AN INVESTIGATION OF THE EFFECTS OF OZONE IN A RECIRCULATING AQUACULTURAL PRODUCTION SYSTEM

by

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(ABSTRACT)

The use of ozone in a recirculating aquacultural production system on a commercial scale was investigated. Ozonation was responsible for statistically significant differences in all test parameters, including: heterotrophic plate count, total coliforms, fecal coliforms, total solids, and total volatile solids. Results indicated ozone had a significantly positive effect in the recirculating aquacultural production system. Further study is recommended, however, to better understand the effects of ozone and to justify economically the use of ozone in commercial systems.

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Table of Contents

List of Figures

List of Tables

INTRODUCTION

Recirculating aquaculture systems were developed in an effort to intensify aquacultural production. Many factors have influenced and even necessitated the intensification of aquaculture. The commercial fishing industry has been historically the dominant source of fish and other seafood. However, concerns for a higher quality product and more dependable supply have resulted in more intensive production systems, such as the catfish production system in ponds in the southern United States. These pond production systems have had some problems including poor water quality under some circumstances and high costs of production. Many of these problems have been successfully addressed and the production systems are now functioning more profitably.

The next logical step in the intensification of aquacultural production is the effort to move production systems indoors. Greater system control made possible by a controlled indoor environment has several theoretical advantages that benefit producer and consumer. The controlled indoor environment allows the producer greater control of production factors, such as temperature and water quality. Temperature control encourages optimum growth on a year-round basis and improved feed-to-gain ratios. Water quality control provides an environment where diseases and taste and odor problems are minimized or more effectively

treated. Most importantly, the consumer is rewarded with a higher quality product that has not been exposed to the contaminated environment often found in open systems.

Development of indoor systems has brought more problems to light as the intensity of production has increased. As fish density increases, the amount of flow-through water must also be increased to provide a means of carrying the increased waste load away from the fish. However, this increased water volume substantially increases the amount of energy needed to heat the incoming flow to the optimum growth temperature. At some point, the higher energy costs exceed the value of the increased production. Thus, one of the primary goals of the intensive indoor system is to maximize fish production while minimizing water use. Recirculating aquacultural production systems theoretically allow for the reuse of almost 100 percent of the water in the system, but this is not technically achievable at the present time. However, as the demand for high quality water increases, the value of the water itself will increase and recycling of water will become an economic necessity.

Recycling necessitates water treatment in the system to remove harmful contaminants from the recycled flow. Ammonia is one of the most significant contaminants produced as the fish metabolize feed for growth. Removal of ammonia can be accomplished through chemical means, but these are not

practical in aquaculture systems because they generally require large shifts in the pH of the recirculating flow and introduction of chemicals potentially toxic to the fish. Nitrification, biological conversion of ammonia to nitrate, is more appropriate for nitrogen removal because the system utilizes a natural biological system, nitrifying bacteria, to convert the toxic ammonia to nitrate, which is not nearly as harmful to the fish. As a result, recirculating production systems have two interdependent biological systems that coexist and rely on each other for survival. In essence, an equilibrium is necessary for each biological system to thrive. Unfortunately, the environment provided for the nitrifying bacteria may also encourage the growth of other microbiological organisms that may not be beneficial to the system. In fact, these latter microorganisms can pose a threat to the fish in the form of diseases or to the producer in the form of taste and odor problems.

The recirculating flow in these systems provides a broth of nutrients for the growth of potentially harmful organisms and a means to spread infections. Therefore, some level of disinfection is required to curtail the harmful organisms without destroying the beneficial ones. Logically, the fish should be able to tolerate the exposure to small numbers of the harmful organisms which coexist in natural environments with the fish.

RESEARCH OBJECTIVES

The overall purpose in this research was to investigate the use of ozone in a high rate recirculating aquacultural production system to assess its potential role in commercial aquaculture production. Specific objectives of this research included:

1. Show that ozone injection in a recirculating aquaculture system will produce a statistically significant reduction in microbiological activity as measured by total coliform, fecal coliform, and heterotrophic plate count tests.
2. Show that ozone injection in a recirculating aquaculture system will produce a statistically significant reduction in volatile solids while not significantly affecting total solids.

LITERATURE REVIEW

Increased incidence of diseases is one of the major problems encountered as aquaculture production systems intensify. As more fish are crowded into fixed volumes of water, the need for disinfection in the system dramatically increases (Wheaton, 1987). Traditionally, disinfection is applied to the system influent to prevent the makeup water from introducing diseases to the system and to the system effluent to prevent the release of diseases from the system.

Several potential disinfection methods exist for aquaculture. Heat is an effective disinfection method often employed in the food processing industry, but its main disadvantage is high energy cost (Wheaton, 1987). In a recirculating system the temperature must remain constant to minimize stress on the fish. The flow must be heated, held for a period of time at the required disinfecting temperature, and then cooled before being reintroduced to the fish, requiring even more energy input.

Chlorination is a popular disinfection method in many water and wastewater treatment facilities. An additional advantage in water treatment systems is that a chlorine residual can be maintained throughout the distribution system to provide additional disinfecting power. This residual chlorine can be toxic to the fish in the recirculating aquacultural production system, thus chlorine

residual removal through absorption, filtration, or chemical methods may be required to protect the fish. Chlorine also readily combines with nitrogen and organic compounds to form chloramine or chloroform and intensifies the taste and odor causing properties of phenols (APHA, 1989).

Ultraviolet light shows promise for disinfection in aquaculture because disinfection can be achieved without the addition of chemicals to the system. The disadvantages center on the variability of water quality in the system resulting in variable treatment and maintenance problems as water quality deteriorates (Colberg et al., 1977).

Ozone is often viewed as a more effective disinfectant than chlorine due to lower required dosages and shorter required contact times. Ozone also rapidly degrades to oxygen and decomposes complex substances, both organic and inorganic. However, ozone requires expensive onsite generation equipment and is very corrosive (Wheaton, 1987).

The use of ozone in water and wastewater treatment has grown as a result of increased demands on the industry and greater understanding of the usefulness of ozone (Evans, 1975). Water treatment systems usually incorporate ozone as a disinfectant, but the technology has grown hesitantly due to the economic favorability of other disinfectants, such as chlorine. However, ozone appears to be the disinfectant of choice in Europe. Wastewater treatment utilizes ozone for

its capacity to degrade complex substances that impart taste, color, and odor to water. Ozone in aquaculture draws from both applications to address disease control in recirculating systems and take advantage of the other potentially positive effects of ozone treatment.

With an oxidation potential of 2.07 volts, ozone is the most powerful practical oxidant in use for water treatment systems (Montgomery, 1985). Ozone is a triatomic molecule of oxygen formed when oxygen gas is excited by an electrical charge. The oxygen molecules separate and some of them reform as triples, creating the ozone molecule. This molecule has a molecular weight of approximately 48 g/g-mole and a boiling point of approximately -112 °C at standard atmospheric pressure (Weast, 1988). Unstable as a gas, ozone degrades to molecular oxygen with half-life estimates varying widely from minutes to hours, depending on the specific circumstances (Katz, 1980). Ozone is more soluble in water than oxygen and is considered corrosive and potentially explosive if concentrations in air approach 20 percent (Katz, 1980). However, the gas exhibits a noticeably penetrating odor at concentrations as low as 0.01 ppm and normal operational conditions preclude potentially explosive conditions.

Measurement of ozone concentration in gaseous or liquid phases, primarily air or water, is difficult (Rice et al.,

1986). The instability of the gas and propensity to decompose in any solution makes accurate measurement nearly impossible. As a result, comparison of results from various research efforts is difficult and has even caused bitter disputes between ozone researchers. The search for an ozone specific test method resulted in the proposed indigo colorimetric method (APHA, 1989). This method replaces previous methods which measured total oxidant and relied on the principle that indigo was rapidly decolorized by ozone in an acidic solution. Alternatively, many commercial monitoring systems take advantage of another ozone specific characteristic, whereby light in the short wavelength region between 200 and 300 nanometers is absorbed by ozone gas with peak absorption at 253.7 nanometers (Rice, 1986).

The history of ozone can be traced back to the late eighteenth century when, in 1785, the air around Van Marum's electrostatic machine exhibited a noticeable odor after a series of electric sparks. Electrolysis of water in 1801 by Cruickshank yielded a similar odor. Eventually, in 1840, Schonbein associated the term ozone with the substance he thought caused this distinct smell and also suggested the natural occurrence of ozone in the atmosphere. (Katz, 1980)

Commercial ozone generation is accomplished through the excitation of oxygen molecules exposed to 100 to 200 nanometer ultraviolet radiation or by passing the molecules

through a high voltage corona discharge, with the vast majority of generators falling into the latter category (Wheaton, 1987). The corona discharge system can be traced back to the mid-nineteenth century when Siemens, in 1857, passed air between coaxial glass tubes that were coated internally and externally with tin foil (Katz, 1980). Cooling of the system to increase efficiency has been the major improvement over the original ozonator in modern generators, along with very complicated electronics to modify the voltage and frequency of the electrical current which excites the oxygen molecules.

Production variables include the feed gas composition; the pressure, temperature, and flow of the feed gas; and the dimensions and orientation of the gap between the charged surfaces. Very dry feed gas is required to minimize corrosion in the system and the use of pure oxygen doubles the concentration of ozone for equal power consumption when compared to air. Typical concentrations of ozone range from 0.5 to 10 percent with efficiencies dropping rapidly as ozone concentrations increase (Wheaton, 1987).

Applications of ozone have varied widely and have generally been successful. The first large scale treatment systems for disinfecting drinking water supplies date back to the late nineteenth century in Europe. In 1893, ozone was utilized in Holland and in 1901, Germany began utilizing

ozone treatment. In 1906, Nice, France, began using ozone as a disinfection treatment and has done so ever since. The facility in Nice, France, is generally considered the "birthplace" of ozone disinfection for treatment of drinking water (Katz, 1980).

Interest in ozone for disinfection in the United States was somewhat limited during the first half of the twentieth century, but the increased environmental awareness of the 1960's and 1970's renewed research efforts to seek new methods of disinfection and applications for ozone (Evans, 1975). Numerous authors described the usefulness of ozone for disinfection (Kinman, 1975; Venosa, 1975; Legeron, 1984), degradation of organics (Hoigne, 1982), control of taste and odor problems (Nebel and Forde, 1976; Richard and Brener, 1984), and wastewater treatment (Robson and Rice, 1985).

The use of ozone to improve water quality in aquaculture has been an important area of interest for both research and commercial aquaculturists. Applications to date have varied in size from bench top studies to commercial production facilities (Wheaton, 1987; Blogoslawski et al., 1978; Colberg et al., 1977; Rosenlund, 1975; Sander and Rosenthal, 1975; Stopka, 1975) with disinfection driving most applications and decomposition of organics, color, and turbidity as desirable secondary

effects. One of the most extreme examples of water reuse was a closed system trout facility that could maintain the fish for three months without addition of supplemental water to the system (Stopka, 1975).

Ozone for disinfection (Roselund, 1975) was used in a hatchery application in Arizona on the incoming water from a surface water supply. Disinfection, defined as zero colonies/Ml, was verified with ozone residual of 0.01 mg/L in the post treatment flow. However, the fish in the pilot study experienced 100 percent mortality within 4 hours. The researchers had to develop an ozone removal protocol for the ozone residual following disinfection before the pilot study was successful. Aeration was found to remove the residual ozone effectively. A minimum of 11 minutes detention time was required in an aerated detention basin to remove the residual ozone and protect the fish.

Another hatchery pilot experiment was conducted to compare ozone and ultraviolet radiation system (Colberg et al., 1977). The researchers concluded that the ozone system provided more consistent disinfection and required less maintenance than the ultraviolet system. Cost analysis showed that the ozone system was more expensive to purchase and operate, but the improved performance justified the additional costs. An additional experiment was conducted wherein the ozone pilot plant was installed as part of the

recirculating flow in the hatchery. This system was shown to be very effective in improving general water quality. The ozone also killed algae in the system, thereby enhancing biological treatment processes. An attempt to overdose the system with ozone demonstrated that the system had a very high tolerance to potential ozone overdoses. The ozone treatment of the recirculating flow also stabilized highly fluctuating BOD levels.

Given the positive potential of ozone in aquaculture just discussed, the next logical step is an experimental design to test the usefulness of ozone in a commercial recirculating aquaculture production system.

METHODS AND MATERIALS

Experimental Design

The experimental design employed in this research took into account the needs of the researcher as well and the needs of the commercial production facility, Blue Ridge Fisheries, Inc., where the research was conducted. This complicated the research, as research and production needs were often divergent. Two tanks were utilized in the research. One tank was a control without ozone treatment and the other was treated with ozone.

Criteria for the selection of two tanks required that each tank was nearly identical with respect to initial water quality conditions, fish load, and feeding rates. Two tanks, located side-by-side (#14 and #15) roughly in the center of the facility, were selected as the best tanks that met the criteria of the experiment. Each contained approximately 6800 kilograms (15000 pounds) of growing channel catfish, Ictaluris punctatus. Each had similar initial water quality conditions.

The fish were fed a ration of approximately 90 kilograms (200 pounds) per day, which was divided into two feedings per day (8 am and 8 pm). This was roughly one-half the normal ration of approximately 180 kilograms (400 pounds) per day. The reduced ration allowed the experiment to be conducted without excessive organic demand being

placed on the ozone. With the exception of the reduced ration, each tank was treated just like any other tank in the facility.

Each system had four basic components and contained approximately 200 cubic meters (56000 gallons) of water. The fish were contained in the first component, the fish tank, which comprised approximately one half of the total system volume. The remaining system volume was divided between the sump/clarifier which was used for solids (organics) removal and comprised approximately one sixth of the system volume and the rotating biological contactor (RBC) which was used for nitrification and utilized most of the remaining volume. The fourth basic component was the u-tube aerator, where oxygen and ozone injection occurred. One tank was injected with ozone slightly upstream of the u-tube, while the other tank received only the pure oxygen injection in the u-tube necessary to maintain the oxygen level in the tank. An air lift pump recirculated the water at a rate of 3400 liters per minute (900 gpm).

A dissolved oxygen level of 85-100 percent saturation or about 6.9 to 8.0 mg/L oxygen at normal operating temperatures was maintained in the system. The ozone and oxygen were delivered to each tank via identical injection systems. The gas was introduced into the system as the recirculating flow entered the u-tube aerator. The ozone

rapidly decomposed to oxygen, thereby killing microorganisms and decomposing any complex substances contacted. The combined liquid and gas flowed to a depth that caused approximately a 1 atmosphere increase in hydrostatic pressure which caused supersaturation of the gases into the flow. The residence time in the u-tube was approximately 30 seconds before returning to the tank.

The ozone generator was a Griffin Technics, Inc. (Lodi, New Jersey) model GTC-2B utilizing pure oxygen as the feed gas and operating to produce a concentration of ozone of approximately 3 percent in the oxygen delivered to the ozonated tank. The oxygen was supplied from an onsite liquid oxygen storage system. The resulting ozone dosage to the recirculating flow was approximately 0.5 mg/L.

The hypothesis to be tested in this research was that the water quality in the ozone tank would improve relative to the tank without ozone over 10 days of ozonation. After 10 days, the ozone was to be terminated in the first tank and started in the second tank (former control). Water quality was expected to improve in the second tank over the next 10 days and deteriorate in the first tank. Total commitment of each tank to the experiment was not to exceed three weeks and thus not significantly interfere with the production schedule of the commercial facility.

Sample Collection and Analysis

A 400-500 mL water sample was taken from each tank in presterilized sample bags (Whirl-paks), stored on ice, and shipped overnight to the Virginia Tech Agricultural Engineering Water Quality Laboratory for analysis within 24 hours of the sample collection. The sample collection point was at the surface of the water in the fish tank. Since the fish tank was considered to be a homogeneously mixed reactor, any location would be acceptable.

Tests for total coliforms, fecal coliforms, heterotrophic plate count, total solids, and total volatile solids were run in triplicate for each test on a daily basis. These tests were performed in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA, 1989).

Upon arrival at the lab, each sample was removed from the ice, shaken vigorously (approximately twenty-five times), and allowed to equilibrate back to room temperature (approximately 25-27 °C). During this time, the work area was cleaned, disinfected, and necessary equipment was autoclaved. Dilutions were made using 100 mL milk dilution bottles with 10 mL of sample diluted to 100 mL using autoclaved dilution water. Before each dilution, each sample was shaken vigorously. Three to five dilutions were plated to ensure an appropriate colony count on the plates.

The pour plate method was utilized for the heterotrophic plate count (section 9215 B.; APHA, 1989) and the membrane filter technique was utilized for the total and fecal coliform tests (sections 9222 B. and D.; APHA, 1989). Presterilized disposable 50 by 9 mm petri dishes were used for all tests.

The heterotrophic plate count utilized plate count agar. Plates were incubated at 35°C for 48 hours in a forced-air incubator. Dilutions were selected to yield 30-300 colony forming units (cfu) per plate from a 1 mL sample.

The total coliform test utilized M-Endo medium placed on presterilized absorbent pads. The plates were incubated at 35°C for 24 hours in a forced-air incubator. Dilutions were selected to yield 20 to 80 pink to dark red colonies with a metallic sheen and not to exceed a total of 200 colonies per plate from a 1 mL sample.

The fecal coliform test utilized M-FC medium on presterilized absorbent. The plates were incubated at 44.5°C for 24 hours in a water-bath incubator. Dilutions were selected to yield 20 to 60 blue colonies per plate from a 1 mL sample.

For the coliform tests, a presterilized 47 mm, 0.45 micrometer gridded filter was placed on the autoclaved filter apparatus. The vacuum system was activated. One milliliter of diluted sample was filtered and the filtering

apparatus was rinsed out. The membrane was then transferred to an appropriately labeled petri dish. This procedure was repeated in triplicate for each type of test and a new autoclaved filter apparatus was used for the next test. The heterotrophic plate count samples were conducted in triplicate with the appropriate dilutions in the same manner as the coliform samples.

The total solids test (section 2540 B.; APHA, 1989) and total volatile solids (section 2540 E.; APHA, 1989) were conducted in triplicate after incubation of the microbiological samples began. Porcelain 90-mm evaporating dishes were first ignited for at least 1 hour at 550°C and stored in a desiccator until they cooled to room temperature. The dishes were then weighed and 100 mL of sample was placed in each dish. The dishes were then placed in a drying oven at 104°C for 24 hours. The dishes were removed, allowed to cool to room temperature in the desiccator, and reweighed. The increase in weight represented the total solids contained in each sample. The dishes were then placed in a muffle furnace at 550°C for 20 minutes. The dishes were removed, allowed to cool to room temperature in a desiccator, and reweighed. The decrease in weight represented the total volatile solids contained in each sample. This roughly approximated the amount of organic matter contained in the water.

Qualitative visual observations of the tanks were taken to monitor system performance. Observable parameters included the color and clarity of the water; the amount of visible suspended solids; the behavior of the fish, especially during feeding; and the circulation characteristics of the tank. These parameters provide the most immediate indications of general water quality in the system and the health and safety of the fish.

The system was also monitored for the presence or absence of ozone residual in the water. The Hach company (Loveland, Colorado) provided a variation of the DPD chlorine test (Section 4500 Cl G.; APHA, 1989) as an ozone test kit. The advantage of this test method was its sensitivity to a wide variety of oxidants, as evidenced by the large number of interfering compounds. Preliminary tests conducted on the system when operated without ozone showed that no oxidants were measurable in the system. Once ozone was applied to the system, the ozone could potentially degrade certain substances in the system to create other oxidants, such as peroxides, that would also be detected by this test method. The test kit was very simple to use and Hach specified an accuracy of ± 0.05 mg/L ozone. The disadvantage of this test method was the inaccuracy in measuring true ozone residual. This was not a problem in the present system because a true ozone residual was not

desired. A true ozone residual would probably yield significant fish mortality in the tank. The Hach test was intended to indicate the presence of increasing levels of total oxidants before a true ozone residual occurred. This would allow the ozone dosage to be reduced before fish mortality. None of these eventualities was expected, but in the event that total oxidant levels increased and the fish appeared to be experiencing discomfort, the ozone application rate could be reduced or cut off. In a situation such as this, the welfare of the fish and the profits of the commercial facility would take precedence over the needs of the experiment; i.e. the research would be sacrificed in favor of the fish should that decision need to be made.

Statistical Methods

The null hypothesis for each of the five test parameters was the same: there was no difference between the control tank and the ozone tank. To accomplish the objectives set forth, the null hypothesis must be accepted for the total solids test and rejected for the other four test parameters.

Linear regression was performed on each set of data using MiniTab statistical software (Release 6.1.1.; MiniTab, Inc.; State College, Pennsylvania) to determine the slope of

the trend line. Comparison of the slopes would indicate whether there was a statistically significant difference in the microbiological populations and solids between the two tanks. If the null hypothesis is correct, the slopes for each tank will not be significantly different. If the null hypothesis is incorrect, the ozone had a positive effect by reducing the growth rate of bacteria and solids content.

The test statistic used to evaluate the null hypothesis was the Welch t-test (Ott, 1988):

$$t' = \frac{\beta_1 - \beta_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where,

t' = *Welch approximate t-statistic.*

β_1, β_2 = *slopes of control and ozone, respectively.*

s_1, s_2 = *standard deviation of slopes.*

n_1, n_2 = *number of samples.*

The approximation was accomplished by modifying the degrees of freedom (df) used to set the rejection region for the null hypothesis:

$$df = \frac{(n_1-1)(n_2-1)}{(n_2-1)c^2 + (1-c)^2(n_1-1)} \quad \textit{where,} \quad c = \frac{\frac{s_1^2}{n_1}}{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

The Welch approximation was necessary due to differences in the variances between the two sample populations. In some cases, the variances differed by greater than a factor of three. The Welch t-test reduces to the standard t-test when the variances between the sample populations are equal and is thus a conservative approach.

RESULTS AND DISCUSSION

The first water sample was taken and ozone was introduced to tank 15 on ozone on June 4, 1990, at 2 pm. Each system was then operated normally, i.e. just like any other tank in the building. The fish were fed a daily ration of 90 kilograms (200 pounds) in two, approximately equal, portions at 8 am and 8 pm. The experiment followed expected guidelines for the first nine days. Visual observations revealed an improvement in the water clarity in the ozone tank (#15), while the control tank (#14) became increasingly turbid and took on a deepening brown tint.

At the end of the ninth day, each tank was inadvertently given a potassium permanganate treatment by a plant worker, thus potentially adversely affecting all of the remaining data points. Being a strong oxidizer, potassium permanganate attacks the organics and microbes in a manner similar to ozone. This reduced the quality of the control because it had been, in effect, ozonated through alternate means. This may not have been too serious however, as the ozone is a much more powerful ozidant than potassium permanganate and the potassium permanganate might not have seriously impacted bacteria and solids levels in the tank.

Unfortunately, the fish in the control tank began dying on June 12. The cause of death was ESC, enteric septicemia

in catfish. The tanks were switched with respect to ozone addition on June 14, according to schedule. The experiment was continued in the hope that the ozone treatment on the former control tank (now the ozone tank) would arrest the mortality. With mortality steadily increasing in the ozone tank and the fish in the control tank (former ozone tank) beginning to show similar ESC symptoms, the experiment was prematurely ended on June 17. Treatment procedures, which included antibiotics, were immediately initiated in the control tank and the original ozone tank eventually had to be treated similarly. Therefore, the data considered pertinent to this experiment were limited to the first nine days of the experiment.

Visual observations and measurements of ozone residual did not show any detectable ozone residual or obvious detrimental effects to the fish. The ESC outbreak was not attributable to the experiment as similar ESC outbreaks were occurring in other tanks in the facility. The ozone seemed to delay the ESC outbreak in the original ozone tank (#15), but could not arrest the mortality in the original control tank (#14).

Both the control tank and ozone tank experienced upward trends in all test parameters as shown in Tables 1 and 2, respectively. Although the ozone tank experienced less of an upward trend, the similarity in trends was unexpected.

Table 1. Control tank data (three replicates).

Day	HPC[1] cfu[6]/mL	TC[2] TC/100mL	FC[3] FC/100mL	TS[4] mg/L	TVS[5] mg/L
	61000	60000	9800	560	180
1	154000	110000	11000	560	170
	115000	80000	8900	530	160
	132000	40000	1600	560	270
2	120000	30000	1400	630	330
	103000	20000	900	590	270
	191000	67000	40000	610	250
3	183000	53000	53000	630	220
	202000	59000	49000	630	240
	80000	77000	31000	650	350
4	63000	74000	22000	650	240
	82000	70000	19000	670	270
	190000	130000	19000	730	330
5	184000	150000	20000	750	340
	170000	220000	12000	750	330
	580000	410000	35000	730	320
6	290000	540000	38000	710	370
	400000	480000	48000	720	320
	1590000	610000	130000	760	380
7	1700000	no data	200000	750	300
	1780000	430000	190000	760	350
	1210000	510000	150000	860	540
8	1380000	420000	150000	860	420
	1430000	370000	140000	910	530
	940000	220000	120000	1020	460
9	860000	230000	80000	1070	500
	1020000	330000	50000	1050	530

Notes:
[1]heterotrophic plate count
[2]total coliforms
[3]fecal coliforms
[4]total solids
[5]total volatile solids
[6]colony forming units

Table 2. Ozone tank data (three replicates).

Day	HPC[1] cfu[6]/mL	TC[2] cfu/100mL	FC[3] cfu/100mL	TS[4] mg/L	TVS[5] mg/L
1	100000	190000	150000	750	270
	330000	200000	110000	700	180
	560000	250000	70000	740	200
2	500000	100000	80000	840	300
	590000	110000	50000	800	270
	520000	120000	60000	no data	no data
3	340000	1500000	390000	710	250
	240000	1600000	450000	710	340
	260000	1400000	510000	740	320
4	129000	570000	170000	810	430
	114000	630000	150000	770	340
	103000	420000	60000	800	320
5	49000	270000	10000	860	360
	67000	240000	15000	810	290
	59000	280000	16000	820	360
6	95000	390000	260000	700	260
	60000	310000	130000	730	240
	81000	340000	180000	790	300
7	380000	260000	46000	800	320
	370000	250000	60000	840	320
	380000	230000	71000	860	340
8	193000	210000	41000	960	580
	176000	270000	29000	980	540
	201000	300000	59000	1020	610
9	710000	170000	52000	970	370
	620000	160000	40000	910	350
	420000	170000	21000	1020	440

Notes:
[1]heterotrophic plate count
[2]total coliforms
[3]fecal coliforms
[4]total solids
[5]total volatile solids
[6]colony forming units

The significance of the difference in the trends was analyzed by comparing the slopes of the linear regression lines obtained from the raw data (Tables 1 and 2) for each test parameter. This analysis (Table 3) showed highly significant differences between the slopes for each test parameter indicating that the ozone was affecting tank microbiological activity and solids levels.

The heterotrophic plate count in the control tank was steady during the first four days and experienced increased growth during the latter half of the experiment (Figure 1). This trend contrasted with the ozone tank, which had fairly stable HPC's throughout the experiment. Statistically, the heterotrophic plate count showed the greatest degree of confidence that the slopes were different when compared to the other test parameters as evidenced by the highest t' value (Table 3). The difference in the slopes was highly significant ($p < 0.01$).

The control tank total coliforms (Figure 2) displayed a pattern similar to the control tank heterotrophic plate count (Figure 1); the values were fairly steady during the first four days and dramatically increased during the last four days. The ozone tank total coliforms (Figure 2) mirrored the ozone tank heterotrophic plate count (Figure 1) except for the two extreme values on the second and third days. The results were highly significant ($p < 0.01$).

Table 3. Statistical results for the Welch t-test (t') used to determine significant differences between the regressed slopes of trend lines for the control and ozone systems.

Parameters	HPC[1] cfu[6]/mL	TC[2] TC/100mL	FC[3] FC/100mL	TS[4] mg/L	TVS[5] mg/L
Slope(units/day)					
control	172156	51900	16636	52.8	36.5
ozone	2167	-40611	-17817	28.4	25.4
n (sample size)					
control	27	26	27	27	27
ozone	27	27	27	26	26
std. deviation of slope					
control	28483	9713	3250	4.15	3.85
ozone	15233	30404	9488	4.94	6.21
Welch t-test					
t'	27.35	15.03	17.85	19.44	7.79
df	39	31	32	48	41
results	**	**	**	**	**

Notes:
[1]heterotrophic plate count
[2]total coliforms
[3]fecal coliforms
[4]total solids
[5]total volatile solids
[6]colony forming units
** indicates 0.01 significance level

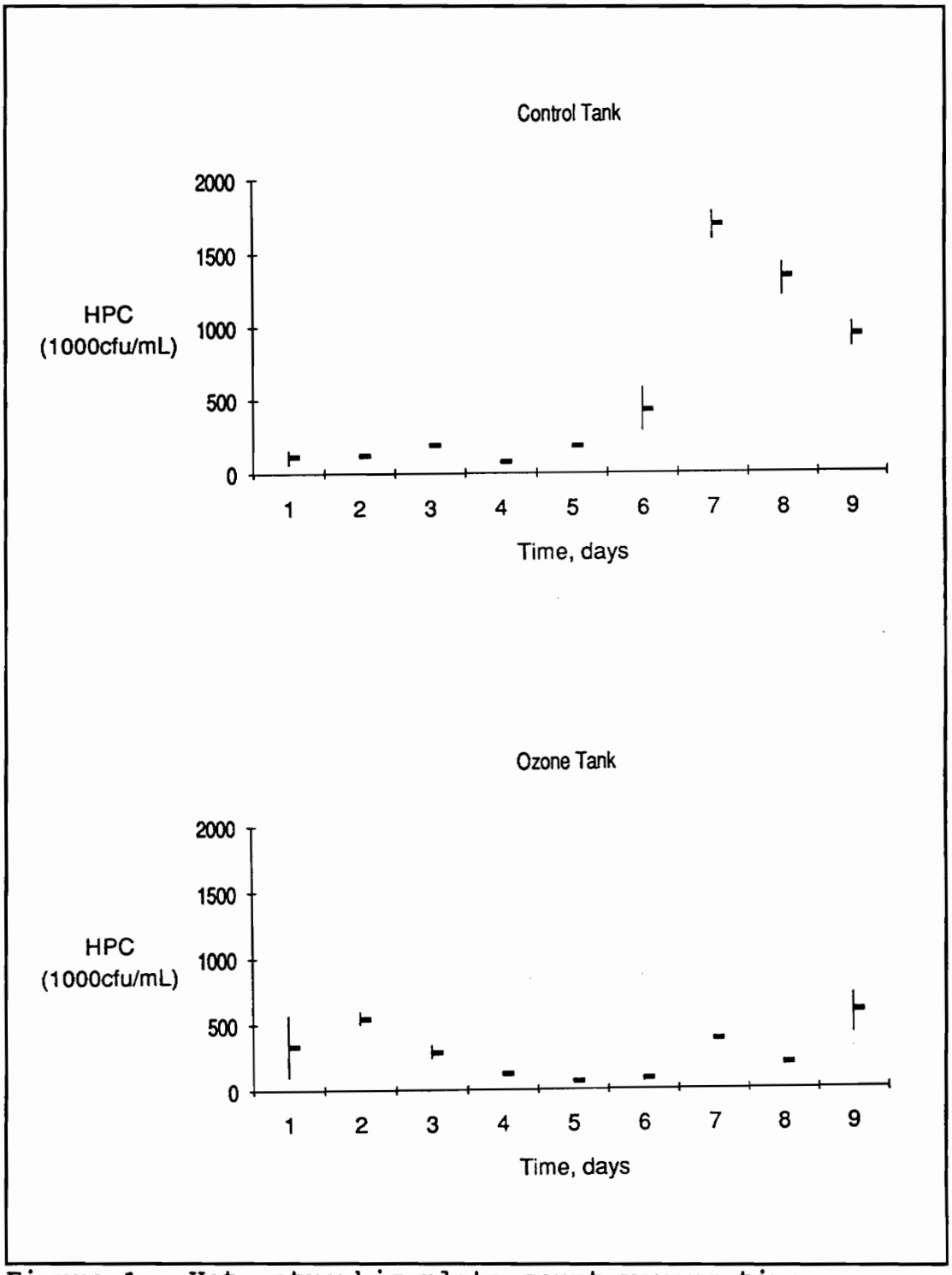

Figure 1. Heterotrophic plate count versus time.

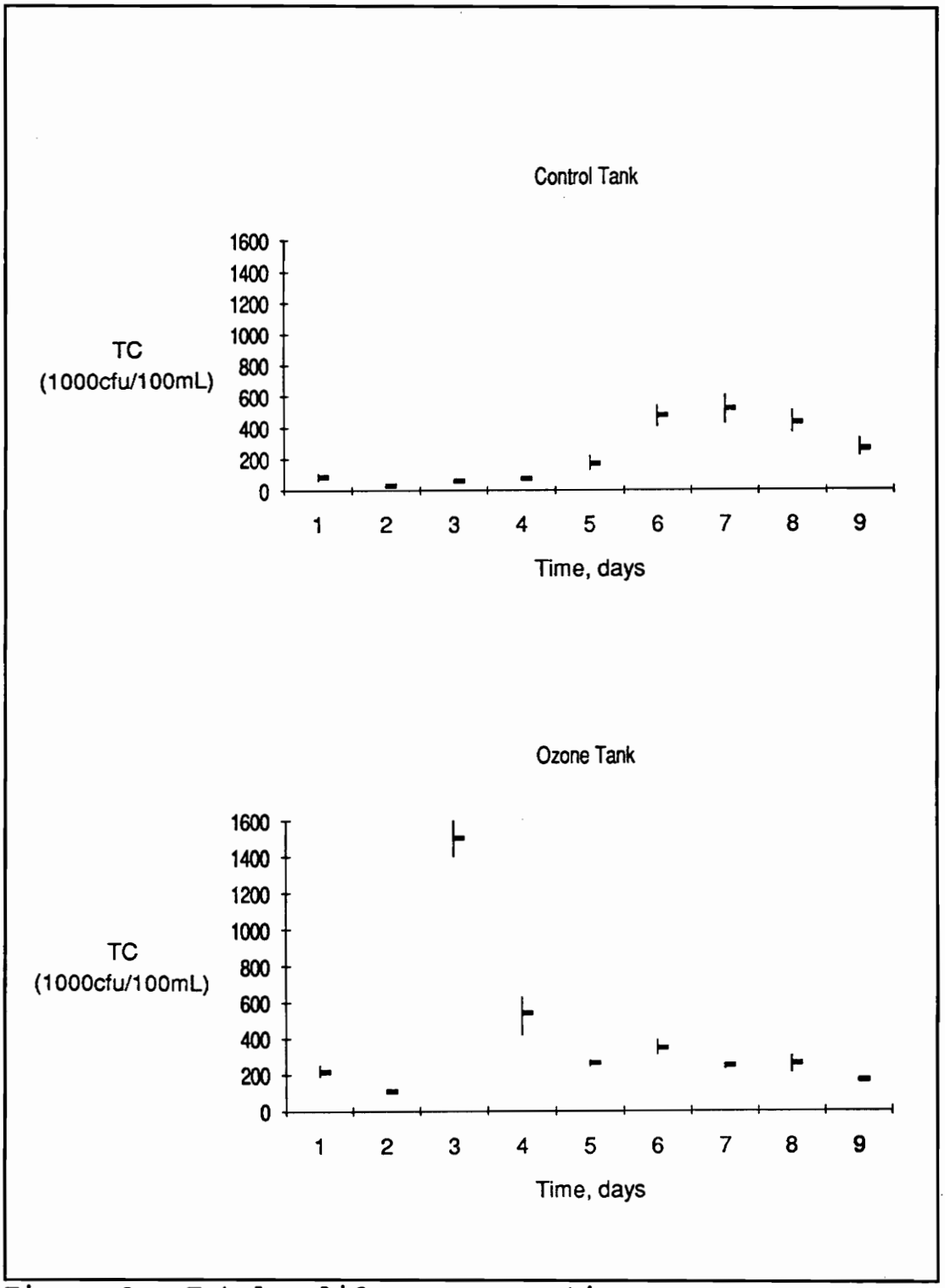

Figure 2. Total coliforms versus time.

The control and ozone tank fecal coliforms (Figure 3) displayed similar trends when compared to the control and ozone total coliforms (Figure 2). Although fecal coliforms are a subset of total coliforms and similarity was expected, both procedures were performed to determine whether one would be a better indicator of microbiological activity in the system than the other. No differences were noted and there was no advantage to performing one procedure over the other. Statistically, the fecal coliform test had a slightly greater degree of confidence that the control tank slope was greater than the ozone tank slope when compared to the total coliforms (Table 3), but both parameters indicated that the differences were highly significant ($p < 0.01$).

Of the three microbiological tests considered, the heterotrophic plate count (Figure 1) followed the most logical trend. Intuitively, the heterotrophic plate count would be expected to be a better measure of general microbiological activity because it measures many types of bacteria rather than just coliforms. The specificity of the coliform tests had no benefit in this research and it is recommended that the heterotrophic plate count be used in the future as the sole indicator of general levels of microbiological activity in the system.

Control tank solids increased steadily (Figure 4), while the ozone tank solids had a much smaller rate of

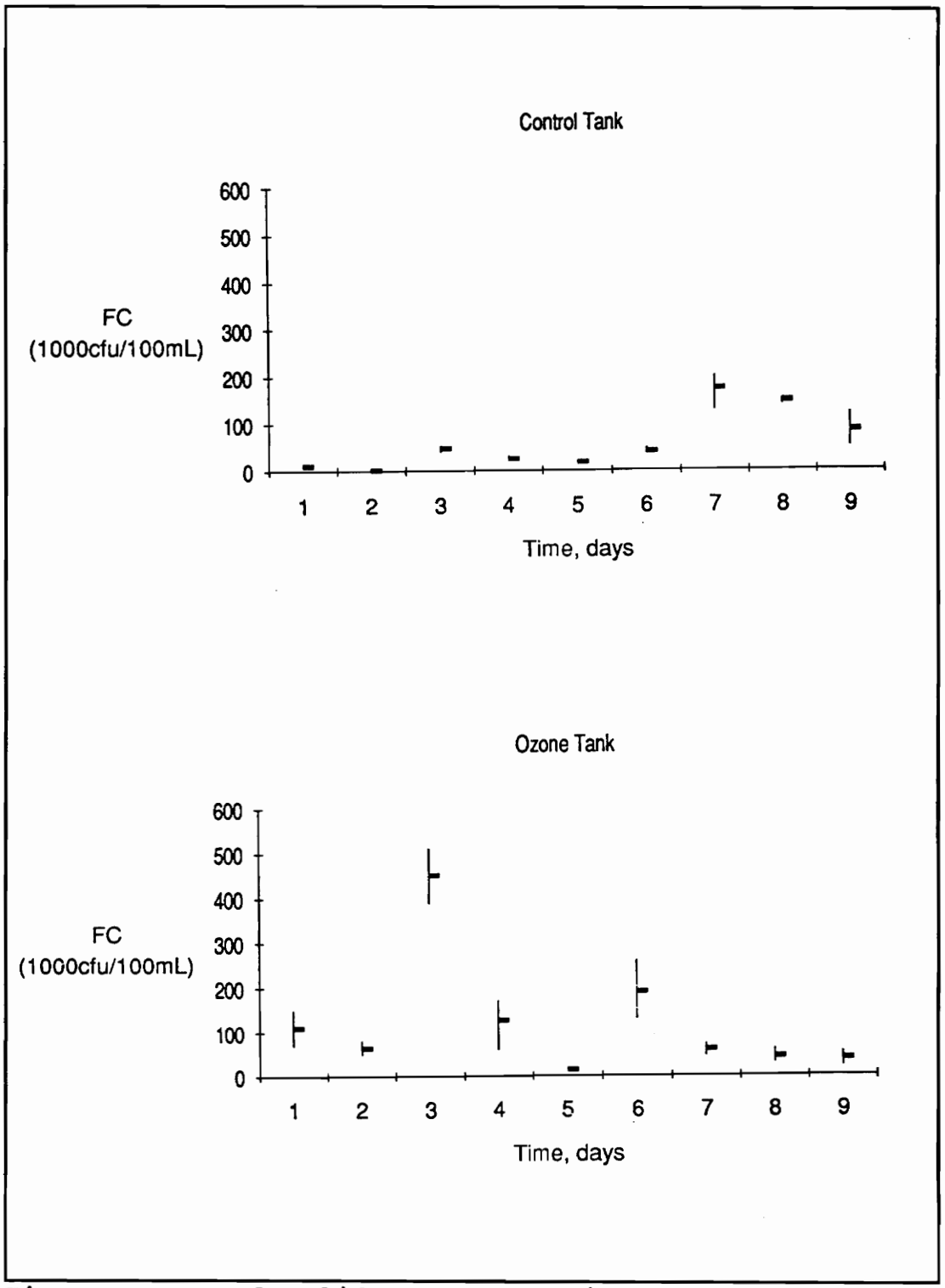

Figure 3. Fecal coliforms versus time.

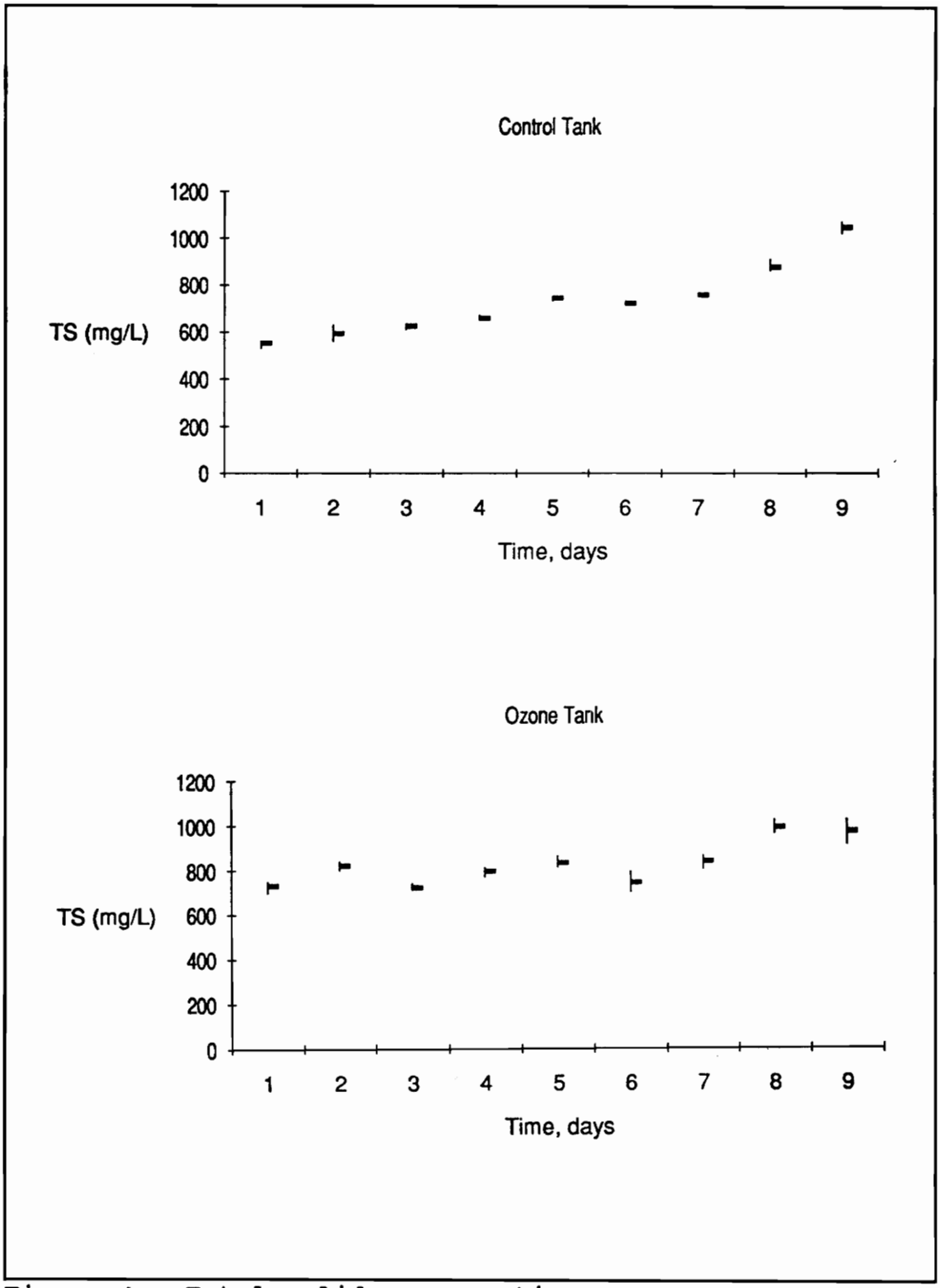

Figure 4. Total solids versus time.

increase, which was approximately one half the rate of increase of the control (Table 3). The results of the total solids were highly significant ($p < 0.01$). The upward trends in total solids (Figure 4) were not expected and were possibly an indication of inadequate solids removal by the clarifier. The statistically significant difference in total solids in the tanks (Table 3) is an indication of improved solids removal in the ozone tank. Perhaps the ozone improved the settling characteristics of the solids or partially oxidized the solids allowing more rapid microbiological utilization of the dissolved solids.

As previously stated, visual observations of the tank water found that differences in the water clarity and color increased progressively throughout the experiment. Statistically, the total volatile solids test showed the least degree of confidence about the significance of the differences in slope (Table 3). Even though the results were highly significant ($p < 0.01$), this lesser degree of confidence, taken in context with the visual and microbiological results, implies that the ozone had a positive effect on color and microbiological activity without reducing organic matter levels. This implication violates the previously held hypothesis that the ozone would reduce the organics and color before affecting microbiological activity.

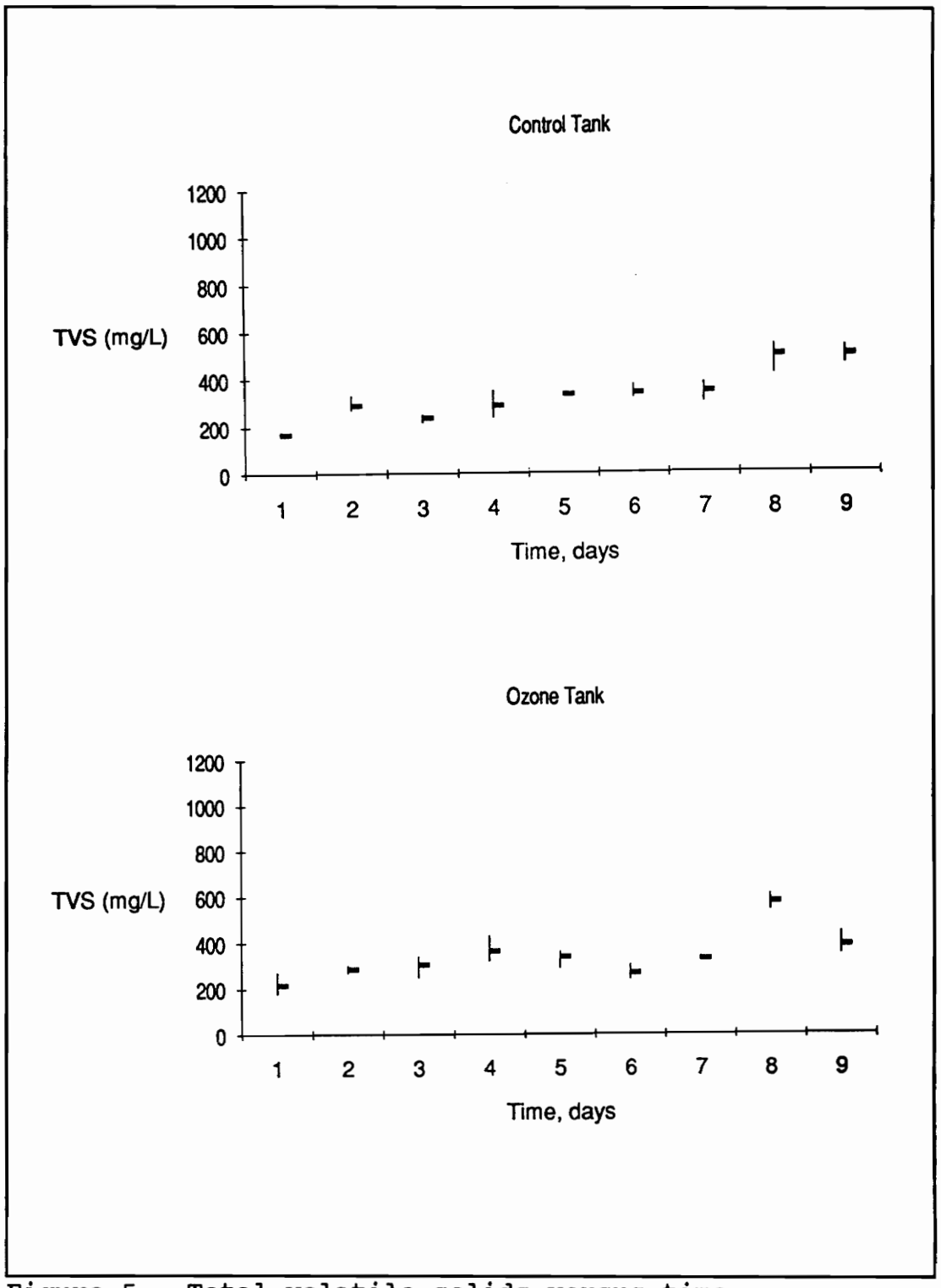

Figure 5. Total volatile solids versus time.

Essentially, ozone had a similar statistically significant effect on all test parameters. While the ozonehad a positive effect in the recirculating system; the ozone generator could not generate enough ozone to produce reductions of the magnitude initially hypothesized. As mentioned previously, the ozone generator employed could only produce a 3 percent concentration of ozone in the injected oxygen stream. Higher ozone concentrations, up to about 6 percent, are possible with other generator models. A 6 percent concentration would double the ozone dosage to the system and achieve a greater degree of disinfection.

This 6 percent upper limit also highlights what may be a basic flaw in the application of ozone via the u-tube in this system. Inherently, the amount of ozone that can be applied is limited by the amount of oxygen which can be applied to the system concurrently. Lack of ozone may harm the fish by allowing water quality to degrade, but excessive super saturation of the water with oxygen may also harm the fish by damaging their gills or causing gas bubble disease. The only recourse under these circumstances would be to strip the excess oxygen from the water before adding it to the fish tank or ozonate the water prior to the u-tube to prevent excess oxygenation of the water. Either option would waste oxygen, but the value of the additional ozone in the system may outweigh the value of the lost oxygen.

Under some operational circumstances, the u-tube injection of ozone should be adequate, but ozone demand will likely exceed the dosage capabilities of the u-tube injection system as greater stocking densities and feed rations are attempted. Therefore, u-tube injection of ozone will limit the production potential of the recirculating aquacultural production systems unless a means of increasing ozone dosage without a corresponding increase in oxygen levels is incorporated into the system. Perhaps a separate device could be designed as an "on-demand" component in the treatment train that would be activated when needed, thus reducing unnecessary operating costs when the "extra" ozone is not needed by the system.

Many other problems also limit the production potential of the systems, particularly solids removal, disease control, and stress management. Ozone can play an important role in solving these problems, but the application of the ozone has revealed another set of problems with recirculating aquacultural production systems which must be addressed.

SUMMARY AND CONCLUSIONS

Ozone had a beneficial effect in this experiment as it resulted in a significantly lower rate of growth of bacteria as measured by heterotrophic plate count, total coliforms, and fecal coliforms. The rate of increase in total solids and total volatile solids was also significantly less in the ozone tank than in the control. Although the experiment could not be fully completed due to circumstances beyond reasonable control, valuable insight into the application of ozone in recirculating aquacultural production systems was gained.

Microbiological activity was adequately measured by the heterotrophic plate count. The other microbiological tests only served to verify the conclusions drawn from the heterotrophic plate count. Suspected inadequacies in the solids removal system were detected, but the application of ozone appeared to enhance solids removal. Also, assuming that the tanks started with equal susceptibility to ESC, the application of ozone appeared to delay the onset of the ESC infection in the ozonated tank.

Unfortunately, the amount of ozone applied to the system was insufficient to disinfect the system to the extent desired. Increasing the ozone concentration in the ozone/oxygen stream added to the tank will improve the disinfection capabilities of the u-tube injection system,

but the amount of ozone which can be added with this system will be limited by the maximum amount of oxygen which can be added to the tank without adversely affecting the fish. Further study should be conducted under similar conditions with an ozone generator capable of producing a higher concentration of ozone to determine if the amount of ozone required for adequate disinfection can be supplied without overloading the fish tank with oxygen. Should the u-tube injection system be found to be an inadequate delivery and contact system, an alternate means of delivering additional ozone must be developed that will not threaten the fish.

Although ozone appears to have a promising future in aquaculture, ozonation systems have very high capital and operating costs. Ozonation may not be appropriate in every production scenario. However, as the demand for good quality water increases the value of the water itself, the economic feasibility of ozone treatment improves. If the major goal of recirculating aquacultural production systems is to maximize fish production while minimizing water use, ozone treatment will eventually become an integral component in water treatment processes for recirculating aquacultural production systems.

RECOMMENDATIONS FOR FUTURE RESEARCH

To overcome some of the problems encountered in this research, future research efforts should utilize a more traditional experimental design. Replications would allow more powerful statistical conclusions to be drawn. Unexplained differences in performance of apparently identical systems could be accounted for with three or four replicates of control and test systems. The time frame of the research should be increased to include at least one complete growth cycle for the fish. System performance may vary during the growth cycle and experiments that address the entire cycle will be able to identify how critical stages in the development of the fish are affected by the research being conducted.

In addition, an ozone generator is needed which will deliver at least a 6 percent concentration of ozone over the full oxygen flow demand range. With this type of generator, the full potential of the u-tube injection system can be investigated. Alternate or supplemental ozone contact systems must also be investigated or developed.

Finally, the economics of current and future systems must be addressed. At present, the technology exists to grow fish in the recirculating systems. Research efforts must now be directed toward improving the economic feasibility of the recirculating aquaculture systems.

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VITA

William P. Johnson II was born December 26, 1965 in Lynchburg, Virginia. He grew up on a farm in northern Pittsylvania county, Virginia and graduated from Gretna Senior High School in June, 1984.

He enrolled at Virginia Tech the following fall and selected agricultural engineering as his major. As an undergraduate, he participated in the cooperative education program at Virginia Tech. He worked for Schnabel Engineering Associates in Bethesda, Maryland as a geotechnical technician; Moore Golf, Inc. in Culpeper, Virginia as a project estimator; and Dewberry and Davis in Danville, Virginia mainly as a construction inspector. In addition to financing part of his education, the co-op program provided valuable "real-world" experience.

Graduate school provided the opportunity to combine his farming background with his water quality interests in the form of recirculating aquacultural production systems. Wanting to do applied research, he migrated to Blue Ridge Fisheries, Inc. in Martinsville, Virginia. At Blue Ridge, he conducted his master's research on ozone and accepted a full-time position as an environmental engineer for the company. At the present time, he and his wife, Jo Ellen, reside in Ridgeway, Virginia.