Chlorine Dioxide, Part 1

A Versatile, High-Value Sterilant for the Biopharmaceutical Industry

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Historically, chlorine dioxide (CD) became important in sanitation because of municipal water treatment concerns about halomethanes and chloramines generated during industrial chlorine-based water treatment. The American Water Works Association (**1, 2**) details the chemical properties of CD, with gas generator designs and the history and applications of CD in water treatment. ERCO Worldwide (www.ercoworldwide.com) provides extensive background material including recent literature, patents, and microbiology on a dedicated website: www.clo2.com.

To date, limitations in CD gas generation technology have kept this attractive product from many applications for which its properties would be advantageous. Several novel technologies may bring it into the mainstream of biopharmaceutical manufacturing and maintenance operations.

PRODUCT FOCUS: BIOPHARMACEUTICALS

PROCESS FOCUS: MANUFACTURING AND RESEARCH

WHO SHOULD READ: PROCESS DEVELOPMENT, MANUFACTURING, LABORATORY MANAGERS, AND QUALITY CONTROL

KEYWORDS: CLEANING VALIDATION, DISINFECTION/SANITATION, PRODUCTION, DOWNSTREAM PROCESSING, DISPOSABLES

LEVEL: INTERMEDIATE

In its aqueous phase, the same basic CD supply system can be used as a starting point for the entire range of biopharmaceutical applications: sanitization, sterilization, and routine or emergency disinfection. CD is as useful as a sanitizer for utility water systems and surface decontamination as for process applications. Few technologies are as easy and convenient to use while providing value for such a wide range of applications. CD has been studied in-depth for many years. For example, Young and Setlow (**3**) compare CD and bleach, focusing on sporicidal aspects. Mittelman's series (**4–6**) discusses growth and destruction of biofilms in purified water systems. As the industry becomes more familiar with CD, it could become the choice for most if not all operational sanitization, disinfection, and sterilization applications in biopharmaceutical manufacturing facilities.

Comparing CD with Other Sterilants: Table 1 summarizes key properties of oxidizing biocides to consider in choosing a sanitizing/sterilizing agent. As shown, CD is not as aggressive an oxidizer (oxidation potential data) as chlorine, ozone, peracetic acid, peroxide, or bleach — and it should be noncorrosive to common materials of construction. A high oxidation capacity (seeking five electrons rather than two), however, suggests that CD is a most efficient reagent when oxidation proceeds to completion.

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Choosing a sanitizing agent depends on the philosophy of an organization as well as particular process requirements. Clean steam is the best known sterilant for process systems. However, it is expensive because of the necessary specialized generation equipment and the high cost of water-for-injection (WFI). An important, sometimes overlooked feature of CD is that it exists as a neutrally charged gas in aqueous solution, which allows it to penetrate pores, cracks, and crevices to reach microbial contaminants. Also, most plastics and polymers will not absorb it.

Table 2 compares CD with other well-known sanitization agents and sterilants used in gaseous form for space-fumigation applications. Among these, only CD is demonstrated to sterilize as both a liquid and a vapor. Only the vapor-phase attributes are compared. In the table, "+" symbols indicate that an agent is generally favorable for a given criterion; "–" symbols mean it is unfavorable.

The *unfavorable* rating of CD for the *cost* criterion assumes that an equipment-based generator produces CD gas. Using membrane-sachet technology with a sparging technique to generate the gas involves a relatively small capital investment and lower operating costs. Thus, CD generated that way would receive a "+" entry for cost.

Paraformaldehyde will not be widely used in the future because of concerns about its toxicity, residues, and unpredictability. The National Research Council (**7**) has reported on formaldehyde's need for neutralization with ammonium carbonate, as well as the need for careful venting with this Group B1 carcinogen. Over time, vapor-phase peroxide (VHP) has found a niche in the bioprocessing industry. But VHP is of limited use because of careful preconditioning required, long aeration times for removal, and its aggressiveness toward rubbers and some polymers. The aeration time requirements have been a nagging issue with VHP — in some cases requiring four to eight hours to reduce it to a safe level in real-world systems.

Actual aeration times for CD in isolators and similar closed systems are very close to the theoretical air-exchange period expected (**8, 9**). Both gas and aqueous-phase treatments benefit from CD's remarkable ability to penetrate into dead areas and porous materials. It can thus penetrate and disrupt the plaque buildups associated with many microorganisms. For effective vapor-phase cycles, CD introduction must be accompanied by humidification of the air to about 70% relative humidity (RH).

Proven Applications

Decontamination of Isolators: Eylath et al. (**8**) documented use of gaseous CD to sterilize a large (240 ft^3), hard-wall isolator made of grade 316 stainless steel (SS), Lexan brand polycarbonate resin (GE Plastics), and other polymers. The unit contained two half-suits, which are known to present a sterilization challenge. The isolator was humidified and sterilized for 15–60 min with CD for a total exposure time of less than two hours, and excellent results were indicated by biological indicator (BI) analysis (**8**).

Czarneski and Lorheim (**9**) reported on gaseous CD decontamination testing of isolators in several different configurations. They also compared the effectiveness and repeatability of their results with those obtained in other testing using VHP. The authors concluded that because CD is a true gas, it produced superior performance over vaporous agents that can condense during the decontamination process. CD gas can be evacuated more quickly as well, and it produces more repeatable, reproducible results.

Tests were conducted in a transfer isolator fully packed with media or components and in a train including two isolator systems and an autoclave. For three configurations (isolator with media load, isolator with component load, and isolator train) total cycle times of 83 min (both loaded scenarios) and 115 min (isolator train) gave conclusive decontamination results. Cycle times were better than for VHP, for which three- to five-hour cycle times were observed. Total cycle times included 30 min for conditioning, 30–35 min for exposure to CD, and 15 min for aeration down to OSHA-acceptable levels. Only 12–15 air changes were required to meet regulatory standards.

Sterilization of Process Vessels: Eylath et al. (**10**) then used CD gas to sterilize two conventional biopharmaceutical 316 SS vessels with normal connections and agitators. Those process vessels were relatively small (100 L and 500 L), but the reported technique could easily be used for larger vessels such as those typical in media and buffer preparation. The authors claim sterilization with CD treatment cycles of 10–30 min, similar to the isolator study.

In evaluating those results, capital and operating costs should also be considered. Increased capital cost for clean steam (the current industry standard) comes from required vessel pressure ratings, so it is modest for small vessels but substantial for large ones. Savings can be substantial when using CD for sterilization in typically large media and buffer tanks. Operating costs for steam primarily came from generating clean steam and the WFI used as feedstock. The operating cost of using CD for the same purpose can be as little as one fifth of those for clean steam (**11**). Additionally, Bioprocess Associates has shown that sterile water and clean steam prepared using CD are substantially less costly than those prepared by conventional means (**12**).

Table 1: Summarizing key properties of oxidizing biocides to consider in choosing an agent to sanitize or sterilize a system — compiled data from several sources (SELECTIVE MICRO TECHNOLOGIES, WWW.SELECTIVEMICRO.COM)

Biocidal Agent	Oxidation Potential (volts)	Oxidation Capacity (electrons)
O_3 (ozone)	2.07	2e–
CH_3COOOH (peracetic acid)	1.81	2e–
H_2O_2 (peroxide)	1.78	2e–
NaOCl (sodium hypochlorite bleach)	1.49	2e–
ClO_2 (chlorine dioxide)	0.95	5e–

In field testing performed by Selective Micro Technologies, CD solution was generated in a partially filled water storage tank. After 60 min total CD generation and soak, swab samples showed zero cfu remaining at three locations tested, one of which was the top surface of the tank (in the vapor space above the level of the liquid contents). Before treatment, levels of 1.01×10^3 to 7.26×10^3 cfu were recorded. So the liquid does not need to directly contact all surfaces to be effective.

Ultrafiltration (UF) Membrane Sanitization: Selective Micro Technologies and NCSRT (www.ncsrt.com) (**13**) have applied aqueous CD to the sterilization of a 5-m^2 polysulfone UF membrane system in testing at Wageningen University Research in The Netherlands. Their membrane module was used to process *Pichia pastoris* fermentation broth. Dilute CD was circulated through the system while both retentate and filtrate streams were recycled for about

Advantages of CD

CD benefits for the biopharmaceutical industry include

- Broad range of biocidal and sporicidal properties
- Rapid acting, effective at ambient temperature and atmospheric pressure
- Nontoxic, nonhazardous, environmentally friendly, and non–skin-sensitizing at normal use concentrations in water
- Effective as aqueous solution or gas
- Easily and quickly inactivated (purging/aeration, ultraviolet light, or chemical inactivation) and removed from process areas and equipment
- Residue free, easily detectable and measurable
- Noncorrosive to construction materials commonly used in the biopharmaceutical industry
- Less costly (based on efficacy) than other broad-spectrum, high-performance sterilants (e.g., vaporous hydrogen peroxide)
- Versatile: can be used in many applications, minimizing the number of agents that must be stored.

an hour at room temperature. Two separate tests were conducted, with targeted final CD concentrations at 100 ppm and 50 ppm. Concentrations were monitored using CD test strips and spectrophotometry.

Microbial inactivation in the crossflow module was achieved after one hour of exposure at either CD concentration. Samples were cultured using standard plating techniques, with all colonies identified. Following treatment, no growth was detected in samples taken at all UF module openings. No changes in membrane performance or expected membrane life were detected through integrity testing (forward-air diffusion rates at 5 psig). When compared with a sanitization regimen originally used in Wageningen for the same system, significant improvements in total cycle times (from 24 hours to two hours) and completeness of sanitization were observed.

Water System Sanitization: Wise (**14**) used CD for sanitization of reverse-osmosis (RO) membranes, which are widely used in WFI water preparation. The most common material of construction is cellulose acetate (CA), although sophisticated multilayer membranes may displace that in the future. For CA membranes, chlorine cannot be used as a sanitizing agent; in many industrial systems, microflora can grow to unacceptable levels. RO units must be taken off-line for extended cleaning. In using CD to sanitize the system, Wise was careful to show that at low levels it does not damage the membranes to cause unwanted salt breakthrough. Even at a 1 mg/L CD level with a two-hour treatment cycle (93 ppm-minutes), he saw reductions of 77% (permeate) and 96% (concentrate) of the mixed flora typical in such systems.

Selective Micro Technologies used CD (generated using the company's microreactor) to sanitize a complete USP water loop, including the RO membrane unit (**15**). The water system and distribution loop (Figure 1) included two 125-gallon storage tanks plumbed in parallel. Those tanks store RO or DI water that feeds a distribution loop. CD was generated directly in the storage tanks. DI units were bypassed and UV light turned off for that portion of the testing.

The loop was charged with 40-ppm CD, which circulated overnight (~16 hours). Storage tanks were then drained and refilled to 40% with RO-quality water, which went through the distribution system with the return line directed to a drain. Finally, all valves were flushed with RO water until their measured CD concentration was <1 ppm. Total time required to flush the system of residual CD was only a matter of minutes.

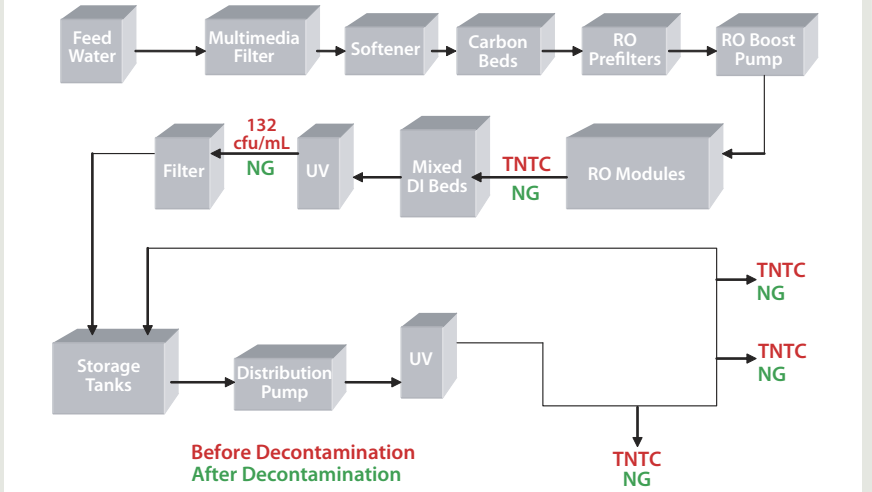
At the same time, RO membranes were also sanitized with a CD solution of about 50 ppm. This CD was generated using a single microreactor sachet in a covered container and injected into the RO feed with a dilution pump. Because CD does not ionize, it can pass through RO membranes, which allows simultaneous decontamination of both the feed and permeate sides of RO membranes. The RO unit was a thin-film composite type supplied by TriSep Corporation (www.trisep.com). CD was visually detected in the RO reject water within a minute. After 10 min, CD concentrations on both sides of the membrane were essentially equal. The system was then isolated and allowed to soak with treatment conditions held for about an hour before CD was flushed from the system. After ~10 minutes of flushing, CD concentrations in both the product and reject lines were measured at less than 1 ppm.

The entire USP system was then returned to service. Before the test it had been heavily contaminated, with

Table 2: Comparing attributes of three biocidal agents — formaldehyde (CH_2O), hydrogen peroxide (H_2O_2), and chlorine dioxide (ClO_2) (HENRY S. LOFTMAN, PHD, MICRO-CLEAN, INC., WWW.MICROCLN.COM)

Issue	CH_2O Gas	H_2O_2 Gas	ClO_2 Gas
Sporocidal effectiveness	+	+	+
Effective through HEPA filters	+	?	+
Noncarcinogenic	–	+	+
Toxicity (TWA PEL, ppm)	0.75	1.0	0.1
Nonexplosive (at normal use concentrations)	–	–	+
Relative humidity requirement	60–90%	30% (Steris) or ambient (Bioquell)	65–90%
No residue	–	+	+
Noncorrosive	+	+ (dry), ? (condensed)	+ (– with Cl_2)
Method of removal	Neutralizer	Catalytic breakdown	Scrubbing
Development effort	+	–	+
Low cost	+	–	–

Figure 1: Ultrafiltration membrane sanitization



most samples showing microbiological counts too numerous to count (TNTC) and positive counts even in water sampled directly downstream of an in-line UV light. No microbial contamination was detected after 24 hours of normal operation following the CD treatment cycle.

Hard Surface Disinfection: Laboratories, especially those involved in animal testing, need to be disinfected both routinely and in periodic emergencies to prevent potential infections by adventitious organisms. Apel discusses such applications for the produce industry (**16**). Hard surfaces can be treated with a CD liquid or foam, but the foam is more easily applied to ceilings. Many other successful applications of CD within the food industry have been published.

Cleanroom Decontamination: The use of CD to disinfect entire rooms and suites has been convincingly demonstrated by several authors. Luftman used CD to disinfect a very large facility (170,000 ft^3) at the Widener ICU Animal Hospital (**17**). The treatment cycle used <0.5 mg/L, (400 ppm) for about an hour, with additional time for humidification and venting. All details (e.g., sealing the room, HVAC circulation, and training) proved straightforward. (Anecdotal evidence indicates that CD does not harm furniture, most plastics, or computers and electronics under the usual treatment conditions.) After the CD cycle, the room was simply exhausted to the outside air. No EPA permits were needed because CD is not considered an environmental pollutant.

The results were a 5–6 log kill of test spores and target bacteria (*Geobacillus stearothermophilus*). Those results would not have been very different with *Bacillus subtilis niger* or its variant *Bacillus globiggi*. The extremophile *G. stearothermophilus* is a model organism used to test worst-case scenarios for steam sterilization. *B. subtilis* is a common spore-former found in soil. CD's activity against spore-formers is an unusual and valuable property.

CD is economical and effective in cases of accidental microbial contamination. Contaminated piping (especially vents and drains), vessels, and HVAC systems can benefit from CD exposure.

Applications with High Potential

Below are applications in the biopharmaceutical industry for which CD could improve on traditional methods. Testing is already in progress for some of these.

Production of Sterilized Water from USP Grade Water: In the absence of published data, the term *WFI* is purposely avoided here; *sterilized water* is used instead, referring to water free of biological activity and having endotoxin levels below typical detection limits. Preliminary tests indicates that CD at very low concentrations (<1 ppm) can effectively inactivate endotoxin in a few minutes. Depending on microbiological conditions of feed water, CD oxidation reactions will produce some level of salts (mostly chlorides). The quantity of salts produced may lead to resistivity values that fall outside the range of acceptability for classification as USP or WFI quality. It can be stated with some level of certainty, however, that the product water will be free of microorganisms, which in and of itself could add significant value in certain applications currently using more costly WFI (e.g., noncritical and intermediate wash downs). For feed water with lower levels of microorganisms present, CD treatment should lead to WFI quality levels. In other situations, there may be other ways to treat water in the deionization-sterilization sequence for more favorable economics than traditional approaches. More work must be done.

Improved Sanitization of Chromatography Columns, Resins, and Membranes: Testing is currently under way to define protocols and determine the effectiveness and suitability of CD for capacity recovery and sanitization of packed-bed chromatography columns. Even at 100 ppm CD solutions appear to have no detrimental effect on even the most sensitive of common stationary phases. The effectiveness of CD for sanitizing membranes is established. If column testing is successful, it should be relatively straightforward to demonstrate CD's effectiveness in membrane chromatography technologies, which may play a significant role in the future of bioprocessing.

Biowaste Kill: Warriner (**18**) compared CD with ozone and chlorine as a liquid-phase treatment for wastewater. Quantitative testing involved seeded polio virus and typical coliform bacteria. Of the three agents tested, CD was most effective at typical concentrations. Because the challenges in typical biowaste kill systems for biopharmaceutical facilities are similar to those in municipal systems (except for scale), CD potentially provides an economically attractive alternative that is effective at ambient temperatures and displaces more dangerous, toxic, and/or flammable chemicals. Laboratory testing on specific waste samples from

Proven Effectiveness

Here are some organisms for which chlorine dioxide's effectiveness has been proven. Testing for bacteria, viruses, and algae/fungi was performed at an EPA-certified laboratory. DATA FROM SELECTIVE MICRO TECHNOLOGIES (WWW.SELECTIVEMICRO.COM)

Bacteria: *Staphylococcus aureus,* Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa, Salmonella choleraesuis,* multiple drug resistant *Salmonella typhimurium* (MDRS), tuberculosis, *Escherichia coli* 0157:H7 and ATCC 11229, Vancomycin-resistant *Enterococcus faecalis* (VRE), *Klebsiella pneumoniae,* and *Bacillus subtilis* (a spore-forming bacterium)

Viruses: Coronavirus, human immunodeficiency virus, hepatitis A, rotovirus, feline calici virus, and poliovirus

Algae/Fungi: *Phormidium boneri,* T-mentag (athlete's foot fungus), *Penicillium digitatum, Botrytis* species, and *Fusarium solani*

Yeasts: *Saccharomyces cerevisiae* and *Pichia pastoris*

a particular process or facility is a good starting point for those contemplating CD use in this application.

Sterilization of Disposable Processing Systems: Disposable systems offer many advantages, as the biopharmaceutical industry is slowly but surely recognizing. These technologies are expected to be widely adopted over the next five to 10 years. Generally, components of such systems are gamma-irradiated. Once components are linked together to form systems, the sterile condition of that system (if required) is in jeopardy. Extraordinary measures must often be taken for tubing connections. Some processors irradiate their entire systems. Preliminary testing indicates that CD treatment could be the quickest, most economic, and most effective method available for presterilization of disposable systems.

Once processing is complete, disposable materials must be eliminated. Depending on the nature of that processing, it may be necessary to decontaminate those materials or treat them as medical-grade waste, requiring destruction in a specialized, costly way (e.g., incineration at a certified facility). CD could provide a low-cost decontamination approach that saves time and eliminates special handling and destruction challenges. Testing continues, but preliminary results indicate a total kill is possible in under five minutes, with an additional 10–20 min required for system evacuation.

Looking Ahead

As the industry becomes more familiar with CD, it could become an attractive choice for many operational sanitization, disinfection, and sterilization applications in biopharmaceutical manufacturing. Next month, Part 2 of this article will discuss validation and economic issues and examine methods of making CD for local use. Because the US Department of Transportation will not permit manufactured CD to be transported, generation must be performed on-site. That is a major reason why CD has not been widely used in biopharmaceutical manufacturing — but new production methods are changing things.

References

1 *Water Disinfection with Ozone, Chloramines, or Chlorine Dioxide* (Report #20152). American Water Works Association: Denver, CO, December 1980.

2 Gates DJ. *The Chlorine Dioxide Handbook* (Water Disinfection Series). American Water Works Association: Denver, CO, June 1998.

3 Young SB, Setlow P. Mechanisms of Killing of *Bacillus subtilis* Spores By Hypochlorite and Chlorine Dioxide. *J. Appl. Microbiol.* 95(1) 2003: 54–67.

4 Mittelman MW. Biological Fouling of Purified Water Systems: Part 1, Bacterial Growth and Replication. *Microcontamination* 3(10) October 1985: 51–55, 70.

5 Mittelman MW. Biological Fouling of Purified Water Systems: Part 2, Detection and Enumeration. *Microcontamination* 3(11) November 1985: 42–58.

6 Mittelman MW. Biological Fouling of Purified Water Systems: Part 3, Treatment. *Microcontamination* 4(1) January 1986: 30–40, 770.

7 National Research Council. *Reopening Public Facilities after a Biological Attack: A Decision Making Framework.* The National Academies Press: Washington, DC, 2005; 147–8; www.nap.edu/openbook/0309096618/html/147.html.

8 Eylath A, et al. Successful Sterilization Using Chlorine Dioxide Gas: Part One — Sanitizing an Aseptic Fill Isolator. *BioProcess International* 1(7) 2003: 52–56.

9 Czarneski MA, Lorheim P. Isolator Decontamination Using Chlorine Dioxide Gas. *Pharm. Technol.* April 2005: 124–133; www.pharmtech.com/pharmtech/data/articlestandard/pharmtech/172005/156880/article.pdf.

10 Eylath A, et. al Successful Sterilization Using Chlorine Dioxide Gas: Part Two — Cleaning Process Vessels. *BioProcess International* 1(8) 2003: 54–56.

11 Wintner B. Net Present Value Analysis of Chlorine Dioxide Versus Clean Steam (unpublished report), April 2005.

12 Wintner B. Using CD to Get to Sterile Water (unpublished report), March 2005.

13 Kopf H, O'Neill G. Chlorine Dioxide Solution for Microbial Inactivation in Crossflow Filtration Systems (unpublished report). Selective Micro Technologies: Beverly, MA, April 2005.

14 Wise B, et al. Membranes: Effectiveness of Chlorine Dioxide in Sanitizing Thin Film Membrane Systems. *Ultrapure Water* September 2004: 12–16.

15 O'Neill G. Field Trial: Decontamination of USP Water Purification System and Distribution Loop (unpublished report). Selective Micro Technologies: Beverly, MA, April 2005.

16 Apel G. Chlorine Dioxide. *Tree Fruit Postharvest J.* 4(1): 12–13.

17 Luftman HS, Regits MA. Chlorine Dioxide Gas Decontamination of the University of Pennsylvania's George D. Widener Large-Animal Hospital Intensive Care Unit. Presented at the *47th Annual Biological Safety Meeting,* San Antonio, TX, October 2004 (American Biological Safety Association, Mundelein, IL, www.absa.org).

18 Warriner R, et al. Disinfection of Advanced Wastewater Treatment Effluent By Chlorine, Chlorine Dioxide, and Ozone. *Water Res.* 19(12) 1985: 1515–1526.

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