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Biological Stability of Drinking Water Through Ozonation at the Croton Water Supply in New York City



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Prepared for Reclamation Under Agreement No. 1425-5-FC-81-20450

by

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Bureau of Reclamation
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The study was carried out at the Jerome Park Water Treatment Demonstration Plant owned and operated by NYCDEP. The plant personnel constructed all the attached growth rate estimate (AGRE) units used in the study, monitored the operating parameters of the plant processes, and maintained the plant. The assimilable organic carbon (AOC) and heterotrophic plate count (HPC) analyses were carried out by the staff of the Central NYCDEP laboratories at LeFrak City.

The City College group consisted of graduate research assistants of the Department of Civil Engineering involved in collection and analysis of samples for chemical water quality parameters during the process experiments. The attached bacterial colony counts on disks were carried out by Minna G. Duarte of the Department of Biology at City College.

Acronyms

AGRE	attached growth rate estimate
AOC	assimilable organic carbon
BDOC	biodegradable dissolved organic carbon
CaCO ₃	calcium carbonate
CDC	Center for Disease Control and Prevention
Ct	contact time
CuSO ₄	copper sulfate
DEF	diatomaceous earth filtration
DOC	dissolved organic carbon
ft ²	square foot
gpm	gallons per minute
HPC	heterotropic plate count
JPWTDP	Jerome Park Water Treatment Demonstration Plant
L/hr	liters per hour
MCL	maximum contaminant level
mg	milligrams
mgd	million gallons per day
mg/L	milligrams per liter
min/L	minutes per liter
NO ₃ -N	nitrates
NYC	New York City
NYCDEP	New York City Department of Environmental Protection
ppb	parts per billion
psi	pounds per square inch
SFP	El Sobrante Filtration Plant
TOC	total organic carbon
TTHM	total trihalomethanes
USEPA	U.S. Environmental Protection Agency
UV	ultraviolet
μ	τ / t
/cm ²	per square centimeter
°C	degrees Celsius
%	percent

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1. Executive Summary

The effect of ozonation and diatomaceous earth filtration (DEF) on the potential of a water supply to support growth of bacteria was investigated for the Croton water supply of New York City. This is a phenomenon that occurs following disinfection and within the distribution system independent of size. Waters that do not contain the necessary nutrients and, thus, cannot support bacterial growth have low bacterial regrowth potential; while waters that support post disinfection growth have high regrowth potential. The study reported herein evaluated the effect of ozonation, a disinfection technology widely used in Europe and steadily gaining ground in the United States on post-disinfection bacterial regrowth potential of drinking water. Currently, the procedures used to determine the bacterial growth potential in water are based on liquid phase procedures even though bacterial regrowth is known to occur as attached biofilm on the inner walls of distribution pipes. Biostability, in this study, was evaluated using the attached growth rate estimate (AGRE) method which relies on measurement of growth rates of attached bacterial cells. This novel approach is considered much simpler than those currently employed by water utilities. The study was carried out jointly by the Environmental Engineering Group at City College of the City University of New York and the Division of Drinking Water Quality, Bureau of Water Supply, Quality and Protection of the New York City Department of Environmental Protection (NYCDEP). The ozonation experiments were carried out at the Jerome Park Water Treatment Demonstration Facility, owned and operated by NYCDEP.

Croton water presents levels of organic carbon that could be conducive to significant biological activity after ozonation at Ct (disinfectant residual concentration•contact time) values greater than 0.5 milligrams (mg)•minutes per liter (min/L). DEF does not reduce biological regrowth potential in ozonated Croton water to the levels found in raw Croton water. Factors that could not be fully explained in the experiments carried out in this study, influenced filtration to produce finished water of highly variable quality. Biological filtration or other similar processes appear necessary to reduce the biological regrowth potential of the DEF effluent and avoid expected problems in the distribution system.

The attached growth rate as measured by the AGRE method was reduced by ozonation of the tested water through reduced presence of heterotrophic plate count (HPC) bacteria and reduced bacterial attachment efficiencies. Although the exact reasons for decreased attachment to rotating disks could not be explained, it is surmised that bacterial injury and the high total organic carbon (TOC) concentrations in tested waters may have played a role. Scatter in both assimilable organic carbon (AOC) and AGRE results was significant, suggesting that difficulties were experienced with the experimental methods and indicating the complexity of assessing biological stability of drinking water.

Experiments should be designed to determine the dynamic behavior of DEF and to better understand its effectiveness when it is used in combination with biological filtration. Such a study would provide valuable information that can be used to predict the biological regrowth potential of finished Croton water after ozonation is employed as a treatment process.

2. Introduction

Chlorine is the most common secondary disinfectant used in potable water disinfection. Research has shown that maintenance of a chlorine residual cannot be relied on to totally prevent bacterial occurrences in distribution systems. Nagy et al. (1982) observed biofilm growth in the Los Angeles aqueduct with a chlorine residual of 1-2 milligrams per liter (mg/L). Episodes of excessive bacterial presence in distribution systems have also been well documented (Ridgway and Olson, 1981; Characklis, 1988). Furthermore, pipe surfaces in distribution systems are heavily colonized by microorganisms, up to concentrations as high as 10^8 cells per square centimeter ($/\text{cm}^2$) (Donlan and Pipes, 1986), and popular diversity (or the number of different species that are part of the bacterial population) has been shown to increase as water flows from the treatment plant through the distribution system (LeChevallier et al., 1987). Characklis (1988) calculated that drinking water with 5 mg/L total organic carbon (TOC) flowing through a pipe could provide the sufficient amount of nutrients to support a large bacterial population attached to the wall ($10^5 - 10^6$ cells/ cm^2), even though nutrient concentrations are very small.

Excessive number of bacteria have been associated with operational problems, such as pipe corrosion (Victoreen, 1984), reduced fluid flow (Characklis and Cooksey, 1983), and nonbacteriological water quality problems, in particular, taste, odor, and appearance problems (van der Wende and Characklis, 1990). Moreover, if large numbers of coliform bacteria are detected, the water utility may be in violation of drinking water standards, and a potential health hazard may exist. The increase in waterborne diseases in recent years is often attributed to opportunistic pathogenic bacteria, such as *Mycobacterium*, *Pseudomonas*, *Aeromonas sp.* (LeChevallier and McFeters, 1985).

The problems associated with bacterial regrowth in drinking water distribution systems have been brought to focus in recent years by the latest regulatory developments. The very nutrients (particularly total organic carbon), which may stimulate biofilm regrowth in a distribution system pipe, may also combine with the disinfectant utilized and create undesirable disinfection byproducts. Many water utilities will have to modify their disinfection practices in response to recently promulgated and future water quality regulations like the Surface Water Treatment Rule (U.S. Environmental Protection Agency [USEPA], 1989a) and the Coliform Rule (USEPA, 1989b) which imposed stricter standards on microbiological parameters of water quality with inclusion of not only coliform bacteria but also heterotrophic microorganisms. These requirements will call for lower bacterial counts which, however, cannot be met by simply increasing the dose of chlorine, the most popular disinfectant, since the levels of chlorination byproducts will be strictly regulated in the proposed Disinfectants and Disinfection Byproducts Rule (USEPA, 1994).

The concerns presented in the previous section call for assessment of the carrying capacity of drinking water and for evaluation of its potential to support bacterial growth in a distribution system. Such growth, often termed “regrowth,” can be defined as an increase of viable bacterial cell concentrations as a result of growth in the distribution system downstream of the disinfection process (Characklis, 1988). Factors influencing microbial growth include temperature, pH, disinfectant residual, hydrodynamic conditions, and availability and type of nutrients in the water. To reduce or eliminate bacterial regrowth, water entering a distribution system should be “biologically stable,” or low enough in nutrients so as to repress bacterial growth.

The objectives of this study were:

- Evaluation of the effect of ozonation on the potential for biological regrowth in the Croton water supply of New York City using the attached growth rate estimate (AGRE) method.
- Correlation of water quality parameters with the biological growth potential in the water ozonated under various experimental conditions.

3. Conclusions and Recommendations

The conclusions and recommendations of the study are as follows:

- Croton water presents levels of organic carbon that could support significant biological activity after ozonation at contact time (*Ct*) values greater than 0.5 milligrams (mg)•minute per liter (min/L).
- Diatomaceous earth filtration (DEF) does not reduce biological regrowth potential in ozonated Croton water to the levels found in raw Croton water. Factors that could not be fully explained in the experiments, carried out in this study, influenced filtration to produce finished water of highly variable quality. Biological filtration or other similar processes appear necessary to reduce the biological regrowth potential of the DEF effluent and avoid expected problems in the distribution system.
- AGRE was reduced by ozonation of the tested water through reduced presence of heterotrophic plate count (HPC) bacteria and reduced bacterial attachment efficiencies. Although the exact reasons for decreased attachment to rotating disks could not be explained, it is surmised that bacterial injury and the high TOC concentrations in tested waters may have played a role.
- Scatter in both assimilable organic carbon (AOC) and AGRE results was significant, suggesting that difficulties were experienced with the experimental methods and indicating the complexity of assessing biological stability of drinking water.
- Experiments should be designed and carried out to determine the dynamic behavior of DEF and to understand in which ways it should be implemented in combination with biological filtration. Such a study would provide valuable information that can be used to predict the biological regrowth potential of finished Croton water after ozonation is employed as a treatment unit.

4. Biological Stability of Drinking Water

In recent years, several methods have been developed to evaluate the potential of drinking water to support biological activity. These methods have been reviewed by Huck (1990). They can be divided into two broad categories depending on the parameter measured. In the first group, measuring AOC, a sample of water is inoculated with bacteria and incubated (van der Kooij et al., 1982; Werner, 1984; Kemmy et al., 1989). After incubation, the number of bacterial cells grown in suspension is determined through standard microbiological procedures such as plate counts, direct counts, or turbidity measurements. The second group of methods (Joret and Levi, 1986; Servais et al., 1989) uses biodegradable dissolved organic carbon (BDOC) as an indicator of water biostability. In this approach, a sample of water is contacted with a bacterial population. The amount of organic matter assimilated by bacteria is determined from the difference between initial and final dissolved organic carbon (DOC) concentrations.

4.1 “Attached Growth” Methods in Biostability Evaluations

Despite the importance of bacterial association with surfaces, none of the methods for assessment of water biostability described in the previous section—namely AOC or BDOC—directly addresses this issue. Rather, they either rely completely on activities of suspended-growth cells; or if attached biomass is employed, its activity is deduced from water phase measurements. However, microbial activities in drinking water distribution systems take place almost exclusively on surfaces. Environmental conditions in a drinking water distribution system are unfavorable to microbial growth in suspended form due to factors such as low nutrient concentration, relatively short detention time, and presence of disinfecting agents. Rather, it is through attachment to and growth on surfaces, such as pipe walls, sediments, or suspended particles, tubercles, or flocs, that bacterial populations can survive and colonize drinking water distribution systems. By attaching to surfaces, microorganisms are protected from washout and can survive and colonize drinking water distribution systems. Attached bacteria also appear to be less affected by disinfecting agents (LeChevallier et al., 1984; Herson et al., 1987). This could be attributed to reduced mass transfer and chemical oxidation of extracellular polymeric substances that form a matrix binding the cells to the surface. Thus, microbial attachment and surface colonization are the most important factors contributing to the microbiological degradation of drinking water between the points of disinfection and consumption. An alternative method for assessment of biostability in water that relies on bacterial counts on surfaces rather than the liquid phase is the AGRE method (Hermanowicz et al., 1991) described below. This approach was

utilized throughout the study. Other methods, such as the “annular reactors” used in the evaluation of factors influencing biofilm growth in distribution systems (Camper, 1996), also can be used for the same purpose.

4.2 The AGRE Method for Assessment of Drinking Water Stability

The AGRE method, proposed by Hermanowicz et al. (1991), is based on direct determination of attached growth rates of bacteria indigenous to the particular distribution system in the assessment of biostability in drinking water. The bacteria grow attached to the surface of a rotating disk made of the same material as the pipes of the distribution system. The description of the experimental apparatus, a summary of the experimental protocol, and modelling of bacterial growth is given below (Hermanowicz et al., 1991).

4.2.1 Experimental Apparatus

It consists of a disk, 2.25 inches in diameter, mounted at the end of the shaft of a variable speed motor. The shaft extends into a 2-gallon cylindrical autoclavable polypropylene jar which is fitted with an inlet at the bottom and an outlet at the top for continuous flow operation. The volume of the water in the jar is about 1.8 gallons, and the flow rate is about 0.01 gpm (2.3 L/hr). The disk is rotated at appropriate speeds to ensure laminar flow regime, while the hydrodynamic shear stress varies between zero at the center to what it would be on the walls of the pipe in the distribution system in question at the edge of the disk.

4.2.2 Experimental Protocol

The disks are thoroughly washed with detergent, rinsed five times in deionized water, and soaked in an acidic solution of potassium dichromate overnight to oxidize any organic material on the surface. The disks are again rinsed five times with deionized water, autoclaved for 30 minutes at 121 degrees Celsius (°C) and stored in closed sterile containers. The disks are then mounted in a rotating disk system and exposed to the water for the test for 48 hours. Longer contact times resulted in excessive growth on the disk which rendered enumeration of the colonies difficult. Following the testing period, the disks were removed from the experimental apparatus, and the attached bacterial cells were enumerated.

4.2.3 Enumeration Method

The surface of the disk is stained with acridine orange solution for 5 minutes and then air dried. One-, two-, and four-cell bacterial colonies in randomly selected viewfields on a section of the disk are then counted under ultraviolet (UV) light through an epifluorescent microscope. Hermanowicz et al. (1991) indicated that the only problems encountered during the tests were in a few

cases when surface colonization progresses to such extent that even individual cells were too numerous to count.

4.2.4 Modeling of Attached Bacterial Growth Using the AGRE Method

The model proposed by Caldwell et al., (1983) for microbial colonization was expanded by the authors from steady state to transient conditions, and made suitable for estimation of microbial growth. According to the model, the rate of change of one-cell colonies (C_1) can be expressed by the difference between the attachment rate A of single cells and their growth rate μ (μ):

$$dC_1/dt = A - \mu C_1$$

Similarly, the rate of change of numbers of two-cell colonies (C_2) is equal to the difference between the growth rate of single-cell colonies and the growth rate of two-cell colonies which are transformed into four-cell colonies:

$$dC_2/dt = \mu C_1 - \mu C_2$$

Analogous equations can be developed for the number of four-cell colonies (C_4), eight-cell colonies (C_8), and so on. This set of equations allows the calculation of the number of colonies of various sizes as a function of time for specified attachment rate A , bacterial growth rate μ , and initial number of cells on the surface. The ratios of C_2/C_1 ; C_4/C_1 ; C_8/C_1 ; . . . ; C_i/C_1 are only dependent on dimensionless time $\tau = \mu t$, and are independent of the attachment rate as depicted in figure 1 of Appendix II. Thus, by examining observed ratios C_i/C_1 (in practice limited to two- and four-cell colonies) after a period of time (t), it is possible to find a corresponding value of τ for figure 1 and to estimate the bacterial growth rate μ ($\mu = \tau / t$). If the C_i/C_1 ratios are available for two different colony sizes (e.g., C_2/C_1 and C_4/C_1), two estimates of μ are also available.

The AGRE method has been used to assess biostability of drinking water (Hermanowicz et al., 1991; Price et al., 1992), to study the effects of full- and pilot-scale biological filtration (Jolis et al., 1992; Hermanowicz et al., 1992), and to monitor water biostability in distribution systems. Hermanowicz et al (1991), compared the estimated growth rates obtained with the AGRE approach with AOC measurements of settled water, ozone contactor, and biological filter effluents using the van der Kooij method (van der Kooij et al., 1982) with both *Pseudomonas fluorescens* P17 and Spirillum sp. NOX strains. The samples for AOC analyses were taken at the beginning of each week immediately before the start of the AGRE test. A good correlation was obtained between the growth rate μ and averaged AOC *Pseudomonas fluorescens* P17 concentrations for nonozonated filters and for ozonated filters. In comparing growth rates supported by ozonated and nonozonated filter effluents, Hermanowicz et al., (1991) observed that, for the same AOC_{P17} concentrations, the ozonated effluents yielded

significantly higher growth rates. This can be attributed to the breakdown of organic matter to more readily biodegradable forms as a result of ozonation.

5. The Croton Water Supply System of New York City

The daily drinking water consumption in New York City (NYC) is about 1.5 billion gallons. The sources of the water are the Croton, Catskill, and Delaware watershed areas located to the north and northwest of NYC and cover approximately 2,000 square miles. The water flows to NYC through aqueducts; 97 percent reaches homes and businesses through gravity alone, and only 3 percent must be pumped to its final destination.

5.1 The Croton System

Among the three watershed-aqueduct systems, the Croton system is located just north of NYC and consists of 12 reservoirs with a total storage capacity of 93.4 billion gallons of water with safe yield of about 240 million gallons per day (mgd). The flows between reservoirs are in open streams which affect quality of the water. Croton water is used generally in low-lying parts of the Bronx and Manhattan, but some is pumped to the high-level service areas normally supplied by the Catskill and Delaware systems. Croton water is also used extensively by several communities in Westchester County.

The proximity of the Croton water system to NYC has made it vulnerable to water quality problems. Population growth and increasing residential development on the Croton watershed in the suburban counties of Westchester and Putnam, combined with the age and trophic state of its reservoirs, has led to slow, steady deterioration of the water quality. Increasing turbidity, color, taste, and odor levels have indicated the need for further treatment of the water prior to distribution. Although the quality of the Croton water system is generally good, additional treatment will be necessary to meet increasingly stringent water quality standards. NYC and State health agencies, recognizing the potential future water quality problems, agreed upon the need for treating the Croton water further. To investigate the effectiveness of the proposed treatment processes on the Croton water, a demonstration pilot plant was constructed adjacent to the Jerome Park Reservoir in the Bronx where extensive studies were carried out between 1989 and 1991.

5.2 The Distribution System

Through a series of riser shafts along the NYC tunnels, water is distributed in NYC from the tunnels to a 425-mile network of trunk mains ranging in size from 24 to 48 inches in diameter and supplying a 5,700-mile network of distribution mains which range in size from 6 to 20 inches. Water is distributed to the

approximately 800,000 buildings and 97,000 fire hydrants at a nominal pressure at the curb of about 40 pounds per square inch (psi).

Croton water has always met all Federal and State primary drinking water standards. Historically however, there have been quality concerns of aesthetic nature involving color, taste, and odor that occur between July and September each year. The Croton system is nutrient rich and naturally productive. Many of the reservoirs, including the terminal New Croton Reservoir, are eutrophic. Their productivity reaches a maximum during the summer months which coincides with the peak demand period and the most frequent consumer complaints. In recent years, the reservoir had to be taken out of the system entirely as a result of the aforementioned occurrences. Seasonal changes in the Croton water quality characteristics are relatively constant and can be anticipated from year to year. However, short-term upsets attributed to increase in the rate of consumption or unexpected occurrences in the watershed or within the reservoir itself can result in sudden changes in water quality and perhaps violations of drinking water standards usually manifested as higher color and turbidity readings. Such events are unpredictable and are attributed wholly or in part to the periodic presence of excessive quantities of plankton and amorphous matter. Finally, quarterly analyses of Croton water revealed that under current disinfection practices, the revised maximum contaminant level (MCL) for total trihalomethanes (TTHM) of 80 parts per billion (ppb) will be met, while the MCL of haloacetic acids (HAA5) of 60 ppb will most likely not be met. To use Croton water throughout the year, the aforementioned observations have to be confirmed during the high productivity period at the New Croton Reservoir.

5.3 Current Treatment and Disinfection Practices in the Croton System

Croton water is soft (total hardness of 60 mg/L as calcium carbonate [CaCO₃]), has low alkalinity and near neutral pH (6.8-7.3) and is subjected to in-line treatment at various locations of the system. Croton Fall and Muscoot Reservoirs have provisions for copper sulfate (CuSO₄) addition to curtail or prevent algae growth in the water. At New Croton Lake Reservoir, the water is disinfected with chlorine at a dose of 2 mg/L right before entering the gravity aqueduct. There is also provision for copper sulfate treatment at this location. The water is fluoridated at Dunwoodie (Yonkers) at a level of 1 mg/L and flows about 24 miles through the New Croton Aqueduct to Jerome Park Reservoir in the Bronx where there are provisions for further CuSO₄ treatment. The water is re-chlorinated at a level of 1 mg/L prior to distribution to NYC. Furthermore, the water is treated with 1 mg/L of ortho-phosphate for lead and copper corrosion control.

5.4 Bacterial Growth Incidents in the New York City Water Distribution System

The MCL for drinking water samples which are positive for coliform bacteria is 5.0 percent (%) of the required monthly compliance samples collected within the distribution system. Under normal conditions in NYC, the percentage that test positive for coliform bacteria is usually less than 1.0%; and since August 1985, the monthly percentage has never exceeded the MCL. However in the summer months, between June and August, the number of samples which are positive for coliform bacteria in NYC's distribution system increases. Three years—1986, 1991, and 1993—had 1 month each which exceeded the 3.0% level. In 1994, both July and August had 2.5% of the compliance samples testing positive for coliform bacteria.

In June 1993, New York City Department of Environmental Protection (NYCDEP) began to study bacteria in biofilms within the distribution system of New York City. In preliminary samples obtained by high velocity flushing of a water main, four species of environmental bacteria were identified: *Pseudomonas fluorescens*, *Acinetobacter iwofii*, *Comamonas testosteroni*, and *C. acidovorans*. The most common genera of bacteria found in biofilms exposed to free chlorine are *Pseudomonas*, *Comamonas*, and *Hydrogenophaga* (LeChevallier et al., 1993). Coliform bacteria, including *E. coli*, have been isolated from biofilms (LeChevallier, 1990). However, no coliform bacteria were identified in these samples.

An initial attempt to isolate biofilms directly from a water main pipe was unsuccessful. On August 12, 1993, a section of 8-inch-diameter, 70-year-old water main pipe was excised from Bay Ridge, Brooklyn, where 10 days previously, a positive *E. coli* event was reported. The pipe was sent to the New York State Department of Health laboratories in Albany for analysis. No microorganisms were detected on this pipe. The techniques utilized probably were not adequate to isolate the organisms. In August 1994, twice (nonconsecutive) positive *E. coli* samples were collected in Coney Island, Brooklyn. On September 7, 1994, a section of an 8-inch water main was excised. Samples were collected aseptically in the field to minimize the possibility of losing the biofilm or contaminating it during transport of the pipe to the laboratory. Out of 24 samples collected, 3 samples were positive for coliform bacteria, and identified by the NTF system as *Aeromonas hydrophila* (one sample) and the CDC group iv c-2 (two samples). This was the first confirmation of coliform bacteria in any biofilm within the distribution system of New York City.

5.5 The Jerome Park Water Treatment Demonstration Plant (JPWTDP)

The JPWTDP consists of three separate 1-mgd ozone/diatomaceous earth filter/biologically active carbon (O₃/DEF/BAC) trains for a total flow of 3 mgd. Table 2 lists the principal components of the plant. Each train consists of six ozone contactors in series designated with letters A through F, one DE filter, and one biologically active carbon (BAC) column, as shown in figure 2 of Appendix II. Train 1 has ozone contactors that operate with turbine mixers for ozone diffusion, while trains 2 and 3 employ ceramic disc diffusers to introduce ozonated air to water. The piping and flow controls allow for routing the process air and water flow through as many columns as needed. All experiments were carried out in train No. 3. The contactors are of stainless steel, 22 feet high and 3 feet in diameter. When operated at 12-foot depth and 1-mgd flow, each contactor provides a residence time of 1 minute; while at a water depth of 20 feet, the contact time increases to 1.5 minutes. Thus, the total contact time through the ozonation system can vary between 6 to 9 minutes at 1 mgd and can be increased even further by reducing the feed flow to the train. The off-gas from all ozone contactors in a train are directed by a common discharge line to a catalytic ozone destruction unit prior to discharge to the atmosphere. The DE filters (U.S. Filter), studied to a limited extent herein, are pressured horizontal tanks, 260-square-foot (ft²) vertical leaf type and are capable of treating 1-mgd flow at a liquid loading rate as high as 2.7 gallons per minute per square foot. The filtration run is halted when the pressure drop across the filter reaches 25 psi.

6. Experimental Methods

An experimental run consisted of operating treatment Train No.3 for 24 or 48 hours. The principal process equipment of treatment Train 3 is listed on table 1 in Appendix I. The air preparation system for the ozone generators was started several hours prior to each run. The combinations of contact columns that received ozonated air were varied during each run to achieve a wide range of Ct (ozone residual x contact time) levels. Steady-state operation of the process was achieved within a few hours. During this period, frequent analysis of liquid phase ozone residual was carried out in ozonated water withdrawn from the contact columns while the gas phase ozone monitors on the ozone feed line were attended to, ensuring constant production of ozone for the duration of the experiment. Despite occasional disruptions of the air drying system, for the most part, the plant operation was consistent.

An AGRE unit was positioned in the effluent line of the contactors of interest following establishment of steady-state operation. Figure 3 in Appendix II is a schematic of the diagram of an AGRE unit operating in continuous flow mode. A 10-liter holding tank, operating on overflow basis and a residence time of about 30 minutes, was placed between the ozone contactors and the AGRE units to ensure complete decay of ozone residual in the feed water stream prior to entering the AGRE unit and avoid the influence of active ozone residual on the growth rate estimate. A peristaltic pump (Masterflex, Cole-Parmer) maintained the flow into the AGRE unit at the desired level. The effluent of the AGRE unit and of the holding tank were discharged to the drain. Process performance was continuously monitored by frequent measurement of temperature and ozone residual at each AGRE location.

Water samples were collected at a minimum of twice a day from all locations connected to an AGRE unit for:

- Microbiological analyses that included AOC, HPC, as well as total and *E coli*, all carried out by NYCDEP microbiology personnel
- Conventional water quality parameters that included pH, chlorine residual, alkalinity, conductivity, turbidity, nitrite, nitrate, ammonia, TOC, and total phosphorous

Sample collection, preservation, handling, and analysis were carried out according to standard operating procedures of NYCDEP as well as the “Standard Methods” (American Water Works Association- [AWWA] American Public Health Association- [APHA] Water Environment Federation [WEF], 1992). Identification of the AGRE units was made by assigning a location number to each one for each experiment. This designation has been used in all figures and

tables presented at the end of the report. For the nine process experiments carried out in this study, the AGRE identification was as follows:

Experiment No. 1: (Results shown in tables 3, 12, and figure 4)

Location No.	Description
1	Plant Influent (Jerome Reservoir Water)
2	Effluent of Contactort C
3	Effluent of Contactor E
4	Effluent of Contactor F
5	Effluent of DE Filter

Experiments No. 2 and 3: (Results shown in tables 4, 5, 13, 14, and figures 5, 6)

Location No.	Description
1	Plant Influent (Jerome Reservoir Water)
2	Effluent of Contactor B
3	Effluent of Contactor C
4	Effluent of Contactor D
5	Effluent of Contactor E
6	Effluent of Contactor F
7	Effluent of DE Filter

Experiments No. 4 through 9: (Results shown in tables 6 through 11, 15 through 20, and figures 7 through 12).

Location No.	Description
1	Plant Influent (Jerome Reservoir Water)
2	Effluent of Contactor A
3	Effluent of Contactor B
4	Effluent of Contactor C
5	Effluent of Contactor D
6	Effluent of Contactor F
7	Effluent of DE Filter

The experiments were carried out between February and August 1996, thus covering the water quality conditions of Croton water as well as the late summer case where most of the high color and turbidity incident occur.

7. Effect of Ozonation on the Biostability of Croton Water

The operating conditions in treatment Train No. 3 during each of the nine experiments are summarized in table 2 of Appendix I. Experiments 1, 2, and 3 were carried out for 48 hours, while 4 through 9 were run for 24 hours at water flow rates ranging between 620 and 900 gpm (0.9-1.3 mgd). Tables 3 through 11 in Appendix I list the results of analyses for conventional water quality parameters on at least two samples collected in the duration of each experiment. Tables 12 through 20 in Appendix I list the results of microbiological analyses for experiments 1 through 9, respectively. One sample per experiment was subjected to microbiological analyses. Finally, the variation of AOC, TOC, HPC, μ , and Ct through ozonation and DEF are shown in figures 4 through 12 of Appendix II, respectively.

7.1 Influent Croton Water

The AOC measurements for raw Croton water (RCW) ranged from 0 to 47 $\mu\text{g/L}$, and the AGRE ranged from 0 to 0.676 d^{-1} , with no correlation detected between the two parameters confirming observations reported earlier in other similar studies (Hermanowicz, et al., 1991). Readings of zero AOC and zero AGRE were discarded before further analysis based on ample evidence that waters with organic carbon concentration in the nanogram level repeatedly showed AOC and AGRE measurements different than zero (Jolis, 1992), thus suggesting that zero readings were either the result of laboratory mishandling of the sample/test, ozone interfering with the bioassay, or other unidentified problems, but not representative of the true carrying capacity of the tested waters. Although dynamic conditions in the Croton water system will presumably change over time, averaging AOC and AGRE observations is appropriate as detection of the effect of treatment is being sought (i.e., ozonation) which should occur regardless of water quality or at least not much modified by it. In other words, effects of treatment are exerted on stable, comparable conditions, that of the particular RCW quality existing during each experiment.

The modified set of AOC ranged between 11 and 47 $\mu\text{g/L}$ while AGRE ranged from 0 to 0.676 d^{-1} , with the following average values for the period tested (between February and August 1996):

$$\begin{aligned} \text{AOC}_{\text{avg}} &= 26.7 \pm 12.9 \mu\text{g/L} \\ \text{AGRE}_{\text{avg}} &= 0.311 \pm 0.174 \text{d}^{-1} \end{aligned}$$

These results indicate a moderate capacity for RCW to sustain biological activity. Median AOC concentrations of 25 µg/L were reported in a survey of Dutch surface waters (van der Kooij, et al., 1982) with TOC contents comparable to those found in RCW, while AOC concentrations below 20 µg/L were found in settled water at the El Sobrante Filtration Plant, California (Jolis, 1992) which treats Sierra Nevada water with reportedly very low concentrations of organic matter. Moreover, AOC levels indicative of biostability in finished (drinking) water have been determined in the range of 10 to 15 µg/L. The substantial scatter in the data indicates that dynamic conditions in the system are quite mutable, although the inherent variability in the experimental methods must be taken into account when interpreting results.

7.2 Effluent of Ozonation Process

The effect of ozonation on the biological activity in Croton water was also analyzed. Data from Tables 12 to 20 have been analyzed in three groups of Ct as follows:

$$\begin{aligned} Ct \leq 0.5 & \quad \text{mg} \cdot \text{min/L} \\ 0.5 < Ct \leq 1.0 & \quad \text{mg} \cdot \text{min/L} \\ Ct > 1.0 & \quad \text{mg} \cdot \text{min/L} \end{aligned}$$

These three groupings were selected to assess possible ozonation effects at different Ct values and to ensure that enough data points were available in each range. The AGRE tests were affected by the low levels of the HPC bacteria in ozonated waters, but some trends became apparent from the available results. For $Ct \leq 0.5 \text{ mg} \cdot \text{min/L}$ range, the average AGRE value is:

$$\text{AGRE}_{\text{avg}} = 0.192 \pm 0.103 \text{ d}^{-1}$$

which is substantially lower than the average value for RCW and presents much higher scatter. Similarly for AOC, the average of all nonzero values within the same ozone range was determined to be:

$$\text{AOC}_{\text{avg}} = 23.2 \pm 18.2 \text{ } \mu\text{g/L}$$

which is only slightly smaller than the average AOC value for RCW and statistically not discernable from it due to the scatter in the results. These findings suggest that for Croton water, ozone applied at $Ct \leq 0.5 \text{ mg} \cdot \text{min/L}$ failed to increase the biodegradability of the organic components present to a point that was measurable with either of the two experimental methods employed. This is in apparent disagreement with previously published experimental results that showed ozonated water promoting more biological activity than the same nonozonated water. However, a linear relationship between AOC and ozone doses below 1.5 mg/L has been reported (van der Kooij et al., 1982), suggesting

that ozone effects at low ozone doses may not be detectable in the field and which would help explain some of the data generated in this work. In addition, the conditions at which AGRE tests were carried out affected results when ozonated water was tested, as AGRE values were consistently lower after rather than before ozonation.

Similar analyses were also performed for the other two Ct ranges with the following results when all nonzero points are used:

For $0.5 < Ct \leq 1.0 \text{ mg} \cdot \text{min} / \text{L}$

$$\begin{aligned} \text{AOC}_{\text{avg}} &= 31.8 \pm 15.4 \text{ } \mu\text{g/L} \\ \text{AGRE}_{\text{avg}} &= 0.334 \pm 0.327 \text{ d}^{-1} \end{aligned}$$

For $Ct > 1.0 \text{ mg} \cdot \text{min} / \text{L}$

$$\begin{aligned} \text{AOC}_{\text{avg}} &= 36.4 \pm 19.7 \text{ } \mu\text{g/L} \\ \text{AGRE}_{\text{avg}} &= 0.294 \pm 0.092 \text{ d}^{-1} \end{aligned}$$

A positive progression is apparent on AOC averages with increasing Ct values, and a less clear cut trend is also noticeable for AGRE results (in AGRE, the effect of ozone on the bioassay must be taken into account; and as ozone Ct values increase, the number and extent of injuries on bacteria would most likely be greater. Thus, the time for recovery and subsequent growth reduces the AGRE_{avg} for higher Ct values indicating that ozone increased the potential for biological activity in Croton water as tested for $Ct \geq 0.5 \text{ mg} \cdot \text{min} / \text{L}$. Furthermore, the AOC values do not consistently peak over the RCW measurements for $Ct \leq 1.0 \text{ mg} \cdot \text{min} / \text{L}$. Less obvious but parallel observations were also made for AGRE.

7.3 Effluent of Diatomaceous Earth Filtration (Postozonation)

AGRE tests on DEF effluent failed in many cases to produce results due to lack of attachment of bacteria to the rotating disk. The exact reason for this problem is not known, but it can be surmised that the presence of residual ozone played an important role on the outcome. Indeed, for the majority of the experiments, the first injection of ozone brought about a sharp decline in HPC recovered from tested waters (to values ranging from 1 to 3 colony forming units per milliliter), some of which may have impaired attachment ability due to injury (Pringle and Fletcher, 1983). The net effect would be a very slow rate of bacterial attachment that prevented the consistent observation of biomass on the randomly chosen locations on the rotating disks. Similar problems have been reported when running AGRE tests on ozonated water. Inoculation with indigenous bacteria was necessary to get consistent results (Hermanowicz, et al., 1991).

Despite the experimental problems, three AGRE readings are available. Two of them are high (0.79 and 1.25 d⁻¹) and associated with higher than average HPC concentrations, perhaps suggesting that biomass sloughed off the filter or broke through, being present at high enough numbers to attach and grow on the disk surface. Conditions of higher than average temperature, TOC, or turbidity were looked for but did not occur in combination with high AGRE measurements, thus suggesting that abnormal water quality could not be held accountable for increased biological activity in tested water. The other AGRE value is low (0.131 d⁻¹) and comparable to levels encountered in RCW. The data suggest that AGRE tests were interfered with by the application of ozone to tested water even though the AGRE setup provided a 30-minute contact time for the feed stream prior to entering the AGRE unit to destroy the active ozone residual. Thus, the last data point underestimates the real carrying capacity of DEF effluent which would then be higher than the reading for RCW. AGRE results measured on ozonated waters ranged between 0.47 to 1.12 d⁻¹ at the El Sobrante Filtration Plant (SFP), California (Jolis, 1992)—results that show considerable agreement with AGRE measurements on ozonated Croton water. However, unlike in this work, the AGRE at SFP were always higher than measurements gained with settled, nonozonated water. Perhaps the fact that the RCW has a higher TOC content than the SFP effluent could help explain the lesser impact of ozonation on biological activity, as the background (prior to ozonation) bacterial growth was already substantial, and potential increases due to ozonation could not be successfully detected with the experimental method employed.

The AOC test was not so much affected by DEF water characteristics, and there are more data points available—two of which are zero readings and are discarded from the analysis. The range of AOC in the DEF effluent is 6-66 µg/L, with an average value of 31.5 ± 19.6 µg/L. Although the average values of AOC in the RCW and DEF effluent might not be statistically different due to the large scatter in the data, all but one AOC measurements in DEF effluent are higher than the AOC levels observed in RCW in the same experiments. These findings strongly suggest (and are supported by AGRE results) that DEF effluent is less biologically stable than RCW, and that DE filtration is not sufficient in itself to reverse the multiplying effect of ozone on the biodegradability of the organic components in this water. Moreover, the potential for extreme AOC and AGRE values is greater in DEF than RCW, indicating that DE filter performance was not uniform during the course of the experimental period and that unknown water and filter operation related factors affected it in ways that are unpredictable. Finally, comparing AOC values from the effluent of DEF with average AOC levels for the later two *Ct* ranges, it is confirmed that DE filtration does not reduce biological activity potential to pre-ozonation levels.

Therefore, the potential for bacterial growth in DEF is higher than with RCW, but the dynamic variations are large and, thus, more difficult to predict and control. The exact reasons for this cannot be derived from the current experiments, but the small number of HPC most often recovered from tested waters suggests that bacterial colonization of DE filters failed to occur, circumstance that may be

attributable to the filter itself, to the fact that ozonated water was being filtered, to the dynamics of filtration, or to combinations of all the above. Be that as it may, given the available evidence, it is recommended that biological filtration (e.g., an activated carbon filter where an attached consortium of HPC bacteria has evolved) be included after the DE filter to reduce the potential for problems with bacterial regrowth in the distribution system that should otherwise be expected.

The large scatter in the data together with the problems encountered with experimental protocols as reflected with many zero AOC and AGRE readings are indicators of the difficulties in assessing biological stability even in fairly controlled conditions as was the case in the study reported herein. No correlation is apparent between any of the two measurements of biological activity in Croton water and standard water quality parameters that include TOC, nitrates ($\text{NO}_3\text{-N}$), total P, pH, alkalinity, temperature, or turbidity. Similar conclusions were reported in a survey of biological activity in several United States drinking water distribution systems (LeChevallier, et al., 1988). Observed changes in total P (one experiment), $\text{NO}_3\text{-N}$ (three experiments), and turbidity (three experiments) did not correlate with the AGRE measurements.

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Appendix I — Tables

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Table 1. Principal Process Equipment at the Jerome Park Water Treatment Demonstration Facility

Description	Quantity	Size	Capacity
Raw Water Pumps	4	15hp	385-700 gpm
Air Preparation	2		9.6 scfm
Ozone Generators (Positive Pressure)	2		50 lbs/day @5.5% by wt
Ozone Contact Columns	6	18 ft (ID = 3 ft)	
Ozone Destruct Unit	1	1.5 hp	90 scfm
Diatomaceous Earth (DE) Filters	3	280 sf 1-2.5 gpm/sf	200-700 gpm
Carbon Adsorption Columns	6	ID = 7 ft H = 14 ft	125 gpm 3 gpm/sf
DE Recovery Unit lbs/3hrs	1	20 cyclones	1,400
Vacuum Filter	1	ID = 3 ft	2,100 lbs/5hrs

Table 2. Operational Conditions of Ozonation Experiments

Exper. No	Date	Water flow rate (GPM)	Ozone conc. in feed gas (% by weight)	Gas flow rate (CFM)	Average residual ozone in columns (mg/L)					
					A	B	C	D	E	F
1	02.27.96-02.29.96	800	0.42	28	-	-	0.20	0.20	0.20	0.22
2	03.19.96-03.21.96	700	0.65	27	-	0.20	0.20	0.40	0.25	0.50
3	04.30.96-05.02.96	700	0.10	25	-	0.01	0.00	0.01	0.00	0.10
4	06.04.96-06.05.96	700	0.37	22	0.35	0.30	0.22	0.21	-	0.16
5	06.05.96-06.06.96	700	0.42	22	0.40	0.39	0.26	0.25	-	0.19
6	06.18.96-06.19.06	700	0.26	30	0.25	0.25	0.16	0.19	-	0.14
7	07.09.96-07.10.96	900	0.50	19	0.32	0.33	0.20	0.21	-	0.18
8	07.23.96-07.24.96	700	0.30	30	0.33	0.34	0.18	0.20	-	0.15
9	08.14.96-08.15.96	620	0.44	28	0.70	0.62	0.54	0.50	-	0.45

Table 3 Water Quality Characteristics at Jerome Demonstration Plant in Ozonation Experiment No. 1

Date of Sample	Location	Field Temp (°C)	Sampling Time	Chlorine Residual (mg/L)	pH	Alkalinity as CaCO ₃ (mg/L)	Total P (mg/L)	Conductivity (µmhos/cm)	NH ₃ -N (mg/L)	NO ₂ -N (mg/L)	NO ₃ -N (mg/L)	Total Carbon (mg/L)	Total Inorganic Carbon (mg/L)	Total Organic Carbon (mg/L)	Turbidity (NTU)
2/27/96	1	5.5	8:00PM	BDL	7.15	40.7	0.100	240	0.03	BDL	0.45	15.73	12.03	3.70	0.7
	2	5.0	9:10PM	BDL	7.24	41.0	0.092	230	0.02	BDL	0.47	15.82	12.0	3.82	1.0
	3	5.0	9:15PM	BDL	7.25	40.9	0.108	235	0.03	BDL	0.47	15.62	12.02	3.60	0.4
	4	5.0	9:20PM	BDL	7.27	40.9	0.091	238	BDL	BDL	0.49	15.76	12.16	3.60	0.2
	5	5.5	9:30PM	BDL	7.25	40.9	0.102	250	0.01	BDL	0.49	15.34	11.82	3.52	0.2
2/28/96	1	9.5	10:30AM	BDL	7.14	40.6	0.095	255	0.05	BDL	0.48	15.41	12.26	3.15	0.3
	2	5.5	10:30AM	BDL	7.15	40.1	0.097	265	0.06	BDL	0.50	15.89	12.60	3.29	0.65
	3	5.8	10:35AM	BDL	7.14	39.3	0.107	275	0.04	BDL	0.47	15.22	11.93	3.29	0.7
	4	5.5	10:40AM	BDL	7.18	40.1	0.101	260	0.04	BDL	0.50	15.53	12.25	3.28	0.5
	5	5.5	10:45AM	BDL	7.14	40.3	0.105	265	0.04	BDL	0.48	15.44	12.13	3.31	0.01
2/28/96	1	6.0	4:40PM	BDL	7.21	42.5	0.046	238	0.02	BDL	0.48	15.15	12.19	2.96	0.6
	2	5.0	4:45PM	BDL	7.16	40.9	0.079	245	0.03	BDL	0.48	15.39	11.97	3.42	1.0
	3	5.0	4:48PM	BDL	7.18	41.2	0.094	250	0.03	BDL	0.47	14.41	11.95	2.46	0.7
	4	5.0	4:52PM	BDL	7.15	41.5	0.086	240	0.04	BDL	0.48	14.84	11.74	3.10	0.3
	5	6.0	4:57PM	BDL	7.18	41.4	0.112	240	0.04	BDL	0.48	14.27	11.71	2.56	0.1

Table 3. Water Quality Characteristics at Jerome Demonstration Plant in Ozonation Experiment No. 1 (continued)

Date of sample	Location	Field Temp (°C)	Sampling Time	Chlorine Residual (mg/L)	pH	Alkalinity as CaCO ₃ (mg/L)	Total P (mg/L)	Conductivity (µmhos/cm)	NH ₃ -N (mg/L)	NO ₂ -N (mg/L)	NO ₃ -N (mg/L)	Total Carbon (mg/L)	Total Inorganic Carbon (mg/L)	Total Organic Carbon (mg/L)	Turbidity (NTU)
2/29/96	1	10.0	10:25AM	BDL	7.18	40.8	0.098	235	0.04	BDL	0.48	14.59	12.16	2.96	0.5
	2	4.5	10:25AM	BDL	7.21	41.3	0.096	260	0.05	BDL	0.48	15.24	11.87	3.37	0.6
	3	6.5	10:30AM	BDL	7.15	41.1	0.099	260	0.04	BDL	0.42	15.59	11.90	3.69	1.0
	4	4.5	10:35AM	BDL	7.18	40.8	0.098	255	0.05	BDL	0.50	15.74	12.00	3.74	0.4
	5	6.0	10:40AM	BDL	7.22	40.5	0.108	260	0.05	BDL	0.49	14.53	11.84	2.69	0.01
2/29/96	1	5.0	3:00PM	BDL	7.22	41.5	0.098	260	0.04	BDL	0.46	14.91	11.72	3.19	0.85
	2	5.0	3:02PM	BDL	7.18	40.8	0.096	250	0.03	BDL	0.48	15.33	12.48	2.85	1.15
	3	6.0	3:07PM	BDL	7.19	42.1	0.101	245	0.05	BDL	0.45	13.03	10.06	2.97	1.05
	4	5.0	3:10PM	BDL	7.19	41.3	0.095	258	0.01	BDL	0.49	15.13	12.44	2.69	0.9
	5	5.0	3:15PM	BDL	7.25	41.7	0.098	265	0.03	BDL	0.49	14.90	11.72	3.18	0.52

Location 1: Jerome Park Demonstration Plant Influent
 Location 2: Croton Water at Ozonation Column C
 Location 3: Croton Water at Ozonation Column E
 Location 4: Croton Water at Ozonation Column F
 Location 5: Croton Water at Effluent of Diatomaceous Earth Filtration Process
 BDL: Below detectable limit

Table 4. Water Quality Characteristics at Jerome Demonstration Plant in Ozonation Experiment No. 2

Date of Sample	Location*	Field Temp (°C)	Sampling Time	Chlorine Residual (mg/L)	pH	Alkalinity as CaCO ₃ (mg/L)	Total P (mg/L)	Conductivity (µmhos/cm)	NH ₃ -N (mg/L)	NO ₂ -N (mg/L)	NO ₃ -N (mg/L)	Total Carbon (mg/L)	Total Inorganic Carbon (mg/L)	Total Organic Carbon (mg/L)	Turbidity (NTU)	
3/19/96	1	5.0	2:45PM	0.04	6.83	30.6	0.112	290	0.02	BDL	0.62	15.57	10.98	4.59	0.72	
	2	5.0	3:20PM	BDL	6.90	36.0	0.100	295	0.03	BDL	0.62	13.95	11.27	2.68	1.10	
	3	5.0	3:40PM	BDL	6.92	37.2	0.116	285	0.03	BDL	0.64	13.97	10.74	3.23	1.02	
	4	5.0	3:50PM	BDL	6.89	37.2	0.105	290	0.02	BDL	0.64	13.87	10.78	3.09	1.19	
	5	5.0	3:55PM	BDL	6.78	36.5	0.109	285	0.02	BDL	0.62	14.57	10.82	3.75	0.97	
	6	5.0	3:10PM	BDL	6.91	37.2	0.101	290	0.04	BDL	0.64	13.23	10.96	2.27	0.82	
	7	5.0	3:25PM	BDL	6.88	35.8	0.100	289	0.02	BDL	0.64	13.90	10.43	3.47	0.05	
3/20/96	1	5.0	10:15AM	BDL	6.91	34.7	0.108	280	0.03	BDL	0.54	15.05	10.43	4.62	1.48	
	2	5.0	10:20AM	BDL	6.87	35.8	0.102	290	0.02	BDL	0.64	14.14	10.69	3.45	0.90	
	3	5.0	10:25AM	BDL	7.01	38.1	0.108	285	0.03	BDL	0.65	14.66	10.17	4.49	0.95	
	4	5.0	10:30AM	BDL	6.88	37.5	0.112	290	0.04	BDL	0.64	15.26	10.26	5.00	0.97	
	5	5.0	10:35AM	BDL	6.90	36.8	0.108	290	0.03	BDL	0.62	12.46	8.76	3.70	0.92	
	6	5.0	10:40AM	BDL	6.95	37.8	0.105	290	0.03	BDL	BDL	BDL	13.29	9.49	3.80	1.02
	7	5.0	10:45AM	BDL	6.82	36.2	0.102	290	0.03	BDL	0.66	14.00	9.00	5.00	0.27	
3/20/96	1	5.0	4:15PM	BDL	6.84	32.7	0.109	255	0.10	BDL	0.65	13.33	9.13	4.20	0.57	
	2	5.0	4:20PM	BDL	6.81	32.4	0.092	275	0.03	BDL	0.65	13.40	10.20	3.20	0.79	
	3	5.0	4:25PM	BDL	6.82	32.8	0.092	280	0.05	BDL	0.66	13.52	9.72	3.80	0.90	
	4	5.0	4:30PM	BDL	6.78	33.1	0.107	275	0.03	BDL	0.66	12.52	9.42	3.10	0.52	
	5	5.0	4:35PM	BDL	6.88	32.6	0.095	285	0.05	BDL	0.65	12.40	9.50	2.90	0.58	
	6	5.0	4:40PM	BDL	6.78	32.3	0.110	280	0.06	BDL	0.67	13.20	9.40	3.80	0.62	
	7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 4. Water Quality Characteristics at Jerome Demonstration Plant in Ozonation Experiment No. 2 (continued)

Date of Sample	Location*	Field Temp (°C)	Sampling Time	Chlorine Residual (mg/L)	pH	Alkalinity as CaCO ₃ (mg/L)	Total P (mg/L)	Conductivity (µmhos/cm)	NH ₃ -N (mg/L)	NO ₂ -N (mg/L)	NO ₃ -N (mg/L)	Total Carbon (mg/L)	Total Inorganic Carbon (mg/L)	Total Organic Carbon (mg/L)	Turbidity (NTU)
3/21/96	1	5.0	9:45AM	BDL	6.78	33.0	0.108	270	0.03	BDL	0.65	13.68	9.68	4.00	1.25
	2	5.0	9:50AM	BDL	6.84	32.8	0.101	280	0.04	BDL	0.65	12.81	9.61	3.20	1.42
	3	5.0	9:55AM	BDL	6.82	32.2	0.100	278	0.03	BDL	0.65	12.62	9.62	3.00	1.12
	4	5.0	10:00AM	BDL	6.86	32.3	0.096	280	0.04	BDL	0.66	12.71	9.81	2.90	1.03
	5	5.0	10:05AM	BDL	6.85	32.9	0.098	270	0.02	BDL	0.65	13.32	9.42	3.90	1.20
	6	5.0	10:10AM	BDL	6.85	33.2	0.101	280	0.04	BDL	0.66	12.83	9.70	3.13	1.12
	7	5.0	10:15AM	BDL	6.87	32.8	0.102	280	0.03	BDL	0.65	12.54	9.72	2.82	0.50
3/21/96	1	5.0	2:00PM	BDL	6.92	33.3	0.099	245	0.04	BDL	0.63	12.99	9.48	3.51	0.98
	2	5.0	2:05PM	BDL	7.03	33.3	0.102	252	0.02	BDL	0.62	12.39	9.49	2.90	1.23
	3	5.0	2:10PM	BDL	7.02	34.1	0.103	252	0.03	BDL	0.62	12.19	9.34	2.85	0.68
	4	5.0	2:15PM	BDL	6.83	34.1	0.105	245	0.02	BDL	0.64	12.14	9.49	2.65	1.15
	5	5.0	2:20PM	BDL	7.13	34.2	0.098	260	0.04	BDL	0.57	12.64	9.37	3.25	1.10
	6	5.0	2:25PM	BDL	7.04	33.8	0.110	255	0.02	BDL	0.65	11.96	9.21	2.75	1.02
	7	5.0	2:30PM	BDL	6.93	33.8	0.109	252	0.01	BDL	0.66	11.89	9.21	2.68	0.15

Location 1: Jerome Park Demonstration Plant Influent

Location 2: Croton Water at Ozonation Column B

Location 3: Croton Water at Ozonation Column C

Location 4: Croton Water at Ozonation Column D

Location 5: Croton Water at Ozonation Column E

Location 6: Croton Water at Ozonation Column F

Location 7: Croton Water at Effluent of Diatomaceous Earth Filter

N/A: Not available

BDL: Below detectable limit

Table 5. Water Quality Characteristics at Jerome Demonstration Plant in Ozonation Experiment No. 3

Date of Sample	Location	Temp (C) field	Sampling Time	Chlorine residual (mg/L)	pH	Alkalinity as CaCO3 (mg/L)	Total P (mg/L)	Conductivity (μ mhos/cm)	NH ₃ -N (mg/L)	NO ₂ -N (mg/L)	NO ₃ -N (mg/L)	Total Carbon (mg/L)	Total Inorg. Carbon (mg/L)	Total Organic Carbon (mg/L)	Turbidity (NTU)
05.01.96	1	8	9:30am	0.02	6.9	36.6	0.234	270	0.01	BDL	0.52	17.86	13.62	4.24	0.54
	2	8	9:35am	0.08	6.6	41	0.226	265	0.05	BDL	0.52	18.07	14.46	3.61	0.78
	3	8	9:40am	0.05	6.9	38.5	0.08	265	0.01	BDL	0.52	18.47	13.57	4.9	0.52
	4	8	9:45am	0.05	7	39	0.341	270	0.07	BDL	0.53	18.98	14.86	4.12	0.54
	5	8	9:50am	0.08	6.9	38.5	0.097	265	0.02	BDL	0.51	18.12	14.3	3.82	0.6
	6	8	9:55am	0.02	6.9	37.9	0.163	265	BDL	BDL	0.52	18.87	14.47	4.4	0.3
	7	8	10:00am	0.02	6.9	38.2	0.086	265	0.03	BDL	0.53	18.1	13.98	4.12	0.07
05.01.96	1	8	6:00pm	0.05	6.9	38.8	0.222	250	0.01	BDL	0.53	12.92	9.84	3.08	0.5
	2	8	6:05pm	0.06	7	39.4	0.181	250	BDL	BDL	0.53	12.66	10.11	2.55	0.7
	3	8	6:10pm	0.06	7	38.8	0.16	250	0.01	BDL	0.54	13.07	10.34	2.73	0.5
	4	8	6:15pm	0.05	7	39.5	0.12	245	BDL	BDL	0.54	12.43	10.17	2.26	0.5
	5	8	6:20pm	0.05	7	40.9	0.116	250	BDL	BDL	0.46	12.73	9.98	2.75	0.3
	6	8	6:25pm	0.05	7	40.2	0.296	250	BDL	BDL	0.53	12.77	10.46	2.25	0.4
	7	8	6:30pm	0.05	7	39.9	0.16	250	BDL	BDL	0.54	12.72	9.98	2.68	0.1
05.02.96	1	8	9:30am	0.06	6.9	38.5	N/A	265	0.01	BDL	0.53	12.56	9.53	2.97	0.45
	2	8	9:35am	0.06	6.9	38.8	0.113	263	BDL	BDL	0.53	13.17	9.24	3.87	0.4
	3	8	9:40am	0.04	6.9	38.8	0.11	263	0.01	BDL	0.54	12.47	9.98	2.49	0.375
	4	8	9:45am	0.04	6.9	38.9	0.151	263	0.01	BDL	0.53	12.99	10.05	2.94	0.15
	5	8	9:50am	0.04	6.9	39.2	0.075	260	BDL	BDL	0.46	13.12	10.5	2.62	0.5
	6	8	9:55am	0.04	7	40.2	0.012	250	BDL	BDL	0.53	12.49	9.84	2.65	0.25

Location 1: Jerome Park Demonstration Plant Influent
 Location 2: Croton Water at Ozonation Column B
 Location 3: Croton Water at Ozonation Column C
 Location 4: Croton Water at Ozonation Column D
 Location 5: Croton Water at Ozonation Column E
 Location 6: Croton Water at Ozonation Column E
 Location 7: Croton Water at Effluent of Diatomaceous Earth Filter
 BDL: Below detectable limit
 N/A: Not available

Table 6. Water Quality Characteristics at Jerome Demonstration Plant in Ozonation Experiment No. 4

Date of Sample	Location	Field Temp (°C)	Sampling Time	Chlorine Residual (mg/L)	pH	Alkalinity as CaCO ₃ (mg/L)	Total P (mg/L)	Conductivity (µmhos/cm)	NH ₃ -N (mg/L)	NO ₂ -N (mg/L)	NO ₃ -N (mg/L)	Total Carbon (mg/L)	Total Inorganic Carbon (mg/L)	Total Organic Carbon (mg/L)	Turbidity (NTU)
06.04.96	1	15.0	2:40pm	BDL	6.73	40.0	0.091	170	0.014	BDL	0.49	13.03	10.52	2.51	*
	2	11.0	2:50pm	BDL	6.72	41.0	0.083	170	0.016	BDL	0.49	13.94	10.67	3.27	
	3	12.0	3:00pm	BDL	6.72	40.0	0.170	165	0.016	BDL	0.49	13.47	10.85	2.62	
	4	12.0	3:05pm	BDL	6.88	40.0	0.096	173	0.047	BDL	0.49	13.48	10.42	3.06	
	5	12.0	3:10pm	BDL	6.86	41.0	0.160	170	0.008	BDL	0.49	13.48	10.33	3.15	
	6	12.0	3:15pm	BDL	6.75	40.5	0.136	180	0.007	BDL	0.49	13.98	10.78	3.2	
	7	12.0	3:20pm	BDL	6.82	41.0	0.103	180	0.002	BDL	0.49	13.31	11.16	2.15	
06.05.96	1	17.0	10:30am	BDL	6.86	41.0	0.136	185	0.006	BDL	0.48	13.71	10.73	2.89	
	2	12.0	10:35am	BDL	6.88	41.0	0.155	185	0.005	BDL	0.49	13.29	10.96	2.33	
	3	12.0	10:40am	BDL	6.90	41.5	0.195	170	BDL	BDL	0.51	13.44	10.58	2.86	
	4	12.0	10:45am	BDL	6.92	41.3	0.148	180	0.014	BDL	0.53	13.44	10.01	3.43	
	5	12.0	10:50am	BDL	6.95	41.5	0.219	180	0.008	BDL	0.5	13.11	9.99	3.12	
	6	13.0	10:55am	BDL	6.91	41.5	0.057	190	0.006	BDL	0.5	13.72	10.35	3.37	
	7	12.0	11:00am	BDL	6.92	41.5	0.117	190	0.006	BDL	0.52	14.38	10.45	3.93	

Location 1: Jerome Park Demonstration Plant Influent

Location 2: Croton Water at Ozonation Column A

Location 3: Croton Water at Ozonation Column B

Location 4: Croton Water at Ozonation Column C

Location 5: Croton Water at Ozonation Column D

Location 6: Croton Water at Ozonation Column F

Location 7: Croton Water at Effluent of Diatomaceous Earth Filtration Process

* Turbidity measurements were not carried out

Table 7. Water Quality Characteristics at Jerome Demonstration Plant in Ozonation Experiment No. 5

Date of Sample	Location	Field Temp (°C)	Sampling Time	Chlorine Residual (mg/L)	pH	Alkalinity as CaCO ₃ (mg/L)	Total P (mg/L)	Conductivity (µmhos/cm)	NH ₃ -N (mg/L)	NO ₂ -N (mg/L)	NO ₃ -N (mg/L)	Total Carbon (mg/L)	Total Inorganic Carbon (mg/L)	Total Organic Carbon (mg/L)	Turbidity (NTU)
06.05.96	1	17.0	3:10pm	BDL	6.72	38.5	0.282	170	0.002	BDL	0.49	13.05	10.43	2.62	0.46
	2	13.0	3:10pm	BDL	6.85	41.0	0.144	170	0.004	BDL	0.50	13.66	10.73	2.93	0.42
	3	14.0	3:10pm	BDL	6.84	41.0	0.151	170	BDL	BDL	0.50	12.95	10.88	2.07	0.32
	4	14.0	3:10pm	BDL	7.02	42.0	0.159	170	0.042	BDL	0.50	13.27	10.53	2.74	0.20
	5	14.0	3:15pm	BDL	6.95	41.0	0.192	170	0.009	BDL	0.49	13.33	10.24	3.09	0.20
	6	13.0	3:15pm	BDL	6.99	41.5	0.140	175	0.002	BDL	0.51	13.62	10.83	2.79	0.10
	7	13.0	3:15pm	BDL	7.05	41.0	0.141	175	0.052	BDL	0.50	13.57	10.63	2.94	0.05
06.06.96	1	15.5	2:10pm	BDL	6.91	41.0	0.132	180	BDL	BDL	0.48	14.40	11.17	3.23	0.30
	2	11.0	2:15pm	BDL	6.97	41.3	0.147	185	BDL	BDL	0.50	13.91	11.04	2.87	0.10
	3	10.5	2:20pm	BDL	6.99	41.0	0.168	175	BDL	BDL	0.50	13.90	11.04	2.86	0.10
	4	11.0	2:25pm	BDL	6.99	41.1	0.125	180	BDL	BDL	0.50	14.03	11.45	2.58	0.05
	5	11.0	2:30pm	BDL	7.02	42.0	0.140	180	BDL	BDL	0.50	13.98	10.45	3.53	0.10
	6	11.5	2:35pm	BDL	7.01	42.0	0.149	190	BDL	BDL	0.50	13.61	11.48	2.13	0.23
	7	11.0	2:40pm	BDL	7.05	41.7	0.122	190	BDL	BDL	0.50	13.54	11.04	2.50	0.10

Location 1: Jerome Park Demonstration Plant Influent

Location 2: Croton Water at Ozonation Column A

Location 3: Croton Water at Ozonation Column B

Location 4: Croton Water at Ozonation Column C

Location 5: Croton Water at Ozonation Column D

Location 6: Croton Water at Ozonation Column F

Location 7: Croton Water at Effluent of Diatomaceous Earth Filtration Process

BDL: Below detectable limit

Table 8. Water Quality Characteristics at Jerome Demonstration Plant in Ozonation Experiment No. 6

Date of Sample	Location	Field Temp (°C)	Sampling Time	Chlorine Residual (mg/L)	pH	Alkalinity as CaCO ₃ (mg/L)	Total P (mg/L)	Conductivity (µmhos/cm)	NH ₃ -N (mg/L)	NO ₂ -N (mg/L)	NO ₃ -N (mg/L)	Total Carbon (mg/L)	Total Inorganic Carbon (mg/L)	Total Organic Carbon (mg/L)	Turbidity (NTU)
06.18.96	1	14.0	3:10pm	BDL	6.71	40.0	1.116	180	BDL	BDL	0.47	13.26	10.77	2.49	0.4
	2	13.0	3:10pm	BDL	6.76	42.0	0.515	190	BDL	BDL	0.49	12.81	10.66	2.15	0.37
	3	13.0	3:10pm	BDL	6.84	41.5	0.393	190	BDL	BDL	0.49	12.22	10.64	1.58	0.02
	4	13.0	3:10pm	BDL	6.96	42.0	0.304	192	BDL	BDL	0.48	13.11	10.55	2.56	0.03
	5	13.0	3:15pm	BDL	6.99	43.0	0.271	190	BDL	BDL	0.48	12.95	10.64	2.31	0.10
	6	13.0	3:15pm	BDL	6.98	42.5	0.169	190	BDL	BDL	0.48	12.87	10.62	2.25	0.03
	7	13.0	3:15pm	BDL	7.00	43.0	0.180	192	BDL	BDL	0.48	13.03	10.56	2.47	0.10
06.19.96	1	19.5	2:10pm	BDL	7.16	41.5	0.137	175	BDL	BDL	0.48	12.93	10.69	2.24	0.53
	2	14.0	2:15pm	BDL	7.03	42.0	0.115	175	BDL	BDL	0.48	12.73	10.40	2.33	0.10
	3	14.0	2:20pm	BDL	7.28	44.0	0.127	180	BDL	BDL	0.48	13.16	10.53	2.63	0.06
	4	14.0	2:25pm	BDL	7.50	43.1	0.100	180	BDL	BDL	0.48	12.88	10.55	2.33	0.10
	5	14.0	2:30pm	BDL	7.33	43.5	0.107	180	BDL	BDL	0.49	13.34	10.72	2.62	0.30
	6	14.0	2:35pm	BDL	7.23	43.2	0.091	180	BDL	BDL	0.49	13.33	10.70	2.63	0.50
	7	14.0	2:40pm	BDL	7.07	42.0	0.081	185	BDL	BDL	0.49	13.90	10.25	3.65	0.02

Location 1: Jerome Park Demonstration Plant Influent

Location 2: Croton Water at Ozonation Column A

Location 3: Croton Water at Ozonation Column B

Location 4: Croton Water at Ozonation Column C

Location 5: Croton Water at Ozonation Column D

Location 6: Croton Water at Ozonation Column F

Location 7: Croton Water at Effluent of Diatomaceous Earth Filtration Process

BDL: Below detectable limit

Table 9. Water Quality Characteristics at Jerome Demonstration Plant in Ozonation Experiment No. 7

Date of Sample	Location	Field Temp (°C)	Sampling Time	Chlorine Residual (mg/L)	pH	Alkalinity as CaCO ₃ (mg/L)	Total P (mg/L)	Conductivity (µmhos/cm)	NH ₃ -N (mg/L)	NO ₂ -N (mg/L)	NO ₃ -N (mg/L)	Total Carbon (mg/L)	Total Inorganic Carbon (mg/L)	Total Organic Carbon (mg/L)	Turbidity (NTU)
07.09.96	1	16.0	2:30pm	BDL	6.84	48.5	0.174	240	BDL	BDL	0.46	12.49	10.48	2.01	0.60
	2	14.5	2:35pm	BDL	6.84	48.2	0.236	245	BDL	BDL	0.47	13.09	10.56	2.53	0.10
	3	14.5	2:40pm	BDL	6.84	48.2	0.225	245	BDL	BDL	0.47	13.41	10.99	2.42	0.37
	4	14.5	2:45pm	BDL	6.89	47.5	0.200	250	BDL	BDL	0.47	13.58	10.90	2.68	0.50
	5	15.0	2:50pm	BDL	6.95	47.5	0.157	250	BDL	BDL	0.47	13.46	10.97	2.47	0.30
	6	15.0	2:55pm	BDL	6.84	46.5	0.158	250	BDL	BDL	0.48	13.66	11.03	2.63	0.46
	7	15.0	3:00pm	BDL	6.84	46.5	0.167	250	BDL	BDL	0.46	13.48	11.01	2.47	0.40
07.10.96	1	15.5	10:00am	BDL	6.73	38.5	0.221	255	BDL	BDL	0.46	12.84	10.80	2.04	0.77
	2	14.0	10:05am	BDL	6.67	41.0	0.208	250	BDL	BDL	0.48	13.06	10.30	2.76	0.77
	3	14.0	10:10am	BDL	6.64	42.5	0.191	255	BDL	BDL	0.48	13.88	11.24	2.64	0.40
	4	14.0	10:15am	BDL	6.63	45.5	0.183	255	BDL	BDL	0.47	13.12	10.56	2.56	0.60
	5	14.0	10:20am	BDL	6.76	46.0	0.165	250	BDL	BDL	0.48	13.22	10.75	2.47	0.53
	6	14.0	10:25am	BDL	6.89	47.0	0.229	255	BDL	BDL	0.48	13.48	10.99	2.49	0.53
	7	14.0	10:30am	BDL	6.90	48.0	0.141	255	BDL	BDL	0.48	13.57	10.85	2.72	0.40

Location 1: Jerome Park Demonstration Plant Influent

Location 2: Croton Water at Ozonation Column A

Location 3: Croton Water at Ozonation Column B

Location 4: Croton Water at Ozonation Column C

Location 5: Croton Water at Ozonation Column D

Location 6: Croton Water at Ozonation Column F

Location 7: Croton Water at Effluent of Diatomaceous Earth Filtration Process

BDL: Below detectable limit

Table 10. Water Quality Characteristics at Jerome Demonstration Plant in Ozonation Experiment No. 8

Date of Sample	Location	Field Temp (°C)	Sampling Time	Chlorine Residual (mg/L)	pH	Alkalinity as CaCO ₃ (mg/L)	Total P (mg/L)	Conductivity (µmhos/cm)	NH ₃ -N (mg/L)	NO ₂ -N (mg/L)	NO ₃ -N (mg/L)	Total Carbon (mg/L)	Total Inorganic Carbon (mg/L)	Total Organic Carbon (mg/L)	Turbidity (NTU)
07.23.96	1	16.5	2:30pm	BDL	6.58	42.5	0.262	200	0.02	BDL	0.4	22.87	17.0	5.87	0.9
	2	15.2	2:35pm	BDL	6.60	45.5	0.204	230	0.01	BDL	0.42	22.57	16.76	5.76	0.9
	3	15.2	2:40pm	BDL	6.65	45.0	0.184	240	0.02	BDL	0.41	22.39	16.86	5.50	0.6
	4	15.2	2:45pm	BDL	6.67	45.3	0.190	245	0.02	BDL	0.42	22.07	16.68	5.39	0.6
	5	15.2	2:50pm	BDL	6.68	46.2	0.195	250	BDL	BDL	0.41	22.62	16.63	5.99	0.8
	6	15.2	2:55pm	BDL	6.74	47.0	0.180	250	0.01	BDL	0.41	22.10	16.60	5.50	0.77
	7	15.5	3:00pm	BDK	6.82	46.5	0.176	250	0.01	BDL	0.41	21.66	16.28	5.38	0.4
07.24.96	1	16.0	10:00am	BDL	6.64	44.5	0.215	230	0.01	BDL	0.39	22.62	16.66	5.96	1.67
	2	15.5	10:05am	BDL	6.67	46.0	0.206	240	BDL	BDL	0.41	21.20	16.05	5.15	0.87
	3	15.2	10:10am	BDL	6.63	45.0	0.215	250	BDL	BDL	0.41	21.58	16.12	5.46	0.6
	4	15.2	10:15am	BDL	6.80	46.0	0.212	250	BDL	BDL	0.41	21.18	15.91	5.27	0.8
	5	15.5	10:20am	BDL	6.74	46.2	0.232	250	BDL	BDL	0.41	20.86	16.11	4.75	0.5
	6	15.5	10:25am	BDL	6.74	46.2	0.207	250	BDL	BDL	0.41	21.17	15.96	5.21	0.57
	7	15.5	10:30am	BDL	6.77	46.0	0.193	250	0.02	BDL	0.41	21.04	15.76	5.28	0.27

Location 1: Jerome Park Demonstration Plant Influent

Location 2: Croton Water at Ozonation Column A

Location 3: Croton Water at Ozonation Column B

Location 4: Croton Water at Ozonation Column C

Location 5: Croton Water at Ozonation Column D

Location 6: Croton Water at Ozonation Column F

Location 7: Croton Water at Effluent of Diatomaceous Earth Filtration Process

BDL: Below detectable limit

Table 11. Water Quality Characteristics at Jerome Demonstration Plant in Ozonation Experiment No. 9

Date of Sample	Location	Field Temp (°C)	Sampling Time	Chlorine Residual (mg/L)	pH	Alkalinity as CaCO ₃ (mg/L)	Total P (mg/L)	Conductivity (µmhos/cm)	NH ₃ -N (mg/L)	NO ₂ -N (mg/L)	NO ₃ -N (mg/L)	Total Carbon (mg/L)	Total Inorganic Carbon (mg/L)	Total Organic Carbon (mg/L)	Turbidity (NTU)
08.14.96	1	18.0	9:30am	0.31	6.35	39.2	0.208	150	BDL	BDL	0.32	18.45	14.54	3.91	1.0
	2	17.1	9:35am	0.13	6.40	37.3	0.230	160	BDL	BDL	0.35	16.21	13.62	2.59	1.0
	3	17.1	9:40am	0.11	6.42	37.7	0.170	150	BDL	BDL	0.35	17.15	14.48	2.67	1.0
	4	17.5	9:45am	0.12	6.47	38.0	0.180	190	BDL	BDL	0.36	16.90	14.69	2.21	1.0
	5	16.9	9:50am	0.11	6.46	38.4	0.176	175	BDL	BDL	0.35	17.15	14.59	2.56	1.0
	6	17.7	9:55am	0.13	6.47	40.0	0.137	200	BDL	BDL	0.35	17.91	14.99	2.92	1.0
	7	17.8	10:00am	BDL	6.57	39.2	0.166	150	BDL	BDL	0.35	16.82	13.81	3.01	1.0
	8	17.0	10:05am	BDL	6.75	34.8	0.144	190	BDL	BDL	N/A	N/A	N/A	N/A	N/A
08.15.96	1	18.5	5:00pm	0.24	6.51	39.0	0.217	220	BDL	BDL	0.33	16.95	15.1	1.85	0.43
	2	17.8	5:05pm	0.07	6.46	39.2	0.179	220	BDL	BDL	0.36	17.80	15.01	2.79	0.43
	3	17.5	5:10pm	0.07	6.44	38.0	0.214	210	BDL	BDL	0.36	17.85	14.55	3.30	0.3
	4	17.6	5:15pm	0.10	6.54	40.4	0.157	220	BDL	BDL	0.36	18.32	14.86	3.46	0.5
	5	17.2	5:20pm	0.06	6.46	39.2	0.146	215	BDL	BDL	0.36	17.66	15.1	2.56	0.3
	6	17.9	5:25pm	BDL	6.48	41.1	0.007	221	BDL	BDL	0.36	18.95	15.82	3.13	0.3
	7	18.0	5:30pm	BDL	6.70	41.5	0.096	219	BDL	BDL	0.36	18.33	14.35	3.98	0.2
	8	18.5	5:35pm	BDL	6.89	35.3	0.176	190	BDL	BDL	N/A	N/A	N/A	N/A	N/A

Location 1: Jerome Park Demonstration Plant Influent

Location 2: Croton Water at Ozonation Column A

Location 3: Croton Water at Ozonation Column B

Location 4: Croton Water at Ozonation Column C

Location 5: Croton Water at Ozonation Column D

Location 6: Croton Water at Ozonation Column F

Location 7: Croton Water at Effluent of Diatomaceous Earth Filtration Process

Location 8: Croton Water at Effluent of Biological Activated Carbon Process

BDL: Below detectable limit

N/A: Not available

Table 12. Microbiological Water Characteristics in Ozonation Experiment No. 1 at Jerome Park Water Treatment Demonstration Plant

Date of Sample	Location	Field Temp (°C)	Sampling Time	AOC (µg/L)	Total Coli (colonies/100mL)	E.Coli (colonies/100mL)	HPC1 (CFU/mL)	HPC2 (CFU/mL)	Growth Rates μ (d ⁻¹)
2/27/96	1	5.5	8:00PM	-	*	0	35	43	*
	2	5.0	9:10PM	250		0	1	0	
	3	5.0	9:15PM	250		0	0	0	
	4	5.0	9:20PM	61		0	1	0	
	5	5.5	9:30PM	11		0	0	0	
2/28/96	1	9.5	10:30AM	47		0	12	14	
	2	5.5	10:30AM	0		0	1	0	
	3	5.8	10:35AM	8		0	1	0	
	4	5.5	10:40AM	7		0	0	0	
	5	5.5	10:45AM	0		0	1	0	
2/29/96	1	10.0	10:25AM	0		0	54	62	0.354161 ± 0.332815**
									0.648986 ± 0.00000***
	2	4.5	10:25AM	9		0	6	3	
	3	6.5	10:30AM	0		0	2	4	0.117572 ± 0.00000**
									0.21267 ± 0.095098***
4	4.5	10:35AM	22		0	1	0		
5	6.0	10:40AM	0		0	1	0		

- Location 1: Croton Water at Jerome Park Demonstration Plant
- Location 2: Croton Water at Ozonation Process C
- Location 3: Croton Water at Ozonation Process E
- Location 4: Croton Water at Ozonation Process F
- Location 5: Croton Water at Effluent of Diatomaceous Earth Filtration Process

* Blank boxes represent total Coli and growth rate not measured

** Upper disc

*** Lower disc

Table 13. Microbiological Water Characteristics in Ozonation Experiment No. 2 at Jerome Park Water Treatment Demonstration Plant

Date of Sample	Location	Field Temp (°C)	Sampling Time	AOC (µg/l)	Total Coli (colonies/100mL)	E.Coli (colonies/100mL)	HPC 1 (CFU/mL)	HPC 2 (CFU/mL)	Growth Rates μ (d ⁻¹)	
									Upper disk	Lower disk
3.21.96	1	5.0	2:00PM	46	<1	*	1	2	0.065 ± 0.000	0.0000
	2	5.0	2:05PM	0	1		1	1	0.211 ± 0.032	0.217 ± 0.000
	3	5.0	2:10PM	0	<1				0.156 ± 0.086	0.181 ± 0.114
	4	5.0	2:15PM	0	<1				0.087 ± 0.000	0.0000
	5	5.0	2:20PM	46	<1		0	1	0.381 ± 0.000	0.0000
	6	5.0	2:25PM	7	<1		0	0	**	0.0000
	7	5.0	2:30PM	8	<1					

Location 1: Croton Water at Jerome Park Water Treatment Demonstration Plant

Location 2: Croton Water at Ozonation Column B

Location 3: Croton Water at Ozonation Column C

Location 4: Croton Water at Ozonation Column D

Location 5: Croton Water at Ozonation Column E

Location 6: Croton Water at Ozonation Column F

Location 7: Croton Water at Effluent of Diatomaceous Earth Filter

* Blank boxes represent E.Coli and HPC not measured

** No attachment

Table 14. Microbiological Water Characteristics in Ozonation Experiment No. 3 at Jerome Park Water Treatment Demonstration Plant

Date of sample	Location	Field Temp (°C)	Sampling Time	AOC (µg/l)	Total Coli (colonies/100mL)	E.Coli (colonies/100mL)	HPC1 (CFU/mL)	HPC2 (CFU/mL)	Growth Rates μ (d ⁻¹)
05.01.96	1	8	9:30am	0	21	21	1	0	0.330465 ± 0.294211
	2	8	9:35am	0	<1	*	3	1	0.108076 ± 0.051928
	3	8	9:40am	17	3	2	2	1	0.363392 ± 0.230983
	4	8	9:45am	0	<1		0	2	0.105173 ± 0.063725
	5	8	9:50am	16	<1		22	34	0.123092 ± 0.093072
	6	8	9:55am	8	<1		2	0	0.178924 ± 0.000000
	7	8	10:00am	14	<1		34	38	0.790000 ± 0.165325

Location 1: Croton Water at Jerome Park Water Treatment Demonstration Plant

Location 2: Croton Water at Ozonation Column B

Location 3: Croton Water at Ozonation Column C

Location 4: Croton Water at Ozonation Column D

Location 5: Croton Water at Ozonation Column E

Location 6: Croton Water at Ozonation Column E

Location 7: Croton Water at Effluent of Diatomaceous Earth Filter

* Blank boxes represent E.Coli not measured

Table 15. Microbiological Water Characteristics in Ozonation Experiment No. 4 at Jerome Park Water Treatment Demonstration Plant

Date of Sample	Location	Field Temp (°C)	Sampling Time	AOC (µg/l)	Total Coli (colonies/100mL)	E. Coli (colonies/100mL)	HPC1 (CFU/mL)	HPC2 (CFU/mL)	Growth Rates μ (d ⁻¹)
06.05.96	1	17.0	10:30am	11	<1	*	38	51	0.239774 ± 0.199148
	2	12.0	10:35am	8	<1		1	2	0.275260 ± 0.138998
	3	12.0	10:40am	10	<1		1	1	0.045124 ± 0.019001
	4	12.0	10:45am	21	<1		5	7	0.202537 ± 0.062281
	5	12.0	10:50am	30	<1		0	0	0.233850 ± 0.203265
	6	13.0	10:55am	31	<1		4	4	0.161471 ± 0.00000
	7	12.0	11:00am	0	<1		0	0	

- Location 1: Croton Water at Jerome Park Demonstration Plant
 - Location 2: Croton Water at Ozonation Column A
 - Location 3: Croton Water at Ozonation Column B
 - Location 4: Croton Water at Ozonation Column C
 - Location 5: Croton Water at Ozonation Column D
 - Location 6: Croton Water at Ozonation Column F
 - Location 7: Croton Water at Effluent of Diatomaceous Earth Filtration Process
- * Blank boxes represent E.Coli not measured

Table 16. Microbiological Water Characteristics in Ozonation Experiment No. 5 at Jerome Water Treatment Demonstration Plant

Date of Sample	Location	Field Temp (°C)	Sampling Time	AOC (µg/l)	Total Coli (colonies/100mL)	E.Coli (colonies/100mL)	HPC1 (CFU/mL)	HPC2 (CFU/mL)	Growth Rates μ (d ⁻¹)
06.06.96	1	15.5	2:10pm	17	<1	*	104	131	0.263731 ± 0.179566
	2	11.0	2:15pm	22	<1		0	0	0.391790 ± 0.000000
	3	10.5	2:20pm	33	<1		0	0	1.015270 ± 0.000000
	4	11.0	2:25pm	23	<1		0	0	0.000000
	5	11.0	2:30pm	17	<1		0	0	0.000000
	6	11.5	2:35pm	N/A	<1		1	1	**
	7	11.0	2:40pm	24	<1		70	53	1.249350 ± 0.000000

Location 1: Croton Water at Jerome Park Demonstration Plant

Location 2: Croton Water at Ozonation Column A

Location 3: Croton Water at Ozonation Column B

Location 4: Croton Water at Ozonation Column C

Location 5: Croton Water at Ozonation Column D

Location 6: Croton Water at Ozonation Column F

Location 7: Croton Water at Effluent of Diatomaceous Earth Filtration Process

N/A: Not available

* Blank boxes represent E.Coli not measured

** No attachment

Table 17. Microbiological Water Characteristics in Ozonation Experiment No. 6 at Jerome Water Treatment Demonstration Plant

Date of Sample	Location	Field Temp (°C)	Sampling Time	AOC (µg/l)	Total Coli (colonies/100mL)	E.Coli (colonies/100mL)	HPC1 (CFU/mL)	HPC2 (CFU/mL)	Growth Rates μ (d ⁻¹)
06.19.96	1	19.5	2:10pm	0	<1	*	>5700	>5700	0.282109 ± 0.169290
	2	14.0	2:15pm	0	<1		0	0	0.174258 ± 0.112467
	3	14.0	2:20pm	0	<1		0	0	0.238367 ± 0.142364
	4	14.0	2:25pm	0	<1		1	0	0.166910 ± 0.115239
	5	14.0	2:30pm	0	<1		3	3	0.913740 ± 0.60054
	6	14.0	2:35pm	0	<1		0	0	0.272803 ± 0.144399
	7	14.0	2:40pm	0	14	<1	11	7	0.130783 ± 0.086783

Location 1: Croton Water at Jerome Park Demonstration Plant

Location 2: Croton Water at Ozonation Column A

Location 3: Croton Water at Ozonation Column B

Location 4: Croton Water at Ozonation Column C

Location 5: Croton Water at Ozonation Column D

Location 6: Croton Water at Ozonation Column F

Location 7: Croton Water at Effluent of Diatomaceous Earth Filtration Process

* Blank boxes represent E.Coli not measured

Table 18. Microbiological Water Characteristics in Ozonation Experiment No. 7 at Jerome Water Treatment Demonstration Plant

Date of Sample	Location	Field Temp (°C)	Sampling Time	AOC (µg/l)	Total Coli (colonies/100mL)	E.Coli (colonies/100mL)	HPC1 (CFU/mL)	HPC2 (CFU/mL)	Growth Rates μ (d ⁻¹)
07.10.96	1	15.5	10:00am	11	20	2	0	0	0.138927 ± 0.082923
	2	14.0	10:05am	17	<1	*	0	0	1.24935 ± 0.000000
	3	14.0	10:10am	17	<1		0	0	0.196987 ± 0.065882
	4	14.0	10:15am	20	<1		0	0	0.471992 ± 0.290023
	5	14.0	10:20am	18	<1		1	0	0.308309 ± 0.111290
	6	14.0	10:25am	20	TNTC	<1	3	5	0.202268 ± 0.102081
	7	14.0	10:30am	21	<1		1	0	**

Location 1: Croton Water at Jerome Park Demonstration Plant

Location 2: Croton Water at Ozonation Column A

Location 3: Croton Water at Ozonation Column B

Location 4: Croton Water at Ozonation Column C

Location 5: Croton Water at Ozonation Column D

Location 6: Croton Water at Ozonation Column F

Location 7: Croton Water at Effluent of Diatomaceous Earth Filtration Process

* Blank boxes represent E.Coli notmeasured

** No attachment

Table 19. Microbiological Water Characteristics in Ozonation Experiment No. 8 at Jerome Water Treatment Demonstration Plant

Date of Sample	Location	Field Temp (°C)	Sampling Time	AOC (µg/l)	Total Coli (colonies/100mL)	E.Coli (colonies/100mL)	HPC1 (CFU/mL)	HPC2 (CFU/mL)	Growth Rates μ (d ⁻¹)
07.24.96	1	16.0	10:00am	33	<1	*	10	14	0.00000
	2	15.5	10:05am	70	<1		4	1	0.00000
	3	15.2	10:10am	47	<1		0	0	0.043698 ± 0.014595
	4	15.2	10:15am	40	N/A		N/A	N/A	0.156151 ± 0.099946
	5	15.5	10:20am	60	<1		8	12	0.640379 ± 0.000000
	6	15.5	10:25am	40	<1		58	64	0.00000
	7	15.5	10:30am	66	<1		1	1	**

Location 1: Croton Water at Jerome Park Demonstration Plant

Location 2: Croton Water at Ozonation Column A

Location 3: Croton Water at Ozonation Column B

Location 4: Croton Water at Ozonation Column C

Location 5: Croton Water at Ozonation Column D

Location 6: Croton Water at Ozonation Column F

Location 7: Croton Water at Effluent of Diatomaceous Earth Filtration Process

* Blank boxes represent E.Coli not measured

** No attachment

N/A: Not available

Table 20. Microbiological Water Characteristics in Ozonation Experiment No. 9 at Jerome Water Treatment Demonstration Plant

Date of Sample	Location	Field Temp (°C)	Sampling Time	AOC (µg/L)	Total Coli (colonies/100mL)	E.Coli (colonies/100mL)	HPC1 (CFU/mL)	HPC2 (CFU/mL)	Growth Rates μ (d ⁻¹)
08.14.96	1	18.0	9:30am	33	<1	*	1	0	**
	2	17.1	9:35am	68	<1		1	3	**
	3	17.1	9:40am	-	9	<1	1	1	0.000000
	4	17.5	9:45am	100	<1		0	0	0.000000
	5	16.9	9:50am	50	<1		1	0	**
	6	17.7	9:55am	47	<1		0	0	**
	7	17.8	10:00am	50	<1		0	0	**

- Location 1: Croton Water at Jerome Park Demonstration Plant
- Location 2: Croton Water at Ozonation Column A
- Location 3: Croton Water at Ozonation Column B
- Location 4: Croton Water at Ozonation Column C
- Location 5: Croton Water at Ozonation Column D
- Location 6: Croton Water at Ozonation Column F
- Location 7: Croton Water at Effluent of Diatomaceous Earth Filtration Process

* Blank boxes represent E.Coli not measured

** No attachment

Appendix II — Figures

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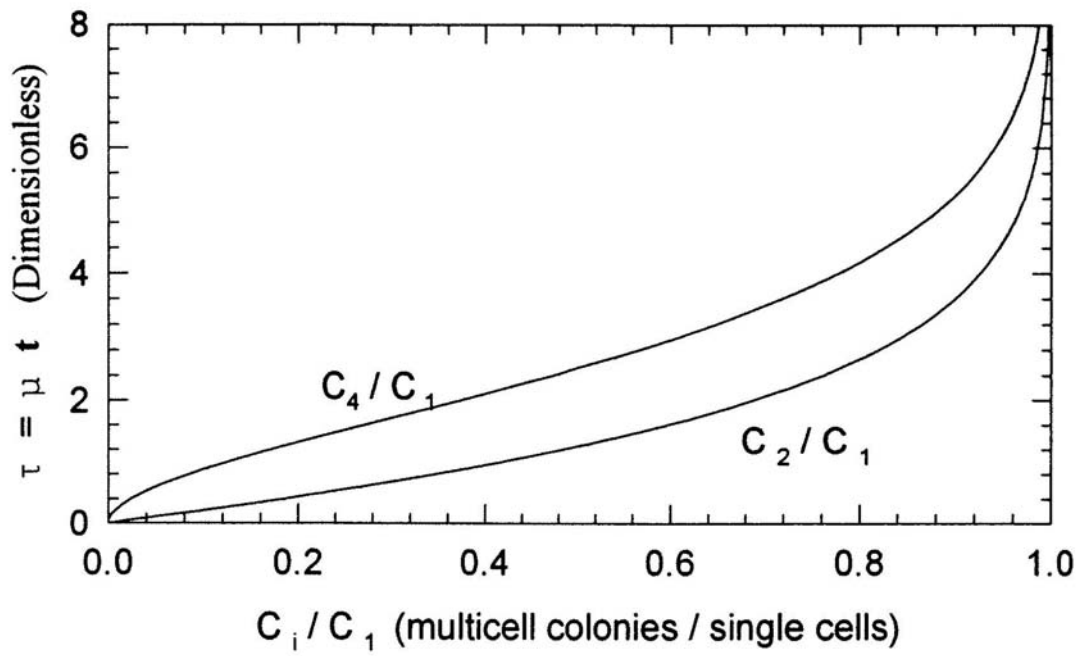


Figure 1. Dimensionless attached bacterial growth rate curves.

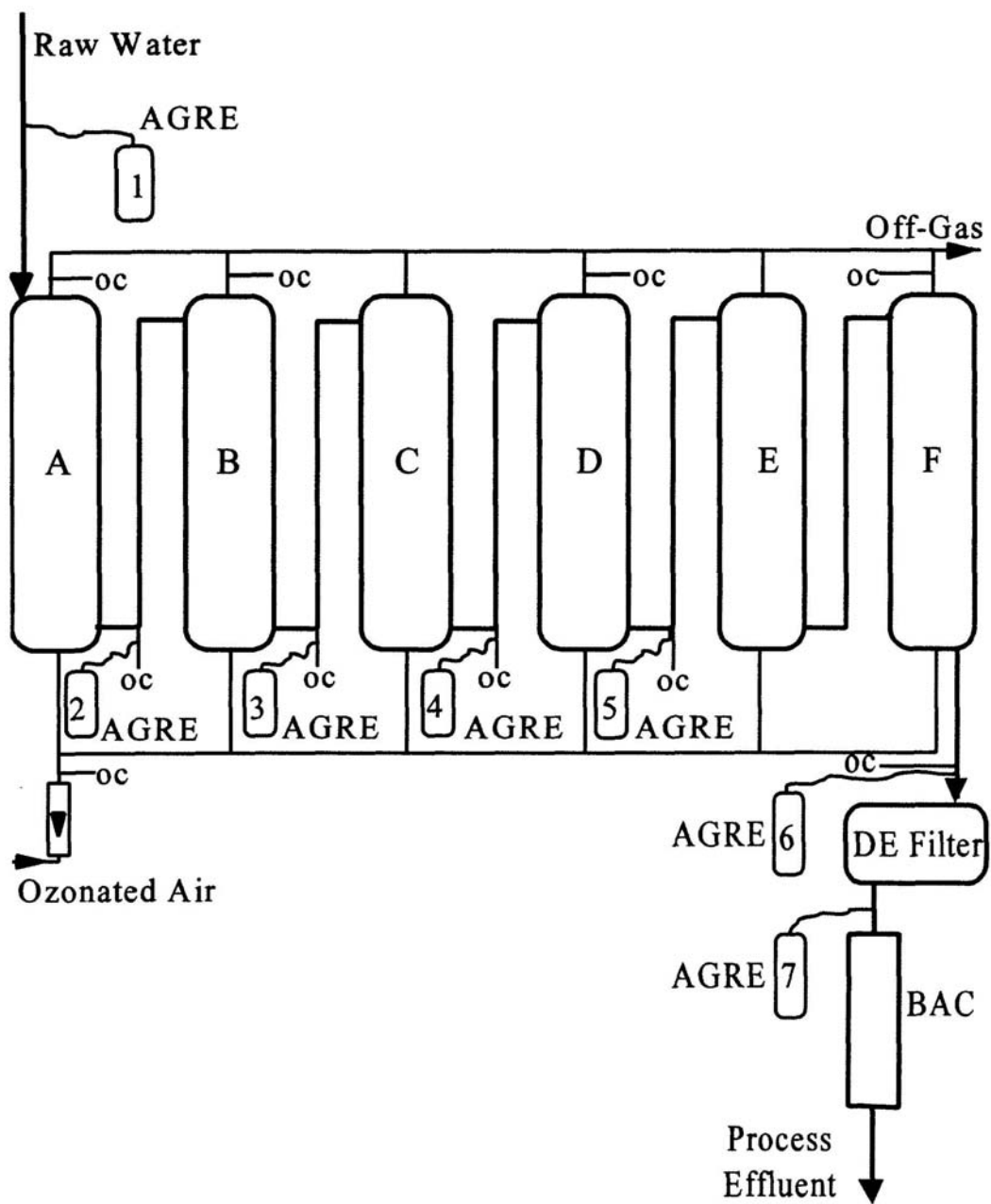


Figure 2. Flow schematic of the water treatment train at Jerome Park Water Treatment Demonstration Plant.

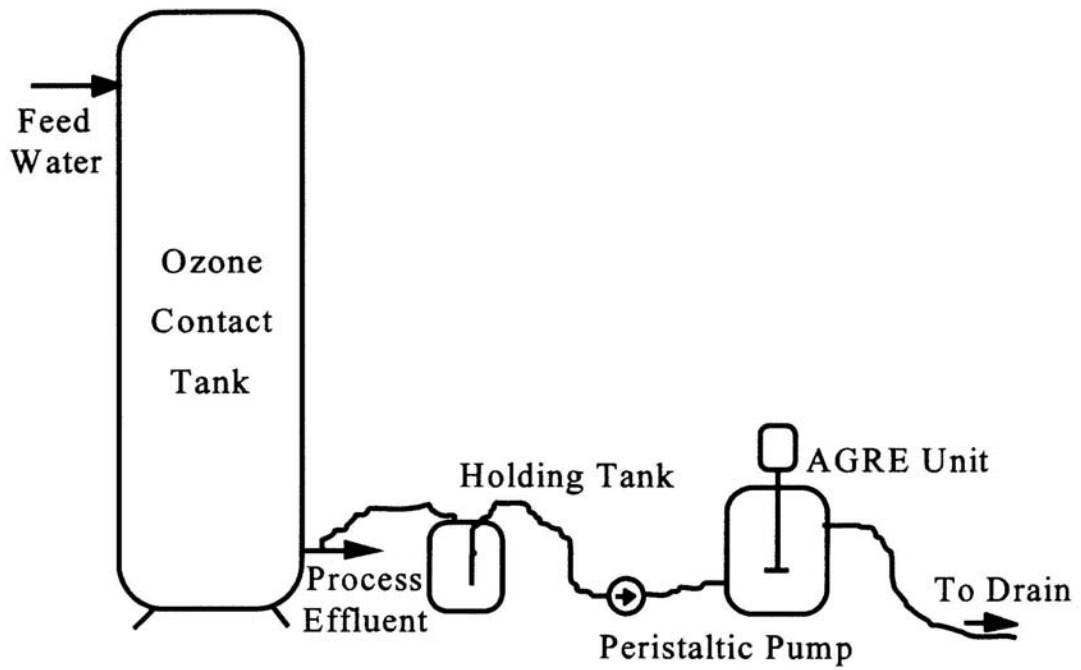


Figure 3. Flow schematic of an AGRE unit operating in continuous flow mode.

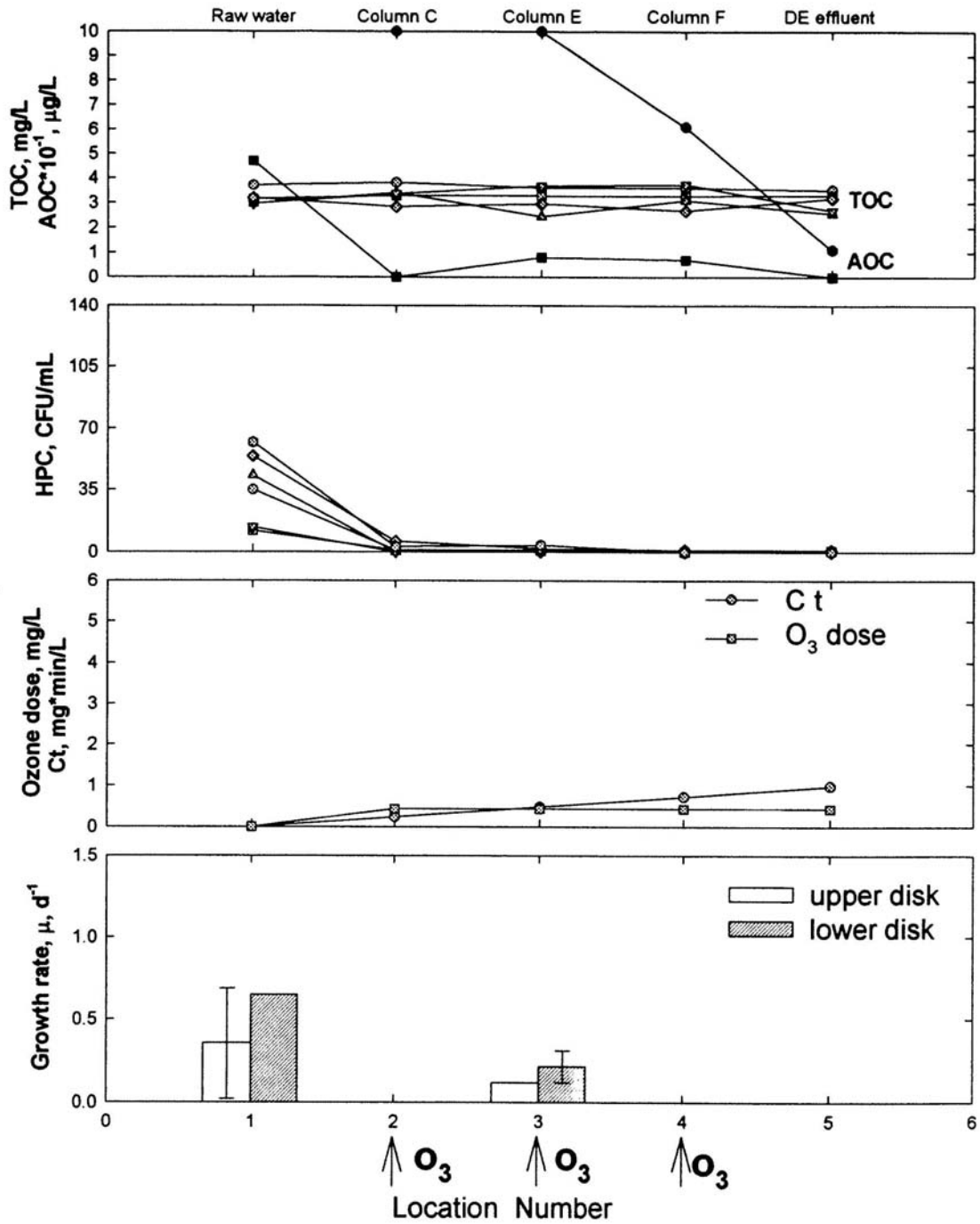


Figure 4. Effect of ozone and diatomaceous earth (DE) filtration on the biostability of Croton water. Results of experiment No. 1.

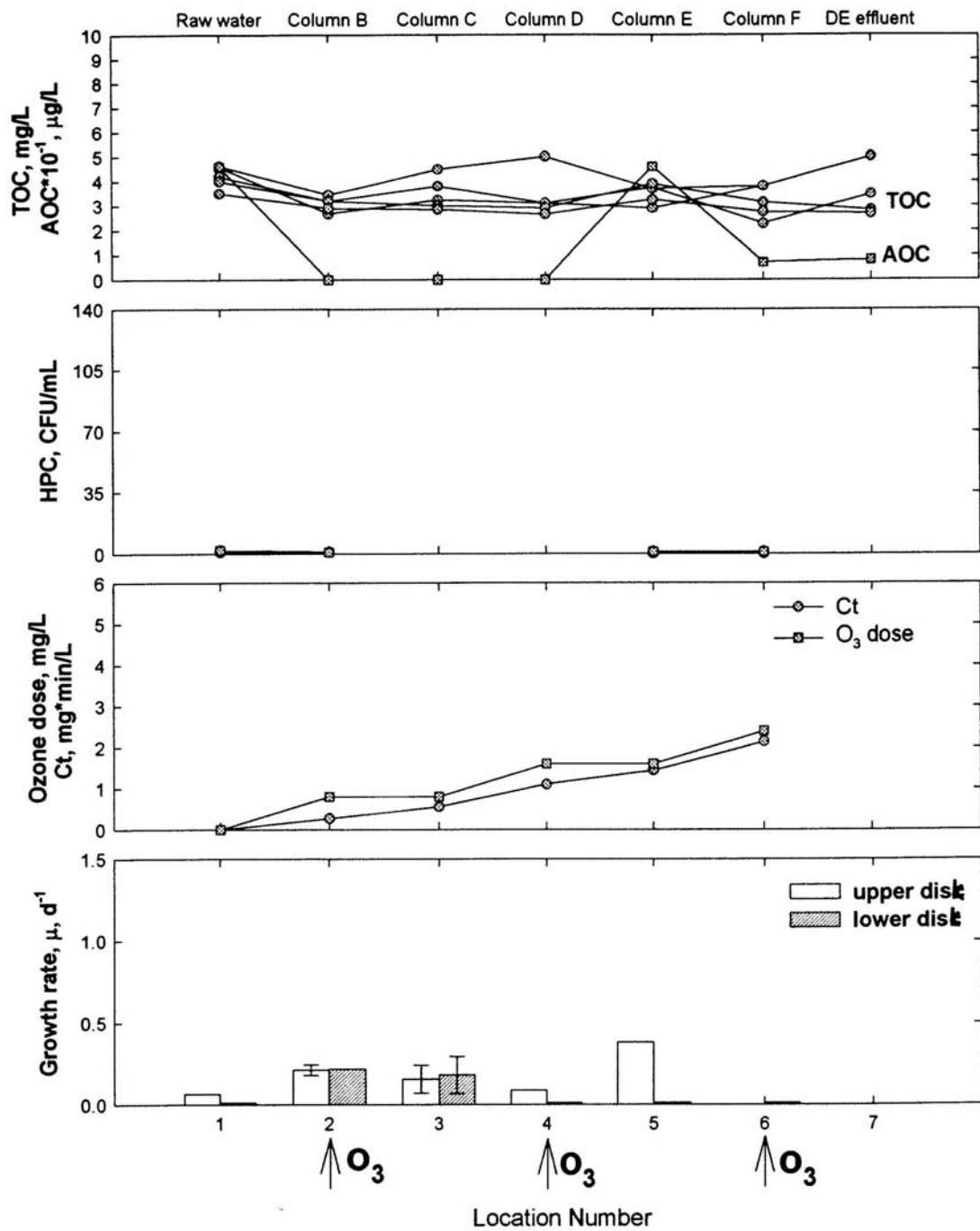


Figure 5. Effect of ozone and DE filtration on the biostability of Croton water. Results of experiment No. 2.

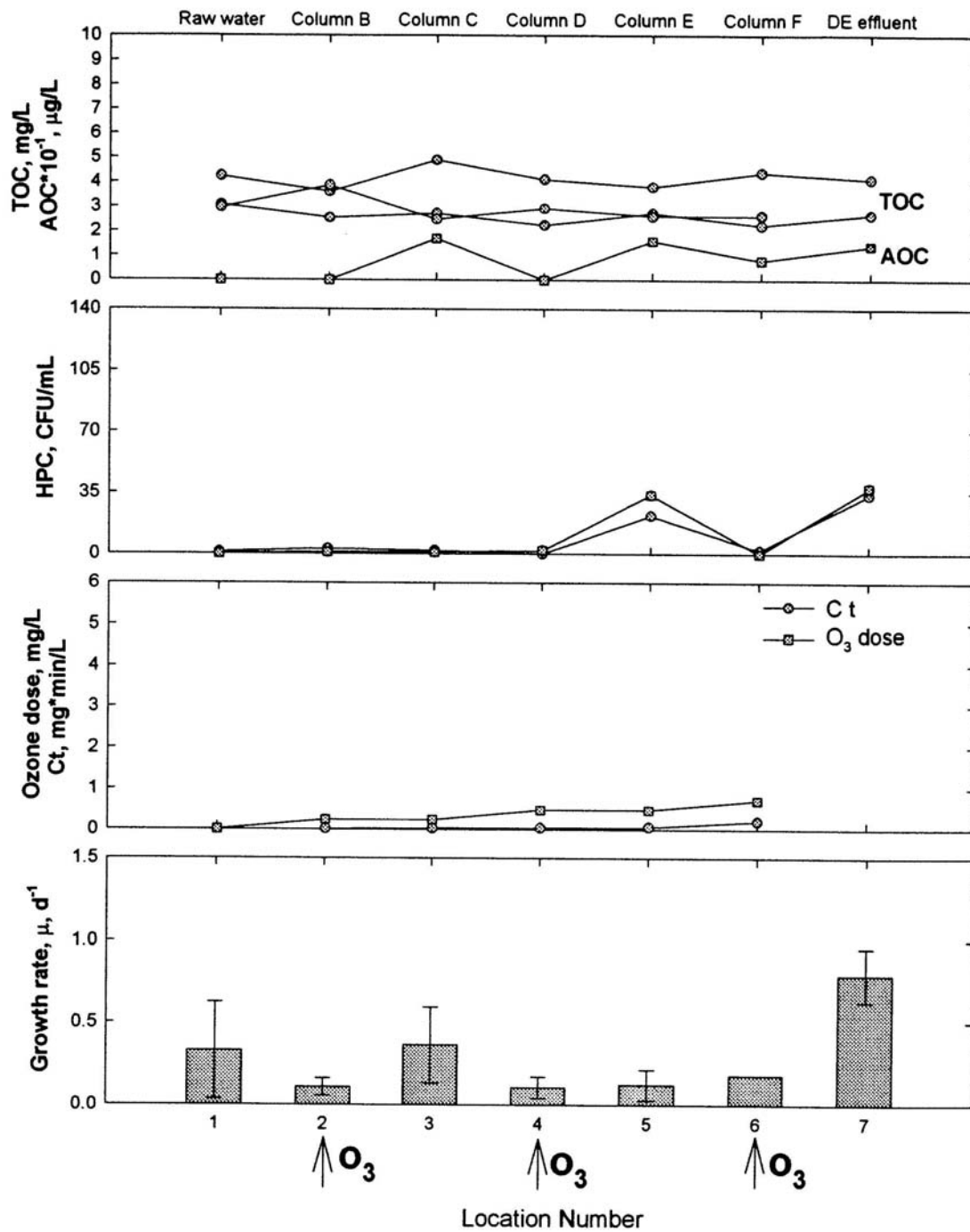


Figure 6. Effect of ozone and DE filtration of the biostability of Croton water. Results of experiment No. 3.

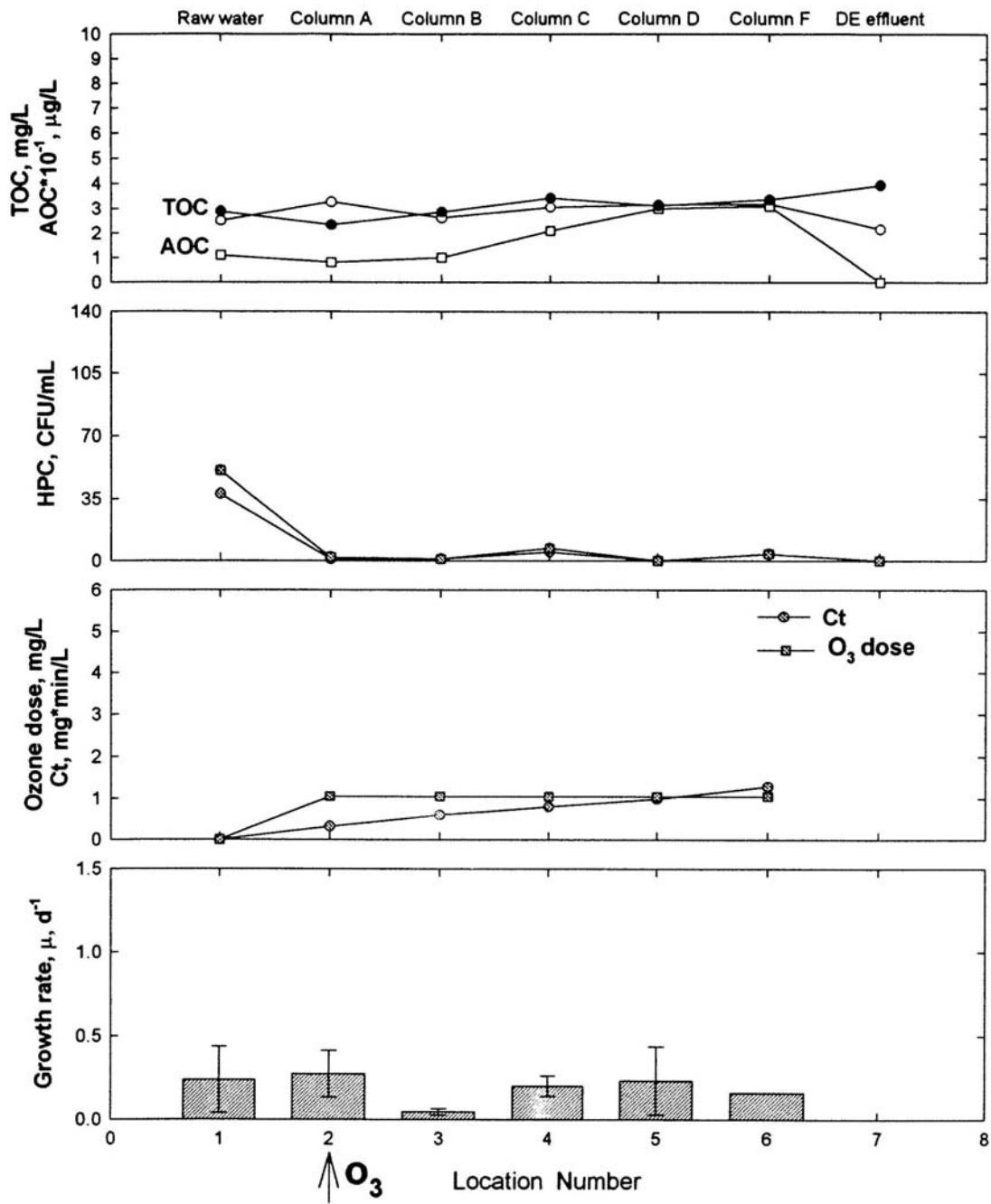


Figure 7. Effect of ozone and DE filtration on the biostability of Croton water. Results of experiment No. 4 (blank and full points represent the start and end of the AGRE test, respectively).

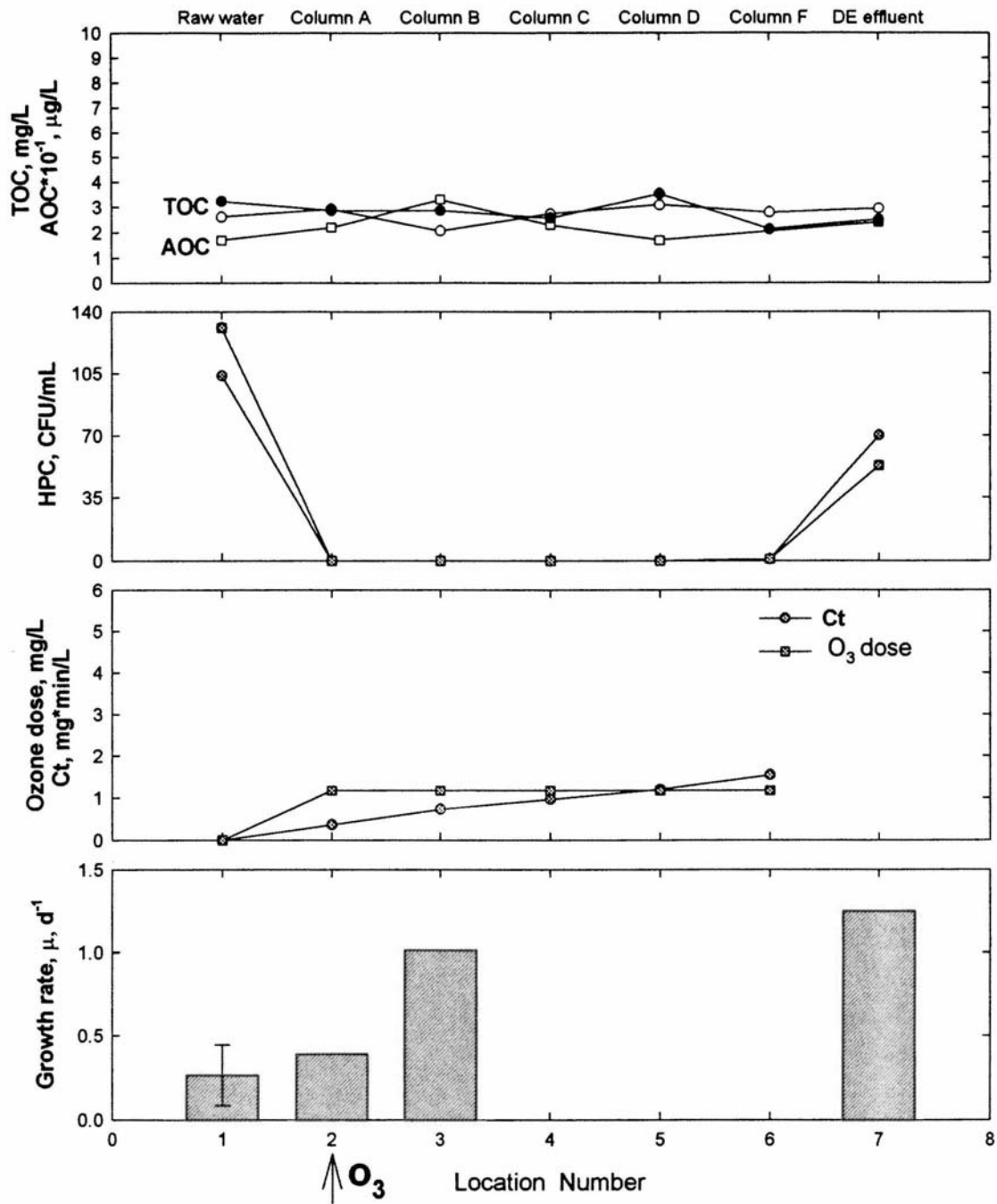


Figure 8. Effect of ozone and DE filtration on the biostability of Croton water. Results of experiment No. 5 (blank and full points represent the start and end of the AGRE test, respectively).

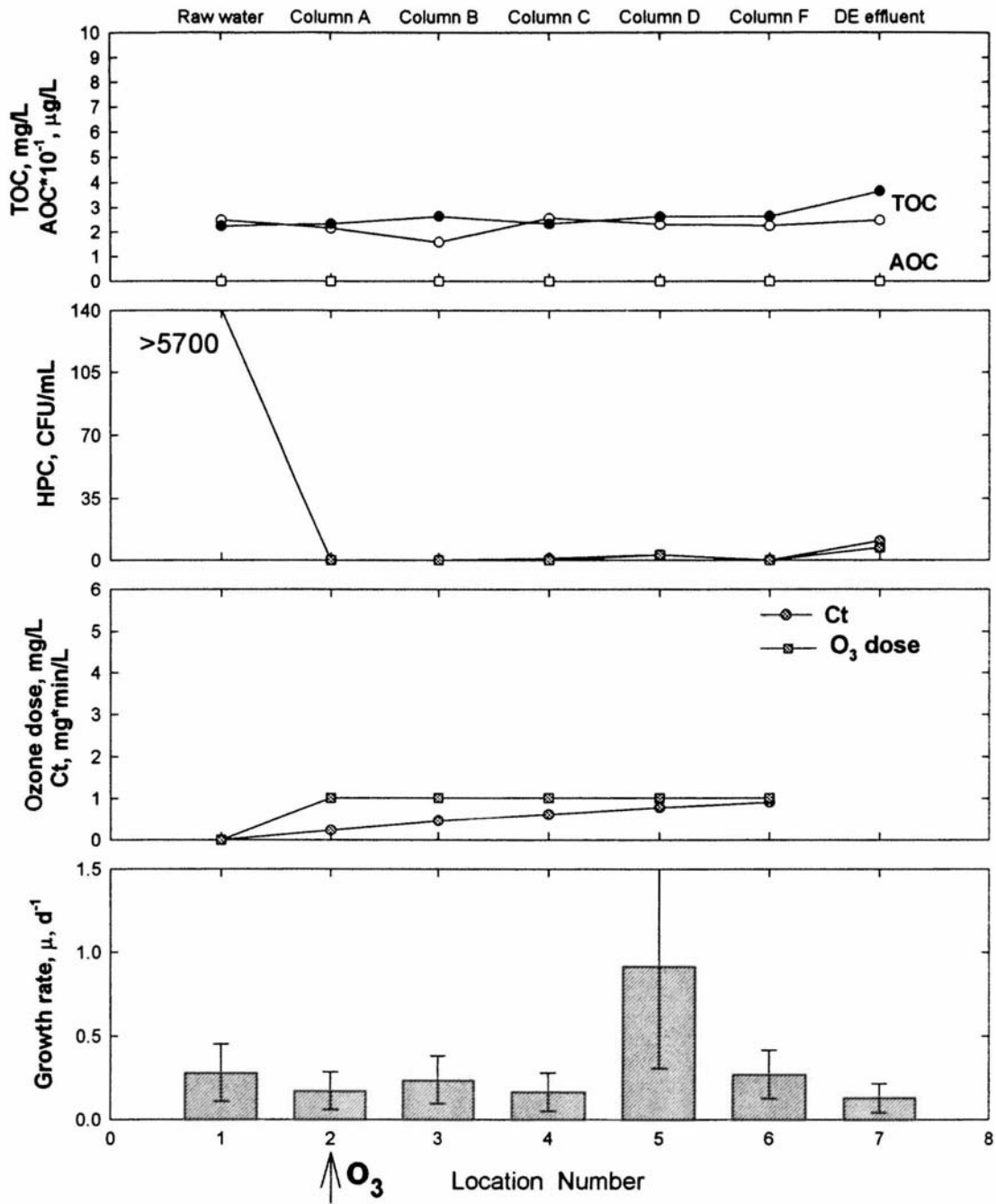


Figure 9. Effect of ozone and DE filtration on the biostability of Croton water. Results of experiment No. 6 (blank and full points represent the start and end of the AGRE test, respectively).

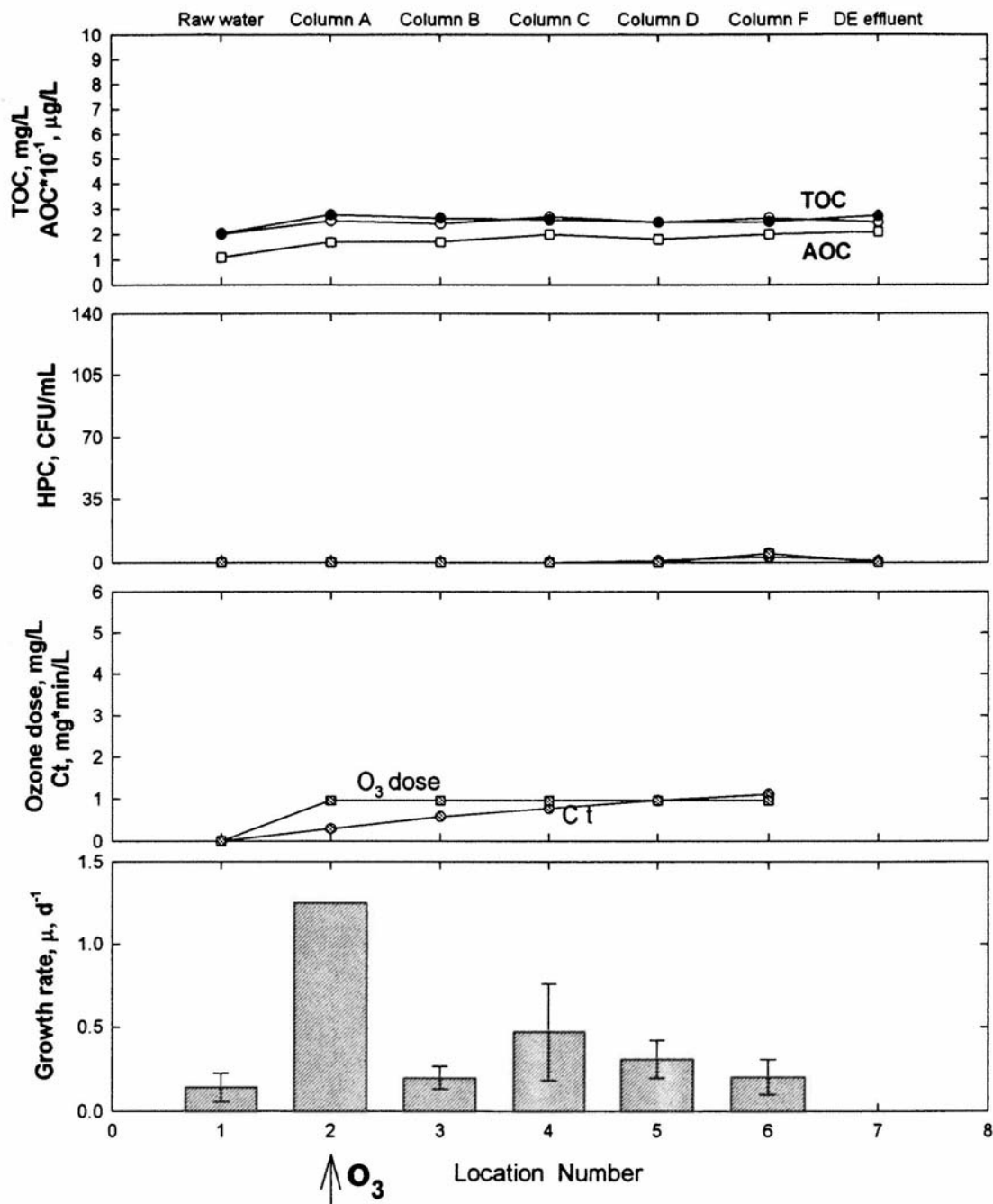


Figure 10. Effect of ozone and DE filtration on the biostability of Croton water. Results of experiment No. 7 (blank and full points represent the start and end of the AGRE test, respectively).

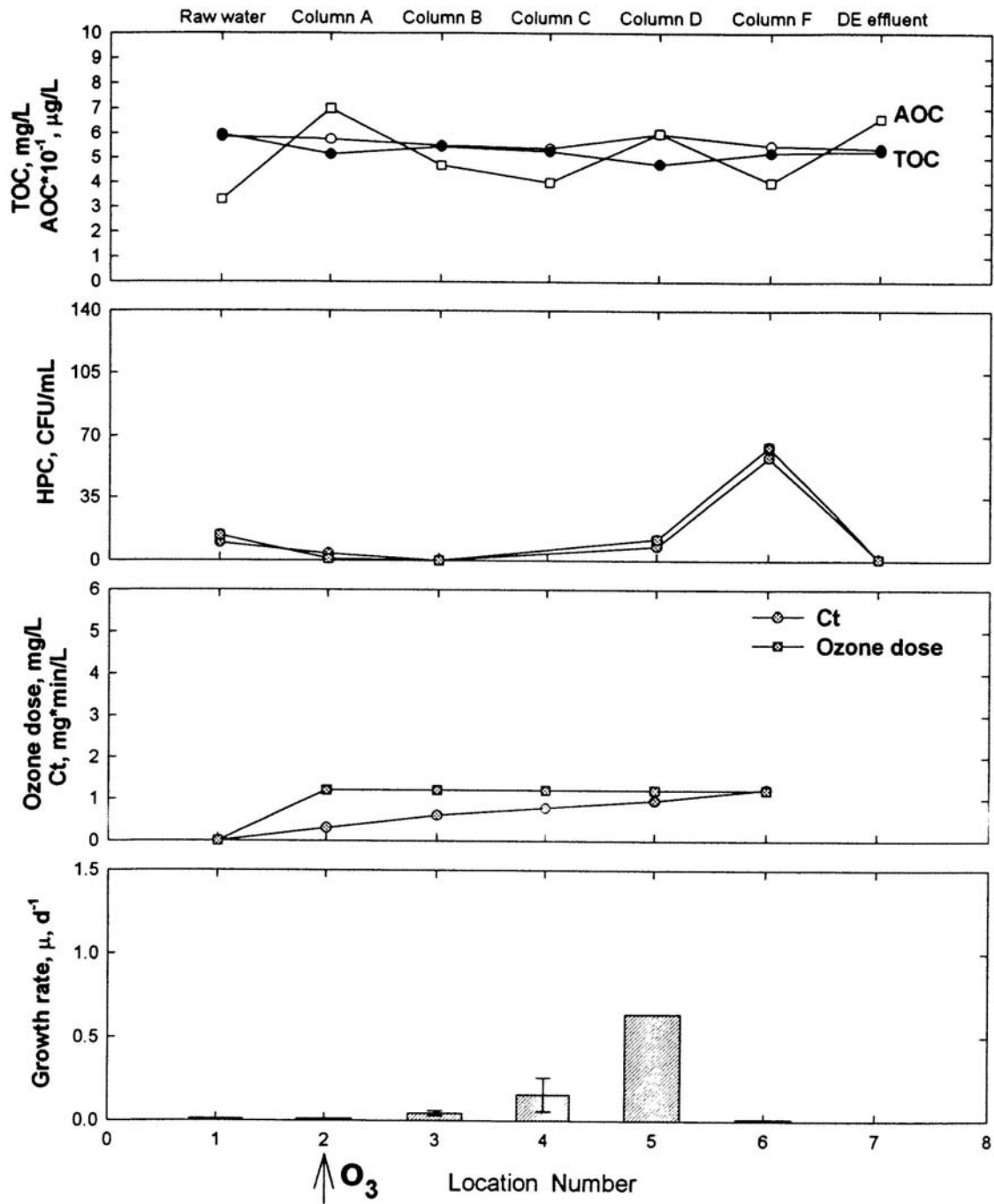


Figure 11. Effect of ozone and DE filtration on the biostability of Croton water. Results of experiment No. 8 (blank and full points represent the start and end of the AGRE test, respectively).

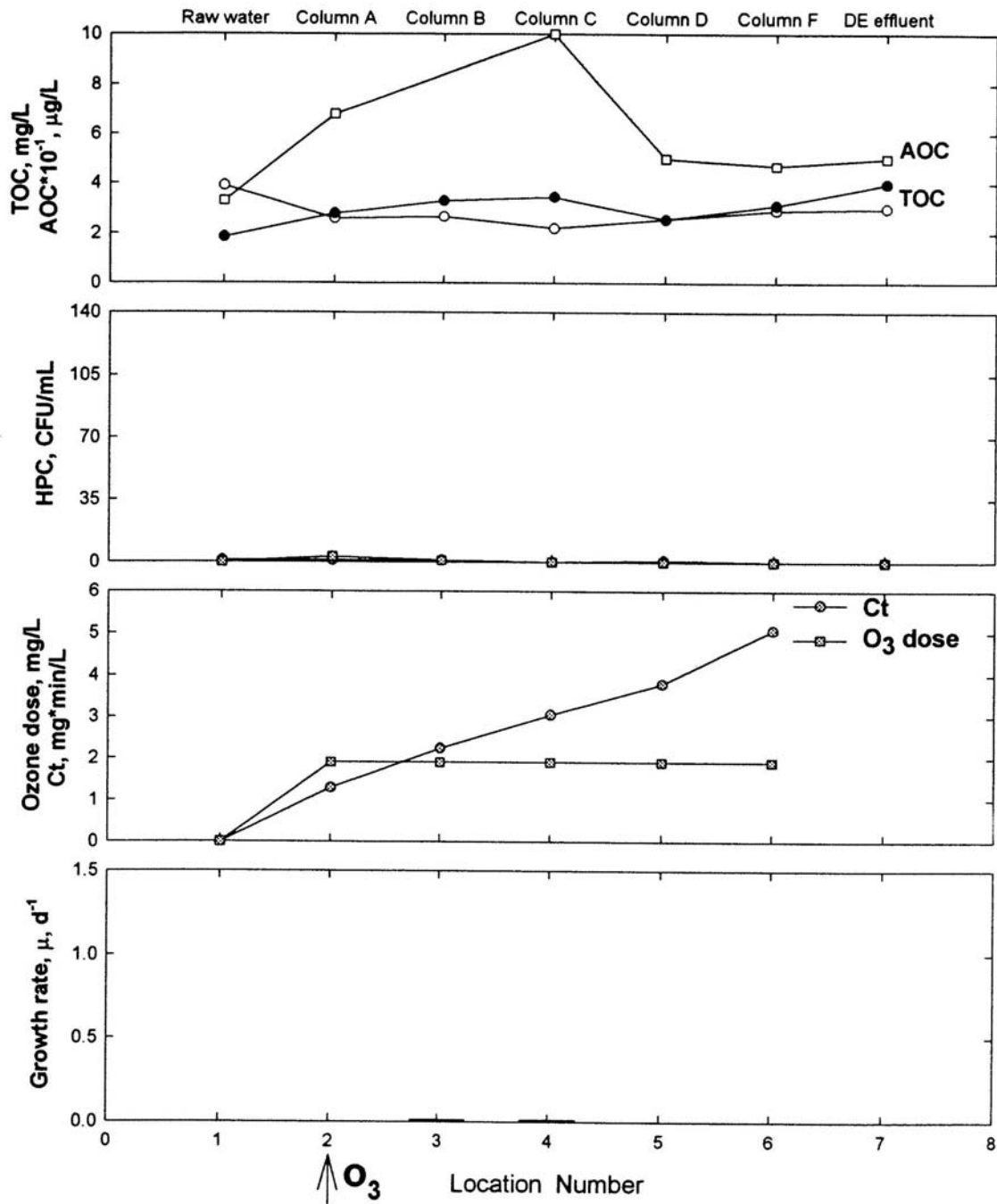


Figure 12. Effect of ozone and DE filtration on the biostability of Croton water. Results of experiment No. 9 (blank and full points represent the start and end of the AGRE test, respectively).

Appendix III — Analytical Methods

Assimilable Organic Carbon (AOC) Analysis

A modification of the standard method for AOC (AWWA-APHA-WEF, 1992) was utilized. An axenic stock culture of *Pseudomonas fluorescens*, which was isolated from the New York City distribution system, is maintained at the New York City Department of Environmental Protection's (NYCDEP's) Central Laboratory. Utilizing a known concentration of acetate-carbon, maximum colony counts were measured and a standard curve determined. A volume of 35 milliliters (mL) of sample water is dispensed aseptically into a sterile 45-mL vial then covered with a teflon-lined cap. This tube is pasteurized in an autoclave at 70 degrees Celsius (°C) for 30 minutes, then cooled to room temperature. A known concentration and volume of the stock culture of *Pseudomonas fluorescens* is added to the vial, and 0.1 mL is plated onto nutrient agar to determine the initial colony count in the vial. Duplicates, controls (50 micrograms per liter [$\mu\text{g/L}$] acetate-carbon) and blanks (deionized water) are run simultaneously on each sample. All vials are kept at 20 °C with plate counts performed daily until the culture reaches stationary phase. The highest colony count is utilized in the assimilable organic carbon (AOC) determination.

Equipment

1. 45-mL vials with teflon- (TFE) lined septa caps
2. Sterile pipets
3. R₂A filled petri dishes

Preparation of reagents

A. Sodium acetate stock solution:

- i. Prepare 400 milligrams (mg) acetate (C/L) stock solution: dissolve 2.267 grams (g) $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$ (FW 136.1) in 1 liter (L) of organic carbon-free, deionized water.
- ii. Transfer acetate stock to 45-mL vials, fill to shoulder (approximately 40 mL).
- iii. Autoclave vials making sure septa lids are tightly capped to prevent the acetate from escaping into the air.
- iv. Store at 5 °C in tightly capped vials up to 6 months.

B. Sodium persulfate solution – 10 percent (%) (w/v):

- i. Dissolve 100 g $\text{Na}_2\text{S}_2\text{O}_8$ in 1L deionized water. (This is an oxidizing solution which binds to carbon element and produces CO_2 .)

C. Sodium thiosulfate solution:

- i. Dissolve 13.2 mg $\text{Na}_2\text{S}_2\text{O}_3$ in 1 L deionized water.

D. Mineral salts solution:

- i. Dissolve 171 mg K_2HPO_4 (FW 174.2) and 767 mg NH_4Cl (FW 53.49) and 1.444g KNO_3 (FW 101.1) in 1L carbon-free water.

E. Bacterial acetate stock buffer with Sodium Acetate Carbon Source:

For each liter of 2 mg C/L, sodium acetate stock solution (see A, above) add:

- i. 7.0 mg K_2HPO_4 (FW 174.2)
- ii. 3.0 mg KH_2PO_4 (FW 136.1)
- iii. 0.1 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (FW 246.5)
- iv. 1.0 mg $(\text{NH}_4)_2\text{SO}_4$ (FW 132.1)
- v. 0.1 mg NaCl
- vi. 1.0 μg FeSO_4 (ferrous sulfate has only heptahydrate form w/FW 278).

Preparation of incubation vessels

A. 45-mL vials:

- i. Wash with detergent.
- ii. Rinse with hot water twice.
- iii. Rinse with 0.1 N HCl twice (removes trace metals and dissolves organic debris; i.e, makes them more soluble in water).
- iv. Rinse with deionized water three times.
- v. Cap with foil and heat to 550 °C for 6 hours.

B. TFE-lined silicone septa:

- i. Soak septa in 10% sodium persulfate solution.
- ii. Heat the solution to 60 °C for 1 hour.
- iii. Rinse the septa with deionized water three times.

Preparation of stock culture

A. Transfer bacterial culture

- i. Streak the bacteria culture from an agar slant to a R₂A plate (make sure to get isolated colony growth).
- ii. Incubate at room temperature (<20 °C) for 3 to 5 days.

B. Preparation of the bacterial stock culture

- i. Inoculate an isolated colony into 50 mL (100 mL) of sterile, chlorine-neutralized tap water (neutralize tap water by adding 100 µL of sodium thiosulfate solution to every 50 mL water sample then sterilize the tap water by either filtration or by autoclaving) placed in an autoclaved 125 mL ground glass stopper Erlenmeyer flask.
- ii. Incubate tap water at room temperature for 7 days.
- iii. Inoculate an aliquot (0.1 mL) of the tap water-adapted bacterial culture into 50 mL (100 mL) sodium acetate stock buffer solution.
- iv. Incubate the bacterial stock solution at room temperature for 7 days.

C. Bacterial enumeration of the stock solution:

- i. Prepare 10^{-1} , 10^{-2} , and 10^{-3} serial dilutions of the bacterial stock solution.
- ii. Transfer 0.1 mL of the respective concentrations of stock solution to R₂A plates (each concentration should be done in triplicate).
- iii. Incubate R₂A plates at 15 °C for a period of 3-5 days.
- iv. Enumerate R₂A plates and calculate bacterial abundance as the number of colony forming units (CFU) mL⁻¹.
- v. Bacterial abundance should be in the range of 10^6 CFU/mL. (We have calculated 8.2×10^6 and 2.4×10^7 CFU/mL for strains P-17 and NOX, respectively)
- vi. Store sodium acetate bacterial stock culture at 5 °C for up to 6 months.
- vii. This sodium acetate solution is to be used as a working stock culture to inoculate AOC experiments.

Procedure

A. Collection and preparation of water sample:

- i. Pour 35 mL of the water sample into a 45-mL U.S. Environmental Protection Agency (USEPA) approved, testing vial with the teflon (TFE) side of the TFE-silicone septa facing down on the water surface.
- ii. Securely tighten the screw cap of the vial.
- iii. Neutralize the water sample with 100 microliters (μL) of sodium thiosulfate solution (a reducing agent used to deactivate the chlorine in the water).
- iv. Pasteurize each vial (tighten cap) in a 70- $^{\circ}\text{C}$ autoclave for 15 minutes.

B. Inoculation of water sample with the bacterial strain:

- i. Inoculate water sample in each vial with 500 colony forming units (CFU)/mL from the bacterial stock culture.
- ii. Incubate vials at 15 $^{\circ}\text{C}$ (20 $^{\circ}\text{C}$) for 7 days
- iii. From day 7 to day 9, transfer an aliquot (0.1 mL) of water sample from each vial for the 3 consecutive days. Transfer of sample is completed as follows:
 - a. Shake vials vigorously for 1 minute.
 - b. Remove 1-mL sample with a sterile pipet and prepare serial dilutions for each concentration (10^{-2} , 10^{-3} , 10^{-4}).
 - c. Plate 0.1 mL^{-1} of each dilution (10^{-2} , 10^{-3} , 10^{-4}) on R_2A plates in duplicate.
 - d. Incubate R_2A plates at 25 $^{\circ}\text{C}$ (20 $^{\circ}\text{C}$) for 3 to 5 days.

C. Calculations:

- i. After incubation, enumerate plates calculating CFU mL^{-1} for each bacterial strain.
- ii. Calculate AOC concentration using the following equation:

$$\text{AOC } \mu\text{g C L}^{-1} = \frac{\text{P-17 CFU mL}^{-1} (\text{max dens}) + \text{NOX CFU mL}^{-1}}{4.1 \times 10^6 \text{ CFU mL}^{-1} \quad 1.2 \times 10^7 \text{ CFU mL}^{-1}}$$

Standard curve

A. Preparation of water sample:

- i. Pour 40 mL of the carbon free stock buffer solution into 10 45-mL organic carbon-free glass vials with teflon-lined silicon septa.
- ii. Securely tighten caps and autoclave vials for 20 minutes using 30 pounds per square inch (psi) at 121 °C.
- iii. After vials have cooled, pipette the respective volumes of sodium acetate stock buffer solution to give final concentrations of 20 µg C/L, 40 µg C/L, 60 µg C/L, 80 µg C/L, 100 µg C/L, 150 µg C/L, 200 µg C/L, 300 µg C/L, and 400 µg C/L. Leave one vial empty for the control. Each concentration should be done in duplicate.
- iv. Inoculate each vial with 500 CFU of the bacterial strain *Pseudomonas fluorescens* from the sodium acetate stock culture. This will be DAY ONE of the bacterial enumeration procedure.

B. Enumeration:

- i. On DAY ONE:
 - a. Shake each vial for 1 minute.

Aseptically transfer 1 mL of water sample from each vial and prepare serial dilutions for concentrations of 10^{-2} , 10^{-3} , 10^{-4} .
- ii. Plate 0.1 mL^{-1} of each dilution (10^{-2} , 10^{-3} , 10^{-4}) on R₂A plates in duplicate.
- iii. Incubate R₂A plates at 25 °C (20 °C) for 3 to 5 days.
- iv. After incubation, enumerate plates calculating CFU/mL.
- v. Bacterial density should be in the range of 10^5 to 10^6 CFU/mL. Adjust dilution factor accordingly to be within this range.
- vi. Continue this procedure each successive day (i.e., from DAY 2 onwards) until bacterial density reaches a maximum and remains constant.

C. Analyses of Data:

- i. Graph #1: Plot Mean Bacterial Density (CFU/mL) versus Time (day) for each AOC concentration.
- ii. From the graph, determine maximum cell density of *Pseudomonas fluorescens* (i.e., peak point in each curve) for each AOC concentration.

- iii. Graph #2: Extract the maximum bacterial density (CFU/mL) for each carbon concentration from Graph #1 and plot Mean Bacterial Density (CFU/mL) versus AOC concentration.
- iv. Draw a best fit line through the data points.

Membrane Filter Technique for Total and Fecal Coliform Counts

Apparatus and Materials:

1. Incubator set at 35 ± 0.5 °C (maintain a high level of humidity). For this, fill up a stainless steel pan with distilled water and leave it in the bottom shelf of the incubator. Also, leave distilled water-filled 1,000-mL beaker on the top shelf of the incubator.
2. Membrane Filtration Units: The filter-holding assembly (stainless steel) consists of a seamless funnel fastened to a base by a locking device; the design permits the membrane filter to be held securely on the membrane filter support screen without damage to the membrane during filtration. Separately, wrap the two parts (funnel and base) of the assembly in heavy paper bags. The funnel is to be wrapped in large (5-pound [lb]) bag which is stapled at the bottom for extra support. The base part is placed sideways in a small (3-lb) bag. The top of the bag is wrapped around the object and stapled securely so that after autoclaving parts of the same are put together. Put the base in one basket and funnel tops in another basket. Put the sterility testing tape on the bags (one piece on each basket). Sterilize by autoclaving at 121 °C for 30 minutes and store in a proper place until use. For filtration, mount the receptacle (rubber stopper) of the filter holding assembly to a six-place hydrolab manifold. Two six-place hydrolab manifolds are used to mount a total of 12 funnels. For filtration, connect the side of the manifold to the flask with dri-rite. Connect a 5-gallon carboy between the flask and the vacuum source to trap carryover water.
3. Membrane Filter: Individually wrapped presterilized 47-millimeter (mm) Gelman type GN6 membrane filters obtained from Millipore or Gelman Scientific.
4. Petri dish with pad: Millipore presterilized 47-mm petri dishes loaded with presterilized 47-mm absorbent pad obtained from millipore or Gelman Scientific.
5. m-ColiBlue24 media. m-ColiBlue24 media comes prepared and packaged in ampules.

6. Dilution water prepared as described in the standard operating procedures (SOP) of buffer preparation.
7. Dilution bottles.
8. Sterilized pipets (1.1 mL and 10 mL) in pipet container.
9. Graduated cylinders covered with aluminum foil and sterilized.
10. Electric vacuum pump.
11. Safety trap flask placed between the filter flask and vacuum source.
12. Alcohol in small mouth jar for sterilizing forceps.
13. Microscope (low power) giving 10-15 X magnification.
14. Forceps: round-tipped, without corrugations on the inner sides of the tips to permit easy handling of filters without damage. Sterilize the forceps before use by dipping in 95% alcohol and flaming.
15. Sterile plate count agar plates prepared as described in the SOP of medium preparation.

Preparations

- A. Clean the working area with Lysol. Put clean paper towels on the table to absorb any spill of the medium. Arrange petri dishes (presterilized with pad) in row, and add 1.8 to 2.0 mL m-ColiBlue24 media to the sterile absorbent pad; saturate but do not flood the pad.
- B. Mark each petri dish to identify the sample number as written on the sample bottle.
- C. Using sterile forceps, place a sterile membrane filter (grid side up) on the grid plate of the filter base.
- D. Carefully place the matched funnel unit to the base of filter unit, taking care not to damage or dislodge the filter and lock in place. The membrane filter is now fitted between the funnel and base. Filter sample under partial vacuum.

Sample Analysis

- A. Using sterile forceps, place sterile membrane filter in all the funnels as described above.

- B. Shake the sample bottles vigorously (25 times) and filter the samples under partial vacuum. Rinse the funnels with three 20- to 30-mL portions of sterile dilution water. The funnels should be rinsed fully (on all sides), particularly in a swirling movement. Upon completion of the final rinse and the filtration process, unlock and remove the funnels.
- C. Place the membrane filters in the premarked (with sample number) plates. Follow a continuous break down of six and six samples to complete the analysis. This is important to check for any possible laboratory error. Repeat the process till all the samples are filtered and filters placed in the petri dishes.

Quality Control

In the morning of the analyses, inoculate cultures with *Escherichia coli* and *Proteus vulgaris*. Incubate the cultures in the incubator. The culture of *E. coli* is used as a positive control and *Proteus vulgaris* is used as a negative control.

Prior to filtering of the samples, with filter still in place, rinse funnels (whole row of 12) by filtering three 100-mL portions of sterile dilution water. The funnels should be rinsed fully (on all sides), particularly in a swirling movement. Upon completion of the final rinse and the filtration process, unlock and remove the funnel.

Holding the funnel in one hand, immediately remove the membrane filter with the other hand with sterile forceps. Put the funnel back on the base. Open the premarked m-ColiBlue24 plate with one hand and place the membrane filter with the grid side up in the sterile plate in a rolling motion to avoid entrapment of air. Reset the membrane filter if nonwetted areas occur due to air bubbles. Follow the same steps to place the rest of the filters from the funnels to the m-ColiBlue24 plates. These 12 plates marked as B1 through B12 are the controls to check the sterility of the funnels as well as the rinse water.

To check for the sterility of plastic sample bottles, add 100 mL of sterile distilled water in two plastic sample bottles. Using 1.1-mL sterile pipet, plate out 1-mL sample on a plate count agar plate. Filter the rest of the sample, and place the membrane filters, from two plastic sample bottles, on the two sterile m-ColiBlue24 plates. Then process the samples.

After the last water samples are processed for analysis, the final control steps are to be initiated. Follow the same quality control steps as described earlier, i.e., 12 filters in each of the 12 funnels, rinsing with dilution water and placing the filter on the plates. These plates are marked as A1 through A12. Also repeat the medium control steps.

Place the filters in the filter assembly. Use the left side of the manifold for analysis of the raw samples and right side for the *E. coli* and *Proteus vulgaris* controls. Analyze raw samples as regular water samples.

For organism control, use the culture tubes of *E. coli* and *Proteus vulgaris* which were inoculated in the morning. Prepare the samples by serial dilution technique. It is important to dilute the culture to get few colonies on the plate. Using a sterile 1-mL pipette, prepare initial dilution by pipetting 1 mL of culture into an already made dilution bottle containing 100 mL of dilution buffer. Vigorously shake the bottle and pipet 1 mL into additional dilution bottles. Follow the technique for two more dilutions, thus making a total of four dilutions. A new sterile pipet must be used for each transfer, and each dilution must be thoroughly mixed before removing an aliquot for subsequent dilutions. Also, when an aliquot is removed, the pipet tip should not be inserted more than 2.5 centimeters below the surface of the liquid. The last dilution bottle is used as the organism control sample.

A separate funnel is used for organism control. The first organism to be used is *Proteus vulgaris*. Follow the same steps as described in analysis of a water sample carefully adding a 1.0-mL aliquot of the diluted culture from the last dilution bottle into the funnel with the filter. Follow the rinsing steps as described earlier. Remove the filter and place in the petri dish marked *Proteus* (PV). The empty funnel is rinsed with 70% ethanol followed by subsequent washes twice with buffered dilution water. Place a new membrane filter in the funnel and proceed with the *E. coli* sample. Follow the steps as described above. Mark the plate as EC. After the organism control run, the funnel is washed once with Lysol, rinsed with tap water and followed by distilled water.

Cleaning of Funnel Assembly

After all the runs are over, the funnels are rinsed with 70% ethanol followed by three washes with enough distilled water to get rid of traces of alcohol. Funnels are then repacked as described earlier and left for sterilization.

Once a week (Monday morning), funnels are cleaned to remove any metal tarnish. Funnels are immersed in hot water and air fresh metal polish is applied to all parts of the funnel with a wet towel under sink. Funnels are rinsed with warm water in the bucket and then with warm tap water. This is followed by rinsing with distilled water. Funnels are placed in the numerical order, dried, inspected for any remaining polish, and packed in paper bags (as described earlier) for sterilization. Rewash the funnel if you see any polish.

Air Monitoring of Work Area

It is important to monitor the work area because the number of microorganisms in the laboratory air is directly proportional to the amount and kind of activity. The number and type of airborne microorganisms can be determined by exposing a petri dish for a specified time at points where inoculating, filtering, plating, and transfer work are done.

Pour three petri dishes (air monitor) with plate count agar and allow to harden. Store poured plates in the refrigerator if they are not used on the same day.

For air monitoring of the work area, remove the petri dish covers and place the exposed plates for 15 minutes at (1) table opposite the manifold, (2) the shelf above the manifold, and (3) railing by the wall. These plates are marked as table, shelf, and ledge, respectively. Also, write the time of exposure (from-to) on the plates. Replace cover, incubate plates at 35 °C for 48 hours. Count the colonies with the help of a colony counter. Report the result as the number of organisms per minute (count the total number of colonies and divide by 15, the time of exposure). Record the results in the appropriate quality control record book labeled air monitor.

Incubation

For incubation, use plastic boxes with lid. Place a moist terry cloth towel (not paper towel) to provide a humid atmosphere. Arrange the plates in the box in such a way that it would be easy to take them out for counting. Plates are placed in an upside down flat position (first row), and all subsequent rows of plates are leaned upside down in a diagonal fashion. The maximum number of plates in the box should be between 60-68 plates. Incubate the petri dishes in an inverted position for 24 ± 2 hours at 35 ± 0.5 °C in an incubator with 100% humidity.

Counting

The typical coliform colony has a red color, and a blue color indicates *E. coli*. Count colonies visually or with the aid of a low power (10 to 15 X) binocular wide-field microscope. The grid line can be used in counting colonies.

Spread Plate Method for Total Heterotrophic Bacterial Count

Apparatus and Materials:

1. Incubator set at 35 ± 0.5 °C.
2. Sterile bacteriological pipet (1.1 mL).
3. Sterile petri dishes (15 x 100 mm).
4. Colony counter.
5. Plate Count Agar (PCA) medium.
6. Alcohol burner or Bunsen/Fisher type burner.

Preparation of Plates

Pour 15 mL of PCA medium into sterile 15- x 100-mm petri dishes and let agar solidify. Invert the plates and place the plates at 35 °C overnight for next day's use. Check the plates; discard the plates if there is any growth on the agar plates.

Procedure

Wipe the work area before and after use with Lysol. Prepare duplicate plates for each examined sample; label each with sample number, date, and any other necessary information. Shake the bottle vigorously (about 25 times) to disperse the bacteria. During the shaking, close cap tightly to prevent leakage of sample.

Lift cover of petri dish just high enough to insert the pipet. Slowly release 1 mL of sample from pipet onto surface of predried agar plate by using a 1.1-mL bacteriological pipet. Distribute inoculum over surface of the medium by rotating the dish by hand until the inoculum is even on the surface of the agar plate. Let inoculum be absorbed completely into the medium; invert plates and incubate the plates at 35 ± 0.5 °C for 48 hours.

Sterility Control

For air control, remove three covers of petri dishes, leave them open for 15 minutes and incubate them at appropriate temperature and time.

Standardized Micro-Method Bacterial Identification Systems

DWQC utilizes three standardized micro-method bacterial identification systems: API 20E, NFT, and Biolog. Any colonies which grow on the m-ColiBlue24 media will be re-isolated and grown on tryptic soy agar, gram stained, and analyzed by the API 20E system, which consists of 23 biochemical tests. Any bacteria that are unable to be identified, and are nonfermentative bacteria, then will be analyzed by the NFT system, which consists of 20 biochemical and assimilation tests. The Biolog system, which consists of 95 biochemical tests, will be utilized when both the API 20E and the NFT fail to identify the bacteria. The standardized procedures and controls will be followed when utilizing any of the three identification systems.

The Attached Growth Rate Estimate (AGRE) Test

The background for the AGRE test was given in section 4 of the main body of the report.

Preparation of Rods, Disks and Carboys for AGRE Experiment

The various components of the AGRE unit were prepared as follows:

Polyvinylchloride (PVC) disks: Attached bacterial growth was observed through the use of 2.5-inch diameter PVC disks. The bacteria attached to the bottom of the rotating disk were of interest. Each disk was mounted onto a stainless steel (316S) rod with stainless steel shaft collars affixed to the rod on both sides of the disk with allen keys. Prior to running an experiment, each object was treated as follows:

Rods and shaft collars: Soak in 99% isopropyl alcohol for 24 hours. Attach PVC disk using collars.

Polyvinyl Chloride Disks:

1. Scrub with detergent
2. Rinse well with water
3. Soak in isopropyl alcohol for 24 hours
4. Mount disk at the tip of the treated rod with two shaft collars at the top and bottom
5. Wrap the entire unit in aluminum foil. Take care not to leave any openings.

Sterilize in the autoclave at 250 °C and 15 psi for 20 minutes at “slow exhaust” to prevent the disks from warping.

Recycling PVC Disks:

Following each experiment, the disks were recycled using the following procedure:

1. Remove microscope cover slips and discard.
2. Scrub disk with detergent to remove immersion oil (best done right after observation under the microscope).
3. Rinse well with water.
4. Soak in chlorine (bleach) for 2 hours.
5. Rinse well with sterilized water.
6. Soak in 99% isopropyl alcohol for 24 hours.

7. Mount disk on treated rod with two shaft collars.
8. Cover entire unit with aluminum foil.
9. Sterilize in autoclave at 250 °C and 15 psi at slow exhaust for 20 minutes.

Preparation of Carboys:

The 2.5 gallon high density polypropylene jars used in both batch and continuous flow experiments were prepared as follows:

1. Place carboys and carboy covers into an acid bath consisting of potassium dichromate and concentrated sulfuric acid for 24 hours.
2. Transfer carboys to a distilled water bath for rinsing (water to be changed daily).
3. Place carboy with cover into an autoclave bag and seal bag.
4. Sterilize in autoclave at 250 °C and 15 psi for 20 minutes.

Preparation of Tubing for Liquid Transfer:

Tubing consisted of three parts for each carboy used in continuous flow tests. The first and third parts, 7-15 and 2.5 feet, were teflon tubing that fit inside pressure fittings located at the water source, as well as the inlet and outlet of the carboy, thus connected the carboy to the source and directed the carboy effluent to the drain. The second part was soft rubber tubing (2.5 feet) that was placed through the pumphead of a peristaltic pump which regulated the flow into the carboy. The tubing was prepared as follows:

1. All pieces of tubing were soaked in soapy water for 24 hours.
2. Soapy water was drawn from the tubing using a 60-mL syringe with a pipette tip attached to it.
3. Fresh soapy water was filled into a second 60-mL syringe and driven through the tubing.
4. A third 60-mL syringe was used to drive sterile water through the tubing.
5. A fourth 60-mL syringe was used to drive 99% isopropyl alcohol through the tubing.
6. Sterilized water was again driven, for a last time, through the tubing.
7. The tubing was placed in small autoclave bags and sealed with autoclave tape.

8. Sterilized in autoclave at 250 °C and 15 psi for 20 minutes.

Attached Bacteria Enumeration Procedures:

Three positions were randomly identified on the surface of the disk, each at $r/4$, $r/2$ and $3r/4$ (r is radius of disk) distances from the center of the disk. At each position, 10 viewfields, also randomly selected, were inspected using an epifluorescent microscope to count single, dual, and quadruple colonies. The results were used to determine the $C2/C1$ and $C4/C1$ ratios at each viewfield and position which in combination with figure 1 was used to determine μ , or the attached growth rate estimate.

Conventional Water Quality Parameters

pH: Standard Method 4500-H-B (American Water Works Association [AWWA], American Public Health Association [APHA], and Water Environment Federation [WEF], 1992).

Temperature: Standard Method 2550 B (AWWA-APHA-WEF, 1992).

Temperature will be measured using with American Society for Testing Materials (ASTM) with either an approved mercury-filled or alcohol-filled thermometer with a minimum precision of 0.1 °C and markings etched on the capillary glass.

Conductivity: Standard Method 2510 B (AWWA-APHA-WEF, 1992).

The laboratory method for measuring conductivity will be used. The measurement involves rinsing the conductivity cell with one or more portions of sample, temperature adjustment to about 25 °C and measurement of conductance or resistance on the meter. The temperature will be recorded to the nearest 0.1°C.

Alkalinity: Standard Method 2320-B (AWWA-APHA-WEF, 1992).

Procedure

A 100-mL sample will be added to a 200-mL erlenmeyer flask followed by a 0.2-mL (5 drops) indicator solution. The titration will be carried out on a white surface to a persistent color change characteristic of the equivalence point using 0.02N sulfuric acid as titrant and a precision burette. The titration end-points will be determined using bromocresol green (pH: 4.5), and metacresol purple (pH: 8.3) indicators.

Interference Control

Chlorine residual that may be present in water will be destroyed by adding 0.05 mL (1 drop) 0.1M $\text{Na}_2\text{S}_2\text{O}_3$ solution, or by exposing it to ultraviolet

(UV) radiation. The color at both pH endpoints will be checked by adding the same concentration of indicator used with sample to a buffer solution at the designated pH.

Nitrite-Nitrate: Standard Methods 4500-NO₂-C/NO₃-C (AWWA-APHA-WEF, 1992).

Both nitrite and nitrate were measured by ion chromatography with chemical suppression of eluant conductivity.

Apparatus and Materials

1. Ion chromatograph (Dionex, 4500i)
2. Anion separator column
3. Guard column
4. Membrane suppressor
5. Sample injector syringes (1-10 mL)
6. Volumetric flasks (100, 200, 500 mL)
7. Strip chart recorder
8. Various reagents

Procedure

Operated ion chromatograph in the “anion” mode. Adjust eluant flow to 2.0 milliliters per minute (mL/min) and regenerant flow to about 3 mL/min. Turn on conductivity detector and equilibrate system for about 20 minutes. Equipment ready to use when a steady baseline is observed at detector setting of 3 microsemens. Calibrate the system by injecting several working standards (at least four) for both nitrite and nitrate and develop linear dynamic range for detection using peak height and attenuation. Remove all particulates from the sample using membrane filters. Recalibrate whenever the detector setting, eluent or regenerant is changed.

Ammonia: Standard Method 4500-NH₃-F (AWWA-APHA-WEF, 1992)

Apparatus and Materials

1. Electrometer (capable of reading millivolt [mV])
2. Ammonia-selective electrode
3. Magnetic stirrer and teflon coated stirring bars

4. Beakers and erlenmeyer flasks

Procedure

Prepare a series of standard solutions covering the concentration range of 1,000, 100, 10, 1 and 0.1 mg/L as NH₃-N. Place 100 mL of each standard solution in a 150-mL beaker. Immerse electrode in standard of lowest concentration and mix with a magnetic stirrer and raise the pH to 11 using 10N NaOH. Keep electrode in solution until a stable millivolt reading is obtained. Use readings for all standards to develop a calibration curve. Use dilutions whenever necessary to bring the sample concentration to the linear dynamic range of the calibration curve. In samples with NH₃-N < 1 mg/L wait for at least 5 minutes before recording millivolts.

Total Organic Carbon (TOC): Standard Method 5310-C (AWWA-APHA-WEF, 1992)

Apparatus and Materials

1. Total Organic Carbon Analyzer (Tekmar-Dohrman 90)
2. Injection syringes (0 to 50; 0 to 250 microliter; and 0-1 mL fitted with a blunt-tipped needle).

Procedure

Follow manufacturer's instructions for assembly, testing, calibration, and operation of TOC analyzer. Homogenize samples that contain particulates. Prepare operating standards over the range of organic carbon concentrations expected in the samples. Inject standards and blanks and record analyzer's response as peak area. Subtract appropriate blank's peak area from those for each sample and determine organic carbon from the standard curve.

Total Phosphorus: Standard Method 4500-P (AWWA-APHA-WEF, 1992)

Apparatus and Materials

1. Spectrophotometer
2. Acid-washed glassware
3. Various reagents

Procedure

Pipet 50.0-mL sample into a flask and add one drop of phenolphthalein indicator. Discharge any red color using several drops of 5N H₂SO₄ and add 8.0 mL combined reagent and mix thoroughly. Measure absorbance

at 880 nm after 10 but no later than 30 minutes. For highly colored or turbid waters, prepare a blank as described in the Standard Methods and subtract its absorbance from the absorbance of each sample.

Chlorine Residual: Standard Method 4500-Cl-F (AWWA-APHA-WEF, 1992)

Apparatus and Materials

1. pH meter
2. Precision burette
3. Magnetic stirrer
4. Teflon coated 1-inch stirring bars
5. Erlenmeyer flasks - 100 and 200 mL
6. Assorted volumetric pipets.
7. Various reagents

Procedure

Place 5 mL each of buffer reagent and DPD indicator in a titration flask; then add 100-mL sample, or diluted sample, and mix. Add a few crystals of KI and titrate rapidly with standard FAS until red color is discharged. Each mL of standard FAS is equivalent to 1 mg/L of chlorine residual as Cl₂.

Ozone Residual: Standard Method 4500-O₃-B (AWWA-APHA-WEF, 1992)