. The waste water tank was dumped on a daily basis with a sample of the effluent being obtained about halfway through the dump cycle. This sample was cultured to obtain the residual contamination in the waste water tank. Special media were used to eliminate background contamination. Continuous tests lasted for six days.

TABLE 5-4

SENSITIVITY OF E. COLI TO SILVER IN SWEAT CONDENSATE
(STATIC TESTS USING SILVER NITRATE)

Date (1966-67)	Conditions of Test	Viable Count Reduction					
		With Silver			Control		
		Time (Min)	Viable Count (org/cc)	Kill (%)	Time (Min)	Viable Count (org/cc)	Kill (%)
11-4	E. coli in sweat condensate	0	4.9×10^5	---	0	7.5×10^5	---
	50 ppb silver	60	1	>99.999	60	4.9×10^5	35
	pH = 7.08	120	<1	>99.999	120	2.9×10^5	61
	(control is sweat condensate)	240	<1	>99.999	240	7.6×10^3	98.99
11-9	E. coli in sweat condensate	0	2.2×10^5	---	0	8.6×10^5	---
	50 ppb silver	40	<1	>99.999	40	8.7×10^4	89.9
	pH = 7.45	60	<1	>99.999	60	4.7×10^4	94.5
	(control is sweat condensate)	90	<1	>99.999	90	1.58×10^4	98.16
11-11	E. coli in sweat condensate	0	5.9×10^5	---	0	7.3×10^5	---
	50 ppb silver	15	6.6×10^3	98.88	15	3.9×10^5	46.0
	pH = 7.50	30	4.6×10^1	99.992	30	1.2×10^5	83.7
	(used both distilled water and	45	<1	>99.999	45	7.3×10^4	90.0
	sweat condensate as controls)	60	<1	>99.999	60	4.7×10^4	93.56
					Distilled Water Control		
					0	6.8×10^5	---
					15	4.3×10^5	36.7
					30	3.4×10^5	50
					45	1.3×10^5	80.8
					60	4.4×10^4	93.5

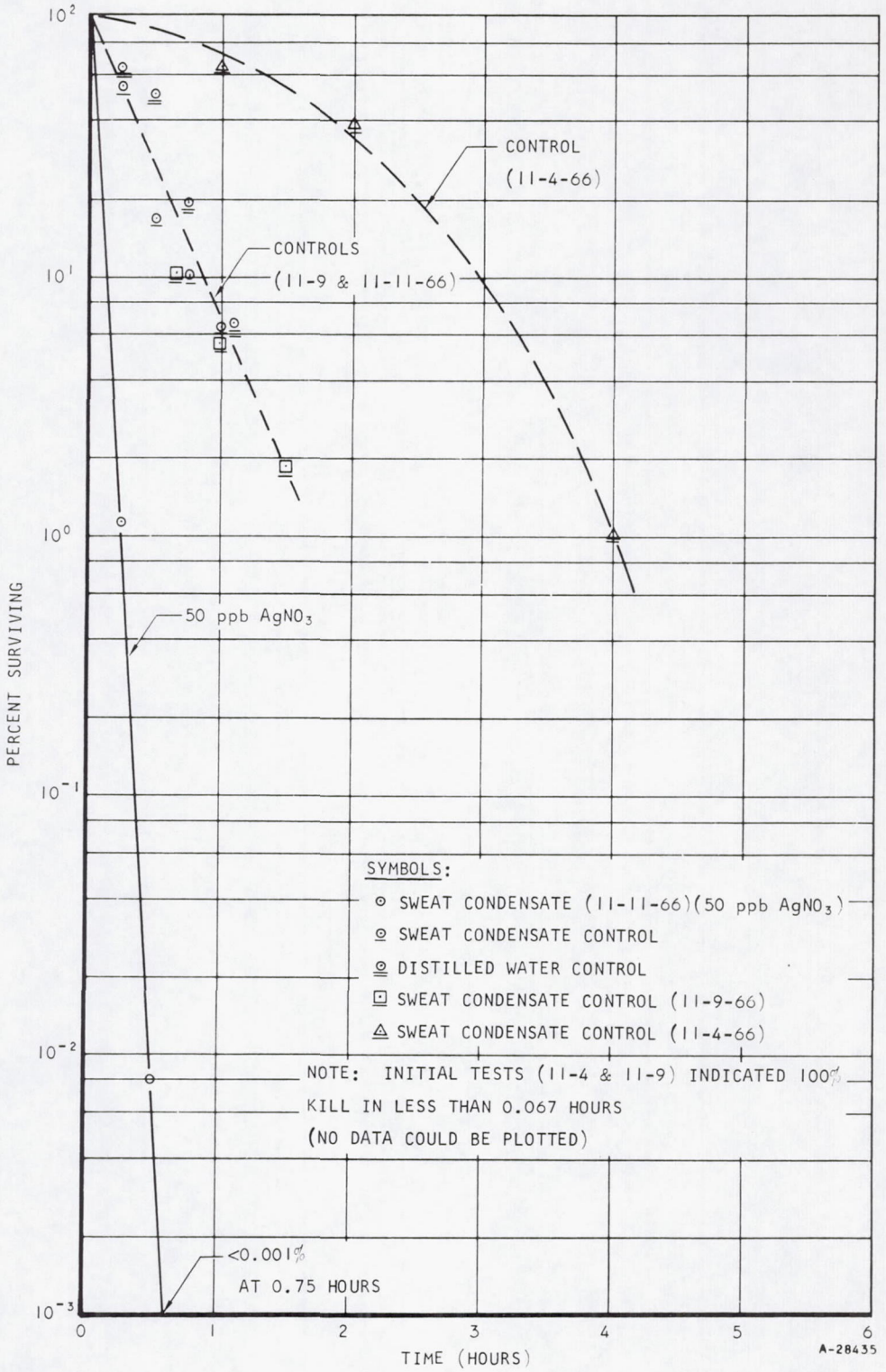


Figure 5-1. Results of Static Tests Using Inoculated (E. coli) Sweat Condensate and Silver Nitrate Solution

TABLE 5-5

SENSITIVITY OF S AUREUS TO SILVER IN SWEAT CONDENSATE
(STATIC TEST USING SILVER NITRATE)

Date (1966-67)	Conditions of Test	Viable Count Reduction					
		With Silver			Control		
		Time (Min)	Viable Count (org/cc)	Kill (%)	Time (Min)	Viable Count (org/cc)	Kill (%)
2-8	S aureus in sweat condensate	0	4.5×10^5	---	0	4.2×10^5	---
	50 ppb silver	30	2.9×10^5	35.5	30	3.8×10^5	---
	pH = 5.3	60	2.9×10^5	35.5	60	4.5×10^5	---
	(low pH due to CO_2 adsorption	90	2.7×10^5	40.0	90	4.1×10^5	---
	during storage of condensate	120	2.38×10^5	47.2	120	5.1×10^5	---
	in dry ice)	180	1.0×10^5	77.8	180	3.14×10^5	25.2
	Control is sweat condensate	24 Hr	<1	>99.999	24 hr	4.0×10^2	99.0

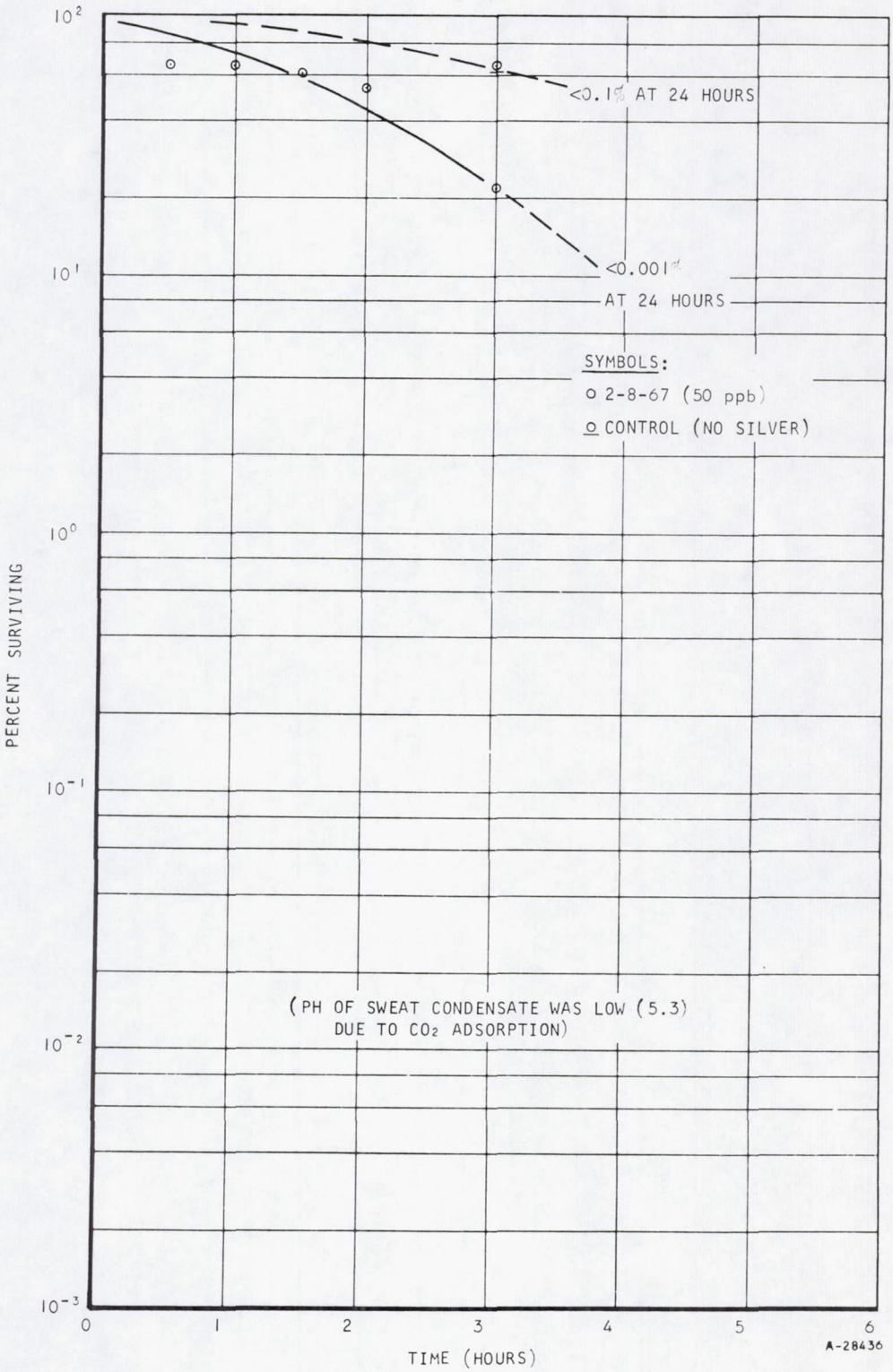
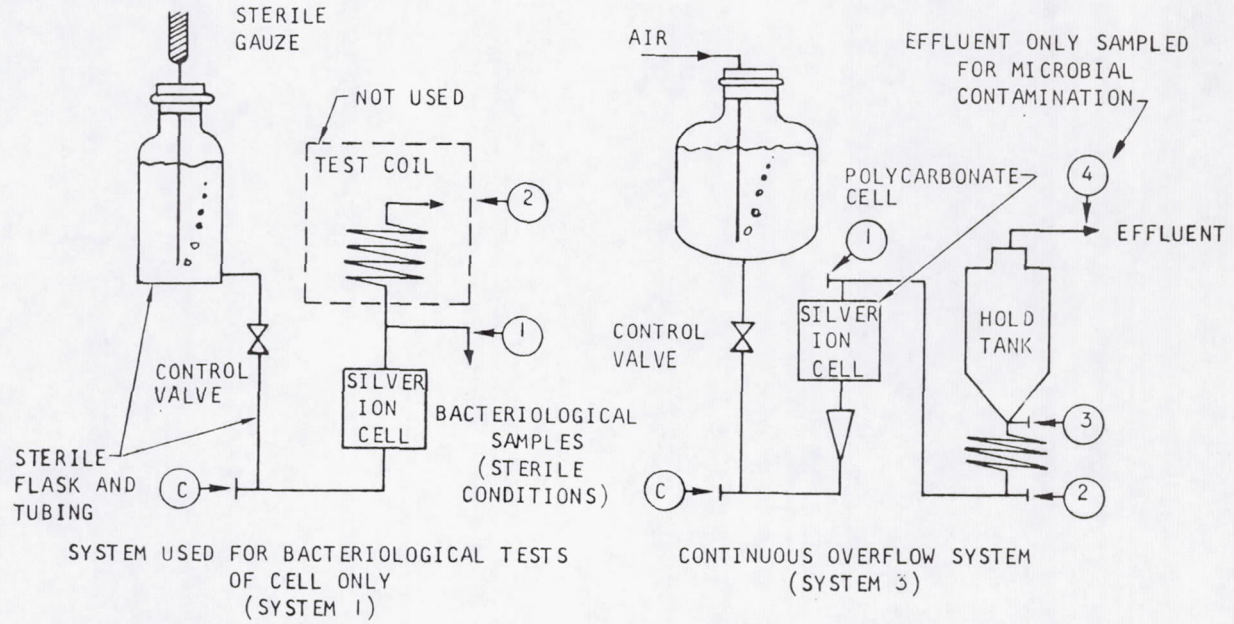


Figure 5-2. Result of Static Test Using Inoculated (S. aureus) Sweat Condensate and Silver Nitrate Solution



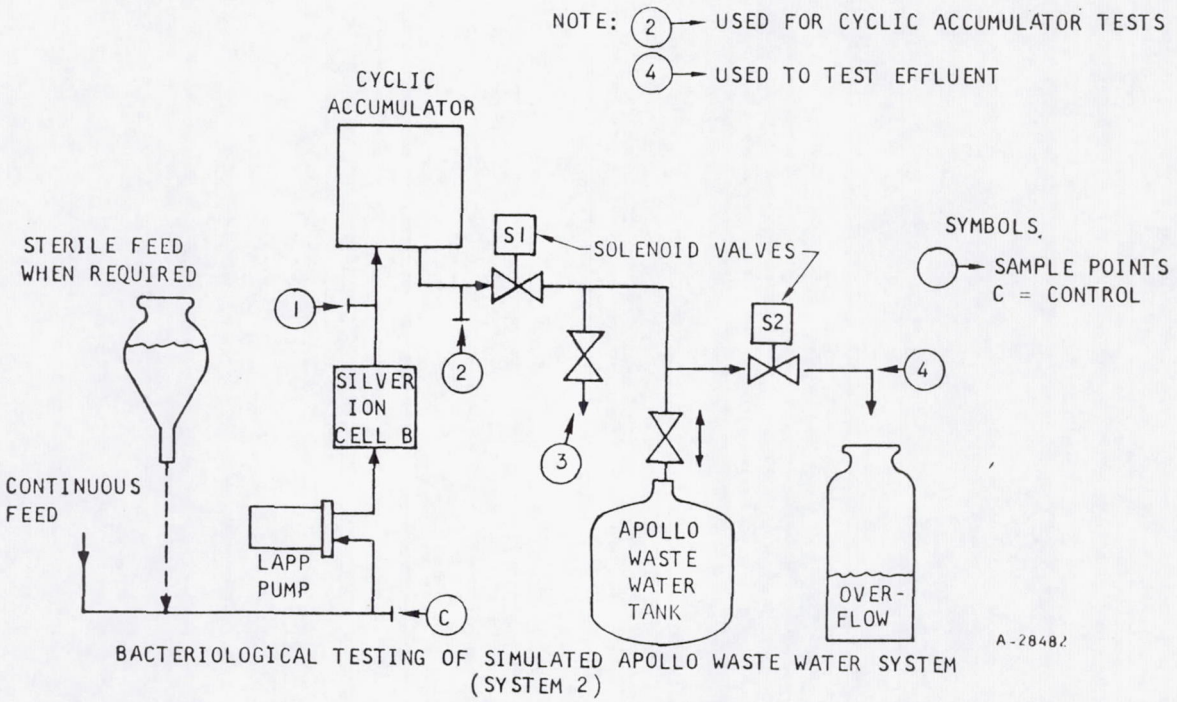


Figure 5-3. Systems Used for Testing the Bactericidal Effectiveness of Silver-Ion Generators

Table 5-6 and Figures 5-4 and 5-5 present data obtained with E. coli, using System I, Figure 5-3, and either Prototype cell A or Prototype cell 150. Both theoretical and measured values are given for the silver-ion concentration. In the initial experiment (10-28) distilled water was inoculated with the test organism after passage through the cell. In subsequent experiments, the water was inoculated prior to passage through the cell so that a portion of the bacteria was killed before a sample was obtained. Table 5-6 shows two separate time columns for the actual silver test. The left-hand column is based on the time at which the system was inoculated with E. coli; the right-hand column is based on the time at which the E. coli sample was withdrawn from the cell. The sample was collected into a 250-ml Erlenmeyer flask previously equilibrated with silver and cultured periodically to obtain the percent kill.

Examination of the data shows that greater than 99.0 percent kill of E. coli was effected within 30-min contact time with the electrolytically produced silver at silver concentrations between 50 and 100 ppb. Essentially, complete kill was obtained within 90 min. The viable count reduction in the control samples (no silver) was usually in the range of 65 to 75 per cent after two to three hours of testing. The electrolytically produced silver therefore appears to be an effective method for elimination of E. coli.

The more resistant S. aureus was also tested using Prototype Cell A. These data are presented in Table 5-7 and Figure 5-6. Only an 80-percent reduction in viable count was obtained in 180 min at 50 to 120 ppb, but effective kill (99.999 percent) was obtained after 24 hr. These data are in agreement with preliminary tests at moderate pH levels previously reported. Samples were withdrawn in the same manner as for the E. coli tests.

Compared to the control data (no silver), the electrolytically produced silver was effective in killing S. aureus within a 24-hr period. It should be noted that no samples were taken between 4 hr and 24 hr of contact time with silver; therefore, the residence time of 24 hr needed for complete kill may be somewhat exaggerated and may not represent a true value.

The effect of the addition of the cyclic accumulator in conjunction with Cell B (System 2, Figure 5-3), on the kill rate of E. coli was tested both with distilled water plus silver from the cyclic accumulator (Figure 5-7) and by passage of inoculated water through the cyclic accumulator (Figure 5-8). Results are tabulated in Table 5-8.

Distilled water obtained from the cyclic accumulator containing 84.5 and 115 ppb silver (121 and 157 ppb theoretical, respectively) and inoculated with E. coli showed a slight loss in effectiveness in kill, as the time required to achieve 99.99 to 99.999 percent was increased by 30 min over the time required for essentially complete kill when water plus silver was taken directly from the silver-ion generator. Tests in which inoculated distilled water was passed through the cyclic accumulator showed a similar loss in effectiveness at 89.5 and 55 ppb silver (theoretical); however, at 133 ppb silver (theoretical), greater than 99.999 percent kill was obtained within 60 min. This slight loss of efficiency is considered minor.

TABLE 5-6

TESTS OF ELECTROLYTICALLY PRODUCED SILVER WITH E. COLI
(STATIC AND DYNAMIC)

Date (1966-67)	Conditions of Test	Viable Count Reduction						
		With Silver				Control		
		Time (min) [1]	Time (min) [2]	Viable Count (org/cc)	Kill (%)	Time (min)	Viable Count (org/cc)	Kill (%)
11-23	Inoculated distilled water through Cell 150	0	0	6.6×10^7	---	0	6.6×10^7	---
	(three samples removed at	30	0	TNTC (10 -3)	---	30	7.4×10^7	---
	1/2 hr intervals but not	60	0	TNTC	---	60	TNTC	---
	cultured at periodic inter-	90	0	TNTC	---	90	TNTC	---
	vals)							
	53.5 ppb theory, 19.3 ppb measured -Flow = 10 cc/min	TNTC - to numerous to count - diluted.				samples were not sufficiently		
11-30	Inoculated distilled water through cell 150	0	-	1.12×10^6	---	0	1.12×10^6	---
	278 ppb theory	120	0	1.50×10^4	98.652	120	TNTC	---
	400 ppb measured	180	60	<1	>99.999	180	TNTC	---
	Flow = 5 cc/min	210	90	<1	>99.999	210	TNTC	---
12-7	Inoculated distilled water through cell 150	0		9.5×10^5		0	9.5×10^5	---
	67 ppb theory	105	0	6.6×10^4	93.05	105	4.2×10^5	55.8
	71 ppb measured	135	30	2.5×10^3	99.737	135	2.7×10^5	71.6
	Flow = 10 cc/min	165	60	1.3×10^2	99.986	165	2.83×10^5	70.2
		195	90	<1	>99.999	195	2.59×10^5	72.7
10-28	Distilled water from cell A	0		4.8×10^5	---	0	8.3×10^5	---
	pH = 6.4	60		2.3×10^2	99.952			
	85 ppb theory	120		18	99.996			
	51 ppb measured	240		<1	>99.999	240	TNTC (10^2 dil)	0.5

[1] Time after inoculation.

[2] Contact time based on removal of sample from cell.

TABLE 5-6 (Continued)

Date (1966-67)	Conditions of Test	Viable Count Reduction					
		With Silver			Control		
		Time (min)	Viable Count (org/cc)	Kill (%)	Time (min)	Viable Count (org/cc)	Kill (%)
		[1] [2]					
12-14	Inoculated distilled water through cell A	0	7.1×10^5	---	0	7.1×10^5	---
	98 ppb theory	30 0	4.9×10^4	93.09	30	5.1×10^5	28.2
	88 ppb measured	60 30	$<1.0 \times 10^2$	>99.0	60	3.7×10^5	17.8
	(1st sample 30 min after	90 60	5	>99.999	90	2.8×10^5	60.5
	inoculation - 2nd sample	120 90	<1	>99.999	120	2.2×10^5	68.4
	removed 60 min after inoculation)						
		Sample No. 2 - No Control Taken					
		0	7.1×10^5	---			
		60 0	9.2×10^4	87.1			
		90 30	1.7×10^3	99.761			
		120 60	1.6×10^2	99.978			
12-20	Inoculated distilled water through cell A	0	8.4×10^5	---	0	8.4×10^5	---
	94.5 ppb theory	45 0	1.21×10^5	85.6	45	---	---
	86.5 ppb measured	60 15	1.39×10^4	98.34	60	3.5×10^5	58.3
	(1st sample 45 min after	75 30	1.44×10^3	99.828	75	---	---
	inoculation - 2nd sample	90 45	97	99.988	90	2.8×10^5	66.6
	removed 105 min after	105 60	17	99.997	105	---	---
	inoculation)	120 75	<1	>99.999	120	2.8×10^5	66.6
		135 90	<1	>99.999	135	---	---
		Sample No. 2 - Calculated From 1 1/2 - 2 Hour Viable Count on Control - Control Calculated From Original Value					
		105 0	6.4×10^4	77.2	105	2.8×10^5	66.6
		120 15	1.46×10^3	99.478	120	---	---
		135 30	2.2×10^2	99.922	135	1.4×10^4	83.3
		150 45	<1	>99.999	150	---	---
		165 60	<1	>99.999	165	2.49×10^5	70.4

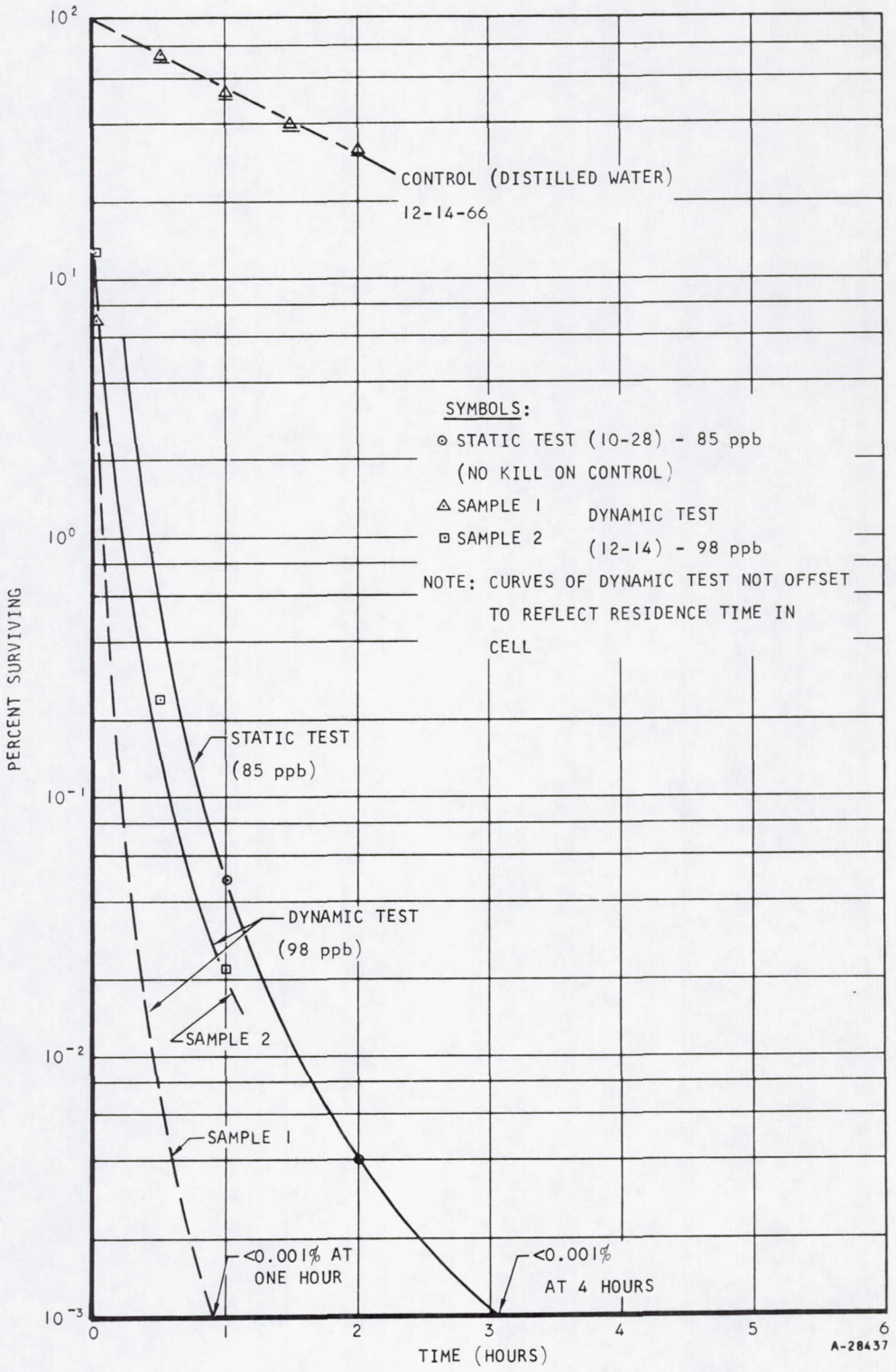


Figure 5-4. Results of Static and Dynamic Tests on Kill Rate of *E. coli* in Distilled Water Using Electrolytic Silver (Cell A)

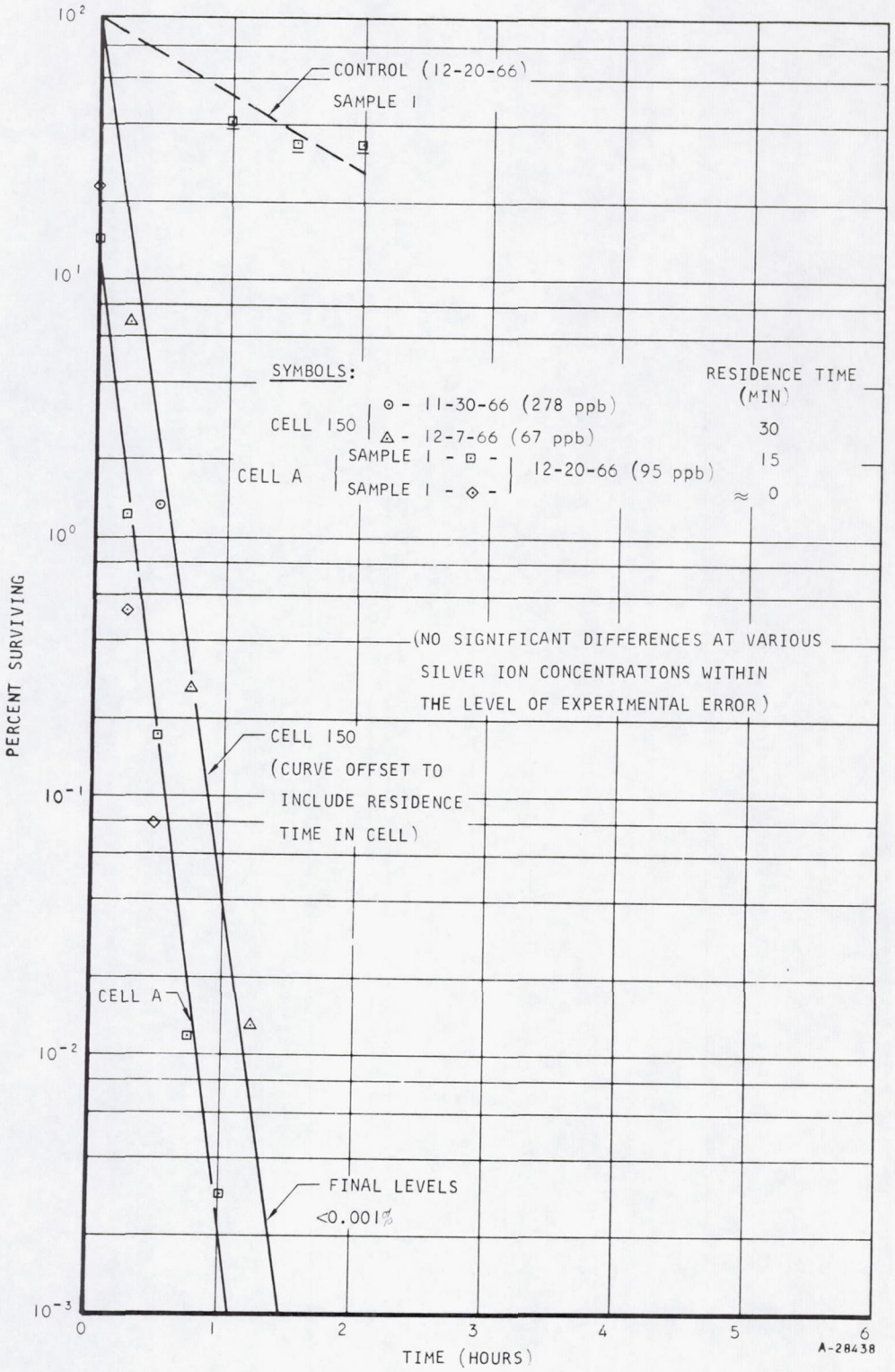


Figure 5-5. Results of Tests Using Inoculated (E. coli) Distilled Water Through Silver Ion Generators

TABLE 5-7

TESTS OF ELECTROLYTICALLY PRODUCED SILVER WITH S AUREUS

Date (1966-67)	Conditions of Test	Viable Count Reduction					
		With Silver			Control		
		Time (min) [1]	Viable Count (org/cc)	Kill (%)	Time (min)	Viable Count (org/cc)	Kill (%)
1-25	Inoculated distilled water through cell A	0 - [2]	5.0×10^5	---	0	5.0×10^5	---
	83.5 ppb theory	60 0	4.5×10^5	---	60	4.5×10^5	---
	78 ppb measured	90 30	4.2×10^5	---	90	4.0×10^5	---
		120 60	4.4×10^5	---	120	4.1×10^5	---
		150 90	1.27×10^5	71.8*	150	1.76×10^5	61.0
		180 120	TNTC	---	180	8.1×10^4	82
		*Based on 4.5×10^5 org/cc initial					
1-27	Inoculated distilled water through cell A	0 -	6.1×10^5	---	0	6.1×10^5	---
	134 ppb theory	60 0	4.9×10^5	19.7	60	6.1×10^5	---
	120 ppb measured	120 60	4.4×10^5	27.8	120	4.7×10^5	23
		180 120	2.5×10^5	58.8	180	---	---
		240 180	9.4×10^4	84.6	240	---	---
		25 Hr	<1	>99.999	25 Hr	3.8×10^4	93.8
1-31	Inoculated distilled water through cell A	0 -	4.7×10^5	---	0	4.7×10^5	---
	59.5 ppb theory	60 0	3.9×10^5	27.8*	60	5.4×10^5	---
	50 ppb measured	120 60	3.8×10^5	29.6	120	5.2×10^5	---
		180 120	3.1×10^5	42.6	180	5.2×10^5	---
		240 180	8.4×10^4	84.5	240	5.0×10^5	---
		24 Hr	<1	>99.999	24 Hr	2.47×10^4	95.4
		*Based on 5.4×10^5 org/cc initial.					

[1] Time after inoculation.
[2] Contact time based on removal of sample from cell.

TABLE 5-7 (Continued)

Date (1966-67)	Conditions of Test	Viable Count Reduction					
		With Silver			Control		
		Time (min)	Viable Count (org/cc)	Kill (%)	Time (min)	Viable Count (org/cc)	Kill (%)
2-1	Inoculated distilled water through cell A	0 [1] - [2]	5.2×10^5	---	0	5.2×10^5	---
	93.5 ppb theory	60 0	5.1×10^5	2	60	5.4×10^5	---
	93.0 ppb measured	120 60	4.7×10^5	9.6	120	6.0×10^5	---
		180 120	3.6×10^5	30.8	180	5.4×10^5	---
		25 Hr	<1	>99.999	25 Hr	3.4×10^4	93.7

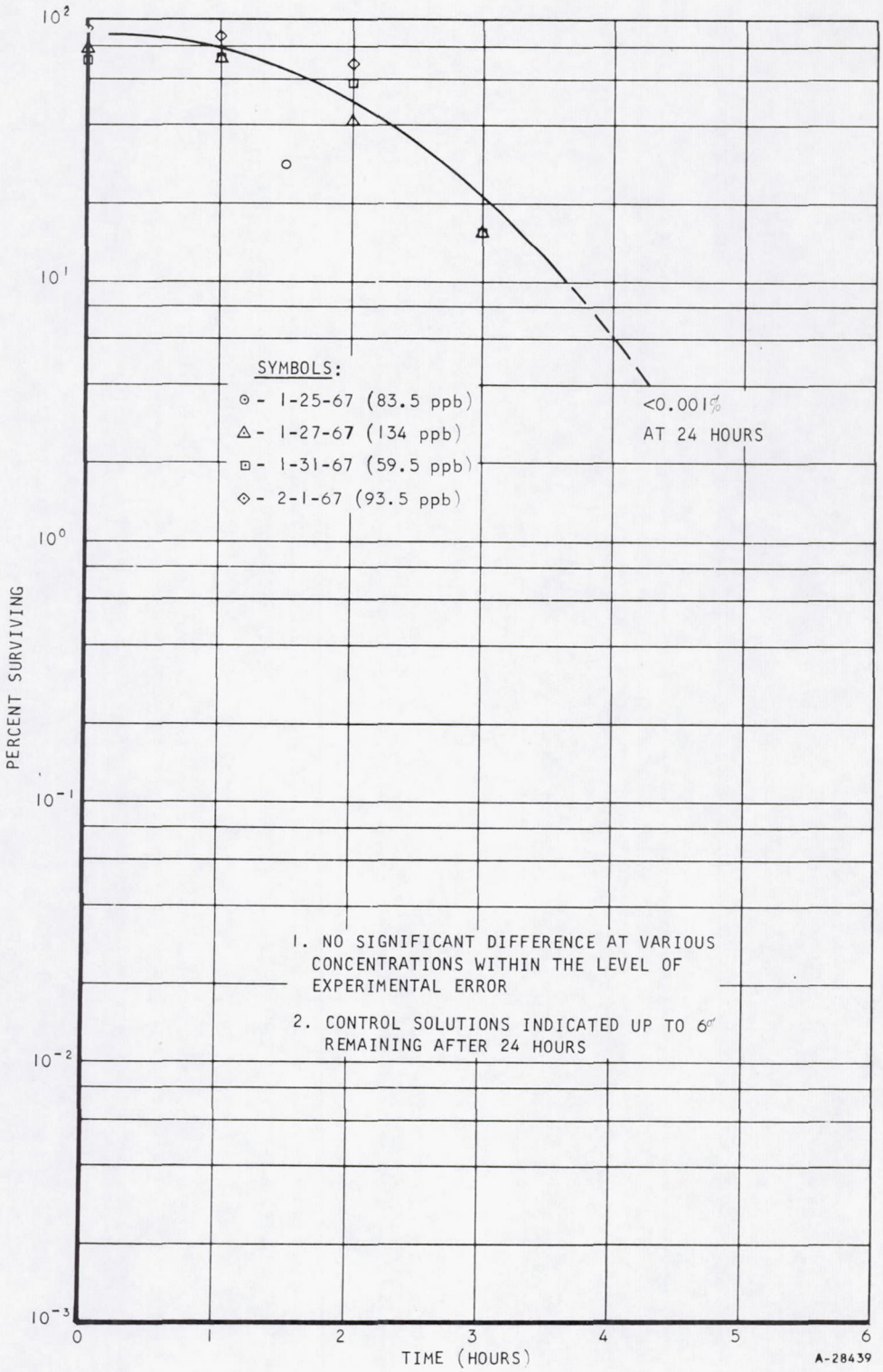


Figure 5-6. Results of Tests Using Inoculated (S. aureus) Distilled Water Through a Silver Ion Generator (Cell A)

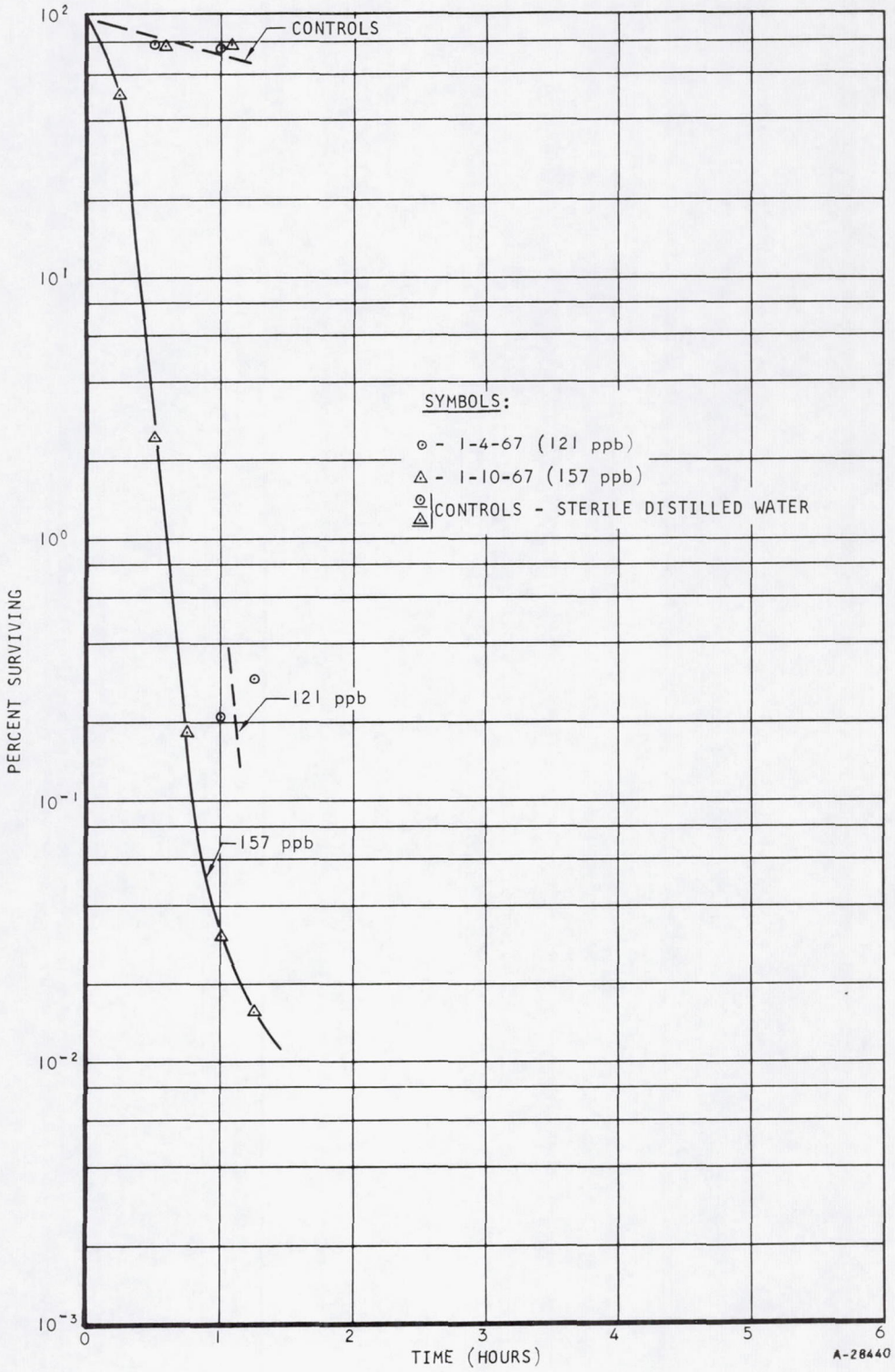


Figure 5-7. Results of Static Tests Using Inoculated (E. coli) Distilled Water Obtained from the Cyclic Accumulator

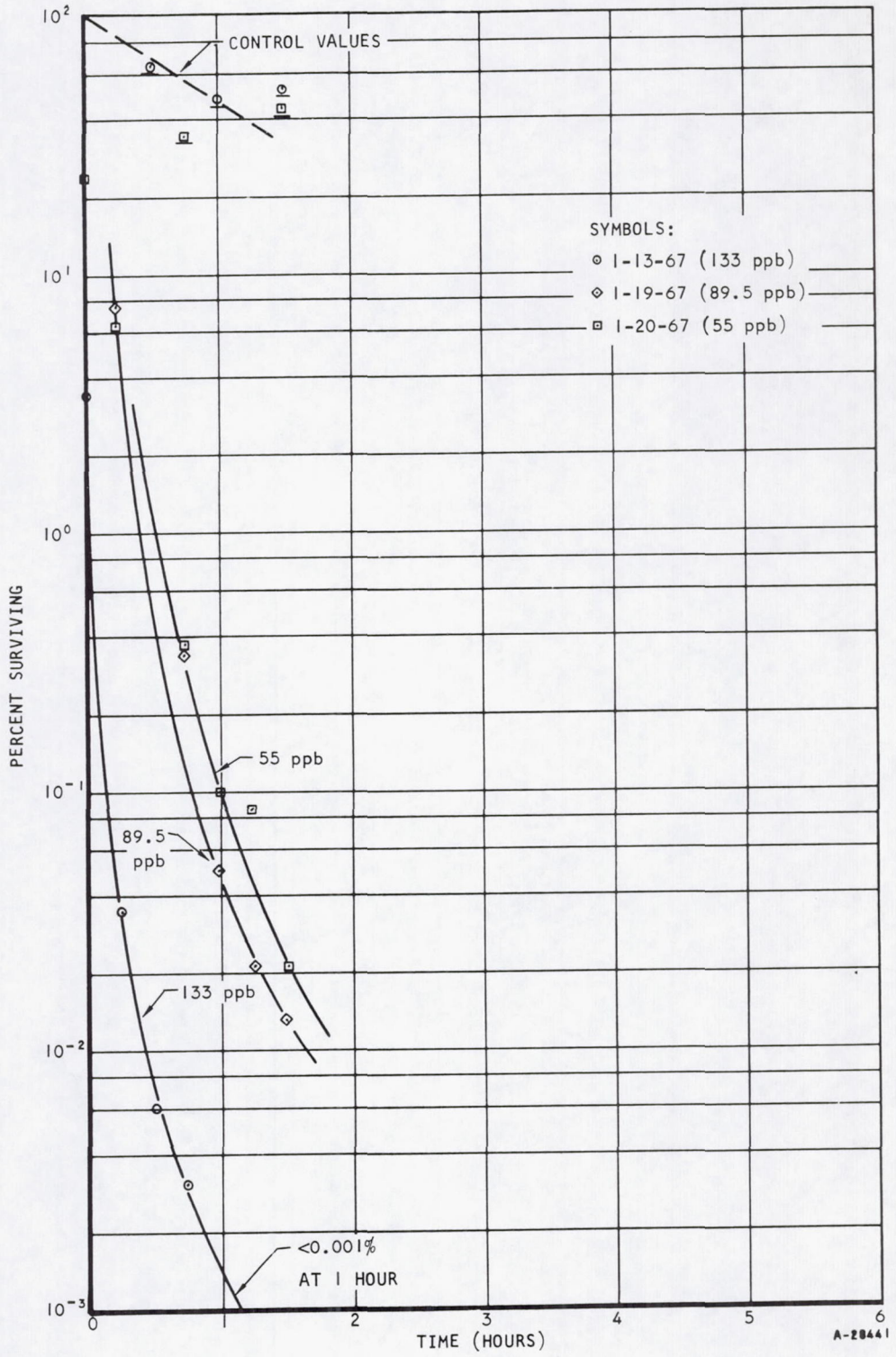


Figure 5-8. Results of Tests Using Inoculated (E. coli) Distilled Water Through Cell B and Cyclic Accumulator

TABLE 5-8

EFFECT OF CYCLIC ACCUMULATOR ON KILL RATE OF E COLI
(Static and Dynamic Tests)

Date (1966-67)	Conditions of Test	Viable Count Reduction					
		With Silver			Control		
		Time (min)	Viable Count (org/cc)	Kill (%)	Time (min)	Viable Count (org/cc)	Kill (%)
1-4	Distilled water obtained from cyclic accumulator	0	1.10×10^6	---	0	1.32×10^6	---
	121 ppb theory	15	TNTC	---	15	---	---
	84.5 ppb measured	30	TNTC	---	30	1.03×10^6	22.0
	(control is sterile distilled water)	45	TNTC	---	45	---	---
		60	2.35×10^3	99.787	60	9.9×10^5	26.4
		75	2.11×10^3	99.708	75	---	---
1-10	Distilled water obtained from cyclic accumulator	0	5.8×10^5	---	0	1.26×10^6	---
	157 ppb theory	15	2.93×10^5	49.5	15	---	---
	115 ppb measured	30	1.42×10^4	97.55	30	9.9×10^5	21.5
	(control is sterile distilled water)	45	1.08×10^3	99.814	45	---	---
		60	1.78×10^2	99.969	60	9.9×10^5	21.5
		75	9.5×10^1	99.984	75	---	---
1-13	Inoculated distilled water	0[1] [2]	4.0×10^5	---	0	4.0×10^5	---
	passed through the cyclic	60 0	1.16×10^4	96.58[3]	60	3.4×10^5	0.0[3]
	accumulator (used 8 minute	75 15	1.2×10^2	99.965	75	---	---
	pump, 2 minute dump cycle)	90 30	2.2×10^1	99.994	90	2.17×10^5	36
	133 ppb theory	105 45	1.2×10^1	99.997	105	---	---
	131 ppb measured	120 60	<1	>99.999	120	1.56×10^5	54
		135 75	<1	>99.999	135	---	---
		150 90	<1	>99.999	150	1.72×10^5	49.5

[1]Time after inoculation.
[2]Contact time based on removal of sample from cyclic accumulator.
[3]Based on 3.4×10^5 org/cc initial.
[4]Control sample (input) removed at time sample was taken from cyclic accumulator.

TABLE 5-8 (Continued)

Date (1966-67)	Conditions of Test	Viable Count Reduction					
		With Silver			Control		
		Time (min)[4]	Viable Count (org/cc)	Kill (%)	Time (min)[4]	Viable Count (org/cc)	Kill (%)
1-19	Inoculated distilled water passed through the cyclic accumulator (used 8 minute pump, 2 minute dump cycle) 89.5 ppb theory 70 ppb measured	0	TNTC	---	0	4.76×10^5	---
		15	3.58×10^4	92.47	15	---	---
		30	TNTC		30	---	---
		45	1.61×10^3	99.662	45	---	---
		60	2.37×10^2	99.950	60	TNTC	---
		75	1.01×10^2	99.979	75	---	---
		90	613×10^1	99.987	90	TNTC	---
1-20	Inoculated distilled water passed through the cyclic accumulator (used 8 minute pump, 2 minute dump cycle) 55 ppb theory 50 ppb measured	0	1.20×10^5	76.4	0	5.1×10^5	---
		15	3.32×10^4	93.49	15		
		30	---	---	30		
		45	1.86×10^3	99.635	45	3.4×10^5	33.4
		60	4.3×10^2	99.901	60	---	---
		75	1.82×10^2	99.916	75	---	---
		90	1.02×10^2	99.979	90	2.9×10^5	43

Silver Sensitivity of S. aureus in Sweat Condensate to Electrolytically Produced Silver

A static test with S. aureus and ionized silver ($AgNO_3$) in sweat condensate (previously reported, Table 5-5, Figure 5-2) indicated an increased sensitivity of S. aureus to this combination. The sweat condensate used for this static test had been stored for an appreciable period of time so that considerable absorption of carbon dioxide had occurred, which lowered the pH of the sweat condensate from a normal value of about 7.0 to 5.3 (Table 5-5, Figure 5-2). Initial studies in distilled water (see Screening report) showed the silver sensitivity of S. aureus to be pH dependent. In the cyclic accumulator studies discussed below the increased pH (above 7.0) was found to considerably enhance the bactericidal properties of silver in sweat condensate.

A series of experiments (Table 5-9, Figure 5-9) were undertaken by passing sweat condensate inoculated with S. aureus through the cyclic accumulator (System 2, Figure 5-3), at increasing silver ion concentrations.

Examination of Table 5-9 shows that the kill rate of S. aureus in sweat condensate at pH levels of 7.6 to 7.75 was enhanced with greater than 99.5 kill being obtained at a theoretical silver concentration of 103 ppb within four hours. At a theoretical concentration of 146 ppb, essentially complete kill was obtained at the four hour sampling time. S. aureus in sweat condensate appears signicantly more sensitive to silver than in distilled water.

Comparable tests were not undertaken with E. coli, due to limitations on availability of sweat condensate. However, the results obtained in static tests with E. coli show them to be extremely sensitive to silver in sweat condensate. The kill rate in the control culture (no silver) was considerably increased over that observed for controls in distilled water.

Continuous Tests in Simulated Apollo System

Prior to continuous bacteriological tests in the simulated Apollo waste water system (System 2, Figure 5-3) microbial contamination of the waste water tank was examined (Table 5-10). No attempt was made to sterilize the Apollo waste water tank, since the effect of various possible sterilizing methods on the polyisoprene bladder was not known. Residual bacterial contamination other than E. coli and S. aureus was measured at about 500 organisms per ml after continuous purging of the tank for over a week with water containing electrolytic silver at 110 to 120 ppb. Following this initial purge, the system was equilibrated at the desired concentration for several days (50 ppb theoretical). A residual quantity of about 10 lb of water containing silver was in the waste water tank at the start of each continuous run.

It was necessary to use differential and selective media for quantitative measurements of kill rates on E. coli and S. aureus to eliminate background contaminants. Mac Conkey's agar was used for the E. coli run and Tellurite-Glycine agar for S. aureus. Such media were not used until after the third day of the E. coli test. A possible silver-resistant organism in the waste

TABLE 5-9

SILVER SENSITIVITY OF S AUREUS IN SWEAT CONDENSATE
THROUGH CYCLIC ACCUMULATOR
(SIMULATED APOLLO WASTE WATER SYSTEM)

Date (1966-67)	Conditions of Test	Viable Count Reduction					
		With Silver			Control		
		Time (min) [1]	Viable Count (org/cc)	Kill (%)	Time (min)	Viable Count (org/cc)	Kill (%)
2-16	Inoculated sweat condensate passed through cyclic accumulator (8 minute pump, 2 minute dump cycle) 58.5 ppb theory 50.0 ppb measured 8.0 cc/min average flow 7.0 ua pH = 7.6	0	5.5×10^5	---	0	5.5×10^5	---
		60 (0)	3.4×10^5	17.1	60	4.1×10^5	0*
		90 (30)	2.4×10^5	41.5	90	---	---
		120 (60)	1.71×10^5	58.3	120	2.9×10^5	29
		150 (90)	8.9×10^4	78.3	150	---	---
		180 (120)	8.8×10^4	28.6	180	1.28×10^5	69
		210 (150)	3.5×10^4	91.5	210	---	---
		240 (180)	2.7×10^4	93.4	240	3.9×10^4	90.5
		24 Hr	<1	>99.999	24 Hr	Contaminated	
		*Used to calculate % kill					
2-17	Inoculated sweat condensate passed through cyclic accumulator (8 minute pump, 2 minute dump cycle) 103 ppb theory 89 ppb measured 8.2 cc/min average flow 12.6 ua pH = 7.6	0 (-)	5.0×10^5	---	0	5.0×10^5	---
		60 (0)	2.0×10^5	35.5	60	3.1×10^5	0*
		90 (30)	1.9×10^5	38.7	90	---	---
		120 (60)	8.1×10^4	73.5	120	1.35×10^5	56
		150 (90)	3.62×10^4	88.3	150	---	---
		180 (120)	2.17×10^4	93.0	180	2.6×10^4	92
		210 (150)	5.0×10^3	98.39	210	---	---
		240 (180)	1.48×10^3	99.522	240	3.8×10^4	98.8
		24 Hr	<1	>99.999	24 Hr	Contaminated	
		*Used to calculate % kill					

[1] Time after inoculation
[2] Contact time based on removal of sample from cell

TABLE 5-9 (Continued)

Date (1966-67)	Conditions of Test	Viable Count Reduction					
		With Silver			Control		
		Time (min)	Viable Count (org/cc)	Kill (%)	Time (min)	Viable Count (org/cc)	Kill (%)
2-21	Inoculated sweat condensate passed through cyclic accumulator (8 minute pump, 2 minute dump cycle) 146 ppb theory 119 ppb measured 8.2 cc/min average flow 19.0 ua pH = 7.65	0 - 60 0 90 30 120 60 150 90 180 120 210 150 240 180 24 Hr	4.9×10^5* 2.12×10^5 1.01×10^5 8.4×10^3 3×10^2 $<1 \times 10^2$ $<1 \times 10^1$ $<1 \times 10^1$ No Test	--- 56.7 79.4 98.29 99.939 >99.9 >99.99 >99.99	0 60 90 120 150 180 210 240 24 Hr	4.9×10^5* 5.3×10^5 --- 4.4×10^5 --- 3.21×10^5 -- 2.34×10^5 No Test	--- --- --- 10.2 --- 34.5 -- 52.2
		*Used to calculate % kill					
2-24	Inoculated sweat condensate passed through cyclic accumulator (8 minute pump, 2 minute dump cycle) 146 ppb theory 125 ppb measured 8.2 cc/min average flow 19.0 ua (repeat of above) pH = 7.75	0 - 60 0 90 30 120 60 150 90 180 120 210 150 240 180 24 Hr	5.5×10^5* 1.22×10^5 2.78×10^4 8×10^2 1.1×10^2 1.1×10^1 <1 <1 No Test	--- 77.8 94.94 99.854 99.98 99.998 >99.999 >99.999 ---	0 60 90 120 150 180 210 240 24 Hr	5.5×10^5 3.8×10^5 --- 4.5×10^5 --- 4.4×10^5 --- 3.27×10^5 No Test	--- --- --- --- --- --- --- --- ---
		*Used to calculate % kill					

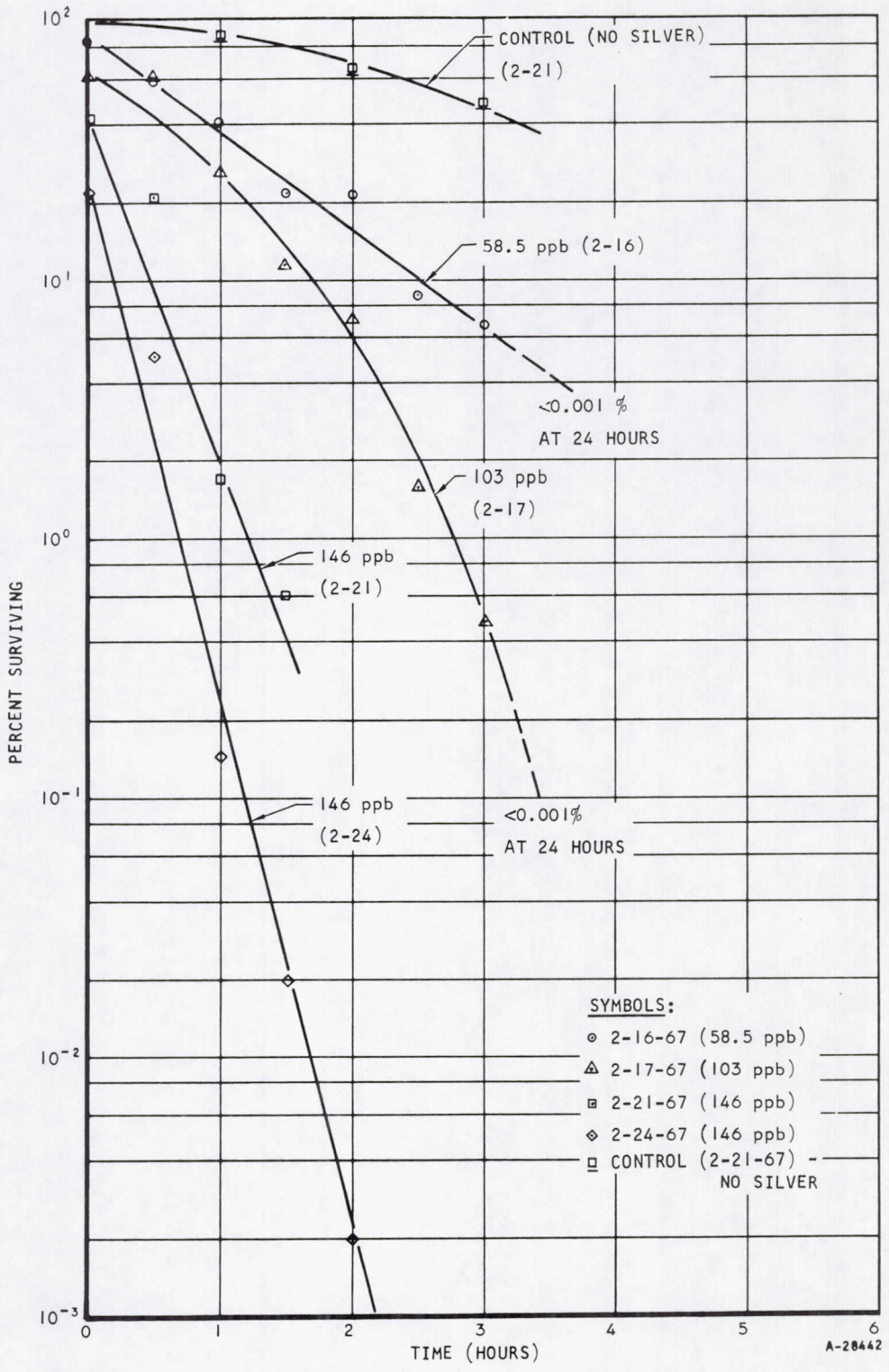


Figure 5-9. Results of Tests Using Inoculated (S. aureus) Sweat Condensate Through Cell B and Cyclic Accumulator

TABLE 5-10

CONTAMINATION OF EFFLUENTS FROM HOLD TANKS OF CONTINUOUS AND SIMULATED APOLLO WASTE WATER SYSTEMS

Date (1966-67)		Time (min)	Viable Count (org/cc)	Kill (%)	Time (min)	Viable Count (org/cc)	Kill (%)
	These tests were undertaken to determine background contamination with silver resistant organisms in hold tanks which had been equilibrated with electrolytically produced silver in the specified concentration						
	Hold Tank of Continuous System						
			Effluent			Inlet	
11-16	92 ppb theoretical input 46 ppb measured		>500 colonies/cc-TNTC			309 colonies/cc	
			Effluent			Inlet	
11-30	200 ppb theoretical input 117 ppb measured		≈50 colonies/cc			(not cultured)	
			Effluent			Inlet	
1-18	200 ppb theoretical input 92 ppb measured		1.5×10^3 colonies/cc			(not cultured)	
	Waste Water Tank Simulated Apollo System						
			Effluent			Inlet	
11-30	Prior to introducing silver into tank - water in tank 6 days prior to culture		≈100 colonies/cc			(not cultured)	
			Effluent			Inlet	
1-18	143 ppb theoretical input 110 ppb by atomic adsorption		5.6×10^2 colonies/cc			(not cultured)	

water tank was isolated and identified as an Alkaligenes sp., probably A. metalcaligenes, an organism found in the human intestinal tract and common in water supplies. The resistance of this organism to silver was not actually tested. In a previous report (Reference 1), data were presented for the kill rate on a closely related bacterium, A. faecalis, using electrolytically produced silver at 93 ppb. These bacteria had predominated in suit-loop condensate and an effective kill of better than 99.999 percent was obtained in 90 min.

In the continuous flow test, E. coli was pumped into the waste water tank for a total period of 144 hours with a theoretical silver ion input concentration of 55 ppb (Table 5-11, Figure 5-10). The water supply tank containing distilled water was reinoculated daily for the first four days to a level of 6 to 9 x 10^5 organisms per ml. Approximately a 99.9 percent kill was observed using Tryptic Soy agar, but on Mac Conkey's agar approximately 99.999 percent reduction was obtained.

Essentially, the same experiment was undertaken with S. aureus (Table 5-12, Figure 5-11) using the same theoretical silver ion concentration (55 ppb). Daily sampling of the hold tank showed greater than 99.9 percent kill for each 24-hr period. On the fourth day of sampling, a five-log reduction of viable bacteria was obtained, complete kill being obtained on the sixth day. It should be noted that after the third day of the run, reinoculation of the reservoir was discontinued. Higher silver ion concentrations (up to 100 ppb) would probably effect complete kill within a 24-hr period.

In summary, both continuous runs were successful in that the kill rates and percentage reductions in viable count were similar to those obtained for tests run in systems employing only the silver-ion generator or the silver-ion generator in series with the cyclic accumulator. The die-off rate for S. aureus in the reservoir was consistently low over each 24-hr period, the same being true for E. coli. The presence of electrolytically produced silver effected essentially complete kill for E. coli and S. aureus.

Bladder Water Studies

The Apollo waste storage tank contains an inflatable bladder which is in contact with the water in the waste tank. It therefore became important to determine the effect that the bladder itself, or materials leaching from it, might have on the bactericidal properties of silver.

Small strips of the bladder material (1 in. by 2-1/2 in.) were placed in sterile distilled water and allowed to soak for several days to allow leaching of any of the bladder material into the water. This water (bladder water) was then used as a suspending medium for ionized silver ($AgNO_3$) at concentrations of 50 ppb. A control test was included and consisted of bladder water only. Both E. coli and S. aureus were tested.

The results of these tests are reported in Table 5-13 and Figures 5-12 and 5-13. The bladder water decreased the kill rate of E. coli, greater than 99.999 percent kill being obtained after 90 min in water in which the bladder material had soaked for only three days. Water in which the bladder

TABLE 5-II

CONTAMINATION LEVELS FOR E. COLI UNDER CONTINUOUS FLOW CONDITIONS
(SIMULATED APOLLO WASTE WATER SYSTEM)

Date (1967)	Elapsed Time (hr)	Reservoir			Hold Tank Effluent			
		Before Inoculation (org/cc)	Reduction in Control (%)	After Innoculation (org/cc)	Viable Count (org/ml)		% Kill	
					Total	E. coli (Mac Conkey's)	Total	E. coli
1-24	0	2.18×10^2	--	8.9×10^5	TNTC	---	---	---
1-25	24	4.94×10^5	45	6.3×10^5	2.58×10^3	---	99.710	---
1-26	48	4.3×10^5	32	6.9×10^5	2.46×10^3	---	99.609	---
1-27	72	5.0×10^5	27	8.0×10^5	1.29×10^3	1.58×10^2	99.813	99.977
1-28	96	5.4×10^5	32	Not inoculated	1.23×10^3	3.5×10^1	99.816	99.996
1-29	120	Not tested	--	Not inoculated	Not tested	Not tested	---	---
1-30	144	TNTC	--	Not inoculated	3.6×10^2	2.0×10^1	99.955	99.998

Test Conditions:

1. Inoculated (E. coli) water through Cell B and cyclic accumulator into waste water tank
2. Tank dumped manually at 24 hour intervals except 1-28 (Saturday) and 1-29 (Sunday)
3. Reservoir inoculated daily with fresh bacteria except as noted. Agitated at ten minute intervals
4. Flow rate at 8.2 cc/min (average). Current at 6.7 ua. Concentration (theory) = 55 ppb
5. Mac Conkey's medium used to differentiate E coli from background contamination
6. Percent kill based on viable count in reservoir for previous date

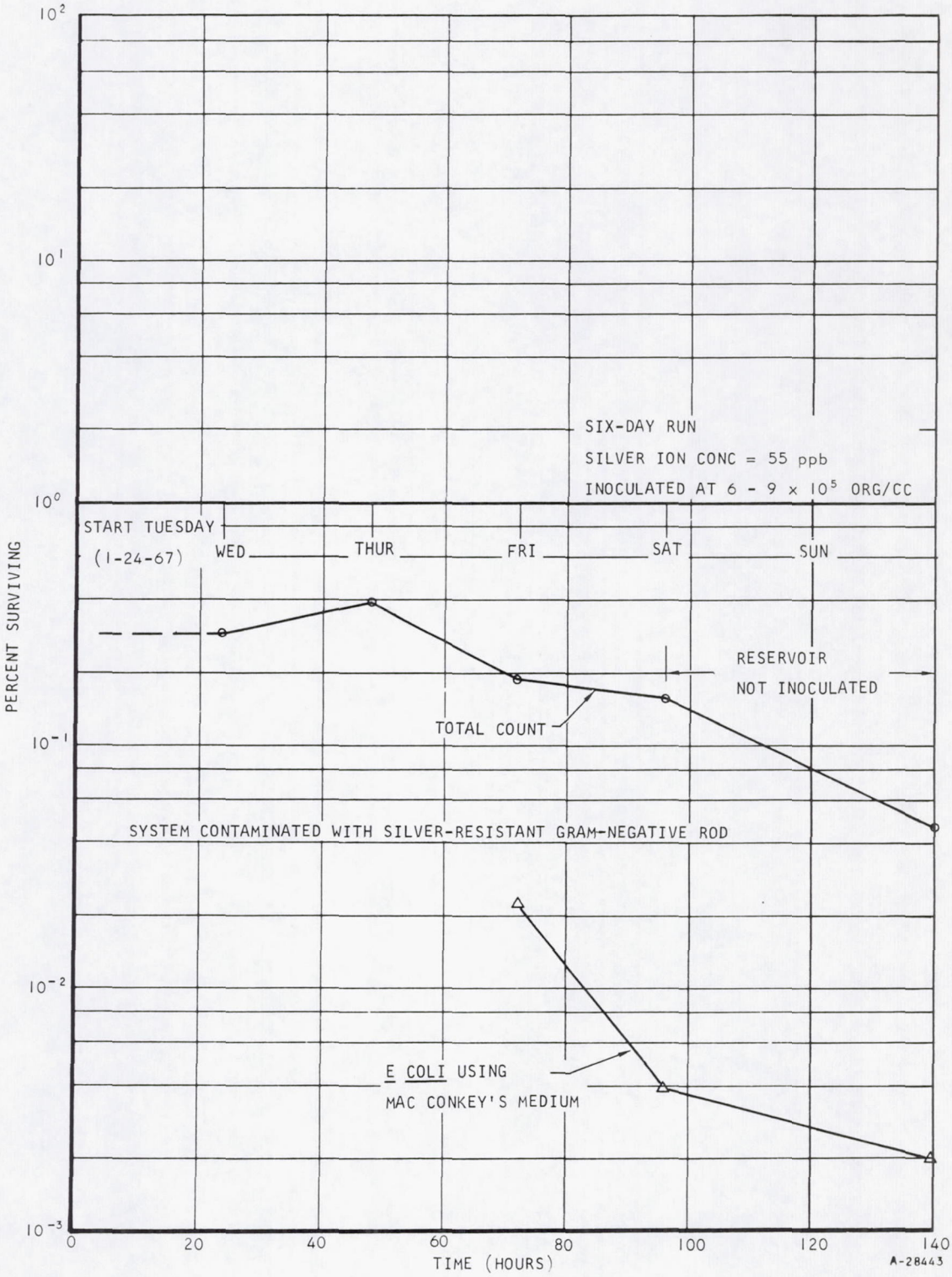


Figure 5-10. Results of Continuous Test Using Inoculated (E. coli) Distilled Water Through the Apollo Waste Water Hold Tank

TABLE 5-12

CONTAMINATION LEVELS FOR S. AUREUS UNDER CONTINUOUS FLOW CONDITIONS
(SIMULATED APOLLO WASTE WATER SYSTEM)

Date (1967)	Elapsed Time (hr)	Reservoir			Hold Tank Effluent			
					Viable Count (org/ml)		% Kill	
		Before Inoculation (org/cc)	Reduction in Control (%)	After Innoculation (org/cc)	Total	S. aureus (Tellurite-glycine)	Total	S. aureus
2-7	0	TNTC	--	4.9×10^5	9.0×10^2	---	---	---
2-8	24	5.5×10^5	Not calculated	4.8×10^5	TNTC	1.45×10^2	---	99.970
2-9	48	3.6×10^5	25	4.9×10^5	3.3×10^3	9.5×10^1	99.313	99.980
2-10	72	4.2×10^3	14	4.8×10^5	4.7×10^3	1.5×10^1	99.041	99.997
2-11	96	2.5×10^3	48	Not inoculated	5.4×10^3	5	98.875	99.999
2-12	120	Not tested	--	Not inoculated	Not tested	Not tested	---	---
2-13	144	6.0×10^3	98.7	Not inoculated	6.8×10^2	<1	99.858	>99.999

Test Conditions:

1. Inoculated (S. aureus) water through Cell B and cyclic accumulator into waste water tank
2. Tank dumped manually at 24 hour intervals except 2-11 (Sat) and 2-12 (Sun)
3. Reservoir inoculated daily with fresh bacteria except as noted. Agitated at ten minute intervals
4. Flow rate at 8.2 cc/min (average). Current at 6.8 ua. Concentration (theory) = 55.5 ppb
5. Tellurite-glycine used to differentiate S. aureus from background contamination
6. Percent kill based on viable count in reservoir for previous date

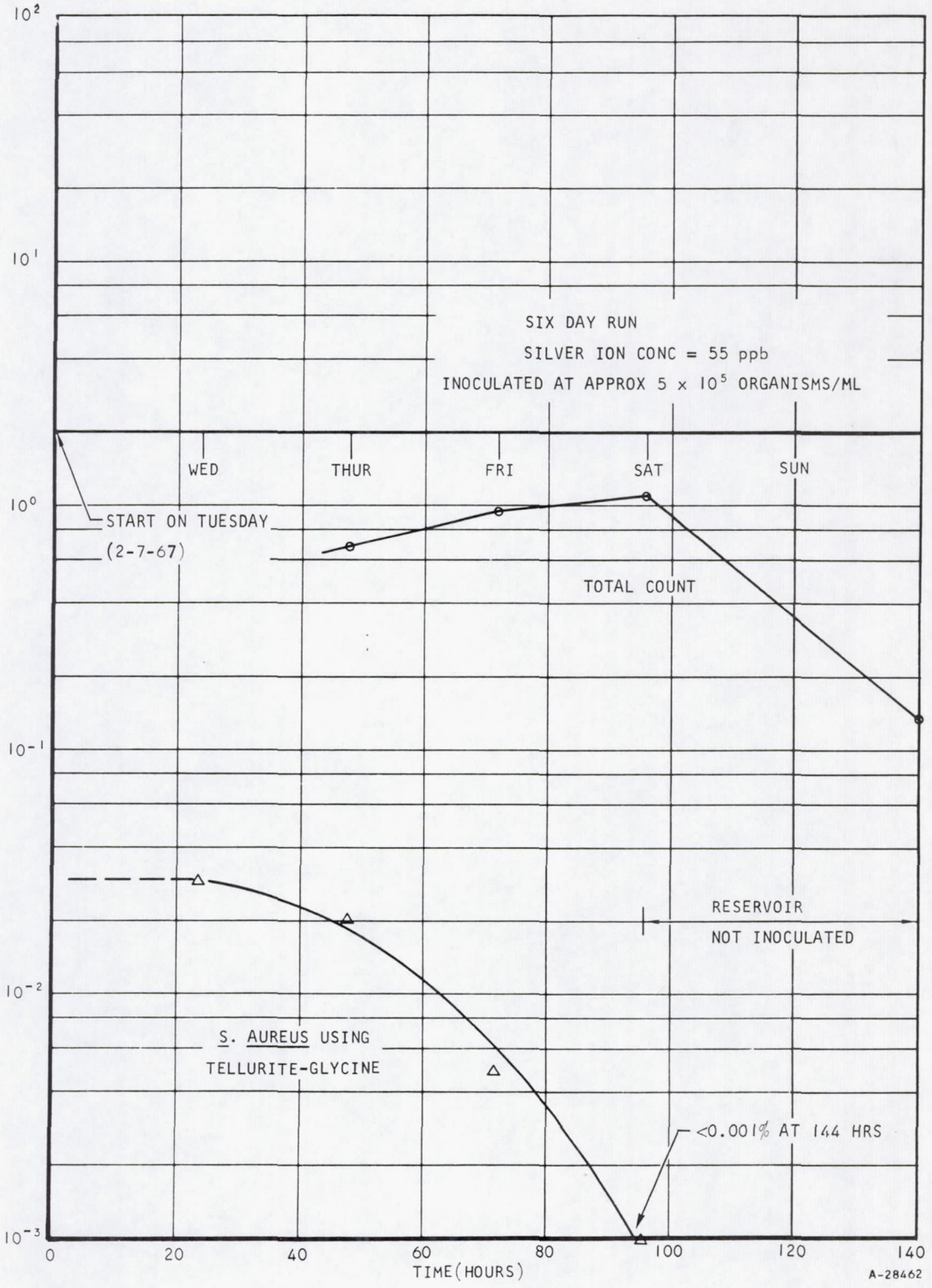


Figure 5-11. Results of Continuous Test Using Inoculated (S. aureus) Distilled Water Through the Apollo Waste Water Hold Tank

TABLE 5-13

SILVER SENSITIVITY OF TEST ORGANISMS TO APOLLO TANK BLADDER WATER
(STATIC TESTS WITH SILVER NITRATE)

Date (1966-67)		Viable Count Reduction					
		With Silver			Control		
		Time (min)	Viable Count (org/cc)	Kill (%)	Time (min)	Viable Count (org/cc)	Kill (%)
10-28	Distilled water containing $AgNO_3$ in contact with polyisoprene bladder for 20 hours prior to inoculation with E coli - 100 ppb initial pH = 6.62 (distilled water used as control pH = 6.65)	0	6.2×10^5	---	0	7.7×10^5	---
		60	2.11×10^4	96.6	60	7.4×10^5	---
		120	8.4×10^2	99.865	120	TNTC	---
		240	<1	>99.999	240	TNTC	---
	The following static tests were undertaken using sterile distilled water (200 cc) which had been in contact with strips (1 in. wide, 2 1/2 in. long, 0.058 in. thick) of polyisoprene bladder for the specified period. A silver nitrate solution (200 ppb) was diluted 3/1 with the above bladder water to give the requisite 50 ppb of $AgNO_3$. The control was bladder water diluted as above with sterile distilled water. The bladder water was inoculated after dilution.						
1-26	Bladder water inoculated with E coli 3 day soak (see above) 50 ppb	0	9.3×10^5	---	0	9.1×10^5	---
		30	TNTC	---	30	9.5×10^5	---
		60	8.6×10^2	99.908	60	9.8×10^5	---
		90	<1	>99.999	90	7.9×10^5	13
		120	<1	>99.999	120	7.9×10^5	13

TABLE 5-13 (Continued)

Date (1966-67)		Viable Count Reduction					
		With Silver			Control		
		Time (min)	Viable Count (org/cc)	Kill (%)	Time (min)	Viable Count (org/cc)	Kill (%)
2-3	Bladder water inoculated with *E. coli* 11 day soak (see above) 50 ppb pH = 6.45	0	6.7×10^5	---	0	6.4×10^5	---
		30	2.0×10^5	70.2	30	6.1×10^5	---
		60	TNTC	---	60	7.0×10^5	---
		90	TNTC	---	90	5.8×10^5	---
1-31	Bladder water inoculated with *S. aureus* 8 day soak (see above) 50 ppb	0	3.0×10^5	---	0	5.4×10^5	---
		60	$<1 \times 10^3$	>99	60	2.6×10^5	51.8
		120	$<1 \times 10^3$	>99	120	5.7×10^4	89.5
		180	$<1 \times 10^2$	>99.9	180	1.7×10^4	96.86
		24 Hr	<1	>99.999	24 Hr	$<1 \times 10^2$	>99.9
2-3	Bladder water inoculated with *S. aureus* 11 day soak (see above) 50 ppb pH = 6.45	0	3.2×10^5	---	0	3.7×10^5	---
		30	1.1×10^3	99.656	30	2.9×10^5	21.6
		60	--- [1]	---	60	2.08×10^5	43.8
		90	1.4×10^1	99.996	90	5.0×10^4	86.5
		120	1.9×10^1	99.994	120	4.2×10^4	88.7
		180	5	99.998	180	4.8×10^3	98.7

[1] Data point lost due to lab accident

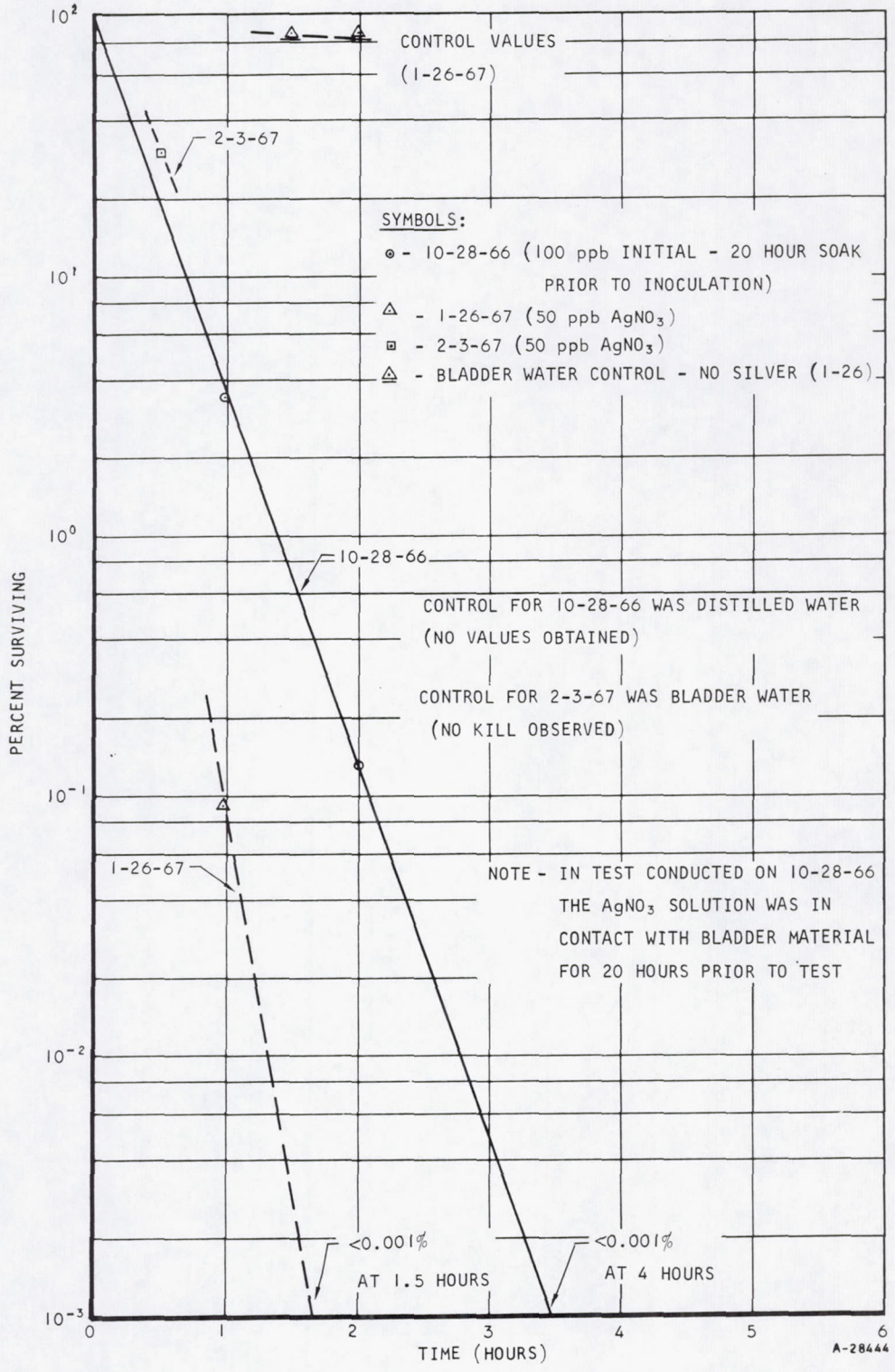


Figure 5-12. Results of Static Tests Using Inoculated (E. coli) Bladder Water and Silver Nitrate Solution

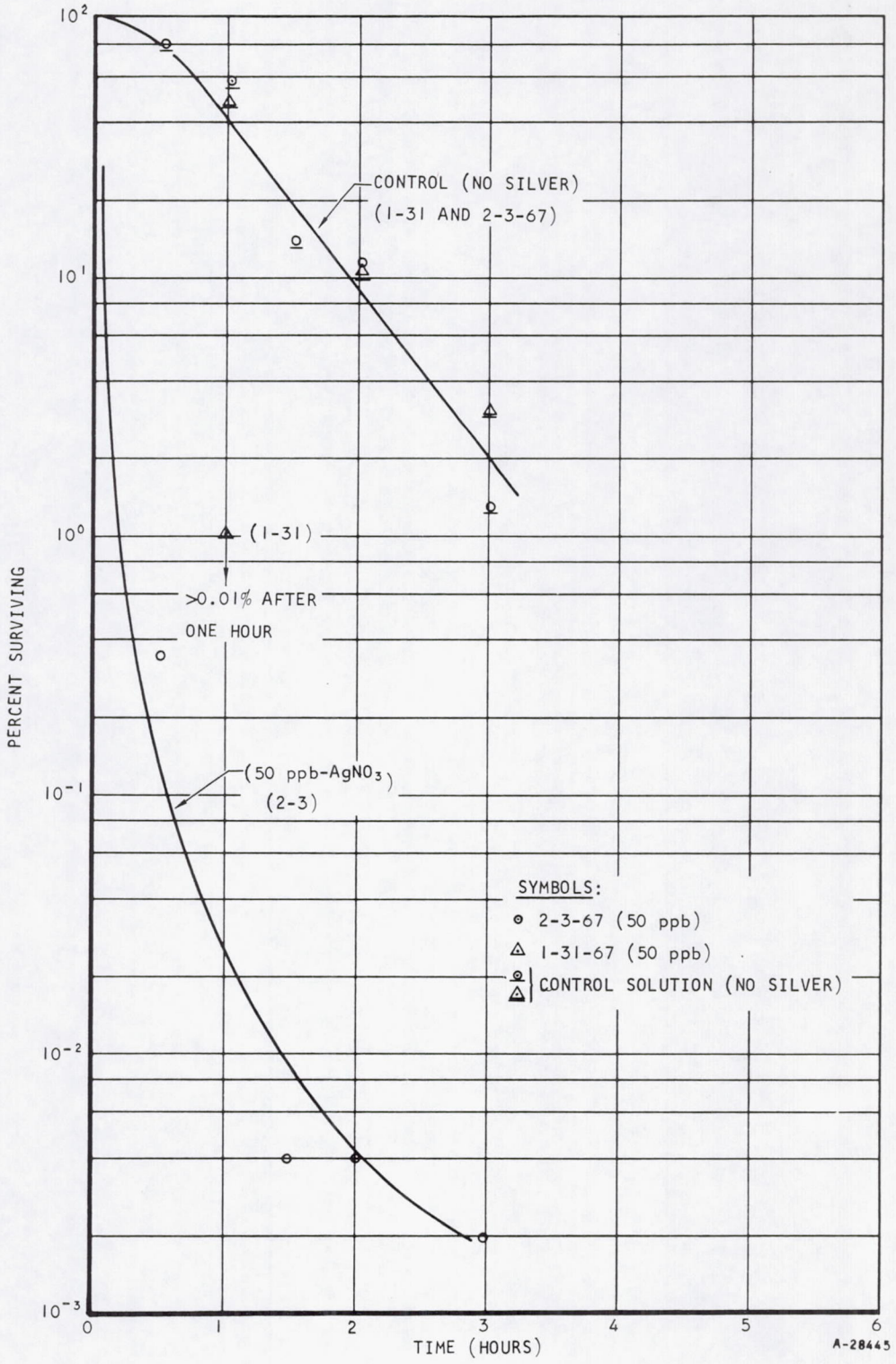


Figure 5-13. Results of Static Tests Using Inoculated (S. aureus) Bladder Water and Silver Nitrate Solution

material had soaked for 11 days appeared to provide even greater protection for the relatively silver-sensitive E. coli. The results using S. aureus were completely opposite to those obtained for E. coli. The results obtained from both test and control samples indicate that the substances leaching from the bladder material are highly toxic to S. aureus. No measurements could be made of quantity or type of compounds obtained in the water by the leaching action. However, further studies would be of extreme interest, especially with the silver resistant Bacillus subtilis var. niger spores. Further testing of the protective effect of bladder water for E. coli should be performed.

SUMMARY AND CONCLUSIONS

Extensive testing was performed to determine the efficacy of silver as a a bactericidal agent in the Apollo water systems. Fluids assumed representative of the Apollo water systems were tested to establish their effect on the efficacy of silver ions, both as silver nitrate and as silver ions generated by an electrolytic silver ion generator. The fluids tested consisted of distilled water, representative of the potable water supply, and sweat condensate, obtained from the suit circuit heat exchanger. The microorganisms used as test organisms were Escherichia coli and Staphylococcus aureus, chosen on the basis of the relative sensitivity of the former and the relative resistance of the latter to silver ions.

The greatest contributor of microbial contamination is expected to be the sweat condensate obtained from the suit heat exchanger, as large numbers of microorganisms are continually shed from the skin and upper respiratory passages. Chemical analysis of human sweat condensate showed this material to be essentially distilled water. Extensive testing of both test organisms, i.e., E. coli and S. aureus, showed that sweat condensate enhanced both the kill rate and the percent kill of both organisms. Even more significant is the enhanced kill of S. aureus at a pH of about 7.6. Thus, it can be concluded that sweat condensate is an excellent suspending medium for silver ions and that sweat condensate by itself (no silver) has bacteriostatic or bactericidal properties.

Tests utilizing silver ions produced by the silver-ion generator showed that electrolytically generated silver ions were highly effective in killing of the test organisms, in both distilled water and human sweat condensate. Although a slight loss in efficiency was observed when the silver-ion generator was in series with the cyclic accumulator, this loss was not considered significant. E. coli, suspended in distilled water, showed essentially complete kill within 90 min. S. aureus, somewhat more resistant to the bactericidal effects of silver, showed essentially complete kill within 24 hr at silver concentrations of 50 to 100 ppb. In sweat condensate at a pH of 7.6 to 7.75, S. aureus was quite sensitive to silver at theoretical silver concentrations of 100 to 146 ppb, a kill of almost 100 percent occurring at the 4-hr sampling point.

Silver ion concentrations of 50 ppb were found effective against both E. coli and S. aureus in continuous tests (six days) using inoculated distilled water, although higher concentrations up to 100 ppb would increase the kill rate and provide more effective sterilization.

The bladder material used in the hold tank was found to release minute quantities of some unidentified compound (possibly dipentamethylene thiuram tetrasulfide) into the hold tank water. This material was found to be highly toxic to S. aureus, enhancing or acting synergistically with the silver ions. Controls (no silver) were also toxic to S. aureus. Bladder water appeared to afford some protection against silver to E. coli. Although conclusions at this point would be premature, the differences in sensitivity to the leached compound or compounds may be a reflection of the gram reaction of these two organisms, E. coli being gram-negative and S. aureus gram-positive. Further investigation is recommended,

Possible silver-resistant contaminants were briefly mentioned in the discussion on the continuous tests. Although they were isolated, no attempt was made to further determine the actual, if any, silver-resistance of these organisms. Such studies must be conducted, since a highly resistant organism would have a selective advantage over sensitive forms and in a very short period of time might grow to a sizable population.

In conclusion, the data show that electrolytically produced silver is an effective bactericidal agent, in both distilled water and human sweat condensate. Some further work is required as outlined above.

Data obtained for the bacteriological tests are summarized in Table 5-14. Kill rates are summarized in Figures 5-14, 5-15, and 5-16, for comparative purposes.

TABLE 5-14

SUMMARY OF BACTERIOLOGICAL TESTS

TEST CONDITIONS	Silver Ion Source (Cell No)	E. coli		S. aureus		Other	
		Table No.	Figure No.	Table No.	Figure No.	Table No.	Figure No.
Static: (Inoculated)							
Distilled Water from Silver Ion Cell	(A)	5-6	5-4	-	-	-	-
Distilled Water from Cyclic Accumulator	(B)	5-8	5-7	-	-	-	-
Sweat Condensate	$AgNO_3$	5-4	5-1	5-5	5-2	-	-
Bladder Water	$AgNO_3$	5-13	5-12	5-13	5-13	-	-
Dynamic: (Sterilized and Inoculated)							
Distilled Water thru Silver Ion Cell only	(150) and (A)	5-6	5-4, 5-5	5-7	5-6	-	-
Distilled Water thru Cyclic Accumulator	(B)	5-8	5-8	-	-	-	-
Sweat Condensate thru Cyclic Accumulator	(B)	-	-	5-9	5-9	-	-
Continuous Flow Test: (6 days - not sterile)	(B)	5-11	5-10	19	5-11	-	-
Contamination Levels in Hold Tanks:							
Continuous System	Polycarbonate	-	-	-	-	5-10	-
Apollo Waste Water Tank							
without silver	-	-	-	-	-	5-10	-
with silver	(B)	-	-	-	-	5-10	-
Artificial Sweat: (Static tests)	$AgNO_3$ (150)	5-2	-	-	-	-	-
Comparison of Chemical Composition	-	-	-	-	-	5-3	-

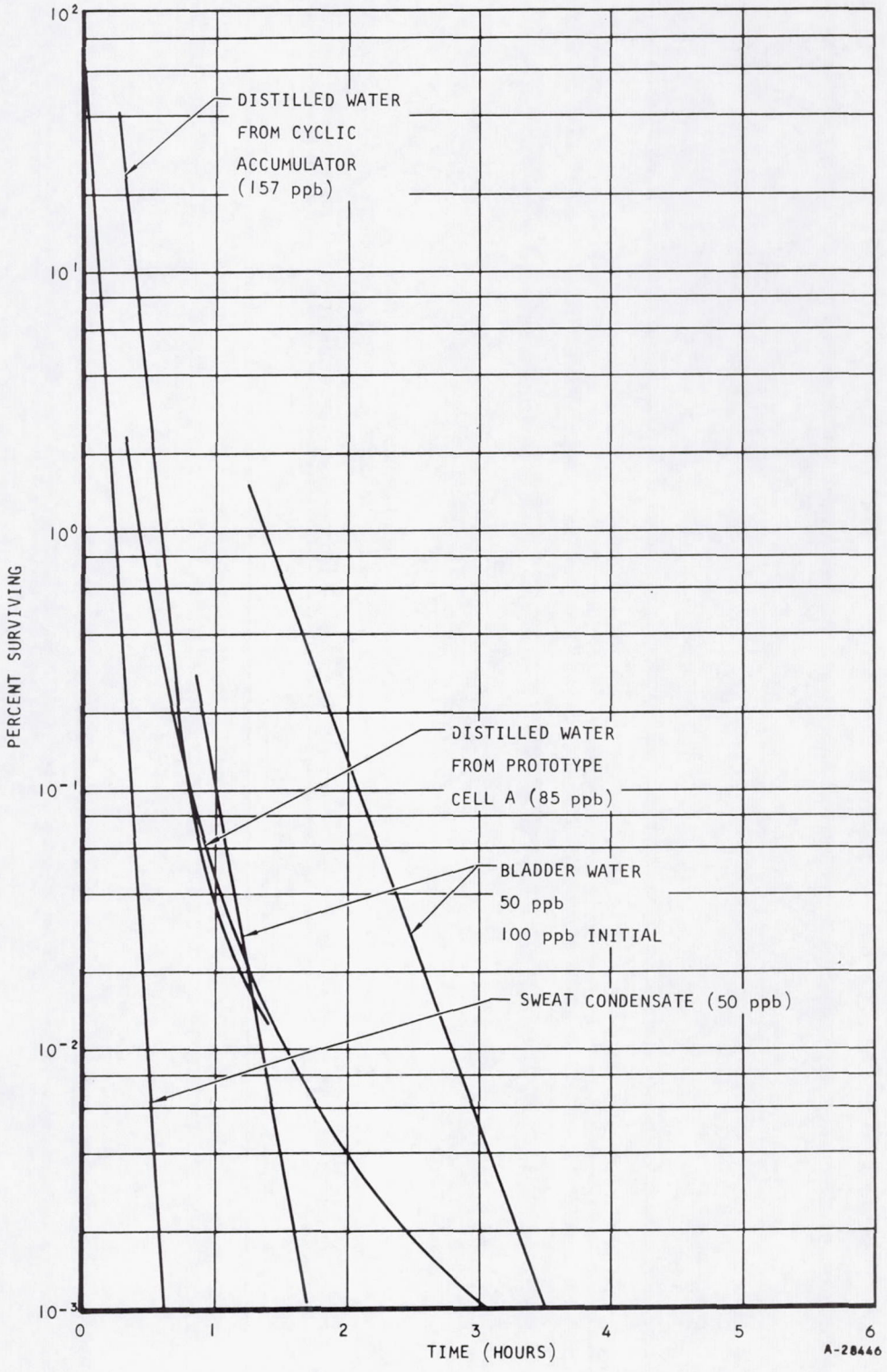


Figure 5-14. Summary of Static Tests with E. coli

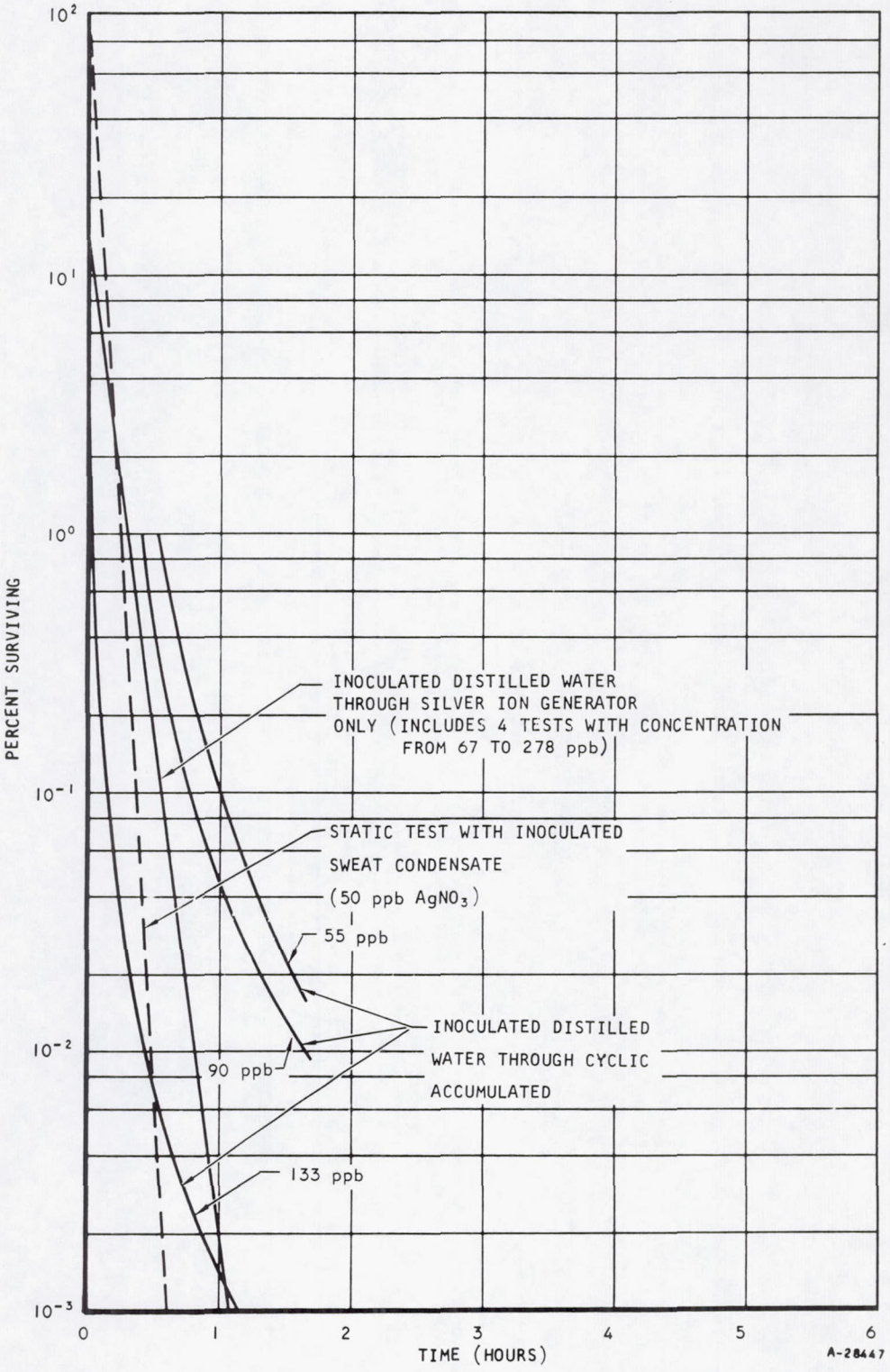


Figure 5-15. Summary of Dynamic Tests with E. coli (Static Test with Sweat Condensate Included for Comparison)

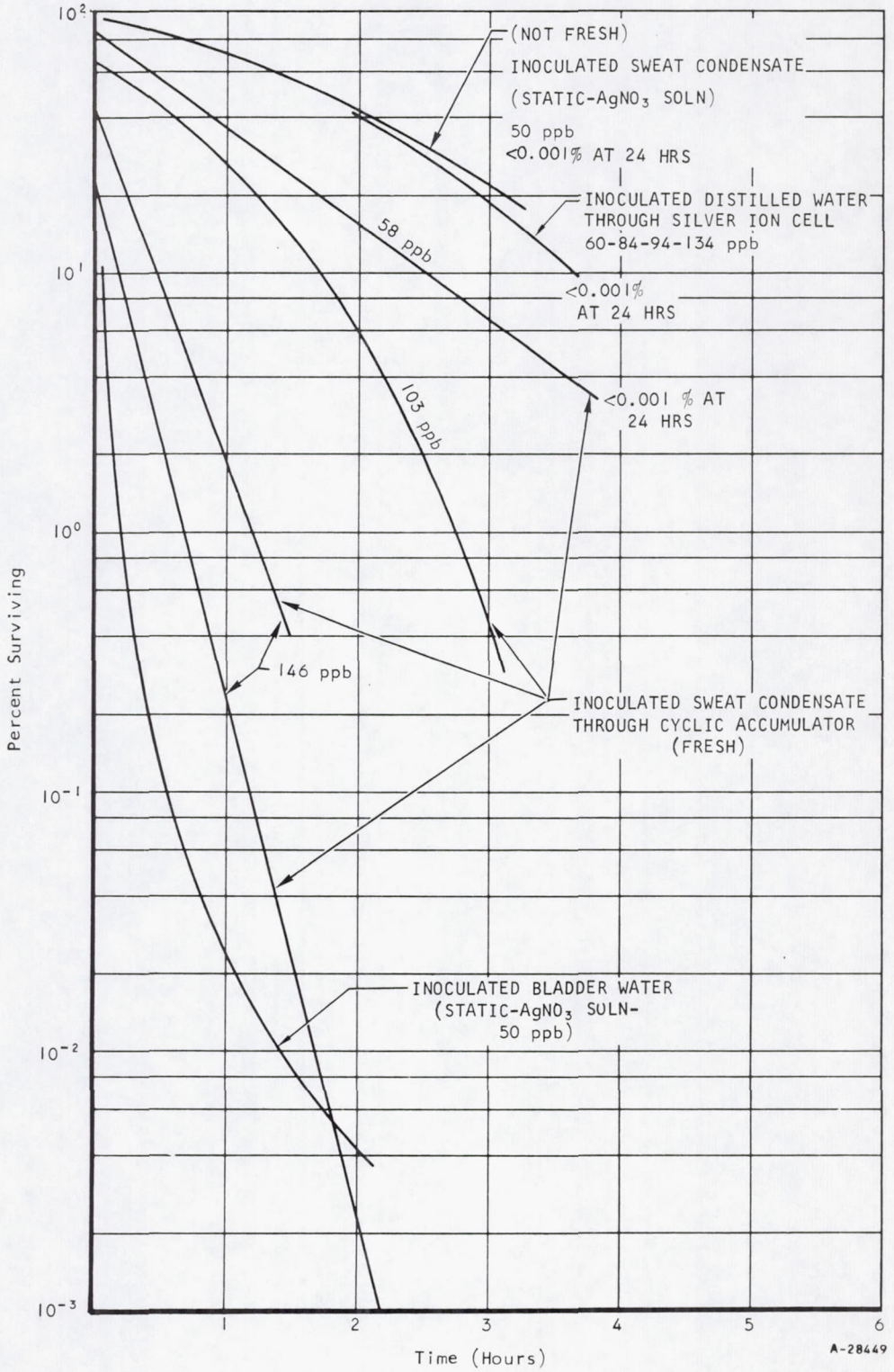


Figure 5-16. Summary of S. aureus Tests (Both Static and Dynamic)

APPENDIX
67-2158

MICROBIOLOGICAL SENSITIVITY TO DISINFECTION BY IONIZED SILVER

INTRODUCTION

This work, carried out under NASA Contract NAS9-3541, is a screening study to evaluate the effectiveness of ionized silver as a bactericidal agent against a variety of microorganisms. A series of six different bacterial species was tested to determine which of them would be used in the second phase of this study to test the effectiveness of an electrolytic silver-ion generator to release controlled amounts of silver ions into the Apollo water system. The present report is concerned only with Phase I of this study--the determination of test organisms to be used in Phase II.

The purpose of the study was to evaluate the efficacy of ionized silver as a bactericidal agent. Death curves for each organism were determined at three different pH values, in the absence and presence of ionized silver. Once pH effects were tested under atmospheric conditions, these pH values were then used to determine the effect of oxygenated and hydrogenated (deoxygenated) water on the bactericidal action of silver. Hydrogenated water was used, since hydrogenation will exist in the Apollo fuel cell water.

Wuhrmann and Zobrist (1958), have reported that the bactericidal properties of ionized silver are increased in increased amounts of oxygen; whereas deoxygenated systems show a decreased effect. Our data showed these trends, however, the percent kill appeared to be more pH dependent, confirming the data of Chambers, et al; 1962.

Examination of the data showed that the gram-negative organisms tested were more sensitive to silver than the gram-positives, though both groups exhibited greater than 99.0 percent kill after four hours of exposure. Bacillus subtilis var. niger spores at pH values of 5.0 and 7.0 showed a decrease in numbers of about 50 percent after 70 hr of testing, both in the control and test flasks. At pH 9.0 in 50-ppb silver, a one-log kill was observed after 22 hr and at 94 hr a two-log kill. Thus, for short term experiments, Staphylococcus aureus and albus were chosen as the test organisms for the second phase of this study. For longer-term studies, the Bacillus subtilis var. niger spores could be used. In addition, an extremely sensitive organism such as E. coli can be used to determine levels of residual organisms for short-term experiments under conditions which do not have an appreciable effect on S. aureus.

MATERIALS AND METHODS

Materials

1. Organisms

Both gram-negative and gram-positive bacteria were used for the screening study. Gram-negative organisms used were Escherichia coli and Alcaligenes faecalis; Bacillus stearothermophilus, Staphylococcus aureus and Staphylococcus albus comprised the gram-positive group. Bacillus subtilis var. niger spores were also used as a test organism.

Cultures of E. coli and B. subtilis spores were obtained from culture collections of Fort Detrick, Maryland. The latter consisted of a dry powder. A. faecalis, S. aureus and S. albus were obtained from the culture collection of the University of Southern California. The B. stearothermophilus culture was obtained from the University of California at Los Angeles.

2. Media

All organisms except the spore suspension were grown in Tryptic Soy Broth (TSB), (Difco) for 10 to 24 hr prior to being used as test organisms. Plate counts were made using Tryptic Soy Agar (TSA), (Difco).

Methodology

1. Preparation of Test Organisms

Prior to each day's testing, the test organism was grown in TSB for 18 to 24 hr at 37^0C in 250 ml. Ehrlenmayer Flasks. B. stearothermophilus was grown at 55^0C. All organisms were grown in standing culture in 50 ml of TSB. Inoculations were made from TSA slants. The culture was then centrifuged, the supernatant was discarded, and the culture was then washed in sterile distilled water and recentrifuged. The organisms were then resuspended in sterile distilled water and adjusted to a predetermined absorbency at 660 mm with a Bausch and Lomb Spectronic 20 spectrophotometer to give a stock suspension. The values for each organism are given in Table I in the results section. The working suspension was then prepared from the stock suspension by a 1/1000 dilution of the stock suspension, yielding approximately 5×10^5 organisms per ml, except where indicated in Table I.

The spore suspension was prepared by adding the dry spore powder to sterile distilled water to make a stock suspension. This stock suspension was then diluted to give the desired absorbency as outlined above. Prior to dilution of the spore suspension, it was heated at 80^0C for 10 min to kill any vegetative calls present.

2. Preparation of Glassware and Silver Solutions

All tests were run at silver-ion concentrations of 50 ppb in distilled water. Determination of silver-ion concentration was made by employing the dithizone method (Reference 1). The glassware used for the silver tests were preconditioned with silver solutions (50 ppb). Flasks and/or test tubes were filled with the silver solution, allowed to stand overnight and then refilled three more times before using, for purposes of equilibrating the glass with the silver/ion concentration to be tested.

3. Preparation and Inoculation of Test and Control Solutions

Each organism tested was run in silver solution and a control solution without silver. The desired pH values were obtained by adjusting the test solutions with 0.1N HCl or 0.05N KOH with a Beckman Zeromatic pH mater with a thermoregulator probe. The test solutions at pH 9.0 were buffered using

0.0025M dipotassium phosphate as the test solution. (Chambers, et al., 1962) Each flask and/or tube was inoculated with the test organism in amounts to give the desired number per milliliter. All testing was carried out at 25° ±2°C.

Oxygen saturation of the test solutions was carried out by bubbling oxygen through the test solutions for a period of 25 to 30 min. At the end of this period, 30 ml of the saturated solution was poured into a sterile test tube which had previously been flushed with oxygen and which was then inoculated with the test organism. The test tube was then stopped with a sterile rubber diaphragm. A 2-1/2-cc sterile disposable syringe with a 20-gauge needle was first flushed with oxygen contained in a two-liter vacuum flask, prior to taking of samples.

Hydrogenation (deoxygenation) was carried out by first bubbling nitrogen gas through the test solutions for a period of 15 to 20 min. Hydrogen gas was then bubbled through the solutions for 25 to 30 min. The flasks were then inoculated with the desired organism and 30 ml was transferred to a test tube previously flushed with hydrogen gas. The test tubes were stoppered with a rubber diaphragm. A two-liter vacuum flask was filled with hydrogen gas, which was used to flush the needle and syringe used to obtain samples.

4. Plating and Quantitization of Samples

Samples for plating were obtained from flasks using 1-ml sterile disposable pipettes (Flacon Plastics). These samples either were used for direct plating or were diluted in serial dilutions of 1/10 in sterile 9.0-cc water blanks and then plated at the desired dilution. Sterile disposable syringes were used to obtain samples from test tubes.

Plating of the organisms was carried out using the pour-plate method. 1.0 cc or 0.1 cc of the desired dilution was pipetted into a sterile disposable petri dish (Falcon Plastics). 15 to 20 ml of TSA cooled to 47°C was poured into the plates containing the sample, rotated to spread the organisms, and then allowed to solidify. All organisms were incubated at 37°C for 72 hr, except B. Stearothermophilus which was incubated at 55°C. Duplicate plates were made for each dilution run.

At the end of the incubation period, the plates were counted with the aid of a Quebec Colony Counter.

Results and Discussion

Each organism to be used for this screening study was standardized to a predetermined absorbency on a Bausch and Lomb Spectronic 20 at 660 mu. In this manner a standard inoculum could be used for each test. Table I summarizes the absorbency and the inoculum size for each test organism used. These values were used throughout the project.

TABLE I

Organism	A660	Org/cc	Dilution for Test
B. subtilis var. niger spores	0.24	4×10^7	0.01/100 cc (4×10^3)
A. faecalis	0.27	5×10^8	0.1/100 cc (5×10^5)
S. albus	0.28	5×10^8	0.1/100 cc (5×10^5)
E. coli	0.32	5×10^8	0.1/100 cc (5×10^5)
S. aureus	0.22	5×10^8	0.1/100 cc (5×10^5)
B. stearothermophilus	0.54	1×10^7	1/100 cc (1×10^5)

The working suspensions of 5×10^5/ml were chosen because no turbidity was produced by this concentration and this is the probable level to be expected, assuming small amounts of organics in the water supply released from dead bacteria. The spore suspension used was 4×10^3. This two-log decrease in concentration was chosen as only few spores would be expected to contaminate the Apollo fuel cell water or the condensate from the heat exchanger.

Also, in studies carried out on the Apollo heat exchanger, microbiological sampling of the cyclic accumulate showed a consistent level of microorganisms to be present in the cyclic accumulate at a concentration of about 5×10^5/cc (Reference 2).

This study was divided into two parts. Part one consisted of determining the percent kill in distilled water (control) and in distilled water containing 50 ppb ionized silver at three different pH values, 5,7, and 9.0. These pH values were selected as they fall at the extremes and within the expected range of the Apollo fuel cell water. Part two was concerned with testing the effect of oxygenated and hydrogenated (deoxygenated) water on the bactericidal properties of ionized silver. Oxygenated water has been shown to enhance the bactericidal properties of ionized silver, thus tests in oxygenated water were run at pH values which showed least amount of kill under normal conditions. In the absence of oxygen, the bactericidal properties of ionized silver are decreased, thus pH values were chosen which showed the greatest percent kill under normal conditions.

Tables II and III show the percent kill obtained with the test organisms using distilled water with and without ionized silver. All tests were run at pH values of 5,7, and 9 except where indicated.

TABLE II

PERCENT KILL OF GRAM POSITIVE AND GRAM NEGATIVE BACTERIA IN DISTILLED WATER AND DISTILLED WATER WITH SILVER

Organism	Total Test Time (hr)	Percent Kill					
		Control pH			Ag (50 ppb) pH		
		5	7	9	5	7	9
Gram Positive							
Staphylococcus albus	4	94.26	84.29	47.1	97.0	>96.0	>99.99
Staphylococcus aureus	3	77.5	63.18	23.5[1]	13.8[2]		
Bacillus stearothermophilus	3	99.97	99.94	99.92	99.96	99.92	99.91
Gram Negative							
Alcalilgenes faecalis	3 1/2	95.7	98.8	99.64	99.995	99.997	>99.9999
Escherichia coli	3	0.00	37.0	73.3[1]	>99.999[2]		

[1] The solutions at pH 9.0 were unbuffered and became acid within 3 hours.

[2] pH 5.6

TABLE III

PERCENT KILL OF B. SUBTILIS VAR. NIGER SPORES
UNDER VARIOUS CONDITIONS

Total Test Time (hr)	Percent Kill					
	Control pH			Ag (50 ppb) pH		
	5	7	9	5	7	9
46	6.1	46.5	31.2	37.6	68.8	96.02
94	24.2	50.0	31.2	54.6	66.2	97.63
Final pH at 94 hr	6.3	6.5	7.6	5.7	6.6	7.6

Examination of the data shows that the percent kill of S. aureus and S. albus appears to be dependant on pH in the control solution. S. albus was most stable at alkaline pH, whereas at pH 5, greater than 94 percent kill was effected. In 50 ppb silver, the greatest skill occurred at pH 9. S. aureus was similar to S. albus, being stable at alkaline pH but showing only slight kill at pH 5.6 in silver; whereas at pH 9.0 greater than 99.9999 percent kill was obtained. B. stearothermophilus showed rapid kill in both control and test conditions. The greatest kill in silver occurred at alkaline pH. Since the percent kill of B. stearothermophilus was not complete, it was suspected that any surviving organisms might be due to the formation of spores. Microscopic examination of a 24-hour culture showed that sporulation was taking place. Thus the vegetative cells are being killed within the first 1 1/2 hours, the numbers then remaining constant for the remainder of the test due to the presence of resistant spores.

Of the two gram-negative organisms tested, A. faecalis showed a rapid and almost complete kill in both the control and test conditions. E. coli appears to be stable at acid and neutral pH. Silver at pH 5.6 showed a percent kill greater than 99.9999 percent. The gram-negative appear to be more sensitive to the bactericidal properties of silver than the gram-positive species.

Thus silver is more affective as a bactericidal agent at alkaline pH for both gram-positive and gram-negative organisms, although the gram-negative species tested were sensitive to silver at acid pH.

Wuhrmann and Zobrist (1958) found that phosphates interfere with the bactericidal properties of silver. Tests run at pH 9.0 were buffered with 0.0025H phosphate buffer. The data obtained do not appear to indicate any interference with the bactericidal action of the ionized silver.

As pointed out in the above discussion, oxygen enhances the bactericidal properties of silver; whereas anaerobic systems decrease the percent kill. Table IV summarizes the percent kill obtained in oxygenated systems with and without 50 ppb silver.

The effect of oxygenation on vegetative cells appeared to enhance the kill, in both control and test systems, especially at the alkaline pH levels. The effect of oxygen on spores decreased the kill rate in both systems. The reason for this anomaly is not apparent at this time, but may be due to the pH at which the test was run. Under anaerobic conditions at pH 9 in the presence of silver, 88.9 percent kill was observed. Thus, it is apparent once again that the percent kill in the presence of silver is in large part dependent on the pH at which the test is carried out. Oxygen appeared to protect S. albus both in the controls and at pH 5 in the silver test, although comparable kills were noted for the more alkaline conditions under both normal and oxygenated conditions. Both gram-negative species tested showed high percent kills under normal and oxygenated conditions. Oxygen increased the percent kill in the control tests, the percent kill increasing as the pH moved to the more alkaline values, once again pointing out the influence of pH on the kill.

As the fuel cell water in Apollo will be hydrogenated, tests were run on the test organisms to observe the effect of anaerobic water on the efficancy of ionized silver under these conditions (Table V). All tests were run at pH 9.0. S. albus showed no decrease in percent kill in relation to tests carried out under normal conditions (see Table II). S. aureus was observed to have a decrease of two logs in kill when compared to tests run at normal conditions. The percent kill of the two gram negative organisms was not affected. A slight decrease in percent kill was noted with the spores in comparison to normal conditions at pH 6.5.

The data included in this report in table form are only a small part of the data collected. The rest of the data are presented in graph form, found at the end of this report.

The test organisms to be used for the second phase of this study were selected on the basis of their sensitivity to ionized silver and the effect of oxygenation and hydrogenation on the bactericidal effects of silver. S. aureus was chosen as it is sufficiently sensitive to silver, but does not show the extreme sensitiveity, either in distilled water or distilled water with silver, that was shown by B. stearothermophilus or by both gram-negative species. For long-term studies, B. subtilis var. niger spores will be used.

E. coli can be used for short term studies under conditions having little effect on S. aureus or S. albus.

TABLE IV

EFFECT OF OXYGEN ON SILVER AS A BACTERICIDAL AGENT

Organism	Total Test Time (hr)	Percent Kill (Oxygenated)					
		Control pH			Ag (50 ppb) pH		
		5	6.5	9.0	5	6.5	9.0
Gram Positive							
Staphylococcus albus	3		66.8	28.3	64.3	>99.9999	99.82
Staphylococcus aureus	3	73.3	89.75	90.45	73.7	>99.9999	99.99
Bacillus steao-thermophilus	3		99.997			>99.9999	
Gram Negative							
Alcaligenes faecalis	3		99.96			99.99	
Eacherichia coli	3	44.7	80.25		99.86	99.99	
Bacillus subtilis var niger spores	46		9.7			12.5	

TABLE V

EFFECT OF HYDROGENATED WATER ON THE PERCENT KILL
OF 50 PPB SILVER AT PH 9.0

Organism	Total Test Time	Percent Kill	
		Control	Ag (50 ppb)
Gram Positive			
Staphylococcus albus	4	11.3	>99.999
Staphylococcus aureus	4	93.56	98.97
Gram Negative			
Alcaligenes faecalis	2	99.28	99.999
Escherichia coli	4	38.2	99.93
Bacillus subtilis var niger spores	46	27.9	88.9

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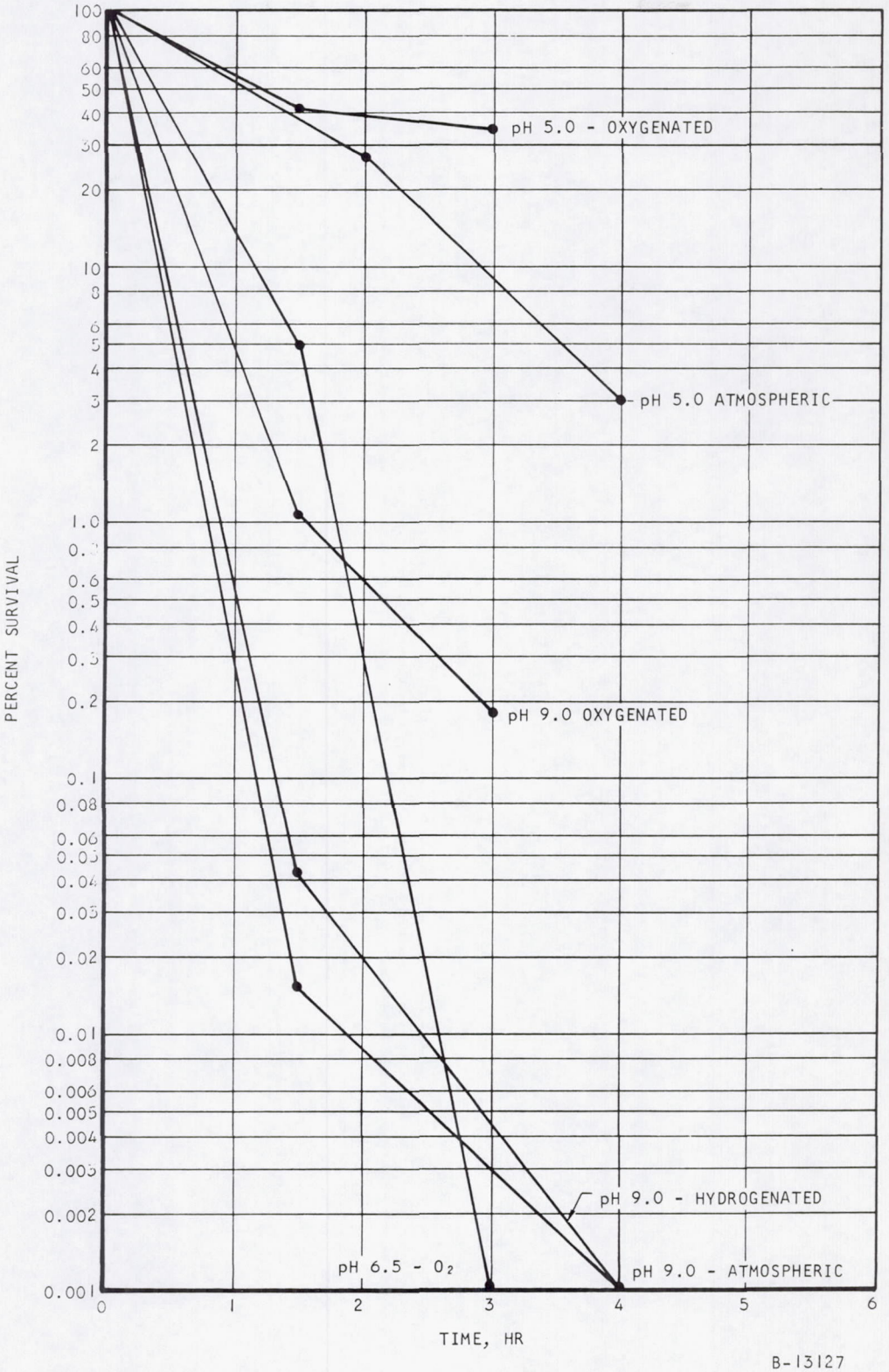
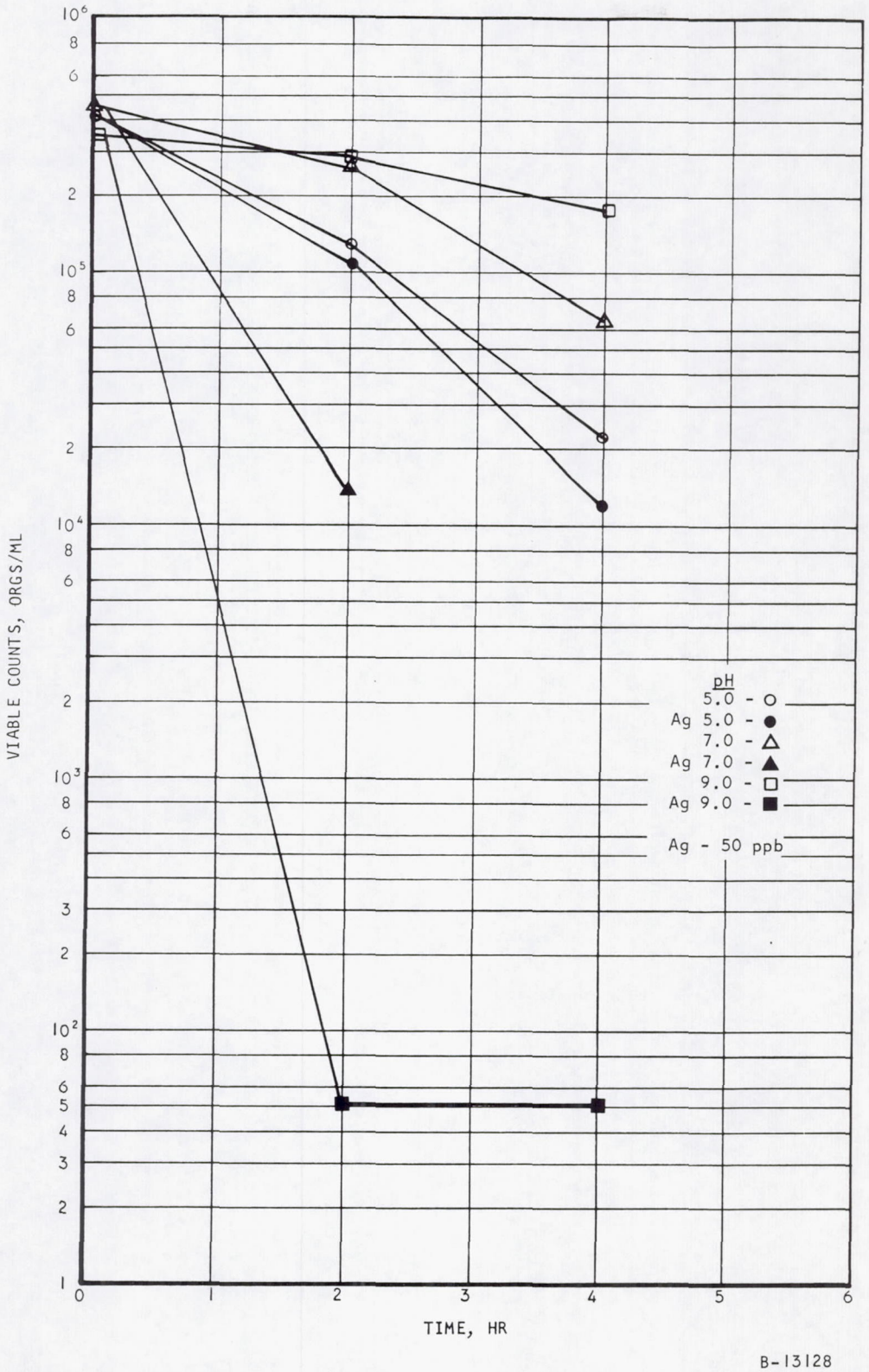
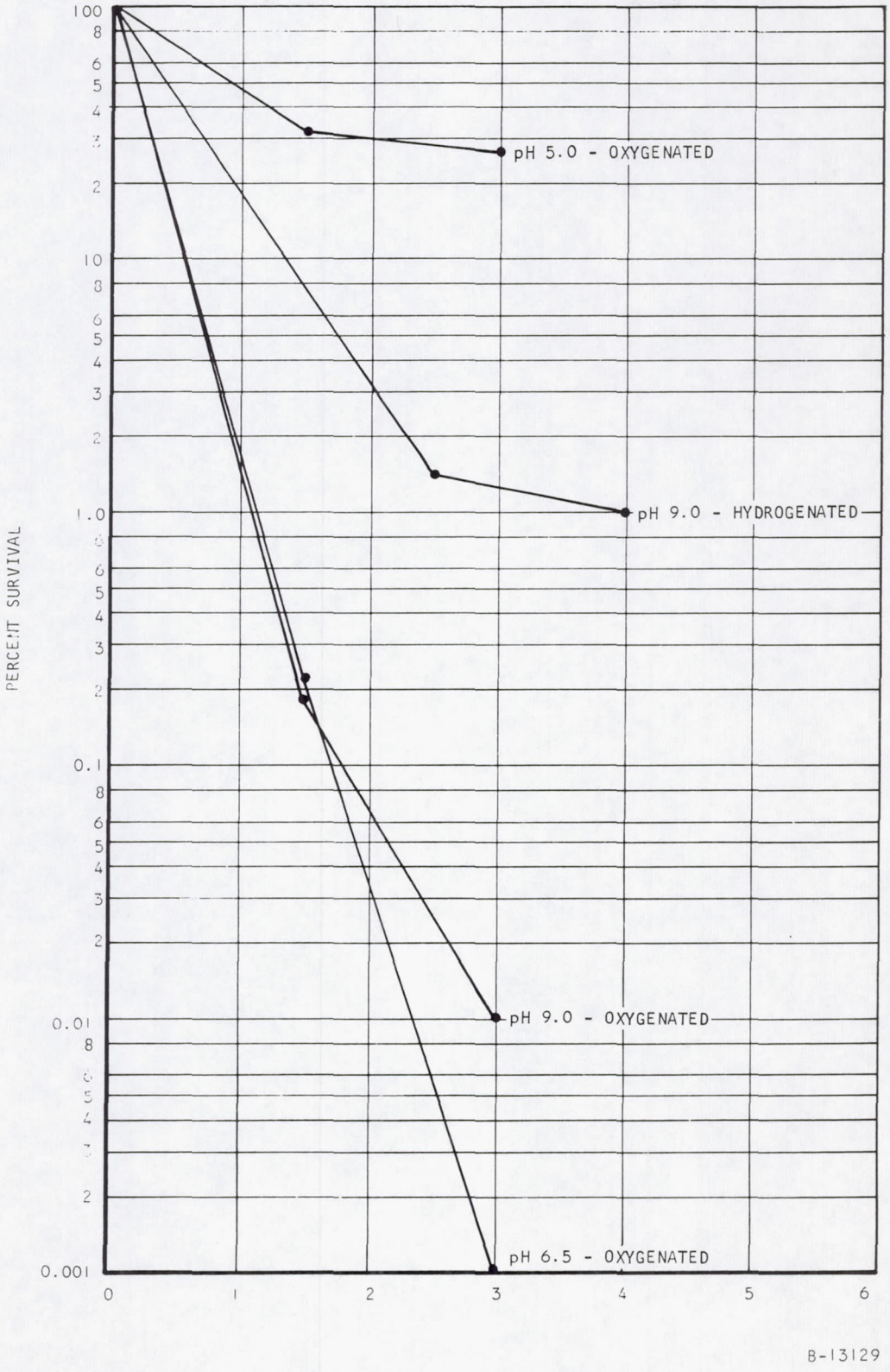


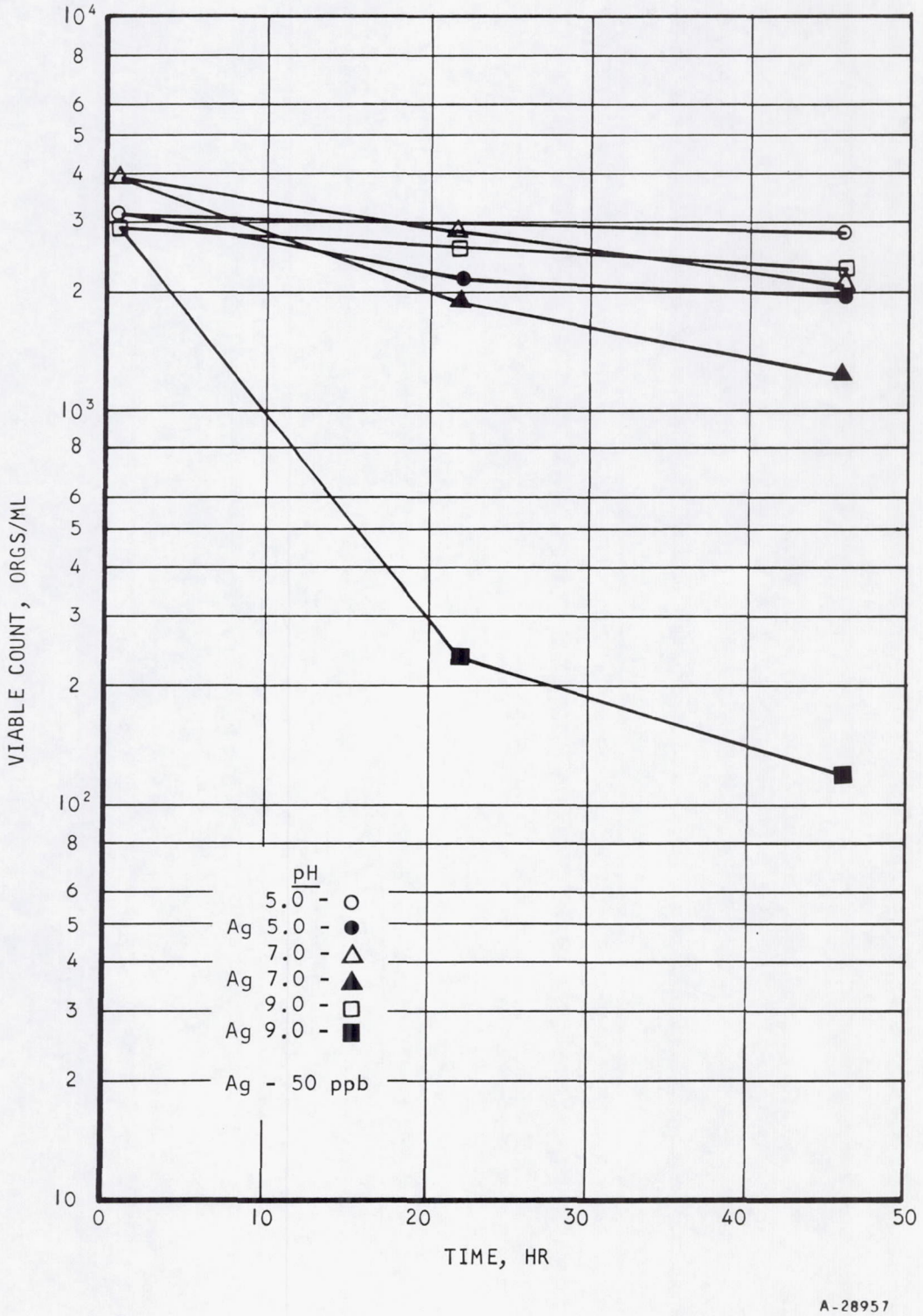
Percent Survival of Staphylococcus albus in 50 ppb Ionized Silver Under Various Conditions



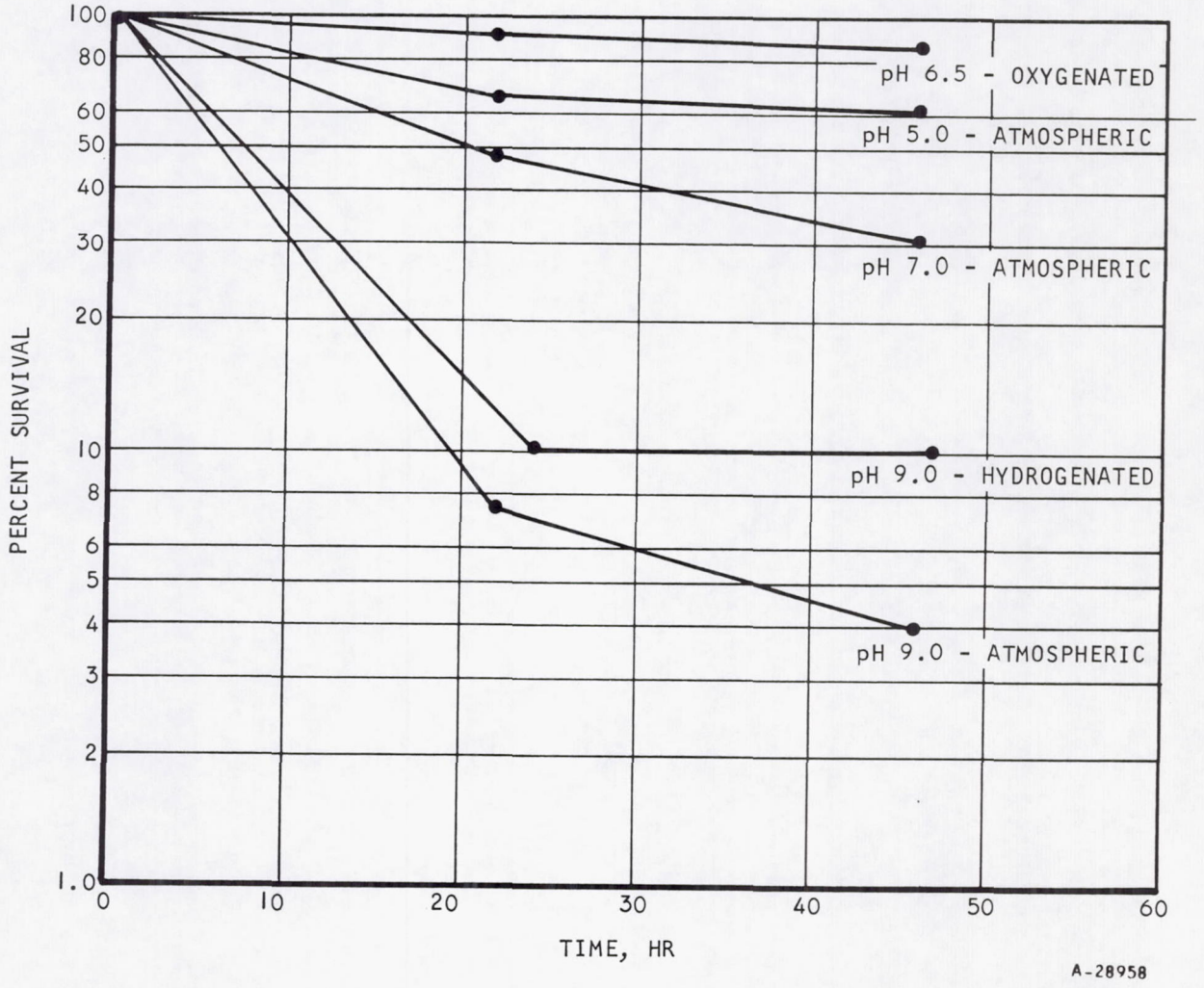
Staphylococcus albus



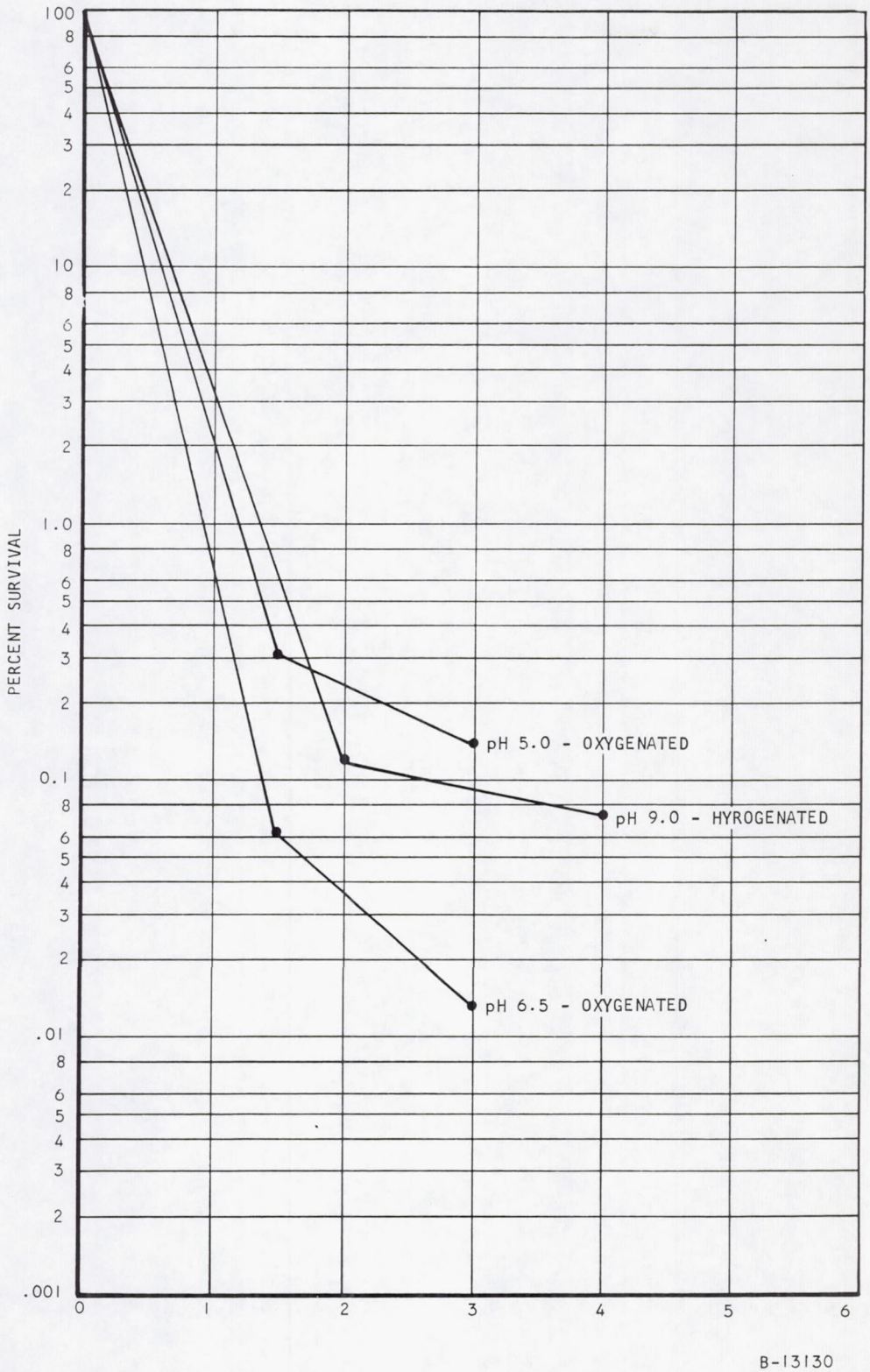
Percent survival of Staphylococcus aureus in
50 ppb. Ionized Silver Under Various Conditions



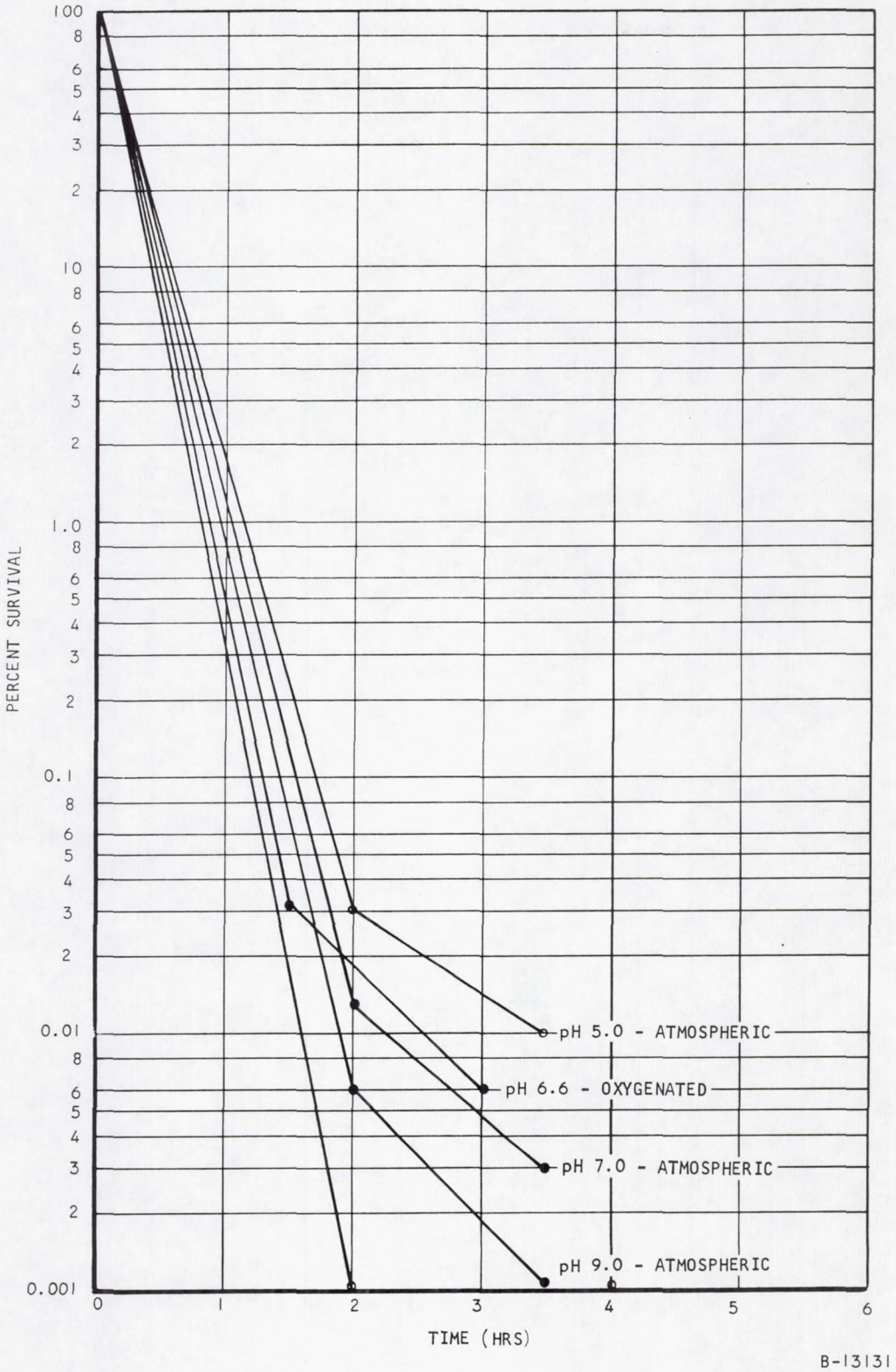
Bacillus subtillis var. niger spores



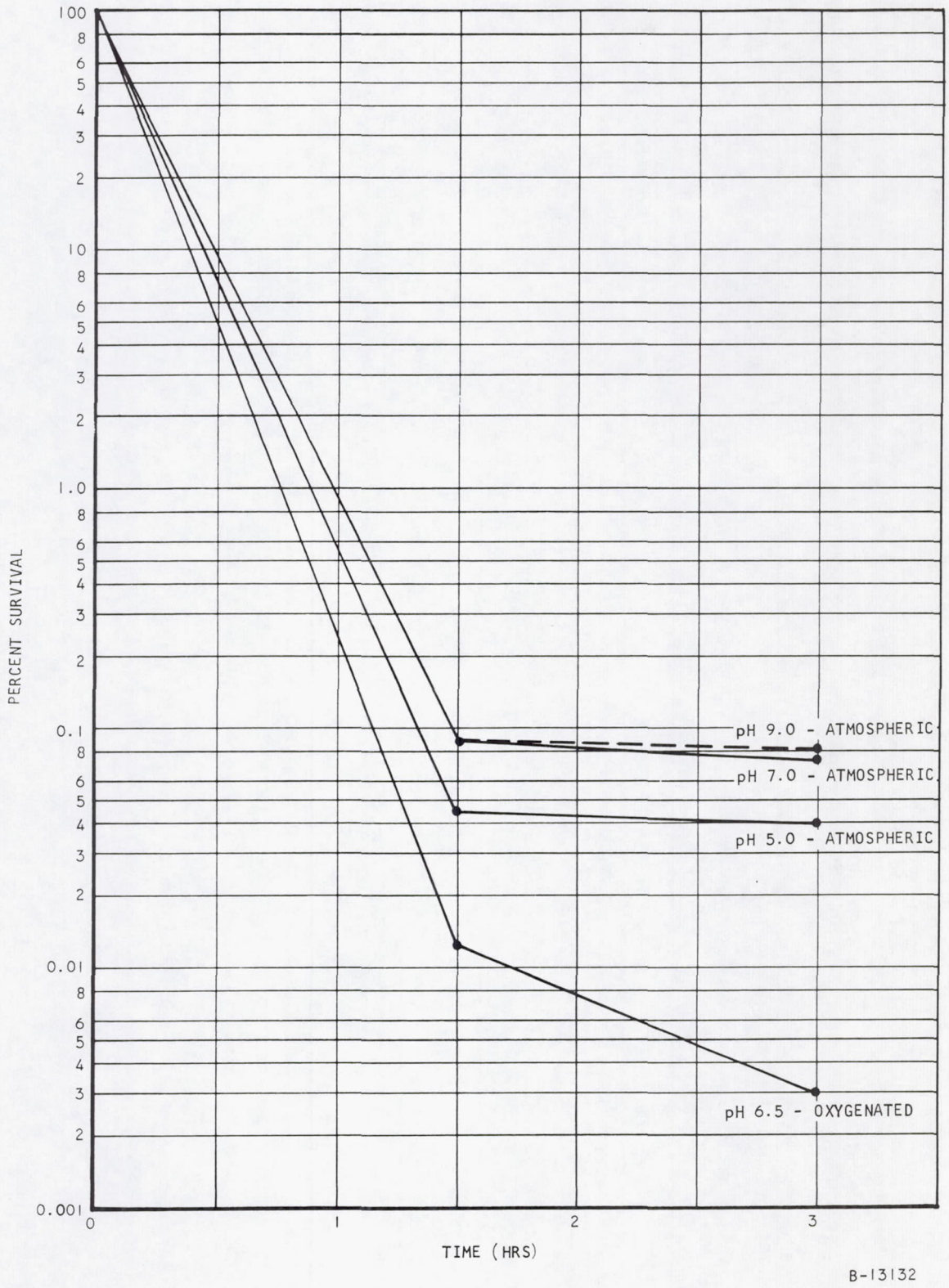
Percent Survival of Bacillus subtillis var. niger Spores
Under Various Conditions in 50 ppb. Ionized Silver



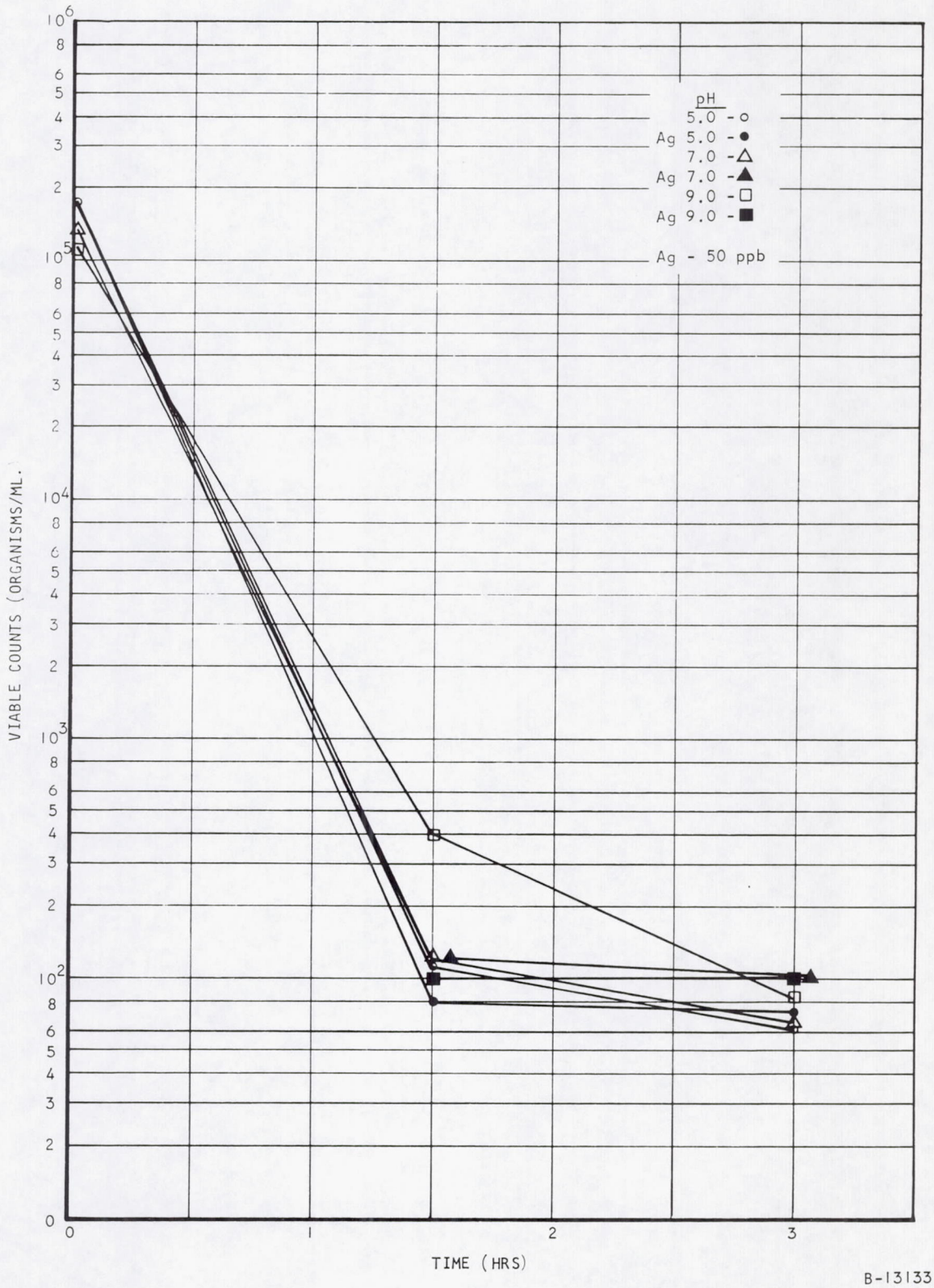
Percent Survival of Escherichia coli in 50 ppb Ionized Silver Under Various Conditions



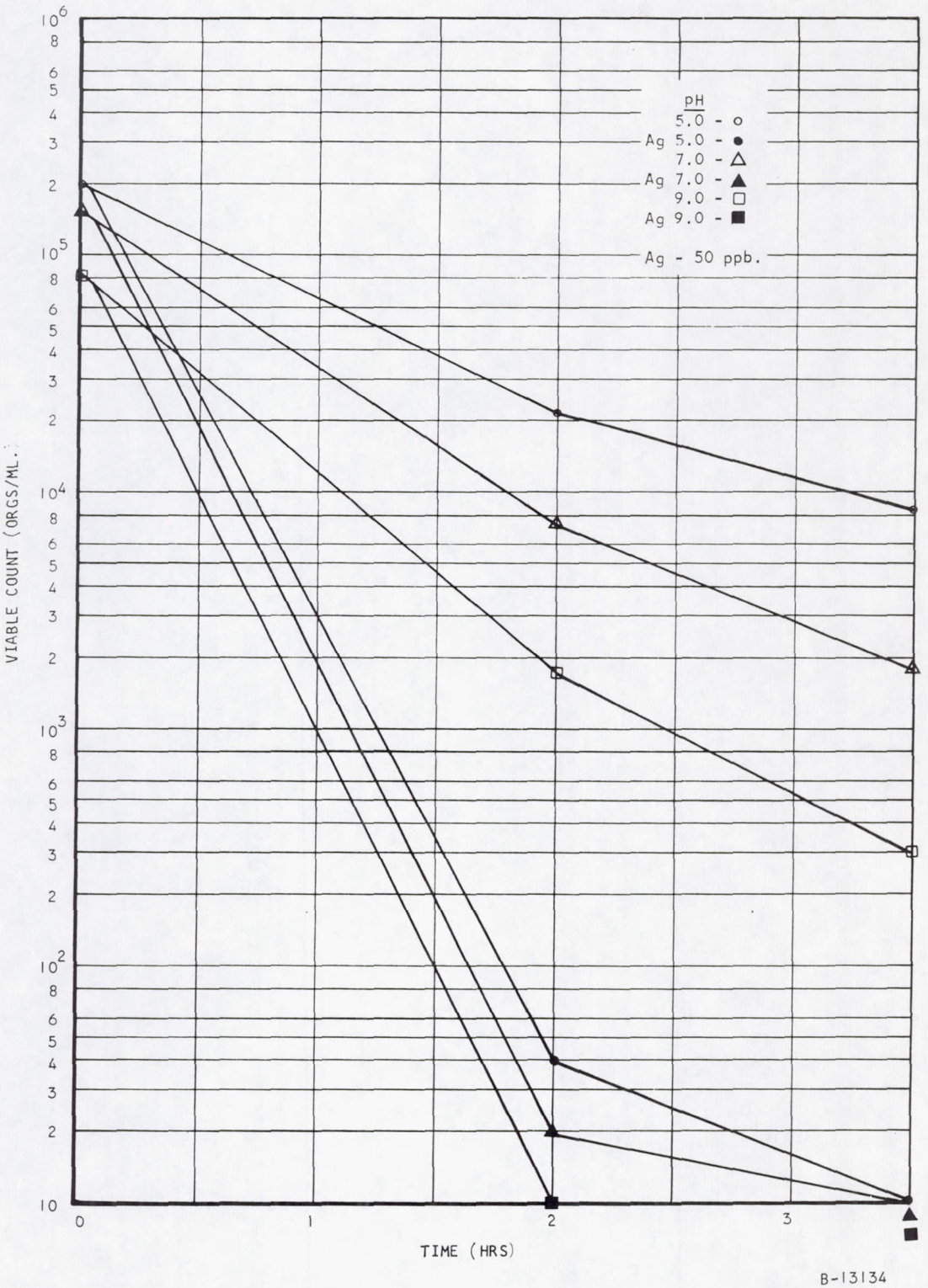
Percent Survival of Alcaligenes faecalis in 50 ppb Ionized Silver Under Various Conditions



Percent Survival of Bacillus stearothermophilus in 50 ppb Ionized Silver Under Various Conditions



Bacillus stearothermophilus



Alcaligenes faecalis

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