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Sporicidal Effect of Peracetic Acid Vapor

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The sporicidal activity of peracetic acid (PAA) vapor at 20, 40, 60, and 80% relative humidity (RH) and 25 C was determined on *Bacillus subtilis* var. *niger* spores on paper and glass surfaces. Appreciable activity occurred within 10 min of exposure to 1 mg of PAA per liter and 40% or higher RH. The sporicidal rate decreased from the optimum at 80% RH to a slight effect at 20% RH. Spores on an impermeable surface were more difficult to kill than those on a porous one, probably because the cells tend to pile up on an impermeable surface and the vapor penetrates poorly through the layer of covering cells.

Numerous studies (3-5, 7, 8, 10) have shown that peracetic acid (PAA) in aqueous solution is an effective germicide against a wide spectrum of microorganisms. On the other hand, information about the vapor-phase bactericidal activity of PAA is very limited and seems to be confined to a brief study by Greenspan et al. (4).

The corrosiveness of PAA has limited its practical application as a disinfectant. However, because PAA itself is not adsorbed onto surfaces and its decomposition products (acetic acid, water, and oxygen) are nontoxic and free-rinsing, PAA has found wide application as a surface decontaminant for foods (5, 6, 9). Spraying dilute solutions of PAA until a fog forms has been an effective means of sterilizing equipment used in gnotobiotic studies (1, 11).

The accumulation of liquid PAA and moisture produced from spraying a dense fog may be undesirable because of corrosion or other damaging effects. Therefore, it is possible that PAA vapor may be used instead of the fog. Because of the extreme reactiveness and considerable volatility of PAA, the study reported here was undertaken to determine the effectiveness of PAA vapor against bacterial spores, on porous and impermeable surfaces, at various relative humidities (RH).

MATERIALS AND METHODS

Preparation of test samples. An aqueous stock spore suspension of *Bacillus subtilis* var. *niger* was used to contaminate filter-paper discs [5/8 inch (1.58 cm) in diameter] and glass squares [0.5 by 0.5 inch (1.27 by 1.27 cm)]. The contaminated samples were conditioned for 4 days in desiccators maintained at 25 C and at constant RH of 80, 60, and 40% by saturated salt solutions of ammonium sulfate, sodium bromide, and chromic acid, respectively. Samples were conditioned at 20% RH in the laboratory during the winter months when the ambient RH was constantly low as indicated by a hygrothermograph.

Exposure to PAA. The 86-liter test chamber shown in Fig. 1 was fabricated for vapor-phase decontamination studies. Prior to spraying PAA, the RH within the test chamber was adjusted to the same RH used to precondition the samples, either by spraying water into the chamber to raise the RH or by blowing dry air in to lower it. No adjustment was needed for tests conducted at 20% RH because the RH in the test chamber and the laboratory was the same. The RH within the test chamber was monitored with a humidity-sensing element (El-tronics, Inc., Warren, Pa.).

The commercial grade of PAA solution is composed of approximately 40% peracetic acid, 5% hydrogen peroxide, 39% acetic acid, 1% sulfuric acid, and 15% water, w/w. A direct spray UCTL atomizer (2) was used to spray 0.5 ml of the undiluted solution into the test chamber. To allow time for the vapor concentration to stabilize, the test samples were not exposed to the vapor until 30 min after spraying. (Note the rapid initial drop in concentration in Fig. 2.) Furthermore, the fan in the test chamber was run throughout the test period to insure uniform distribution of the PAA vapor. For each exposure period, 16 contaminated samples (8 paper, mounted on pins; 8 glass, laid on wire screen) were placed on an aluminum tray then quickly inserted into the middle of the chamber (Fig. 1). All samples were exposed to PAA vapor at 25 ± 2 C and the same RH that was used for conditioning them. Individual exposure periods ranged from 75 sec to 80 min.

PAA concentration. The concentration of PAA in the chamber was determined at periodic intervals during each exposure period by drawing 500 ml of the chamber air through 20 ml of 20% buffered potassium iodide. The optical density was measured at 500 nm with a spectrophotometer, and the concentration was read from a standard curve prepared by plotting optical densities against known weights of PAA. This method is specific for PAA.

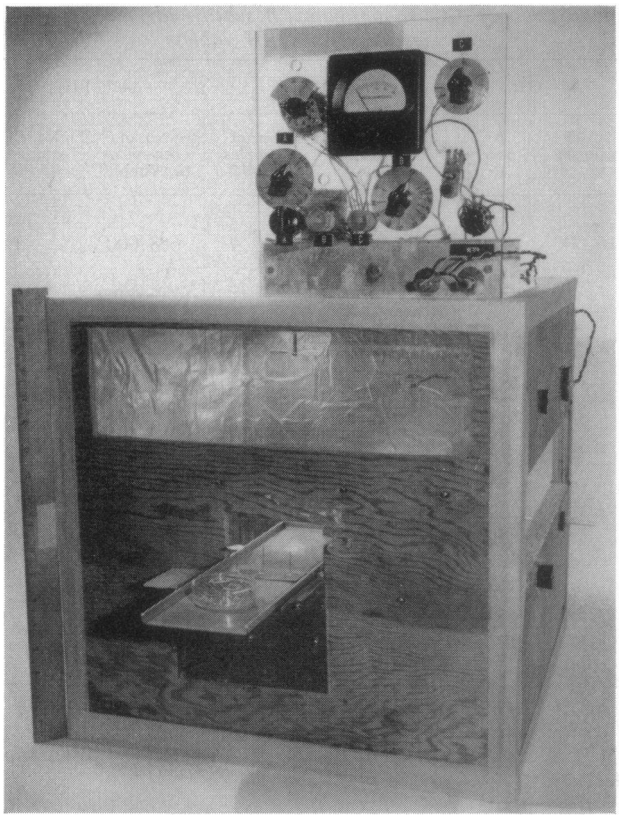

FIG. 1. *Test chamber used for vapor-phase decontamination studies.*

Method of asssy. After exposure to PAA vapor, the samples were transferred to sterile, stoppered plastic tubes containing 10 ml of 0.01% sodium thiosulfate solution, to neutralize excess PAA, with about 0.5 g of sand, to aid in removal of spores; the samples were then shaken vigorously. To insure that an estimate of the number of organisms could be obtained for each exposure period regardless of population size, duplicate 2.5- and 1-ml portions from each tube were plated directly and the remainder was used for serial dilutions. Pour plates were prepared with Trypticase soy agar and were counted after 72 hr of incubation at 32 C. At each exposure period, conditioned samples not exposed to PAA vapor were also assayed in the manner described above. The solution of sodium thiosulfate used in these tests had no observable effect upon the viability of the spores.

The precision of counting a small population is increased by plating a large portion of the sample. One can also be confident that sterility is being attained when no colonies appear on any of the plates prepared from over 70% of the suspending fluid of the sample.

RESULTS AND DISCUSSION

The results are summarized in Table 1. Because an exceptionally high value occasionally appeared among the individual sample counts for given conditions, which were otherwise of the same order of magnitude, the data are expressed by geometric rather than arithmetic means to represent more truly the relationships among the samples. For many of the test samples assayed per exposure period, no organisms were recovered from the part (70%) of the sample that was plated. The frequency of this occurrence is also given in Table 1 as another indication of vapor activity.

Most of the spore population was killed with PAA vapor within a few minutes at 40% or higher RH. At 20%, only a slight reduction occurred on paper and none occurred on glass after 80 min of exposure.

The apparently faster sporicidal action on paper than on glass is probably the result of distribution. The spores in a drop of water spread over a large surface area when placed on a porous material; thus, they are not as likely to pile up on one another as the liquid evaporates. When a similar drop of suspension begins to dry on an impermeable surface, however, there is a tendency for the organisms to collect and pile up

TABLE 1. *Effectiveness of PAA vapor against B. subtilis var. niger spores on paper and glass at various RH values*[a]

Exposure to PAA (min)	80% RH		60% RH		40% RH		20% RH	
	No. of organisms per sample[b]	No. of samples sterile[c]	No. of organisms per samples[b]	No. of samples sterile[c]	No. of organisms per sample[b]	No. of samples sterile[c]	No. of organisms per samples[b]	No. of samples sterile[c]
Paper surface								
0[d]	816,000		831,000		658,000		275,000	
1.25	676	0	—[e]	—	—	—	—	—
2.5	1	5	1,390	0	—	—	—	—
5	<1	12	14	2	7,270	0	—	—
10	<1	14	2	7	24	2	171,000	0
20	0	16	1	8	7	1	117,000	0
40	—	—	<1	12	<1	8	129,000	0
80	—	—	—	—	<1	13	26,000	0
Glass surface								
0[d]	813,000		971,000		709,000		290,000	
1.25	5	7	—	—	—	—	—	—
2.5	2	7	216	1	—	—	—	—
5	<1	13	88	2	33,600	0	—	—
10	<1	13	38	0	1,530	0	218,000	0
20	<1	10	4	6	2,330	0	153,000	0
40	—	—	2	7	623	0	327,000	0
80	—	—	—	—	143	0	267,000	0

[a] Approximately 1 mg of PAA/liter.
[b] Each entry is the geometric mean of 16 samples, except at 20% RH which is based on 8 samples.
[c] Based on plating 70% of each sample. With 80, 60, and 40% RH, 16 test samples were assayed; with 20% RH, 8 test samples were assayed.
[d] Control, no exposure to PAA.
[e] Not tested.

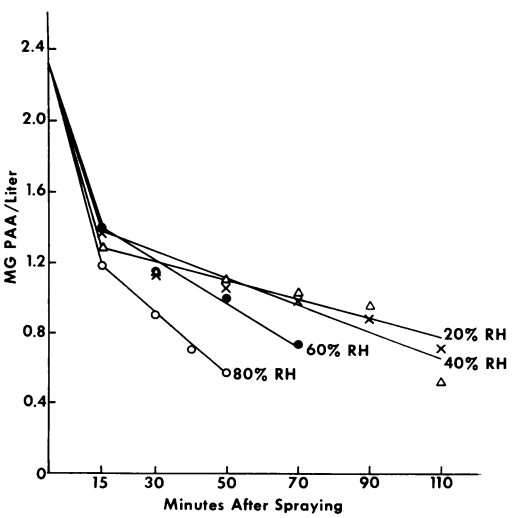

FIG. 2. *Concentration of PAA vapor in test chamber as a function of time and RH.*

at the periphery of the drop. Such a pileup of cells provides protection for the spores underneath from the lethal action of a poorly penetrating vapor like PAA.

The data for the activity of PAA against spores on glass show a rapid initial death rate, then a decrease and leveling off of the curve. The contaminated surfaces were not inserted into the chamber until 30 min after spraying the PAA; by this time, the initial rapid concentration drop (Fig. 2) had taken place and the period of gradual decrease with time had begun. The gradual concentration drop is not sufficient, however, to account for the slower rate of killing with time. Greenspan et al. (4) concluded that PAA vapor-phase sterilization is only dependable with clean surfaces. Trexler and Reynolds (11) believed that dirt was the cause for their difficulty in sterilizing animal cages with a fog of PAA. The surfaces used in this study were clean, but the high concentration of spores on the small sample area of the impermeable surface may have had an effect similar to that of dirt. The outer layer of spores killed during the initial exposure may physically protect the remaining viable spores. Because PAA vapor apparently does not penetrate well, its power as a germicide seems to be limited to exposed microorganisms.

The results of this study indicate that, at RH values between 40 and 80%, PAA vapor can

appreciably reduce spore contamination on both porous and impermeable surfaces within 10 min. However, the optimal sporicidal activity occurs at 80% RH, and no appreciable activity occurs at a low (20%) RH. The level of microbial contamination and the cleanness of the surface, as well as the RH, are undoubtedly factors that determine whether sterility is achieved by treatment with PAA vapor.

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