BACTERIAL DECAY IN SEWAGE TREATMENT PLANT EFFLUENT

by

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APPROVAL BY THESIS DIRECTOR

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Robert A. Phillips — August 14, 1967

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ABSTRACT

Bacterial decay in an activated sludge effluent was studied with the intent of obtaining the following information: effect of temperature on bacterial decay; effect of presence or absence of oxygen on bacterial decay; relative death rates in various bacterial groups; importance of natural decay in the reduction of bacteria by soil filtration; comparison of bacterial reductions in laboratory-scale experiments with those obtained in field oxidation ponds.

Decay curves for coliforms, fecal coliforms, and fecal streptococci were obtained using the membrane filtration technique. Increased decay rates were noted for higher temperatures and also for aerobic conditions. At 20°C and with oxygen (conditions similar to those found in field studies) the relative decay rate was fecal streptococci > coliform > fecal coliform. Natural decay was shown to account for greater than one-half the order of magnitude of the bacterial reduction during soil filtration. Laboratory studies of bacterial decay are shown to be similar to field studies of oxidation ponds.

CHAPTER 1

INTRODUCTION

Statement of the Problem

In areas where sources of fresh water have been depleted, particularly in the semi-arid Southwest, attention has been focused on methods of sewage reclamation as a possible source of water. After adequate treatment this water could then be used for irrigation of edible crops, for recreational purposes, and as a source of domestic water supply. The reclamation methods usually are tertiary treatment methods--that is, additional treatment of a sewage plant effluent which has been subjected to a biological method of stabilization.

One of the most economical methods of tertiary treatment, where land costs are not great, is soil filtration. Effluent from the conventional sewage treatment plant is allowed to percolate through a porous soil, where various chemical and bacteriological transformations occur.

A primary purpose of tertiary treatment is the reduction of the disease-causing organisms in the water. In soil filtration, bacteria primarily are reduced by two mechanisms: adsorption or sedimentation of the bacteria onto the soil particles, and natural bacterial decay.

The purpose of this study is to investigate the decay rates of certain groups of bacteria, and to determine how important these natural death rates are to the overall bacterial reduction which occurs during soil filtration.

Scope of the Problem

Laboratory studies were made on samples of activated sludge effluent. The bacteria groups chosen to be monitored were coliforms, fecal coliforms, and fecal streptococci. Membrane filtration was the method of choice for the bacteriological studies, as it provided a direct enumeration of bacterial numbers in a relatively short time.

The investigations were limited to the following points:

(1) A comparison of the decay rates of the three groups.

(2) The effect of temperature on bacterial decay.

(3) The effects of the presence or absence of oxygen on bacterial decay.

(4) An estimate of the importance of natural decay in the reduction of bacteria by soil filtration.

(5) A comparison of the laboratory study results with those results obtained in oxidation pond studies.

Objectives of the Research

It is hoped that this study will contribute to the knowledge required for:

(1) An evaluation of the possibility of greater use of fecal coliforms and fecal streptococci as indicators of pollution.

(2) Evaluations of soil filtration methods in regard to bacterial removal.

CHAPTER 2

DEFINITIONS

For purposes of limiting unnecessary repetition and avoidance of misunderstandings in this text, some of the terms used are defined (1) (2) (3) or explained as follows:

(1) Algae - the group of microscopic plants having photosynthetic chlorophyll, but lacking definite stems and leaves.

(2) Bacteria - the group of microscopic, unicellular plants, lacking chlorophyll, having no well-defined nucleus, and reproducing by transverse fission.

(3) Coliforms - a group of bacteria including all of the aerobic and facultative anaerobic, Gram-negative, non-sporeforming, rod-shaped bacilli which ferment lactose with gas formation within 48 $\pm$ 3 hours at 35 $\pm$ 0.5°C.

(4) Enzyme - a ferment or cell secretion that acts as a catalyst in speeding or slowing chemical reactions.

(5) Fecal coliforms - a group of bacteria comprising that portion of the coliform group which is characteristically an inhabitant of human and animal intestinal tracts.

(6) Fecal streptococci - a group of bacteria, Gram-positive, spherical, generally occurring in pairs or short chains and characteristically an inhabitant of human and animal intestinal tracts.

(7) Gram-negative - refers to the characteristic of an organism which upon staining by Gram's method takes the color of the counterstain; that is, does not retain the purple color of the crystal violet.

(8) Gram-positive - a characteristic which enables an organism upon staining with Gram's method to retain the purple color of crystal violet in spite of decoloring procedures.

(9) Membrane filter - flat, highly porous, flexible plastic discs about 0.15 millimeters in thickness, with pore size controlled within narrow limits.

(10) Microorganism - any small living plant or animal, microscopic in size.

(11) MFC - membrane filter count, an enumeration of bacteria in a given group as determined by the membrane filtration method.

(12) MPN - most probable number, the number of bacteria in a given group as determined by applying the laws of probability to the results of serial-dilution, multiple tube tests.

(13) Oxidation pond - a large shallow basin into which raw or secondary wastewaters are added and become stabilized through natural biological action. A detention period of 20 to 30 days is normally provided. The terms "stabilization pond" and "sewage lagoon" are often-used synonyms.

(14) Phage - virus particles capable of multiplying within young bacterial cells and thus causing their destruction.

(15) Protozoa - a group of microscopic, unicellular animals, devoid of chlorophyll, and capable of ingesting such solid food as plant cells, bacteria, and other protozoa.

CHAPTER 3

PREVIOUS INVESTIGATIONS

For many years, the bacteriological method of choice for indicating water pollution by excreta of warm-blooded animals has been the fermentation tube technique for the coliform group. The results of this method are used to obtain a Most Probable Number (MPN) value for the water in question. However, there are two major drawbacks in this measure of pollution: certainty that the results indicate harmful pollution, and laboratory time requirements to complete the analysis.

It has been shown by Clark and Kabler (4) that coliform group bacteria "are found in the feces of warm-blooded animals, in the guts of cold-blooded animals, in soils, and on many plants." Kabler and Clark (5) discussed the natural sources and habitats of the coliform group, indicating that Escherichia coli is normally an inhabitant of human and animal intestines, while Aerobacter aerogenes can also be found on vegetation and in the soil. Subgroups of A. aerogenes may be found in feces, but normally in much fewer numbers than E. coli. The fermentation tube technique, as routinely used, does not differentiate between the "fecal" and "non-fecal" types of coliform bacteria. Other groups have been investigated in recent years. Fecal coliforms and fecal streptococci are two groups which have received considerable attention as being more indicative of fecal contamination. Media have been devised (6) (7) which are very selective in the enumeration of these two groups. Since these

groups are restricted primarily to a fecal habitat, it would seem that they would be of greater use as indicators of pollution than would the coliform group.

The acceptance of the fecal coliforms and fecal streptococci as indicators of pollution is hindered because of two major limitations:

(1) The numbers of these organisms in feces as compared to coliform bacteria are small, in the order of one to ten percent of the coliforms present.

(2) The relationship between the disappearance of these organisms as compared to that of enteric pathogens during natural self-purification processes or treatment practices has not been studied sufficiently at this time.

However, the two groups are useful for supplementing coliform data and are a valuable tool in research. Since the presence of coliforms, either fecal or non-fecal in origin, "renders the water potentially unsatisfactory and of unsafe sanitary quality" (2), this group is the one officially used for evaluation of water for potability.

The relatively long time required (two to four days) to obtain results from the fermentation tube method made it desirable to investigate other methods of bacteriological analysis of water. In the last fifteen years, the membrane filter technique has gained a wide degree of popularity. In 1955 a tentative method for coliform analysis by membrane filtration was included in "Standard Methods" (2), and in 1960 the method became official.

Advantages of the membrane filtration method, as stated by Clark et al. (8), are availability of data in 24 hours (48 hours in the case of fecal streptococci), and reduction of space, material, equipment, and labor. In addition, larger and therefore more representative samples can be used. The Membrane Filter Count (MFC) method gives a greater reproducibility than does the fermentation tube method as shown by Hoffman et al. (9). These authors also indicate that "MFC gave results essentially equal to or slightly higher than MPN results for settled, unchlorinated sewage and for sewage-contaminated waters." Limitations on the method are caused by flocs, suspended solids, and algae hindering filtration and colony growth.

A comprehensive search of the literature revealed that no studies were available concerning bacterial death rates in activated sludge effluent. Bacterial reductions in polluted waters have been studied, and the literature is replete with references to oxidation pond studies. Studies have also been made with regards to causes of bacterial decay.

Kittrell and Furfari (10) listed among the causes of decrease in bacterial numbers a change in nutrient balance, bacterial predators such as protozoa, and toxic metabolic (bacterial) products. Carlucci and Pramer (11) demonstrated that phages were active against coliform bacteria in sea water. An increase in temperature has also been shown to increase the rate of bacterial decay, due to more rapid metabolic processes.

In studies of polluted waters, Streeter (12) noted an initial increase in bacterial numbers followed by a continual decrease. Also

the decrease was greater when sedimentation of stream suspended solids was allowed. Kittrell and Furfari (10) presented evidence that this may be an actual increase in bacteria as well as an apparent increase due to dispersion of bacterial clumps. Frost and Streeter (13) in a study of the Ohio River, observed that the bacterial decay rate decreased with increasing time, but that the curve could be considered as "the combined result of two fractions of the coliform population decreasing at individual rates described by straight lines on semi-log paper." Burman (14) found that in shallow wells, there was a tendency for streptococci to outlive E. coli, and in water mains an even greater tendency. In a laboratory study of a one percent dilution of sewage, Hanes et al. (15) found that in a comparison of decay rates at low, normal and high dissolved oxygen, the samples with low dissolved oxygen had lower decay rates, and that at the same dissolved oxygen, fecal streptococci had higher decay rates than did coliforms. Mallman and Mack (16) stated that in ground water, both E. coli and A. aerogenes survived for up to four years, and "retained their cultural, morphological and physiological characteristics throughout the experiments."

Bacterial decay in oxidation ponds, even though occurring under conditions of sunlight and algae growth, can be compared to the laboratory study in regard to overall removal efficiencies. In studies of aerobic stabilization ponds in Wisconsin, Mackenthum and McNabb (17) found a reduction in coliform organisms greater than 98 percent more than 87 percent of the time, and noted a greater reduction in summer than winter. Parker (18), studying multi-unit stabilization ponds following

high-rate trickling filters, found reductions of 99.97 percent for E. coli and 99.95 percent for Streptococcus faecalis during a 7.3 day retention time. Little difference in bacterial counts between summer and winter was observed. A 99.98 percent removal of coliforms was measured by Neel et al. (19) for a raw sewage lagoon with a detention time of 17 days, while in a laboratory study of a well-baffled oxidation pond Malina and Yousef (20) noted a 99.4 percent reduction in coliforms. In an evaluation of the available literature, Fitzgerald and Rohlich (21) stated that "in nearly all instances bacterial counts have been lowered to less than 1 percent of the original concentration" in stabilization ponds. In addition, they found no basis for the theory that algae liberate substances toxic to bacteria, but that long storage with settling and extreme competition are more likely responsible for reduction in bacteria.

CHAPTER 4

LABORATORY PROCEDURES AND ANALYSES

Collection and Treatment of Samples

Four 5-gallon samples of unchlorinated activated sludge effluent were collected from the Tucson Sewage Treatment Plant, two on May 12, 1967 and two on June 6, 1967. The first two samples were lettered A and B; the second two, C and D. Each sample, after collection, was settled overnight at room temperature and then filtered through glass wool and distributed into four washed and sterilized 2.5-l glass bottles.

To promote anaerobic conditions, two bottles were filled full and plugged with rubber stoppers. In addition, two 500-ml Erlenmeyer flasks were filled with the sample and were used for refilling the bottles after microbiological samples were taken. The bottles were submarked 2 and 4 for incubation at 20°C and 30°C respectively. The liquid volume to wetted area ratio for these bottles was 0.90 inches.

For the aerobic samples, two bottles were filled with the sample such that each held 2.0 l. The bottles were submarked 1 and 3 for incubation at 20°C and 30°C respectively. The liquid volume to wetted area ratio for these bottles was 0.95 inches. Rubber stoppers were used to prevent evaporation.

Incubation was accomplished using two environmental modules, one at 20 $\pm$ 0.5°C and the other at 30 $\pm$ 0.5°C. The samples were mixed once

each day by rotating them through a 1-ft. radius circle 25 times in 25 sec. Except during sampling for bacterial analyses, the bottles were in darkness.

Bacteriological Analyses by Membrane Filtration

The four samples (1, 2, 3, and 4) for each run (A, B, C, and D) were analyzed prior to initial incubation and then 6 times at intervals during the following 19 days for coliforms, fecal coliforms, and fecal streptococci.

All bacteriological analyses were performed using the techniques and methods as specified in "Standard Methods" (2). The membrane filters used were Millipore, Type HA (0.45μ). Millipore plastic Petri dishes were used for culture containers. Direct enumeration, rather than enrichment, was selected.

Methodology which was specific for each of the groups tested is described as follows:

(1) Coliforms - M-Endo Broth MF (B·B·L) was used for the coliform culture medium. All colonies that developed a dark, metallic green sheen were counted. Incubation was at 35 ± 0.5°C for 24 ± 2 hours.

(2) Fecal coliforms - MFC Broth Medium (Difco) was used for culturing fecal coliforms. The culture containers were placed in waterproof plastic bags and submerged in a water bath at 44.5 ± 0.5°C for 24 ± 2 hours. All blue colonies that developed were counted.

(3) Fecal streptococci - M-Enterococcus Agar (B·B·L) was used to culture fecal streptococci. Incubation was at 35 ± 0.5°C for 48 ± 3 hours. Colonies appearing light pink to dark red were counted.

Colony counts were accomplished at 20X using a stereomicroscope. Since the colony density was limited by confluency, crowding, competition for nutrients, and differential characteristics of the medium, the following upper limits were used for counting the plates:

(1) Coliforms - 80 colonies.

(2) Fecal coliforms - 60 colonies.

(3) Fecal streptococci - 100 colonies.

A lower limit of 20 colonies was desired to reduce "statistical errors caused by random sampling variations" (6); however, due to the serial dilution technique used for sample volumes, and errors in determining sample volumes, many counts fell outside the recommended range. In these cases, the lower counts were used when available.

CHAPTER 5

DATA PRESENTATION AND DISCUSSION

The method of Frost and Streeter (13) was used to formulate equations for the bacterial decay curves. Each curve, when plotted on semi-log paper, can be mathematically expressed as the sum of two straight lines and can be represented by the equation:

$$y = a(10^{-bx}) + c(10^{-dx}) \qquad (1)$$

where y = percentage of bacteria remaining

a = initial percentage of less resistant bacteria

b = death rate of less resistant fraction, $days^{-1}$

c = initial percentage of more resistant bacteria

d = death rate of more resistant fraction, $days^{-1}$

x = time, days

The curves were drawn by observation. If more data had been obtained, the method of least squares might have been preferred. However, Frost and Streeter (13) state that the method of least squares was not practicable because "the excessive weight given by this method to observations in the upper section of the curve distorts the result giving a curve which entirely fails to fit the actual observations."

Constants for the curves were calculated as follows:

(1) A straight line was drawn tangent to the curve at its lower extremity.

(2) The constant (c) in the equation $y = c(10^{-dx})$ for the straight line was determined graphically at $x = o$ which results in $y = c$.

(3) The constant (d) was determined by substituting into the equation $dx = \log c - \log y$ any coordinate values of (y) and (x) on this straight line.

(4) Since $a + c = 100$ (percent), $a = 100 - c$.

(5) Constant (b) was then determined by selecting three points on the curve, and solving equation 1 for (b) in each case. The arithmetic average of the three results was used as the reported value.

Decay of Coliforms

Decay curves for coliforms are shown in Figures 1 through 8 for the various conditions studied. The calculated constants for these curves are listed in Table 1. These curves show the presence of two fractions of bacteria having different decay rates.

Decay of Fecal Coliforms

Decay curves for coliforms are shown in Figures 9 through 16. Constants for these curves are listed in Table 2. It is noticed that these curves are all straight or nearly straight lines, indicating that the fecal coliforms were uniformly resistant under the conditions of the study.

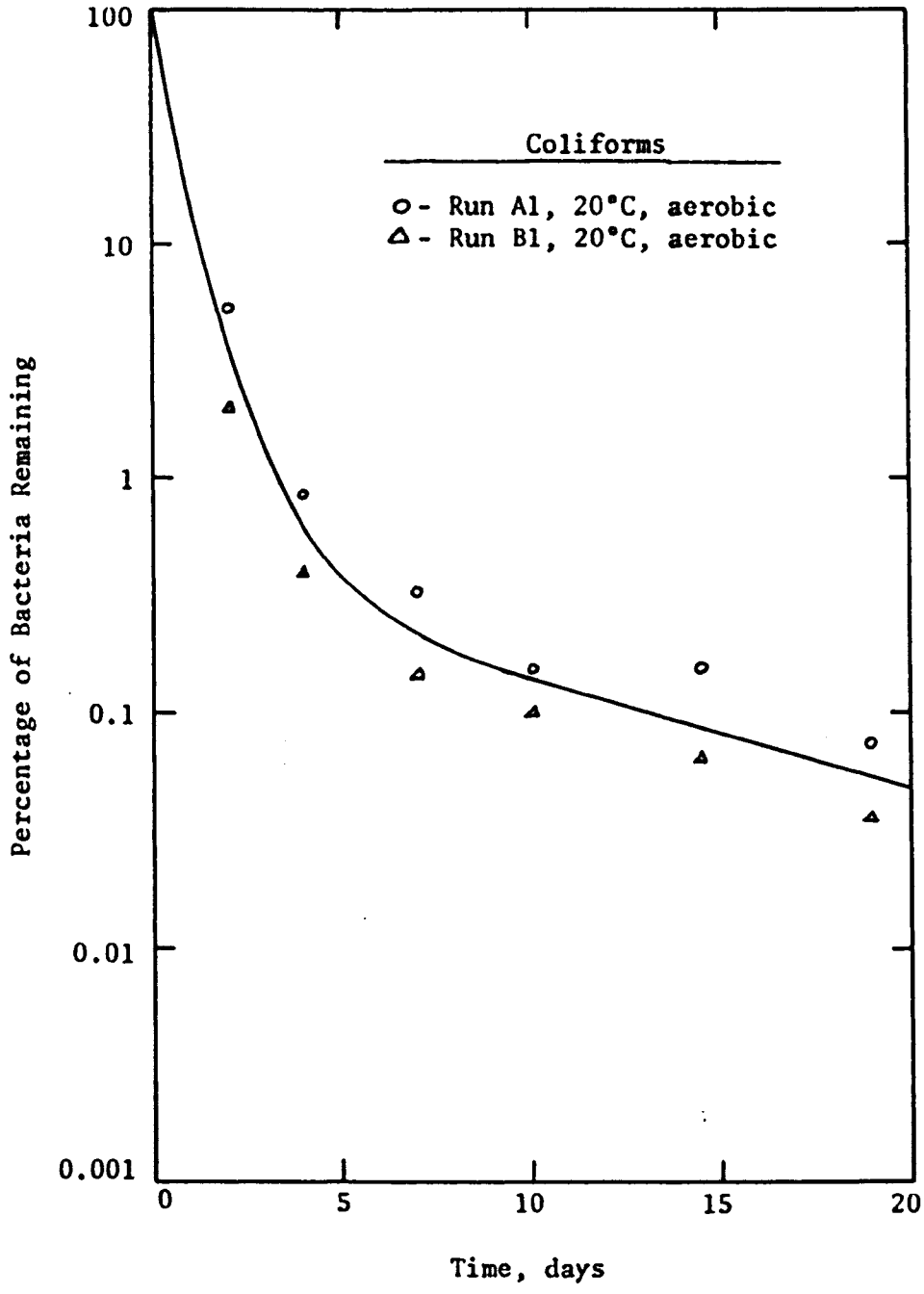

Figure 1. Decay Curve for Coliforms--Runs A1 and B1.

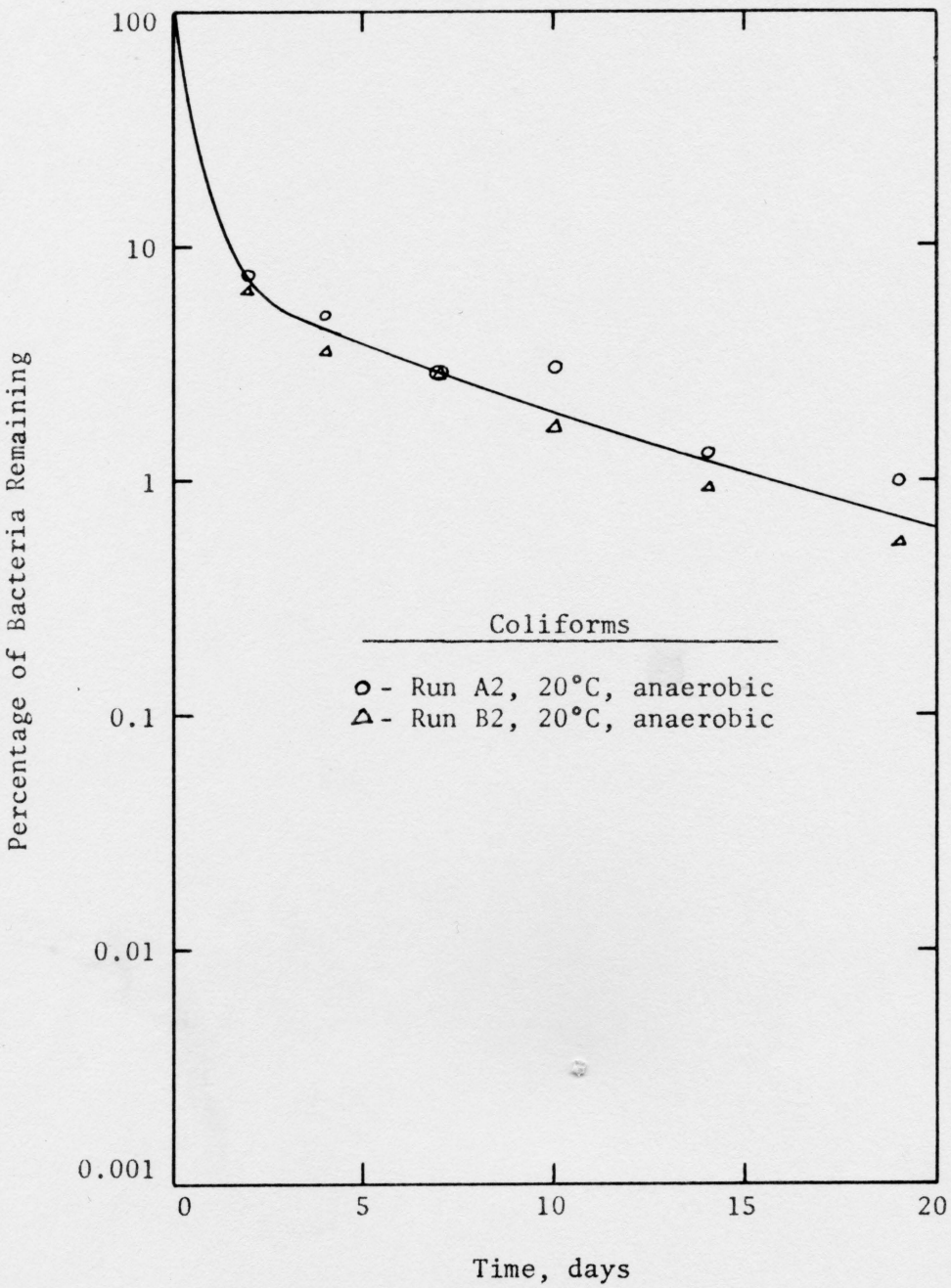

Figure 2. Decay Curve for Coliforms--Runs A2 and B2.

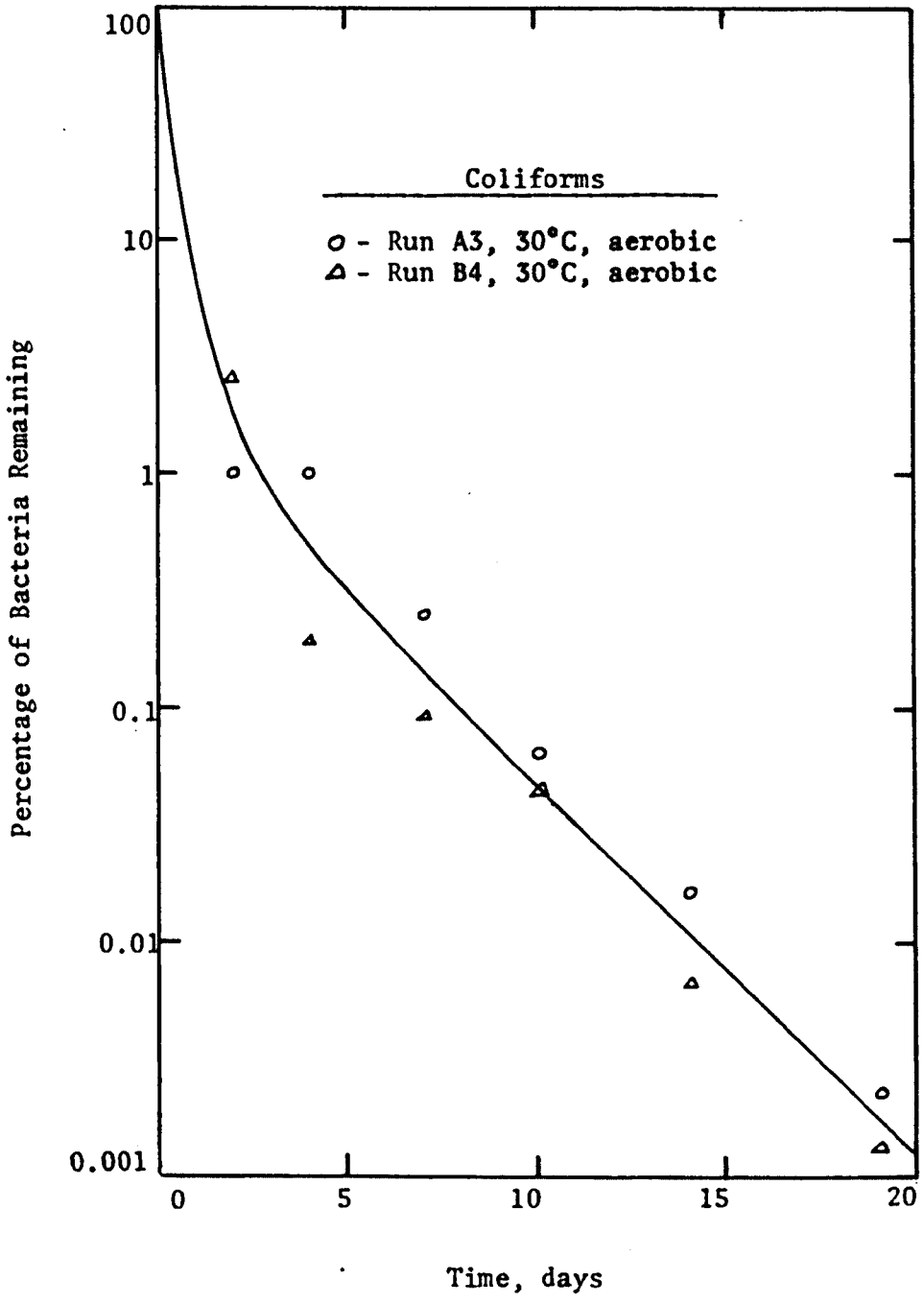

Figure 3. Decay Curve for Coliforms--Runs A3 and B3.

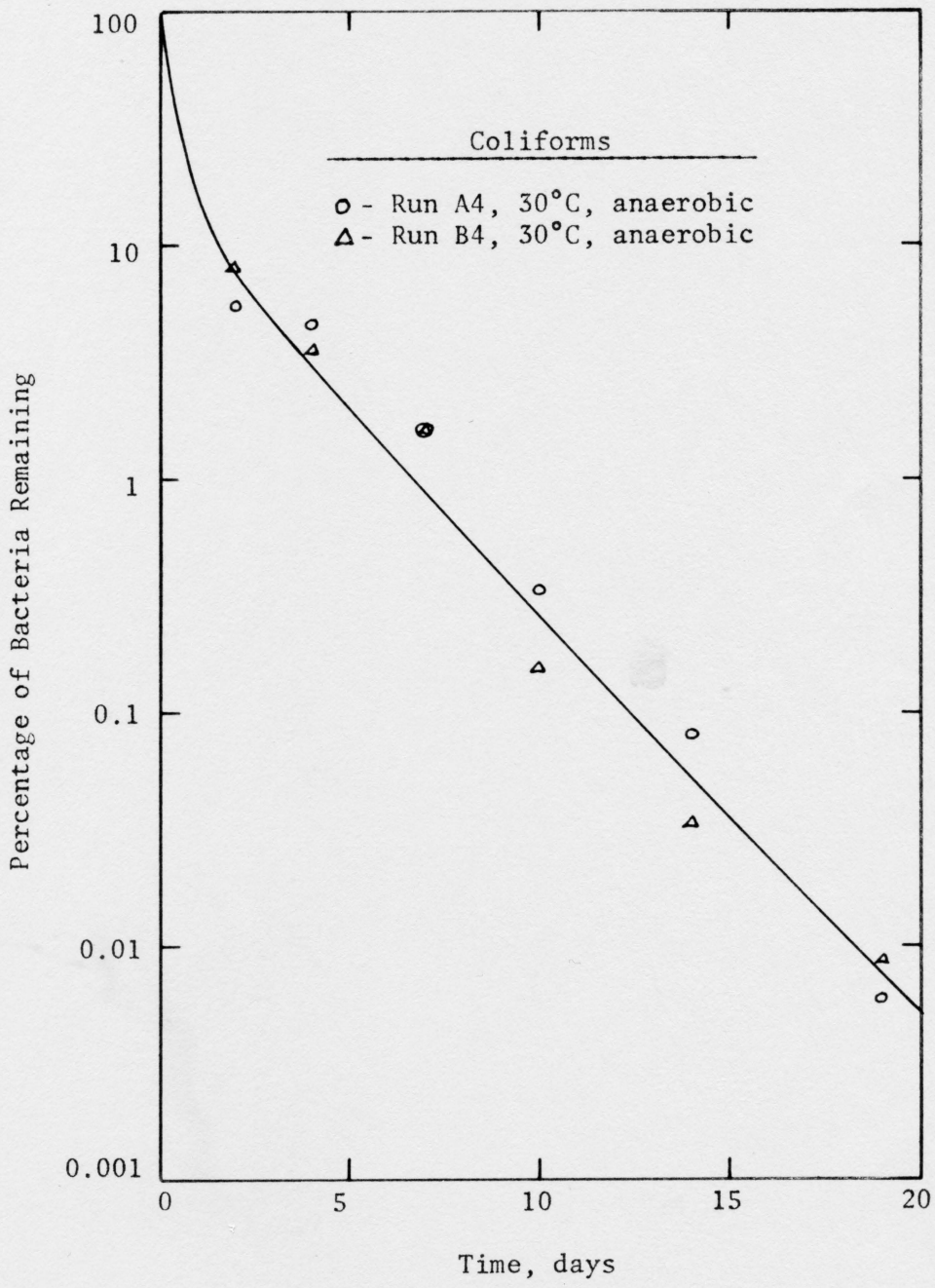

Figure 4. Decay Curve for Coliforms--Runs A4 and B4.

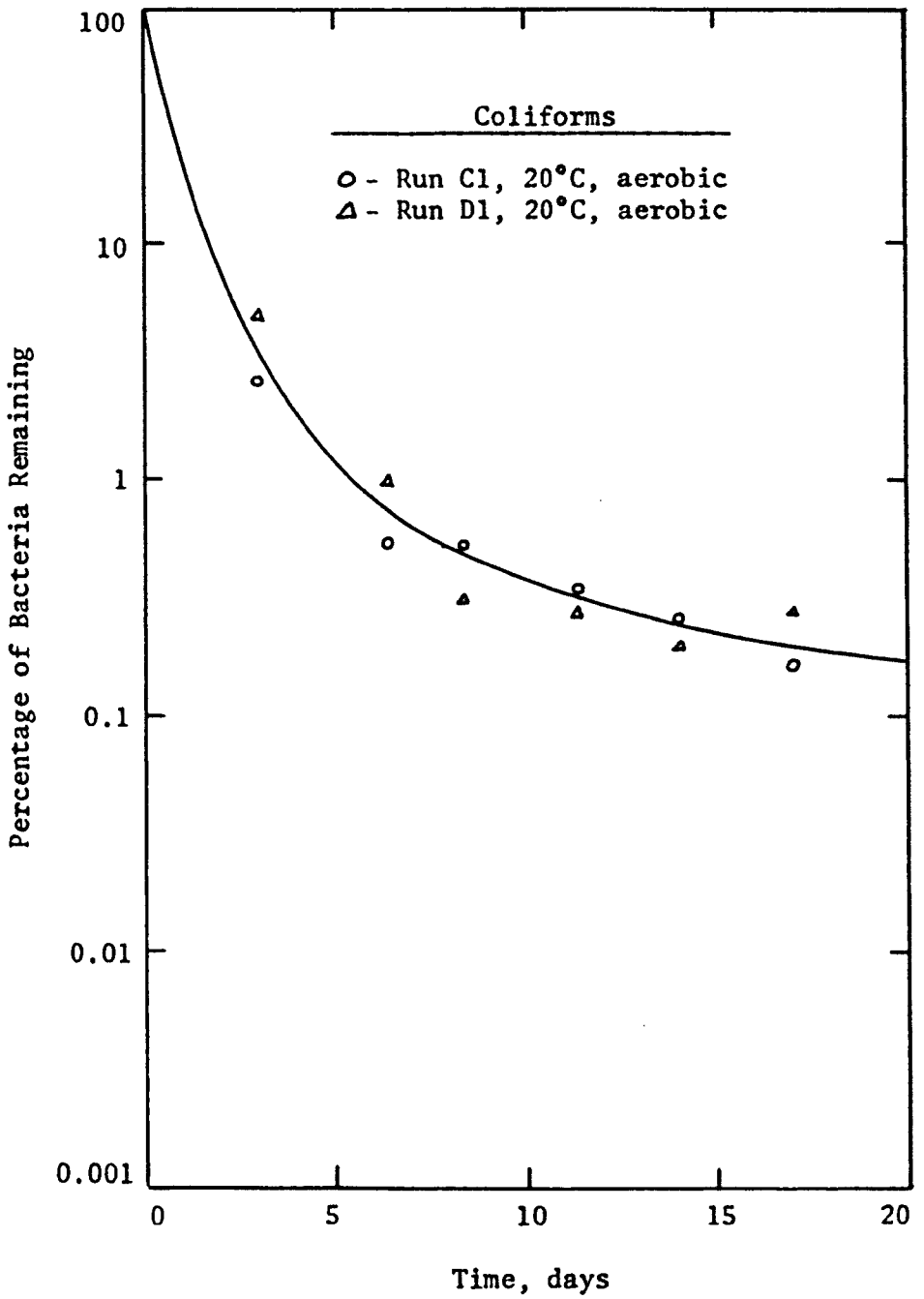

Figure 5. Decay Curve for Coliforms--Runs C1 and D1.

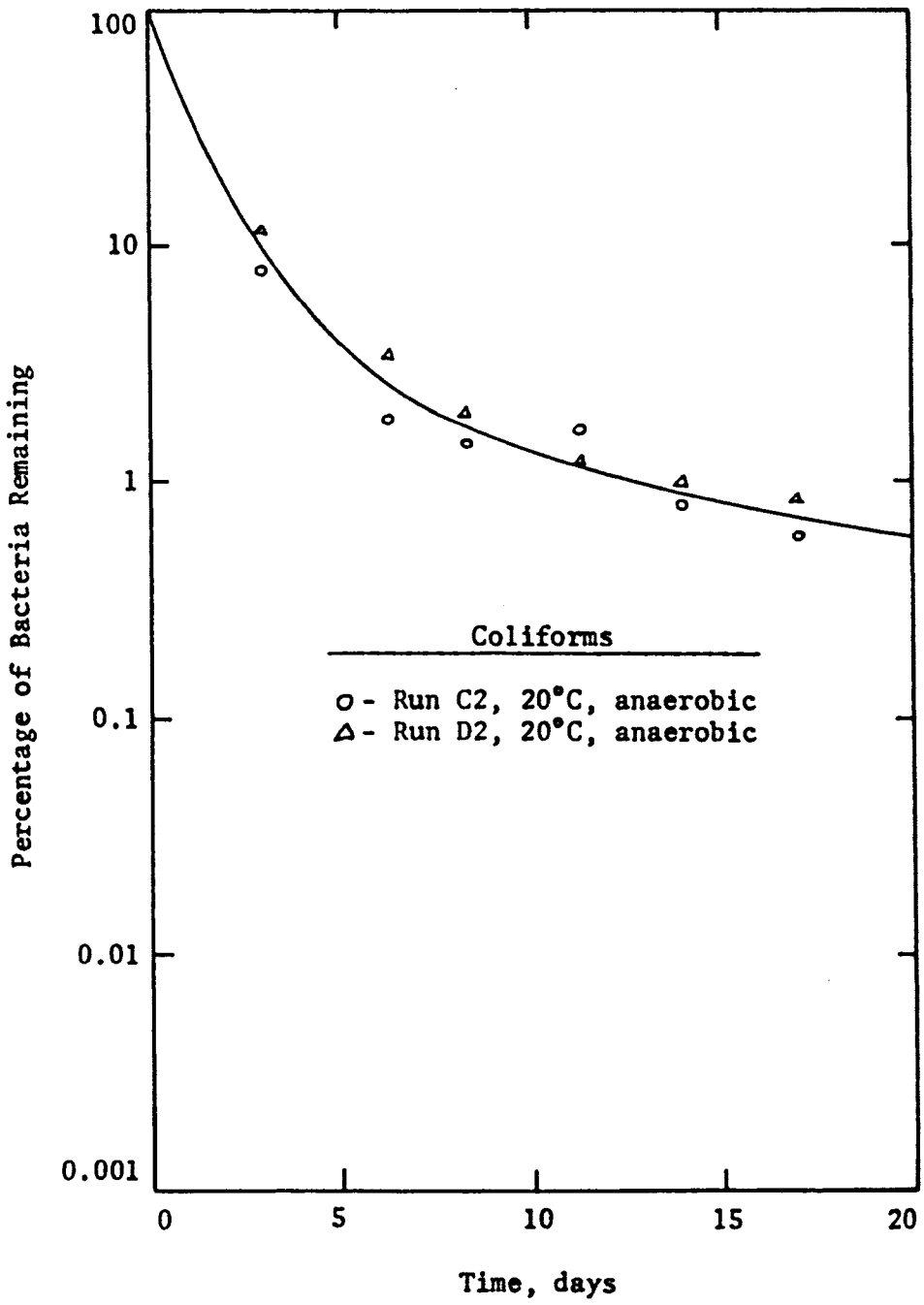

Figure 6. Decay Curve for Coliforms--Runs C2 and D2.

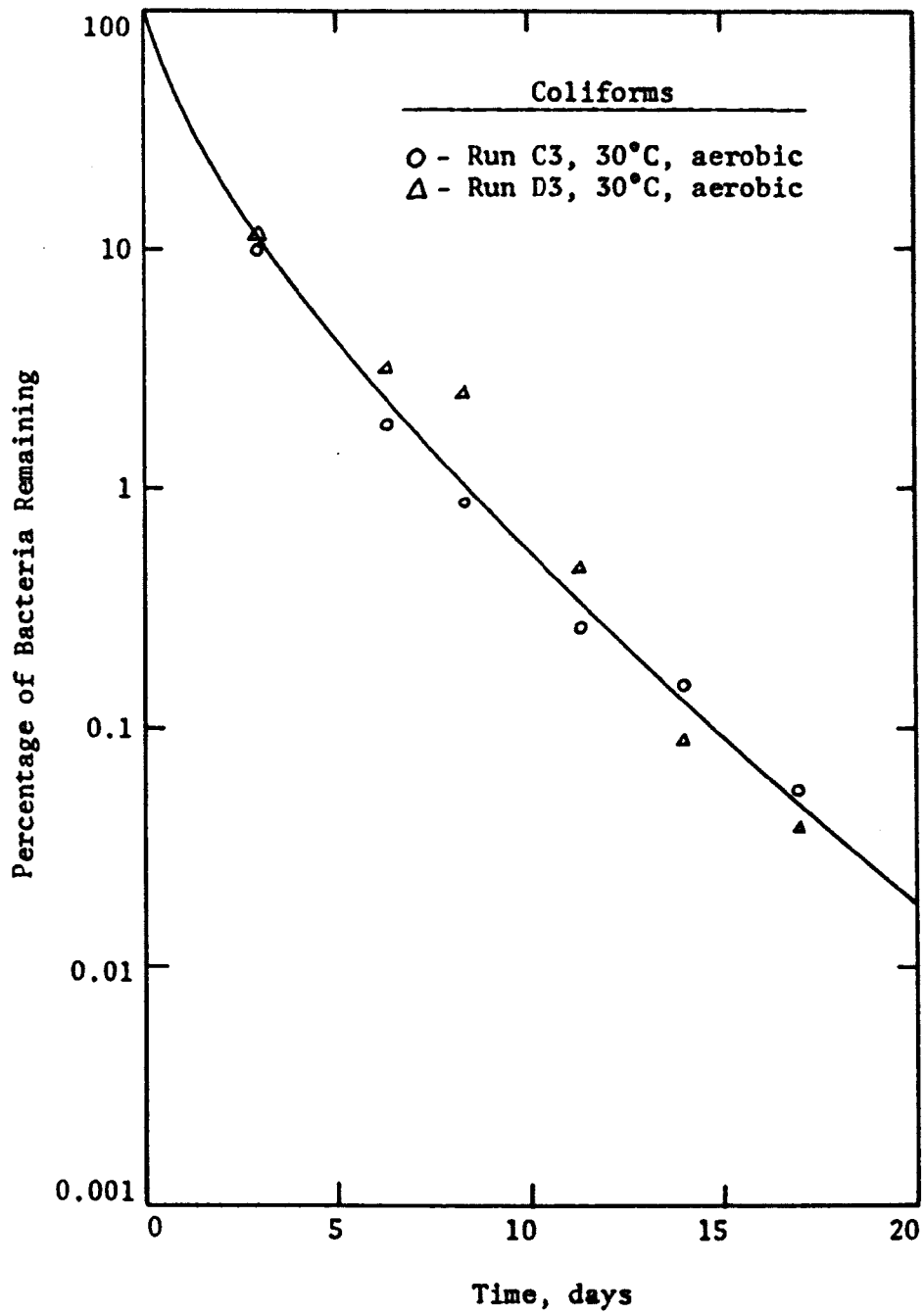

Figure 7. Decay Curve for Coliforms--Runs C3 and D3.

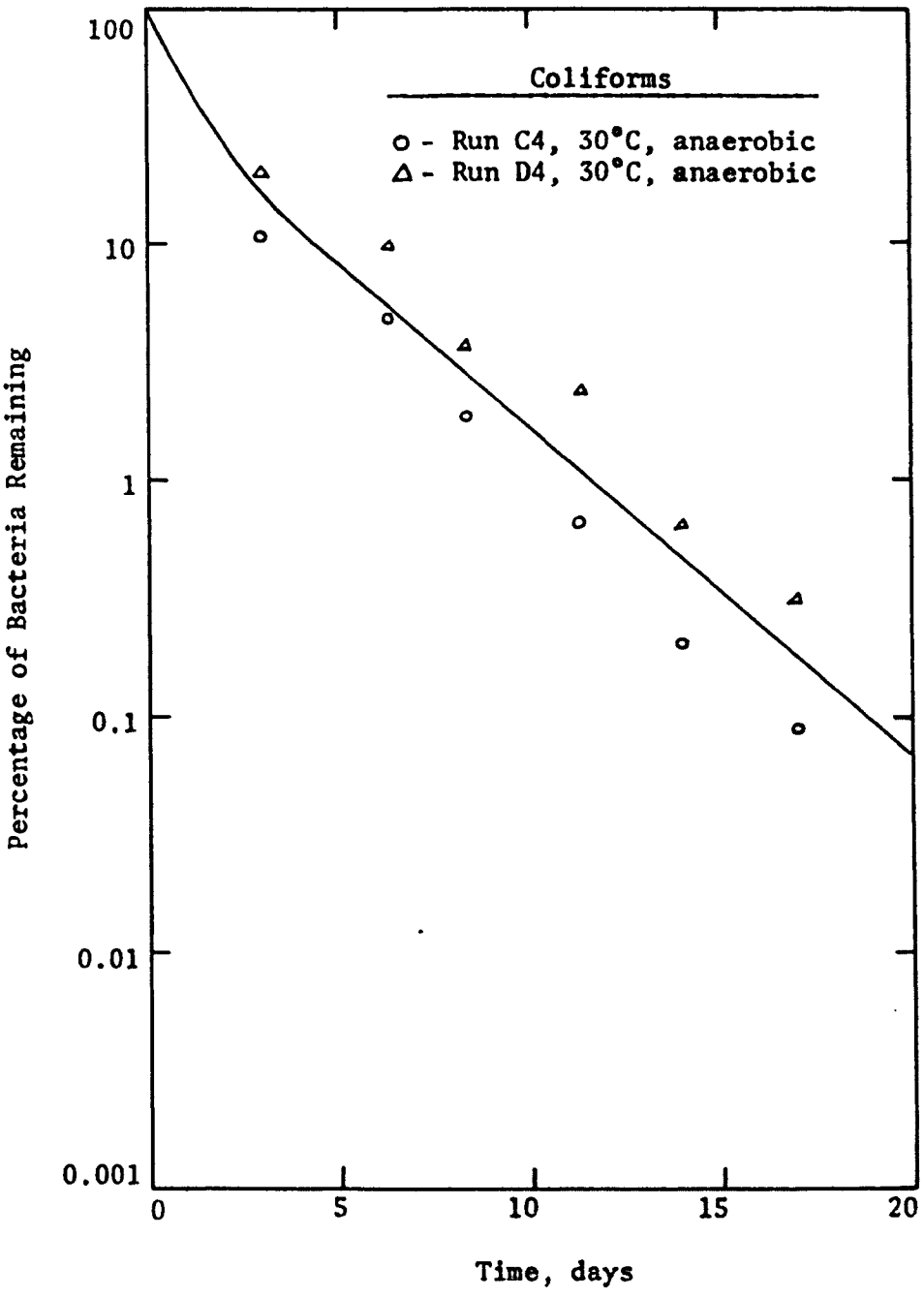

Figure 8. Decay Curve for Coliforms--Runs C4 and D4.

TABLE 1

CONSTANTS OF DECAY CURVES FOR COLIFORMS

Run	Initial Concentration of Bacteria per 100 ml	Constants for Equation 1			
		a	b	c	d
A1	93×10^5	99.63	0.40	0.37	0.045
B1	150×10^5				
A2	93×10^5	94.6	0.73	5.4	0.048
B2	150×10^5				
A3	93×10^5	98.4	0.69	1.6	0.155
B3	150×10^5				
A4	93×10^5	88	0.76	12	0.175
B4	150×10^5				
C1	15×10^5	99.55	0.33	0.45	0.024
D1	8×10^5				
C2	15×10^5	97.9	0.44	2.1	0.029
D2	8×10^5				
C3	15×10^5	88	0.63	12	0.142
D3	8×10^5				
C4	15×10^5	60	0.72	40	0.139
D4	8×10^5				

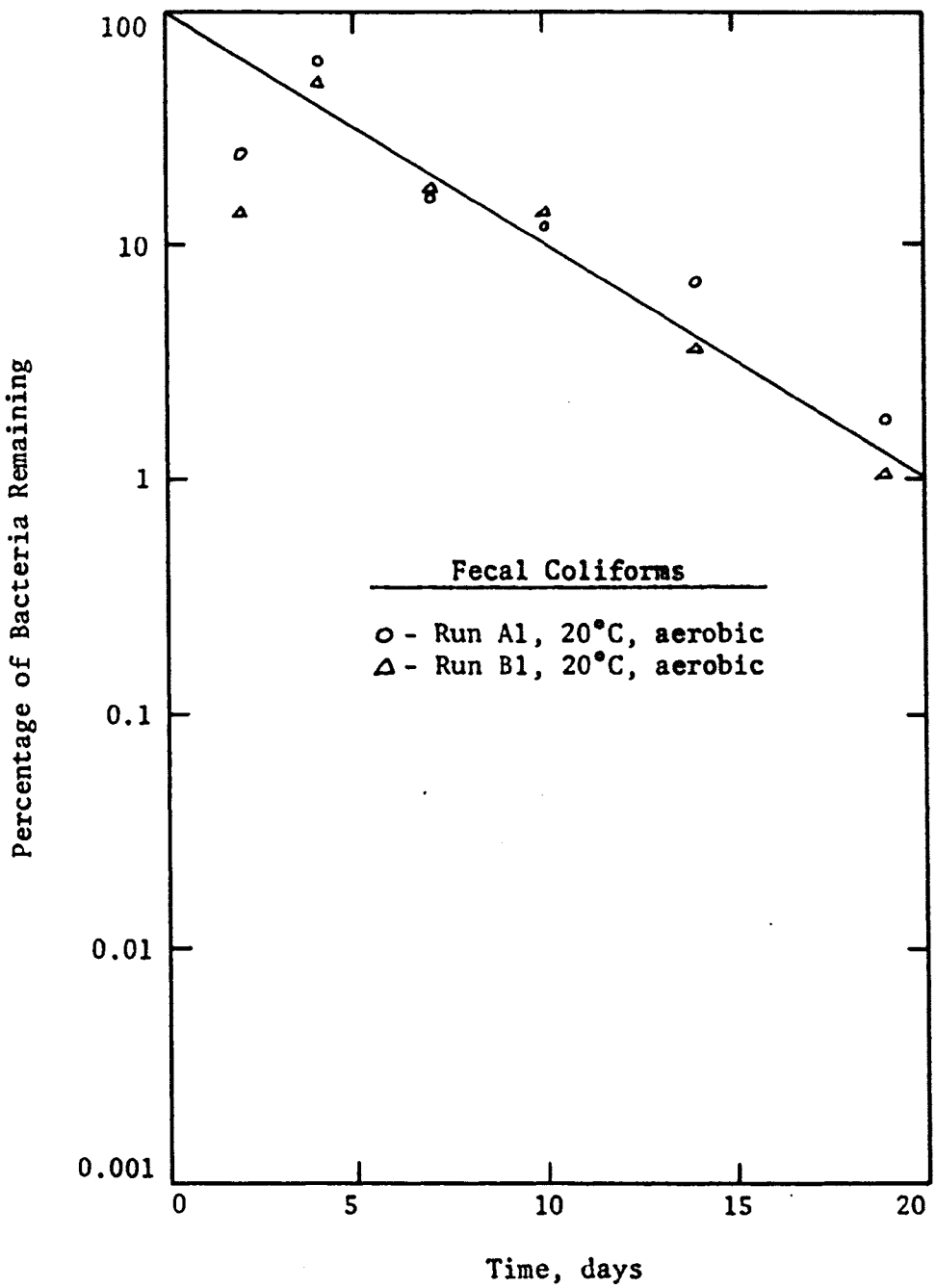

Figure 9. Decay Curve for Fecal Coliforms--Runs A1 and B1.

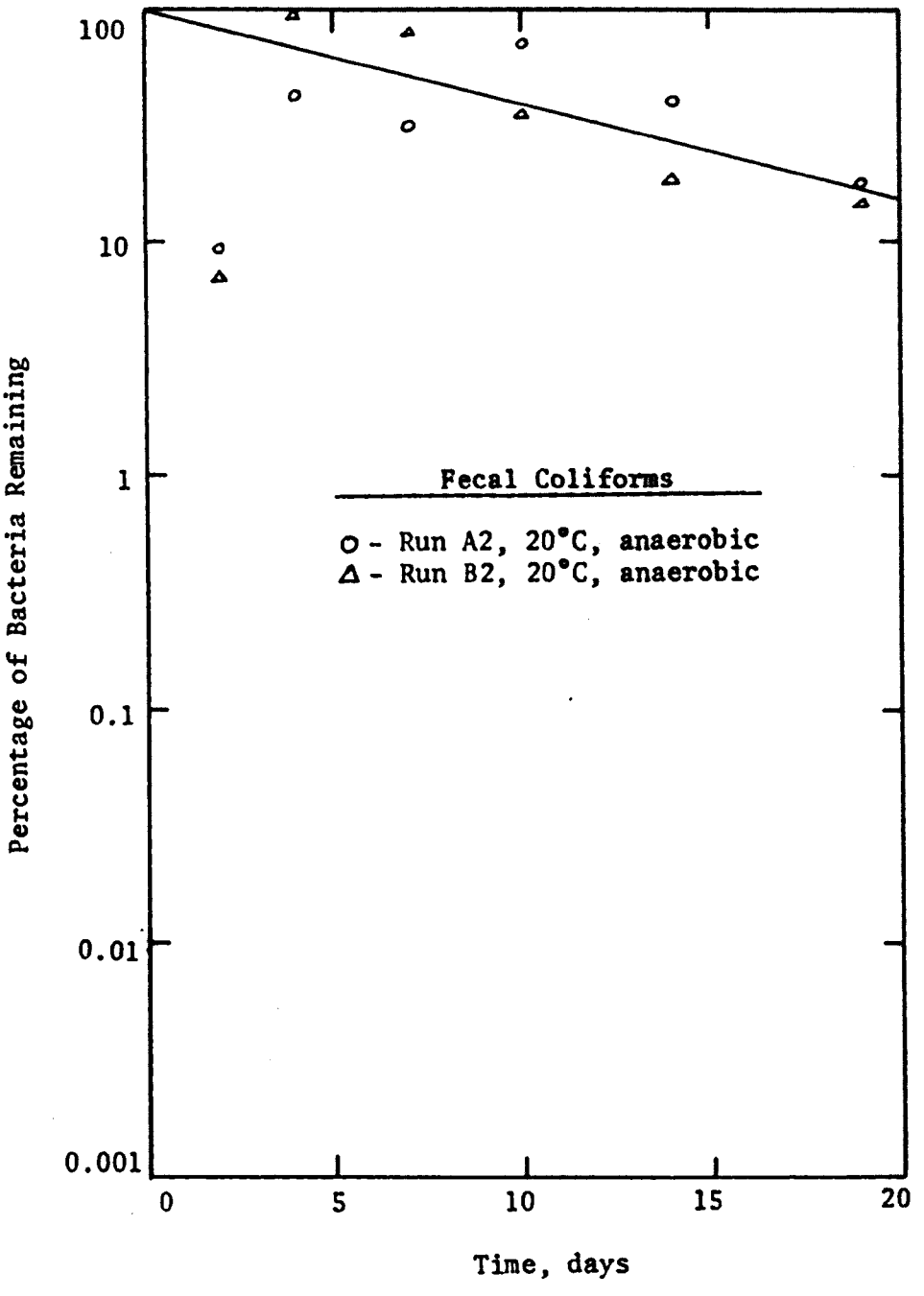

Figure 10. Decay Curve for Fecal Coliforms--Runs A2 and B2.

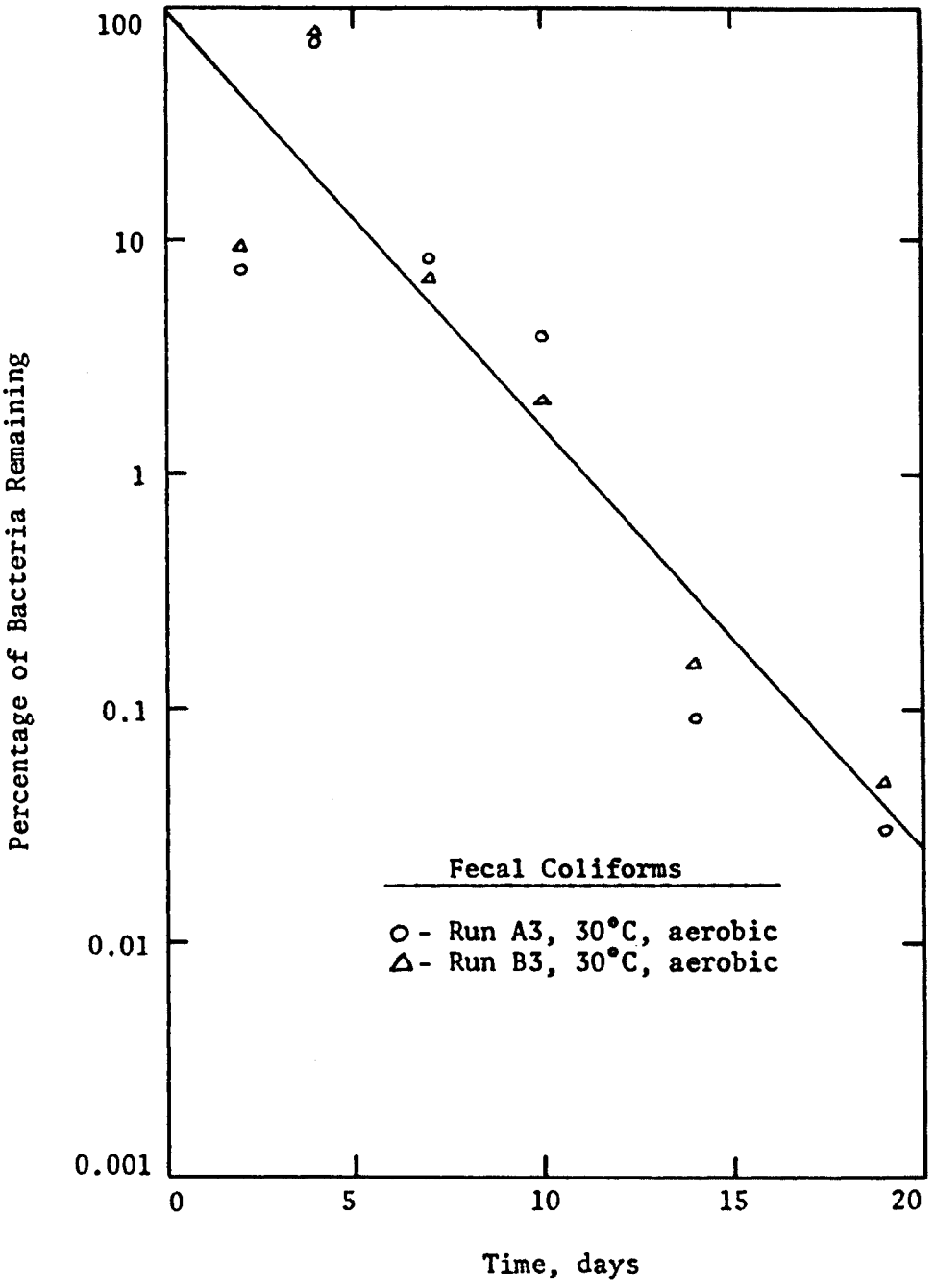

Figure 11. Decay Curve for Fecal Coliforms--Runs A3 and B3.

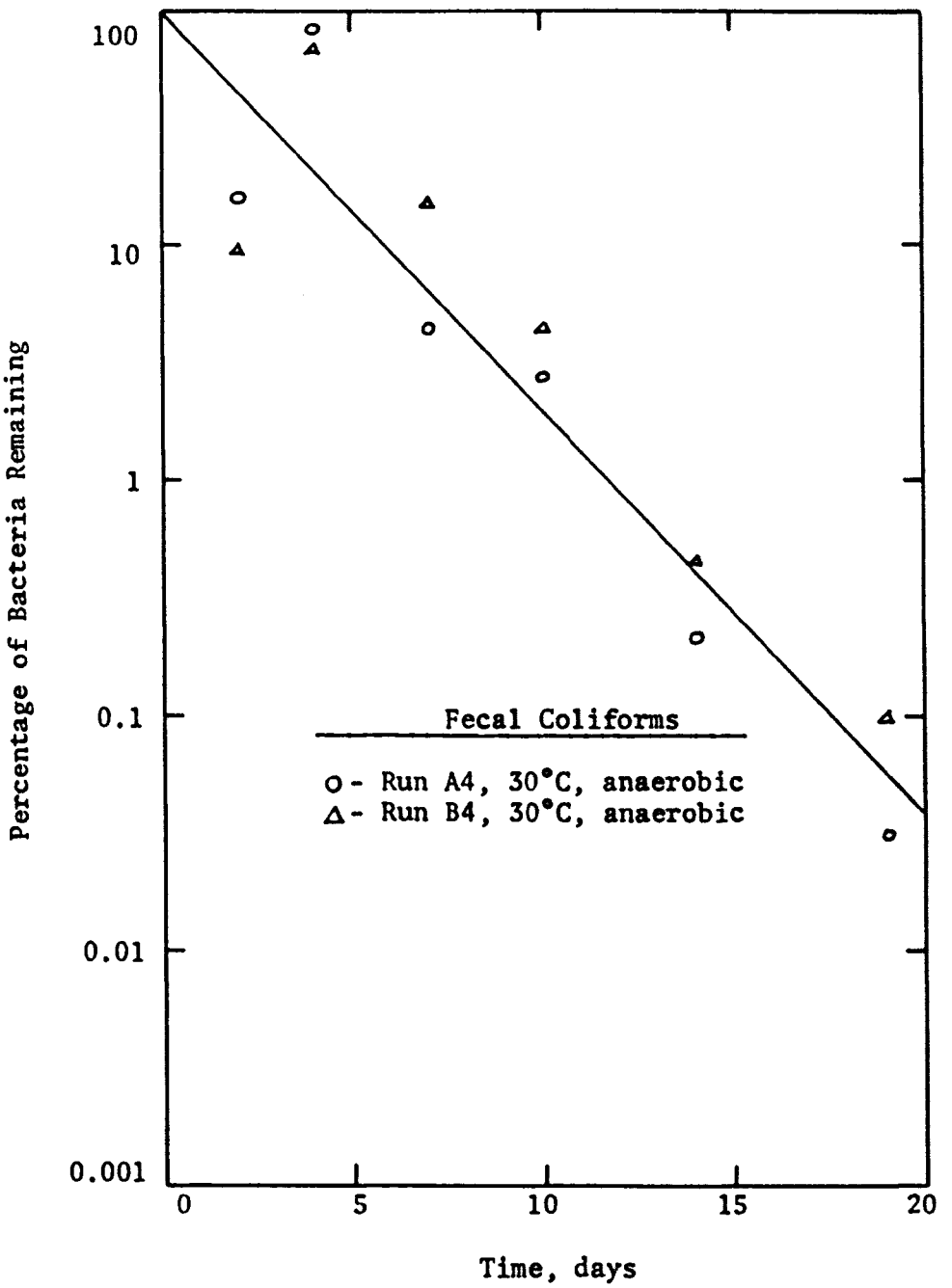

Figure 12. Decay Curve for Fecal Coliforms--Runs A4 and B4.

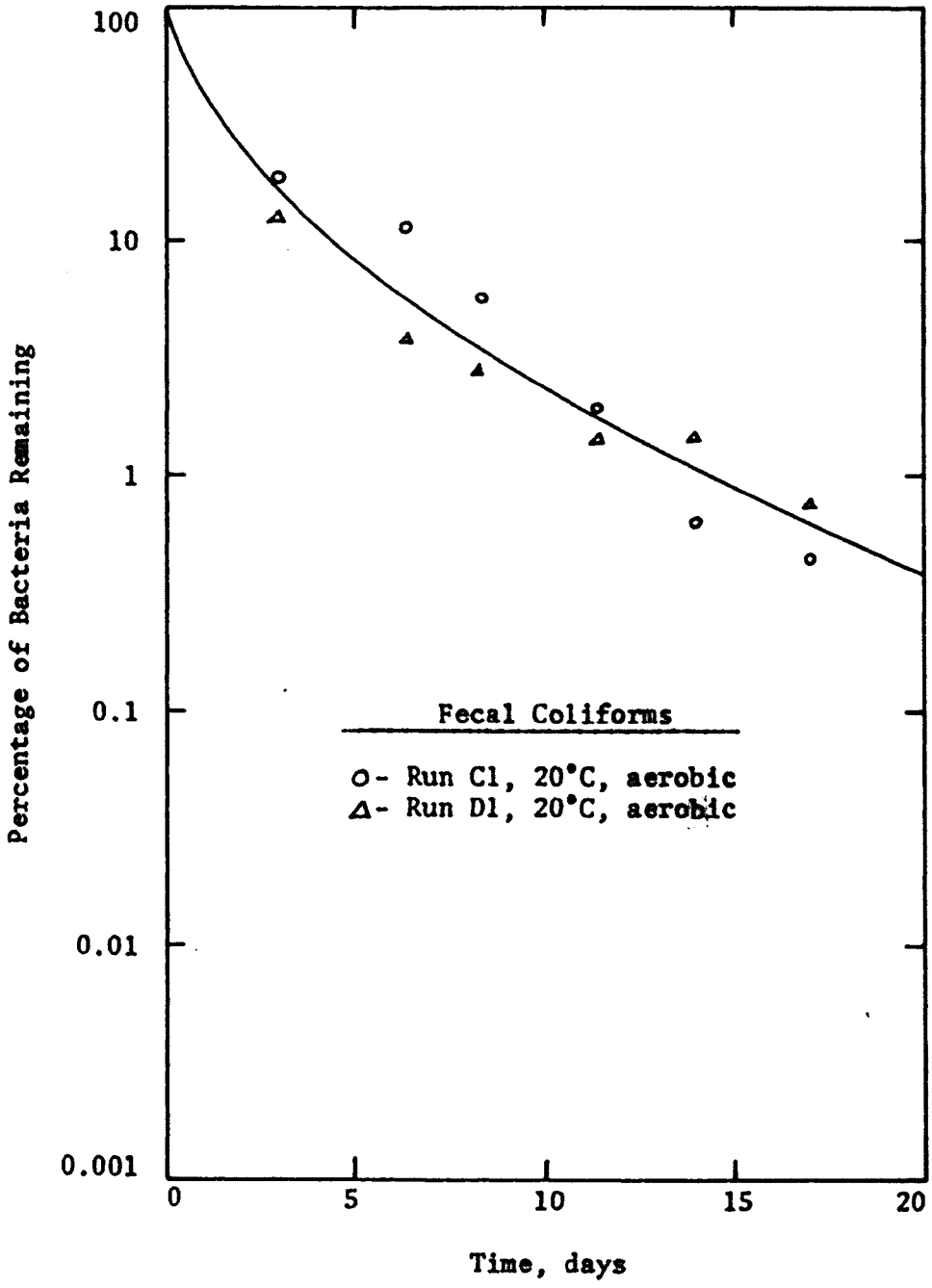

Figure 13. Decay Curve for Fecal Coliforms--Runs C1 and D1.

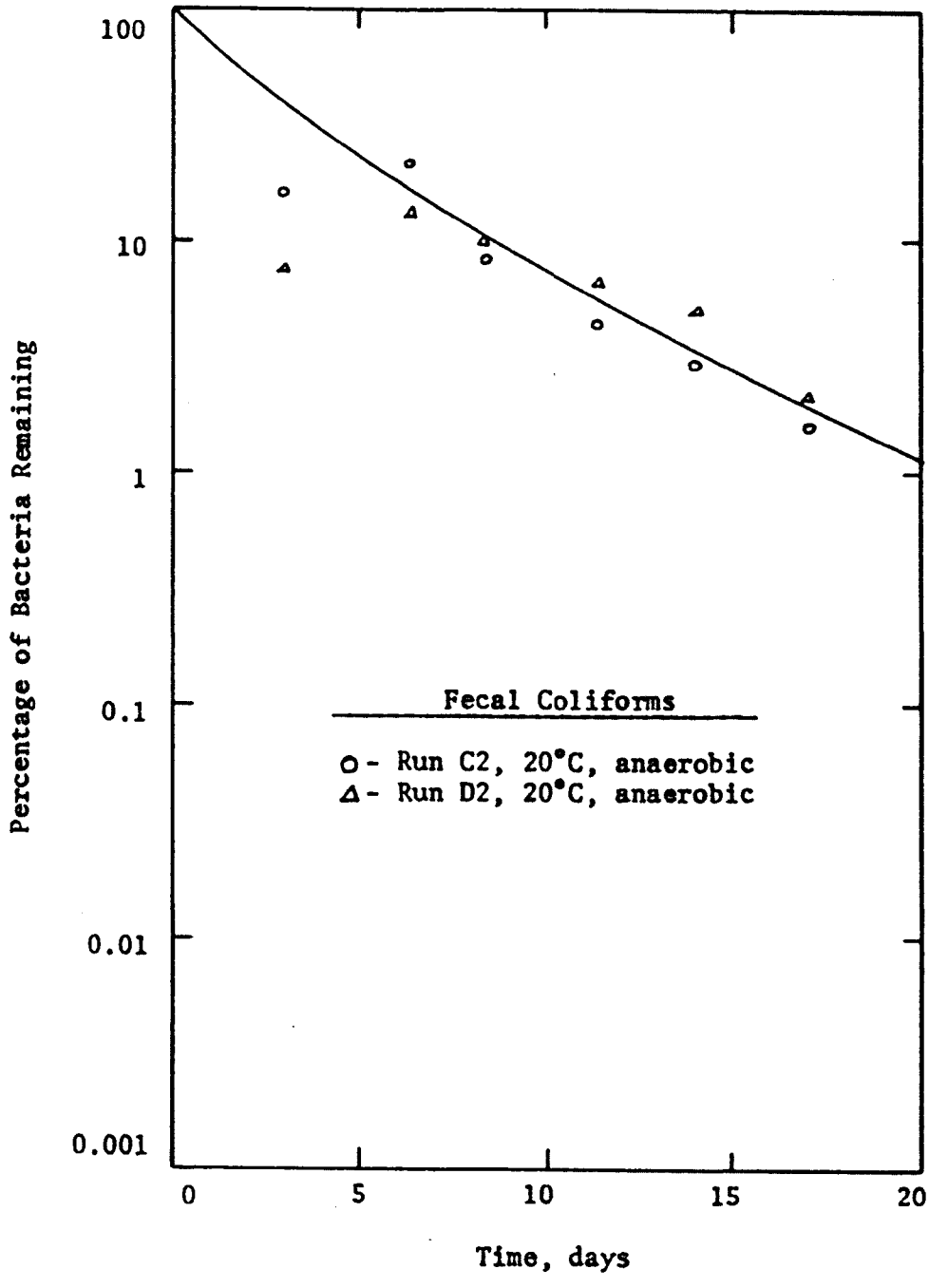

Figure 14. Decay Curve for Fecal Coliforms--Runs C2 and D2.

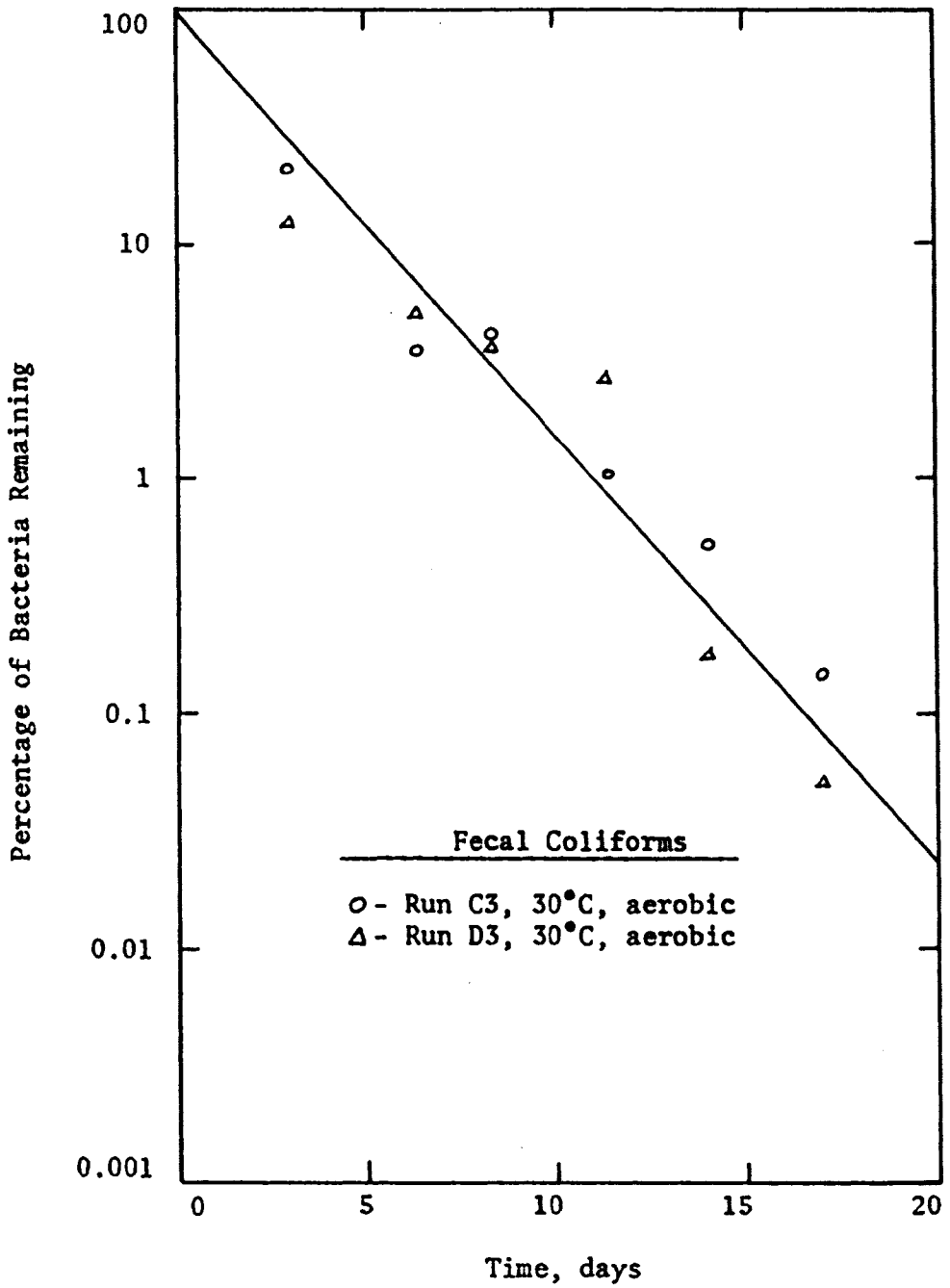

Figure 15. Decay Curve for Fecal Coliforms--Runs C3 and D3.

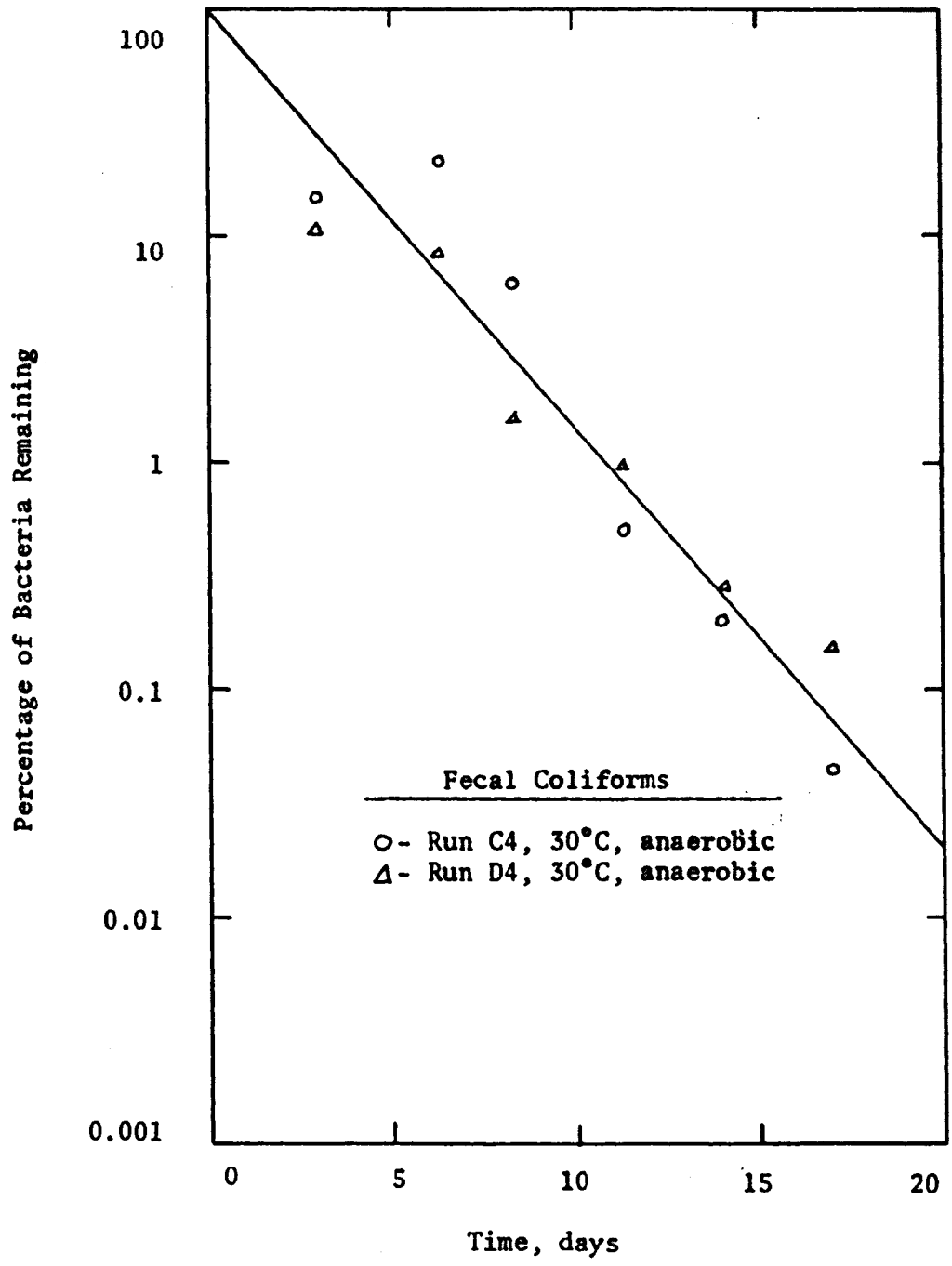

Figure 16. Decay Curve for Fecal Coliforms--Runs C4 and D4.

TABLE 2

CONSTANTS OF DECAY CURVES FOR FECAL COLIFORMS

Run	Initial Concentration of Bacteria per 100 ml	Constants for Equation 1			
		a	b	c	d
A1 B1	$32x10^3$ $20x10^3$	100	0.100	a	-
A2 B2	$32x10^3$ $20x10^3$	100	0.042	-	-
A3 B3	$32x10^3$ $20x10^3$	100	0.181	-	-
A4 B4	$32x10^3$ $20x10^3$	100	0.170	-	-
C1 D1	$70x10^3$ $39x10^3$	89.5	0.62	10.5	0.074
C2 D2	$70x10^3$ $39x10^3$	55	0.65	45	0.083
C3 D3	$70x10^3$ $39x10^3$	100	0.182	-	-
C4 D4	$70x10^3$ $39x10^3$	100	0.187	-	-

a. When the decay curve is a straight line, the equation is $y=a(10^{-bx})$.

Decay of Fecal Streptococci

Decay curves for fecal streptococci are shown in Figures 17 through 22. Constants for these curves are listed in Table 3. These curves indicate that two differently resistant fractions of bacteria are present.

Discussion of Results

Tables 1, 2, and 3 list the initial bacterial concentration for the various runs. As shown by the curves and the calculated constants, the decay rates are dependent upon the initial concentration of bacteria; higher decay rates occur with higher initial concentrations.

In addition to a dependency on initial concentration, a number of conclusions can be made in regard to the study.

A greater decrease in bacterial numbers was noted at 30°C than at 20°C; the decay rates of the more resistant bacterial fractions were much higher at the higher temperature (as shown by Tables 1, 2, and 3). This was to be expected since, at higher temperatures, bacterial metabolism increases and produces a lessened food supply and an increase in toxic metabolic products. In addition, predation is increased.

A greater decrease was observed when the system was aerobic as opposed to anaerobic. This oxygen effect was shown to be less important than the temperature effect for coliforms and fecal coliforms, and diminished with increasing temperature as shown by comparing Figures 1 and 2 with Figures 3 and 4. For fecal streptococci, the reverse was true. A comparison of Figures 17 and 18 with Figure 19 and 20 shows that the oxygen effect was more important than the temperature effect, and was

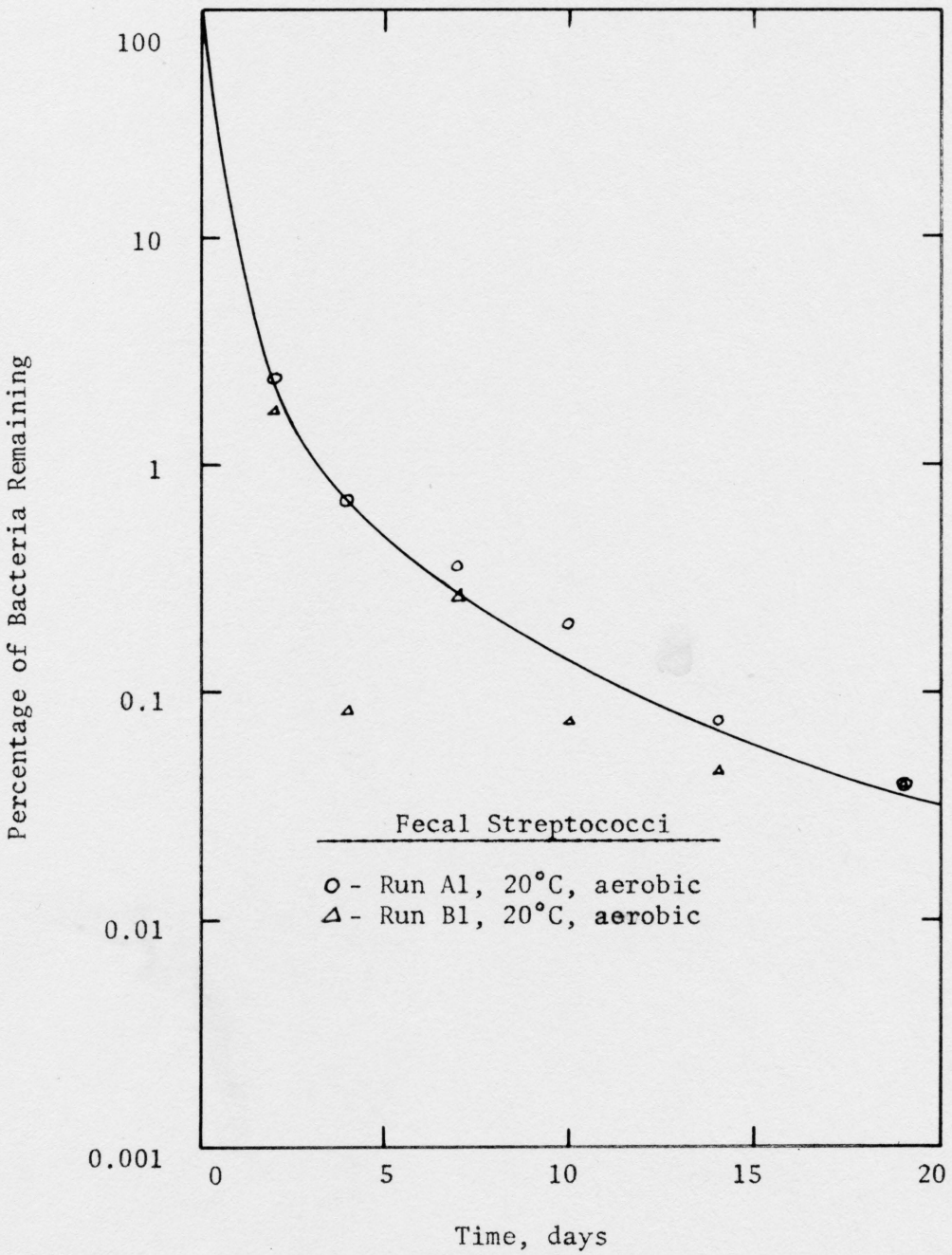

Figure 17. Decay Curve for Fecal Streptococci--Runs A1 and B1.

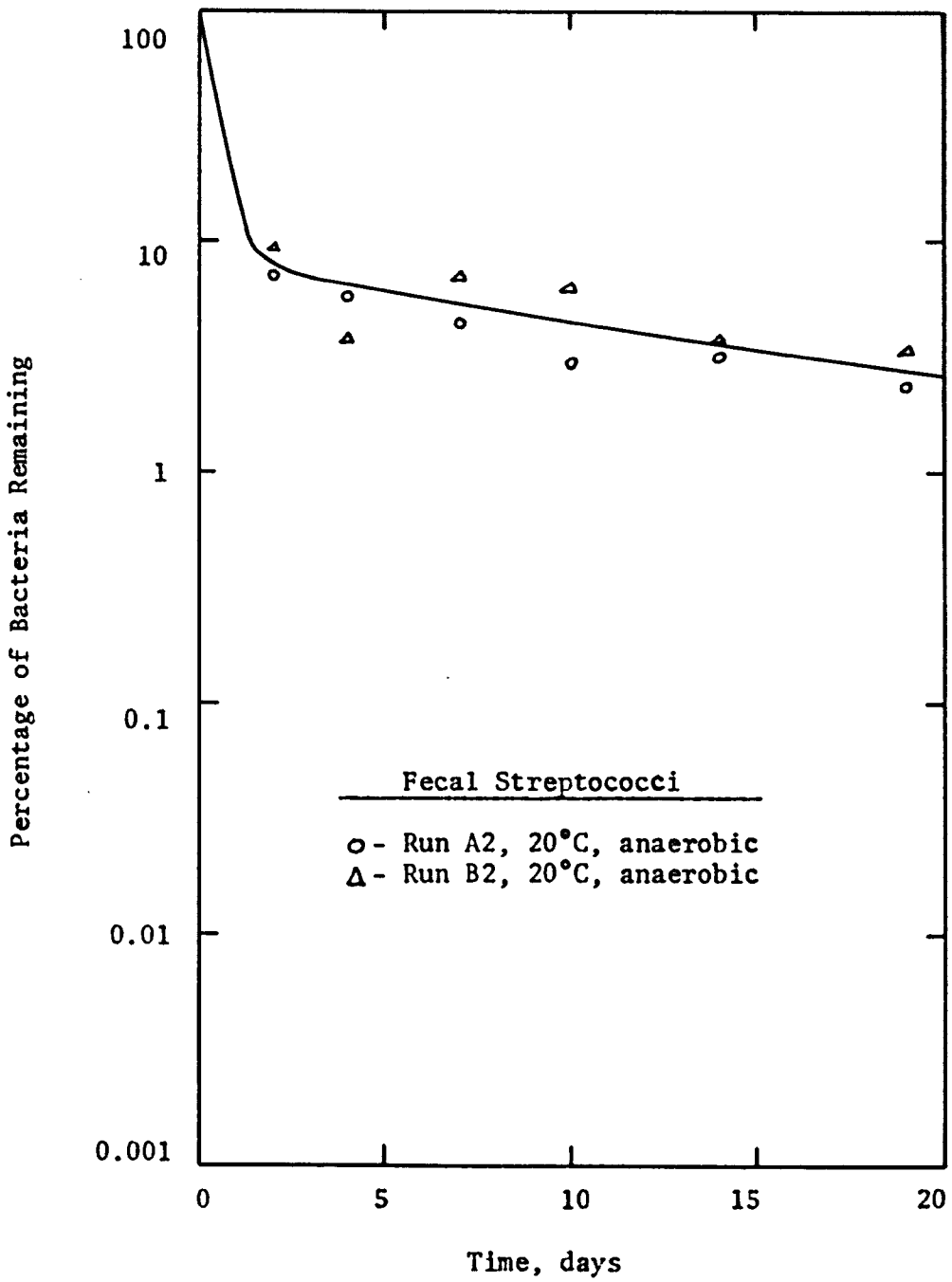

Figure 18. Decay Curve for Fecal Streptococci--Runs A2 and B2.

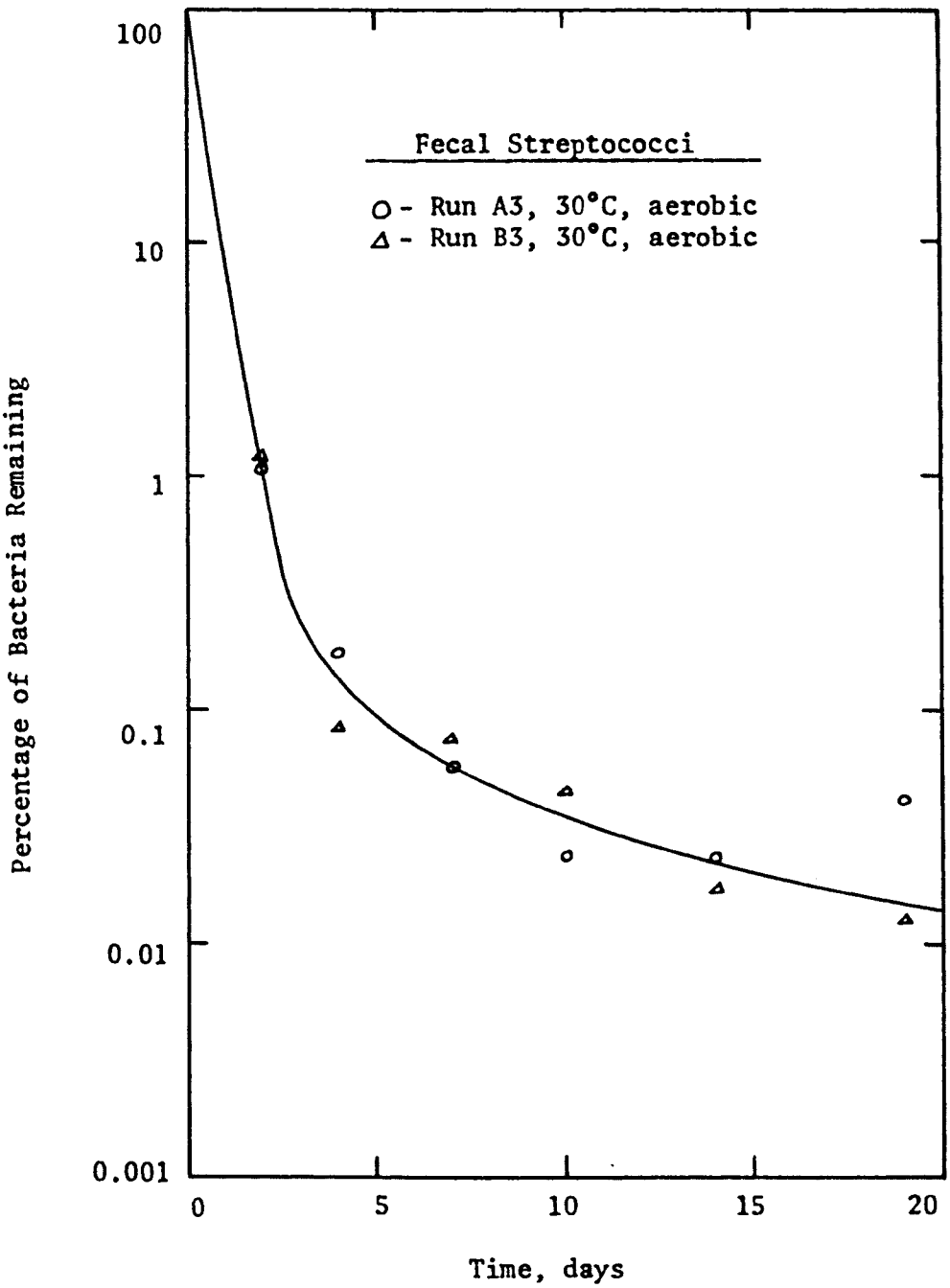

Figure 19. Decay Curve for Fecal Streptococci--Runs A3 and B3.

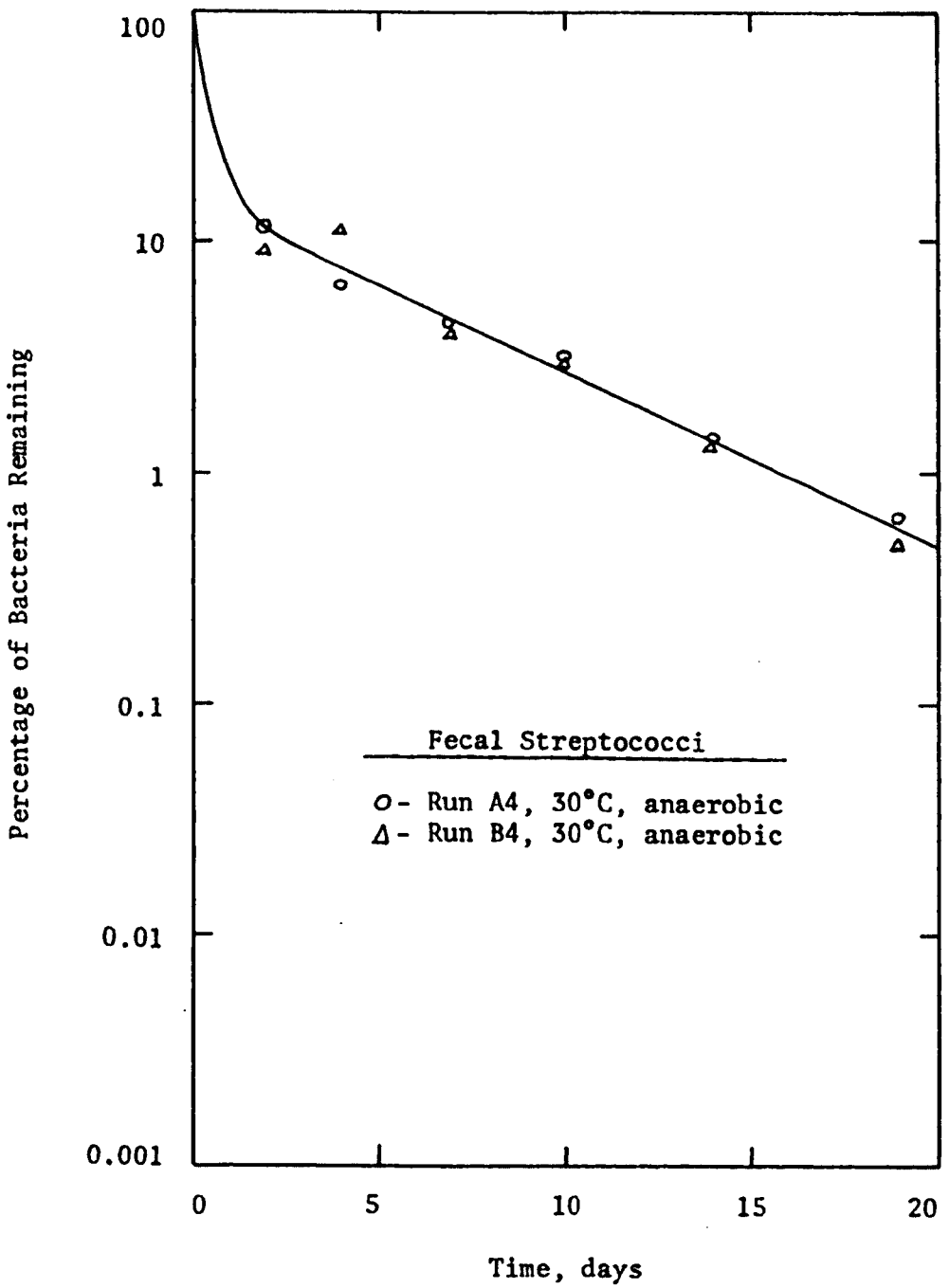

Figure 20. Decay Curve for Fecal Streptococci--Runs A4 and B4.

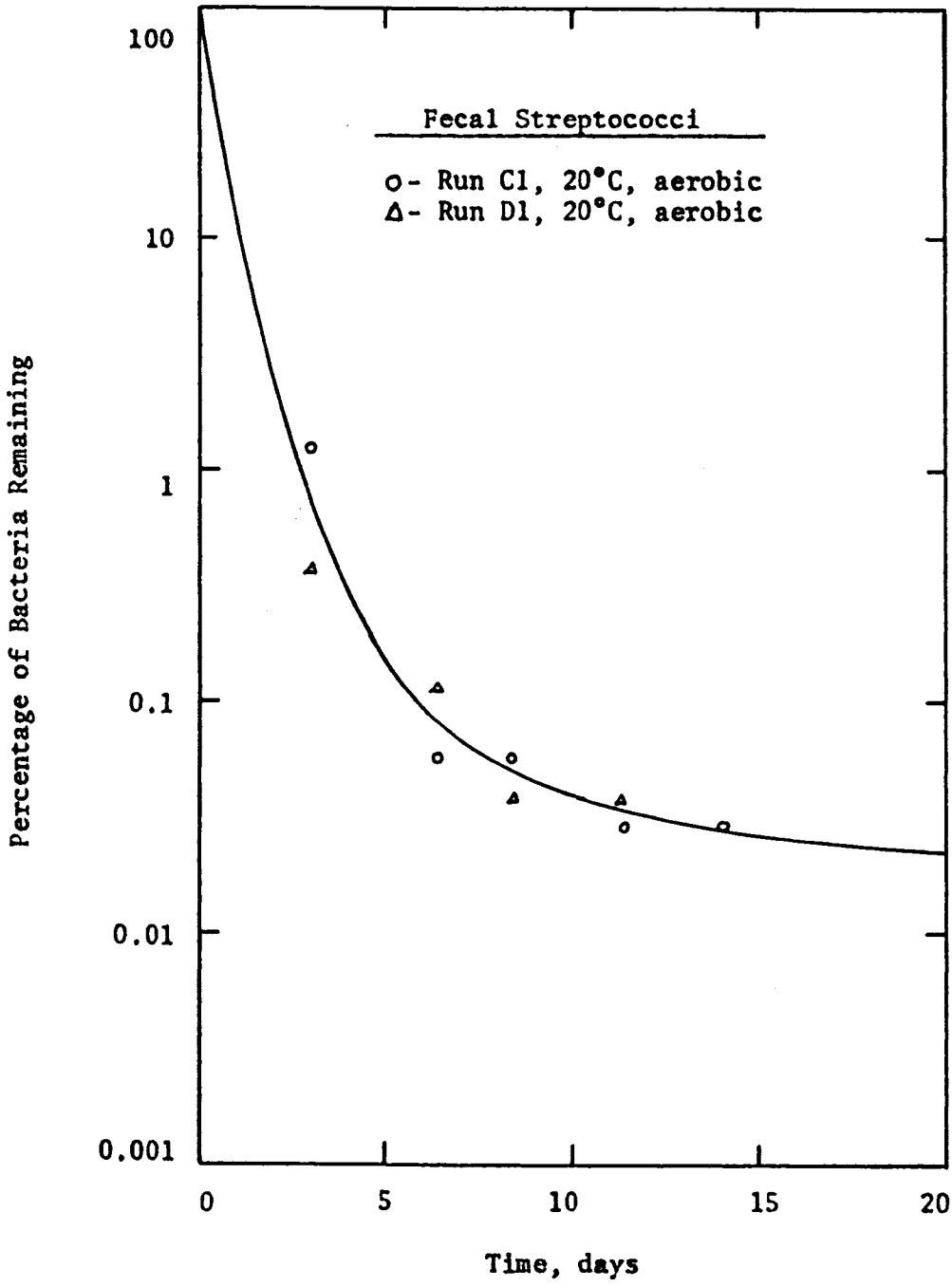

Figure 21. Decay Curve for Fecal Streptococci--Runs C1 and D1.

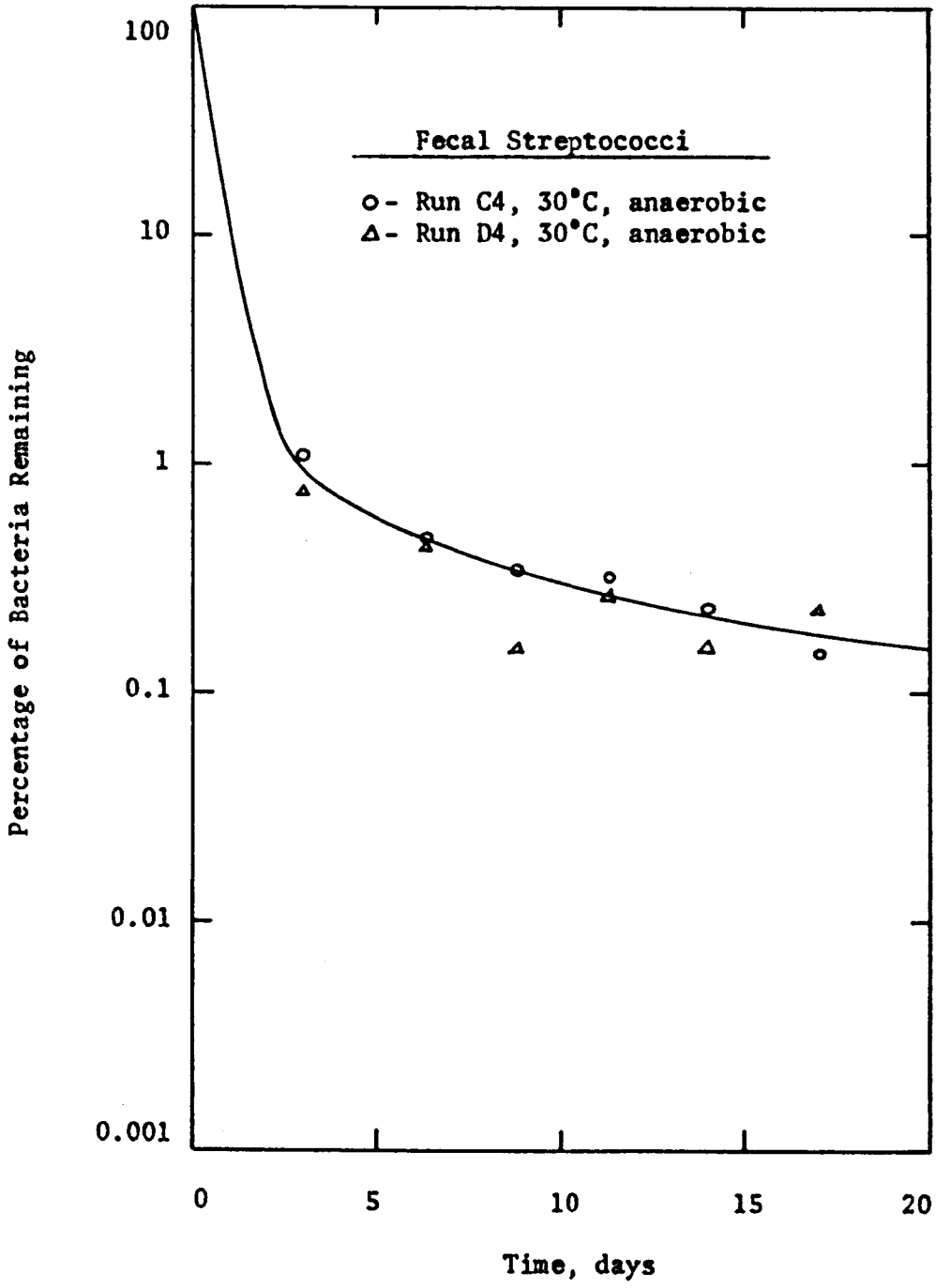

Figure 22. Decay Curve for Fecal Streptococci--Runs C4 and D4.

TABLE 3

CONSTANTS OF DECAY CURVES FOR FECAL STREPTOCOCCI

Run	Initial Concentration of Bacteria per 100 ml	Constants for Equation 1			
		a	b	c	d
A1	170×10^3	99.76	0.41	0.24	0.041
B1	240×10^3				
A2	170×10^3	92.6	0.79	7.4	0.020
B2	240×10^3				
A3	170×10^3	99.944	0.30	0.056	0.029
B3	240×10^3				
A4	170×10^3	84	0.81	16	0.080
B4	240×10^3				
C1	34×10^3	99.96	0.23	0.04	0.016
D1	26×10^3				
C4	34×10^3	99.57	0.51	0.43	0.023
D4	26×10^3				

relatively constant. The probable reason for this variation in effects is that fecal streptococci are organisms which require an aerobic environment, whereas coliforms and fecal coliforms are comprised of both aerobic and facultative anaerobic bacteria and are not so oxygen dependent.

A greater initial decrease, representing the percentage of less resistant bacteria, was observed at aerobic as opposed to anaerobic conditions. Again, this can be explained by the increased metabolism by the bacteria in a more favorable environment. It was also observed that there was a greater initial decrease at the lower temperature; the reason for this apparent anomaly is not evident.

A comparison of the percentage decrease during twenty days can be made for the three bacterial groups. At 20°C and aerobic conditions, the relative decrease was fecal streptococci > coliforms > fecal coliforms. At both the 20°C and anaerobic condition and the 30°C and aerobic condition, the order was coliforms > fecal streptococci > fecal coliforms. The 30°C and anaerobic condition had mixed results, with the coliforms and fecal coliforms decaying faster than the fecal streptococci.

The results are at variance with decay studies of polluted waters as reported in the literature. The majority of these studies have shown a tendency for fecal coliforms to decay faster than coliforms. In this study, however, only in one run did this occur. It may be that the "non-fecal" coliforms find activated sludge effluent to be much more unfavorable than do the fecal coliforms. Another factor could be the effect of initial bacterial concentration. Since the coliforms are present in

much greater numbers, this would tend to produce an increased death rate.

The disparity between the results of this study as regards the relative decay rates of coliforms and fecal streptococci and the study made by Hanes et al. (15) can possibly be explained in terms of the two different environments (activated sludge effluent versus a one percent dilution of raw sewage) and the two different media used to enumerate the fecal streptococci (M-Enterococcus Agar versus K-Broth).

Results reported from oxidation pond studies can be compared to this laboratory study. At 20°C and with aerobic conditions, an inspection of Figures 5, 9, and 17 indicates removal efficiencies, after 20 days, of approximately 99.8, 99, and 99.96 percent for coliforms, fecal coliforms and fecal streptococci respectively. These values are of the same order of magnitude as those reported for most field studies. With respect to bacterial decay, then, results obtained from laboratory scale studies parallel those occurring in full scale oxidation ponds.

Activated sludge effluent from the Tucson Sewage Plant was applied to a soil filter being used in the Tucson Wastewater Reclamation Project. Under loading conditions between January 10, 1967 and April 10, 1967, fluorescent dye studies indicated an average travel time of the liquid of 17 days from time of application to appearance at the effluent sampling point. Bacteriological studies during this time showed average removals of 99.97, 99.96, and 99.97 percent for coliforms, fecal coliforms, and fecal streptococci respectively. Assuming 20°C and aerobic conditions, removals for the same bacterial groups during the first 17

days of the laboratory study were 99.8, 98, and 99.95 percent. It can be seen from a comparison of these figures that natural decay can account for from one-half to nine-tenths of the order of magnitude of the bacterial changes during soil filtration. For a more exact analysis, the actual conditions of the liquid in the filter would have to be known.

CHAPTER 6

CONCLUSIONS AND SUGGESTIONS FOR FURTHER STUDY

Summary and Conclusions

A laboratory study was made on four samples of unchlorinated activated sludge effluent from the Tucson Sewage Treatment Plant to determine bacterial decay curves. Environmental parameters studied were temperature (20° and 30°C) and oxygen tension.(aerobic and anaerobic conditions). Membrane filtration was used to determine bacterial concentrations of coliforms, fecal coliforms, and fecal streptococci at intervals during a 20 day period.

From this study, the following conclusions can be drawn:

(1) The death rates of the bacterial groups varied with the initial concentration of bacteria; the rates increased when the initial concentration increased.

(2) Higher death rates occurred at 30°C than at 20°C. The percentage of less resistant bacteria was greatest at the low temperature.

(3) All bacterial groups had greater decay under aerobic as opposed to anaerobic conditions. The effect of the presence of oxygen was shown to be less important to coliforms and fecal coliforms at 30°C than at 20°C.

(4) Coliforms decayed faster than fecal coliforms.

(5) Natural decay can be responsible for from one-half to nine-tenths of the order of magnitude of bacterial reduction through a soil filter.

(6) A laboratory study of bacterial decay gives results that correlate well with removals obtained by others in the operation of oxidation ponds.

Suggestions for Further Study

Several problems indicated by this study and requiring further investigation are:

(1) The effect of initial bacterial concentration on the bacterial decay rates.

(2) Effects caused by variations in substrate concentration, within the limits of those normally encountered in the disposal of treated and untreated sewage effluents.

(3) Variations in decay rates with respect to sewage source, especially with regard to the surviving bacterial fraction following chlorination.

(4) Variations in decay rates caused by operational changes in secondary sewage treatment methods.

APPENDIX

TABLE A-1

BACTERIOLOGICAL DATA

Run	Elapsed time, days	Number of Bacteria per 100 ml		
		Coliforms	Fecal Coliforms	Fecal Streptococci
A1	0.00	93×10^5	32×10^3	17×10^4
	2.08	5×10^5	8×10^3	4×10^3
	3.98	8×10^4	199×10^2	12×10^2
	6.97	30×10^3	52×10^2	62×10^1
	10.05	14×10^3	37×10^2	34×10^1
	14.02	15×10^3	23×10^2	13×10^1
	19.09	69×10^2	60×10^1	5×10^1
A2	0.00	93×10^5	32×10^3	17×10^4
	2.08	7×10^5	3×10^3	12×10^3
	3.98	48×10^4	134×10^2	98×10^2
	6.97	27×10^4	10×10^3	72×10^2
	10.05	29×10^4	23×10^3	50×10^2
	14.02	12×10^4	13×10^3	53×10^2
	19.09	95×10^3	590×10^1	39×10^2
A3	0.00	93×10^5	32×10^3	17×10^4
	2.08	1×10^5	24×10^2	2×10^3
	3.98	10×10^4	240×10^2	3×10^2
	6.97	24×10^3	27×10^2	10×10^1
	10.05	60×10^2	13×10^2	4×10^1
	14.02	15×10^2	3×10^1	4×10^1
	19.09	20×10^1	1×10^1	7×10^1
A4	0.00	93×10^5	32×10^3	17×10^4
	2.08	5×10^5	49×10^2	20×10^3
	3.98	41×10^4	278×10^2	11×10^3

TABLE A-1, Continued

Run	Elapsed time, days	Number of Bacteria per 100 ml		
		Coliforms	Fecal Coliforms	Fecal Streptococci
A4	6.97	$14x10^4$	$14x10^2$	$76x10^2$
	10.05	$30x10^3$	$9x10^2$	$53x10^2$
	14.02	$74x10^2$	$7x10^1$	$23x10^2$
	19.09	$55x10^1$	$1x10^1$	$11x10^2$
B1	0.00	$150x10^5$	$20x10^3$	$24x10^4$
	2.09	$3x10^5$	$26x10^2$	$4x10^3$
	3.99	$6x10^4$	$101x10^2$	$2x10^2$
	6.97	$21x10^3$	$35x10^2$	$59x10^1$
	10.07	$15x10^3$	$27x10^2$	$18x10^1$
	14.04	$10x10^3$	$7x10^2$	$11x10^1$
	19.10	$53x10^2$	$22x10^1$	$7x10^1$
B2	0.00	$150x10^5$	$20x10^3$	$24x10^4$
	2.09	$10x10^5$	$14x10^2$	$22x10^3$
	3.99	$54x10^4$	$193x10^2$	$9x10^3$
	6.97	$44x10^4$	$16x10^3$	$17x10^3$
	10.07	$25x10^4$	$7x10^3$	$15x10^3$
	14.04	$14x10^4$	$37x10^2$	$86x10^2$
	19.10	$79x10^3$	$28x10^2$	$79x10^2$
B3	0.00	$150x10^5$	$20x10^3$	$24x10^4$
	2.09	$4x10^5$	$19x10^2$	$3x10^3$
	3.99	$3x10^4$	$161x10^2$	$2x10^2$
	6.97	$14x10^3$	$14x10^2$	$18x10^1$
	10.07	$67x10^2$	$42x10^1$	$11x10^1$
	14.04	$10x10^2$	$3x10^1$	$4x10^1$
	19.10	$19x10^1$	$1x10^1$	$3x10^1$

TABLE A-1, Continued

Run	Elapsed time, days	Number of Bacteria per 100 ml		
		Coliforms	Fecal Coliforms	Fecal Streptococci
B4	0.00	150×10^5	20×10^3	24×10^4
	2.09	12×10^5	21×10^2	22×10^3
	3.99	52×10^4	145×10^2	28×10^3
	6.97	24×10^4	3×10^3	10×10^3
	10.07	22×10^3	9×10^2	70×10^2
	14.04	48×10^2	9×10^1	32×10^2
	19.10	13×10^2	2×10^1	12×10^2
C1	0.00	15×10^5	7×10^4	34×10^3
	2.97	4×10^4	13×10^3	4×10^2
	6.28	8×10^3	80×10^2	2×10^1
	8.23	78×10^2	40×10^2	2×10^1
	11.30	53×10^2	136×10^1	1×10^1
	14.05	39×10^2	44×10^1	1×10^1
	17.00	24×10^2	30×10^1	$<1 \times 10^1$
C2	0.00	15×10^5	7×10^4	
	2.97	12×10^4	11×10^3	
	6.28	27×10^3	15×10^3	
	8.23	21×10^3	59×10^2	
	11.30	24×10^3	301×10^1	
	14.05	12×10^3	21×10^2	
	17.00	88×10^2	111×10^1	
C3	0.00	15×10^5	7×10^4	
	2.97	15×10^4	15×10^3	
	6.28	27×10^3	25×10^2	
	8.23	13×10^3	29×10^2	
	11.30	39×10^2	74×10^1	

TABLE A-1, Continued

Run	Elapsed time, days	Number of Bacteria per 100 ml		
		Coliforms	Fecal Coliforms	Fecal Streptococci
C3	14.05	24×10^2	37×10^1	
	17.00	8×10^2	10×10^1	
C4	0.00	15×10^5	7×10^4	34×10^3
	2.97	16×10^4	10×10^3	4×10^2
	6.28	73×10^3	15×10^3	16×10^1
	8.23	27×10^3	43×10^2	12×10^1
	11.30	10×10^3	35×10^1	11×10^1
	14.05	31×10^2	14×10^1	8×10^1
	17.00	13×10^2	3×10^1	5×10^1
D1	0.00	8×10^5	39×10^3	26×10^3
	2.99	4×10^4	5×10^3	1×10^2
	6.27	8×10^3	15×10^2	3×10^1
	8.23	25×10^2	11×10^2	1×10^1
	11.30	22×10^2	56×10^1	1×10^1
	14.04	16×10^2	60×10^1	$< 1 \times 10^1$
	16.99	23×10^2	29×10^1	$< 1 \times 10^1$
D2	0.00	8×10^5	39×10^3	
	2.99	9×10^4	3×10^3	
	6.27	27×10^3	50×10^2	
	8.23	16×10^3	41×10^2	
	11.30	10×10^3	266×10^1	
	14.04	8×10^3	20×10^2	
	16.99	67×10^2	85×10^1	
D3	0.00	8×10^5	39×10^3	
	2.99	9×10^4	48×10^2	

TABLE A-1, Continued

BACTERIOLOGICAL DATA

Run	Elapsed time, days	Number of Bacteria per 100 ml		
		Coliforms	Fecal Coliforms	Fecal Streptococci
D3	6.27	25×10^3	20×10^2	
	8.23	21×10^3	15×10^2	
	11.30	37×10^2	104×10^1	
	14.04	71×10^1	7×10^1	
	16.99	31×10^1	2×10^1	
D4	0.00	8×10^5	39×10^3	26×10^3
	2.99	16×10^4	40×10^2	2×10^2
	6.27	79×10^3	32×10^2	12×10^1
	8.23	23×10^3	59×10^1	4×10^1
	11.30	19×10^3	37×10^1	7×10^1
	14.04	52×10^2	11×10^1	4×10^1
	16.99	25×10^2	6×10^1	6×10^1

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