EPRI

Ozone Applications in Fish Farming

Technical Report

Ozone Applications in Fish Farming

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REPORT SUMMARY

Ozone is a powerful oxidizing agent with numerous beneficial aquaculture uses. This user's manual presents recommendations for the application of ozone in fish farming as well as the monitoring of ozone in aquaculture systems. The results of bench- and full-scale ozone applications support the effectiveness of ozone as a water treatment tool for aquaculture, particularly when added at the end of a full-system treatment train.

Background

Ozone can improve the quality of aquaculture production water by helping improve solids settling and by reducing nitrite-nitrogen (NO_2-N), color, fine particulate matter, and microbial activity. Ozone shows excellent potential for many aquacultural systems because of its rapid reaction rate, few harmful reaction by-products, and oxygen produced as a reaction end product. Ozone usage for aquaculture began in the mid-1970s and focused on disinfection and color reduction of aquarium system water with low fish densities and low feed loadings. Since then, ozone has been used to improve water quality in various types of aquaculture systems—ranging from flow-through raceway systems for salmonids to indoor recirculating systems. Although ozone has proven effective in the reduction/control of certain water quality characteristics, ozone use is not a one-stop water treatment technology. Because less ozone is needed when supporting water treatment technologies are present, the use of ozone as part of a larger water treatment system maximizes its treatment- and cost-effectiveness.

Objectives

- To perform bench- and full-scale ozone research and develop information for the aquaculture community regarding effective and appropriate methods for applying ozone in aquaculture systems.
- To develop a user's manual that will help aquaculturists understand the basics of how ozone works and what to consider in evaluating ozone for system-specific applications.

Approach

Investigators proposed a series of batch experiments to 1) quantify the effectiveness of ozone at various application rates for improving aquaculture water quality (removal of suspended solids, volatile solids, NO_2-N, and color) and 2) establish the interactive effects of various parameters. As part of the research, Fingerlakes Aquaculture participated in full-scale application of ozone to one of its six 250,000-lb per year fish production systems in order to examine the effects of ozone on both production system water quality and fish health. Investigators used this combination of batch and full-scale information as the basis for developing this user's manual.

Results

Given the results of batch and full-scale studies, it appears that ozone can be an effective water treatment tool for aquaculture. In laboratory batch studies, ozone removed color and NO_2-N from water at concentrations common to aquaculture finfish production. The removal of color and NO_2-N from batch water was rapid, with most removal taking place within the first 30 minutes of exposure to ozone. In the full-scale application of ozone at Fingerlakes Aquaculture's Groton tilapia production facility, both color and foam were effectively removed from production water. Reduction in foam was rapid (within the first few days of ozone addition to the system), with color removal occurring a few days after.

The addition of ozone did not have any discernable effect on total suspended solids or volatile suspended solids in either the laboratory batch studies or the full-scale study. Increased solids settling has been observed by other researchers as a result of increased flocculation. However, in batch and full-scale studies at Fingerlakes Aquaculture it appears that insufficient ozone was applied to cause increased flocculation and suspended solids removal, perhaps due to preferential oxidation of other compounds. Preferential compound oxidation would be expected in an actual production system and especially so in a facility such as Fingerlakes Aquaculture, where fish production water receives large amounts of feed and high concentrations of solids exist.

EPRI Perspective

Based on information gathered at Fingerlakes Aquaculture, several recommendations can be made toward the design and application of ozone in aquaculture systems. First, ozone should be added at the end of a full-system treatment train to obtain maximum effectiveness. Second, ozone should be added to a fish production system where sufficient reaction time is allowed to completely use up the ozone before direct contact with fish, and extreme caution toward fish health should be taken prior to ozone addition. Third, a thorough understanding of potential ozone-oxidized compounds is needed before ozone application. Finally, ozone monitoring can be performed effectively using water samples and simple water chemistry techniques. However, it is especially important to sample fish production water to avoid residual ozone contact with production fish. EPRI believes this research will be particularly beneficial as the fish farming industry evaluates specific ozone applications as part of a larger system treatment regimen.

Keywords

Fish farming
Ozone applications
Aquaculture

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1
INTRODUCTION TO OZONE IN AQUACULTURE

1.1 Background.

Ozone is a powerful oxidizing agent that can be put to numerous beneficial uses within aquaculture. Ozone can improve the quality of aquaculture production water by reducing NO2-N, color, fine particulate matter, microbial activity, and by helping improve solids settling (Hochheimer and Wheaton, 1995; Summerfelt and Hochheimer, 1998; Rosenthal and Kruner, 1985; Christenssen et al 1996; Bablon et al. 1993; Brazil et al. 1996). Ozone shows excellent potential for many aquacultural systems because of its

- rapid reaction rate,
- few harmful reaction by-products, and
- oxygen is produced as a reaction end product, (Hochheimer and Wheaton, 1995).

Ozone usage for aquaculture began in the mid 1970's and focused on sterilization (or disinfection) and color reduction of aquarium system water with low fish densities and low feed loadings. Since then, ozone has been used to improve water quality in various types of aquaculture systems ranging from flow through raceway systems for salmonids to indoor recirculating systems. A bibliography of ozone research is presented in the appendix.

Proper application of ozone requires consideration of four unit processes:

- ozone gas generation,
- gas to liquid absorption,
- contact time for reaction, and
- ozone residual removal (Summerfelt and Hochheimer).

In addition, monitoring of ozone in both the gaseous and aqueous states is often necessary because ozone is toxic to humans and to aquatic organisms at very low levels.

For the most part, ozone research has typically focused on individual target parameter reduction and has not evaluated the compound relationship (either inhibitory or symbiotic) of multi-parameter reduction effects. This is largely due to the complexity of such analysis and the utility of such information being very limited to a site-specific aquaculture applications (i.e. water quality varies for every aquaculture system).

The application rate of ozone may be dramatically different in each aquaculture application depending upon the target parameter, aquaculture species, feed characteristics, and system design, and typical system production water characteristics. For this reason, the design and application of ozone in aquaculture systems is typically performed by aquaculture system design firms based on empirical evidence observed in operating systems and trial and error methodology.

Although ozone has been shown to be effective in the reduction/control of certain water quality characteristics, ozone use is not a one stop water treatment technology and should be used in conjunction with other water treatment technologies. Because less ozone is needed when other supporting water treatment technologies are present (less loading on the ozone), the use of ozone as part of a larger water treatment system maximizes its treatment effectiveness and cost effectiveness.

1.2 Objectives

The primary objective of this project was to perform ozone research at the bench and full-scale levels and develop information for the aquaculture community regarding effective and appropriate methods for the application of ozone in aquaculture. The final product of this project was to be a "Users How To" Manual that would help aquaculturists understand the basics of how ozone works and what to consider in evaluating ozone for their system.

A series of batch experiments was proposed to evaluate the independent and combined effects of ozone on the removal of color, NO_2-N, and solids in aquaculture water. In addition, as part of the research portion of this project, Fingerlakes Aquaculture proposed to purchase and implement, a large-scale ozone production system on a full-scale aquaculture production system. This combination of batch and full-scale information was then to be used as the basis for writing the "How To" Manual

A summary of the proposal objectives is as follows:

- Quantify the effectiveness of ozone application at various application rates on improving water quality (e.g. the removal of suspended solids, volatile solids, NO_2-Nitrogen, and color) and establish the interactive effects of one parameter upon the other (microbes not addressed),
- Develop recommendations for application and design of ozone in aquaculture,
- Develop recommendations on where and how to monitor ozone in aquaculture systems,
- Quantify changes in fish health with and without ozone under commercial conditions, and
- Produce a user's "How To" Manual for the aquaculture industry.

These objectives were accomplished.

2
METHODS AND MATERIALS

FLA performed two (2) sets of ozone experiments. The first set of ozone experiments consisted of bench scale (laboratory) batch studies that examined the effects of ozone addition on individual aquaculture production water characteristics including NO2-Nitrogen, total suspended solids, volatile suspended solids and color. The second set of ozone experiments was full-scale application of ozone to a 250,000 lb per year fish production system (one of 6 such systems at the Fingerlakes Farm) to examine the effects of ozone on both production system water quality and fish health.

2.1 Batch Experiments

Descriptions of parameter-specific batch study procedures are presented below. In general, batch experiments were set up to examine the effects that ozone addition would have on the degradation/removal of specific water characteristics associated with aquaculture. Batch studies were conducted with parameter concentrations typically associated with aquaculture systems. The following water quality parameters were evaluated:

- NO_2-Nitrogen (NO_2-N), measured in mg/L,
- True color (color), measured in platinum cobalt color units,
- Total suspended solids (TSS), measured in mg/L, and
- Volatile suspended solids (VSS), measured in mg/L.

Batch studies were conducted in 500-mL pyrex glass beakers under room temperature conditions (21°C). Stock solutions for both ozone and each test parameter were prepared in distilled/deionized water. Samples were prepared in triplicate for each target concentration to be analyzed. To achieve the proper ozone to mass ratio in each batch experiment, a specific volume of known concentration for each parameter investigated was added to test beakers containing specific volume of ozone solution (concentration known). The concentrations and volumes of mixing the two solutions were then used to determine the initial concentrations of each test sample.

Ozone for the batch studies was generated from a Pacific Ozone Model 3M23 Ozone generator capable of generating 3.2 kg/day (7.1 pounds) of ozone per day from pure oxygen. Ozone solutions were made by bubbling ozone from the generator into filtered, DI water until consecutive Ozone tests showed O_3 concentrations which were: 1) within the range of 1.9 mg/L to 2.5 mg/L; and 2) within 5% of one another. [O_3] was determined by a Hach DR2000 Spectrophotometer.

Application Rate Calculations

.013 - .025 kg ozone/ kg feed fed (Hochheimer and Summerfelt,1997) and (Brazil et al. 1996)

On a pod basis at Fingerlakes Aquaculture's Groton Facility, approximately 272 kg (600 lbs) of feed are fed daily

272	kg/ day *	0.013	kg ozone/ kg feed fed =	3.54	kg ozone/day
272	kg/ day *	0.025	kg ozone/ kg feed fed =	6.80	kg ozone/day

Groton: 80,000 gal or 302400 L (per pod system)

Thus, at Groton, ozone should be applied between:

	0.012	g / L per day	and	0.022	g / L per day	
For an sample:	8	L	0.094	g	to	0.180 G

In order to cover this range and beyond, O_3 application rates of 0.05 mg O_3 / mg contaminant, 0.10 mg O_3 / mg contaminant, 0.5 mg O_3 / mg contaminant were chosen. The following example illustrates how a batch of NO_2-N and O_3 would be mixed:

Desired mix: 0.125 mg/ L NO_2-N and approximately 0.1 mg O_3/ mg NO_2-N

Start with 100 mg/L NO_2-N, add 1.25 mL to a clean, dry beaker, and then dilute to one liter.

0.125 mg NO_2-N * (0.1 mg O_3/ mg NO_2-N) = 0.0125 mg O_3

If the concentration in the O_3 water was found to be 2.21 mg O_3/L, then:

2.21 mg O_3/L * (X liter O_3 water) = 0.0125 mg O_3 → 5.65 mL O_3 water

Final concentrations:

.0125 mg O_3 / 1.00565 L = 0.0124 mg O_3/ L

.125 mg NO_2-N/ 1.00565 l = 0.124 mg NO_2-N/ L

Analysis methods used in the specific parameter batch studies are as follows:

Total Suspended Solids (TSS): Method # 2540 D Standard Methods for the Examination of Water and Wastewater. TSS concentrations are reported in mg/L

Volatile Suspended Solids (VSS): Method # 2540 E of Standard Methods for the Examination of Water and Wastewater. VSS concentrations are reported in mg/L.

NO2-Nitrogen (NO_2-N): Hach DR2000 Spectrophotometer – test # 371. (Diazotization method) - a USEPA-approved methodology: Concentrations are reported in mg/L.

True Color: Hach DR2000 Spectrophotometer – test # 120 (APHA platinum-cobalt or Pt-Co Method): Concentrations are reported in Pt-Co color units.

Ozone (O_3): Hach DR2000 Spectrophotometer test # 450 (N,N-diethyl-P-phenylene diamine method). Concentrations are reported in mg/L.

[NO_2-N] Batch Experiments:

Preparation of NO_2-N stock solutions for batch experiments:

A 100 mg/ stock solution of $NaNO_2^-$ was mixed at the beginning of each day of testing.

Batch experiments:

Ozone concentration was determined as above and 250 mL of ozonated water were added to each of nine numbered beakers (1-9). Oxygen saturated water was added to three more beakers – 10, 11, and 12 – which served as controls. Beakers 1,4,7, and 10 received 0.5 mL of 100 mg/L NO_2-N; beakers 2,5,8, and 11 received 1.5 mL of 100 mg/L NO_2-N; beakers 3,6,9, and 12 received 2.5 mL of 100 mg/L NO_2-N. The [NO_2 - N] was determined as above at times of approximately 30, 60, and 90 minutes after the NO_2-N was added to the ozonated water.

True Color Batch Experiments:

Preparation of Color Solutions for True Color batch experiments:

Two liters of Lipton® Tea were brewed and filtered. The filtrate from this process was collected and served as the color additive for all of the batch reactions. It was determined that adding 25 mL of tea filtrate to filtered, DI water created a solution with color equivalent to 350 Platinum-Cobalt Color Units. Additions of 50 mL and 75 mL to 250 mL of filtered, DI water produced solutions with color equivalent to 650 and 850 Platinum-Cobalt Color Units, respectively.

Procedure

Four sample beakers received 25 mL of tea filtrate; four sample beakers received 50 mL of tea filtrate; and four sample beakers received 75 mL of tea filtrate. Ozone concentration was determined as above and 200 mL of ozonated water were added to each of the first nine beakers (1-9). Oxygen saturated water was added to three more beakers which served as controls. The True Color was determined as above at times of approximately 20,30,and 45 minutes after the ozonated water was added to the tea filtrate, and again after 24 hours.

POD 2 Culture Water Batch Experiments:

Ozone concentration and the [NO_2-N] of the full-scale test system culture water were determined as above. Three test sample beakers received 100 mL of ozonated water and 400 mL of culture water; three test sample beakers received 250 mL of both ozonated water and culture water; and three test sample beakers received 400 mL of ozonated water and 100 mL of culture water. Oxygen saturated water and culture water were added to the control sample beakers in the same proportions that the ozonated water and culture water were added to the first set of test sample beakers. The [NO_2-N] was determined as above at times of approximately 30, 60, and 90 minutes after the NO_2^- was added to the ozonated water. The original culture water and samples from each beaker were taken to Cornell University for solids testing (TSS and VSS); the filtrate from this testing was analyzed for True Color on the following day. Mathematical corrections for dilution were performed before results were compared to the original sample.

2.2 Full-Scale Ozone Application Study

The full-scale ozone application study was performed at the Fingerlakes Aquaculture (FLA) Groton production facility in Groton, NY. Ozone for this study was generated from a Pacific Ozone Technologies Model 3M23 ozone generator using pure oxygen (approximately 3.2 kg {7.1 pounds} of ozone output per day of continuous duty maximum production). Ozone was administered into one of FLA's three operating production systems (referred to as 'Pods") through a low head oxygenator unit (or LHO) for a period of 30 days.

A flowchart of the unit processes associated with each Fingerlakes Aquaculture production system (including where ozone was added into this system) is presented in Figure 2-1. The two (2) other Pod production systems were run without ozone and were considered control systems for the full-scale application study.

Prior to the actual full-scale experiment, the Pacific Ozone Generator was plumbed into place by Fingerlakes Aquaculture personnel. Much consideration to entry gas blending and highly resistive valve and flowmeter equipment was used in plumbing the ozone generator into the LHO system of Pod 3. In addition, the ozone generator was placed in a high air mixing zone within the fish production building with an ozone sensor/monitor nearby to ensure worker safety. It should be noted that the ozone generator used in the full-scale test was operated at 2/3 capacity as one of the three ozone generator units within the larger unit box would not stay "lit". This failure to stay lit was attributed by the manufacturer of the ozone generator to an electrical short in the system perhaps caused by the excessive wear of the metal plates or past storage procedures (the ozone generator was purchased and used by Fingerlakes Aquaculture two years ago in anticipation of this project.

During full-scale testing, water samples were collected on a daily basis from each Pod production system after culture water had passed through a settling chamber but before the biofiltration process (see Figure 2-1). Sample water was swirled vigorously prior to sampling to avoid the inclusion of flocculent material in the sample. The samples were immediately tested for NO_2-N and then taken to Cornell University for TSS and VSS analysis. The filtrate from this process was collected and analyzed the next day for the True Color of the water. In addition to NO_2-N/TSS/color sampling and analysis process indicated above, daily water collection and analysis of ammonia, pH, NO_2-N, and alkalinity were also conducted by fish farm personnel as part of routine fish farm operation. This data was gathered everyday both before and after the ozone experimentation period in Pod 3 and can be used as baseline data.

Sample analyses for NO_2-N, TSS, VSS, and color for the full-scale application study were those used for the batch experiments and are described in the previous section (Section 1.2.1).

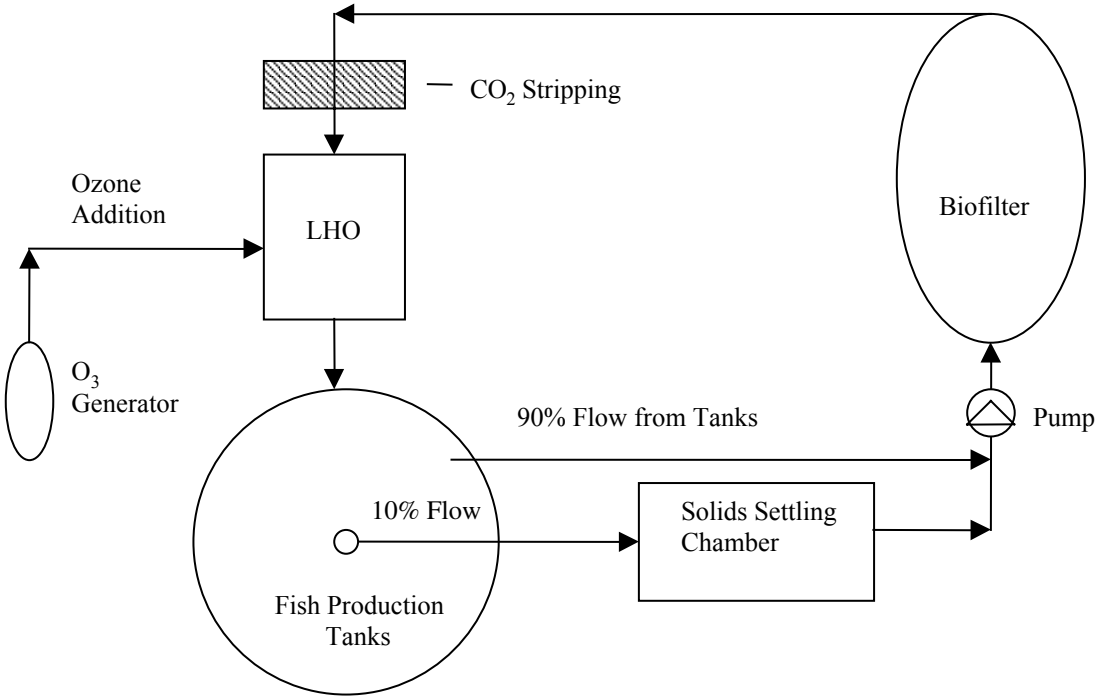

Figure 2-1
Unit Process Flow Diagram depicting Ozone Administration Location

3
EXPERIMENT RESULTS

3.1 Results of Batch Experiments

Results of the individual and combined parameter batch studies are presented in the following section. It should be noted that the batch studies were tedious in nature due to the highly reactive nature of ozone (repeatability of concentrations) and the difficulty in measuring ozone in water.

3.1.1 Color Experiments

Results of Experiments Treated with 2.17mg/L Ozone:

Three independent color experiments were run with ozone treatment concentrations of 2.17 mg/L representing a range of color found in aquaculture production systems. Color levels used in the 2.17 mg/L ozone batch experiments were 350, 650, and 850 Pt-Co Color units and are identified by the following respective experiment titles: Color 1-350, Color 1-650, and Color 1-850.

Data for experiment Color 1-350 (first 45-minutes) are presented in Figures 3-1 and 3-2. These figures show color concentration versus time for both the first 45 minutes and 1440 minutes (24 hours) of exposure to ozone. Analysis of Figure 3-1 data (the first 45 minutes) indicates that color was reduced significantly during the first 45 minutes from the application of 2.17 mg/L ozone compared to the control sample in which no ozone was added. Reduction of color in this experiment ranged from 78 to 126 color units within the triplicate samples after 45 minutes of exposure to ozone. The average percent of color removal within the ozone treated samples was 30.7% compared to 2.3% in the control sample. On a mass basis, the average color removal was 247.3 color units per mg ozone added. As indicated graphically in Figure 3-1, the majority of color reduction within the treated samples (79% of the total removal) took place within the first 20 minutes of exposure to ozone. This would suggest a design value for required contact time.

Figure 3-2 indicates that at 1440 minutes (24 hours) of exposure to ozone, color had recovered (increased) slightly in all three of the experimental samples compared to the color levels present at 45 minutes. The amount of color recovery for the experiment samples ranged from 48 to 60 color units with an average color gain of 55 units between 45 and 1440 minutes. Even though color increased in the experiment samples between 45 and 1440 minutes, overall color was still less in the experimental samples that received ozone compared to the control sample that received no ozone. A final average reduction of 53 units (15.0 %) was achieved in these samples after 1440 minutes compared to a 7 unit (2.0 %) color reduction in the control sample.

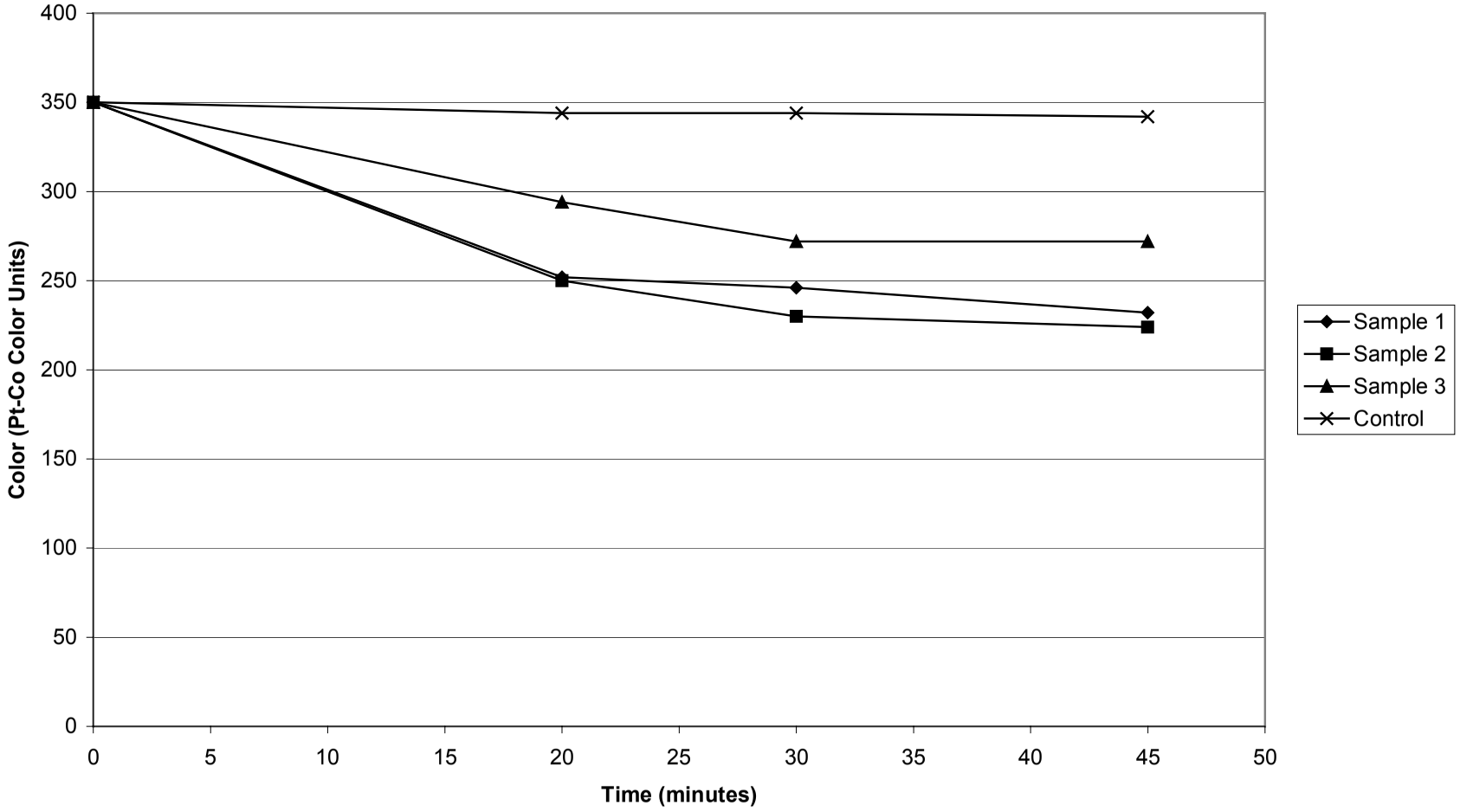

Figure 3-1
Color vs. Time (2.17 mg/L Ozone Application) Co = 350 color units

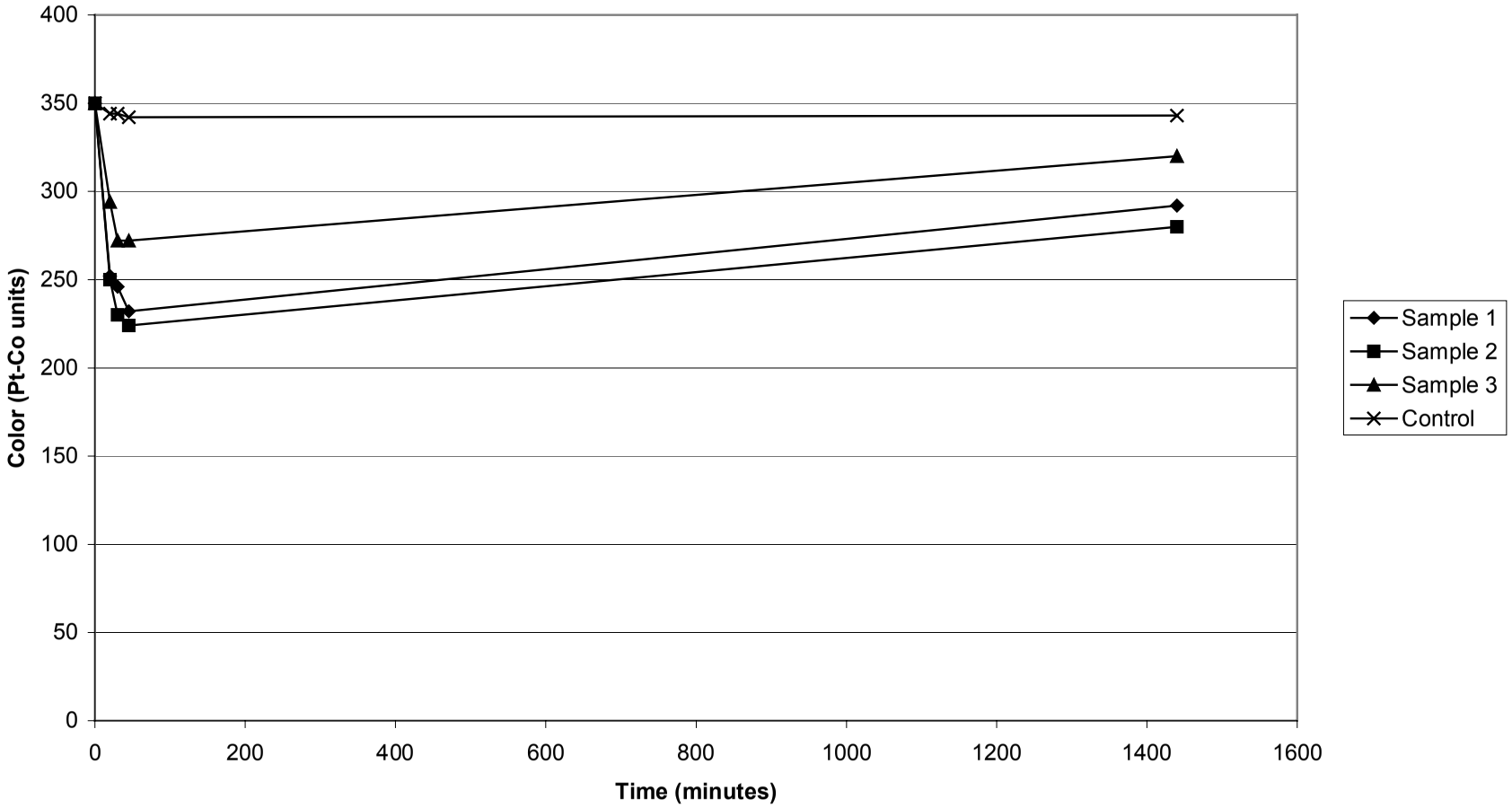

Figure 3-2
Color vs. Time (2.17 mg/L Ozone Application) Co = 350 color units

Data for experiment Color 1-650 are presented in Figures 3-3 and 3-4. Reduction of color in the ozone treated triplicate samples of this experiment ranged from 148 to 156 color units after 45 minutes of exposure to ozone and was significant compared to the control sample where only 1 color unit was reduced during this time period (see Figure 3-3). The average percent of color removal within the ozone treated samples was 22.9 % compared to 0.2 % in the control sample during the first 45 minutes of exposure to ozone. On a mass basis, the average color removal of this set of test samples was 342.5 color units per mg ozone added. The majority of color reduction within the treated samples of Experiment Color 1-650 (81% of the total removal) took place within the first 20 minutes of exposure to ozone during this period (see Figure 3-3).

Figure 3-4 indicates that at 1440 minutes (24 hours) of exposure to ozone, color had recovered (increased) slightly in all three of the experimental samples compared to the color levels present at 45 minutes. The amount of color recovery for the experiment samples ranged from 78 to 101 color units with an average color gain of 87 units between 45 and 1440 minutes. Even though color increased in the experiment samples between 45 and 1440 minutes, overall color was still slightly less in the experimental samples that received ozone compared to the control sample that received no ozone. A final average reduction of 62 units (9.5 %) was achieved in these samples after 1440 minutes compared to a 1 unit gain in color (+0.2 %) in the control sample.

Data for experiment Color 1-850 are presented in Figures 3-5 and 3-6. Analysis of this data indicates that ozone treatment reduced color in the treated samples of this experiment compared to the control sample in which no ozone was added. Based on the precision of data seen in earlier experiments, it appeared that Sample 2 of this experiment should be treated as an outlier (its data was up to 21.2 % different from Samples 1 and 3). With Sample 2 as an outlier, reduction of color in this experiment ranged from 138 to 170 color units within the triplicate samples after 45 minutes of exposure to ozone. The average percent of color removal within the ozone treated samples was 18.1 % compared to 0.1 % in the control sample during this time period. On a mass basis, the average color removal of this set of test samples was 375.6 color units per mg ozone added. As indicated graphically in Figure 3-5, the majority of color reduction within the treated samples took place within the first 20 minutes of exposure to ozone.

Figure 3-6 indicates that at 1440 minutes (24 hours) of exposure to ozone, color had recovered (increased) significantly in the experimental samples compared to the color levels present at 45 minutes. The amount of color recovery for the experiment samples was 88 and 124 color units for Samples 1 and 3, respectively between 45 and 1440 minutes. Even though color increased in the experiment samples between 45 and 1440 minutes, overall color was still slightly less in the experimental samples that received ozone compared to the control sample that received no ozone. A final average reduction of 48 units (5.8 %) was achieved in these samples after 1440 minutes compared to a 2 unit reduction in color (0.2 %) in the control sample.

Results of Experiments Treated with 2.46mg/L Ozone:

Three additional color experiments were run at the slightly higher ozone treatment concentration of 2.46 mg/L (compared to 2.17 mg/L) to determine how added ozone would affect the speed and completeness of color removal in batch experiments. Color levels used in the 2.46 mg/L ozone batch experiments were 350, 650, and 850 Pt-Co Color units (for comparison to the first set of tests) and are identified by the following respective experiment titles: Color 2-350, Color 2-650, and Color 2-850.

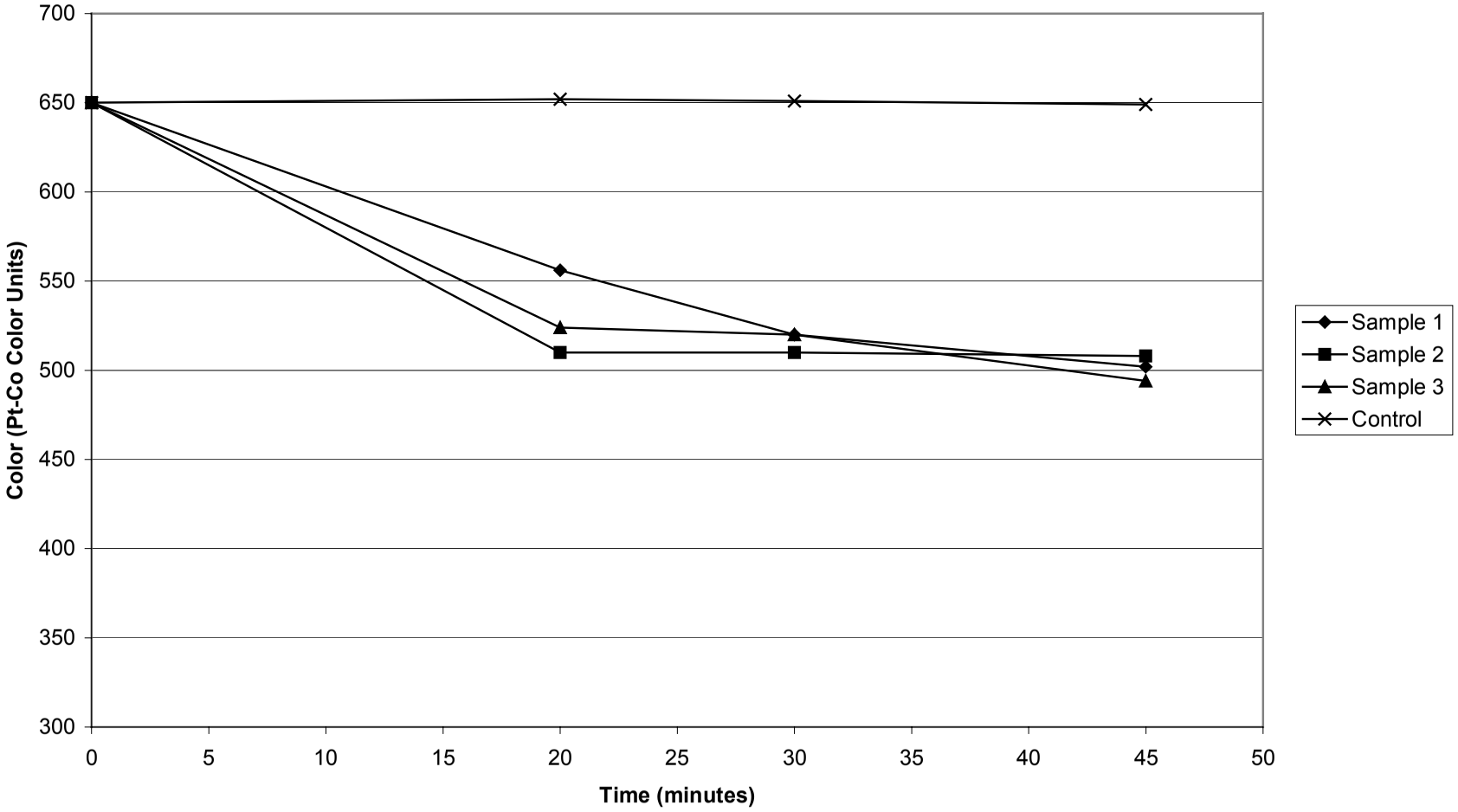

Figure 3-3
Color vs. Time (2.17 mg/L ozone application) Co = 650 color units

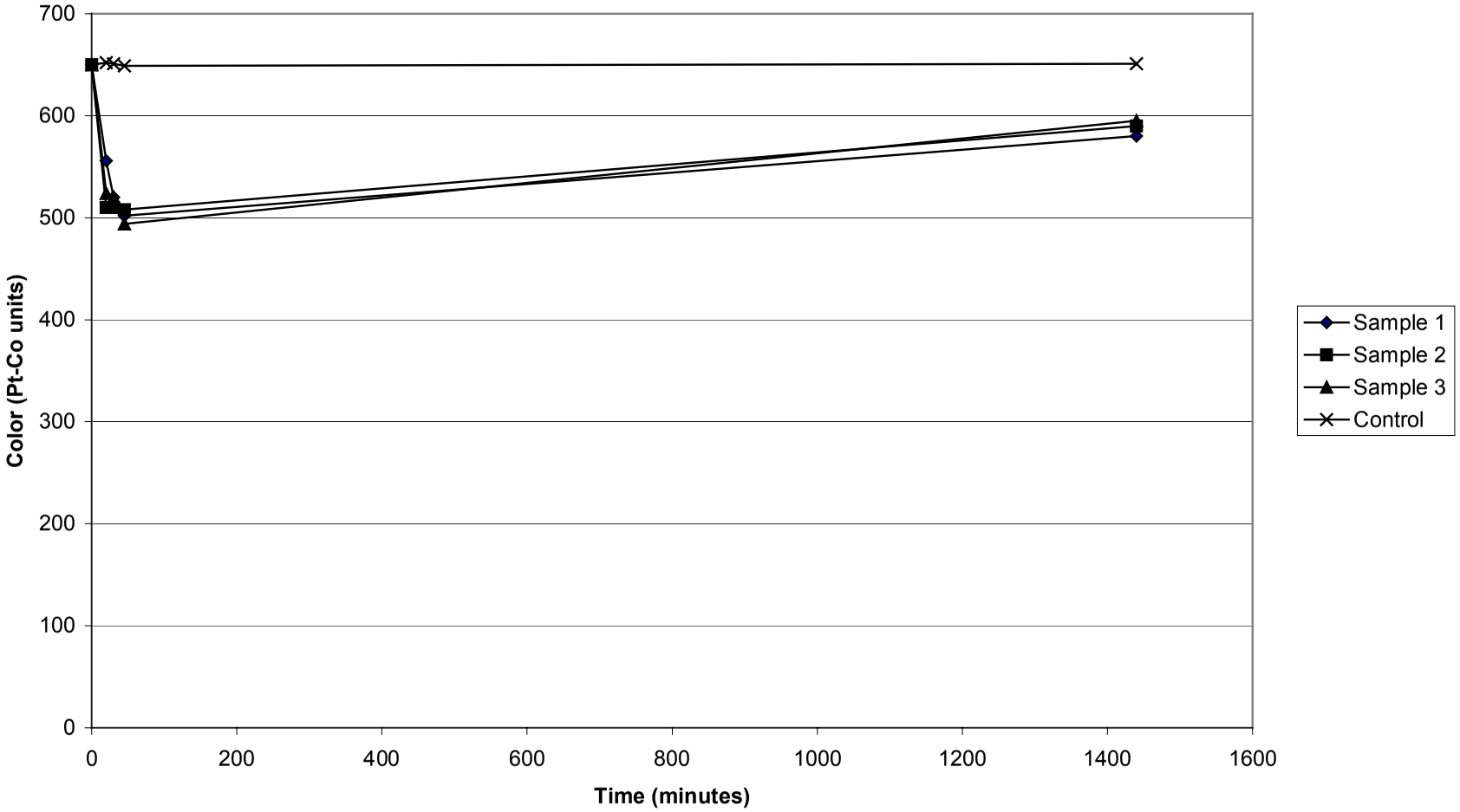

Figure 3-4
Color vs. Time (2.17 mg/L ozone application) Co = 650 color units

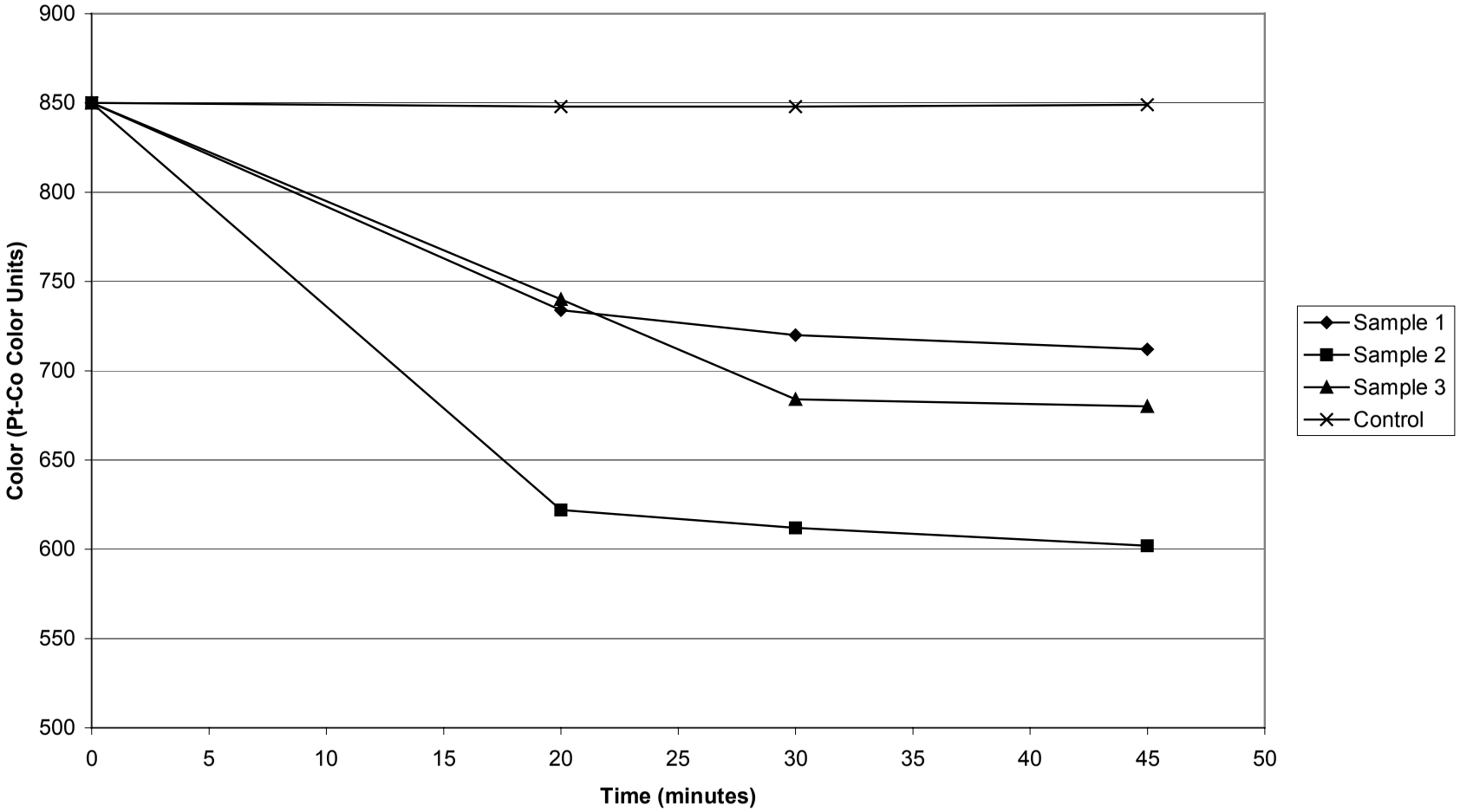

Figure 3-5
Color vs. Time (2.17 mg/L ozone application) Co = 850 color units

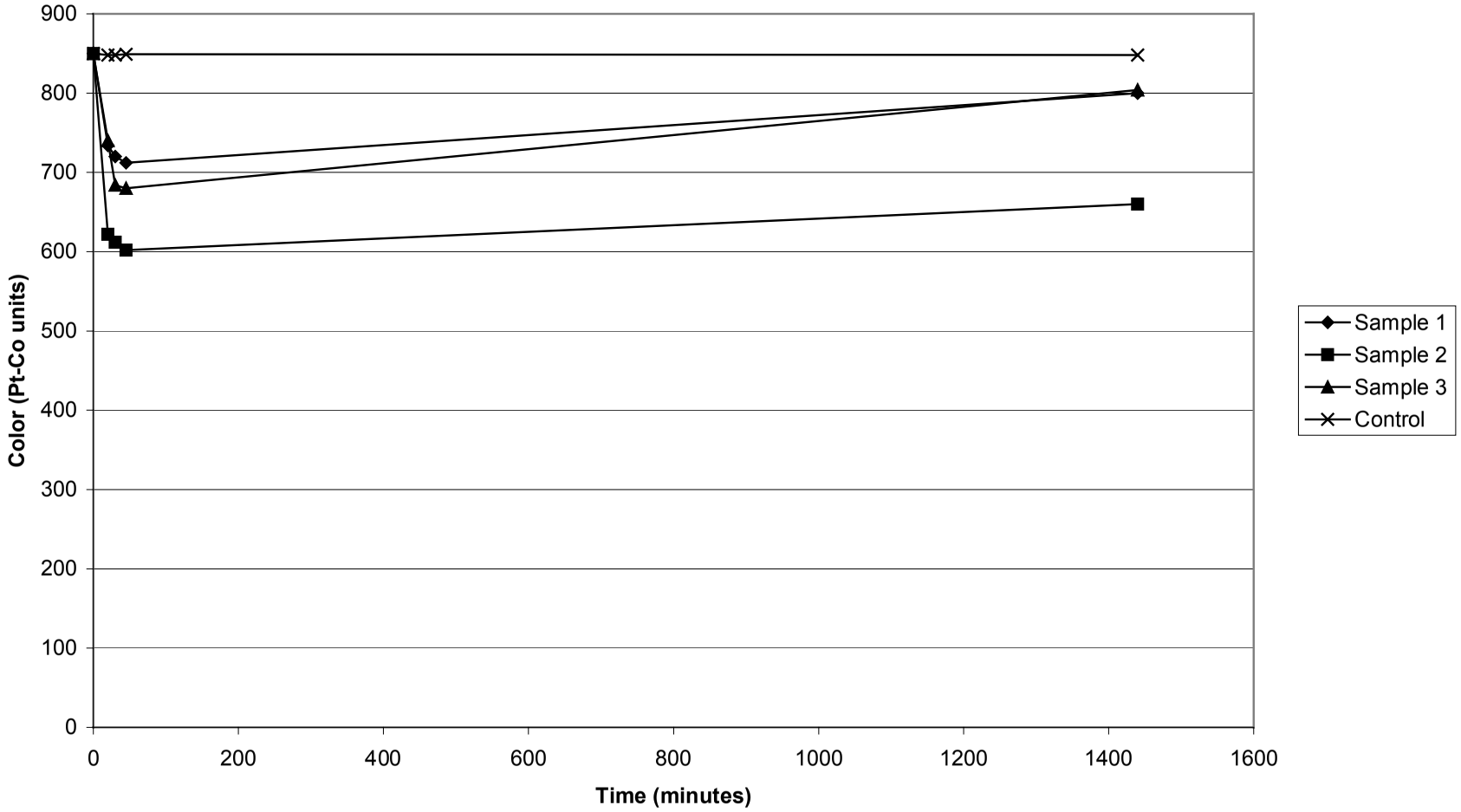

Figure 3-6
Color vs. Time (2.17 mg/L ozone application) Co = 850 color units

Data for experiment Color 2-350 are presented in Figures 3-7 and 3-8. Analysis of this data indicates that color was reduced by the application of 2.46 mg/L significantly compared to the control sample in which no ozone was added. Reduction of color in this experiment ranged from 105 to 136 color units within the triplicate samples after 45 minutes of exposure to ozone (see Figure 3-7). The average percent of color removal within the ozone treated samples was 36.8 % compared to 1.1 % in the control sample during this time period. On a mass basis, the average color removal of this set of test samples was 261.7 color units per mg ozone added. As indicated graphically in Figure 3-7, the majority of color reduction within the treated samples (83% of the total removal) took place within the first 20 minutes of exposure to ozone.

After 1440 minutes (24 hours) of exposure to ozone, color had recovered (increased) somewhat in the experimental samples compared to the color levels present at 45 minutes (see Figure 3-8). The amount of color recovery for the experiment samples ranged from 47 to 50 color units with an average color gain of 49 units between 45 and 1440 minutes. Even though color increased in the experiment samples between 45 and 1440 minutes, overall color was still slightly less in the experimental samples that received ozone compared to the control sample that received no ozone. A final average reduction of 80 units (22.9 %) was achieved in these samples after 1440 minutes compared to a color reduction of 5 units (1.4 %) in the control sample.

Data for experiment Color 2-650 are presented in Figures 3-9 and 3-10. Reduction of color in the ozone treated triplicate samples of this experiment ranged from 172 to 205 color units after 45 minutes of exposure to ozone and was significant compared to the control sample where only 5 color units was reduced during this time period (see Figure 3-9). The average percent of color removal within the ozone treated samples was 29.7 % compared to 0.8 % in the control sample. On a mass basis, the average color removal of this set of test samples was 392.5 color units per mg ozone added. The majority of color reduction within the treated samples of Experiment Color 2-650 (90% of the total removal) took place within the first 20 minutes of exposure to ozone (see Figure 3-9).

After 1440 minutes (24 hours) of exposure to ozone, color had recovered (increased) somewhat in the experimental samples compared to the color levels present at 45 minutes (see Figure 3-10). The amount of color recovery for the experiment samples ranged from 44 to 91 color units with an average color gain of 73 units between 45 and 1440 minutes. Even though color increased in the experiment samples between 45 and 1440 minutes, overall color was still significantly less in the experimental samples that received ozone compared to the control sample that received no ozone after 1440 minutes of exposure. A final average reduction of 120 units (18.5 %) was achieved in these samples after 1440 minutes compared to a color reduction of 5 units (0.8 %) in the control sample.

Data for experiment Color 2-850 are presented in Figures 3-11 and 3-12. Analysis of this data indicates that ozone treatment significantly reduced color in the treated samples of this experiment compared to the control sample in which no ozone was added. Based on the precision of data seen in earlier experiments, it appeared that Sample 2 of this experiment should be treated as an outlier (its data was up to 19 % different from Samples 1 and 3). With Sample 2 as an outlier, reduction of color in this experiment ranged from ranged from 195 to 238 color units within the triplicate samples after 45 minutes of exposure to ozone. The average percent of color removal within the ozone treated samples was 25.4 % compared to 0.4 % in the control sample.

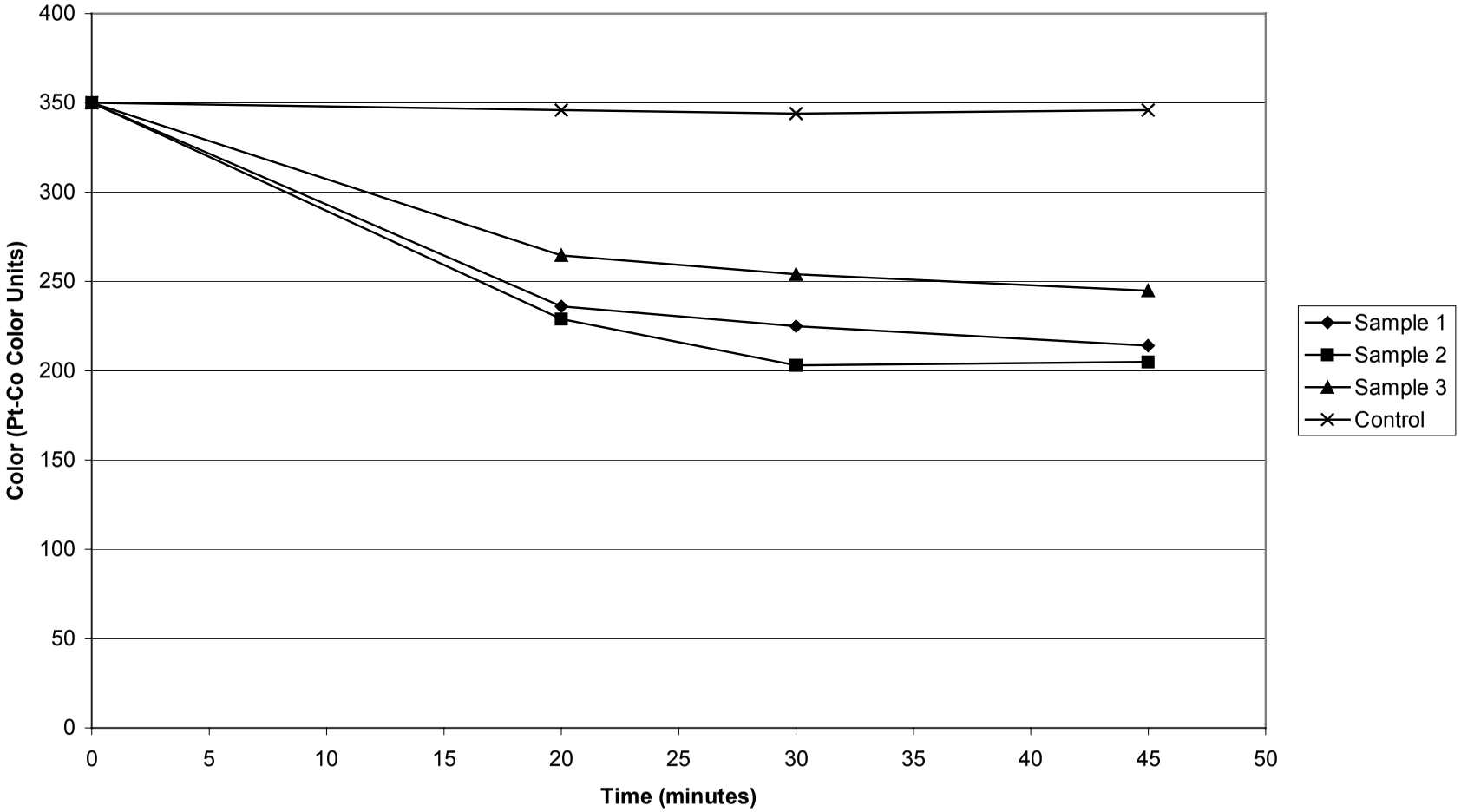

Figure 3-7
Color vs. Time (2.46 mg/L ozone application) Co = 350 color units

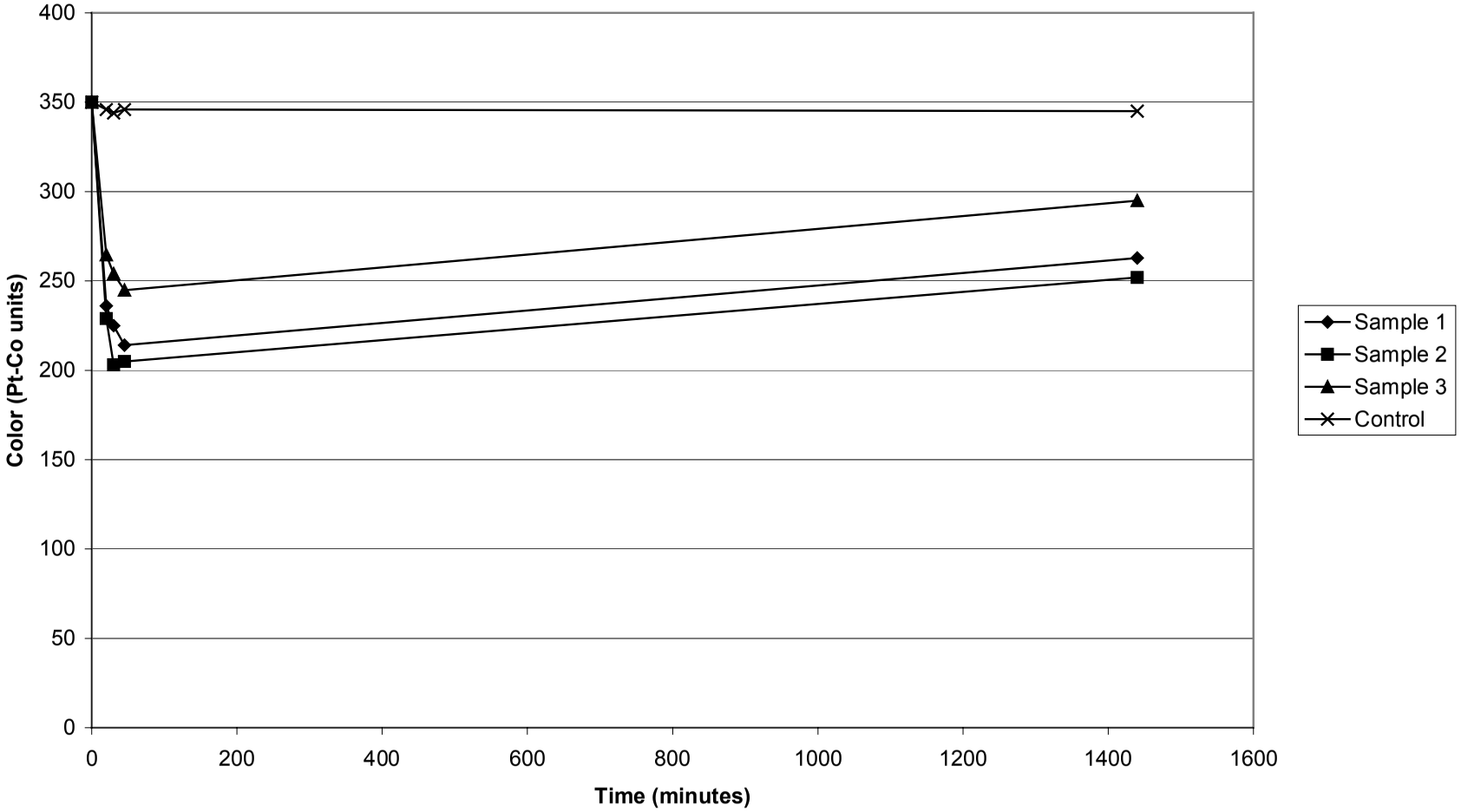

Figure 3-8
Color vs. Time (2.46 mg/L ozone application) Co = 350 color units

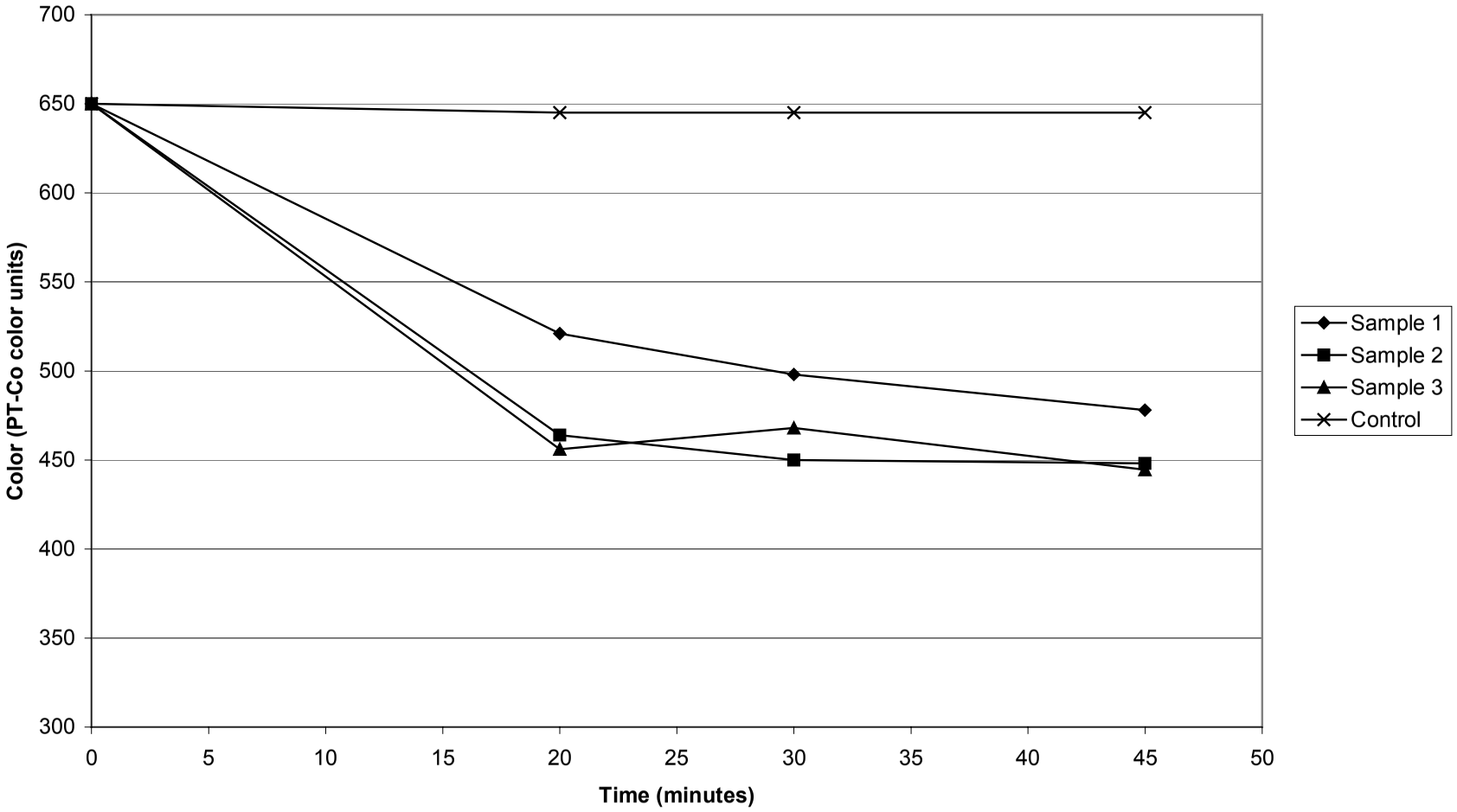

Figure 3-9
Color vs. Time (2.46 mg/L ozone application) Co = 650 color units

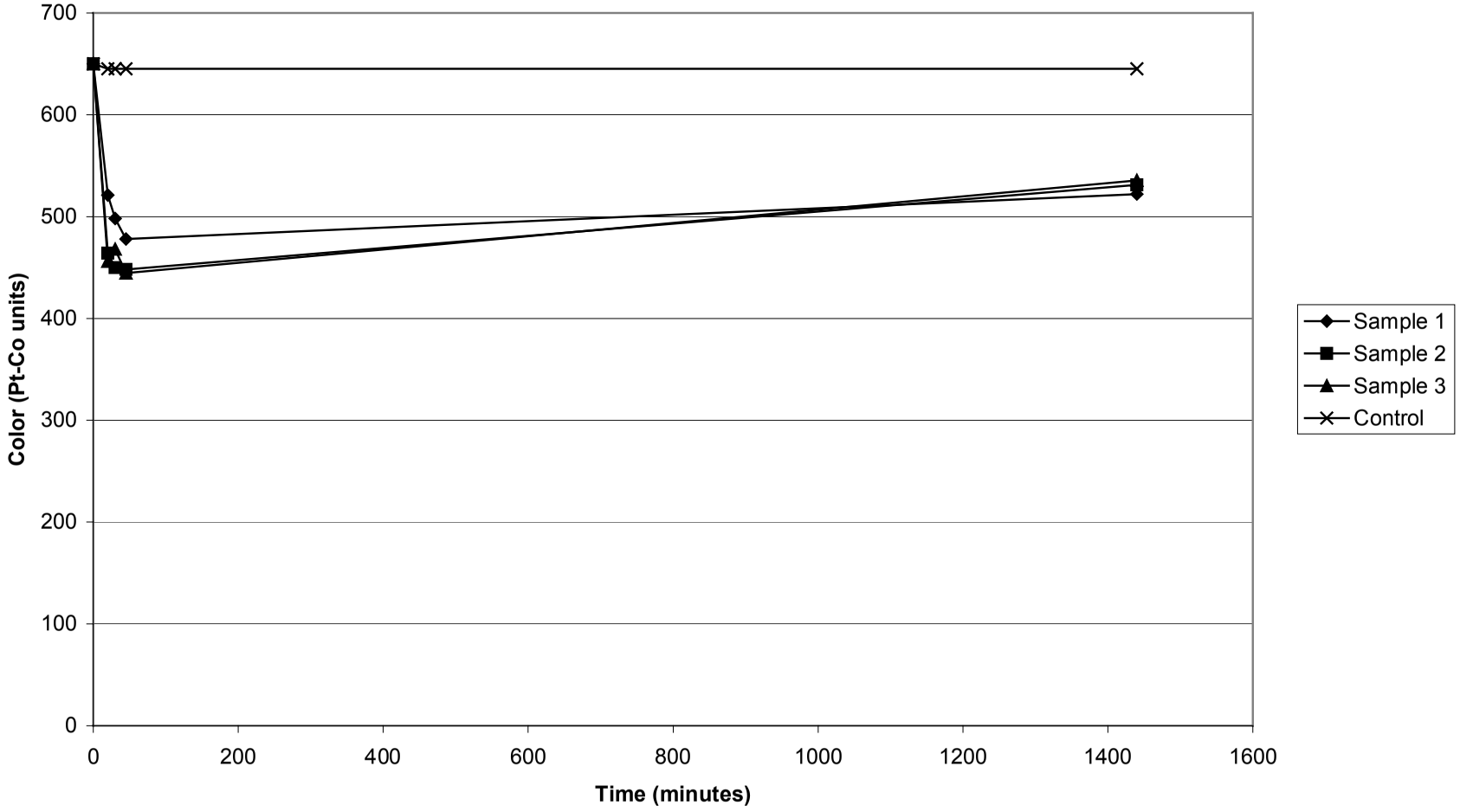

Figure 3-10
Color vs. Time (2.46 mg/L ozone application) Co = 650 color units

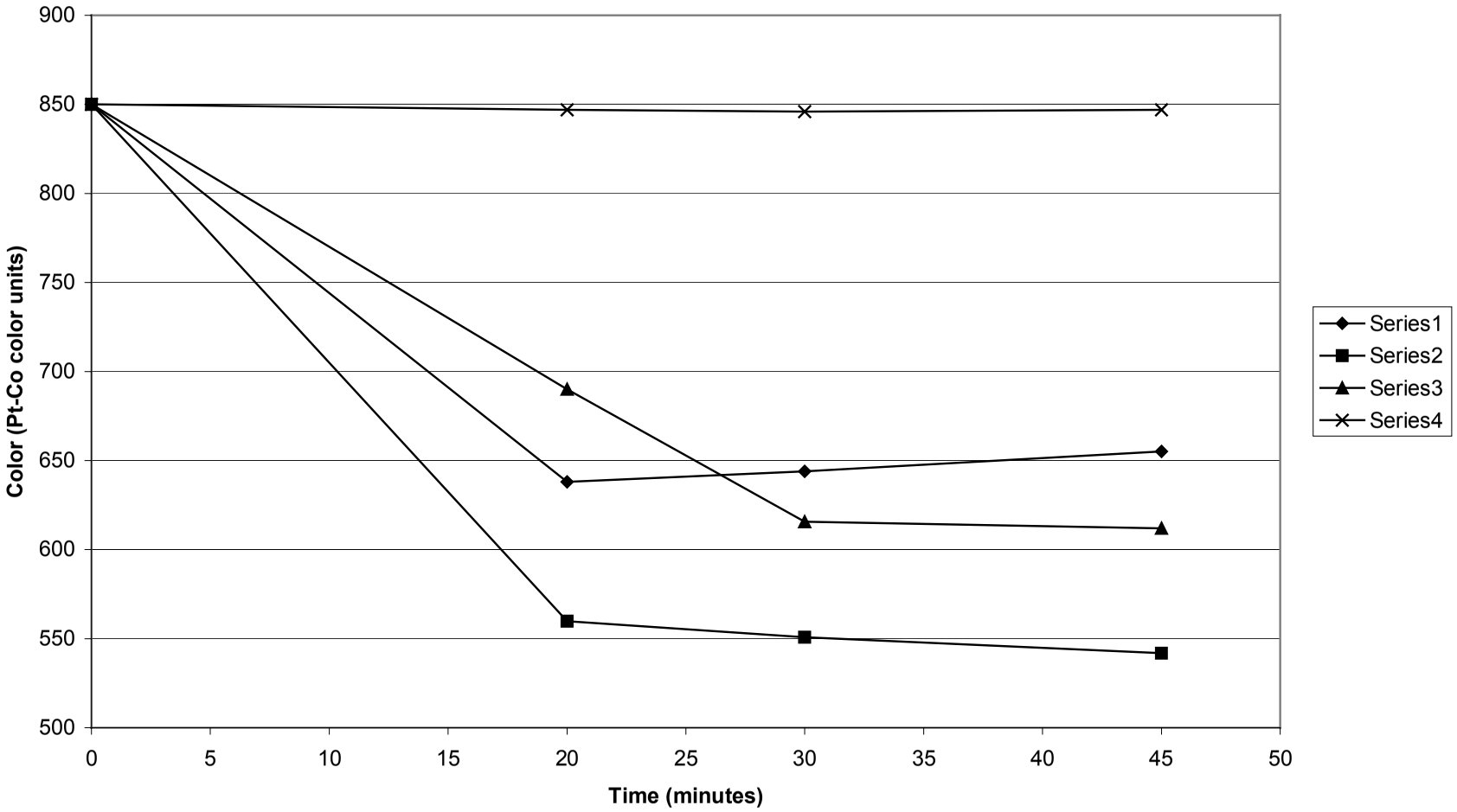

Figure 3-11
Color vs. Time (2.46 mg/L ozone application) Co = 850 mg/L

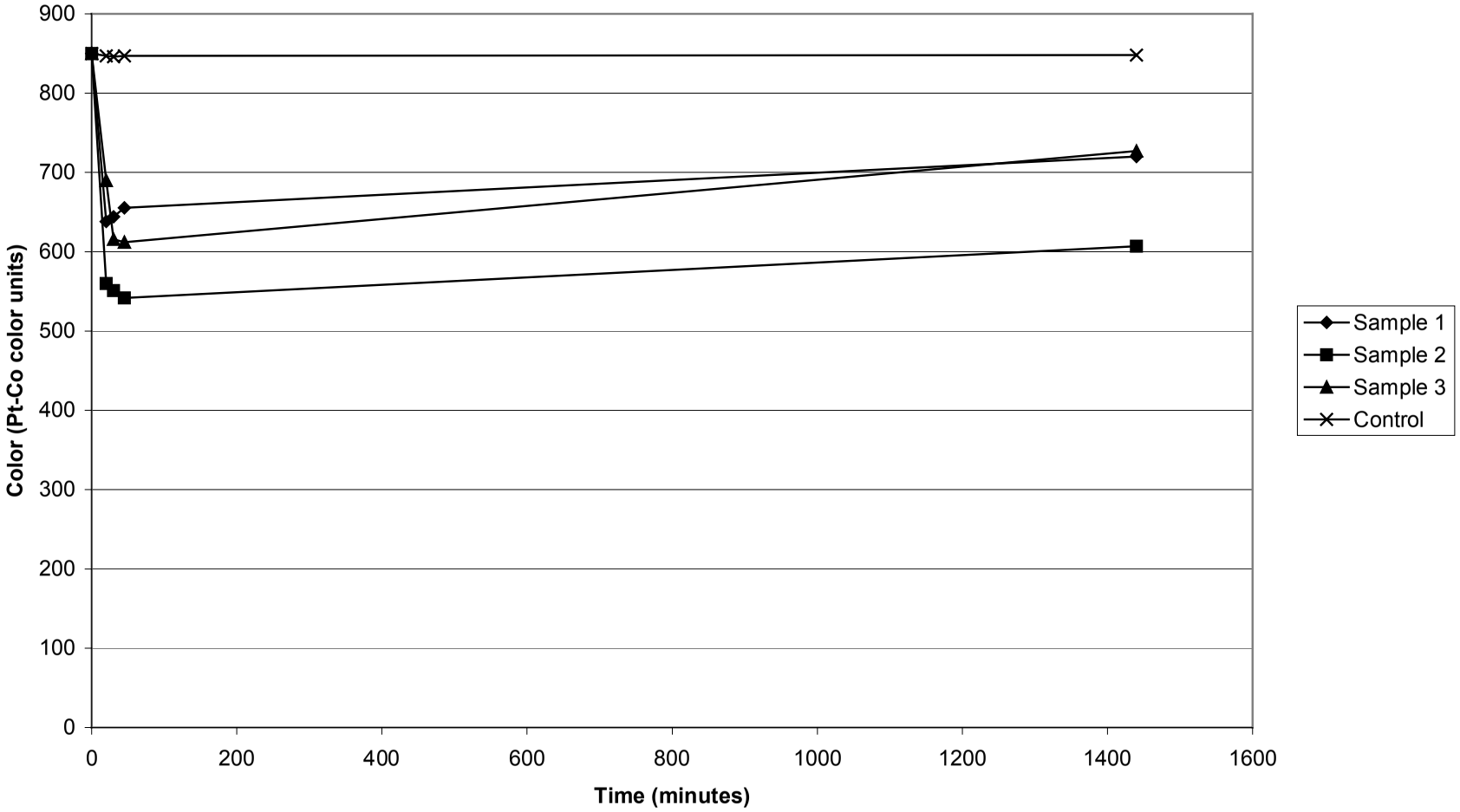

Figure 3-12
Color vs. Time (2.46 mg/L ozone application) Co = 850 mg/L

On a mass basis, the average color removal of this set of test samples was 439.8 color units per mg ozone added. As indicated graphically in Figure 3-11, the majority of color reduction within the treated samples (89% of the total removal) took place within the first 20 minutes of exposure to ozone.

Figure 3-12 indicates that at 1440 minutes (24 hours) of exposure to ozone, color had recovered (increased) significantly in the experimental samples compared to the color levels present at 45 minutes. The amount of color recovery for the experiment samples was 65 color units each for both Samples 1 and 3 between 45 and 1440 minutes. Even though color increased in the experiment samples between 45 and 1440 minutes, overall color was still significantly less in the experimental samples that received ozone compared to the control sample that received no ozone. A final average reduction of 126 units (14.9 %) was achieved in these samples after 1440 minutes compared to a 2 unit reduction in color (0.2 %) in the control sample.

Discussion of Color Experiments

Results of the batch experiments for color removal indicate that the application of ozone effectively and rapidly reduces the amount of color in water. Total color removal in these batch studies ranged from 29% to 36% during the first 45 minutes to 5 % to 28 % at 24 hours of exposure to ozone with increased removal occurring at both higher initial color concentrations and higher ozone treatment concentrations. The average amount of color removed per mg ozone added was 332.6 color units per mg ozone added for the first 45 minutes of time. Change in color was not noted in control samples.

Several interesting observations were made in these sets of experiments in regards to color reduction over time. The first observation pertains to reaction time. In comparing color removal data for the first 45 minutes of all ozone treated water samples, it appears that most color removal in the color batch experiments occurred within the first 20 minutes of exposure to ozone (80 to 90% of total removal), indicating that ozone treatment occurs rapidly. Based on average color removal data for the two ozone application rates, slightly more rapid removal took place in the batch experiments with the higher ozone application rates (79% to 82% of total removal occurring during the first 20 minutes of the 2.17 mg/L ozone application experiment compared to 83% to 89% of total removal occurring during the first 20 minutes of the 2.46 mg/L ozone application experiment.

The second observation was that although color was reduced in all ozone treated samples during the first 45 minutes, an increase in color also occurred in each of these samples between 45 and 1440 minutes. The reason for the subsequent increase (or recovery) of color during these experiments is not known. One possible cause of the color increase is biological activity, whereby the effectively of ozone was used up in the first 45 minutes or so of exposure, but then biological activity (availability of nutrients?) caused color to increase again. In samples where no ozone was added, color did not increase.

According to Bablon et al, (1991), the ozone decomposition rate is assumed to be a first order kinetic equation that is contant linear function of pH. To determine if the rate of color removal was first order in the batch experiments with respect to time (or dependant upon concentration of color present), the natural logarithm (ln) of color was plotted versus time for Experiments Color

1-350 and Color 1-850 (see Figures 3-13 and 3-14). If the color removal rate was dependant upon concentration, then the line connecting the data points on a ln C vs. time plot would be a straight line (with the slope being the kinetic coefficient of decay). However, upon analysis of the graphical data in Figures 3-13 and 3-14, it appears that the removal rate of color in these experiments does not decrease at a first order kinetic rate for the three data points taken within the first 45 minutes. This is not to say that the removal is not first order, in fact first order data could fit a first order degradation pattern with a tailing off of removal at t = 20, 30, and 45 minutes, however using the time points tested, the data does not fit a first order pattern. To determine color removal is truly first order in rate, more data will need to be gathered during the first 20 minutes of exposure to ozone where most of the color removal is likely taking place.

To determine whether a relation exists between total color removal and initial color concentration (independent of ozone added), total color removal (initial color level minus 45-minute color level) was plotted against initial color concentrations for both the 2.17 and 2.46 mg/L ozone treatments. Removal versus initial color concentration data are presented in Figures 3-15 and 3-16. Initial observance of this data seems to indicate that in general, increased color removal occurs for higher initial color concentrations for the same amount of ozone treatment. Regression analysis of this data indicates however, that although a general trend of increased color removal at higher initial color concentrations took place in the batch studies, this trend is not statistically significant. This factor is reflected in low R^2 values (less than 0.90) assigned to the statistical trend lines in Figures 3-15 and 3-16 which mean that the data do not 'fit' the line very well (support the trend).

To determine whether a relationship exists between total color removal and the amount of ozone added, total color removal (initial color level minus 45-minute color level) was plotted against initial ozone treatment concentrations for all three initial color levels (350, 650, and 850 color units). Removal versus initial ozone treatment concentration data are presented in Figure 3-17. Note that there are three different groupings of data in this graph representing the three initial color concentrations and the amount of removal occurring for each of these initial color concentrations at the three amounts of ozone added (0 mg/L, 2.17 mg/L and 2.46 mg/L). Analysis of this removal versus ozone treatment data indicates that in general, increased color removal occurs with higher ozone treatment. Regression analysis supports this interpretation and although there are only three sets of data points for this analysis (many blanks to fill for lower ozone treatment levels) the trend of increased color removal with increased ozone treatment appears valid (see R^2 values on trend lines in Figure 3-17).

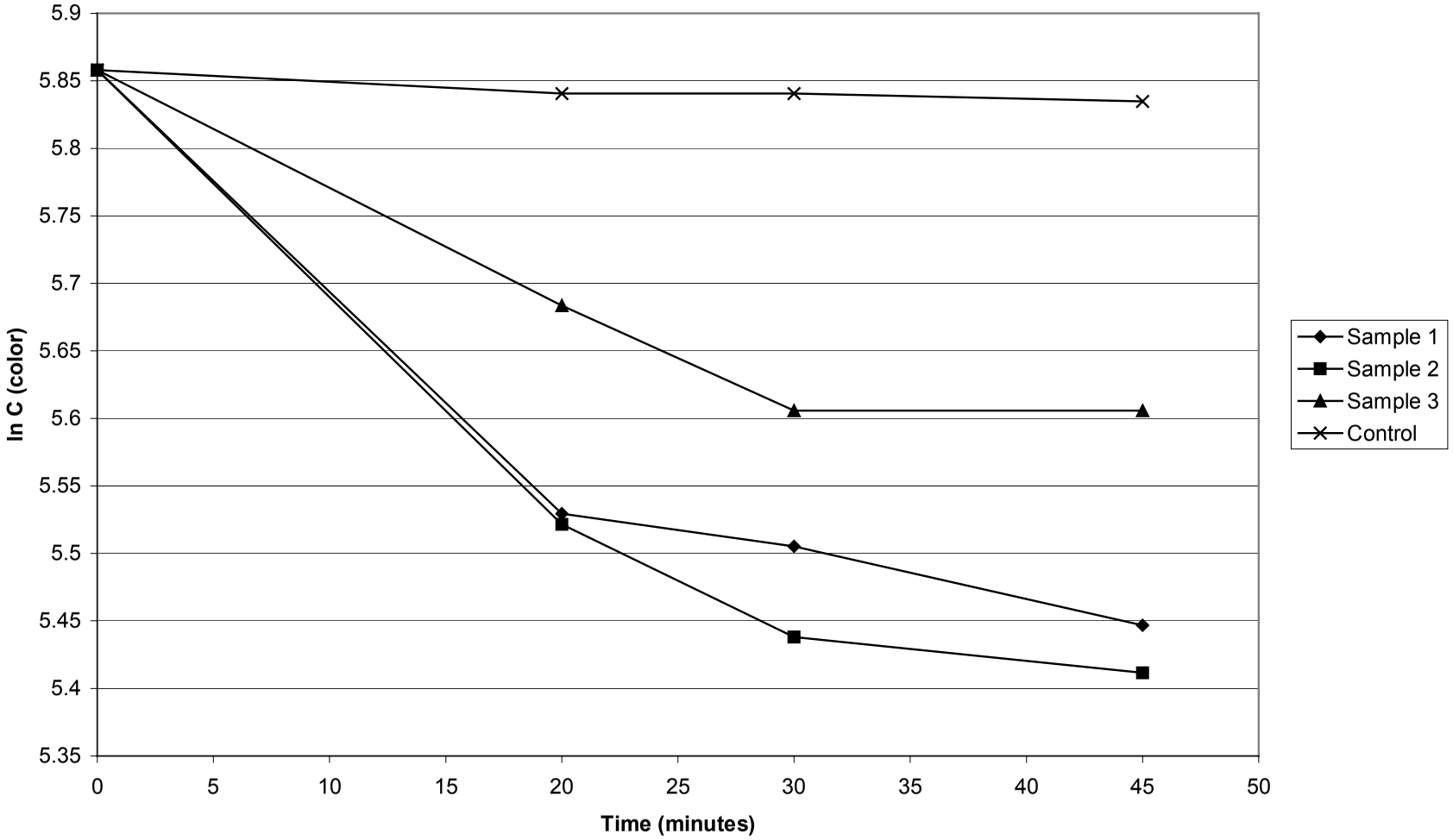

Figure 3-13
ln conc vs. time for Experiment Color 1-350 2.17 mg/L Ozone, Co = 350 Pt-Co Color Units

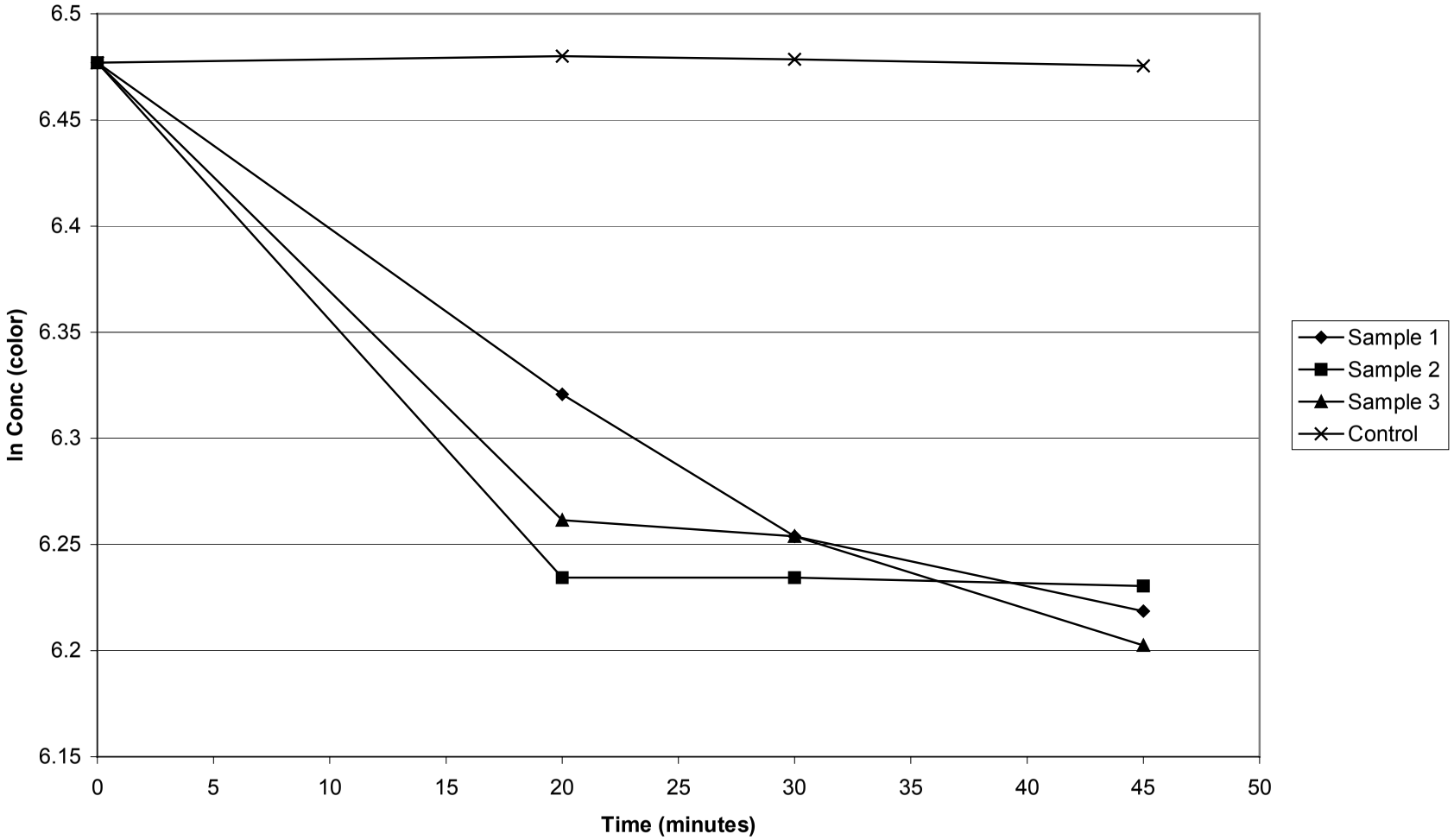

Figure 3-14
ln C vs. Time Experiment Color 1-850 2.17 mg/L Ozone, Co = 850 Pt-Co Color Units

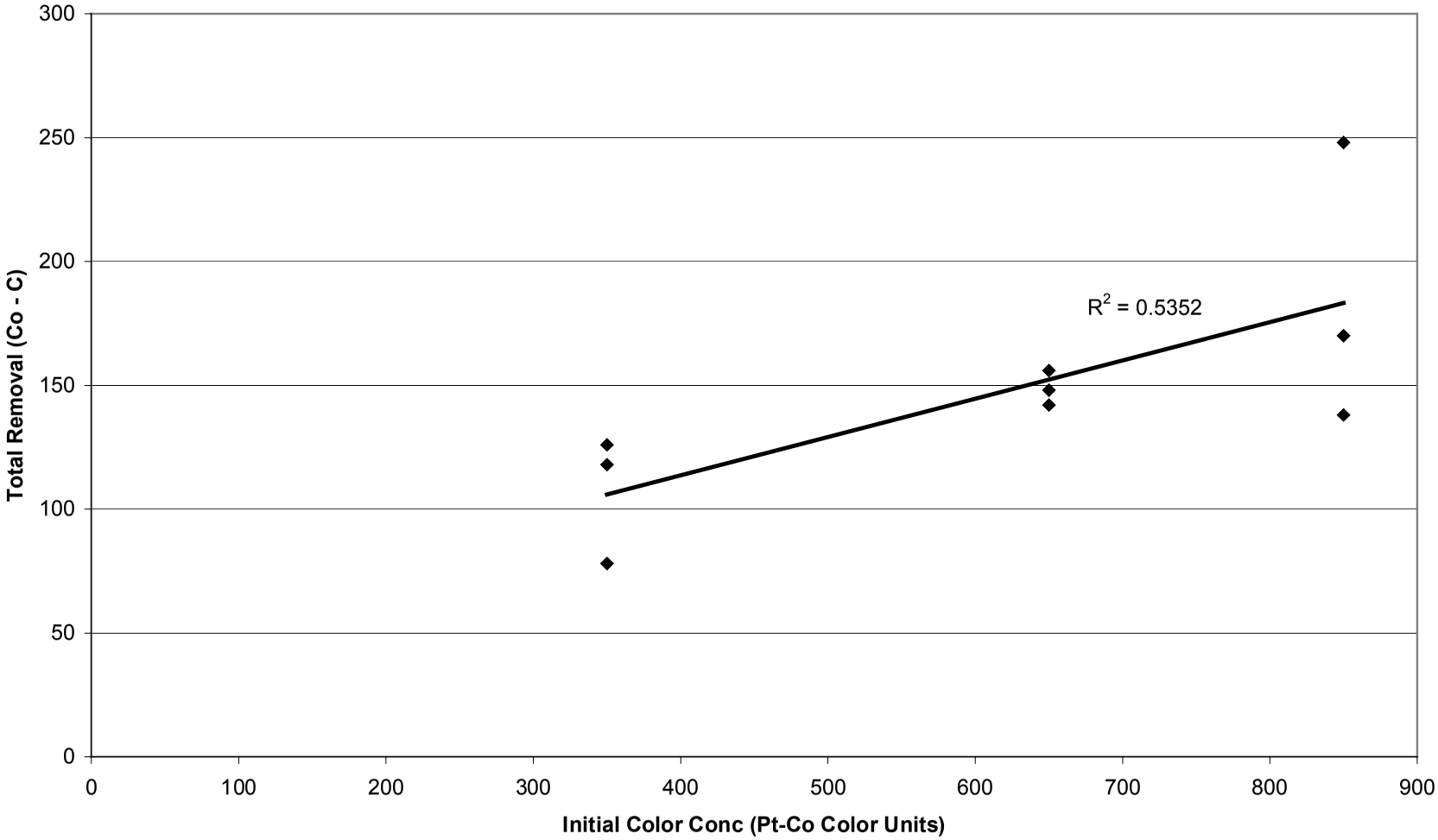

Figure 3-15
Total Removal vs. Initial Concentration 2.17 mg/L Ozone Treatment Experiments

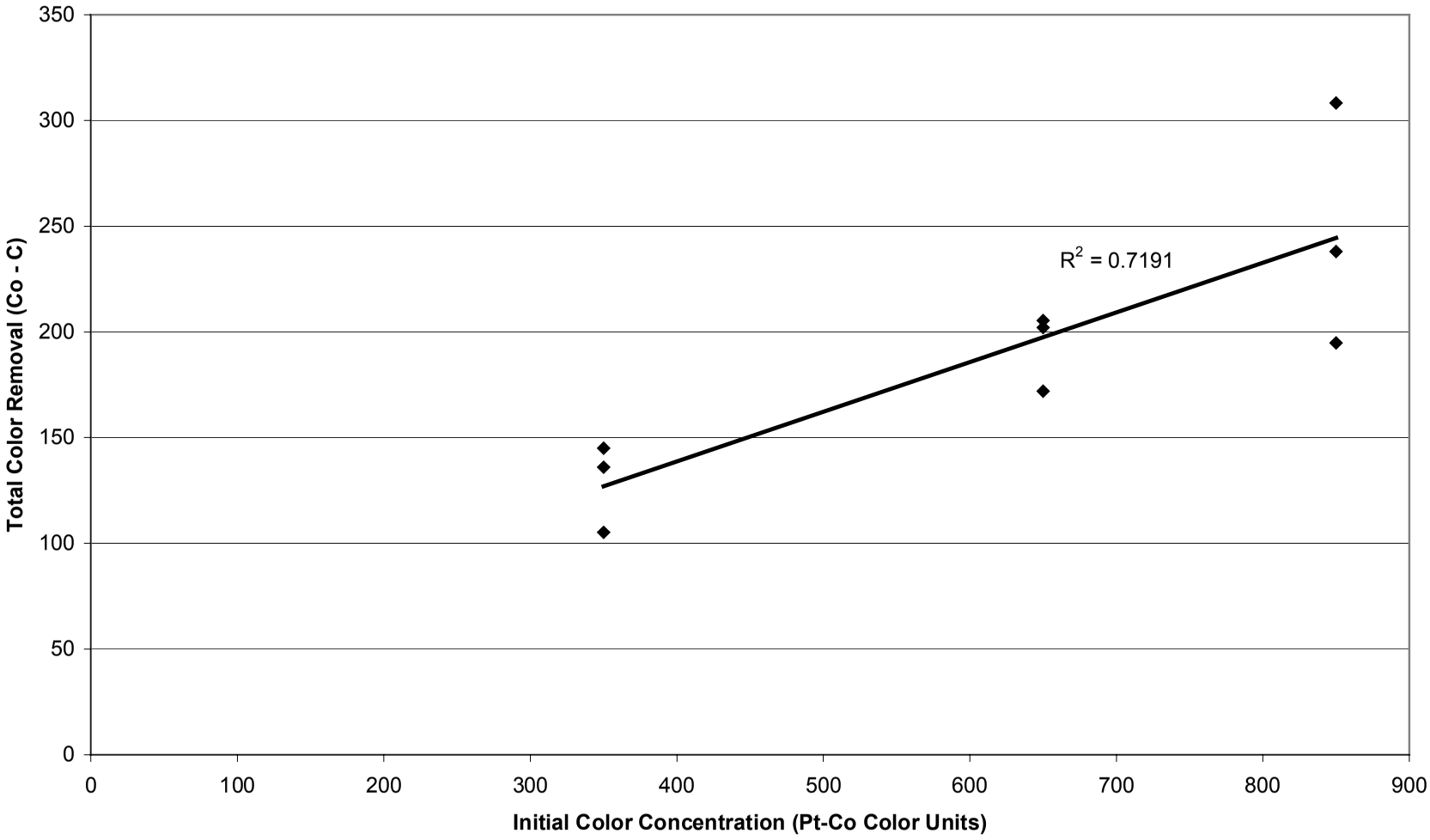

Figure 3-16
Removal vs. Initial Concentration 2.46 mg/L Ozone Treatment Experiments

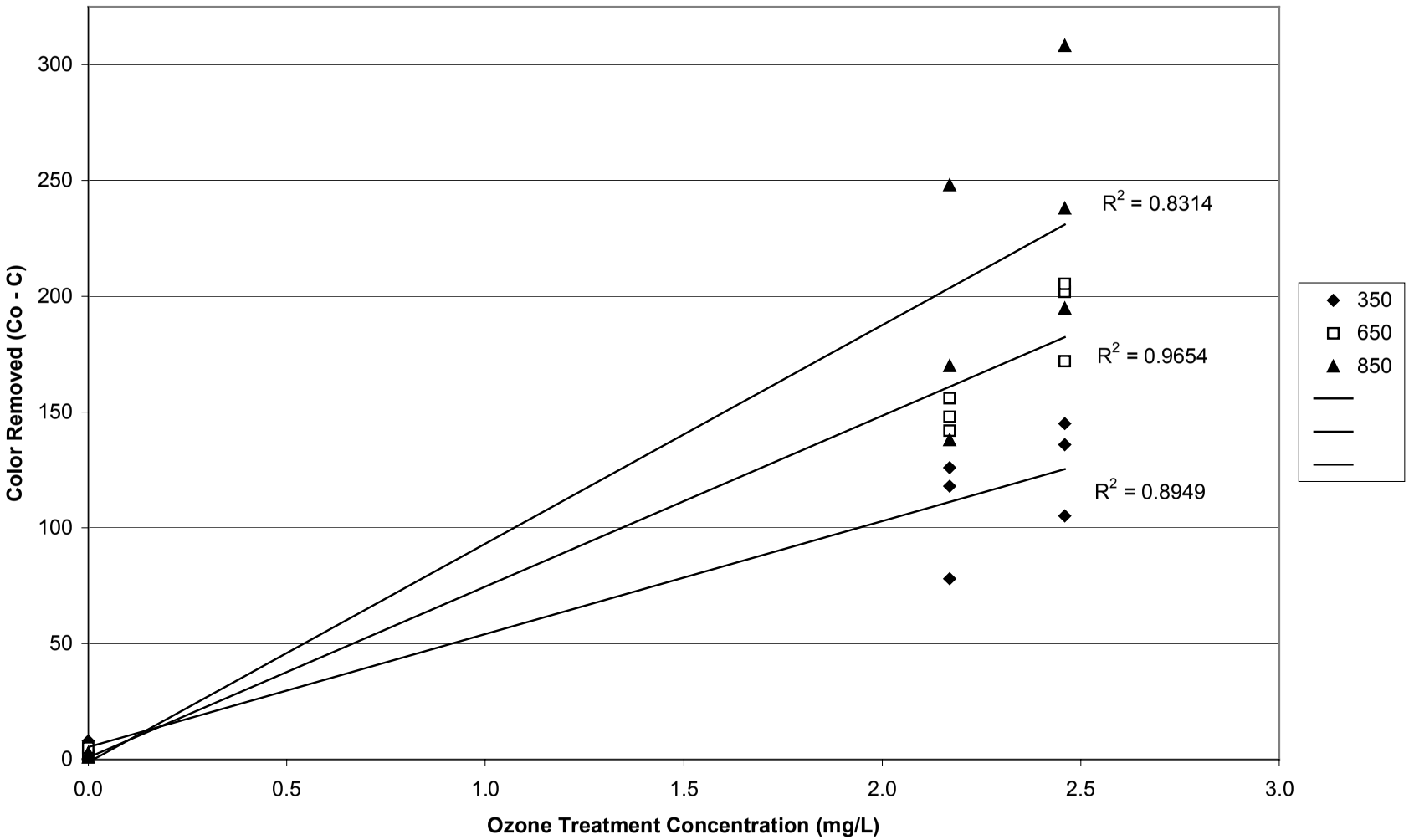

Figure 3-17
Color Removal vs. Ozone Treatment

Analysis of the number of color units removed per g ozone added for all samples where ozone was added yielded the following color removal information: 332.6 +/- 78.5 color units per mg of ozone added, with higher removal taking place at higher initial color concentrations (as described above).

In summary, results from the color batch experiments were as follows:

a. ozone effectively removed color from discreet samples of water

b. increased color unit removal was achieved with increasing ozone concentrations,

c. increased color unit removal occurred at higher initial color concentrations,

d. color removal using ozone was rapid with most color removal taking place within the first 20 minutes of exposure to ozone, and

e. more rapid color removal occurred with higher ozone applications.

3.1.2 Nitrite-Nitrogen Experiments

Results of Experiments Treated with 2.15 mg/L Ozone:

Three independent nitrite-nitrogen (NO_2-N) experiments were run with ozone treatment concentrations of 2.15 mg/L over a range of NO_2-N found in aquaculture production systems. NO_2-N levels used in the 2.15 mg/L ozone batch experiments were 0.2, 0.6, and 0.99 mg/L NO_2 and are identified by the following respective experiment titles: N 1-0.2, N 1-0.6, and N 1-0.99. NO_2-N analyses were performed at 30, 60, and 90 minutes after exposure to ozone.

Data for experiment N 1-0.2 are presented in Figure 3-18. Analysis of this data indicates that NO_2-N was reduced by the application of 2.15 mg/L significantly compared to the control sample in which no ozone was added. Reduction of NO_2-N in this experiment ranged from 0.194 to 0.196 mg/L within the triplicate samples after 90 minutes of exposure to ozone. The average percent of NO_2-N removal within the ozone treated samples was 98 % compared to 5 % in the control sample. As indicated graphically in Figure 3-18, the majority of NO_2-N reduction within the treated samples (89% of the total removal) took place within the first 30 minutes of exposure to ozone.

Data for experiment N 2-0.6 are presented in Figure 3-19. Reduction of NO_2-N in the ozone treated triplicate samples of this experiment was 0.592 mg/L for all three samples after 90 minutes of exposure to ozone and was significant compared to the control sample where only 0.05 mg/L NO_2-N was reduced during this time period. The average percent of NO_2-N removal within the ozone treated samples was 99 % compared to 8 % in the control sample. The majority of NO_2-N reduction within the treated samples of Experiment N2-0.6 (96% of the total removal) took place within the first 30 minutes of exposure to ozone (see Figure 3-19).

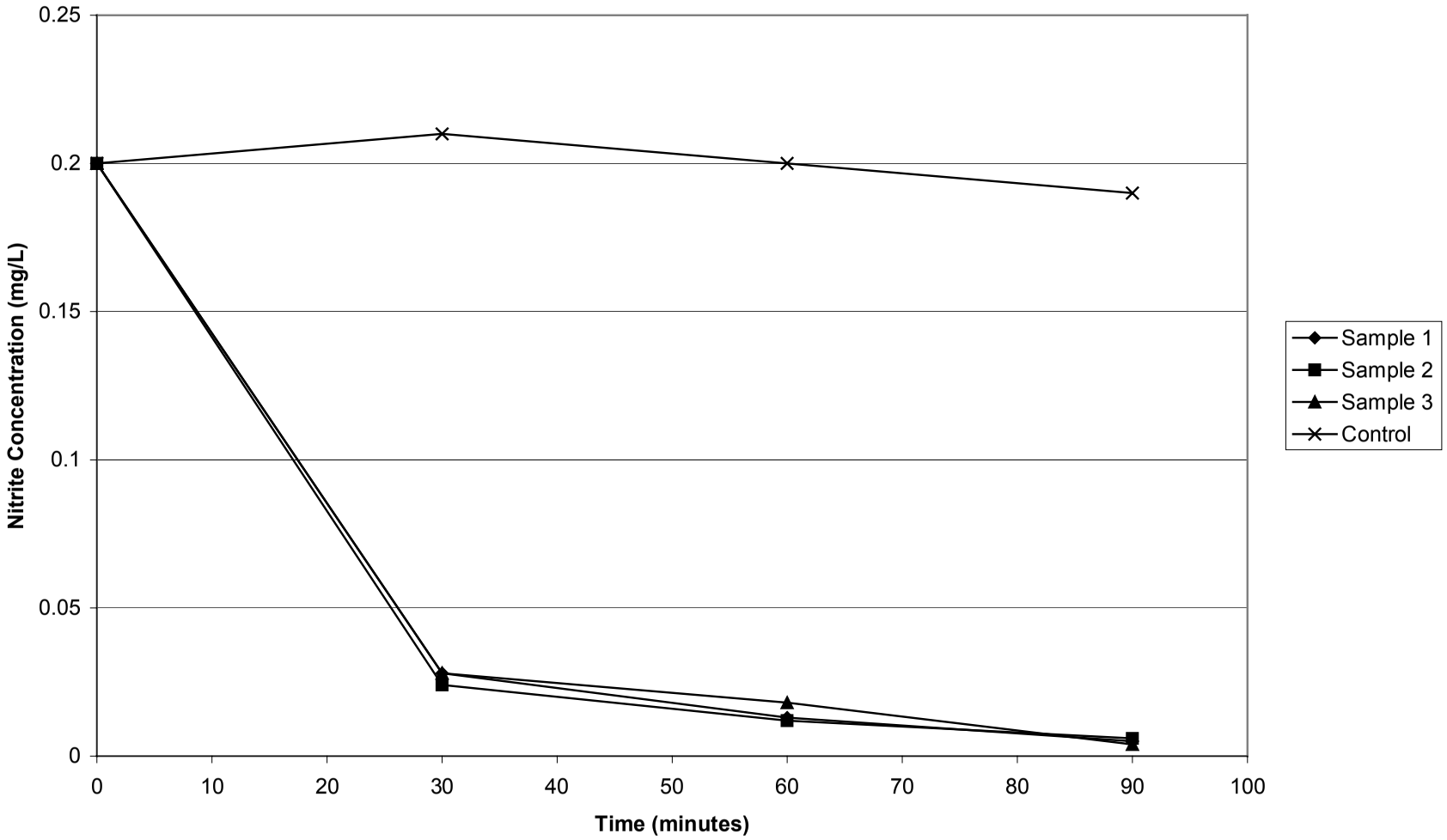

Figure 3-18
Nitrite Concentration vs. Time 2.15 mg/L Ozone, Co = 0.2 mg/L

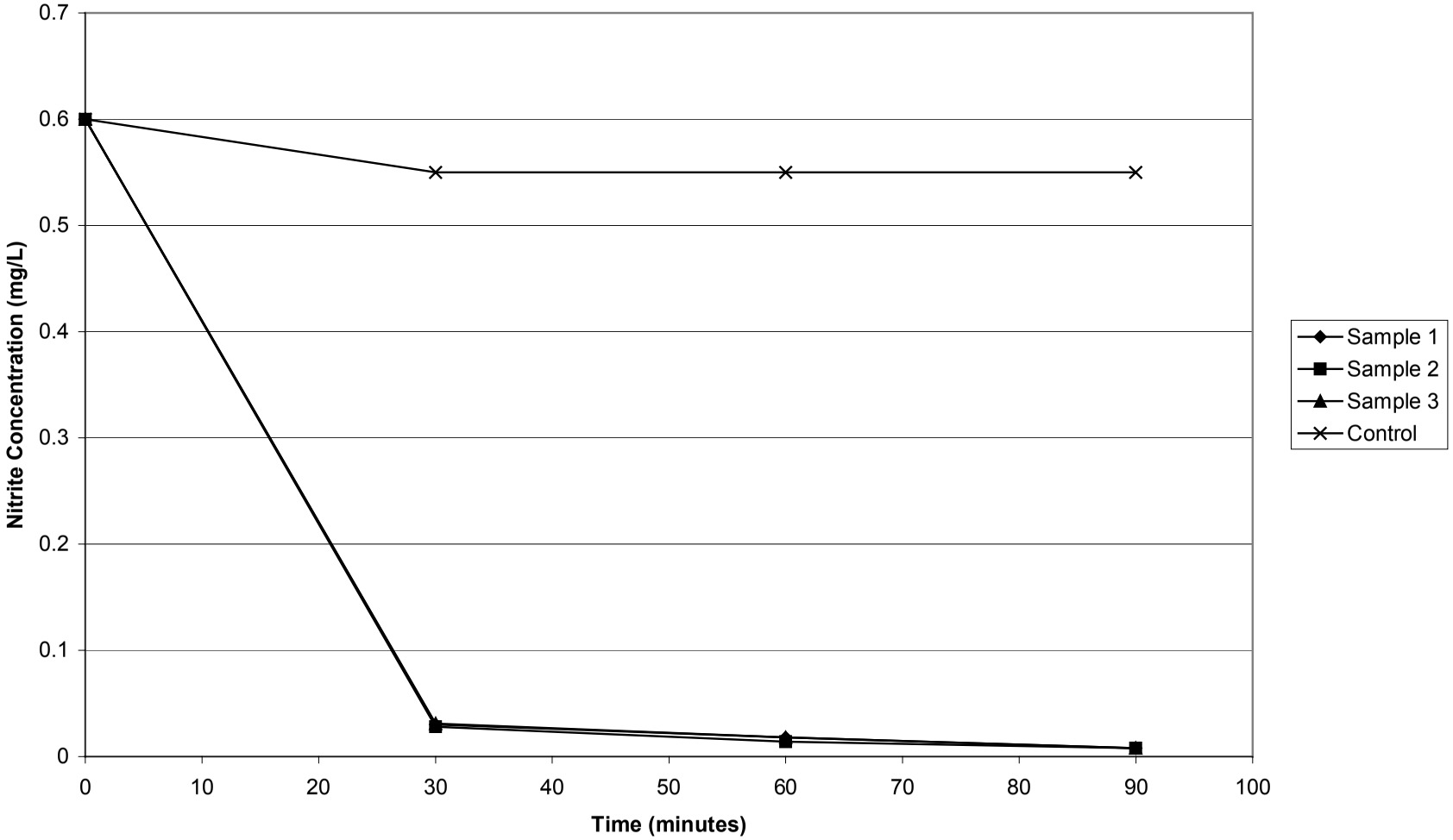

Figure 3-19
Nitrite Concentration vs. Time Co = 0.6 mg/L Nitrite, 2.15 mg/L Ozone

Data for experiment N 2-0.99 are presented in Figure 3-20. Analysis of this data indicates that ozone treatment significantly reduced NO_2-N in the treated samples of this experiment compared to the control sample in which no ozone was added. Reduction of NO_2-N in this experiment ranged from 0.979 to 0.981 mg/L within the triplicate samples after 90 minutes of exposure to ozone. The average percent of NO_2-N removal within the ozone treated samples was 99 % compared to 0.2 % in the control sample. As indicated graphically in Figure 3-20, the majority of NO_2-N reduction within the treated samples (97% of the total removal) took place within the first 30 minutes of exposure to ozone.

Results of Experiments Treated with 2.32mg/L Ozone:

Three additional NO_2-N experiments were run at the slightly higher ozone treatment concentration of 2.32 mg/L (compared to 2.15 mg/L) to determine how added ozone would affect the speed and completeness of NO_2-N removal in batch experiments. NO_2-N concentrations used in the 2.32 mg/L ozone batch experiments were 0.2, 0.6, and 0.99 mg/L (for comparison to the first set of tests) and are identified by the following respective experiment titles: N 2-0.2, N 2-0.6, and N 2-0.99.

Data for experiment N 2-0.2 are presented in Figure 3-21. Analysis of this data indicates that NO_2-N was significantly and rapidly reduced by the application of 2.32 mg/L ozone compared to the control sample in which no ozone was added. Reduction of NO_2-N in this experiment ranged from 0.194 to 0.196 mg/L within the triplicate samples after 90 minutes of exposure to ozone. The average percent of NO_2-N removal within the ozone treated samples was 98% compared to 10% in the control sample. As indicated graphically in Figure 3-21, the majority of NO_2-N reduction within the treated samples (93% of the total removal) took place within the first 30 minutes of exposure to ozone.

Data for experiment N 2-0.6 are presented in Figure 3-22. Reduction of NO_2-N in the ozone treated triplicate samples of this experiment ranged from 0.592 to 0.594 mg/L after 90 minutes of exposure to ozone and was significant compared to the control sample where only 0.02 mg/L was reduced during this time period. The average percent of NO_2-N removal within the ozone treated samples was 99 % compared to 0.3 % in the control sample. The majority of NO_2-N reduction within the treated samples of Experiment N 2-0.6 (97% of the total removal) took place within the first 30 minutes of exposure to ozone (see Figure 3-22).

Data for experiment N 2-0.99 are presented in Figure 3-23. Analysis of this data indicates that ozone treatment significantly and rapidly reduced NO_2-N in the treated samples of this experiment compared to the control sample in which no ozone was added. Reduction of NO_2-N in this experiment ranged from 0.98 to 0.982 mg/L within the triplicate samples after 90 minutes of exposure to ozone. The average percent of NO_2-N removal within the ozone treated samples was 99 % compared to 0.2 % in the control sample. As indicated graphically in Figure 3-23, the majority of NO_2-N reduction within the treated samples (98% of the total removal) took place within the first 30 minutes of exposure to ozone.

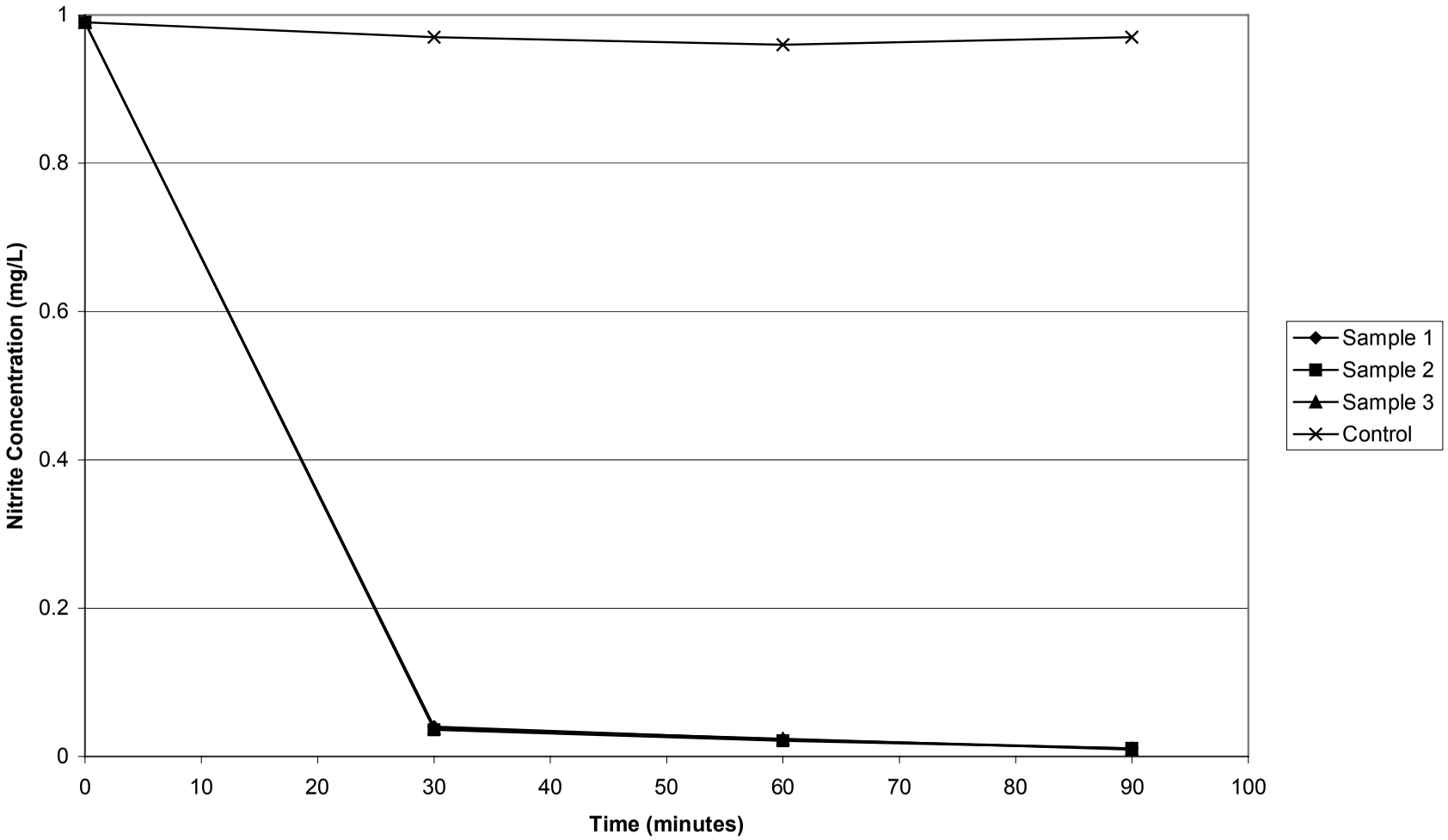

Figure 3-20
Nitrite Concentration vs. Time Co = 0.99 mg/L Nitrite, 2.15 mg/L Ozone

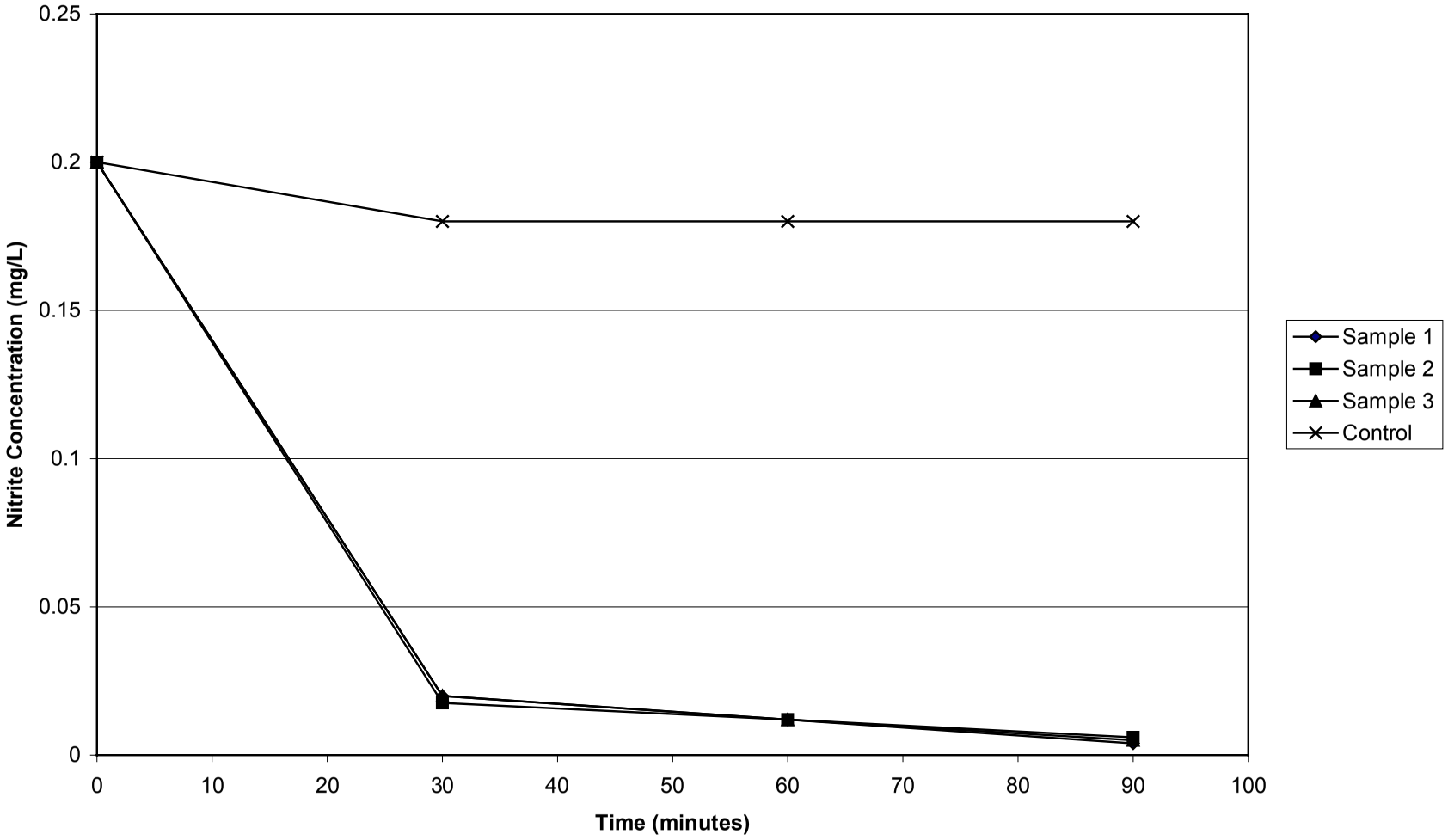

Figure 3-21
Nitrite Concentrations vs. Time Co = 0.2 mg/L Nitrite, 2.32 mg/L Ozone

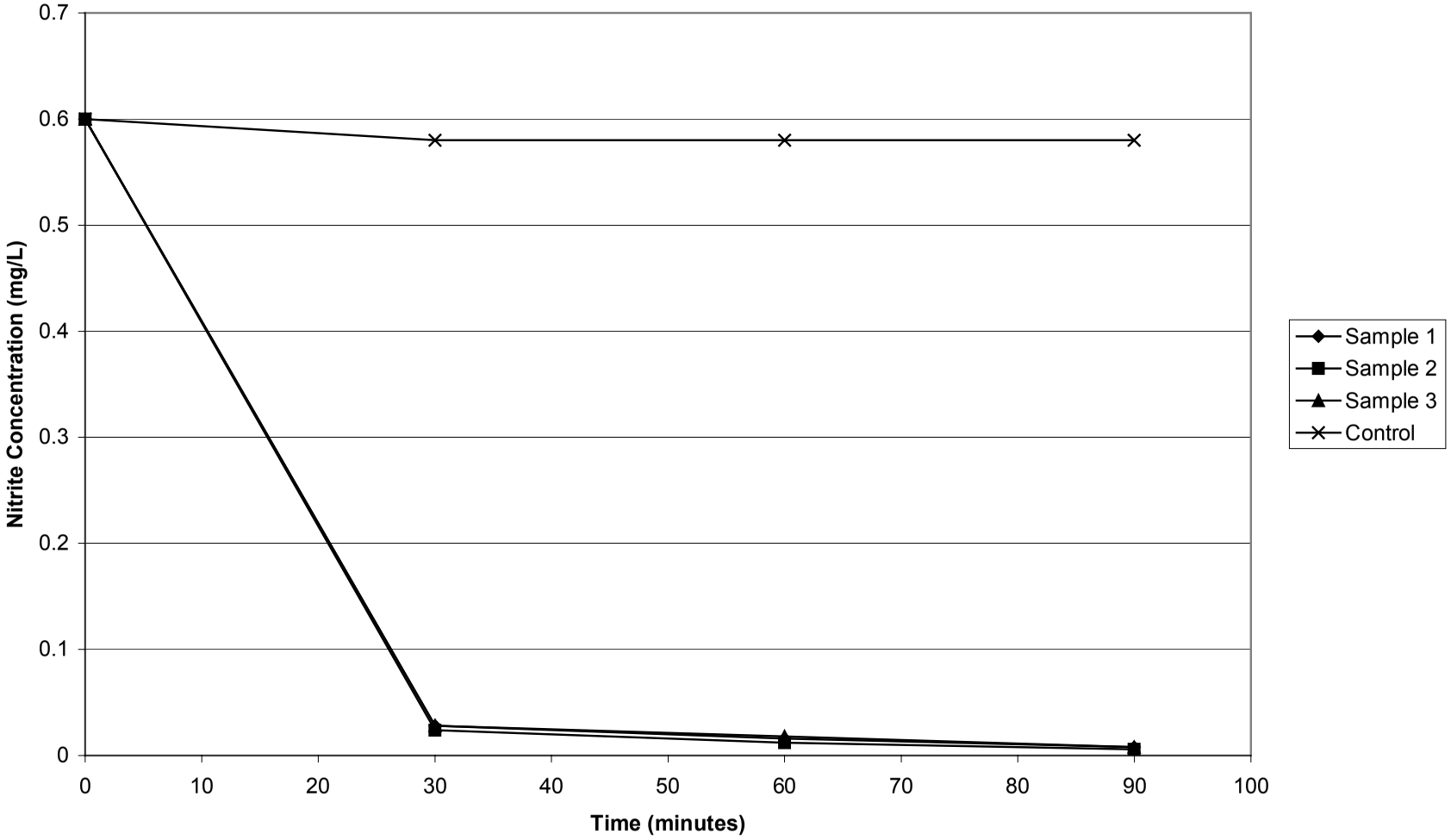

Figure 3-22
Nitrite Concentration vs. Time Co = 0.6 mg/L Nitrite, 2.32 mg/L Ozone

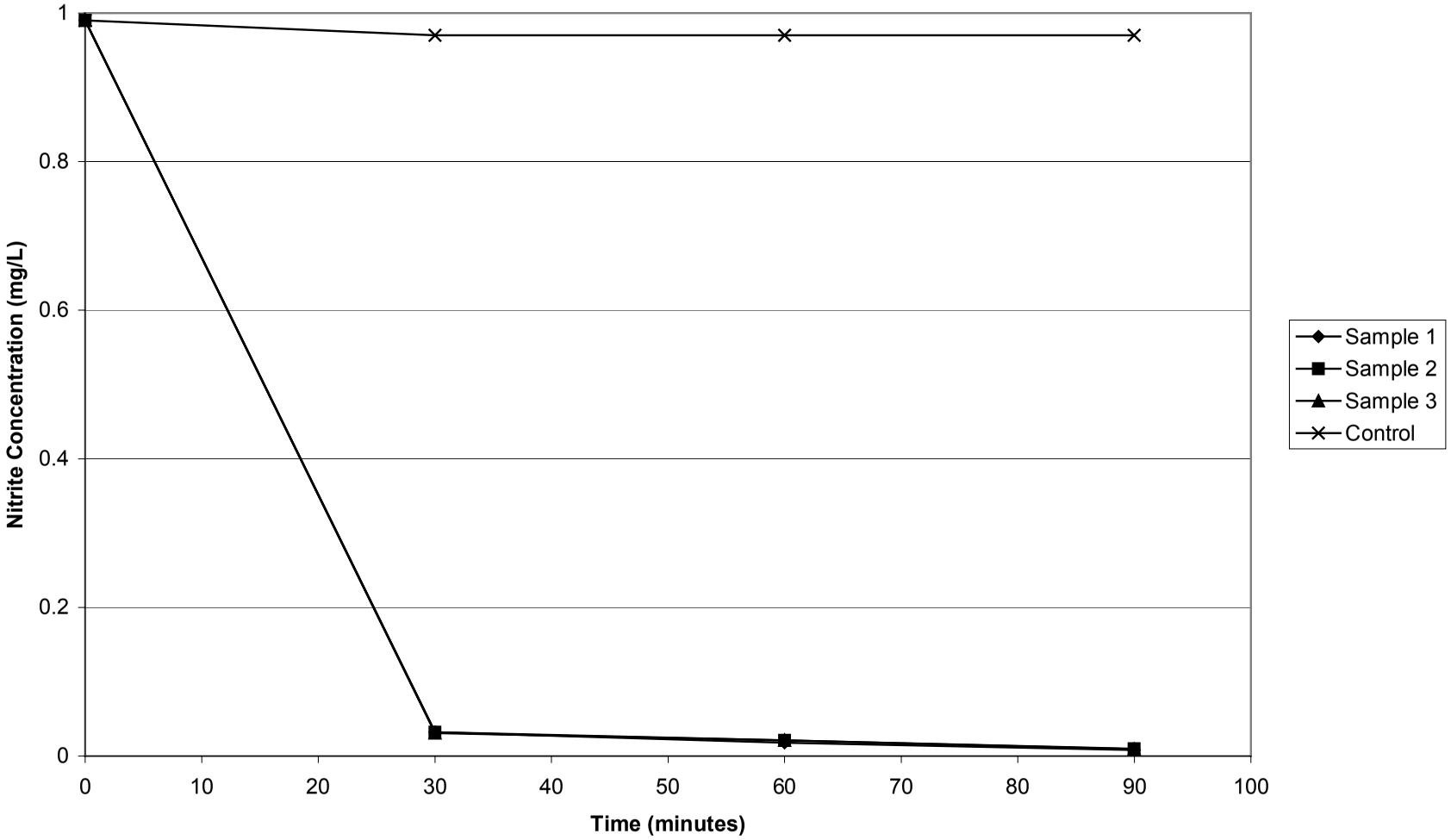

Figure 3-23
Nitrite Concentration vs. Time Co=0.99 mg/L Nitrite, 2.32 mg/L Ozone

Discussion of Nitrite Experiments

Results of the batch experiments for NO_2-N removal indicate that the application of ozone effectively and rapidly reduces NO_2-N in water. Total NO_2-N removal in these batch studies ranged from 97% to 99% and was significant compared to samples where no ozone was added.

The natural logarithm of NO_2-N was plotted vs. time for two sets of nitrite removal data, (Experiment N1-0.2 and N1-0.99) to determine if NO_2-N removal was a first order reaction rate (Figure 3-24). Results from this data indicate that the removal of NO_2-N from the batch samples was not first order with respect to time. As in the case with the color experiments, in the nitrite experiments, it appears that most of the removal has already taken place by t = 30 minutes, and the 30, 60, 90-minute data does not form a good first order curve by themselves (more data would be needed during the first 30 minutes).

Total NO_2-N removal (initial NO_2-N minus 90-minute NO_2-N concentrations) was plotted against initial NO_2-N concentrations for both the 2.15 and 2.32 mg/L ozone treatments to determine whether a relationship could be established between total NO_2-N removal and initial NO_2-N concentration (independent of ozone added),. Removal versus initial NO_2-N data is presented in Figure 3-25. Analysis of this data indicates that increased NO_2-N removal occurred for higher initial NO_2-N concentrations for the same amount of ozone treatment during these batch experiments. Regression analysis supports this observation (see R^2 value of 1 for the trend line) however this appears to be a result of the fact that nearly all of the NO_2-N was removed in all of the samples of these experiments (i.e. there was more than enough ozone to treat all the NO_2-N in these samples).

Total NO_2-N removal (initial NO_2-N level minus 90-minute NO_2-N level) versus ozone treatment concentrations for all three initial NO_2-N levels (0.2, 0.6, and 0.99 mg/L NO_2) and three ozone treatment levels (0, 2.15, and 2.32 mg/L ozone) is presented in Figure 3-26. Due to near complete removal of NO_2-N in the ozone samples and near no removal in the control samples, no relationship could be established through this analysis. Without additional lower ozone treatment experiments, it is not clear whether there is linear or asymptotic removal of NO_2-N as more ozone is added.

In regards to reaction time, most NO_2-N removal in the NO_2-N batch experiments occurred within the first 30 minutes of exposure to ozone (87% to 98% of total removal) indicating that ozone treatment of NO_2-N occurs rapidly. Based on average NO_2-N removal data for the two ozone application rates, no discernable increase in NO_2-N removal took place when slightly higher ozone was used (2.46 mg/L ozone application compared to 2.15 mg/L ozone - see Table N1).

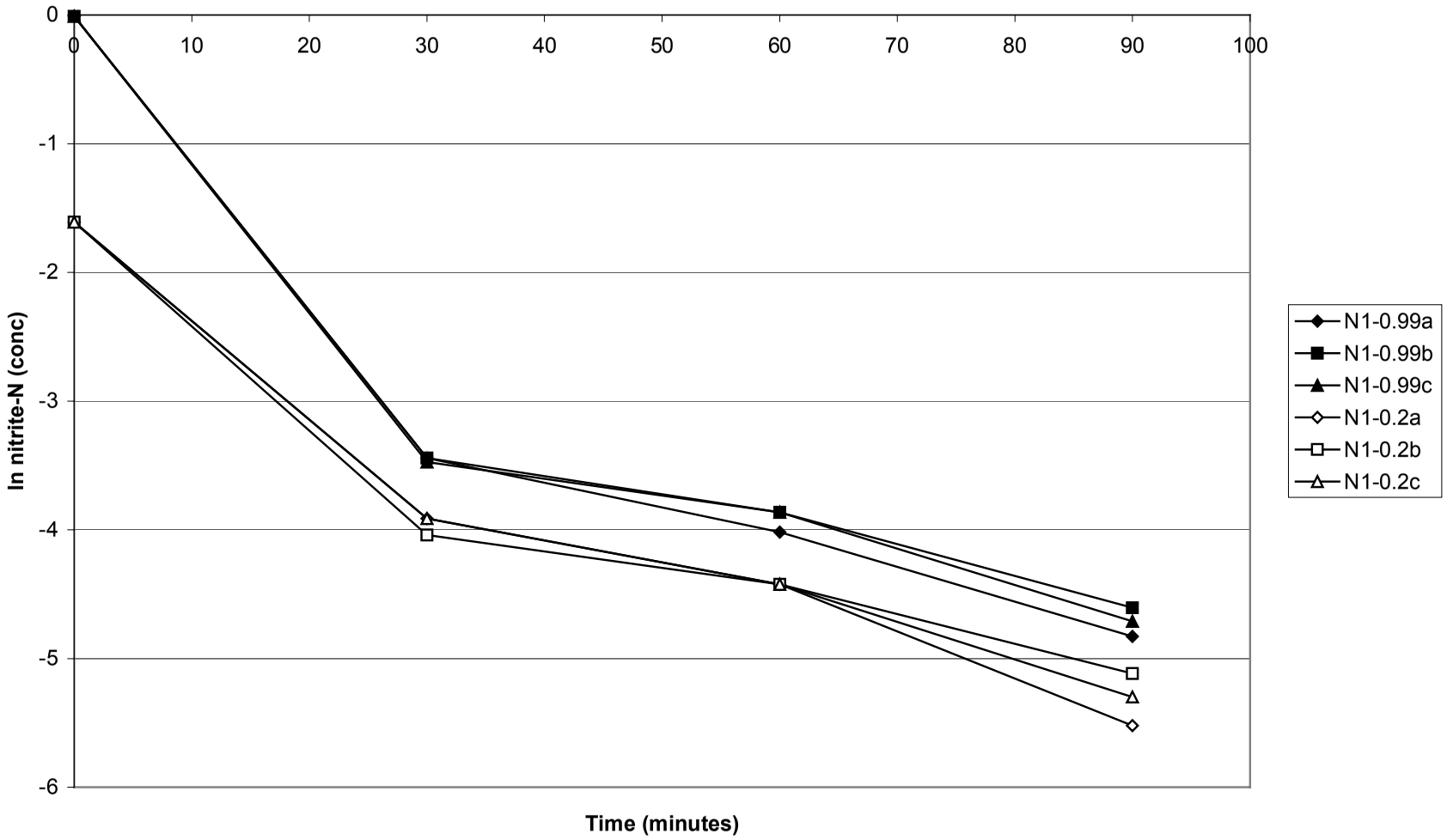

Figure 3-24
ln N vs. Time Experiments N1-0.2 and N1-0.99

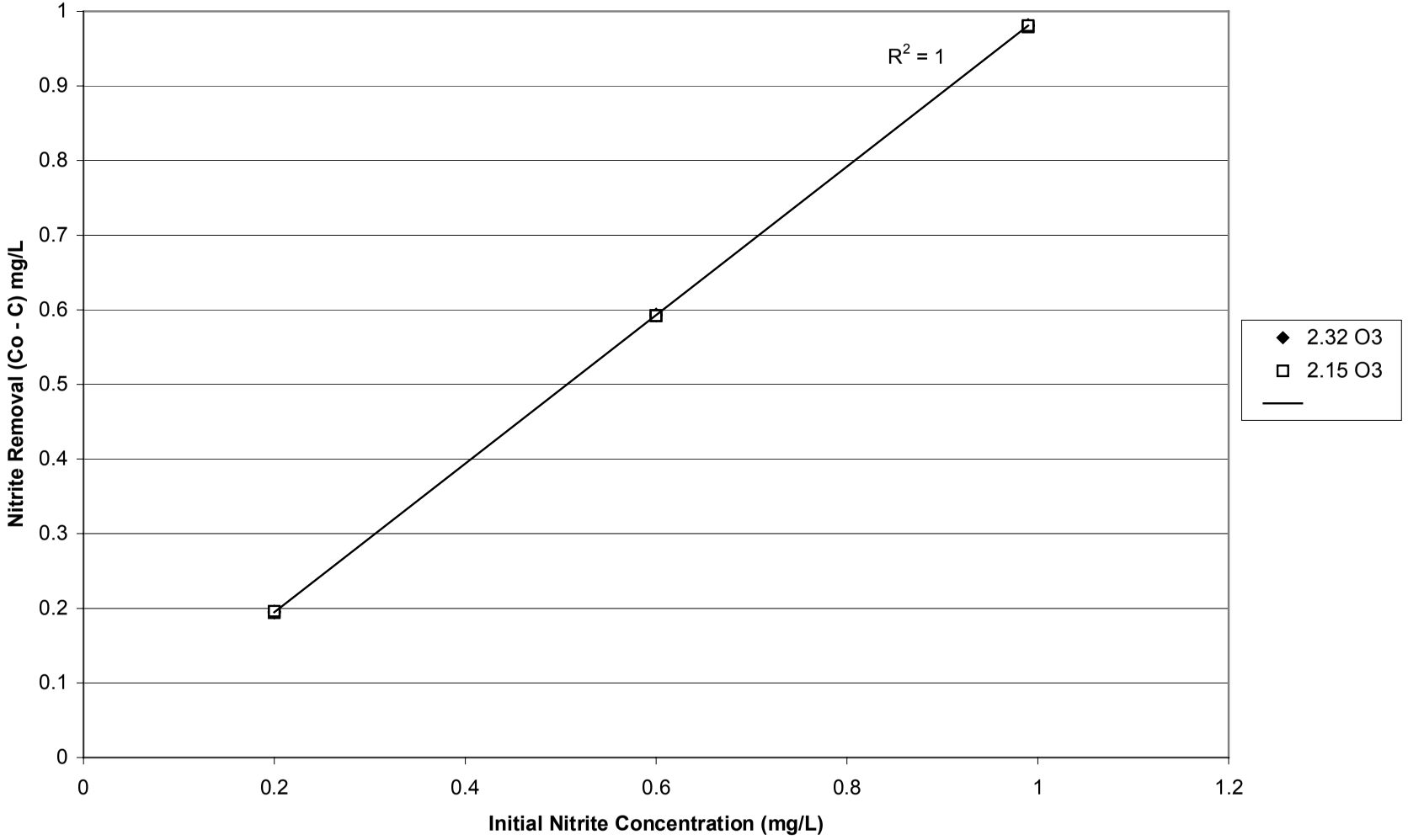

Figure 3-25
Nitrite Removal vs. Initial Concentration Nitrite Batch Studies

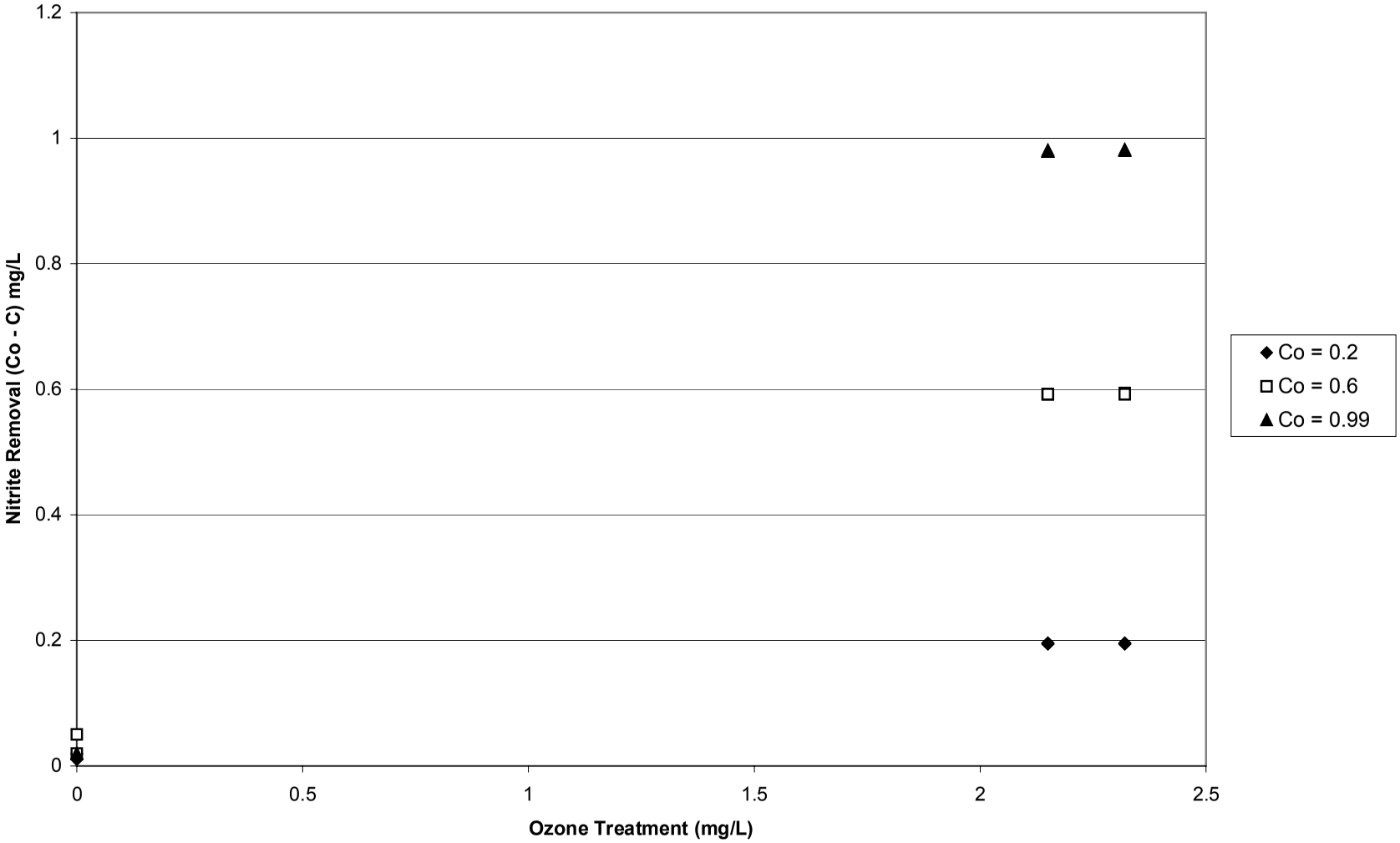

Figure 3-26
Nitrite Removal vs. Ozone Treatment Nitrite Batch Studies

In summary, results from the NO_2-N batch experiments were consistent with the literature in that

a. ozone applications at concentrations greater than 2.17 mg/L effectively removed NO_2-N (up to 99%) from water in concentrations commonly found in aquaculture,

b. increased NO_2-N removal (in terms of concentration removed) occurred at higher initial NO_2-N concentrations, and

c. NO_2-N removal using ozone was rapid with most NO2-N removal taking place within the first 30 minutes of exposure to ozone.

3.1.3 Mixed Batch Experiments

Results of Mixed Batch Samples Treated with 2.12 mg/L Ozone solution:

Three independent NO_2-N, color, TSS and VSS experiments were run with ozone treatment solution of 2.12 mg/L under mixed batch culture conditions. The three dilutions of the 2.12 mg/L ozone solution used in the first mixed batch experiments resulted in ozone application rates of 0.42 mg/L, 1.06 mg/L, and 1.70 mg/L ozone.

The resulting NO_2-N, color, TSS, and VSS concentrations resulting from the dilution of the 2.12 mg/L ozone solution batch experiments and presented in Table 3-1 and are identified by the following respective experiment titles: M 1a, M 1b, and M 1c.

Table 3-1
Initial Concentrations of Test Parameters in Mixed Batch Study Test 1

Sample No.	Ozone (mg/L)	color (Pt-Co color units)	NO_2-N (mg/L)	TSS (mg/L)	VSS (mg/L)
M-1a	0.42	648	0.78	86	76
M-1b	1.06	405	0.49	54	48
M-1c	1.70	162	0.20	22	19

Experiment M-1a (application of 0.42 mg/L ozone)

NO_2-N data for experiment M-1a are presented in Figure 3-27. Analysis of this data indicates that NO_2-N was initially reduced by the application of 0.42 mg/L ozone during the first 30 minutes of testing compared to the control sample in which no ozone was added. This average initial reduction of NO_2-N was 0.25 mg/L for the triplicate samples after 30 minutes of exposure to ozone, representing a reduction of 32% compared to 0.3% for the control sample. After the first 30 minutes of exposure to ozone however, NO_2-N concentrations increased in all three M-1a test samples at both 60 minutes of treatment and at 90 minutes of treatment. The average amount of NO_2-N removal that occurred at the end of 90 minutes of exposure period was 0.32 mg/L, representing an average overall reduction of 13% (compared to 0.4% for the control sample).

The color data for experiment M-1a are presented in Figure 3-28. Reduction of color in the ozone treated samples of this experiment averaged 66 color units for all three samples after 24 hours of exposure to ozone, representing an overall color reduction of 10%. In contrast, the control sample saw negligible reduction of color (one color unit or 0.2%).

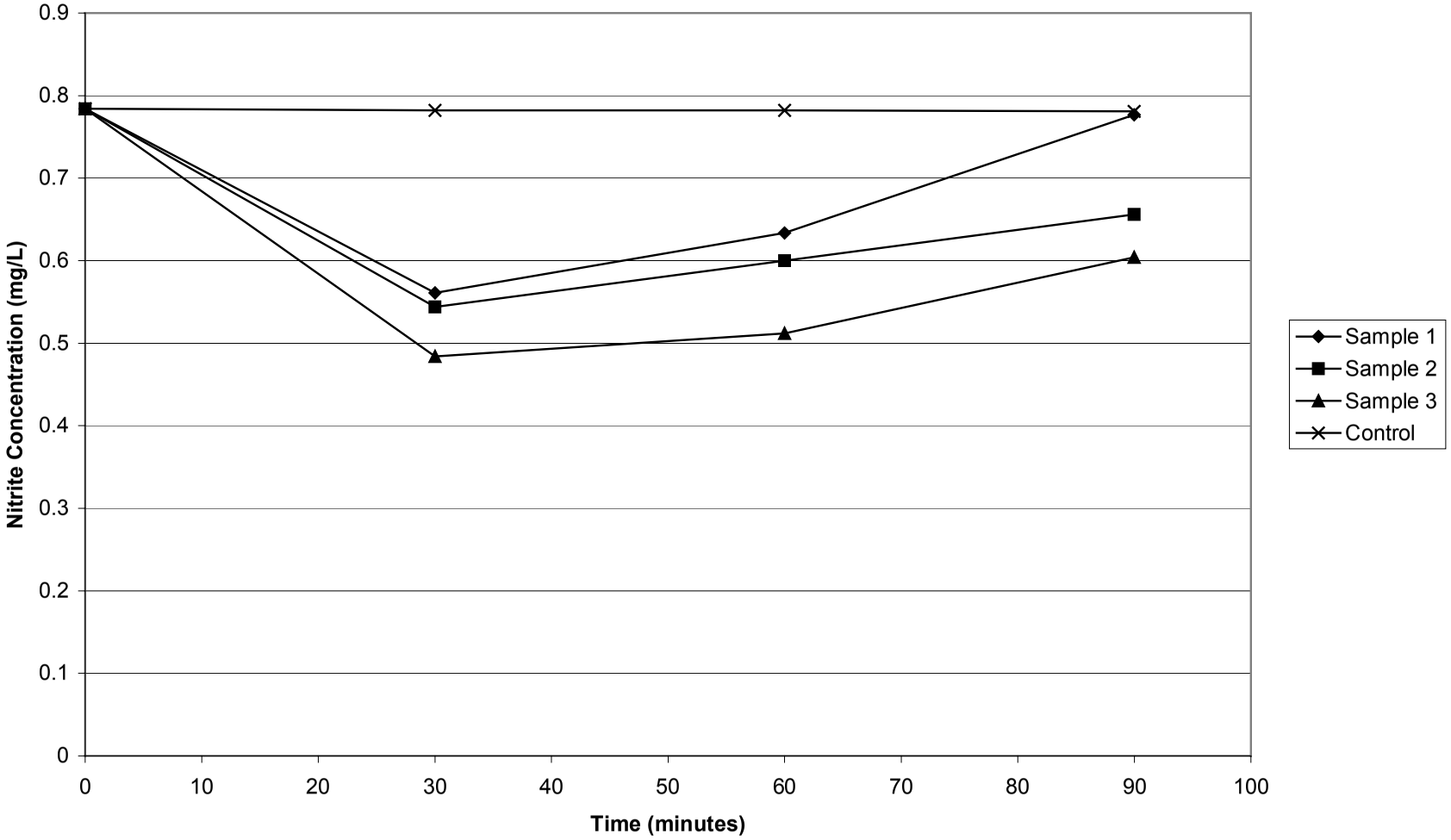

Figure 3-27
Nitrite vs. Time - Mixed Batch Study Co = 0.78 mg/L NO2 + 0.42 mg/L Ozone

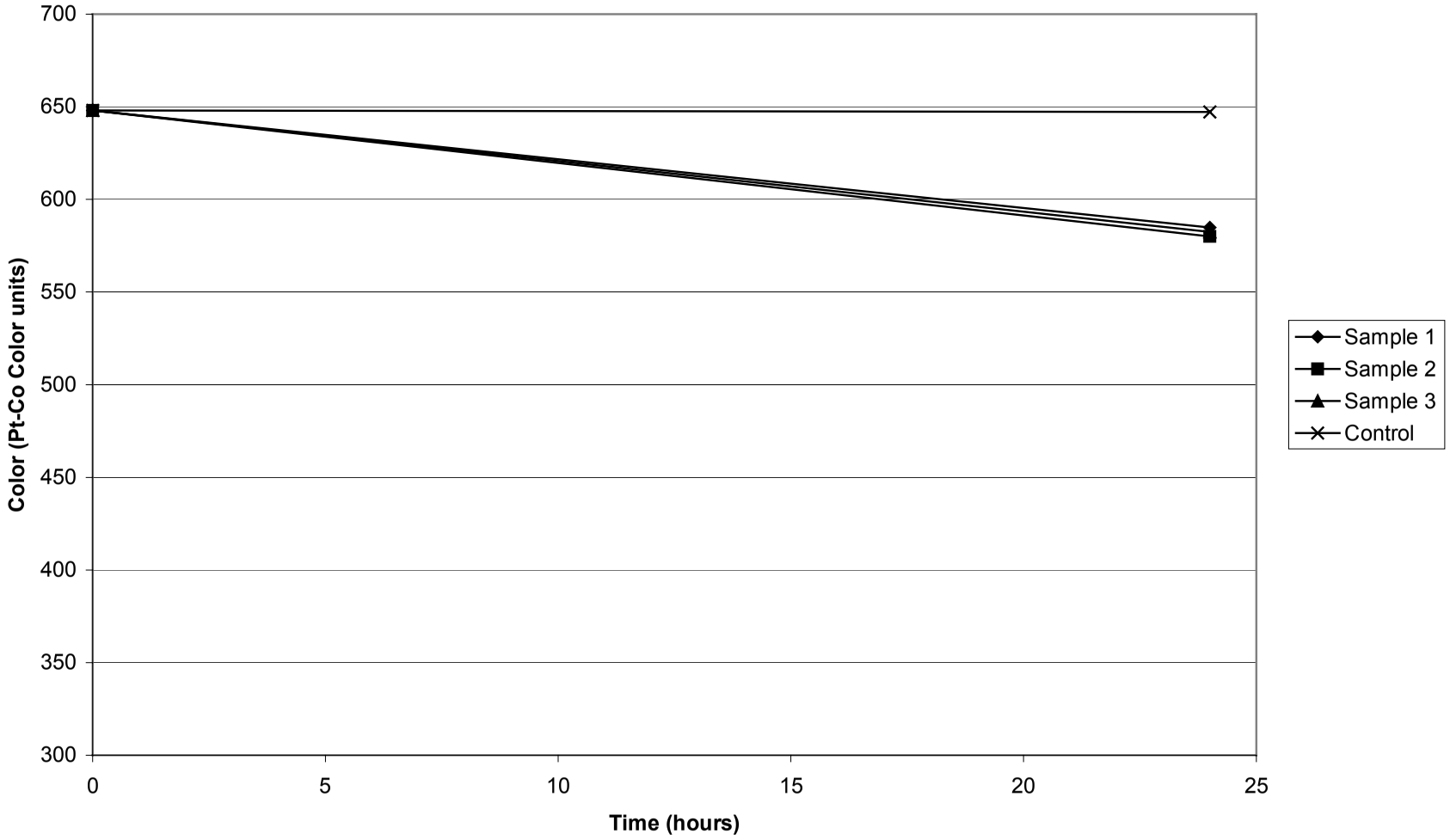

Figure 3-28
Color vs. Time - Mixed Batch Study Co = 648 color units + 0.42 mg/L Ozone

The TSS data for experiment M-1a are presented in Table 3-2 below. Analysis of this data indicates that ozone treatment did not reduce total suspended solids in the treated samples of this experiment compared to the control sample in which no ozone was added. Total reduction of TSS for both the treated and untreated control samples was negligible (less than 4%) for the 24 hours of treatment.

Table 3-2
TSS and VSS Concentrations in Mixed Batch Study M-1a

Sample	TSS (mg/L) T = 0 hours	TSS (mg/L) T = 24 hours	VSS (mg/L) T = 0 hours	VSS (mg/L) T = 24 hours
M-1a – 1	86	88	76	73
M-1a – 2	86	86	76	78
M-1a – 3	86	84	76	73
Control M1a	86	87	76	81

The VSS data for Experiment M-1a are also presented in Table 3-2. Analysis of this data indicates that ozone treatment did not reduce volatile suspended solids concentrations in the test samples (change of less than 4% during the 24 hour treatment period) and that total reduction for all samples was negligible.

Experiment M-1b (application of 1.06 mg/L ozone)

NO_2-N Data for experiment M-1b are presented in Figure 3-29. Analysis of this data indicates that NO_2-N was initially reduced by the application of 1.06 mg/L ozone during the first 30 minutes of testing compared to the control sample in which no ozone was added. This average initial reduction of NO_2-N was 0.42 mg/L for the triplicate samples after 30 minutes of exposure to ozone, representing a reduction of 83% compared to 0.6% for the control sample. After the first 30 minutes of exposure to ozone however, NO_2-N concentrations increased slightly in all three M-1b test samples at both 60 minutes of treatment and at 90 minutes of treatment. The average amount of NO_2-N removal that occurred at the end of 90 minutes of exposure period was 0.36 mg/L, representing an average overall reduction of 73% (compared to 0.6% for the control sample).

The color data for experiment M-1b are presented in Figure 3-30. Reduction of color in the ozone treated samples of this experiment averaged 64 color units for all three samples after 24 hours of exposure to ozone, representing an overall color reduction of 16%. In contrast, the control sample saw negligible reduction of color (3 color units or 0.6%).

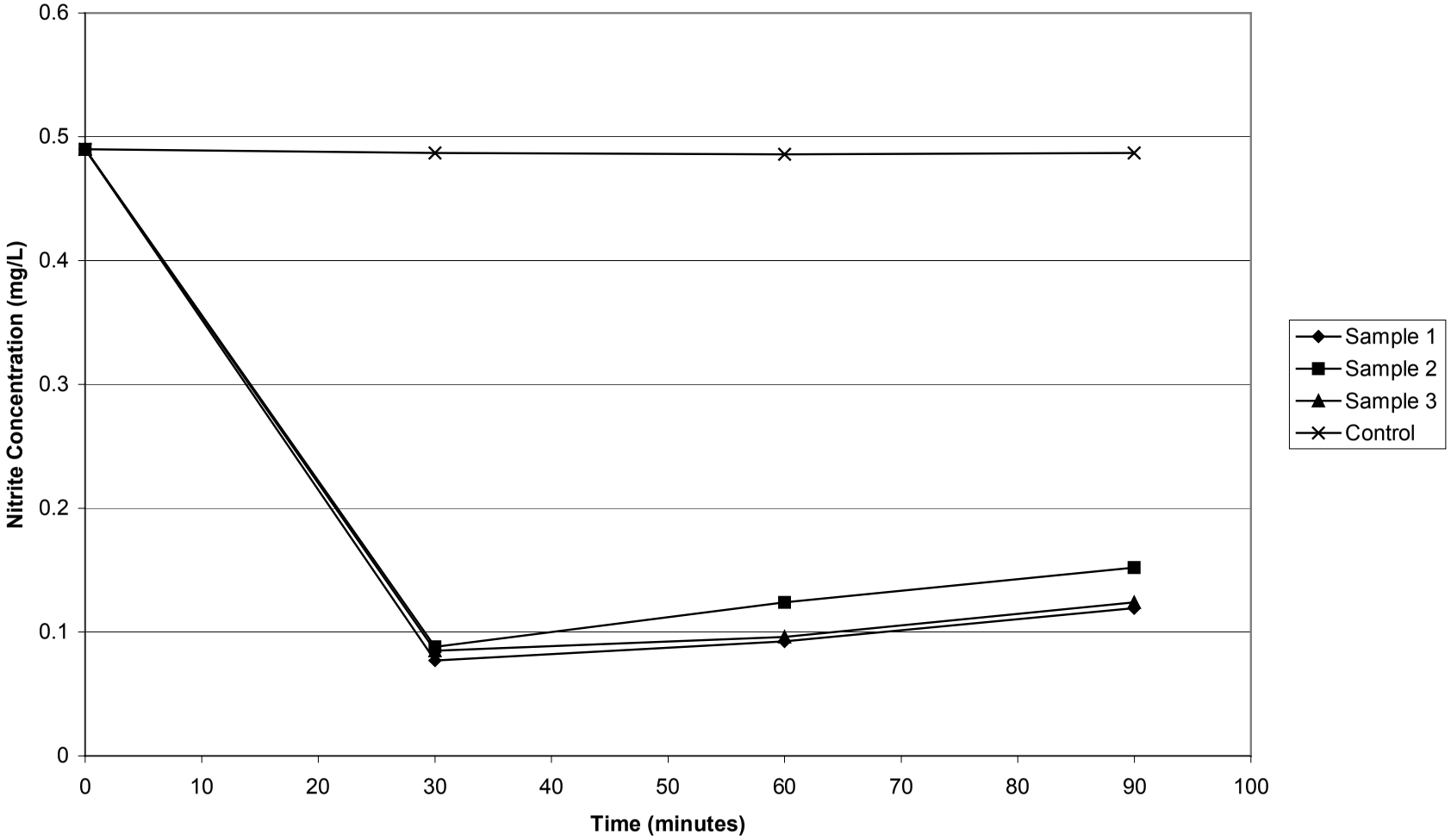

Figure 3-29
Nitrite vs. Time - Mixed Batch Study Co = 0.49mg/L NO2 + 1.06 mg/L Ozone

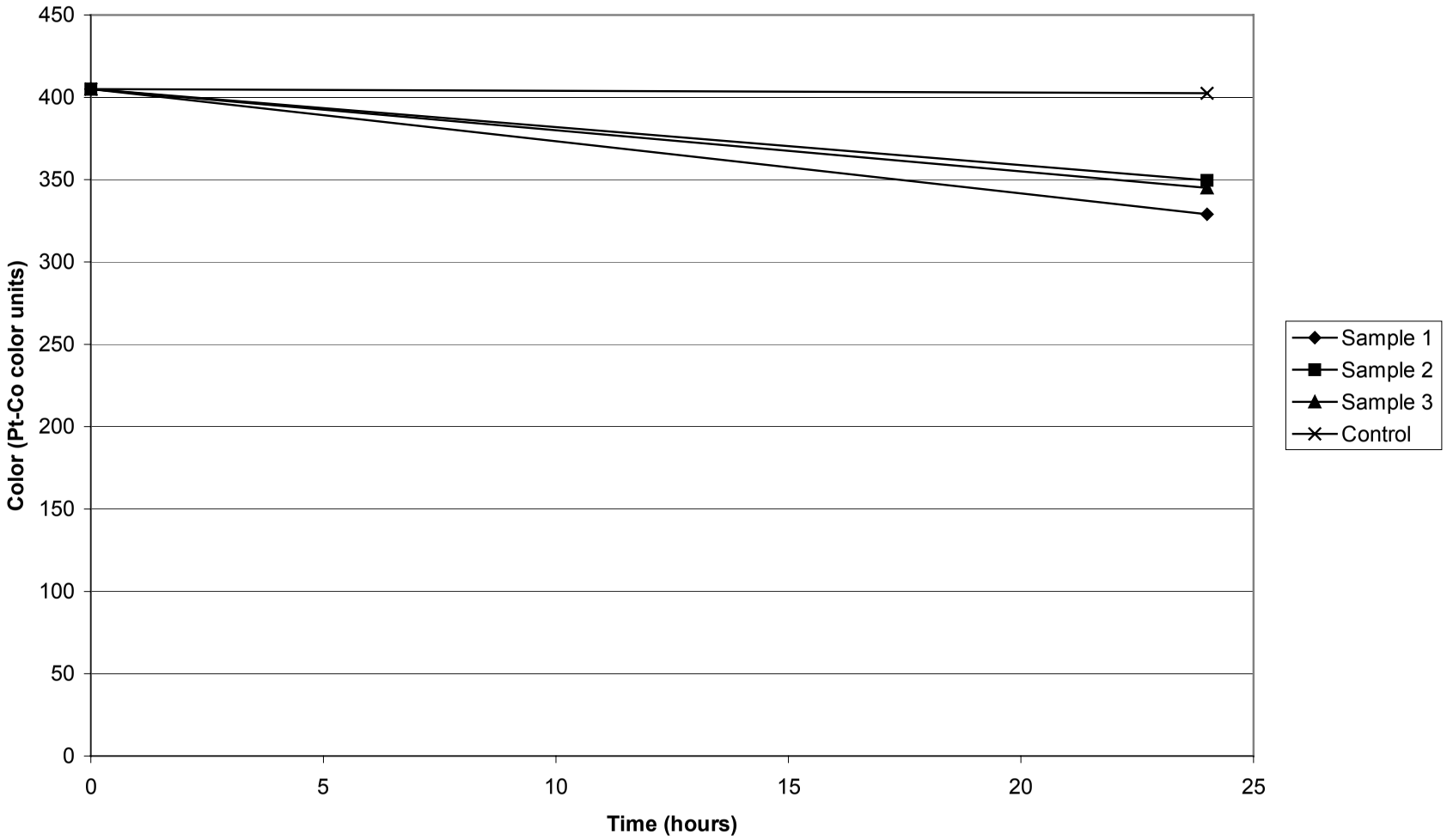

Figure 3-30
Color vs. Time - Mixed Batch Study Co = 405 color units + 1.06 mg/L Ozone

The TSS data for experiment M-1b are presented in Table 3-3 below. Analysis of this data indicates that ozone treatment did not reduce total suspended solids in the treated samples of this experiment compared to the control sample in which no ozone was added. Total reduction of TSS for both the treated and untreated control samples was negligible (less than 4%) for the 24 hours of treatment.

Table 3-3
TSS and VSS Concentrations in Mixed Batch Study M-1b

Sample	TSS (mg/L) T = 0 hours	TSS (mg/L) T = 24 hours	VSS (mg/L) T = 0 hours	VSS (mg/L) T = 24 hours
M-1b – 1	54	54	48	48
M-1b – 2	54	54	48	49
M-1b – 3	54	52	48	47
Control M1b	54	52	48	47

The VSS data for Experiment M-1b are also presented in Table 3-3. Analysis of this data indicates that ozone treatment did not reduce volatile suspended solids concentrations in the test samples (change of less than 4% during the 24 hour treatment period) and that total reduction for all samples was negligible.

Experiment M-1c (application of 1.7 mg/L ozone)

NO_2-N Data for experiment M-1c are presented in Figure 3-31. Analysis of this data indicates that NO_2-N was rapidly and significantly reduced by the application of 1.70 mg/L ozone compared to the control sample in which no ozone was added. The average initial reduction of NO_2-N was 0.187 mg/L for the triplicate samples after 30 minutes of exposure to ozone, representing a reduction of 96% compared to 0% for the control sample. After the first 30 minutes of exposure to ozone, NO_2-N concentrations remained stable in the M-1b test samples at T=60 min or T=90 min, and no NO_2-N concentration increases occurred. The average amount of NO_2-N removal that occurred at the end of 90 minutes of exposure period remained stable at 0.186 mg/L, representing an average overall reduction of 95% (compared to 0% for the control sample).

The color data for experiment M-1c are presented in Figure 3-32. Reduction of color in the ozone treated samples of this experiment averaged 69 color units for all three samples after 24 hours of exposure to ozone (original color was 162 color units), representing an overall color reduction of 43%. In contrast, the control sample saw negligible reduction of color (2 color units or 1%) during the 24 hour test period.

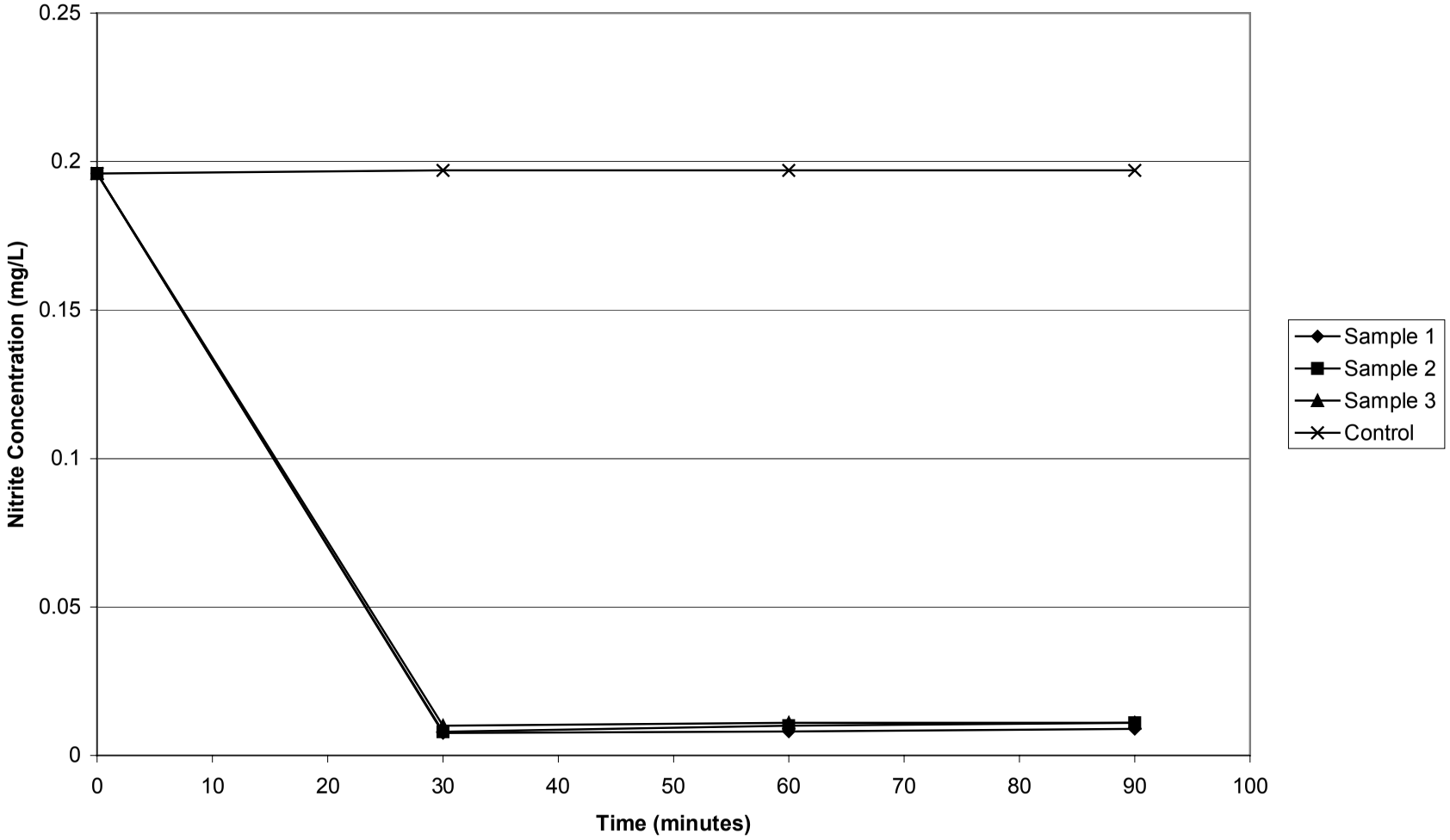

Figure 3-31
Nitrite vs. Time - Mixed Batch Study Co = 0.2 mg/L NO2 + 1.7 mg/L Ozone

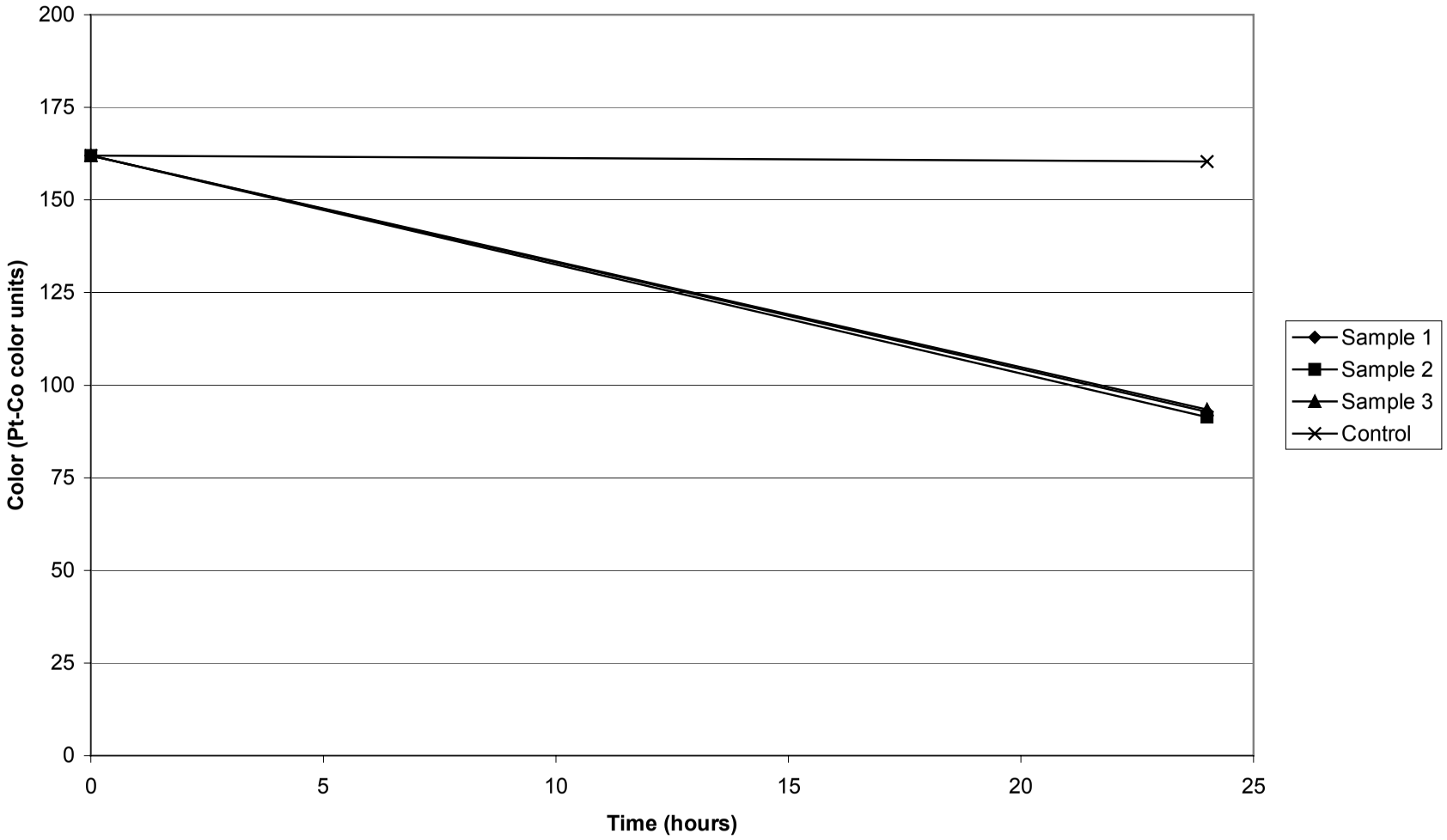

Figure 3-32
Color vs. Time - Mixed Batch Study Co = 162 color units + 1.7 mg/L Ozone

The TSS data for experiment M-1c are presented in Table 3-4 below. Analysis of this data indicates that ozone treatment did not reduce total suspended solids in the treated samples of this experiment compared to the control sample in which no ozone was added. Total reduction of TSS for both the treated and untreated control samples was negligible for the 24 hours of treatment.

Table 3-4
TSS and VSS Concentrations in Mixed Batch Study M-1c

Sample	TSS (mg/L) T = 0 hours	TSS (mg/L) T = 24 hours	VSS (mg/L) T = 0 hours	VSS (mg/L) T = 24 hours
M-1c – 1	21	21	19	19
M-1c – 2	21	22	19	19
M-1c – 3	21	21	19	19
Control M1c	21	21	19	19

The VSS data for Experiment M-1c are also presented in Table 3-4. Analysis of this data indicates that ozone treatment did not reduce volatile suspended solids concentrations in the test samples and that total VSS reduction for all samples was negligible.

Results of Experiments Treated with 2.23 mg/L Ozone Solution:

Three additional mixed batch experiments were run with a slightly higher ozone treatment concentration of 2.23 mg/L (compared to 2.12 mg/L solution) to determine how additional ozone would affect the removal of color, NO_2-N, TSS, and VSS in batch experiments with actual fish production water. The three dilutions of the 2.23 mg/L ozone solution used in the first mixed batch experiments resulted in ozone application rates of 0.45 mg/L, 1.12 mg/L, and 1.78 mg/L ozone.

The resulting NO_2-N, color, TSS, and VSS concentrations resulting from the dilution of the 2.13 mg/L ozone solution batch experiments are presented in Table 3-5 and are identified by the following respective experiment titles: M-2a, M-2b, and M-2c.

Table 3-5
Initial Concentrations of Test Parameters in Mixed Batch Study Test 1

Sample No.	Ozone (mg/L)	color (Pt-Co color units)	NO_2-N (mg/L)	TSS (mg/L)	VSS (mg/L)
M-2a	0.45	648	0.78	86	76
M-2b	1.12	405	0.49	54	48
M-2c	1.78	162	0.20	22	19

Experiment M-2a (application of 0.45 mg/L ozone)

NO_2-N Data for experiment M-2a are presented in Figure 3-33. Analysis of this data indicates that NO_2-N was initially reduced by the application of 0.45 mg/L ozone during the first 30 minutes of testing compared to the control sample in which no ozone was added. This average initial reduction of NO_2-N was 0.17 mg/L for the triplicate samples after 30 minutes of exposure to ozone, representing a reduction of 22% compared to 0.1% for the control sample. After the first 30 minutes of exposure to ozone however, NO_2-N concentrations increased in all three M-2a test samples at both 60 minutes of treatment and at 90 minutes of treatment. The average amount of NO_2-N removal that occurred at the end of 90 minutes of exposure period was 0.05 mg/L, representing an average overall reduction of 6% (compared to 0.4% for the control sample).

The color data for experiment M-2a are presented in Figure 3-34. Reduction of color in the ozone treated samples of this experiment averaged 77 color units for all three samples after 24 hours of exposure to ozone, representing an overall color reduction of 11.9%. In contrast, the control sample saw negligible reduction of color (one color unit or 0.2%).

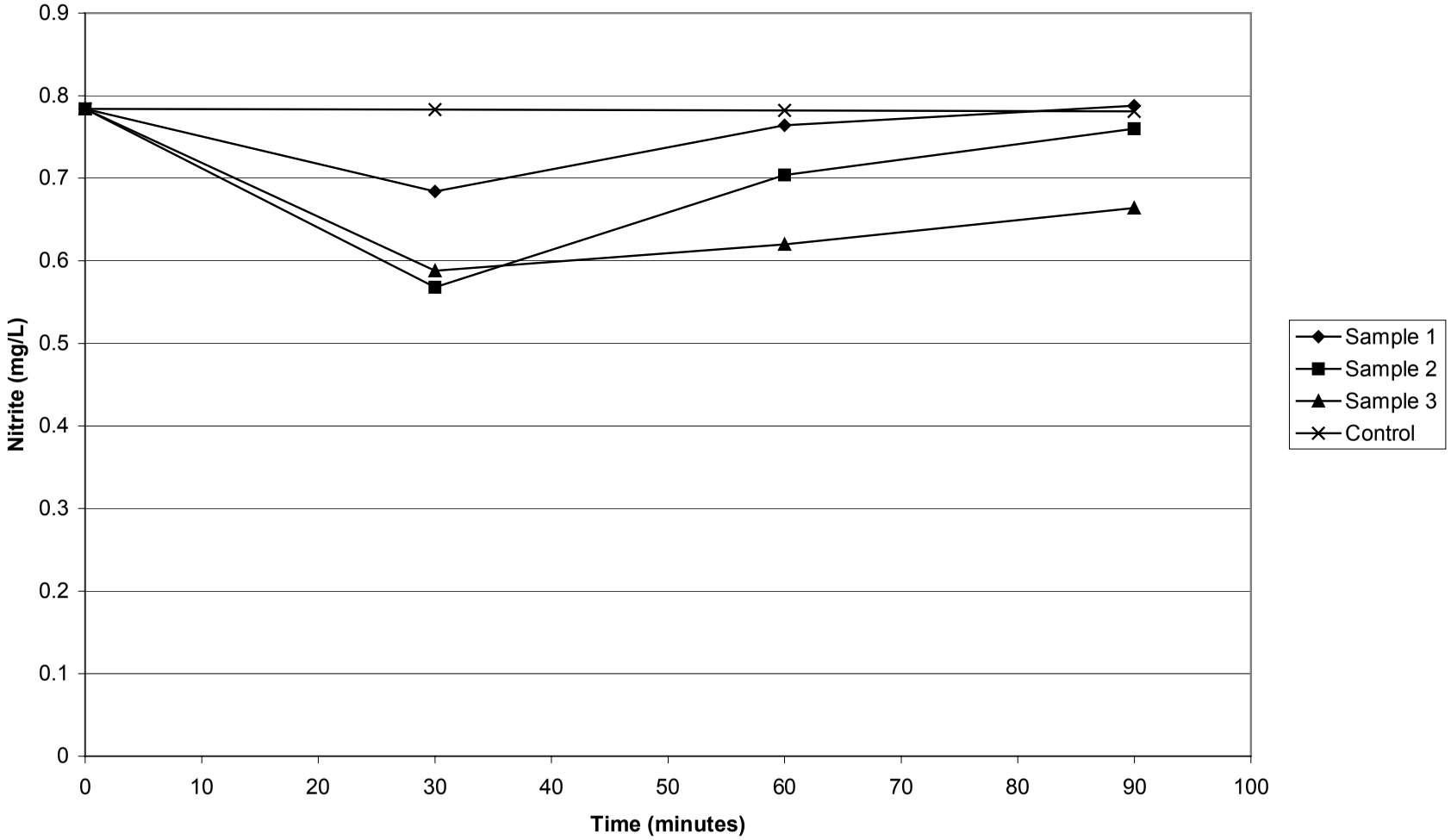

Figure 3-33
Nitrite vs. Time - Mixed Batch Study Co = 0.78 mg/L N + 0.45 mg/L Ozone

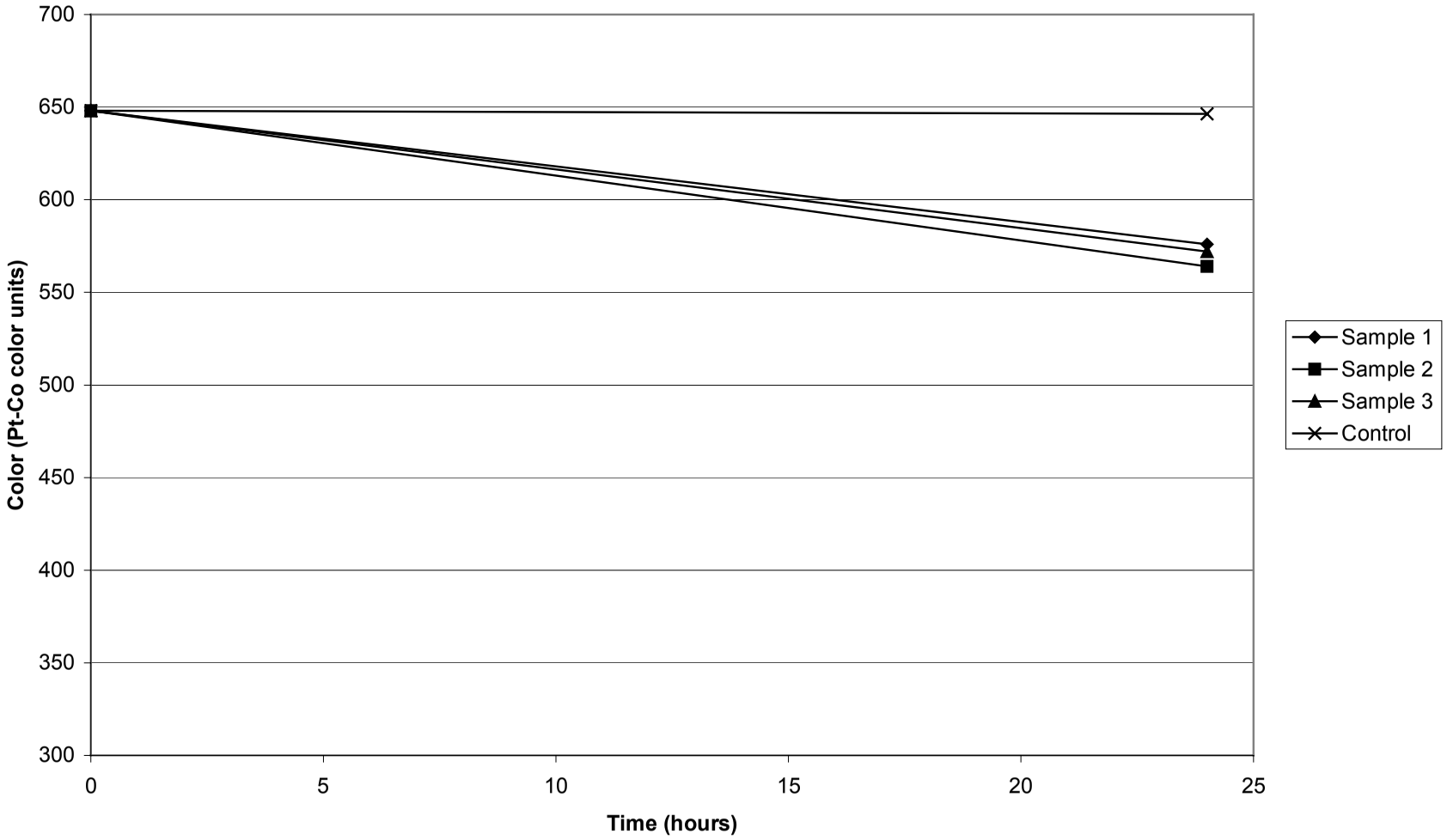

Figure 3-34
Color vs. Time - Mixed Batch Study Co = 648 color units + 0.45 mg/L Ozone

The TSS data for experiment M-2a are presented in Table 3-6 below. Analysis of this data indicates that ozone treatment did not reduce total suspended solids in the treated samples of this experiment compared to the control sample in which no ozone was added. Total reduction of TSS for both the treated and untreated control samples was negligible (average less than 3%) for the 24 hours of treatment.

Table 3-6
TSS and VSS Concentrations in Mixed Batch Study M-2a

Sample	TSS (mg/L) T = 0 hours	TSS (mg/L) T = 24 hours	VSS (mg/L) T = 0 hours	VSS (mg/L) T = 24 hours
M-2a – 1	86	85	76	78
M-2a – 2	86	85	76	77
M-2a – 3	86	81	76	72
Control M2a	86	86	76	79

The VSS data for Experiment M-2a are also presented in Table 3-6. Analysis of this data indicates that ozone treatment did not reduce volatile suspended solids concentrations in the test and that total reduction for all samples was negligible.

Experiment M-2b (application of 1.12 mg/L ozone)

NO_2-N Data for experiment M-2b are presented in Figure 3-35. Analysis of this data indicates that NO_2-N was initially reduced by the application of 1.12 mg/L ozone during the first 30 minutes of testing compared to the control sample in which no ozone was added. This average initial reduction of NO_2-N was 0.4 mg/L for the triplicate samples after 30 minutes of exposure to ozone, representing a reduction of 82% compared to 1.4% for the control sample. NO_2-N concentrations increased in the M-1b test samples at both 60 minutes of treatment and at 90 minutes of treatment. The average amount of NO_2-N removal that occurred at the end of 90 minutes of exposure period was 0.36 mg/L, representing an average overall reduction of 73% (compared to 1.6% for the control sample).

The color data for experiment M-2b are presented in Figure 3-36. Reduction of color in the ozone treated samples of this experiment averaged 72 color units for all three samples after 24 hours of exposure to ozone, representing an overall color reduction of 18%. In contrast, the control sample saw negligible reduction of color (1.5 color units or 1.2%).

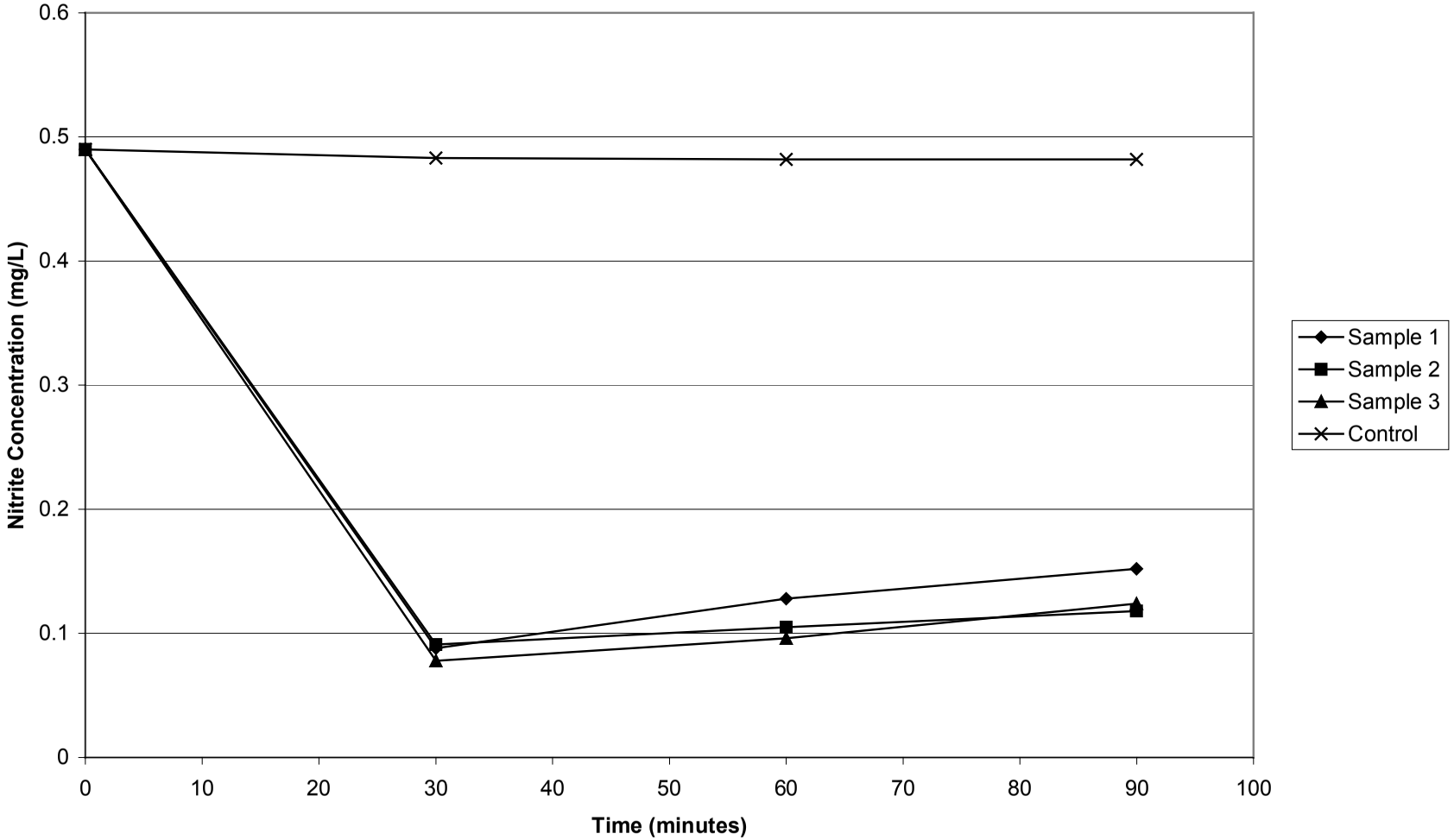

Figure 3-35
Nitrite vs. Time - Mixed Batch Study Co = 0.49 mg/L N + 1.12 mg/L Ozone

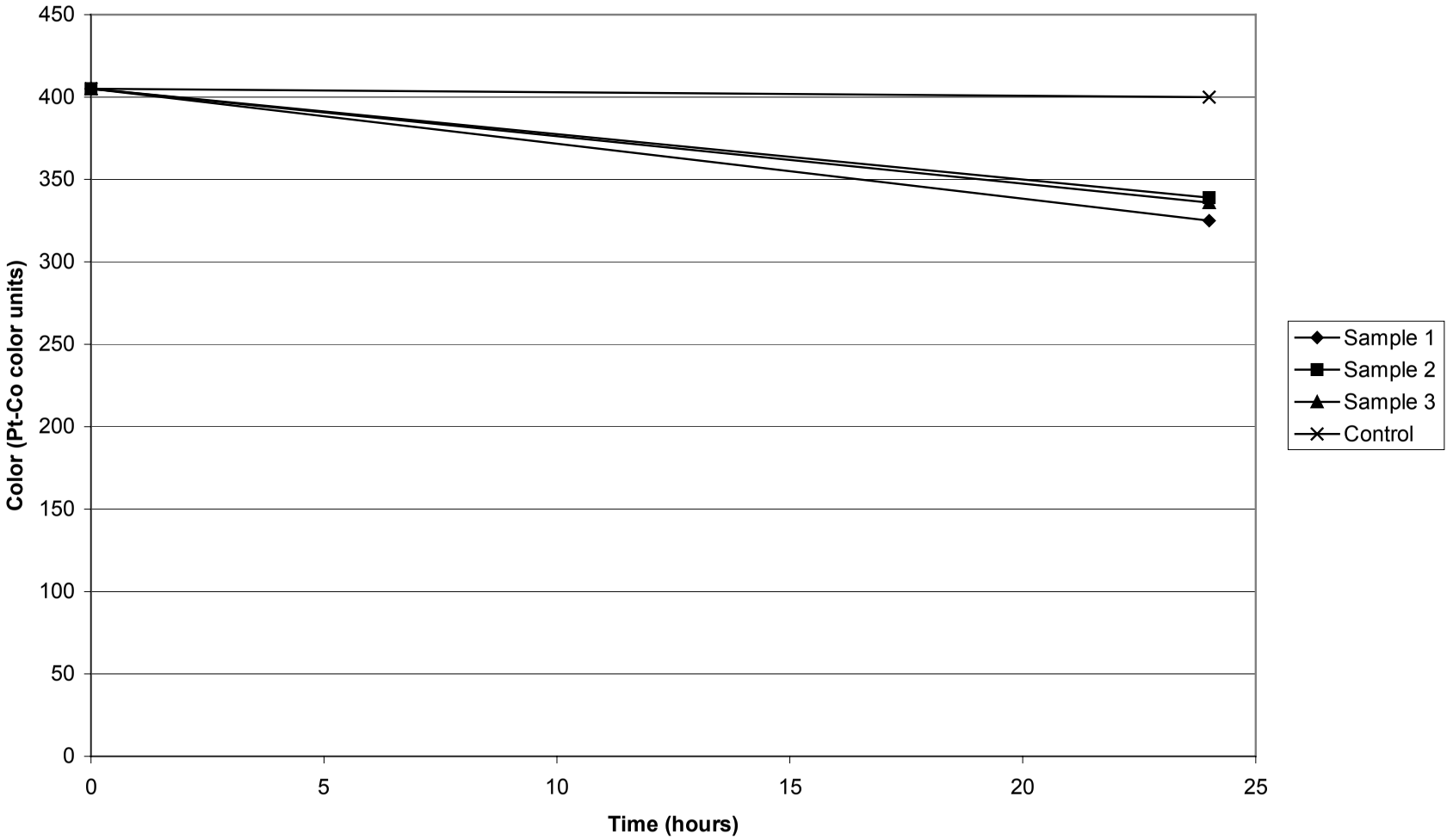

Figure 3-36
Color vs. Time - Mixed Batch Study Co = 405 color units + 1.12 mg/L Ozone

The TSS data for experiment M-2b are presented in Table 3-7 below. Analysis of this data indicates that ozone treatment did not reduce total suspended solids in the treated samples of this experiment compared to the control sample in which no ozone was added. Total reduction of TSS for both the treated and untreated control samples was negligible (less than 4%) for the 24 hours of treatment.

Table 3-7
TSS and VSS Concentrations in Mixed Batch Study M-2b

Sample	TSS (mg/L) T = 0 hours	TSS (mg/L) T = 24 hours	VSS (mg/L) T = 0 hours	VSS (mg/L) T = 24 hours
M-2b – 1	54	56	48	50
M-2b – 2	54	53	48	48
M-2b – 3	54	52	48	46
Control M2b	54	52	48	46

The VSS data for Experiment M-2b are also presented in Table 3-7. Analysis of this data indicates that ozone treatment did not reduce volatile suspended solids concentrations in the test samples and that total reduction for all samples was negligible.

Experiment M-2c (application of 1.78 mg/L ozone)

NO_2-N Data for experiment M-2c are presented in Figure 3-37. Analysis of this data indicates that NO_2-N was rapidly and significantly reduced by the application of 1.78 mg/L ozone compared to the control sample in which no ozone was added. The average initial reduction of NO_2-N was 0.187 mg/L for the triplicate samples after 30 minutes of exposure to ozone, representing a reduction of 96% compared to 1.5% for the control sample. After the first 30 minutes of exposure to ozone, NO2-N concentrations remained stable in the M-1b test samples at T=60 min or T=90 min, and no NO_2-N concentration increases occurred. The average amount of NO_2-N removal that occurred at the end of 90 minutes of exposure period remained stable at 0.186 mg/L, representing an average overall reduction of 95% (compared to 2% for the control sample).

The color data for experiment M-2c are presented in Figure 3-38. Reduction of color in the ozone treated samples of this experiment averaged 74 color units for all three samples after 24 hours of exposure to ozone (original color was 162 color units), representing an overall color reduction of 46%. In contrast, the control sample saw negligible reduction of color (1.4 color units or 0.9%).

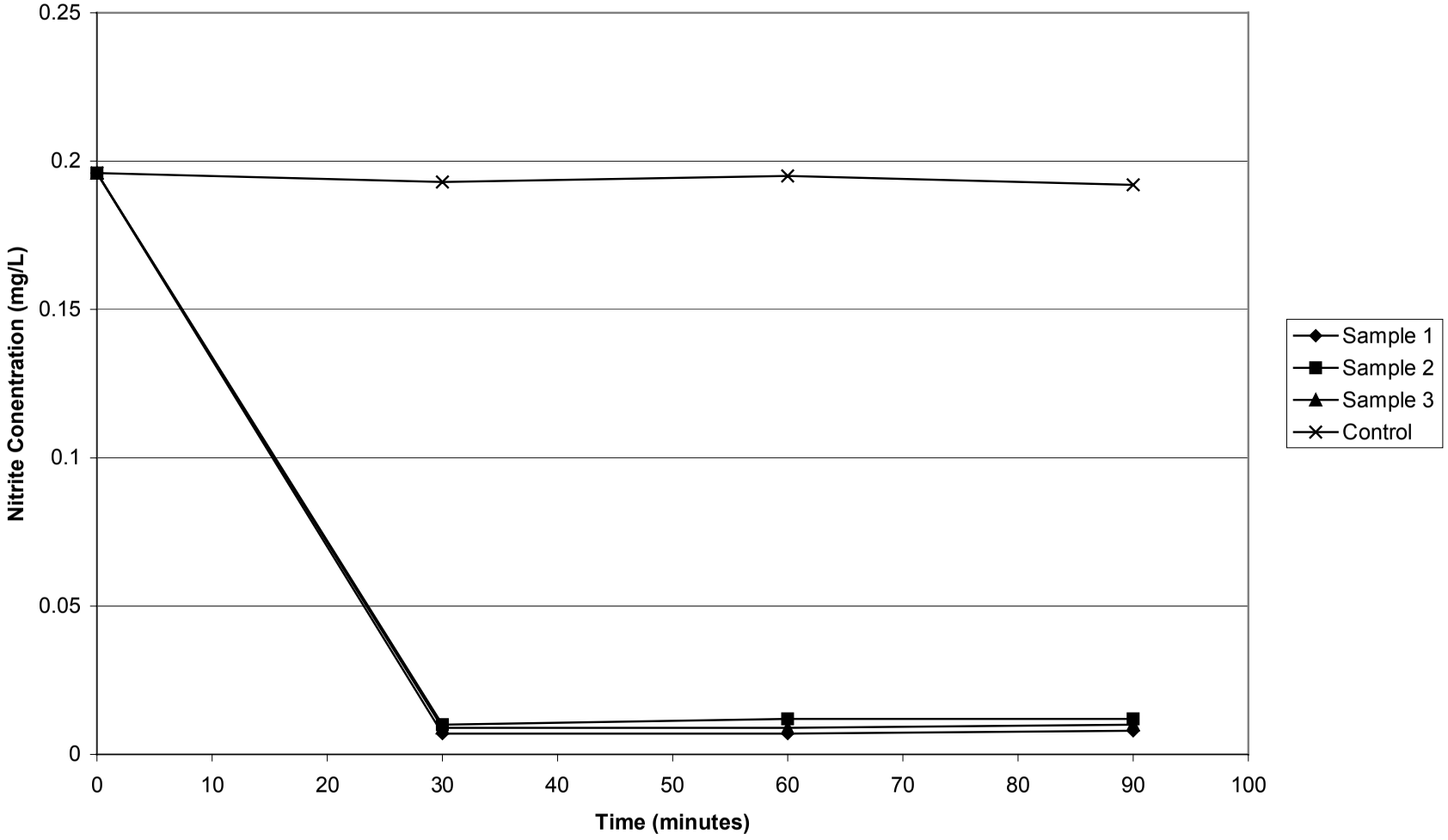

Figure 3-37
Nitrite vs. Time - Mixed Batch Study Co = 0.2 mg/L N + 1.78 mg/L Ozone

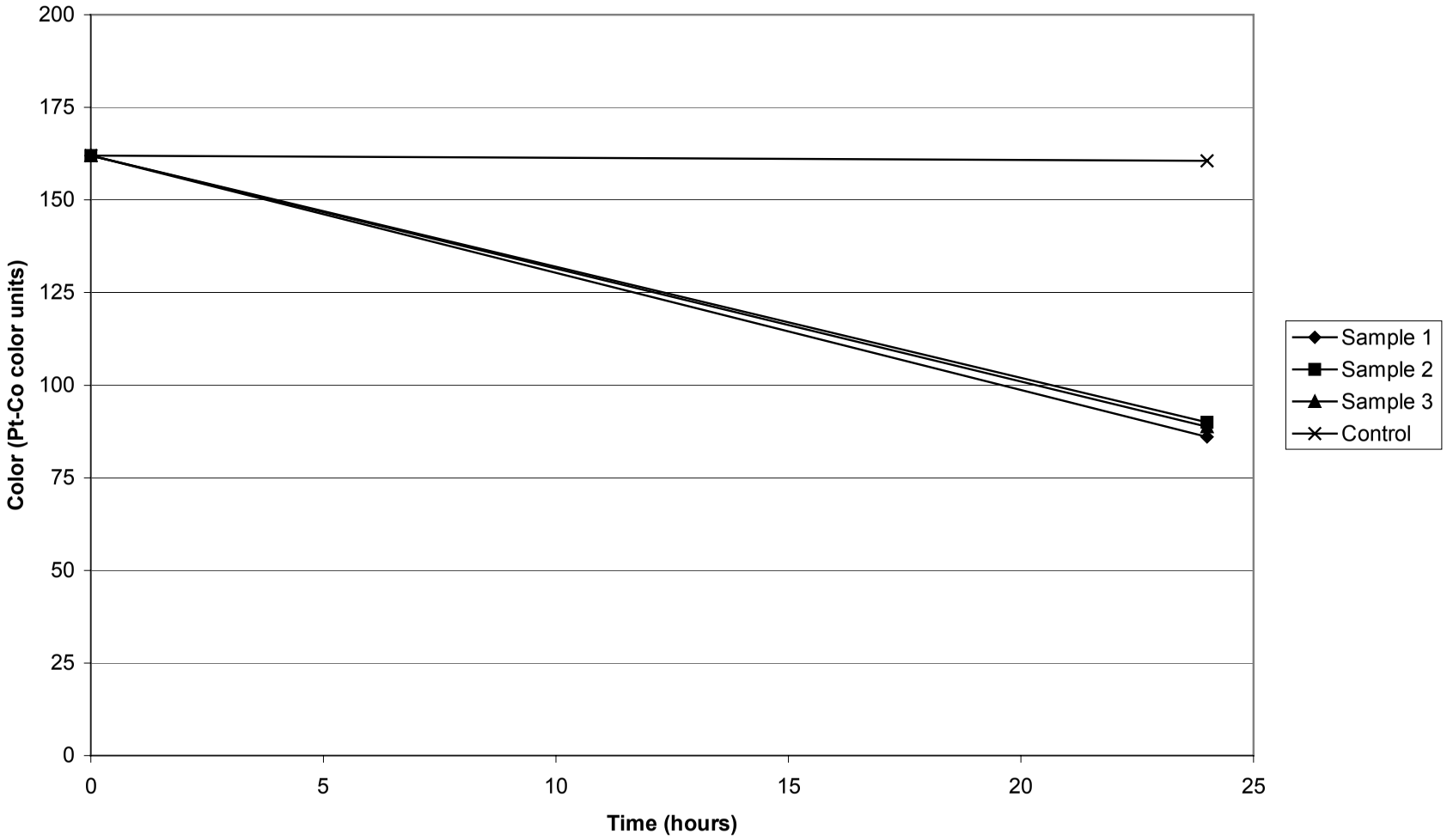

Figure 3-38
Color vs. Time - Mixed Batch Study Co = 162 color units + 1.78 mg/L Ozone

The TSS data for experiment M-2c are presented in Table 3-8 below. Analysis of this data indicates that ozone treatment did not reduce total suspended solids in the treated samples of this experiment compared to the control sample in which no ozone was added. Total reduction of TSS for both the treated and untreated control samples was negligible for the 24 hours of treatment.

Table 3-8
TSS and VSS Concentrations in Mixed Batch Study M-2c

Sample	TSS (mg/L) T = 0 hours	TSS (mg/L) T = 24 hours	VSS (mg/L) T = 0 hours	VSS (mg/L) T = 24 hours
M-2c – 1	21	21	19	18
M-2c – 2	21	22	19	19
M-2c – 3	21	22	19	19
Control M2c	21	22	19	19

The VSS data for Experiment M-1c are also presented in Table 3-3. Analysis of this data indicates that ozone treatment did not reduce volatile suspended solids concentrations in the test samples and that total VSS reduction for all samples was negligible.

Discussion of Mixed Culture Batch

Results of the mixed batch culture water experiments for NO_2-N removal indicated that the application of ozone is effective in reducing NO_2-N in fish culture water under laboratory conditions. The total effectiveness of NO_2-N removal in these mixed culture water batch studies varied from 3% to 96%, depending upon the initial NO_2-N concentrations present in the water as well as the amount of ozone added to the sample.

The first pattern of interest regarding NO_2-N reduction occurred in the two experiments (M-1a and M-2a) where there was low initial ozone test concentrations and high NO_2-N test concentrations. In these experiments, significant NO_2-N removal occurred during the first 30 minutes of treatment (average of 22% and 32% removal), however the NO_2-N concentrations in the samples then steadily increased through the next two time points (60 minutes and 90 minutes), reducing the overall NO_2-N removal in these experiments to between 6% and 13% by 90 minutes. It is not known why the NO_2-N levels in these first two experiments dropped then gradually increased again, however it can be estimated that the increase in NO_2-N concentrations occurred as a result of further biological activity within the experimental samples.

The biological activity explanation for the increase in NO_2-N concentrations during Experiments M-1a and M-2a is supported by the NO_2-N concentration patterns in the experiments with more ozone. The middle-strength samples, where more ozone was applied, exhibited a similar, yet less significant recovery of NO_2-N concentrations during the second and third periods of analysis (60 and 90-minute readings). In contrast, the highest strength ozone treated samples (experiments M-1c and M-2c) showed no recovery in NO_2-N concentrations during the course of the experiment, indicating that perhaps as more ozone was applied, more complete inactivity of biological activity occurred in the samples.

The amount of color removed (initial concentration – final concentration) and the completeness of color removal (total percent removed) were plotted against the level of ozone treatment (mg/L) for each batch culture experiment to determine if a relationship existed between color removal and the amount of ozone added at time = 30 minutes,. Data from these relationships are presented in Figures 3-39 and 3-40. Analysis of this data is somewhat difficult due to the fact that in these batch studies both the amount of ozone added and the initial nitrite concentrations changed for each sample. With this limitation in mind, two very general observations can be made. One is that the total amount of color removed for all batch study experiments ranged from 55 to 85 color units, and was independent of initial color concentrations. It appears that slightly higher color removal took place when higher ozone treatments were used for a given initial color concentration, however the two concentration data points for each initial color concentration were not enough for a statistical analysis. This can be seen in the slight upwards shift of the right-most set of data (3 of 6 data points) for each initial color concentration in Figure 3-40.

Similarly, (the second observation) although it appears that percent color removal increases with increasing ozone treatment (see trend of Figure 3-40), this claim can not really be statistically supported as only two sets of data points exist for each initial color concentration, {i.e. one can really only consider the right 3 points for each set of 6 points representing each initial color concentration}. One should note that although Figure 3-40 indicates that increased percentage removal takes place at lower

In regards to reaction time, all the NO_2-N removal that occurred in the mixed culture water batch experiments took place within the first 30 minutes of exposure to ozone. This outcome was slightly different from the NO_2-N batch studies where some additional NO_2-N removal took place after the first 30 minutes of ozone treatment. This data would seem to support the notion that ozone treatment should be designed for shorter reaction times in actual culture water situations and not for long-term ozone residual based reactions.

Color Data for the mixed culture water batch studies indicated that ozone was effective in reducing color in mixed culture water. Because of the fact that suspended solids must be removed prior to color analysis, and suspended solids were not removed until after 24 hours of exposure to ozone in these experiments, rate of color removal could not be determined in the mixed culture water batch experiments. Total color increased over time during the color-only experiments however it is not clear as to whether the same pattern would have occurred under mixed culture conditions.

According to the TSS and VSS data in the mixed culture water batch studies, it appeared that ozone had no effect on reducing either of these parameters. This is consistent with the literature in that ozone is not known to reduce solids, but acts more as a bactericide or reactant with color or dissolved chemical compounds.

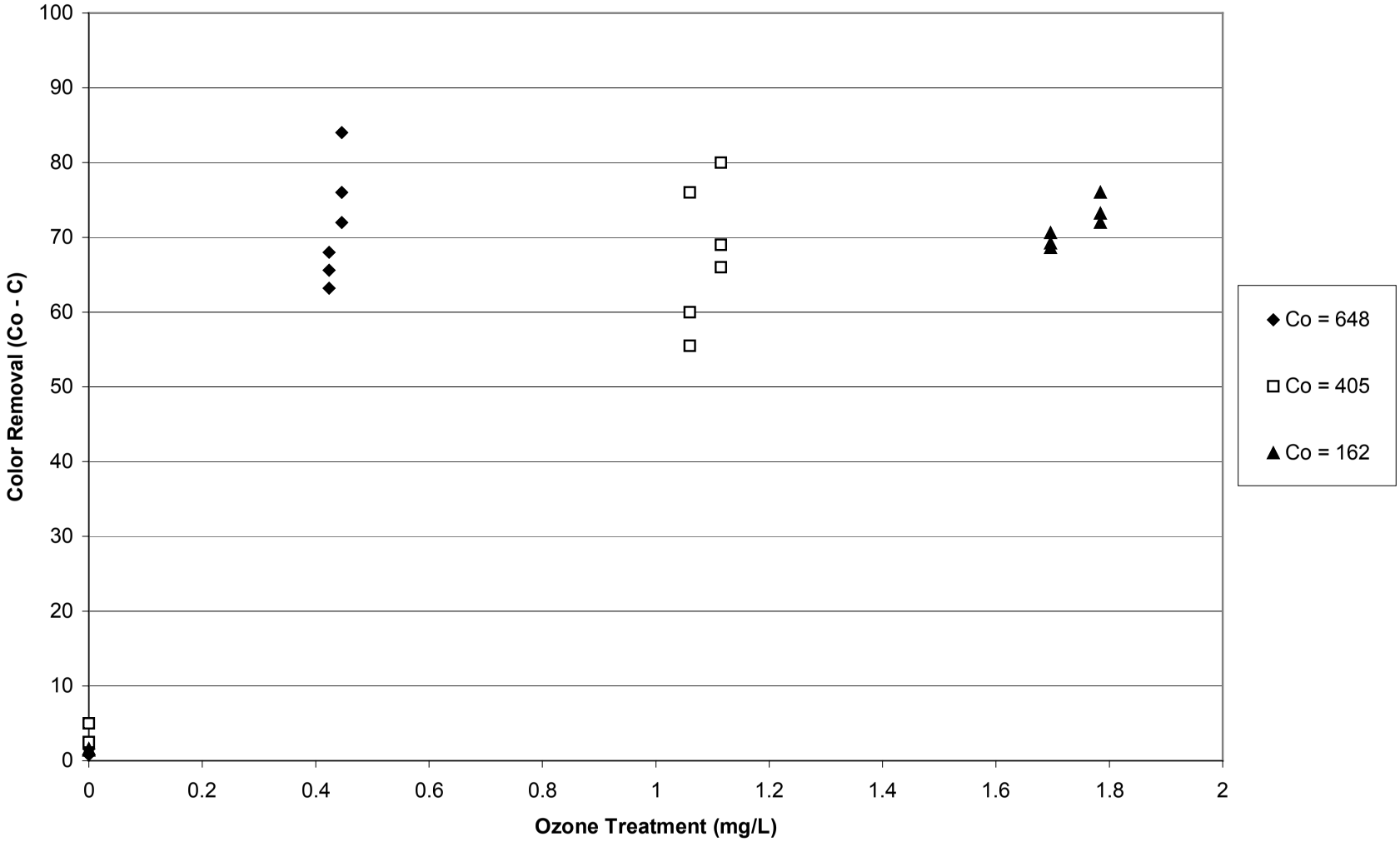

Figure 3-39
Color Removal vs. Ozone Treatment Mixed Batch Studies

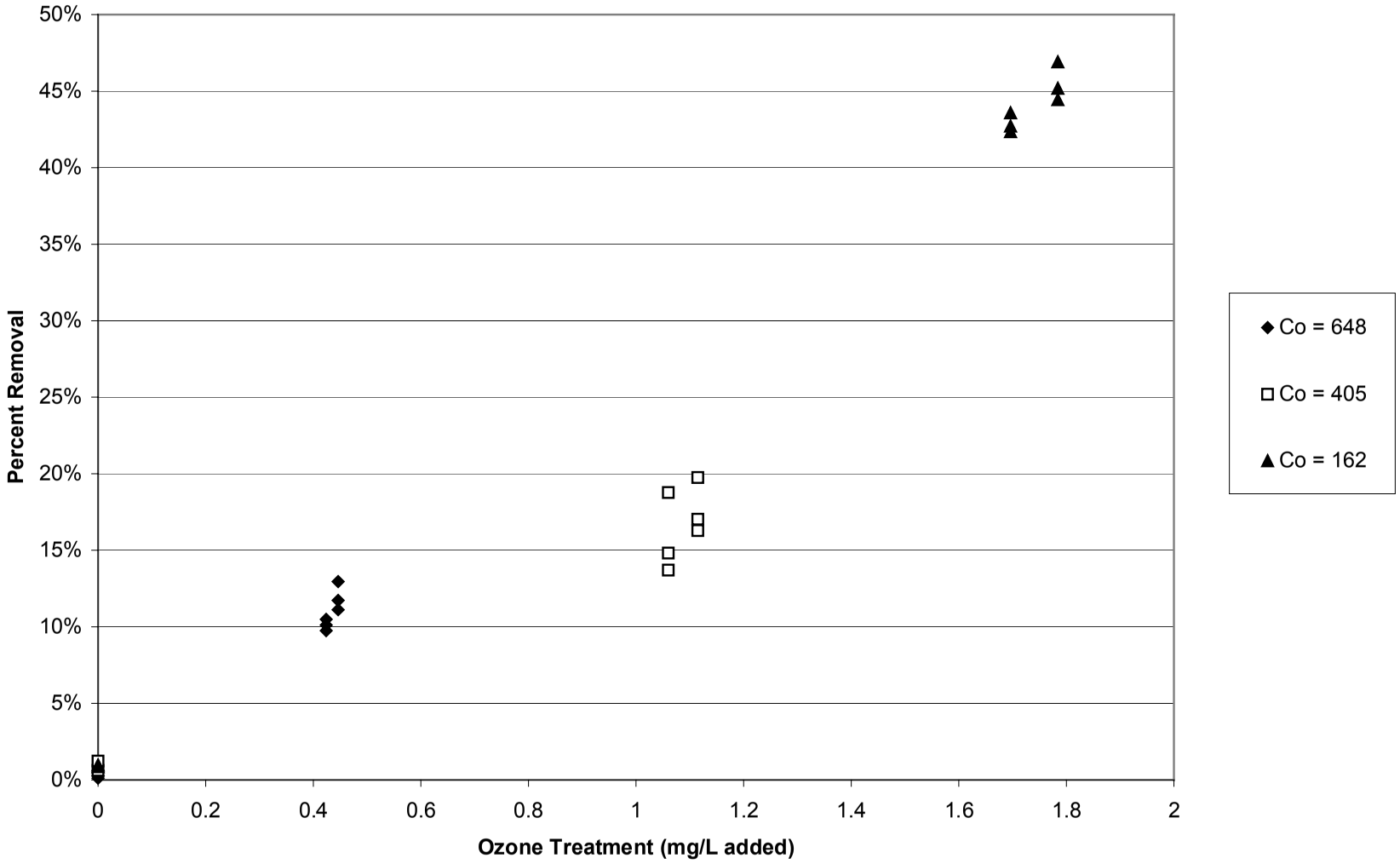

Figure 3-40
Percent Color Removal vs. Ozone Treatment Mixed Batch Studies

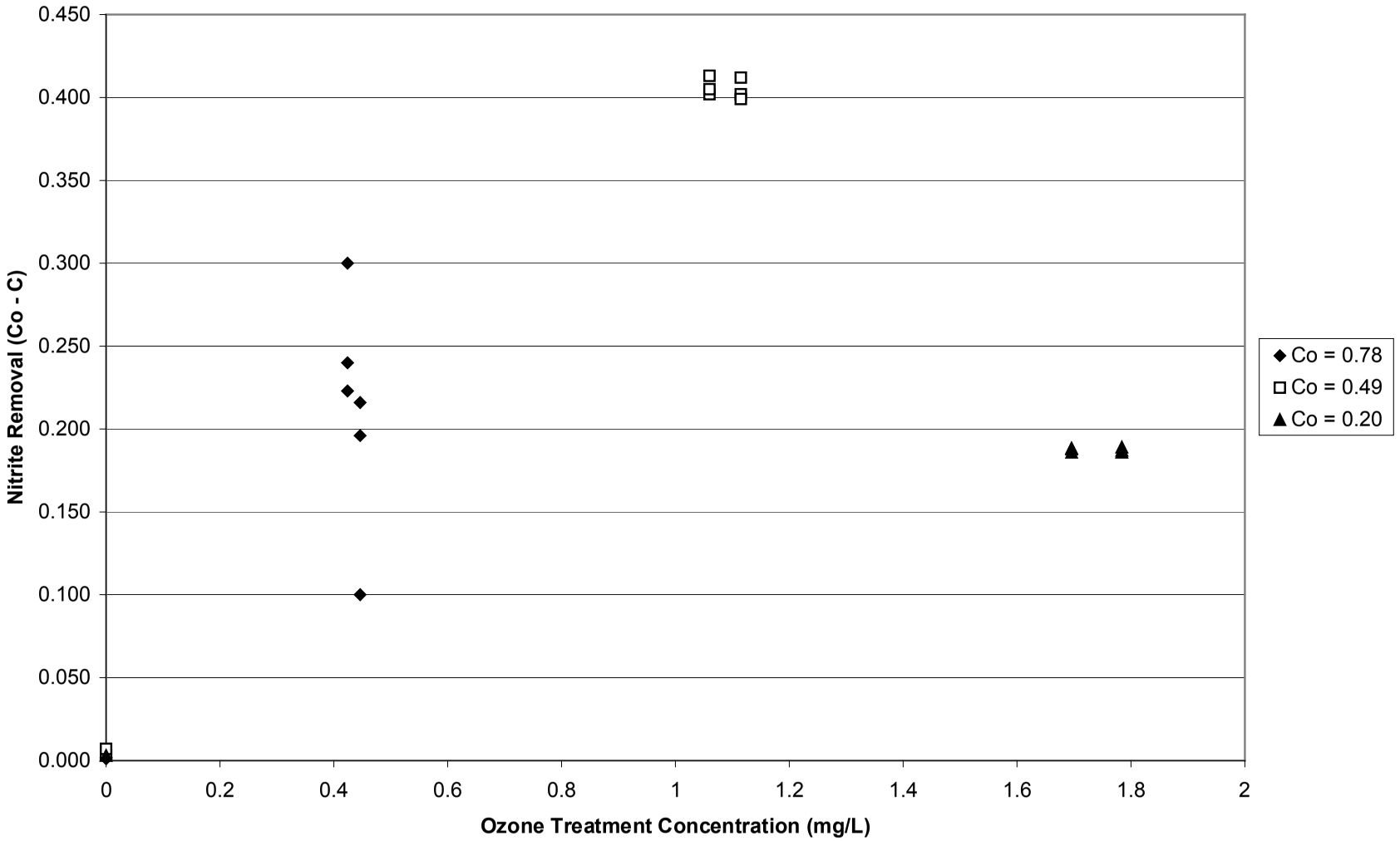

Figure 3-41
Nitrite Removal vs. Ozone Treatment Mixed Batch Studies

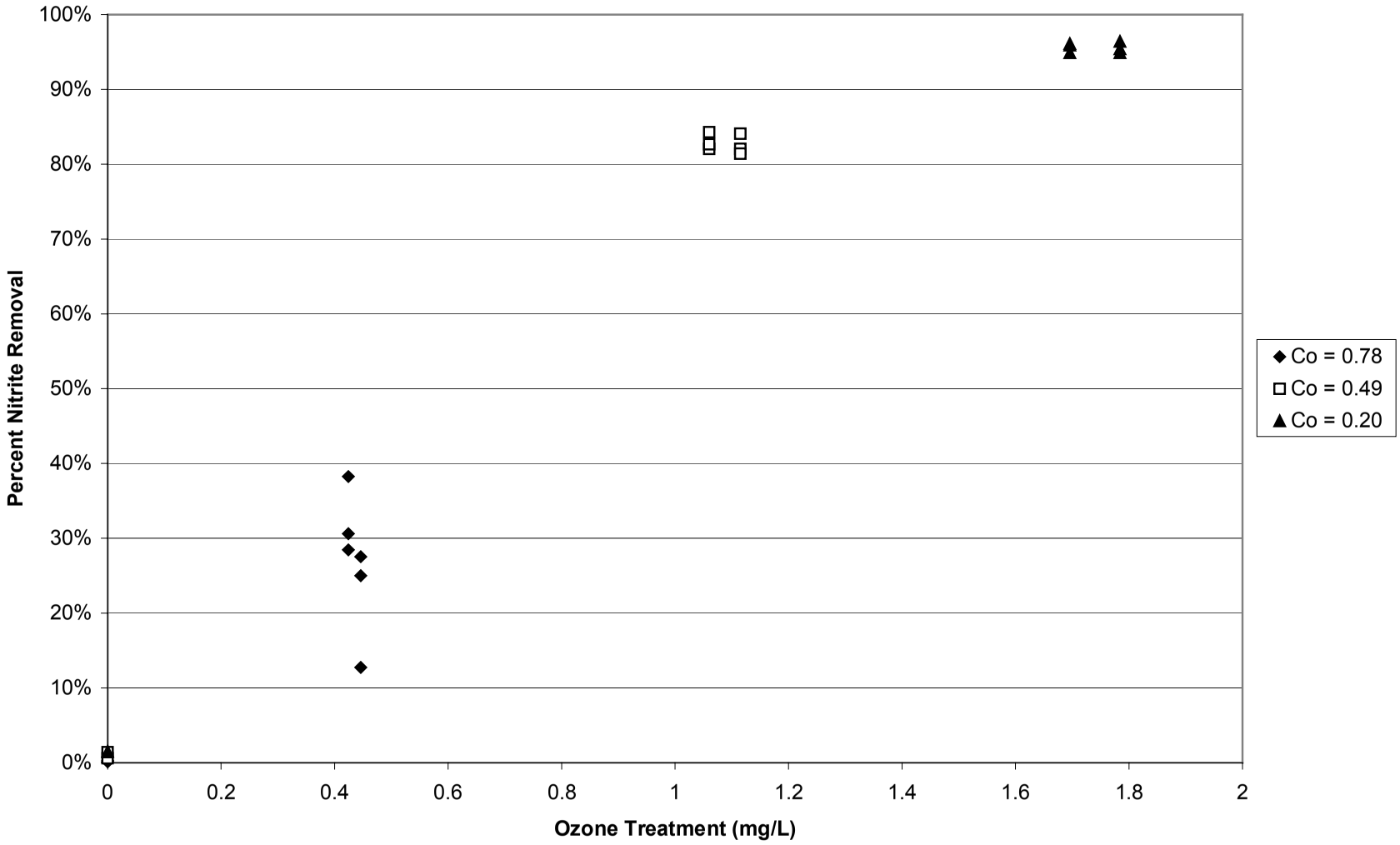

Figure 3-42
Percent Nitrite Removal vs. Ozone Concentration Mixed Batch Studies

In summary, results from the mixed culture water batch experiments were consistent with the literature in that

a. ozone effectively removed NO_2-N (up to 99%) in aquaculture production water,

b. the percentage of NO_2-N removal generally increased with increased ozone treatment concentrations,

c. ozone effectively removed color in aquaculture production water,

d. ozone did not reduce TSS in aquaculture production water, and

e. ozone did not reduce VSS in aquaculture production water.

3.2 Results and Discussion of the Full-Scale Application Study

3.2.1 Water Quality Data

Ozone was applied to Fingerlakes Aquaculture's production Pod 3 from 12/4/00 to 1/7/01. There are two sets of data depicting water quality during this time. The first set of data was collected by Fingerlakes Aquaculture personnel as part of routine farm operation, while the second set of data were collected specifically for the ozone test.

The first set of data include the amount of feed fed, ammonia-nitrogen, NO_2-N, pH, and alkalinity measured in each of the three production Pods from 11/16/00 to 1/22/01. These data are presented in Figures 3-43 through 3-48.

Feed fed in pounds per day for each production Pod is presented in Figure 3-43. This data indicates that all three production pods, and especially Pod 3, were fed a considerable amount of feed during this period of time (typically between 136 and 300 kg {300 - 650 pounds} of fish feed per day per production pod). The average amount of feed fed to Pod 3 between 11/16/00 and 1/22/01 was 204 kg (450 pounds) per day. It should be noted that when this much feed is fed to a production system, there is considerable impact to water quality in terms of color, ammonia-nitrogen, and suspended solids. The total impact depends upon the treatment components of the fish production system, especially the solids removal component. At Fingerlakes Aquaculture's Groton facility, suspended solids are removed using a passive, gravity settling method. Because gravity settling is not an especially effective way to remove fine solids (solids less than 60 micron in diameter) from water, there is a considerable suspended solids load in Fingerlakes Aquaculture's fish production systems (up to 150 mg/L TSS). The impact of this suspended solids and its resulting biological oxygen demand is discussed further in this section.

Ammonia and NO_2-N data for all three production pods from 11/16/00 to 1/22/01 are presented in Figures 3-44 and 3-45. These data indicate that in general, the ammonia and nitrite nitrogen loadings of Pod 3 were typically higher than those of either Pod 1 or Pod 2. This trend continued even during the period of ozone administration into Pod 3 (12/4/00 through 1/7/01) indicating that the addition of ozone into Pod 3 did not have a pronounced effect on its water quality in

comparison to the other full-scale production pods. Slightly higher ammonia and NO_2-N would be expected in Pod 3 than either Pod 1 or Pod 2 because in general, it was fed more feed than Pods 1 or 2. A correlation of nitrite to feed can be seen in the tailing off of NO_2-N in Pod 1 data (see Figure 3-45) which follows the tailing off of feed fed to Pod 1 beginning in late December (see Figure 3-43).

Feed, ammonia and NO_2-N data for just Pod 3 is presented in Figure 3-46. This data was gathered to determine if a pattern could be established between ammonia and NO2-Nitrogen present in Pod 3 before, during, and after ozone addition, when compared to the amount of feed fed to the system. Analysis of the ammonia and NO_2-N data for Pod 3 is inconclusive. One would expect for the NO_2-N concentrations to decrease during the period of time 12/4/00 through 1/7/01 as a result of the ozone addition to the system as was seen the batch studies. No such trend was observed during the full-scale ozone study, however, indicating that the application of ozone in this particular system was ineffective in reducing NO_2-N.

Additional background data for Pods 1, 2, and 3 regarding pH and alkalinity are presented in Figures 3-47 and 3-48. It should be noted that alkalinity is maintained in Fingerlakes Aquaculture's fish production pods by adding sodium bicarbonate to the fish production water before system water is pumped to the biofilter. According to Bablon et al. (1991), bicarbonate, carbonate, alkyl groups, tertiary alcohols, and humic substances are common inhibitors to the free-radical decomposition of ozone.

The second set of data for the full-scale study were taken expressly during the ozone application period. This data includes a second set of NO_2-N, color, TSS and VSS for the time period 12/4/00 through 1/7/01.

The second set of NO_2-N data are presented in Figure 3-49. This data, like the NO_2-N data presented earlier in this section, confirms that in general the NO_2-N concentrations in Pod 3 were slightly higher than the NO_2-N concentrations in Pods 1 an 2. This trend of slightly higher nitrite concentrations continued throughout the test period, even after ozone was applied to Pod 3, indicating that the addition of ozone did not have a pronounced effect on the NO_2-N in Pod 3.

Color data for Pods 1, 2, and 3 are presented in Figure 3-50. Analysis of this data indicates that for the first week of ozone application the color in Pod 3 water was significantly higher than the color in the water of Pods 1 and 2 (see Figure 3-50). This trend of higher color in Pod 3 would be expected due to the higher feeding rates associated with Pod 3 compared to Pods 1 and 2. After one week however, the color level in Pod 3 decreased significantly, and for the most part was below the level of that in Pods 1 or 2. Because the only difference between these Pods was the introduction of ozone, it appears that ozone effectively removed color in Pod 3 production water under the full-scale conditions. This reduction of color caused by ozone was significant (up to 400 color units less during the last 3 weeks of application compared to the first week of operation).

Based upon the ozone application rate of 10 g ozone/Kg feed added, in this full-scale study, the color removal observed in this study are similar to those observed by Christensen et. al. (1996) who found color removal of ozone in a continuous recirculation system to be on the order of 7-16 g ozone to remove the color of each Kg feed added.

TSS data for Pods 1, 2, and 3 are presented in Figure 3-51. This data indicates that the TSS in Pods 1, 2, and 3 were typically in the range of 80 to 120 mg/L TSS. VSS data for Pods 1, 2, and 3 are presented in Figure 3-52. VSS in Pods 1, 2, and 3 were typically in the range of 80 to 120 mg/L throughout the duration of this study. Although not readily apparent in the Figures, the average percentage of the TSS that were volatile (VSS) was 89% (for all production pods). Based upon this data, it appears that the application of ozone had no perceptible effect on the removal of suspended solids in fish production water under full-scale conditions. The addition of ozone also did not have an effect on the percentage relationship between volatile solids and total suspended solids (i.e. the percentage of solids that were volatile did not change due to the application of ozone).

In addition to the data presented in Figures 3-43 through 3-52, there were also several qualitative observations made by Fingerlakes Aquaculture personnel during the full-scale ozone application study. These qualitative observations include

a. the reduction of foam production in Pod 3 during the ozone study, and

b. the improvement of water clarity in Pod 3 during the ozone study.

The first observation made by Fingerlakes Aquaculture personnel was the removal of foam in Pod 3 after ozone was applied. Foaming occurs in indoor recirculation aquaculture systems due to a build up of proteins from fish feed and the digestion of feed by fish. The reduced foam production from Pod 3 was pronounced after the addition of ozone to this Pod, not only in comparison to before ozone was applied to Pod 3 (foaming was consistent before ozone was added), but also in comparison to Pods 1 and 2, where foam persisted during the entire period of study.

The second observation made by Fingerlakes Aquaculture personnel was that the clarity of water in Pod 3 was also improved after ozone was applied. Water clarity is a function of both water color and the concentration of suspended solids present. Because the TSS of Pod 3 was not affected by the addition of ozone (please see Figure 3-51), it appears the improvement in water clarity observed by Fingerlakes Aquaculture personnel during the ozone study was due primarily to the removal of color. This observation is supported by the color data of Figure 3-50.

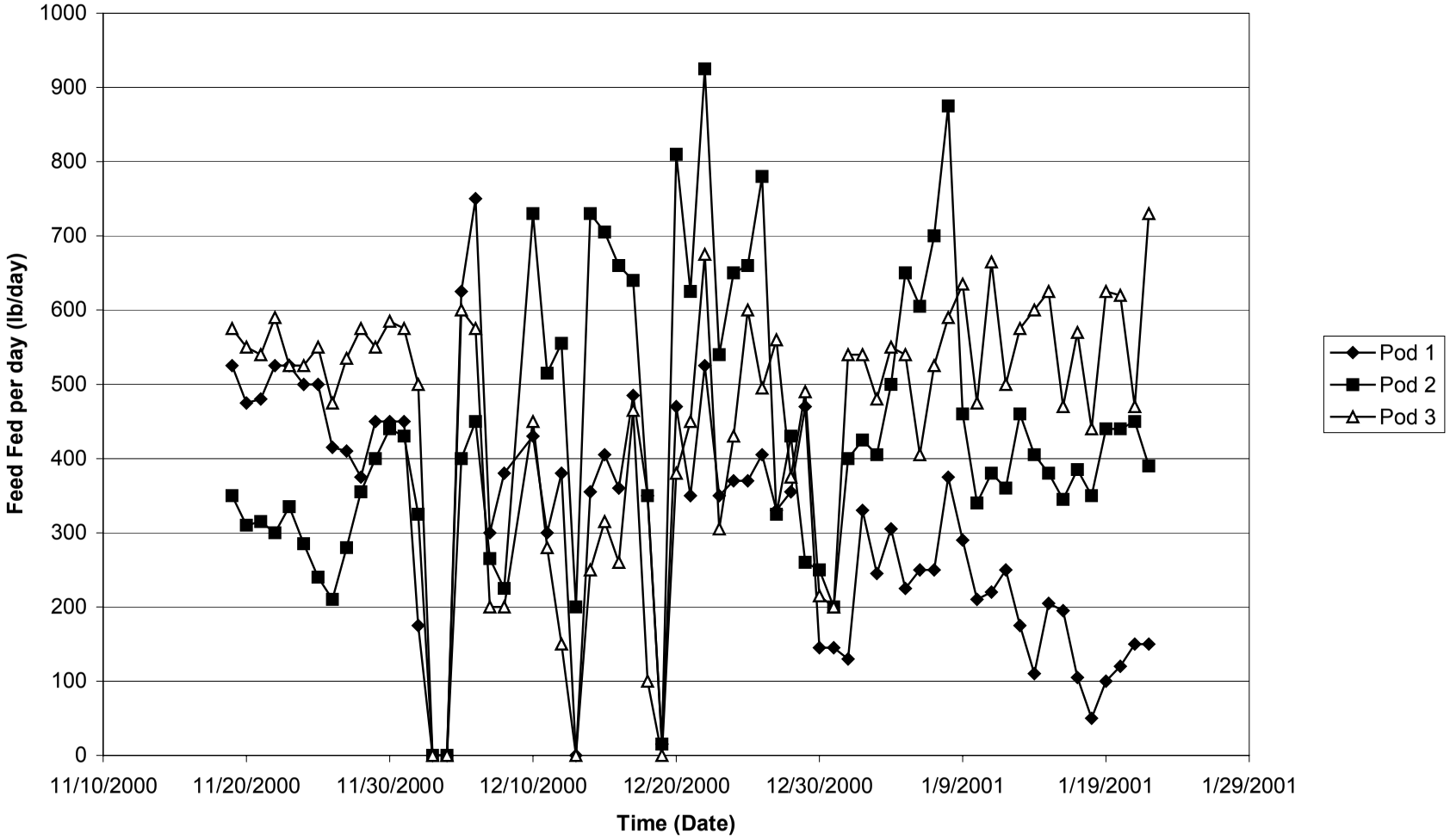

Figure 3-43
Feed Fed vs. Time for All 3 Pods Ozone applied 12/4/00 - 1/7/01 in Pod 3

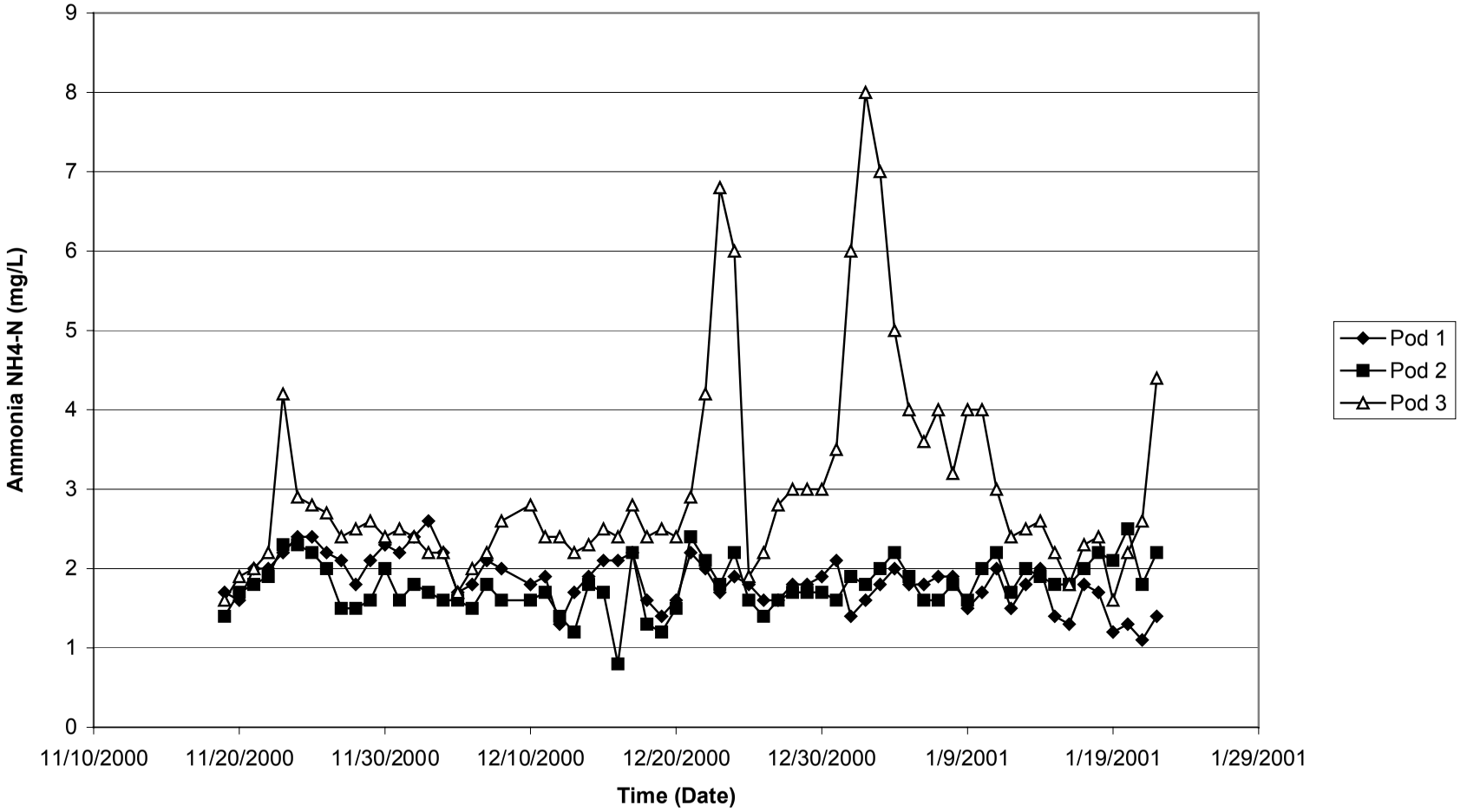

Figure 3-44
Ammonia vs. Time Full-Scale Study - 3 Production Pods Ozone applied 12/4/00 - 1/7/01 in Pod 3

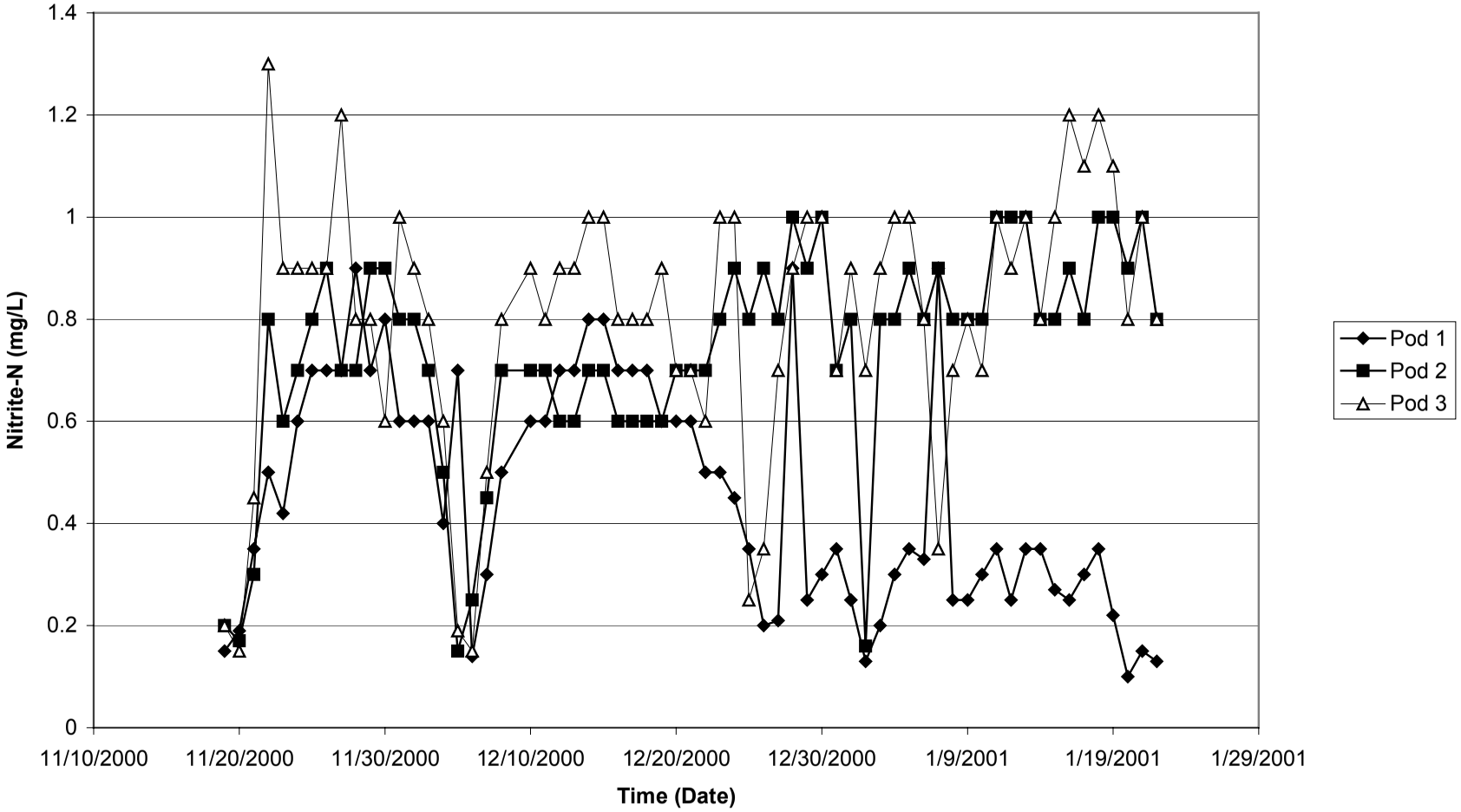

Figure 3-45
Nitrite-N vs. Time Full-Scale Study - 3 Production PodsOzone applied 12/4/00 - 1/7/01 in Pod 3

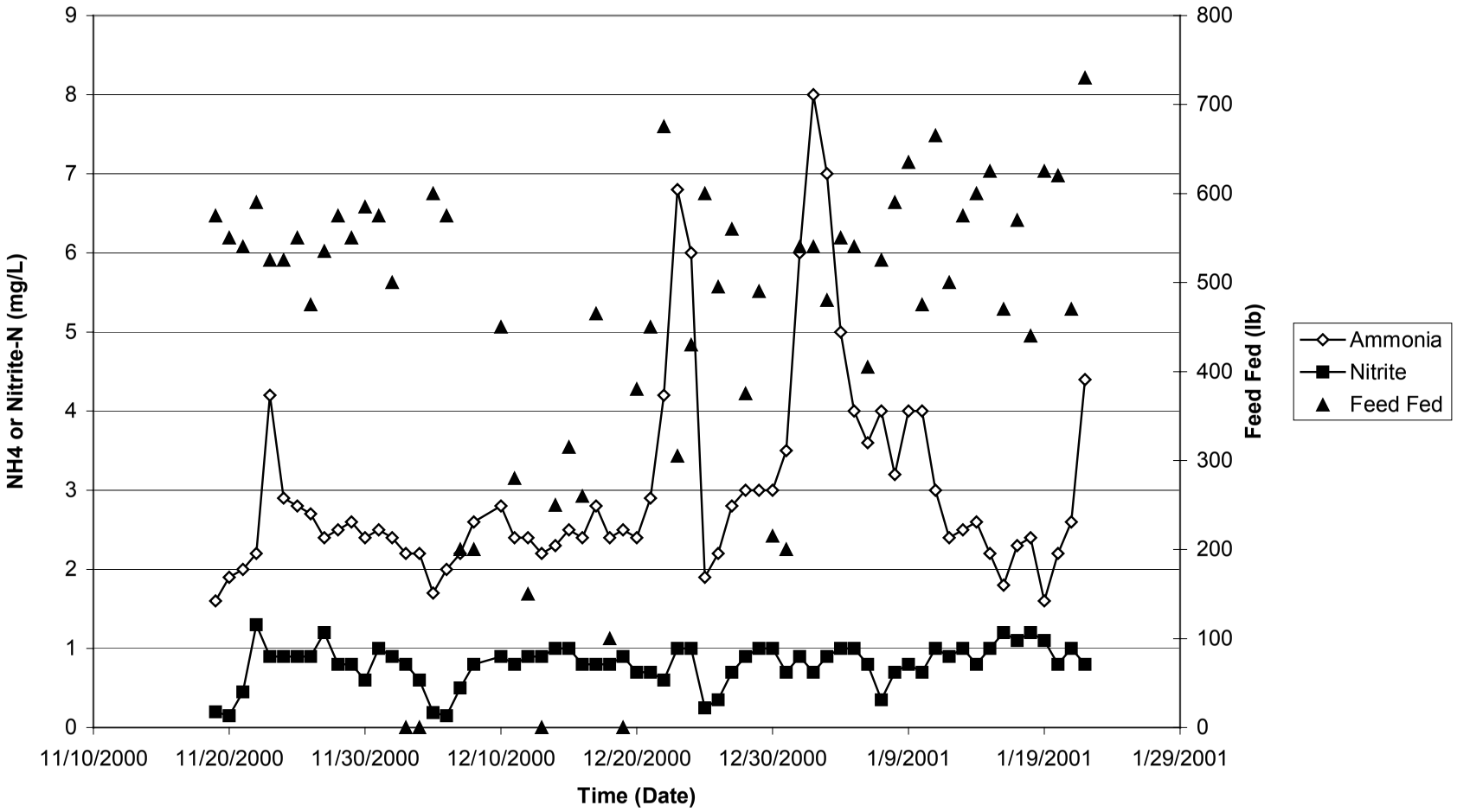

Figure 3-46
Feed and Nitrification vs. Time Pod 3 Full Scale Study Ozone applied 12/4/00 - 1/7/01

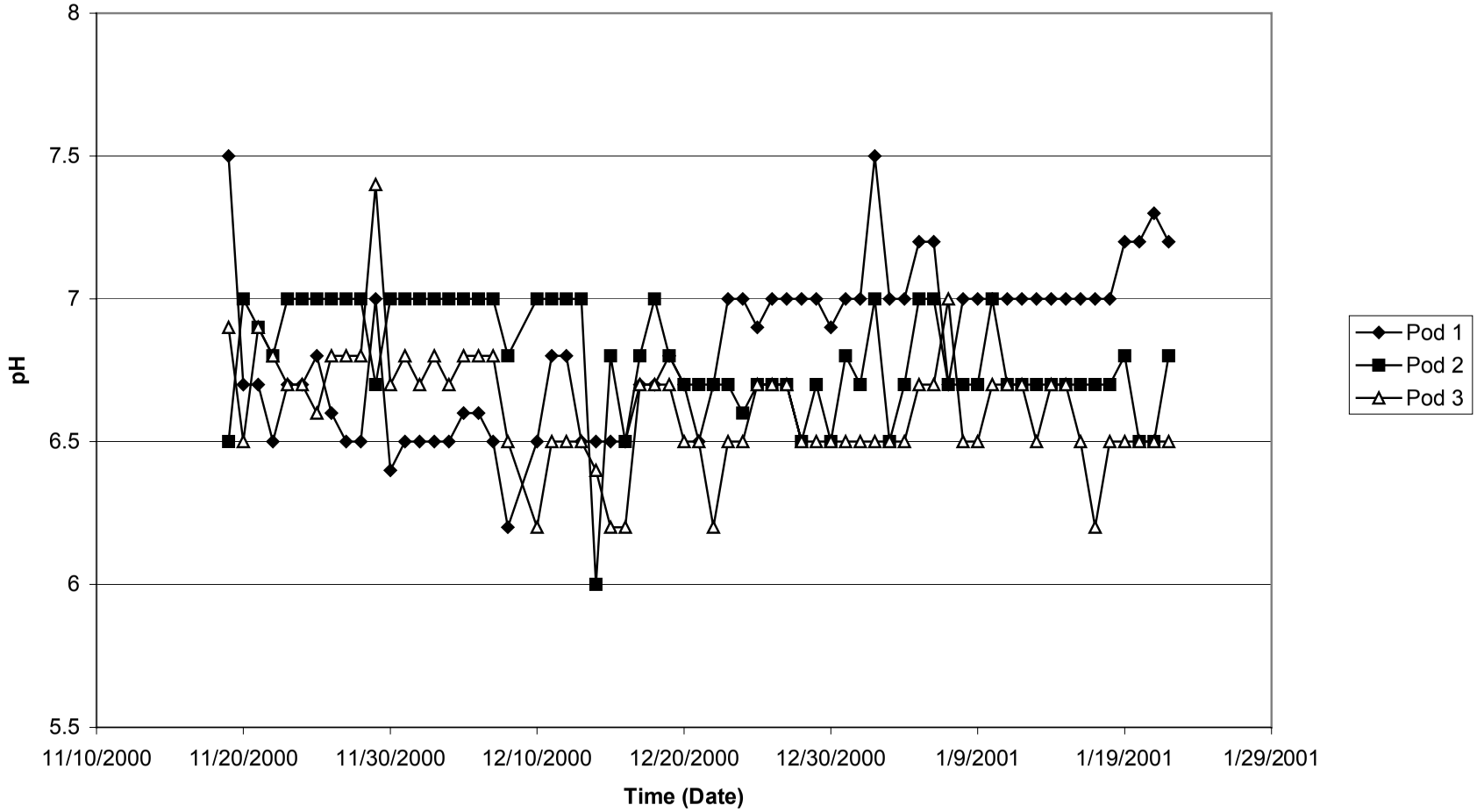

Figure 3-47
pH vs. Time: Full-Scale Study - 3 Production Pods Ozone applied 12/4/00 - 1/7/01 in Pod 3

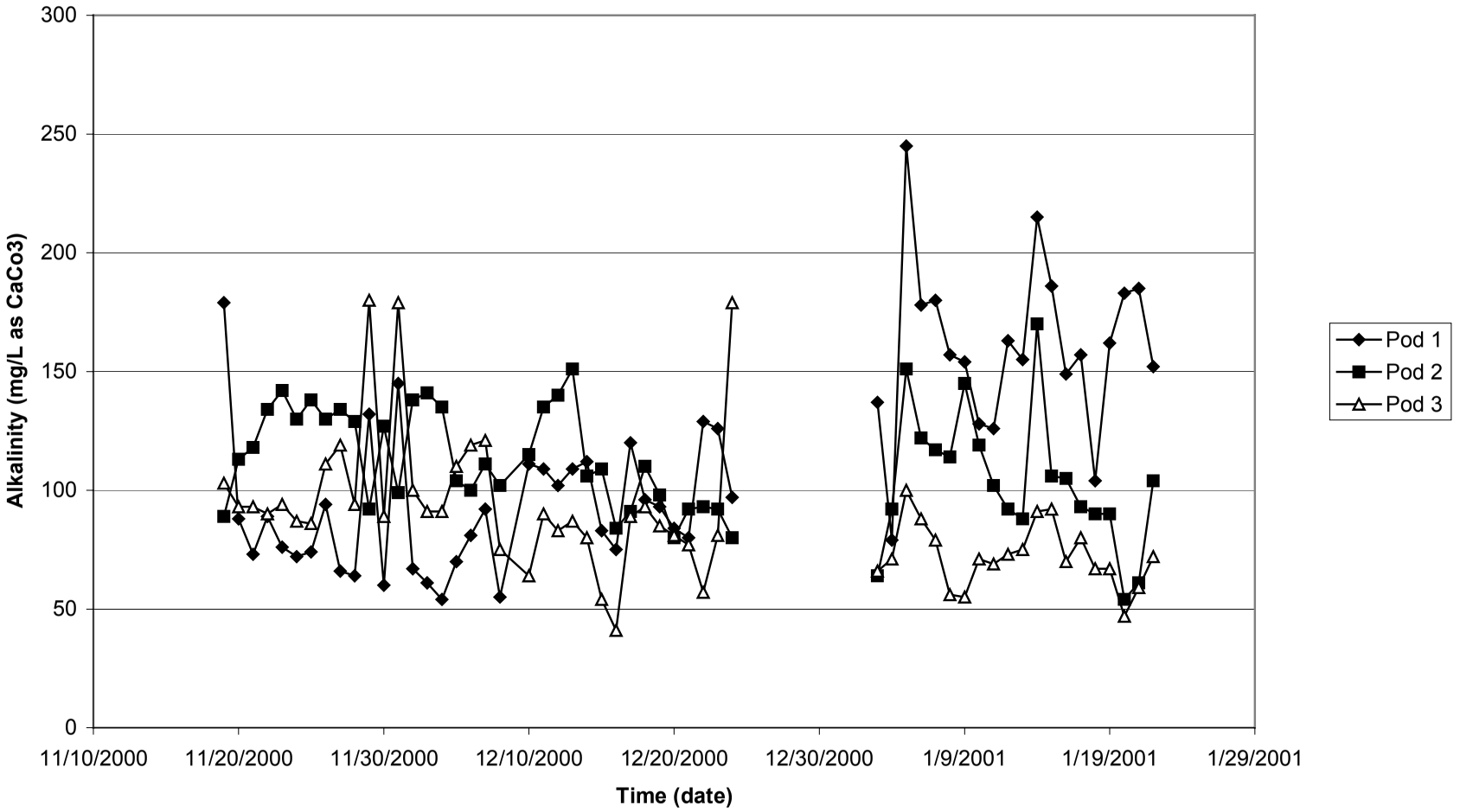

Figure 3-48
Alkalinity vs. Time Full-Scale Study - 3 Production Pods Ozone Applied 12/4/00 - 1/7/01 in Pod 3

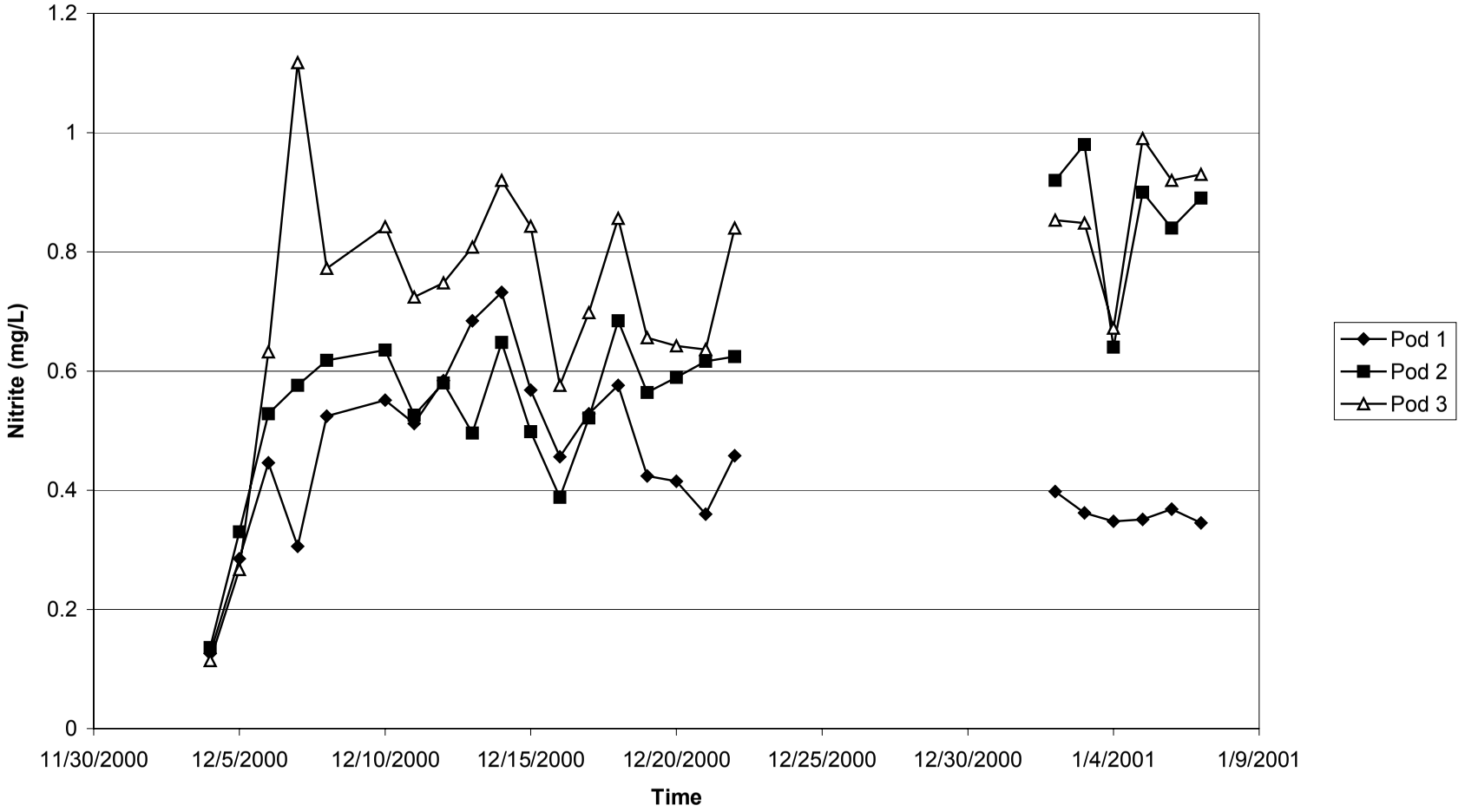

Figure 3-49
Nitrite vs. Time Full-Scale Study - 3 Production Pods Ozone applied 12/4/00 - 1/7/01 in Pod 3

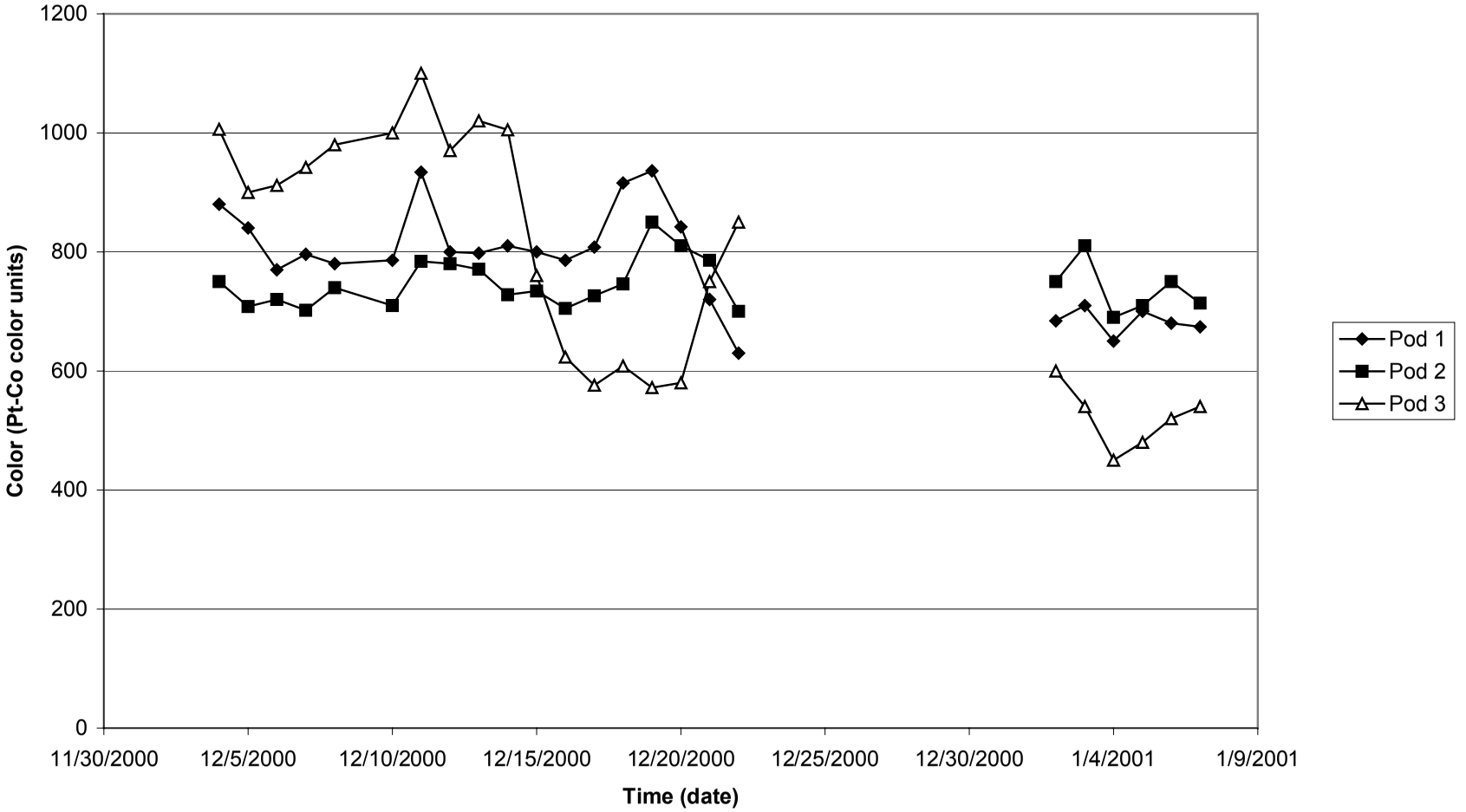

Figure 3-50
Color vs. Time Full-Scale Study - 3 Production Pods Ozone applied 12/4/00 - 1/7/01 in Pod 3

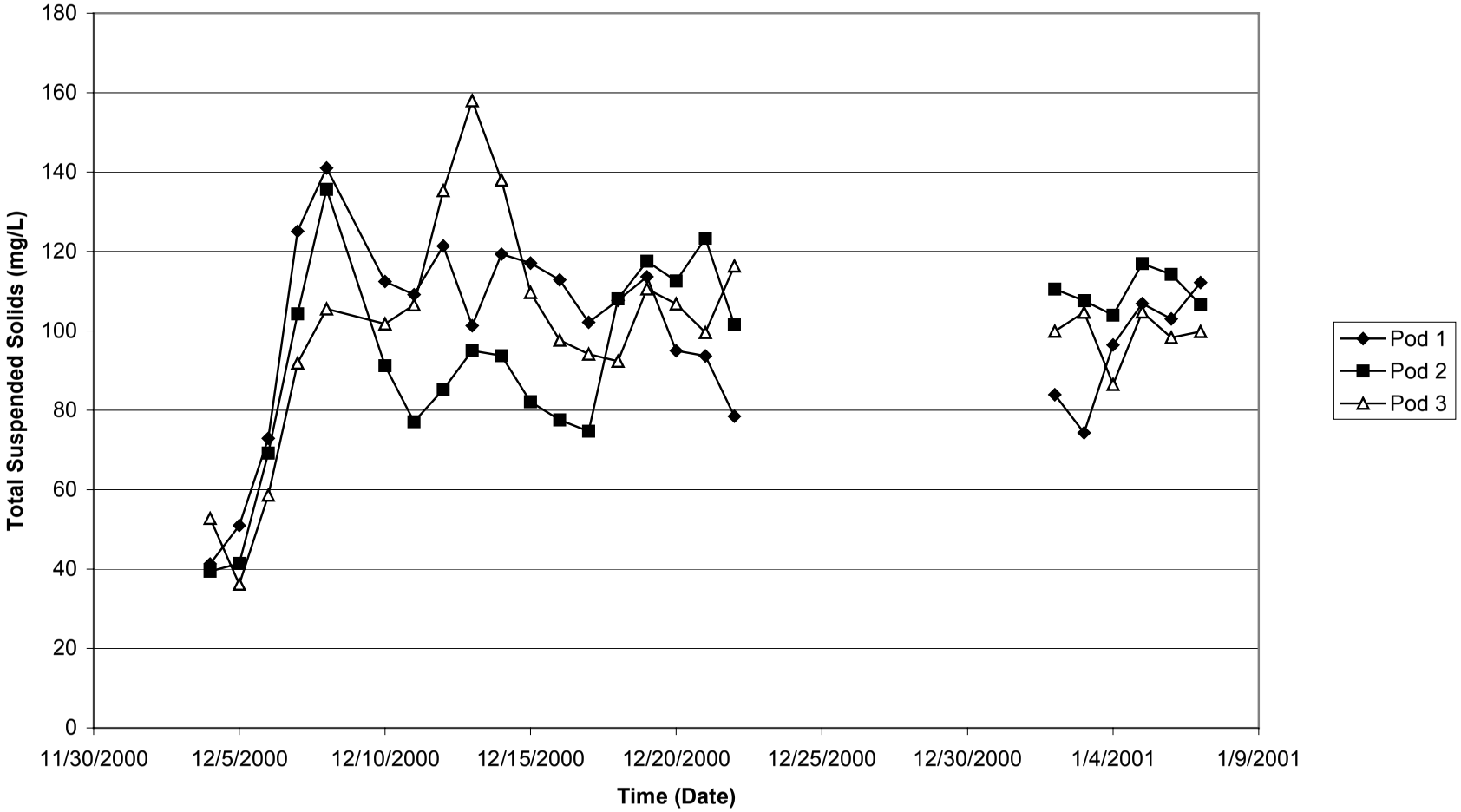

Figure 3-51
TSS vs. Time Full-Scale Study - 3 Production Pods Ozone Applied 12/4/00 - 1/7/01 in Pod 3

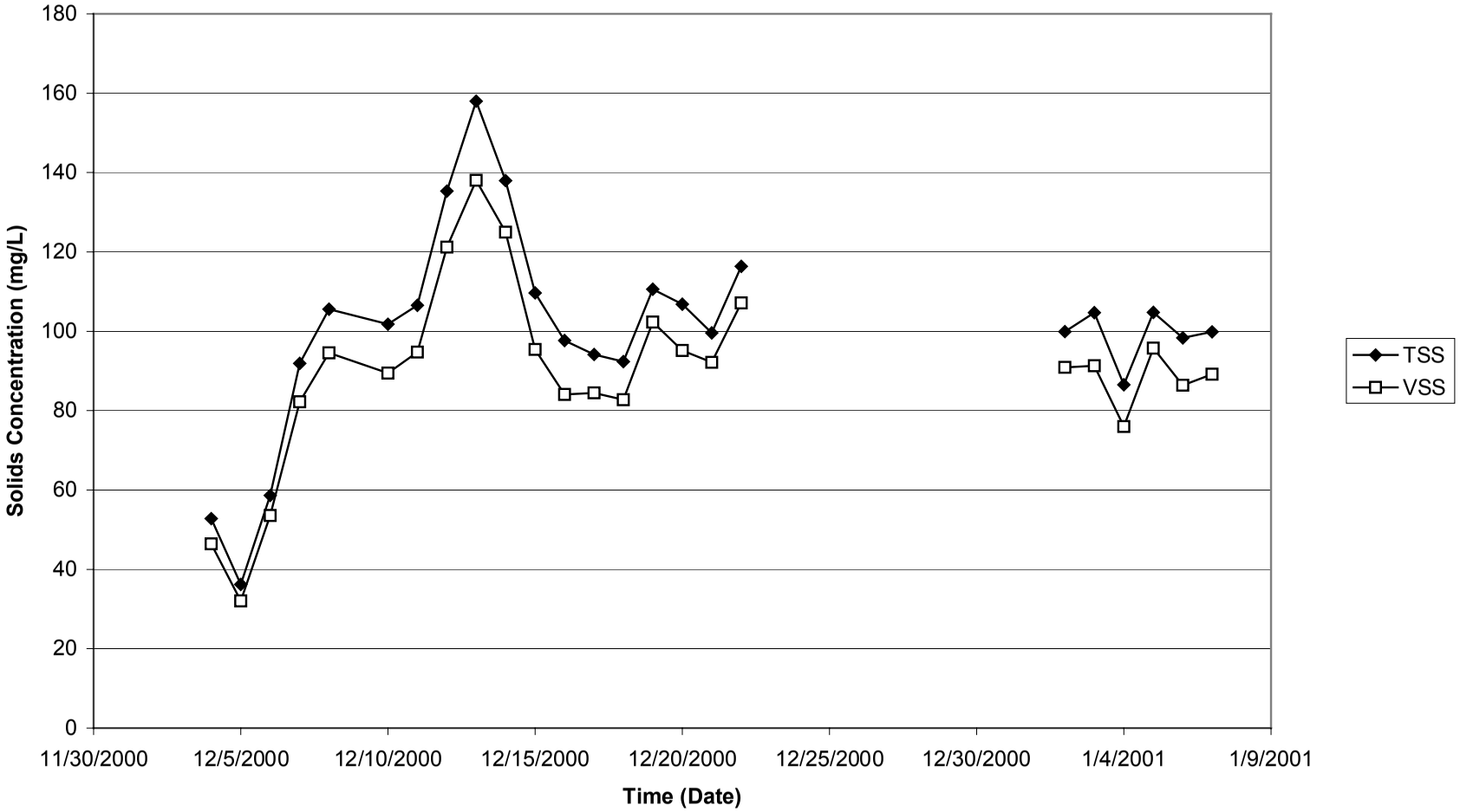

Figure 3-52
TSS and VSS vs. Time Full Scale Study - Pod 3 Only Ozone Applied 12/4/00 - 1/7/01

Summary of Full-Scale Research

Based upon the data collected during the full-scale study, it appears that the addition of ozone had a positive effect on water clarity (reduction of color) and foam reduction in a full-scale application at Fingerlakes Aquaculture's Groton facility. The addition of ozone did not have a significant effect on the removal of NO_2-Nitrogen, ammonia-nitrogen, or suspended solids (total or volatile). Because both color and foam were removed from Pod 3 production water during the course of study and NO_2-N, a relatively-easily degradable (oxidizable) compound by ozone was not, it can be hypothesized that the ozone added to Pod 3 during the full-scale study preferentially consumed select compounds within the full-scale system. Preferential compound oxidation would be expected within an actual production system and especially so in a production facility such as Fingerlakes Aquaculture, where fish production water receives large amounts of feed and high concentrations of solids exist.

It is further hypothesized that greater improvement of water quality would have occurred in Pod 3 if either a) the water quality was improved by other treatment systems prior to adding ozone or b) more ozone was added to the system. Water quality improvement could be achieved through several different pathways including decreased feed rates, improved solids separation, increased water exchange (more fresh make-up water), and increased biofiltration. Such methods would lower overall loading upon the system and allow the ozone added to oxidize other target parameters within the system including NO_2-N.

The recommended level of ozone addition is 10-20 g ozone per kilogram of feed added or between 1 and 2% ozone to feed ratio (Summerfelt and Wheaton, 1999). Fingerlakes Aquaculture added approximately 4.6 pounds of ozone per day to Pod 3 during this study which is equivalent to 10.3 g of ozone per kilogram of feed added (the lower limit of this recommended range). Based upon both the results achieved in the full-scale test in color and foam removal, and the results achieved in the color and nitrite batch studies, it is estimated that increased ozone addition would have had a greater impact on water improvement on Pod 3 production water. It should be noted that although additional ozone would improve water quality in terms of nitrite and color removal, additional ozone into Fingerlakes Aquaculture's Pod 3 production system might be deleterious towards fish health as discussed in the next section.

3.2.2 Fish Health Analysis: Blood Chemistry and Histological Examination of Ozone-treated tilapia (Oreochromis niloticus)

Material and Methods

Tilapia were sampled in January, 2001 from the Fingerlakes Aquaculture production facility. Thirty fish were taken from the production system that was incorporating ozone treatment (ozone-treated fish), and 2 days later another 30 were taken from a production system not using ozone (control fish). Blood samples were collected in lithium-heparin treated tubes to prevent coagulation. Subsamples were taken for hematocrit determination. Blood sampled were spun, and 500 uL plasma samples were submitted to Clinical Pathology Lab in College of Veterinary Medicine, Cornell University. Thirty-two parameters were then measured and calculated using Boehringer Mannheim/Hitachi 911 system.

Gill arches were saved for histological examination from randomLy selected 10 fish for each group. Eosinophilicgranular cells (EGCs) were counted using the standardized protcol: EGCs were counted from 5 gill filaments. The gill filaments were randomLy chosen from those sectioned at the plane which cartilage can be seen in the middle. EGCs located in the region within 20 consecutive gill lamellae were counted. We only counted EGCs presented in epithelium of gill filament, and exclude those presented in gill lamellae.

Results and Discussion

The results for the control group and ozone-treated system tilapia are listed in Table 3-9. These data are compared to other groups we have sampled before at Fingerlakes Aquaculture's Groton facility.

Compared to 'healthy' control fish sampled in January 2001, ozone-treated fish have lower hematocrit, plasma sodium, chloride, magnesium, total protein, albumin concentrations, total iron binding capacity(TIBC), and higher bicarbonate, alanine aminotransferase(ALT), creatinine kinase(CK) concentration. These differences are significant ($P<0.01$).

Lower hematocrit can be seen in fish during infection, under handling stress, or stress caused by pollutant. Decreased plasma sodium and chloride concentrations also have been associated with pollutant-induced gill damages. Pollutant in the aquatic environment can increase permeability to Na^+ and Cl^- in gill epithelial cell, and leads to decreased concentrations in freshwater fish and increases in salt water fish. Albumin is the major component of plasma proteins. Decrease in albumin can be seen in acute phase response during infection, or can due to hepatic disease. Magnesium is tightly regulated in fish, the decrease of magnesium might be of some importance. But so far it has not been associated with specific disease condition.

Higher ALT is associated with damage in skeletal muscle, heart muscle and liver damages in other animals. But here the healthy group has lower ALT activity compared to other healthy groups we sampled last year, and ozone-treated group is not significantly different from other healthy groups. Same situation happened in plasma bicarbonate concentrations. High CK is associated with damage in cardiac muscle or skeletal muscle. The importance of these parameters in fish is again unclear.

If we compare both healthy and ozone-treated groups to other healthy groups we sampled earlier last year, some common changes can be identified. Both groups have higher plasma potassium concentration, alkaline phosphatase, bilirubin, amylase activities, and higher levels of lipemia and hemolysis. Increase in amylase activity in circulation could be an indication of pancreatic damage in some animal species, but its importance in fish is unclear. It has also been reported in animal with liver or renal diseases. Elevated potassium concentration has been observed in tilapia cultured in the presence of copper. Higher concentration of bilirubin and higher level of hemolysis suggest that red blood cells were more vulnerable in both groups. Also the lipemia might be an indication of imbalanced diet.

Examination of gill histology revealed there is no abnormality in ozone-treated tilapia, except for higher level of EGCs. The EGC counts are higher in ozone-treated fish comparing to healthy control ($P = 0.028$). Some fish in ozone treated group had normal level of EGCs, but others had relatively numerous EGCs. We didn't see EGC in gill lamellae in healthy fish, but that appeared

to be not uncommon in ozone-treated fish. EGC has been reported to react to stimulants in the environment. And some fish might react more dramatically to ozone treatment and then more EGC aggregated in their gills.

Table 3-9
Comparison of blood chemistry parameters between healthy and ozone-treated tilapia. P values are calculated using 1-tail T test.

		Jan, 2001 *healthy fish*		Jan, 2001 *Ozone-treated fish*		P value	Interpretation
		Mean	*SD*	*Mean*	*SD*		
sample size		30		30			
weight	**g**	324.47	99.21	256.20	61.38	0.001	significantly lower than healthy group
hematocrit	**%**	42.40	3.25	37.36	3.03	0.000	significantly higher than healthy group
Blood Chemistry Parameters							
sodium	**mEq/L**	163.47	4.19	157.50	4.59	0.000	significantly lower than healthy group
potassium	**mEq/L**	5.52	1.26	5.43	1.14	0.193	
chloride	**mEq/L**	134.53	3.62	124.07	6.25	0.000	significantly lower than healthy group
bicarbonate	**mEq/L**	16.17	2.78	21.87	2.40	0.000	significantly higher than healthy group
anion gap	**mEq/L**	18.33	3.06	17.03	2.30	0.017	
sodium : potassium		30.93	6.21	30.17	6.02	0.157	
urea nitrogen	**mg/dL**	<2.17	--	<2.10	--	*NC	
creatinine	**mg/dL**	0.34	0.16	<0.21	--	NC	
calcium	**mg/dL**	17.16	7.28	14.65	2.54	0.021	
phosphate	**mg/dL**	9.74	2.31	9.09	1.43	0.049	
magnesium	**mEq/L**	2.87	0.35	2.54	0.32	0.000	significantly lower than healthy group
total protein	**g/dL**	4.14	0.43	3.83	0.47	0.002	significantly lower than healthy group
albumin	**g/dL**	1.37	0.15	1.24	0.12	0.000	significantly lower than healthy group
globulin	**g/dL**	2.78	0.31	2.59	0.37	0.009	significantly lower than healthy group
albumin/globulin		0.49	0.04	0.48	0.04	0.095	
glucose	**mg/dL**	90.23	25.42	84.00	14.76	0.063	
ALT/P5P	**U/L**	74.70	46.37	114.40	67.43	0.003	significantly higher than healthy group
AST/P5P	**U/L**	228.20	212.74	345.43	263.48	0.016	
alkaline phosphatase	**U/L**	27.17	8.06	27.93	4.80	0.164	
GGT	**U/L**	< 3.00	--	< 3.07	--	NC	
total bilirubin	**mg/dL**	0.03	0.05	0.04	0.05	0.073	
direct bilirubin	**mg/dL**	0.00	0.00	0.00	0.00	NC	
indirec bilirubin	**mg/dL**	0.03	0.05	0.04	0.05	0.073	
amylase	**U/L**	64.44	131.54	138.45	178.15	0.020	
cholesterol	**mg/dL**	235.10	62.22	249.63	80.76	0.110	
creatine kinase	**U/L**	4672.63	4707.90	11437.67	9367.37	0.000	significantly higher than healthy group
iron	**ug/dL**	198.10	238.89	132.33	35.07	0.037	
total iron binding capacity	**ug/dL**	591.33	66.01	531.00	85.34	0.001	significantly lower than healthy group
% satuation	**%**	26.20	6.19	25.03	5.55	0.111	
lipemia		29.17	9.61	26.93	12.70	0.114	
hemolysis		82.87	52.93	112.11	74.59	0.023	
icterus		0.00	0.00	0.04	0.19	0.082	

*NC: not calculated

Additional Observations by Fingerlakes Aquaculture Personnel

In addition to the analysis performed by Dr. Paul Bowser's Fish Veternary team at Cornell University, personnel at Fingerlakes Aquaculture also noted changes to fish appearance and behavior in Pod 3 when ozone was applied to the tank. The primary additional observations by Fingerlakes Aquaculture personnel include

a. an increased redness in the head belly regions of the tilapia

b. absence of mucus membranes around the fish,

c. inflamed fin margins and

d. an increased irratability or jumpiness of all the fish in the entire Pod.

These observations were qualitative in nature but seemed to indicate that the addition of ozone physically irritated a significant number of fish in Pod 3, when it was being applied to this pod.

Summary of Fish Health Analysis

Based upon both the blood serum chemistry performed by Cornell University and the qualitative visual observations collected by Fingerlakes Aquaculture, it appears that the ozone applied to Fingerlakes Aquaculture's Pod 3 during the full-scale ozone study had an irritating effect on the tilapia present in this pod. The cause for the irritation is not known, however it can be assumed that it is due to residual ozone not readily consumed in the LHO's to which they were administered.

4
CONCLUSIONS AND RECOMMENDATIONS

Based upon the information gathered in the batch studies and full-scale studies, it appears that ozone can be an effective water treatment tool for aquaculture. In laboratory batch studies, ozone effectively removed color and nitrite from water at concentrations common to aquaculture finfish production. In a full-scale application of ozone at Fingerlakes Aquaculture's Groton tilapia production facility, both color and foam were effectively removed from production water. The removal of color and nitrite from batch water was rapid with most removal taking place within the first 30-minutes of exposure to ozone. Reduction in foam in the full-scale study was also rapid (within the first few days of ozone addition to the system) with color removal taking place a few days after. This data supports what other researchers have found in that although ozone is an effective and rapid oxidizer, it preferentially degrades compounds when exposed to many compounds simultaneously (Bablon et al, 1991, Hochheimer and Wheaton, 1995.

The addition of ozone did not have any discernable effect on total suspended solids or volatile suspended solids in either the laboratory batch studies or the full-scale study. Increased solids settling has been observed by other researchers (Pak et al. 1981, Rice, 1986) as a result of increased flocculation, however in the case of batch and full-scale studies at Fingerlakes Aquaculture it appears that not enough ozone was applied to cause increased flocculation and suspended solids removal (perhaps due to preferential oxidation of other compounds).

Laboratory blood serum analysis and visual inspection of tilapia present in the full-scale production pod to which ozone was added, indicated that tilapia were irritated/stressed by the introduction of ozone to the recirculating aquaculture system. This stress was measured as an increase in redness in the head and belly regions, absence of mucus membranes, and inflamed fin margins, as well as lower hematocrit levels and higher ALT levels in the blood of the tilapia. The cause of this stress is likely due to residual ozone present in the fish production system resulting from inadequate reaction time in the Low Head Oxygen units before entering the fish production systems.

Based upon the information gathered at Fingerlakes Aquaculture several recommendations can be made towards the design and application of ozone in aquaculture systems including:

- Ozone should be added at the end of a full-system treatment train to obtain maximum effectiveness (e.g. after solids separation and ammonia-nitrification),
- A thorough understanding of potential ozone-oxidized compounds is needed before ozone application,

- Ozone should be added to a fish production system where enough reaction time is allowed to completely use up the ozone before coming in direct contact with fish.
- Extreme caution towards health of fish should be considered prior to ozone addition (Fingerlakes Aquaculture only used 10 g per kg and experienced highly stressed tilapia)

This work also showed that ozone monitoring can be performed effectively using water samples and simple water chemistry techniques. It is especially important to sample fish production water to avoid residual ozone contact with production fish.

5
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A
A USER'S MANUAL FOR OZONE IN AQUACULTURE

A User's Manual for Ozone in Aquaculture

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Introduction

Ozone is a powerful oxidizing agent that has many potential uses for water quality improvement within the aquaculture industry.

Ozone is an unstable double bonded form of oxygen represented by the formula: O_3

Ozone itself is a powerful oxidizing agent, capable of reducing several unwanted characteristics within aquaculture water (e.g. nitrite nitrogen). In addition, because ozone is unstable, it readily breaks down under normal water conditions to form ozone radicals, which also act as powerful oxidizing agents (can reduce or degrade other chemical compounds) [1,2,6,10,16].

When administered properly, ozone can be used within aquaculture production systems to degrade unwanted chemical compounds that hinder good aquaculture species growth. Some of the beneficial effects that can be produced by ozone in aquaculture systems include the reduction of color and nitrite-N and improved solids settling (important in recirculating aquaculture systems) [1,2,3,9,12,16]. In addition, ozone can also act a sterilizing agent and reduce microbial activity within water, which is important for replacement water in recirculating aquaculture systems [10,16,17].

Although ozone can be an effective tool in aquaculture, much consideration should be used before applying ozone to an aquaculture system. The primary areas of concern with ozone use include human and aquatic health (ozone can be harmful to both if used improperly) and cost (ozone generation can be expensive).

This manual is thus designed to introduce the concept of applying ozone to beginning aquaculturists and to establish some basic guidelines to consider before applying ozone. In this manual, we shall explain how ozone works within aquaculture systems, important design criteria to consider including dosages and application methods, describe costs of applying ozone, and finally, where to obtain additional ozone information and equipment.

Background/Use of Ozone in Aquaculture

The application of ozone in aquaculture began in the 1970's when researchers were evaluating the use of ozone to maintain aquarium systems. This usage was primarily one for sterilization whereby ozone proved effective in reducing microbial activity in systems that had low fish densities and low feed loading rates. Since this time, ozone research has been applied to all types of aquaculture production systems (from flow-through raceways to near complete recirculation systems) and has been evaluated for many different chemical compounds.

A brief listing of some of the more recent ozone research papers is presented in the References of this manual. It is recommended that the aquaculturist familiarize himself/herself with this literature prior to designing his or her own ozone application.

Ozone Generation

Ozone can be produced from any process that dissociates molecular oxygen into oxygen radicals [1,6,16]. Although there are several ways to dissociate molecular oxygen, including high-voltage corona discharge, cheminuclear sources, electrolytic processes, and UV light at wavelengths less than 200 nm, most commercial ozone generators used in the aquaculture industry produce ozone by the corona discharge technique[6,16]. In this technique, oxygen is passed through a gap formed by a high voltage electrode, dielectric and ground electrode[10]. The plasma formed in the gap is known as a Corona Discharge, and it is here that some of the oxygen molecules split and recombine to form ozone[10]:

O_2 + energy -> O + O

$O + O_2$ -> O_3

Because the resulting ozone molecule is so unstable (one of the reasons why it is an effective oxidizer) ozone must be generated on-site at an aquaculture facility.

Ozone can be produced from either an ambient air or pure oxygen. Both sources of ozone have advantages and disadvantages. Production of ozone is more efficient from pure oxygen (nearly twice the ozone produced per unit time[6,16]) as air is approximately 20% oxygen. Because of the cost of oxygen however, the use of oxygen for ozone generation may make the most sense when oxygen is already being used in the aquaculture system (more intense aquaculture applications). The ultimate decision factor for the ozone source however depends upon the amount of ozone needed for the aquaculture application and the economic optimization of the entire aquaculture system

Ozone Chemistry

Ozone has been shown to degrade and oxidize compounds both directly (ozone itself) and indirectly [1,2,6,10,16]. Ozone chemistry can be quite complex depending upon the particular compounds present within the water system and the characteristics of the water itself (e.g. pH and temperature).

Ozone and it's decomposition products oxidize many organic compounds at the chemical bond level, which make the compounds more susceptible to further biological degradation and increase settling properties [6,9,10]. In this way, ozone is not really a direct destruction technique, although it does act in that capacity for certain compounds, but rather as a facilitator for further degradation. This concept further supports using ozone as part of a treatment train and not as a stand alone technology for aquaculture systems.

Ozone oxidation reaction rates are rapid in typical aquaculture systems. The more contaminants present in the water (TOC), the more rapid the reaction rate [6,16]. Hydraulic retention time of ozone in the ozone reaction area of an aquaculture system is an important factor in design of ozone application systems because all the ozone should be 'used' up (fully reacted) with water contaminants prior to reintroduction to the fish culture water or the biological filter. This is because ozone residuals (partially reacted ozone) are toxic to aquatic organisms including fish [2,4,8,17].

Application Guidelines

The intended usage of ozone may differ for different aquaculture operations. Thus, the first consideration for the use of ozone in an aquaculture system should be the type of contaminant to remove. For example, a hatchery operation may choose to use ozone for microbial reduction/disinfection purposes, while a koi producer may wish to simply reduce color in water.

In general, ozone has been shown to be effective in producing the following effects:

- Reduction of color,
- Reduction of Nitrite-N (NO2) concentrations,
- Improved solids settling/separation
- Reduction of organic carbon, and
- Disinfection/microbial reduction.

A brief summary of findings on the above effects is as follows:

Reduction of Color:

- Water color is produced in aquaculture operations due to the addition of feed.
- Color can be an unwanted water characteristic of aquaculture systems because it decreases visibility.
- Ozone can be a very effective agent in reducing color
- Fingerlakes Aquaculture (2001) saw the reduction of up to 400 (potassium cobalt) color units in a full-scale recirculating aquaculture system through the addition of 10 mg ozone per Kg feed applied.
- Christensen et. al. (1996) at Louisiana State University measured the accumulation of color in a 1,500 liter fish production system due to feed and the subsequent destruction of color with the application of ozone. They determined the destruction rate of ozone to be approximately 7 – 16 g ozone per Kg of feed to remove the color produced by feed.
- Schwartz and Moncrieff (1976) used ozone at 4mg/L to remove turbidity and color from a drinking water supply with acceptable results.

Reduction of Nitrite Nitrogen

- Nitrite-N nitrogen is a by product of the biological oxidation of ammonia by bacteria. Nitrite-N is unwanted in aquaculture systems as it is toxic to most fish species at low concentrations.
- Ozone has been shown to be effective in reducing nitrite-N nitrogen in aquaculture systems.
- In general, ozone has not been very effective in reducing ammonia nitrogen at normal application levels

- Fingerlakes Aquaculture (2001) showed that concentrations of 2.1 and 2.3 mg/L ozone removed nitrite-nitrogen concentrations of 0.2, 0.6 and 0.99 mg/L by 89% to 99% in laboratory experiments.
- Rosenthal and Kruner (1985) showed in laboratory flow tests that ozone concentrations of between 4 mg/L and 20 mg/L were effective in removing nitrite nitrogen concentrations of up to 7 mg/L.
- Paller and Lewis (1988) found that ozone in doses up to 6 mg/L enhanced the performance of granular carbon biological filters in the removal of ammonia and nitrite by enhancing the bacterial removal of ammonia and directly oxidizing nitrite.

Improved solids settling:

- Solids is introduced into aquaculture production systems through feeding or through influent water,
- Solids are unwanted in aquaculture systems because they can be a source of unwanted dissolved nutrients, increased bacteria, increased biological oxygen demand on the system, and decreased water visibility,
- Several researchers have shown that ozone affects the surface chemistry of colloidal particles which in turn caused increased flocculation and settling (Hochheimer and Wheaton, 1995; Rice, 1986)
- In laboratory and full-scale studies by Fingerlakes Aquaculture (2001), the introduction of ozone into fish production water at a concentration of 10 g ozone/Kg feed did not reduce total suspended solids present in the water column.
- Research at the Freshwater Institute has shown that adding ozone at a rate of 25g ozone per Kg feed improved water quality and microscreen filtration (Summerfelt and Hochheimer, 1999).
- Results from studies by Pak et al., 1981 and Rice, 1986, using ozone for reducing suspended solids indicated that pilot studies may need to be performed in aquacultural systems to determine if the application of ozone will be effective.

Reduction of organic carbon

- Organic carbon is introduced into aquaculture production systems through feed application or influent water.
- Organic carbon is unwanted in aquaculture system because it can be a source of unwanted dissolved nutrients, increased bacteria and increased biological oxygen demand.
- Krumins et al., (2000), showed that ozone application rates of 10, 15, and 30 g ozone per Kg feed effectively removed up to 10 mg/L of TOC in laboratory recirculating systems.
- Rosenthal and Kruner (1985) showed that ozone concentrations of between 4 mg/L and 20 mg/L effectively removed biological oxygen demand (usually proportional to total organic carbon) of up to 4 mg/L in laboratory studies.

Microbial Reduction

- Excessive microbial activity can be deleterious to both target biofiltration and target species production.
- Microbial reduction is largely limited by the ability to maintain certain dissolved ozone concentrations for a given amount of time (Summerfelt and Hochheimer, 1999; Langlais et al. 1991)
- Several different researchers have shown that ozone can be effective at disinfecting a wide range of microbial species common to aquaculture systems such as Aeromonas salmonicida, Vibrio anguillarum, Vibrio salmonicida, and Yersinia ruckeri.
- For disinfection, the required residual ozone concentrations have been shown to be between 0.1 and 1.0 mg/L with hydraulic retention times of 0.5 to 20 minutes (Langlais et al., 1991; Lilved et al. 1995; Colbeg and Lingg 1978; Summerfelt and Hochheimer, 1998).

System Location and Design Consideration Recommendations

According to Summerfelt and Hochheimer (1999), the four factors that are used to determine ozone design are:

1. ozone gas generation,
2. gas to liquid absorption,
3. contact time for reaction, and
4. ozone residual removal

Recommendations:

A list of recommendations pertaining to the design and installation of ozone systems and the four points listed above is as follows:

Ozone Generation and Transmission

- There are several suppliers of commercial ozone generators capable of producing a range of ozone suitable for many different aquaculture applications. A partial list of commercial ozone generator manufacturers is included at the end of this manual.
- The amount of ozone produced by any commercial ozone generator depends upon the purity of influent air passing between the dialectric plates of the ozone generator. For highest output of ozone, pure oxygen is recommended.
- Pre-treatment of the ozone generator influent air or oxygen stream (filtration) to remove moisture, dust, and other impurities, is often recommended by ozone manufacturers and distributors to prevent fouling electrode surfaces of ozone generators.

- Hydrocarbon contamination is also a concern for dialectric plate discharge fouling (i.e. be sure to use pure source of air or oxygen to generate ozone),
- Some ozone generator distributors for ozone generation claim that very pure oxygen causes corrosion of the electrode plate (this claim is not supported by research or the manufacturer). To prevent corrosion, these ozone generator distributors recommend diluting very pure oxygen influent streams with up to 5% with nitrogen gas.
- Due to the highly corrosive nature of ozone, only ozone-resistant tubing, valves and fittings should be used in the ozone transfer system between the ozone generator and the ozone transfer device.
- Significant air ventilation is recommended in the vicinity of an ozone generator for human safety reasons,
- Ozone monitors and alarms are also recommended near ozone generators for human safety

<u>Ozone Transfer Devices</u>

- Ozone absorption, contact time, and residual removal are all taken into consideration when designing the ozone transfer device.
- The common methods for ozone transfer are the same methods used for oxygen transfer. Examples of ozone transfer devices include:
  - Bubbling chambers,
  - Mazzi Injectors,
  - Low-head oxygenators,
  - Oxygen cones, and
  - Membrane diffusers.
- Depending upon the location, the easiest way to apply ozone to an aquaculture system is through the existing oxygen transfer device (assumes one currently exists).
- The most efficient transfer devices will get the most ozone into the aquaculture production system
- An example layout of a full-scale ozone transfer device used in a full-scale fish production system (600-pound per day of feeding) is presented in Figure A-1.
- The removal of residual ozone should take place outside of the aquaculture production system to avoid undue stress on the target production species.

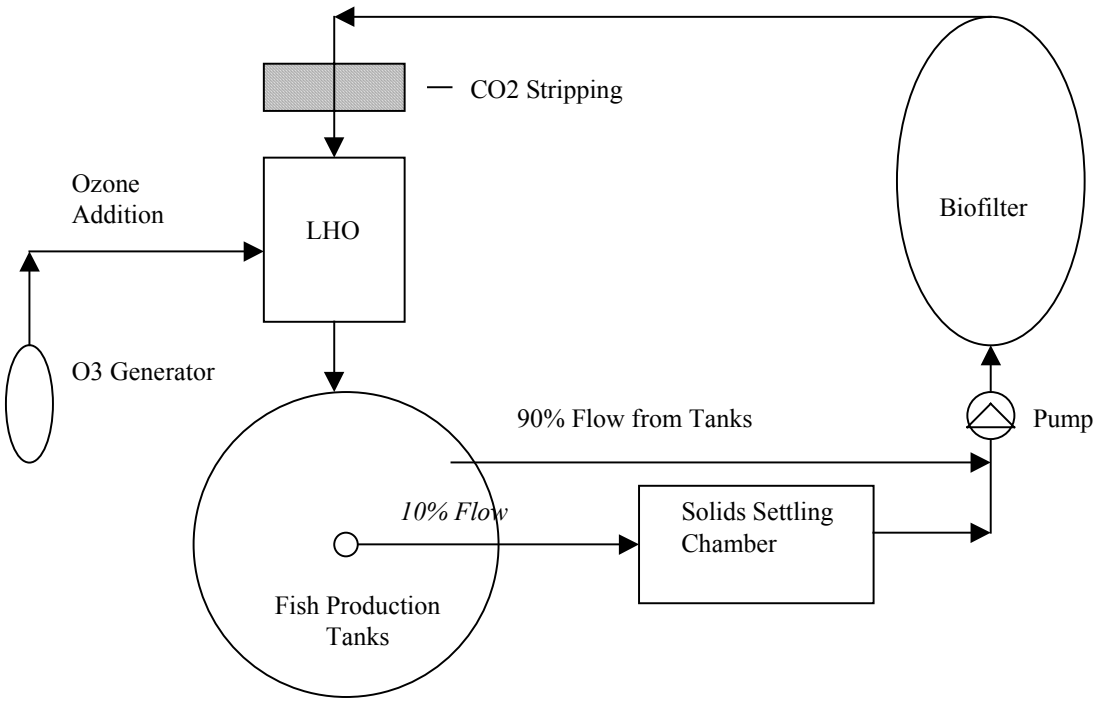

Figure A-1
Unit Process Flow Diagram depicting Ozone Administration Location

Conclusions

Based upon research conducted by Fingerlakes Aquaculture and other aquaculture researchers, there are four points from this Guidebook for aquaculturist that are considering the use of ozone in his/her aquaculture production system:

1. The application of ozone can be an effective means of water treatment in aquaculture production systems.

2. Ozone is most effective when applied as part of a larger water treatment system for end-of-treatment water polishing.

3. Great consideration of the target water quality parameter needs to be made prior to the decision to use ozone.

4. In addition to target water quality parameter, risk to both target production species and human health need to be considered prior to introducing ozone to an aquaculture production system.

Ozone Manufacturer/Distributor List (Partial Listing)

Ozonair International
1519 Brandywine Road, San Mateo, CA 94402

Ozonia, North America
491 Edward H. Ross Dr., Elmwood Park, NJ 07407

Pacific Ozone Technology
730 Concord Ave., Brentwood CA 94513

Stargate Intenational Inc.
10235 S. Progress Way, Units 7&8, Parker, CO 80134

Tri O_3 Industries Inc.
2254 North US1, Ft. Pierce, FL 34946

Yanco Industries, Inc.
390 Silver Queen Road, Burton, B.C. V0G IE0

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