

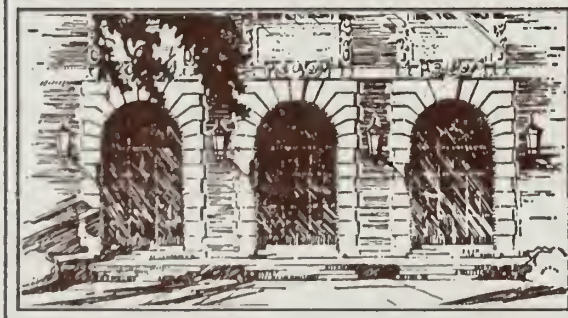
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ENVIRONMENTAL CONTAMINATION RESULTING FROM LAND RECLAMATION WITH ANAEROBIC DIGESTED SLUDGE

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CHAPTER I

INTRODUCTION

The high population growth rates in our urban centers presents the sanitary engineer with a very challenging problem; how to most efficiently dispose of the voluminous quantities of domestic waste produced by man. Present technology has made it possible to treat domestic wastewater so as to make it fit for human consumption. However, so far we have not been able to implement successful processes to dispose of the large amounts of solids originally present in the waste or resulting from treatment processes. Past or present methods of disposal have failed to comply with one or more of the four attributes of a successful sludge disposal operation as proposed by the Metropolitan Sanitary District of Greater Chicago [1].

These attributes are as follows:

1. The process must be compatible with the environmental standards of the area; i.e., no land, air or water pollution;
2. Must be one which solves the problem into perpetuity;
3. Must be economical;
4. Must conserve the sludge constituents for beneficial use.

The method of land reclamation by disposal of sludge is thought by many to come closest to achieving these ends. Many communities, both in the United States and abroad, are presently using the method of land disposal of sludge, both raw and digested, and either the dried, incinerated or liquid form. Some towns have set up their own business operations to package and sell dried sludge as fertilizer. Some of these operations proved successful, but many were a failure due to insufficient demand. A number of the experts feel that disposal of sludge in the liquid form on land is the most advantageous from an economic viewpoint [2,3,4,5]. The

philosophy of this method is not necessarily to provide a source of income, but to dispose of the sludge on land as a means of reducing overall operating costs of the water pollution control plant. Land disposal of sludge either in the dried or liquid form carries with it inherent problems to public health. Prior anaerobic digestion may help alleviate this problem [6]. In addition, it will help to stabilize the waste. Even with the digestion step, land disposal of sludge in the liquid form is felt to be the most economical. The Chicago Sanitary District estimates a two-thirds reduction in sludge disposal costs will be realized by so doing [1]. The validity of the belief that anaerobic digestion will help eliminate any dangers to public health, from a biological viewpoint, will be examined in the next chapter, but for now let it suffice to conclude that anaerobic digestion will not completely solve the problem. There are always some pathogenic organisms able to pass this treatment process relatively unharmed.

This research project dealt with contamination of the environment by pathogenic bacteria, by land disposal of anaerobically digested domestic sludge. Contamination of the environment can come about by contamination of the soil, surface water, and ground water by land runoff and leaching from land utilized for sludge disposal. Some comment on these aspects will be made in subsequent sections of this thesis.

This project was in no way a comprehensive study of the biological contamination of the environment resulting from land disposal of liquid digested sludge. Contamination by viral, protozoan or helminth agents is also possible. Only bacterial contamination was investigated. Again, even this was not all inclusive. The bacterial flora of domestic sewage is quite varied. Pathogenic as well as non-pathogenic species have the

potential for being present. Because of this and for reasons presented in Chapter III, an indicator group of organism, fecal coliforms was used in all bacterial studies reported herein.

CHAPTER II

LITERATURE REVIEW

A. The Bacterial Flora of Domestic Waste and Its Significance

As was previously stated, the bacterial flora of domestic sewage is quite varied. All of the species of bacteria present in the digestive tract of man have the potential for being present. This includes all of the enteric pathogens which plague man. The most common enteric pathogens are species of the genera *Shigella* and *Salmonella*. Gastro-intestinal complications caused by *Shigella* are usually mild in nature in the temperate areas of the world with a fatality rate of less than 1 percent [7]. In addition to this, disease produced by *Shigella* infection are not of common occurrence except in institutions or in crowded environments. This possibility indicates that a more common mode of conveyance is by direct transmission, i.e., fecal-oral transmission, rather than by water contamination. The diseases caused by bacteria of the genus *Salmonella*, however, are generally of a more serious nature. Case fatality rates for some of these disorders may be as high as 10 percent, i.e., typhoid fever [7]. The occurrence of *Salmonella* caused infections are also more common than those caused by *Shigella* [8]. In addition to this, only rarely do carrier states result from *Shigella* infections, whereas the carrier state is quite common after *Salmonella* diseases. A common mode of transmission of *Salmonella* infection is by contaminated water supplies. Thus, the presence of *Salmonella* in sewage is of more concern than is the presence of *Shigella*.

3. Survival of *Salmonella* in Waste Treatment Processes

The presence of *Salmonella* in the digester effluent is directly related to their ability to survive the various treatment processes up to and including anaerobic digestion. Generally speaking, *Salmonella*

organisms are moderately fastidious. They have to be supplied with quite an elaborate array of nutrients. In fact, disease is produced when parasitic pathogens, e.g., *Salmonella*, derive these essential nutrients from the host animal at the host's expense [9]. However, in domestic waste, these essential metabolites are limiting as *Salmonella* is unable to synthesize many of them, while the less fastidious organisms in the waste are able to synthesize these required nutrients. Therefore, conditions present in the various biological waste treatment processes may be quite lethal to the *Salmonella* bacteria. A discussion of their survival in these various processes follows:

1. Survival in activated sludge process

The survival of *Salmonella typhosa* in activated sludge was the subject of study by Ruchhoft [10] using activated sludge from the Calumet treatment plant in Chicago. With 5.5 hours of aeration, a reduction of 66 percent of *Salmonella typhosa* was obtained. Green and Beard [11] were able to obtain removal percentages ranging from 91 to 99 percent in laboratory experiments with activated sludge. Bruns and Sierp [12] found a reduction of 96.8 percent of typhoid organisms in activated sludge which was aerated for about 6 hours. After only 3 hours aeration, a reduction of 96 percent was noticed. Two tests with paratyphoid organisms gave decreases of 97 and 98 percent, respectively, after 3 hours aeration. In contrast to this, Pesch and Sauerborn [13] were able to obtain only about 74 percent reductions of paratyphoid B after 6 hours aeration. Courmont and Rochaix [14] qualitatively report that typhoid and paratyphoid bacteria were still present in activated sludge after aeration for 6 hours. These results are summarized in Table 1. It should be noted that these values for percent reduction are in the mixed liquor, i.e., prior to final

sedimentation. Ruchnoft [10] investigated the effects of final settling of activated sludge on the presence of S. typhosa. With one hour of settling following 5.5 hours of aeration, an additional reduction of 94.4 percent was obtained. Thus, giving a total reduction of 99.23 percent. Although these removal percentages are quite high, considerable numbers may still remain, depending on the initial number of organisms present. With final settling in the activated sludge process, it is evident that large numbers of Salmonella organisms may be concentrated in the sludge phase. Thus, if anaerobic digestion is the method employed for sludge conditioning, large numbers of Salmonella bacteria may find their way to the digesters. Since we are interested in the microbiological hazards of digested sludge for land reclamation, this is quite significant.

TABLE I
SURVIVAL OF SALMONELLA IN ACTIVATED SLUDGE PROCESS

Organism	Hours of Aeration	Percent Reduction	Investigator
<u>S. typhosa</u>	5.5	86	Ruchnoft [10]
<u>S. typhosa</u>	6	91-99	Green and Beard [11]
Typhoid group	6	96.8	Bruns and Sierp [12]
Typhoid group two	3	96	Bruns and Sierp [12]
Paratyphoids	3	97 and 98	Bruns and Sierp [12]
Paratyphoid B	6	74	Pesch and Sauerborn [13]
Typhoid and Paratyphoid bacteria	6	present	Courmont and Rochaix [14]

2. Survival in the trickling filter process

Little information on the survival of Salmonella bacteria in trickling filtration is available in the literature. However, the information which was available will be reviewed.

McCoy [15] has found that the trickling filter was effective in removing from 84 to 99 percent of S. paratyphosa B. The efficiency of removal of S. typhosa by the trickling filter was investigated by Green and Beard [11]. The results of their study is shown in Table 2.

In the trickling filter process, many of the removed organisms are present in the microbial slime on the surface of the filter media. This slime is subject to sloughing and is removed with final sedimentation. Therefore, considerable numbers of Salmonella bacteria may be concentrated in the settled sludge.

TABLE 2

SURVIVAL OF SALMONELLA TYPHOSA IN THE TRICKLING FILTER PROCESS

Dosage (MGAD)*	Percent Removal
3.6	99
5.4	96
6.6	99
12	95

*Million gallons per acre per day.

3. Survival in anaerobic digestion

Mom and Schaeffer [16] investigating the effect of sludge digestion in the Imhoff tank on the survival of S. typhosa found conditions to be quite lethal to this bacteria. The study was conducted under laboratory conditions with a digestion period of one month and a temperature of 27°C. With an initial inoculum of 700 S. typhosa per cc., 8 days was sufficient to destroy all of the organisms. Even with a very heavy infection of 116,000 per cc. all of the typhoid organisms were eliminated after only 11 days.

Ruchnoft [17] found retention time and temperature of digestion to be determining factors in the survival of S. typhosa. He found a 95.5 percent destruction of S. typhosa in about 10 days at a digestion temperature of 20 to 25°C. No viable organisms were isolated after 14 days. At lower digestion temperatures of 10 to 15°C., 91.5 percent of the organisms were destroyed in about 10 days, while after approximately 20 days, 96.3 percent were eliminated. Thus, lower temperatures have the effect of increasing the longevity of the typhoid organism. However, modern day digesters are operated at 35°C. which would increase the rate of elimination.

In another investigation conducted by Langley, McKinney and Campbell [6], a reduction of 84 to 92.4 percent in the numbers of S. typhosa was obtained for retention times of 6 and 20 days, respectively. No tendency for bulldup was noticed after the first 24 hours of digestion. A very comprehensive biochemical analysis indicated that competition for the essential amino acid, trptophan, was the main cause for the rapid destruction.

C. Fate of Salmonella in the Environment.

The results of the investigations cited above indicates that present biological treatment practices are effective in destroying a

large percentage of Salmonella bacteria in domestic waste. However, when dealing with pathogens the percentage reduction is not necessarily the important factor. The numbers of organisms remaining is of greater interest. Thus, even with a large percentage reduction in the population of a given pathogen through the treatment process, a lethal number may still remain in the sludge. These organisms, when discharged into the environment in the disposed anaerobically digested sludge may present serious hazards from a public health viewpoint. Therefore, an investigation of the survival of Salmonella organism in the environment seems warranted.

1. A mathematical approach

Chick's law [18] is often used to mathematically explain the survival kinetics of microorganisms when exposed to adverse conditions. In essence, the law states that the rate of destruction of the microorganisms follows a first order relationship. Stated mathematically:

$$\frac{dy}{dt} = -K (N_0 - Y) \quad (1)$$

where:

Y = Number of organisms destroyed

N_0 = Original number of organisms

K = Rate constant, 1/t

t = Time

By integration equation (1) becomes

$$\ln \frac{N_0 - Y}{N_0} = -Kt \quad (2)$$

And because $N_0 - Y = N$, the number of organisms remaining,

$$\ln \frac{N}{N_0} = -Kt \quad (3)$$

And equation (3) can be reduced to

$$\frac{N}{N_0} = e^{-Kt} \quad (4)$$

$$\text{or } \frac{N}{N_0} = 10^{-kt} \quad (5)$$

where k = rate constant to base 10

If equation (5) is plotted on semi-logarithmic paper with time plotted on the abscissa and N/N_0 plotted on the ordinate, a straight line will result if the kinetics of the biological system follow Chick's law. The slope of this straight line is the rate constant, k . Thus, by knowing the value of k and the number of organisms initially present we will be able to predict the number of organisms remaining at some future time for a given biological system. This could be a very handy tool in our assessment of bacteriological contamination of the environment resulting from land reclamation with liquid digested sludge. Of course, this will only hold if the kinetics of our system is found to follow a first order relationship.

2. Removal of Salmonella by percolation through porous media

The discussion which follows is directed toward bacteria in general but can be applied to Salmonella bacteria specifically. An investigation of groundwater contamination resulting from latrine trench leaching was conducted by Stiles, Crohurst and Thompson [19]. In this study, Escherichia coli was used as the tracer organism. The resulting data indicated that the E. coli traveled at nearly the rate of groundwater movement in the vicinity of the trench, but the rate of travel diminished with time. In this study, it was also found that chemical pollution traveled nearly twice as far as did bacterial contamination. Caldwell and Parr [20] investigated groundwater contamination from a bored hole

latrine. They noted a gradual regression of the bacterial front practically to the latrine after a maximum travel distance had been reached. They attributed this retreat to clogging of soil voids by sewage solids and microbial activity which acted as an effective straining mechanism in the vicinity of the latrine hole. The mechanisms of removal of bacteria on percolation through porous media was studied by Krone, Orlob and Hodgkinson [21]. They found the two main mechanisms of removal to be straining and sedimentation. Straining takes place at the point of contact of soil grains and sedimentation occurs on the grain surfaces. The region of greatest removal was found to be at the soil surface. The reason for this is that the greatest number of bacteria are available for removal from the applied liquid in this region. As bacterial cells accumulate, the straining mechanism becomes more efficient. However, as the bacterial agglomeration increase in size at the soil straining sites, they reach a point of instability and portions break off as clusters which are subsequently removed by straining or sedimentation at some lower depth. This type of activity continues as agglomerations grow and become unstable. At the lower depths, substrates are limiting due to their removal by the organisms near the surface. Thus, it seems that there will be decreasing bacterial concentrations as depth increases. And with a given rate of application of liquid laden with bacteria and substrates, there is a lower limit of depth beyond which bacterial contamination will not exist. However, the initial application of the sludge, prior to the settling out and accumulation of particulates and bacteria at the surface, presents the most critical period. This is so because some bacteria may be able to migrate to considerable depths during this period with resulting groundwater contamination.

3. Survival in soil

The availability of meaningful data on the survival of S. typhosa in soil is quite limited. However, Beard [22] has conducted a worthwhile investigation into the longevity of S. typhosa in various soils under different climatic conditions. The results of some of his work are shown in Table 3. It is apparent that two governing factors in the survival of this organism are availability of moisture and organic matter.

TABLE 3
TIME FOR 99.9 PERCENT KILL OF
SALMONELLA TYPHOSA IN VARIOUS SOILS

Soil Type	Rainfall Amounts (Inches)			
	10.1	3.2	2	0.7
Adobe	64	21	14	13
Adobe-peat	65	24.5	17	16
Loam	67	27.5	13	14
Sand	5	3.5	1.5	1.5
Peat		< 1	< 1	
Loam-sand	47.5			8

The maximum time for 99.9 percent destruction was in loam soil during a 2-month rainy period with 10.1 inches of rain, while peat exhibited the shortest time for 99.9 percent removal, less than one day. However, the main cause of death in peat is attributable to the very low pH that existed (3 to 4). The next shortest time was approximately 1.5 days in sand with 0.7 inches of rain in a 1-month period.

The effect of sunlight was also investigated by Beard [22]. Death rates were found to be increased by 30 percent when exposed to sunlight. In another report, Beard [23] summarized the factors which determine the survival of typhoid bacteria in soil as moisture retaining capacity of the soil, temperature, humidity, rainfall, pH, and the presence of other organisms. Loam, which best supported S. typhosa, has a high organic content as well as a high moisture retaining capacity. These are probable reasons for the extended survival period in this type of soil. However, this is not the case with sand which has a low organic content and a low moisture retaining capacity.

OBJECTIVES AND PROCEDURES

A. Objectives

The possibility of pathogenic bacteria being present in anaerobic digested sludge was discussed in the previous chapter. Also discussed were the factors which may determine the survival of pathogenic organisms in soil as reported by Beard, et al. [22,23]. These are moisture retaining capacity of the soil, temperature, humidity, rainfall, pH, and the presence of other organisms. If sludge were disposed of on sandy soil, the organic concentration, as well as the moisture retaining capacity, could be increased. Thus, this soil would then be better able to support microbial life. An attempt was made to determine the longevity of pathogenic bacteria at various moisture concentrations in sandy soil, reclaimed with anaerobic digested sludge.

B. Procedures

1. Use of indicator organisms

It has been the practice of sanitary microbiologists to resort to the use of non-pathogenic indicator organisms to predict the behavior of pathogenic organisms. This is because the procedure for detection of pathogenic organisms are quite involved and requires much skill and time. The chosen indicator organism should therefore be easily detectable and the method of detection should be one which is simple and quickly performed. In addition to this, the indicator organism should also be slightly more resistant to adverse conditions than are the pathogenic organisms. The reason for this is when the indicator organism is at a very low density or completely destroyed, we can be reasonably certain that the pathogenic organism will not be present.

.

The group of organisms most commonly used as an indicator of fecal contamination, and thus the possible presence of pathogenic bacteria, is the coliform group of bacteria. Included in this group are not only bacteria of enteric origin, but also some species of bacteria normally found in soil. The most common soil coliform is Aerobacter aerogenes. Thus, in a given sample of material, the presence of coliform bacteria may not necessarily be an indication of fecal contamination. In order to alleviate this difficulty, the fecal coliform most probable number (MPN) test according to Standard Methods [24] was used in all bacterial analysis. With this method conditions are such that coliforms of fecal origin are able to survive while non-fecal strains are destroyed. By using fecal coliforms as the indicator bacteria, a more realistic idea of the behavior of enteric bacteria in the environment is obtained.

2. Experimental methods

The type of soil used in this study was Plainfield sand. Table 4 illustrates the physical properties of the sand. A sample of this soil was obtained and placed in a large plastic tub, 18-1/4 inches in diameter. In the bottom of this container was placed about 3 inches of graded gravel. On top of this gravel was placed 2 feet of the soil sample. The bottom of this tub was provided with a means for drawing a vacuum. Thus, in essence a lysimeter was constructed. Samples of liquid digested sludge were obtained from the Champaign-Urbana, Illinois, Sewage Treatment Plant. This sludge was found to have an average moisture concentration of about 97 percent. The soil in the lysimeter was "conditioned" with this sludge so that a situation would be obtained similar to that which would occur after repeated heavy applications of sludge to land. This was accomplished by applying a one-inch depth of sludge to the surface of the lysimeter. The liquid

phase of the sludge was allowed to leach through the soil and was drawn off by the vacuum provided. After the liquid had disappeared from the surface, the remaining solids were mixed into the upper 6 to 9 inches of soil. This was similar to the sludge being disced into the upper most region of the soil in the field. This process took about one day. A second application was made about 3 days hence and the above process was repeated. A third and final application was made in another 3 days and again this process was repeated. Thus, a total of 3 inches of sludge was added in about 9 days.

TABLE 4
PHYSICAL PROPERTIES OF PLAINFIELD SAND

Particle Size (mm)	Percent Finer (%)
1.000	99.9
0.500	89.5
0.250	50.8
0.100	13.6
0.050	6.9
0.020	4.2
0.002	2.8

Following the conditioning phase, the upper 9 inches of the soil in the lysimeter was removed to a second tub of the same size as that used in constructing the lysimeter. To this soil was added one-half inch of fresh liquid digested sludge. Also enough rainwater was added to adjust the moisture concentration to the lowest of the four different moisture concentrations used in this study. The moisture concentrations used were

5, 10, 15 and 20 percent moisture. The lower limit of 5 percent moisture was chosen as it was felt that this would most closely represent average field moisture concentrations. At moisture concentrations greater than 20 percent, the saturation capacity of the soil had been exceeded. It was felt that tests on such samples would not be meaningful. After adding enough rainwater to obtain 5 percent moisture, a sample was removed to a 10-3/4 inch by 13-1/4 inch by 3-1/2 inch plastic pan. The depth of soil in the pan was adjusted to one inch. More rainwater was added to the remaining soil in the tub to obtain the next moisture concentration. Again, a sample was removed to a second plastic pan. This procedure was repeated for the remaining moisture concentrations desired. In a fifth plastic pan was placed 1/2 inch of liquid digested sludge. The plastic pans were then covered with Saran Wrap and the edges sealed with Scotch tape. This was done to prevent the loss of moisture with the resultant decrease in moisture concentration. Saran Wrap is quite well suited for this application as it has the advantageous feature of allowing oxygen to pass through while not permitting the loss of moisture. Thus, it was possible to maintain a fairly constant moisture concentration and also aerobic conditions in the atmosphere above the soil.

Next the survival capabilities of the fecal coliforms were tested at these four soil moisture concentrations and the sludge. Initial fecal coliform densities were obtained in soil samples and sludge from the pans described above. The densities were also found at varying times after this; every day at first and then at longer periods as time progressed. Samples were taken by coring the soil with a glass tube and removing ten grams of soil. The entire one-inch depth of soil was cored to obtain the samples. It was required that a number of cores be taken to obtain ten grams of soil. The cores were taken in different areas of the pan. The ten

grams of soil obtained was then mixed with 90 milliliters of phosphate buffer water, giving a one to ten dilution by weight. The phosphate buffer water was made according to Standard Methods [24]. This could then be further diluted in the same manner to higher dilutions depending on the anticipated density. The sludge sample was tested by using a volumetric dilution, but this may still be compared with the soil samples as 1 ml of sludge weighs approximately 1 gram. The fecal coliform MPN was then run on the diluted sample. A check of the moisture concentration in the pans was made at various times to insure proper experimental conditions. Some of the test pans required periodic moisture concentration adjustments. This was done by sprinkling rainwater on the surface of the soil as uniformly as possible.

CHAPTER IV

RESULTS AND DISCUSSION

The results of the investigation of the survival of fecal coliforms in sludge conditioned soil at 5 percent moisture are shown on Figure 1. The plotted points shown as squares represent the actual values obtained while the circles represent the 95 percent confidence interval. The 95 percent confidence intervals are as given in Standard Methods [24]. An initial sharp rise in the fecal coliform population will be noted. The reason for this increase was not investigated. However, it might be explained by the fact that in the anaerobic digestion process acid forming bacteria, which include the fecal coliforms, degrade the organic material into short chain fatty acids and other intermediates. These intermediates are in turn used as a substrate by another group of bacteria the methane formers. The methane formers are strict anaerobes. Thus, when the anaerobic sludge was removed from the digester to an aerobic environment, the methane group of bacteria were destroyed. This now leaves the short chain fatty acids available to the fecal coliforms as a food source under aerobic conditions. Thus, new cells are able to be synthesized. Approximately a 100-fold increase in numbers was realized.

The general trend exhibited by the data plotted on Figure 1 does not follow a definite straight line as predicted by Chick's law. Instead, the trend seems to indicate a decreasing rate of die off. This may possibly be due to the elimination of less resistant bacteria which were competing with the fecal coliforms for the organic substrates.

Figure 2 shows the survival of fecal coliforms in sludge conditioned soil at 10 percent moisture. Again, an initial period of growth is evident. The fecal coliform density was increased by about 10 fold. A straight line fit seems best to represent the final trend.

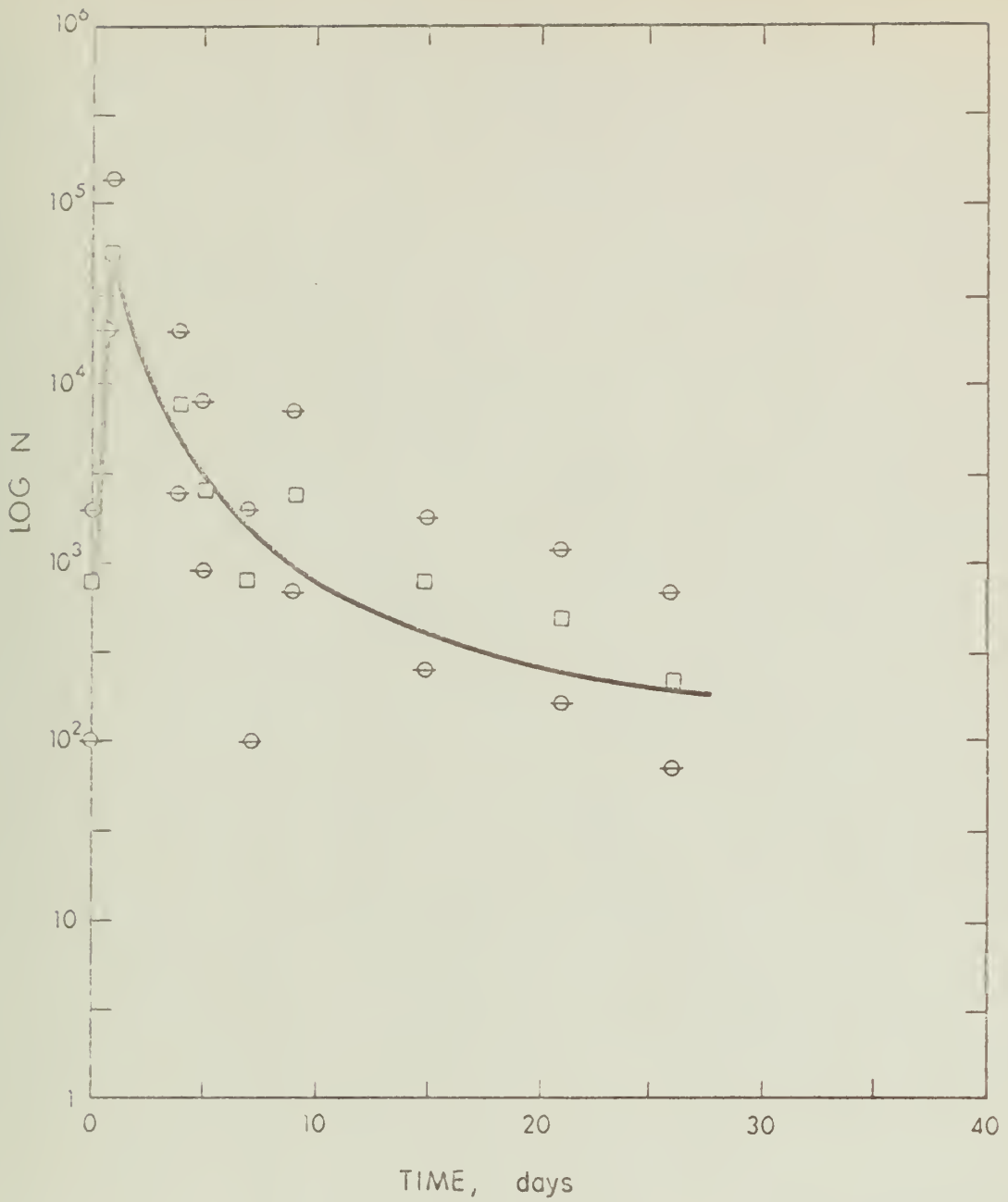


FIGURE 1
FECAL COLIFORM SURVIVAL
IN SLUDGE CONDITIONED
SOIL AT 5% MOISTURE

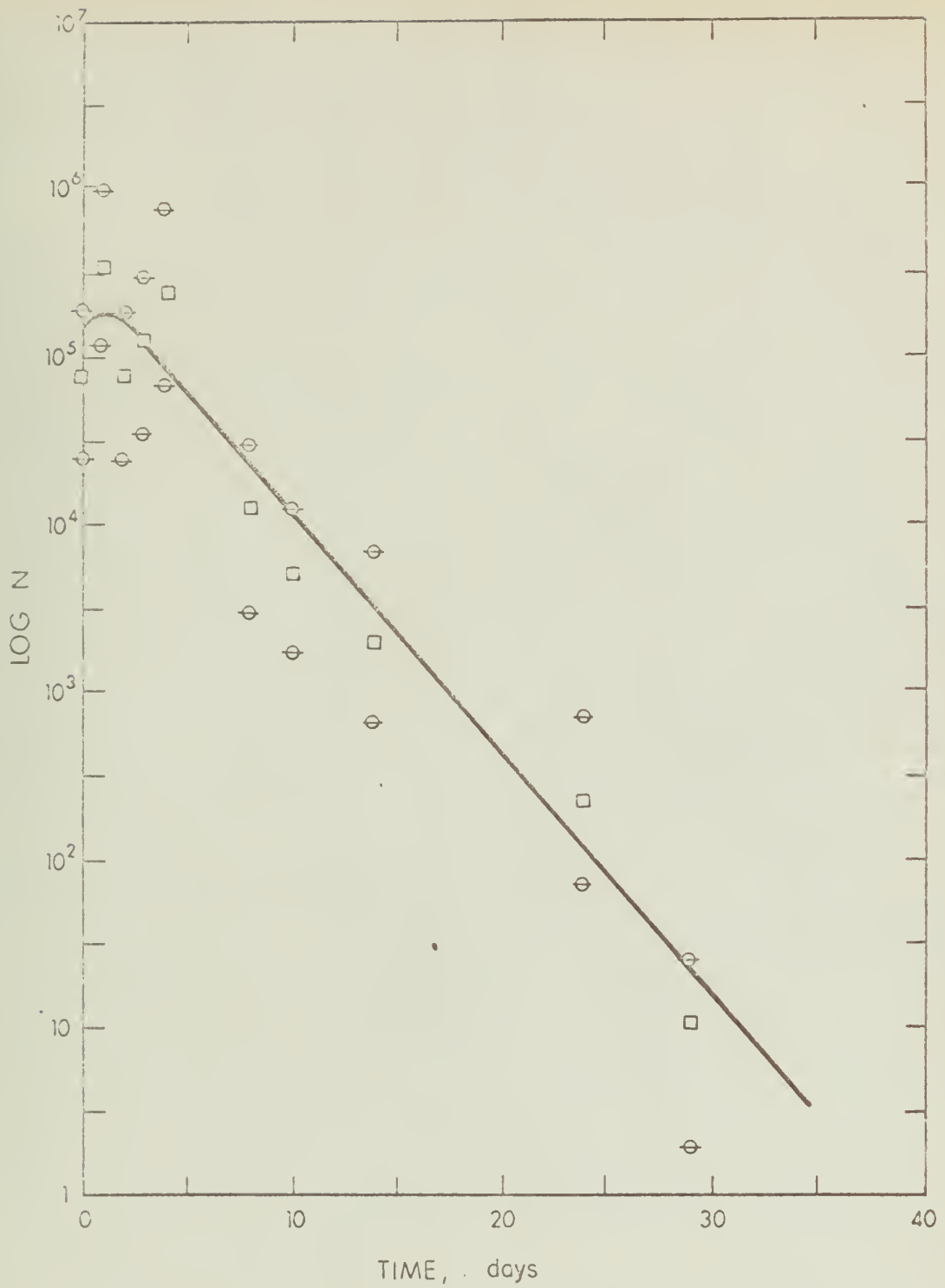


FIGURE 2.
 FECAL COLIFORM SURVIVAL
 IN SLUDGE CONDITIONED
 SOIL AT 10% MOISTURE

The fecal coliform survival in sludge conditioned soil at 15 percent moisture is shown on Figure 3. At this moisture concentration, a 10-fold increase in fecal coliform density is again realized. The final trend here seems also to be best fitted by a straight line.

Figure 4 illustrates fecal coliform survival at 20 percent moisture in sludge conditioned soil. No initial growth phase is exhibited by the data for this curve. This is probably due to the fact that at this high moisture concentration the saturation capacity of the soil was exceeded. Thus, essentially anaerobic conditions were maintained. For this reason, the organic intermediates of anaerobic digestion are not readily available to the fecal coliforms as a food source. Instead they were utilized as a food source by the methane forming bacteria, which are strict anaerobes. It was also evident that anaerobic conditions existed in the soil by the appearance of the soil mass. A black color could be noticed which was evident of precipitation of metal sulfides due to anaerobiasis. The plotted data exhibit a great deal of scatter. However, the general trend can be fitted very well by a straight line.

The trend of the data plotted on Figure 5 for fecal coliform survival in sludge seems to better fit a straight line than any of the previous data. Again, as in sludge conditioned soil at 20 percent moisture, no initial phase of growth was realized. The reason for this is again probably due to the maintenance of anaerobic conditions, with the resulting inavailability of the intermediates of anaerobic digestion to the fecal coliforms as a food source.

Table 5 shows the average and range of percent die off of fecal coliforms after approximately 30 days. To arrive at the figures shown in column (2), the experimental MPN values were used. Thus, the percentages shown represent a sort of average value.

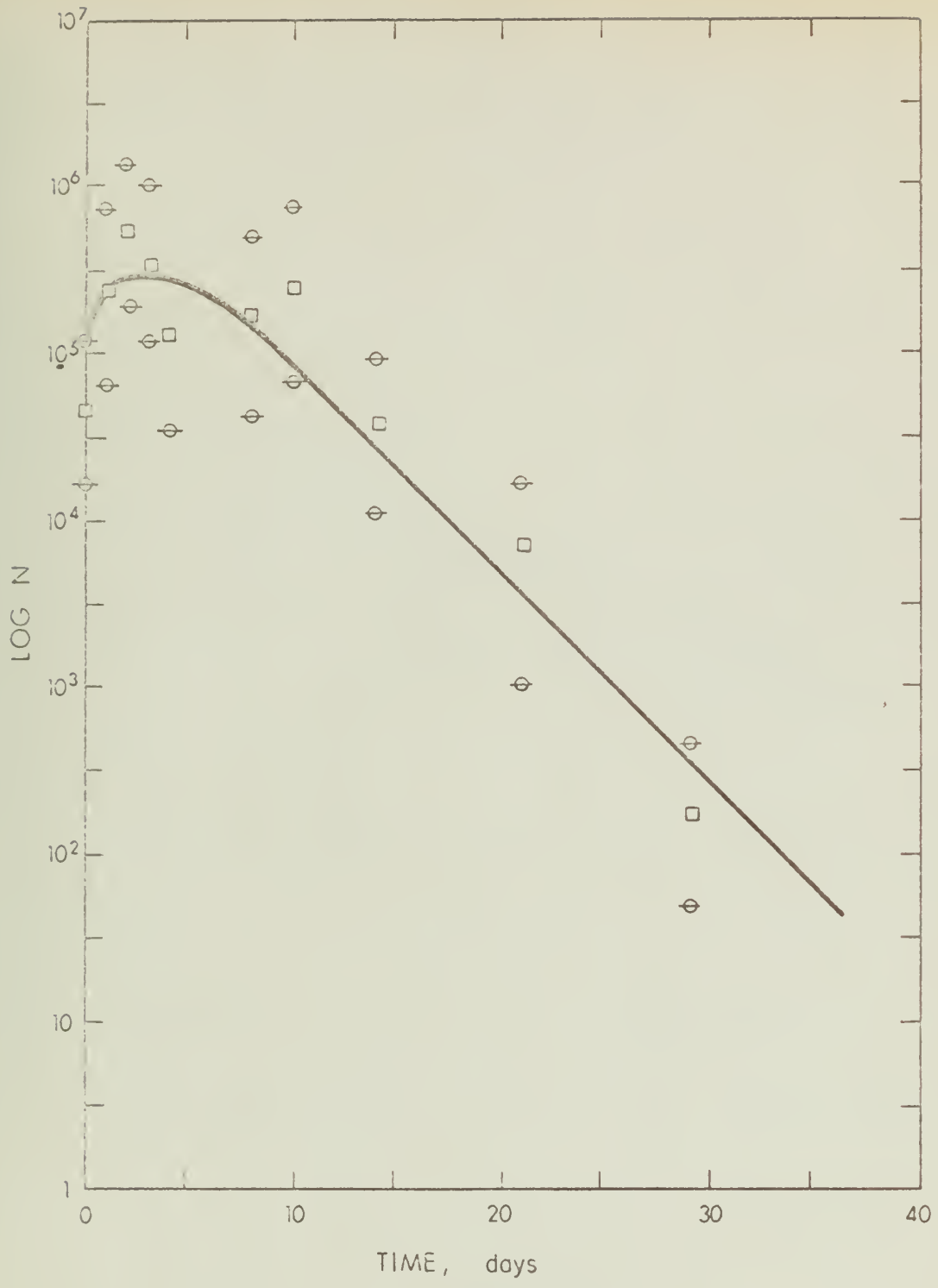


FIGURE 3.
FECAL COLIFORM SURVIVAL
IN SLUDGE CONDITIONED
SOIL AT 15% MOISTURE

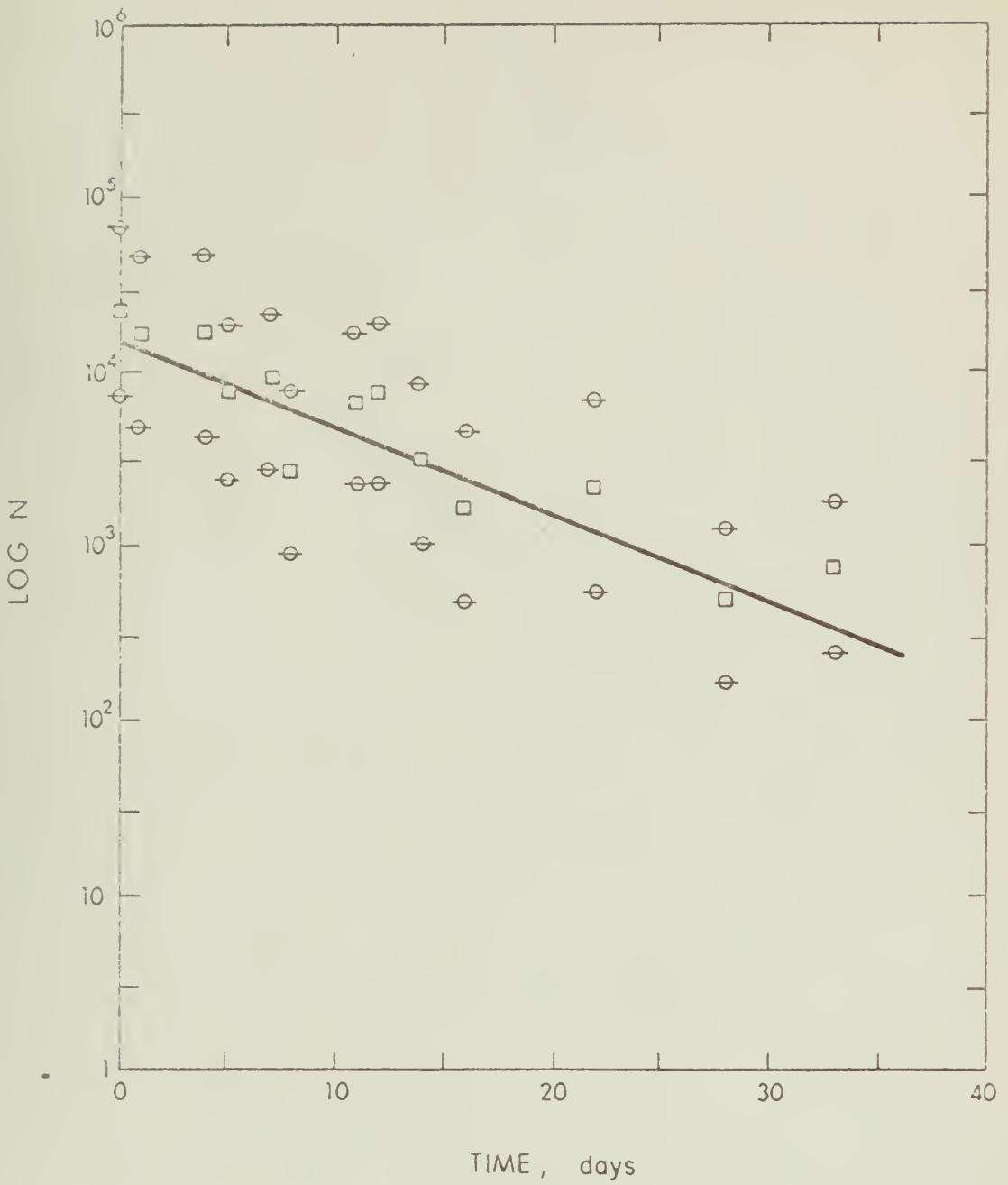


FIGURE 4
FECAL COLIFORM SURVIVAL
IN SLUDGE CONDITIONED
SOIL AT 20% MOISTURE

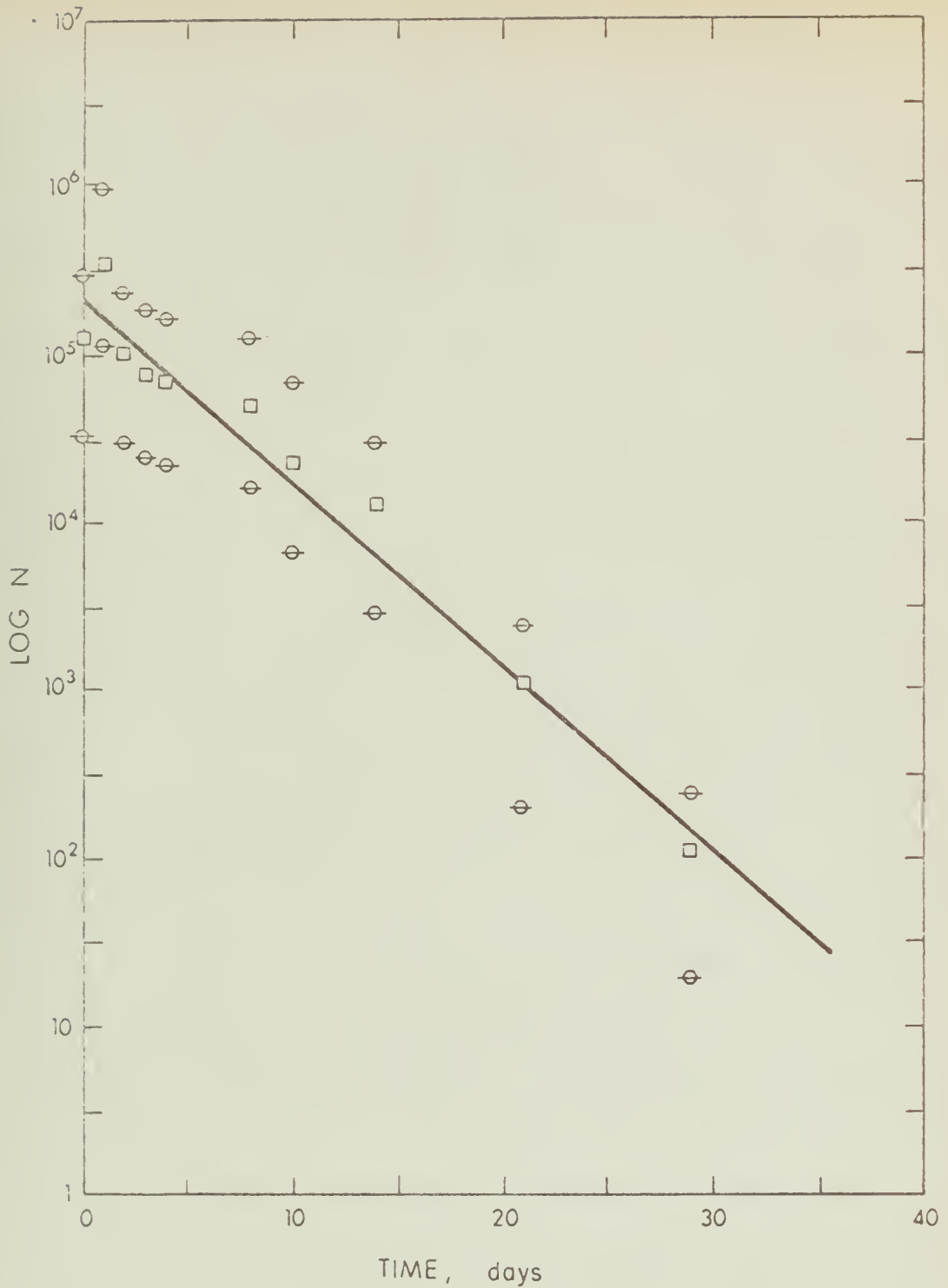


FIGURE 5.
FECAL COLIFORM SURVIVAL
IN SLUDGE AT 98% MOISTURE

TABLE 5
SURVIVAL OF FECAL COLIFORMS IN SOIL AND SLUDGE

Percent Moisture (1)	Average Percent Fecal Coliforms Destroyed in Approximately 30 Days (2)	Range of Fecal Coliforms Destroyed in Approx. 30 Days (Percent) (3)
5	72.50	57.0 (increase) to 96.0
10	99.99	99.90 to 99.99+
15	99.63	97.12 to 99.96
20	96.56	72.80 to 99.64
Sludge	99.91	99.29 to 99.99

Column (3) indicates the range of percent die off at the various moisture concentrations in approximately 30 days. The figures shown in this column were computed by using the final number at the lower end of the 95 percent confidence interval and the original number at upper end of the interval to compute the highest possible percent die off. To compute the lowest possible percent die off, the original number at the low end and the final number at the high end of the confidence interval were used.

Table 6 illustrates the die off rate constants (see equation 1) for the four soil moisture concentrations investigated, as well as the sludge sample at 97 percent moisture. For those moisture concentrations where an initial growth phase was realized, the constants indicate the rate of die off following the period of growth. It was not possible to compute a "k" value for die off at 5 percent moisture due to the decreasing rate of kill. It must be realized that these rate constants were determined from the line that best fit the 95 percent confidence intervals. The rate constants are subject to some variation due to the variation of possible slopes

the line could assume between the limits of the confidence intervals. However, the rate constants reported in Table 6 are still indicative of the rates of die off of fecal coliforms under the conditions investigated. It should be noted that the lowest die off rate constants occurred at 20 percent moisture and for the sludge sample, this is disregarding the results at the 5 percent moisture concentration when a decreasing rate of die off was noticed. The reason for the lower die off rates at the 20 percent moisture concentration and in the sludge sample is probably due to the maintenance of anaerobic conditions in these samples. This provided more favorable conditions for survival of the fecal coliforms.

TABLE 6
DIE OFF RATE CONSTANTS

Percent Moisture	Die Off Rate Constant
5	Decreasing Rate
10	0.18
15	0.13
20	0.05
Sludge	0.11

Examination of the plotted data along with the data of Tables 5 and 6 indicates that at 5 percent moisture concentration the fecal coliiforms are best able to survive. This is indeed surprising as one would expect a faster rate of die off at lower moisture concentrations due to the inavailability of moisture. However, two possible reasons can be cited for the better survival at the 5 percent moisture concentration in this study. These are:

1. As previously stated, the possible elimination of less resistant bacteria that were competing with the fecal coliforms for the organic substrates. These less resistant competitors were able to survive at the higher moisture concentrations. It should also be noted here that it is likely that the fecal coliforms also are not very resistant to conditions in the soil environment.
2. Bhaumik and Clark [25] have hypothesized that there exists an "aeration porosity limit." This is the point at which there exists the most favorable balance between moisture concentration and aeration. Possibly this was the condition at or near 5 percent moisture in this study.

As was discussed, in most cases the trend of the data seemed to follow first order kinetics. This was so within a 95 percent confidence interval. Thus, variations from first order kinetics may actually occur, although the trend can be represented by a straight line. This is what one would actually expect. The work conducted by Chick to derive her expressions for bacterial die off was done under closely controlled conditions. Only one organism was used, a chemical disinfectant was used, and the test was conducted in a completely liquid medium. In the investigation described herein, a very diverse microbial population was present, many factors are contributing to the destruction of the fecal coliforms, e.g., dessication, competition, and disinfection, and the tests were conducted in a relatively dry medium, thus, contact between the fecal coliforms and any nutrients in the system was limiting.

CHAPTER V

CONCLUSIONS

The results of this study indicate that, in most cases, except for an initial period of growth at the lower moisture concentrations, the general trend of die off follows first order kinetics, although no strict adherence to Chick's law can be inferred. This is true except at the 5 percent moisture concentration where the trend does not follow first order kinetics. Instead, at this moisture content the trend shows a decreasing rate of die off following an initial sharp rise in numbers. Also, the fecal coliforms were able to survive for extended periods of time at all moisture concentrations. At 5 percent moisture it is even possible to realize a sizable increase in fecal coliform numbers. This is of particular interest as this is probably closest to the average moisture concentration found in the surface layers of the soil in the field. These slow rates of die off may be significant for two reasons. One is the possibility of leaching of the bacteria into the groundwater. However, this may not be of major concern.

Instances cited in Chapter II indicated that the bacteria are retained fairly well in the upper regions of the soil. But perhaps more important, runoff from these contaminated soils may contribute pathogenic organisms to the receiving stream. These two problems should be subjected to further investigation to establish if there actually is a problem, and if so, what is the magnitude of the problem. Such a study should consist of an extensive lysimeter investigation. This would provide a check of the bacterial removal capacity of the soil. In addition to this, it would also substantiate reports in the literature of efficient bacterial removal in the upper regions of the soil. The study should also include a

bacterial analysis of runoff from sludge reclaimed land. This part of the study should be conducted under field conditions.

Although the results of this study show a slow rate of die off of fecal coliforms in the laboratory, in the field, sunlight would play a very important role of eliminating many bacteria from the surface layers of the soil. Thus, rates of die off shown in the report may, in actuality, be higher.

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