Microbiological Aspects of Ethylene Oxide Sterilization

IV. Influence of Thickness of Polyethylene Film on the Sporicidal Activity of Ethylene Oxide

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Spores of *Bacillus subtilis* var. *niger*, dried on nonhygroscopic and hygroscopic surfaces, were enclosed in one of four thicknesses of low-density polyethylene film (2, 4, 6, and 20 mils). The surfaces were then placed in a specially designed thermochemical death rate apparatus and exposed to an ethylene oxide concentration of 600 mg/liter (at 54.4 °C) and 50% relative humidity. Survival data, including both spore survivor curves and decimal reduction values (expressed as D values at 54.4 °C-600 mg of ethylene oxide per liter), demonstrated similar survivor patterns when the *B. subtilis* var. *niger* spores were enclosed in low-density polyethylene films 2, 4, and 6 mils thick. A comparison of these patterns with those of spores enclosed in glassine and subjected to identical exposure conditions revealed only slight variations. The use of 20-mil polyethylene film greatly increased the time required to kill *B. subtilis* var. *niger* spores under the exposure conditions.

Items to be sterilized by ethylene oxide are usually wrapped or packaged to maintain sterility after sterilization. It is important, however, that the material selected meet a major requirement of being permeable to ethylene oxide and moisture (7).

Plastic films are widely used for packaging such items as foods, hardware, clothing, toys, and many disposable medical and surgical supplies (3, 10, 12). Many manufacturers of these films have tested them for gas permeability and moisture transmission. However, very few manufacturers test the films with ethylene oxide and, for this reason, it is difficult to learn which films are satisfactory for gas sterilization. Only limited information has been published on the permeability of the more commonly used plastic films to ethylene oxide, moisture, and to other gases (2, 8, 9, 11).

Of the commercially available plastic films for wrapping or packaging items to be gas sterilized, polyethylene and (more recently) nylon are the most widely used (10, 12). Polyethylene meets almost all requirements for an ideal wrapper and is especially desirable because of its transparency. There are three density grades of polyethylene film: low, medium, and high. For gaseous sterilization, the low density, 1 to 4 mils (0.001 to 0.004 inches) thick grade is recommended because of its higher permeability to ethylene oxide and moisture (1).

Because of the increased usage of plastic films for packaging articles to be gas sterilized, we present this report of spore-inoculated carriers enclosed in polyethylene film of various thicknesses and exposed to ethylene oxide.

**MATERIALS AND METHODS**

**Spore preparation and carriers.** *Bacillus subtilis* var. *niger* spores dried on hygroscopic and nonhygroscopic carriers were used. Preparation of the spore suspension and inoculated carriers was described in the preceding papers (4-6).

**Polyethylene film.** Four thicknesses of polyethylene film, 2, 4, 6, and 20 mils (Zendel Polyethylene Film, Union Carbide Corp., Cartersville, Ga.), were used. Two and 1.27-cm squares of the plastic film were cut and folded to form envelopes. These were heat sealed on three sides. The envelopes were placed in open glass jars and sterilized with ethylene oxide. After sterilization, the glass jars were loosely capped and the plastic envelopes were allowed to degas for at least 48 hr. The spore-inoculated carriers were then aseptically transferred to the sterile plastic envelopes and the envelope openings were heat sealed.

**Ethylene oxide exposure.** The thermochemical death rate apparatus and exposure procedures previously described (4) were employed. The exposure conditions were as follows: ethylene oxide concentration, 600 mg/liter; relative humidity, 50%; temperature, 54.4 ± 3 °C. The exposure periods varied.

**Recovery and enumeration of survivors.** The recovery and plating procedures were described in previous publications (4, 5).
apoehygroscopic and polyethylene film 2, 4, and 6 mils thick. Symbols: X, 2 mils; □, 4 mils; ○, 6 mils.

RESULTS AND DISCUSSION

When B. subtilis var. niger spores, dried on nonhygroscopic and hygroscopic surfaces, were enclosed in polyethylene films 2, 4, and 6 mils thick and were exposed to the aforementioned conditions, the spore survival patterns (Fig. 1) varied little from one another. This was also reflected in the decimal reduction values (expressed as D at 54.4°C-600 mg of ethylene oxide per liter) shown in Table 1.

If one compares the survival patterns of B. subtilis var. niger spores dried on the same surface types but enclosed in glassine envelopes and exposed to the same conditions (6), only slight variations will be noted. This supports the findings (1) that the low-density grade film does not appreciably affect the diffusion or sporicidal effect of ethylene oxide. The recommendations of other authors (1, 2) to use polyethylene film, approximately 1 to 4 mils thick, are readily appreciated when one notes the extended survival times (Fig. 2) and higher decimal reduction values for B. subtilis var. niger spores sterilized in 20-mil polyethylene film.

This investigation confirmed the permeability of polyethylene film to ethylene oxide, and no radical alteration in the sterilizing ability of the gas as a result of the plastic film barrier was found. This was demonstrated by similar survival patterns for B. subtilis var. niger spores with and without (6) the film enclosure. However, if a thick polyethylene film (20 mils) is used, its thickness must be compensated for by a longer exposure period to allow complete diffusion of the sterilant.

Information concerning the suitability of a packaging material for gas sterilization (whether it is permeable to ethylene oxide and moisture

Fig. 1. Resistance of B. subtilis var. niger spores to ethylene oxide when dried on nonhygroscopic (a) and hygroscopic (b) surfaces and enclosed in polyethylene film 2, 4, and 6 mils thick. Symbols: X, 2 mils; □, 4 mils; ○, 6 mils.

Fig. 2. Resistance of B. subtilis var. niger spores to ethylene oxide when dried on nonhygroscopic (a) and hygroscopic (b) surfaces and enclosed in polyethylene film 20 mils thick.

Table 1. Decimal reduction values of B. subtilis var. niger spores dried on nonhygroscopic and hygroscopic surfaces enclosed in various thicknesses of polyethylene film.

<table>
<thead>
<tr>
<th>Thickness of polyethylene film</th>
<th>Nonhygroscopic surface</th>
<th>Hygroscopic surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mils</td>
<td>3.75</td>
<td>2.15</td>
</tr>
<tr>
<td>2</td>
<td>3.00</td>
<td>3.40</td>
</tr>
<tr>
<td>4</td>
<td>3.30</td>
<td>3.45</td>
</tr>
<tr>
<td>6</td>
<td>3.10</td>
<td>3.84</td>
</tr>
<tr>
<td>20</td>
<td>15.00</td>
<td>13.10</td>
</tr>
</tbody>
</table>

* Values are expressed as D at 54.4°C-600 mg of ethylene oxide per liter.

Glassine envelope.
and whether it can be employed as a wrapping material) should be sought from the manufacturer. Such information should be readily available from all manufacturers of packaging materials for sterilization applications.

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LITERATURE CITED