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Enteric Virus Survival in Package Plants and the Upgrading of the Small Treatment Plants Using Ozone

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Research Report No. 98

ENTERIC VIRUS SURVIVAL IN PACKAGE PLANTS AND THE UPGRADING OF THE SMALL TREATMENT PLANTS USING OZONE

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November, 1976

ABSTRACT

Post-chlorinated effluent collected with a portable viral concentrator from four treatment plants in Jefferson County, Kentucky, yielded infective viral particles from three plants from spring through late fall. The pH, chlorine, turbidity, and coliform levels of these effluents indicated that viral persistence was correlated with inefficient processing which produced effluent environments that inhibited disinfection by chlorine. The disinfection potential of ozone was tested on secondary effluent and finished water seeded with poliovirus and *Escherichia coli.* Low doses of ozone inactivated viruses and bacteria in treated water, but not in effluent. The inactivation of bacteria by ozone does not appear to be caused by cell lysis. Inability of poliovirus to form plaques correlated with inhibition of capsid penetration. Electron micrographs revealed that ozone degrades capsids. Ozonation produced low levels of COD and TOC reduction in package plant effluent. Since the reaction rates were not a simple function of COD levels and ozone dose it would be difficult to standardize dose rates. The relative inefficiency of ozone in reduction of biological and non-biological pollutants in effluents, combined with its high cost, does not favor a recommendation for ozonation as a tack-on process to upgrade these plants.

*Enteric virus *Ozonation *Wastewater Treatment Plants, Water Quality

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The Kentucky Department of Human Resources provided auxiliary funds which made it possible to obtain the Virus Concentrator, and their assistance is gratefully acknowledged. The Jefferson County Department of Health maintained a keen interest in the study from its beginning, and its personnel were extremely helpful. The Metropolitan Sewer Distric personnel also provided invaluable assistance. The owners and operators of the plants never failed to provide us with the cooperation required to obtain samples at their plants. Dr. Louis A. Krumholz and Dr. Stuart E. Neff, Water Resources Laboratory, were always generous with the use of their facilities.

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CHAPTER 1

INTRODUCTION

1.1 Objectives of the Study. This study was designed to determine if enteric viruses are released in infective form in the effluent of sewage treatment package plants in Jefferson County, Ky., and to evaluate the feasibility of ozonation as a third stage treatment process to improve the effluent quality of existing small wastewater plants. The purpose of the study was to obtain data on the quality of the effluents of the plants as they currently operate, and to provide information useful for future decisions in the design and operation of these plants.

1.2 Relevance of the Research

1.2.1 Waterborne Viruses. Raw sewage containing human excreta will contain enteric viruses. Whether these viruses constitute a hazard depends upon the extent of removal by treatment processes and upon the probability that virulent particles which persist in the effluent will be ingested in minimum infective doses by the hosts. Most standard secondary treatments remove large numbers of viruses, but they all leave a residue of infective particles (3, 32). Halogens are capable of inactivating residual viruses, but the complexity of the reaction of the disinfectants in varying effluent environments makes it difficult to predict the efficacy of the chemical treatment as a virucide (3, 21, 26). It is assumed that the typical sewage treatment facility, particularly the smaller plants which are commonly operated in a sub-optimal mode, yield viruses in the effluents. However, the inquiry into the problem

of viruses in water is relatively new, and there have been few field studies on the survival of viruses in these small plants under routine operating conditions.

Epidemic occurrences of waterborne-viral diseases have been documented. The best known in modern times was the outbreak of 300,000 cases of infectious hepatitus in Delhi, India, in 1955. Craun and McCabe (8) recently published a compendium of waterborne disease outbreaks which shows a total of 72,358 cases in the United States from 1946 to 1970, but of these only 1,849 can be attributed solely to viruses (infectious hepatitis and poliomyelitis), since the remainder listed are associated with non-viral agents (e.g., typhoid, Shigellosis), or may be caused by non-viral organisms (e.g., "gastroenteritis"). The problems of obtaining valid epidemiological evidence, coupled with the technical difficulties of studying viruses in water, have made it difficult to ascertain if waterborne viruses are an important vector of human disease. Therefore, there is now a controversy over the significance of residual viruses in effluents. Dr. E. H. Lenette, Chief of the Biomedical Laboratories of the California State Department of Health, recently stated that these is little evidence that waterborne viruses have epidemiological significance in clinical infections, and that there is no empirical or theoretical reason to assume that the presence of viruses in wastewater is hazardous (24). Others disagree with this perspective, and share Berg's view that "even small amounts of viruses in water are important" (5), and that there is "sufficient justification for seeking the total removal of viruses from any waters which man might consume." (2). There are persuasive arguments in favor of the latter view, including the facts that (1) very low doses of viruses have been shown to

be infective for man, (2) minimum infective doses may produce subclinical disease which results in proliferation of the viruses and subsequent excretion in clinically-significant numbers (5); (3) some viruses assumed to be harmless have been shown to generate varieties producing clinical disease, while other viruses once considered benign have been shown to be hazardous by virtue of latent-virus and slow-virus syndromes.

The question of a standard for maximum viral particles in wastewater is as uncertain as their relevance as a health hazard and as practical as the methods for discerning viruses in water. The small minimum infective dose established for poliovirus and the apparent need for a standard has led to proposals for maximum viral residues (4). Others believe the uncertainty over the hazard and the technological problems of viral assay makes it impractical (and unreasonable) to establish any standard (24).

Since today's health officials cannot await tomorrow's evidence, their decisions will be made partly upon the extent of public demand for pure water, and partly upon their conservatism in balancing the risks vs the benefits in the costly technology required to assay and reduce viral particles.

1.2.2 Non-Microbial Pollutants. In addition to infective agents, non-microbial pollutants may be dispersed from sewage treatment facilities. These include a variety of organic and inorganic constituents which may destroy the esthetic value of water with noxious odors, tastes, and visible debris; which may be hazardous to the aquatic ecosystems; and which may be a hazard to human health. In particular there is interest in the persistence of carcinogenic and teratogenic chemicals which

may survive both wastewater and finished water treatment. Investigations of the removal of such impurities by treatment processes parallels those concerned with the removal of microorganisms. There is agreement that most current practices diminish but do not eliminate many noxious components. There is uncertainty over the optimum system for maximum reduction, the extent to which certain residues are a hazard, and a rational standard for emissions of specific contaminants. The problem is exacerbated by the variety of synthetic products which are now added to residential and industrial influents.

1.3 Treatment facilities in Jefferson County, Kentucky. Jefferson County, Kentucky, shares with other metropolitan areas the problems of waste disposal from suburban areas which developed after the metropolitan sewer and treatment facilities had been established. To cope with this, over 300 substations treating approximately 2.0 mgd are maintained in areas peripheral to the major treatment plant in Louisville. About 1/4 of these substations are located in suburban housing developments. Most are self-contained "package plants" designed on the principles of activated sludge treatment followed by chlorination. Many are owned and operated privately, and the extent of routine supervision and maintenance of the facilities varies.

Since 1975, the Jefferson County Board of Health has monitored the plants for coliform counts, chlorine residuals, and other parameters of effluent quality. Effluent standards are specified in the Louisville and Jefferson County Board of Health Sanitary Code, Chapter III, Sec. 303.8, which includes maximum permissable fecal coliforms, BOD, and suspended solids, and the permissable range of pH and chlorine residuals. Due to limitations of personnel and laboratory facilities,

the most frequent testing possible for any one plant is once every 30 days, and most plants are assayed less frequently. The limited testing which has been completed over the past years indicates that many of these plants produce a product which cannot meet rigorous effluent quality standards. This study is the first assay for viruses in the effluents of these plants in Jefferson County, Ky., and one of the few studies of indigenous viruses in the effluent of package plants in this country.

The site of the effluent drainage of the plants serving subdivisions is typically close to the plant site, where the emitted water joins storm-runoff, traverses an extensive series of open drainage ditches, and eventually flows into county streams which drain into the Ohio River. Many of these drainage ditches are adjacent to heavily populated areas, and the ditches commonly overflow onto residential property after even moderate rainstorms.

1.4 Specificity of the Potential Hazards. The presence of pollutants in wastewater in concentrations sufficient to evoke clinical toxicity is a nonspecified hazard unless there is a probability of effective contact with the effluents. There are many considerations which enter into this assessment. The most important factors involve the probability of significant dilution to nontoxic concentrations prior to contact with the effluent, and the persistence of the toxic properties of the pollutants.

Since the effluents of the treatment plants are not always collected in sewage pipes, but flow into open ditches close to the plants, an immediate problem may occur for the population adjacent to the plant. It is common for children to use these ditches for wading pools. The

water frequently overflows onto residential yards, many of which have growing edible vegetables on which some human viruses and certain chemicals may persist for weeks. A number of enteric viruses may persist in small animals, such as the racoon, which share the suburban environment with man. Recent studies show that viruses discharged in effluent may persist for prolonged periods in soil, and show vertical and lateral movement through soils (40).

As the effluents flow toward county streams these hazards spread to larger numbers of individuals. When they drain into the receiving stream they join the pollutants from other plants in the watershed, and may be hazardous to individuals who work and live adjacent to the streams. Eventually some portion of the pollutants, depending on their original concentration and the environmental factors, will reach the Ohio River, adding to the burden of hazardous material constantly entering that major waterway. Thus, there are many opportunities for human contact with these discharges which support a realistic concern over the potential hazard of pollutants from the numerous wastewater treatment plants.

The quality of the effluents of these plants in Jefferson County constitutes an important aspect of the Kentucky water management problem, and is a major issue in the state's current plans to comply with Public Law 92-500 (Water Pollution Control Act Amendments, 1972). In July, 1974, a Water Quality Management Plan ("201 Plan") was prepared for the Kentukiana Regional Planning and Development Agency (KIPDA), and included an analysis of the existing package plant facilities and the streams accepting their wastes (21). This study was used to formulate a Master Plan Expansion Program which proposed that ultimately

wastewater in Jefferson County will be treated in 3 major facilities:

(1) Morris Forman, the existing major treatment facility in Louisville, which is being upgraded to comply with effluent standards;

(2) West County — a proposed facility to serve West County, Pond Creek, and Floyds Fork currently served primarily by package plants;

(3) North County — a proposed plant to serve northeastern Jefferson County, currently served primarily by package plants.

There are major problems associated with the existing package plants in the implementation of the Master Plan. First, the public has voiced strong opposition to the assessment of private owners for sewer hookups. The estimated cost of collectors to private dwellings, $220,903,000, which is more than half the total estimated cost, is not funded by the federal government. It is estimated that the average cost to each homeowner for collectors will be from $2,000 to $2,500. As a result of the vociferous objections from property owners in affected areas, an Alternate Phase I of the Master Plan Expansion Program was prepared, part of which would defer construction of collector sewers. A second problem involves the elimination of private ownership of plants. These plants represent a substantial financial investment and income for a number of private owners and operators, and the law is unsettled on the rights of the Metropolitan Sewer District to assume domain over the facilities. There is a case pending in the courts testing the right to exert such domain.

1.5 Ozonation for the Treatment of Wastewater. The disinfectant properties of ozone have been recognized for a century, and treatment of water by ozonation has been adopted in many European countries. Interest in ozonation facilities for wastewater treatment in the United

States is recent. The concern over dwindling water resources, nondegraded pollutants, and potentially hazardous byproducts of chlorination, have evoked an interest in ozonation to remove noxious and dangerous contaminants in water. Consequently, there has been a sudden proliferation of research in this area, but the available data are too contradictory and incomplete to permit a uniform and coherent analysis useful for the rational application of ozonation in the field.

It is apparent that the dose response data for ozonation is subject to significant alteration by many factors and a contemplated facility should be based upon studies individualized for the specific systems. An example of these problems involves the calculation of reactive dissolved ozone, which cannot be estimated accurately solely on the basis of the applied ozone dosage. This unpredictability is attributable to the fact that the reactivity of ozone with the diverse and complex constituents of wastewater is unknown. This and the problems of the analysis of data on ozone is the subject of several recent reviews (14, 21, 29, 36).

This investigation was designed to generate data on the feasibility of using ozone to reduce microorganisms and non-biological pollutants in the effluents of small package plants in Jefferson County, Ky., and to investigate the validity of certain assumptions that have been made regarding the mechanisms of inactivation of bacteria and viruses by ozone. The former is intended specifically for the use of the Louisville Metropolitan Sewer District, the Jefferson County Board of Health, the Kentucky Department of Natural Resources and Environmental Protection, and other Kentucky agencies responsible for the quality of water in this region. The latter is intended to add to the basic science of ozonation,

which still requires extensive contributions before this process may be used reliably for water purification.

CHAPTER 2

RESEARCH PROCEDURES

2.1. Experimental Design. The experimental design is shown diagramatically in Figure 1. The quality of the effluent of 4 sewage treatment package plants was characterized according to 4 common parameters of effluent quality: chlorine residual, pH, turbidity, and coliform levels. Chlorine residual, turbidity and pH were assessed each time samples were taken for bacterial or viral assay. Coliform counts were performed during the first year of the study at selected time intervals. The Health Department's records of these plants were obtained for comparison with the values derived in our laboratory. The COD and TOC of one plant, Villa Ana, was tested at intervals. Samples were collected for viral assay beginning August, 1974, and ending February, 1976.

Ozone was applied to viruses and bacteria seeded into plant effluent in a laboratory reactor. Inactivation curves in effluent were compared to the inactivation of viruses and bacteria in finished water. Experiments were performed to test several current hypotheses of the mechanism of the disinfectant action of ozone. In addition, plant effluent was ozonated to measure reduction in TOC and COD levels. The alteration of the pH of effluent, and the half life of ozone, were measured to assess two of the potential hazards of ozonation. These data on ozone doses and effectiveness were then used as part of a preliminary cost analysis study of ozone as a tack-on process for package treatment plants.

Figure 1

Experimental Design

V I R U S E S I N E F F L U E N T	O Z O N A T I O N O F E F F L U E N T
Quality of Effluent	Inactivation bacteria and viruses as Function of Water Quality
Chlorine Residual	Reduction of COD and TOC
pH	Mechanisms of Disinfection
Turbidity	Ozone Residuals
Coliform Levels	Alteration of pH
Enteric Virus Persistence	Cost Analysis

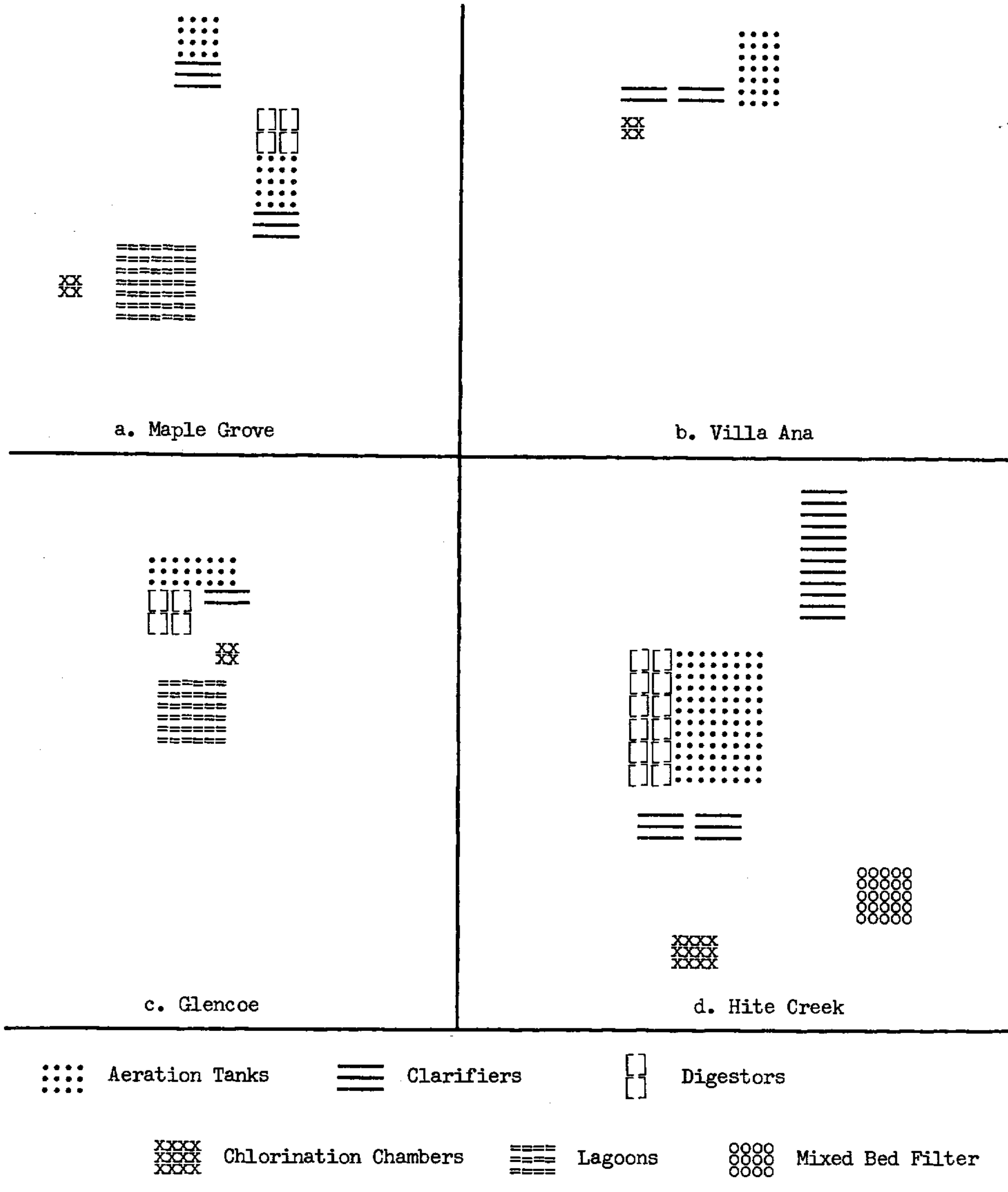
2.2. Viral Assays of the Plant Effluent.

2.2.1. Plants Tested. The 4 plants selected for assays were "Maple Grove #5", "Glencoe", "Villa Ana", and "Hite Creek". The first 2 are privately owned and operated, and the latter 2 are owned and operated by the Metropolitan Sewer District. Maple Grove, Glencoe, and Villa Ana are representative of approximately 80 small package plants which treat subdivision wastewater, with virtually no industrial influent. These plants are all operated similarly, employing clarifiers, activated sludge treatment, and chlorinators. Maple Grove and Glencoe have lagoons which receive the chlorinated secondary effluent. The design capacity of Villa Ana is 0.170 mgd, Glencoe is 0.100 mgd, and Maple Grove #5 is 0.110 mgd. The effluent from Villa Ana flows into a tributary of Mill Creek; Glencoe's effluent is received by a tributary of Fern Creek, and Maple Grove's effluent empties into the Pennsylvania Run of Floyd's Fork. All of these streams flow into the Ohio River. The Hite Creek plant is a large modern facility with a design capacity of 2.10 mgd wet weather flow, 1.82 mgd dry weather flow. The plant provides tertiary treatment with mixed media filters. Approximately one-half the influent is residential and the remaining is industrial, primarily from a Ford Motor Company Truck Assembly Plant. The effluent empties into Hite Creek, which flows into the South Fork of Harrod's Creek. The 3 smaller plants are supervised by personnel who visit the plants for brief periods, but the Hite Creek plant is supervised by MDS personnel stationed at the plant. Figure 2 a-d are schematic diagrams of these 4 plants.

The samples taken for analysis of viruses, bacteria, COD, and other parameters of water quality were all collected after the

Figure 2

Treatment Facilities of Plants Studied



chlorination chamber, prior to entry into the lagoons, mixed bed filter, or outflow pipe.

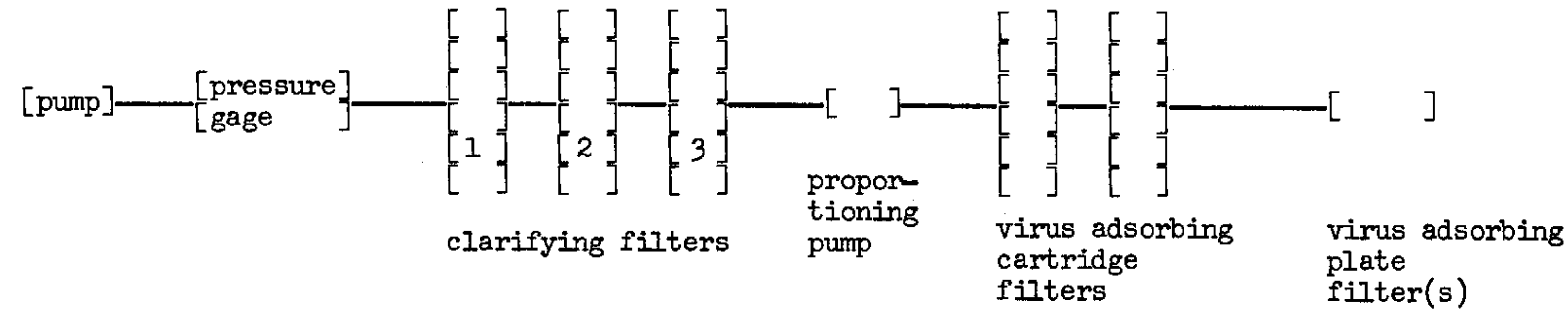
2.2.2. <u>Collection and Concentration Techniques for Viral Assays</u>. From August, 1974 to December, 1974 grab samples were collected from the plant and transported to the laboratory, where several concentration techniques were tried, including the polyethylene glycol dehydration method (7). In December, 1974, we obtained an Aquella Virus Concentrator (Carborundum Co., Niagra Falls, N. Y.). The equipment had been marketed only a brief time prior to this investigation, and this project was the first extensive use of the apparatus for the study of viruses in package plant effluents derived from unseeded influent. A diagram of the apparatus is shown in Figure 3. The procedures used were in accordance with the methods standardized by the Carborundum Company. The details of the apparatus and the techniques for recovery of seeded virus from tap water and sewage are explained in detail in several recent reports (17, 38).

The concentrator has a series of wound cartridge clarifying filters which remove particles that would interfere with viral adsorption; however, these filters do not adsorb viruses. The clarifying filters are followed by wound cartridge filters which do adsorb the viruses, and a membrane plate filter on which viruses are reconcentrated after elution from the cartridge filters. Additional components include a variable speed pump, a flow meter, and a proportioning pump which automatically adds reagents to the samples at preset volumes. The pH of the sample during clarification, concentration, and elution is critical, and was monitored with a portable field pH meter.

The sample is pumped at approximately 1 gpm through the 3 pretreated

Figure 3

Major Components of Viral Concentrator



clarifying filters, from which it flows toward the concentrating depth filter. Proximal to the concentrating filters, the proportioning pump adds to the preclarified water an acidified solution of aluminum chloride ($AlCl_3$-HCl) in a ratio of 1 part to 20 parts of water, to yield an aluminum concentration of 0.0006M and a pH of 3.5. Chlorine will interfere with accurate results, and sodium thiosulfate was added at a ratio of 1:100 to eliminate chlorine. The polyvalent ions and acidic pH enhance the adsorption of viruses onto the viral adsorbing filter. The viruses, which do not adsorb at high pH, are eluted from the wound filter with 0.05 M glycine at pH 11.5. This eluent, which may inactivate viruses, is forced quickly through the filter, and neutralized to pH 7 by the addition of a solution of 0.05M glycine at pH 1.5-2.0. The same eluting procedures were repeated on the clarifying filters to test for viruses that may have adsorbed to the particles adhering to these filters.

The standard procedure specifies that the viral particles in the neutralized glycine solution are then reconcentrated on membrane filters according to the same principles of enhanced viral adsorption at low pH in the presence of polyvalent ions. The eluted solution is acidified to pH 3.5 with 0.1M HCl, and $AlCl_3$ is added at a rate of 1 ml, 0.5M $AlCl_3$ per liter. This solution is forced by pressure through the membrane filters, from which the adsorbed particles are reeluted with 0.05M glycine at pH 11.5. The final concentrate is passed through a 0.2μ sterilizing filter, neutralized with 0.1M HCl, and tonicity adjusted with NaCl. If the concentrate is not assayed for viruses immediately, it is preserved by freezing after addition of fetal calf serum.

The first samples collected in the field with the Concentrator, at Villa Ana on December, 1974, included effluent seeded with poliovirus. The reconcentration process was completed as described, and the concentrate assayed for virus. The results indicated that the standardized procedure was suitable for this project. However, when this method was subsequently applied to samples beginning February, 1975, there were severe difficulties in reconcentrating the glycine eluates, since a viscous gel formed. The Carborundum Company informed us that similar problems were occurring at other sites. The problem was attributed to excessive organic contaminants in the effluents. The Company devised an alternate reconcentration technique. By this method, $AlCl_3$ was added to the eluate, followed by neutralization to pH 7.0 by addition of 1M Na_2CO_3. The supernatant fluid containing the virus was separated from the floc by decanting. The viruses were concentrated by centrifugation, and the particles in the pellet were resuspended in equal volumes of fetal calf serum, and 0.1M EDTA at pH 11.5. The mixture was recentrifuged, the supernatant neutralized, and the sample frozen until it was assayed for virus. Prior to adopting this technique, the eluates concentrated from two effluent samples were seeded with poliovirus I and reconcentrated by this alternate method. The results are shown in Table 1.

Table 1

Recovery of Exogenous Poliovirus by Gel Reconcentration

	Sample 1		Sample 2	
	Expected pfu/ml	Observed pfu/ml	Expected pfu/ml	Observed pfu/ml
Initial eluate, seeded	300	245	300	370
Discarded supernatant	0	15	0	0
Reconcentrated eluate	1.25×10^4	1.3×10^4	1.25×10^4	8.9×10^3

These data indicated that this technique was suitable for the assay of our samples, and it was used to generate all data reported in the results of this project.

2.2.3. Viral Assays of Concentrates. Buffalo Green Monkey Cells (BGM) which are very sensitive to enteroviruses, were used to assay viruses in all our studies. Dr. Gerald Berg, United States Environmental Protection Agnecy, Cincinnati, Ohio, kindly supplied us with a culture of these cells, which were subsequently propagated on Medium 199 (Gibco), supplemented with 5% calf serum. The plaque assay method was used to detect indigeneous viruses in effluent concentrates and to titrate seeded poliovirus in samples. Most assays were performed in a virology laboratory at the University of Louisville Department of Microbiology and Immunology. The Carborundum Company generously assayed a number of our samples in order to provide verification of our results.

2.3. Coliform Analysis of Effluents. The technique for detecting coliform bacteria was a modification of the standard confirmed test, with enumeration based on the MPN prodecure (1). Grab samples were apportioned into 10.0 ml, 1.0 ml, 0.1 ml, and 0.01 ml aliquots and 3 concentrations were selected for inoculation into 5 tubes of Brilliant Green Lactose Bile Broth (Difco) prepared in accordance with the manufacturer's specifications. The tubes were incubated at 35 C, and gas production within 48 hours was considered presumptive evidence of coliforms. These results were used with MPN tables to calculate the numbers of Gram negative lactose fermenters, presumptively the total coliform count. Samples from positive tubes were inoculated into EC medium (Difco) incubated at 44.5 C for 24 hours, and onto EMB agar (Difco) incubated at 35 C for 24 hours. The colonial morphology on EMB was

used to confirm the presence of coliforms, and gas production in EC medium at the elevated temperature was used to indicate that the organisms were fecal coliforms. Grab samples were collected at the plants at 7 a.m., 9 a.m., 11 a.m., and 1 p.m. in order to determine the time of maximum coliform load in the effluent and to assess the efficiency of the disinfectant procedures. The time of maximum coliform counts was used as guide for the collection of samples for viral assays.

2.4. Ozonation Studies. The second facet of this study concerned the feasibility of using ozonation as an alternate or tack-on process to upgrade the quality of the effluent from package treatment plants. The experiments tested the ability of ozone to inactivate residual bacteria and viruses and to reduce the TOC and COD levels of plant effluent.

2.4.1. Application of Ozone. The ozone was generated by a Welsbach M-816 ozonator. Oxygen is fed between 2 charged electrodes, and ozone is produced by application of high voltage alternating current to the electrodes. The concentration of ozone was regulated by varying the voltage applied to the electrodes. Determination of the ozone was based on the oxidation of iodide (I^-) to iodine (I_2) by ozone. The oxidized molecule in solution is measurable by titration with thiosulfate, using starch as the indicator. The procedure used was in accordance with the Standard Methods (1), and was used for determination of the ozone concentration in the aqueous phase (after the ozone dissolved in the liquid in the reactor) and the gas phase (as the generated gas bubbles were passed through potassium iodide [KI] solution).

The applied gas-phase ozone was measured by passing the gas stream through collection bottles containing 2% KI. A dose rate expressed as

the amount of ozone in the gas applied to the liquid in the reactor per unit time (mg O_3/l-min = mg ozone gas per liter liquid per minute) was used. Since ozone was dispersed through ozone diffusers, the potential decomposition of ozone during dispersion to the liquid was considered. The diffuser stones were placed into KI solution, and the amount of ozone passing out of the diffuser was compared to the amount of ozone applied to the stone. These data showed no decomposition of ozone by the diffusers, and the dose rate diffusing from the stone was thereafter assumed to be equivalent to the dose applied to the diffuser stone.

Direct measurement of dissolved ozone by the KI method did indicate residual ozone when the applied dosage was large, and this was used to determine the decomposition rate of ozone in the reactor. However, attempts to measure residual ozone in the reactor following inactivation studies of viruses and bacteria, in which low dosages were used, were unsuccessful. The concentration of dissolved ozone following these low rates must have been below the detectable concentration, 0.001 mg O_3/l. There are 3 explanations for the lack of detectable ozone: one is that the ozone was used completely in its reactions with the substrates; a second is that it decomposes rapidly in solution; and a third is that it escapes into the gas phase at the upper liquid-gas interface. To test the last possibility, the off-gases were passed through KI solutions and analyzed for ozone. Ozone was detected in these off-gases only when the highest ozone dosage was applied. Therefore, it was assumed that the inability to detect ozone in solution was not caused by loss into the atmosphere, but resulted from the rapid dissociation of the ozone to concentrations below

detectable limits, or complete utilization in the inactivation process. Since the ozone dosage used for the inactivation studies was based on a level which produced useful survival curves, doses that would yield a higher concentration of dissolved residual ozone were not used for these studies.

The results of the experiments on recovery of ozone from the stone diffusers, direct measurement of dissolved ozone, and ozone in the off-gas, indicated that the only feasible measurement of ozone dosage rate in these experiments was the ozone in the gas phase applied to the liquid phase (mg O_3/l-min). The maximum concentration of dissolved ozone in the liquid phase was estimated from the distribution coefficient of ozone between oxygen and water (mg O_3/l H_2O)/(mg O_3/l O_2) (13, 41), and a value of 0.35 at 25 C was assigned for the maximum concentration under the experimental conditions.

2.4.2. The Reactor. The ozone reactor was constructed by one of the graduate Research Assistants, Jerry Perrich. It was a plexiglas chamber 90 cm long x 15 cm wide x 60 cm deep, separated into 9 similar chambers by parallel baffles. Each chamber has a capacity of 7.4 liters. A removable top permitted isolation of individual chambers. The reactor was fitted into a wooden support, and a framework of metal dowels and wing nuts permitted a tight seal between the chambers and their tops.

Inlet and outlet water ports were provided by drilling holes into the reactor. Each chamber had a sample collection port which permitted rapid withdrawal of samples without interrupting the gas flow. Gas inlets regulated by a set of 9 valves connected to inlet tubes entering the chambers were constructed to permit independent flow into each chamber. The gas was dispersed through 2.54 cm diffuser stones attached

to each inlet tube. A gas rotameter attached to the frame was connected to the gas supply for measurement of total gas flow and flow into each chamber. Gas outlets were constructed on the top of the chambers for collection of off gas. The reactor is depicted diagramatically in Figure 4.

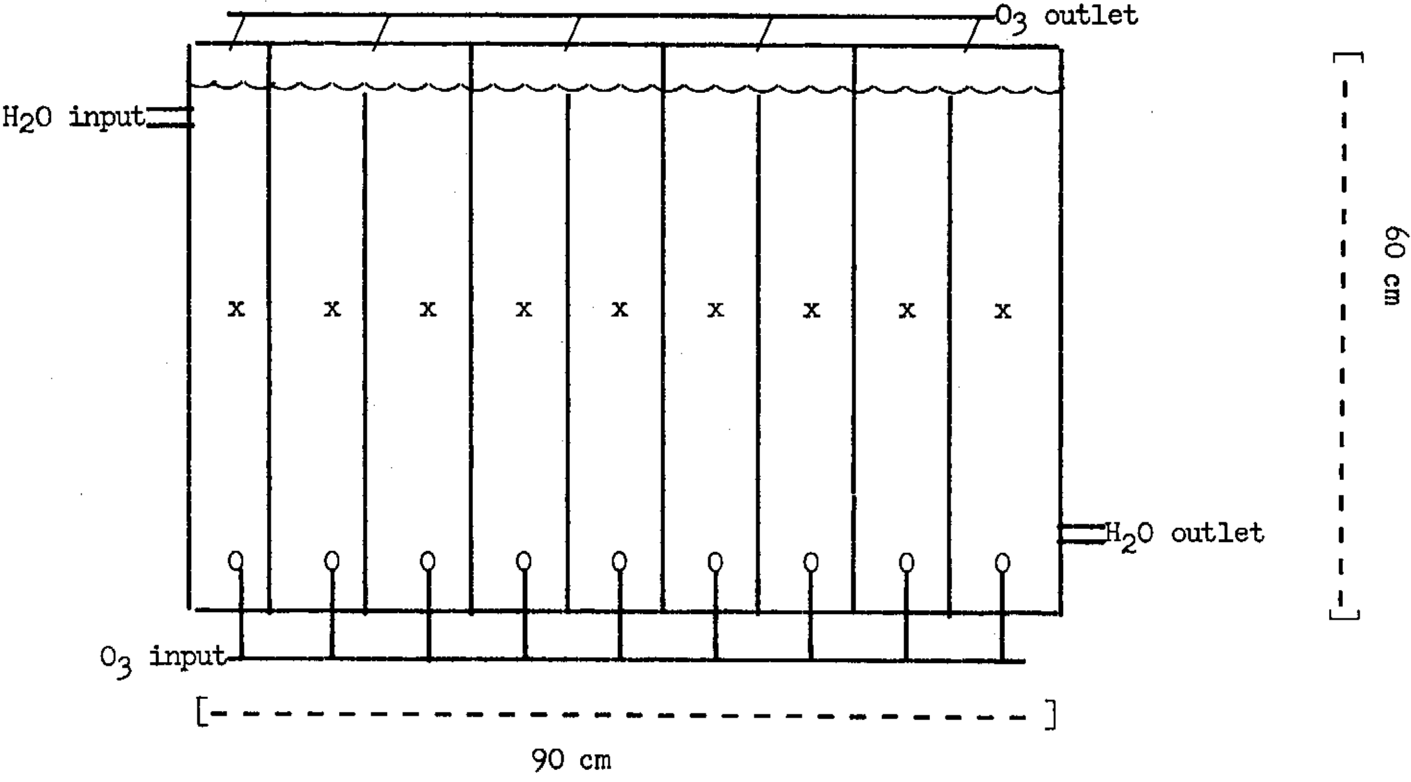
2.4.3. Characteristics of the Aqueous Phase of the Reactor. The liquids in the reactor are identified as "Finished water", "Effluent", or "Simulated Effluent". Finished water consisted of tap water deionized and buffered to pH 7.2 with 0.3 mM potassium phosphate. Effluent was collected from the Villa Ana treatment plant, transported to the laboratory, and kept refrigerated until use. Simulated effluent consisted of tap water altered by complex undefined or simple defined constituents.

Inactivation curves of bacteria were obtained in all three systems. Simulated effluent for these experimental runs consisted of the growth medium in which the cells had been propagated, which was added together with the cells. For the experimental runs in finished water and effluent, the cells were washed free of culture medium prior to addition to the reactor.

Inactivation curves of viruses were obtained in plant effluent, and in simulated effluent prepared by adding dextrose or alanine. Dextrose was added at a concentration of 36 mg/l, which is equivalent to a BOD of 40 mg/l. Alanine was added at a concentration of 15 mg N/l, which is comparable to high levels of organic nitrogen expected in actual effluent. It was not feasible to purify viruses completely from all traces of the tissue culture medium, so none of the viral inactivation curves were obtained in identical conditions to bacterial survival in "finished water". The tissue culture medium was added in

Figure 4

Schematic Diagram of the Reactor



O inlets to stone diffusers
x sample ports

higher concentrations in some experimental runs to permit a comparison with runs with small quantities of the contaminants.

The purpose of these tests was to compare inactivation curves obtained without wastewater components which might exert an ozone demand to those obatined when such substances were present. This is essential to assess the inhibition of disinfection by ozone in actual effluent, and one of the drawbacks in applying data from the literature is that inactivation is commonly studied in purified water.

2.4.4. Mode of Operation of the Reactor. The reactor permitted continuous flow operation, in which ozone is dispersed into the chambers while the sample flows through the reactor. Since the chambers could be isolated, the reactor also could be used in a batch and semiflow batch mode operation. In the former, ozone is applied to the liquid in a chamber before the sample is added, and in the latter, the sample is added before the ozone is applied. Dispersion of the sample throughout the chamber in the semiflow batch mode is accomplished by the currents created by the gas bubbles rising from the diffuser. Dispersion of the sample in the batch mode requires mechanical agitation. A preliminary test with India ink as the dispersion agent indicated that, in the semiflow batch mode, the sample is distributed evenly within 20 seconds.

Most of the studies were performed in the semiflow batch mode. Continuous flow operation was not used and the batch mode was used only for several experiments to assess the survival of bacteria in water that had been pre-ozonated without additional ozonation after the cells were added to the reactor.

2.4.5. Bacterial and Viral Cultures used in Ozonation Studies. Escherichia coli K-12 lactose^{+} was used for all studies on the inactivation of bacteria. The cells were propagated in Tryptone medium (Difco) at 35 C with aeration. For studies utilizing cells free of extraneous culture medium and other noncellular debris, a 12-hour culture was washed twice by centrifugation and resuspended in phosphate buffer.

Poliovirus 2 was used for all inactivation studies. The viruses were propagated and assayed on BGM cells, as described in Sec. 2.2.3. Particles were suspended in a maximum of 10 ml Medium 199 for addition to the reactor for inactivation studies in the reactor.

2.4.5.1. Enumeration of Bacteria and Viruses. The enumeration of viruses for inactivation curves utilized the plaque assay method, described in Sec. 2.2.3. The titer of the initial viral population was obtained prior to inoculation into the reactor. Samples were obtained through the outlet ports at specified intervals during ozonation.

The bacteria were enumerated for inactivation curves by the membrane filter technique. Millipore membrane filters and m-Endo medium were used. Dilutions were made in sterile buffered water. The initial concentration of bacteria was obtained after the cells had been inoculated into the reactor. The bacterial counts in experimental runs on effluent included indigenous coliforms and the seeded stock culture. The counts reported are the averages of duplicate or triplicate counts at each interval.

2.4.6. Total Organic Carbon and Chemical Oxygen Demand. The COD was determined by the technique recommended in Standard Methods (1). The accuracy of this method was analyzed with potassium acid phthalate

standards corresponding to an initial COD of 25 mg, 40 mg, 50 mg and 70 mg/l. The error ranged from 2.72% to 4.56% with the largest error in the smaller COD concentrations. This corresponds with the predicted accuracy of this method, which becomes less reliable at values below 30 mg COD/l (1).

TOC was monitored with a Beckman model 915 Total Organic Carbon Analyzer. Total carbon and total inorganic carbon were measured in separate channels, and the TOC was calculated as the difference between these values. The concentration of organic carbon was determined from a calibration curve.

2.4.7. Decomposition of Ozone. To avoid interference by complex reactive contaminants, ozone decomposition was studied in finished water at an ozone dose of approximately 1.838 mg/l-min, which provided sufficient residual ozone for analysis. A 13.6 liter sample was ozonated at this dosage for 30 minutes in a semiflow batch run. After the ozonation was stopped, 800 ml samples were collected at 10 minute intervals up to 60 min., 20 min. intervals from 60 to 180 min., and at 240 min. after ozonation. Dissolved ozone was purged by passing pressurized atmospheric air through the samples for 8 minutes at a rate of 1 lpm. The ozone was collected from a gas outlet in the sealed collection vessel, passed through tubing into a KI scrubber, and analyzed for the concentration of ozone.

2.4.8. Effect of Ozonation on the pH of Wastewater. The effect of ozonation on the pH of effluent was assessed by measuring the pH of plant effluent before and after samples were ozonated for 60 minutes at 6 dosages, ranging from 0.440 to 1.933 mg O_3/l-min. The pH was measured with a Corning Expanded Scale pH meter.

2.4.9. Mechanism of Inactivation of Bacteria and Viruses by Ozone. There have been many theories suggested to explain the disinfectant action of ozone on bacteria, but few theories to explain the inactivation of viruses by ozone, and no conclusive evidence to support any of the suggested mechanisms. As part of this study we performed several experiments to test one of the theories of the bacteriocidal effects of ozone; i.e., that it causes lysis of the cell envelope. In addition, studies were done to assess the damage to the protein capsid of viruses by ozone.

The test for lysis of the bacterial cells was based on the principle that bacterial cells dispersed in liquid behave as colloidal suspensions, scattering and absorbing light as a function of the size and numbers of the particles. In general, the optical density of a bacterial suspension increases as a function of cell numbers and is reduced by a decrease in viability due to leakage and cell lysis. This is the principle underlying the standard turbidimetric estimation of cell counts. If ozonated bacteria lose viability due to lysis there should be a correlation between the turbidity of the suspension and the decrease in viable cells. This was tested by obtaining the optical density of a suspnesion of bacterial cells at intervals following ozonation, and comparing the measurement of turbidity with plate counts taken from identical samples. As one control, the optical density of a non-ozonated sample of the same culture was followed at the same intervals. As a second control, a culture of *E. coli* B was infected with virulent T4 phage, which causes lysis of the bacterial cells. The optical density of the infected culture was recorded at time 0 (immediately after adding the phage) and at intervals up to 60 minutes following infection. All

turbidometric measurements were made with a Bausch and Lomb Model 20 Spectrophotometer.

The effect of ozone on the capsid of poliovirus was assayed by determinations of the penetration of treated particles on susceptible tissue culture cells and by electron microscopy of ozonated viral particles.

The effect on poliovirus penetration was studied by assaying the intracellular radioactivity of BGM cells inoculated with labelled poliovirus 2. The viral capsid was labelled with ^{14}C reconstituted yeast protein hydrolysate (5uCi/ml media). Because of the relatively low titer of the virus stock and the hazards of the isotope, the reactor for this experiment consisted of a disposable flask with one liter of liquid. Labelled virus suspended in medium was added to deionized water at a ratio of 30 ml stock, 3.4×10^4 pfu/ml, to 1 liter water. Ozone was then applied at a rate of 2.9 mg O_3/l-min. This relatively large dosage was necessary because the large quantities of medium with calf serum interferred with inactivation of the viruses.

Aliquots of the suspension were withdrawn at intervals from 2 to 10 minutes. Each sample was assayed for plaque forming units as a test of viability. Duplicates of the samples were inoculated onto a monolayer of BGM cells and permitted to adsorb for 2 hours. Non-adsorbed particles were then removed by washing in phosphate buffered saline, and the BGM cells were tested for intracellular radioactivity by liquid scintillation.

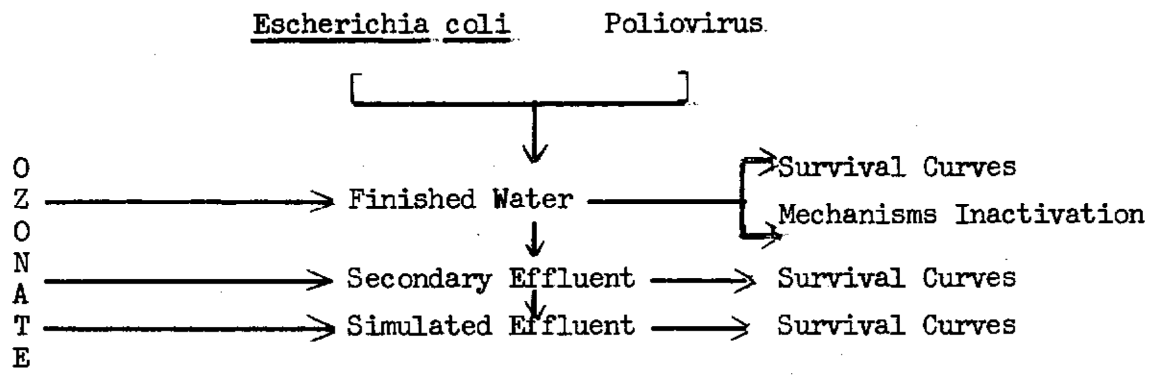
The visualization of ozone-treated poliovirus 2 particles was performed with a Seimens Elmskop IA electron microscope at a screen magnification of 40,000 x and a 100,000 volt accelerating potential.

The viruses were purified by banding on a sucrose gradient followed by a CsCl gradient prior to visualization. Saturated uranyl acetate was used as a negative stain. Figure 5 is a diagram of the experimental protocol for these ozonation studies.

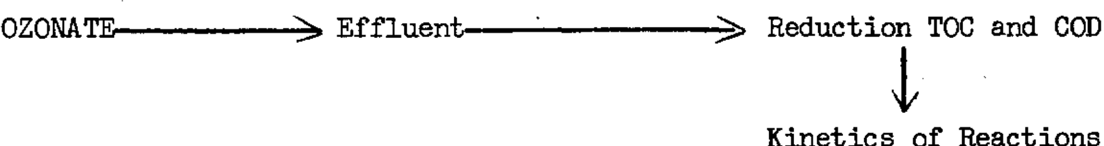
Figure 5

Experimental Protocol Ozonation Studies

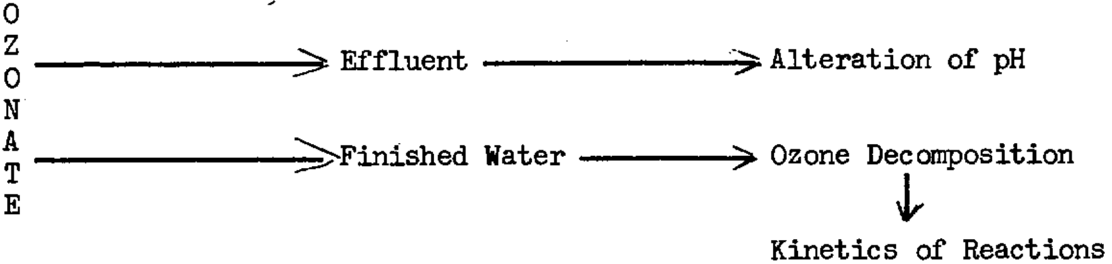
I. REDUCTION OF INFECTIVE BACTERIA AND VIRUSES



II. REDUCTION TOC AND COD



III. ALTERATION OF AQUATIC SYSTEMS BY OZONE



CHAPTER 3

RESULTS

3.1. Quality of the Plant Effluents. Two of the plants, Maple Grove and Glencoe, consistently produced effluents which failed to meet the minimum standards for chlorine residual, pH, and coliform levels. The residual chlorine of the Glencoe plant varied from 0 to 1.1 ppm. There was no chlorine detectable in 3 samples and less than 0.5 in 50% of the samples tested. The chlorine was less variable in the Maple Grove effluent, ranging from 0.1 ppm to 0.6 ppm. Conversely, the pH of the Glencoe water was within acceptable limits for effluent, ranging from 6.7 to 7.4, while the pH of the Maple Grove effluent frequently exceeded the permissable alkaline limit of 9.0. On 6 tests the pH of the plant was from 9.2 to 9.6. These values obtained by our laboratory were similar to those reported by the Health Department Laboratories. However, our estimations of the turbidity of these 2 plants varied from the Department's reports, which recorded the effluents as clear. In 90% of the assays in the Maple Grove plant, the effluent was observably turbid, and the high particulate matter was verified by the rapid deposition of particles on the clarifying filters. In 80% of the assays in the Glencoe plant, the effluent was also visibly turbid and produced heavy depositions on the filters. Cakes of sludge were frequently floating on the surface of the baffled area of the lagoon.

There was also a wide variation in the coliform count of the effluents of these 2 plants. Six samples taken at 11 a.m. on 6 different

days, yielded the following values (coliform/100 ml; TNTC = Too numerous to count):

Maple Grove: 800, 40, 200, TNTC, 20, 2,300

Glencoe: TNTC, TNTC, 92,000, 200, TNTC, 35,000.

Our results on coliform bacteria were not compared with those obtained by the Health Department Laboratory, since their assays are performed on samples collected at various times of the day. We found differences in coliform counts as great as 10^5/100 ml in samples collected at 2-hour intervals, and comparisons are not valid on samples collected at differing periods of time.

There was no apparent relationship between residual chlorine values and the coliform counts. The Glencoe plant, with consistently higher coliform levels, also had consistently higher concentrations of residual chlorine. There is a suggested relationship between the bacterial counts and the pH of the effluent in the Maple Grove plant, since the pH reached alkaline levels incompatible with the growth of coliforms.

The effluent of the Villa Ana plant, judged by residual chlorine, pH, turbidity, and coliform counts, was of higher quality than the Glencoe and Maple Grove plants. There was little variation in the chlorine residual. The values ranged from .1 ppm to .4 ppm on 18 tests, and registered .6 ppm on 1 assay and .8 ppm on another. The pH of the effluent ranged from 6.3 to 6.8 for 19 tests, and was 6.0 on one assay. The turbidity of the effluent was variable, but the effluent was relatively clear approximately 50% of the time assays were performed. The coliform counts on samples collected at the same time of day over an interval of time in spring, 1975, were variable, but were consistently lower than the Glencoe plant. The results of 6 tests performed on

samples collected at 11 a.m. during a period comparable to that shown for Maple Grove and Glencoe, were: 35,000, 20, TNTC, 2,300, TNTC, 4,000.

The Hite Creek plant had a consistently high quality effluent, although the chlorine residual varied from .1 ppm to 1.0 ppm. The pH ranged from 6.8 to 7.1. The effluent was clear and never yielded the rapid deposition of particles on the clarifying filters obtained from the other plants. Four assays for coliforms were performed at 11 a.m. over a one week period in late summer, 1975, and the results (coliform/100 ml) were: 40, 20, 300, and 30.

Table 2 summarizes the characteristics of the effluents of these four plants:

Table 2

Characteristics of the Effluents of the Sewage Treatment Plants

Plant	Turbidity	pH	Cl (ppm)	Coliform (cells/100 ml)
Maple Grove	consistently high	7.2-9.6	.1-.6	variable (20 - TNTC)
Glencoe	consistently high	6.7-7.4	0-1.1	consistently high (200 - TNTC)
Villa Ana	variable	6.0-6.8	.1-.6	variable (20 - TNTC)
Hite Creek	consistently low	6.8-7.1	0-1.0	consistently low (20 - 300)

It should be noted that these characteristics refer to the effluents shortly after they flow from the chlorine contact chambers. We were interested in the relative efficiency of the plants to this point. Complex variables must be considered in determining the characteristics of the effluents as they are emitted from the plant. In the case of

Maple Grove and Glencoe these effluents would be retained for varying periods in the lagoons; in the case of Hite Creek the effluents receive tertiary treatment in the mixed bed filter; in Villa Ana there is an outflow pipe through which the effluent flows for several hundred feet, which provides varying periods of detention before the effluent is released. Therefore, these characteristics of the plant effluent are the basis for a comparison of the operation of the clarifiers, aeration tanks, and chlorination facilities of these 4 plants as they are currently operated.

3.1.1. Detection of Viruses. Table 3 summarized the total numbers of samples assayed, the number of these samples which yielded viruses, and the months during which the samples contained detectable viruses. These data were obtained with the Aquella Viral Concentrator, using the gel reconcentration technique.

Table 3

Recovery of Viruses in 4 Sewage Treatment Plants

Plant	Positive Tests/Total No. Tests	Months Virus Detected
Maple Grove	5/10	July, Aug, Sept, Nov
Glencoe	5/12	May, July, Aug, Sept
Villa Ana	3/14	July, Aug
Hite Creek	0/7	-

The range of pfu/ml was 0.5 to 8.0. There was no apparent correlation between the pH and chlorine residual of the effluents and the presence or numbers of viruses. In Maple Grove viruses were recovered from effluents with a pH as low as 7.6 and as high as 9.5, and with chlorine residuals of 0.1 ppm and 0.6 ppm. In the Glencoe plant, 2

samples taken from effluents with a chlorine residual of .7, pH 7.0 yielded viruses, but 4 samples with identical chlorine and pH values did not produce viruses.

Since the effluents from Maple Grove and Glencoe were trubid during most assays, it is not possible to correlate the detection of viruses with the clarity of the effluent in these plants. However, all samples which yielded viruses from the Villa Ana plant were rated as highly turbid, and it is noted that no viruses were detected at Hite Creek, which has a consistently clear effluent.

There was a correlation between the climate and viral recovery. Viruses were not detected during December to April. All plants which yielded viruses did so in July and August, viruses were recovered from 2 of the plants in September, and from only one plant in November.

3.2. The Inactivation of Bacteria by Ozonation. The initial doses of ozone used to obtain inactivation curves of bacteria were based on prior studies reported in the literature, which indicated there was a threshold concentration of 1 to 2 mg/l ozone for inactivation. Therefore, the first inactivation studies utilized an applied dose of .25 mg O_3/l-min. At this dosage there were no viable cells detectable at the end of 30 seconds. The ozone dosage was then reduced until a survival curve was obtained which extended inactivation of the population to approximately 20 minutes. This rate of inactivation occurred at ozone dosages of approximately .005 mg O_3/l-min. Similar preliminary studies on the survival of *E. coli* in plant effluent indicated that these low dosages would not inactivate the bacteria in wastewater and a dosage rate of .29 mg O_3/l-min was determined as appropriate for these studies.

Tables 4, 5, 6, and 7 show the inactivation data for *E. coli* at

4 ozone doses in finished water when the reactor was operated in the semiflow batch mode. These data are plotted in Fig. 6. In these studies washed bacterial cells suspended in buffer were added to the reactor and mixed for 2 minutes by air flow. A sample was taken for enumeration, and the precalibrated ozone gas flow was started. The ozone was applied continuously at the same rate, and samples were collected at intervals for enumeration of viable cells. At the conclusion of each run the reactor was drained and the ozone diffusing through the stones was measured to confirm the consistency of the applied dose. Figure 6 also indicated the numbers of bacteria surviving during a comparable period when air flowed through the reactor at a rate of .3 l/min.

The differences in initial concentration (2×10^6 to 4.1×10^6) were not considered a significant difference with regard to determining the influence of the numbers of cells in the initial population. All the curves were sigmoid, with a shoulder and tail separated by an exponential decrease in viability. It was not the purpose of these experiments to establish a dose-response relationship between ozone concentration and death rate. However, the slope of the curve at the lowest dose (.0043 mg O_3/l-min) compared to the highest dose (.0077 mg O_3/l-min) indicates that the death rate in the exponential phase increases with ozone dose.

Tables 8 and 9 show the inactivation data obtained in finished water when the reactor was operated in the batch mode at 2 doses of ozone. In these studies, the ozone was diffused into the reactor for 20 minutes prior to adding washed cells suspended in buffer. The ozone flow was stopped immediately prior to adding the bacterial suspension,

the cells were mixed in the reactor mechanically, and samples were collected for enumeration from 30 sec to 20 min after the ozone flow stopped. Assuming that the ozone dissolved rapidly and the solubility characteristics assumed for the experimental conditions obtained, the maximum dissolved ozone would have been .047 mg O_3/l at the applied dose of .0054 mg O_3/l-min. These data are also shown plotted in Figure 7. The inactivation was linear through the origin and there was no tail. There was very little inactivation of the bacteria in this preozonated water at doses similar to those which produced rapid inactivation when ozone was supplied continuously after the cells were added. Since other conditions were substantially the same in both experiments, the reduction in inactivation must be attributable to a rapid decrease in the dissolved ozone.

Tables 10 and 11 and Figure 8 show the inactivation of bacteria in simulated effluent with the reactor operated in a semiflow batch mode. The simulated wastewater was obtained by adding the growth medium in which the bacteria were grown, which produced an initial COD of 12 mg/l in the reactor. These curves did not have a shoulder or tail, which is unlike the sigmoid curve obtained in finished water, but similar to the shape of the curves in effluent (Fig. 9). The slope of the curve indicates a slower rate of reaction in this simulated effluent than in the exponential death phase in finished water (Fig. 6) but a more rapid rate than was obtained in natural effluent (Fig. 9).

Tables 12 and 13 and Figure 9 show the inactivation data for bacteria in plant effluent at 2 ozone doses with the reactor operated in a semiflow batch mode. The effluent was collected at the Villa Ana treatment plant, and added to the reactor immediately prior to the

experimental run. These bacterial counts included seeded and indigenous coliforms. There was insignificant inactivation of the bacteria in effluent at the same magnitude of ozone which produced a marked decrease in bacterial numbers in finished waters. At a high dose rate (.29 mg O_3/l-min) there was inactivation of bacteria in effluent, but the decrease was less than 3 logs in 20 minutes, and the rate of death was slower than obtained at low doses (.0043 - .077 mg O_3/l-min) in finished waters.

3.3. Inactivation of Viruses by Ozonation. The ozone dosage rates applied to suspensions of viruses were determined by the same general procedure used for the bacterial studies. The initial viral concentration was approximately 10^3 pfu/ml. Since a minimum of 10 virions per ml were necessary for accurate quantification, and an interval of 2 minutes between samples was desirable, the criterion for the appropriate dosage was an inactivation of approximately 2 decades in 20 minutes. Initial studies, using suspensions of viruses in medium 199 added to buffered water and operating the reactor in a semiflow batch mode, indicated that the viral population was inactivated within 15 seconds at dosage rate of .06 mg O_3/l-min. There was almost no inactivation at dosages less than .003 mg O_3/l-min. Figure 10 shows an inactivation curve under these experimental conditions with an ozone dose of .023 mg O_3/l-min.

The inactivation of poliovirus in simulated effluent (alanine dextrose added to finished water) is shown in Figure 10. Ozone was applied at a dosage of .023 mg/l-min. The data show no significant difference between inactivation in finished water compared to water containing either the carbohydrate or amino acid. As shown in Figure

11, a comparable ozone dosage applied to plant effluent did not affect the survival of the viruses over the 20 minute test period. A higher dose rate, 0.96 mg O_3/l-min, yielded 4 logs of inactivation in 20 minutes in this plant effluent.

3.4. Summary of the Characterisitcs of the Inactivation Curve. These inactivation data did not show the threshold or "all-or-none" phenomenon reported by others. The results confirm the relative resistance of both bacteria and viruses to the disinfection properties of ozone in the complex milieu of wastewater compared to finished water, but they also indicate that low doses of ozone are effective in inactivating both viruses and bacteria in finished water. Higher doses were required to inactivate viruses than bacteria.

There was a difference between the shape of the survival curves of bacteria in finished water compared to their survival in plant effluent. In the finished water the curves were sigmoid, with a shoulder and a tail, but in plant effluent or simulated effluent the inactivation was linear through the origin and did not yield a tail. The inactivation curves of the viruses in finished water compared to natural plant effluent exhibited different slopes, but the shape was the same in both cases, with inactivation linear through the origin.

3.5. Ozone Decomposition by Stone Diffusers. Figure 12 shows the results of the studies on the levels of ozone detectable after the ozone was passed through the stone diffusers into a KI solution. The measured ozone dissolved in the solution added to the measured ozone recovered from flushing the stones gave a total recovery approximating the applied ozone dosage. Therefore, there does not appear to be significant decomposition of ozone in the stone diffusers, and the estimation of residual

ozone was calculated as a function of the applied dose and the distribution coefficient of ozone, without compensation for loss in the diffusers.

3.6. Mechansims of Inactivation of Viruses and Bacteria. Figure 13 shows the inactivation curve of washed *E. coli* cells at an ozone dosage of .09 mg O_3/l-min compared to the optical density of a comparable suspension during the disinfection process. The optical density of a control suspension of cells at the same initial concentration is also illustrated. This survival curve has no shoulder, and there was a relatively slow rate of inactivation at a high rate of ozone. These differences may be attributed to the fact that the initial concentration of bacteria (1.9×10^9) was 10^3/ml higher than that used for the other studies. This concentration was used in order to obtain an optical density that could be measured readily. The small magnitude of change in the optical density of the suspension does not indicate that lysis is a primary cause of cell death. In 160 minutes there was a change in viability of ozonated cells of eight decades. The final bacterial count was 4.8×10^1/ml. The O.D. of the ozonated sample decreased from .49 to .29, while the O.D. of the control sample decreased from .49 to .45. This may be compared to the change in turbidity of the *E. coli* cells infected with a T-phage, in which the O.D. dropped from .59 to .15 in 60 minutes due to cell lysis. The O.D. of an unozonated culture with approximately 5×10^1 cells/ml was <0.1.

The effect of ozonation of viruses on their uptake into BGM cells is shown in Figure 14. The percent decrease of intracellular capsid protein in susceptible cells is correlated with the percentage decrease in the ability to form plaques in the same cell line by ozonated virus.

When there is a 95% inactivation of the viruses, as measured by plaque formation, there is a 75% decrease in intracellular capsular material. Figure 15 is a micrograph of the untreated poliovirus as revealed by the negative stain at a magnification of 400,000x. The capsid of these intact viruses, with their cubic symmetry, is clearly discernible. By contrast, the viruses in Figure 16 examined under the same magnification after ozonation, are not detectable as intact particles. The irregular clumps are interpreted as aggregated viral particles which have lost their symmetry as a result of disintegration of the capsid. This degradation of the capsid protein by ozonation correlates with the diminished capacity to penetrate the host cells. The experimental rate of inactivation without an initial shoulder is typical of disinfectants which require only a single "hit" to complete inactivation. Since the infectivity of the virus requires integrity of the capsid, it is feasible to assume that a single disruptive event could evoke a "domino effect" on the symmetry of the capsid.

3.7. Effect of Ozonation on COD. Table 14 presents data obtained from 6 experiments in which Villa Ana effluent was ozonated at an applied dosage of .007 mg O_3/l-min to 1.933 mg O_3/l-min for 40 to 60 minutes at each dosage. These samples were collected on 6 different days and are referred to as "heterogeneous effluent samples". They illustrate the variation in the COD of effluents, as well as the alteration in the COD as a function of the initial COD and ozone dosage rate.

Table 15 presents the alteration of COD of 4 aliquots derived from the same effluent sample, referred to as "homogenous effluent samples". Ozone was applied at different dose rates for 60 minutes. When these data are plotted as $-\ln (COD)_t/(COD)_0$ vs time (Figures 17 and 18), it

is apparent that the reduction in COD approximates a first order reaction with respect to COD:

$$-d(COD)/dt = k_1(COD) \qquad (3.1)$$

$$-\ln (COD)_t/(COD)_o = k_1 t \qquad (3.2)$$

Table 16 shows the first order rate constants for the 6 heterogeneous samples, and Table 17 shows these rate constants for the homogeneous samples.

These data indicate that the apparent reaction rate coefficients are not a simple function of ozone dose, since the rate for lower doses was sometimes higher than the constant for higher doses on heterogeneous samples. The relatively lower rate coefficients obtained in the experiments with heterogeneous samples with lower initial COD (Table 16, ozone dosages .066 mg O_3/l-min, .904 mg O_3/l-min, and 1.933 mg O_3/l-min) suggest that the reaction rate might be affected by the initial COD. However, the low value of k in the homogeneous samples, which had high initial COD levels, contradicts this interpretation. An alternate explanation is that the specific composition of the organic molecules which are not distinguished in the COD test constitutes a dependent variable in the overall decomposition rate of the COD. It is unlikely that the composition of the effluents collected at different times would be the same. A second possibility is that the reaction rate is influenced by a mass transfer of ozone from the gas to the liquid phase, which will be influenced by the concentration of the ozone in the particular system, ozone concentration in the gas, and gas flow rate.

The apparent dependence of COD reduction on the molecular composition of the organic components is indicated also by the positive correlation between ozone dosage and apparent reaction rate when both

the COD and the specific composition of the water was held constant (in the experiments utilizing increasinq ozone dosages on homogeneous samples). In these experiments, the reaction rate increased consistently with an increase in ozone dosage.

A relatively large proportion of the organic constituents resisted oxidation, even at high ozone dose rates applied for 60 minutes. Table 18 gives the percentage reduction of COD obtained in 60 minutes at 8 of the doses shown in Figures 17 and 18 for heterogeneous and homogeneous samples. The comparable data on a 9th sample at a very high dosage rate is also shown. These figures reflect the same lack of correlation between dosage rate and reaction rate constant, and indicate further the inability to predict the effect of ozone on variable waters from wastewater treatment plants. Table 19 shows the initial COD values obtained from 15 different samples of effluent collected from the Villa Ana plant. These values emphasize the variability of plant effluents.

3.8. Effect of Ozonation on TOC Reduction. Table 20 shows the effect of 3 different ozone dosage rates on the reduction of TOC in plant effluent. As with COD reduction, the specific composition of the chemicals appear to have a greater influence on the reaction rate than the initial concentration of TOC. The percentage reduction of TOC was considerably less than the percentage reduction of the COD, which is explainable in terms of the requirement to oxidize completely the organic carbon molecules to inorganic derivatives in order to lower the TOC levels.

3.9. Ozone Decomposition. Table 21 depicts the decomposition of ozone from 10 to 240 minutes in finished water at pH 6.2 at 25° C following ozonation at a rate of 1.838 mg O_3/l-min. Applying the method of

integration of rate equations given by Walas (37) for identifying integral reaction orders, these data fit a reaction rate for ozone decay of three-halves:

$$\frac{-d(O_3)}{dt} = K_o(O_3)^{3/2} \qquad (3.3)$$

$$\left(\frac{1}{(O_3)_t} - \frac{1}{(O_3)_0} \right)^{1/2} = -1/2\ k_o t \qquad (3.4)$$

As shown in Figure 19, a plot of $(1/(O_3)_t - 1/(O_3)_o)^{1/2}$ vs time gives the straight line predicted for this reaction rate. The rate constant, determined from this slope, is .0189 $(mg/l)^{-1/2}$ $(min)^{-1}$.

The half life for ozone based on a 3/2 rate of decay is calculable from the equation:

$$t_{1/2} = .8284\ k_o^{-1}(O_3)_o^{-1/2} \qquad (3.5)$$

The rate constant is a function of temperature and pH. For the conditions of this experiment, the half life was 20.07 minutes. This rate of decay of dissolved ozone was also inferred by the inability to detect measurable amounts of dissolved ozone when small doses were applied, and by the failure to obtain significant inactivation of bacteria by preozonation (Tables 8 and 9, Fig. 7).

3.91. Effect of Ozonation on pH. Table 22 shows the results of 60 minutes ozonation on the pH of secondary wastewater at 6 ozone dosages. The maximum alteration obtained was from pH 6.8 to pH 7.31. The magnitude of these changes, and the final pH of the samples, suggest that ozonation would not in itself produce pH changes of physiological significance. The randomness of the magnitude and direction of the changes related to ozone dosage and initial pH again point to the differences in the reactivity between ozone and the reactive species in wastewater.

3.92. Preliminary Cost Feasibility Study. The economics of

operating an ozonater at a small treatment plant was examined by estimating ozone production costs for dosages from .1 kg O_3/hr to 100 kg O_3/hr, and then comparing this cost to the cost of chlorination. Time value of money was not included in these preliminary estimates.

The capital cost of the ozonation equipment was calculated according to the formula:

$$\text{Annual cost} = (M/m)(P-L) \qquad (3.6)$$

where M is the number of ozonaters, m is the life expectancy in years, P is the purchase price, and L is the salvage value. M was calculated by dividing the amount of ozone required by the capacity of the generator, and increasing the value to the next integer. A value of 10 years was assigned to m, and L was assumed as 0. Values of P were based on estimates supplied by the Welsbach Corporation for 3 of their generators: Model CLP-19-D19L, .333 kg O_3/hr, $38,000.00; Model CLP-68-D19L, 1.189 kg O_3/hr, $73,000.00; Model CLP-258-D19L, 4.511 kg O_3/hr, $60,000.00.

The operating cost of ozonation was calculated according to the formula:

$$\text{cost/year} = MFC_1H + EC_2HR \qquad (3.7)$$

where M is as above, F is the cooling water flow rate in liters/hr, C_1 is the cost of the cooling water, H is the numbers of hours per year, C_2 is the cost of electricity, E is the electricity requirement for O_3 production, and R is the ozone used as kg O_3/hr.

For equation 3.7, M was obtained as described previously, except that the value was not raised to the next integer. F for the 3 models listed is 817.65, 2,922.34, and 11,083.69 respectively: C_1 is currently 1.823×10^{-4}/liter; H is 8,760; C_2 is currently $.0205/KWH; E is 25.53 KWH/kg O_3.

The total cost was then calculated for ozone production rates of .1 to 100 kg O_3/hr according to the formula:

$$\text{Total cost/year} = M(P/m) + MF\ (1.5969) + R(4585.67) \quad (3.8)$$

using the appropriate data for the ozonator required for each dose.

Figures 20, 21, and 22 are graphs obtained by plotting total cost per year vs the ozone production rate. The slopes yield the cost per kg of ozone:

$$\text{cost/kg } O_3 = (\text{slope, cost-hr/yr kg } O_3)(\text{yr}/8760) \quad (3.9)$$

The results are shown in Table 23. The excessive value for dosages from 10 to 100 kg O_3/hour results from the proportionately larger range of R; if R is plotted in increments of 10 kg O_3/hr, a series of lines with smaller slopes would result, bringing the production cost in these ranges in accord with the values below 10 kg/hr. The ozone production costs were therefore estimated as \$0.95/kg O_3 to \$1.05/kg O_3.

The total cost of ozonation at specified ozone dose rates for given volumes of wastewater (mg O_3/l-min) may then be calculated by applying the cost of ozone production (R), using the following formula for R:

$$R = DV(60\ \text{min/hr})(1 \times 10^{-6}\text{kg } O_3/\text{mg } O_3) \quad (3.10)$$

where D is mg O_3/l-min and V is the volume of water in liters. The ozone dosage rate for any value of R may be determined by:

$$D = R/V(60)(1 \times 10^{-6}) \quad (3.11)$$

These calculations were applied in comparing the costs of chlorination and ozonation. The chlorination costs were based on a capital outlay of \$10,000.00, depreciated according to the same schedule as the ozonating apparatus, and an operating cost of \$0.01/3785.41 liters wastewater Eckenfelder (11). The chlorination costs per year for 37.854 liters per day to 1,892,706 liters per day and the ozone production

available for an equivalent cost per unit volume were calculated. The results are shown in Table 24. These results indicate that, for package plants of the size considered in this study, the dosage of ozone available at a cost equivalent to chlorine would be well below the dosage found necessary in this study for either disinfection or degradation of chemical oxygen demand. These comparisons do assume that the chlorine levels used would meet disinfection requirements.

CHAPTER 4

CONCLUSIONS AND DISCUSSION

4.1. The Persistence of Viruses in Effluents of the Package Plants. Infective viruses were detected in the post-chlorinated effluent of 3 of 4 of the treatment plants studied in Jefferson County, Ky. The quantity of viruses recovered in the samples, and the preponderance of the viruses in the summer months is in general accord with the several other studies reported on treatment plants (18, 23, 40). These results suggest that the fate of these infective agents in the small package plants in Jefferson County is subject to interpretation by the current theories on virus survival and detection in the sewage treatment process.

First, the relatively low numbers of viruses recovered in most samples in which the effluent was extremely turbid may be explained as the result of the relative inefficiency of detection methods in ioslating viral particles from aqueous systems which contain debris to which viruses may adsorb. We did not detect viruses in washes of the clarifying filters, but since the methods used were identical to the concentration techniques on the post-filter flow, there may have been persistent viruses which were not detected.

However, the yield of viruses, including the absence of detectable viral particles in one plant may also be explained by the reduction in the numbers of such particles by the activated sludge process and disinfection process used in all of the plants studied. Most primary treatment methods remove large numbers of viruses and the activated

sludge process is particularly effective (3, 30, 31). However, the common treatment methods do not remove all viruses, and disinfection is required to insure that viruses are absent from effluents. Chlorine is used in most plants in this country, and all the plants in this study utilized this disinfectant. There is no standard for the chlorine level required to inactivate viruses, but it is agreed that chlorine will remove viruses if it is applied properly. The requirement for a proper environment for the halogen suggests an explanation for the presence of viruses in the post-chlorinated effluent of these treatment plants.

The efficacy of chlorine as a disinfectant depends upon a number of factors which affect the reactive form of chlorine in solution. In aqueous solutions the Cl_2 molecule hydrolyzes to yield hypochlorous acid (HOCl). This acid may in turn yield hypochlorite ions (OCl^-) by protolysis. The reaction constant of this reaction ($HOCl \rightleftarrows OCl^-$) varies as a function of temperature and pH. At pH 5 the equilibrium favors the formation of HOCl, at pH 7.5 OCl^- predominates, and above 9.5 virtually all the chlorine is present as the hypochlorite ion. This is of practical importance, since HOCl is a more potent microbicide than OCl^-, including the effect on both bacteria and viruses (9, 39).

The presence of reactive substances may remove both HOCl and OCl^- from solution. Reaction with chlorine yields "combined chlorine" compounds, and the best known examples are reactive nitrogen compounds. The nitrogen may be available in many forms, but ammonia nitrogen is of particular significance. In either the neutral (NH_3) or ionic (NH_4^+) form, there may be a reaction with the dissociated chlorine products, OCl^- or HOCl, to produce mono-, di-, or trichloramines or

nitrogen trichloride.

These chloramines present several problems. Of particular importance in the consideration of our data, the chloramines are not effective disinfectants (9, 12). The prevalence of these products is a function of the relative concentrations of chlorine and ammonia-nitrogen, the temperature, and the pH. The point at which free residual chlorine is present in such systems corresponds to the point at which the reactive interfering compounds will no longer combine with the chlorine. The point at which the chlorine demand has been met is commonly known as the "breakpoint".

In addition to nitrogeneous compounds, other organic substances common in wastewater exert a chlorine demand, including charbohydrates and phenols, as do some inorganic contaminants, such as H_2S, iron, and manganese (39). The turbidity of the water is important and Culp (9) recommends a maximum of <.1 Jtu for maximum disinfection by chlorine. He notes that viruses may be protected from the disinfectant by entrapment in the particles of turbid water. Also, the particles contributing to the turbidity might combine with the chlorine, or otherwise effect the distribution of the HOCl molecules. The pH is obviously important since it governs the equilibrium of the reactions. Sufficient contact time of the chlorine at microbicidal levels is essential.

A comparison of the characteristics of the effluents in the plants studied with the model suggested by the requirements for viral removal by chlorination of an activated sludge effluent, suggests obvious reasons for the presence of viruses in 3 of these plants. Fifty percent of the samples tested at Glencoe had chlorine residuals from 0 to <.5 ppm. The chlorine residual of the other plant from which most viruses

were recovered, Maple Grove, was also usually low. In addition, in the Maple Grove plant, with an alkaline pH, the chlorine would exist predominantly in the less effective OCl^- form. The turbidity of both of these plants was consistently high, which would also interfere with the disinfecting efficacy of the chlorine. By comparison, the Villa Ana plant, from which viruses were recovered in smaller quantities, has an effluent which more closely fits a model for adequate disinfection. While the chlorine residuals were low, there were no samples without detectable chlorine. Further, the pH was slightly below neutrality, and would favor a greater concentration of HOCl than would be available at the higher pH of the other 2 plants. The effluent was usually less turbid than the other 2 plants.

The Hite Creek plant, from which no viruses were detected, fits very closely a model plant with regard to efficient disinfection. Half of the samples had a chlorine residual of at least 1 ppm. The pH was below the point at which OCl^- ions predominate, and the effluent was never turbid.

A similar comparison may be made of the coliform levels of the 4 plants' effluents. The consistently low counts at the Hite Creek plant, the consistently high counts at the Glencoe plant, and the variable levels at Villa Ana reflect the absence of viruses at the first plant, the high levels of viruses at the 2nd, and the lower yield at the 3rd. Only the Maple Grove plant was inconsistent in the relationship between the usual coliform levels of the effluent and viral levels of the samples tested. However, the high pH of the Maple Grove plant suggests that the specific compounds contributing to the alkalinity, as well as an alkalinity of >9, might have a bacteriocidal effect.

(These comparisons between effluent quality, coliform counts, and viral counts, are intended only to indicate the microbicidal environment in the effluents, and are not meant to suggest that the coliform count was tested as a reliable index of the viral load. There was no feasible way to correlate the 2 counts accurately in this study, since the viruses were concentrated from samples collected over a period of one to two hours, and there is a pronounced relationship between the time of collection and the coliform count. In addition, there are substantial theoretical reasons why coliform levels should not be used as an indicator of viruses (4).)

The study of viral inactivation in settled wastewater and secondary effluent by Lothrop and Sproul is pertinent to the interpretation of these data (26). They concluded that chlorine might give a high level of inactivation of viruses but it required a combined chlorine residual of 40 mg/l with a 30 min contact time to inactivate poliovirus in settled wastewater, and a free chlorine residual of 0.2 to 0.4 mg/l for inactivation of this virus in secondary effluent. The extreme difference between these figures reflects the differences in the breakpoint as a function of the chlorine demand. The settled wastewater had an average of 21.3 mg/l ammonia-N, and the secondary effluent an average of <1.0 mg/l. This point is also made by Culp, who includes pretreatment as a factor affecting proper chlorination, since such treatment reduces the viral load in addition to eliminating the need for breakpoint chlorination (9).

In summary, the study of the survival of viruses in package plants in Jefferson County, Kentucky, indicates that:

1. The *in-situ* concentration of wastewater using the Aquella

Viral Concentrator yielded samples from which viruses were detected by routine assay. The high organic contamination of the water necessitated the use of alternate techniques for the final concentration of the viruses. Seeded poliovirus samples produced a high rate of recovery by this method, but there is the possibility that large numbers of viral particles entering in the raw wastewater may escape detection in the final effluent. The correlation between the presence of viruses and the characteristics of the effluent considered relevant to viral presistence suggests that the method is accurate for indicating relative numbers of viral particles. However, it would not be economically feasible to adopt this system for routine viral monitoring for the large number of plants in the test area. In addition to the relatively high cost of the apparatus, filters, and chemicals, it required approximately 4 hours and 2 technicians to process each sample.

2. The conditions which yielded viruses in the effluents are compatible with the current theories regarding the persistence of viruses in wastewater. The plants whose pre-chlorination processes produced a high quality effluent yielded fewer viruses than the plants which produced an effluent that would be judged below standard by routine criteria of quality. Since the persistence of viruses in wastewater is dependent on complex interactions between the environmental components it is not possible to suggest a single measurable factor that would be a reliable indicator for viruses.

3. These data suggest that the small package plants in Jefferson County could be upgraded without extensive modification of the current design of the plants. First, the results indicate that the proper operation of a plant, such as the small Villa Ana facility and the

large Hite Creek plant, can produce high quality effluents despite the diversity of the details of plant design. Secondly, all of the plants in this study, and most of the treatment facilities in Jefferson County, utilize activated sludge which is considered by Berg and others to be the most effective conventional method for the removal of viruses (3). This was reaffirmed in a recent study which reported 95% removal of seeded viruses in the high-rate activated sludge module of a multi-stage pilot plant (31). Further, there appears to be a reasonable latitude in the requirements for the operating efficiency of the activated sludge process. Malina et al. reported that viruses were inactivated within the range of suspended solids and aeration time of the normal sludge system, which in his study varied from 1,940 mg/l to 2,710 mg/l suspended solids, and 5.1 to 15.4 hours aeration (28). Thirdly, all of the plants in the test area are required to utilize a disinfectant, and adequate levels of chlorine in the proper environment will inactivate residual viruses. This favorable environment is available in the effluent from secondary treatment facilities which are operated properly.

4.2. Ozone-Feasibility Studies.

4.2.1. Inactivation of Bacteria and Viruses. These data are in general agreement with other studies of the disinfectant potential of ozone to the extent that they indicate that ozone may be a potent agent for the inactivation of bacteria and viruses. The conclusions permitted by this study regarding the application of ozone for wastewater treatment follow:

4.2.1.1. Ozone Dosage. Venosa's review of the literature on ozone indicates that most studies utilized dosages from 1 mg/l to 2 mg/l to achieve microbicidal effects (36). In their recent study on inactivation

kinetics of ozone in ozone-demand-free-systems Katznelsen et al. (19) employed dosages from 1.02 to 1.55 mg/l for inactivation of *E. coli* and .07 to 2.5 mg/l for inactivation of poliovirus 1. The differences in the ozone dosages required for inactivation by various researchers cannot be nationalized, since many of the reports lack sufficient detail to permit comparisons. It seems apparent that the doses found effective in this study for rapid inactivation of bacteria and viruses in finished buffered water were significantly lower than those reported by others. Our initial inactivation studies which utilized doses in the ranges 1 mg/l to 2 mg/l, resulted in such rapid inactivation it was not possible to obtain usable survival data as a function of exposure time to ozone.

These results differ also from others in that no detectable threshold of ozone was required prior to onset of inactivation, and the "all-or-none" effect was absent. A minimum effective dose was required, since there were levels of ozone tested which yielded no inactivation. The shoulder on the inactivation curves of bacteria in treated water might also suggest the requirement of a minimum residual of dissolved ozone. However, neither of these effects, which are common to most disinfection data, are necessarily related to the concept of a threshold dose at it has been used by Katznelsen and others.

There is also a wide spectrum in the assessment of the influence of contaminants on the effectiveness of ozone as a disinfectant. Most studies agree with the early work of Gubelman and Scheller (14) who assign importance to the water quality, with diminished microbicidal effects in raw wastewater. Majumdar et al. did not find significant differences in the inactivation of viruses as a function of the water

quality (27). Their survival data differed as a function of dosage but were similar for distilled water, secondary effluent, and primary effluent. This study shows the nature of the water is an essential variable in determining effective ozone doses to inactivate both bacteria and viruses. We achieved <3 logs inactivation of *E. coli* in 20 minutes with a dosage of .29 mg/l-min in plant effluent compared with >5 logs inactivation in 20 minutes with a dosage of .007 mg/l-min in finished water. Similarly, >2 logs inactivation of poliovirus were achieved in 20 minutes at an ozone dosage of .023 mg/l-min, but the same ozone dosage did not affect the virus survival in effluent. A dosage of approximately 1 mg O_3/l-min was required to achieve 4 logs inactivation in 20 minutes in the effluent, suggesting that doses may have to be increased by a magnitude of 25 to 50 to achieve virucidal effects in wastewater compared to treated water. Figure 23 shows a plot of the rate of inactivation vs COD, using the rate constants for 4 experiments on bacterial inactivation in effluent. While these data are scattered, they indicate an inverse relationship between COD levels and the rate constants for inactivation. The scattering may be explained on the basis of the differences in the behavior of ozone in heterogeneous effluents with the same COD levels (Sec. 3.7).

This study did not indicate the specific contaminating compounds which may inhibit ozone disinfection. The prolongation of inactivation in the experiments concerned with cell lysis indicates that initial concentration of cells is a critical variable, since the only essential difference in this experiment was the increase in the initial number of cells. This "organism-demand-effect" was also found by Suchkov, who reported the dose of ozone required for inactivation of bacteria

increased in proportion to the number of bacteria in the system (33). The complex ingredients present in the growth medium in the runs using unwashed cells and in those with high concentration of medium 199 and calf serum did inhibit the inactivation by ozonation, but these additives are not readily definable. There was no significant difference in the inactivation of viruses in finished water compared to water with the defined additives alanine or dextrose. Neither of these molecules emulated the wastewater constituents which exerted an ozone demand that interfered with disinfection.

4.2.1.2. The Mechanisms of Action and Reaction Kinetics. The shape of the inactivation curves differed as a function of (1) the initial concentration of cells in the case of bacteria, and (2) the characteristics of the aqueous phase in the case of the bacteria, but not the viruses. The slope of the curves varied as a function of (1) initial concentration of bacteria and viruses, (2) nature of the water, (3) ozone dosage.

All the viral inactivation curves were in accordance with Chick's law which described the process of disinfection as analogous kinetically to a first order chemical reaction:

$$\frac{d(\ln F)}{dt} = -k, \tag{4.1}$$

where F = fraction of survivors, k = the inactivation rate constant, t = time. As shown in the integrated form:

$$\ln F = -kt, \tag{4.2}$$

the curves should be exponential when survival is plotted against time if disinfection is a first order reaction. The inactivation of bacteria at high concentrations (Fig. 13) and in wastewater (Fig. 9) also yielded an exponential curve linear through the origin, suggesting an apparent

first order reaction.

The survival curves of bacteria in finished water were sigmoidal, with a shoulder and a tail. It is possible to interpret the linear portion of the curve as resulting from a first order reaction, although this is not the exclusive interpretation. The shoulder and tail, which is not atypical of many disinfectants, is subject to numerous alternate interpretations. The most common explanations include: (1) there is more than one critical site required for inactivation, and the shoulder results from the time required for this multiple number of sites to react with the agent; (2) a minimum contact time is required before the agent is effective, which would result in a shoulder even if there were only one critical site; (3) the applied dose of a disinfectant does not always result in equivalent residuals, and the tail might result from a lower effective concentration in contact with the cells; (4) the cells or particles might exist in clumps in the initial population, or clumps may be generated during the process, which would yield reaction kinetics emulating the multihit model even if only a single hit per particle were required for inactivation; (5) populations of cells are not genetically identical with respect to their susceptibility to some disinfectants, and there is a high probability that resistant cells from the initial population, or those developing from spontaneous mutation, will prolong the inactivation curve to produce a tail; (6) there is a mass transfer effect with respect to the transport of the disinfectant. This is a particularly compelling explanation in the case of ozonation, considering the time required to transport the ozone from the gas phase to the effective site of the particle in the liquid phase. The coefficient of this transfer phase would alter the apparent rate constant,

since the rate of transport as well as the inactivation kinetics of the reactive species would be included collectively; (7) there may be more than one reaction mechanism, and the specific mechanism may occur as a function of the environment. Each of these might yield its own reaction rate. A single plotting of the data as survival vs time would not yield an exponential curve from the origin if the reaction rates or the reaction orders differed for several operative mechanisms.

An experiment pertinent to one of these theories was performed. The bacterial cells were subjected to high speed turbulence in a blender prior to ozonation. This inactivation curve was sigmoidal (unpublished data). This process should have disrupted aggregates, which suggested that the shoulder was not caused by clumping. In addition, stained slides of the cultures after growth in the pre-inoculation environment did not reveal aggregated cells in the magnitude required to explain the shape of the curve. This may be contrasted with the reported synergistic effects of sonication and ozonation on viral and bacterial inactivation. Burleson et al. (6) concluded that simultaneous sonication and ozonation of the particles in secondary effluent reduced the contact time required for inactivation. Katznelson et al. stated that sonication of poliovirus prior to ozonation dramatically increased the effectiveness of the ozone. In the first study, the simultaneity of the treatment makes it compelling to explain the results in terms of enhanced ozone contact (reduced mass transfer limitations). In the second study the failure to show that sonication did not increase the titer of the inoculum prior to treatment makes it difficult to conclude that the enhanced inactivation was due to dispersion of clumps.

It is not essential that the mechanisms explaining the kinetics

of experimental data be known before the kinetics can be applied to the use of a disinfectant in a particular reactor, although an understanding of the basic mechanisms will facilitate rational use. In a steady-state, continuous process, the linear portion of the inactivation curve can be made to occur for the duration of the required contact period. Hence, the shoulder may be of more theoretical than practical concern. The existence of a tail would be important in determining the final product of disinfection, but routine practices of monitoring effluent for coliforms would indicate the persistence of organisms.

The experiments which traced the optical density of a suspension during ozonation indicates that lysis of the cell is not the immediate cause of loss of viability. The experiments on ozonated poliovirus uptake indicate that inactivation of the virus involves either the inhibition of adsorption of the virus on the host, or of active uptake into the host cell. Either process would involve an alteration of the protein capsid. The electron micrographs of the ozonated particles are confirmatory evidence that the ozone does degrade the capsid. A severe effect, such as degradation of the capsid, is compatible with the fact that inactivated particles do not recover their infectivity after ozonation (unpublished data). This suggests that the calculation of ozone doses may be based on the assumption that ozonation is virucidal and not virustatic.

4.2.2. Reduction of Non-Biological Pollutants. The scope of the experiments on COD and TOC reduction was confined to an assessment of the feasibility of ozone to upgrade the effluent of small treatment plants by improving these parameters of effluent quality. This assessment was based on the direct measurement of the levels of COD and TOC

in the effluent of a plant during normal operation after the application of realistic doses of ozone. It would be valuable to test the validity of these data by comparison with related studies. However, the critical review by Kinman (22) points to the same problems in interpreting the reports of reduction of non-biological contaminants as in the literature of ozone as a disinfectant. Our data on the modification of organic compounds, and the decomposition of ozone, are generally compatible with reports based on similar experimental conditions (15, 16).

This study confirms that ozone reduces the COD and TOC levels of wastewater effluent. However, these data do not favor a recommendation that ozone would be suitable as a tack-on process to upgrade the existing quality of the effluents. This is based on: (1) the resistance of substantial portions of the contaminants to oxidation at feasible ozone doses, and (2) the inability to predict accurately the dose-response relationship between ozone and oxidation of normal wastewater contaminants. There was a predictable relationship in reaction rate and ozone dose in homogeneous samples, and in the rates on both ends of a broad spectrum of doses applied to heterogeneous samples. This proportionate response in heterogeneous samples diminishes as the doses converge, and in some cases lower ozone doses produced higher reaction rates than higher doses. This problem is complicated by the fact that the reaction rate was not dependent upon the initial COD levels. Since normal use of an ozonater would require calculation of rates applicable to an effluent with continually varying composition, it would be impossible to predict accurately the appropriate dose to achieve an established standard of COD and TOC reduction.

The relative reduction of COD was in the same range as that reported

in a study in pre-chlorinated effluents from the Coral Gables Sewage Treatment Plant (35). His data show a consistent relationship between percentage reduction and initial COD in 3 different samples. However, he used a single applied ozone dose, and it was relatively high. Hewes and Davidson obtained much higher rates of COD reduction in effluents by ozonation (16). Their results are not comparable to this study because the water was pretreated extensively by chemical and physical methods to remove all colloidal and suspended solids before ozonation. This pretreatment approximately halved the COD. This study is of interest because it suggests that ozonation probably is effective if there has been pretreatment to remove substances from the effluent which interfere with the ozonation process.

4.2.3. Safety of the Ozonated Product. The experiments concerning residual ozone and alteration of pH suggest that the process of ozonation should not yield products in the water toxic to the environment at the lower ozone doses used by most investigators. There was no significant change in the pH. The decomposition rate of the ozone in finished water at a pH similar to that of effluents would result in a substantial reduction of ozone by the time the effluent joined the receiving stream. This decomposition rate of 3/2 under the experimental conditions agrees with the reports of Hewes and Davidson (16). However, the variation in kinetics in complex wastewater must be considered a potential variable in the decay process, and a determination of this aspect of safety should not be made until extensive studies are done on decomposition in normal effluents.

The information which is available prompts a conclusion that the major hazard in ozonation might be an unwarranted reliance on its ability

to diminish biological and non-biological pollutants.

4.2.4. Cost of Ozonation. Our estimation of the cost of ozonation was a part of the evaluation of the feasibility of using ozone as a disinfectant and to reduce COD and TOC in treatment plants. This report expresses the costs in terms of a comparison with the cost of chlorination. There are other current economic analyses for ozonation. The inconsistencies in these estimates derive to some extent from different values used for the capital cost and the water and electricity rates, which are a major factor in operating costs. There are also large differences derived from different assumptions in the factors which must be considered. Some calculations have been based on operating costs, omitting capital outlay. Others include assumptions regarding differences in labor costs if ozonation were used instead of chlorine. Our calculations are based on capital outlay and the operating costs without considering the factors of maintenance or labor for any of the processes.

Our estimates show that ozonation would cost much more than chlorination, which is in agreement with other workers (22, 42). The amount of ozone available for the same cost as chlorination would not provide effective ozone doses for improving the quality of the plant effluents.

If ozonation were used in plants representative of those in this study, it would not be feasible to simply add an ozonater to replace the chlorinator, since the ozonated effluent would not meet effluent standards, which includes a disinfectant residual. In fact, not only would chlorination still be required, but it would probably be necessary to alter the design and/or operation of the plants to produce a clearer effluent suitable for ozonation. Therefore, the actual costs of adding ozonation as a tack-on process to these plants would include

the costs of chlorine, ozonation, and the additional processes required to improve the current secondary treatment procedures.

4.3. Recommendations

1. It is recommended that the regulatory agencies of the state and county persist in the efforts to eliminate substandard effluents from small sewage treatment plants as a source of pollution in Kentucky waterways. This study confirms that these small wastewater treatment plants in Jefferson County, Ky. may be a source of hazardous pollutants to the residents near the plants and to the aquatic ecosystems of the state. The investigation revealed that in addition to the release of coliforms and non-biological hazards to aquatic systems, viruses are being released from some of the effluents.

2. It is recommended that the MSD and Board of Health consider that viruses are probably present in larger quantities in plants with poor quality effluent than in those with high quality effluents. "Poor quality" includes high turbidity, high levels of coliform, insufficient chlorine residuals, and a pH which removes HOCl as the predominant chlorine product in solution.

The cost and expertise required for assessments of the viral loads in effluents would prohibit the routine analysis of the numerous wastewater plants in Jefferson County. There is no single indicator among the routine measurements made in these plants which was found to be a reliable index for viruses. However, when the routine parameters, including chlorine residuals, coliform counts, pH, and turbidity, were combined to assess the quality of the effluent, there was a correlation between plants producing a poor effluent and the viral load.

3. It is recommended that ozonation should not be considered as

the solution to the problems of existing plants. Ozone was extremely effective in removing bacteria and viruses from treated waters, but was not reliable for removal of biological and non-biological contaminants in normal secondary plant effluent. If ozonation were merely added on as a tack-on process chlorination would still be required to insure disinfection. Further, it would not be possible to establish ozone doses that would assure meeting preset standards for quality, because effluents of varying composition alter the required dose. It would still be necessary to improve the quality of the water by other mechanisms prior to ozonation if the ozonation process were to be made reliable and predictable. Further, the costs of ozonation would be prohibitive. This suggests the paradox that ozonation is feasible for improvement of wastewater quality in systems which already meet effluent standards, but not feasible for treatment of wastewaters which require improvement.

4. It is recommended that upgrading the operation of the existing plants is the most feasible mechanism for eliminating the release of hazardous effluents. The operation of the plants tested was a more important factor than plant design.

5. It is recommended that the Health Department improve its facilities for routine monitoring of small treatment plants. This improvement should include more frequent sampling, a reassessment of the methods used to test each parameter, and a schedule which accounts for the large temporal differences in the constituents of the wastewater throughout a 24-hour period.

Implementation of the recommendations of this study, i.e.; improvement in the operation of the plants as currently designed, would require

diligence in the routine monitoring of these plants, since an assessment of the effluent is the only reliable measurement of the proper operation of the facility. The author appreciates that the MSD and Department of Health currently perform maximally with the facilities which have been provided, and that an improvement in the monitoring would require a committment to expand the facilities of these agencies.

APPENDIX

Tables 4 – 24

Figures 6 – 23

TABLE 4

Inactivation of *Escherichia coli* in Finished Water,

0.043 mg O_3/l-min, Semiflow Batch Mode

Time (min)[a]	CFU/ml[b]	Fraction Surviving
0	2.0×10^6	1.0
2	1.9×10^6	9.5×10^{-1}
4	1.3×10^6	6.5×10^{-1}
7	1.9×10^6	9.5×10^{-1}
10	1.8×10^5	9.0×10^{-2}
18	3.0×10^2	$.5 \times 10^{-4}$
22	4.4×10^2	2.3×10^{-4}
26	1.2×10^2	6.0×10^{-5}
20	1.0×10^1	5.0×10

[a]Time after ozonation started

[b]Survivors, enumerated as colony forming units per ml

TABLE 5

Inactivation of *Esherichia coli* in Finished Water,

0.0067 mg O_3/l-min, Semiflow Batch Mode

Time (min)	CFU/ml	Fraction Surviving
0	2.6×10^{6}	1.0
2	2.0×10^{6}	7.7×10^{-1}
4	1.9×10^{6}	7.3×10^{-1}
7	1.4×10^{4}	5.4×10^{-3}
10	6.3×10^{2}	2.4×10^{-4}
15	2.0×10^{2}	7.7×10^{-5}

TABLE 6

Inactivation of *Escherichia coli* in Finished Water,

0.007 mg O_3/l-min, Semiflow Batch Mode

Time (min)	CFU/ml	Fraction Surviving
Run 1		
0	3.3×10^6	1.0
5	2.9×10^6	8.8×10^{-1}
10	1.0×10^4	3.0×10^{-3}
15	1.1×10^2	3.3×10^{-5}
20	3.8×10^1	1.2×10^{-5}
Run 2		
0	2.0×10^6	1.0
2	1.3×10^6	6.5×10^{-1}
5	1.8×10^5	9.0×10^{-2}
10	1.7×10^5	8.5×10^{-2}
15	8.3×10^1	4.2×10^{-5}
20	1.1×10^1	5.5×10^{-6}
25	9.0×10^0	4.5×10^{-6}

TABLE 7

Inactivation of *Escherichia coli* in Finished Water,

0.0077 mg O_3/l-min, Semiflow Batch Mode

Time (min)	CFU/ml	Fraction Surviving
0	4.1×10^{6}	1.0
2	3.4×10^{6}	8.3×10^{-1}
4	1.6×10^{6}	3.9×10^{-1}
7	3.3×10^{3}	8.0×10^{-4}
10	2.8×10^{2}	6.8×10^{-5}
16	6.3×10^{1}	1.5×10^{-5}
23	1.4×10^{1}	3.4×10^{-6}
30	9.0×10^{0}	2.4×10^{-6}

TABLE 8

Inactivation of *Escherichia coli* in Finished Water,

0.0054 mg O_3/l-min, Batch Mode

Time (min)[a]	CFU/ml	Fraction Surviving
0	4.4×10^{6}	1.0
1	3.7×10^{6}	8.4×10^{-1}
4	3.7×10^{6}	8.4×10^{-1}
8	2.9×10^{6}	6.6×10^{-1}
14	1.5×10^{6}	3.4×10^{-1}
20	1.2×10^{6}	2.7×10^{-1}

[a]Time after bacteria added to preozonated reactor

TABLE 9

Inactivation of *Escherichia coli* in Finished Water,

0.0076 mg O_3/l-min, Batch Mode

Time (min)	CFU/ml	Fraction Surviving
0	7.9×10^5	1.0
10	3.4×10^5	4.3×10^{-1}
20	1.9×10^5	2.4×10^{-1}

TABLE 10

Inactivation of *Escherichia coli* in Simulated Effluent,

0.0034 mg O_3/l-min, Semi-flow Batch Mode

Time (min)[a]	CFU/ml	Fraction Surviving
0	2.0×10^5	1.0
5	1.2×10^4	6.0×10^{-2}
8	4.2×10^3	2.1×10^{-2}
15	2.9×10^1	1.5×10^{-4}
20	6.0×10^0	2.0×10^{-5}

[a]Time after ozonation started, cells added with growth medium prior to ozonation

TABLE 11

Inactivation of *Escherichia coli* in Simulated Effluent,

0.0038 mg O_3/l-min, Semiflow Batch Mode

Time (min)	CFU/ml	Fraction Surviving
0	2.2×10^{3}	1.0
5	4.9×10^{2}	2.2×10^{-1}
15	3.0×10^{1}	1.4×10^{-2}

TABLE 12

Inactivation of *Escherichia coli* in Plant Effluent,

0.0063 mg O_3/l-min, Semiflow Batch Mode

Time (min)	CFU/ml	Fraction Surviving
0	9.6×10^5	1.0
10	4.3×10^5	4.5×10^{-1}
20	3.9×10^5	4.1×10^{-1}
40	2.0×10^5	2.1×10^{-1}

TABLE 13

Inactivation of *Escherichia coli* in Plant Effluent,

0.29 mg O_3/l-min, Semiflow Batch Mode

Time (min)	CFU/ml	Fraction Surviving
0	1.7×10^{3}	1.0
2	1.4×10^{3}	8.2×10^{-1}
4	9.8×10^{2}	5.8×10^{-1}
8	4.7×10^{2}	2.8×10^{-1}
12	9.1×10^{1}	5.4×10^{-2}
20	7.0×10^{0}	4.1×10^{-3}

TABLE 14

Chemical Oxygen Demand Reduction,

Heterogeneous[a] Effluent Samples

Initial COD (mg/l)	Ozone Dosage Rate (mg O_3/l-min)	Reaction Time (minutes)	Chemical Oxygen Demand (mg/liter)
51.82	.007	10	49.00
		20	49.69
		40	45.41
28.37	.066	50	26.08
		60	25.79
54.79	.413	10	50.38
		20	45.41
		40	37.28
		60	31.26
37.29	.904	20	32.59
		30	31.14
		40	28.84
		60	25.08
55.86	1.627	10	49.44
		20	39.69
		30	37.55
		40	37.36
		50	36.06
		60	36.84
22.58	1.933	10	20.02
		20	15.97
		30	12.34
		40	12.25
		50	12.90

[a] Each sample collected on a different day

TABLE 15

Chemical Oxygen Demand Reduction

Homogeneous[b] Effluent Samples

Initial COD (mg/l)	Ozone Dosage Rate (mg O_3/l-min)	Reaction Time (minutes)	Chemical Oxygen Demand (mg/liter)
83.05	.210	10	80.78
		20	83.05
		40	82.99
		60	72.91
82.84	.732	10	77.95
		20	76.44
		40	70.67
		60	56.38
78.15	1.406	10	71.30
		20	68.00
		40	50.87
		60	43.04
71.10	2.511	10	67.41
		20	53.94
		40	50.26
		60	32.45

[b]Single sample divided into 4 portions

TABLE 16

Reaction Rate Constants for Heterogeneous

Effluent Samples

Ozone Dosage Rate (mg O_3/l-min)	First Order Rate Constant (minutes $^{-1}$)
.007	.0033
.066	.0014
.413	.0103
.904	.0056
1.627	.0201
1.933	.0201

TABLE 17

Reaction Rate Constants for Homogeneous

Effluent Samples

Ozone Dosage Rate (mg O_3/l-min)	First Order Rate Constant ($minutes^{-1}$)
.210	.0010
.732	.0043
1.406	.0075
2.511	.0082

TABLE 18

Maximum COD Reduction in 60 Minutes,

Heterogeneous and Homogeneous Samples

Ozone Dosage Rate (mg O_3/l-min)	Initial COD (mg COD/liter)	COD 60 min Ozonation (mg COD/liter)	Percent Reduction
0.210	83.05	72.91	12.21
0.413	54.79	31.26	42.95
0.732	82.84	56.38	31.95
0.904	37.29	25.08	32.75
1.406	78.15	43.04	44.93
1.627	55.86	36.84	34.05
1.933	22.58	12.90	42.87
2.511	71.10	32.45	54.37
7.165	26.54	11.18	57.87

TABLE 19

COD Values of Secondary Effluent

Date	COD[a] (mg/liter)
9-20-75	51.98
9-27-75	52.51
10-11-75	41.54
10-13-75	36.11
10-25-75	51.82
11-15-75	64.87
12-21-75	54.79
12-23-75	55.86
12-24-75	41.66
12-28-75	22.58
12-29-75	26.02
1-8-76	37.29
1-12-76	28.37
1-21-76	22.80
1-31-76	26.54

[a]Initial COD values of unozonated secondary effluent

TABLE 20

Total Organic Carbon Reduction of Secondary Effluent

Ozone Dosage (mg/l-min)	Sample Time (minutes)	TOC (mg/liter)	TOC Reduction (%)
.0069	0	10.50	0
	20	8.50	19.05
	40	8.50	19.05
.2272	0	9.83	0
	20	8.17	16.89
	40	6.83	30.52
.4110	0	11.50	0
	20	11.50	0
	40	9.88	14.09

TABLE 21

Ozone Decomposition in Finished Water

Time (minutes)	Dissolved Ozone Concentration (mg/liter)[a]	$(1/(O_3)_t - 1/(O_3)o)^{1/2}$ $(liter/mg)^{1/2}$
10	3.469	.280
20	2.754	.392
30	2.274	.480
40	1.720	.610
50	1.444	.695
60	1.272	.759
80	.833	.995
100	.696	1.108
120	.502	1.335
140	.401	1.511
160	.321	1.705
180	.261	1.903
240	.156	2.490

[a]Ozone applied at 1.838 mg O_3/l-min, pH 6.2, 25 C

TABLE 22

Effect of Ozonation on pH of Secondary Effluent

Ozone Dosage (mg/l-min)	Reaction Time (minutes)	Initial pH	Final pH
.440	60	6.90	6.80
.648	60	6.80	7.31
.791	60	7.05	7.19
1.627	60	6.70	6.40
1.876	60	6.98	7.34
1.933	60	7.29	7.25

TABLE 23

Ozone Production Costs, .1-100 kg O_3/hr

Ozone Production Range (kg O_3/hr)	Cost ($/kg O_3)	Cost ($/lb O_3)
.10 - .33	.97	.44
.34 - 1.0	.95	.43
1.2 - 1.5	.97	.44
1.6 - 4.5	1.03	.47
4.7 - 9.0	1.05	.48
9.2 - 10	.97	.44
10 - 100	1.38	.63

TABLE 24

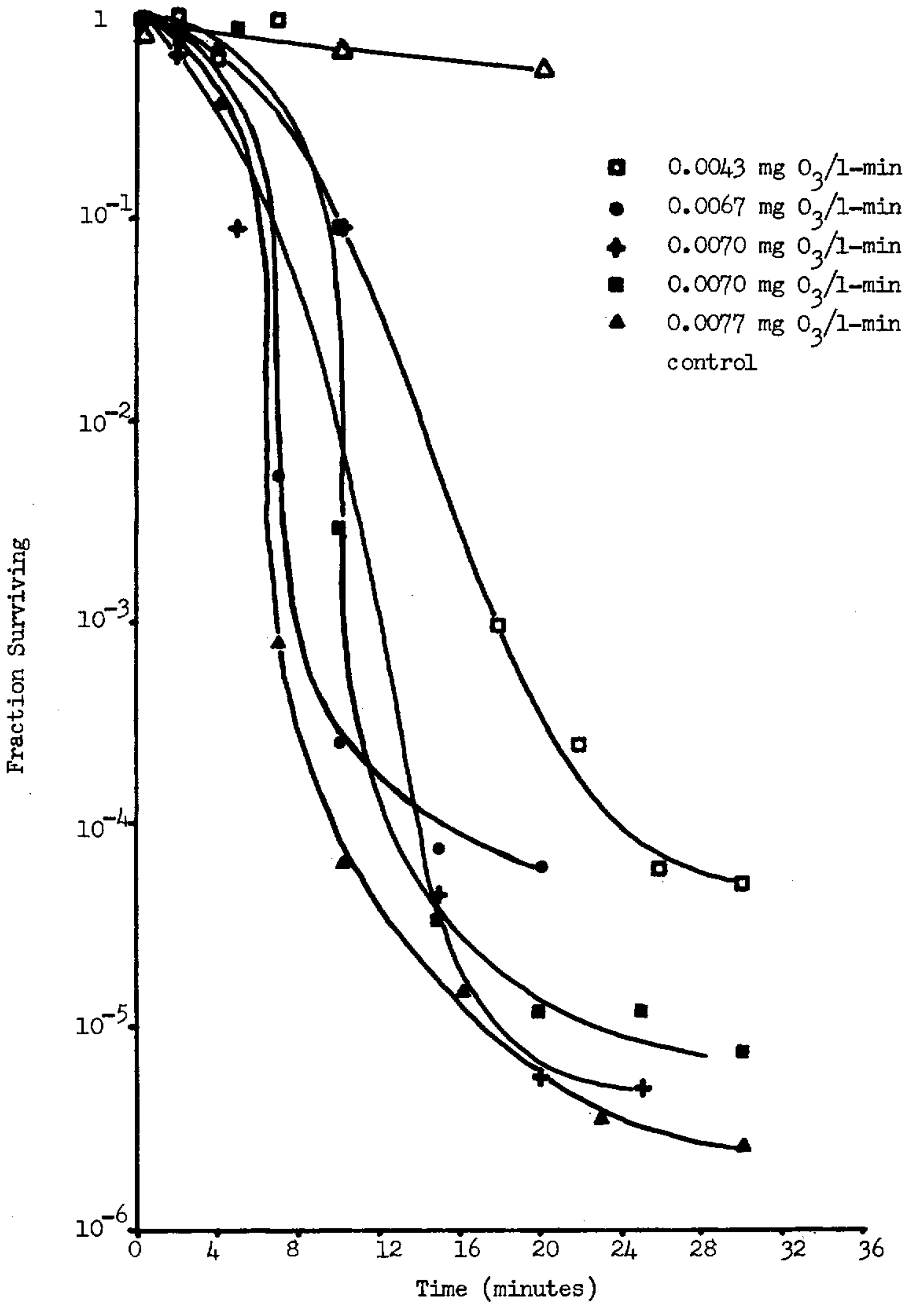
Comparison of Ozone and Chlorine Treatment

Liters of Water/day	Chlorination Cost per Year (dollars)	R[a] (kg O_3/hr)	Equivalent Ozone Dosage Rate[a] (mg O_3/min-hr)
37,854	1,138	less than .1	negligible
189,270	1,691	less than .1	negligible
378,514	2,382	less than .1	negligible
757,082	3,763	less than .1	negligible
1,135,624	5,145	.15	6.03×10^{-6}
1,514,165	6,527	.32	9.65×10^{-6}
1,892,706	7,908	.34	8.20×10^{-6}

[a]Assuming an ozone treatment system with an equivalent cost to chlorination.

Figure 6

Bacterial Survival Curves in Finished Water, Semiflow Batch Mode



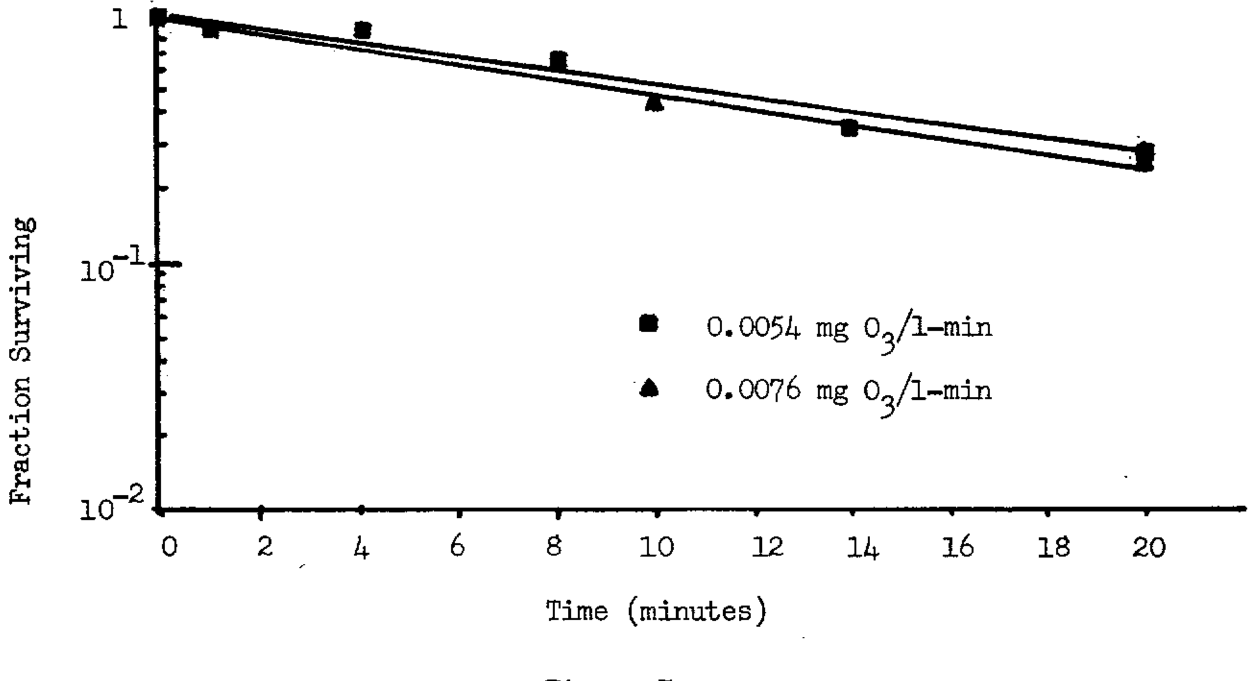


Figure 7

Bacterial Survival Curves in Finished Water, Batch Mode

Ozone was applied for 20 minutes prior to inoculation

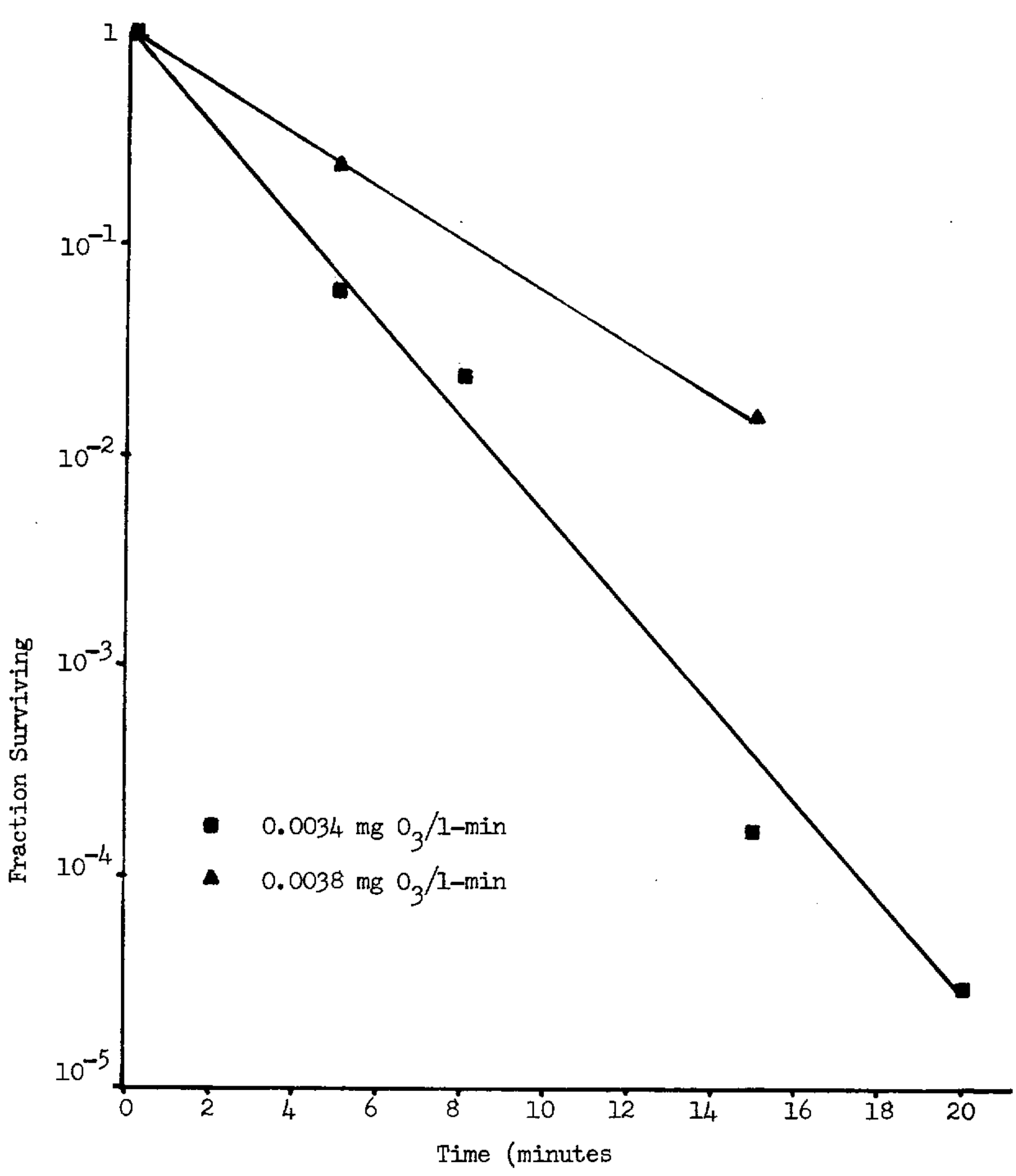


Figure 8

Bacterial Survival Curves in Simulated Effluent,Semiflow Batch Mode
Tryptone growth medium was added with the bacteria

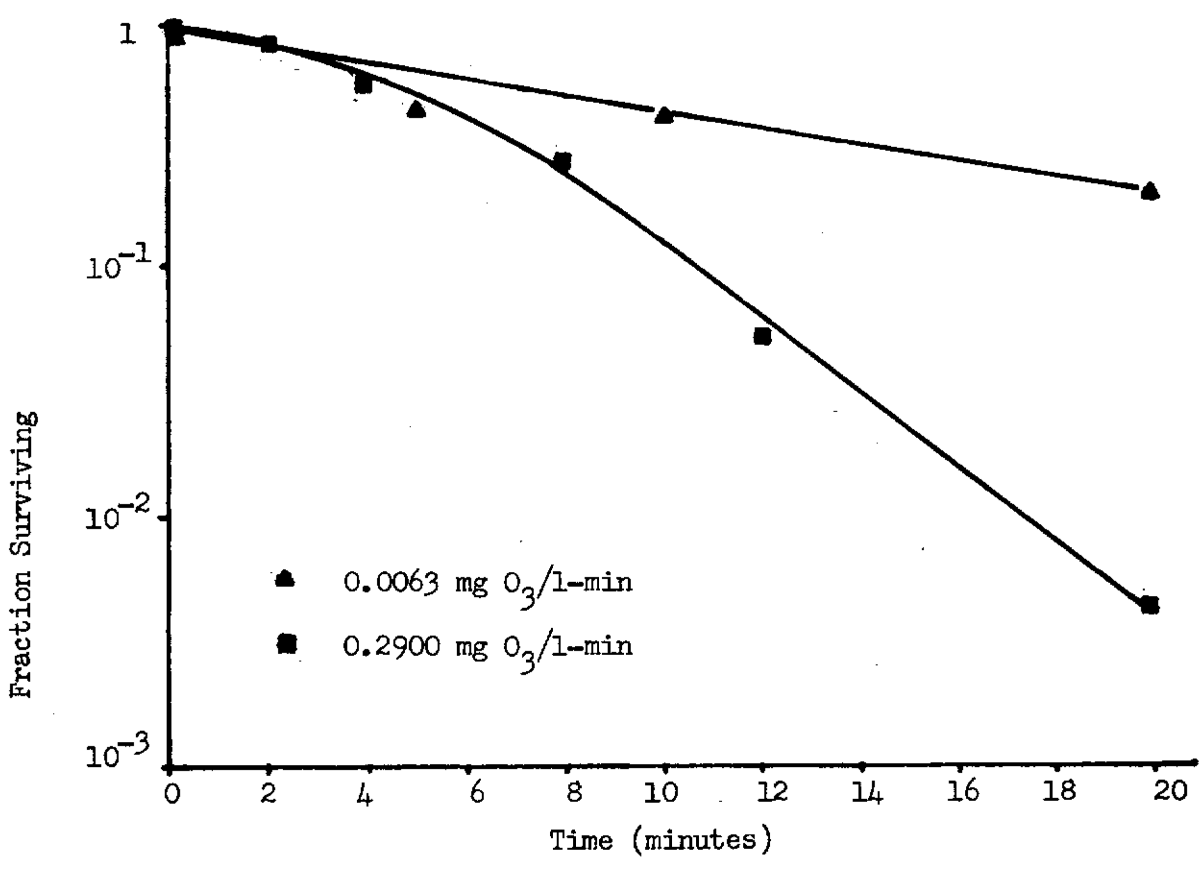


Figure 9

Bacterial Survival Curves in Package Plant Effluent, Semiflow Batch

Bacterial cell counts include the seeded and indigenous coliform cells

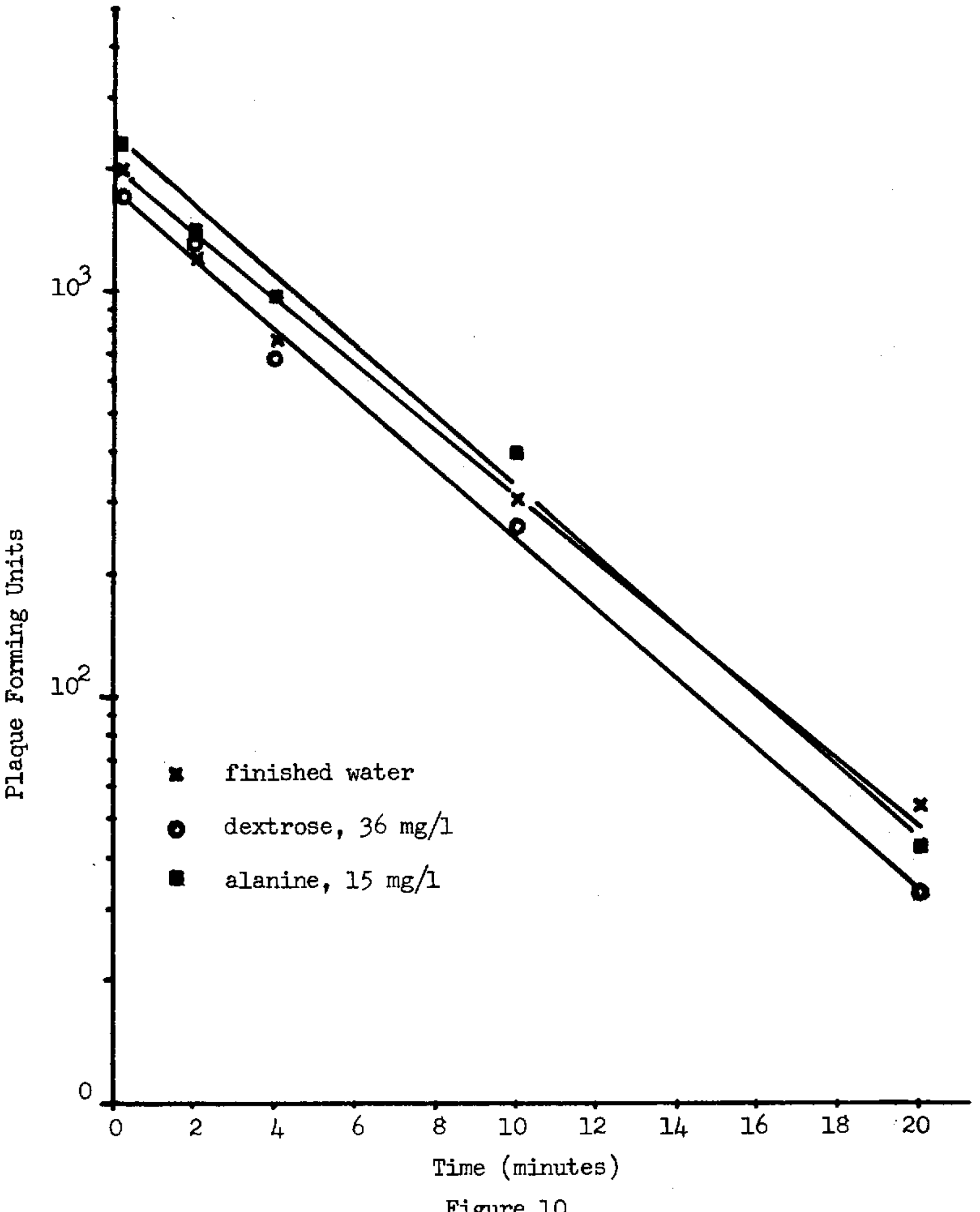


Figure 10

Poliovirus Survival Curves in Finished Water and Simulated Effluent, Semiflow Batch Mode, Ozone Applied at .023 mg/l-min.

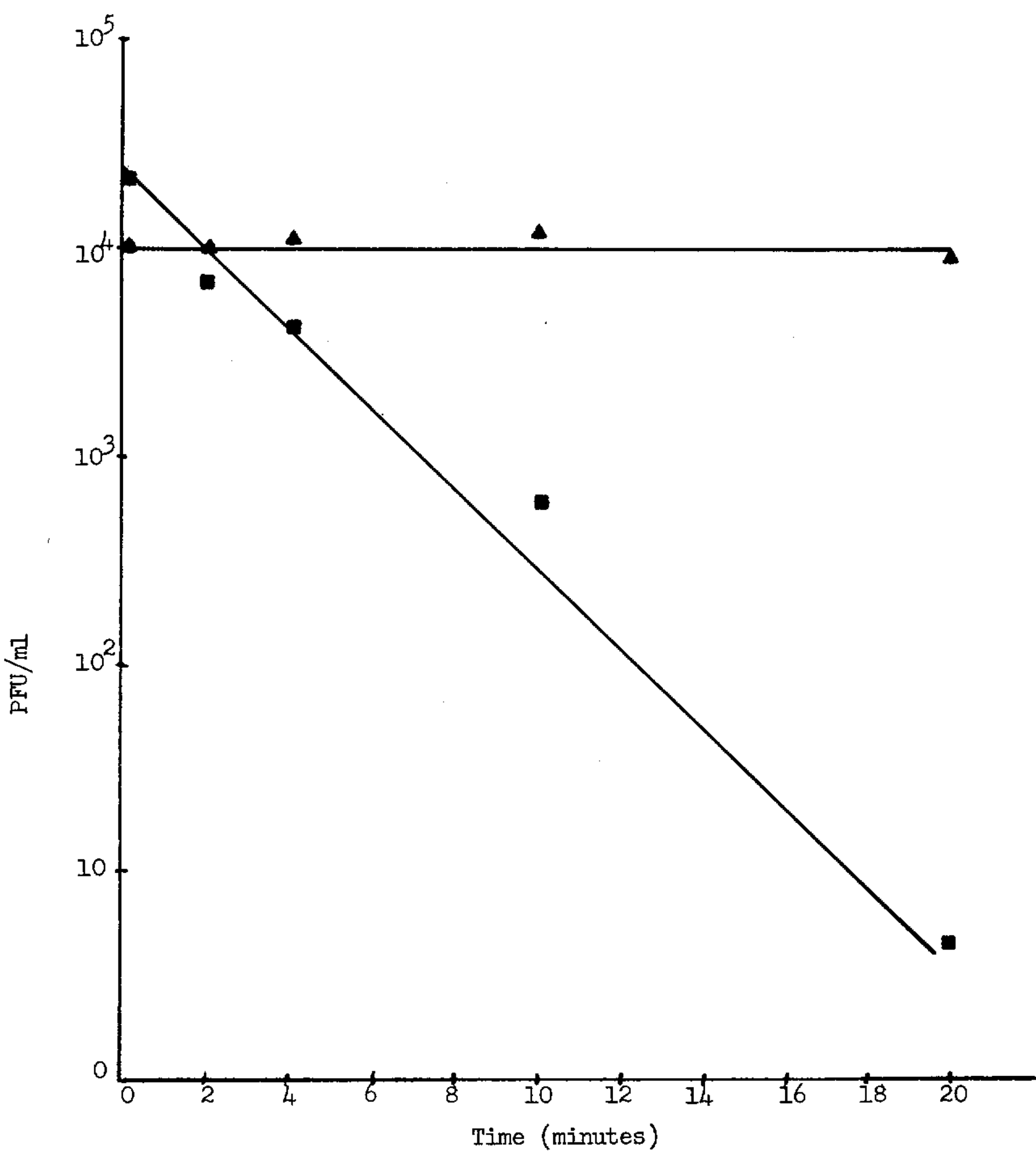


Figure 11

Poliovirus 2 Survival Curves in Package Plant Effluent, Semiflow Batch Mode

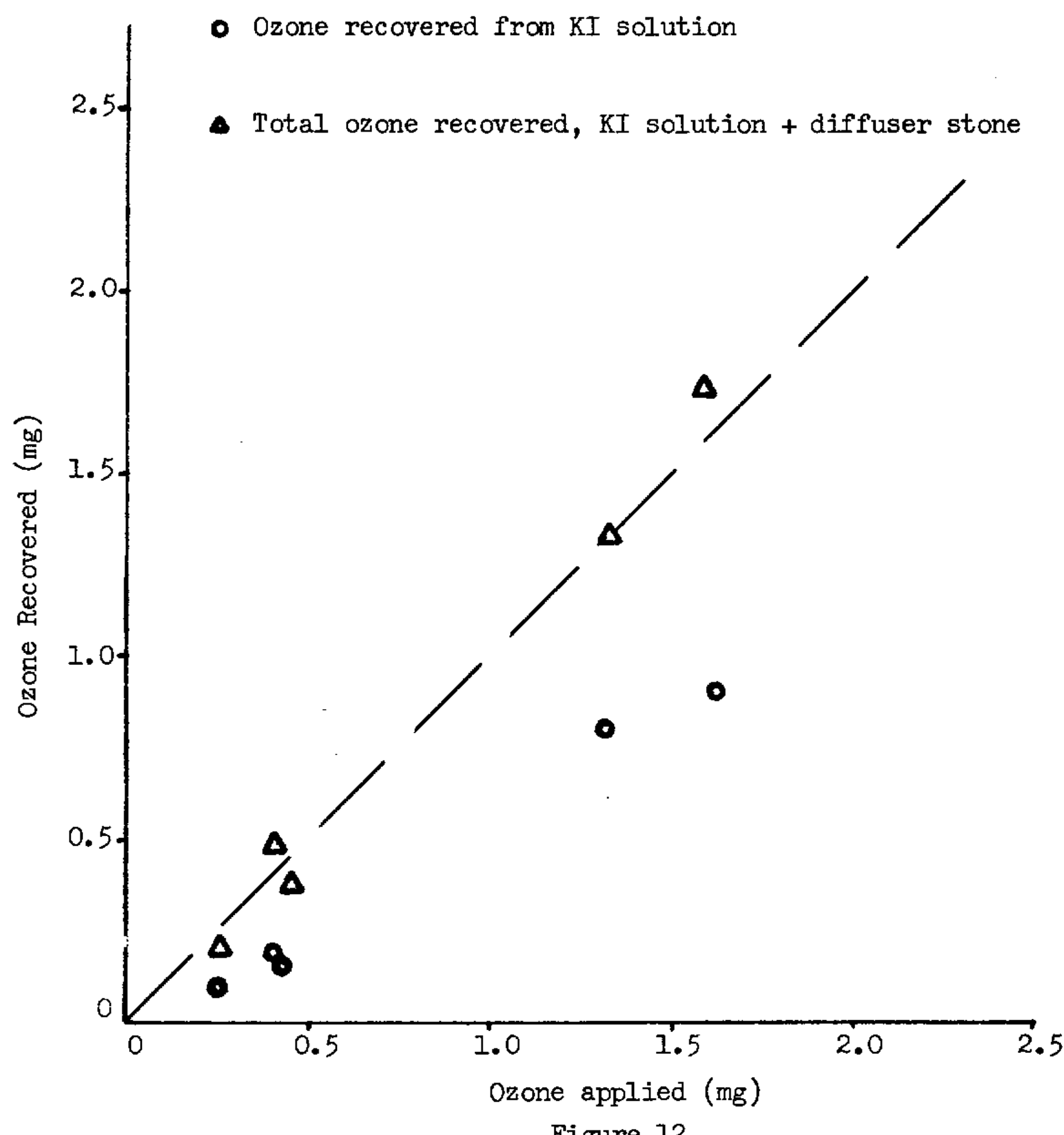


Figure 12

Recovery of Ozone Dispersed by Stone Diffusers

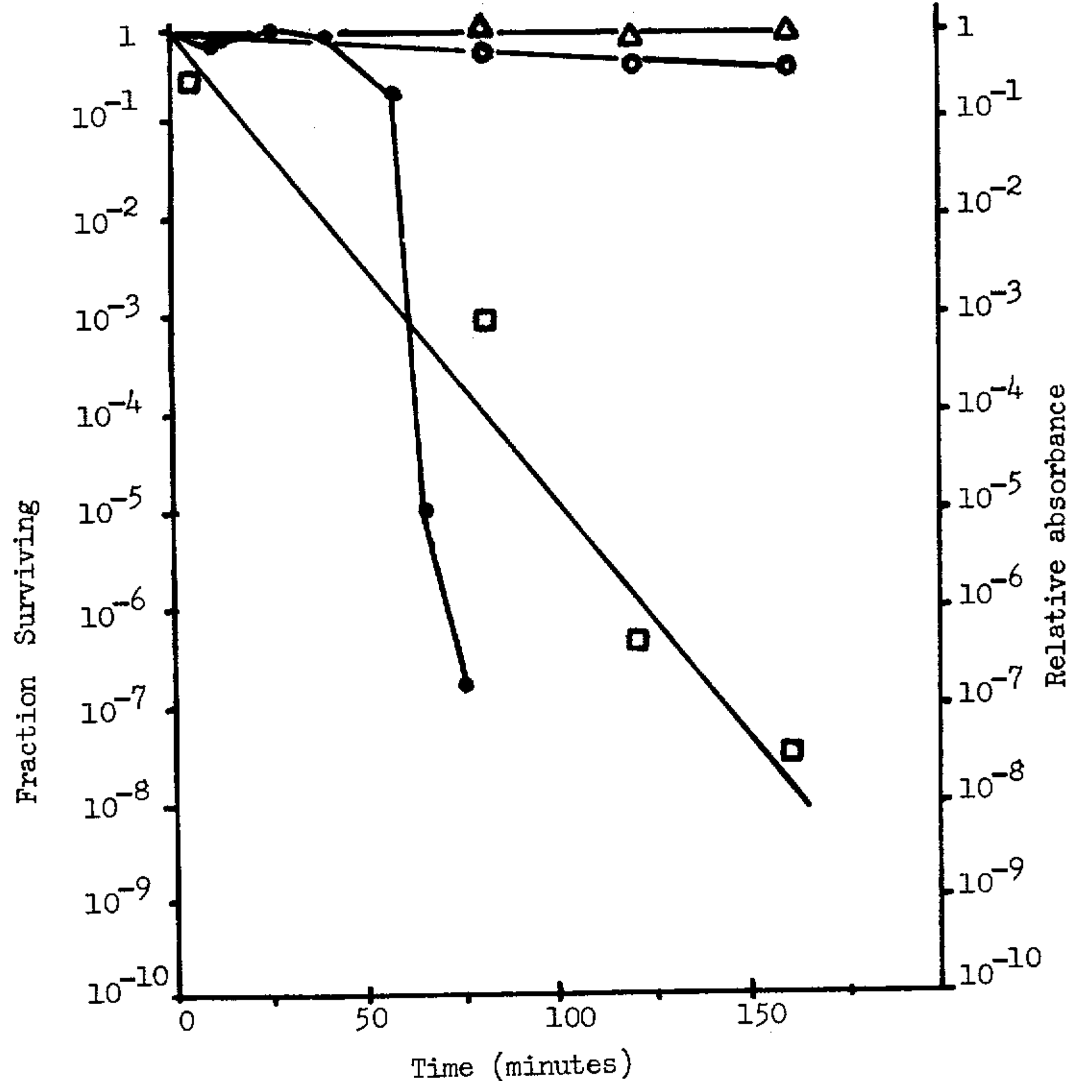


Figure 13

The Optical Density of a Bacterial
Suspension During Inactivation by Ozone

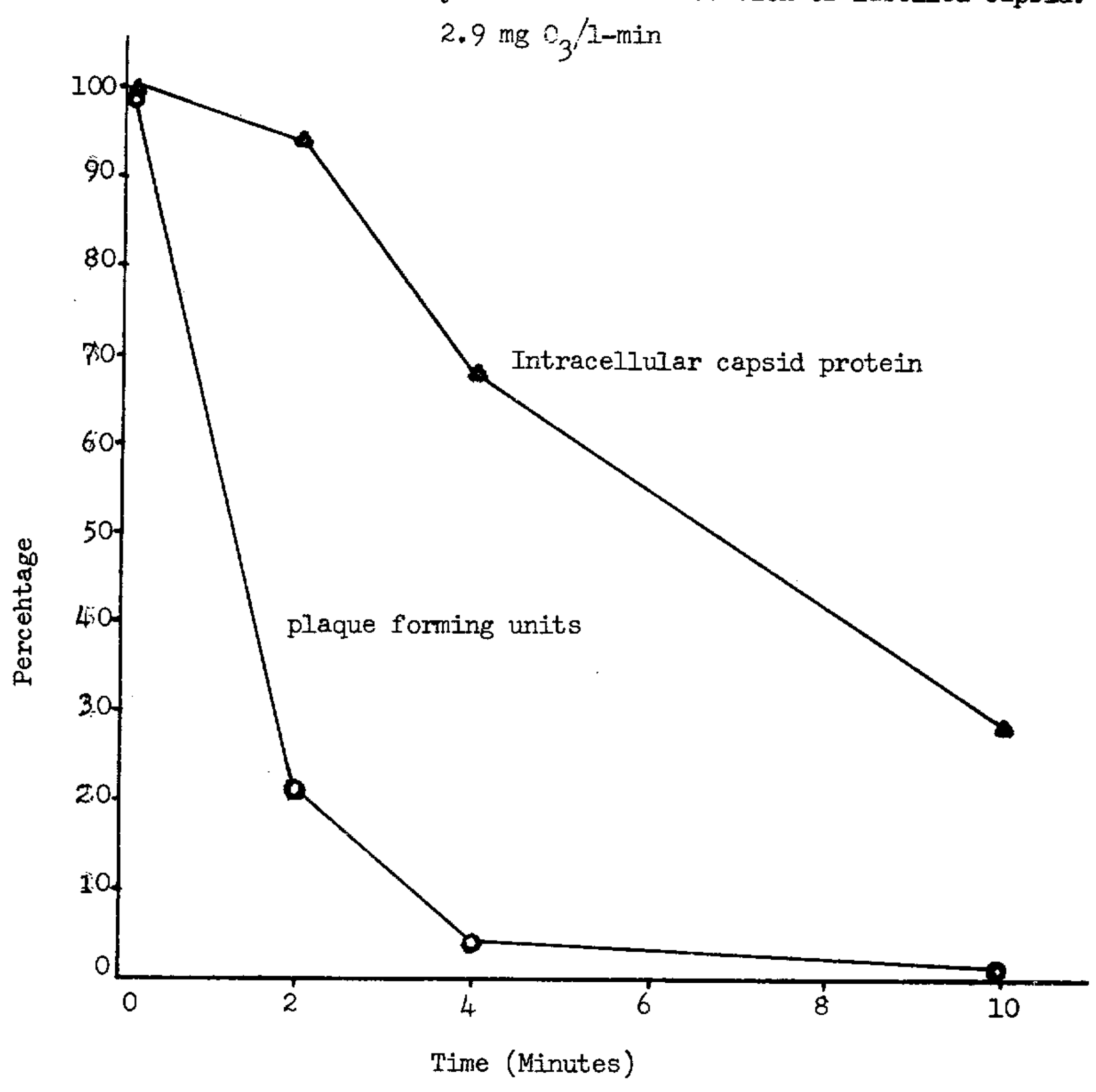
- ◘ survivors, ozonated culture
- ⊙ absorbance, ozonated culture
- ● absorbance, phage-infected cells
- △ absorbance, control suspension

Figure 14

Effect of Ozone on Poliovirus Penetrance and

Plaque Formation in BGM Cells

Viability was tested by ability to form plaques, and penetration was tested by intracellular location of labelled **capsid.**



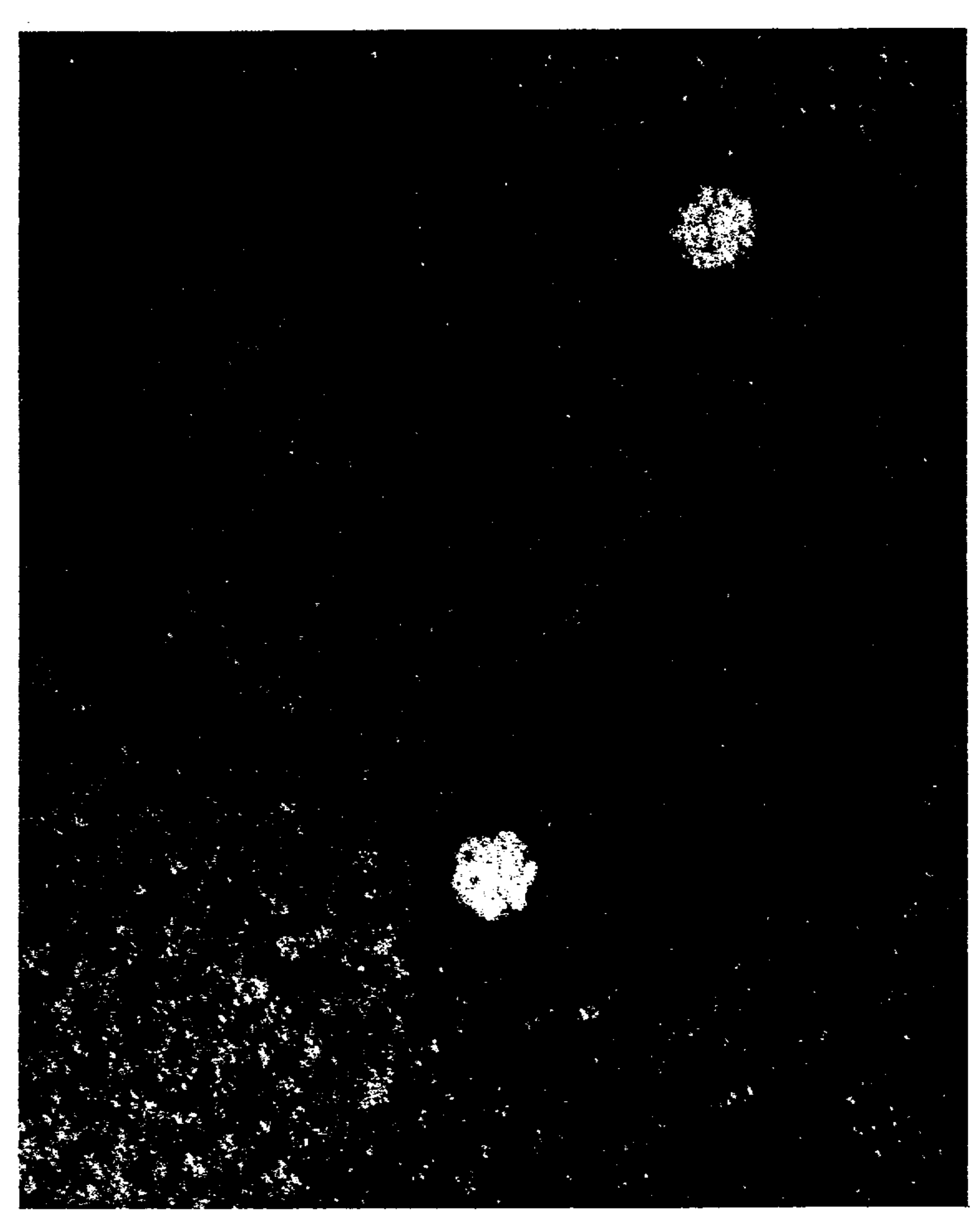


Figure 15

Electron Micrograph of Untreated Poliovirus

Magnification 40,000x

Figure 16

Electron Micrograph of Ozonated Poliovirus

The Poliovirus was exposed to 2.9 mg O_3/l-min for 10 minutes
Magnification 40,000x

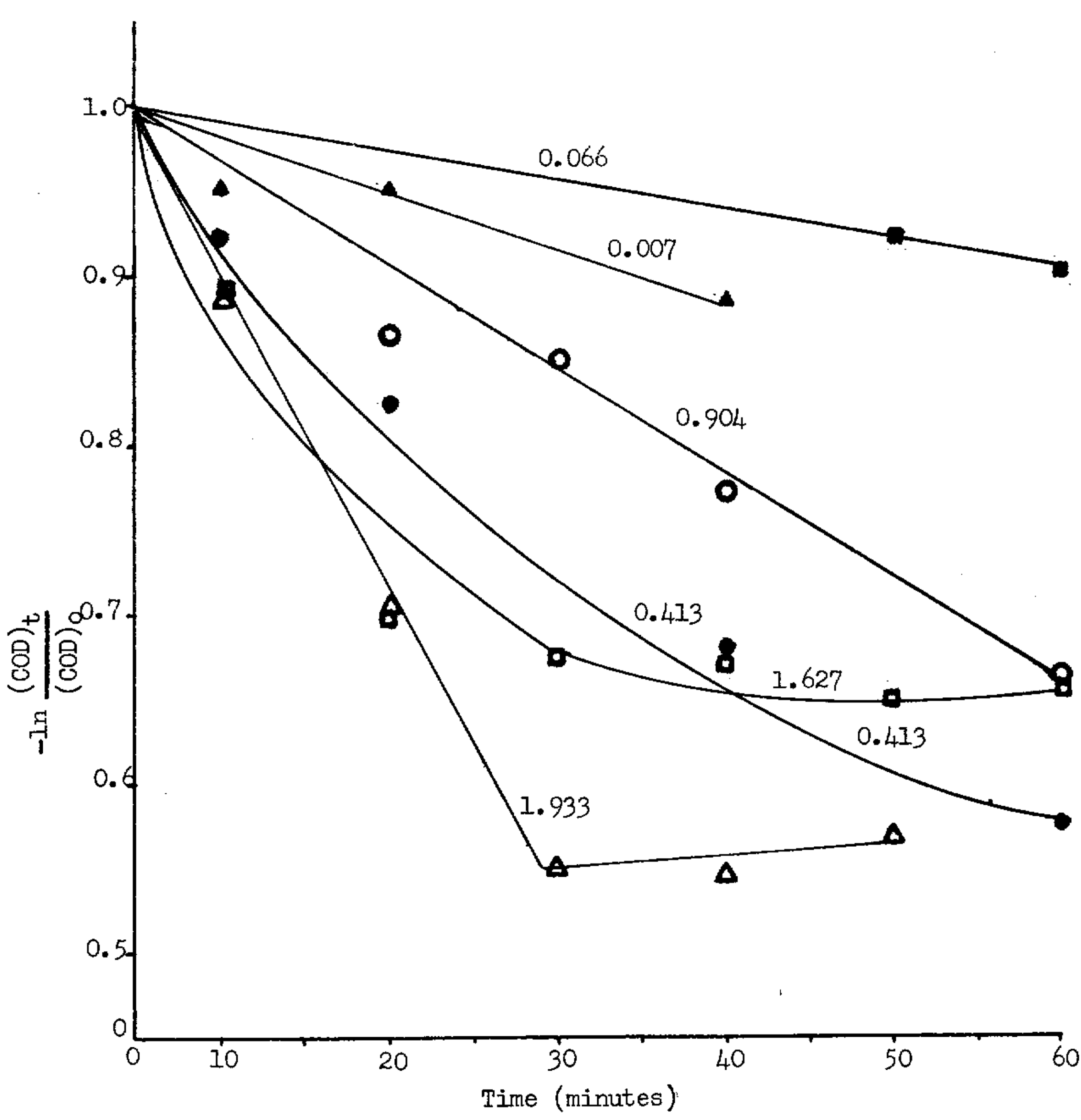


Figure 17
Effect of Ozone Dosage on COD Reduction
of Heterogeneous Effluent Samples

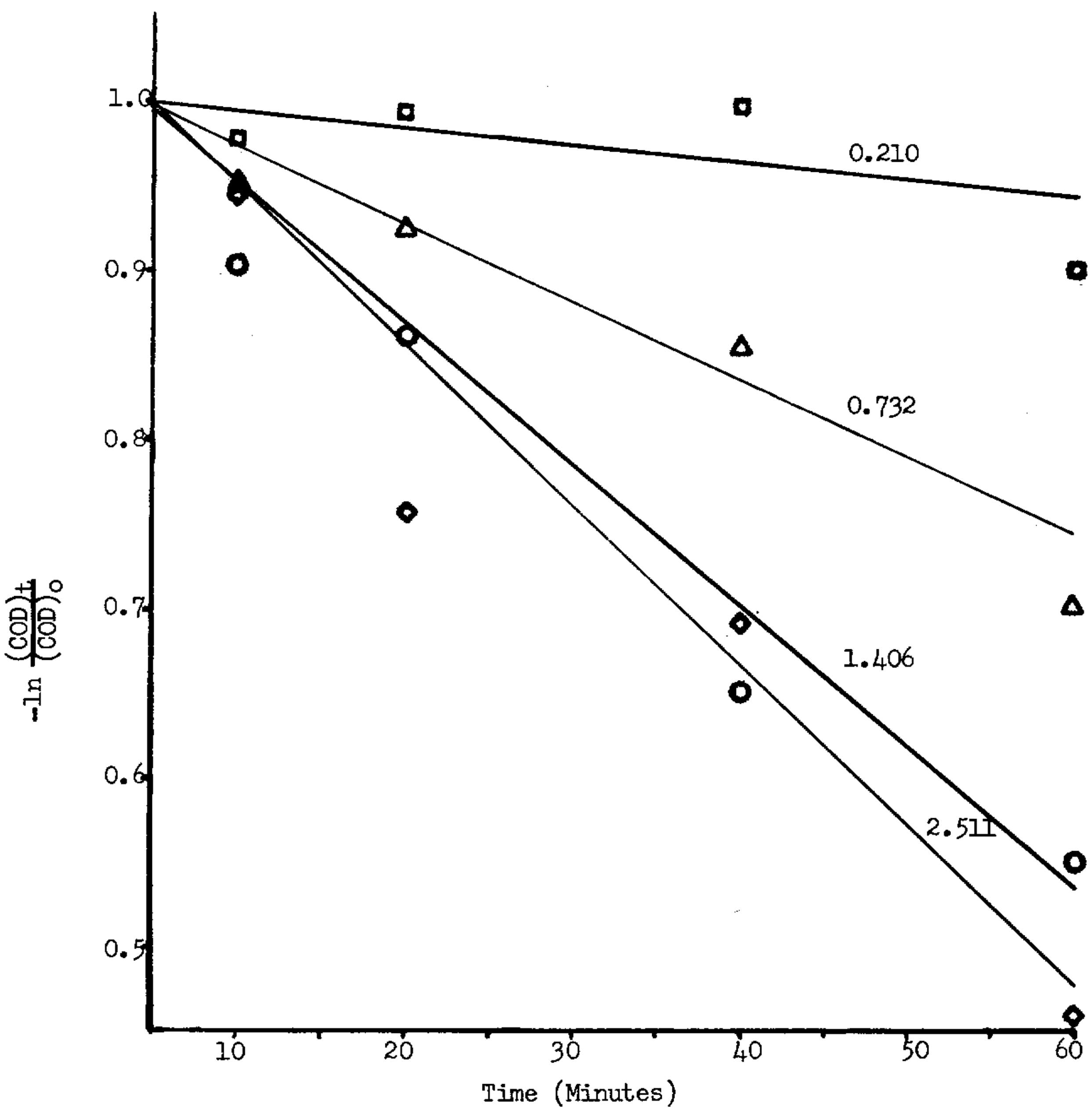


Figure 18. Effect of Ozone Dosage on COD Reduction of Homogeneous Effluent Samples

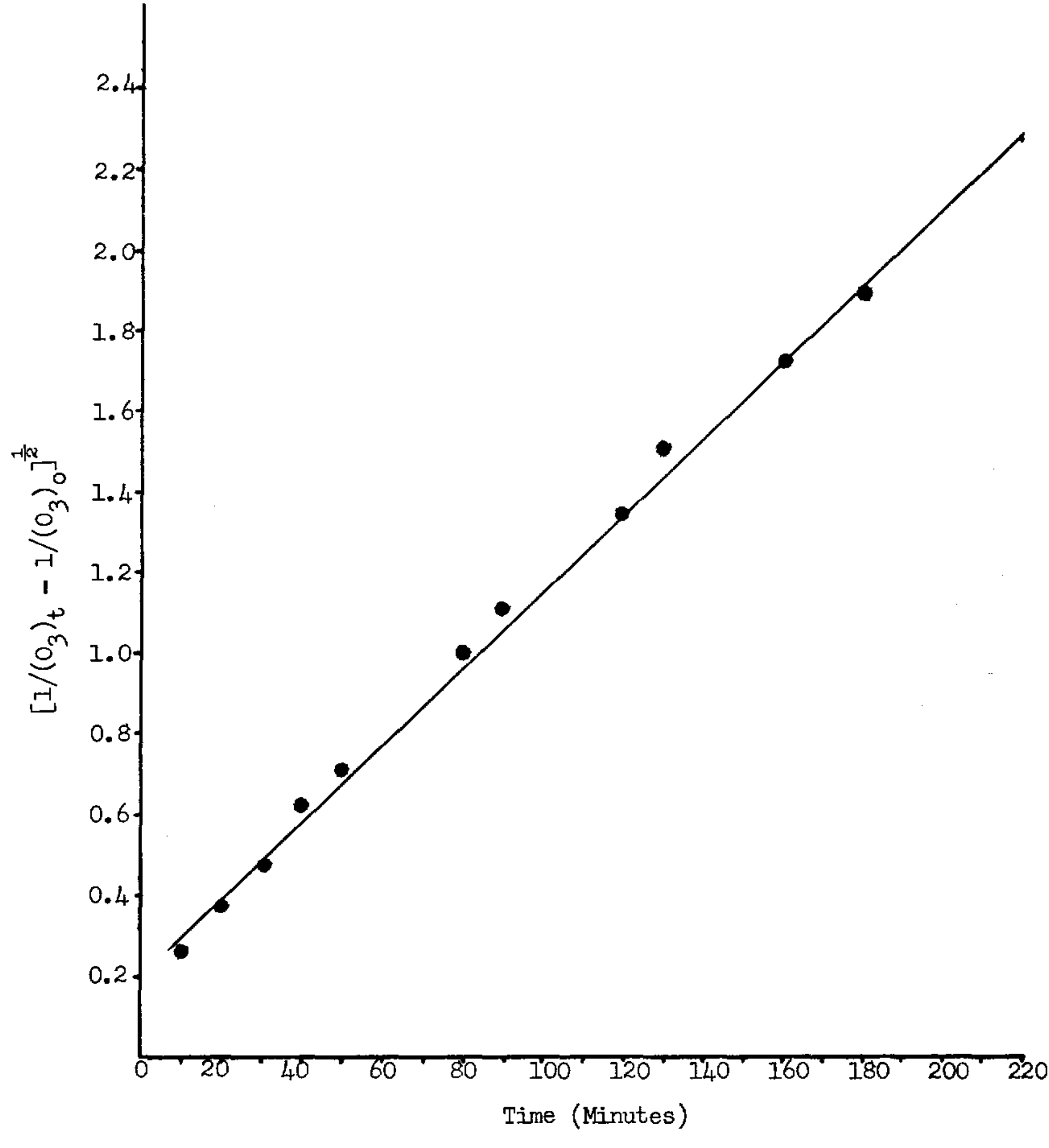


Figure 19

Ozone Decomposition

The decomposition of ozone, finished water, pH 6.2, 25°C, 1.838 mg O_3/l-min

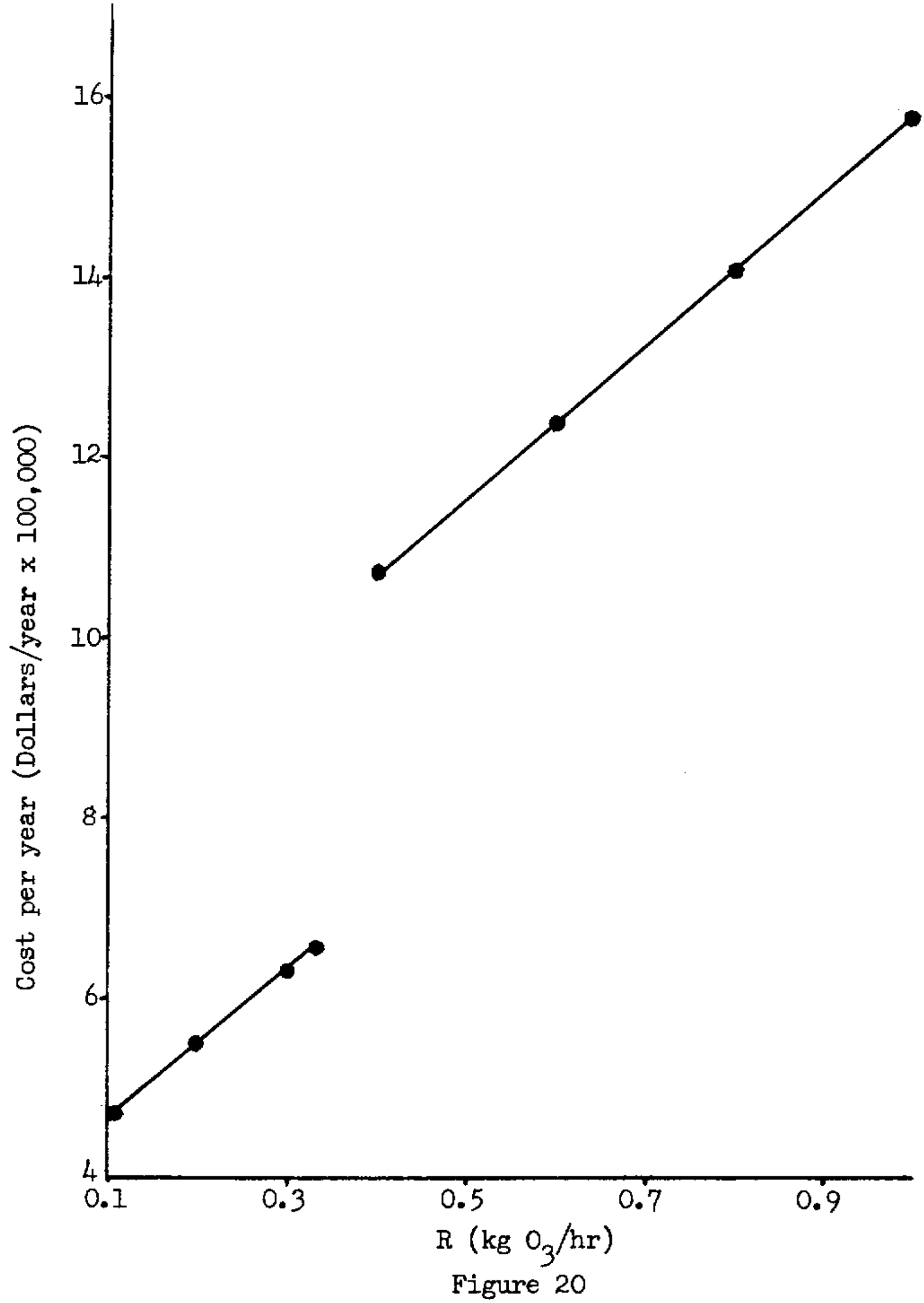


Figure 20

Cost of Ozone for 0.1 to 1.0 kg O_3/hr

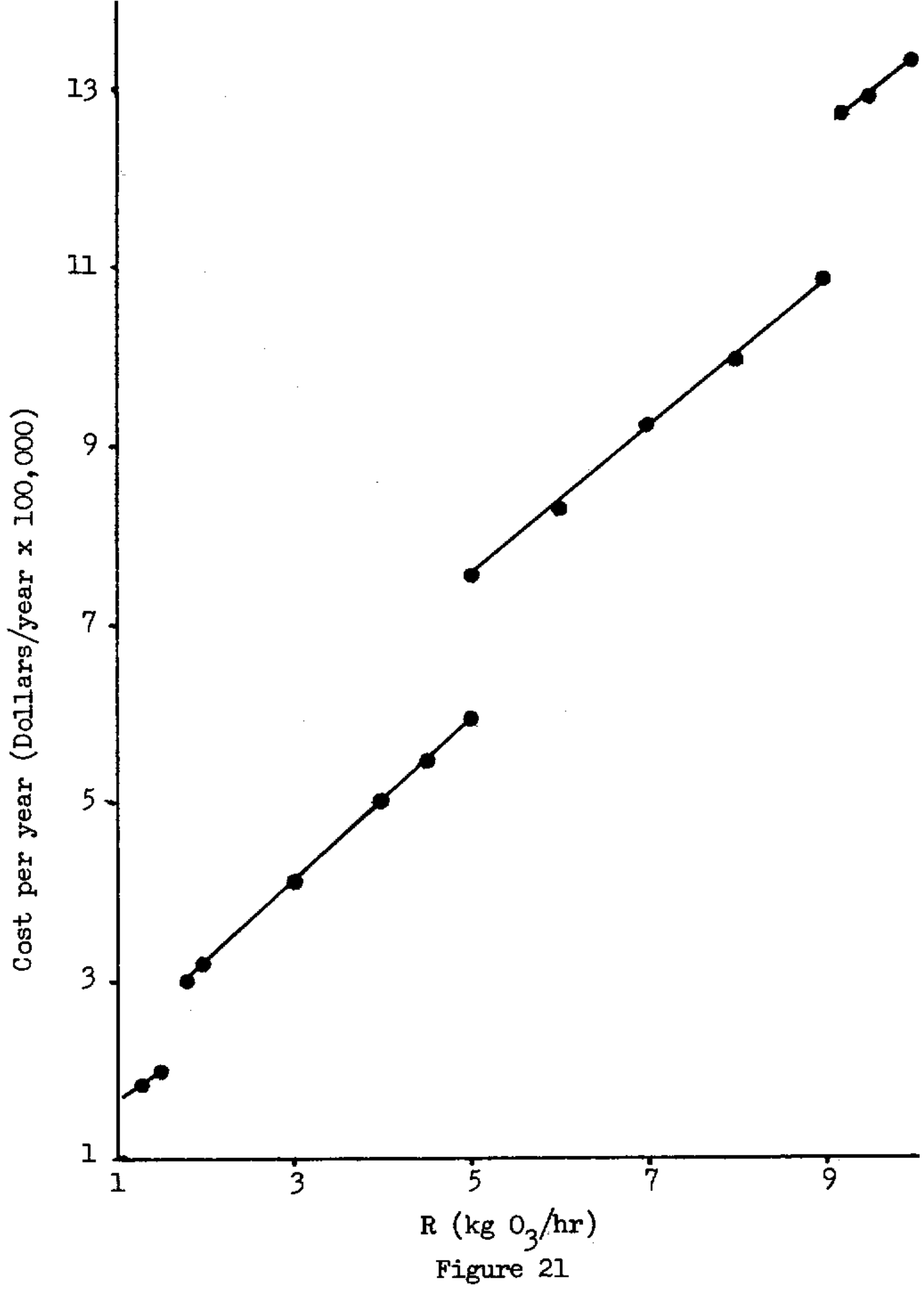


Figure 21

Cost of Ozone for 1 to 10 kg O_3/hr

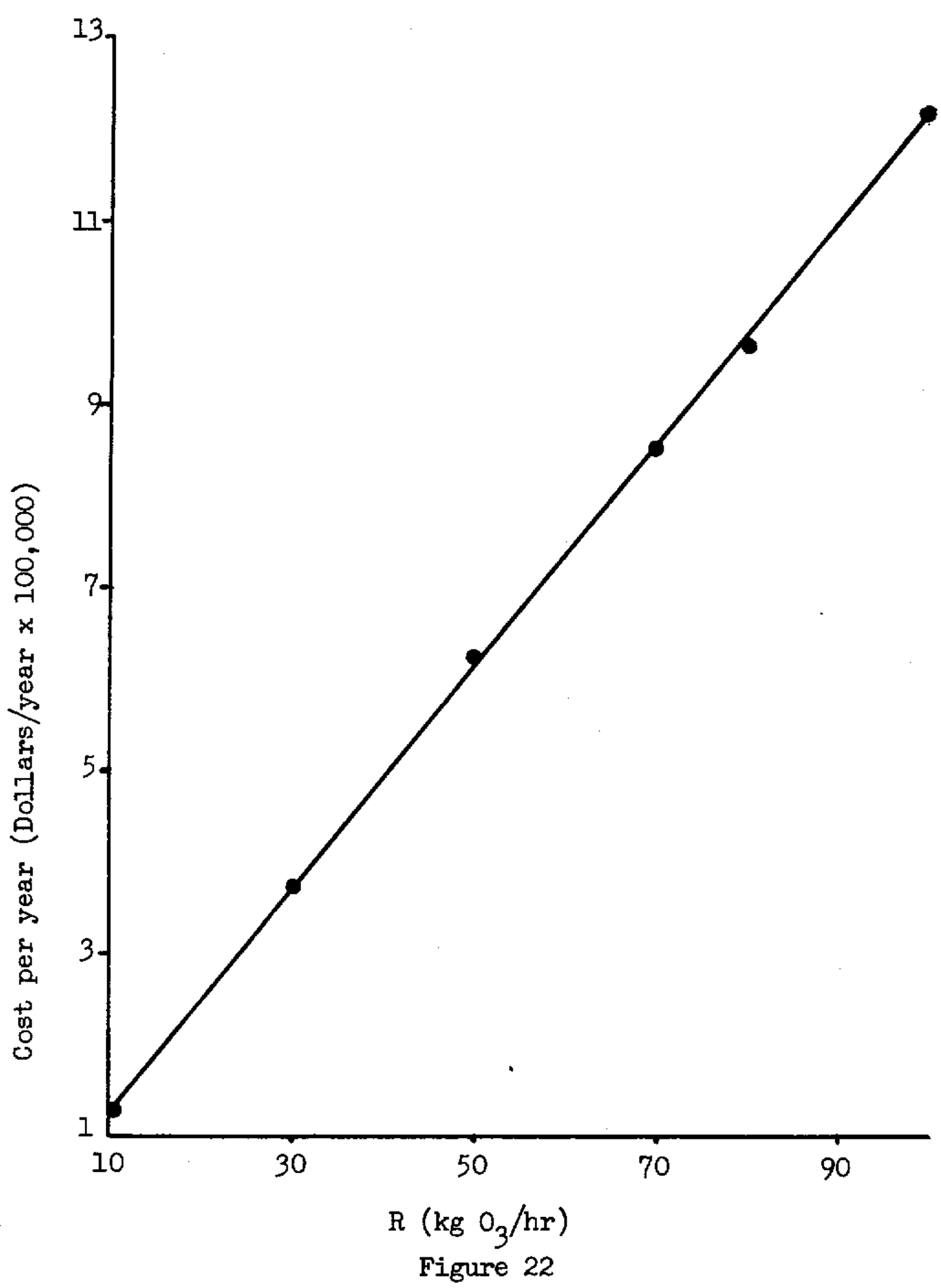


Figure 22

Cost of Ozone for 10 to 100 kg O_3/hr

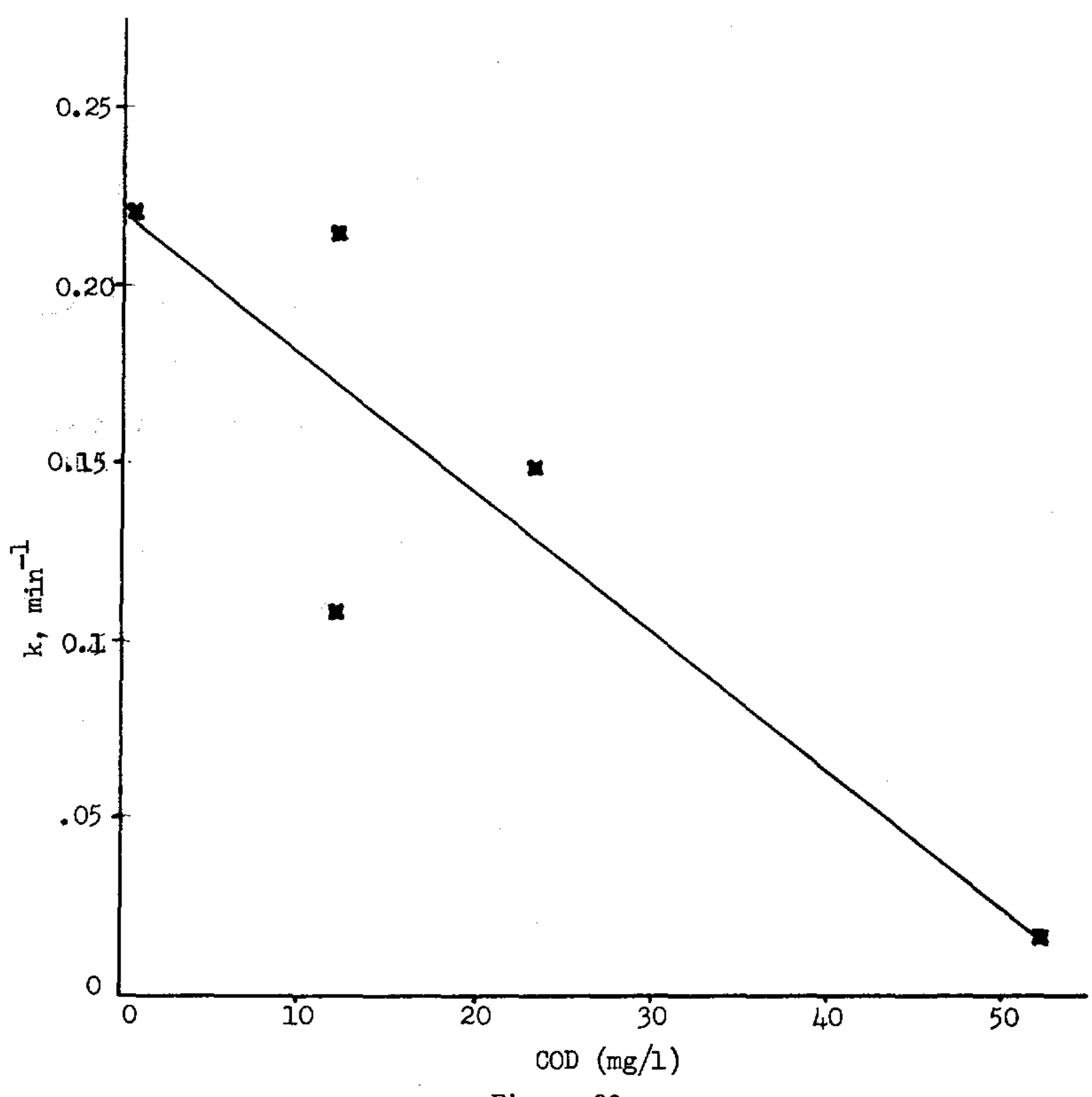


Figure 23

Effect of COD on the Inactivation Rate Constant

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