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Planetary Quarantine Unit
Phoenix Field Station Section
Ecological Investigations Program
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1. Statistical analysis of the data from the study of the probability of release of spores (Bacillus subtilis var. niger) from fractured lucite has been completed by Dr. Richard Cornell at Florida State University. The following mathematical model was developed theoretically to relate probability of release, area of surface exposed by fracturing, and the concentration of spores in the lucite.

$$P = 1-e^{-kAC}$$

where P = probability of release of spores from fractured lucite

A = area of surface exposed by fracturing

C = concentration of spores in lucite

k = proportionality constant determined empirically

The model described the data well considering the variables inherent in the experimental procedure. Using a high speed computer and employing the maximum likelihood technique the value of k was estimated for each of four sets of data based on four different areas of exposed surface. Graphical presentations of the experimental data and the curves based on the estimated values of k are provided in figures 1 through 4. The consistency of k based on these data suggested that the overall value of k constituted a valid constant for purposes of estimating probability of release from combinations of spore concentration and surface area exposed by fracturing. This value was calculated to be 3.68×10^{-4} with a standard error of $.078 \times 10^{-4}$

An industrial abrasive unit has been obtained to investigate its possible application in a study of the release of microbial contamination from solids as the result of erosion by airborne abrasives.

2. A promising modification of the Sandia Corporation technique for the microbiological sampling of surfaces with the vacuum probe was developed. The modified technique employs a membrane filter (Gelman, Alpha-6) that withstands insonation and a shortened probe tip and conical section. After sampling a surface, both the filter and the probe tip-conical section unit are aseptically submerged in 200 ml sterile rinse solution and insonated for 2 minutes. The rinse solution is then assayed to determine the number of microorganisms removed from the surface. This technique was compared with the standard strip assay procedure for recovering naturally occurring airborne microbial contamination on the surface of stainless steel fallout strips. Results are presented in table 1. It appeared that the two methods were comparable both in overall recovery and in detecting the variation in natural contamination.

An effort was made to evaluate this probe technique on less variable surface contamination by using strips inoculated with known numbers of Bacillus subtilis var. niger spores in (1) a water aerosol, (2) a pipetted water suspension and (3) a pipetted ethanol suspension. However, the spores could not be adequately removed with the probe. The mean removal

from inoculated strips was only 34% compared to 98% removal of natural contamination. Efforts are now being made to simulate natural particulate fallout tagged with spores of Bacillus subtilis var. niger for the purpose of further evaluating the vacuum probe technique.

3. Efforts to standardize the inoculation, storage and transportation of stainless steel strips for use both as split samples and as biological indicators of the effectiveness of ultrasonic baths in the certification of laboratories were continued. In earlier studies of strips inoculated with aerosolized Bacillus subtillus var. niger spores, an increase with time in the mean number of spores recovered from strips inoculated simultaneously was observed. Similar tests were repeated and on two occasions significantly higher values were observed on day 7 than on day 1. However, on two occasions no significant differences were observed over a 21 day period. In all tests the strips were stored at ambient conditions in aluminum pans covered with aluminum foil. No apparent explanation exists for the inconsistent results.

   A variation of these experiments was repeated by storing stainless steel strips for 14 days in containers that could be used for shipping purposes. The strips were inoculated with an ethanol suspension of Bacillus subtilis var. niger spores using a pipette and stored in (1) sterile covered petri plates, (2) sterile flasks sealed with untreated black rubber stoppers, (3) sterile sealed polyethylene packets and (4) sterile flasks sealed with aluminum foil covered black rubber stoppers. Table 3 presents the results of these experiments. In summary, it was found that (1) storage in petri plates resulted in no significant change in the spore population, (2) storage in flasks sealed with black rubber stoppers resulted in a significant decrease in the spore population, (3) storage in polyethylene packets resulted in a significant decrease in the spore population and (4) storage in flasks sealed with aluminum foil resulted in no significant change in the spore population. Studies are currently underway on a polyethylene mailing tube that contacts the strips only on the edges.

4. Studies were continued on the kinetics of dry heat inactivation of naturally occurring spore populations. As reported previously (Report No. 20), the major factor contributing to polyphasic dry heat survivor curves of naturally occurring spore populations in soils was the presence of two or more individual groups of spores differing in heat resistance. Initial assays of the soils and subsequent dry heat survivor procedures employed thin layers of spore suspensions applied to the surfaces of stainless steel strips which were suspended for various intervals in a forced air oven at 125 C (Report No. 19). A model system of two clean spore isolates from a soil suspension exhibiting a diphasic survivor curve at 125 C (Report No. 20) was used to demonstrate the lack of protective effects by soil (Figs. 5 and 6). Survivor curves of the two isolates in pure cultures of spores were monophasic and unaffected by the presence of soil (Isolate X-1, D_{125C} = 5 min., Isolate XA, D_{125C} = 56 min.). Slight variations in D_{125C} values were noted among runs and were attributed to such factors as plate count error due to spreading growth and also day by day fluctuations in ambient relative humidities during heat processing.

   Current controversy regarding the calculation of dry heat sterilization cycles stems not from the lack of agreement that polyphasic survivor curves do, in fact, exist but disagreement concerning the causes of these curves.

The rationale of some investigators supporting the assumption of logarithmic death in the calculation of sterilization cycles is summarized by R. R. Ernst (Ernst, R. R. 1968, Sterilization by heat, p. 707-708. In C. A. Lawrence and S. S. Block (Ed.), Disinfection, Sterilization and Preservation. Lea and Febiger, Philadelphia). He states,

> "The value of D is indicated, based on the slope of the straight line connecting the starting number (10^6 spores) with the end point (one viable spore or less).
>
> In the actual case a relatively few (represented by 100 in this case) heat-resistant spores were undoubtedly protected in some way in the soil menstruum, since isolates of the same spores were found to be not nearly so heat-resistant as the soil treatments indicated." (See Fig. 7)

Ernst concluded that a D value determined by the point of extinction (end point) of a population would provide "nothing in fact other than the largest possible value for the slope" and "an adequate safety factor for the subsequent extrapolations." We cannot agree with either of these statements. From our findings that the presence of soil has no appreciable effect upon the polyphasic survival character of the mixed populations model system and that naturally occurring spores seem to lose much heat resistance upon subculture, it is concluded that dry heat sterilization cycles may possibly be in error when they are based primarily on the type of extrapolations described by Ernst and others. Consequently, it is suggested that a re-evaluation of the rationale used for the current spacecraft sterilization cycles should be undertaken.

Studies have been undertaken to obtain comparative D_{125C} values of soils and spore isolates from these respective soils in order to substantiate the observation that spores seem to loose much heat resistance upon subculture. Information concerning the magnitude of losses in heat resistance may be derived from such a study. For example if there are somewhat constant or predictable losses in heat resistance among various sporeformers, calculation of sterilization cycles may be approached with greater degrees of confidence. In this vein, profiles of heat resistance at 125 C of sporeformers isolated from spacecraft will be helpful in formulating parameters for sterilization cycles, since the current reference organism, Bacillus subtilis var. niger, does not appear to be the "worst case condition".

In the past, spore crops have been prepared using an "active culture" technique on TAM Sporulation Agar (Difco) supplemented with calcium and magnesium. However, it has been found that the majority of sporeformers isolated from spacecraft show good growth but do not sporulate on this medium. Results of a search for a medium providing optimum sporulation and heat stability of these isolates will be reported later.

5. Studies concerning the use of ultrasonics for recovering microorganisms from surfaces were continued. As reported earlier (Report No. 20), a modification of the Sandia Corporation technique for recovering natural

and artificial contamination from stainless steel strips was evaluated and has been adopted for use in the next edition of the "NASA Standard Procedures". The technique consists essentially of placing a contaminated stainless steel strip face down in a 250 ml Erlenmeyer flask in the presence of 50 ml of sterile rinse fluid. The container is then insonated for 2 min while being suspended in the middle of a full ultrasonic bath. It was concluded that this method, from the standpoint of removal, is significantly better than the previous procedure (Report No. 20).

In order to allow a certain degree of flexibility with respect to the container used in the above mentioned procedure, a study was conducted to determine if using 125 ml in place of 250 ml Erlenmeyer flasks had any appreciable effects on the assay. Stainless steel strips (1" x 2") were inoculated with an ethanol suspension of B. subtilis var. niger spores (ca. 3×10^2 spores per strip) and stored in vacuo over silica gel for 16 hr. Sets of strips were insonated 2 min in 125 and 250 ml flasks employing the "single-suspended" method with 0.02% Tween 80 in buffered distilled water as the rinse fluid. After insonation, strips were aseptically removed from the flasks, gently rinsed with sterile distilled water and plated with TSA. Entire 50 ml portions of rinse fluid were plated with double strength TSA. Colony counts were made on strips at 24 hr and on plates after 48 hr incubation at 32 C. Results showed removal in both types of vessels to be 99.8% and not significantly different ($P > 0.5$).

In the assay procedures described in the newly revised "NASA Standard Procedures", rinse solutions from samplings of stainless steel fallout strips and environmental surfaces are pour-plated in various dilutions in order to obtain countable plates. Variations in agar and nutrient concentration are often considerable. Consequently, a study was undertaken to assess the effects of representative TSA concentrations upon plate counts of natural (fallout) and artificial (B. subtilis var. niger spores) contamination. Concentrations, experimental design, and results are shown in table 4 One ml portions of the rinse fluids and appropriate amounts of diluent and TSA were added to petri plates, and the plates were swirled to distribute the inocula evenly. After the plates inoculated with naturally occurring contamination had solidified they were overlayed with regular strength TSA (5 ml for 100 mm plates and 15 ml for 150 mm plates). Incubation was at 32 C and counts were made at 24 and 48 hr. Results showed the varied concentrations of TSA to have no significant effect upon plate counts of natural or artificial contamination. The plates containing naturally occurring contamination seemed to have less spreading growth as the TSA concentration increased.

6. The bioclean room met performance specifications during a one-week test period and was accepted from Westinghouse Corporation. A pass-through autoclave is currently being installed. Upon completion of this modification the room will be placed into service.

7. Studies on the levels of microbial contamination in the intramural environments of the Hangar AO cleanroom, area 60-A sterilization and assembly laboratory and the Surveyor fuel loading room were terminated in December.

A complete listing of the microbial contamination detected on the surfaces of the Surveyor 7 spacecraft, Adapter, Shroud, Altitude Marking Radar (AMR) and

the Retro rocket is shown in table 5. An 89 percent reduction in aerobic mesophilic microorganisms were noted after final cleaning. Table 6 shows the types of aerobic mesophilic microorganisms isolated from the surface of the Surveyor 7 spacecraft.

Studies were continued in the Apollo facilities. Two new areas, the Lunar Module and Command Module High Altitude Test Chambers, were added to those reported in the last quarterly report (Report No. 20). The Apollo 6 spacecraft was sampled for microbial contamination in all intramural environments that it was exposed to during assembly and testing. The types of microorganisms isolated on exterior and interior surfaces of the Command module (spacecraft 20) are shown in table 7. Results obtained from studying the service module are shown in table 8. Table 9 shows levels of aerobic and anaerobic spores found on the exterior of the command module as it was moved from one environmental area to another. An increase in the number of spores recovered was noted as the spacecraft moved from the Manned Spacecraft Operations Building to Launch Complex 39A. A representative number of microorganisms isolated from the command and service modules were gram stained and observed microscopically. Results are shown in table 10. A comparison of microbial contamination detected on the surfaces of the Apollo command and service modules and Surveyor spacecraft 5, 6 and 7 are shown in table 11. The number and percentage of molds isolated from exterior and interior surfaces of the command module and from the exterior surface of the service module are shown in tables 12 and 13. An increase in percentage of molds isolated in sample number 3 could have been due to the moving of the command and service modules to Integrated Test Stand #2 where the two modules were mated.

Four rooms were assigned to the Sterility Control Laboratory in the Vehicle Assembly Building (VAB). These rooms will be converted into a microbiological assay laboratory. Two trailer sites also were assigned near the VAB and at Launch Complex 39A. Modifications of the trailer laboratory for a mobile microbiological assay laboratory are progressing and should be completed by the next quarter.

TABLE 1. COMPARISON OF THE STANDARD STRIP ASSAY PROCEDURE WITH THE VACUUM PROBE IN RECOVERY OF NATURALLY OCCURRING AIRBORNE MICROBIAL CONTAMINATION FROM SURFACES OF STAINLESS STEEL FALLOUT STRIPS

Experiment No.	No. of Strips	Standard Strip Assay Procedure		Vacuum Probe	
		Mean No. Microorganisms Recovered	Coefficient of Variation	Mean No. Microorganisms Recovered	Coefficient of Variation
1	6	278	53%	473	44%
2	8	988	72%	620	53%
3	9	61	59%	114	50%
4	8	337	42%	226	93%
5	9	111	59%	143	55%
Overall	40	355	57%	315	59%

TABLE 2. CHANGES WITH TIME IN BACILLUS SUBTILIS VAR. NIGER SPORE POPULATIONS ON STAINLESS STEEL STRIPS STORED UNDER AMBIENT CONDITIONS

Experiment No.	Mean No. Spores Per Strip (10 Strips Per Experiment)				
	Day 1	Day 3	Day 7	Day 14	Day 21
1	176	- -	216[1]	- -	- -
2	332	328	368[2]	- -	- -
3	527	- -	524	530	475[3]
4	118	- -	115	115	102[3]

[1] 216 significantly higher than 176 ($P < .05$).

[2] 368 significantly higher than 332 and 328 ($P < .05$).

[3] No significant differences between values.

TABLE 3. CHANGES IN *BACILLUS SUBTILIS* VAR. *NIGER* SPORE POPULATIONS ON STAINLESS STEEL STRIPS STORED FOR 14 DAYS IN VARIOUS CONTAINERS

Experiment No.	Mean No. Spores Per Strip (10 Strips Per Experiment)				
	Initial Inoculum	Storage in Petri Plates	Storage in Flasks w/Black Rubber Stoppers	Storage in Polyethylene Packets	Storage in Flasks w/Aluminum Covered Stoppers
1	1002	1040	875	639	- -
2	356	356	235	232	- -
3	339	356	- -	278	322

TABLE 4. EFFECTS OF AGAR AND NUTRIENT CONCENTRATIONS ON PLATE COUNTS OF NATURAL AND ARTIFICIAL CONTAMINATION

Rinse Fluid[1] (ml)	Sterile Diluent[2] (ml)	Single Strength TSA[3] (ml)	Double Strength TSA[4] (ml)	TSA Concentration[5]	Mean Plate Count[6]	Mean Plate Count[7]
1	0	20	0	95%	179	38
1	9	30	0	75%	173	40
1	24	0	50	133%	181	39

[1] Natural Contamination: Three fallout strips were placed in a 250 ml Erlenmeyer flask with 25 ml of sterile 0.02% Tween 80 in buffered distilled water (pH 7.2). The flask was shaken vigorously for 2 min, the strips were aseptically removed, and the rinse fluid was insonated for 12 min to break up clumps of microorganisms. On milliliter portions were distributed to each petri plate before adding the appropriate amount of diluent. Replicate series of 8 were run for each TSA concentration.

Artificial Contamination: One milliliter portions of a 0.02% Tween 80 in buffered distilled water suspension of B. subtilis var. niger spores (ca. 1.8×10^2 spores/ml) were distributed to each petri plate before adding the appropriate amount of diluent. Replicate series of 10 were run with each TSA concentration.

[2] 0.02% v/v Tween 80 (polyoxyethylene sorbitan monooleate) in buffered distilled water, final pH 7.2± .1.

[3] Trypticase Soy Agar, molten (50C).

[4] Same as (3) except twice the usual amount of TSA per volume of distilled water, molten (50C). For this part of the test 150 mm petri plates were used.

[5] Assuming the concentration of single strength TSA to be 100%.

[6] B. subtilis var. niger, 48 hr incubation at 32 C.

[7] Natural contamination, 48 hr incubation at 32 C.

TABLE 5. MICROBIAL CONTAMINATION DETECTED ON THE SURFACES OF THE SURVEYOR 7 SPACECRAFT, ADAPTER, SHROUD, AMR[1], AND RETRO ROCKET

Part Sampled	Date	Area[2] sq. in	Aerobes	Anaerobes	Aerobic spores	Anaerobic spores
Spacecraft	11-13-67	80	25	10	0	10
Spacecraft	11-27-67	80	670	30	20	5
Adapter	11-27-67	40	260	50	25	10
Shroud	11-27-67	32	70	50	5	0
Spacecraft	12-01-67	80	690	180	80	5
Adapter	12-01-67	40	365	45	45	10
Shroud	12-01-67	32	5	0	10	0
AMR[1]	12-21-67	80	845	205	25	0
Retro Rocket	12-21-67	68	7280	1610	35	0
(Prior to Final Cleaning)						
Spacecraft	12-30-67	80	1565	795	85	45
Adapter	12-30-67	40	2040	350	110	15
Shroud	12-30-67	32	130	30	0	0
(After Final Cleaning)						
Spacecraft	1-02-68	80	175	5	0	0
Adapter	1-02-68	40	780	145	20	0
Shroud	1-02-68	28	10	10	5	0

[1] AMR - Altitude Marking Radar

[2] Swab-Rinse Technique

TABLE 6. TYPES OF AEROBIC MESOPHILIC MICROORGANISMS ISOLATED FROM THE SURFACE OF THE SURVEYOR 7 SPACECRAFT

Types of Microorganisms	Number	Percent
Staphylococcus epidermidis	81	45.0
Staphylococcus aureus	10	5.6
Micrococcus spp.	16	8.9
Bacillus spp. (spore-formers)	12	6.7
Brevibacterium-Corynebacterium group	23	12.8
Gram Negative *Bacilli*	13	7.2
Molds	18	10.0
Yeast	1	0.6
Actenomycetes	1	0.6
Miscellaneous	5	2.8
TOTAL	180	

TABLE 7. MICROBIAL CONTAMINATION ISOLATED ON EXTERIOR AND INTERIOR SURFACES OF THE APOLLO COMMAND MODULE (SPACECRAFT 20)

Source	Date	Area[1] (Sq. In.)	Aerobes	Anaerobes	Aerobic Spores	Anaerobic Spores
Exterior[2]	12-1-67	80	3655	470	40	40
Exterior[2]	12-6-67	80	5850	705	75	45
Exterior[2]	1-3-68	80	1265	265	310	55
Exterior[3]	1-26-68	80	2225	1055	65	35
Exterior[4]	2-15-68	68	6330	1205	180	15
Exterior[4]	2-29-68	76	16860	2280	265	55
Exterior[4]	3-22-68	80	6545	2280	340	155
Interior[3]	1-11-68	80	1740	3675	40	5
Interior[3]	1-26-68	80	5585	2005	50	5
Interior[4]	2-15-68	68	3350	2265	20	5

[1] Swab-rinse technique.

[2] Samples taken while command module was located in the Manned Spacecraft Operations Building.

[3] Samples taken while command module was located in the Vehicle Assembly Building.

[4] Samples taken while command module was located at Launch Comples 39A.

TABLE 8. MICROBIAL CONTAMINATION ISOLATED ON SURFACES OF THE APOLLO SERVICE MODULE (SPACECRAFT 20)

Date	Area[1] (Sq. In.)	Aerobes	Anaerobes	Aerobic Spores	Anaerobic Spores
12-1-[illegible][2]	136	6596	2355	75	65
12-[illegible]-67[2]	140	8530	3365	110	75
1-3-68[2]	136	7760	3040	540	195
1-18-68[3]	140	6890	2055	380	145
2-1-68[3]	80	2810	1080	105	65
2-23-68[4]	80	3505	1025	120	35
3-8-68[4]	80	3730	1470	115	30

[1] Swab-rinse technique.

[2] Samples taken while service module was located in the Manned Spacecraft Operations Building.

[3] Samples taken while service module was located in the Vehicle Assembly Building.

[4] Samples taken while service module was located at Launch Complex 39A.

TABLE 9. COMPARISON OF THE AEROBIC AND ANAEROBIC SPORES RECOVERED FROM THE SURFACE OF THE COMMAND MODULE WHILE LOCATED IN VARIOUS AREAS

Source	Date Sampled	Area[1] (Sq. In.)	Spores Per Square Inch		TOTAL SPORES PER SQ IN
			Aerobic Spores	Anaerobic Spores	
Exterior[2]	12-1-67	80	0.50	0.50	1.0
Exterior[2]	12-6-67	80	0.94	0.55	1.49
Exterior[2]	1-3-68	80	3.87	0.69	4.56
Exterior[3]	1-26-68	80	0.81	0.44	1.25
Exterior[4]	2-15-68	68	2.65	0.22	2.87
Exterior[4]	2-29-68	76	3.49	0.72	4.21
Exterior[4]	3-22-68	80	4.25	1.94	6.19

[1] Swab-rinse technique.

[2] Samples taken while command module was located in the Manned Spacecraft Operations Building.

[3] Samples taken while command module was located in the Vehicle Assembly Building.

[4] Samples taken while command module was located at Launch Complex 39A.

TABLE 10. STAINING AND MORPHOLOGICAL CHARACTERISTICS OF MICROORGANISMS ISOLATED FROM THE SURFACES OF THE APOLLO COMMAND AND SERVICE MODULES

Spacecraft	Number	Staining and Morphology	Percent
Apollo Command and Service Modules[1]	207	Gram Positive cocci	60.4
		Gram Positive rods	10.1
		Gram Negative rods	6.3
		Actinomycetes	1.0
		Yeast	2.4
		Molds	19.8
Apollo Command Module[2]	159	Gram Positive cocci	56.0
		Gram Positive rods	25.2
		Gram Negative rods	13.2
		Actinomycetes	0.0
		Yeasts	0.6
		Molds	5.0

[1] Spacecraft was located at Manned Spacecraft Operations Building.

[2] Spacecraft was located at Launch Complex 39A.

TABLE 11. COMPARISON OF MICROBIAL CONTAMINATION DETECTED ON SURFACES OF THE APOLLO COMMAND AND SERVICE MODULES AND SURVEYORS 5, 6 & 7

Spacecraft	Area[1] Sampled (Sq. In.)	Microorganisms Per Square Inch			
		Aerobes	Anaerobes	Aerobic Spores	Anaerobic Spores
Apollo					
Command Module (Exterior)	544	78.6	15.2	2.3	0.7
Command Module (Interior)	228	46.8	34.8	0.5	0.07
Service Module	792	50.3	18.2	1.8	0.8
Surveyor 5	320	28.8	7.1	0.3	0.09
Surveyor 6	240	20.0	1.0	0.4	0.0
Surveyor 7	320	9.1	3.2	0.6	0.2

[1] Swab-rinse technique.

TABLE 12. MOLD CONTAMINATION ISOLATED ON EXTERIOR AND INTERIOR SURFACES OF APOLLO COMMAND MODULE (SPACECRAFT 20)

Sample No.	Source	Date	Area[1] Sampled (sq. in.)	Molds	No. Per sq. in.	Percent[5]
1	Exterior[2]	12-1-67	80	40	0.50	1.09
2	Exterior[2]	12-6-67	80	20	0.25	0.30
3	Exterior[2]	1-3-68	80	30	0.38	2.37
4	Exterior[3]	1-26-68	80	35	0.44	1.57
5	Exterior[4]	2-15-68	68	30	0.44	0.47
6	Exterior[4]	2-29-68	76	60	0.79	0.36
7	Exterior[4]	3-22-68	80	50	0.63	0.76
1	Interior[3]	1-11-68	80	10	0.13	0.57
2	Interior[3]	1-26-68	80	10	0.13	0.18
3	Interior[4]	2-15-68	68	15	0.22	0.45

[1] Swab-rinse technique.

[2] Samples taken while command module was located in the Manned Spacecraft Operations Building.

[3] Samples taken while command module was located in the Vehicle Assembly Building.

[4] Samples taken while command module was located at Launch Complex 39A.

[5] Percentage of aerobic mesophilic microorganisms.

TABLE 13. MOLD CONTAMINATION ISOLATED ON SURFACES OF THE APOLLO SERVICE MODULE (SPACECRAFT 20)

Sample No.	Date	Area[1] Sampled (sq. in.)	Molds	No. Per sq. in	Percent[5]
1	12-1-67	136	50	0.36	0.76
2	12-6-67	140	15	0.11	0.18
3	1-3-68	136	245	1.80	3.16
4	1-18-68	140	290	2.07	4.21
5	2-1-68	80	5	0.06	0.18
6	2-23-68	80	100	1.25	2.85
7	3-8-68	80	10	0.13	0.27

[1] Swab-rinse technique.

[2] Samples taken while service module was located in the Manned Spacecraft Operations Building.

[3] Samples taken while service module was located in the Vehicle Assembly Building.

[4] Samples taken while service module was located at Launch Complex 39A.

[5] Percentage of aerobic mesophilic microorganisms.

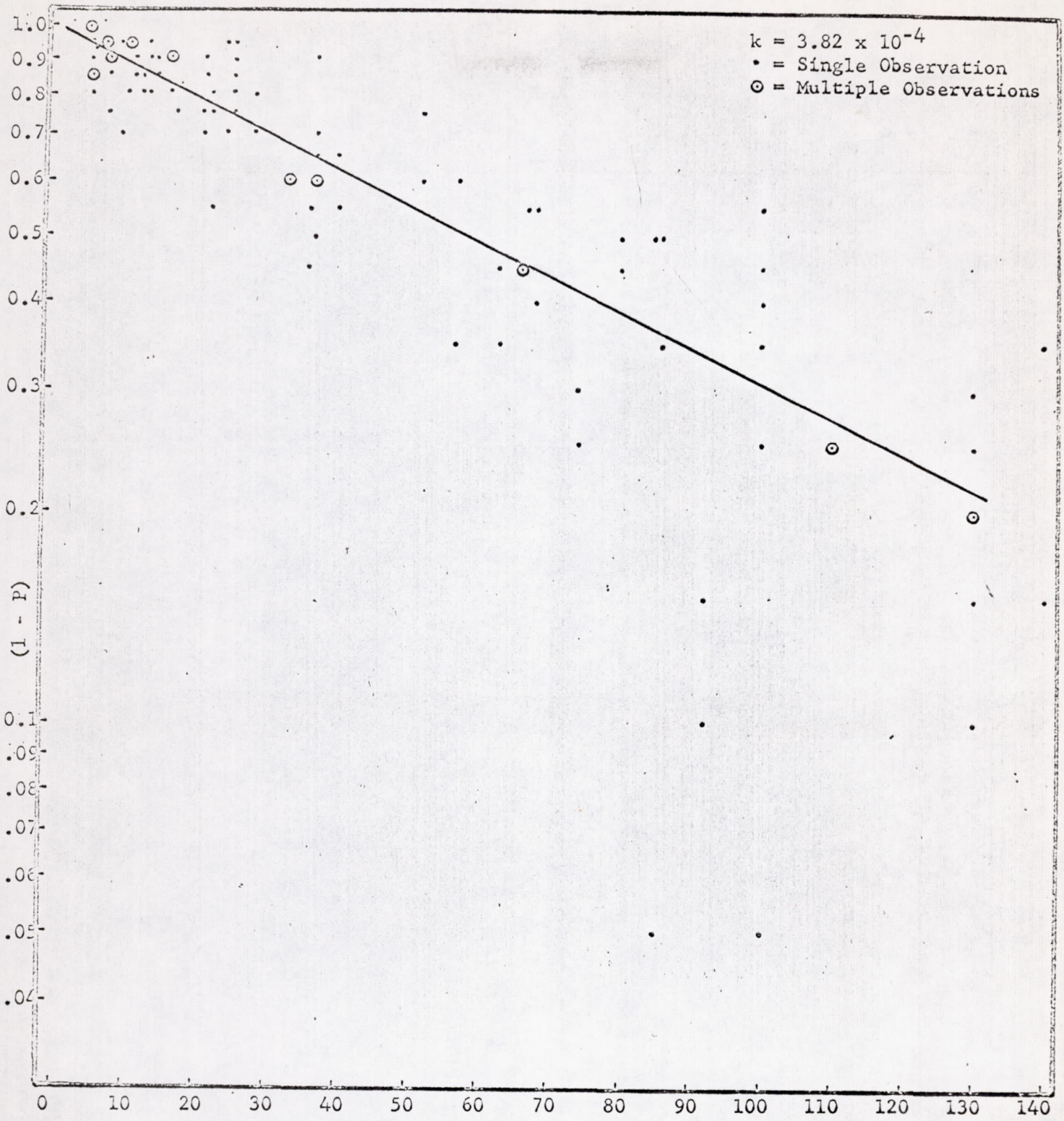

FIGURE 1. SPORE CONCENTRATION (spores/gm. lucite x 100)

EXPERIMENTAL RESULTS WITH 30.6 mm^2 SURFACE AREA EXPOSED BY FRACTURING

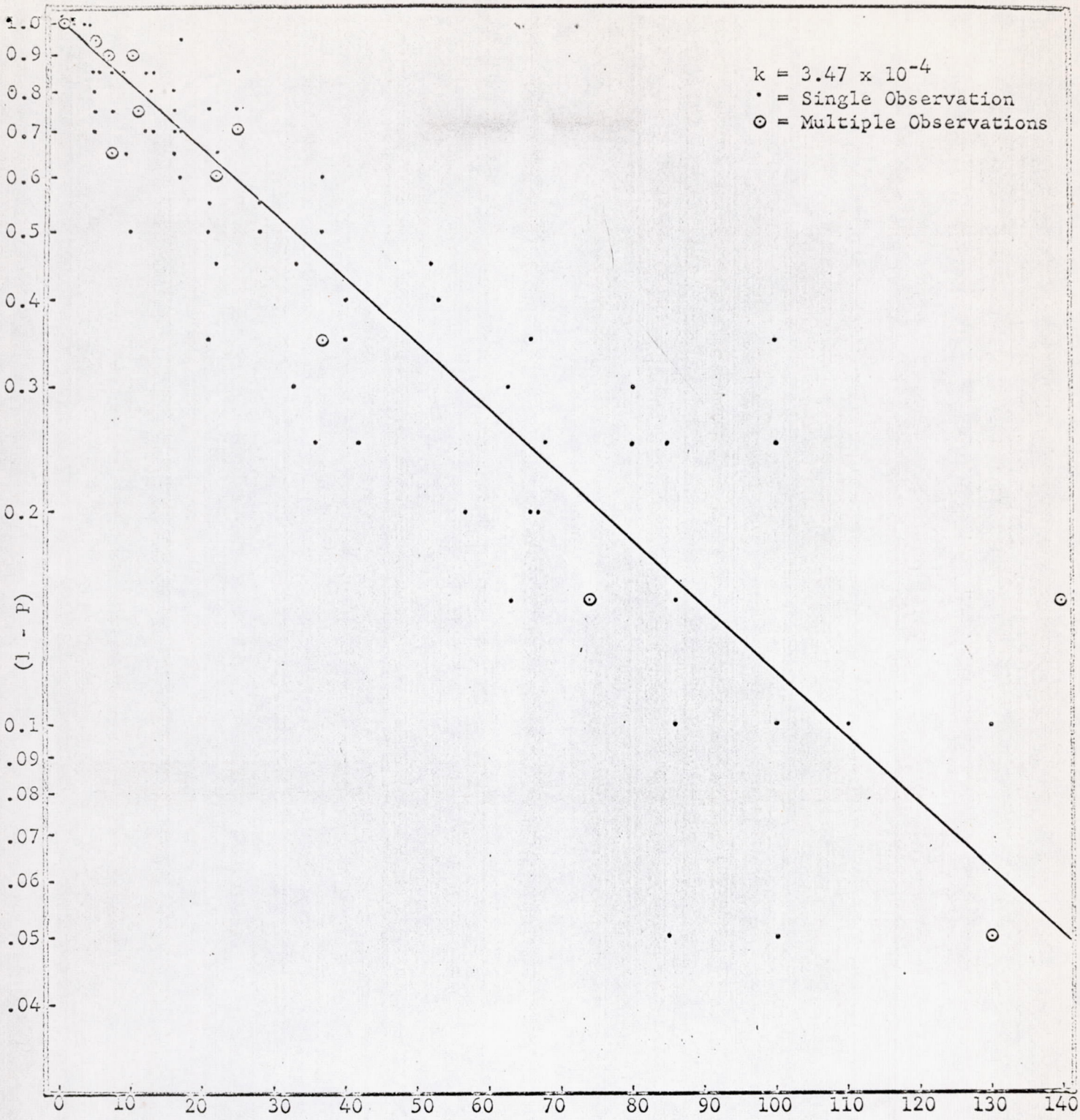

FIGURE 2. SPORE CONCENTRATION (spores/gm. lucite x 100)

EXPERIMENTAL RESULTS WITH 61.2 mm^2 SURFACE AREA EXPOSED BY FRACTURING

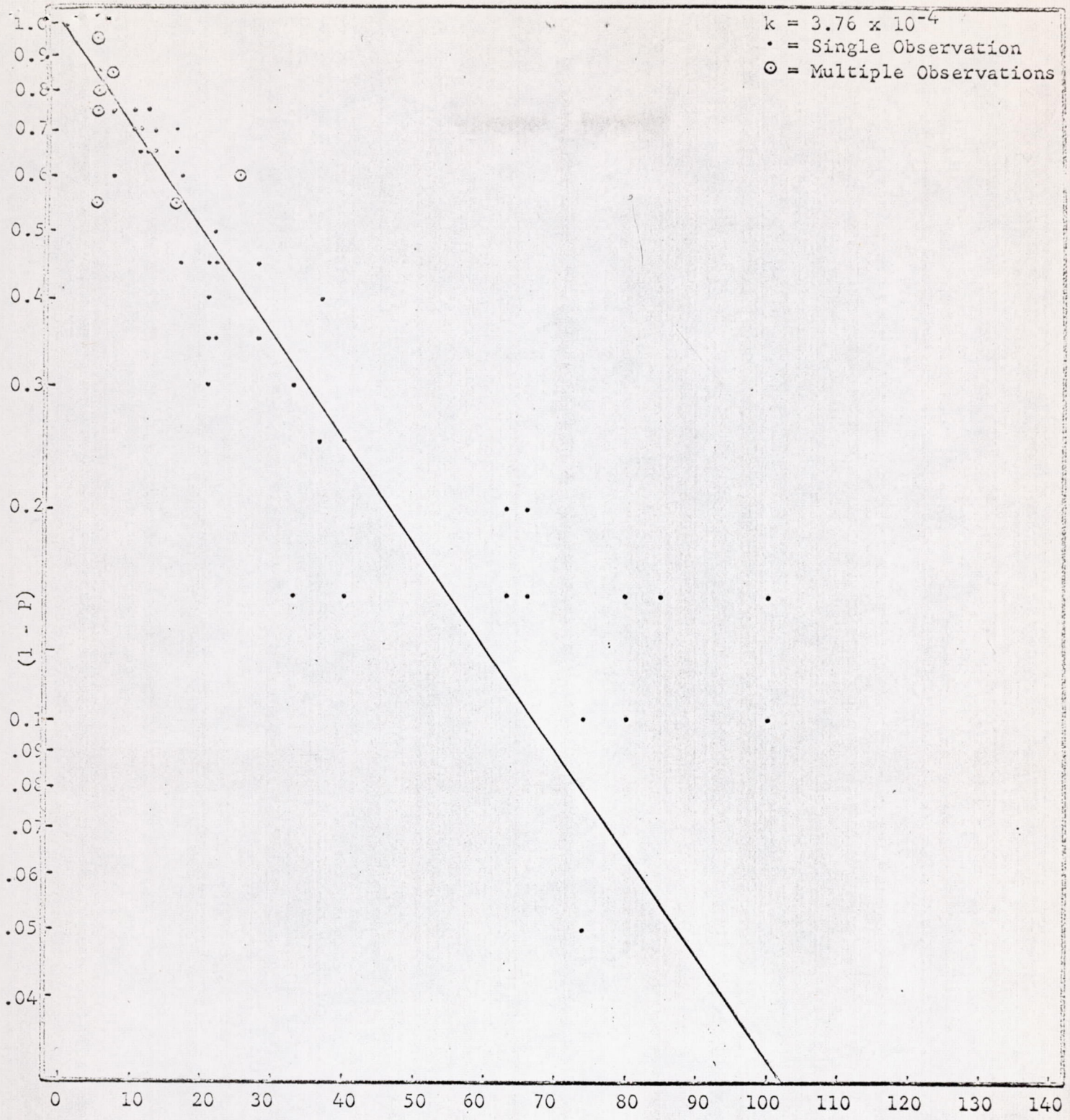

FIGURE 3. SPORE CONCENTRATION (spores/gm. lucite x 100)

EXPERIMENTAL RESULTS WITH 91.8 mm^2 SURFACE AREA EXPOSED BY FRACTURING

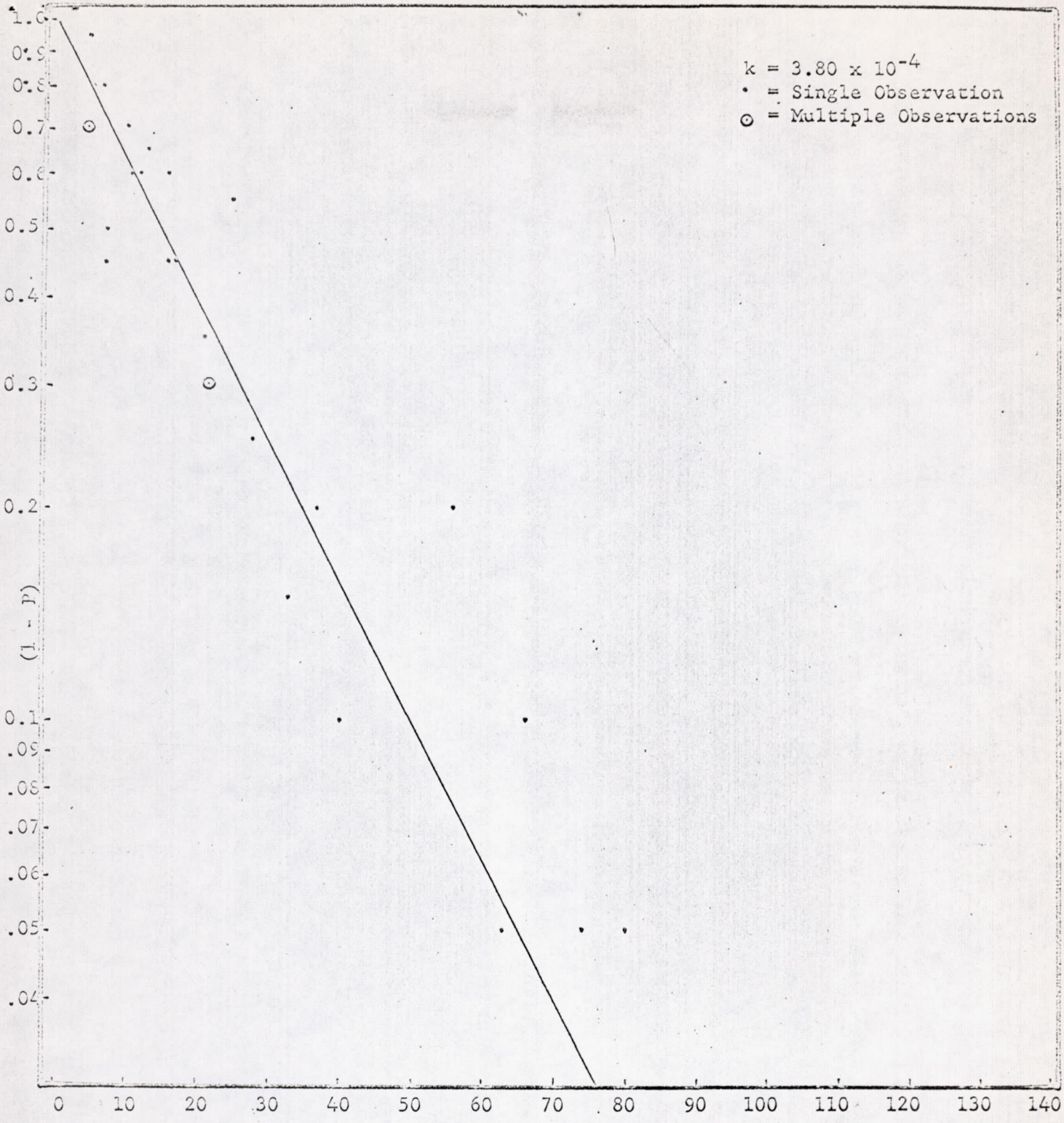

FIGURE 4. SPORE CONCENTRATION (spores/gm. lucite x 100)

EXPERIMENTAL RESULTS WITH 122.4 mm^2 SURFACE AREA EXPOSED BY FRACTURING

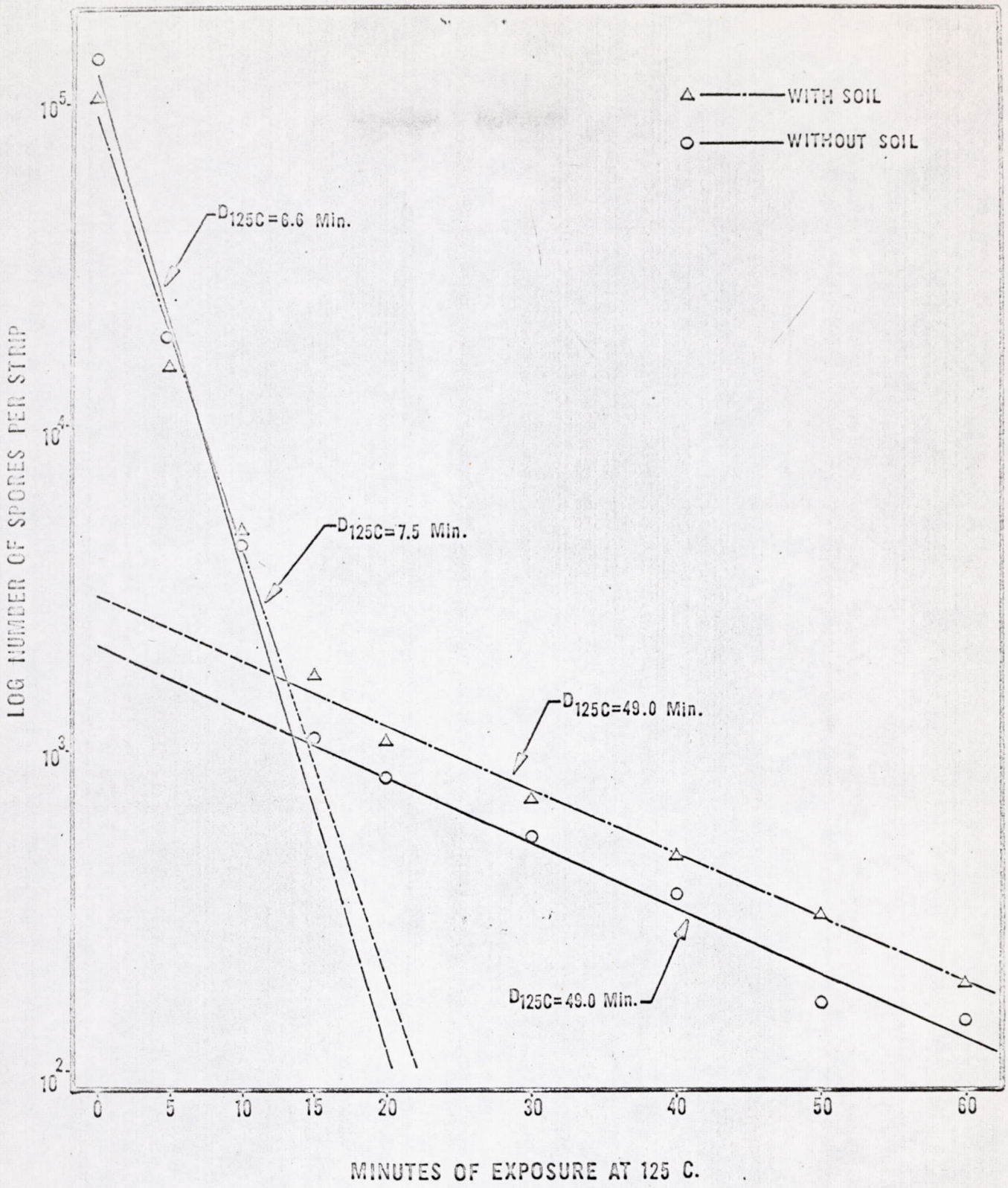

FIGURE 5. DIPHASIC SURVIVOR CURVE OF A MIXTURE OF ISOLATE X-1 AND ISOLATE XA SPORES IN THE PRESENCE AND ABSENCE OF PARENT SOIL. EXPERIMENT 1.

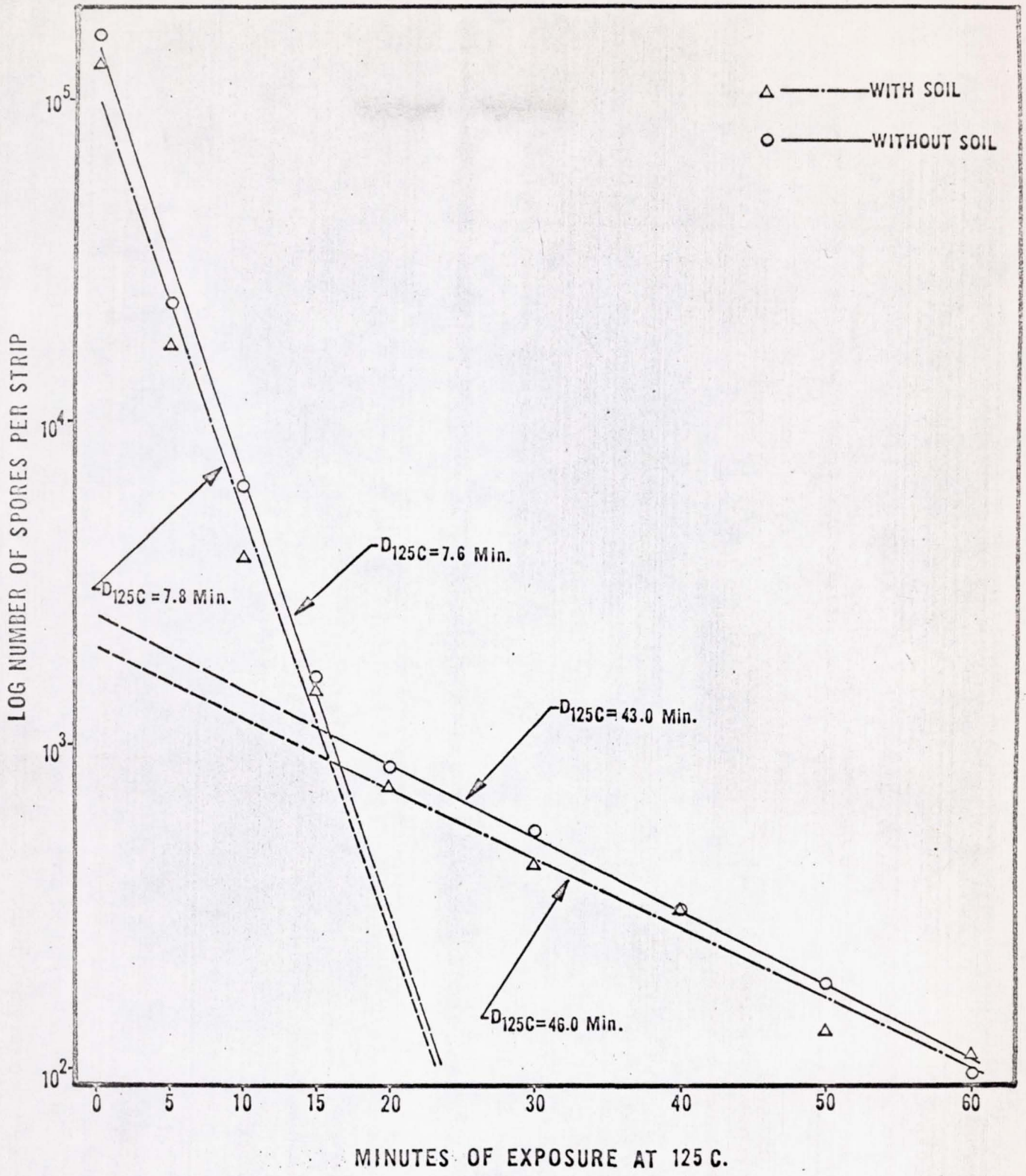

FIGURE 6. DIPHASIC SURVIVOR CURVE OF A MIXTURE OF ISOLATE X-1 AND ISOLATE XA SPORES IN THE PRESENCE AND ABSENCE OF PARENT SOIL. EXPERIMENT 2.

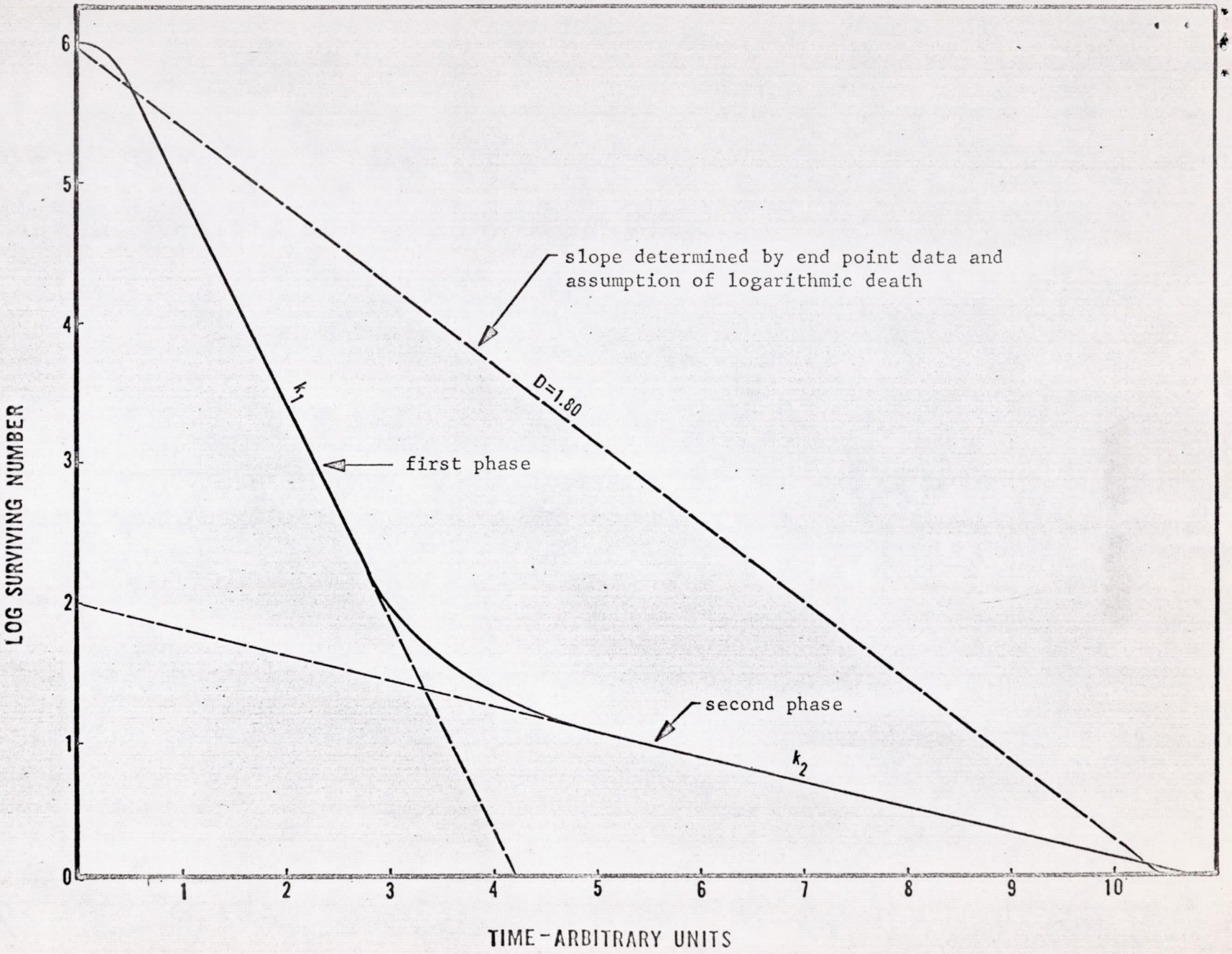

FIGURE 7. DEATH OF NATURALLY OCCURRING SPORES IN SOIL BASED ON ACTUAL DATA, R. R. ERNST, 1968 (SEE COMPLETE REFERENCE IN TEXT).