Deactivation of Single-Use Bioprocessing Systems Using Aqueous Chlorine Dioxide

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The biopharmaceutical industry has found multiple process uses for disposable containers. They include flexible containers with integral mixers such as the "one-touch" mixing technology from HyNetics brand (www.hynetics.com) that can be used to prepare media, buffers, IV solutions, and homogeneous suspensions such as adjuvant vaccines. Systems are available to accomodate 30–10,000-L flexible bag sizes.

As with any other innovation, new challenges are identified as our industry proceeds through the adoption life cycle for disposable processing systems. One set of challenges is associated with the deactivation of these systems.

But aren't these requirements exactly what we are trying to avoid through the use of disposables? The answer is yes, of course. But it is also true that in some circumstances drug processors will require or prefer to perform some biological deactivation if only for specific unit operations in a process. One example is the final deactivation of certain materials before disposal.

Depending on the nature of materials used in bioprocessing, users may choose terminal deactivation of disposables used in specific processing steps. Some relevant traditional methods include gamma irradiation, shredding followed by chemical inactivation, and treatment (incineration at a certified facility) as hazardous medical waste. Higher-temperature treatments (e.g., steam sterilization and autoclaving) are not an option with single-use components because of the polymeric materials generally used in their fabrication. When evaluating deactivation options, important performance parameters include biocidal effectiveness, cost, time requirements, convenience, and risk mitigation. Relevant risk considerations include handling and shipping of contaminated materials. Consider the current practice of autoclaving components that leave a classified area, for example.

When confronted with those challenges, HyNetics scientists looked at the alternatives and decided to test deactivation using a novel chlorine dioxide (ClO_2) generation technology. This appeared to offer superior performance opportunities in the following areas: proven biocidal effectiveness (ClO_2 is a broad-based disinfectant proven effective against bacteria, yeasts, viruses, and spore-formers); low operating costs after minimal if any capital investment; quick kill (typically a matter of seconds or minutes); in-place decontamination before removal of disposable processing components from their support infrastructure; and the ability to quickly flush-out (evacuate) the sanitizing agent without leaving hazardous residuals. The testing described herein was scheduled to verify those perceptions.

Test Methods and Results

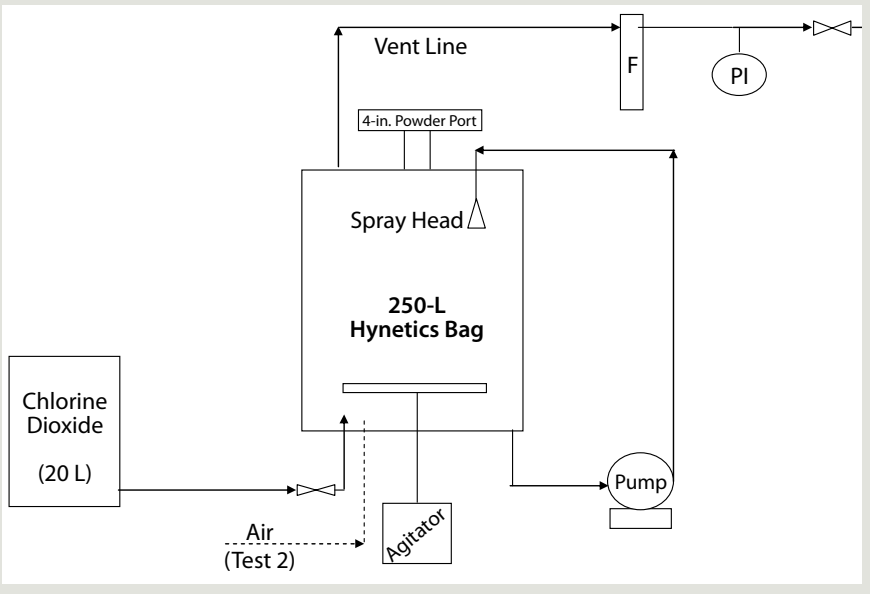
Two tests were conducted on 250-L HyNetics bags to determine the efficacy of ClO_2 as a disinfectant for typical industrial-scale disposables. The bag was suspended in a HyNetics holding vessel. ClO_2 was prepared using 12G Selectrocide sachets from Selective Micro Technologies (www.selectivemicro.com), each of which produce 12 grams of pure ClO_2. The

sachets were immersed in water in a 20-L plastic carboy on the day before the actual test runs. Figure 1 is a schematic of the test system.

One advantage of the sachet generation approach is storage. A gas at standard conditions, ClO_2 can be generated and stored in aqueous format in advance of its activation and use. The activated aqueous solution has a shelf life of over two weeks. For applications such as final decontamination of single-use systems, using the aqueous form rather than ClO_2 gas provides the added benefit of accomplishing a physical wash-down of soiled surfaces.

For our tests, we used ClO_2 concentrations of 300–700 mg/L. First, we placed *Bacillus subtilis* (10^6) spore strips (Cat. No. NA008 from Steris Corp of Mentor, OH, www.steris.com) and ClO_2 wide-range test strips (from Selective Micro Technology, used for measuring concentration) at strategic locations inside disposable liner bags. Figure 2 shows their locations in tests 1 and 2. They were also placed inside the cap of the container's powder addition port, which is considered to be the most difficult location in a disposable bag to decontaminate. Additional test strips were placed in vent line tubing just upstream of the vent filters (creating a dead-end situation for the closed system of Test 1).

Figure 1: Schematic of test system configuration



With the spore strips and test strips in place, we pumped an initial charge of ClO_2 solution into each bag. The solution was recirculated through each integral spray nozzle at 13 L/min through a peristaltic pump and recirculation manifold constructed of disposable tubing. We determined ClO_2 concentrations in the liquid by drawing samples from the recirculation lines at specific time intervals and immediately performing a colorimetric analysis using model

Chlorine Dioxide Summary

Biological Effects: Chlorine dioxide's moderate oxidation potential and existence as an uncharged gas allow it to selectively oxidize compounds that are essential to microorganism survival. ClO_2 kills bacteria, fungi, viruses, and spores over contact times ranging from a few seconds to a few minutes. A gas in its natural state, it is as effective as 10× the concentration of bleach (sodium hypochlorite, NaOCl), extremely soluble in water, material-compatible with polymers, and effective over a broad pH range.

Health and Safety: ClO_2 is characterized by a low human toxicity. Selective Micro Technologies has sponsored independent laboratory toxicity testing of ClO_2 solutions (at 650–700 ppm concentration) generated using the company's microreactor technology. This testing determined that the single-dose acute oral LD_{50} of a ClO_2 solution is greater than 5 g/kg of bodyweight in both male and female rats. Similarly, for inhalation, the LC_{50} of the activated solution is >2.07 mg/L in both male and female rats. The testing also found that the solution is not considered a contact (dermal) sensitizer. ClO_2 is widely used in both large-scale municipal water treatment and food processing. ClO_2 vapor itself requires careful handling because its OSHA-determined short-term exposure limit is 0.3 ppm and its time-weighted average is 0.1 ppm. This is rarely a restriction because ClO_2 is generally handled in closed systems and is readily controlled by standard environmental techniques. Any excess can be readily scrubbed, adsorbed, or vented depending on the quantities involved.

Application Notes: As generated by "micro-reactor" technology, ClO_2 has been found to be compatible with commonly used bioprocessing materials, including stainless steels, plastics, and elastomers. Selective Micro Technologies has detailed data for common materials. When present in confined spaces (e.g., closed equipment), ClO_2 in aqueous solution can kill microorganisms on surfaces that come into contact only with gas that is in equilibrium above the liquid. This provides an added level of assurance in cases where it may be difficult to confirm that all targeted surfaces can be wetted with the liquid solution. ClO_2 remains as an undissociated gas in water, providing for quick, residue-free flush-out and evacuation once it has completed microbial deactivation.

"Micro-reactor" (Sachet) Generators: For the testing reported herein, ClO_2 was generated by a novel membrane technology developed by Selective Micro Technologies to selectively permit diffusion of ClO_2 gas into aqueous solution. Raw materials are enclosed in a proprietary reaction-controlling membrane sachet and activated by its immersion in ambient water. Reaction residuals and byproducts (e.g., chlorates, chlorites, and remaining acids used in the generation process) are denied passage into the water. The membrane (sachet) serves a secondary purpose of containing raw materials and residues both during and after ClO_2 generation.

Table 1A: Spore strip deactivation results from Test 1

Spore Strip Number	Location	Exposure Time (minutes) 0	15	30
1	Powder Port		negative	negative
2	Liner		negative	negative
3	Liner		negative	negative
4	Liner		negative	negative
5	Liner		negative	negative
6	Liner		negative	negative
7	Liner		negative	negative
8	Liner		negative	negative
9	Liner		negative	negative
10	Vent Tubing (filter outlet)		positive	positive

"Negative" indicates zero spore growth (a total kill).
"Positive" indicates that viable organisms remained on the spore strip.

DR/890 colorimeter from Hach Company, of Loveland, CO (www.hach.com). After each test, the remaining ClO_2 solution was discarded to a sewer, and the tank liners were disposed of through normal solid waste channels.

Test 1: We prepared 20 L of an aqueous solution containing 332 mg/L of ClO_2. The test period began once the solution was inside a bag and circulation was established using an integral spray device. Spore strip and test strip samples were taken after 15 and 30 minutes (Table 1A). Final ClO_2 concentration in the liquid was measured at 183 mg/L (Table 1B). Pressure within the bag was kept slightly positive and constant during the test period.

Table 1b: Liquid concentration data (Test 1)

Time (minutes)	ClO_2 Concentration (mg/mL)
0	332
5	203
20	188
30	183

Table 1c: Evacuation data (Test 1)

Time (minutes)	ClO_2 Concentration at Vent (ppm)
0.0	N/A
2.1	240
5.0	100
10.0	55
15.0	18
20.0	5
25.0	1
31.4	0

Visual inspection of the test strips at the conclusion of the test indicated (by a dark brown coloration) that all surface areas within the liner bag and vent tubing had been exposed to significant quantities of ClO_2. Subsequent laboratory analysis showed that all spore strips in the tank liner — including those within the powder port — tested negative, with zero spore growth, indicating a total kill. But those located in the vent tubing were not totally killed during this run.

The final phase for a typical application is evacuation of ClO_2 down to acceptable levels. For this test, air was introduced at a single point in the bottom of the system at 1 scfm. No attempt was made to minimize evacuation times by using higher purge air flows or multiple inlets.

Test 2: For the second test, we prepared 20 L of fresh solution containing 686 mg/L ClO_2. The test period began once the solution was inside a bag and circulation was established using the integral spray device. For this test, we bubbled a small quantity of air (150 cc/min) through the liquid to provide a continuous airflow through the vent line and filter.

Spore strip and test strip samples were taken after five, 15, and 30 minutes. Again the test strips confirmed that all surfaces had been exposed to significant quantities of ClO_2. ClO_2 concentrations in the liquid were measured periodically. The final liquid concentration was 376 mg/L. Results are recorded in Tables 2A–C.

At the five-minute exposure point, all spore strips tested negative (indicating total kill) — including those within the powder port, but not those located in the vent line tubing. After 15 and 30 minutes, even those strips tested negative. Apparently the increased concentrations (compared with Test 1 conditions) and the sparged air were sufficient to achieve total kill in the vent system upstream of the filter. Again, the pressure within the bag was held slightly positive (less than or equal to three inches of water-column) and relatively constant throughout the test period.

The results of evacuation monitoring for this test appear in Table 2C. Air was again introduced in a single point at a rate of 1 scfm, at the top of the system this time, and it was removed from the bottom. Quicker evacuation was accomplished with this arrangement — probably because ClO_2 is denser than air.

Results and Conclusions: Tables 1A–C and 2A–C summarize the results for this test program. We conclude that aqueous ClO_2 provides a quick, effective, and economic option to one challenge (final decontamination) hindering the widespread adoption of single-use processing technologies in the biopharmaceutical industry. We performed effective decontamination cycles of 30 minutes or less without serious effort toward cycle

Table 2A: Spore strip deactivation results from Test 2 (purge air flow = 1 scfm)

Spore Strip Number	Location	Exposure Time (minutes) 0	5	15	30
1	Powder Port		negative	negative	negative
2	Liner		negative	negative	negative
3	Liner		negative	negative	negative
4	Liner		negative	negative	negative
5	Liner		negative	negative	negative
6	Liner		negative	negative	negative
7	Liner		negative	negative	negative
8	Liner		negative	negative	negative
9	Liner		negative	negative	negative
10	Vent Tubing (filter outlet)		positive	negative	negative

"Negative" indicates zero spore growth (a total kill).
"Positive" indicates that viable organisms remained on the spore strip.

Table 2B: Liquid concentration data (Test 2)

Time (minutes)	ClO_2 Concentration (mg/mL)
0	686
5	426
20	424
30	376

Table 2C: Evacuation data (Test 2) with purge air flow at 1 scfm

Time (minutes)	ClO_2 Concentration at Vent (ppm)
0	N/A
5	66
10	15
15	3
20	1
21	0

optimization. Using the microreactor ClO_2 generation technology instead of other decontamination options minimizes capital and operating costs. Generation of the sanitizing agent can be conveniently scheduled, and cycle time requirements can be minimized.

Validation and Future Testing

Disposables decontamination is an area that will undoubtedly require future pronouncements from validation and regulatory compliance experts. Below are a few general comments that summarize our opinions regarding validation of decontamination operations for disposables.

The ClO_2 decontamination cycle can be readily validated on-site to demonstrate microbial inactivation. Cycle times for decontamination of single-use systems using aqueous ClO_2 should be somewhat shorter (and less costly) than thermal treatment of traditional stainless steel systems, as demonstrated by these test results. To validate such a process in an industrial setting, specific protocols will be needed. The procedure outlined here provides general guidance. A parametric release philosophy supported by in-plant testing (several successful inactivation efforts of appropriately positioned spore strips) similar to that described above seems a prudent and potentially effective approach.

Presterilization of Single-Use Systems: Another challenge for users of disposables is sterilization of systems and/or components before processing. This is particularly relevant as disposables move into bioreaction and fermentation applications. Testing has begun in this area with very good preliminary results. Data will be reported in an upcoming article.

For Further Reading

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Figure 2: Spore strip locations for Tests 1 and 2 (diagrams do not include filter outlet strips)