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Isolator Decontamination Using Chlorine Dioxide Gas

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The authors argue that chlorine dioxide (CD) is a safe and effective decontaminating agent that can be used for challenging applications. The effectiveness of CD gas for sterilizing complex isolator systems is studied.

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The use of isolation technology in the United States and Europe is beginning to show signs of widespread acceptance and growth. With the increased use of isolators for critical processes comes a demand for better and quicker decontamination methods. Fueling the growth of chlorine dioxide (CD) in the pharmaceutical and medical device industries are its properties as a true gas at ambient temperatures, its capacity to be unaffected by temperature variations and gradients, and its tight process consistency because concentrations can be precisely monitored and controlled.

CD properties and background

CD is a single-electron, transfer-oxidizing agent. The gas has a chlorine-like odor and a green–yellow color, which enables it to be monitored with an ultraviolet (UV)–*vis* spectrophotometer. This monitoring can help provide tight process control of the decontamination cycle from beginning to end. The properties of CD are as follows:

- Chemical formula: ClO_2,
- Molecular weight: 67.45 g/mol,
- Melting point: –59 °C,
- Boiling point: +11 °C.

CD is a true gas at normal-use temperatures and thus is not affected by temperature gradients that can cause condensation with vapors.

Since the 1920s, CD has been known for its disinfecting properties. It was recognized as a chemosterilizing agent in 1984 and in 1988, it was registered with the US Environmental Protection Agency (US EPA) for use as a sterilant. In 1985, Rosenblatt *et al.* developed the use of CD as a chemosterilizing agent to sterilize surfaces commonly used in the pharmaceutical and medical sciences (1). Five years later, Jeng and Woodworth reported the sporicidal activity of CD gas with an experimental sterilizer used for medical instruments (2). More recently, Eylath described a process of using CD gas to decontaminate a 240-ft^3 aseptic fill isolator and pharmaceutical processing vessels (3–4).

Research has shown that CD in gaseous and aqueous phases is an effective sanitizing agent with both broad and high biocidal effectiveness. Aqueous CD has been reported to be effectively inactivate pathogens such as bacteria, spores, viruses and algae. Gaseous CD, however, has been shown to be more effec-

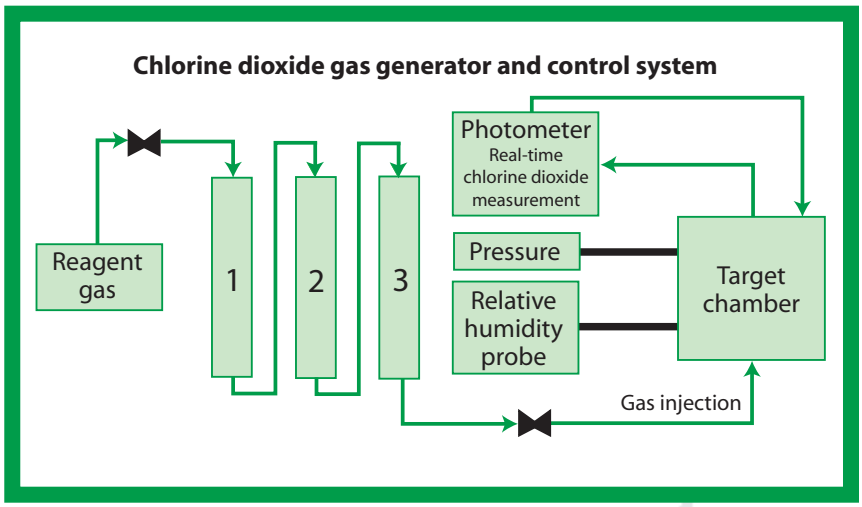

Figure 1: A diagram of a chlorine dioxide gas generator and control system.

Figure 2: Transfer isolator fully packed with media (23-ft^3 316 SS transfer isolator with 3 gloves, 2 circulation fans, high-efficiency particulate air filters, and a pressure blower). Cycle time is 83 min.

Figure 3: Transfer isolator fully packed with components (23-ft^3 316 SS transfer isolator with 3 gloves, 2 circulation fans, high-efficiency particulate air filters, and a pressure blower). Cycle time is 83 min.

tive than its liquid form when applied in equal concentrations and times.

In addition, CD in gas form can decontaminate areas where the consequences of ineffective decontamination would be severe. For example, the US EPA used gaseous and aqueous phases of CD to decontaminate *Bacillus anthracis* in areas of the Hart Senate office building and the Brentwood postal sorting facility in Washington, DC (5). After examining various decontaminated agents, the US EPA selected CD gas because of its proven track record of effective decontamination of anthrax-causing bacteria from building-type structures (6).

Table I: Chlorine dioxide D-values.

Concentration (mg/L)	D-value (min)
5	1.6
10	0.75*
20	0.27*
30	0.12*

*see reference 8.

Because of its success in the anthrax cleanup of several governmental buildings, researchers tested the efficacy of CD on various materials. Han *et al.* demonstrated that CD was highly efficacious in reducing *B. subtillis* spores on paper, plastic, epoxy-coated stainless steel, and wood surfaces (7). In addition, no corrosion was observed when using pharmaceutical-type materials such as 316 and 304 series stainless steel, Lexan, and other commonly used plastics including Delrin, Teflon, UHMWPE, Viton, and PVC (8). In a separate study, postexposure rinses of 304 stainless steel coupons in water-for-injection showed no residual CD when measured with a high-pressure liquid chromatographic method for chloride detection (3). In addition, many studies have demonstrated that CD gas is highly effective (0.5 log reductions) in reducing foodborne pathogens (*Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella*) on fruit and vegetable surfaces (9–17) and spoilage microorganisms on food-contact surfaces (18).

CD process description

CD is not stable enough to be generated, bottled, and shipped and therefore, it must be generated on site as needed. CD is not prepared by the vaporization of a solution, but rather as a true gas at the point of use. The gas can be generated by using a method in which solid sodium chlorite contained in small plastic cartridges is exposed to a chlorine–nitrogen (2:98%) gas mixture (see Figure 1). The reaction produces pure CD in nitrogen without any of the byproducts that occur with liquid CD generation methods.

$$Cl_2\,(g) + 2NaClO_2\,(s) \rightarrow 2ClO_2\,(g) + 2NaCl\,(s)$$

CD cycle

CD cycle is similar to other decontamination cycles that use humidity or moisture and a specific gas concentration for sporicidal efficacy. The CD cycle can be carried out from negative pressures (2 KPa) to slightly above atmospheric pressures. Negative pressures (vacuum) generally are used in applications such as lumens, syringes, and small-necked bottles.

The steps used in a CD cycle are preconditioning, conditioning, charging, exposure, and aeration.

Figure 4: A train of isolators (total 279 ft^3): a workstation isolator (a 150-ft^3 316 SS workstation isolator with 2 half suits, 6 circulation fans, high-efficiency particulate air [HEPA] filters, and a pressure blower), an autoclave interface isolator (a 90-ft^3 316 SS workstation isolator with 1 half suit, 4 circulation fans, HEPA filters, pressure blower, and pressure control [disabled in interface isolator]), and an autoclave (a 39-ft^3 316 SS autoclave). Total cycle time is 2 h.

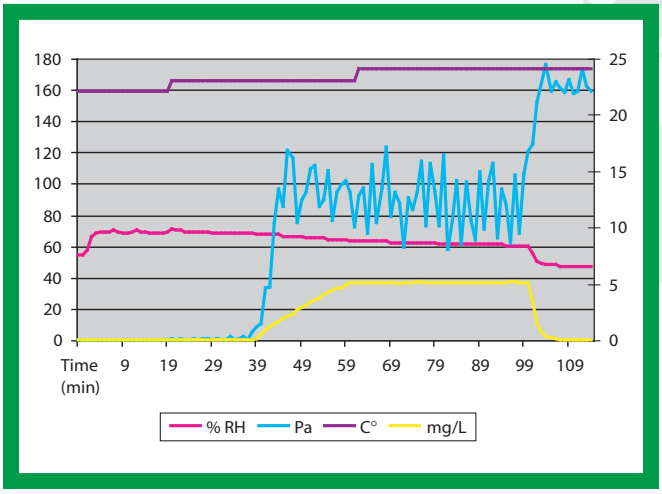

Figure 5: Cycle time for the train of isolators shown in Figure 4 (115 min total).

Preconditioning. When using any sterilant, it is a good practice to perform a chamber leak test before each decontamination cycle to ensure chamber integrity. For an isolator or other atmospheric chambers, the pressure is raised to a suitable level for the target chamber and held static for a period of time to determine whether the system has any leaks.

Once it has been determined that the chamber is leak free, it can be brought to the proper relative humidity set point (typically 70%). Humidity can be generated using a variety of methods such as steam, fine particle-size atomizers, hot plates, or foggers. Steam offers the quickest, cleanest, and most efficient way to raise humidity.

Conditioning. Once the humidity is at the proper level (60–75%), the cycle can begin its conditioning time (typically 30 min), during which the relative humidity (RH) is monitored continuously. If the RH drops by any significant amount (5%), moisture must be added to the chamber. This step conditions the spores and prepares them for the charging step.

Table II: Aeration time for component loaded isolator.*

Air exchange**	CD (mg/L)	CD (ppm)	Time (min)
1	2.5000	892.50	1.03
2	1.2500	446.25	2.07
3	0.6250	223.13	3.10
4	0.3125	111.56	4.13
5	0.1563	55.78	5.17
6	0.0781	27.89	6.20
7	0.0391	13.95	7.23
8	0.0195	6.97	8.27
9	0.0098	3.49	9.30
10	0.0049	1.74	10.33
11	0.0024	0.87	11.37
12	0.0012	0.44	12.40
13	0.0006	0.22	13.43
14	0.0003	0.11	14.47
15	0.0002	0.05	15.50

*Chamber volume is 31 ft^3; target concentration is 5 mg/L; exhaust rate is 30 cfm; and amount of CD in chamber is 4.39 g.
**For each air exchange, half the CD is removed. The safe level is 0.1 ppm (time weighted average).
Abbreviations: CD is chlorine dioxide.

Charging. During charging, CD gas is generated and introduced into the chamber through a small tube to achieve a set gas concentration. The target concentration is dependent upon various factors including cycle time, consumable life, amount of reagent gas, ambient pressure cycle, and vacuum chamber cycle. If the cycle time is extremely important, a higher concentration is sometimes selected to achieve a faster kill (15–30 mg/L). At higher concentrations, the D-values are much quicker, thereby shortening the overall cycle (see Table I). If a site has limited consumables or reagent gas, a lower concentration can be used to preserve consumables (1–15 mg/L), but the exposure time must be extended accordingly.

Because CD is measured easily in real time, the target concentration can be achieved each and every time in a straightforward manner, thus ensuring a repeatable and reproducible decontamination cycle. When gas concentration reaches the target concentration, the cycle proceeds to the next step.

Exposure. During exposure, the CD gas concentration is monitored and maintained at the selected concentration for the entire exposure time (typically 20–30 min). In addition, if the gas concentration drops during the cycle because of CD absorbance by celluoistic materials, CD gas is added to ensure the required CD concentration is maintained during the entire decontamination exposure step.

Aeration. During aeration, CD gas is removed from the chamber by allowing clean air into the chamber and removing CD to outside exhaust. Table II provides an example of the time required for the aeration of a 31-ft^3 isolator (at a 30-cfm exhaust rate) and the time required to bring the CD concentration in the chamber to a safe level (0.1 ppm).

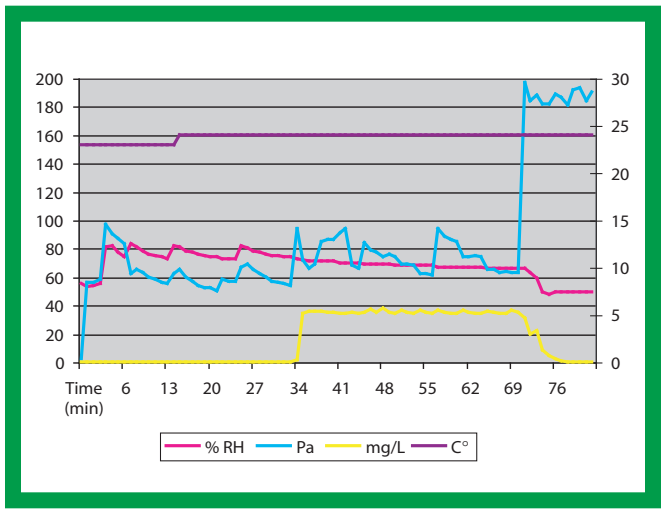

Figure 6: Media load cycle time (83 min total).

Figure 7: Component load cycle time (83 min total).

Table III: Chlorine dioxide (CD) decontamination cycle parameters.*

	Condition SP (% RH)	Condition time (min)	CD charge concentration (mg/L)	CD exposure time (min)	Aeration time (min)**	Decontamination cycle time (min)	Results BIs positive/BIs tested (per run)
Train of isolators	70	30	5.0	35	15	115	0/24
Transfer isolator media load	75	30	5.0	30	15	83	0/25
Transfer isolator component load	75	30	5.0	30	15	83	0/25

*Parameters are the same for all isolators.
**At the end of aeration, the CD level in the chamber was less than the Occupational Safety and Health Administration recommended amount of 0.1 ppm (time weighted average).
Abbreviations: SP is set point and BIs are biological indicators.

CD applications

CD gas is becoming more widely used because of its efficacy in some difficult and challenging applications. Though it is effective in all isolator and room decontamination applications, more-challenging applications demonstrate CD's superior sterilization capabilities.

If an isolator contains several types of complex equipment, such as in a filling line, it is difficult for nongaseous decontaminating agents to reach all the surfaces and crevices effectively. For isolators that are densely packed and have impeded circulation (Figures 2 and 3), CD exhibits better distribution than other sterilants such as hydrogen peroxide. For isolators that are trained together (see Figure 4), the good distribution of CD gas is apparent.

The benefit of the thorough distribution of CD gas becomes apparent when the sterilization of complex isolator systems (2 or more chained together) is required. CD can pass through high-efficiency particulate air filters for effective decontamination while maintaining a short aeration time. This attribute is possible because CD is a true gas as opposed to a vapor, which can condense to some extent on various isolator surfaces. As a gas, CD can be exhausted with only ~12–15 air exchanges.

Thorough aeration also is a strong factor in the use of CD. When temperature gradients are observed, which are usually more pronounced in rooms and larger isolator systems, vapor methods can have unequal condensation and effectiveness throughout the entire area. Because CD is a gas, temperature gradients do not affect its concentration. When concentration accuracy is preferred or the documentation of the sterilant concentration is required, a UV-*vis* photometric monitoring system can provide positive control to ensure a precise, repeatable cycle.

Train of isolators. When a train of isolators is used, CD disseminates throughout the train without requiring multiple injection ports (see Figure 4). In this example, CD was only introduced into the workstation isolator. Gas permeated throughout this isolator, into the autoclave interface isolator, and to the far side of the autoclave. The total cycle time for this isolator train was less than 2 h. Table III provides the cycle times and kills for the train of isolators (each trial is done in triplicate) and Figure 5 is an example of a cycle chart from one trial.

The cycle efficacy was confirmed by the distribution of *B. subtillis* biological indicators (BIs) on paper carriers wrapped in Tyvek/mylar pouches. The BIs were placed on various isolator surfaces and throughout the autoclave, including the autoclave's door well. All 24 BIs placed throughout the chambers were killed.

When compared with vapor-phase hydrogen peroxide (VHP),

the CD decontamination cycle time is much shorter. For example, a cycle time of a little more than 3 h was described in the literature for VHP decontamination of a 100-ft^3 isolator (19). This comparison is not exactly equivalent because the isolator described in the literature was only a single isolator. However, the comparison is still valid because a train of isolators is more complex and more difficult to decontaminate than a single chamber. Moreover, the decontamination time with CD for the train of isolators was strikingly shorter than the single-chamber isolator decontamination with VHP.

Transfer isolator. An example of the decontamination capacity of CD gas is demonstrated by its ability to decontaminate the interior surfaces and components of densely packed three-glove transfer isolators in the sterility test laboratory (see Figures 2 and 3). CD was chosen to obtain a more-repeatable cycle, to have a shorter decontamination cycle time (80 min), and to obtain a reproducible 6-log sporicidal reduction. Table III shows the cycle times and kills for the two dense loads (each trial was done in triplicate) and Figures 6 and 7 are examples of cycle charts for each trial type (dense load). The cycle efficacy was confirmed by the distribution of 25 *B. subtillis* BIs on paper carriers wrapped in Tyvek/mylar pouches. The BIs were placed on hard-to-reach areas of the isolator (*e.g.,* corners) throughout the loads to demonstrate the penetration ability of the gas. These total cycle times using CD gas are much lower than those reported in the literature, such as decontamination systems of 3–5 h for the "VHP 1000ED" (Steris) and 3–3.5 h for the "Clarus 'C'" (Bioquell) H_2O_2 generator (20). In these examples, the chamber sizes and loads, and the makeup of the isolators (316SS) were similar. This testing was performed to determine which VHP generator had a quicker cycle, thus both cycles were optimized to generate the shortest possible cycle times.

Conclusion

Decontamination with chlorine dioxide (CD) is a very effective and repeatable method. Because it is a true gas at normal-use temperatures, CD can get into crevices and other hard-to-reach areas by quickly and evenly dispersing to reach the same concentration throughout the isolator. As a gas, CD will not condense in colder areas or reduce concentration in warmer areas, as typically occurs with vapors such as hydrogen peroxide. This property eliminates the concerns about condensation and uneven concentration that can occur with vapor systems. This distinction is important because temperature gradients occur in every chamber and adversely affect vapors. If temperature is not tightly controlled, condensation is uneven, causing an uneven distribution of the vapor.

In addition, CD gas aerates quickly because it does not condense and does not require evaporation from surfaces before it can be effectively removed. Finally, CD gas can be accurately monitored using an ultraviolet-visible spectrophotometer, which ensures true process control of the decontaminating agent's concentration, thus enabling a repeatable and reproducible cycle each time. In conclusion, although the use of gaseous CD has been in practice for decades, newer technology and application methods underscore its efficacy and safety as an excellent decontaminating agent.

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