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Propylene Oxide Sterilization of Media Containing Bacterial Populations[1]

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Abstract. Propylene oxide sterilization of media containing soil bacterial populations occurred after 11 hours exposure in a closed system. Population reaction to the sterilant was characterized by inhibition intervals in the sterilization process. These stages represent minimal survival rates of 70%, 40%, and 7% respectively. A sterilization threshold (less than 1% survivors) occurred between 8-10 hours. Homogenous populations of *Bacillus subtilis* showed survival values in the first inhibition interval until the 4th hour. Lethality was noted at the 5th hour of exposure and other stages observed in heterogeneous populations did not occur.

Previous work has demonstrated that sterilization of microbiological media by exposure to propylene oxide occurs within 24 hours at ambient conditions and that the nutritive value of the media is not altered (1,4,6,7,8). The present study was undertaken to obtain information on population characteristics within a heterogeneous population versus a homogenous population (*Bacillus subtilis*) when exposed to propylene oxide in a closed system.

PROCEDURE

Heterogeneous populations of microorganisms were obtained by serial dilution (10^5) of soil samples. Nutrient agar was poured into sterile petri plates which had been previously inoculated with one ml aliquots. *B. subtilis* was used in parallel for a study of a homogeneous population of spore-forming bacteria. Twenty-four hour nutrient broth stock cultures were serially diluted 10^4 and 1 ml was used as the inoculum.

After inoculation, the plates were placed in a closed system consisting of an inverted four-liter battery jar with 6 ml of propylene oxide in a 25 x 70 mm glass vial. Glass plates were used for bases and the system made air-tight by coating the jar lip with petroleum jelly.

All test series were conducted at ambient conditions. The inoculated plates were exposed to propylene oxide for 1-6 hours (*B. subtilis*) and 1-11 hours (soil population). Twenty plates were used for each treatment and colony counts made at 24, 48, and 72 hour intervals. Chamber controls consisted of a series of twenty plates each. Controls were terminated with the corresponding treatment interval.

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Discussion

Analysis of surviving fractions in the heterogenous population shows a straight line correlation (slope: 0.345) until the fourth hour. A straight line relationship (slope: 2.864) is reestablished between the fifth and ninth hours, with sterilization occurring at the eleven hour interval (Figure 1). This data is in opposition with that of Goss and Marr (1) which indicates a straight line relation from the second to the sixth hours on plates exposed to air contaminants.

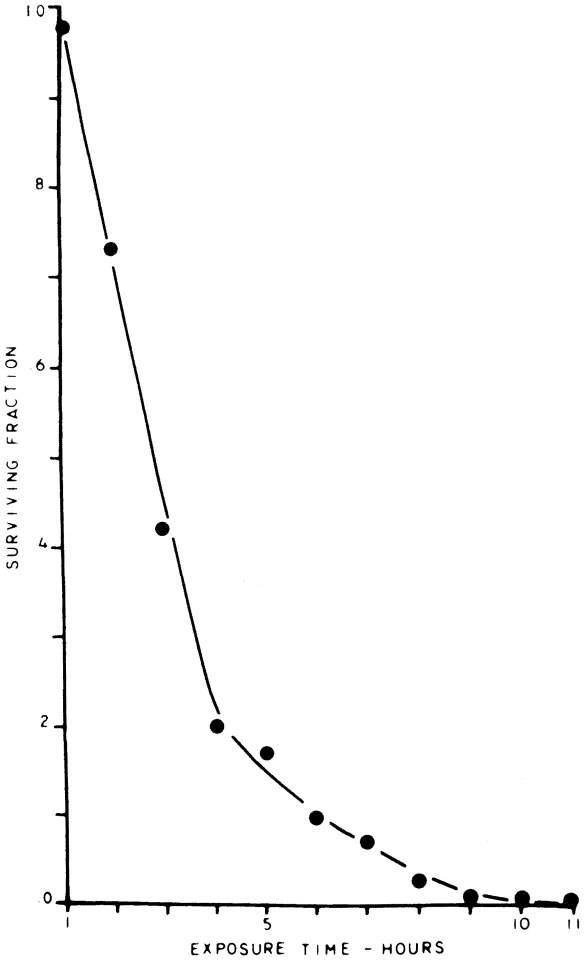

Figure 1. Surviving fractions of heterogeneous populations of soil bacteria exposed to propylene oxide in closed systems.

Step phenomena implies the existance of an underlying population survival threshold. Plotting the treatment as a function of the control was used to compensate for population variables. Intervals which appear to represent stages where new population factors become operative may be delineated on an argument whose value remains constant under the conditions of the experiment (Figure 2).

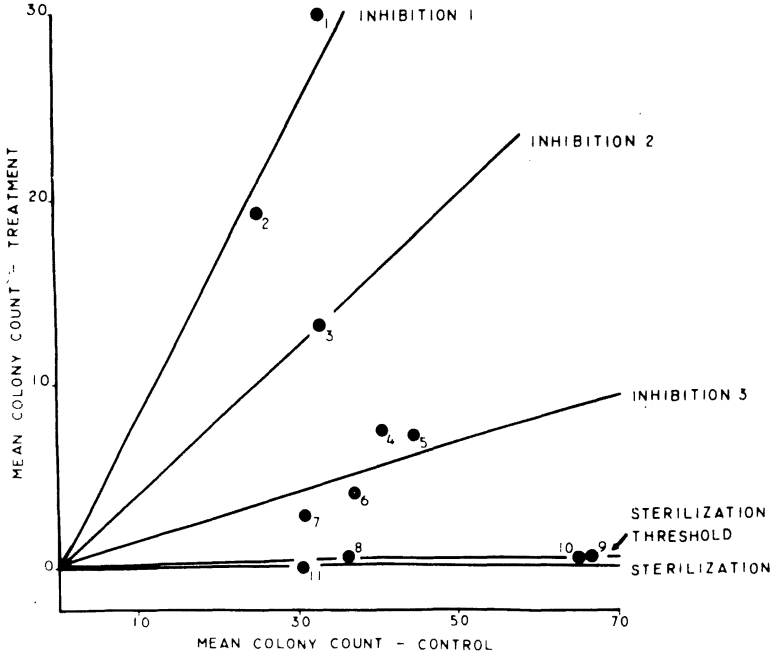

Figure 2. Step phenomena occurring in the sterilization process when populations of soil bacteria are exposed to propylene oxide in closed systems.

Data from the 72-hour counts can be interpreted as demonstrating several distinct chemical reactions of a lethal nature within the propylene oxide systems. These reaction stages may be separated into inhibition 1, inhibition 2, inhibition 3, and sterilization. An intermediate interval designated as the sterilization threshold occurred between hours 8 and 10.

Conversion of the surviving fraction values to precentage survival showed that Inhibition 1 was characterized by a minimum survival rate of 70% of the population. Data from the 3-hour interval indicated that about 40% of the population had recovered and was designated as Inhibition 2. Survival values of 7-20% were noted at Inhibition 3 (hours 4-7). The exposure intervals from

8-10 hours exhibit surviving fractions of less than 1% and have been designated as representative of the sterilization threshold.

Sterilization was interpreted as ocurring when no colonies developed after 72 hours of incubation at ambient conditions following removal from the propylene oxide system. Under the conditions of the experiment, this stage was reached between 10 and 11 hours in the chambers. Populations of soil bacteria exposed for 11 hours showed no growth when subcultured to nutrient broth. This was interpreted as indicating that the propylene oxide system was lethal to the bacteria and that the petri plates and their contents were sterile.

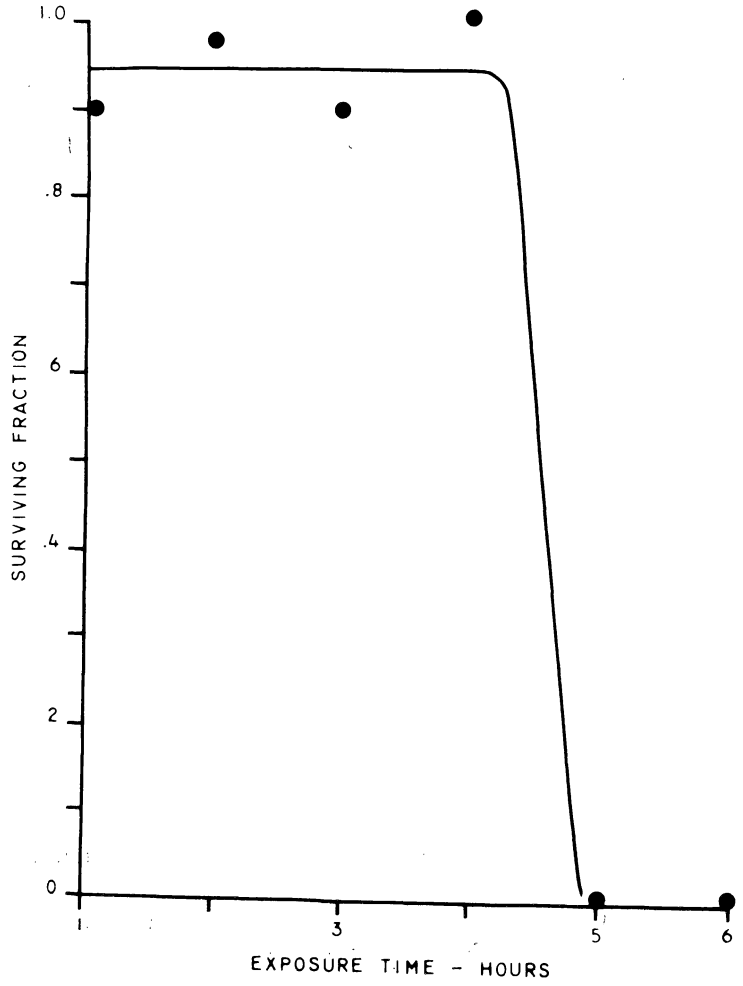

Figure 3. Surviving fractions of a homogeneous population of *B. subtilis* exposed to propylene oxide in a closed system.

The progressive lethality shows that the mortality reflects a lag phase near the end point. Jordan and Jacobs (2,3) described this lag phenomenon as a function of environmental conditions relative to simultaneous multiplication-rapid death population dynamics.

Increased colony counts at 48 and 72 hours in the heterogeneous population may prove to be a characteristic of species activation which is reflected by the surviving fraction. Other research (5) demonstrates (i) metabolic retardation at sublethal exposures to bactericides with recovery occurring after removal from the sterilant system, (ii) adaptation by induction may result in modified metabolic activities which show increased numbers of organisms, and (iii) cells may demonstrate an increased sensitivity at certain stages in their growth cycle.

There was no detectable sensitivity of *B. subtilis* to propylene oxide for hours 1-4 (Figure 3). Sterilization occurred between the fourth and fifth hours. Nutrient broth subcultures incubated for 72 hours at 37°C and petri plates incubated 15 days after 5 and 6 hours of exposure showed no growth. This is interpreted as indicating that the mortality threshold lies between 4 and 5 hours and that the 5th hour interval represented a lethal exposure for this organism (Figure 4).

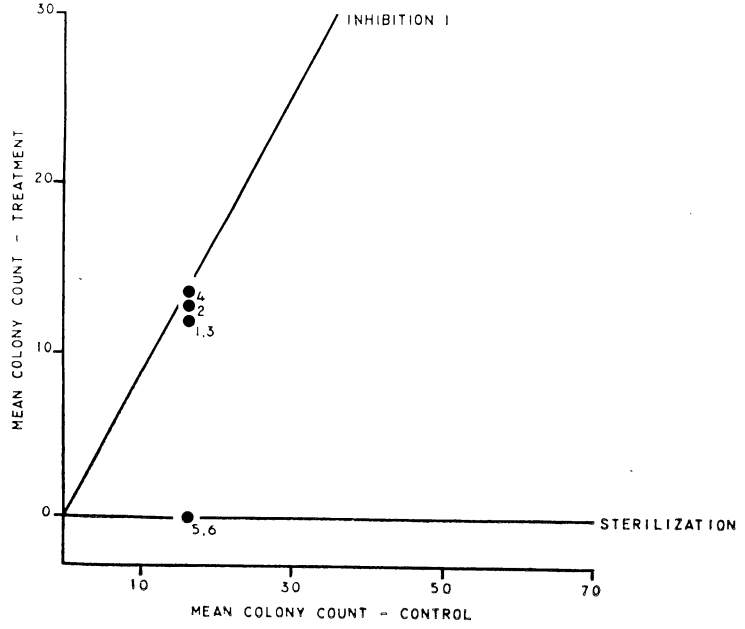

Figure 4. Comparison of the survival of *B. subtilis* exposed to propylene oxide in closed systems to the interval phenomena found in heterogeneous populations of soil bacteria.

Comparison of the homogenous population to the heterogeneous population data showed (i) that colonies of *B. subtilis* can be expected to appear within 24 hours and will make no further contribution to the overall colony population trends, and (ii) the surviving fraction and threshold phenomena observed in figures 1 and 2 is a species characteristic.

The sterilization lag phenomena noted in the heterogeneous studies was not demonstrated in the 4-5 hour interval for *B. subtilis*. It is speculated that the organism's sensitivity is greatest at this point and that the lag occurs over a matter of minutes.

Gaseous sterilants and disinfectants exert their influence by first dissolving in the moisture surrounding he cell (5). Organisms exposed to propylene oxide may be protected from its lethal effects by (i) reaction of the oxide with the chemical environment, (ii) adsorption of the sterilant to organic material in the system, and (iii) formation of protective films around the cell.

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