Successful Sterilization Using Chlorine Dioxide Gas

Part Two: Cleaning Process Vessels

Amnon Eylath, Sushil Madhogarhia, Emilio Rivera, Paul Lorcheim, and Marc Czarneski

Chlorine dioxide (CD) is an oxidizing agent used extensively in solution for bleaching pulp, treating water systems, and disinfecting food processing equipment and food including fresh fruits and vegetables. It has also been used in the formulations of disinfectant mouthwashes and toothpaste (1, 2). Most visibly in recent years, chlorine dioxide in both its gaseous and aqueous forms was used to successfully decontaminate the Hart Office Building and Brentwood postal sorting facility in Washington, DC, in response to their contamination with Anthrax spores (3–5). Chlorine dioxide gas is approved for use as a sterilant by the United States Environmental Protection Agency (US EPA). Its sporicidal effects are well documented and can be compared with those of vaporized hydrogen peroxide and formaldehyde, agents commonly used for surface disinfection in the pharmaceutical industry.

In the past, gaseous CD was typically prepared by vaporization of a solution (6). For our study, CD was generated at the point of use. This is accomplished by using a method in which solid sodium chlorite contained in small plastic cartridges is perfused by a 2% chlorine–98% nitrogen gas mixture. The reaction products are CD and sodium chloride.

Our target concentration in the vessel to be cleaned at 5 and 10 mg/L, with 10–30 minutes exposure once that target concentration was reached. The prototype CD generator makes up to 8,000 L chlorine dioxide at 100 mg/L at a typical pressure of 50 Pascals above atmospheric. Once used, the gas is disposed of by aeration under positive pressure assist using 0.22 µm-filtered pharmaceutical grade air through an exhaust fan into general atmosphere at EPA-approved limits for exhaust of CD. No corrosion is observed when using pharmaceutical-type materials such as high-grade 316 and 304 stainless steel, Lexan, and various other plastics such as Delrin, Teflon, and UHMWPE (7). Neither were any effects observed on the exhaust system. In a separate experiment, postexposure rinses of 304 stainless steel coupons in water for injection (WFI) showed no residual CD as measured using a high-pressure liquid chromatographic (HPLC) method for detection of chlorides (8).

In the July issue of *BioProcess International*, we described the development of that process for disinfecting the surfaces of an isolator using CD (8). That approach to developing sanitization cycles was modeled after cycle development in more typical sterilization and disinfection procedures (such as steam sterilizers and aseptic isolators) for pharmaceutical equipment. This month, we conclude our two-part article by reporting the use of

Photo 1: Isodox chlorine dioxide gas generator

PRODUCT FOCUS: PARENTERAL PRODUCTS

PROCESS FOCUS: ASEPTIC PROCESSING

WHO SHOULD READ: QUALITY, MANUFACTURING, VALIDATION, AND ENVIRONMENTAL MONITORING PERSONNEL

KEYWORDS: STERILIZATION, CLEANING METHODS, ASEPTIC PROCESSING, PROCESS VESSELS

LEVEL: INTERMEDIATE

chlorine dioxide for disinfecting the internal surfaces of pharmaceutical process vessels. The approach to testing the sanitization cycles was modeled after cycle development for typical sterilization and disinfection procedures for pharmaceutical vessels using steam in place.

Because of the materials and design of pharmaceutical processing equipment, saturated steam has been the method of choice for decontamination and sterilization. The advantages for using steam are that it is made from the same purity of water (typically USP-grade WFI) used to clean and rinse the vessels and piping associated with process systems. Pure steam leaves no residue, and its corrosive nature is mitigated by passivation of the exposed stainless steel surfaces with acids and chelating regents. Enriching the surface with chromium protects it from corrosion by WFI or steam or other materials. The disadvantages of using steam are in its high energy cost of production, dependance on elaborate WFI systems, and a need for costly pressure-rated vessels and piping.

Materials and Methods

The "Materials" sidebar details the materials and equipment used in our study. CD produced by the Isodox generator with integral steam humidification capabilities (Photo 1) was tested for its sporicidal activity on a 100-L stainless steel process vessel (Photo 2) and a 500-L stainless steel process vessel (Photo 3). Biological indicators were used as the microbial challenge for each test.

Those biological indicators were placed throughout each vessel on representative surfaces (Figures 1 and 2). Each indicator was removed from its individual glassine envelope and transferred into an individual Tyvek/film pouch. The gas impedance of the Tyvek was found to be negligible for the diffusion of CD — unlike for glassine, which exhibited slower diffusion of gas into the envelope. The data presented herein came from the Tyvek-enclosed biological indicators, which were considered to represent a realistic yet rigorous challenge.

We ran two sterilization cycles based on prior experience using CD for the high-level decontamination of isolators (**8**). Our approach to cycle development was based on the fact that spore inactivation rates are directly related to CD concentration and length of exposure. Humidification before exposure also affects inactivation kinetics, so we fixed the relative humidity prior to exposure at 70% based on previously published work (**1**).

Test Results

The exposure cycles tested were shown to be successful in sterilizing the interior surfaces of the test equipment using biological indicator spore strips with a population of 10^6 spores each, as seen in Table 1. To assist in the distribution of the gas, the mixing impellor (manufactured by Lightnin, www.lightnin-mixers.com) in the 500-L vessel was turned on during the complete sterilization cycle. Significantly, CD penetrated into the deadlegs at the bottom of that larger vessel, which showed complete inactivation of the biological indicators at depths of up to 5 inches.

Materials Used

100-L stainless steel process vessel

500-L stainless steel process vessel

Chlorine dioxide gas generator, prototype; Advanced Sterilization Products, Irvine, CA (www.sterrad.com), now fabricated by ClorDiSys Solutions, Inc., Lebanon, NJ (www.clordisys.com)

CD-Cartridge set; Advanced Sterilization Products, Irvine, CA (www.sterrad.com), now fabricated by ClorDiSys Solutions, Inc., Lebanon, NJ (www.clordisys.com)

2% chlorine–98% nitrogen compressed gas, 200 SCF/cylinder; Praxair, Inc., Oxnard, CA (www.praxair.com)

Amsco biological indicators (*Bacillus subtilis*), paper, 10^6 spores/carrier; Steris Corporation, Mentor, OH (www.steris.com)

Vis-U-All II heat seal Tyvek pouches for repackaging biological indicators, Steris Corporation, Mentor, OH (www.steris.com)

Heat impulse sealer HS-8; Fetpak Inc., Commack, NY (www.fetpack.com)

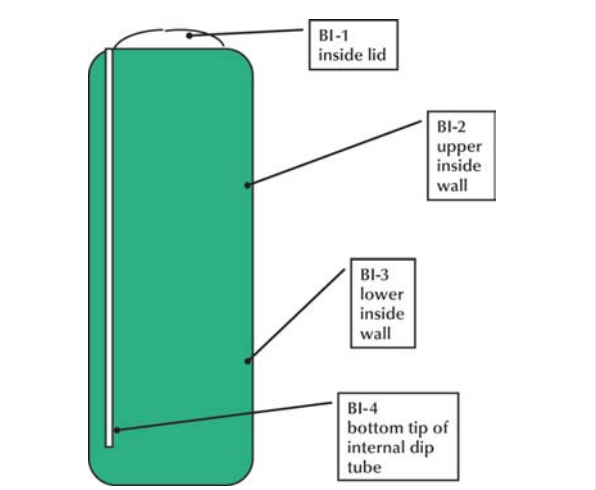
Photo 2: 100-liter pharmaceutical process vessel

Photo 3: 500-liter pharmaceutical process vessel

Table 2: Disinfection cycle parameters and results using *B. subtilis* spore strips (10^6 population) as biological indicators (BIs)

Vessel	CD Concentration	Exposure Time	BI Test Results
100 L	5 mg/L	30 min.	All negative
100 L	5 mg/L	20 min.	All negative
100 L	10 mg/L	15 min.	All negative
100 L	10 mg/L	10 min.	All negative
500 L	5 mg/L	30 min.	All negative
500 L	10 mg/L	15 min.	All negative

Figure 1: Location of biological indicators in the isolator, as detailed in Table 1



Our Findings

We used CD generated by the prototype chlorine dioxide gas generator to disinfect the interior surfaces of two GMP process vessels. Several exposure cycles were successful. Additionally, the CD penetrated into deadleg and hard-to-reach areas of the vessels, such as in the domes and at various depths along the vessel walls. CD has proven itself to be a practical and effective method for sterilizing process vessels as demonstrated by the high-level spore reduction achieved.

CD is easy to use. It has a distinct odor, making even minor leaks self-alerting, which constitutes a significant safety feature. And because the gas is green, direct measurement of CD concentration is readily performed using a spectrophotometer. In two separate studies, we have found it to be useful in GMP sterilization applications for the pharmaceutical and biotechnology industries.

Acknowledgments

The authors would like to thank all who assisted in this project, especially Dave Thatcher and Donald Wilson for their support and expertise.

References

1 Battisti, DL. Commercial Applications of Chlorine Dioxide. In *Development of a Chlorine Dioxide Sterilization System*. Doctor of Philosophy dissertation; University of Pittsburgh, Pittsburgh, PA, 2000; pp. 21–28.

2 Knapp, JE; Rosenblatt, DH; Rosenblatt, AA. Chlorine Dioxide As a Gaseous Sterilant. *Med. Dev. Diagn. Ind.* 1986, 48–51.

3 Heilprin, J. EPA Completes Fumigating Senate Hart Office Building for Anthrax Spores. *Associated Press,* 31 December 2001.

4 Senate Building Reopens After Anthrax Cleanup, US Department of State, International Information Program, 22 January 2002.

5 Brier, HJ. Post Office Takes Fumigation Step. *The Washington Times,* 15 December 2002.

6 Woodworth, AG; Jeng, DK. Chlorine Dioxide Sterilization Under Square-Wave Conditions. *Appl. Environ. Microbiol.* 1990, 56, 514–519.

7 Kowalski, JB. Sterilization of Medical Devices, Pharmaceutical Components, and Barrier Isolator Systems with Gaseous Chlorine Dioxide. In *Sterilization of Medical Products.* Morrissey, RF; Kowalski, JB, Editors; 1998, Polyscience Publications Inc.; Champlain, NY, pp. 313–323.

8 Eylath, A, et al. Successful Sterilization Using Chlorine Dioxide Gas: Part One — Sanitizing an Aseptic Fill Isolator. *BioProcess Intl.* 2003, 1(7).

Amnon Eylath *developed this article when he was manager of isolator technology and project manager in the filling and packaging engineering group at Amgen, Inc. Currently, he is development quality manager at Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, 317-276-2000, fax 317-277-4708, eylath_amnon_a@lilly.com, www.lilly.com.* ***Sushil Madhogarhia*** *is filling and packaging engineering manager, and* ***Emilio Rivera*** *is senior director and general manager of Puerto Rican operations at Amgen, Inc. Corresponding author* ***Paul Lorcheim*** *is director of operations and* ***Marc Czarneski*** *is director of technology at ClorDiSys Solutions, Inc. PO Box 138, Lebanon, NJ 08333, 908-236-4100, fax 908-236-2222, paullorcheim@clordisys.com.*

Figure 2: Example of chlorine dioxide process data (concentration profile)