Flexible-Chamber Ethylene Oxide Sterilization Systems: Part 1—Determination of Spore Lethality at Low Ethylene Oxide Gas Concentrations at Atmospheric Pressure

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Abstract

The use of a flexible chamber as part of an ethylene oxide (EO) sterilization system was first described in 1959. Since the 1960s, flexible-chamber EO sterilization systems have been in continuous use in the United States and around the world. Ethylene oxide flexible-chamber systems offer distinct advantages, including efficient gas usage, very low risk of harm to the sterilization load (no steam injection or deep vacuum), and the ability to process small lots of product efficiently.

This paper describes spore lethality at low EO concentrations in flexible-chamber EO sterilization systems and is Part 1 of two recent studies on the characteristics of this sterilization method.

Introduction

Flexible chambers have been used for ethylene oxide (EO) sterilization for more than 50 years. During this time, most published EO sterilization studies focused on traditional fixed chambers, whereas little has been published regarding relative humidity (RH), temperature, and EO concentration control and uniformity in flexible-chamber systems. In October 2010, the AAMI Ethylene Oxide Sterilization Working Group began developing a Technical Information Report (TIR) on flexible-chamber EO sterilization systems. In the course of this development work, a number of questions arose regarding the science behind this sterilization method. In answer to those questions, a series of studies were undertaken to provide more data on the lethality and the unique characteristics of flexible-chamber EO sterilization.

The flexible chamber consists of a sterilization bag manufactured from either a permeable or impermeable plastic film. Technicians place the product to be sterilized into the flexible bag, along with a humidity-control device (if used). Ethylene oxide gas can either be injected into the sealed bag or dispensed from a unit-dose device containing a known amount of EO which is placed into the flexible bag before it is sealed. The flexible bag is then placed within a sterilization cabinet designed to maintain the bag contents at sterilization temperature. EO is removed from the bag either by a passive diffusion process, in permeable bag systems, or by mechanical means when impermeable bags are used. Depending on the size of the cabinet, one or more flexible sterilization bags can be processed simultaneously.

Flexible-chamber EO systems are characterized by very small load volumes and serve an important niche in the processing of

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small lots, very delicate or custom devices that require strict control of temperature and/or humidity, and applications in which in-house processing of small batches is essential. This paper explores a number of the critical parameters of flexible-chamber systems and offers a mathematical model to calculate cycle lethality. Specifically, this article will demonstrate the following:

1. That the relationship between EO concentration and spore lethality within a flexible sterilization bag is identical to the lethality kinetics within a traditional fixed chamber.
2. That low concentrations of EO in a flexible sterilization bag can inactivate spores (i.e., bioburden).
3. That there is a correlation between low EO concentration and cycle lethality (expressed as the reciprocal of D-values).
4. That a mathematical model can be used to predict total log kill within a sterilization cycle using three variables: gas concentration (milligrams per liter [mg/L]), temperature (°C), and time (minutes).
5. That the mathematical model can be applied to an existing validated flexible-chamber EO sterilization process in order to calculate a theoretical log reduction of bioburden over the full exposure cycle.

When D-values are determined at different EO gas concentrations, total spore log reduction (SLR) can be calculated using the mathematical model, and the mathematical model can be applied to an established, validated cycle.

Background

The Role of Relative Humidity

A relative humidity of greater than 30% is typically used for effective EO sterilization. However, studies have shown that it is the hydration of the spores prior to exposure (EO injection) that affects the rate of inactivation rather than the RH during EO exposure.1 It has also been demonstrated that although RH is a factor in EO sterilization efficiency, its effect on cycle lethality is considered to be constant when it falls between 30% and 90%.2 For all D-values determined in this study, the relative humidity within the flexible sterilization bags was maintained within this range.

Temperature

Temperature is a critical factor in establishing and defining the D-value of the sterilization process. Because the exposure phase within the flexible sterilization bag begins at room temperature, and the sterilization load might take up to two hours to reach 50 °C, it is important to consider the impact of warm-up time on the process D-value.

The Q10 Temperature Coefficient

The Q10 temperature coefficient is a measure of the rate of change of a biological or chemical system as a consequence of increasing or decreasing the temperature by 10 °C. Q10 values have been demonstrated to range between 1.8 and 2.7 for the inactivation of Bacillus atrophaeus spores in EO systems, depending on the substrate. For the sake of this study, a conservative Q10 factor of 2.7 was chosen. For every 10 °C decrease in temperature (from 50 °C), the D-value was assumed to increase by a factor of 2.7. (For example, a D-value of 20 minutes at 50 °C corresponds to a D-value of 146 minutes at 30 °C). Experimentally, this is in agreement with the published literature.3

The Andersen BIER ("A-BIER") Vessel

A conventional BIER vessel was not appropriate for this study because it draws a deep vacuum and then controls EO concentration by injecting EO to a fixed pressure, whereas in a flexible sterilization bag EO diffuses from the dispenser throughout the flexible bag at atmospheric pressure. The Andersen BIER vessel utilizes a SEC Signature Process Gas Analyzer attached to the end of an EO-impermeable flexible sterilization bag, permitting direct EO measurement (Figure 1). The impermeable sterilization bag allows for the creation and maintenance of a static EO environment at atmospheric pressure.

In the A-BIER vessel, the spore strips or biological indicators (BIs) are sealed in glass ampoules. The sealed glass ampoules allow the conditions within the sterilization bag to equilibrate before the operator breaks the neck of the ampoules, exposing the indicators to the environment in the sterilization bag.

Using EO cartridges of varying sizes, each introducing a different volume of EO into the sterilization bag, it is possible to establish

D-values at different EO gas concentrations.

Materials and Equipment

- The A-BIER vessel was created using an Andersen AN1004 impermeable EO sterilization bag (Lot 110232) surrounding a 36-liter (L) sterilization tote (Sterilite 1629).
- EO-filled ampoules were used to introduce the sterilant into the flexible sterilization bag, and a Humidichip® (Lot 111122) was used to maintain RH between 30% and 90%.
- Cycle temperature and RH were measured using temperature and %RH data loggers traceable to the National Institute of Standards and Technology (NIST).
- Direct EO measurement was achieved using an SEC EO analyzer (P/N 142-0597—s/n 30341) attached to the aluminum assembly sealed into the sterilization bag.
- D-values were calculated using Bacillus atrophaeus spore strips (SGM Biotech Lot RGS 256 and NAMSA Lot N28630) sealed into ampoules using a Cozzoli ampoule sealer. Population counts of both BI lots were verified by Presque Isle Laboratories. Different populations were used to increase the likelihood of achieving a fractional result.
- The Andersen sterilizer cabinet was used to heat the A-BIER vessel to 50°C.
- After EO exposure, spore strips were removed from the A-BIER vessel in a fume hood and then were transferred into soybean casein digest culture medium (SGM Biotech Lot RM 256) in a Labconco Bio-hood.

Methods

The A-BIER vessel was loaded into a heated sterilization cabinet with a series of B. atrophaeus spore strips (104 or 106 organisms per strip) sealed into glass ampoules (EO cannot penetrate glass). Using a range of starting spore populations increases the probability of finding a fraction-negative time point. The sealed glass ampoules containing the spore strips were inverted and attached to the wall of an empty 36-L rigid container, which provided a static framework within the sterilization bag. The framework was then inserted into the impermeable sterilization bag. An SEC Signature Process Gas Analyzer was attached to the other end of the sterilization bag, permitting direct EO measurement within the exposure environment. The EO gas cartridge was activated and left for 2 to 3 hours to permit the EO concentration and temperature within the flexible sterilization bag to stabilize. Once the EO concentration had achieved a plateau, the temperature was 50°C ± 2°C, and the relative humidity was 60% ± 10%, the ampoule necks were broken so that the spore strips were exposed to the sterilant. At the conclusion of the exposure phase, the spore strips were removed from the load, immediately transferred to growth medium, and incubated for 7 days. D-values were

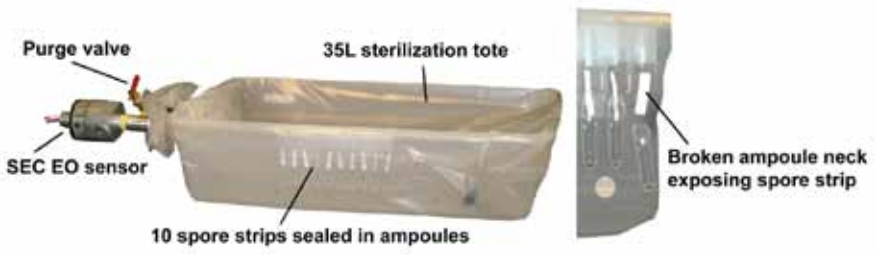

Figure 1. The "A-BIER Vessel"

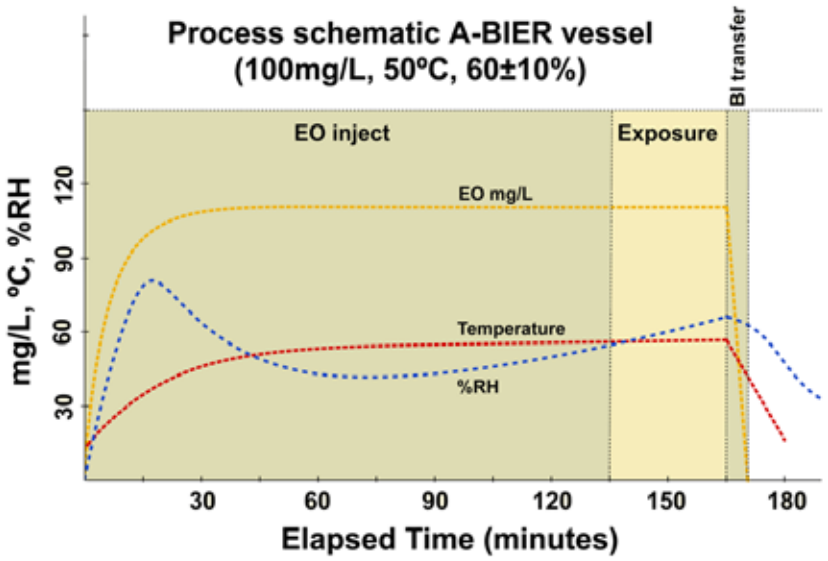

Figure 2. Schematic of the "A-BIER" Vessel Exposure Cycle (Gas Concentration, Temperature, and Relative Humidity)

$$\text{D-Value} = \frac{U}{\log N_o - \log N_u}$$

U = gas exposure
N_o = Initial spore population
N_u = 2.303 (log n/r)
n = total number of tests
r = number of sterile tests

Figure 3. Calculation of D-value by the Stumbo Murphy and Cochran Procedure (SMCP)

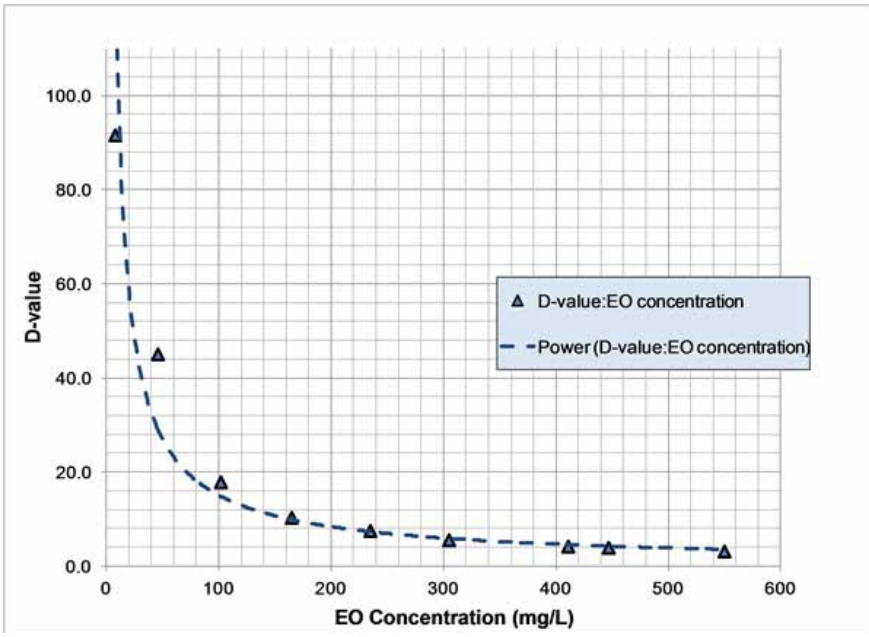

Figure 4. Bacillus atrophaeus D-value vs. EO Concentration (8 mg/L to 550 mg/L)

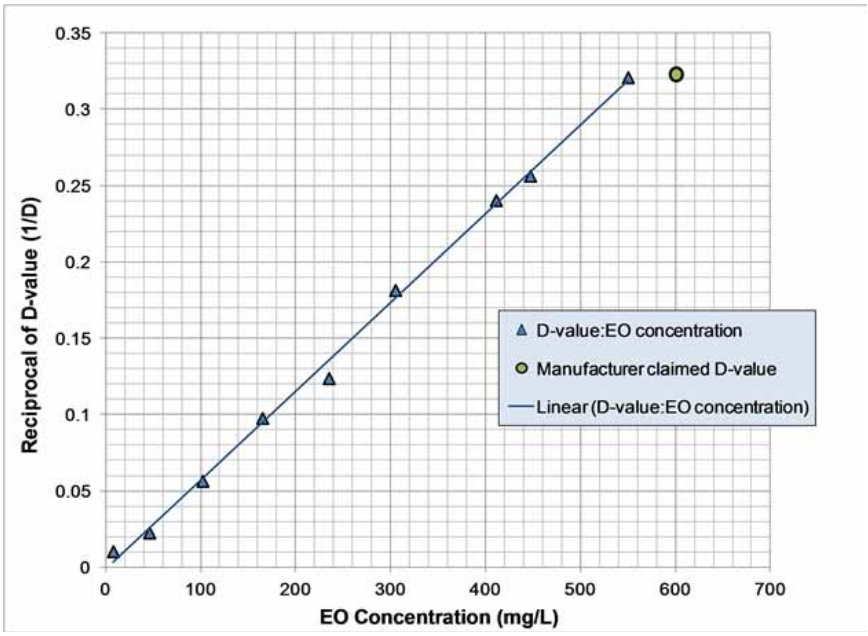

Figure 5. Bacillus atrophaeus D-value vs. EO Concentration (8 mg/L to 550 mg/L); Log Plot

[i] Concentration of EO in mg/L ± 3 mg/L (recorded SEC EO sensor values).

[ii] Note that the SEC sensor used in this study has an accuracy of ±30 mg/L, making it difficult to measure gas concentration accurately on the lower end of the scale. In the 8 mg/L D-value study, the calculated concentration, based on the weight of EO added to the A-BIER vessel, was closer to 21 mg/L. Still, it is clear that EO concentrations at or below 50 mg/L will inactivate bacterial spores.

[iii] Figures 4, 5, and 6 were generated from a previously validated process using sutures as the product load.

EO (mg/L)	Average exposure temp. (°C)	Average exposure %RH	Spore lot #	D-value (minutes)
550	50.8	53.7	N28630	3.1
447	51.4	55.3	RGS-256	3.9
406	51.7	58.4	RGS-256	4.2
306	49.5	63.5	RGS-256	5.5
235	49.5	64.5	RGS-256	8.1
165	51.3	54.3	RGS-256	10.3
102	50.9	57.2	RGS-256	17.8
46	51.2	57.4	N28630	45.0
8	51.2	63.7	RGS-256	91.6

Table 1. Summary of Exposure Conditions and Calculated D-values

calculated at a variety of EO concentrations by varying the ampoule weights of EO introduced into the sterilization bag. D-values were calculated using the Stumbo Murphy and Cochran Procedure (SMCP) (Figure 3).

Results

A range of D-values were calculated at different gas concentrations, ranging from 8 mg/L to 550 mg/L (Table 1 and Figure 4). Plotting the gas concentration in mg/L against the reciprocal of the D-value (lethal rate) reveals a direct relationship between the two. This inverse relationship is illustrated in Figure 5 and demonstrates a strong correlation with an r2 of 0.99. The manufacturer's claimed D-value is annotated on the graph, and was calculated using a traditional BIER vessel. As the EO gas concentration increases, the reciprocal of D-value increases (D-value decreases). The nature of the plot allows use of the slope as an established standard, from which any EO gas concentration between 8 mg/L and 550 mg/L can be correlated to a D-value, assuming that the exposure temperature is maintained at 50°C. Not only can the D-value be predicted at any gas concentration, but it can also be adjusted for any temperature using the Q10 factor. This makes it possible to calculate the level of lethality achieved within an exposure environment in which the gas concentration and temperature are changing.

The data in Figure 6 demonstrate the results obtained within the A-BIER vessel combined with previously published data using traditional fixed EO chambers. All

studies demonstrated that at a fixed temperature, D-values are linearly related to ethylene oxide gas concentration.

Predicting Cumulative Log Kill with the Spore Reduction Lethality Equation

For the purposes of this study, the total sterilization time (16 hours) was divided into 192 equal 5-minute intervals. Measuring the temperature and ethylene oxide gas concentration during each interval enables the theoretical log reduction for each 5-minute time interval to be calculated. The total log reduction throughout the exposure cycle (L) is determined by summing the 192 calculated 5-minute interval log reductions.

To calculate the theoretical total cycle spore reduction (L), a mathematical equation was developed using the Riemann sum shown in Figure 7. This equation considers the temperature (°C) and EO gas concentration (mg/L) for each 5-minute interval. The first part of the equation simply states that the 16-hour exposure cycle was subdivided into 192 equal time intervals, where gas concentration, temperature, and therefore D-value are known. (The D-values are based on an experimentally determined relationship to EO concentration, as seen in Figure 5.)

The second part of the equation states that the log reduction occurring is computed as rate of kill (f) multiplied by time. The third part of the equation is an observation that the rate of kill (f) (log reductions per minute) is the reciprocal of minutes per log reduction.

The Use of the Lower Bound: A Conservative Approach

The temperature (T) and concentration (C) that are used to compute f are not measured across each entire interval. However, because these parameters and f are monotone across each interval, a lower bound for f (ti) can be established for each interval by noting:

$$f(t_i) \geq \min\{f(t_{start}), f(t_{end})\} = f_i$$

And then concluding:

$$L = \sum_{i=1}^{192} f(t_i)\Delta t \quad \geq \quad \sum_{i=1}^{192} f_i \Delta t = L_{bound}$$

The measured (T) and (C) for the start and end of each interval is used to compute the corresponding Dval and f, and then f_i for each interval L_{bound} is computed from the above formula.

The computation of Lbound is asserted to be a lower bound of the true log reduction (L) because of the following conservative policies in the computation:

1. A kill rate of zero was used when the gas concentration dropped below 50 mg/L.
2. A Q10 value of 2.7 was chosen to calculate log reduction at lower temperatures.

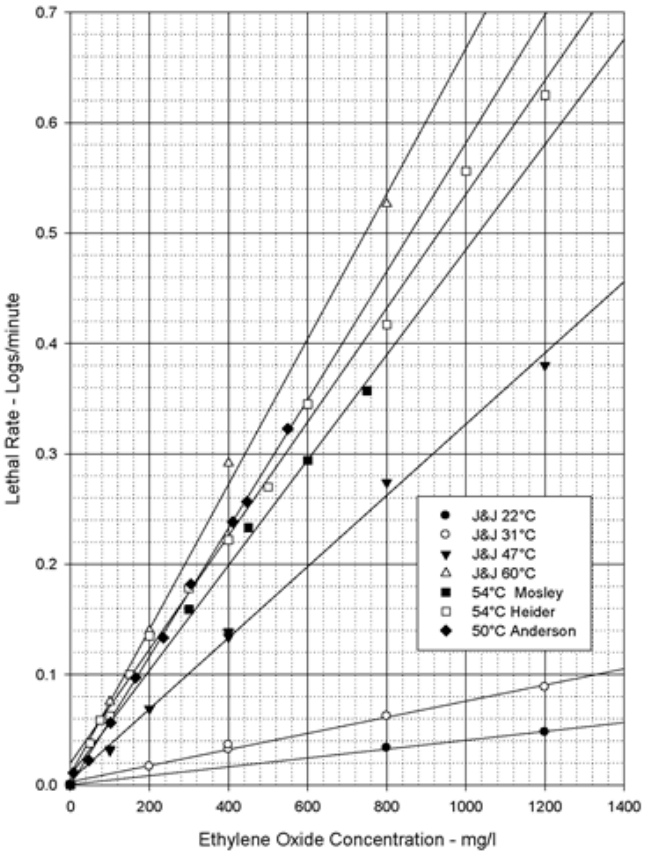

Figure 6. Bacillus atrophaeus D-values vs. EO Concentration (8 mg/L to 1,200 mg/L) and Temperature (22°C to 60°C); Multiple Sources

$$L = \sum_{i=1}^{192} \Delta L_i = \sum_{i=1}^{192} f(t_i)\Delta t = \sum_{i=1}^{192} \left(\frac{1}{\mathrm{Dval}(T(t_i), C(t_i))} \right) \Delta t$$

L = total log reduction over the 960 minute sterilization
i = index referring to each of 192 specific 5 minute intervals in the 960 minute sterilization
ΔL_i = the log reduction achieved in the ith interval
t_i = moment of time t in the ith interval
f = rate of log reduction per unit time, measured in log reductions per minute
Δt = 5 minutes, the duration of each interval
T = temperature
C = concentration of gas
Dval = D-value; the number of minutes required to achieve a one log reduction in the population

Figure 7. The Lower-Bound Analysis for Log Reduction of Population

Applying the Formula to an Established Cycle

For the purposes of this study, an Andersen Sterilizers, Inc., EOGas 3 system was used. Unlike traditional EO sterilization performed in fixed metal chambers, the EOGas 3 system uses flexible sterilization bags at atmospheric pressure. The load is placed into the flexible sterilization bag along with an EO gas cartridge. The sterilization bag is hermetically sealed and placed into the sterilizer cabinet, the cartridge is activated within the bag, and the sterilizer cabinet door is closed. Once

[iv] Formula generated by C. Bray, Ph.D., Assistant Associate Professor of the Practice, Department of Mathematics, Duke University.

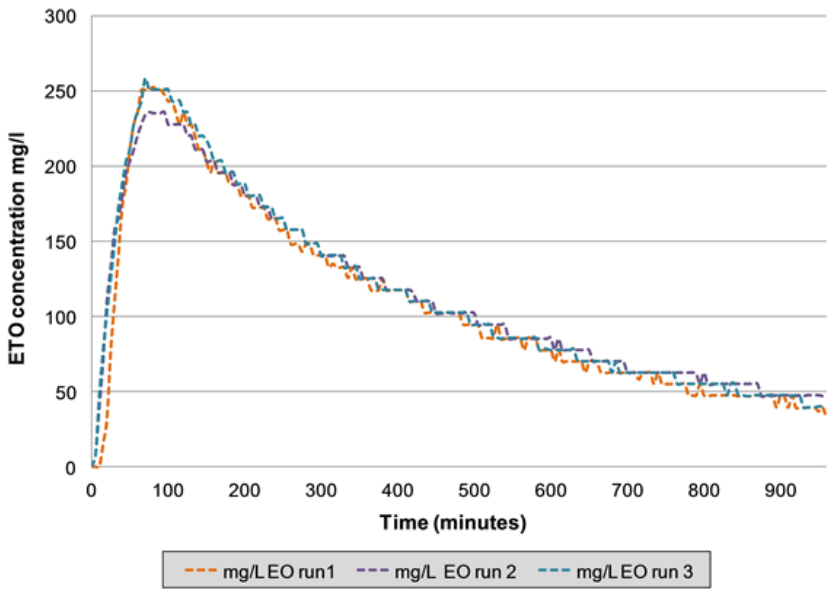

Figure 8. Three Consecutive EO Concentration Profiles (mg/L over 16 Hours)

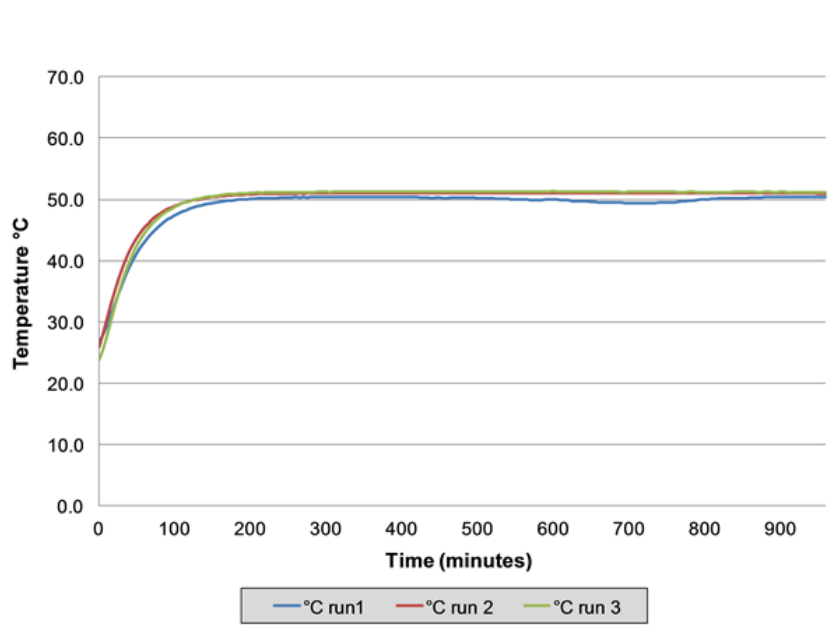

Figure 9. Three Consecutive Temperature Profiles (°C over 16 Hours)

	Average temperature (°C)	Average %RH	Average EO concentration (mg/L)
Run 1	48.8	53.1	113
Run 2	49.9	46.5	108
Run 3	49.9	49.2	113

Table 2. Summary from Three Consecutive Sterilization Processes (Figures 8, 9, and 10)

Exposure cycle	Calculated total spore log reduction (L)
Run 1	51.7
Run 2	48.4
Run 3	58.5

Table 3. The Theoretical Log Reduction Calculated Using the Riemann Sum Formula

the sterilization bag is inside the sterilizer, the load temperature equilibrates, and the EO diffuses throughout the load (peaking at 1 to 2 hours following the start of the cycle). The EO gas diffuses through the permeable sterilization bag over the course of a 16-hour cycle. As a result, the concentration within the sterilization bag peaks rapidly and then slowly declines.

The mathematical model described in the previous section was applied to a series of three consecutive exposure cycles performed using the EOGas 3 process. Each sterilization load was kept constant: a single sterilization bag containing 1080 sutures, an AN2018 EO gas cartridge (containing 17.6 g ± 5% EO), two Humidichips (providing relative humidity to the exposure cycle as the sterilization load is heated to 50°C) and an NIST-traceable temperature and relative humidity data logger. The three consecutive profiles demonstrate a consistent EO concentration (Figure 8), temperature (Figure 9), and relative humidity (Figure 10) profile. Table 2 is a summary of the three profiles.

By applying the Riemann sum formula to the three consecutive profiles, one can estimate the capability of the cycle by calculating the lower-bound log reductions of all time intervals and then totaling them to establish the total lower-bound log reduction (Lbound). The total log reduction for runs 1, 2, and 3 is shown in Table 3.

Conclusions

1. At given EO concentrations and temperatures within EO flexible sterilization bags, spore inactivation is identical to traditional fixed EO chambers.
2. Inactivation of B. atrophaeus spores was achieved at EO concentrations as low as 8 mg/L (measured by SEC sensor, or 21 mg/L by weight/volume calculation). At this concentration, spore populations were reduced by one log (90%) every 91.6 minutes, achieving a sterility assurance level of 10-6 in less than 19 hours (92 minutes x 12 logs).
3. A linear relationship between gas concentration and D-value (reciprocal of lethality) exists between 8 mg/L and 550 mg/L, allowing known EO gas concentrations to be expressed as rates of kill (SLR/minute).
4. Using measured gas concentration and

temperature during sterilization cycles, the Spore Log Reduction equation predicts cumulative log kill. The cumulative log kill is calculated from the known D-values along the curve of ethylene oxide gas concentration versus time, with a correction applied (Q10 value) to adjust for temperature variation.

The formula and spore lethality data presented in this article have been developed to assist those involved in validating flexible chamber EO sterilization cycles.

References

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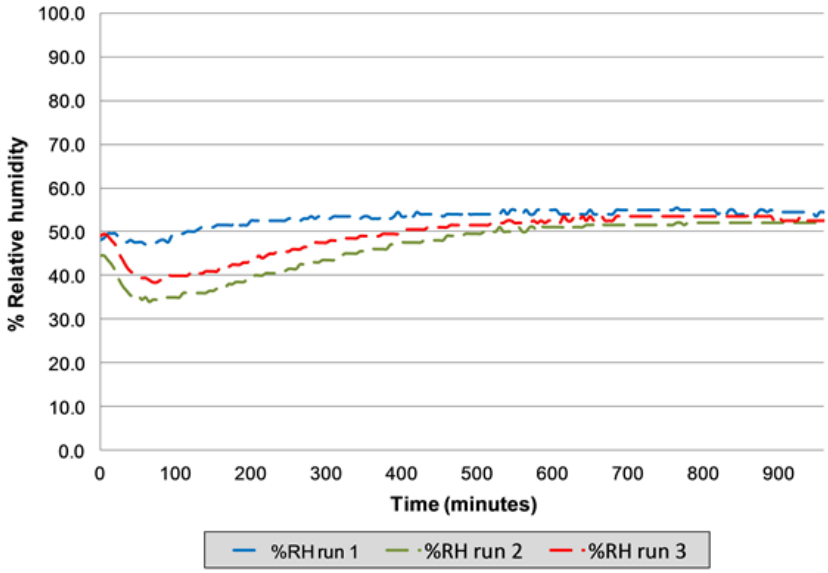

Figure 10. Three Consecutive Relative Humidity Profiles (% RH over 16 Hours)