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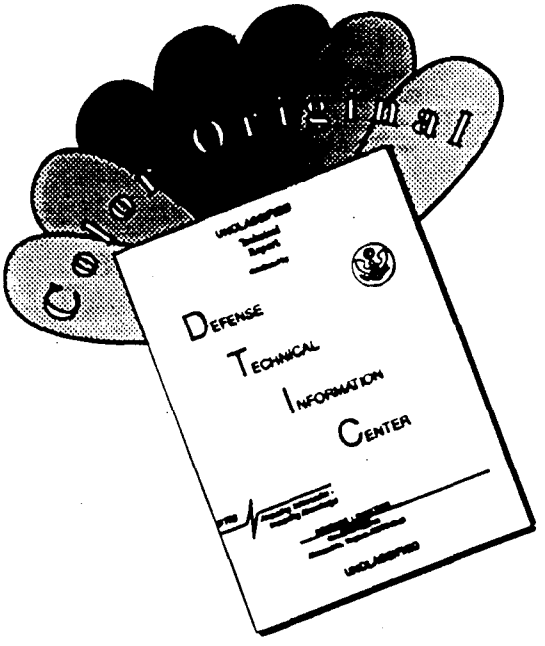

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13. ABSTRACT ***(Maximum 200 words)***

This project focuses on the utilization of high concentration ozone as an alternative to ethylene oxide for use in medical sterilization systems. Evaluations of high concentration, electrochemically generated, ozone clearly demonstrated that: 1) it is an effective medical sterilant with a D-value of less than 10 minutes for bacterial spores, 2) high humidity (90-95% r.h.) is critical for optimum ozone sporicidal activity, and 3) the application of pressure (5 to 15 psig) increases the sporicidal activity of ozone. The findings provided the foundation for the design and fabrication of a prototype high concentration ozone sterilizer for field use.

The prototype ozone sterilizer has a cycle time of 1 hour or less, and a maximum surgical pack throughput of three trays per sterilization cycle. The prototype design is modularized into the following six subsystems: 1) power, 2) ozone generation, 3) cassette, 4) thermal control, 5) electronics, and 6) ozone destruct. The operation of these six subsystems is controlled by an Intel 80c188 microprocessor operating at 16 MHz. The microprocessor continuously monitors the subsystems and controls all aspects of sterilizer operation. The prototype will provide high quality sterilization of medical equipment with minimum logistical requirements and training of personnel.

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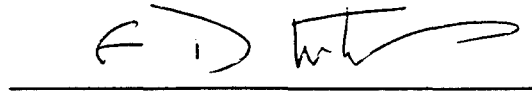

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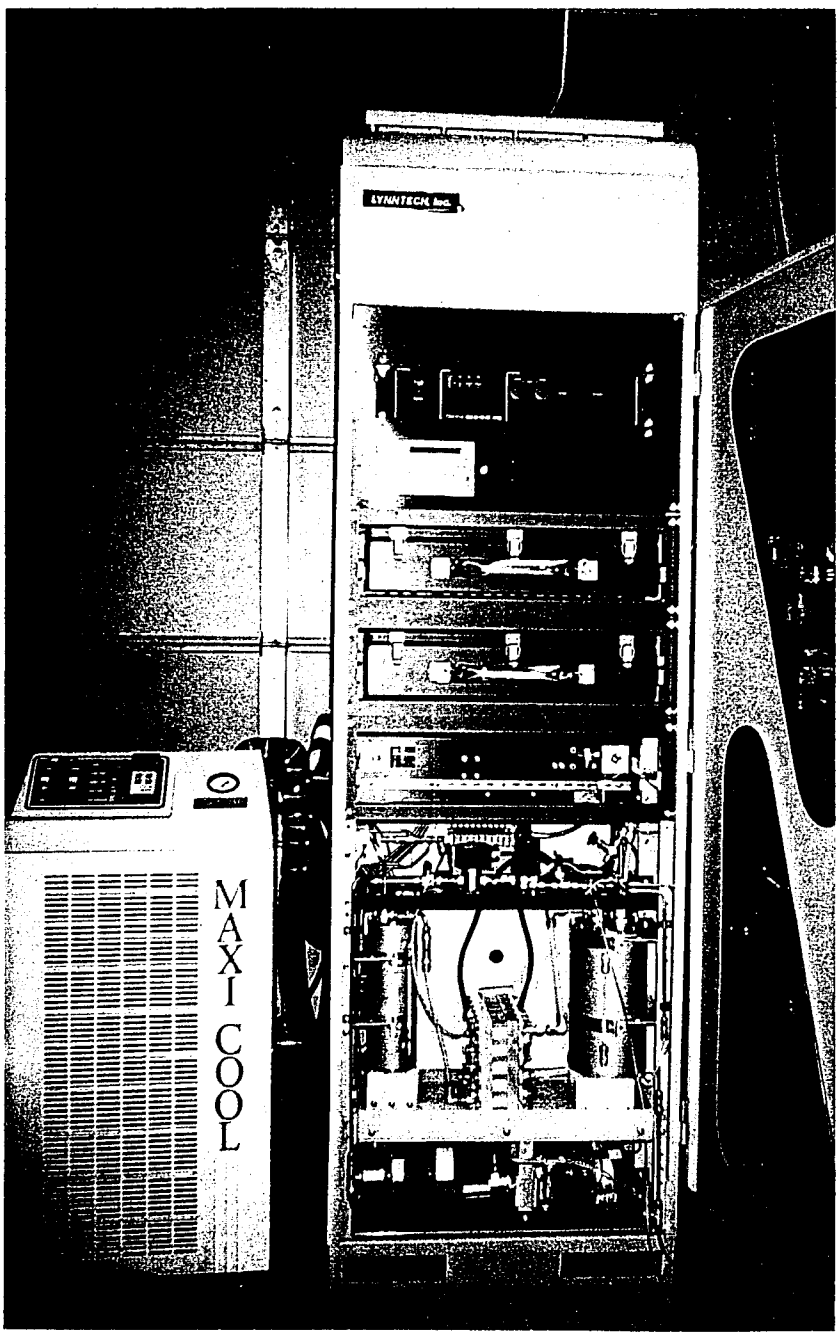

Lynntech's Ozone Sterilizer (the *Sterizone*) utilizes eclectrochemically generated ozone (11-16 wt%) as a highly efficient microbiological sterilant. Three cassette-type sterilizing units (shown in the mid-section of the console) can be sterilized at the same time or in sequence, with a turn-around time of 60 to 90 minutes (including air wash). An important feature of this technology is that ozone degrades to oxygen, thus eliminating any hazardous byproduct.

SECTION I

INTRODUCTION

The aim of the two-year SBIR Phase II project is the development and demonstration of a mobile, prototype gaseous ozone based sterilizer. For military field hospital applications, fast sterilizer cycle times and the ability to operate with few logistical constraints are critical requirements. Ethylene oxide (EtO) is the primary gas used by hospitals and industry to sterilize items that cannot be sterilized by steam or radiation. A chief disadvantage of EtO is the excessive processing time (approx. 24 hours) required[1-3]. A second major disadvantage of EtO is that it must be transported in pressurized cylinders which present logistic difficulties. Furthermore, an increasing emphasis toward personnel safety and the potential for environmental impact related to EtO usage has stimulated the need for new approaches to gas sterilization.

Ozone (O_3) is an antimicrobial agent that is widely used for disinfecting drinking water[4]. The main advantages of ozone gas as a sterilant are its low processing temperatures and easy decomposition into the non-polluting effluent (oxygen). To date, the use of ozone gas as a sterilant has not been feasible due to the limitations of the existing ozone generating technology (the corona discharge process). This process is complex and has several undesirable features[5,6]. Only low gas phase ozone concentrations can be generated by this method (1-2 percent by weight using an air-feed corona, or 2-6 wt% using an oxygen feed corona[7]). Also the feed gas to the corona unit must be extremely dry (i.e., moisture removal to a minus 40°C dew point or lower) which is especially counter productive as the sporicidal activity of ozone is impaired at low humidity levels [8].

This report describes the development of a sterilization system which uses ozone gas generated by electrochemically splitting water. Very high ozone concentrations are generated by the electrochemical process, up to 10 times greater than the concentration that can be obtained from an air-feed corona discharge unit. The logistical requirements are minimal, only a source of relatively pure water and electrical energy are needed to produce ozone by this method. Furthermore, ozone is easily decomposed into oxygen thus reducing personnel safety and environmental concerns. The proposed ozone gas sterilizer is innovative because it combines electrochemical ozone generation with a broad-based new approach to gas sterilization as a direct result of the high concentration of ozone which can be produced on demand and generated from water.

The detailed evaluations of ozone as a medical sterilant conducted during Year 1 and the first quarter of Year 2 have provided Lynntech with a strong foundation upon which to design a prototype gaseous ozone medical sterilizer. This work also provided specific insights into environmental factors which impact on the effectiveness of gaseous ozone as a medical sterilant. Primary among these factors is humidity. High humidity was shown to enhance the effectiveness of gaseous ozone as a sterilant.

With this information in hand, the focus of the present report and Year 2 of the project has been the design, fabrication, and testing of a computer controlled automated prototype ozone sterilization unit. The major action areas involved with this goal were hardware development and fabrication, and software control systems program development.

SECTION II

SCOPE

Electrochemical ozone production is an emerging technology with a wide range of potential applications including gaseous sterilization, water purification, and disinfection. In Phase I of this SBIR project, electrochemically generated ozone was used for cold sterilization of surfaces to produce pharmaceutical grade water (i.e., Water for Injection) and to decompose pyrogens and other chemical contaminants in water. The positive results of development, testing and application of electrochemical ozone, coupled with the recognized effects of ozone as a sterilant provide a strong technical base for Phase II follow-on research and development of an ozone gas sterilizer.

The initial task of Year 1 of Phase II was to establish, through parametric tests, the conditions required to achieve high kill rates with ozone gas (e.g., temperature, humidity, pressure inside the chamber). The need for this phase of testing arises because there is relatively little published information on using ozone gas as a sterilant. Karlson[9,10] describes an ozone sterilization system but gives few details. Studies on use of ozone as a means of disinfecting clean rooms have demonstrated that the sporicidal properties of ozone are enhanced at high relative humidity[8,11].

A second task was to assemble a breadboard sterilizer system to provide a basic understanding of the ozone requirements for different sized sterilizing chambers. This apparatus served as a test bed for evaluating hardware and control items necessary to execute a sterilization cycle rapidly and safely.

The efforts in Year 2 focused primarily on the engineering and fabrication of the prototype. The breadboard sterilizer was used to conduct more extensive evaluations of various sterilization process conditions, which provided critical information needed to finalize the design requirements for the prototype sterilizer unit. Once the design requirements were identified, the design and fabrication of the unit began.

The design aspects of this work encompassed the identification of safety and performance requirements, the selection of subsystem critical components, and the defining of all hardware interfaces. Engineering drawings were developed for all components which were not commercially available. Fabrication efforts have involved the construction of the prototype's enclosure, electrochemical cell stack, and sterilization chambers.

The deliverable for this SBIR project is an automated ozone sterilizer, termed the *Sterizone* (see photograph, inside front cover). The Sterizone demonstrates the results of the research conducted for this SBIR.

SECTION III

ELECTROCHEMICAL OZONE GENERATION SYSTEM DESCRIPTION

A. Principle.

Figure 1 depicts the principle of the electrochemical ozone generation process. Sources of electrical power and water are the only requirements for producing ozone electrochemically. Further, the need for gas drying is eliminated and there are no toxic byproducts formed. The reactions depicted are made to occur by applying DC electricity between the anode and cathode which are positioned on either side of a Nafion® 117 (DuPont) proton-exchange membrane. Water is fed to the anode side where two water oxidation reactions take place. The thermodynamically favored oxygen (O_2) evolution reaction Equation (1) and the ozone formation reaction Equation (2).

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \quad E^\circ = 1.23\ V \qquad (1)$$

$$3H_2O \rightarrow O_3 + 6H^+ + 6e^- \quad E^\circ = 1.51\ V \qquad (2)$$

Utilization of high overpotentials (i.e., anode potentials much higher than 1.23 Volts) and certain electrode materials enhance ozone formation at the expense of O_2 evolution. The majority of the O_3 and O_2 formed is evolved as a gas. The water oxidation reactions yield protons and electrons which are recombined at the cathode. Electrons are conducted to the cathode via the external electronic circuit.

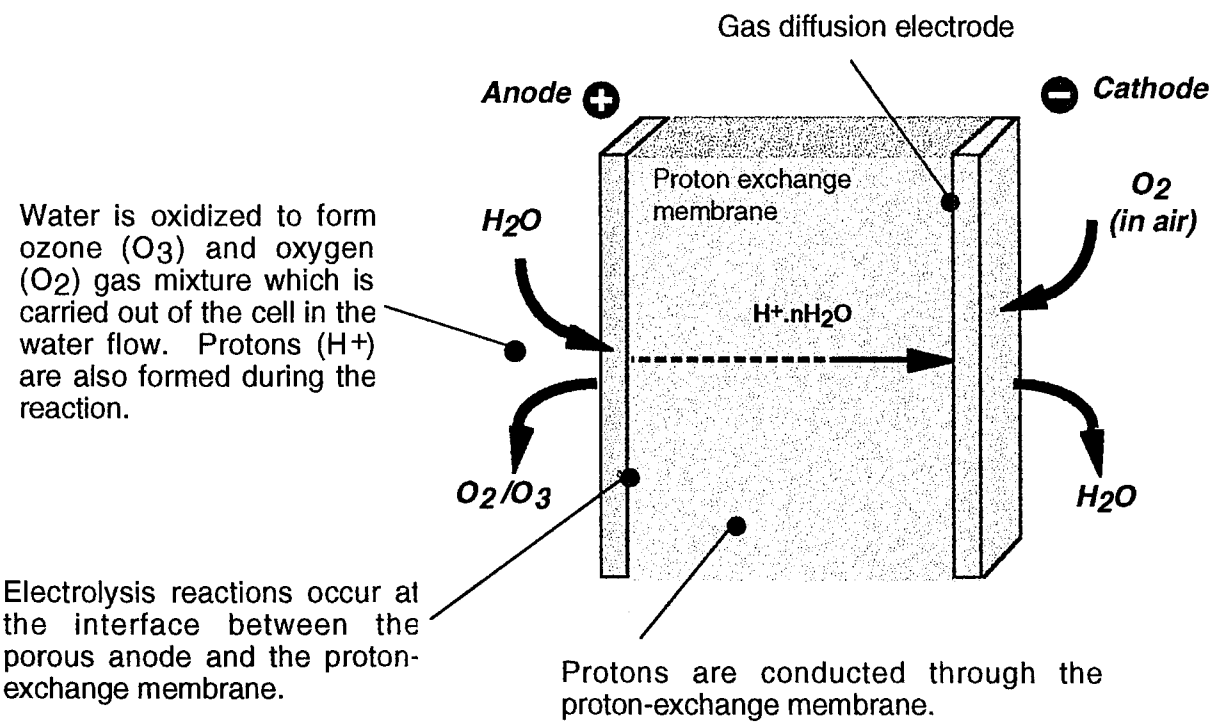

Figure 1. Proton Exchange Membrane Reactor Concept for Ozone Generation.

A Nafion® (DuPont) proton-exchange membrane (PEM) solid electrolyte, containing SO_3^- ion-exchange sites, serves as the proton conducting pathway between the two electrodes. The use of a Nafion® PEM instead of a liquid electrolyte offers several advantages: 1) fluid management is simplified and the potential for leakage of corrosive liquids is eliminated; 2) the membrane serves as a separator between the anode and cathode; and, 3) the Nafion®/anode interface provides a chemical environment which is suited to the electrochemical ozone formation reaction. As a fluoropolymer, Nafion® displays a very high resistance to chemical attack. The cathodic reaction is the reduction of oxygen. Air serves as the oxygen source. This reaction is represented by Equation (3).

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad E°=1.23\ V \tag{3}$$

Specialized gas diffusion electrodes are required for the oxygen reduction reaction to occur efficiently. The layer of bonded carbon particles serves as a three-dimensional microporous structure for diffusion of the reactant gas (air) into the electrode structure. The presence of oxygen at the cathode suppresses the hydrogen (H_2) formation reaction. Furthermore, the oxygen reduction reaction is thermodynamically favored over hydrogen formation, hence, the reduction of oxygen reduces the overall cell voltage (i.e., the energy required to drive the system).

B. Electrochemical Cell Design.

Figure 2 is a photograph of the ozone generating cell stack. The cell components are positioned between two titanium end plates that incorporate ports for liquids and gases to enter and leave the cell. The anode half cell frame and gasket material are made of Teflon. The cell frame incorporates an internal manifold system for distribution of water to the anode and air to the cathode. The components are held in place by 16 tie rods. Typically the geometric area of each electrode is 100 cm^2. These ozone generators can be scaled to any desired ozone output by incorporating several cells within the end plates in a filter press-type arrangement and where each cell is connected electrically by a bipolar plate.

C. Operating Characteristics.

The electrochemical ozone generator is operated by circulating deionized water through a heat exchanger and into the anode side of the cell. The water then goes through a small tank which separates the O_3/O_2 gas mixture from the circulating water. *Most (i.e., >95%) of the ozone is formed in the gas phase (it is released from the anode in the form of gas bubbles).* The ozone concentration in this gas stream exiting the separator is measured spectrophotometrically (λ = 254 nm); the total rate of gas generation is also monitored. A diagram of the apparatus used to monitor the performance of the ozone generation system is shown in Figure 3. The measurements given in Figure 4a-b characterize the performance of a 5-cell stack after 2350 hours of continuous operation. The graph presented in Figure 4a shows the total voltage drop across all five cells for different applied currents (the current is expressed in amps per unit area of electrode). A voltage is generated because the current is passed through each cell consecutively which is equivalent to a resistor. Figure 4a also shows that the current efficiency increases with current density. The current efficiency is the proportion of the current

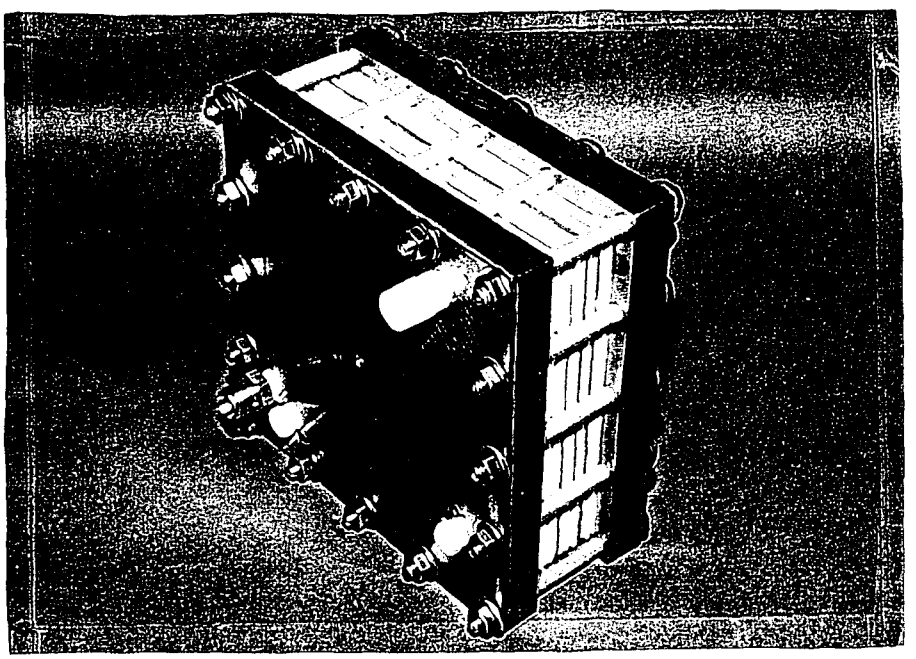

Figure 2. Photograph of the Ozone Generating Cell.

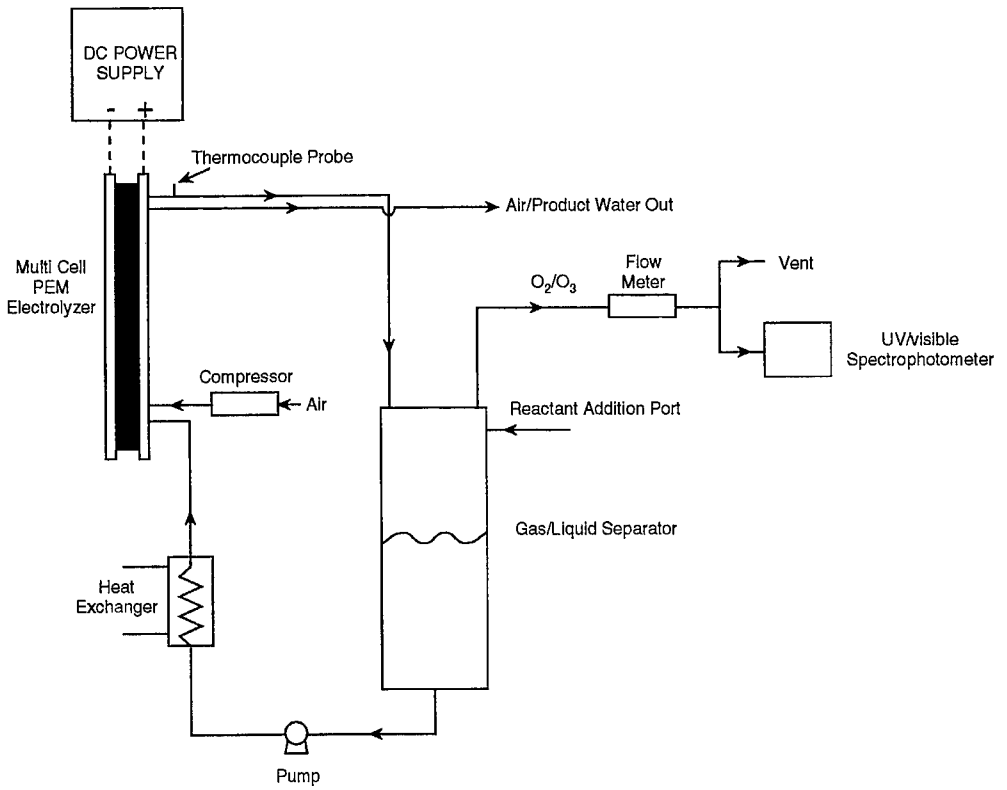

Figure 3. Apparatus used to Monitor the Performance of Electrochemical Ozone Generators.

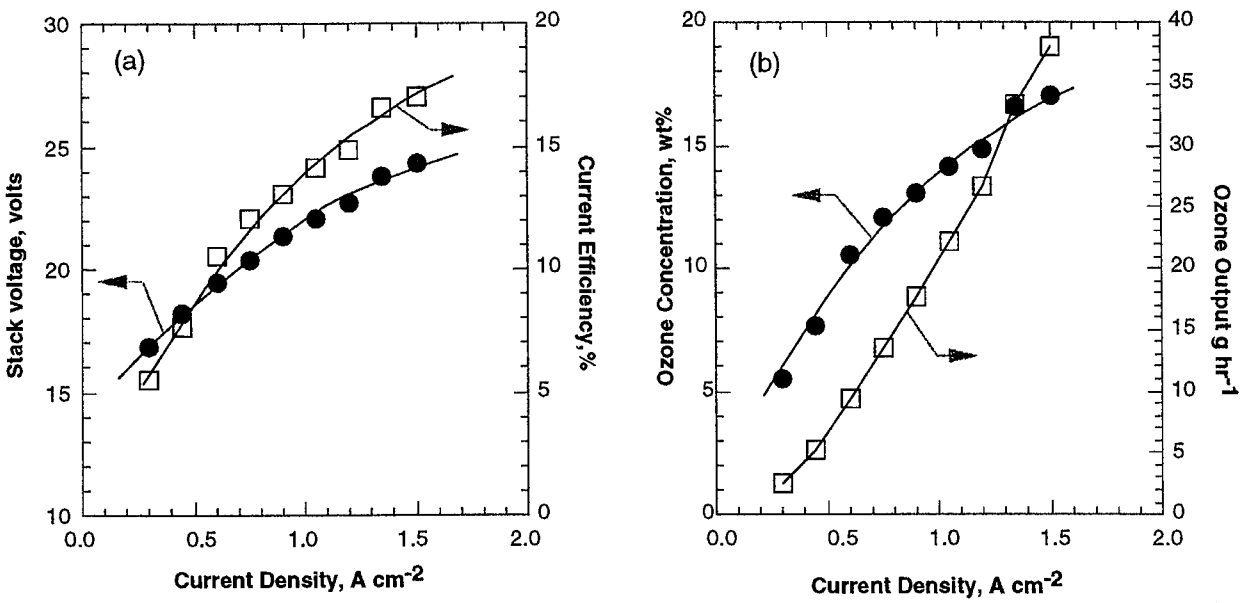

Figure 4. Electrochemical Ozone Generator Test Data. (a) The stack voltage and current efficiency versus current density; and, (b) the gas phase ozone concentration and overall ozone output versus current density.

supplied to the cell that goes towards the formation of the desired product (ozone). The gas phase ozone concentration increases with increasing current density in much the same way as does the current efficiency. Figure 4b is the ozone concentration in the gas phase as a function of current density which maximizes over the same range of current density as does the current efficiency. Figure 4b also shows the ozone production rate as a function of current density. Approximately 40 ml of deionized water is consumed for every hour of operation of the five cell stack.

Table 1 gives comparative information on electrochemical and corona discharge methods for ozone generation. It is apparent from the information given that electrochemically-generated ozone can form the basis for a novel process for sterilization of medical equipment.

Table 1. Ozone Generation From Different Sources (adapted from ref. 7)

Ozone Source	**Energy** (kWh/lb)	**Gas Phase Concentrations** (mg/l air)
Air fed Corona 1 wt%	12	12.1
Air fed Corona 2 wt%	20	24.2
Air fed Corona >6 wt%	not possible to generate	
Electrochemical @ 18 wt% (this work)	25-30	217.8

SECTION IV

TESTING OF OZONE GAS AS A STERILANT

A. Introduction.

This section of the report describes experiments that examine the sporicidal activity of ozone under different conditions. From previous work it has been shown that elevated humidity is critical for efficient sterilization with ozone. A follow up study is described here showing the effects of: 1) a continuous feed of ozone gas into the sterilization chamber; 2) pressurization of the sterilization chamber; and, 3) static ozone gas treatment.

B. Description of the Breadboard Ozone Sterilizer.

An ozone sterilization chamber was built by completely refitting a surplus AMSCO EtO sterilizer (Model AM-23, American Sterilizer Company, Erie, PA), with Teflon® gas lines, electrical wiring, new cycle timers, Teflon® gaskets, and a vacuum air purge system. The only portions of the original device that were salvaged were the stainless steel chamber and the exterior heating blanket (this required a new controller). A 400 cm^2 active electrode area ozone generator with a gas feed rate of 2.2 liters per minute (lpm) was used for the breadboard sterilizer. The ozone concentration in the output from the generator was between 10 and 12 wt%. The ozone generator had a power consumption of 2.4 kilowatts. Total volume of the sterilizer chamber was 40 liters. The ozone/oxygen mixture generated by the electrochemical reactor is in a near-water saturated state when it leaves the ozone generator. Further humidity in the chamber was achieved by keeping an open container of distilled H_2O inside the chamber while cycling the sterilizer. The air exhaust system utilized a piston air pump. The ozone concentration was measured spectrophotometrically at 254 nm using a Shimadzu UV spectrophotometer (Model UV-2101PC, Shimadzu Corp., Japan), integrated with a computer for data evaluation.

C. "Flow Through" Operation of the Ozone Sterilizer.

The sterilizer is normally operated with a continuous feed of ozone gas into (and out of) the chamber; (i.e., **"flow through"** mode). There are three phases of the sterilization cycle: 1) evacuate; 2) sterilize; and, 3) flush. The gas flow configuration during each phase is shown by Figures 5a-c. The changes in conditions inside the chamber during cycling are illustrated by Figure 6. Each phase of the cycle is described below.

1. Evacuate

After samples have been loaded and the chamber door is secured, the evacuation is manually initiated. The flow configuration during "evacuate" is shown in Figure 5a. At this time, the vacuum block valve opens, the vacuum pump is turned on, and the chamber air is evacuated through the vacuum pump and out the air purge valve. After approximately 5 minutes, a vacuum of 30" Hg is reached. Figure 6a shows the changes in vacuum pressure in the chamber during sterilizer cycling.

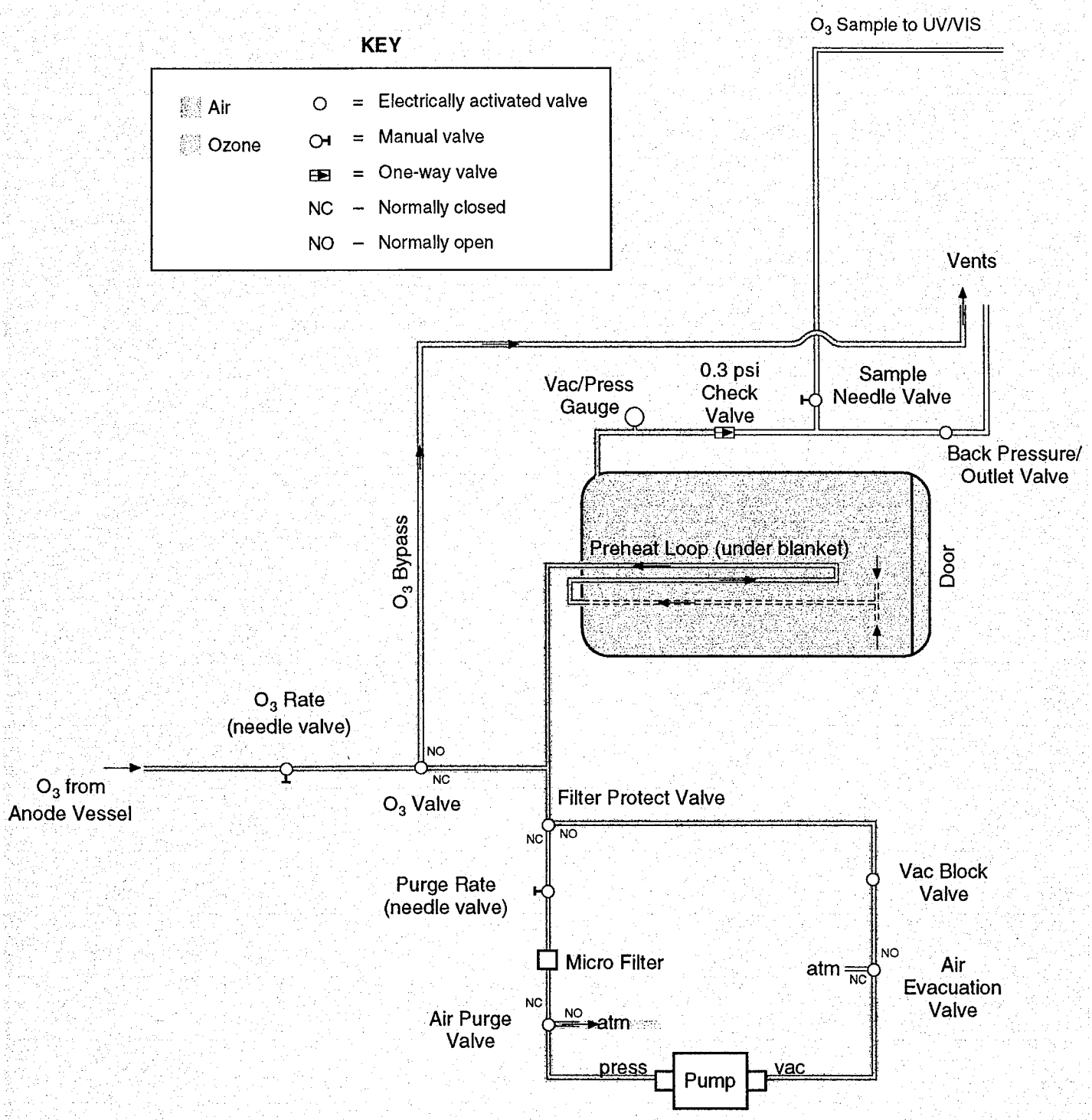

Figure 5a. Breadboard Ozone Sterilizer Design and Operations Schematic: Evacuation Phase of the Cycle.

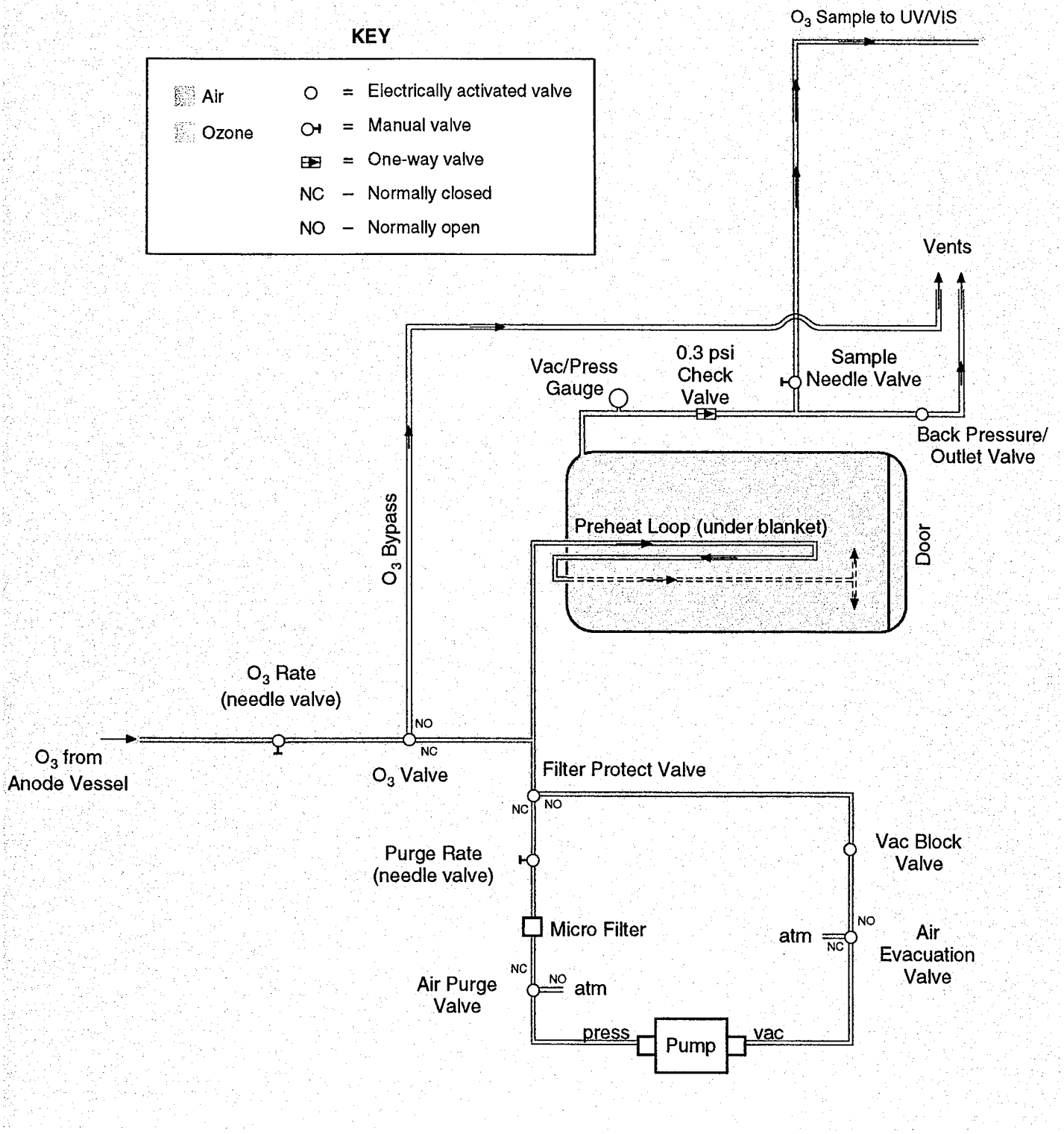

Figure 5b. Breadboard Ozone Sterilizer Design and Operations Schematic: Sterilize Phase of the Cycle.

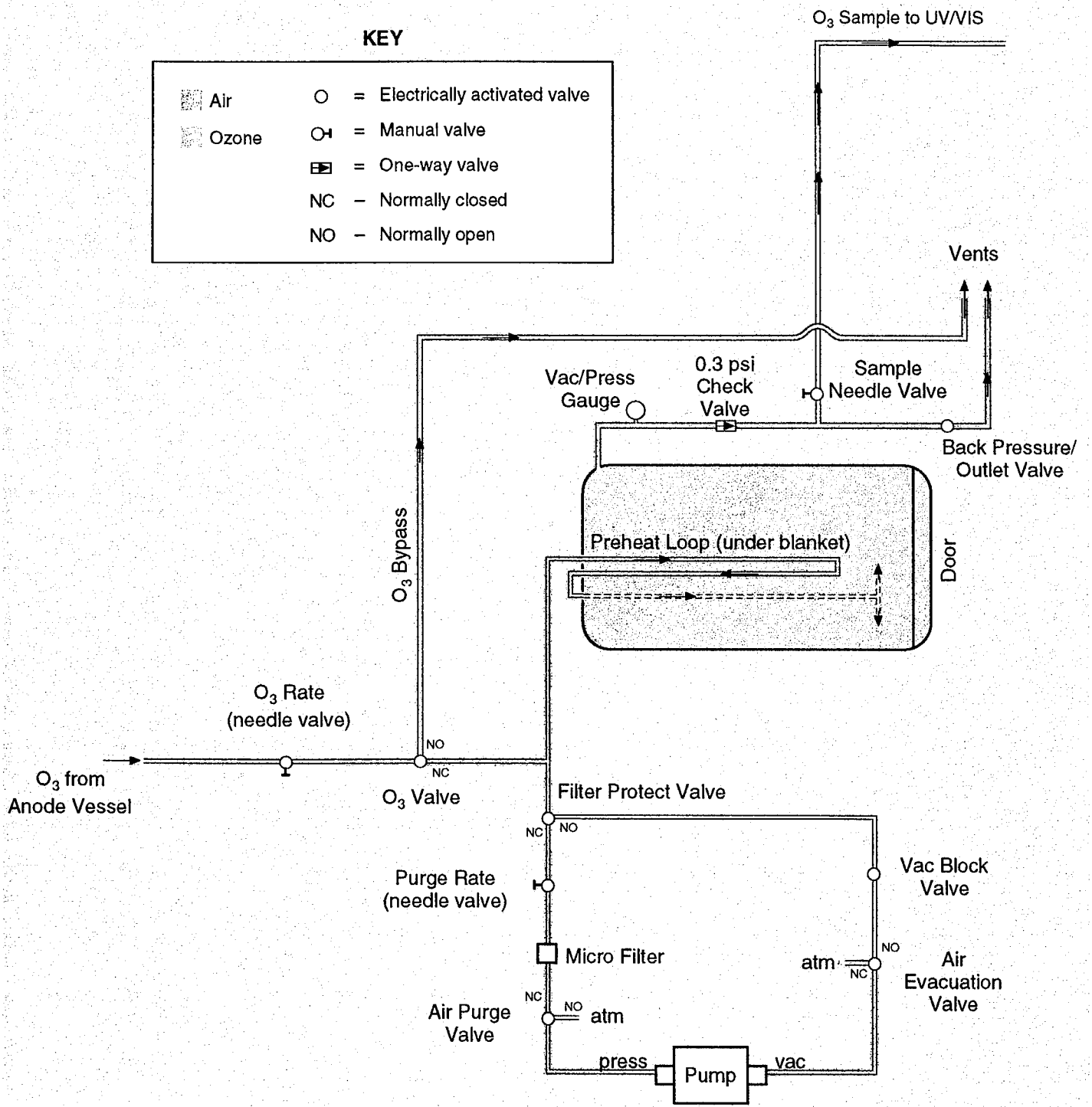

Figure 5c. Breadboard Ozone Sterilizer Design and Operations Schematic: Flush Phase of the Cycle.

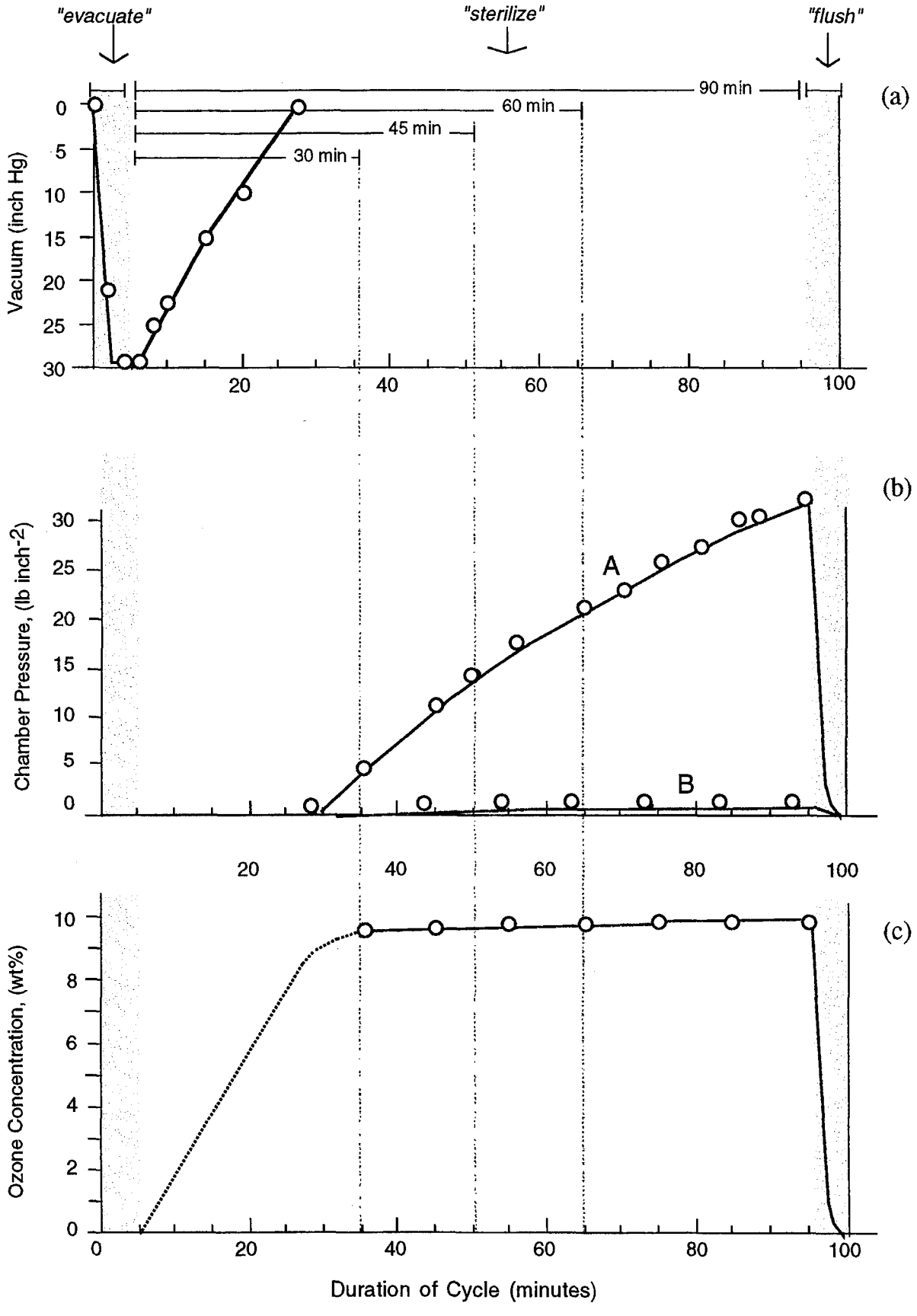

Figure 6. Chamber Conditions During Breadboard Sterilizer Operations.

2. Sterilize

Sterilization is manually initiated after 5 minutes of evacuation. The vacuum pump is turned off, the vacuum block valve is closed, the ozone valve diverts ozone into the chamber. The gas flow regime is illustrated in Figure 5b. Figure 6b shows that after approximately 25 minutes the vacuum within the chamber is broken and at approximately the same time, the 0.3 psi check valve allows ozone to be vented through the sample needle valve. At this time, the ozone concentration reaches a value of 10 wt% (see Figure 6c). For the remainder of the sterilize phase, ozone gas flows continuously through the chamber at approximately 2 lpm. The pressure in the chamber remains close to atmospheric. The length of the sterilize phase is also the duration of ozone exposure of the samples in the chamber. Testing of the sterilizer was conducted using four sterilize times (i.e., 30, 45, 60 and 90 minutes).

3. Flush

At the end of the preset ozone treatment period, the flush phase of the cycle is automatically initiated. The timer engages and the air evacuation valve is opened, the air purge valve is closed, the filter protect valve is opened, and the vacuum pump is turned on for a timed microfiltered air purge period. At the same time, the ozone valve bypasses the ozone to vent. At the end of the sterilization cycle, all solenoid valves switch off and the complete light is illuminated. The chamber can be opened and the samples removed.

D. "Pressure" and "Single Fill" Operation of the Ozone Sterilizer.

The operating procedure and cycle events described above were modified in some experiments to investigate the effects of pressurizing the chamber during ozone treatment (i.e., **"pressure"** mode). The difference from "continuous flow" mode was that immediately at the start of the sterilize phase, ozone was diverted into the sterilization chamber but with the chamber outlet closed (i.e., the back pressure outlet valve was closed). This meant that once the vacuum inside the chamber was broken, the pressure inside the chamber built up. The time course of this build-up is given in Figure 6b, trace 'A'. This meant that the samples were subjected to an increased ozone gas pressure during the sterilization phase of the cycle. The chamber was flushed in the normal way.

Static ozone gas treatment was investigated by subjecting samples to a single chamber filling of ozone (i.e., **"single fill"** mode). The time course of this mode is shown in Figure 6b, trace 'B'. This single fill was accomplished by modifying the sterilize phase so that once vacuum was broken and the ozone concentration in the chamber was 10 wt%, both the inlet and outlet valves were closed. This seals the ozone in the chamber with no further gas inflow, hence the samples are maintained in a static ozone atmosphere for the remainder of the sterilize phase. The flush phase was accomplished in the usual way.

E. Sample Treatment.

Paper spore strips (Duo-Spore, Propper Manufacturing Co., Inc., Long Island City New York) containing *Bacillus stearothermophilus* and *Bacillus subtilis* var. *niger* were used for all tests. Spore strips were tested both with and without glassine envelopes. The strips were placed on a metal rack at a height mid-way between the top and the bottom of the chamber. A minimum of four spore strips were placed in the chamber for each run. For each run, two non-

ozone treated spore strips were used as controls. They were asceptically removed from their envelope and placed immediately in sterile nutrient broth (NB). All control strips showed vigorous growth in NB within 24 hours.

Following treatment, spore strips were asceptically removed from the chamber and placed in individual test tubes containing 9 ml of sterile NB. The strips were incubated at 37 °C for 21 days. If no growth was visible after 21 days, the strips were heat shocked. Heat shocking was accomplished by placing the tubes containing the strips into an 80 °C water bath for 20 minutes. The tubes were then returned to the incubator for an additional 14 days. Any strips not showing growth at the end of this time were classified as "sterile".

F. Results.

Figures 7a and 7b illustrate the results obtained from over 80 evaluations of the sporicidal activity of ozone under different chamber conditions and exposure times. A minimum of 4 replicate spore strips were used for each evaluation. Typically each spore strip contained in excess of 10^6 spores (e.g., 6.0 X 10^5 spores of *Bacillus stearothermophilus* and 5.0 X 10^6 spores of *Bacillus subtilis* var. *niger*). The results of these evaluations are expressed in terms of "% of spore strips sterilized" for each exposure time period and each operational mode. Overkill was achieved when 100% of the spore strips were sterilized.

Figure 7a contains the results of tests conducted on spore strips enclosed in glassine envelopes. Figure 7b shows the results of tests conducted on spore strips which had been removed from the glassine envelopes. These data clearly show that the glassine envelopes had a small but observable negative effect on the sporicidal activity of ozone. The glassine envelopes represented a physical barrier which delayed the contact and penetration of ozone into the spores, thus decreasing the overall sporicidal efficiency of the ozone.

The results seen in Figures 7a and 7b clearly demonstrate that the "pressure" mode of operation was the most effective. An increase of chamber pressure significantly enhanced sporicidal activity, and overkill was achieved after a 45 minute exposure. The "flow through" scheme was the next most effective mode of operation. This operational mode displayed a significantly lower level of spore inactivation at the 30 and 45 minute exposure times than did the "pressure" mode. With this operational mode, overkill was achieved after a 60 minute exposure. The "single fill" was the least effective operational mode. A 90 minute ozone exposure was required to achieve overkill.

These results are in agreement with what might be expected from simple reaction rate arguments. In the "single fill" experiment, the chamber is filled once with ozone with no subsequent addition and no mechanism to promote convection. As a result, the ozone concentration at the surface of the spore would be expected to decrease as the ozone reacts with the spore coat since additional ozone can be supplied only by natural diffusion of the gas. In the "flow through" mode, gas is continuously pumped into the chamber without an increase in overall chamber pressure. This inflow of gas would provide a continuous supply of ozone at the spore surface through convection. A shorter reaction time than that of the "single fill" experiment would be expected and, is indeed observed. In the "pressure" mode experiment, the

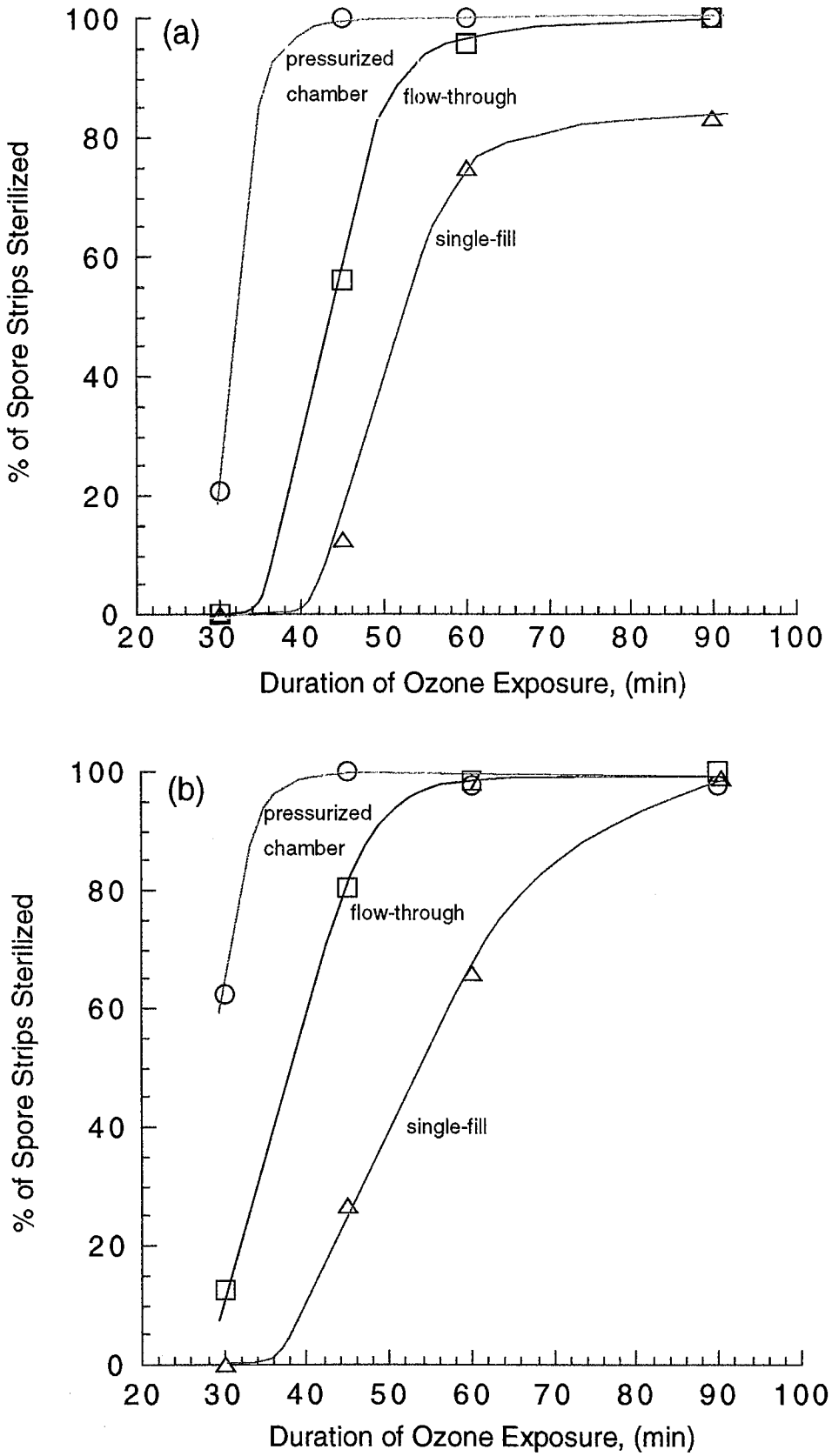

Figure 7. Graph Showing Percentage of Spore Strips Sterilized Versus Ozone Exposure Time; (a) with glassine envelope; (b) without glassine envelope.

reaction chamber is filled to a positive pressure of ozone and gas is introduced continuously into the system. In this case, both the overall concentration of ozone within the chamber is increased and a mechanism for convection is present which would lead to the faster reaction rate and the minimum reaction time, as observed.

The lower efficiency of the "single fill" operational mode could also result from a stratification of the ozone in the chamber. Ozone is heavier than air and without sufficient mixing of the internal chamber environment, the ozone becomes stratified into the lowest portion of the chamber, thus limiting its availability at the spore surface.

A D-value is defined as the amount of time required by a sporicidal agent to effect a one log reduction in the number of viable spores present in a sample. The D-values given for the experiments conducted during this work were extrapolated from the overkill time requirements. The extrapolation was based on the following assumptions: 1) individual spore strips contained in excess of 10^6 spores; and 2) the time required to achieve overkill (100%) represented a six log reduction. Based on these assumptions, the "pressure" mode with an overkill time requirement of 45 minutes yielded an extrapolated D-value of 7.5 minutes. The "flow through" mode achieved overkill at 60 minutes, this yielded an extrapolated D-value of 10 minutes. While the "single fill" mode required 90 minutes to achieve overkill, resulting in an extrapolated D-value of 15 minutes.

Ishizaki et. al.[8] also examined the sporicidal activity of gaseous ozone. Ishizaki et. al.[8] used spores that were prepared in-house, dried for only 2.5 hours and then exposed to conditioning humidity. The D-values reported by Ishizaki, et. al.[8] exclude a substantial portion of the ozone exposure time which is reported as the "lag phase". This "lag phase" was the time required to neutralize the reactive compounds within the spore coat, prior to initiation of spore inactivation. For example, Ishizaki, et. al.[8] reported an average D-value of 37 minutes for spores exposed to 0.2 wt% ozone at 80% r.h. However, the initial 108 minute "lag phase" portion of the ozone exposure time was excluded from this D-value calculation. If this ozone exposure lag phase were included in the calculations, the new D-value becomes 146 minutes (2.4 hours) which provides a substantially different view of the effectiveness of this treatment.

Commercially impregnated spore strips were used in the experiments described in this report. These spore strips were prepared by desiccation of the spores onto the paper strip prior to enclosure in glassine envelopes. This dehydration of the spores could result in an increased resistance to inactivation. Extrapolated D-values for the "pressure", "flow through" and "single fill" modes, evaluated by us, were 7.5, 10 and 15 minutes respectively using 10 wt% ozone. The D-values reported by Ishizaki et. al.[8] are substantially longer however, the ozone concentration was 50 times lower. This reinforces the importance of a high ozone concentration for rapid inactivation of spores.

SECTION V

DESCRIPTION OF THE AUTOMATED OZONE STERILIZER ("STERIZONE")

Year 1 of Phase II of the SBIR project focused on the determination of optimum operating parameters for ozone-based sterilization, presented in the preceding sections of this report. This section presents the work of Year 2, which focused on the design and fabrication of an automated prototype demonstrating the results of Year 1.

A. Theory of Operation.

The Sterizone is designed to provide sterilization in field medical situations with a short turnaround time and minimum logistical requirements. A humidified oxygen/ozone mixture containing a high concentration of ozone (typically 9-15 wt. %) is used as the sterilant. The high concentrations of ozone, approximately 10 times greater than a conventional ozonizer (1 to 2%), contribute to shortened sterilization cycles (kill rate is proportional to ozone concentration). Ozone is a highly effective sterilant against all classes of microorganisms, with an oxidation potential (2.07 V) that is higher than that of ethylene oxide (0.95 V). Ozone decomposes to oxygen, so there are no hazardous residuals associated with ozone sterilization. Short aeration times are possible since ozone can be rapidly destroyed catalytically.

The Sterizone generates ozone electrochemically by splitting water with electric current (electrolysis) to form hydrogen, oxygen and ozone gas. The ozone cell uses from 12 to 20 volts at 100 amps of dc current. The cathode side of the cell is fed with air, and hydrogen is transported from the anode to the cathode through a proton exchange membrane where it combines with oxygen in the air (air depolarization), and a mixture of air and water exits the cathode side. Water is fed to the anode side of the cell, and a stream of water, oxygen, and ozone exits the anode side to a water/gas separator. The ozone and oxygen are then separated from the water, and the resulting ozone/oxygen gas mixture is then sent to a larger accumulator where it is stored until it is required for the sterilization process. The ozone accumulator system is designed so that the accumulator is continuously replenished with fresh ozone thus maintaining high ozone concentration for 'on-demand' filling of the cassettes. All ozone is collected and passed through an "ozone destruct module" which catalytically converts the ozone to oxygen which is then vented to the atmosphere. The cathode exhaust (air and water) is passed through a "hydrogen recombiner" to eliminate any traces of hydrogen which might have escaped the cell. Thus the inputs to the system are air, water, and power, and the outputs of the system are air, water, and heat.

The unit is operated by loading a small 'cassette' with a tray containing the items to be sterilized, and inserting the cassette into any of three cassette slots in the Sterizone cabinet. Upon command the cassette is evacuated of air (to facilitate rapid filling), and then filled with ozone. Periodically, fresh ozone is pulsed into the cell to ensure that a high concentration is maintained during the sterilization portion of the treatment cycle. When sterilization is complete, the cassette is purged with air to remove all traces of ozone, and the cassette is then available to the user.

B. System Overview.

The Sterizone consists of 6 primary subsystems: the Cabinet, the Ozone Generation subsystem, the Cassette Stack, the Power Distribution subsystem, the Thermal Control subsystem, and the Control and Data Acquisition subsystem. A system flow diagram appears in Figure 8.

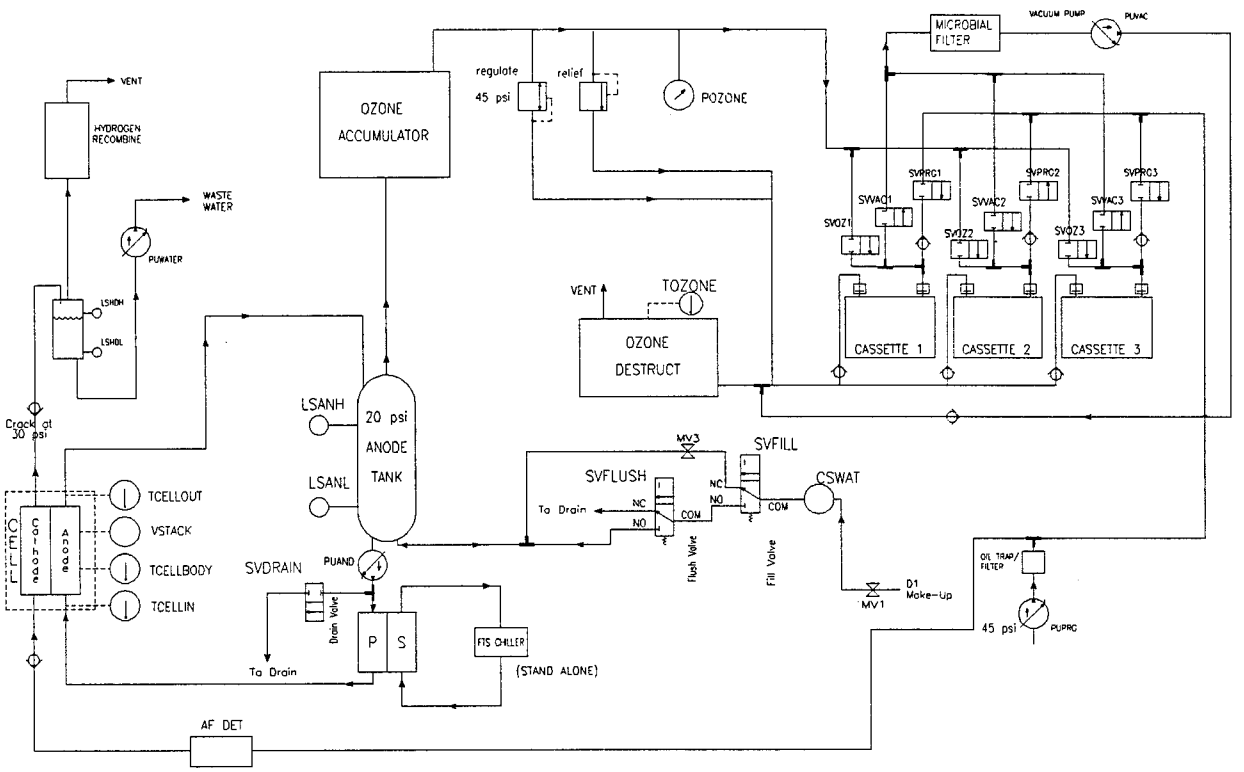

Figure 8. System Flow Diagram.

C. Control and Data Acquisition.

System control for the Sterizone is provided by an Intel® 80c188 microcontroller mounted in a single board computer manufactured by Midwest Micro-Tek (MMT). The microcontroller has a 16 bit internal bus and an 8 bit external bus, and operates at 16 MHz. The system software resides on a single 64k x 8 EPROM. The process control loop operates roughly 400 times per second. The MMT is connected to the MMT Expansion Board, the Digital Buffer Board, the data logging serial port, and the display panel.

The MMT Expansion board is an 8 channel analog data acquisition and two channel analog output board. The analog inputs include the four temperature transducers, the ozone pressure transducer, the ozone stack voltage, the ozone stack current, and the water conductivity signal. The eight input channels are multiplexed to a single 8 bit analog to digital converter with a fixed input range of 0 to 5 volts. Each channel is sampled 16 times at a frequency of 960 Hz and then the DC component of the signal is calculated by a simple average of the 16 values. The sample set size and sampling frequency were chosen to allow effective filtering of any 60 Hz noise

components on the input channel (the sample set size of 16 samples at 960 Hz covers one period of a 60 Hz signal). The analog output channels can generate 0 to 5 volts with 12 bit resolution. Only the first channel is used by this application, sending a control signal representing the desired cell current flow to the Sorensen DC power supply.

The digital buffer board serves to increase the signal strength of the digital output lines coming from the MMT so that they can activate the output modules on the digital I/O mounting rack. The MMT digital outputs can only drive about 3 milliamps of current, and the I/O modules require a minimum of 15 milliamps.

The first of the two serial ports of the MMT is a data logging port, and is connected to DB-9 RS-232C connector mounted on the patch panel. The second serial port connects to the Intelligent Instrumentation microterminal, referred to as the *display panel* within this document (Figure 9).

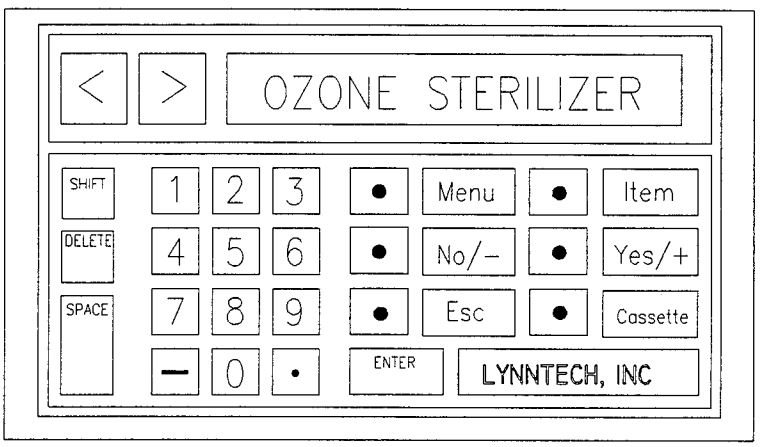

Figure 9. Sterizone Display Panel.

The first function of the display panel is to display system messages and to receive operator commands. The second function is to provide 8 channels of digital output and 8 channels of digital input, provided through the *auxiliary* connector on the back of the unit. The microcontroller can send special commands through the serial cable to set the states of the digital output channels and to query the present states of the digital input channels. These digital I/O lines travel through a ribbon cable to a passive interface connector where they are then connected to the various components.

D. Cabinet.

The Sterizone cabinet contains all of the functional modules needed to operate, except the stand-alone chiller. The cabinet layout is illustrated in Figure 10 and in Figure 11. The cabinet contains a number of fans for cooling and air circulation, air filters, cable and hose connections, and has several important physical features:

- The front door can be removed simply by lifting upward and off of its hinges.
- The cabinet bottom will accept a fork lift to facilitate moving.
- The lower rear panel has 2 chiller connections (lower for input, upper for output), and a louvered air exhaust. On the inside of the lower rear panel is an activated charcoal filter, and 2 exhaust fans. Any ozone which might leak in the cabinet should be trapped by this filter.
- An exhaust fan is located inside the cabinet top to provide cooling for the power supplies.
- An air input fan is located on the main rear panel.
- the main rear panel can be removed by taking out the retaining bolts on both sides of the panel.
- The upper rear of the cabinet (angled between the top and the main rear panel) is the patch panel. It has the main power cord, a power output cord to a chiller, the main circuit breaker, and a serial port to allow connection to a remote data logger.

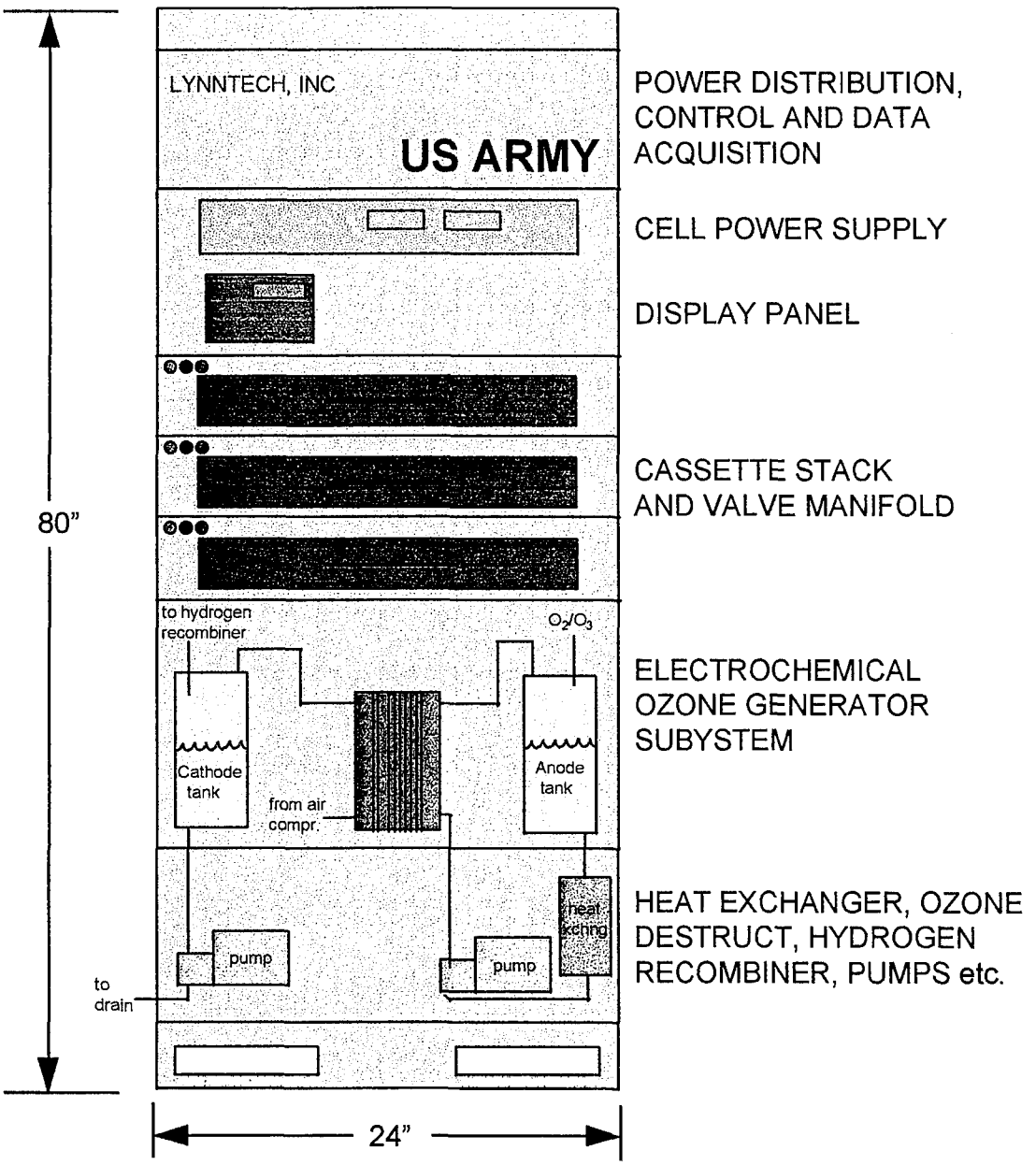

Figure 10. Cabinet Layout - Front View.

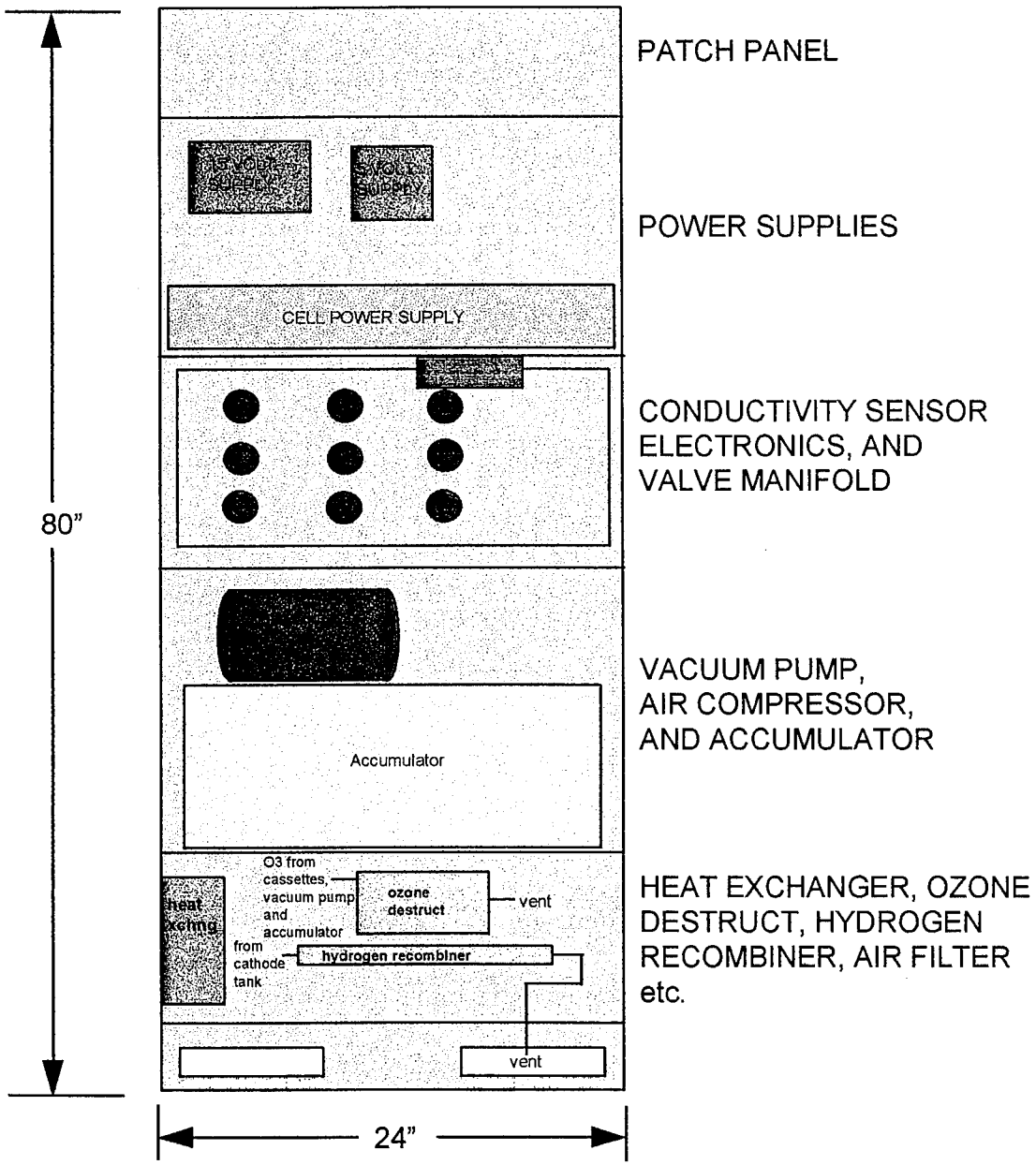

Figure 11. Cabinet Layout- Rear View.

E. Ozone Generation.

The ozone generation subsystem consists of: the **Deionized Water Supply**, the **Ozone Cell**, the **Anode Flow Circuit**, the **Accumulator**, the **Ozone Destruct Unit**, the **Cathode Flow Circuit**, the **Hydrogen Recombiner**, the **Cell Power Supply**, the **Cell Thermal Control Subsystem**.

1. Deionized Water Supply.

When makeup water is needed by the anode tank, care must be taken to ensure that it is clean water, and care must be taken in the method of reading the make-up water conductivity sensor. As water sits in a de-ionizing canister, the water will actually become ionized (because of equilibrium forces). Therefore, it is necessary to flush the water through the filter for a short amount of time before even expecting to see good water.

If after flushing for 10 seconds the conductivity does not rise above 10 MΩ•cm, then the deionizer will be suspected of being faulty, and the Sterizone will issue a "Bad Water" warning. If the water condition improves, then the firmware will accept the water and disable the warning. If the water condition does not improve over the next 30 minutes, then the firmware will initiate a system shutdown.

2. Ozone Cell.

The electrochemical ozone generator is the core component of the Sterizone. It is a stack of five cells which when operated at 100 amps produces approximately 2.1 lpm of ozone/oxygen gas mixture with an ozone concentration of 9 - 15 wt. %. The inputs are deionized water to the anode, and air to the cathode. The anode outputs a mixture of water, oxygen and ozone, and the cathode outputs air and water, and the cell produces up to 2 kilowatts of heat. The cell temperature is controlled by maintaining anode water temperature such that the cell operates between 30 and 35 °C (the optimum temperature for producing ozone).

3. Cell Anode Flow Circuit.

Anode circuit hardware:- Three major components make-up the anode flow circuit: the anode circulation pump, the anode water heat exchanger, and the anode reservoir. The **anode pump** supplies water to the cell. The **anode water heat exchanger** cools the anode water to maintain optimum cell temperature. The **anode reservoir** and associated valving maintains the proper water level, and separates the ozone/oxygen gas from the recirculating water supply. The anode reservoir has high level (80%) and low level (20%) sensors to control filling (or draining) with water as needed. The anode reservoir can also be filled manually.

Anode circuit control algorithm:- Water is drawn from the anode side to the cathode side of the cell as it produces ozone. Additionally, the ozone-rich gas mixture leaving the anode tank will be saturated with water vapor. Thus, the water level in the anode tank will drop with time as ozone is produced. The anode circuit control algorithm is responsible for maintaining the proper water level in the anode tank and for circulating the deionized water through the anode side of the ozone cell. The algorithm maintains the water level in the anode tank near the upper level sensor, keeping the tank as full as possible. This is done because the extra water volume serves to

dampen the thermal control for the ozone cell and also because the small dead space above the water level helps to force the ozone generated by the cell to flow into the accumulator. Furthermore, water make-up from the deionized water source may be very slow; therefore it is beneficial to keep a large volume in reserve.

Whenever the ozone generation subsystem transitions from the inactive to the active state, the control algorithm first checks the anode water tank level. If it is below the lower level sensor water is requested from the deionized water source, and the remainder of the subsystem is inhibited until the water level rises above the lower level sensor. Once the water is above the lower level sensor, the algorithm turns on the anode pump. The ozone cell power is inhibited from activating, however, for a short amount of time, allowing the water to completely fill the tubing, heat exchanger, and ozone cell first. The request for water is continued until the water level is sensed by the upper level sensor. In a relatively short amount of time, the water level will drop below the upper level sensor. In order to prevent frequent, short, requests for deionized water, there is a five minute delay in which there will be no request for water.

There are two diagnostic messages that can be generated by this algorithm; the **Anode Tank Low** message and the **Bad Hydro Tank Level Sensors** message. The first is generated when the anode tank water level falls below the lower level sensor at any point *after* the ozone generation subsystem is fully active. The second error occurs when the upper level sensor senses water while the lower sensor does *not* sense water.

4. Accumulator.

Since the ozone cell produces a steady stream of ozone/oxygen, but the usage rate varies, the ozone/oxygen mixture is retained in an accumulator. The accumulator is a pressure vessel with an internal volume of 27.5 liters. The accumulator is continuously replenished with fresh ozone/oxygen gas via the anode flow circuit. A back-pressure regulator regulates the pressure in the accumulator at approximately 30 psig. A pressure relief valve is placed in parallel with the back-pressure regulator to prevent over pressurization due to regulator failure. The outputs of both the safety relief valve and the back pressure regulator are connected to the ozone destruct unit. Thus a high concentration of ozone is maintained in spite of ozone decomposition to oxygen. The volume and pressure of the accumulator ensure that rapid filling of the three cassettes will be possible whenever needed.

5. Ozone Destruct Unit.

The ozone destruct subsystem is two 1 inch diameter by 6 inch long stainless steel tubes packed with Pt/Pd catalyst. The tube is wrapped with a nichrome heater, and the temperature is controlled by a bi-metal switch. The temperature of the ozone destruct is maintained between 100 and 130°C to allow full decomposition of the ozone and to prevent water condensation. The output of the ozone destruct is humidified oxygen which is vented to the atmosphere.

The temperature of the ozone destruct unit is constantly monitored by the Sterizone firmware. If, after an initial warmup period of 20 minutes, the temperature of the ozone destruct unit is below 80 °C, or above 140 °C, the system is shutdown and an error message is displayed.

6. Cell Cathode Flow Circuit.

The Cathode flow circuit has 4 main components; the air compressor, the air flow detector, the cathode tank, and the water pump. The **air compressor** provides compressed, filtered (with a microbial filter) air to both the ozone cell cathode and the cassettes. At least 45 SLPM air flow must be maintained to fully depolarize the cathode (oxygen from the air combines with hydrogen from the cell). The **air flow detector** will detect any reduction of the air flow to the cathode below 45 SLPM. The **cathode tank** separates the exhaust air from the exhaust water. The exhaust air flows to the hydrogen recombiner. The water pump is used to remove excess water from the cathode tank according to the cathode tank control algorithm. The cathode tank has high level (80%) and low level (20%) water sensors to control water pumping.

Water saturated vapor flows from the cathode side of the ozone cell to the cathode tank, which serves as a gas liquid separator. The hydrogen gas flows out through the top of the tank to the catalytic recombiner, and the water condenses and collects in the bottom of the tank. The cathode tank control algorithm monitors the two level sensors in the cathode tank. When the water level rises above the upper level sensor, the algorithm turns on the water pump and drains until the level drops to just below the lower level sensor.

If a membrane within the ozone cell tears, an excessive amount of water will flow from the anode side, through the tear and into the cathode side of the cell. This will result in an unusually quick filling of water within the cathode tank. The Sterizone firmware detects this failure condition using the following safety algorithm: whenever the tank is drained to the lower level sensor, the present time is recorded. Under normal conditions, the tank should require at least an hour before the water level rises to the upper level sensor. If, however, the fill time is less than 10 minutes, the system shuts down and displays the error message **Excessive Water Gen** on the display panel.

7. Hydrogen Recombiner.

The hydrogen recombiner is a 12 inch long, 1 inch diameter stainless steel pipe that is packed with a platinum catalyst. The exhaust air from the cathode can potentially contain small amounts of hydrogen (well below the lower explosive limit). Any hydrogen present in the exhaust will combine with oxygen to form water when passed through the hydrogen recombiner. The output is air and water vapor which are vented to the atmosphere.

8. Cell Power Supply.

DC power for the ozone cell is supplied by a Sorensen Model DCS 20-150, which can produce up to 150 amps at up to 20 volts. The supply has been configured to run in current control mode, in which it will attempt to produce a current flow between 0 and 150 amps that is proportional to a 0-5 volt control signal originating from an analog output of the controller board. During normal operation, the supply will provide 100 amps of current. This will require a driving voltage from 12 to 20 volts, depending on the condition of the ozone cell. If the measured current differs from the desired current flow by more than 5 amps, then the error **Cell Curr. Err** is generated and displayed.

The Sorensen power supply has built-in overtemperature protection circuitry. If the internal temperature of the supply rises above its safety threshold, the unit will stop supplying current and will signal its shutdown condition by setting its thermal overtemperature digital output to the +5 volt state. This output is monitored by the controller board, and when detected, the firmware will shut down the rest of the Sterizone and display the message **Sorensen Overtemp**. Additionally, the supply also has built-in over voltage protection circuitry. The voltage is set at Lynntech to its maximum value, approximately 25 volts, which is more than the unit can produce. However, if the power leads going to the ozone cell become unconnected, the voltage could float above 25 volts. If the Sorensen detects a voltage higher than this threshold across its leads, it will shut down and set its over voltage alarm digital output to the +5 volt state. This signal is also monitored by the Sterizone firmware, and, when detected, will cause the Sterizone to shut down and to display the message **Sorensen Over-Voltage**.

The measured stack voltage is examined to try to determine if there is a short in the wiring or internally to the cell. If the voltage is below 2 volts, the shutdown message **Low Cell Voltage** is issued.

Note that a certain amount of delay time in alarming is required when measuring some of the analog values. Specifically, the dc current error and the minimum stack voltage comparison must be in the error state for at least 5 seconds before a shutdown message is triggered.

9. Cell Thermal Control Safety Algorithm.

Three temperatures are monitored by the Sterizone firmware to ensure the correct operation of the thermal control subsystem and to prevent damage to the ozone cell: cell inlet temperature, cell body temperature, and cell outlet temperature. If any of these temperatures exceeds 50 °C, the firmware will display the message **Cell Inlet Hot**, **Cell Body Hot**, and **Cell Outlet Hot**, respectively.

F. Cassette Stack.

1. Cassettes.

The cassette is basically a shallow rectangular box with a hinged lid, two protruding fittings on the back, and two pin brackets (a bracket with a pin) also in the back.

The two fittings mate with receptacles in the back of the cassette bay when the cassette is in the locked position. The fitting on the user's right (when facing the front of the cassette) is the fill port, the fitting on the left is the exhaust port. A leak tight seal is obtained due to the two o-rings on each fitting.

The cassette has a special o-ring seal around the lip of the lid to prevent ozone leakage during sterilization. The cassette is sealed by 3 clamps located along the front. Care should be exercised not to damage any of the seals for the cassettes.

Note that the cassette is not air-tight when it is out of the cassette locked position, so sterile instruments should not be stored in an unlocked cassette!

2. Cassette Interlock Mechanism.

There are three cassette bays in the Sterizone, each with its own safety interlock system. Each cassette interlock consists of two motor-actuated cams which mate with the two pin brackets on the back of each cassette, and a three-LED display.

The cam is driven by a small DC gear motor. The gear motor has enough torque to engage/disengage the double o-ring seals on the inlet and outlet of each cassette. The cam pulls the cassette in approximately 1/2" to the locked position, and pushes it approximately 1/2" out to the unlocked position.

Each bay has three LED's that indicate the status of that bay (see Table 2). The blue LED indicates that a cassette is present and detected by the interlock system. The red LED indicates that the cassette is in the locked position. The green LED indicates that the sterilization cycle is complete for that cassette.

Table 2. LED Indicators.

BLUE	RED	GREEN	DESCRIPTION
off	off	off	no cassette present, or cassette not slid all the way in.
on	off	off	cassette present but not locked.
on	on	off	cassette present and locked.
on	on	on	cassette present, locked and sterilization complete.
off	on	don't care	cassette not present, but interlock is closed. Either: 1) 'cassette present' switch failure, or 2) interlock closed with no cassette present.
off	off	on	display panel error.

3. Valve Manifold.

The three cassette bays are serviced by a valve manifold, located in the back of the cabinet just above half-way, which controls the flow of gases. Each cassette bay has three dedicated solenoid valves on the valve manifold, and two check valves (a total of 9 solenoid valves and 6 check valves. For each bay, one valve connects to vacuum, one valve connects to ozone (from the accumulator), and one valve connects to purge air (from the compressor). All valves are controlled by the system microcontroller, and at most one valve (for each bay) is on at a time. A check valve is located after each purge valve to prevent backflow of ozone, and a check valve is located in each exhaust line for the same reason. Exhaust from the cassettes and from the vacuum pump is sent to the ozone destruct module.

Vacuum is provided as needed by a Jun-Air vacuum pump, with a capability of about 27 inches vacuum (Hg), which is located in the back of the cabinet about half-way up (just above the accumulator). The air drawn from the cassettes is passed through a microbial filter prior to reaching the vacuum pump.

Ozone is provided by a solenoid valve attached to the outlet of the accumulator.

Compressed air is provided at approximately 45 psig by an oil-less air compressor located just behind the ozone cell.

G. Power Distribution.

Three phase, 208 VAC 60 Hz power with separate neutral and ground wires is supplied to the Sterizone through a connector located at the patch panel. This power is connected directly to a 3 phase circuit breaker also mounted on the same patch panel. One leg of the three phases, which is normal 120 VAC, is wired through a lighted switch located on the front panel and then to the 5 volt power supply. When the front switch is closed, the 5 volt power supply is energized, which in turn energizes the microcontroller board, the display panel, and cassette subsystem electronics. The remainder of the Sterizone components are not energized until the main 3 phase solid state relay (SSR) has been energized, an action that is controlled by a digital output of the microcontroller. Thus the microcontroller has full control over the power of the non-5 volt components through the main SSR.

Once the main SSR has been energized by the microcontroller, the 15 volt power supply will be functional, which in turn supplies power to the cassette interlock motors and the temperature transducers. Additionally, the Sorensen DC power supply will be energized, which is the only component in the Sterizone that requires the 3 phase power. Single phase power will be made available to the two single phase SSRs which control the refrigeration compressor and the vacuum pump, respectively.

H. Chiller.

The ozone cell generates a large amount of heat (up to 2 kilowatts) which is removed by providing chilled water on the anode side of the cell. The anode water is recirculated through the anode heat exchanger, which is cooled by a stand alone chiller. The heat exchanger is located in the bottom of the cabinet on the back left hand side (when facing the back of the cabinet), and has permanent connections to the lower back panel on the back of the cabinet. The chiller is attached to these two bulkhead feed-throughs by insulated flexible hoses. The chiller re-circulates ethylene glycol at approximately 15 °C through these hoses to the heat exchanger.

SECTION VI

DISCUSSION

The results from Year 1 of the Phase II project demonstrated that electrochemically generated gaseous ozone was ideally suited for use as a cold or ambient temperature sterilant for bacteria; specifically bacterial endospores. Specific ozone generation and application methods and parameters were experimentally established. First, it was determined that evacuating the sterilization chamber of all air before adding ozone increased the sporacidal effectiveness. Secondly, three methods of ozone flow (pressurized continuous flow, atmospheric continuous flow, and no flow (single fill)) were evaluated based on their sporicidal effectiveness. The pressurized continuous flow was the most effective, but complexities in fabrication lead to the choice of the second most effective approach, atmospheric flow. Additionally, it was determined that an ozone exposure time of 60 minutes at ambient pressure would result in overkill of the spores, and that an optimum current density for ozone generation by an electrochemical cell would is 1.5 mA / cm^2.

The focus of Year 2 centered on determining the best means for implementing these parameters and methods to develop a highly effective, safe, and automated medical instrument sterilizer. Two features were added to increase sterilization cycle turn-around time: 1) an ozone accumulator for storing ozone generated during non-peak usage and allowing for rapid filling of an evacuated chamber, and 2) a three-cassette system, allowing three separate sterilizations to be performed concurrently while also minimizing post sterilization contamination.

The goal of the Phase II project was to develop an ozone gas sterilizer suitable for operation in field medical situations. In the field, fast sterilizer cycle times and the ability to carry out sterilization with few logistical constraints are critical requirements. The Sterizone (see picture inside front cover), based on electrochemical ozone generation, offers significant technology and logistic advantages over the conventional ethylene oxide (EtO) method (see Table Table 3). Ethylene oxide is the primary gas used by hospitals and industry to sterilize items that cannot be sterilized by steam or radiation. The chief disadvantage of EtO is that lengthy periods (24 hours) are required to sterilize an object effectively and further time is required to permit evaporation of any residual before use. The Sterizone has a cycle time of approximately 1 hour. An increasing emphasis toward personnel safety and the potential for environmental impact related to EtO usage further accent the benefits of ozone. There are no hazardous residual associated with ozone because it can be rapidly decomposed catalytically. Ethylene oxide must be transported to the sterilizer from the site where it is generated; the Sterizone will generate ozone from water on demand hence the logistical requirements are minimal. Furthermore, Ozone is a highly effective sterilant against all classes of microorganisms; its oxidation potential (2.07 V) is higher than that of ethylene oxide (0.95 V).

Table 3. Comparison of the *Sterizone* to Ethylene Oxide Sterilization.

Attribute	Ethylene Oxide	Sterizone
Sterilization Time	24 hours	1 hour
Oxidizing Potential	0.95 V	2.07 V
Inputs	Ethylene Oxide, transported in cylinders	Air, Water, Electricity
Destruction of Gas	Complex, Time Consuming	Simple Catalytic Reduction

SECTION VII

CONCLUSIONS

During the Phase II Small Business Innovation Research project, a new method for treating and decontaminating hospital instruments was successfully demonstrated. The method employed was ozone generated by a new electrochemical method. Significant conclusions from the project are given below.

1) Electrochemically generated ozone is an effective gaseous sterilant.

2) High ozone concentration generated in this process gives enhanced kill rates over what can be expected from ozone generated by conventional methods.

3) Electrochemical ozone generation systems can be automated and integrated with gaseous sterilization chambers to provide a completely automated gaseous sterilizer.

4) Logistical requirements of an ozone sterilizer are significantly less extensive than conventional gaseous sterilization systems.

5) The ozone sterilizer provides substantially increased sterilization throughput compared to ethylene oxide.

6) To fully assess the system's potential, further evaluation is required under realistic (field) operating conditions.

SECTION VIII

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US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

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SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for the following Awards.

DAMD17-91-C-1105	ADB206577
DAMD17-93-C-3081	ADB202797
DAMD17-94-C-4049	ADB190895
DAMD17-95-C-5033	ADB206103
DAMD17-95-C-5035	ADB231081
DAMD17-95-C-5036	ADB208058

Request the limited distribution statement for Accession Document Numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Virginia Miller at DSN 343-7327 or by email at Virginia.Miller@det.amedd.army.mil.

FOR THE COMMANDER:

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management