Chlorine Dioxide, Part 1
A Versatile, High-Value Sterilant for the Biopharmaceutical Industry

Barry Wintner, Anthony Contino, Gary O’Neill

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.

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.

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 ft²), 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).

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 × 10³ to 7.26 × 10⁷ 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-m² 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
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$_2$O), hydrogen peroxide (H$_2$O$_2$), and chlorine dioxide (ClO$_2$) (HENRY S. LOFTMAN, PHD, MICRO-CLEAN, INC., WWW.MICROCLN.COM)

<table>
<thead>
<tr>
<th>Issue</th>
<th>CH$_2$O Gas</th>
<th>H$_2$O$_2$ Gas</th>
<th>ClO$_2$ Gas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporicidal effectiveness</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Effective through HEPA filters</td>
<td>+</td>
<td>?</td>
<td>+</td>
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<tr>
<td>Noncarcinogenic</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Toxicity (TWA PEL, ppm)</td>
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<tr>
<td>Nonexplosive (at normal use concentrations)</td>
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<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Relative humidity requirement</td>
<td>60–90%</td>
<td>30% (Steris) or ambient (Bioquell)</td>
<td>65–90%</td>
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<tr>
<td>No residue</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Noncorrosive</td>
<td>+</td>
<td>+ (dry), ? (condensed)</td>
<td>(+ with Cl$_2$)</td>
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<tr>
<td>Method of removal</td>
<td>Neutralizer</td>
<td>Catalytic breakdown</td>
<td>Scrubbing</td>
</tr>
<tr>
<td>Development effort</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Low cost</td>
<td>+</td>
<td>–</td>
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</tbody>
</table>
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 ft³) 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 globigii*. 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 indicate 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

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


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Chlorine Dioxide, Part 2
A Versatile, High-Value Sterilant for the Biopharmaceutical Industry

Barry Wintner, Anthony Contino, and Gary O’Neill

Last month, Part 1 of this article discussed some proven applications of chlorine dioxide (CD) gas and solutions as cleaning/sterilizing agents for isolators, process vessels, filter membranes, water systems, hard surfaces, and cleanrooms. Potential applications were also described, including the production of sterilized water from USP-grade water; sanitization of chromatography columns, resins, and membranes; biowaste kill; and sterilization of disposable process systems. This month, Part 2 discusses economic and validation issues as well as methods of production.

Application Versatility
CD has been demonstrated effective in both the aqueous and gas phases, which is highly advantageous for biopharmaceutical applications. It is easy to change CD from one state to the other. One benefit of the membrane sachet technology (see below) is that it can be used to generate an aqueous solution that can be stored until needed. To release the CD for gas-phase use, the stock solution is sparged with air or nitrogen and resulting vapor directed to the work area. When an equipment generator is used to produce gaseous CD, aqueous solutions can be formed by exposing the gas to water in some type of contacting device (e.g., a packed column). For gaseous uses, depending on the volume and configuration of the treatment area, fans may be needed to circulate the CD vapor.

Information is extensive on using CD as an aqueous-solution sanitizer in food applications. CD is unique in that it attacks and erodes the structure of microbial plaques as well as individual microbes. So it can penetrate and breakdown microflora deposits and then assault the microflora themselves.

CD is easy to apply as an aqueous solution, fog, or foam. The best method will depend on the individual application. If timing is tight, gas is likely to provide the best results in pure sanitization of large spaces. There are no liquids or other chemicals to complicate and possibly interfere with the reactions. Foggling is a means of distributing a liquid uniformly over a large area and can be used to disinfect surfaces in air-handling systems and equipment. Foams require an active agent but can be valuable in the presence of small nooks or woven materials. CD liquid is best for hard surfaces, such as in many process applications in biopharmaceutical manufacturing. As stated, in a closed vessel the liquid phase need not contact all surfaces to accomplish sterilization. Liquid also provides enhanced cleaning capability. CD acts as an oxidizer for organic compounds and could displace caustic (NaOH) for certain clean-in-place (CIP) tasks. Time savings for a complete CIP–sterilization cycle could be significant. Liquids generally provide soil removal because of their convective energy and viscosity, which cannot be duplicated by low pressure gas.

Comments on Validation
One concern when introducing novel methods and materials into a biopharmaceutical facility involves validation. In the case of CD, such concerns are minimal. Available literature indicates that standard methods of BI testing work well for both sanitization and sterilization applications. Traditional validation should be appropriate because of the simplicity of CD generation, the ability to detect CD at very low concentrations, and the ease of evacuating CD once disinfection is accomplished. Berry provides a complete outline for the pharmaceutical industry (19). BIs have received a great deal of attention in harmonization of international standards. Part one of ISO 1138 covers general requirements; part two...
is specific for BIs for EtO sterilization processes; and part three deals with BIs for moist-heat sterilization processes (20, 21). Table 4 summarizes the European standardization committee’s standards series. The FDA’s recent openness to process improvements that improve manufacturing economics to lower drug prices makes this an ideal time to transition to CD technologies.

**The Value Proposition**
The pharmaceutical industry has come under pressure in recent years to find ways to reduce drug prices. Expectations are that the situation will continue. Manufacturing is one area that has been targeted as having considerable potential for cost savings. CD use in biopharmaceutical applications identified herein should translate into economic advantages for implementers. The “Economic Advantages” box lists some of these.

**Risk Minimization:** Quality is the major issue for biopharmaceutical operations. The cost of contaminated product lots (whether or not they reach patients) far outweighs the costs associated with any sanitizing agent. Because of its effectiveness, speed of kill, and ability to enter the smallest spaces and quickly diffuse into liquid-filled deadlegs, CD could very well decrease instances of microbiologically contaminated batches. Potential benefits include elimination or mitigation of the following:

- Cost of rework and disposal of contaminated batches
- Loss of future revenues
- Legal costs and payouts to consumers
- Impact on corporate reputation and company valuation (stock price).

**Economic Advantages Over Clean Steam:** The value of a sanitizing agent depends on the specific problem it is intended to solve. Both operating factors and engineering factors come into play. Clean steam is not an inexpensive option if operating factors are taken into account. It must be prepared from WFI at the end of a complex water treatment sequence. In many biopharmaceutical processes, scheduling of sterilization steps becomes challenging because of a desire to sterilize an entire train of equipment before feed operations begin. Long sequences and lengthy delays for equipment preparation must be included in the manufacturing cycle plan.

With CD, especially in a stock solution setup, equipment can be sterilized as it becomes available: The CD is prepared as an aqueous solution at several times its use strength. A portion of that liquid is then diverted to the system or vessel to be sterilized and mixed with water to provide the proper concentration. For vapor-phase treatment, the stock solution is sparged to produce the vapor, which is routed to an area for treatment. Effective at ambient temperature and atmospheric pressure, CD sterilization requires no heat-up or cool-down periods, nor must process equipment be pressure-rated as is needed for steam service.

Another challenge for steam sterilization is an inability to ensure that all surfaces are exposed to the appropriate temperature in a two-phase condensing system. This is especially true for deadlegs and small spaces. CD does not depend on temperature–time exposure (although its effectiveness depends on concentration–time). Doing its job as a liquid, gas in equilibrium with liquid, or as a totally gaseous input, CD can easily handle this challenge.

Disposal of steam after its use is also time consuming. Depending on the application, a vessel or system may be vented or gas-purged—or vacuum and air may be applied in sequence to displace the steam. With CD in an aqueous-phase application, the system is merely drained and rinsed with one to 1.5 times the system volume of WFI. Drying may or may not be required. In a vapor-phase treatment, CD is purged to atmosphere with compressed air or nitrogen or by using normal vent or HVAC fans.

Finally, clean steam (especially clean steam condensate), can be very corrosive even to high-grade stainless steels. CD’s high activity at ambient temperature eliminates the effects of repeated heating and cooling cycles. The resulting lower maintenance is another useful and valuable attribute.

Interrelated engineering factors revolve around time cycles. For a novel agent such as CD, the potential time

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Test Organisms</th>
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<tbody>
<tr>
<td>Sodium hypochlorite 1</td>
<td><em>P. aeruginosa</em></td>
</tr>
<tr>
<td>Sodium hypochlorite 2</td>
<td>820</td>
</tr>
<tr>
<td>Stabilized chlorine dioxide 1 2</td>
<td>310</td>
</tr>
<tr>
<td>Stabilized chlorine dioxide 2 3</td>
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<tr>
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<tr>
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<td>Peracetic Acid 3</td>
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</tr>
<tr>
<td>Selective Micro Clean 4</td>
<td>5</td>
</tr>
</tbody>
</table>

2 Lactic acid activation
3 Citric acid activation
4 Reported by Vergagene Ltd. (Bolton, UK)
5 Testing performed by Microbiotest, Inc. (Sterling, VA)

**Table 4:** Biological indicator (BI) standards for sterilization from the European Committee for Standardization (www.cenorm.be) (8)
savings could make a difference in process equipment scale, complexity, and cost. The simplicity of CD operation (over VHP) in sterilizing isolators reduces costs in time, labor, or both. Table 5 compares other important attributes of CD and clean steam.

**CD Production Technologies**

If not handled properly, CD gas can become unstable and is potentially explosive. Aqueous solutions are much more stable. The US Department of Transportation will not permit manufactured CD to be transported. So generation must be performed on-site. This is a major reason CD has not been widely used in biopharmaceutical manufacturing until now.

Active CD can be generated safely in two ways: by equipment-based gas generators and membrane sachets for aqueous CD. Both share the common benefit of simplified waste disposal because CD is environmentally friendly, dissipating rapidly in the gas or aqueous phase upon exposure to UV light.

**Equipment-Based Generators:**

Today’s equipment-based CD gas generating processes have evolved from small chemical sub-processes involving several unit operations. Older versions were characterized by low-purity product, severe material-compatibility issues, and poor controls. Many improvements have been made over the years. Today gas-generation systems are based on several different chemical reaction routes that consistently produce high-purity CD. Each technology has advantages in certain applications.

In general, gas generators are appropriate for making large quantities. These generators have been offered to the pharmaceutical industry, but they may be a more appropriate fit for large, continuous users such as municipal water systems. The purity of CD produced is variable depending on the generator design: high for pharmaceutical units and moderate for municipal water units. Some technologies involve compressed, dilute chlorine as a raw material, which makes safety a consideration. ClorDiSys Solutions Inc. (www.clordisys.com) is a provider of CD gas generators appropriately sized and designed for the biopharmaceutical industry.

For biopharmaceutical companies, equipment-based gas generators offer:

- semibatch CD generation
- self-contained systems including generator, controls, and monitoring provisions
- ability to generate larger quantities of CD.

When compared with membrane sachets, operating and capital costs favor the sachet method (below), as do space requirements. But based on cost per gram of CD produced, the gas generation raw material cost is lower.

**Membrane Sachets:** CD can be generated by several different chemical reaction routes through the reaction of dry chemicals with water. The quality (purity) and yield is highly variable across these technologies, so their individual utility to the biopharmaceutical industry must be determined. Some products make CD from “stabilized chlorine dioxide” solutions. Such wet-chemical processes produce less effective and more corrosive form of CD (due to low pH and relatively high concentrations of undesirable byproducts) unsuitable for most biopharmaceutical applications.

In some formats, CD precursors are supplied as a number of dry chemicals inside a reaction-controlling membrane sachet. These sachets are immersed in water to generate the CD.

Selective Micro Technologies, LLC (www.selectivemicro.com) uses a patented membrane system to generate CD in water at room temperature. With this approach, liquid water never contacts reactant material inside the sachet (called a microreactor) because the membrane is gas-permeable, allowing only water vapor inside. Only pure CD gas is transferred across the membrane and out of the sachet. This approach has advantages over other liquid and gas systems because it rapidly generates concentrated CD of the highest purity at neutral pH. With no impurities released, corrosion is minimal or nonexistent for stainless steel, plastics, and other materials commonly used in biopharmaceutical facilities.

Using that technology, a solution of CD is available after 1–10 hours, depending on the sachet size, number of sachets used, and desired concentration. These sachets can be used to make a concentrated stock solution that is diluted and added to substrate in a target vessel, or the solution can be used directly for applications such as laboratory surface decontamination. For gas-phase applications, the stock solution can be sparged to produce humidified CD gas. CD can be stripped from such aqueous solutions in a matter of minutes using modest gas sparge rates.

The microreactor approach is attractive because it offers:

- Rapid cycles producing a >99% pure aqueous solution of CD
- Minimal capital cost and equipment space requirements
- Maximum flexibility of location and scale of generation
- Minimal storage requirements, low operating cost
Table 5: Comparison of chlorine dioxide and clean steam attributes

<table>
<thead>
<tr>
<th>Attribute</th>
<th>ClO₂ Gas</th>
<th>Steam Vapor</th>
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<td>Sporicidal effectiveness</td>
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<tr>
<td>Relative humidity requirement</td>
<td>65–90%</td>
<td>N/A</td>
</tr>
<tr>
<td>No residuals</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Noncorrosive (to biopharm MOC)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Removal</td>
<td>Venting</td>
<td>Venting vacuum</td>
</tr>
<tr>
<td>Application development effort limited</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Raw material cost</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Capital cost</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

- Self-regulating CD generation without chlorine gas or dependence on control systems
- Nonhazardous waste products

**RELEVANT PROPERTIES**

Although CD is soluble in organics and moderately soluble in water, it is easily transferred from aqueous solution to vapor phase because of weak hydrogen bonding. Its Henry’s Law coefficient is sufficiently low to ensure a relatively high concentration in the liquid phase. The usual concentrations for CD application are at the mg/L level. Gas-phase sterilization applications are generally optimum at a relative humidity of about 70%.

With its fast kinetics against microbes, CD is remarkable for its stability and low interaction (22). Numerous investigators report D-values of a few minutes (23) across a wide range of microorganisms, even against virus and spore formers (3). The National Research Council has developed extensive documentation on chlorine dioxide for drinking water treatment (24).

Chlorine dioxide most likely inactivates microorganisms (as listed in the “Proven Effectiveness” box) through direct oxidation of tyrosine-, methionyl-, or cysteine-containing portions of their proteins, interfering with key structural regions of sensitive metabolic enzymes or membrane components (2).

CD is classified as an irritant rather than a toxic agent. It can be mildly irritating to mucous membranes with direct exposure over time. Conventional carbon respirators have been effective. In a series of animal tests at certified laboratories, CD generated by microreactor sachets has been shown to be nontoxic for liquid ingestion and inhalation at concentrations well above those for recommended use.

**AN EMERGING SOLUTION**

Although it has been in industrial use for some time, CD’s reputation as a sanitizer, disinfectant, and sterilant has improved considerably in recent years. One reason is that modern generation technologies can produce higher purity, “user-friendly” CD. Pricing (and profitability) pressures are likely to continue, forcing bioprocessors to continue searching for technologies that will improve manufacturing economics.

CD appears to have the potential to make a significant contribution in that regard. A number of biopharmaceutical industry applications have been targeted following successes in analogous applications elsewhere as well as some direct successes in applications unique to bioprocessing. Results to date have been impressive, with industry acceptance, implementation, and testing proceeding at a rapid rate.

**REFERENCES**

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Note: References 1 and 4-18 appear in Part One of this article, published in December 2005.