Efficacy of Oxonia Active Against Selected Spore Formers†

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ABSTRACT

Alternatives to hydrogen peroxide are being sought for use in aseptic packaging systems because this sterilant is efficacious at temperatures higher than some of the newer packaging materials can tolerate. Earlier in this century, peracetic acid was known to be bactericidal, sporicidal, and virucidal but was not widely used because of handling, toxicity, and stability problems. Sanitizer suppliers have capitalized on the efficacy of hydrogen peroxide, acetic acid, and peracetic acid stabilized with a sequestering agent. Formulations have been improved and marketed as Oxonia Active, and its use as an alternative sterilant to hydrogen peroxide merits evaluation. Oxonia was assessed at a concentration of 2% and a temperature of 40°C against a number of spore-forming organisms, including foodborne pathogens. Spores tested in aqueous suspension showed an order of sensitivity (least to greatest) to Oxonia as follows: Bacillus cereus > B. subtilis A > B. stearothermophilus > B. subtilis var. globigi > B. coagulans > Clostridium sporogenes (PA3679) > C. butyricum > C. botulinum type B (nonproteolytic) > C. botulinum type B (proteolytic) = C. botulinum type A = C. botulinum type E. B. subtilis A and B. stearothermophilus spores tested in the dry state were less sensitive to Oxonia than when tested in aqueous suspension. B. cereus, a foodborne pathogen, proved to be markedly less sensitive to Oxonia under the described test conditions. The decreased sensitivity to Oxonia by the foodborne pathogen B. cereus raises concern about the efficacy of the sterilant for aseptic packaging of low-acid foods. Further work will be needed to determine if this decreased sensitivity is an inherent property of the organism that affords unusual protection against Oxonia or if the challenge parameters selected were at the minimum conditions for efficacy.

Hydrogen peroxide (30 to 35%) is the only approved chemical sterilant for use in aseptic systems (21 Code of Federal Regulations (CFR) §178.1005). However, alternatives are being sought because hydrogen peroxide is efficacious at temperatures too high for some of the packaging materials in use today. Peracetic acid has been found to be sporicidal at low temperatures and in the presence of organic matter (4). Block (4) has summarized much of the work, noting inactivation of Bacillus spp. in 15 s to 15 min at concentrations between 0.05 to 3% at room temperature. Although the efficacy of peracetic acid has been long known, the chemical was not widely used because of handling, toxicity, and stability problems. Sanitizer suppliers have capitalized on the efficacy of hydrogen peroxide, acetic acid, and peracetic acid beginning in 1975 with commercialized product in Europe formulated with a stabilizer (6) and introduced into the United States in 1986. More recent formulations on the market are Oxonia Active (hereafter referred to as Oxonia), an equilibrium mixture of hydrogen peroxide, peracetic and acetic acids, and 1-hydroxyethylidene-1,1-diphosphonic acid, which is currently approved for use as a sanitizer under 21 CFR §178.1010, and a microbialic wash for fruits and vegetables, Tsunami 100, approved under 21 CFR §173.315. Information on Oxonia as a bactericidal agent is limited in the literature and most studies on sporicidal properties are proprietary, so this study was designed to assess the efficacy of 2% Oxonia as a sterilant against a number of spore formers.

METHODS AND MATERIALS

Culture sources. Tables 1 and 2 list the organisms used in the experiments, their National Food Processors Association (NFPA) collection number, and their source, if known. Spore suspensions were prepared according to procedures in the Food and Drug Administration (FDA) Bacteriological Analytical Manual (2). Exceptions were Clostridium butyricum that was grown anaerobically for 2 weeks at 30°C on strained pea agar (strained peas, 4.5 oz; calcium carbonate, 3 g; agar, 8 g; distilled water, 1,000 ml); B. cereus grown on a sporulation medium of 0.8% (per 1,000 ml) nutrient broth, yeast extract (0.4%), 0.1 ml of 3.8% (per 100 ml) manganese sulfate, and 2% agar; and B. subtilis A grown on beef extract (1%), proteose peptone (0.55%), glucose (0.01%), manganese sulfate (0.005%), iron sulfate (0.005%), and agar (2%). Suspensions of many crops were previously prepared and were held at 4°C. Crops made fresh were B. cereus and the following Clostridia: C. butyricum, OS1A, 62A, AZK3, AVL1, 4896A, NCA1B, 32B, and 73-211B. Stock suspensions were heat shocked before inoculation on sporulation media. After a heavy spore population was verified, the crops were harvested in sterile distilled water and washed twice by centrifuging the spores, pouring off the supernatant, and resuspending in water. Crops were stored under refrigeration and portions tempered before use.

Oxonia challenge testing. The 2% solution was prepared from concentrated Oxonia (Ecolab Inc., St. Paul, Minn.). The hy-
TABLE 1. Some test organisms used in the Oxonia experiments

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number (Source)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis A</td>
<td>N1002 (Busta, University of Minnesota)</td>
</tr>
<tr>
<td>B. subtilis var. globigii</td>
<td>N1018 (Toledo, University of Georgia)</td>
</tr>
<tr>
<td>B. cereus</td>
<td>N1009 (Vanilla ice cream)</td>
</tr>
<tr>
<td>B. cereus</td>
<td>N1012 (Unknown)</td>
</tr>
<tr>
<td>B. cereus</td>
<td>SC112 (Milk)</td>
</tr>
<tr>
<td>B. cereus</td>
<td>SC114 (ATCC 7065)</td>
</tr>
<tr>
<td>B. stearethermophilus</td>
<td>FS1518/N1088 (Unknown)</td>
</tr>
<tr>
<td>B. coagulans</td>
<td>43-P/N1062 (Unknown)</td>
</tr>
<tr>
<td>C. sporogenes</td>
<td>PA3679 (Unknown)</td>
</tr>
<tr>
<td>C. butyricum</td>
<td>87-409 (Blueberries)</td>
</tr>
</tbody>
</table>

drogen peroxide concentration was determined by titration with 0.1 N potassium permanganate and peracetic acid concentration by back titration with 0.1 N sodium thiosulfate in the presence of starch and potassium iodide according to the procedures supplied by Ecolab Inc. Working concentrations of hydrogen peroxide throughout the many experiments varied between 4,788 and 5,413 ppm (0.48 to 0.54%), while peracetic acid was between 973 and 1,096 ppm.

Portions of spore suspensions were pipetted into Erlenmeyer flasks containing 100 ml of Oxonia held in a Haake water bath at 40°C ± 0.1°C. Final concentration of spores in the Oxonia was between 8 x 10^6/ml and 2 x 10^7/ml. Additional spore testing was done at a final concentration between 8 x 10^7/ml and 2 x 10^8/ml. At designated time intervals, 1 ml of the resulting suspension was removed and pipetted immediately into a 9-ml neutralizing solution of 0.1% thiosulfate and 1% peptone in distilled water (autoclaved) to which was added daily, fresh, filter-sterilized catalase (2,000 U per tube) prepared in 0.05 M of monopotassium phosphate buffer adjusted to pH 7.0 with 1 N NaOH or 1 N HCl. After approximately 5 min, the neutralizing solution with spores was transferred in its entirety to 90 ml of recovery broth, either dextrose tryptone for Bacillus spp. or T-Best for Clostridium spp. The latter medium was reported (17) to be an excellent recovery broth for Clostridium spp. At least 2 replicate experiments of each species or strain were recorded.

All recovery broths were incubated at 30°C, except those used for B. stearethermophilus, which were incubated at 55°C. Cultures were incubated for up to 21 days to allow for outgrowth of injured spores. Growth was indicated by turbidity. Positive cultures were streaked to confirm the presence of rods.

Controls were conducted to ensure viability of each organism in the medium and efficacy of the neutralizing solution. One type of positive control consisted of the challenge concentration of the organism pipetted into the neutralizing medium and then transferred into the recovery broth before incubation. A second type of positive control consisted of addition of the challenge concentration of the organism to neutralized Oxonia (to simulate the experimental). The medium with neutralized Oxonia was then transferred to the recovery broth and incubated to ensure growth. The negative control for each experiment consisted of the recovery broth plus unincoculated neutralizing medium.

Spores of B. subtilis A, B. stearethermophilus, B. cereus, and C. sporogenes (PA3679) were inoculated onto Whatman no. 1 paper strips approximately 2.5 x 1 cm. Whatman no. 1 was used because it is highly porous, wicks easily, and has long been used by Becton Dickinson and Co., Paramus, N.J., as the carrier for biological indicators for ethylene oxide sterilizers. Final spore concentration was between 8 x 10^5/ml and 2 x 10^6/ml. After at least 1 h of drying time, the strips were spirally wound and placed in test tubes held in the water bath at 40°C. One milliliter of Oxonia warmed to 40°C was added by pipet to each tube, and neutralizer was added at appropriate intervals to stop the reaction. Experimental samples were then handled as described previously. Paper substrate in neutralizing solution was transferred in its entirety.

In one method, sterile 0.1% Tween 80 (a surfactant) was added to the Oxonia before spore inoculation into the Erlenmeyer flask and before challenge of spores dried onto Whatman no. 1 paper strips. It was anticipated that Tween might keep the spores evenly distributed in suspension, thereby preventing clumping.

In a second method, the spore crop, harvested in sterile water, was diluted for wet state testing such that the final concentration of spores in Oxonia was between 8 x 10^3/ml and 2 x 10^5/ml. This approach was to address the possibility that higher concentrations, but not lower concentrations, of spores aggregated. In a third method, spores at a concentration between 8 x 10^5/ml and 2 x 10^6/ml were inoculated onto 0.45-μm microporous filters and tested immediately in Oxonia. This approach was taken to limit clumping.

**Hydrogen peroxide testing.** Food-grade hydrogen peroxide standardized to 35% was supplied by Ecolab Inc. Wet spores of B. cereus organisms were challenged against 35% hydrogen peroxide in a method similar to Oxonia and at temperatures of 40°C and 85°C. The neutralizer consisted of 1% peptone (no thiosulfate) and 12,000 U of catalase per 1 ml of hydrogen peroxide (0.012 M). The neutralizer was dispensed into 8-in tall tubes to allow for the anticipated reaction of hydrogen peroxide and catalase.

A control was prepared to verify that the catalase was neutralizing the peroxide such that growth of B. cereus could occur. An amount of B. cereus equal to that used in the experiment was added to neutralizer. One milliliter of hydrogen peroxide was then added to the neutralizer. After the reaction subsided, the mixture was transferred to recovery broth and incubated at 30°C. The organism grew out overnight.

**Morphological examination.** Spores of B. cereus and B. subtilis A were stained by Ashby’s spore stain method as detailed

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TABLE 2. A listing of composite suspensions of C. botulinum used in the Oxonia experiments

<table>
<thead>
<tr>
<th>Organism</th>
<th>Composite suspensions (Source)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. botulinum type A</td>
<td>62 A Soil</td>
</tr>
<tr>
<td></td>
<td>AZK3 Smelt</td>
</tr>
<tr>
<td></td>
<td>AVL1 Homemade ham</td>
</tr>
<tr>
<td></td>
<td>4896A Unknown</td>
</tr>
<tr>
<td></td>
<td>OS1A Onions</td>
</tr>
<tr>
<td>C. botulinum type B</td>
<td>NCA1B Asparagus</td>
</tr>
<tr>
<td>(proteolytic)</td>
<td>32B Soil</td>
</tr>
<tr>
<td></td>
<td>73-21B Mushroom brine</td>
</tr>
<tr>
<td></td>
<td>213B Unknown</td>
</tr>
<tr>
<td></td>
<td>RH19 CDC, Atlanta, Ga</td>
</tr>
<tr>
<td>C. botulinum type B</td>
<td>17-B Unknown</td>
</tr>
<tr>
<td>(nonproteolytic)</td>
<td>Kapchunka</td>
</tr>
<tr>
<td></td>
<td>Kapchunka, FDA</td>
</tr>
<tr>
<td></td>
<td>2129B Unknown</td>
</tr>
<tr>
<td>C. botulinum type E</td>
<td>Alaska Stomach contents</td>
</tr>
<tr>
<td></td>
<td>Saratoga Herring and rice cakes</td>
</tr>
<tr>
<td></td>
<td>Whitefish Smoked whitefish</td>
</tr>
</tbody>
</table>
TABLE 3. Summary of wet spore trials using $10^6$ spores per ml of Oxonia

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Strain</th>
<th>End point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus</td>
<td>cereus</td>
<td>N1009</td>
<td>$X &gt; 720$ s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N1009</td>
<td>$30 s &lt; X &lt; 45$ s</td>
</tr>
<tr>
<td></td>
<td>coagulans</td>
<td>—</td>
<td>$60 s &lt; X &lt; 90$ s</td>
</tr>
<tr>
<td></td>
<td>subtilis</td>
<td>A</td>
<td>$150 s &lt; X &lt; 240$ s</td>
</tr>
<tr>
<td></td>
<td>subtilis var. globigii</td>
<td>—</td>
<td>$90 s &lt; X &lt; 120$ s</td>
</tr>
<tr>
<td></td>
<td>stearothermophilus</td>
<td>—</td>
<td>$90 s &lt; X &lt; 150$ s</td>
</tr>
<tr>
<td>Clostridium</td>
<td>botulinum type A composite</td>
<td>62A</td>
<td>$X &lt; 5$ s</td>
</tr>
<tr>
<td></td>
<td>botulinum</td>
<td>AZK3</td>
<td>$X &lt; 5$ s</td>
</tr>
<tr>
<td></td>
<td>botulinum type B (proteolytic)</td>
<td>AVL1</td>
<td>$X &lt; 5$ s</td>
</tr>
<tr>
<td></td>
<td>botulinum type B (nonproteolytic)</td>
<td>OS1A</td>
<td>$X &lt; 5$ s</td>
</tr>
<tr>
<td></td>
<td>butyricum</td>
<td>—</td>
<td>$5 s &lt; X &lt; 15$ s</td>
</tr>
<tr>
<td></td>
<td>sporogenes (PA3679)</td>
<td>—</td>
<td>$15 s &lt; X &lt; 20$ s</td>
</tr>
</tbody>
</table>

X is the time in seconds (s) to reach end point (no survivors) with 2% Oxonia at 40°C.

RESULTS

Oxonia challenge testing. Table 3 presents the results of the trials using spores inoculated into Oxonia at a final concentration of $10^6$/ml. Bacillus spp. were less sensitive to Oxonia (survival time $> 60$ s) than Clostridium spp. (survival time $< 20$ s). C. sporogenes (PA3679) was consistently the least sensitive to Oxonia among the clostridia tested; although in one test OS1A did show sensitivity equal to PA 3679.

The order of sensitivity (least to greatest) was: B. cereus $> B. subtilis$ A $> B. stearothermophilus$ $> B. subtilis$ var. globigii $> B. coagulans$ $> C. sporogenes$ (PA3679) $> C. butyricum$ $> C. botulinum$ type B (non proteolytic) $> C. botulinum$ type B (proteolytic) $= C. botulinum$ type A $= C. botulinum$ type E.

B. cereus was markedly less sensitive to Oxonia. The principal organism (N1009) was tested seven times at increasing end point times. The parameters of the study were such that the end point of the organism in Oxonia could not be determined. Table 3 notes only the final trial, which resulted in an end point beyond 12 min (720 s). In one trial the organism did respond to Oxonia treatment with an end point (no surviving organisms) less than 45 s; the reason for this is unknown. Challenges on N1012 and strains SC112 and SC114 consistently demonstrated the marked lack of sensitivity of this organism. Results of duplicate testing on these strains showed resistance to be consistently in excess of 5 min under the conditions tested.

B. cereus spores tested at a concentration of $10^2$/ml showed sensitivity to Oxonia similar to that at the higher spore concentration, with an end point in excess of 4 min. Testing was done in duplicate.

Three different species of Bacillus were tested after air drying onto Whatman no. 1 paper strips. Results are summarized in Table 4. Triplicate challenges of B. subtilis A showed lack of sensitivity to Oxonia to be in excess of 10 min. Four challenge tests with B. stearothermophilus showed varying sensitivities, ranging from 1 min to less than 2½ min. B. cereus showed resistance in excess of 10 min in triplicate experiments.

PA3679 was tested dry. In one experiment, resistance was between 3 and 4 min. In a second experiment, resistance exceeded 5 min.

In order to determine whether the low sensitivity of B. cereus was an artifact due to clumping, various approaches to test this were tried. The addition of Tween 80 did not facilitate the reduction of end point time for B. cereus. Although only one test each was conducted on wet and dry state spores, resistance was in excess of 5 min for wet spores and 60 min for spores dried onto Whatman no. 1 paper strips.

Dilution of B. cereus spores such that the final concentration in Oxonia was $10^2$/ml did not augment kill rate.

FIGURE 1. (A) Spores of B. cereus from the stock suspension evenly dispersed in water. (B) B. cereus after reaction at room temperature with 2% Oxonia. Magnification is $\times1,000$ (no oil).
TABLE 4. Summary of dry state spore trials using 10^6 spores

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Strain</th>
<th>End point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus</td>
<td>stearothermophilus</td>
<td>1 m &lt; X &lt; 2.5 m</td>
<td></td>
</tr>
<tr>
<td></td>
<td>subtilis</td>
<td>A</td>
<td>X &gt; 10 m</td>
</tr>
<tr>
<td></td>
<td>cereus</td>
<td>X &gt; 10 m</td>
<td></td>
</tr>
<tr>
<td>Clostridium</td>
<td>sporogenes (PA3679)</td>
<td>3 m &lt; X &lt; 4 m</td>
<td>X &gt; 5 m</td>
</tr>
</tbody>
</table>

a X is the time in minutes (m) to reach end point (no survivors) with 2% Oxonia at 40°C.

b Individual experiments are listed for PA3679.

Resistance time was found in triplicate experiments to be in excess of 4 min. B. cereus spores inoculated onto 0.45-

μm membranes, such that the final concentration was 10^2 spores per membrane, maintained low sensitivity to Oxonia
despite the dilution. In duplicate experiments, the end point exceeded 5 min. The use of membranes did not enhance
Oxonia efficacy.

**Hydrogen peroxide (35%) challenge testing.** Because there are no published data on the efficacy of hydrogen
peroxide alone against wet state spores of B. cereus, some testing was undertaken to compare it with Oxonia.
End point times from two experiments at 40°C varied: 60

s < X < 2 min (1) and 15 s < X < 30 s (2). (Numbers in
parentheses indicate the number of replicate experiments.)
A positive result occurred later at 1.5 min in one experiment.
No other positive results were noted during the
testing time, which lasted 5 min. The positive result could have been due to clumping of the spores.
Two experiments were conducted at 85°C. In the first, no samples were taken before 30 s of exposure to hydrogen
peroxide. Thus, when no outgrowth of B. cereus occurred
after exposure to hydrogen peroxide for times ranging be-
tween 30 s and 15 min, a second test was run with samples
taken every 5 s up to 30 s. No outgrowth was noted in any
of the samples.

**Morphological examination.** An inoculating loopful of B. cereus from the stock suspension added to a droplet of
2% Oxonia on a glass slide yielded some interesting visual results. Clumping was quite obvious to the unaided
eye in the presence of the chemical. Under the light micro-
scope, Oxonia-treated B. cereus spores were seen in pairs and in groups, including at least 1 large clump (Fig. 1A
and 1B). B. subtilis A did not react similarly. Spores re-
mained evenly dispersed. The clumping effect was not not-
eted with B. cereus in the presence of 35% hydrogen per-
oxide; some clumping was seen with peracetic acid alone
(1,000 ppm), although not as strongly as with Oxonia. Fur-
ther study of this phenomenon was not made.

**DISCUSSION**

In this study, aqueous suspensions of spores proved to be more sensitive to chemical treatment than those tested
dry. Dried B. subtilis A survived for more than 10 min in
Oxonia versus a wet end point time of 150 to 240 s. C.
sporogenes had a dry end point time of greater than 3 min
and a wet state time between 15 and 20 s. B. stearother-
mophilus showed similar end point times in wet versus dry
testing. End point time for B. cereus was not determined,
either wet or dry, because of the longer sterilant residence
time required for this organism against Oxonia. The liter-
ature does not present unequivocal answers to the question
of wet versus dry state resistance of spores to chemicals.
Bacterial spores are known to be less sensitive to dry heat
than wet heat (15). Ito et al. (9) found C. botulinum to be
slightly less sensitive in the dry state when the organism
was tested against 20% hydrogen peroxide. Portner and
Hoffman (13) found when peracetic vapor was used to kill
B. subtilis var. niger spores that sporicidal activity was op-
timum at 80% relative humidity and lower at 20%. Leaper
(11) found wet and dry state resistance of B. subtilis SA22
to 29.5 and 35.4% hydrogen peroxide at 30°C to be similar,
but at 40°C the dry spores were at least twice as sensitive
as wet spores. Her data substantiate the suggestion of Smith
and Brown (15) that, under certain conditions, dry spores
may be less sensitive to hydrogen peroxide than wet spores.

All the Bacillus spp. (in the wet state) proved to be
less sensitive to Oxonia as compared with Clostridium spp.
This pattern matches that seen in the work of Mottishaw
et al. (12) in which various concentrations of hydrogen per-
oxide plus peracetic acid were used to challenge B. subtilis
SA22 and C. sporogenes. Even though the peracetic acid
concentration was higher for B. subtilis SA22 versus C.
sporogenes, B. subtilis was less sensitive. In the present
study, destruction of 10^6 wet spores of the bacilli was not
achieved quickly at 40°C under the test conditions desirable
for use in an aseptic system. Particularly problematic was
the resistance of the foodborne pathogen B. cereus. The
effect of Oxonia on this organism did not appear to be
related to cell concentration or aided by the addition of
TWEEN. To eliminate the possibility of clumping, micropor-
ous filters were used to separate individual spores during
challenge tests and 0.1% Tween 80 was added to Oxonia
during testing. Unfortunately, neither of these approaches
proved effective. Giffel et al. (8) state that sporicidal effect
of Oxonia against B. cereus was affected considerably by
the sporulation medium. For these experiments, B. cereus
was grown on only one medium. The organism was not
tested against peracetic acid alone, which reportedly inac-
vitates 10^6 to 10^7 spores of B. cereus (5). Because B. cereus
was so markedly less sensitive to Oxonia, the organism was
tested against 35% hydrogen peroxide. The longest time to
kill 10^6 organisms at 40°C was between 1 and 2 min. At
85°C (a commonly used temperature for aseptic sterilization
using hydrogen peroxide), survival time was less than 5 s.
B. subtilis A is less sensitive to hydrogen peroxide than B.
cereus (NFPA unpublished data). The contrast between Ox-
onia and hydrogen peroxide is intriguing. Key to the con-
trast, presumably, is the presence of peracetic acid in Ox-
onia, which causes changes to B. cereus that result in or-
ganism aggregation, as noted under the light microscope.
The aggregation may be the result of the highly hydropho-
bic nature of B. cereus (7, 14).

Information was limited on B. cereus, possibly because
much of the historical data on sterilants for aseptic pack-
aging systems were collected when the organism was emerging as a pathogen. According to Johnson (10), not until 1950 was sufficient information gathered by Steinar Hauge in Norway to prove its pathogenesis. In the United States, the first well-characterized outbreak of foodborne illness due to *B. cereus* occurred in 1969. Although a large number of cases were involved in outbreaks in 1978, *B. cereus* is relatively uncommon in the United States, although common in England and Hungary. In the 5-year period between 1988 and 1992, the Centers for Disease Control and Prevention (CDC) reported 21 outbreaks in the United States (3). This number represented 0.9% of the diagnosed outbreaks for which the etiologic agent was confirmed. In contrast, between 1972 and 1978 only 13 outbreaks were reported (10). According to Johnson, the increase in outbreaks can be attributed to increased surveillance by the CDC because the FDA by the early 80s considered the organism to be an emerging pathogen. As for the significance of *B. cereus* in packaging, Finnish researchers (16) have noted this organism to be common in paperboard and of possible significance if the board is used in milk packaging.

Because of the resistance of the pathogen *B. cereus*, the present work brings into issue the utility of Oxonia as a sterilant for aseptic systems packaging low-acid foods that require low-temperature sterilization of packaging materials. Temperature conditions selected for this study were admittedly low, as was the percentage of sterilant and the number of challenge organisms high. In commercial practice, Oxonia concentration may be as high as 4% and the temperature as high as 60°C. Further work will be needed to determine if this resistance is a result of hydrophobicity that affords unusual protection against Oxonia or if the challenge parameters selected were near the minimum conditions for efficacy.

**ACKNOWLEDGMENTS**

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