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23. Sterilization of overwrapped foil suture packages with gaseous chlorine dioxide

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SUMMARY

Gaseous chlorine dioxide (ClO_2) was investigated for the sterilization of overwrapped foil suture packages (secondary sterilization). Using a simulated production load that contained *Bacillus subtilis* biological indicators (BIs) and actual product samples, incremental ClO_2 exposures were made. The sterilization conditions covered the range of 24–28°C, 45–70% relative humidity (RH) and 10–50 mg/l ClO_2. The load was also preconditioned for 24 h at 18–24°C, 70–85% RH prior to being placed in the sterilization chamber. At lower gas concentrations (20 and 10 mg/l), the BIs and product samples were sterile after 60 and 120 min of exposure, respectively. At 40 mg/l of ClO_2, the sterilization rate was more rapid; the BIs and product samples were sterile after 30 and 15 min of exposure, respectively. ClO_2 appears to be an effective sterilant for overwrapped foil suture packages.

INTRODUCTION

Exploration of the use of ClO_2 as an alternate sterilizing gas was prompted by the difficulty and expense of the use of ethylene oxide (EO) as a sterilant [2]. Ethylene oxide is commonly supplied as a mixture of EO and chlorofluorocarbon (CFC) gases and used in a pressure chamber at high concentrations (600–1000 mg/l). Because of its toxicity and potential carcinogenicity [1], costly controlling and monitoring equipment is required to keep personnel exposure and environmental emissions to a minimum. The CFC gas component, due to its deleterious effect on the stratospheric ozone concentration, will shortly become regulated [6] and require costly recovery equipment. In addition, because of the high concentrations required, there has been concern about residual EO and EO by-product levels in the final products.

In contrast, ClO_2 is generated just prior to use in the sterilizer, used at slightly less than atmospheric pressure and at low concentrations (< 50 mg/l). Although it is also toxic [7], the sterilization chambers will tend to leak 'in' and the maximum permissible exposure levels correspond to the odor detection level, so personnel monitoring is of less concern. At the completion of the cycle, the ClO_2 is absorbed from the exhaust by a simple cartridge system eliminating hazardous emissions. Finally, because of the low concentration, residual levels are not expected to present a significant problem. As with any process change, however, the effect of ClO_2 on the product will have to be investigated over the product shelf life.

Exploratory studies with gaseous ClO_2 performed with biological indicators (BIs) [3,5] confirmed the lethality of low concentrations of ClO_2 at ambient temperatures. The purpose of this report is to present data from several experiments conducted to evaluate the effect of ClO_2 gas concen-

tration on production-type loads containing overwrapped foil suture packets and spore strip BIs.

MATERIALS AND METHODS

Chlorine dioxide sterilizer

The sterilizer is an unheated, unjacketed stainless steel chamber. A simplified schematic diagram of the sterilizer system is given in Fig. 1.

Sterilization with gaseous ClO_2 is similar to EO processes: an initial vacuum is drawn, gas is admitted, a sterilization dwell time elapses, and the gas is removed from the chamber by evacuation. The ClO_2 is not obtained from a cylinder in the usual manner, but is generated just prior to its introduction into the chamber by passing a mixture of chlorine in nitrogen through dry sodium chlorite contained in a reactor column [5]. The concentration of ClO_2 that is produced is controlled by varying the chlorine concentration in the feed gas. The ClO_2 that is evacuated from the chamber after a timed exposure is passed through a chemical 'scrubbing' column which absorbs the ClO_2 gas. The sterilizer can be operated manually, or in a microprocessor-controlled automated mode.

Experimental load

Simulated production-type loads consisted of 40 suture boxes, each containing one dozen sealed foil suture packets overwrapped in a gas-permeable Tyvek/film pouch. An assembled and secured load was approximately an 11-inch cube, with a volume displacement of 0.8 cubic feet. The loads were fitted with internal (inside of suture boxes) and external (outside the suture boxes) BIs consisting of Tyvek-overwrapped *Bacillus subtilis* (ATCC 9372) spore strips in glassine wrappers (AMSCO Medical Products). Product test pieces, represented by overwrapped foil suture packets, were also placed inside suture boxes. Completed loads were humidified for 24 h in an environmental chamber maintained at 18–24°C and 70–85% RH. Prehumidification is required for efficient sterilization [5]. Each load was triple-wrapped immediately after prehumidification with a moisture barrier plastic film (Saran Wrap) which was removed just prior to ClO_2 exposure.

Exposure of loads to ClO_2

Initial evaluations of ClO_2 lethality were carried out using the manually operated sterilizer (experiments I and II). Exposure times were 0, 15, 30, 60 and 120 min at a target dose of 40 mg l ClO_2, and 0, 30, 60, 120 and 240 min at a target dose of 20 mg/l. A zero exposure was achieved by admitting the ClO_2 gas to the target pressure and then immediately exhausting the chamber, with zero dwell time at sterilizing conditions.

The ClO_2 concentration in the sterilizer during a run was determined by removing samples of the chamber atmosphere with a gas-tight syringe and injecting them into accurately measured quantities of a standardized chlorphenol red solution. The

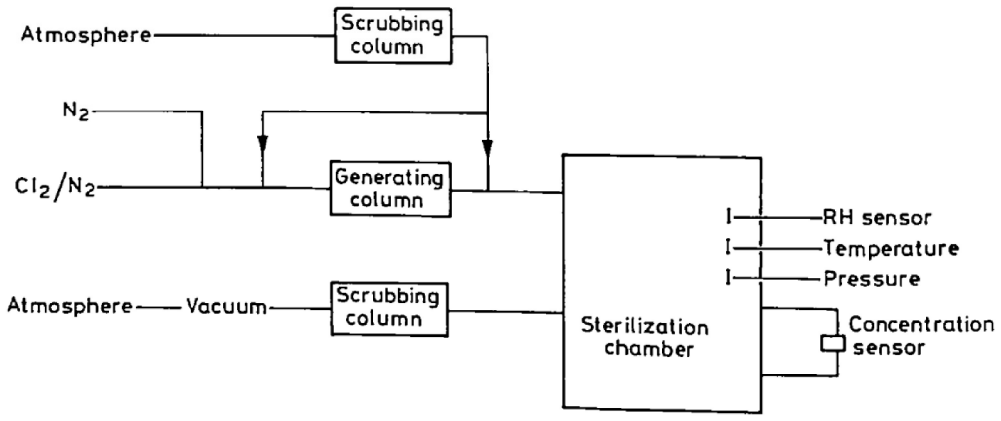

Fig. 1. Schematic diagram of the ClO_2 sterilizer system. → represents a pressure relief valve.

amount of ClO_2 present in a sample was determined by the extent of the decolorization of the test solution as estimated by measuring the optical density at 575 nm.

In subsequent studies (experiments III and IV), an automated sterilizer unit was employed, at a gas concentration of 20 mg/l and exposure times of 0, 15, 30 and 60 min and a gas concentration of 10 mg/l with exposure times of 30, 60, 90, 120, 180 and 240 min. Chlorine dioxide was monitored continuously during runs by measuring the characteristic ClO_2 absorbance peak at 360 nm. All trials were conducted at ambient temperature; relative humidity was not controlled in the sterilizer chamber.

The simulated production loads contained a complement of 20 internal and 12 external spore strip BIs (10^6 cfu per strip) and 20 internal product test pieces, dispersed at various locations. In experiments III and IV, an extra set of 20 internal and 12 external spore strips (10^3 cfu per strip) was included in each load.

The loads were exposed to an overwrap sterilization cycle in the ClO_2 sterilization chamber. The chamber was evacuated to a pressure of 5–13 inches Hg, and the residual air displaced with ClO_2 gas while maintaining the low vacuum. The chamber was then allowed to fill with gas to near atmospheric pressure. At the conclusion of the designated exposure period, an exhaust/purge cycle was initiated which consisted of a multiple series of low-vacuum draws followed by 0.2-μm-filtered-air flushes.

Sterility testing

The BIs and product test pieces were sterility-tested under aseptic conditions in a laminar flow hood within 24 h after removal from the degassed load. Spore strips were transferred into 40 ml of trypticase soy broth (TSB) and incubated at 30–35°C for 7 days. Product test pieces were transferred into 160 ml of TSB and incubated at 20–25°C and 30–35°C for 14 days (10 samples at each temperature).

RESULTS AND DISCUSSION

Exposure and sterility test data obtained with the use of the manually operated sterilizer are presented in Tables 1 and 2. In experiment I, ClO_2 gas concentrations were greater than the 40 mg/l target. All BIs were sterile after a 30-min exposure. The

Table 1

Experiment I: manual sterilizer exposures (target concentration: 40 mg/l ClO_2)

Exposure time (min)	ClO_2 concentration (mg/l)		RH (%)		Temperature (°C)		Sterility test results[a]			
	initial	final	initial	final	initial	final	spore strips (10^6 cfu) external	spore strips (10^6 cfu) internal	product samples (30–35°C)[b]	product samples (20–25°C)[b]
0	64	64	53	53	25	25	3/12	19/20	1/10	1/10
15	50	46	52	55	27	27	0/12	1/20	0/10	0/10
30	52	37	51	52	25	25	0/12	0/20	0/10	0/10
60	40	22	52	55	26	26	0/12	0/20	1/10[c]	0/10
120	46	26	49	54	25	24	0/12	0/20	1/10[d]	0/10

[a] No. nonsterile/total tested.
[b] Incubation temperature.
[c] *B. megaterium*.
[d] *Corynebacterium* species.

Table 2

Experiment II: manual sterilizer exposures (target concentration: 20 mg/l ClO_2)

Exposure time (min)	ClO_2 concentration (mg/l)		RH (%)		Temperature (°C)		Sterility test results[a]			
	initial	final	initial	final	initial	final	spore strips (10^6 cfu)		product samples (30–35°C)[b]	product samples (20–25°C)[b]
							external	internal		
0	30	30	43	47	27	27	12/12	20/20	0/10	0/10
30	11[c]	7	45	54	26	26	12/12	20/20	0/10	0/10
60	21	11	53	55	25	25	0/12	0/20	0/10	0/10
120	22	15	56	56	26	26	0/12	0/20	0/10	0/10
240	22	20	55	55	24	26	1/12[d]	0/20	0/10	0/10

[a] No. nonsterile total tested.
[b] Incubation temperature.
[c] Reactor column failure.
[d] *B. subtilis*.

foil suture packet product samples were sterile at 15 min, but scattered instances of bacterial growth were observed after 60 and 120 min. At a lower gas concentration (experiment II, 20 mg/l target) all the BIs and product samples were sterile after a 60-min exposure. Insufficient gas generation, caused by a depleted sodium chlorite column, eliminated the 30-min exposure as a datapoint for the inactivation of the BIs in experiment II. The cause of the nonsterile samples at the later time periods in experiments I and II is not clear. Laboratory contamination may have occurred during the testing of the product samples. As there is no circulation system in the vessel, gas stratification is also suspected. With the automated sterilizer (see below), which has automatic make-up gas addition, no 'late' nonsterile results were seen at a 10 or 20 mg/l ClO_2 concentration.

Exposures at the 20 mg/l gas concentration target were repeated, employing an automated sterilizer (experiment III). One of the attributes of the automated sterilizer is a system which meters gas into the chamber during a sterilization exposure. As indicated in Table 3, initial and final ClO_2 concentrations for all exposures were very close to the target concentration. The inactivation rates for externally and internally placed BIs, as extracted from the positive data fraction, are similar. This implies that the diffusion of ClO_2 into a bulky and compact load is rapid and complete. The BIs and product samples were sterile at the 60-min exposure period; this confirmed the results obtained in experiment II. The reproducibility of the 60-min ClO_2 cycle was demonstrated by three successful exposure runs.

The results obtained at a concentration of 10 mg/l, shown in Table 4, also exhibit consistent concentration levels throughout the run. To ensure total inactivation of the BIs and sterility of the product samples, 120 min was required. The longer sterilization time required for the lower gas concentration is consistent with what would be expected of gas sterilization.

The results of this investigation indicate that gaseous ClO_2, operating at low concentrations and temperature, is an effective sterilizing agent for foil suture packets in a Tyvek/film pouch. Further investigations will assess the ability of ClO_2 to sterilize natural and synthetic suture materials, and the chemical and physical effects of ClO_2 on product and packaging component integrity.

Table 3

Experiment III: automated sterilizer exposures (target concentration: 20 mg/l ClO_2)

Exposure time (min)	ClO_2 concentration (mg/l)		RH (%)		Temperature (°C)		Sterility test results[a]					
	initial	final	initial	final	initial	final	spore strips (10^3 cfu)		spore strips (10^6 cfu)		product samples (30–35°C)[b]	product samples (20–25°C)[b]
							external	internal	external	internal		
0	20	20	62	62	26	26	12/12	20/20	12/12	20/20	1/10	0/10
15	21	20	66	68	26	26	1/12	2/20	5/12	19/20	0/10	0/10
30	22	20	67	70	26	26	0/12	0/20	2/12	4/20	0/10	0/10
60	21	20	66	70	27	27	0/12	0/20	0/12	0/20	0/10	0/10
60	23	20	60	70	26	26	0/12	0/20	0/12	0/20	0/10	0/10
60	25	21	68	70	26	26	0/12	0/20	0/12	0/20	0/10	0/10

[a] No. nonsterile/total tested.

[b] Incubation temperature.

Table 4

Experiment IV: automated sterilizer exposures (target concentration: 10 mg/l ClO_2)

Exposure time (min)	ClO_2 concentration (mg/l)		RH (%)		Temperature (°C)		Sterility test results[a]					
	initial	final	initial	final	initial	final	spore strips (10^3 cfu)		spore strips (10^6 cfu)		product samples (30–35°C)[b]	product samples (20–25°C)[b]
							external	internal	external	internal		
30	9	10	72	80	26	28	2/12	20/20	12/12	20/20	0/10	0/10
60	9	10	73	82	27	28	0/12	0/20	1/12	6/19	0/10	0/10
60	10	10	68	78	25	25	2/12	7/20	2/12	11/20	0/10	0/10
90	10	10	80	85	26	27	0/12	0/20	0/12	3/20	0/10	0/10
90	11	10	71	78	25	25	0/12	0/20	0/12	0/20	0/10	0/10
90	10	10	72	85	25	26	0/12	0/20	0/12	0/20	0/10	0/10
120	10	9	72	80	25	25	0/12	0/20	0/12	0/20	0/10	0/10
180	10	9	73	82	26	26	0/12	0/20	0/12	0/20	0/10	0/10
240	10	10	70	79	25	25	0/12	0/20	0/12	0/20	0/10	0/10

[a] No. nonsterile/total tested.

[b] Incubation temperature.

REFERENCES

1 Glaser, Z.R. Special Occupational Hazard Review with Control Recommendations for the Use of Ethylene Oxide as a Sterilant in Medical facilities: DHEW (NIOSH) Publication No. 77-200. 1977. National Institute for Occupational Safety and Health, Rockville, MD. (Also published in part as: 'Ethylene oxide: toxicology review and field study results of hospital use. 1979. J. Environ. Pathol. Toxicol. Oncol. 2: 173–208.)

2 Jorkasky, J.F. 1987. Medical product sterilization: changes and challenges. Med. Device Diagn. Ind. 9: 32–37.

3 Knapp, J.E., D.H. Rosenblatt and A.A. Rosenblatt. 1986. Chlorine dioxide as a gaseous sterilant. Med. Device Diagn. Ind. 8: 48–51.

4 Rosenblatt, D.H., A.A. Rosenblatt and J.E. Knapp. 1985. Use of chlorine dioxide gas as a chemosterilizing agent. United States Patent No. 4,504,442.

5 Rosenblatt, D.H., A.A. Rosenblatt and J.E. Knapp. 1987. Use of chlorine dioxide gas as a chemosterilizing agent. United States Patent, No. 4,681,739.

6 Thomas, L.M. 1987. Protection of stratospheric ozone. Fed. Regist. 40CFR Part 82. 52 (239): 47486–47523.

7 Wojtowicz, J.A. 1979. Chlorine oxygen acids and salts. In: Encyclopedia of Chemical Technology (Kirk, R.E. and Othmer, D.F. eds.), 3rd Edn., Vol. 5, pp. 612–620, John Wiley and Sons, New York.