Chlorine Dioxide Gas Decontamination of a Blow/Fill/Seal Machine

How chlorine dioxide was used to decontaminate equipment in a specially built 6000 ft³ microbial challenge room.

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Introduction

Cardinal Health, Inc. (Woodstock, IL), a contract manufacturing facility that utilizes Blow/Fill/Seal (BFS) technology to aseptically package liquid pharmaceutical and healthcare products, has undertaken a research program to further the understanding of the impact that the environment surrounding a BFS machine has upon the microbiological quality of the processed product [1]. A dedicated facility (Challenge Room) has been constructed, and is qualified to provide for the production and containment of dispersions of microorganisms in the air of a room housing an operating BFS machine of a given style and configuration (Figure 1).

During a particular challenge study, microorganisms are dispersed in the air at varying concentrations while an operating Blow/Fill/Seal machine produces containers filled with a medium that supports the growth of the challenge microorganism. The resulting data may then be used to establish a relationship between the levels of airborne microorganisms in the machine's operating environment and the extent of product contamination.

Following these challenge studies, there is a need to decontaminate the Challenge Room and its contained BFS machine. Given the nature of the Challenge Room and the complex construction of the BFS machine, chlorine dioxide gas was chosen for decontamination purposes. To determine the efficacy of chlorine dioxide gas in this function, three independent qualification runs were performed with biological indicators. On the assumption that linear or near-linear inactivation kinetics hold at low probabilities of spore survival, a minimum of 15 log spore reduction was identified as effective decontamination since, in future challenges of the BFS technology, the room could be contaminated with up to 10^{12} spores.

Current Room Decontamination Methods

Current decontamination methods for rooms include manual spray and wipe, or soaking techniques using liquid disinfecting solutions, formaldehyde gas, hydrogen peroxide vapor, and chlorine dioxide gas. Manual spray and wiping and formaldehyde gassing are by far the primary methods but each has significant drawbacks.

Figure 1. Room housing an operating BFS machine

Manual spray and wiping methods are the least effective methods of decontamination because they are subject to missing areas because of human error. The personnel performing the decontamination must spray each and every surface with the decontaminating agent, allow the agent to remain on the surface for the specified amount of time without drying (typically 10-20 minutes), then wipe the surface. While this may sound easy, it is actually the more difficult task, especially in hard to reach areas such as corners, the under side of HVAC grills, floor drains, beneath components, etc.

Sprayers, foggers, and misters are an improvement upon the manual spraying and wiping by removing the person from the process, but also are limited in their ability and coverage. These methods create small size droplets that are heavier than air and settle downwards when sprayed or injected. While they get cov-

erage on the walls, they do not get coverage on the underside of components or behind equipment. Additionally, when spraying in odd shaped rooms or rooms containing equipment, it becomes impossible to reach all surfaces and thereby a complete decontamination can not be achieved.

Formaldehyde is a very effective method that has been used longer than all of the other "gassing" methods. It is understood and has an effective cycle. It is effective against a broad range of organisms, is low in cost, and can effectively reach all surfaces. The major concern with decontaminating rooms, buildings, or vessels with formaldehyde is that it is listed as carcinogenic [2].

As of June 2004, the International Agency for Research on Cancer classified formaldehyde as carcinogenic to humans [3]. Furthermore, formaldehyde requires additional steps which include neutralization and manual wiping of the neutralization byproduct. A residue is commonly left after such treatment, consisting of polymerized formaldehyde (paraformaldehyde) and the neutralization product (methenamine). The removal of such a residue was considered challenging for this application. There was additional concern that residual formaldehyde from off-gassing would also be problematic, both because of its odor and its perceived toxicity. This residue removal increases the amount of time involved, amount of personnel involved, and cost in labor and time.

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Furthermore formaldehyde concentrations are not monitored during the process thereby not having the assurance of complete coverage throughout the room.

Vapor hydrogen peroxide is somewhat effective under ideal conditions, but falls short in real-life applications where there are even slight temperature gradients or an assortment of materials affecting the process. It is effective against a broad range of organisms but celluloid materials such as flooring, boxes, ceiling tiles, paperwork, etc. absorb the peroxide thereby scavenging it from other areas [4]. Vapor hydrogen peroxide is a vapor, not a gas, which upon delivery to the room, wants to transform back to its normal state (liquid). Any cool ➤

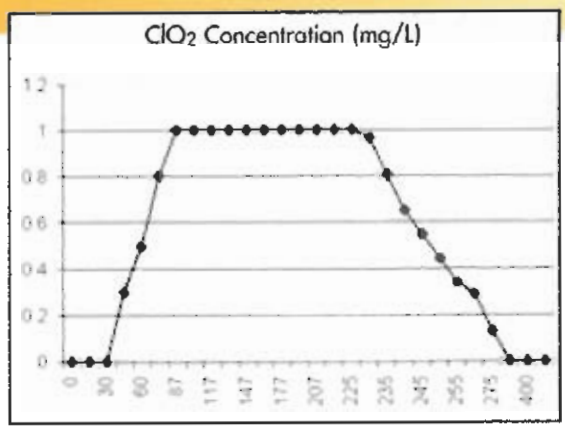

Figure 2. Chlorine Dioxide Cycle Chart

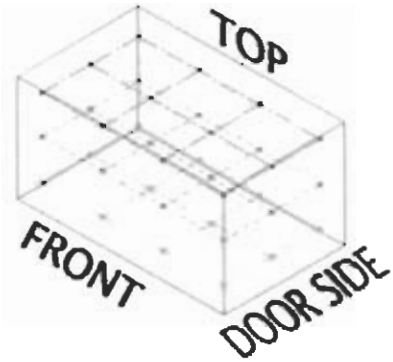

Figure 3. Biological Indicator Placement

surfaces or temperature gradients cause this to occur as condensation. Such condensation on cooler surfaces caused by temperature gradients creates an uneven distribution of the decontaminating vapor. This creates areas with a greater amount of the decontaminating agent and areas with less or little; thereby not getting good distribution or more importantly, good kill. Additionally vapor hydrogen peroxide has challenges in reaching difficult areas such as floor drains, HVAC grills, beneath furniture and components, the inside of cabinets, hinges, instruments, and components. Vapor hydrogen peroxide was believed too problematic for the current application, particularly the ductwork and the many surfaces with various temperature differentials that exist in any large enclosed space.

Chlorine Dioxide

Chlorine dioxide (ClO_2) is a greenish-yellow gas and a single-electron-transfer oxidizing agent with a chlorine-like odor. Pure chlorine dioxide is an unstable gas and therefore is generated as needed. Although chlorine dioxide has "chlorine" in its name, its chemistry is radically different from that of chlorine. Chlorine dioxide oxygenates products rather than chlorinating them and thus trihalomethane (THMs) formation does not occur [5]. Therefore, unlike chlorine, chlorine dioxide does not produce environmentally undesirable organic compounds containing chlorine.

Chlorine dioxide is a true gas at normal use temperatures and therefore is not susceptible to condensation issues and temperature gradients such as vapors. It has a yellowish-green color which allows its concentration to be precisely monitored and controlled by a UV-VIS spectrophotometer. This gives the ability to provide tight process control from beginning to end.

> The decontamination process using chlorine dioxide gas was determined to be an unqualified success.

Chlorine dioxide has been recognized since the beginning of the century for its disinfecting properties. Gaseous chlorine dioxide has even been shown to be more effective than liquid chlorine dioxide when applied in equal concentrations and times [6]. Chlorine dioxide in both its gaseous and aqueous phase is a strong oxidizing agent and has about 2.5 times the oxidation capacity of chlorine. Additionally, ClO_2 gas has been approved for use as a sterilant/decontaminate by the United States Environmental Protection Agency (US EPA). In addition, both gaseous and aqueous phase chlorine dioxide has been shown to be an effective sanitizing agent that has broad and high biocidal effectiveness. Aqueous ClO_2 has been reported to effectively inactivate bacteria [7, 8] including pathogens [9, 10, 11], viruses [12, 13], bacterial spores [14, 15] and algae [16].

Chemical Formula:	ClO_2
Molecular Weight:	67.45 g/mole
Melting Point (°C):	-59
Boiling Point (°C):	+11
Density:	2.4 times that of air

Table 1. Chlorine dioxide characteristics

Gaseous ClO_2 has been proven to be an effective disinfectant especially in the medical science areas. Rosenblatt, et al. [17] developed the use of gaseous ClO_2 to sterilize surfaces, especially the gas-impermeable surfaces of implements commonly employed in medical sciences, such as those made of porcelain, ceramics, metal, plastics, and glass. Jeng and Woodworth [18] reported the sporicidal activity of ClO_2 gas under square-wave conditions within an experimental sterilizer used for medical implements. ClorDiSys Solutions, Inc. [19, 20] described the development of a process using ClO_2 gas for sterilization of aseptic fill isolators and pharmaceutical process vessels.

Recently, gaseous ClO_2 has successfully been used to decontaminate B. anthracis contaminated areas of the Hart Senate Office Building and the Brentwood postal sorting facility in Washington, DC. Han et al. [21] also reported high efficacy of ClO_2 gas in

reducing Bacillus spores on paper, plastic, epoxy-coated stainless steel, and wood surfaces. Additionally, numerous research has demonstrated that ClO_2 gas is highly effective (greater than 5 log reductions) in reducing foodborne pathogens (E. coli O157:H7, Listeria monocytogenes, and Salmonella) on fruits and vegetables surfaces [22, 23, 24, 25, 26, 27, 28, 29], and spoilage microorganisms on food-contact surfaces [30].

About 5% of large water treatment facilities (serving more than 100,000 people) in the United States use chlorine dioxide to treat drinking water. In communities that use chlorine dioxide to treat water for drinking uses, chlorine dioxide is permitted to be present at low levels in the tap water [31]. It is estimated that about 12 million people may be exposed in this way to chlorine dioxide. It is also estimated that there were 743,015 pounds (337,026 kg) of chlorine dioxide released to the atmosphere from over 100 manufacturing, processing, and waste disposal facilities in 2000 [2].

Decontamination

The chlorine dioxide gas decontamination process consists of five steps: Pre-Condition, Condition, Charge, Exposure, and Aeration. The first step of Pre-Condition consists of raising the humidity to a target level of 65-75%. In our application the humidity was raised to 70-75% using the room HVAC system. Once this target humidity was reached it was maintained for 30 minutes (Condition). When the condition time was completed the Charge step occurred next. At this point, chlorine dioxide gas was generated by passing 2% chlorine gas (98% nitrogen) over sodium chlorite cartridges that convert the chlorine gas to pure chlorine dioxide with no byproducts.

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

$$2ClO_2\,(g) + 2NaCl\,(s)$$

This pure chlorine dioxide gas was then introduced into the 6000 cubic foot room to achieve a target concentration of 1mg/L (Figure 2). The charging time to achieve the target concentration was slightly less than 60 minutes. This charge time was what was expected from the calculated time but was also confirmed by a photometric concentration monitor. Once the concentration was achieved it was held for 120 minutes (exposure) to achieve an extreme overkill cycle. However, typical exposures range from 30-60 minutes.

During the qualification studies, thirty-six paper biological indicators (BIs) with a nominal spore population of 106 Bacillus atrophaeus ATCC 9372 were suspended around the room in a grid pattern (Figure 3), twelve of them, one foot from the floor, twelve one foot from the ceiling and twelve in the middle of the room. One BI was placed in the HVAC duct work. Additionally, two sample loops, designated north and south, were utilized to perform a series of 24 minute exposures to gelatin filters loaded with a nominal spore population of 106 Bacillus subtilis NCIMB 8649. Spores of this organism are to be used as the primary challenge test organism.

During the exposure time, the contaminated gelatin filters, located separately in filter housings (north and south) were attached to the air sampling system. The 120 minute exposure time was broken into four quarters of 30 minutes each. During each quarter two (north and south) gelatin filters were exposed to the room environment containing 1mg/L of chlorine dioxide gas by drawing a continuous sample over the filters for 24 minutes for a total of eight exposures during the 120 ➤

Study	Results BIs Positive / BIs Tested
1	0/37
2	0/37
3	0/37

Table 2. Results of B. atrophaeus 10^6 (ATCC 9372) Suspended in Room

minute exposure time. At the end of the sample period, the filter housing was detached from the port and air flowed through the assembly to free it of ClO_2 gas. This study along with the suspended BIs was performed three times.

Finally, once the exposure was complete, the room was aerated by exhausting to the atmosphere. Aeration of the room took approximately 2.5 hours using the room exhaust system (500CFM).

Results

Results of the suspended BIs yielded a complete inactivation of all 37 biological indicators of Bacillus atrophaeus ATCC 9372 (Table 2).

Results of the north and south sample loops yielded acceptable results with a cumulative spore log reduction of greater than 15 (Tables 3,4,5).

This work qualified a low concentration (1mg/L) gaseous chlorine dioxide process for use in the decontamination of a Microbial Challenge Room having a volume of 6000 ft^3. In three independent studies, it was demonstrated that inactivation levels remained constant over an extended two-hour period and each trial yielded a complete kill of all biological indicators suspended around the room and a cumulative Spore Log Reduction of greater than 15.

Conclusion

The decontamination process using chlorine dioxide gas was determined to be an unqualified success. Complete inactivation of the suspended paper BIs was achieved for each trial. Spore inactivation, as quantitatively assessed in the gelatin filters loaded with spores of

Quartile Number	Exposure Start Time	Exposure End Time	Exposure Time	Actual Spore Log Reduction	Average Spore Log Reduction (24 min)	Predicted Spore Log Reduction (30 min)
1	0	24	24	3.50	4.01	5.01
1	1	25	24	4.52		
2	32	56	24	2.54	2.99	3.74
2	33	57	24	3.44		
3	63	87	24	3.19	2.585	3.23
3	64	88	24	1.98		
4	95	119	24	3.13	3.74	4.68
4	96	120	24	4.35		
				Cumulative Spore Reduction		16.66

Table 3: Study 1 Results of B. subtilis 10^6 Gelatin Filters (NCIMB 8649) In Sample Loop

Quartile Number	Exposure Start Time	Exposure End Time	Exposure Time	Actual Spore Log Reduction	Average Spore Log Reduction (24 min)	Predicted Spore Log Reduction (30 min)
1	0	24	24	5.57	5.725	7.16
1	1	25	24	5.88		
2	32	56	24	3.65	4.27	5.34
2	33	57	24	4.89		
3	63	87	24	3.54	3.56	4.45
3	64	88	24	3.58		
4	95	119	24	4.80	3.675	4.59
4	96	120	24	2.55		
				Cumulative Spore Reduction		21.54

Table 4: Study 2 Results of B. subtilis 10^6 Gelatin Filters (NCIMB 8649) In Sample Loop

Quartile Number	Exposure Start Time	Exposure End Time	Exposure Time	Actual Spore Log Reduction	Average Spore Log Reduction (24 min)	Predicted Spore Log Reduction (30 min)
1	0	24	24	4.66	4.755	5.94
1	1	25	24	4.85		
2	32	56	24	4.00	3.8	4.75
2	33	57	24	3.60		
3	63	87	24	3.30	4.15	5.19
3	64	88	24	5.00		
4	95	119	24	2.52	3.87	4.84
4	96	120	24	5.22		
				Cumulative Spore Reduction		20.72

Table 5: Study 3 Results of B. subtilis 10^6 Gelatin Filters (NCIMB 8649) In Sample Loop

B. Subtilis (NCIMB 8649) for each trial, provided a predicted cumulative spore log reduction of greater than 15 for the 120 minute exposure period. This data is evidence that chlorine dioxide is an effective decontamination agent at the concentration of 1mg/L, and that the cycle provides reproducible results. The real time monitoring of gas concentration allows for the system to respond to concentration drops by adding additional gas. However this was not necessary in any of the qualification runs since chlorine dioxide does not condense on surfaces or react with any of the materials present in the room. The total cycle time, from beginning to end of seven hours, was designed for the particular application discussed in this article. Cycle times may be optimized based on the level of decontamination determined to be necessary, and by adjusting gas concentration to lower the exposure period.

Also of note is that post-aeration, the room was available for immediate reuse with no need for wiping or rinsing of surfaces, as might be required had a formaldehyde or aqueous decontamination process been employed.

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