

THE TOXICITY OF OZONATED SECONDARY-TREATED MUNICIPAL
WASTE EFFLUENT TO BLUE-GILL SUNFISH

by

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS	ii
LIST OF TABLES	iv
LIST OF FIGURES	v
I. INTRODUCTION	1
II. LITERATURE REVIEW	3
III. METHODS AND MATERIALS	15
IV. RESULTS	27
V. DISCUSSION	44
VI. SUMMARY AND CONCLUSIONS	56
VII. BIBLIOGRAPHY	58
VIII. APPENDIX	61
APPENDIX A	61
APPENDIX B	64
IX. VITA	82

LIST OF TABLES

Table	Page
I. Waste Characterization Data for Influent to Bioassay Chambers--Control Run	28
II. Dissolved Oxygen Concentration and Temperature of Bioassay Medium at Intervals During Control Run	29
III. Effects of Ozonation on Several Chemical Characteristics of Secondary Sewage Effluent Used in Run #2	32
IV. Dissolved Oxygen Concentration and Temperature of Bioassay Medium at Intervals During Run #2	33
V. Effects of Ozonation on Several Chemical Characteristics of Secondary Sewage Effluent Used in Run #3	36
VI. Dissolved Oxygen Concentration and Temperature of Bioassay Medium at Intervals During Run #3	37
VII. Effects of Ozonation on Several Chemical Characteristics of Secondary Sewage Effluent Used in Run #4	40
VIII. Dissolved Oxygen Concentration and Temperature of Bioassay Medium at Intervals During Run #4	41
IX. Application Rates and Dosages of Ozone Used in This Investigation Compared to Those Used in Actual Waste Treatment Practice	48
X. Physical and Chemical Changes in Sewage Caused by Ozone Treatment: A Comparison Between Results of This Investigation and Those Obtained at Redbridge, England	49

LIST OF FIGURES

Figure	Page
1. Ozone Production and Standardization System	17
2. Continuous Flow--Pilot Ozone System	19
3. Ozone Contact Chamber	21
4. Hypothetical Ozone Production Pattern Resulting From Moisture Breakthrough and Replacement of Drying Filters . . .	26

APPENDIX FIGURES

Figure	Page
1. Bactericidal Activity of Ozone and Chlorine	62
2. Disappearance of Residual Ozone	63

I. INTRODUCTION

Ozone was introduced into sanitary engineering practice in 1906 at Nice, France, for disinfecting water. Results were so encouraging that ozone was adopted as the predominant method of disinfection throughout France and in much of Europe. Today, there are over two thousand water and wastewater treatment plants around the world that utilize ozone disinfection.

Despite the apparent success of ozone treatment in Europe, authorities in the United States, until recently, have displayed little interest in its use. The reasons for this lack of interest center on the fact that ozone is more costly than chlorine for disinfection and does not provide for residual action. Increased emphasis upon tertiary treatment of wastewaters and the development of new concepts in ozone treatment have stimulated renewed interest in ozone in the United States.

Ozone is now viewed as a tertiary treatment agent with a variety of uses that range from disinfection to color removal and oxidation of trace organic material. Several authorities have stated that through the proper application of ozone to waste treatment an effluent capable of limited recycle could be produced. These views, coupled with an increasing use of ozone in waste treatment, pose a serious and unanswered question as to the possible toxic effects of an ozonated effluent upon a receiving stream. Previous research has shown conclusively

that both chlorine residuals and chlorinated compounds in waste effluents exhibit a toxic effect upon a receiving stream. If ozone treatment is to become common for wastes then it is desirable to determine what effect the effluents will have on aquatic life.

The objective of this research project was to determine whether toxicity of ozonated secondary-treated, municipal-sewage effluents to fish could be demonstrated, and, if so, to establish a range of ozone dosages that would bracket the dose lethal to fifty percent (TLM_{50}) of the specimens. Due to restrictions of time and equipment, an accurate TLM_{50} was not established by the study. Bluegill sunfish, a common bioassay organism, were selected for testing in ozonated secondary effluents from the municipal waste treatment plant at Blacksburg, Virginia.

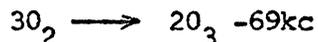
During the toxicity study, several waste parameters were monitored so that the effects of ozone on the waste could be evaluated. If the ozonated effluents proved toxic, then the data might aid in determining the mode of toxicity. Otherwise, the data would be useful, as stated before, in evaluating the effects of ozone on waste effluents. With the results from this preliminary toxicity study the application of ozone to waste treatment practices can be made more rationally, knowing some of the effects it is having on the receiving stream.

II. LITERATURE REVIEW

The literature was surveyed for information relative to the toxicity of ozonated waste effluents. Only one pertinent article was found. Because little information was found, a review of the recent literature concerning the chemistry of ozone and its reactions with organic matter was undertaken to provide a background for understanding the sources and possible reasons for any toxicity that might be demonstrated. Also, as an aid in developing a study with practical implications, the various uses of ozone in sanitary engineering practice were reviewed. The following summary of the literature surveyed, therefore, is divided into three parts. Ozone chemistry will be discussed first, as it will aid in understanding the other two sections on toxicity and sanitary engineering applications.

Ozone Chemistry

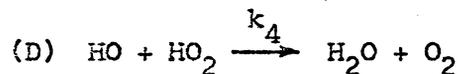
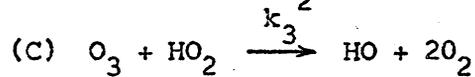
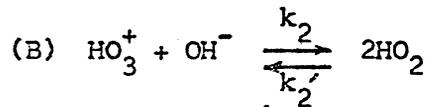
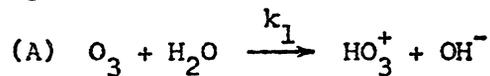
Ozone is a gas formed by the polymerization of the oxygen molecule as described in the following reaction (1):



The polymerization occurs when a stream of oxygen is exposed to a high energy electrical discharge or to certain wave lengths of ultraviolet light. Ozone has a density one and a half times that of oxygen, imparts a characteristic odor, and is ten times more soluble in water than oxygen. In the triatomic state, ozone is naturally unstable and

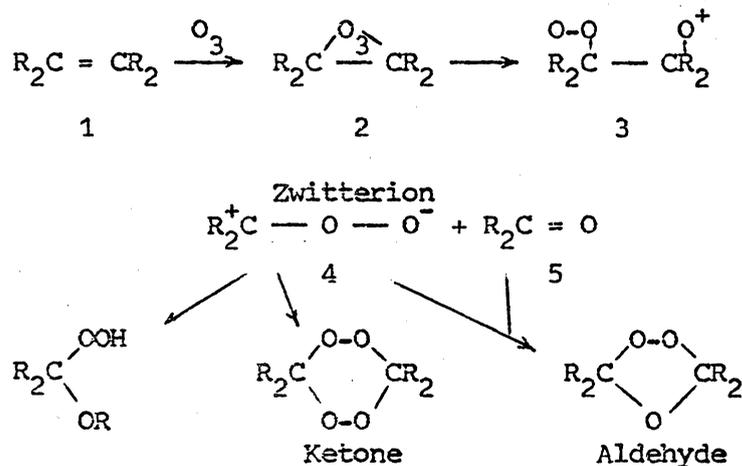
and will slowly decompose to O_2 and nascent oxygen. The decomposition can be accelerated by heat, or it can be catalyzed by several compounds such as water, chlorine, soda lime, and manganese dioxide (2).

Ozone's high solubility and rapid decomposition in water serves as the basis for its utilization in sanitary engineering practice. Much research has been done in recent years in an attempt to describe the relationship between the decomposition of ozone in water and subsequent oxidizing reactions with organics. A summary of research on ozone decomposition in water reveals that ozone is broken down through a series of four reactions. These four reactions are generally accepted as the following:



The ozone decomposition described above occurs at exponential rate resulting in the dissipation of any ozone residual in 8-10 minutes (5). Both temperature and pH have a significant influence upon the rate of these reactions (3) (4). The reported rates of ozone decomposition vary from first to second order reactions over a pH range of 1.0 to 8.0 and a temperature range of 0 to 60 degrees Centigrade (4). At the pH and temperature ranges normally encountered in sanitary engineering practice, recent research indicated that ozone decomposition is an order between second and three halves (4) (5).

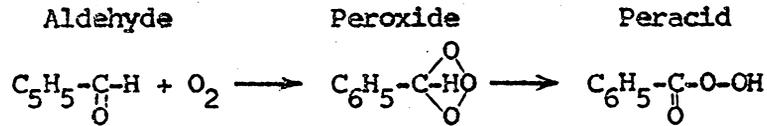
Ozone and its intermediate products of decomposition are all oxidizing agents capable of producing a series of instantaneous reactions when in contact with oxidizable substances (1) (6). These reactions can be classified as one of three possible types--ozonolysis, auto-oxidation, and conventional oxidation. Ozonolysis is defined as the cracking of unsaturated organics by the attachment of an ozone molecule at a double bond. The resulting compound, an ozonide, is also unstable and will break down through a series of reactions to an acid or aldehyde (5) (7). An example of the ozonolysis reaction, as explained by Crigee (7), is described below:



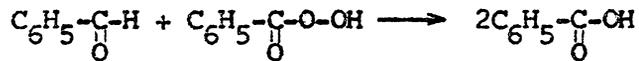
Ozonide formation and ozonolysis reactions are not instantaneous and usually require the presence of an excess of ozone (5).

Auto-oxidation, catalytic ozonation, influences the number of oxygen molecules involved in an oxidation reaction. Some authorities explain the reaction as some type of chain-reaction mechanism that requires the presence of free active ozone (5). During auto-oxidation ozone acts as both a catalyst and as an oxidizing agent. By observing a wide range of auto-oxidation reactions, more oxygen was found to be

used with ozone present than in ozone's absence. Another observation from studies with ozone and auto-oxidation was that the accelerating action of ozone increases to a point in dilute concentrations. An example of an auto-oxidation reaction is given below (8):



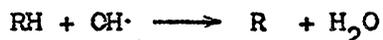
Through subsequent steps the peracid reacts with an aldehyde to produce an acid:



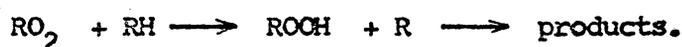
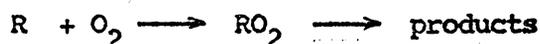
The third type of reaction resulting from ozone decomposition, conventional oxidation, occurs as a result of the cleavage of ozone into an oxygen molecule and an atom (5) (9). High energy oxidation is stimulated because this cleavage causes conventional oxidation reactions to occur faster and more efficiently through a free radical mechanism. Unlike ozonolysis and auto-oxidation, conventional oxidation does not require an excess of ozone and relatively long contact times (5).

In considering the application of ozone to waste treatment, the reactions of ozone with organics is of particular interest. Research has proven conclusively that through the proper application of ozone to organic wastes, significant reduction in the chemical oxygen demand (COD) can be obtained (4) (10) (11) (12). The amount of COD removal has been shown to be proportional to the ozone decomposition rate. From this fact, it can be concluded that COD reduction is a result of organic compounds reacting with short-lived ozone decomposition products

such as free radicals. Because the reaction is dependent upon the ozone decomposition rate, COD removal can be enhanced by accelerating decomposition through optimizing pH, temperature, contact time, and ozone concentration. The reaction of the OH radical with an organic can serve as an example of the many possible reactions resulting from ozone decomposition (4):



followed by auto-oxidation



Some organic compounds which react with ozone in wastewaters are macro-molecules in the colloidal state. The ozone reactions discussed previously can act to break down hydrophilic groupings and peptizing agents combined with hydrophobic particles which are responsible for stability of colloidal dispersions. Ozone, therefore, causes the precipitation of some dispersed organic particles. At the same time, however, it is possible for other ozone reactions to produce colloids by the formation of negatively charged micelles (11) (12).

Toxicity

Despite the fact that the toxicity of ozone in air has been studied extensively, there have been almost no reported investigations of the possible toxicity of ozone or its by-products in water. To date, the only reported toxicity study involving ozone in water was conducted at the University of Michigan in 1930 (13). The objective of the study was to determine if water purification processes involving nascent oxygen produced a water supply unfit for the maintenance of "delicate

fishes". Because ozone decomposition is one of the primary sources of nascent, or atomic, oxygen ($O_3 \longrightarrow O_2 + O$), ozone was one of the substances studied. Several varieties of minnows were tested in a static bioassay utilizing tap water dosed with various concentrations of ozone.

Results of the study indicated that ozone residuals in water were highly toxic at extremely low concentrations. All of the fish tested showed signs of irritation, even in traces of ozone too small to detect by the chemical methods available. Some of the minnows were killed in solutions containing ozone in concentrations of .03, .08, and .16 milligrams per liter (mg/l). There was a marked difference in the susceptibility of individuals of the same species to various concentrations of ozone. Certain individuals exposed to non-toxic doses of ozone exhibited some degree of resistance at higher, toxic levels.

By observing the responses of the fish to toxic concentrations of ozone, the effects of ozone were found to be cumulative. Initially, the fish showed altered locomotor and respiratory movements when subjected to even sublethal doses of ozone. Soon afterward, at lethal doses, there was a slight loss of equilibrium marking the end of the reversible toxic effects. Fish removed from the ozone solutions at this point recovered quickly while those further exposed all died. Following this loss of equilibrium, death soon occurred but was preceded by alternating periods of erratic, wild dashing and unbalanced rest. Unfortunately, examinations of the dead specimens did not produce a clue as to the mechanism of toxicity (13).

Sanitary Engineering Applications

Ozone, with its powerful oxidizing capabilities, has found a wide range of uses in sanitary engineering practice. From the first use of ozone as a disinfectant in water treatment, the uses of ozone have expanded to include supplementary water treatment, tertiary treatment and disinfection of municipal wastes, and some specific applications to industrial wastes. Despite the variety of applications for ozone, the principles of production and dosing are basically the same for all uses.

The most economic method for producing ozone is to pass a stream of air or oxygen between two electrodes across which there is a high voltage potential. Before passing between the electrodes, the air or oxygen must be filtered to remove dust and dried to a dew point of -40°C to -50°C to prevent corrosion of the electrodes. A typical ozone generation system, therefore, consists of an air filter, a refrigeration and/or dessication unit, and a high voltage transformer or ozone generator (1) (11) (12) (14) (15).

Application of the ozone gas to water is usually done with the idea of emulsifying the ozone in water to promote a more efficient reaction. This emulsifying action is best accomplished by means of either porous diffusers or injectors. Porous diffusers are usually used in conjunction with a contact column employing counter current feed of the water and gas. Injectors utilize the principle of drawing the ozonized air stream into a low pressure section of a water stream and then mixing the emulsified stream with the bulk of the water in a contact column. Contact times for either of the methods employed will

depend upon the specific objectives of the treatment (1) (11) (12) (14) (15).

While ozone is just beginning to gain acceptance as a tool in waste treatment practices, it has long been an acceptable agent for use in water treatment. From its initial introduction as a disinfectant, the role of ozone in water treatment has expanded greatly to include such areas as taste, odor, color, and iron and manganese control (1) (2) (3) (5) (11) (14) (15). Disinfection, though, is still the primary purpose for its application in water treatment.

Most authorities on disinfection agree that only free residual chlorine can compare to ozone in germicidal power. Numerous researchers have proven that concentrations of ozone less than 0.5 mg/l at contact times of less than one minute destroy all pathogenic organisms in water. Ozone has been shown to be highly effective against all forms of microorganisms including viruses at these low concentrations (1) (2) (3) (5) (11) (15) (16). Higher doses of ozone, .5-10 mg/l, and longer contact times, 2-10 minutes, are usually employed for practical use because organic matter and other substances will exert an ozone demand preventing the full utilization of ozone for disinfection (5) (11) (12) (15) (16) (17) (19) (20) (21). There is some disagreement between French and American authorities as to the effect of temperature on ozone demand, but researchers generally agree that pH has little effect (1) (3) (5) (15) (16) (20).

In comparing ozone and chlorine as disinfectants, there are several notable differences. Ozone has been shown to act between 600 and 3000 times more rapidly than chlorine in the destruction of bacteria.

This dramatic difference in disinfection rates is due to the difference in the mode of each agent. Chlorine acts in a progressive manner, killing a larger fraction of organisms as the dose and the contact time are increased. In contrast, ozone exhibits an all-or-none effect, which means that ozone has little effect until a threshold concentration is reached at which time its action is sudden and total (Appendix Figure 1) (1) (2) (3) (15) (16). A comparison of the modes of action of each agent shows that ozone acts as a protoplasmic oxidant, while chlorine exhibits selective injury to the cytoplasm (3) (15).

The fact that ozone residuals can not be maintained through the distribution system is often cited as one disadvantage in adopting ozone disinfection. Analysis of the chlorine residuals maintained in distribution systems indicates that most are not adequate to deal with any accidental contamination. After over fifty years of experience in utilizing ozone disinfection in Europe, there has been no deterioration in water quality noted (1) (5) (15) (20) (22).

In addition to being a disinfectant, ozone is also a popular agent for supplementary water treatment. One characteristic of ozone accounting for its popularity in Europe is its ability to alleviate taste, odor, and color problems caused by organic compounds and to reduce problems with gases such as hydrogen sulfide and carbon dioxide. Ozone processes are well suited to deal with these problems because, in addition to their powerful oxidizing ability, they add appreciable aeration. Compounds such as phenols, which produce tastes and odors accentuated by chlorine, are effectively oxidized and removed by ozone treatment. Treatment of taste and odor problems can usually be accomplished with

low ozone doses of 1-2 mg/l, but the dose required for color removal will vary depending upon the ozone demand exerted by the color intensity (1) (5) (14) (15) (18) (20).

Some other areas of supplementary water treatment in which ozone is effective are iron and manganese removal and algae control. Again, the powerful oxidizing capability of ozone serves in some plants to oxidize iron and manganese to their insoluble state for precipitation and removal. Algae blooms, a seasonal problem at many water treatment plants, have proven responsive to ozone treatment in some instances (1) (4).

The powerful oxidative capacity of ozone, which made it applicable to water treatment practices, also makes ozone a desirable agent for waste treatment practices. With increased emphasis placed on tertiary treatment of municipal wastewaters, ozone is gaining increased use and support in the waste treatment field. Some authorities have suggested that through the proper use of ozone an effluent capable of limited recycle can be obtained (1) (11) (12).

As a result of the success of ozone as a disinfectant in water treatment, it has recently begun to be utilized for the same purpose in waste treatment. Dosages required for disinfection of waste streams will vary from 2-60 mg/l at contact times varying from 5-30 minutes. The actual dosage required for a particular waste stream will depend upon the organic content, color, turbidity, and the degree of disinfection required. Generally authorities agree that doses in the 10-20 mg/l range at 5-10 minutes will kill greater than 99+ percent of the organisms (1) (2) (11) (12) (23). Another reason ozone is gaining

support as a disinfectant for effluents is the belief that, unlike chlorinated effluents, ozonated effluents are not toxic to aquatic life. Most of this reasoning is based on the lack of residual action by ozone (1) (2) (4).

Possible ozone applications to tertiary waste treatment have stimulated a great deal of interest and research in recent years. Research cited in the discussion of ozone reactions with organic compounds has indicated that it is possible to obtain reductions in COD on the order of 50-70 percent (4) (10). Nebel of the Welsbach Corporation has stated that ozone was capable of higher reductions of residual biochemical oxygen demand (BOD) and total organic carbon (TOC) than are possible by the use of activated carbon (26). The results of several recent projects indicated that an effluent with about 15 mg/l COD can be produced from an influent with a COD of 50 mg/l or less (4) (25). To obtain significant COD reductions, ozone doses ranging from 20-200 mg/l at contact times from 20-60 minutes have been used (1) (4) (11) (12) (25).

Reduction in COD was not the only benefit of tertiary treatment with ozone. At the high ozone doses used, oxidation of refractory organics, such as organic pesticides, readily occurred. Combined with the froths created in the contact chamber, high ozone doses have broken down and removed surface active agents. Also, color- and odor-causing substances usually were totally oxidized. Another often unrecognized advantage of ozone treatment was the saturation or super saturation of the effluent with dissolved oxygen (1) (11) (12) (27).

A project conducted a few years ago in England utilized ozone in conjunction with other tertiary treatment processes in an attempt to produce an effluent capable of limited recycle (11) (12). Effluent from a treating-humus tank, containing a BOD of about 18 mg/l and a suspended solids content of 50 mg/l, was applied to microstraining, ozonation, and sand filtration. The treatment produced a final effluent clear, colorless, and comparable in appearance to the "best potable supplies" (11) (12). Ozone was responsible in the process for significant reductions in BOD, color, turbidity, phenolic compounds, certain pesticides, and surfacants. Microbiological investigations showed the final effluent to be superior to river waters and suitable for a variety of industrial purposes with further treatment (11) (12).

Industry has also found ozone's oxidative capabilities useful in solving some of its special waste problems. Oxidation of cyanide wastes by ozone has proven as effective as the traditional chlorine treatment (1) (28) (29) (30). Wastes from refineries and coke works, containing up to 2000 mg/l of phenol, have been effectively treated using a two step biological and ozone oxidation process. Phenol concentrations in the treated effluent have been less than 1 mg/l (1) (31). Other industrial wastes, which have proved treatable by ozone processes, were biorefractory organics, acid mine wastes, and some unsaturated organic compounds (1).

III. METHODS AND MATERIALS

A continuous-flow bioassay system was chosen as the means of investigating the possible toxicity of an ozonated, secondary municipal effluent. The experimental apparatus and procedures were designed to be conducive to repetition of results and easy comparison with similar studies. Most of the materials and procedures utilized were selected in accordance with guide lines listed in Standard Methods (32) for a four-day, continuous-flow bioassay. Other tests employed to monitor the effects of ozone on the secondary effluent also followed, when possible, procedures outlined in Standard Methods (32). Some materials and procedures employed, not outlined in Standard Methods (32) and uncommon to general research practices, were recorded in detail to satisfy the requirements of repetition and comparison.

Ozonation System

The source of ozone for the continuous-flow system was an air-cooled Airox ozonator (Model 2P-3C-2). For proper and consistent operation of the ozonator a clean, oil-free air supply with a -40°F dew point, to insure the absence of moisture, was required. Because of the extended periods of operation and the large volumes of air required it was infeasible to utilize bottled air. Therefore, a system of drying and filtering columns was devised to insure the required air quality from a laboratory air source.

The air filter system consisted of four filter columns in series attached to a laboratory air source. Each of the four filters was contained in a piece of galvanized steel pipe, 1.5 inches in diameter and 14 inches long, fitted with tapered ends for the attachment of Tygon tubing. Glass wool was packed in both ends of the pipes and acted as a dust filter. The first filter contained 12 x 40 mesh activated carbon, to remove oil from the air. Silica gel, molecular sieves, and indicating-Drierite were the respective media of the succeeding three columns. These media dried the air to a -40°F dew point. Twice a day the column containing indicating-Drierite was checked for breakthrough of the absorption capacity of the filter system. Checks were not made more often because each check required shutting down the ozone generator and opening the columns.

Various production rates of ozone were controlled and regulated by varying the pressure and flow of the air stream and the voltage applied at the electrodes. Because the ozonated air was bubbled through a column of liquid, the liquid back pressure created problems in regulating the flow and pressure of the gas stream. These problems were solved by splitting the gas flow from the ozonator with a "Y" connection and wasting part of the stream. A pinch clamp was then attached to the gas waste line to act in conjunction with the ozonator controls in regulating the pressure and flow (See Figure 1).

Before beginning the actual bioassay investigation, it was necessary to calibrate the ozonator to determine the range and accuracy of ozone production. Because no direct means of measuring ozone production was available, the iodometric method for determining ozone,

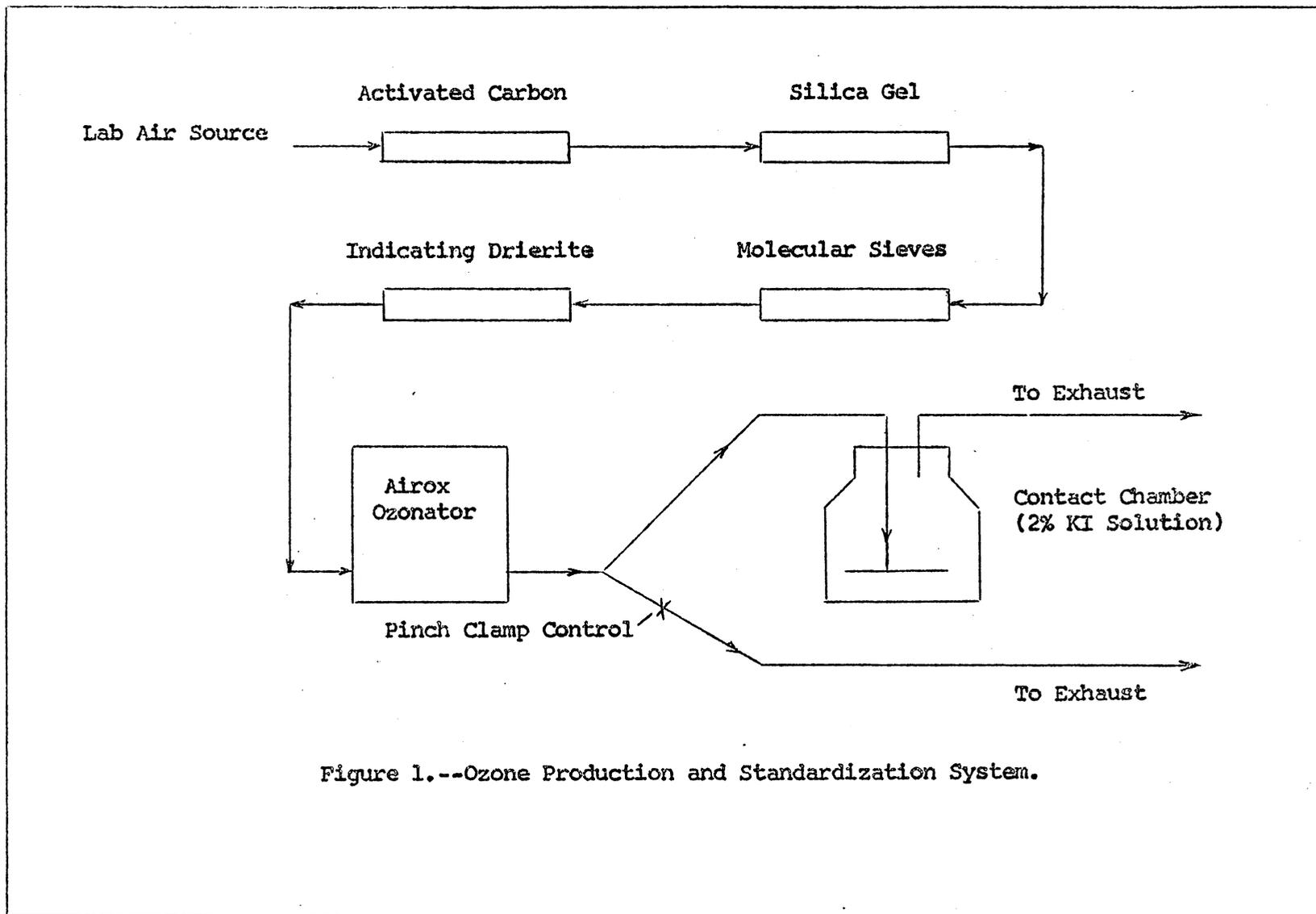


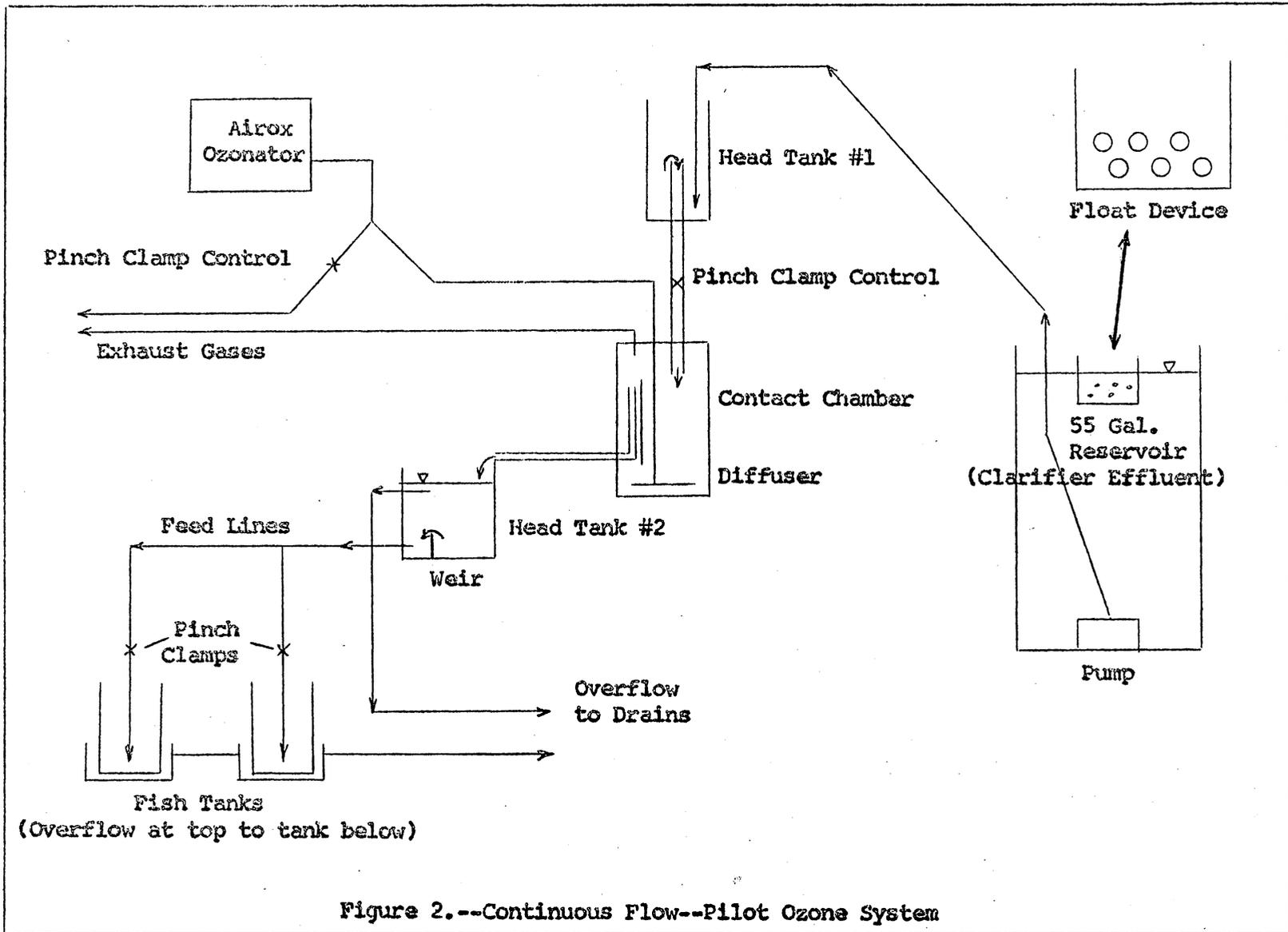
Figure 1.--Ozone Production and Standardization System.

outlined in Standard Methods (32) was modified for that purpose. The ozonated air was bubbled through a contact chamber containing 500 milliliters of a two percent potassium iodide solution for one minute. Iodine was liberated in a one-to-one molar relationship by the ozone in the gas stream. Sodium thiosulfate titration was used to measure the amount of liberated iodine from which the ozone concentration could be calculated. At each combination of settings for pressure, flow, and voltage, three values of ozone production were obtained and analyzed for a mean and standard deviation.

Continuous-Flow--Pilot Ozone Treatment System

The design of the continuous-flow system utilized in the investigation was based upon a combination of two sets of criteria. Requirements and guide lines outlined in Standard Methods (32) for a continuous-flow bioassay were followed in creating the flow system used to deliver the ozonated effluent to the test fish. It was necessary to inject ozone continuously to insure a continuous supply of ozonated by-products in the flow of secondary effluent. Production of the ozonated effluent was governed by the rapid decomposition of ozone and the subsequent by-product reactions requiring the continuous production of the possible toxicant.

To satisfy the above criteria a pilot operation, modeled to stimulate a possible tertiary treatment operation, was constructed as shown in Figure 2. The system was designed to test one dilution of the possible toxicant, as shown, or to be easily converted to a system that would test several dilutions at once. Flow through the system



was calculated on the basis of a fish oxygen consumption rate of 0.2 mg oxygen per gram of fish per hour and the requirement that the solution volume equal the container volume every four hours. Because two twenty-liter containers were used for each test, the flow to each tank was maintained at about 100 ml/min. to satisfy the above requirement. Aeration was not utilized to aid in meeting the oxygen requirement because it might affect some of the ozone decomposition reactions. Except for the pump in the waste effluent reservoir, the system was operated by gravity flow to minimize mechanical dependency.

Ozonation in the contact chamber employed the diffuser-counter current principle discussed in the literature review (See Figure 3). To permit flexibility in converting from a one-dilution system to a multiple-dilution system, the volume of the contact chamber was chosen to provide for a maximum flow of 400 ml/min., producing a five-minute contact time. Retention time in the contact chamber was maintained at 5-10 minutes for the investigation because restrictions imposed by the available equipment prevented utilizing contact times of 30-60 minutes. However, the range of contact times utilized was typical of those used for disinfection and most tertiary treatment uses of ozone.

Other than acting to maintain constant flow through the system, the two head tanks served other functions. The first related to the dissipation of ozone residuals. As the objective of the study was to investigate ozonated effluents and not ozone residuals, detention time in the head tanks was designed to be sufficiently long to allow ozone residuals in effluents from the contact chamber to dissipate. Time in the head tank varied from 18-24 minutes, about three times that reportedly

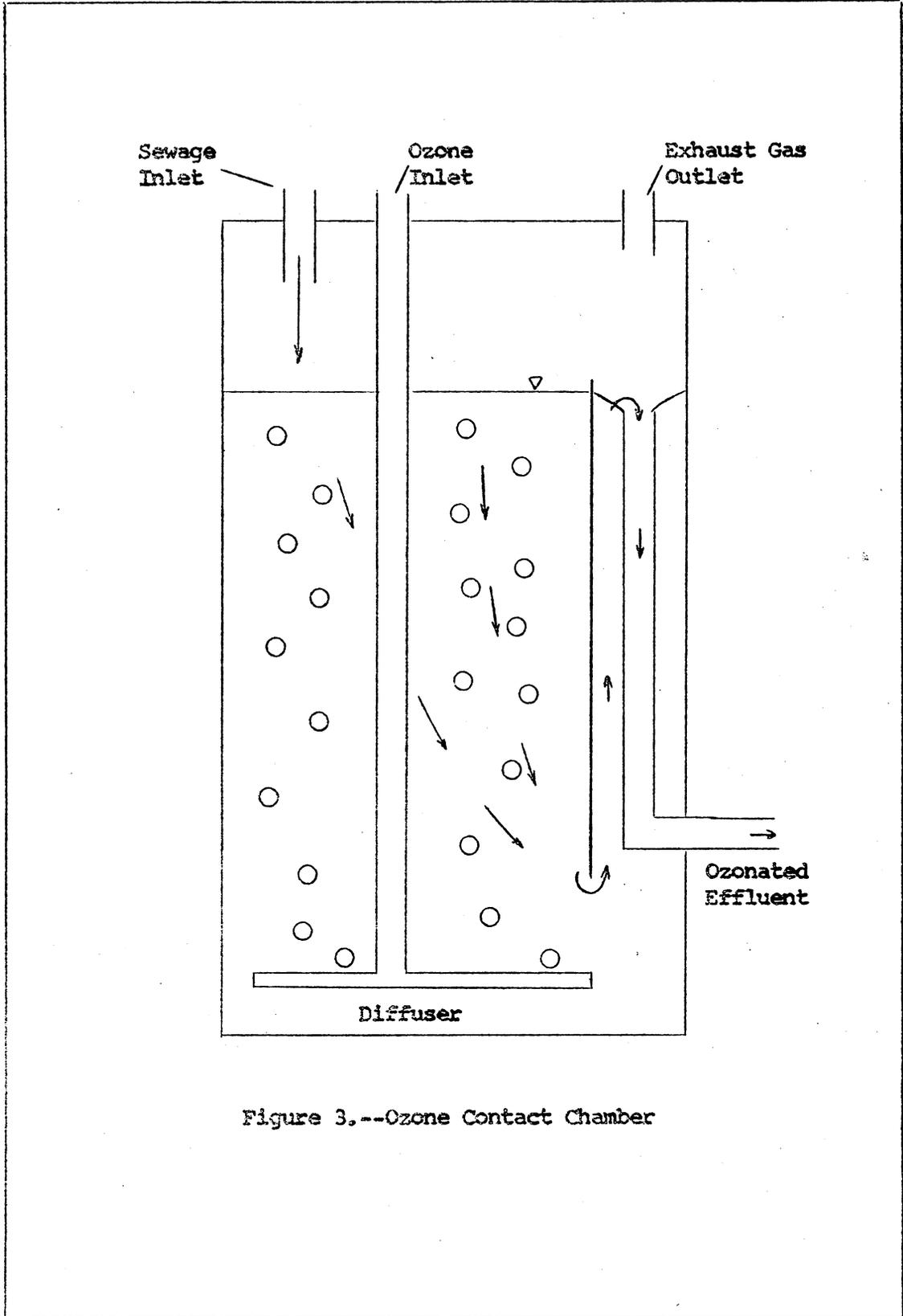


Figure 3.--Ozone Contact Chamber

required for the dissipation of ozone residuals (Appendix Figure 2). The second problem alleviated in the head tanks was the accumulation of solids in the continuous-flow system. Both tanks, as shown in the diagram (Figure 2), were partially baffled to provide some retention of solids in each tank.

General Tests and Procedures

Chlorinated effluents have proven to be toxic to fish. Therefore, the waste utilized in this investigation was taken from the effluent of the final clarifier prior to chlorination at the Blacksburg municipal sewage treatment plant (trickling filter). Effluent was composited three times per day in a fifty-five-gallon reservoir. The reservoir was filled by pouring the waste into a float device (shown in Figure 2) to minimize the resuspension of settled solids. During one part of the investigation, the fourth run, the initial solids concentration of the clarifier effluent was reduced by filtering the effluent through glass wool.

Ten bluegill sunfish meeting the size requirements outlined in Standard Methods (32) were exposed to ozonated sewage for four days. The fish were replaced each time the ozone dosage level was changed. The fish were acclimated to continuous-flow, laboratory conditions for more than 30 days prior to the study. Two twenty-liter containers housed the fish during each test to prevent over-crowding.

A number of traditional waste parameters were monitored for the purpose of characterizing and monitoring the waste effluent and its changes through the system. These tests also served to insure that

that any toxicity demonstrated was the result of ozonation and not due to some undetermined characteristic of the waste. Generally these tests could be classified in two groups: those necessary to evaluate changes resulting from ozonation and those necessary to evaluate any toxic characteristics of the waste unrelated to ozonation. All of the parameters were measured twice a day: once, about one hour after the storage reservoir was filled in the morning, and again, after refilling in the afternoon.

Changes in the waste resulting from ozonation were monitored by measuring COD, pH, suspended solids, alkalinity, dissolved oxygen, BOD, and ammonia nitrogen in the influent and effluent. Hardness was measured in the influent only. Ammonia nitrogen and BOD were not tested twice a day, but, instead, were measured in random grab samples during the course of the exposure period. Procedures from Standard Methods (32) were used. Dissolved oxygen was measured with a YSI oxygen probe (Yellow Springs Instrument Company, Yellow Springs, Maryland) and pH was measured with a Beckman pH meter (Beckman Instruments, Fullerton, California).

Parameters monitored to assess possible toxicity by causes unrelated to ozonation of the sewage were: the dissolved oxygen, temperature, and pH of the fish tanks, and the ozone residual in the feed. Ozone residuals were measured at the beginning of each bioassay and then periodically during the remainder of the test. The iodometric method for determining ozone residuals was used, as outlined in Standard Methods (32). Dissolved oxygen and pH were measured, as mentioned previously, by instruments.

To further insure that toxicity was the result of ozonation, a bioassay was performed, the test solution being clarifier effluent without ozonation. The effluent in this run was aerated in the contact chamber with an air stream containing no ozone. This test provided a control to insure that ozonation was the only significant variable in the investigation. All parameters mentioned previously were monitored during the control run.

The ozone dosage for the first bioassay was arbitrarily set at approximately 18.9 mg/l to provide some basis for adjusting dosages in subsequent tests. Adjustments were based on the number of fish killed during the initial test. Unfortunately, no information was found in the literature on which to base a range of ozone dosages to be applied. In subsequent tests, the ozone dose was varied in increments of approximately 10 mg/l to determine a range in which the TLM_{50} would fall.

Method of Expressing Ozone Dosage

As was mentioned previously, moisture in the air stream reduced the efficiency of ozone production by the ozonator. There was no method available for determining precisely when the ozone generation rate began to decline due to the breakthrough of moisture from the filters. Therefore, the filters were replaced with reconditioned ones at intervals arbitrarily selected to keep the efficiency of the ozone production as high as possible. During any one 96-hour bioassay, the filters were changed at fixed intervals, whole number multiples of which would exactly equal 96--i.e., 12, 24, 48 hours.

The ozone production rate was measured at the beginning of each run and again at the end. The value obtained at the end of the run

was assumed to be that for the rate of ozone production at the end of each interval during the run immediately before the drying filters were replaced. Figure 4 is a hypothetical ozone production pattern that was assumed for purposes of arriving at an average ozone dosage for any given bioassay. The line on the figure denoting the average was derived on the assumption that the decrease in ozone production rate was constant during any given interval. Though this assumption was most likely not accurate, frequent measurements of ozone production that would be needed to better define the curve were not possible without disruption of the bioassay equipment.

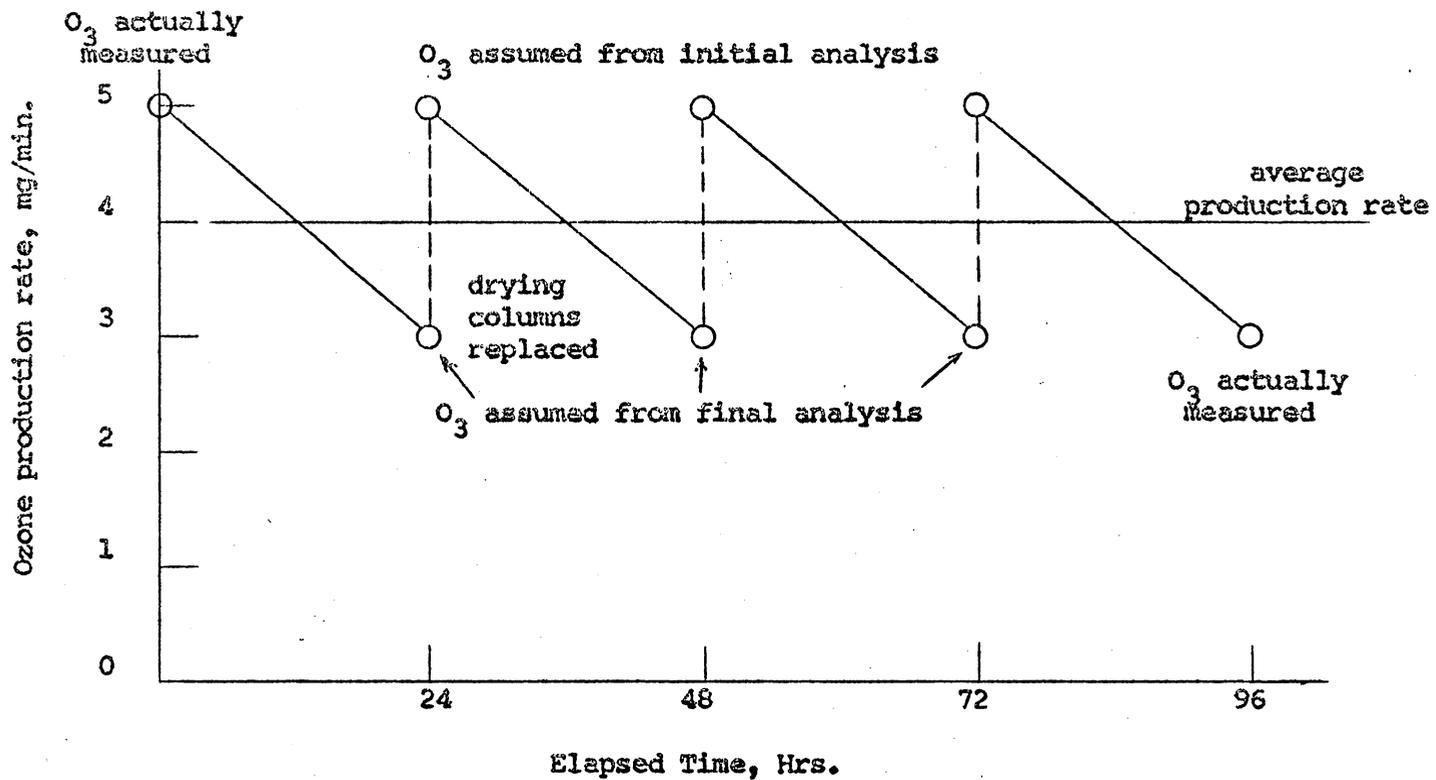


Figure 4.--Hypothetical Ozone-Production Pattern Resulting From Moisture Breakthrough and Replacement of Drying Filters.

IV. RESULTS

At the end of four runs enough data had been collected to give a clear indication as to the toxicity of an ozonated, secondary treated municipal effluent. Though much of the data was quantitative in nature, a significant portion included qualitative observations made during each test run. These observations complemented the quantitative data and aided in the interpretation of results.

Run #1 (Control Run)

Only one fish died during the control run with aerated clarifier effluent. Death occurred some time during the night of the third day before the 66th hour of the run. The nine survivors exhibited no adverse effects of exposure to the sewage. This is not to say that the fish were unaffected by the clarifier effluent. Throughout the first 36 hours the fish began to surface frequently, and during the last 48 hours they spent nearly all their time grouped at the surface.

Results of the analyses of waste parameters monitored and the dissolved oxygen measurements are summarized in Tables I and II, respectively. A problem developed in maintaining the dissolved oxygen concentrations in the fish tanks. On four occasions during the last 48 hours of the run, the dissolved oxygen fell significantly below 5 mg/l, the concentration generally accepted for maintenance of fish life. On these occasions the liquid in the fish chambers was aerated to bring the dissolved oxygen level above 5 mg/l.

TABLE I
WASTE CHARACTERIZATION DATA FOR INFLUENT
TO BIOASSAY CHAMBERS--CONTROL RUN

Parameter	Concentration
COD (mg/l)	59
Suspended Solids (mg/l)	26
pH	7.5
Alkalinity mg/l as CaCO ₃	152
Hardness mg/l as CaCO ₃	163

TABLE II

DISSOLVED OXYGEN CONCENTRATION AND TEMPERATURE OF BIOASSAY
MEDIUM AT INTERVALS DURING CONTROL RUN

Parameter	Vessel	Elapsed Time, Hrs.							
		2	18	26	42	50	66 ⁺	74	90
D.O. (mg/l)	1	6.0	5.0	5.0	2.5*	4.5*	2.5*	4.5*	4.5*
	2	6.0	5.0	5.0	3.0*	5.0	2.5*	4.5*	5.0*
	1 & 2	22	22	22	22	23	23	23	22

* Sewage aerated for 30 minutes at these times.

+ Dead fish discovered.

Operation of the continuous-flow system required little maintenance until about the end of the second day. Some time between 35 and 42 hours the pump failed, and all flow stopped. By the time the pump was replaced, the sewage had developed a strong odor, and the dissolved oxygen in the fish tanks had fallen below 3 mg/l. The fish tanks were aerated and fresh sewage was provided.

Another operational problem that occurred with increasing frequency during the last 48 hours was the maintenance of constant flow rates. Accumulation and growth of solids in the feed lines, especially at the pinch clamps, created obstructions that continually interfered with flow. Frequent flushing of the feed lines was necessary to maintain a constant flow rate.

Run #2 (Ozone Treatment)

During the second 96-hour bioassay, ozone was applied to the clarifier effluent at an average rate of 16.9 mg/l. The drying filters were replaced at the end of 48 hours, and ozone production had decreased from 18.9 mg/l to 14.9 mg/l during the interval.

Ten fish were used in this bioassay, eight of which had been used previously in the control run. The other two fish, while never having been exposed to sewage, had been exposed for a brief period to 0.1-0.2 mg/l chlorine.

While these fish were inappropriate for a valid scientific bioassay, there were no acclimated specimens available at the time. The test was initiated with the belief that if no deaths occurred in the 96-hour test period, one might assume that sewage ozonated with approximately 19 mg/l ozone was non-toxic. Then dosages for future tests could be adjusted accordingly.

This first bioassay with ozonated sewage resulted in the death of four fish. These fish died sometime during the night of the second day but were not noted until 46 hours had elapsed. Though the other six fish survived the succeeding 50 hours of exposure, three of the six died within 24 hours after the completion of the run. Two of the three fish died while still in tanks containing the ozonated sewage, about one hour after the termination of the run. The third post-test death occurred about 24 hours after the fish had been removed from the ozonated sewage.

Behavior of the fish during the test run paralleled that observed during the previous control run to some degree. Initially the fish remained near the bottom of the tanks, rarely venturing into the upper half. About the time the four deaths were noted, the fish were observed gathering at the top of the tanks and over the last 48 hours, they avoided the lower half of the tank. Other than this pattern of behavior, the fish exhibited nothing unusual to indicate toxicity or irritation.

However, the fish displayed an alteration in physical appearance not noted during the control run. Each fish, even the survivors, had red spots around their gills and eyes that appeared to be hemorrhaging. The time the hemorrhaging actually first appeared was not observed, but the spots were evident on dead specimens examined after 48 hours.

The summary of monitored waste parameters and dissolved oxygen concentrations, shown in Tables III and IV, respectively, summarizes the observed effects of ozone upon the clarifier effluent. Effluent from the ozone contact chamber was colorless, though not clear, in

TABLE III
EFFECTS OF OZONATION ON SEVERAL CHEMICAL CHARACTERISTICS
OF SECONDARY SEWAGE EFFLUENT USED IN RUN #2

Parameter	Influent	Effluent	% Change
COD (mg/l)	50	38	-25
S.S.	34	26	-23
pH	7.5	8.0	+ 7
Alkalinity (mg/l as CaCO ₃)	123	120	- 2
Hardness (mg/l as CaCO ₃)	157		
Ozone Dose: Beginning of Bioassay		18.9 mg/l	
End of Bioassay		14.9 mg/l	
Average		16.9 mg/l	

TABLE IV
DISSOLVED OXYGEN CONCENTRATION AND TEMPERATURE OF BIOASSAY
MEDIUM AT INTERVALS DURING RUN #2

Parameter	Vessel	Elapsed Time, Hrs.							
		0	6	22	30	46	54	70	78
D.O., (mg/l)	Fish Tank #1	7.4	8.1	8.0	7.8	5.2	7.0	6.0	6.5
	Fish Tank #2	7.4	8.1	8.0	7.8	5.1	7.0	5.0	6.5
	Head Box #2	7.9	8.1	8.3	8.4	7.8	7.7	8.1	7.3
	Saturation	7.9	7.9	7.8	7.7	7.8	7.8	7.8	7.8
Temperature °C		23.5	23.5	24.0	24.5	24.0	24.0	24.0	24.0

contrast to the brown, turbid character of the influent. An odor in the ozonated sewage that could best be described as "super-fresh" had replaced the stale odor of the influent sewage. Foam formed on top of the sewage in the contact chamber and at the point of discharge to the head box. Upon close examination and comparison of an influent and effluent sample the character of the suspended solids appeared to have been altered. While influent suspended solids were comparatively large and settled readily, the ozonated effluent suspended solids were almost microscopic and settled very slowly.

Problems in operation of the continuous-flow system began to develop by the end of the second day. At the time the four dead fish were discovered, the oxygen concentration in the tanks had dropped to below 5 mg/l, requiring aeration of the test chambers as was necessary in the control run. Additional aeration periods were not required. The low dissolved oxygen levels were attributed to failure of the ozonation system because moisture breakthrough was detected in the drying columns after 48 hours of operation. Ozone production was halted for about an hour while the filter media were changed.

Maintenance of flow rates and the deposition of solids again produced some of the same problems encountered in the control run. Initially, difficulty was encountered in maintaining constant flow in the feed lines to the fish tanks because of the aggregation of gas bubbles in these lines. Frequent flushing of the lines and resettling the flow rates solved this problem. After about 24 hours the gas-bubble deposition and aggregation gradually ceased, but then deposition and accumulation of solids in the feed lines and head boxes began to be significant, affecting the flow system as had been observed in the control run.

As in the control run, frequent cleaning of the tubing at the sites where there were clamps allowed adequate flow to be maintained. A survey of the total system at the end of the run revealed large deposits of solids in both head and fish tanks as well as in the feed lines.

Run #3 (Ozone Treatment)

The ozone dose was increased for the second bioassay to an average output of 22.4 mg/l, while the time between changes in the drying filter was decreased to 24 hours between replacement of the filters to 12.1 mg/l. The ten fish utilized in this run were new specimens, fully acclimated and not previously used for any bioassay.

After 96 hours of exposure to the ozonated sewage, none of the specimens were dead. However, the signs of hemorrhaging around the eyes and gills, noted in the previous run, appeared on every test fish within 24 hours. Behavior patterns of the fish were apparently unaffected by this hemorrhaging or by the exposure to the ozonated effluent. In fact, for the large part of this run the behavior of the test fish paralleled that of the fish in the acclimation tanks. That is to say, the test fish spent most of their time in the lower part of the tanks, coming to the surface infrequently for short periods of time. During the last twenty-four hours of the test, though, the fish began appearing at the surface more frequently and for longer periods of time.

Summarized in Tables V and VI are the data derived from waste parameters and dissolved oxygen concentrations. In addition to waste parameters measured in Run #2, ammonia nitrogen concentrations were determined in random samples collected during Run #3. As was true in

TABLE V
EFFECTS OF OZONATION ON SEVERAL CHEMICAL CHARACTERISTICS
OF SECONDARY SEWAGE EFFLUENT USED IN RUN #3

Parameter	Influent	Effluent	% Change
COD (mg/l)	66	59	- 13
S.S. (mg/l)	27	26	- 4
pH	7.1	7.4	+ 6
Alkalinity (mg/l as CaCO ₃)	115	113	-1.7
Hardness (mg/l as CaCO ₃)	140		
NH ₃ --N	6.0	6.0	+13.3
Ozone Dose: Beginning of Bioassay . . .		32.8 mg/l	
End of Bioassay		12.1 mg/l	
Average		22.4 mg/l	

TABLE VI
DISSOLVED OXYGEN CONCENTRATION AND TEMPERATURE OF BIOASSAY
MEDIUM AT INTERVALS DURING RUN #3

Parameter	Vessel	Elapsed Time, Hrs.							
		4	20	28	44	52	68	76	92
D.O., (mg/l)	Head Box #2	7.9	7.9	7.9	7.8	7.9	7.4	7.5	7.3
	Fish Tank #1	7.1	5.1	6.0	5.5	5.5	5.1	5.0	4.1
	Fish Tank #2	7.1	5.1	5.8	5.5	6.0	5.1	5.0	4.1
	Sewage						3.3	3.8	3.6
	Saturation	7.9	7.9	7.8	7.7	7.8	7.8	7.8	7.8
Temperature °C		23.5	23.5	24.0	24.5	24.0	24.0	24.0	24.0

Run #2, the ozonated effluent appeared to be low in turbidity and the flow rate had decreased, and the effluent from the contact chamber was almost clear. An analysis of the suspended solids concentrations during one of these periods showed the influent and effluent solids to be approximately 10 mg/l. As in the previous run, the influent suspended solids were comparatively large and easily settled, while the effluent suspended solids were almost microscopic and difficult to settle.

Difficulties arose in attempting to maintain a fairly constant level of ozone production. Breakthrough of moisture in the filter columns occurred in less than twenty-four hours, necessitating that the filter media be changed every day. Controlling the moisture content of the columns was difficult because breakthrough occurred so suddenly. Periodic checks of the dessicant did not provide adequate warning. To complicate matters the ozonator overheated on the morning of the second day, necessitating a shutdown for about two hours. In all, a total of about six hours of ozone production was lost, not to mention the decreases in ozone production from moisture breakthrough.

Solids deposition again presented problems in maintaining consistent flow rates through the system. As in the two previous runs, the problem was overcome by frequent flushing of the feed lines and resetting the flows. Accumulation of a large quantity of solids in the fish tanks was prevented by siphoning the solids from the bottom of the tanks once a day. Solids were not removed from the tanks during the two previous runs, though such action might have prevented oxygen depletion in the tanks.

Run #4 (Ozone Treatment)

The last bioassay run was made utilizing an average ozone dose of 46 mg/l. At the beginning, the dose was 52 mg/l but declined to 40.1 mg/l during the time between changes in the drying filters. Some procedures not employed in the previous runs were adopted during this final run. First, the exposure period lasted only 48 hours instead of 96 hours, as was used previously. Another alteration in previous test procedures was that the clarifier effluent was filtered through glass wool prior to ozonation to remove large suspended solids. Also, the flow rate into the contact chamber was reduced from 400 ml/min. to 250 ml/min. to provide more contact time than in previous runs, a period of approximately eight minutes.

All of the test fish survived the exposure period. Within eight hours of the beginning of the run, though, eight of the fish exhibited signs of hemorrhaging around the eyes and gills. After twenty-four hours the other two fish began to show signs of hemorrhaging also. Throughout most of the 48-hour run the behavior of the fish was regarded as normal. An exception occurred during the morning of the second day. The five fish in the second tank were discovered grouped at the top of the tank, but after three or four hours, they returned to the lower part of the tank.

The effects of ozonation, during the fourth run, on the waste characteristics and on the dissolved oxygen concentrations are summarized in Tables VII and VIII, respectively. In addition to the measurement of COD, a BOD measurement was made for comparison. Changes in ammonia nitrogen were also evaluated. Physical characteristics of

TABLE VII

EFFECTS OF OZONATION ON SEVERAL CHEMICAL CHARACTERISTICS
OF SECONDARY SEWAGE EFFLUENT USED IN RUN #4

Parameter	Influent	Effluent	% Change
COD (mg/l)	51.8	46.4	-14.3
BOD (mg/l)	60	42	- 30
S.S. (mg/l)	23.8	24.8	+ 4
pH	7.4	7.8	+ 5
NH ₃ --N (mg/l as N)	4.3	5.0	+12.7
Ozone Dose:	Beginning of Run	52.0 mg/l	
	End of Run	40.1 mg/l	
	Average	46.0 mg/l	

Note: BOD and NH₃--N determined by grab samples.

TABLE VIII

DISSOLVED OXYGEN CONCENTRATION AND TEMPERATURE OF BIOASSAY
MEDIUM AT INTERVALS DURING RUN #4

Parameter	Vessel	Elapsed Time, Hrs.				
		1	8	25	32	48
D.O., (mg/l)	Head Box #2	7.7	7.4	7.6	7.6	7.0
	Fish Tank #1	7.0	6.7	6.5	5.8	5.0
	Fish Tank #2	7.0	6.4	4.0	4.7	4.4
	Sewage	7.0	5.9	4.0	6.6	5.4
	Saturation	7.7	7.7	7.7	7.7	7.7
Temperature °C		25	25	25	25	25

the ozonated effluent were the same as those in the two previous ozone runs with one exception. The turbidity of the influent was much less (due to pre-filtration) than in previous runs, and there was no discernible turbidity in the effluent. When compared to a sample of tap water, the ozonated effluent could not be identified solely by physical appearance.

The media in the filter columns were changed twice a day in an attempt to maintain a more constant ozone dose. With the filter media changed so often, no significant moisture breakthrough occurred. Ozone dosage was affected, however, by a break in the connection between the contact chamber and ozonator. The break occurred some time during the evening of the first day and was discovered and repaired that evening. The failure in the system lasted a maximum of four hours.

Deposition and accumulation of solids presented few problems during this run. At the end of the run, the feed lines to the contact chamber and the first fish tank were virtually free of solids and the flow rate required only minor adjustments. On the other hand, the feed line to the second tank was observed to have a large accumulation of solids, and frequent adjustments were necessary during the last 24 hours of the run. Few solids were noted in either of the head tanks.

There were two observations concerning ozone, in each of the experimental runs that were regarded as significant. First, ozone was never detected in any of the samples collected at random during any experiment. Second, ozone could be detected (by its odor) in the exhaust from the contact chamber. The concentration of ozone in the exhaust was not determined because no instrument was available and

other available procedures were extremely tedious. Therefore, there was no way to estimate what fraction of the applied ozone was actually consumed by the sewage, but it was evident that not all the ozone produced actually was retained in the system.

V. DISCUSSION

Despite the fact toxicity of ozonated sewage was not demonstrated in this investigation, the analytical results and observations were analyzed to determine to what extent the objective of this study had been realized. Results of the effects of ozonation on the clarifier effluent were useful in interpreting the bioassay data. In addition, the analytical data indicated the levels of treatment that tertiary treatment processes with ozone (similar to the pilot process in this study) might achieve. The continuous-flow system and the many problems encountered during the investigation were carefully evaluated in assessing the results. From this analysis of the continuous-flow system, information also was obtained that was useful for recommending improvements for similar studies in the future.

The Effects of Ozonated Sewage on Fish

Response of the fish during each test run was the first aspect of the study that was evaluated. One fish died during the control run, but its death was not considered significant for two reasons. First, nine other fish survived, and second, the oxygen concentration in the tanks was only 2.5 mg/l when the dead fish was discovered (See Table II). So death probably was caused by a lack of oxygen.

Lack of oxygen also was the probable cause of death of four fish in the first bioassay with ozonated effluents. However, another factor

that may have affected the results was that the test fish had been used in previous tests, though none of them had been exposed to ozonated effluents. Because there were no deaths observed in the succeeding two bioassays, which utilized higher doses of ozone, the ozonated effluent was ruled out as the cause of death. An analysis of the oxygen concentrations observed during the run revealed that oxygen levels in the tanks prior to the discovery of the fish kill dropped (in about sixteen hours) from approximately 8 mg/l to 5 mg/l.

During each bioassay, the only behavioral abnormality observed was that the fish tended to gather at times, at the surface of the tanks. This pattern of fish behavior often is attributed to a lack of oxygen in the water. During short periods of oxygen deficiency, the fish were provided with a better chance of survival by taking advantage of the constant gas exchange between the air and water surfaces. A review of the oxygen concentrations in the fish tanks during periods when the fish were at the water surface revealed that the oxygen level was either below or barely above 5 mg/l. Because the aforementioned behavior pattern also was exhibited during the control run, lack of oxygen was ruled as the cause of the behavior instead of ozonated sewage.

An analysis of the dissolved oxygen data (Tables II, IV, and VIII) revealed that the effluent from the contact chamber was always saturated, or supersaturated. On the other hand, the dissolved oxygen in the fish tanks was near saturation at the beginning of any one bioassay, but decreased gradually during the run. The reason for oxygen depletion in the test chambers most probably was caused by the oxygen demand of the solids that accumulated with time. As was mentioned, when these

solids were removed periodically, maintenance of adequate dissolved oxygen levels was no longer a problem.

Hemorrhaging around the eyes and gills of the test fish during the three bioassays in which ozonated sewage was used was attributed to an effect known as "gas-bubble disease". The hemorrhaging apparently was not serious because during the last two bioassays all the fish survived even though they displayed physical symptoms of the gas-bubble disease. Conditions favorable for the existence of the gas-bubble effect, the saturation or supersaturation of a gas in water, occurred during all three ozonation runs. In the first 24 hours of each bioassay, when the hemorrhaging was observed, the oxygen concentrations in the fish tanks generally were near saturation. Whether this gas-bubble effect would manifest itself in a stream receiving ozonated effluent was not resolved because bioassays were not performed with dilutions of the treated waste. Bioassays with dilutions were not performed because the probability of conditions existing in actual sewage treatment practices that would induce gas-bubble disease was considered remote.

The Effects of Ozonation on Chemical and Physical

Characteristics of Sewage Effluents

The application of the data obtained during this investigation to actual field practice depends upon the degree to which the operation of the pilot-scale system corresponds to that in the larger field units. As stated in a previous section, the contact chamber was designed on the basis of the diffuser-countercurrent techniques of ozone application.

Diffuser-countercurrent techniques along with injector techniques were the two most popular means of ozone application mentioned in the literature review.

Ozone dosages and contact times utilized during the investigation compared favorably with those reported for most tertiary treatment practices involving ozone practices. Table IX shows a comparison of data collected during this investigation with values reported in the literature. Two uses of ozone where the doses did not compare favorably were for COD reduction and for high dosage disinfection. Only in one instance was the high dosage for disinfection mentioned (2). All others cited dosages in the 2-20 mg/l range. Direct comparison of COD-reduction data between this study and those reported in the literature was not possible because contact times of 20-60 minutes were not possible in this investigation.

Additional evidence that the pilot-scale treatment used in this investigation simulated most plant-scale applications was found from an analysis of the results of the monitored waste parameters. A comparison of the analytical data derived during the three ozonation runs with those obtained during the Redbridge experiments of Boucher and Truesdale is presented in Table X. Boucher and Truesdale's work, as cited in the literature review involved the ozonation of a filtered secondary effluent to produce a treated effluent which could be recycled for limited uses. From the comparison it can be seen that the results were quite similar for all parameters except BOD reduction. The reason BOD reduction was not attained in the Redbridge experiments probably was because of an extremely low influent BOD of 10 mg/l.

TABLE IX

APPLICATION RATES AND DOSAGES OF OZONE USED IN THIS
INVESTIGATION COMPARED TO THOSE USED IN
ACTUAL WASTE TREATMENT PRACTICE

Application of Ozone	Dose (mg/l)	Ozone Contact Time (minutes)
Pilot Study (This Investigation)		
Bioassay #2	14.9-18.9	5-10
Bioassay #3	12.1-32.8	5-10
Bioassay #4	40.1-52.0	5-10
Summary	12.1-52.0	5-10
Disinfection		
(References (1) (2) (11) (23))	2-20 50-60	5-10 10-30
Color, Odor, Turbidity, and Surfactant Removal		
(References (1) (11) (12) (15) (18) (27))	2-20	5-10
COD Reduction		
(References (1) (4) (12) (25))	20-200	20-60

TABLE X

PHYSICAL AND CHEMICAL CHANGES IN SEWAGE CAUSED BY OZONE TREATMENT:
 A COMPARISON BETWEEN RESULTS OF THIS INVESTIGATION
 AND THOSE OBTAINED AT REDBRIDGE, ENGLAND
 (REFERENCES 11,12)

Parameter	Percent Change ⁺			
	Redbridge O ₃ = 20-25 mg/l	Run #2 (Table III) O ₃ = 16.9 mg/l	Run #3 (Table V) O ₃ = 22.4 mg/l	Run #4 (Table VII) O ₃ = 46.0 mg/l
COD	-17.2	-24.9	-13	-14.3
BOD	0	---	---	- 30
S.S.	- 5	-23.3	- 4	+ 4
pH [!]	+ .2	+ .5	+ .5	+ .5

+ Negative sign before number designates a decrease after ozonation, and a positive sign designates an increase.

! Change expressed in terms of pH units.

TABLE X (Continued)

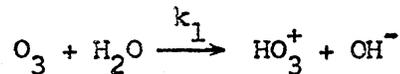
Parameter	Percent Change ⁺			
	Redbridge O ₃ = 20-25 mg/l	Run #2 (Table III) O ₃ = 16.9 mg/l	Run #3 (Table V) O ₃ = 22.4 mg/l	Run #4 (Table VII) O ₃ = 46.0 mg/l
NH ₃ --N	+10.4	---	+13.3	+12.7
Color	- 83	No visible color*	No visible color*	No visible color*
Turbidity	- 63	Dramatic decrease*	Dramatic decrease*	Dramatic decrease*
D.O., % Saturation in effluent	99	100	100	104

+ Negative sign before number designates a decrease after ozonation, and a positive sign designates an increase.

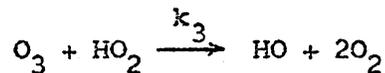
* Color and turbidity were not measured for test runs but change was determined by qualitative observations.

Reductions in COD attained during the test runs in this investigation fell far short of those cited in the literature from other studies. On the average, the degree of COD reduction observed during the test runs was only 25 to 50 percent of the often-quoted values of 50 to 70 percent COD reduction with ozone. The principal difference between this study and other studies involving COD reduction by ozonation was that the contact time used in this study (5-10 min.) was much shorter than that used in other studies (20-60 min.).

Ammonia nitrogen and pH were two parameters that significantly increased as a result of ozonation, but these parameters were not discussed at any length in the literature. Boucher and Truesdale (11) (12) indicated that some increase in ammonia nitrogen occurred during their experiments but offered no explanation as to a cause. A review of the literature concerning ozone chemistry also failed to produce an explanation for the ammonia increase. However, a possible reason for the pH increase was found in the ozone decomposition reaction. The two reactions responsible for the disappearance of ozone in water:



and



both produced hydroxyl ions, a possible explanation for the pH increase.

The most readily observable effects of ozone on the sewage were the reductions in color, odor, and turbidity. Similar effects have been reported by others (1) (11) (27) with much lower doses of ozone than those used in this investigation. Only qualitative data were recorded to express the observed changes in these three waste characteristics,

but the extent of the reductions probably was as great as those observed during the Redbridge Study (Table X). The authors state:

The most obvious effect of ozonation (dose about 20 mg/l) was to remove colour of the effluent, giving a product . . . which was clear and sparkling and was comparable in appearance with the best potable supplies.

Their description of the treated waste is similar to one recorded during Run #4 in this investigation:

Effluent from the contact chamber is crystal clear and fresh smelling--comparable to tap water in appearance.

Reductions were noted in turbidity (by visual comparison of influents and effluents) even though no decrease in suspended solids concentrations were measured. It is possible that particles imparting "turbidity" were too small to be trapped on the glass fiber filter used in the solids determination, but were attacked by ozone to reduce them to a size too small to impart turbidity to the water. Boucher and Truesdale (11) (12) alluded to this phenomenon, which they described as "micellization". The process apparently changes the structural aspects of suspended matter (whether visible or not) to smaller invisible particles known as micelles.

One other fact that became apparent when analyzing the chemical and physical data was that similar results were obtained from all three experimental runs. This fact was interpreted as an indication that the ozone dosages used during this investigation were excessive. In terms of actual treatment applications for the removal of color, turbidity, and odor from clarifier effluents much smaller ozone dosages would have been sufficient. Before an optimum ozone dose for COD reduction could have been recommended, further tests would have been required

utilizing larger contact times. Because microbiological investigations were not conducted, recommendations of doses required for disinfection were not possible.

Rationally, it would seem that the ozone demand of a waste would be reflected in the reduction of parameters such as COD or suspended solids. However, in all experimental runs, the observed reductions in these parameters were approximately the same, even though the ozone dose was increased in successive runs. Therefore, no statements can be made regarding the ozone demand of these wastes based solely on the analytical data. It is possible that the contact time of ozone with the waste was too short because of losses of ozone to the atmosphere to actually satisfy the ozone demand of the waste.

There were some indications that suspended solids exerted an ozone demand. Effluents from the first two experimental runs were colorless but had noticeable turbidity, while the effluent collected during Run #4 (third experimental run) was colorless and clear. The suspended solids in the influents used during the first two experimental runs were in the form of larger aggregates than those solids in the influent of Run #4. Therefore, it is postulated that an ozone demand could be exerted by large suspended solids being fractured in successive steps into smaller and smaller aggregates without there being a substantial decrease in the suspended solids concentration. Eventually the particle size could become small enough that the particles would not impart turbidity. If this phenomenon could actually occur, the smallest particles would still have to have been trapped on the glass-fiber filter for the suspended solids concentration to have

remained the same. Some mechanism other than physical trapping on the filter would have to be operative for the suspended solids to remain the same.

The Continuous-Flow Bioassay Apparatus

Analysis of the operation of the ozonation system and the continuous-flow bioassay system revealed problem areas that should be considered before conducting similar investigations in the future. Deposition and accumulation of solids was probably the most significant problem encountered in the operation of the flow system. As stated in the results, solids in the feed lines caused variations in the flow rates. One effect of solids, suggested by the data, was the depletion of oxygen in the head tanks, fish tanks, and feed lines. A survey of the oxygen data for all four runs showed that the contact chamber effluent was always saturated with oxygen while the level of oxygen in the fish tanks decreased gradually over the period of the test run. This fact, coupled with the observed accumulation of solids and formation of microbiological films in the tanks and feed lines, indicated that these solids were largely responsible for the observed oxygen depletion.

Modification in the normal operating procedures of the third and fourth runs further substantiated this observation. Oxygen depletion was less dramatic and there was a noticeable decrease in solids accumulation and film formation during the fourth run when the clarifier effluent was filtered prior to ozonation. Siphoning off the solids that accumulated in the fish tanks each day during the third run also resulted in less dramatic oxygen depletion.

Inconsistent production of ozone, caused by the presence of moisture in the air, affected the maintenance of consistent operating conditions within the pilot-scale unit. As was mentioned previously, moisture breakthrough significant enough to decrease ozone production occurred before it could be detected. Because the moisture content of the air source varied during the investigation, maintenance of a constant level of ozone production for a test run was impossible. For some unexplained reason, the decrease in ozone production was inversely related to the initial ozone production rate. Nevertheless, the minimum ozone production rate was still greater than that recommended for most tertiary-treatment applications.

VI. SUMMARY AND CONCLUSIONS

It was obvious from the results of this investigation that ozonated, secondary-treated, municipal sewage was not toxic to bluegill sunfish in a 96-hour period when the ozone was applied at concentrations up to 46 mg/l. Most municipal sewage-treatment facilities that use ozone for disinfection and for tertiary treatment apply ozone in concentrations much less than 46 mg/l, though higher dosages reportedly have been used for COD removal and, in one instance, for disinfection.

Before the conclusion can be made that all ozonated municipal waste effluents are non-toxic, more detailed investigations must be conducted. This study was only a preliminary investigation; therefore, these results can not be regarded as the final answer to the question of ozonated effluents. Bioassays should be run utilizing effluents exposed to higher ozone doses and longer contact times than are required for COD reduction. Future investigations also should be conducted utilizing other test organisms to insure that toxicity is not selective or that a particular organism is resistant.

Results of the effects of ozonation on chemical and physical characteristics of the municipal, secondary-treated effluent confirmed many results cited in other studies regarding the effectiveness of ozone as a tertiary treatment agent. Ozonation produces an effluent that is colorless, fresh smelling, and supersaturated with dissolved oxygen.

Turbidity also is reduced significantly and can be almost totally removed if filtration is used prior to ozonation. Substantial COD reduction is not possible at short contact times of five to ten minutes. Significant increases occur in pH and ammonia nitrogen during ozonation.

The results of this investigation also raised a question that was not resolved. An effect of suspended solids on the effectiveness of ozonation was suggested but was not confirmed by some of the observations made during the investigation. A future investigation of a possible relationship between ozone effectiveness and suspended solids concentration is warranted because such a relationship could have a marked effect on the disinfection properties of ozone.

Finally, from the experience and problems encountered in operating the continuous-flow system some conclusions were reached that might aid future studies. Some procedure should be adopted to prevent the deposition and accumulation of solids in the continuous-flow system. Sewage effluents containing even relatively small concentrations of solids can cause oxygen depletion in the system within 48 hours and interfere with the maintenance of consistent flow rates. Two procedures adopted during this investigation to overcome these problems were the periodic removal of solids from points of accumulation and the filtering of the effluent through glass wool prior to ozonation.

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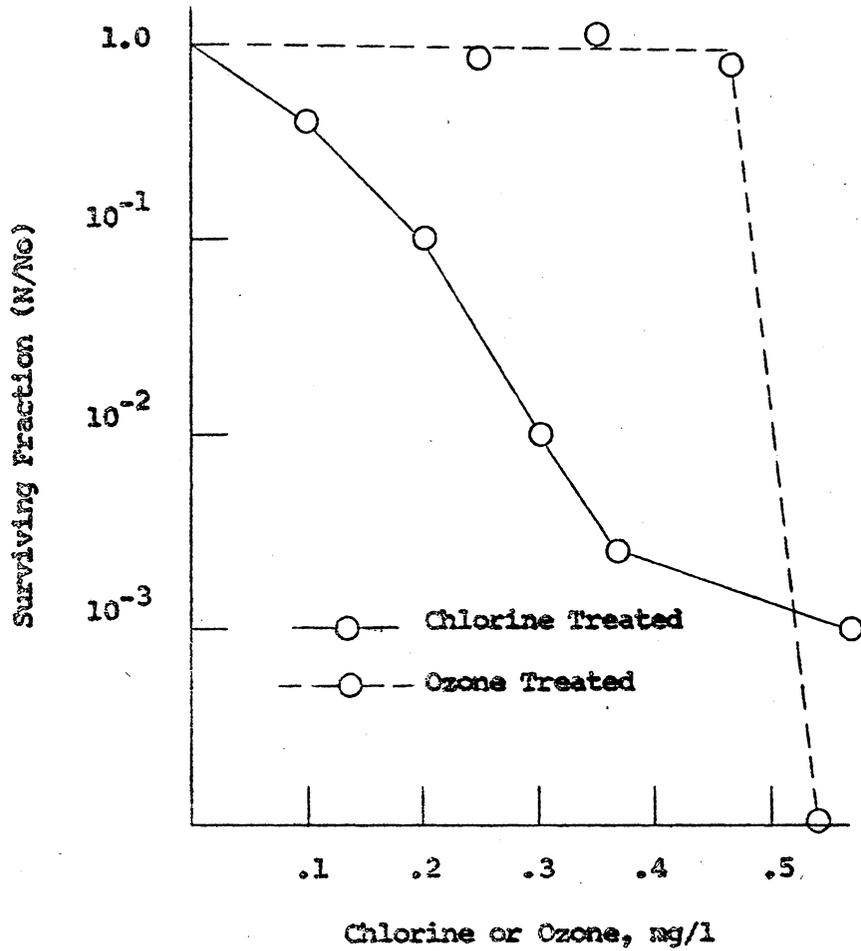
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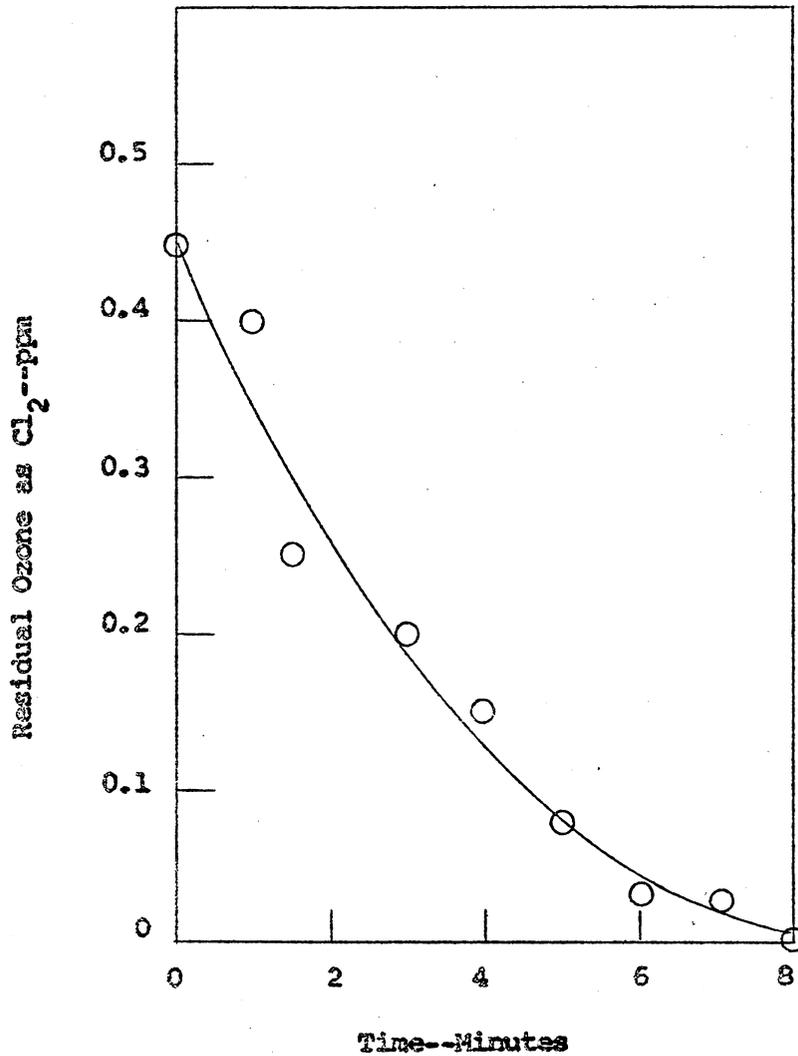
VIII. APPENDIX

APPENDIX A

SUPPLEMENTAL FIGURES ALLUDED TO IN TEXT



Appendix Figure 1.--Bactericidal Activity of
Ozone and Chlorine
(After Fetner and Ingols (3))



Appendix Figure 2.--Disappearance of Residual Ozone.

(After O'Donovan (15))

VIII. APPENDIX (Continued)

APPENDIX B

ANALYTICAL DATA COLLECTED DURING EXPERIMENTAL RUNS

Analytical Data--Run #1 (Control)

Run began--2:00 P.M.--6/27/72

10 fish--100% clarifier effluent

Sample #1--4:00 P.M.--6/27/72

COD	73.1 mg/l
S.S.	26 mg/l
Alkalinity	174.4 mg/l as CaCO ₃ (all bicarbonate)
Hardness	212 mg/l as CaCO ₃
pH	7.7
Dissolved Oxygen	Fish Tanks--7.0 mg/l
Temperature	22°C

Sample #2--8:00 A.M.--6/28/72

COD	63.5 mg/l
S.S.	16 mg/l
Alkalinity	168.5 mg/l as CaCO ₃ (all bicarbonate)
Hardness	166 mg/l as CaCO ₃
pH	7.5
Dissolved Oxygen	Fish Tanks--5.0 mg/l
Temperature	22°C

Analytical Data--Run #1 (Continued)

Sample #3--4:00 P.M.--6/28/72

COD	36.5 mg/l
S.S.	24 mg/l
Alkalinity	150.9 mg/l as CaCO ₃ (all bicarbonate)
Hardness	166 mg/l as CaCO ₃
pH	7.4
Dissolved Oxygen	Fish Tanks--5.0 mg/l
Temperature	22°C

Sample #4--8:00 A.M.--6/29/72

COD	34.6 mg/l
S.S.	12 mg/l
Alkalinity	162.7 mg/l as CaCO ₃ (all bicarbonate)
Hardness	bad sample
pH	7.7
Dissolved Oxygen	Fish Tanks--2.5 mg/l
Temperature	22°C
Observations	Pump stopped--replaced Storage reservoir--refilled with fresh water Fish tanks aerated ½ hour

Analytical Data--Run #1 (Continued)

Sample #5--4:00 P.M.--6/29/72

COD	105.8 mg/l
S.S.	36 mg/l
Alkalinity	150.9 mg/l as CaCO ₃ (all bicarbonate)
Hardness	160 mg/l as CaCO ₃
pH	7.7
Dissolved Oxygen	Fish Tanks--4.5 mg/l (#1), 5.0 mg/l (#2)
Temperature	23°C

Sample #6--8:00 A.M.--6/30/72

COD	53.8 mg/l
S.S.	26 mg/l
Alkalinity	135.2 mg/l as CaCO ₃ (all bicarbonate)
Hardness	166 mg/l as CaCO ₃
Dissolved Oxygen	Fish Tanks--2.5 mg/l
Temperature	23°C
Observations	Fish tanks aerated ½ hour

Analytical Data--Run #1 (Continued)

Sample #7--4:00 P.M.--6/30/72

COD	53.8 mg/l
S.S.	32 mg/l
Alkalinity	147 mg/l as CaCO ₃ (all bicarbonate)
Hardness	132.4 mg/l as CaCO ₃
pH	7.7
Dissolved Oxygen	Fish Tanks--4.5 mg/l
Temperature	23°C
Observations	Fish tanks aerated ½ hour

Sample #8--8:00 A.M.--7/1/72

COD	51.9 mg/l
S.S.	38 mg/l
Alkalinity	127.3 mg/l as CaCO ₃ (all bicarbonate)
Hardness	141.7 mg/l as CaCO ₃
pH	7.3
Dissolved Oxygen	Fish Tanks--4.5 mg/l
Temperature	22°C
Observations	Fish tanks aerated ½ hour

Run ended--2:00 P.M.--7/1/72--9 fish alive

Analytical Data--Run #2 (Ozonation)

Ozone production 7.66 mg/min.
 7.53 mg/min.
 7.32 mg/min.
Average 7.50 mg/min.

Ozone Dosage 18.79 mg/l

Bioassay began--10:00 A.M.--7/3/72

Sample #1--10:00 A.M.--7/3/72

COD Before--21.1 mg/l; After--9.6 mg/l

S.S. Before--12 mg/l; After--8 mg/l

Alkalinity Before--104 mg/l; After--104 mg/l as CaCO₃
(all bicarbonate)

Hardness 138.3 mg/l as CaCO₃

pH Before--7.5; After--7.9

Dissolved Oxygen Fish Tanks--7.3 mg/l
 Head Box----7.8 mg/l

Temperature 22.5°C

Observations Comparison of sewage before and after ozonation--
ozonated sewage much clearer; good reduction in
turbidity. All color removed. Odor is like ex-
tremely fresh air. Some foam on top of contact
chamber and at effluent discharge.

Analytical Data--Run #2 (Continued)

Sample #2--4:00 P.M.--7/3/72

COD Before--42.2 mg/l; After--42.2 mg/l

S.S. Before--34 mg/l; After--22 mg/l

Alkalinity Before--125.3 mg/l; After 117.5 mg/l as CaCO₃
(all bicarbonate)

Hardness 154 mg/l as CaCO₃

pH Before--7.6; After-- 8.1

Dissolved Oxygen Fish Tanks--8.0 mg/l
Head Box----8.0 mg/l

Temperature 23.5°C

Sample #3--8:00 A.M.--7/4/72

COD Before--57.6 mg/l; After--32.6 mg/l

S.S. Before--44 mg/l; After--35 mg/l

Alkalinity Before--117.5 mg/l; After--117.5 mg/l as CaCO₃
(all bicarbonate)

Hardness 157.1 mg/l as CaCO₃

pH Before--7.3; After--7.7

Dissolved Oxygen Fish Tanks--8.0 mg/l
Head Box----8.3 mg/l

Temperature 22.5°C

Observations Comparison of S.S. in before and after samples--
differences in appearance--solids in ozonated
sewage seem to be much finer and more difficult
to settle than raw effluent solids. Almost micro-
scopic in ozonated effluent.

Analytical Data--Run #2 (Continued)

Sample #4--4:00 P.M.--7/4/72

COD Before--57.6 mg/l; After--40.3 mg/l

S.S. Before--34 mg/l; After--28 mg/l

Alkalinity Before--127.3 mg/l; After--119.5 mg/l as CaCO₃
(all bicarbonate)

Hardness 154 mg/l as CaCO₃

pH Before--7.4; After--7.9

Dissolved Oxygen Fish Tanks--7.9 mg/l
Head Box----8.5 mg/l

Temperature 23.5°C

Observations No measurable O₂ residual after test.

Sample #5--8:00 A.M.--7/5/72

COD Before--26.9 mg/l; After--19.2 mg/l

S.S. Before--10 mg/l; After--8 mg/l

Alkalinity Before--123.5 mg/l; After--121.5 mg/l as CaCO₃
(all bicarbonate)

Hardness 163 mg/l as CaCO₃

Dissolved Oxygen Fish Tanks--5.0 mg/l
Head Box----7.8 mg/l

Temperature 23°C

Observations 4 fish are dead; red splotches around eyes and
gills. Fish tanks aerated ½ hour. Filter
media changed--moisture breakthrough.

Analytical Data--Run #2 (Continued)

Sample #6--4:00 P.M.--7/5/72

COD	Before--61.5 mg/l; After--63.5 mg/l
S.S.	Before--24 mg/l; After--27 mg/l
Alkalinity	Before--133 mg/l; After--133 mg/l as CaCO ₃ (all bicarbonate)
Hardness	163 mg/l as CaCO ₃
pH	Before--7.6; After 8.0
Dissolved Oxygen	Fish Tanks--7.0 mg/l Head Box----7.8 mg/l
Temperature	22°C

Sample #7--8:00 A.M.--7/6/72

COD	Before--82.7 mg/l; After--53.0 mg/l
S.S.	Before--82 mg/l; After--56 mg/l
Alkalinity	Before--125.3 mg/l; After--123.5 mg/l as CaCO ₃ (all bicarbonate)
Hardness	169.5 mg/l as CaCO ₃
pH	Before--7.7; After--8.1
Dissolved Oxygen	Fish Tanks--6.0 mg/l Head Box----8.1 mg/l
Temperature	22.5°C
Observations	No measurable ozone residual

Analytical Data--Run #2 (Continued)

Sample #8--4:00 P.M.--7/6/72

COD	Before--50.9 mg/l; After--40.3 mg/l
S.S.	Before--bad data; After--bad data
Alkalinity	Before--127.3 mg/l; After--123.5 mg/l as CaCO ₃ (all bicarbonate)
Hardness	154 mg/l as CaCO ₃
pH	Before--7.7; After--8.1
Dissolved Oxygen	Fish Tanks--6.0 mg/l Head Box----7.3 mg/l
Temperature	22.5°C
Run ended--10:00 A.M.--7/7/72--6 fish alive	
Ozone production	5.94 mg/min. 6.00 mg/min.
Ozone dose	14.9 mg/l
Observations	All six surviving fish had red spots like hemorrhaging around eyes and gills.

Analytical Data--Run #3 (Continued)

Sample #2--8:00 A.M.--7/14/72

COD	Before--62 mg/l; After--52 mg/l
S.S.	Before--17 mg/l; After--12 mg/l
Alkalinity	Before--109.8 mg/l; After--109.8 mg/l as CaCO ₃ (all bicarbonate)
Hardness	141.7 mg/l as CaCO ₃
pH	Before--7.0; After--7.4
Dissolved Oxygen	Fish Tanks--5.0 mg/l Head Box----7.8 mg/l
Temperature	23.5°C
Observations	All 10 fish showed signs of hemorrhaging. Filter media changed at 11:00 A.M. Solids siphoned from bottom of fish tanks.

Sample #3--4:00 P.M.--7/14/72

COD	Before--75.2 mg/l; After--66 mg/l
S.S.	Before--48 mg/l; After--48 mg/l
Alkalinity	Before--125.4 mg/l; After--117.6 mg/l as CaCO ₃ (all bicarbonate)
Hardness	135.5 mg/l as CaCO ₃
pH	Before--7.1; After--7.5
Dissolved Oxygen	Fish Tanks--5.9 mg/l Head Box----7.6 mg/l
Temperature	24°C

Analytical Data--Run #3 (Continued)

Sample #4--8:00 A.M.--7/15/72

COD Before--53.2 mg/l; After--58.0 mg/l

S.S. Before--45 mg/l; After 47 mg/l

Alkalinity Before--115.6 mg/l; After--113.6 mg/l as CaCO₃
(all bicarbonate)

Hardness 141.7 mg/l as CaCO₃

pH Before--7.0; After--7.45

Dissolved Oxygen Fish Tanks--5.5 mg/l
Head Box----7.8 mg/l

Temperature 24.5°C

Observations Filter media changed at 10:00 A.M.

Sample #5--4:00 P.M.--7/15/72

COD Before--69.6 mg/l; After--69.6 mg/l

S.S. Before--45 mg/l; After--45 mg/l

Alkalinity Before--119.6 mg/l; After--117.6 mg/l as CaCO₃
(all bicarbonate)

Hardness 141.7 mg/l as CaCO₃

pH Before--7.2; After--7.6

Dissolved Oxygen Fish Tanks--5.5 mg/l (#1), 6.0 mg/l (#2)
Head Box----7.8 mg/l

Temperature 24°C

Analytical Data--Run #3 (Continued)

Sample #6--8:00 A.M.--7/16/72

COD Before--92.1 mg/l; After--45.1 mg/l

S.S. Before--9 mg/l; After--7 mg/l

Alkalinity Before--107.8 mg/l; After--107.8 mg/l as CaCO₃
(all bicarbonate)

Hardness 141.7 mg/l as CaCO₃

pH Before--7.1; After--7.6

Dissolved Oxygen Fish Tanks--5.1 mg/l
Head Box----7.4 mg/l
Sewage-----3.3 mg/l

Temperature 24°C

Observations Before--Ammonia-N--4.2 mg/l as N
After---Ammonia-N--4.6 mg/l as N
When samples taken flow was about 200 ml/min. or
low enough to have about 10 minutes contact time--
virtually no visible suspended solids.

Sample #7--4:00 P.M.--7/16/72

COD Before--77.1 mg/l; After--74.4 mg/l

S.S. Before--19 mg/l; After--15 mg/l

Alkalinity Before--115.6 mg/l; After--111.7 mg/l as CaCO₃
(all bicarbonate)

Hardness Not measured

pH Before--7.25; After--7.55

Dissolved Oxygen Fish Tanks--5.0 mg/l
Head Box----7.2 mg/l
Sewage-----3.7 mg/l

Temperature 24°C

Analytical Data-- Run #3 (Continued)

Sample #3--8:00 A.M.--7/17/72

COD	Before--23.8 mg/l; After--24.1 mg/l
S.S.	Before--10 mg/l; After--8 mg/l
Alkalinity	Before--90.2 mg/l; After--91.0 mg/l as CaCO ₃ (all bicarbonate)
Hardness	Not measured
pH	Before--7.1; After--7.3
Dissolved Oxygen	Fish Tanks--3.8 mg/l Head Box----7.0 mg/l Sewage-----3.6 mg/l
Temperature	24°C

Run ended--12:00 Noon--7/17/72--10 fish alive

Ozone production	4.90 mg/min. 4.32 mg/min. <u>4.26 mg/min.</u>
Average	4.82 mg/min.
Ozone dose	12.0 mg/l

Analytical Data--Run #4 (Ozonation)

Ozone production 13.8 mg/min.
 12.1 mg/min.

Settings of ozonator the same--production the same

Ozone dose 52.0 mg/l

Flow rate 250 ml/min.

Bioassay began--9:30 A.M.--7/21/72

Sample #1--10:00 A.M.--7/21/72

COD Before--48.5 mg/l; After--35.8 mg/l

S.S. Before--26 mg/l; After--32 mg/l

pH Before--7.4; After--7.8

Dissolved Oxygen Fish Tanks--6.8 mg/l
 Head Box----8.1 mg/l
 Sewage-----6.8 mg/l

Temperature 25°C

Observations Before---Ammonia-N--4.3 mg/l
 After----Ammonia-N--5.0 mg/l
 Before---BOD--60 mg/l
 After----BOD--42 mg/l
 Effluent from the contact chamber is crystal
 clear. Compared to tap water--both look the
 same.

Analytical Data--Run #4 (Continued)

Sample #4--5:00 P.M.--7/22/72

COD	Before--57.8 mg/l; After--51.0 mg/l
S.S.	Before 20 mg/l; After--18 mg/l
pH	Before--7.4; After--7.9
Dissolved Oxygen	Fish Tanks--5.6 mg/l (#1); 4.5 mg/l (#2) Head Box----8.0 mg/l Sewage-----5.2 mg/l
Temperature	25°C

Run ended--9:30 A.M.--7/23/72

Ozone production	10.1 mg/min. 9.9 mg/min.
Average	<u>10.0 mg/min.</u> 10.0 mg/min.
Ozone dose	40.0 mg/l

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THE TOXICITY OF OZONATED SECONDARY-TREATED MUNICIPAL

WASTE EFFLUENT TO BLUEGILL SUNFISH

by

Brian L. Wheeler

(ABSTRACT)

The toxicity of an ozonated, secondary-treated municipal waste effluent to bluegill sunfish was investigated in a continuous-flow bioassay system. Four bioassay experiments were conducted, one with untreated secondary effluent and three with ozonated, secondary effluent. Ozone was applied at dosages between 12.1 and 52.0 mg/l for contact times between five and ten minutes. Several waste parameters were measured periodically throughout the investigation to determine the effects of ozonation on the secondary-treated effluent.

No toxicity was demonstrated during any of the bioassays. Analytical data demonstrated that ozone treatment of secondary effluents resulted in large reductions in color, turbidity and odor, but only small reductions in COD. Increases in pH and ammonia nitrogen concentrations were observed.