Mold Remediation of a Research Facility in a Hospital
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Abstract

A hospital’s life science research facility experienced flooding due to a pipe bursting two floors above. In addition to water damage throughout the facility, mold spores were introduced and distributed throughout. Prior to repairs being made, a full facility decontamination took place to eliminate any mold or mold spores throughout the facility. Chlorine dioxide gas (CD) was chosen as the method of decontamination, which took place over the course of 2 days. Sixteen biological indicators (BI) consisting of Geobacillus stearothermophilus were placed throughout the 145,000 ft³ facility. A target level of 720 parts-per-million hours (PPM-hr) was targeted to achieve a 6-log sporicidal reduction with an actual exposure of approximately 1,000 PPM-hr exhibited. During the decontamination, the surrounding areas of the facility were occupied, so chemical monitoring took place throughout the event to ensure that facility personnel were not exposed to any leakage. Upon completion of the decontamination, the 16 biological indicators exposed, as well as a positive control BI, were tested for growth. After the incubation period, only the positive control exhibited growth, demonstrating that the decontamination was a success and the facility was free of mold and mold spores.

Introduction

In the Spring of 2012, a life science research facility of 145,000 ft³ (4,106 m³) experienced flooding in multiple rooms when a pipe located two floors above the facility burst. Water soaked through the ceiling, affecting multiple rooms and hallways. Environmental testing showed that due to the flooding, the facility became contaminated with multiple types of mold spores, including Aspergillus niger. Rooms within the facility had solid ceilings while the hallways had drop ceilings. The ceilings and ceiling tiles of the affected areas were disposed of but not before mold spores were transferred throughout much of the facility. The facility required decontamination due to the introduction and spread of mold spores. This was to take place after the leak was fixed but prior to the physical repair of the facility. Chlorine dioxide gas was selected as the fumigating agent to decontaminate the facility. Chlorine dioxide gas, registered as a sterilant with the U.S. Environmental Protection Agency (EPA), is capable of killing all viruses, bacteria, fungi, and their spores (List A, 2009). Chlorine dioxide gas provides a more consistent level of kill than hydrogen peroxide vapor across multiple surfaces and locations (Beswick et al., 2011). Chlorine dioxide gas was also more compatible with the epoxy floors within the facility as compared to hydrogen peroxide vapor (Sawyer, 2011). These factors were extremely important as the facility held a variety of surfaces and equipment and all had to be sufficiently treated for mold spores without compromising the equipment and facility.

Materials and Methods

- 9 - Chlorine dioxide gas generators (ClorDiSys Solutions, Inc., Lebanon, NJ)
- 24 - Reagent gas cylinders (Airgas, Malvern, PA)
- 1 - Nitrogen cylinder (Airgas, Malvern, PA)
- 1 - ClorDiSys EMS chlorine dioxide gas-sensing module (ClorDiSys Solutions, Inc., Lebanon, NJ)
- 1 - C16 Portable Gas Leak Detector (Analytical Technology, Inc, Collegeville, PA)
- 50 - Distribution fans (8” fans)
- 30 - Steam humidifiers
- 20 - Extension cords
- 17 - SCD/6 G. stearothermophilus Biological Indicator
- 10⁶ spore strips on paper wrapped in Tyvek (Mesa Laboratories, Bozeman, MT)
- Gas inject and sample tubing
- Plastic sheeting (6 mm)
- Duct tape

Day 1—Site Preparation

The facility contained animal holding rooms, procedure rooms, a laboratory, a cage wash area, storage rooms, necropsy rooms, locker rooms, bathrooms, etc. All the equipment that was not destroyed by the flood was kept in place for the decontamination. This included rodent racks and cages, changing stations, biological safety cabinets, a rack washer, microscopes, water bottles, environmental control systems and sensors, computers, printers, and analytical and electronic equipment. Chlorine dioxide gas has been proven to safely and effectively decontaminate facilities with equipment in them (Czarneski, 2009). The total site preparation was a 1-day process for six people. To prepare the site for decontamination, several things had to be done. During the decontamination itself, the HVAC system in the area was turned off to keep the gas inside for the proper amount of time. Since the facility is within an active hospital, extra care was taken to ensure that no leakage would occur. The outside walls of the area to be treated were checked for penetrations where the gas could escape. The HVAC serviced the two floors of the building, but only the research facility on Level 0 was being decontaminated. The first floor (Level 1) was not a containment area and had little flooding, so this area was not included in the decontamination. The lower floor (Level 0) housed the research facility and was the space requiring decontamina-
tion. This is where the majority of the flood damage occurred and it was also considered a barrier or containment facility. Since the supply and exhaust are common between the two floors, gas would have been able to reach Level 1 after being introduced to Level 0 with the system turned off. To isolate Level 1 and Level 0 from each other, the exhaust and supply ducts were sealed on Level 0 to prevent gas migration to Level 1. Supply and exhaust grills were removed and the ducts themselves were sealed by taping them closed. This decision was made by hospital management as they wanted to limit access to Level 1. The HVAC system was unable to be used to aerate the gas upon completion of the decontamination as Level 0’s ducts could not be remotely opened once sealed. Due to this, two external exhaust lines were put into place to handle the aeration of the gas. An external blower was connected to a 12-inch flexible duct which ran from the facility to the roof via a stairwell. The second exhaust line connected the duct from the facility to the house exhaust system. Both exhaust lines were set up, but not connected to the facility until aeration began the next day.

All of the entry doors into the Level 0 barrier facility (except one used for entry and exit) were sealed with duct tape and plastic. After this, the gas-injection tubing and gas-sampling tubing were run to various locations within the facility (Figure 1). The fans and humidifiers were then placed around the facility. In general, one fan per room was used and several in the hallways were used to aid the distribution of the gas and humidity. The fans were typically placed at the entry to each room to expedite the natural distribution of the gas.

Day 2—Decontamination

On the second day, the biological indicators were placed around the facility. Sixteen biological indicators were placed in rooms and above the ceiling and one control for a total of 17 BIs (Table 1, Figure 1). After placement of the biological indicators, the humidifiers were turned on to raise the relative humidity (RH) to the minimum level of 65%. All spore reduction requires an elevated humidity level to soften and swell the spores, allowing for the sterilitant to enter and kill the spore (Westphal et al., 2003). Raising the humidity also allows for the opportunity to safely check that the HVAC system is shut down and the facility is properly sealed. After raising the RH to a minimum of 65%, the room air was allowed to sit and stabilize. If the RH level decreased quickly, it could signal that the HVAC system was shut down and the facility was properly sealed. During humidification all but one doorway was sealed. This allowed the monitoring of individual rooms for proper RH levels using hand-held sensors that were removed prior to the injection of chlorine dioxide gas. If any room had an RH that was too low, humidifiers were moved to bring it to the desired level. After all the rooms were at the proper RH levels, the last door was sealed and chlorine dioxide gas was introduced.

The target concentration of chlorine dioxide gas was 1 mg/L (363 ppm). As demonstrated by the readings (Figure 2), this target concentration was not reached in all locations. There was some leakage near injection point 17 (Figure 1) and some adsorption of the chlorine dioxide gas into uncoated concrete surfaces above the ceilings. Using chlorine dioxide gas offered the benefit that any leakage is detectable by its odor at its OSHA 8-hour time weighted average (TWA) safety level of 0.1 ppm (Table 2). The entire area surrounding the facility was routinely scanned with a low-level chemical sensor, but the ability to sense any leakage through its chlorine-like smell offered an added level of personal protection. This is different from other common fumigation methods such as formaldehyde and vapor phase hydrogen peroxide, which cannot be smelled at its OSHA 8-hour TWA safety level.

Hospital staff, patients, and all others within the facility would be alerted to any leaks in their vicinity while still at the 8-hour TWA safety level. Although everyone has a varied sense of smell, this presented a major benefit to the hospital as it was to be occupied throughout the decontamination. It had been deemed impractical to provide chemical safety badges or other personal protective equipment to everyone within the hospital; however, the combination of routine facility sweeps with a low-level chemical sensor and chlorine dioxide gas’s low odor threshold was considered to provide sufficient warning if dangerous levels of leakage occurred. Throughout the decontamination, no leaks were detected other than those near injection point 17, which were able to be corrected.

During the decontamination, chlorine dioxide gas concentration measurements were taken continuously within the space being decontaminated. This provided real-time feedback as to the progress and safety of the decontamination. Chlorine dioxide gas concentration samples were taken from 12 locations within the facility. Since the target concentration was not reached in all areas, this impacted the injection and exposure times as the lower concentration of gas needed to be held for a longer period of time. The target dosage for a 6-log sporicidal reduction using chlorine dioxide gas is 720 ppm-hrs. This is measured by accruing the ppm-hours through a calibrated chlorine dioxide gas-sensing module that pulls samples of air from the facility and passes them through a photometric device. When all sample locations exceeded the 720 ppm-hr level, exposure was terminated and aeration of chlorine dioxide gas began (Figure 3).

Exposure finished at 7:30 p.m. and aeration was initiated at 8:15 p.m. Before aeration could begin, the external exhaust lines had to be connected to the facility. Once connected, the external blower was turned on to pull gas from the facility to the outside via the duct that was run-up the stairwell to the roof. The other duct was connected to the house exhaust with an active draw. Both external exhaust lines had to be utilized since the shared HVAC system required that all supply and exhaust vents on Level 0 had to be sealed, preventing use of the shared HVAC system for aeration. Since the gas was being aerated only from two
locations, the overall aeration time was significantly longer than the 1 hour that is typical for a chlorine dioxide gas decontamination (Czarneski, 2009). Once aeration was started, the space was safe to enter at approximately 11:00 p.m. This was determined through the use of both the chlorine dioxide gas sensor module and the portable gas leak detector. The total aeration time was 2.75 hours.

**Discussion**

The targeted chlorine dioxide gas concentration was not reached as identified by the chlorine dioxide gas sensor module. The sensor module pulls samples of air from locations within the facility through a photometric device using a small diaphragm pump. Sample tubing is run from the sensor module to the locations where the samples are taken. This distance can be more than 500 ft (150 m) as chlorine dioxide gas does not condense or become affected by environmental factors over lengths of distance. Since chlorine dioxide gas is a visible yellow-green gas, it can be easily and accurately measured with a photometer. The photometer provides an accurate, repeatable, and real-time measurement of the sterilant concentration within the facility at any number of locations. The concentration monitoring is very

**Figure 1**

Facility layout with generator injector, sample and BI locations.
Table 1
BI Locations, Results, and Pictures

<table>
<thead>
<tr>
<th>BI #</th>
<th>Location</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Room 1, 6 ft off floor in corner of room</td>
<td>No Growth</td>
</tr>
<tr>
<td>2</td>
<td>Room 2, 3 ft off floor in corner of room</td>
<td>No Growth</td>
</tr>
<tr>
<td>3</td>
<td>Room 3, 6 ft off floor in corner of room</td>
<td>No Growth</td>
</tr>
<tr>
<td>4</td>
<td>Room 4, 6 ft off floor in corner under shelf</td>
<td>No Growth</td>
</tr>
<tr>
<td>5</td>
<td>Room 5, 5 ft off floor next to rack</td>
<td>No Growth</td>
</tr>
<tr>
<td>6</td>
<td>Room 6, 2.5 ft off floor under exam table</td>
<td>No Growth</td>
</tr>
<tr>
<td>7</td>
<td>Room 7, 6 ft off floor in corner of room</td>
<td>No Growth</td>
</tr>
<tr>
<td>8</td>
<td>Room 8, 6 ft off floor in corner behind mouse rack</td>
<td>No Growth</td>
</tr>
<tr>
<td>9</td>
<td>Room 9, cage wash area above ceiling</td>
<td>No Growth</td>
</tr>
<tr>
<td>10</td>
<td>Corridor 1, above ceiling</td>
<td>No Growth</td>
</tr>
<tr>
<td>11</td>
<td>Room 10, above ceiling</td>
<td>No Growth</td>
</tr>
<tr>
<td>12</td>
<td>Room 11, above ceiling</td>
<td>No Growth</td>
</tr>
<tr>
<td>13</td>
<td>Room 12, 5 ft off floor above ThermoJet</td>
<td>No Growth</td>
</tr>
<tr>
<td>14</td>
<td>Room 13, 6 ft off floor on Allentown Rack</td>
<td>No Growth</td>
</tr>
<tr>
<td>15</td>
<td>Rooms 14, 5 ft off floor back wall near boxes</td>
<td>No Growth</td>
</tr>
<tr>
<td>16</td>
<td>Room 15, on lab bench/counter top next to sink</td>
<td>No Growth</td>
</tr>
<tr>
<td>17</td>
<td>Positive Control</td>
<td>Growth</td>
</tr>
</tbody>
</table>

Figure 2
Sample CD Gas Readings Chart (mg/L)
Table 2
Odor Threshold and 8-hr TWA of Sporicidal Fumigants

<table>
<thead>
<tr>
<th>Fumigant</th>
<th>Odor Threshold</th>
<th>OSHA 8-hr Time Weighted Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde</td>
<td>0.8 - 1 ppm</td>
<td>0.75 ppm</td>
</tr>
<tr>
<td>Hydrogen Peroxide Vapor</td>
<td>No odor threshold located</td>
<td>1 ppm</td>
</tr>
<tr>
<td>Chlorine Dioxide Gas</td>
<td>Ppm (ref - OSHA web site)</td>
<td>0.1 ppm</td>
</tr>
</tbody>
</table>

Figure 3
Exposure Chart (ppm-hrs)

PPM-Hrs Summary

For a decontamination such as this where the concentration drops over time, a saturated concentration monitor would signal that the cumulative exposure had been reached before it truly was, potentially affecting the efficacy of the decontamination. This is why photometric measurement is significant. The photometric measurement of chlorine dioxide gas is not affected by temperature, humidity, or high or low concentrations and therefore is a reliable measurement device that can accurately monitor the status of the decontamination.

The leak point discovered near injection point 17 created a safety concern and identified a facility issue. Soon after gas injection started, a chlorine dioxide odor was detected near personnel operating the chlorine dioxide gas equipment. A penetration was found between the facility and the hallway, and the gassing was temporarily halted to correct the problem. The leak was temporarily patched with plastic, duct tape, and expanding foam. Gassing was restarted at approximately 4:00 p.m. (Figure 2). Since this was an active hospital with a full contingency of patients, leaks were a primary concern. At no point were hospital
personnel affected by the leak even though some were as close as 15 feet down the hallway from the leakage. Since chlorine dioxide gas has an odor, the leak was able to be detected and then patched, and the decontamination was completed successfully. The penetration that was found was large enough that the facility’s containment was compromised. The gas leak uncovered a containment issue that was previously unknown to management; this was permanently fixed after the decontamination was completed, establishing true containment for the barrier facility.

Conclusions

Chlorine dioxide gas decontamination of the 35-room facility was a qualified success. Figure 3 indicates that the total ppm-hrs all exceeded the minimum of 720 ppm-hours, which is necessary for a 6-log spore reduction. Based upon calculations done after the decontamination, the actual minimum exposure level was just under 1,000 ppm-hrs. Although the desired ppm-hours were reached, the true success factor stems from the results of the BIs after incubation. After the 7 days of incubation, it was noted that all BIs were killed with the exception of the positive controls. Success was also satisfied as there was no physical residue, and no visible indication of material degradation on any of the surfaces within the building, including the ventilated racks, plastic caging, BSCs, etc. Also of note, there was no visible indication of effect to any electronics or measurement devices in the area. The final measurement of success was that no molds were recovered from air exposure plates brought in and opened up within the facility after the decontamination. Plates consisting of malt extract agar (BectonDickenson, Sparks, MD) were left out in 12 locations for 4 hours to allow for settling. After 5 days of incubation, no molds were observed on the agar plates (Table 3). Consequently, chlorine dioxide has proven itself to be a practical and effective method for decontaminating mold problems in large facilities with minimal work.

Acknowledgment

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References


List A: Antimicrobial products registered with the U.S. Environmental Protection Agency (EPA), Office of Pesticide Programs as sterilizers: CSI CD Cartridge, EPA registration number: 80802-1 (2009).

Sawyer M. Got gas? Chlorine dioxide or vaporized hydrogen peroxide: Which one is right for you? Presented at the Midwest Area Biosafety Network Annual Symposium; 2011 Aug 10; Ames, Iowa.