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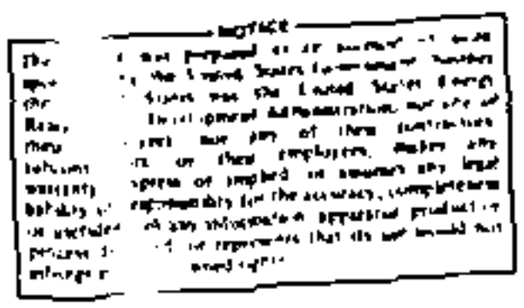

INACTIVATION OF BACTERIA IN SEWAGE SLUDGE BY IONIZING RADIATION, HEAT, AND THERMORADIATION

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ABSTRACT

For purposes of animal feeding or fertilizer usage on edible crops, sewage sludge must be free of pathogenic organisms. Bacterial inactivation by a combination of heat and irradiation is shown to be effective. These results must be viewed in conjunction with those from studies of parasite egg inactivation, virus inactivation, and physical-chemical benefits in order to make a fair assessment of the value of the thermoradiation treatment compared to other possible sludge treatment processes.

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Introduction

Sandia Laboratories has been involved recently in the study of the possible beneficial uses of radioisotopes, particularly cesium-137, from nuclear reactor waste.[1,2] Part of this program has been concerned with the feasibility of treating sewage sludge, the standard handling and disposal of which accounts for approximately 40 percent of the cost of wastewater treatment in the United States.[3] With rising costs of fertilizer and livestock feed, it is likely that municipal sludge from which pathogens have been eliminated will find wide application in agriculture[4,5,6,7] both as feed supplement and as fertilizer. Several studies have been reported which demonstrate the benefits available from treatment of sewage or sewage sludge by ionizing radiation;[8-13] most of these, however, have been concerned with enhancement of settling rates or other changes in physical-chemical properties of sludges. While Sandia Laboratories has been involved somewhat in this aspect as well,[14] our primary effort has been in the area of elimination of pathogenic microorganisms, including parasites,[15] viruses,[16] and bacteria, with optimistic results. Part of our optimism stems from the synergistic behavior of combined heat and radiation in the inactivation of microorganisms such as viruses,[17,18] bacteria,[19,21] spores,[22,23] and others.[15] This report deals with the inactivation of bacteria in sewage sludge, specifically the indicator groups of coliforms and fecal streptococci, subjected to various combination treatments of heat and ionizing radiation.

Materials and Methods

Sludge Sampling

Sewage sludge samples for the inactivation studies were obtained from the Albuquerque Water Reclamation Plant, Number 2, where trickling filters are used for purification of the wastewater. The sludge from the primary settling tanks, trickling filters, and secondary clarifiers is pumped to primary digesters for stablization of biological anaerobic processes and then pumped into secondary digesters for further digestion and holding. Due to odor and other problems, the sludge is pumped into the drying beds only during the winter months; this leads to inconsistencies throughout the year in the makeup and percent digestion in the secondary digesters. For this reason, sludge samples used in the inactivation studies were obtained from the bottom of the primary digesters. These samples have been fairly consistent with solids content ranging from approximately 5 to 8 percent and biological counts typically at 10^6 per milliliter for coliforms and approximately 10^4 for fecal streptococcus bacteria. Since the digester input is essentially continuous, the sludge varies in percent digestion from poorly digested to completely digested. On an average, however, the sludge is fairly well digested, since the average retention time in the primary digesters at the Albuquerque plants is approximately 30 days, which is sufficient time for sludge stabilization by normal anaerobic biological processes.

The sludge, generally fresh for a given set of experiments, was strained through a No. 10 mesh screen to remove large particulate matter. The filtrate was blended in a Waring commercial blender at 15,500 revolutions per minute for 1.5 minutes to provide a more consistent mixture for pipetting. The sludge was then stored at 4° C until used.

Since sewage sludge can be potentially hazardous (pathogenic organisms tend to settle with the solids in the clarification process and are therefore concentrated), all personnel wore surgical gloves and laboratory coats when handling the samples. During blending and straining, face masks were worn in case of aerosolization of the samples. All procedures were performed in a Class 100 clean room or in a fume hood. All apparatus were disinfected thoroughly with a 10 percent bleach solution containing a surfactant. All contaminated glassware, plasticware, and used samples were autoclaved after use, before washing, or discarding.

Irradiation Systems

Cesium-137 has been the primary irradiation source for the inactivation studies. Rods are arranged in a cylindrical array and raised from the storage pool into position around the irradiation chamber (stainless steel). The maximum dose rate was fixed at approximately 30 krads per minute. A system was developed whereby samples could be withdrawn, using remote controls from the irradiation (heat, thermoradiation) chamber. It consisted of spring-loaded syringes activated remotely by electrical signals from a set of timers (see Fig. 1). Samples of 8 - 10 milliliters were withdrawn and cooled in ice until the experiment was completed. This resulted in a considerable saving of time as it was unnecessary to lower and re-raise the source (and the doors to the irradiation cell) between sampling. Sample heating was provided by circulating water of the desired temperature through a jacket between the irradiation chamber and the source. In the experiments at higher temperatures, the slow heat-up profile for the system (Fig. 2) presented a problem, in that the process was not an ideal step function with time. However, in process application such a profile may be realistic, and, additionally, the inactivation curve comparisons remain valid since sampling of heat-treated, radiation-treated, and thermoradiation-treated sludge was consistent in time.

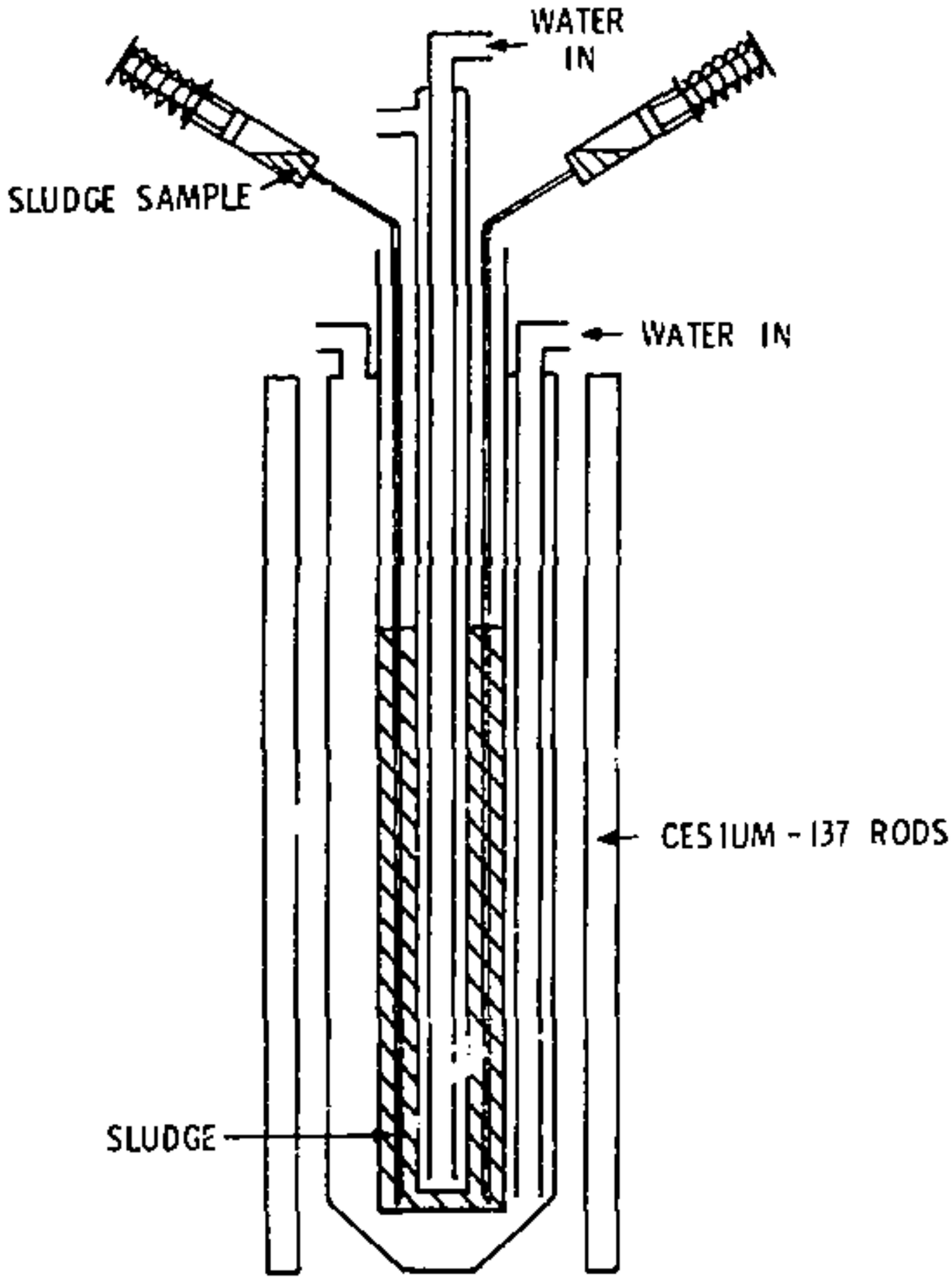

Figure 1. Milliliter irradiation chamber with remote sampling capability

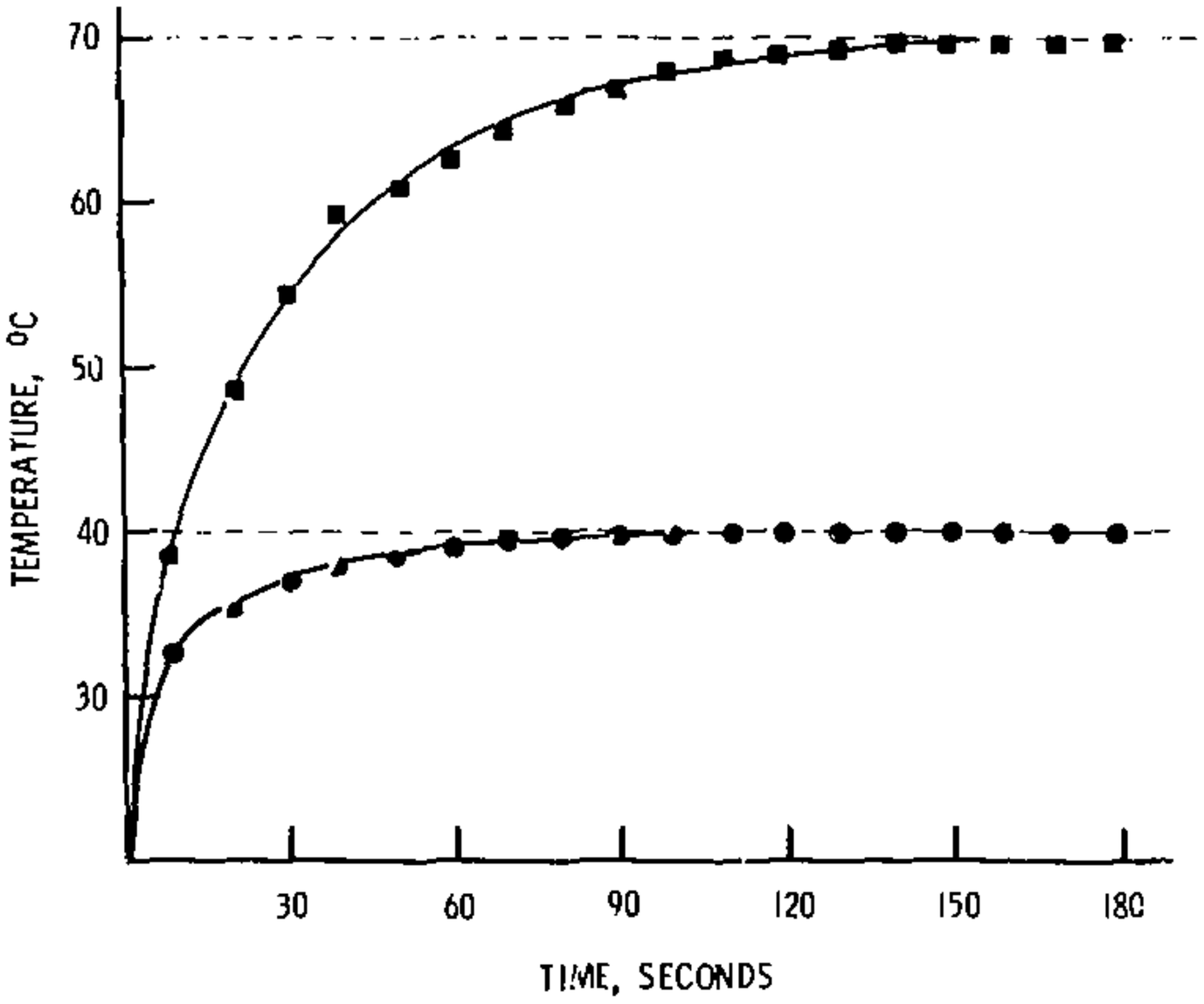

Figure 2. "Heat-up" profiles for the remote sampling system

Cobalt-60 was used as the irradiation source in several experiments, particularly those involving dose rate. Samples in test tubes were placed in the irradiation cell at positions which varied from the center of rectangular array (approximately 60 krads per minute) to several meters away (approximately 1 krad per minute).[24] In all experiments, dosimetry was performed using TLD-400 solid state dosimeters (CaF_2:Mn).

It should be pointed out that the 70° C inactivation of both coliforms and fecal strep bacteria depended strongly on the heat-up profile of the system. Therefore, several experiments were performed in stainless steel metal containers which were rectangular in shape and approximately 1 millimeter thick. The purpose of these experiments was to determine the effects of heat alone on the inactivation at 70 C, and the chambers were designed to provide maximum surface area for minimum heat-up time. The system heat-up profile was approximated using water and a thermocouple placed within the chamber. A typical profile is shown in Fig. 3. Samples were injected and removed with a syringe. Treated samples were immediately placed in ice to inhibit further inactivation.

Biological Techniques

"Colony-forming-ability" was used as a measure of bacterial inactivation following each of the treatment processes. The treated samples (or controls) were serially diluted in physiological (0.85 percent) saline and plated out in petri dishes. The procedure for coliform bacteria is detailed in Appendix A; that for fecal strep is a Standard Method.[25] Coliform colonies have a green metallic sheen and the fecal strep colonies are red and lens-shaped. Both are easily distinguishable. Approximately 150 - 200 plates were required for the determination of a single inactivation curve for either organism since 4 to 20 plates were typically prepared for each serial dilution. Plates

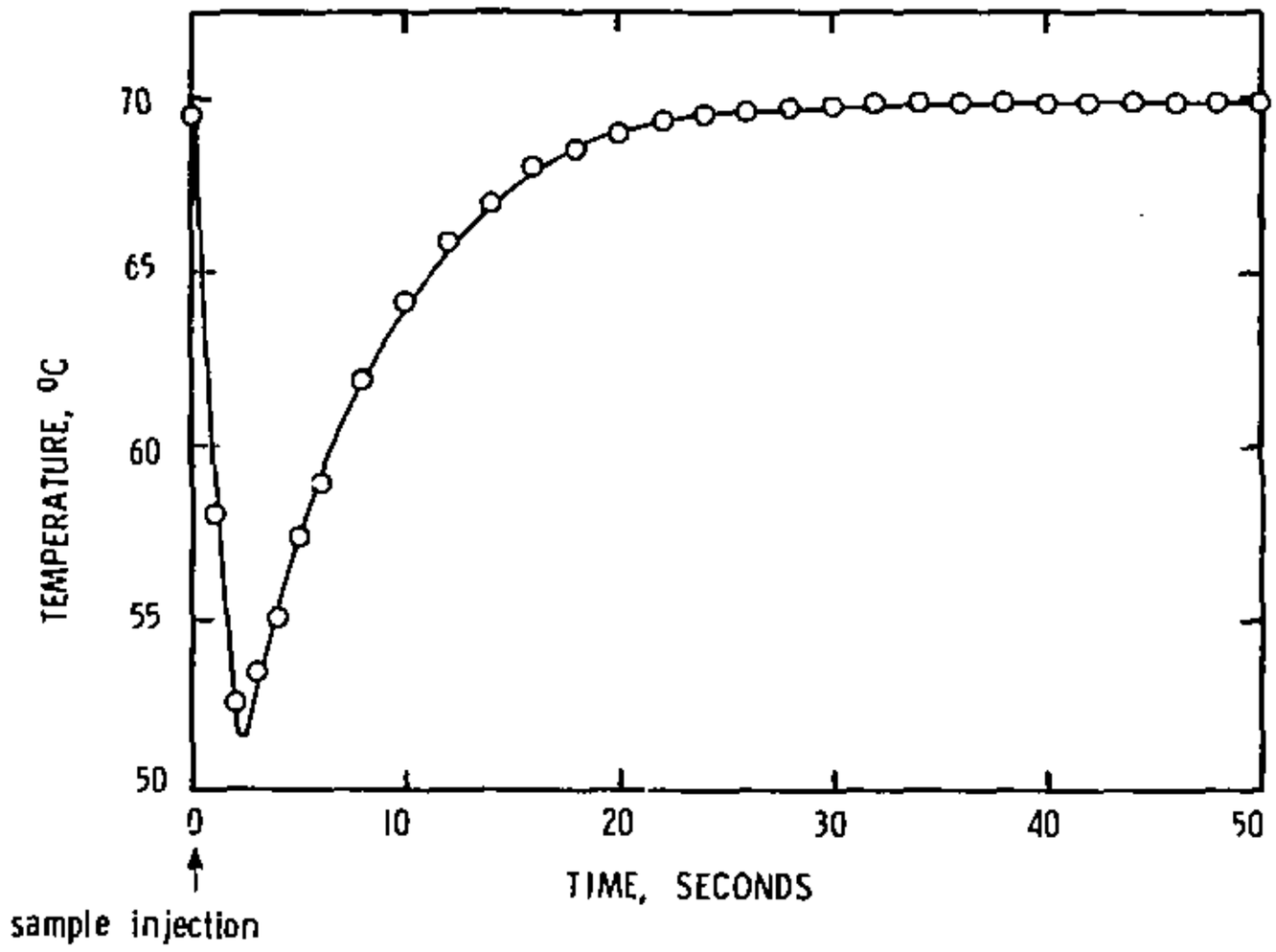

Figure 3. "Heat-up" profile for fast-rise sample chambers

containing more than 300 colonies were considered too numerous and therefore were not used in determining inactivation effectiveness. In all cases, the samples were plated out on the same day as the experiment was performed.

Results and Discussion

Reported sensitivities of various microorganisms to heat and to ionizing radiation are listed in Table I. The viruses tend to be generally radiation-resistant but heat-sensitive. The coliforms are relatively sensitive to either treatment. *Streptococci (and possibly* Salmonella*) appear to be the most* heat- and radiation-resistant groups, aside from spores. Based on this information, much of the work reported herein has been done using fecal streptococcus bacteria as the *microorganism in sludge exhibiting the greatest resistance* to both heat and radiation. There can be differences, certainly, between individual species of any of these groups; in addition, radiation or heat sensitivities may depend on *the medium employed (for example, several types of virus were* reported to be considerably more sensitive to heat when contained in raw milk versus ice cream mix[27]).

A considerable portion of the work was concerned with the total coliform population in sludge because, in a typical wastewater treatment process, the coliforms are nearly always the "indicator" microorganisms monitored.[34]

Figure 4 shows the radiation inactivation at 20° C of coliforms by gamma rays from cesium-137. Different symbols indicate different "batches" of sludge and/or different "runs". Inactivation rate is the important parameter, and the slopes for the four data sets are the same, within experimental error. It should be pointed out that, typically, errors due to plate

TABLE 1.

ORGANISM	KRADS*	MIN @ 60° C*	REFERENCES
ADENOVIRUS	450	0.15	26, 27
POLIOVIRUS	300	1.5	28, 29
COLIFORMS	20	2	30
STAPHYLOCOCCI	22	3.3	31, 32
SALMONELLA	45	7.5	32, 33
STREPTOCOCCI	120-200	15	12, 30

*TREATMENT REQUIRED FOR ONE LOG OF INACTIVATION

Table 1. Sensitivities of various microorganisms to heat and to irradiation

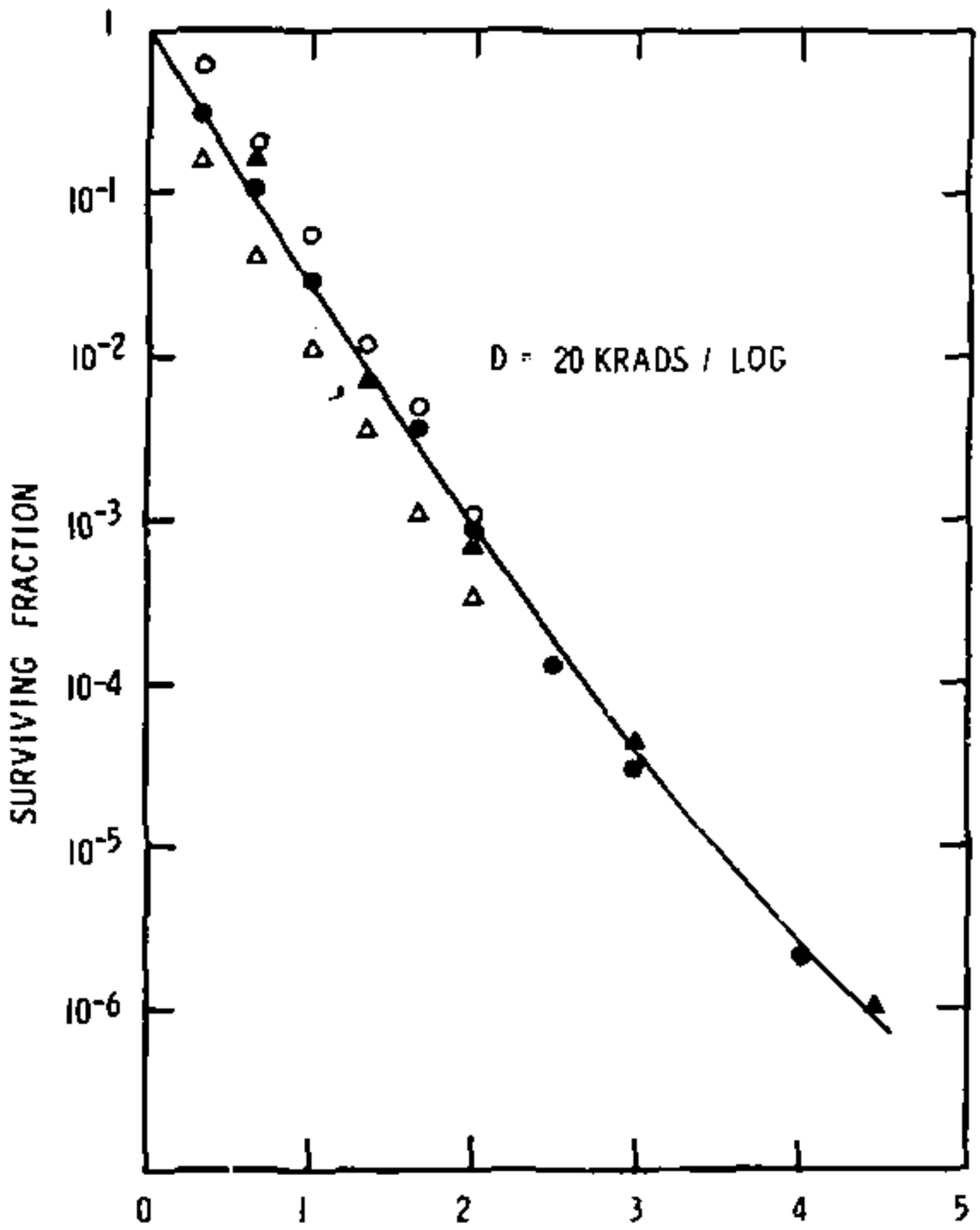

Figure 4. Radiation inactivation of coliforms at 20° C and 30 krads/minute for four separate runs

count variations (dilutions, pipetting) range from 10 - 50 percent, averaging less than 20 percent. On these log plots, however, even factors of 2 and 5 are acceptable in many cases. Subsequent conclusions are not affected significantly by the calculated standard deviations (or 95 percent confidence intervals), and therefore these intervals have not been included on the plots. It is seen that the "D-value" (the absorbed dose required to decrease the bacterial count by one log, or 90 percent) is approximately 20 krads.

Since later studies to be included in this program involve use of an "outer-ring" irradiation chamber, it was important to determine whether any dose rate effects would appear in the inactivation curves. At present, only room temperature irradiation data are available. The dose rate was varied over two orders of magnitude (using cobalt-60, from 1.2×10^{-2} krads per second up to 1.3 krads per second) with no apparent change in the inactivation curve (Fig. 5). This range certainly includes any feasible dose rate considered for application purposes. It is also clear from the slope of the curve in Fig. 5 that, as expected, there is essentially no difference in the effects of cobalt-60 as compared to those of cesium-137, within experimental error.

Data from a complete "run" (i.e., heat, irradiation, and thermoradiation) are presented for one temperature (50° C) in Fig. 6. These are typical curves. It can be seen that at 50° C, heat alone does very little inactivation over this time scale. The inactivation by combination treatment exhibits some synergism (approximately one log), but the effect is considerably less than that observed from some earlier studies, such as those of Escherichia coli in broth.[19] The observed synergism in sludge is consistent, however, and is seen from 40° C to 65° C.

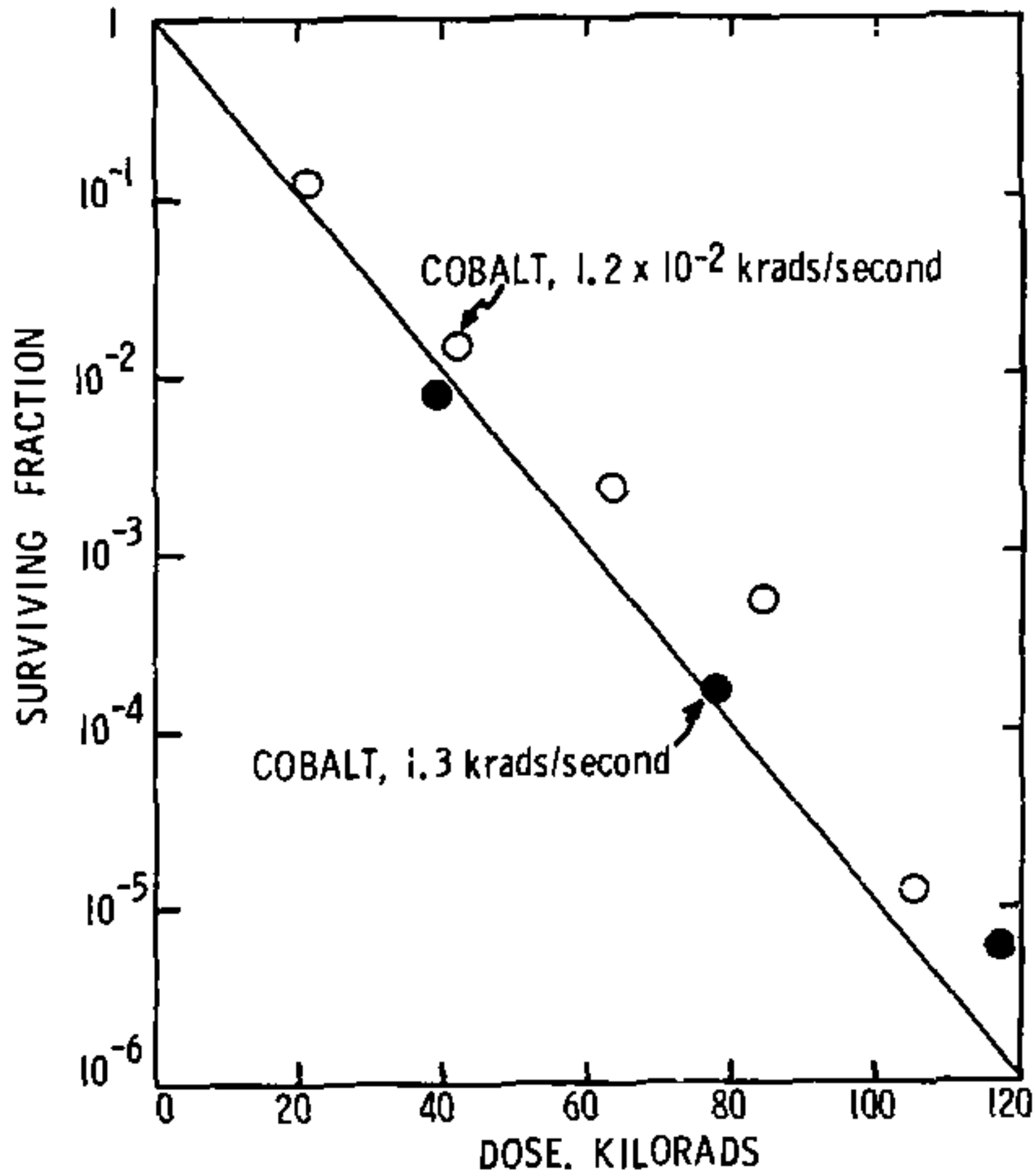

Figure 5. Coliform inactivation at different dose rates (cobalt-60). Solid line is from earlier data (see Fig. 4)

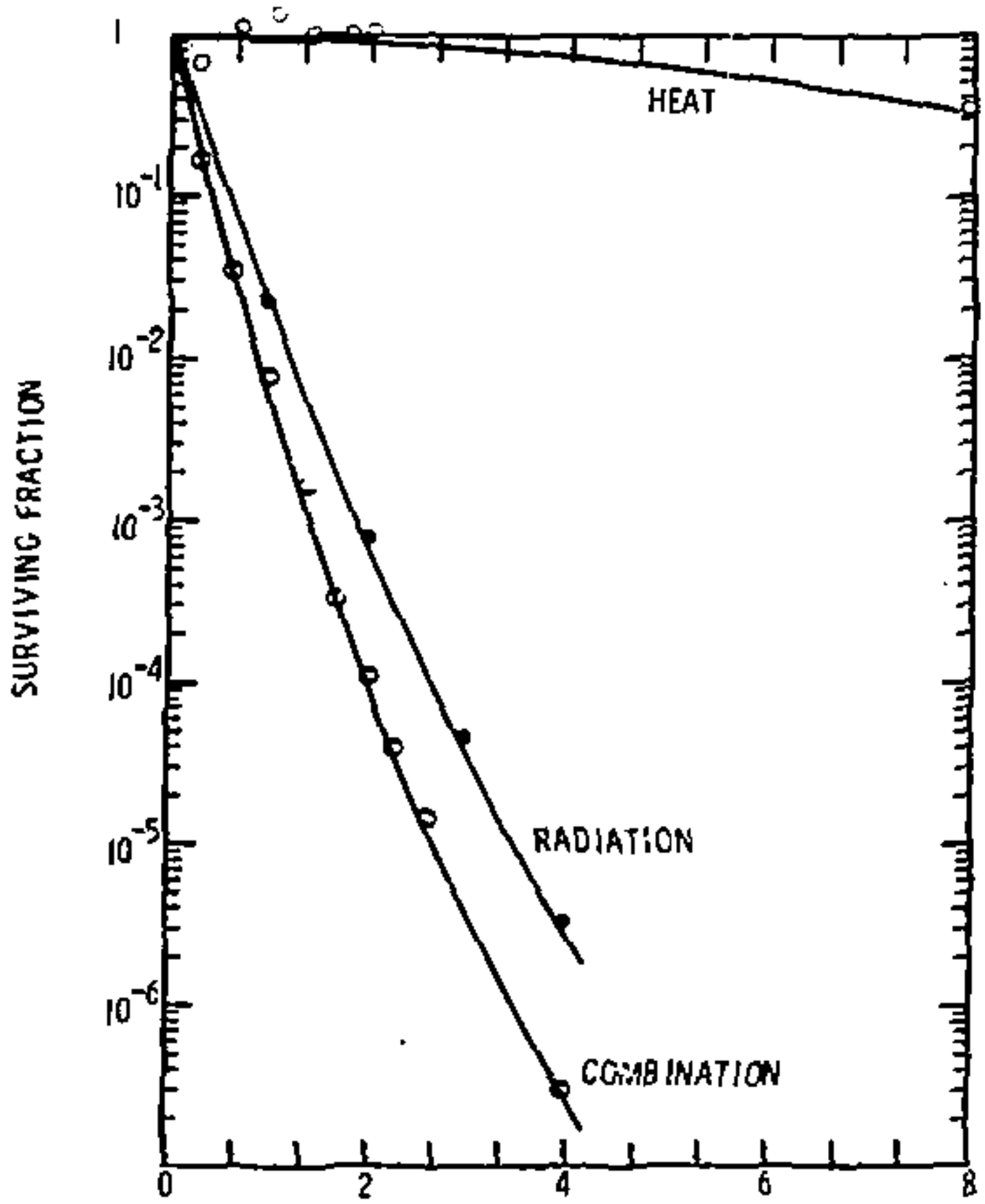

Figure 6. Coliform inactivation by heat and thermo-radiation at 50° C and 30 krads/minute

Heat inactivation curves for total coliforms in sludge are presented in Fig. 7. Only at 55° C and above does heat have an appreciable inactivation effect on this time scale. The results at 60° C and 65° C are inconsistent, possibly due to sludge batch variation or problems resulting from a combination of the heat-up profile and the relatively high heat sensitivity of this heterogeneous group of microorganisms. Included in this figure for comparison purposes are the data obtained at 70° C (dashed line) using the "fast-rise" heat chamber described earlier.

Figure 8 shows a 60° C plot similar to the 50° C run. The radiation D-value at this temperature is seen to be approximately 4.5 krads/log (versus 20 krads/log at 20° C). Similar data have been taken at 5 degree intervals from 40° C to 65° C. In all cases, the heat run and the thermoradiation run were performed on the same day and on the same sludge sample. These data are plotted in Fig. 9 as surviving fraction after one minute of treatment. This time is arbitrarily chosen for demonstration purposes; in fact, the synergism is somewhat greater at longer treatment times. The dotted line in the figure represents the additive effect of heat treatment and radiation treatment, and can be compared with the actual data for combined treatment (thermoradiation). It is seen that the synergism is greater at higher temperatures; however, at lower temperatures, there is some degree of synergism.

Inactivation of fecal streptococcus bacteria in sewage sludge by ionizing radiation is shown for two different runs in Fig. 10. It is observed that the radiation resistance of these microorganisms is approximately 6-fold greater than that of the coliform group (120 krads per log versus 20 krads per log). Over a 60-fold variation in dose rate using cobalt-60, the inactivation curves are dose rate independent (Fig. 11) and, within experimental error, source independent. As in the

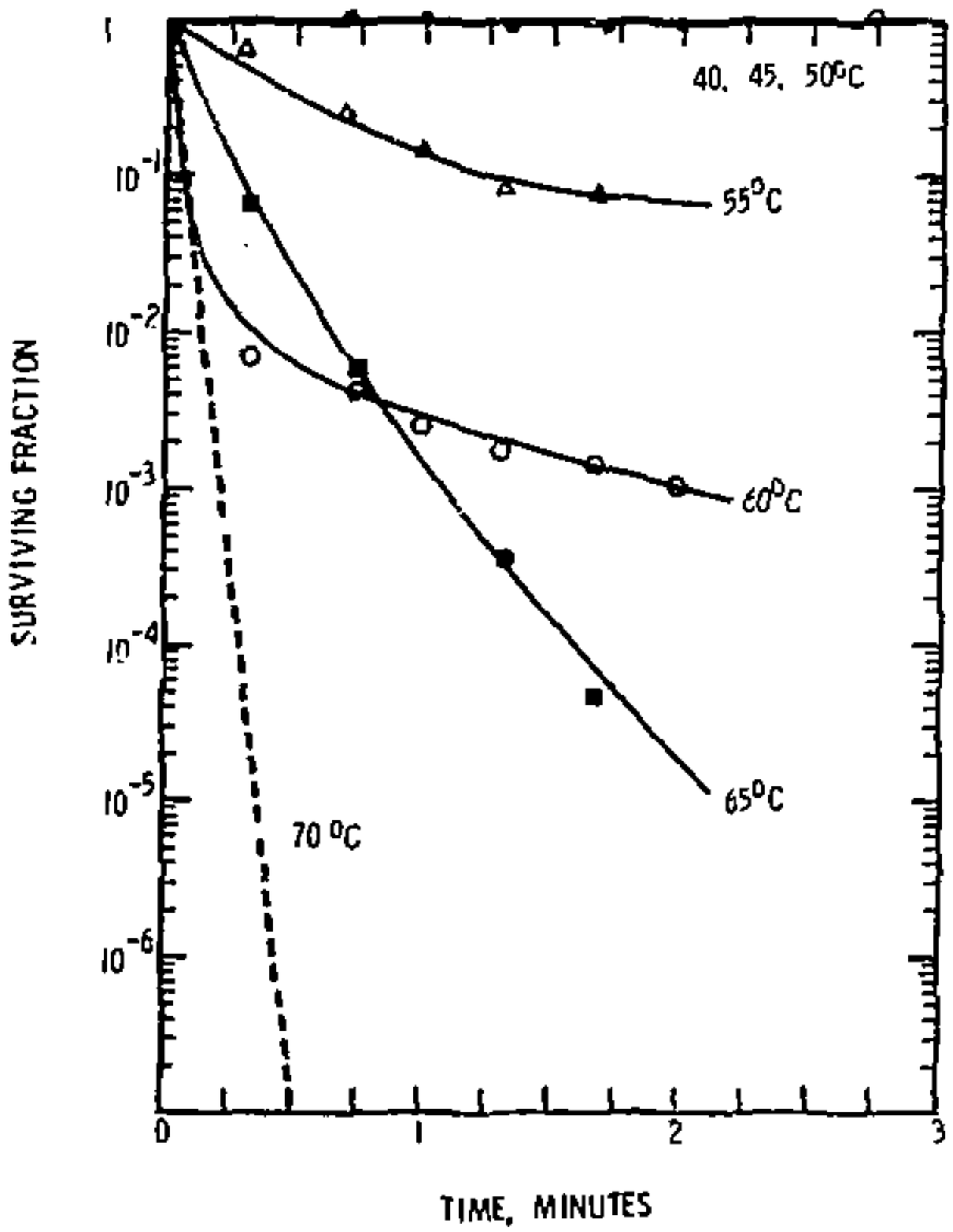

Figure 7. Thermal inactivation of coliform bacteria

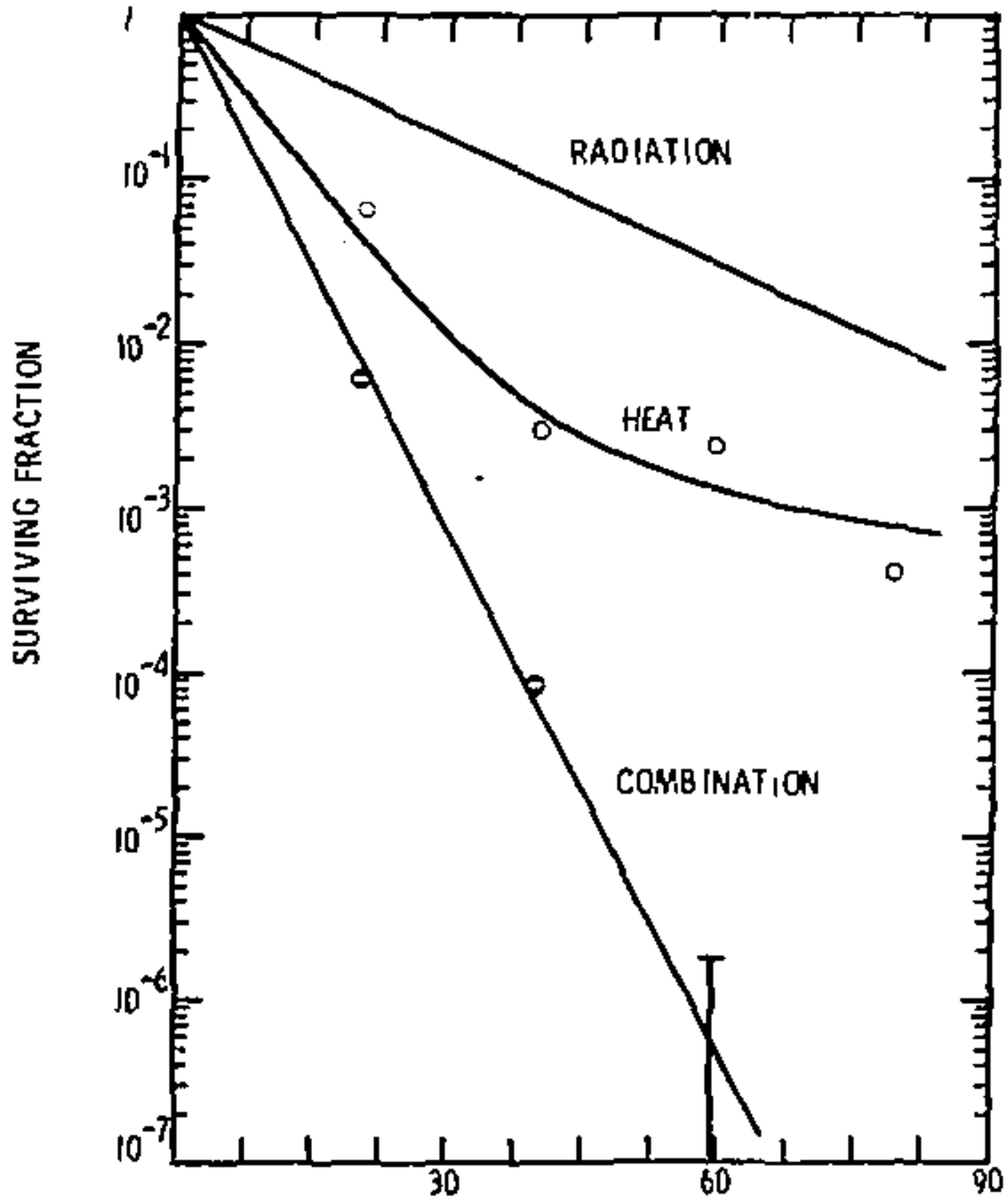

Figure 8. Coliform inactivation at 65° C

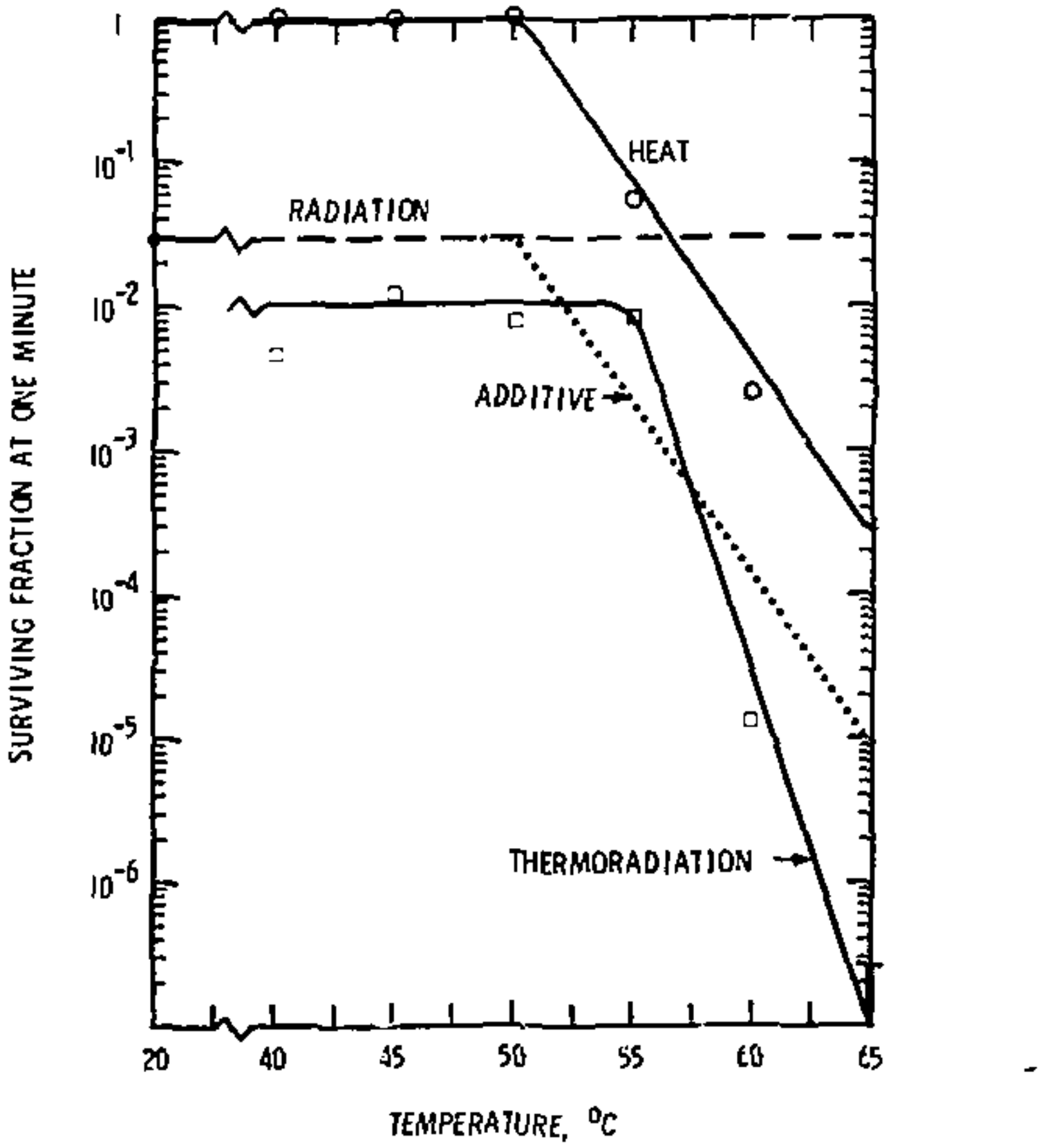

Figure 9. Temperature dependence of coliform survival. Comparison of additive and observed inactivation after one minute of treatment.

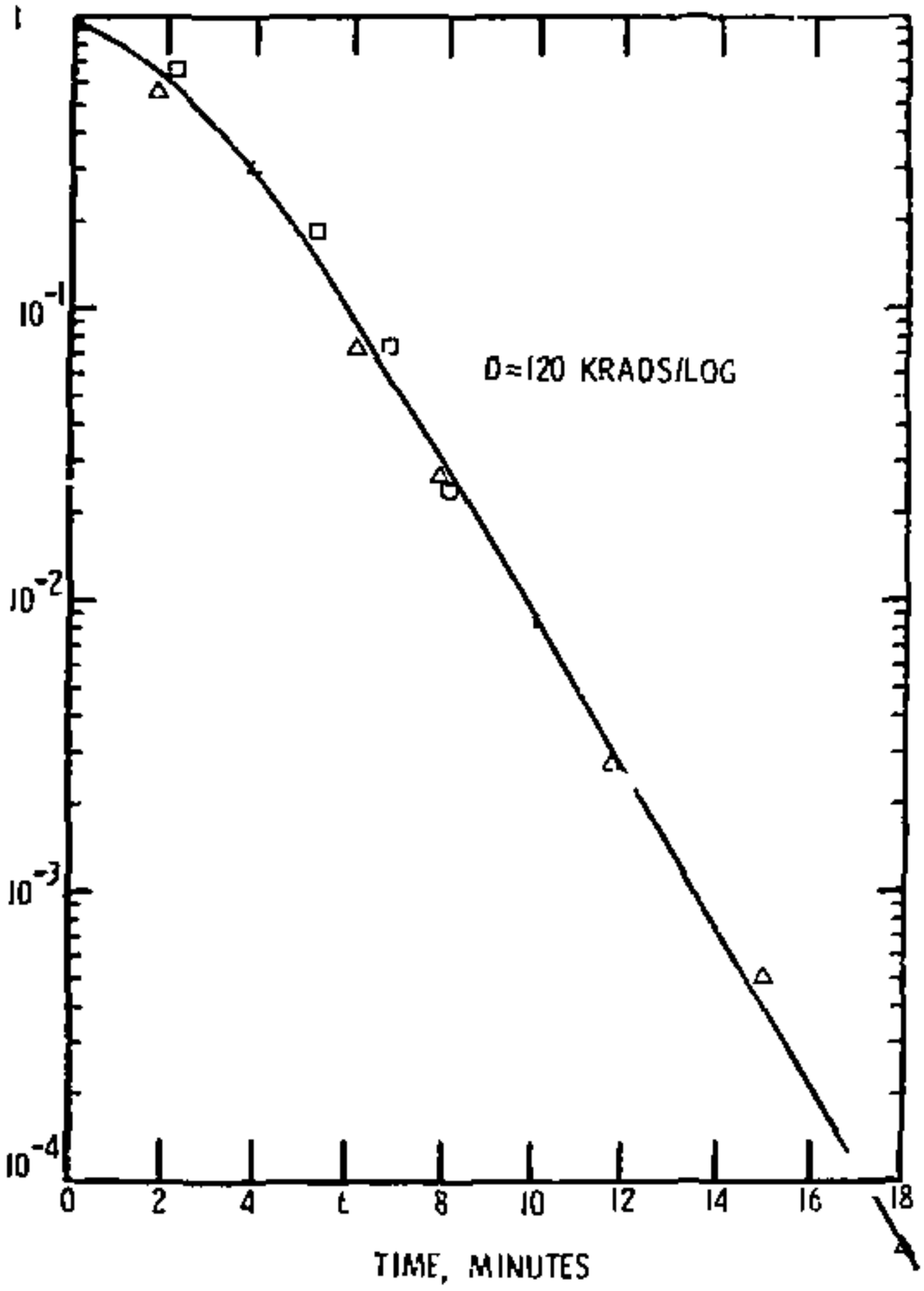

Figure 10. Radiat. inactivation of fecal streptococcus bacteria at 20° C and 30 krads/minute for two separate runs

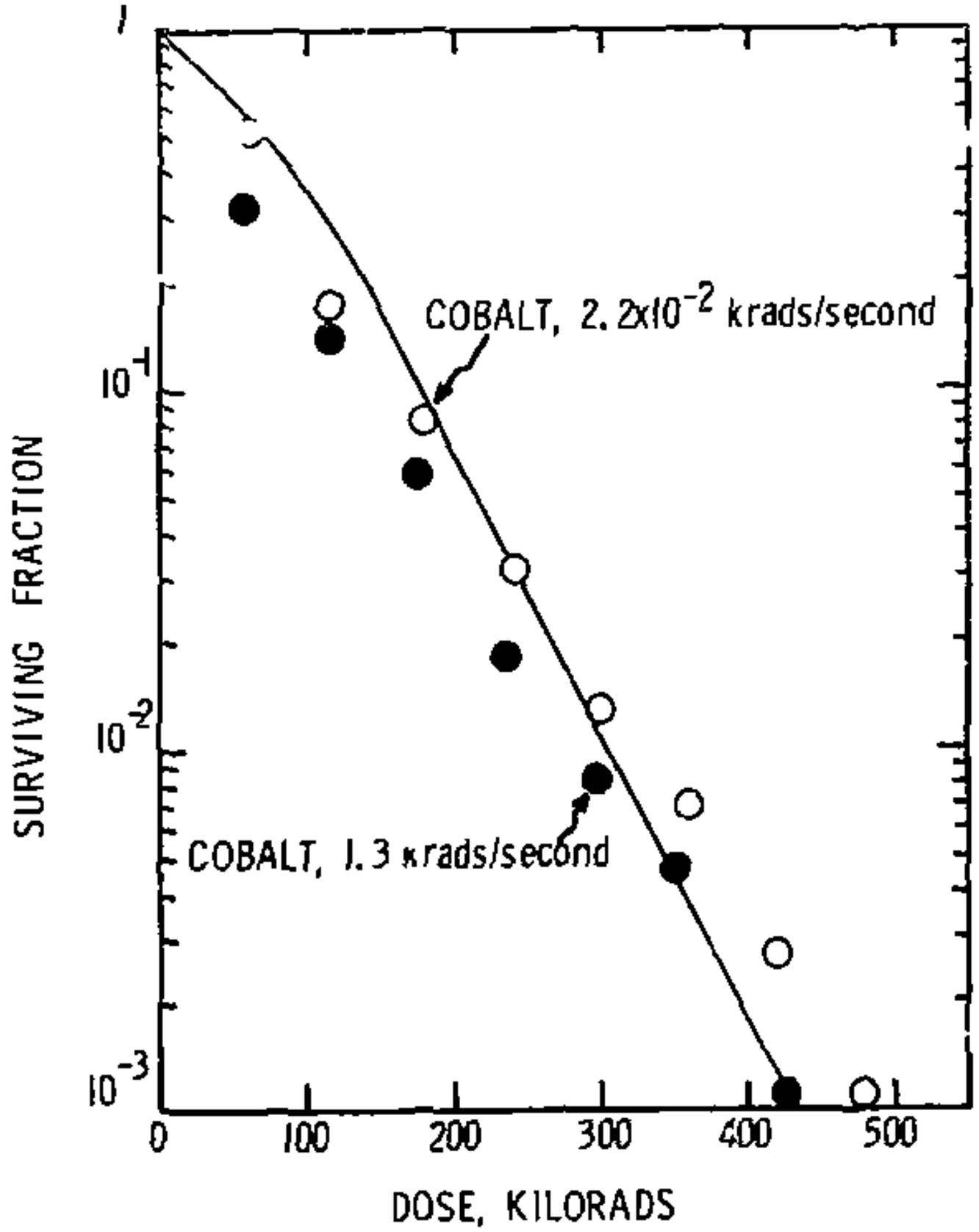

Figure 11. Fecal strep inactivation at different dose rates. Solid line is from earlier data (see Fig. 10).

coliform studies, heat inactivation of fecal strep is minimal below approximately 55° C, as shown in Fig. 12. It should be pointed out that the dashed line in this figure is a heat inactivation curve determined by using the previously described rapid heating system. It is seen that there is a factor of approximately 2 difference in the inactivation rate. The time scale involved in this case is about 3 times that for coliforms. Figures 13 and 14 show that at 45° C and 60° C the synergism exhibited with combined treatment is small but consistent.

The data at all temperatures for the inactivation of fecal strep are summarized in Fig. 15. The sum of the radiation treatment and the heat treatment is shown as a dotted line in this figure. The difference between this line and the solid line for combined treatment is a measure of the "better-than-additive", or synergistic, behavior. It should again be pointed out that the "4-minute" time choice is arbitrary and the synergism is somewhat greater at longer times. The synergism at longer times can be better demonstrated by comparing "final" slopes (in the time frame used) of the inactivation curves for the different treatments. These data for fecal strep in the form of "logs of inactivation per minute of treatment" are presented in Table II for heat treatment (H), radiation treatment (R), and combined treatment (T). Also presented are ratios of thermoradiation data to the "additive" effect (T/H+R), which are a measure of the degree of synergistic enhancement. Similar data are presented for coliforms in Table III.

Conclusions

It is clear that for use as livestock feeding and/or fertilizer on edible crops, sewage sludge must be free of pathogenic organisms. The data presented herein demonstrate

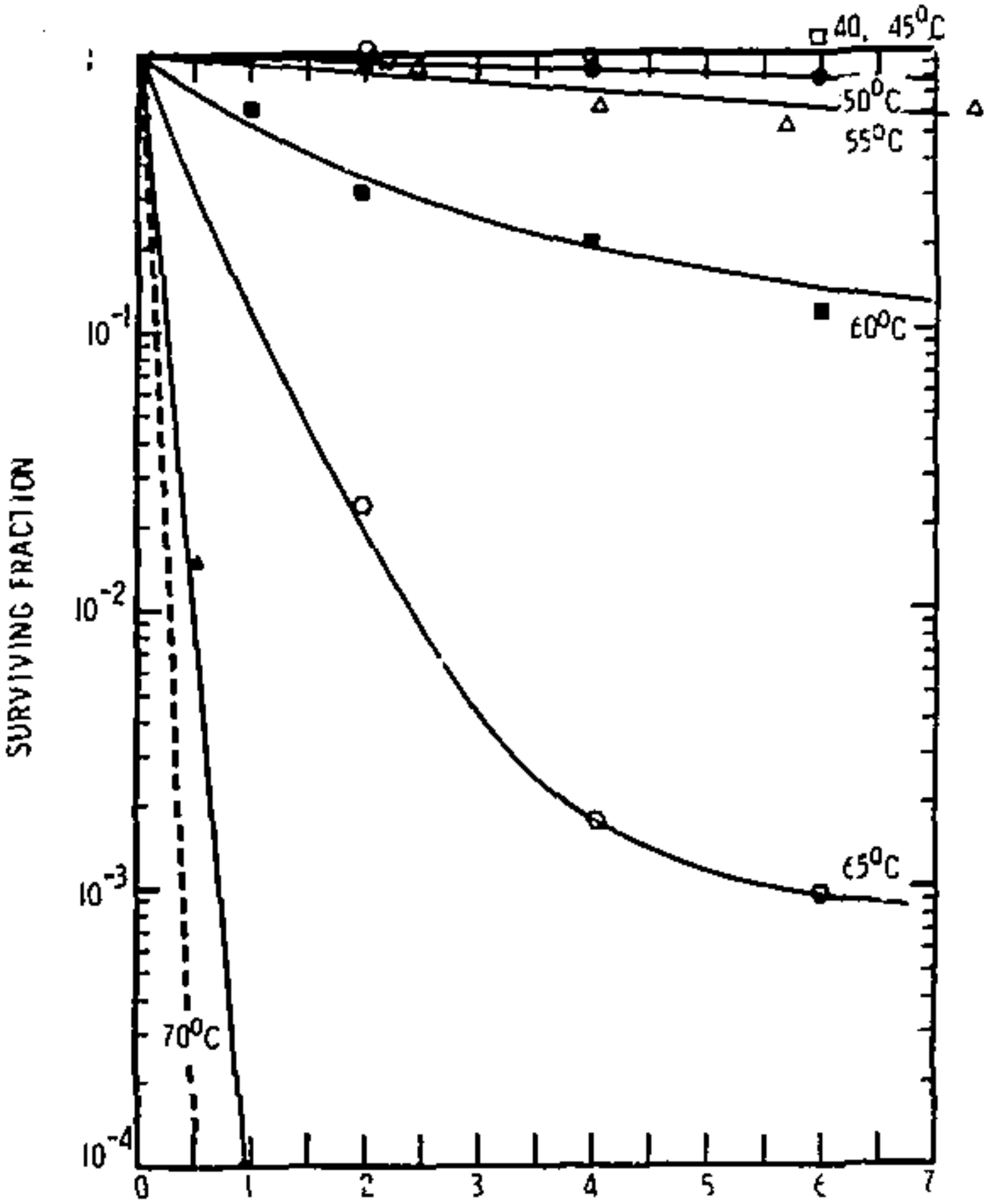

Figure 12. Thermal inactivation of fecal strep

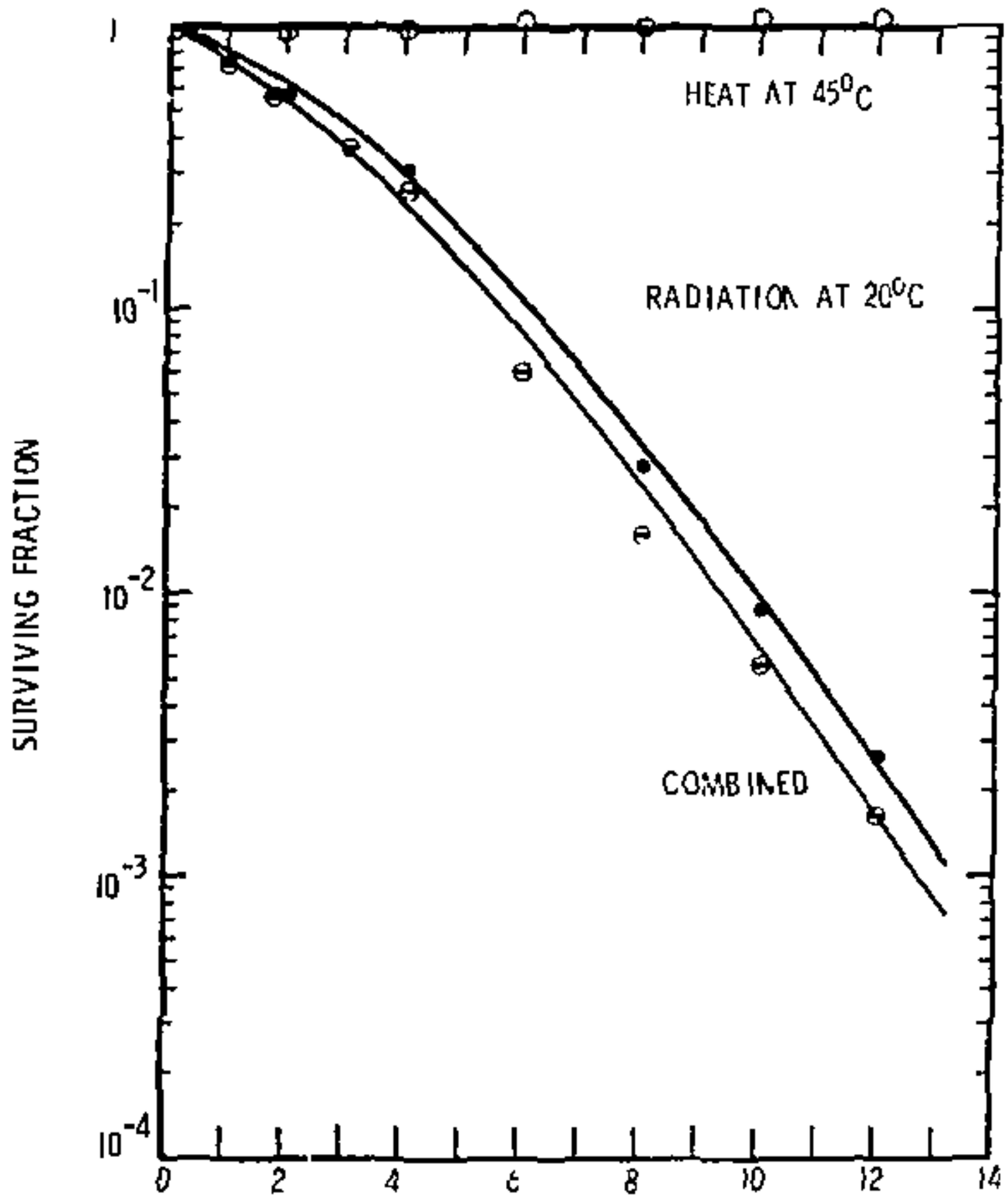

Figure 13. Fecal strep inactivation by heat and thermoradiation at 45° and 30 krads/minute

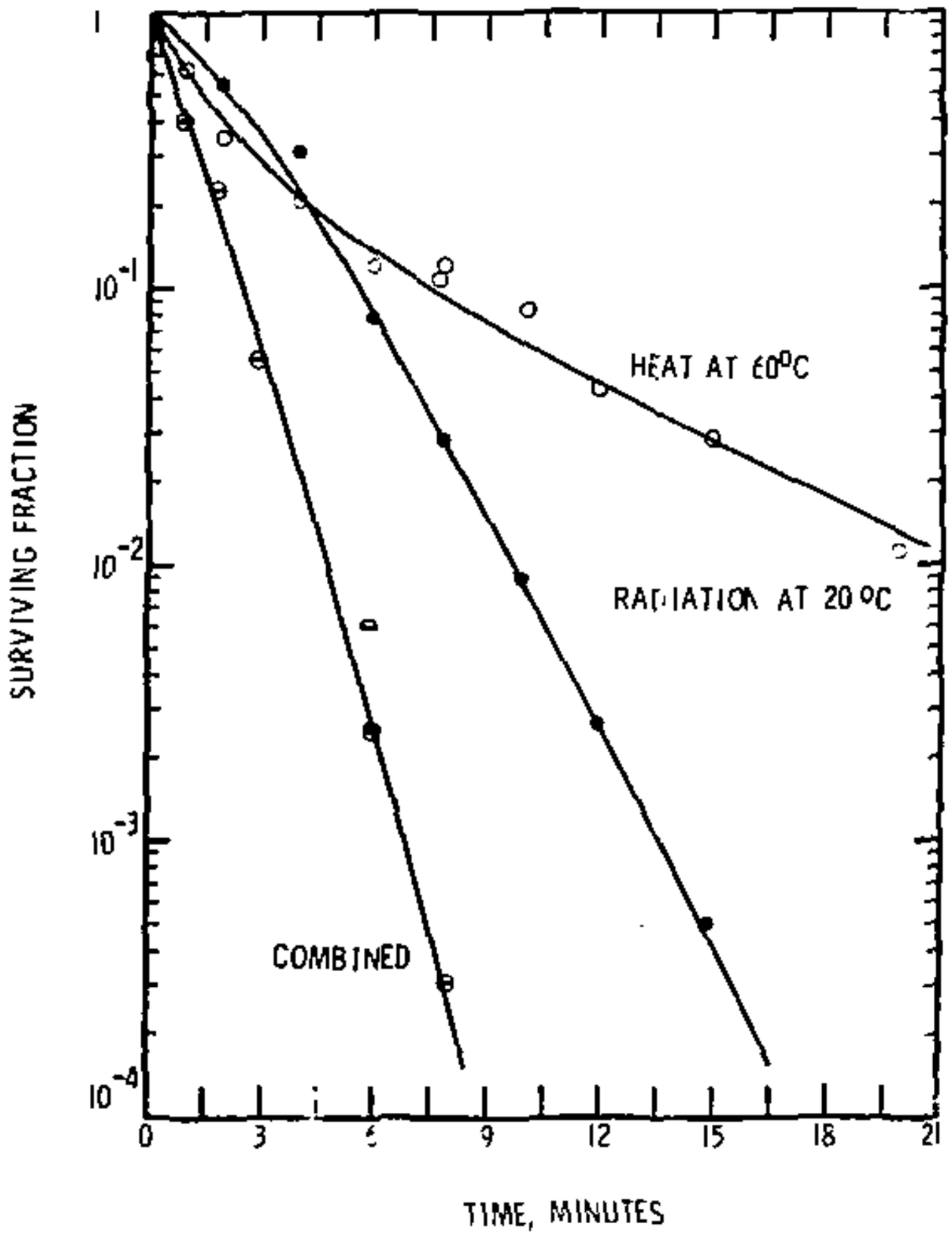

Figure 14. Fecal strep inactivation at 60° C

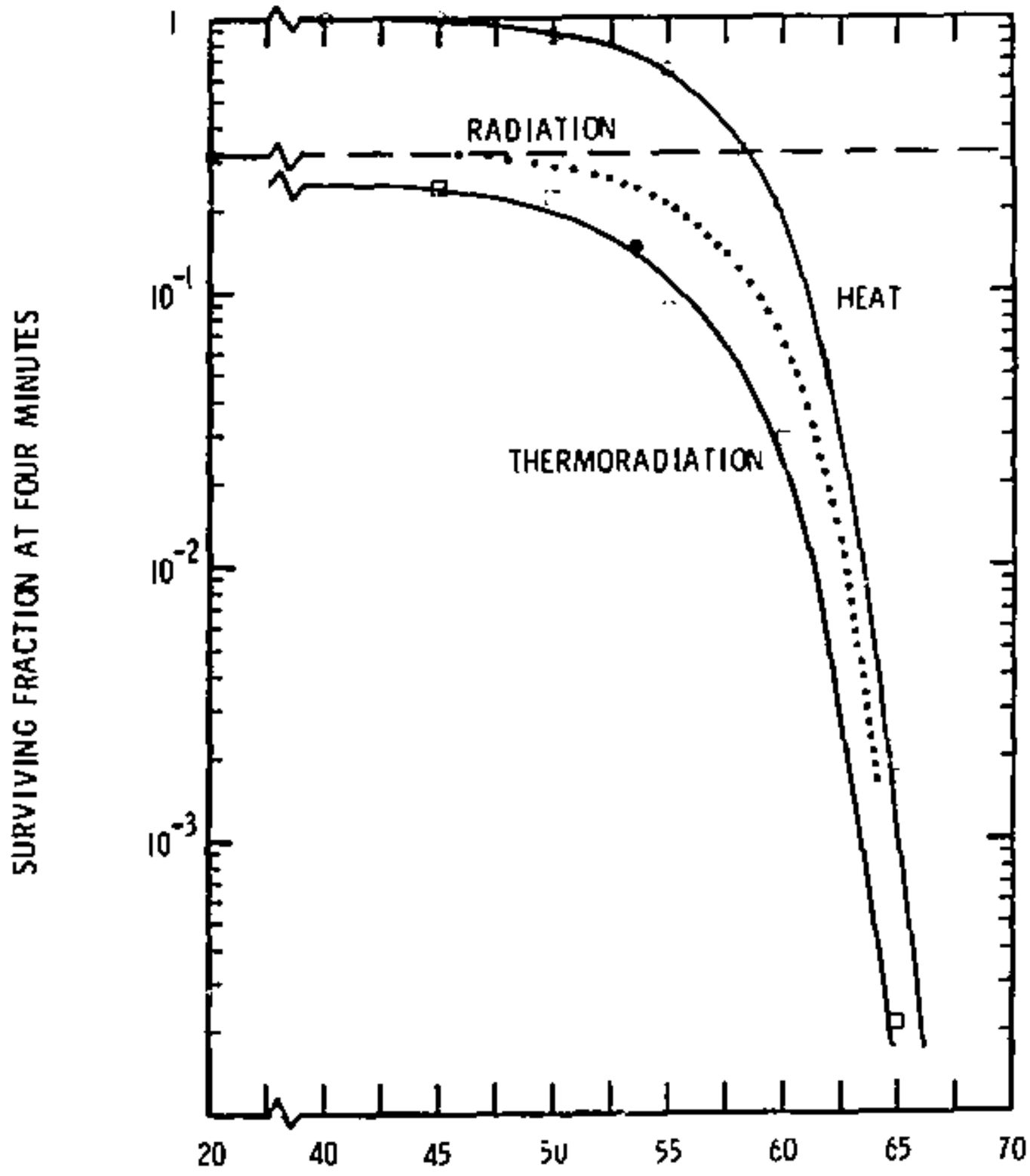

Figure 15. Temperature dependence of fecal strep survival. Comparison of additive and observed inactivation after four minutes of treatment

TABLE II.

TEMPERATURE	SLOPES			SLOPE RATIO
°C	R	H	T	T/(H+R)
20	0.270	0	0.270	1.0
40	0.270	0	0.273	1.0
45	0.270	0	0.273	1.0
50	0.270	0.014	0.320	1.0
55	0.270	0.032	0.409	1.4
60	0.270	0.272	0.500	1.5
65	0.270	0.160	0.756	1.8

Table II. Inactivation rates (final slopes) and ratios for fecal strep bacteria in sludge

TABLE III.

TEMPERATURE	SLOPES			SLOPE RATIO
^{o}C	R	H	T	T / (H+R)
20	1.40	0	1.40	1.0
40	1.40	0	1.40	1.0
45	1.40	0	1.75	1.3
50	1.40	0.06	1.50	1.1
55	1.40	0.19	2.30	1.4
60	1.40	0.46	2.27	1.2
65	1.40	1.93	6.56	2.0

Table III. Inactivation rates (final slopes) and ratios for coliform bacteria in sludge

the effectiveness of bacterial inactivation in sewage sludge by treatment with combined heat and irradiation. These data alone do not support the use of thermoradiation as a cost-effective treatment, since at higher temperatures where the synergism is greatest, an additional 5° rise in temperature will apparently perform at least the same function as the combined treatment (see Fig. 15 at 65° C, for example) on fecal strep or on coliform bacteria. At lower temperatures, a fairly large dose is required to decrease the fecal streptococcus population by a significant amount (approximately 500 krads, for example, would be required to cause a 4-log drop at 20° C, and the synergism observed is minimal below about 55° C). There are two possible positive factors which must be considered. (1) Since a tailing effect is observed in the lower-temperature, heat-inactivation curves, it is possible that such an effect also occurs at higher temperatures, even though the extent of inactivation is sufficient to prevent its observation. No such tailing effect has been observed in combined treatment curves; however, inactivation is sufficiently rapid that the bacterial population may be reduced below detectable levels before tailing effects would appear. This possibility is currently being investigated. The best available measurements (final slope ratios of Tables II and III) show a significant improvement over additive effects (a factor of 2 for fecal strep at 65° C) again subject to linearity in the thermoradiation curve. (2) It is possible that pathogenic species may be eliminated more easily than fecal strep, which was chosen as the "indicator" group for this study, based on its hardiness. This possibility is presently being explored. If indeed pathogenic bacteria are eliminated as easily as fecal streptococci, then 150 - 200 kilorads at 65° C will reduce the population of these species to a sufficiently low level to allow use of the treated sludge as a fertilizer on agricultural land or as a livestock feed supplement. It seems clear that these data must be viewed in conjunction with those from studies of parasite egg

inactivation,[15] virus inactivation,[16] and physical-chemical benefits[14] in order to make a valid assessment of the value of the thermoradiation treatment compared to other possible sludge treatment processes.

In determining cost-effectiveness of thermoradiation treatment, several other factors which have not been addressed in this study may be important. Since "final slopes" of inactivation curves and ratios of slopes (see Tables II and III) are of paramount importance, experiments are under way to extend the sensitivity of these determinations at even longer times. Additionally, it is possible that aeration or oxygenation during treatment may further enhance bacterial inactivation. The possibility also exists of treating sludge in a partially or fully dewatered state, such that much higher doses would be economically feasible. All of these factors are presently being studied.

REFERENCES

1. M. C. Reynolds, R. L. Hagengruber, and A. C. Zuppero, Thermoradiation Treatment of Sewage Sludge Using Reactor Waste Fission Products, SAND 74-0001, Sandia Laboratories, June 1974.

2. H. D. Sivinski, "Treatment of Sewage Sludge with Combinations of Heat and Ionizing Radiation Thermoradiation," IAEA Symposium on the Use of High Level Radiation in Waste Treatment - Status and Prospects, Munich, Germany, March 1975.

3. H. Bernard, "Alternative Methods for Sludge Management," Municipal Sludge Management, Proc. of National Conf. on Municipal Sludge Management, Information Transfer Inc., p. 11, 1974.

4. "Agricultural Utilization of Sewage Effluent and Sludge, *An Annotated Bibliography,*" *Federal Water Pollution Control* Administration, U.S. Dept. of the Interior, 1968.

5. "Recycling Municipal Sludges and Effluents on Land," (Proceedings of the Joint Conference), National Association of State Universities and Land/Grant Colleges, July 1973.

6. E. E. Geldreich and R. H. Bordner, "Fecal Contamination of Fruits and Vegetables During Cultivation and Processing for Market," A Review, J. Milk and Food Technology, V. 34, No. 4, p. 184-195, April 1971.

7. M. Garrard, "Sewage and Wastewater Processing with Isotopic Radiation: Survey of the Literature," Isotopes and Radiation Technology, V. 8, No. 4, p. 429, 1971, and D. S. Ballantine, "Potential Role of Radiation in Waste-Water Treatment," *Isotopes and Radiation Technology,* V. 8, No. 4, p. 415, 1971.

8. P. F. Pratt, F. E. Broadbent and J. P. Martin, "Using Organic Wastes as Nitrogen Fertilizer," California Agriculture, V. 27 (6), p. 10, June 1973.

9. H. N. Lowe, W. L. Lacy, B. F. Surkiewicz, and R. F. Jaeger, "Destruction of Microorganisms in Water, Sewage, and Sewage Sludge by Ionizing Radiations," J. AWWA, V. 48, p. 1363, 1956.

10. Investigation of the Effects of Ionizing Radiation on Synthetic Detergents, Sewage Colloidal Characteristics and Sewage Sedimentation, Final Report, USAEC Report COO-275, Unidynamics, Sept. 6, 1963.

11. W. L. Whittemore, D. M. J. Compton, and S. J. Black, Ionizing Radiation for the Treatment of Municipal Waste Waters, Final Report, USAEC Report Ga-9924, Gulf General Atomic Incorporated, Feb. 1970.

12. G. Baney, "Effects of Gamma Irradiation of Waste Activated Sludge," PhD Thesis, Purdue Univ., University Microfilms, 1970.

13. M. Lapidot, E. Rosenberg, R. Padova, and C. Rosenberg, "Potential Applications of Ionizing Radiation in Environmental Pollution Control," 3rd World Congress of Engineers and Architects in Israel.

14. J. R. Brandon, Physical and Chemical Changes Induced in Sewage Sludge by Ionizing Radiation, Heat, and Thermoradiation, SAND75-0169, Sandia Laboratories, in preparation.

15. J. P. Brannen, D. N. Garst, and S. Langley, Inactivation of Ascaris lumbricoides Eggs by Heat, Radiation, and Thermoradiation, SAND75-0163, Sandia Laboratories, 1975.

16. R. L. Ward, to be published.

17. R. Trujillo and V. L. Dugan, "Synergistic Inactivation of Viruses by Heat and Ionizing Radiation," Biophys. J., V. 12, p. 92, 1972.

18. G. Hotz and A. Muller, "The Action of Heat and Ionizing Radiation on the Infectivity of Isolated OX-174 DNA," Proc. National Acad. Science, U.S., 60, p. 251, 1968.

19. H. D. Sivinski, D. M. Garst, M. C. Reynolds, C. A. Trauth, R. E. Trujillo, and W. J. Whitfield, "The Synergistic Inactivation of Biological Systems," Industrial Sterilization International Symposium, Amsterdam, Duke University Press, 17, p. 305, 1973.

20. A. Anellis, D. Berkowitz, and D. Kemper, "Comparative Resistance of Nonsporogenic Bacteria to Low-Temperature Gamma Irradiation," Applied Micro., V. 25, No. 4, p. 517-523, April 1973.

21. G. E. Stapleton and C. W. Eddington, "Temperature Dependence of Bacterial Inactivation by X-Rays," Rad. Res., V. 5, p. 39-45, 1956.

22. M. C. Reynolds, K. F. Lindell, and N. Laible, A Study of the Effectiveness of Thermoradiation Sterilization," SC-RR-70-423, Sandia Laboratories, June 1970.

23. M. C. Reynolds and O. M. Garst, "Optimizing Thermal and Radiation Effects for Bacterial Inactivation," Space Life Sciences, V. 2, p. 394-399, 1970.

24. J. E. Huston, Absorbed Gamma Dose Rates in the North Cell of the Gamma Irradiation Facility (GIF), SC-RR-72-0212, Sandia Laboratories, March 1972.

25. "Standard Methods for the Examination of Water and Wastewater," 13th Edition, American Public Health Association, p. 691, 1971.

26. R. Sullivan, A. C. Fassolitis, E. P. Larkin, R. B. Read, Jr , and J. T. Peeler, "Inactivation of Thirty Viruses by Gamma Radiation," Appl. Micro., 22, p. 61, 1971.

27. R. Sullivan, J. T. Tierney, E. P. Larkin, R. B. Read, Jr., and J. T. Peeler, "Thermal Resistance of Certain Oncogenic Viruses Suspended in Milk and Milk Products," Appl. Micro., 22, p. 315, 1971.

28. R. Trujillo, Measurement of the Radiation Inactivation of Attenuated Poliovirus by the Plaque Assay Technique, SLA 74-0065, Sandia Laboratories, 1974.

29. G. E. Milo, "Thermal Inactivation of Poliovirus in the Presence of Selective Organic Molecules," Appl. Micro., 21, p. 198, 1971.

30. This work.

31. C. A. Lawrence and S. S. Block, Disinfection, Sterilization, and Preservation, Lea and Ferbiger, Philadelphia, p. 750, 1968.

32. C. T. Thomas, J. C. White, and K. Longree, "Thermal Resistance of Salmonella and Staphylococci in Foods," Appl. Micro., 14, p. 815, 1966.

33. F. J. Ley, Application of Radiation for the Control of Salmonella in Various Foods, IAEA 73/75, 1973.

34. M. A. Benards, "Land Disposal and Sewage Effluent: Appraisal of Health Effects of Pathogenic Organisms," J. AWWA, p. 432, June 1973.

APPENDIX A

DETERMINATION OF COLIFORM NUMBERS IN SLUDGE

I. Preparation of Plates

A. Weigh out M-Coliform broth (BBL), 24 g.

Add: 7 g Bacto Agar (Difco)

10 ml ethanol

500 ml deionized water

B. Mix well; boil for 3 minutes

C. Cool to 45 - 50° C in water bath

D. Pour into petri dishes

E. Use within 4 - 5 hours

II. Plating Samples

A. Samples are prepared from sludge which has been strained through a mesh screen, blended, and stored at 4° C until needed.

B. Proper dilutions are made with physiological saline; typically 0.1 ml aliquots are pipetted onto fresh plates.*

C. Samples are spread over the plate surface with a flamed angled glass rod.

D. Petri dish covers are left ajar until plate surface has dried (3 - 5 minutes); dishes are then inverted and incubated at 35° C for 24 ± 2 hours.

E. All colonies which exhibit the typical green metallic sheen when observed in a Quebec counter are considered to be coliforms.

*Several comparisons were made using 0.1 ml inocula of original sludge versus 1 ml inocula of a first dilution (refer to "Plating' on p. 661 of Standard Methods). No differences were observed