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1. Studies of the naturally occurring bacterial spore population from Cape Kennedy soil were continued. As reported last quarter, the extinction of the population ($N_o \approx 3 \times 10^4$ spores per strip) when exposed to 125 C was beyond 16 hr. Additional preliminary attempts to determine the 125 C end point at 24 and 30 hr were unsuccessful. All broth tubes into which heated strips were placed (QR 31) were positive for growth within 5 days of incubation at 32 C. An equal number of uninoculated control strips included in each run remained negative after 5 days of incubation. After 48 hr of heating, however, 30 strips and 30 uninoculated controls were all negative for growth after 1 week of incubation. An FN-MPN testing system (QR 26) was therefore designed accordingly. Sixty inoculated strips and 60 control strips were suspended in the oven and 10 of each were removed at 1.5-hr intervals out to 48 hr. As strips were removed and cooled, they were placed in tubes of "TSA broth" plus 0.1% soluble starch and 0.2% yeast extract (QR 26) and incubated for 1 week at 32 C. All control strips were negative and the number of positive tubes per replicate of 10 at each heating interval was as follows:

| Hr at 125 C | Tubes positive/10 |
|---|---|
| 40.5 | 6 |
| 42.0 | 3 |
| 43.5 | 2 |
| 45.0 | 4 |
| 46.5 | 4 |
| 48.0 | 1 |

The average $D_{125C}$ value as determined by the Stumbo, Murphy, Cochrane method described by Pflug and Schmidt (QR 26) was 8.9 hr. Positive tubes were streaked onto Trypticase Soy Agar (TSA). All isolates were Gram-positive sporeforming rods.

Figure 1 shows the composite results of two series of experiments. The first was a plating of viable survivors out to 4 hr maximum heating time at 125 C (QR 31). The second was a similar experiment with a maximum heating time of 9 hr. In the 0-4 hr runs, four strips were suspended at each interval on each of 3 days. The 0-9 hr runs were similar except that five strips were suspended at each interval on each of 3 days. The figure shows the averages of duplicate platings of each strip at each interval. The zero and 4-hr intervals are overlappings of the two experiments, and parentheses indicate an additional plate count average at that data point. The $D_{125C}$ value derived from a line best fit to the average value at each interval (excluding $N_o$) was 13.9 hr.

After observing the obvious tailing of the survivor curve, it was decided to investigate the possible protective effects of the soil particles involved. A second sample of soil was collected at Kennedy Space Center (KSC) at the same locations as the initial sample (QR 31). This soil was treated in the same manner as the first except that it was passed

through the sieve series in a completely dry state. The material which passed through the 43-micron screen had a consistency similar to talcum powder. Three 0.6-gm portions of this dust were sterilized--one in a conventional dry air oven (175 C, 3 hr), the second in an autoclave (121 C, 20 min), and the third by ethylene oxide (ETO) (100 F, 45% R.H., 11% ETO in Freon, 10 psi, 16 hr). The three samples, each contained in an open 25 x 125-mm screw capped tube, were allowed to stand at room temperature in a horizontal laminar flow clean bench for 2 days prior to the addition of Bacillus subtilis var. niger spores (BGSSM-10, QR 25) in 95% ethanol (20 ml of $\approx 3 \times 10^5$ spores per milliliter). The resulting suspensions had approximately the same soil and spore concentrations as the original suspension (QR 31). Heat studies of each suspension were conducted in the usual manner, employing four strips at each interval on each of 3 days. In parallel with the B. subtilis var. niger plus each soil, an equal number of strips inoculated with a corresponding dilution of the spores without soil were assayed as controls. Figures 2,3, and 4 show the results. Indicated are the means, ranges of plate counts, best fit lines (ignoring $N_o$) and $D_{125C}$ values of each system. Perhaps the most striking observation of Figures 2 and 3 is the relatively narrow plate count ranges of the spore-soil systems as compared with those of the ethanol controls. During the inoculation procedure, each tube of spore-soil suspension contained a small magnetic stir bar. Each tube was insonated for 12 min prior to inoculation and was kept on a magnetic mixer between fillings of the 0.1-ml Eppendorf pipette. This was necessary in order to maintain a homogeneous suspension of soil. In contrast, the spores in ethanol were insonated and vortexed. Ten strips were inoculated and the tube was revortexed before inoculating the next 10 strips. The constant agitation of the spores with soil probably resulted in more uniform deposition and therefore the narrower plate count ranges. In both the dry-heated and autoclaved soil systems, a slight shoulder was observed during the first 40 min of heating. The soil particles may have been offering a small amount of protection to the spores, perhaps changing the come-up time of the strips or retaining moisture, thereby accounting for the slightly higher D-values. Statistical analysis of these data has not yet been completed, therefore no statement can be made as to the significance of the indicated D-values. The control values and added soil values in both systems are very similar, however, and the soil particles seem to be contributing very little, if any, protection.

Results from heat studies of the spores plus the ethylene oxide-sterilized soil and the corresponding control are shown in Figure 4. No explanation can be given at this time to account for the $D_{125C}$ value of the ETO-sterilized soil system being over twice that of the control value. When compared to the soils sterilized by the other two methods, the ETO has apparently added something to the soil thereby protecting the spores, or else the dry heating and autoclaving have altered some protective mechanism of the soil. Investigation of this phenomenon will continue.

Other workers have suggested that suspension of spores in ethanol as opposed to distilled water may increase resistance. Past observations in our laboratory have indicated that prolonged storage of B. subtilis var. niger spores in 95% ethanol or in water does not affect heat resistance. However, to examine this possibility when dealing with naturally occurring spores, portions of the completely dry-processed KSC soil were suspended separately in filter-sterilized 95% ethanol and in sterile deionized distilled water. Strips were inoculated and heat processed in the same manner as the previous experiments. Figure 5 shows the results when ethanol- and water-suspended soils were run in parallel on each of 3 days. The survivor curves were equivalent and essentially paralleled the survivor curve obtained with the original suspension (QR 31).

From these observations, it was concluded that the relatively high dry heat resistance of the naturally occurring spore population is due primarily to intrinsic characteristics of the spores themselves and not to protection by the soil or by suspension in ethanol.

During the next quarter, additional investigations of the soil inoculum will include isolation, identification, subcultured spore preparation and subsequent dry-heat-resistance testing of survivors from the latter portions of the curve. In addition, the cycle verification tests at KSC (QR 30) will be initiated after preliminary equipment checkout.

2. At the Spacecraft Sterilization Technology Seminar in Williamsburg, Va., Dr. John Brewer pointed out the possibility that the naturally occurring spore inoculum from KSC soil (QR 31) may contain high numbers of thermophilic organisms. Duplicate stainless steel strips were inoculated and processed in the usual manner to obtain an $N_o$ value. Duplicate dilutions from each strip were incubated for 1 week at the following temperatures: 25 C, 35 C, 45 C, and 60 C. Average spore concentrations obtained from the $10^{-3}$ dilutions at the first three incubation temperatures were $4.6 \times 10^4$, $3.6 \times 10^4$, and $3.4 \times 10^4$, respectively. One week of incubation at 60 C resulted in averages of 5 and 2 colonies at the $10^{-2}$ and $10^{-3}$ dilutions, respectively. During the next quarter, incubation temperature studies will be conducted using heat-injured spores from this inoculum.

3. Studies were continued to determine if microbiological air sampling in spacecraft assembly areas can be used to estimate numbers and types of microorganisms on spacecraft surfaces. Experimental work concentrated specifically on the membrane filter field monitor because its small size and ease of operation were considered desirable characteristics for air sampling in and around spacecraft.

A basic question was whether airborne viable particles consisted of similar numbers and types of microorganisms as viable particles found on surfaces. To investigate this question sterile stainless steel strips (60 per tray) were exposed to environmental contamination. Twelve strips were retrieved at intervals between 5 and 17 days of exposure.

Six strips were placed in separate prepoured TSA plates and the strips were then overlaid with TSA. The remaining six strips were placed in each of six sterile 250-ml Erlenmeyer flasks containing 50 ml of sterile standard rinse solution and insonated for 2 min. The entire 50 ml was plated with 50 ml of double strength TSA. All membrane filter air samples were collected on a manifold which held six field monitors (QR 31). Each filter sampled at a flow rate of 1/3 CFM for a period of 30 min. After sampling, three filters from each manifold were placed directly onto the surfaces of TSA plates and the remaining three filters were placed face down in each of three sterile 600-ml beakers containing 50 ml of standard rinse solution. These beakers were insonated for 2 min, and the entire 50-ml portion was plated with 50 ml of double strength TSA. Insonated stainless steel strips and membrane filters were plated to ascertain complete removal of microorganisms from their surfaces. All plates were counted after 24, 48, and 72 hr incubation at 35 C. Table 1 shows the results obtained from the stainless strips and air sampling experiments conducted in the laboratory area. The mean number of microorganisms per viable particle found on the strips was approximately twice that found on the membrane filters. This difference was statistically significant. Table 2 shows the results of the same sampling procedures performed in the Manned Spacecraft Operations Building (MSOB). The mean number of microorganisms per viable particle collected on filters was comparable to the mean on stainless steel strips in this location. However, it was evident from observation of the day-by-day results, as well as the high coefficients of variation associated with the means in both locations, that extreme variation was inherent in the parameter that was measured. When variation of this magnitude exists statistical tests for significant differences are of questionable value. Accordingly, the fact that the means were comparable in the MSOB and differed by a factor of two in the laboratory was understandable when compared, for instance, with an 18-fold difference between stainless steel strip data collected in the MSOB on 7-22-70 and 7-27-70.

A sample of microorganisms from insonated stainless steel strips was isolated and identified. These results are presented in Table 3. It was evident that the frequency with which certain types of organisms appeared on stainless steel strips differed from that observed on the filters. This was not unexpected in view of the differences in resistance of various types of microorganisms to prolonged exposure in the microenvironment of a stainless steel strip surface. However, as a crude measurement system the membrane filter has some value in that it reflects the major components of the microbial population on stainless steel strips.

To determine whether the frequency of occurrence of various types of microorganisms on a direct-plated membrane filter was affected by insonation of the filter, a sample of organisms was identified from direct-plated filters collected at the same time as the insonated filters. These results are presented in Table 4. In both locations a relative

increase in the per cent of nonsporeforming Gram-positive rods and a decrease in the Gram-positive cocci was observed. This change was particularly pronounced in the MSOB. These data suggested that all types of microorganisms are not uniformly distributed among airborne viable particles. Therefore, insonation of the filter with the resulting breakup of particles resulted in a more accurate measurement of the frequency with which each type of microorganism occurred.

An experiment was conducted to determine whether differences in the flow rate at which membrane filter field monitors operate result in different values for the concentration of airborne viable particles. On each of 12 days six samples were collected at a flow rate of 1/2 CFM while 18 samples were collected simultaneously in the same area at a flow rate of 1/6 CFM. Each filter sampled a total of 30 $ft^3$ of air. The results are presented in Table 5 and show that no significant difference was observed between the mean results of the two sampling procedures.

Two membrane filters, one 0.45 micron pore size and one 0.8 micron pore size, were compared for their ability to recover microorganisms from air. The results (Table 6) showed that although the mean recovery value for the 0.8-micron filters was greater than the value for 0.45-micron filters, the difference was not statistically significant. The 0.8-micron filter was then compared with the Reyniers slit air sampler. Two Reyniers samplers and one manifold were placed adjacent to each other and run simultaneously. The 0.8-micron filters were less efficient than the Reyniers air sampler for recovering microorganisms (Table 7). The difference was considered to be significant ($P<.05$). These results were similar to those obtained when a 0.45-micron filter was compared to the Reyniers air sampler (QR 31). Although the membrane filter method recovered 25% fewer viable particles than the Reyniers sampler in the clean room environment of the MSOB, it might be of value as an index of the levels of viable particulate contamination in an environment where the use of a Reyniers air sampler is restricted. The types of microorganisms isolated and identified from samples collected by both methods were similar (QR 31).

Studies were initiated to determine the maximum time a six-stage Andersen sampler could be operated without desiccation of the agar and/or subsequent loss of viable microorganisms. Two samplers calibrated to draw 1 CFM of air were run for 30 min side by side in the intramural environment of the laboratory (66% R.H. and 76 F). One sampler was selected as a control, stopped, and the plates were incubated at 35 C. The other sampler, designated as the test sampler, was momentarily stopped, moved to a laminar flow clean bench and restarted. After running 1 hr in the clean bench, plates were removed and incubated at 35 C. Table 8 shows the results obtained. No significant difference was observed between total plate counts from the two samplers. In a second study, the test sampler was run for 3 hr in the laminar flow clean bench and then moved to a sampling area for a 1-hr run in parallel

with a control sampler. The results are shown on Table 9. Again no significant difference was noted even though at times the agar on the first stage of the test sampler was damaged due to desiccation. Further studies are in progress to observe the effects of incorporating oxyethylene docosanol in the medium to retard desiccation.

4. The study initiated to evaluate certain aspects of the swab-rinse technique was continued during the past quarter. That portion of the study designed to determine the mean per cent removal and recovery of naturally occurring microorganisms from surfaces for each of several "experienced" individuals and the variation associated with these mean values was completed. The methodology was described in QR 31. Results of this portion of the study are presented in Table 10. It was evident that little or no difference existed between the mean values of removal achieved by the three individuals. Furthermore, the coefficients of variation of the removal values were remarkably similar to the three individuals. The mean values of per cent recovery were also quite similar, as were the coefficients of variation associated with these means. As suggested in the last report, the greater coefficient of variation observed for recovery values, when compared with those for removal values, indicated that some factor other than technician variation was responsible for the inconsistency of recovery from strip to strip.

Statistical tests determined that significant differences did not exist between either the removal or recovery values of the three individuals. Further tests showed recovery and removal values to be independent of the concentration of microorganisms on the strips, which ranged from 46 to 407 per 4-sq-in area. Accordingly, it was felt that the combined results from three individuals could be used to calculate valid mean values for individuals experienced in the use of the swab-rinse technique. These mean values were used as a basis for comparison with values obtained in subsequent aspects of this study.

The second phase of this study was designed to determine whether individuals with no experience using the swab-rinse technique achieved significantly different results than "experienced" individuals. Individuals representing a variety of occupations and training were asked to participate in the study provided they had never previously used the swab-rinse technique. The purpose of the experiment was explained to each individual and a brief demonstration of the technique was given. The individual was then permitted to practice the technique on a single strip. The practice period was accompanied by further instructions and corrections of technique. Each individual then swabbed four 2" x 2" strips. All other laboratory procedures and the analysis of data were identical to those described for the "experienced" individuals.

A total of 30 "inexperienced" individuals participated in the study. The results of this study are compared with the results of the three "experienced" individuals in Table 11. It was found that the removal rate of

84% achieved by the "inexperienced" individuals was significantly lower than the rate of 91% for the "experienced" group. Interestingly, the recovery rate of 62% for the "inexperienced" group was found to be significantly higher than the rate of 43% for the "experienced" individuals ($P<.0001$). It also was observed that the coefficient of variation for the removal value achieved by the "inexperienced" group was twice that of the "experienced" group, indicating less consistency in the performance of the "inexperienced" individuals. However, little difference was observed between the coefficient of variation of the recovery values for the two groups. This observation supports the suggestion that the variation in recovery is relatively independent of and much greater than the variation imparted in the swabbing procedure.

It was found that the "inexperienced" group could be further divided into two comparably sized groups on the basis of laboratory experience. A laboratory group of 16 consisted of individuals whose day-to-day tasks involved the performance of microbiological or chemical procedures. The nonlaboratory group of 14 consisted of individuals performing other tasks such as office, maintenance and animal care procedures. Table 12 compares the results achieved by these two groups. It was found that the "inexperienced" nonlaboratory workers had a lower removal rate with a higher coefficient of variation than did the "inexperienced" laboratory worker group. It was also noted that the recovery rate for the nonlaboratory group was higher than that for the laboratory group while the coefficient of variation for recovery values showed no consistent pattern.

The observation of inverse correlation between removal and recovery rates was further tested to determine whether it was significant or simply an artifact. A statistical test for rank correlation between the 30 removal values and the 30 recovery values achieved by the three "experienced" individuals was performed. An inverse correlation between the values was found to be significant at the 95% level. A similar test was applied to the removal and recovery values from the 30 "inexperienced" individuals and again the correlation was inverse and significant at the 98% level. No substantiated explanation can be offered for the consistency with which high removal values are associated with low recovery values.

In response to interest expressed by personnel at the Jet Propulsion Laboratory a study was designed and conducted to determine whether swabbing a surface with a dry cotton swab rather than a moistened cotton swab significantly affected removal and recovery results. Two "experienced" individuals each swabbed five sets of six 2" x 2" strips using dry cotton swabs. Experimental procedures and data analysis were similar to those previously described for swabbing studies. The results achieved with dry swabs are compared with results achieved by "experienced" individuals using moistened swabs (Table 13). The removal value of 95% for dry swabs was significantly higher than the 91% for moistened swabs ($P<.01$). However, the recovery rate of 38% for dry swabs was not

significantly lower than the rate of 43% for moistened swabs. Therefore, for purposes of estimating microbial contamination levels on surfaces exposed to airborne fallout, it would appear that the dry swabbing technique would not produce significantly different results than the standard technique. It was noted that the coefficients of variation for both removal and recovery were lower for dry swabs than for moistened swabs, suggesting greater consistency with this technique.

5. During the past quarter assays for buried microbial contamination were completed on five types of electronic piece parts designed and manufactured for use in spacecraft. Langley Research Center provided 50 units of each component type, along with a general description of the manufacturing process and heat history. Each type of component was tested for its ability to fracture uniformly since such fractures permit the use of a microbial concentration estimation technique developed at this laboratory (QR 18-21). It was found that only one of the five types would consistently produce uniform fracture surfaces. A second type occasionally produced uniform fracture surfaces and components of this type, along with the remaining three types of components, were assayed in ways specifically designed for their respective structures.

The component that fractured uniformly was a cylindrical fixed-composition resistor weighing 2.1 g and having a volume of 0.8 $mm^3$. The part is manufactured using a hot molding process which maintains a temperature of 125 C for several seconds. Fifty resistors were surface-sterilized by exposure to a 2% solution of peracetic acid for 15 min. Ten resistors were tested as surface contamination controls by placing them in individual tubes of TSB and incubating for 20 days at 32 C. All tubes were negative for growth at the end of this period. One tube was then tested for the presence of inhibitory substances by inoculating with approximately $1 \times 10^2$ spores of Bacillus subtilis var. niger and again incubating at 32 C. Turbidity was observed within 24 hr. The remaining 40 resistors were aseptically broken in half using sterile crescent wrenches, and each resistor half was placed in a tube of TSB and incubated for 20 days at 32 C. No growth was detected in any of the tubes. Knowing the proportion of fractured components that was positive for microbial growth and the area exposed by fracturing it was possible to estimate the concentration of microbial contamination in the resistors. Using tables prepared by Exotech, the 95% upper and lower confidence bounds of the estimate based on these data were calcuated to be $5.7 \times 10^3$ and 0.0 organisms per cubic inch of material, respectively.

The second type of component occasionally producing a uniform fracture was a semiconductor rectifier weighing 2.6 g and having a volume of 1.4 $mm^3$. This component is injection molded from a bakelite type material at approximately 95 C. Diodes embedded in the bakelite body experience a manufacturing heat of 180 C. The exact duration of exposure to these temperatures is not known. Ten units of this component were subjected to the surface sterilization and verification procedure described

earlier. It was possible to achieve a uniform fracture in 10 of the remaining 40 units. These fractured units were processed in a manner identical to that for the resistors. No growth was detected and the upper and lower 95% confidence bounds for the microbial concentration in these parts was estimated at $1.0 \times 10^4$ and 0.0 organisms per cubic inch, respectively. Each of the remaining 30 units was aseptically pulverized in a Pica blender mill for 1 min. The resulting powder was placed in a tube and 20 ml of standard rinse solution was added. The tubes were vortexed and then insonated for 2 min, and the tubes were allowed to stand for 3 hr to permit separation of the powder and the rinse solution. Ten milliliters of rinse solution were withdrawn and plated in single strength TSA in 150 mm dia plates. Ten milliliters of double strength TSB were added to the tube containing the powder and remaining rinse solution. All tubes and plates were incubated for 20 days at 32 C, at which time they were observed for evidence of microbial growth. One colony was detected on one TSA plate. Isolation and subculture of this colony was extremely difficult although a variety of techniques and media were used. Sufficient growth for microscopic examination was finally achieved and the organism was classified as a Gram-positive rod with no evidence of spore formation. Biochemical tests were not successful because of lack of growth. All other plates and tubes from the 30 components assayed were negative.

A third type of component that could not be uniformly fractured also was assayed using the pulverization technique described above. This component was an adjustable core coil form weighing 7.8 g and having a volume of 4.0 $mm^3$. The component is hot-molded at 180 C from a nylon-like material. Surface sterility tests were negative for growth as were all plates and tubes from the 40 units that were pulverized.

A fourth type of component was a fixed wire-wound resistor weighing 4.8 g and having a volume of 2.5 $mm^3$. This component consisted of two wire coils that were machine wound in an open factory area and subsequently potted in epoxy at approximately 65 C for 4 to 8 hr. Twenty-seven components were disassembled using sterile hand tools and employing strict product protection techniques in a horizontal laminar flow bench located in a vertical laminar flow clean room. Each disassembled component was placed in a beaker containing 150 ml rinse solution. The beaker was insonated for 2 min and three 50-ml portions of rinse solution were plated in double strength TSA in 150 mm dia plates. After removing the rinse solution the pieces were submerged in 150 ml of TSB. Plates and beakers were incubated for 20 days at 32 C. All surface sterility tests were negative for growth as were all beakers and all but two TSA plates. One surface colony appeared on one positive plate and the organism was isolated and identified as a Bacillus sp. Two submerged colonies appeared on a second plate. However, the same difficulties were experienced in subculturing these colonies as were described earlier. Microscopic examination of the scant growth achieved indicated both organisms were nonsporeforming Gram-positive rods.

The fifth type of component was a mylar capacitor weighing 3.5 g and having a volume of 2.2 $mm^3$. This component consisted of mylar and aluminum films rolled into a cylinder with a plastic tape outer coating and soldered ends. Production was in an open factory area, and the components were not subjected to heat at any time. Assays of these units were conducted in a manner similar to the disassembly procedure described previously. Surface sterility tests were negative for growth. Twenty-seven capacitors were assayed and one TSA plate had a single submerged colony. Subculturing of this colony also proved difficult, and microscopic examination indicated it was a nonsporeforming Gram-positive rod. Five TSB beakers showed visible turbidity and subcultures of the growth showed two beakers with bacilli, one with a coccus, one with a mold and one an actinomycete. The component that produced one colony in TSA was negative in TSB. It was considered of interest that the one type of component having no history of heat in its production process produced the most positive samples.

In the last quarterly report (QR 31) results of tests of the efficacy of the standard peracetic acid decontamination procedure showed that 40% of the experimentally contaminated piece parts remained contaminated after treatment. It was suspected that the concentration of the stock peracetic acid had deteriorated with time. A new supply of acid was obtained and compared to the old acid using four different types of resistors experimentally contaminated to a level of $1 \times 10^5$ spores per part. The results of the first comparison are presented in Table 14 and demonstrated that the new acid was more effective than the old acid but still failed to decontaminate 32% of the parts tested.

The test was repeated using a contact time of 10 min, twice the standard 5-min contact time. The additional contact time resulted in greater effectiveness as shown in Table 15, but 14% of the parts remained contaminated in the new acid. It was also noted that certain types of resistors were more likely to be positive than other types, suggesting some protective effect associated with the type of surface on which the contamination was deposited. The results of these tests re-emphasize the necessity for regular use of surface sterility controls in experiments concerned with buried contamination.

TABLE 1. COMPARISON OF VIABLE MICROORGANISMS PER VIABLE PARTICLE USING STAINLESS STEEL STRIPS AND MEMBRANE FILTER FIELD MONITORS AT THE SPACECRAFT BIOASSAY LABORATORY.

| Date sampled | Exposure of SS strips (days) | Microorganisms per viable particle on stainless steel strips[a] | Microorganisms per viable particle on membrane filters[b] |
|---|---|---|---|
| 5-18-70 | 7 | 2.14 | 2.99 |
| 5-19-70 | 8 | 3.15 | 1.47 |
| 5-20-70 | 9 | 2.49 | 1.08 |
| 5-25-70 | 14 | 2.39 | 2.16 |
| 5-26-70 | 15 | 2.17 | 2.23 |
| 5-27-70 | 16 | 6.81 | 2.06 |
| 6-2-70 | 7 | 2.51 | 1.72 |
| 6-3-70 | 8 | 7.79 | 1.10 |
| 6-9-70 | 13 | 8.44 | 5.40 |
| 6-10-70 | 14 | 3.25 | 5.60 |
| 6-15-70 | 15 | 13.04 | 2.07 |
| 6-16-70 | 16 | 6.43 | 4.08 |
| 6-17-70 | 17 | 11.08 | 2.39 |
| 6-22-70 | 14 | 12.58 | 1.07 |
| 6-23-70 | 15 | 21.78 | 5.79 |
| 6-24-70 | 16 | 4.37 | 1.36 |
| 6-29-70 | 7 | 7.99 | 4.67 |
| 6-30-70 | 8 | 7.64 | 15.31 |
| 7-1-70 | 9 | 19.73 | 2.58 |
| Mean | | 7.67 | 3.42 |
| Coefficient of variation | | 73% | 94% |
| Means are significantly different | | T = 2.778 | |

[a] Average of six strips per day

[b] Average of nine filters per day

TABLE 2. COMPARISON OF VIABLE MICROORGANISMS PER PARTICLE USING STAINLESS STEEL STRIPS AND MEMBRANE FILTER FIELD MONITORS AT THE APOLLO MANNED SPACECRAFT OPERATIONS BUILDING.

| Date sampled | Exposure of SS strips (days) | Microorganisms per viable particle on stainless steel strips[a] | Microorganisms per viable particle on membrane filters[b] |
|---|---|---|---|
| 7-7-70 | 8 | 2.40 | 17.80 |
| 7-8-70 | 9 | 5.99 | 4.28 |
| 7-13-70 | 14 | 5.25 | 3.43 |
| 7-14-70 | 15 | 6.45 | 2.66 |
| 7-15-70 | 16 | 4.27 | 5.23 |
| 7-20-70 | 5 | 15.18 | 8.20 |
| 7-21-70 | 6 | 7.51 | 6.26 |
| 7-22-70 | 7 | 27.92 | 3.88 |
| 7-27-70 | 12 | 1.53 | 6.68 |
| 7-28-70 | 13 | 6.98 | 5.24 |
| 8-3-70 | 7 | 1.16 | 2.65 |
| 8-4-70 | 8 | 1.98 | 16.53 |
| Mean | | 7.22 | 6.90 |
| Coefficient of variation | | 100% | 70% |
| Means are not significantly different | | T = 0.120 | |

[a] Average of six strips per day

[b] Average of nine filters per day

TABLE 3. FREQUENCY OF OCCURRENCE OF TYPES OF MICROORGANISMS IDENTIFIED FROM INSONATED MEMBRANE FILTERS AND STAINLESS STEEL STRIPS.

| Types of microorganisms | Spacecraft Bioassay Laboratory | | MSOB[a] | |
|---|---|---|---|---|
| | Membrane filters % | SS strips % | Membrane filters % | SS strips % |
| Nonsporeforming Gram-positive rods | 44.1 | 17.3 | 71.0 | 46.0 |
| Gram-positive cocci | 31.8 | 50.2 | 24.6 | 41.6 |
| Gram-negative rods | 0.2 | 0.3 | 0.7 | 0.7 |
| Gram-negative cocci | 0.0 | 0.3 | 0.0 | 0.0 |
| Actinomycetes | 8.9 | 2.9 | 1.8 | 4.1 |
| Yeasts | 1.7 | 1.6 | 0.0 | 2.4 |
| Molds | 2.2 | 2.9 | 0.4 | 3.1 |
| Bacillus spp. | 11.1 | 24.5 | 1.5 | 2.1 |
| TOTAL | 100 | 100 | 100 | 100 |
| NUMBER IDENTIFIED | 406 | 376 | 276 | 291 |

[a] Manned Spacecraft Operations Building

TABLE 4. FREQUENCY OF OCCURRENCE OF TYPES OF MICROORGANISMS IDENTIFIED FROM DIRECT-PLATED MEMBRANE FILTERS AND INSONATED MEMBRANE FILTERS.

| Types of microorganisms | Spacecraft Bioassay Laboratory | | MSOB [a] | |
|---|---|---|---|---|
| | Membrane filters % | Membrane filters insonated % | Membrane filters % | Membrane filters insonated % |
| Nonsporeforming Gram-positive rods | 24.1 | 44.1 | 16.5 | 71.0 |
| Gram-positive cocci | 41.2 | 31.8 | 71.3 | 24.6 |
| Gram-negative rods | 0.7 | 0.2 | 0.0 | 0.7 |
| Gram-negative cocci | 0.2 | 0.0 | 0.0 | 0.0 |
| Actinomycetes | 5.2 | 8.9 | 0.5 | 1.8 |
| Yeasts | 5.2 | 1.7 | 2.1 | 0.0 |
| Molds | 4.8 | 2.2 | 6.4 | 0.4 |
| Bacillus spp. | 18.6 | 11.1 | 3.2 | 1.5 |
| TOTAL | 100 | 100 | 100 | 100 |
| NUMBER IDENTIFIED | 420 | 406 | 188 | 276 |

[a] Manned Spacecraft Operations Building

TABLE 5. COMPARISON OF 1/2 CFM FLOW RATE AND 1/6 CFM FLOW RATE PER FILTER IN THE APOLLO MANNED SPACECRAFT OPERATIONS BUILDING.

| Date sampled | Viable particles per $ft^3$ | |
|---|---|---|
| | 1/2 CFM flow rate[a] | 1/6 CFM flow rate[b] |
| 8-24-70 | 0.53 | 0.67 |
| 8-25-70 | 0.62 | 0.68 |
| 8-26-70 | 0.67 | 0.72 |
| 8-31-70 | 0.70 | 0.52 |
| 9-1-70 | 0.60 | 1.02 |
| 9-2-70 | 0.82 | 0.78 |
| 9-14-70 | 0.70 | 0.93 |
| 9-15-70 | 0.47 | 0.65 |
| 9-16-70 | 0.80 | 0.98 |
| 9-21-70 | 1.23 | 1.32 |
| 9-22-70 | 0.95 | 0.71 |
| 9-23-70 | 1.73 | 1.92 |
| Mean | 0.82 | 0.91 |

Means are not significantly different T = 0.599

[a] Average of six filters per day

[b] Average of 18 filters per day

TABLE 6. COMPARISON OF 0.8-MICRON MEMBRANE FILTER WITH 0.45-MICRON MEMBRANE FILTER IN THE APOLLO MANNED SPACECRAFT OPERATIONS BUILDING.

| Date sampled | Viable particles per $ft^3$ | |
|---|---|---|
| | 0.8-micron pore size[a] | 0.45-micron pore size[a] |
| 8-24-70 | 0.67 | 0.49 |
| 8-25-70 | 0.68 | 0.52 |
| 8-26-70 | 0.72 | 0.50 |
| 8-31-70 | 0.52 | 0.69 |
| 9-1-70 | 1.02 | 0.48 |
| 9-2-70 | 0.78 | 0.71 |
| 9-14-70 | 0.93 | 0.72 |
| 9-15-70 | 0.65 | 0.73 |
| 9-16-70 | 0.98 | 0.94 |
| 9-21-70 | 1.32 | 1.40 |
| 9-22-70 | 0.71 | 0.69 |
| 9-23-70 | 1.92 | 1.81 |
| Mean | 0.91 | 0.81 |

Means are not significantly different T = 0.627

[a] Average of 18 filters per day

TABLE 7. COMPARISON OF REYNIERS SLIT AIR SAMPLER AND 0.8-MICRON MEMBRANE FILTERS IN THE APOLLO MANNED SPACECRAFT OPERATIONS BUILDING.

| Date sampled | Viable particles per $ft^3$ | |
|---|---|---|
| | Reyniers sampler[a] | 0.8-micron membrane filter[b] |
| 8-24-70 | 0.75 | 0.67 |
| 8-25-70 | 1.04 | 0.68 |
| 8-26-70 | 0.74 | 0.72 |
| 8-31-70 | 0.78 | 0.52 |
| 9-1-70 | 1.00 | 1.02 |
| 9-2-70 | 1.00 | 0.78 |
| 9-15-70 | 1.00 | 0.65 |
| 9-16-70 | 1.34 | 0.98 |
| 9-21-70 | 1.76 | 1.32 |
| 9-22-70 | 1.28 | 0.71 |
| Mean | 1.07 | 0.81 |

Means are significantly different T = 2.115

[a] Average of six plates per day

[b] Average of 18 filters per day; flow rate per filter 1/3 CFM

TABLE 8. EFFECTS OF LONG-TERM SAMPLING ON RECOVERY OF VIABLE PARTICLES USING THE ANDERSEN SAMPLER.

| Date sampled | Total viable particles recovered | |
|---|---|---|
| | Test sampler[a] | Control sampler[b] |
| 9-17-70 | 93 | 62 |
| 9-18-70 | 165 | 150 |
| 9-18-70 | 61 | 46 |
| 9-18-70 | 87 | 84 |
| 9-21-70 | 71 | 98 |
| 9-21-70 | 175 | 214 |
| 9-21-70 | 270 | 261 |
| 9-28-70 | 147 | 144 |
| 9-28-70 | 103 | 77 |
| 10-2-70 | 173 | 158 |
| 10-2-70 | 131 | 120 |
| 10-2-70 | 63 | 48 |
| Mean | 128 | 122 |
| Means are not significantly different | T = 0.245 | |

[a] 1/2 hr out in room, 1 hr in laminar flow bench

[b] 1/2 hr out in room

TABLE 9. EFFECTS OF LONG-TERM SAMPLING ON RECOVERY OF VIABLE PARTICLES USING THE ANDERSEN SAMPLER.

| Date sampled | Total viable particles recovered | |
|---|---|---|
| | Test sampler[a] | Control sampler[b] |
| 10-20-70 | 60 | 40 |
| 10-21-70 | 101 | 106 |
| 10-22-70 | 50 | 32 |
| 10-23-70 | 75 | 57 |
| 10-26-70 | 96 | 99 |
| 10-27-70 | 52 | 48 |
| 10-28-70 | 26 | 34 |
| 10-28-70 | 23 | 25 |
| 11-2-70 | 23 | 42 |
| 11-3-70 | 34 | 26 |
| 11-4-70 | 65 | 50 |
| 11-5-70 | 130 | 97 |
| 11-6-70 | 50 | 61 |
| Mean | 60 | 55 |

Means are not significantly different T = 0.436

[a] Run 3 hr under laminar flow bench; 1 hr out in room

[b] 1 hr out in room

TABLE 10. COMPARISON OF RESULTS OBTAINED BY THREE INDIVIDUALS WITH SWAB-RINSE TECHNIQUE EXPERIENCE.

| Individual | No. strips | Removal | | Recovery | |
|---|---|---|---|---|---|
| | | Mean % | Coefficient of variation % | Mean % | Coefficient of variation % |
| A | 40 | 90 | 4 | 42 | 31 |
| B | 40 | 90 | 5 | 43 | 27 |
| C | 40 | 92 | 3 | 43 | 33 |

TABLE 11. COMPARISON OF RESULTS OBTAINED BY EXPERIENCED AND INEXPERIENCED INDIVIDUALS USING THE SWAB-RINSE TECHNIQUE.

| Individual | No. strips | Removal | | Recovery | |
|---|---|---|---|---|---|
| | | Mean % | Coefficient of variation % | Mean % | Coefficient of variation % |
| 3 exp. | 120 | 91 | 4 | 43 | 31 |
| 30 inexp. | 120 | 84 | 9 | 62 | 37 |

TABLE 12. COMPARISON OF RESULTS OBTAINED BY LABORATORY AND NONLABORATORY PERSONNEL USING THE SWAB-RINSE TECHNIQUE.

| Individual | No. Strips | Removal | | Recovery | |
|---|---|---|---|---|---|
| | | Mean % | Coefficient of variation % | Mean % | Coefficient of variation % |
| 16 lab. | 64 | 85 | 8 | 59 | 42 |
| 14 nonlab. | 56 | 83 | 11 | 65 | 32 |

TABLE 13. COMPARISON OF RESULTS OF DRY SWAB TECHNIQUE WITH MOISTENED SWAB TECHNIQUE.

| Technique | No. strips | Removal | | Recovery | |
|---|---|---|---|---|---|
| | | Mean % | Coefficient of variation % | Mean % | Coefficient of variation % |
| Dry swab | 60 | 95 | 2 | 38 | 22 |
| Moistened swab | 120 | 91 | 4 | 43 | 31 |

TABLE 14. COMPARISON OF OLD AND NEW PERACETIC ACID USED FOR SURFACE DECONTAMINATION OF ELECTRONIC PIECE PARTS--EXPOSURE TIME OF 5 MINUTES.

| Part | New acid | | Old acid | |
|---|---|---|---|---|
| | No. tested | Per cent positive | No. tested | Per cent positive |
| A | 8 | 88 | 8 | 100 |
| B | 13 | 0 | 13 | 69 |
| C | 4 | 25 | 4 | 25 |
| D | 3 | 33 | 3 | 67 |
| TOTAL | 28 | 32 | 28 | 71 |

Mean level: $1 \times 10^5$ spores per part.

TABLE 15. COMPARISON OF OLD AND NEW PERACETIC ACID USED FOR SURFACE DECONTAMINATION OF ELECTRONIC PIECE PARTS--EXPOSURE TIME OF 10 MINUTES.

| Part | New acid | | Old acid | |
|---|---|---|---|---|
| | No. tested | Per cent positive | No. tested | Per cent positive |
| A | 8 | 38 | 8 | 100 |
| B | 13 | 8 | 13 | 46 |
| C | 4 | 0 | 4 | 0 |
| D | 3 | 0 | 3 | 0 |
| TOTAL | 28 | 14 | 28 | 50 |

Mean level: $1 \times 10^5$ spores per part.

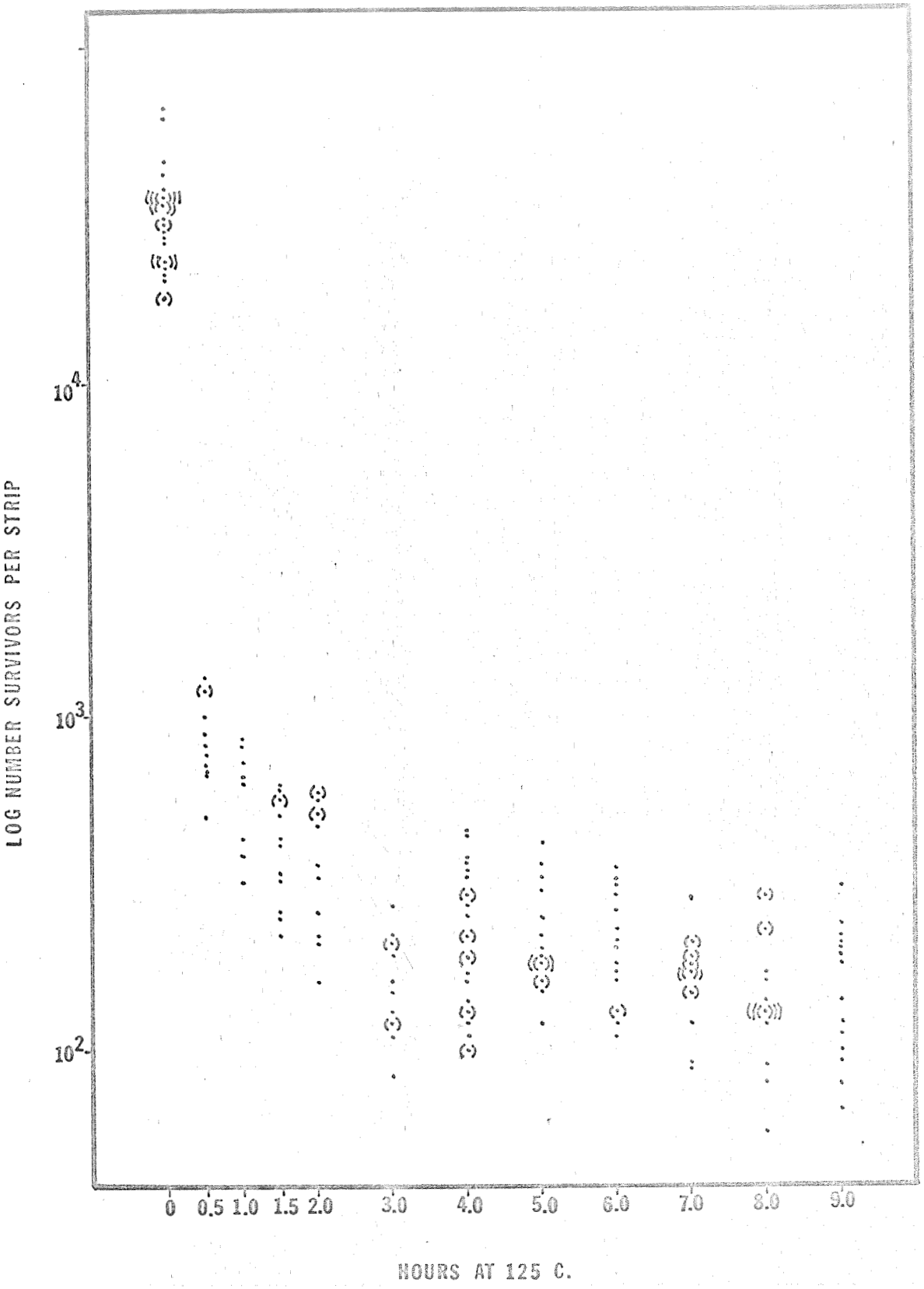


FIGURE 1. KSC SOIL IN 95% ETHANOL.

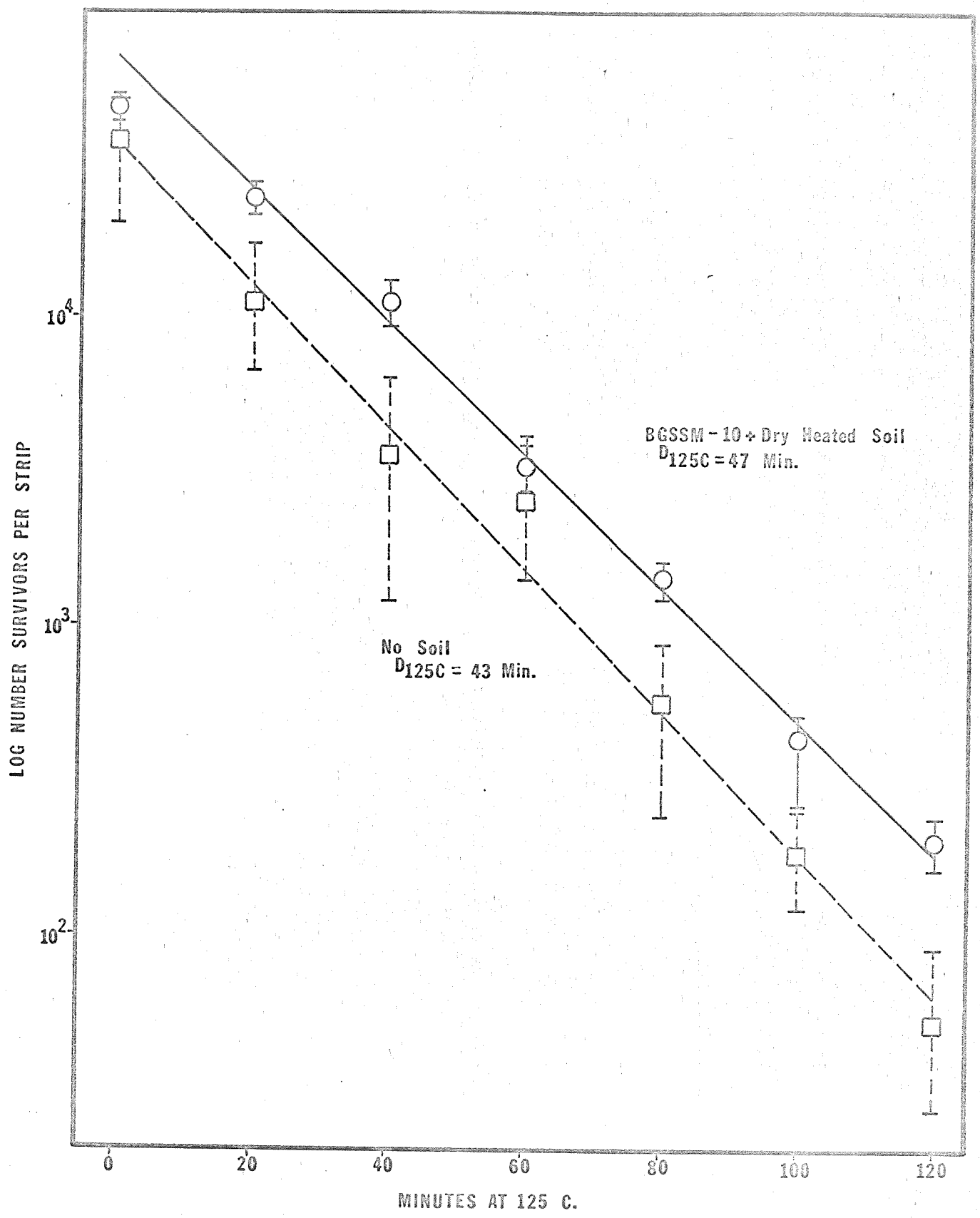


FIGURE 2. BG WITH AND WITHOUT DRY-HEAT STERILIZED KSC SOIL.

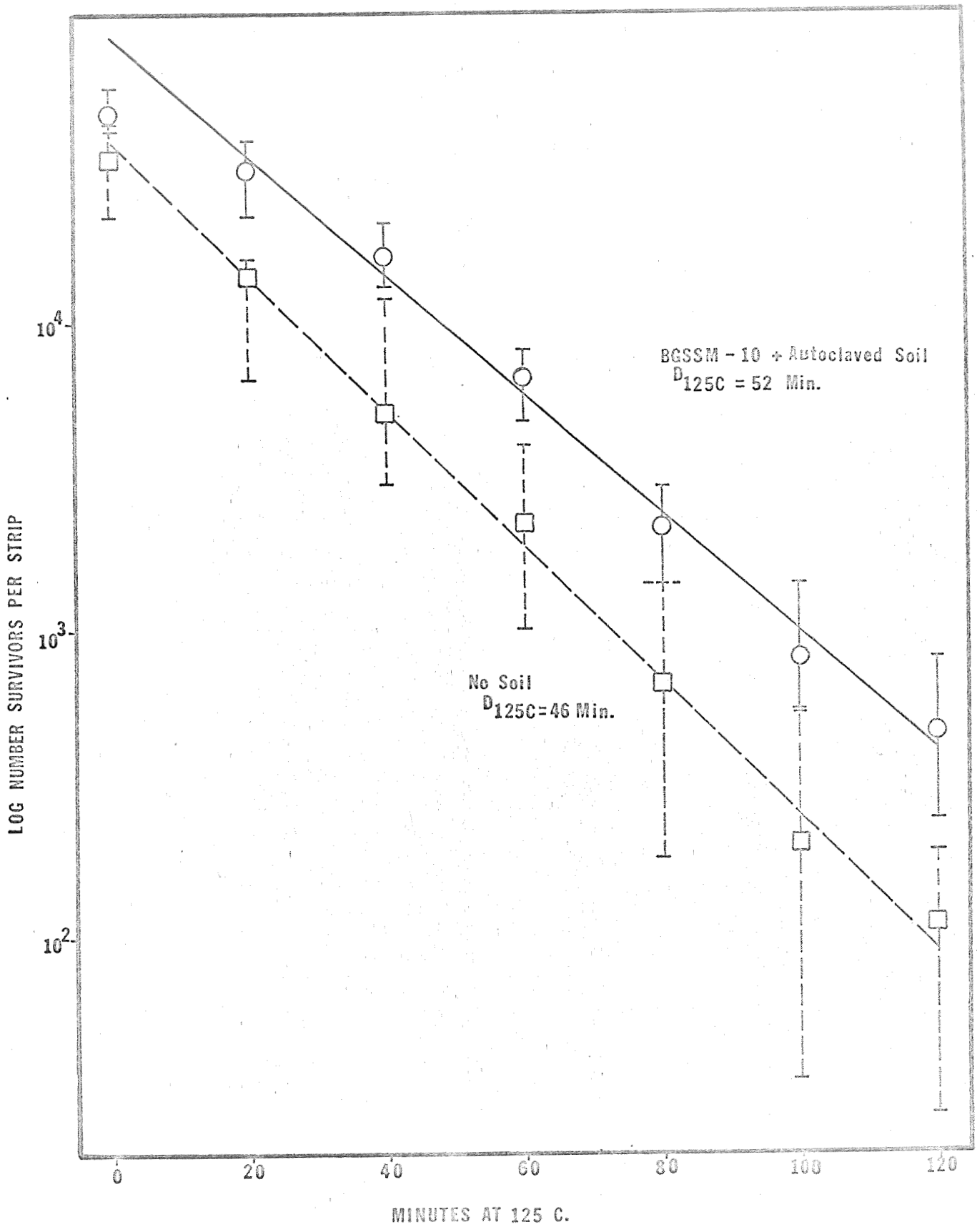


FIGURE 3. BG WITH AND WITHOUT AUTOCLAVED KSC SOIL.

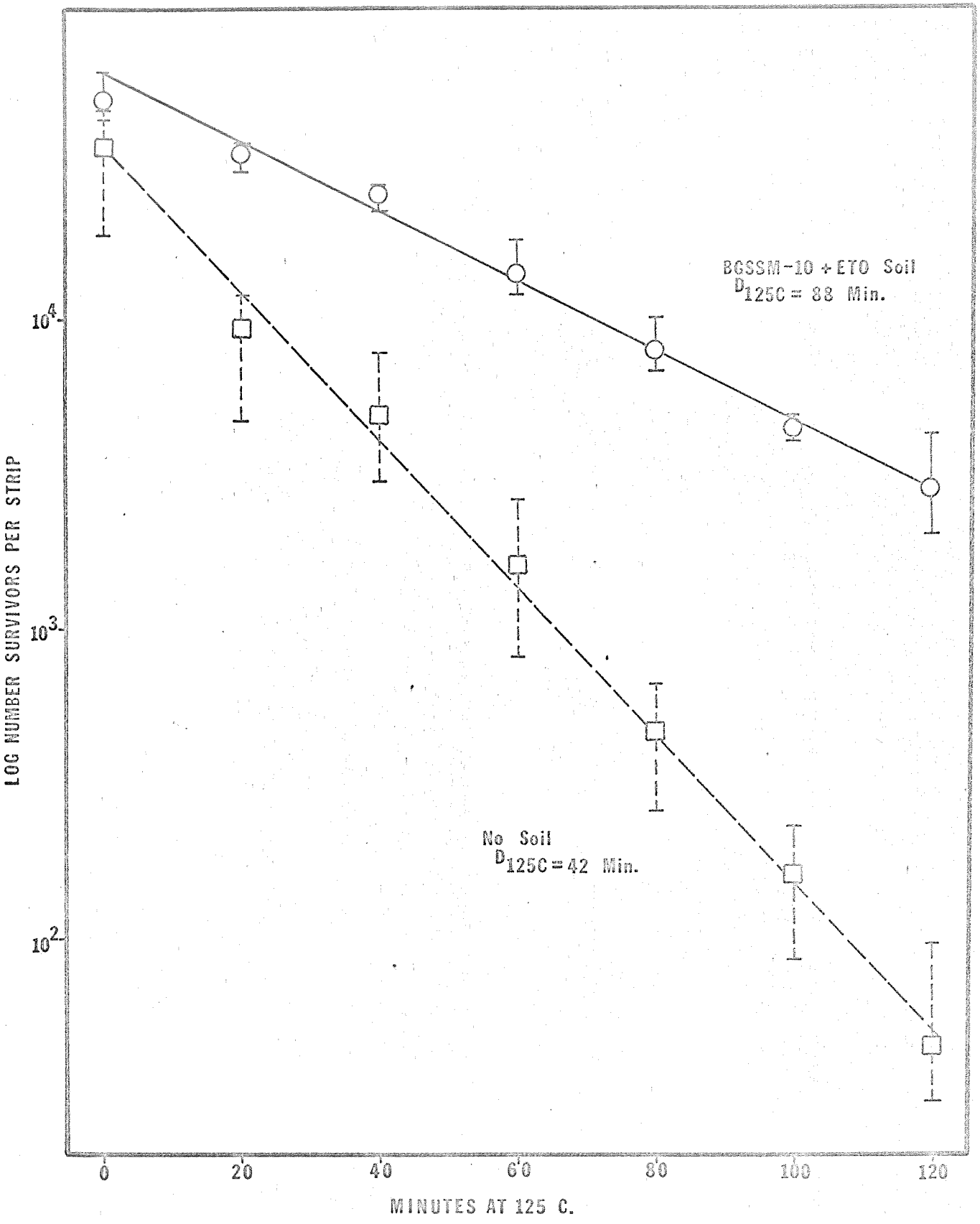


FIGURE 4. BG WITH AND WITHOUT ETO-STERILIZED KSC SOIL.

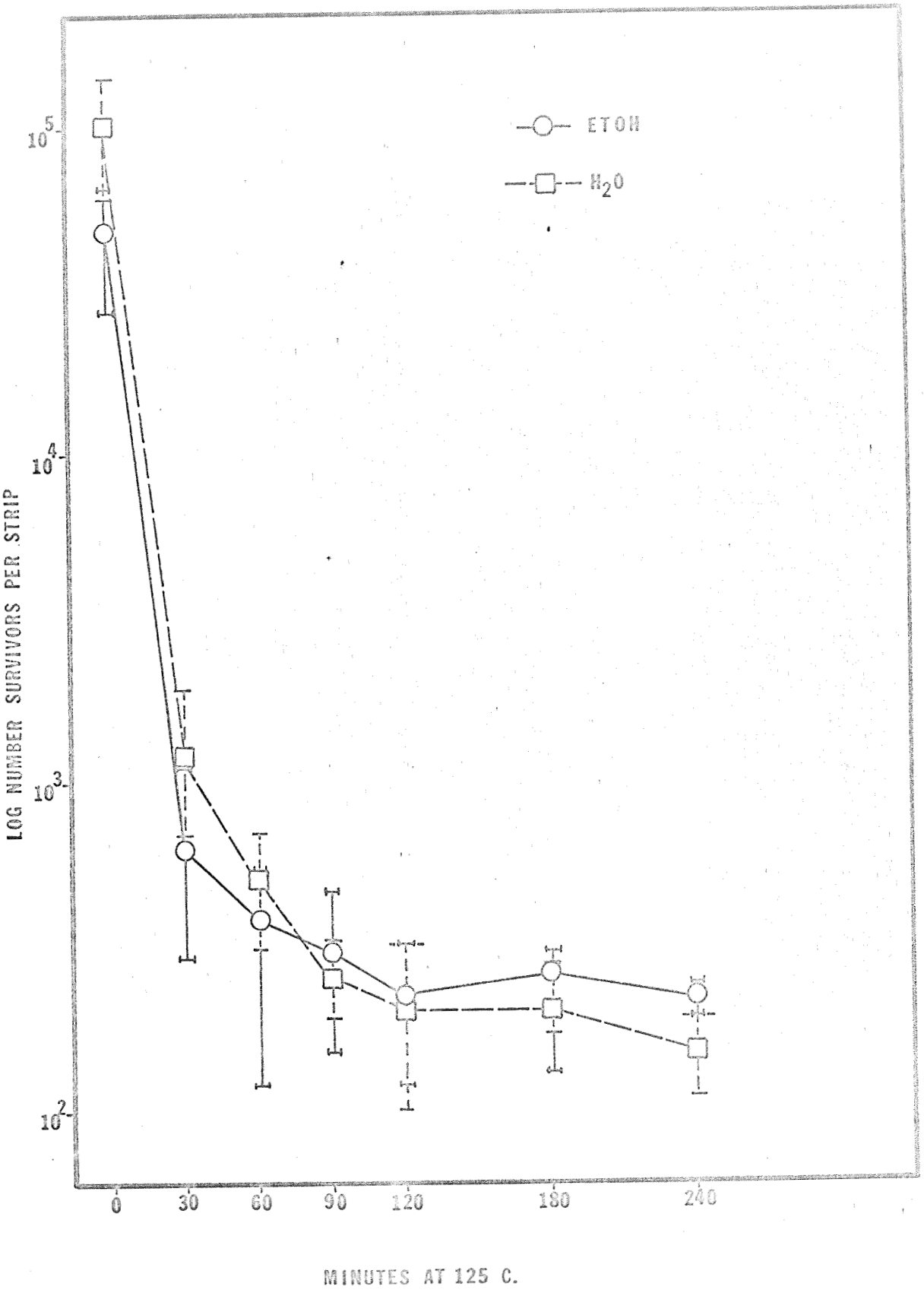


FIGURE 5. KSC SOIL: $H_2O$ vs. ETHANOL SUSPENSION