RESEARCH TECHNICAL COMPLETION REPORT

Project A-053-IDA

OZONATION OF MAKE-UP WATER FOR SALMONID FISH REARING FACILITIES

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ABSTRACT

An ozone pilot plant was installed at the Dworshak National Fish Hatchery to examine the efficacy of sterilizing makeup water entering this recycle hatchery. The pilot plant actually consisted of two separate systems operated together. A recycle system consisting of two fish tanks, a clarifier and biofilter was in operation prior to this study. An ozone system consisting of a Grace ozone generator (later replaced by a Welsbach generator) and a Grace contacting column was installed for this study. The ozone pilot plant supplied the makeup water to the existing recycle system. The pilot plant was run with approximately 125 pounds of cutthroat and one-half pound of steelhead fry. Recycle rate was 30 GPM and makeup rate was 3 GPM.

Plate counts for total bacteria were taken daily from various points in the pilot plant during a continuous (24 hours/day) two week run. Similar plate counts were taken from existing ultraviolet sterilization equipment. Also monitored during the continuous test run were ozone residual levels into and in the recycle system, ammonia, nitrate, nitrite, total organic nitrogen, suspended solids, turbidity and biochemical oxygen demand.

Analysis of the plate counts showed the ozone consistently provided better sterilization of the makeup water than the existing ultraviolet system. Ammonia levels with ozone sterilization showed a 70 percent decrease while nitrate levels showed a 100 percent increase. Nitrite levels remained unchanged. Total organic nitrogen showed a 150 percent increase.

The recycle system was then altered to run 10 percent of the recycle water through the ozone pilot plant. This was done to determine if recycle water could be sterilized and if a general decrease of bacteria in the system could be realized. Process control problems with residual ozone levels caused shutdown after only four days of operation. However, enough data was collected to show that recycle water could be sterilized.

A trial was undertaken to determine the effect of an accidental ozone overdose on the fish in an actual operating system. Thirty percent of the recycle water was dosed with a 3 mg/l residual ozone level for five days in an effort to build up a residual in the system. No residual appeared in the fish tanks after this time indicating the system can tolerate a large accident without fish mortality.

Batch studies were also done on algae growths removed from the hatchery biofilters. These algae growths are so extensive on the biofilters they impair their operation. The batch studies indicated that ozone can effectively destroy algae. Retention times and dosage levels were not determined.

At the conclusion of the pilot plant study, an economic comparison was made of an ozone system and an ultraviolet system. The basis for comparison was a proposed 650 GPM system to be installed at Dworshak. Although the ozone

treatment system requires a capital investment of $164,000 as opposed to $90,000 for an equivalent size ultraviolet system and an annual cost of almost $17,000 as opposed to $12,000 for the UV system, this study demonstrates the increased cost may be justified. The ozone system gave consistently greater sterilization efficiency than the ultraviolet system. It also showed consistently lower ammonia level and more uniform BOD concentrations. All of these effects would enhance fish survival.

In addition to these measured benefits algae destruction was demonstrated. This would result in a lower algae growth rate in the system and enhanced biofilter efficiency. This would also decrease ammonia levels and increase fish survival.

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INTRODUCTION

The Dworshak National Fish Hatchery at Orofino, Idaho, is a recirculation type hatchery. Rearing pond water is treated in biofilters, filtered, aerated and recirculated back through the rearing ponds. A 10 percent portion of the recirculation water is bled off and fresh makeup water added to provide constant flow. The makeup water is presently being treated by filtration with sand filters and sterilization with ultraviolet lights.

Many problem areas exist in the hatchery which could be related to the existing makeup water system. These include:

(1) Algal blooms on the biofilter and in rearing ponds.
(2) Periodic infestation of fish pathogenic protozoans and bacteria.

The ultraviolet light system does not destroy algae entering the fish hatchery in the makeup water. In fact, it may actually promote algae growth. Tubes are often completely covered with an algal growth. During high algae growth rate periods, cleaning of the tubes presents a maintenance problem. If the tubes are not cleaned they become covered with algae and "scum" and their sterilization action on bacteria and viruses is drastically reduced. These ultraviolet tubes also present a problem in that they must be replaced twice a year. This is an expensive, time consuming project involving several hundred tubes and takes personnel from other required work.

The effect of ultraviolet lights on protozoans is not known. These continuing problems have led to the search for a better method of treating the makeup water. A treatment which will completely sterilize the water including protozoans and algae is desired. Ozone treatment in makeup water is the method being evaluated in this study.

Ozone is the second most powerful oxidizing agent known. It is currently in general use in Europe for treatment of potable water. It is starting to come into widespread use in the United States for tertiary treatment of waste water. Unlike chlorine, ozone breaks down naturally with a half-life of 15 minutes[1] to free oxygen. This release of oxygen enhances the water quality rather than leaving a harmful residual. Ozone has also been shown to reduce turbidity, color and odor of water.[2]

Many researchers have studied the properties of ozone in aqueous solution and its effects on various organisms. [1,3,4] A summary of their results indicates ozone is lethal to an average of greater than 96 percent of the test bacteria and virus in less than five minutes contact time and with a residual concentration of less than 0.1 mg/l. Giese and Christensen[5] have shown that ozone is lethal to some protozoans in four minutes while others survived for as long as two hours. All protozoans showed signs of damage in a short time. Whether this damage is sufficient to prevent reproduction, is not know. The effect of ozone on algae is unknown, however, Homan[6] has shown that plant life

is adversely affected by ozone in the air. The plants showed bleaching and wilting.

Few studies have been done to determine the affects of ozone on hatchery recirculation systems or fish. Rosenlund[7] reported in a study at Whiteriver, Arizona, that a residual ozone concentration as small as .01 mg/l produced 100 percent mortality of rainbow trout in four hours. Ozone has also been in continuous use for over one year as primary treatment in a recycle oceanarium in Orlando, Florida.[8] The oceanarium system is entirely closed with no bleed off and makeup is only for leakage and evaporation. The marine mammals and fish have shown no detrimental effects from ozone. BOD levels and bacterial level in this system have remained quite low.

The Fish and Wildlife Branch of the Department of Recreation and Conservation of Canada contracted a study[9] to evaluate the use of ozone for water treatment in a proposed fish hatchery. This proposed hatchery, the Abbotsford Hatchery, is similar in design to the Dworshak Hatchery. It is a recycle hatchery with a constant bleed and makeup of water. Unlike Dworshak, the makeup water is obtained from deep wells. The study consisted of using a Grace Ozone Generator and Grace Contacting Column identical to the ones used for this study. Water was ozonated, mixed with well water to obtain the same dilution as the proposed hatchery, passed through egg baskets and then fish rearing troughs, and released to a nearby creek. This study showed that a sterilization of bacteria of 99.6 percent was normal and that the fish were not harmed. It also demonstrated that any residual ozone was completely destroyed when the ozonized water was mixed with recycle water in a ratio of 1:4. Mortality studies were also run showing that a residual ozone concentration of .01 mg/l caused a 35 percent mortality in 195 minutes.

The primary objective of this study was to operate an ozone test unit to treat the makeup water of a recycle pilot plant, The pilot plant was operated on a recycle basis with clarification of recycle water and ammonia removal by biofilters. Makeup rate was 10 percent and from the North Fork of the Clearwater River, the same as the full scale system at Dworshak. The pilot plant used five to ten inch cutthroat trout in one fish tank to provide the loading and steelhead fry in another tank.

The study was run in this manner to test the effect of ozone on an actual operating recycle system. It is important to run the study in actual hatchery water because the rate of reaction of ozone varies with the quality of the water. As organic and inorganic contaminants in the water increase, more ozone will be consumed in oxidizing these contaminants. Less ozone will remain for sterilization. It is important, then, to determine degree of sterilization in actual hatchery water.

Factors examined during the pilot plant trials are as listed below.

1. Sterilizability of river water by ozone on a continuous basis.
2. Determination of residual level ozone required for sterilization.

3. Ease of operation of an ozone system on a continuous basis.
4. Control of residual levels, in makeup water and in pilot plant system.
5. Effects on nitrifying bacteria.
6. Effects on fish--especially steelhead fry.

On completion of the makeup water treatment run, the system was altered for treatment of part of the recycle stream. The same parameters were then examined.

On completion of the recycle trial a study was run to determine how large an ozone residual can be tolerated without fish mortalities. Also secondary effects of an ozone accident were determined.

Prior to the pilot plant work, laboratory studies were completed to determine rate of decay of ozone in hatchery water. Also studied in the laboratory were the affects of ozone on algae, and the efficacy of ozone as a sterilant.

After completion of the above studies, an economic analysis was made. The analysis determined the capital and operating costs of an ozone treatment system. The system design is based on data collected above. Finally, the cost of an ultraviolet treatment system of equivalent size was compared to the ozone system.

ANALYTICAL PROCEDURES

Bacteriological Examination:

Bacteriological studies were carried out by plate count. Disposable presterilized petri dishes were used with a media of commercial Trypticase Soy agar. The agar was prepared for pouring into the petri dishes by the following method.

> Mix 40 grams powder Trypticase Soy agar with one liter of distilled water in a suitable flask. Cover with aluminum foil and place in an autoclave for twenty minutes at 15 PSIG. After autoclaving and before cooling, place flask in a constant temperature bath at 55 degrees C. When agar starts to thicken, pour into petri dishes. Flame mouth of flask frequently during pouring. Pour as per Standard Methods.[10]

After pouring, the petri dishes were stored at room temperature overnight then placed in a refrigerator for storage until use. While in the refrigerator the petri dishes were inverted and covered to prevent contamination.

Sampling

> The method of sampling for bacterial counts was determined in conjunction with the University of Idaho Bacteriology Department[11] and is follows:
>
> If the sample is from a sample valve, open valve and allow to flush for one minute. If the sample is from a tank, a grab sample is taken. A sterile test tube is rinsed once with sample water then filled. A sterile 1/10 ml/inch pipette is used to withdraw sample from the test tube. One-tenth ml of sample is placed on each of three petri dishes. The sample is spread evenly over the agar surface using a sterile "hockey stick." Care must be used not to tear the surface of the agar.
>
> The plates were incubated for three days at room temperature to prevent overgrowth of colonies in the more contaminated samples and yet allowed detection in the less contaminated samples. During incubation the plates were inverted, covered and kept from drafts. The results were multiplied by ten to obtain bacteria per millimeter.

Ozone in Gas

During the laboratory work, calibration of the ozone generators required a method for determining concentration of ozone in gas. Ozone in oxygen was determined by the standard iodometric method. For this test the ozone containing gas was passed into a washing bottle containing one liter of 0.1N KI solution.

A second washing bottle containing KI was connected in series with the first bottle to insure all ozone was absorbed. The gas discharge volume was measured by a wet test meter. After ozonation, the KI solution was placed in 2,000 ml beaker. The washing bottle was rinsed with distilled water and the rinse water added to the beaker. The sample was then titrated using standardized sodium thiosulfate and starch indicator.

Ozone Dissolved in Water:

A method for measuring ozone dissolved in water was adopted from "Pilot Plant Tertiary treatment of Wastewater with Ozone."[12] This method is as follows:

> Add 80 ml of 0.1N KI and 1 ml of .05N KOH to a 500 ml beaker. Fill beaker to 480 ml with ozonated sample. Place beaker on a magnetic stirrer and while stirring add 20 ml. 0.1N sulfuric acid. Titrate with standardized sodium thiosulfate and starch indicator.

The Following Analysis Was Done as Per Standard Methods Techniques:

> Nitrates in solution (Brucine method)
> Nitrites in solution
> Ammonia in solution (Nesslerization method)
> Biochemical oxygen demand
> Total organic nitrogen (Kjeldahl method)
> Alkalinity
> Dissolved oxygen
> Suspended solids

These samples were taken at Dworshak, placed on ice and returned to University of Idaho for analysis on same day.

Turbidity:

Turbidity of water was determined using a HACH Model No. 2100A turbidimeter which reads directly in Jackson turbidity units.

pH:

pH levels were determined using three different pH meters. First, a Corning Model 610A portable pH meter was used. Subsequent problems caused replacement with a Markson Model 80 digital pH meter. This meter was found to be inaccurate outside of a small band around pH of 7.0. This meter was replaced with a Beckman Zeromatic SS-3. This stopped working after several days. pH was not measured in the field after this.

Dissolved Nitrogen in Solution:

Dissolved nitrogen gas was determined using a Weiss Saturometer Model No. ES-2.

General:

It was also desired to find a method of measuring dissolved ozone which was readily adaptable to field work. This method would then be tried in the field and if successful, would be recommended as useful in a full scale system. In this context the Schechter method[13] was evaluated. After laboratory trials it was decided not to use this method in the field. There were several reasons for this, First, and most important, was that turbidity in the water caused inaccuracies in the results. A waiting time of 30 minutes, to allow color development in the sample was also considered unsatisfactory for field work. This method was, however, used in the laboratory and gave very accurate results within certain limits.

The second method evaluated was the Orthotolidine Manganese-Sulfate Method (OTM)[10] for residual ozone. This method involves use of a chlorine color comparator to find residual "chlorine" level. This is then divided by 1.45 to give residual ozone. This method was found to be less accurate than the Iodometric or Schechter techniques. It is very rapid in that results can be obtained within one minute of sampling. This method has been in satisfactory use at the Whiting, Indiana Potable Water Treatment Plant for many years. It has one major drawback. The chemical Orthotolidine (4,4'-diamino- 3,3'-dimethyl-biphenyl) is the primary reagent. This is a suspected carcinogen and must be handled with extreme caution.

For the majority of this study the Iodometric technique was used, since it is slightly more accurate than the OTM technique. The OTM technique is limited by the color comparator accuracy. The Iodometric technique was difficult to use in the field in that daily standarization of the Sodium Thiosulfate was required. Also, several different chemicals (0.1N $Na_2S_2O_3$, 0.01N $Na_2S_2O_3$, KOH, 0.1N H_2SO_4, Conc. H_2SO_4, 0.1N KI) were required and much (somewhat cumbersome) glassware which needed constant cleaning and rinsing with distilled water was required. Once these nuisance type problems were worked out, the Iodometric technique was satisfactory.

EQUIPMENT DESCRIPTIONS

Numerous equipment was used during this study. A list and description of important pieces is given below, along with appropriate comments. Also included is a description of the pilot plants and their daily operating requirements and sample points.

Grace Ozone Generator Model LG-2-L2: (air cooled, gas flow 10 to 100 SCFH).

This ozone generator was used extensively during the laboratory studies. However, early in the continuous pilot plant run it experienced a major failure. Replacement of electronic components failed to repair the generator. The Union Carbide Company, the manufacturer, was contacted and it was established that warping of the Lowther tube had occurred. This repair took over a week to accomplish and the unit was not placed back in continuous service during the rest of the trial. It was, however, used as a back up unit.

Welsbach Ozone Generator Model C1-D: (water cooled)

This older model Welsbach unit was recovered from a previous installation[2] and rebuilt as a single unit. Even though this unit is simpler in construction than the Grace unit, in that it does not use high frequency current, it produced a higher ozone concentration in the gas. It was used as the primary unit during the continuous pilot plant run and operated continuously for two weeks with no maintenance.

Constant Voltage Source:

To prevent voltage fluctuations from affecting the ozone concentration, constant voltage transformers were connected between the ozone generator and the power source.

Contact Column:

A Grace experimental contacting system was used to contact the ozone into the raw water. This column is shown in Figure 1. The actual method of mixing is Grace proprietary information, but the column, after mixing, consisted of three concentric pipes. The gas, dispersed in liquid mixture, passes down the innermost column, up the middle column and down the outer column. Residence time can be varied by varying the level in the outer column. For this study a standpipe was used which kept the outer column full. A sample valve was installed on the standpipe which allowed sampling at the column outlet.

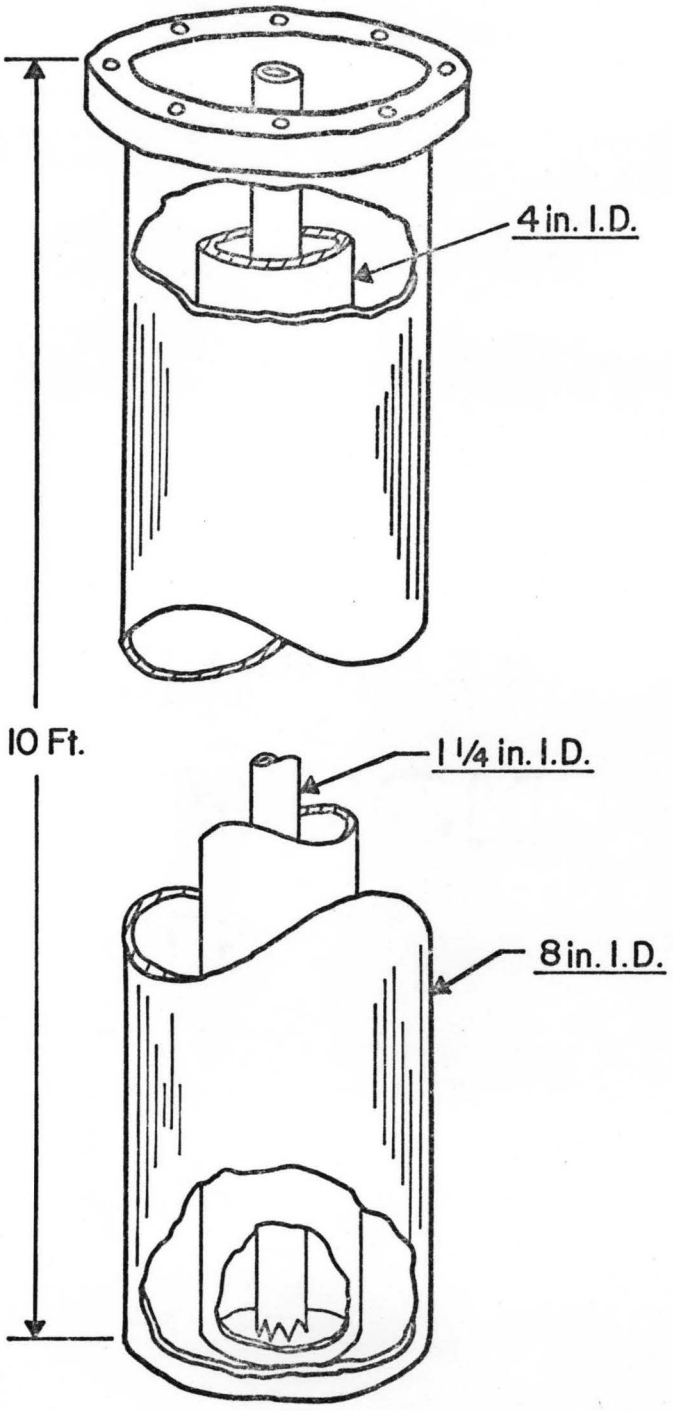

Figure I. Ozone Contact Column

Prior to the continuous run, a test was made to determine overall efficiency of the contact column. For this test the gas washing bottle was connected to the vent of the contact column. The generator was preset to a calibrated concentration and residual out of the column was measured. The concentration in the vent gas was also measured. The test showed that at 3 GPM and 20 SCFH the water was only absorbing about 61 percent of the delivered ozone. The low efficiency probably comes from the low flow rate of liquid. Efficiency was not tested at higher flow rates since they were not pertinent to this study.

Wet Test Meter:

For volumetric measurement of gas, a Precision Scientific Wet Test Meter No. 63125 was used. This meter has a maximum flow rate of 24 Ft^3/Hr and a minimum flow rate of 2 Ft^3/Hr. It was used to calibrate the ozone generators.

Ozone Pilot Plant:

The ozone generating and contacting pilot plant consisted of the generator and contact column. A header for connecting three gas cylinders to the generator supplied the gas. At the set flow of 20 SCFH the three cylinders supplied enough gas for 36 hours of continuous operation.

Once the plant was started for the continuous run it required very little actual attention. Daily operating instructions are listed below:

1. Check residual ozone concentration in makeup water. Adjust ozonator power as necessary to obtain required residual.
2. Check for residual ozone in clarifier (see biofilter pilot plant/flow schematic), inlet and outlet.
3. Bacteria plate count samples (daily):
   Raw water (from sample line)
   Post UV (from incubator water)
   Post ozone (from sample line)
   Clarifier (grab sample)
4. Change gas cylinders

Biofilter Pilot Plant:

The biofilter pilot plant was in existence at Dworshak National Fish Hatchery for several years prior to this study. It was in use primarily by the University of Idaho Department of Civil Engineering for studying various types of media used to grow nitrifying bacteria. The plant was a self-contained recycle system designed to operate in the same manner as the hatchery.

A schematic diagram of the plant is shown in Figure 2. It consisted of:

(4)-500 gallon fiberglass tanks
(1)-biofilter tower--wooden 41" x 44" x 10 feet.
(1)-pump

Two of the fiberglass tanks were connected in parallel and were used as fish ponds. One contained one half pound of steelhead fry (at 500 to the pound). The other contained 150 pounds of cutthroat trout, which were primarily for loading on the biofilter.

The two fish tanks drained by gravity flow to another 500 gallon tank which was used as a clarifier. This tank was baffled to minimize short circuiting, and had an automatic sludge scraper. The sludge drained by gravity flow to a sludge basin. The supernatant liquid flowed out of the clarifier through a weir to the pump reservoir (the last 500 gallon tank). From the reservoir it was pumped to the bottom of the biofilter tower, and passed up through the biofilter media and out to the fish tanks via a weir in the top of the tower. The biofilter media was Norton rings and provided a culture surface for nitrifying bacteria which oxidized ammonia to nitrates.

Total system volume was 3,000 gallons. Recirculation flow rate through the system was 30 GPM. Makeup water flow rate was 3 GPM. Bleed off, out of the pump reservoir by overflow, was also 3 GPM.

The cutthroat fish tank had automatic fish feeders connected above it. These feeders fed the cutthroat approximately 2.3 pound of 1/8" fish pellets per day. The steelhead fry were too small to be fed by automatic feeders and were fed on a daily basis by Dworshak operating personnel.

Daily operating instructions and sampling points are given below:

1. Sample procedure: (see Figure 2).
   Monday, Wednesday and Friday:
   2 liter sample at each of the following: biofilter outlet, fish tank outlet and settling tank outlet.
   Friday: 1 liter sample at base of biofilter.
2. Fish feeding:
   2.3 pounds fish food per day into automatic feeders.
   (steelhead fry fed by hand by Dworshak personnel)
3. Record on chart:
   sludge depth
   water temperature
   Pounds fish feed
4. Drain sludge basin
5. Clear all drain valves by opening momentarily
6. Visually inspect fish for mortality or signs of stress. The water samples were iced and removed to the University of Idaho for measurements as previously described.

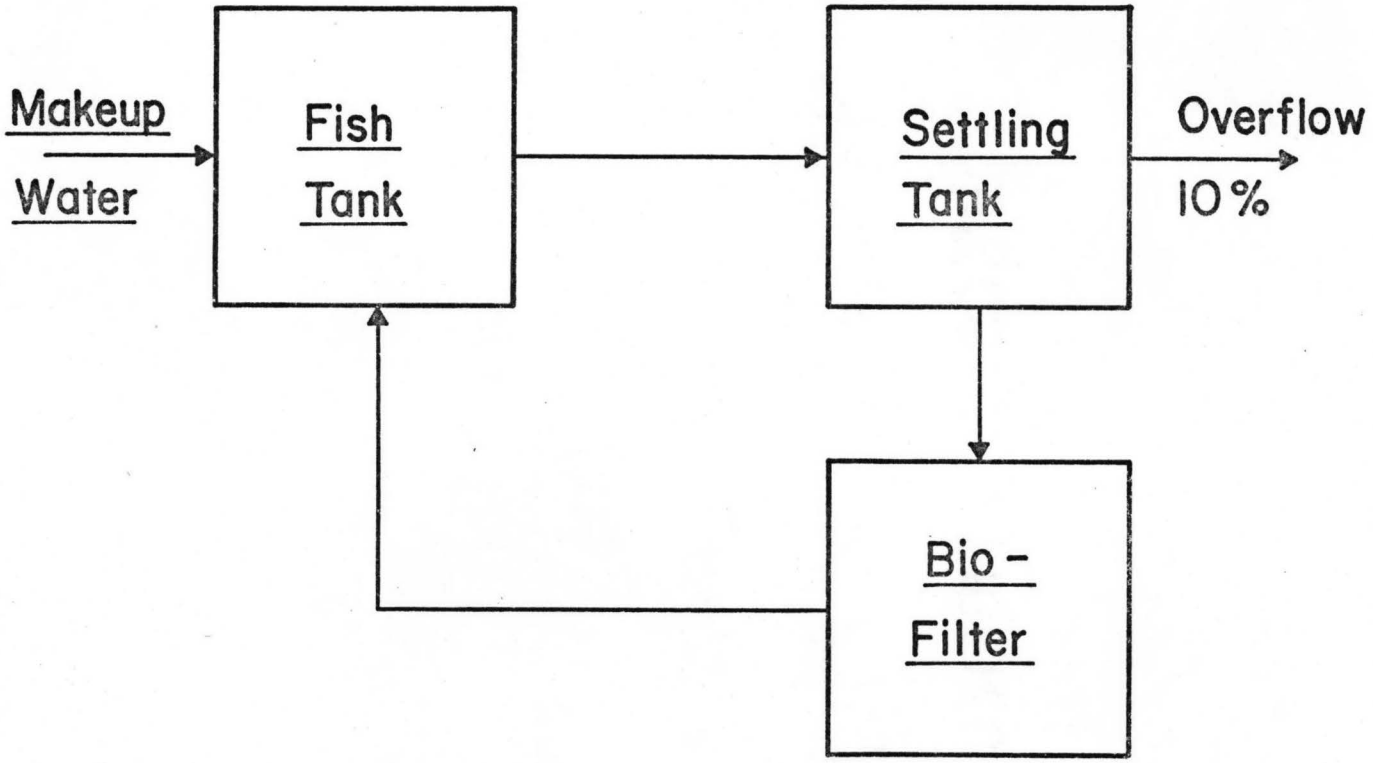

Figure 2. Flow Schematic, Existing Biofilter Pilot Plant At Dworshak

Integrated Pilot Plant:

The pilot plants were combined and operated as a unit for the study. A schematic of the integrated system is shown in Figure 3. This was the system as operated with ozone treated makeup water. Figure 4 shows the integrated pilot plant as it was operated during the ozonation of recycle water.

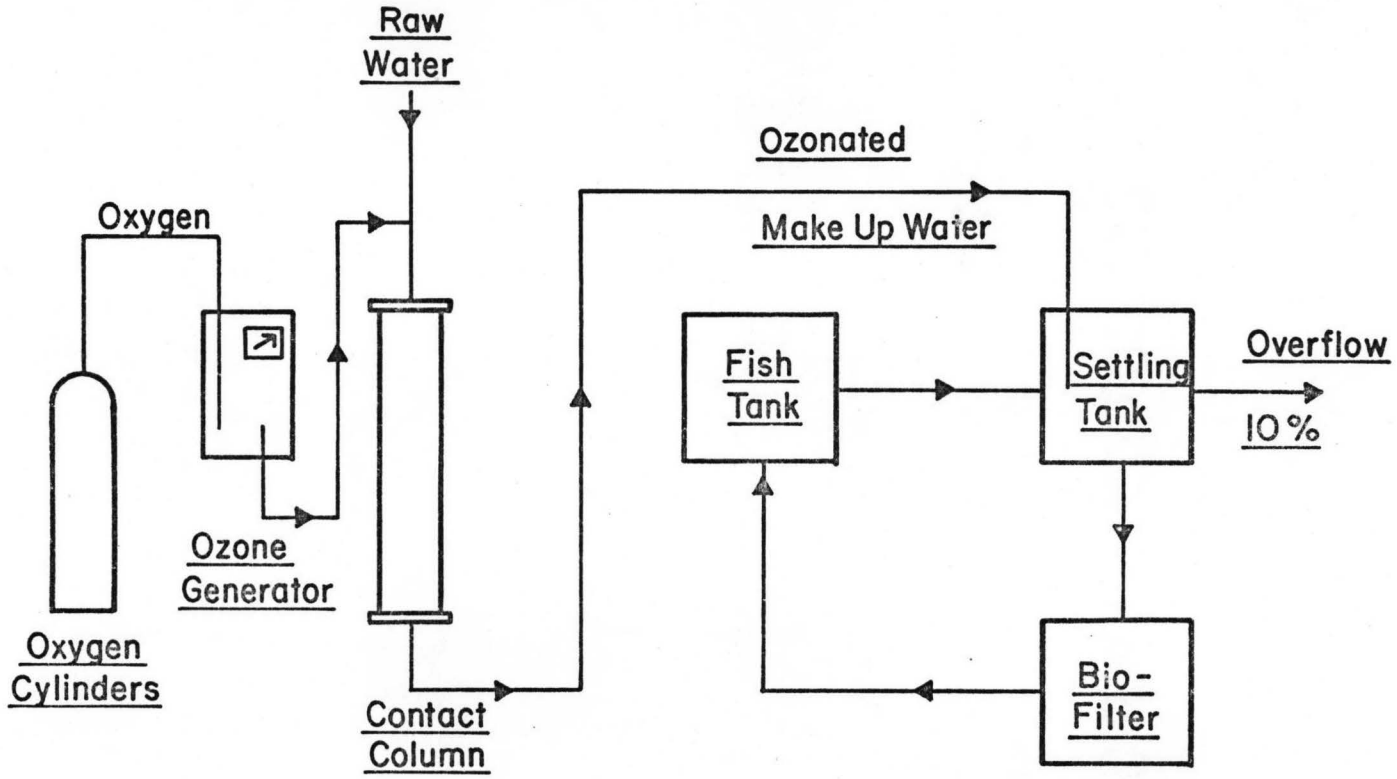

Figure 3. Flow Schematic - Integrated Pilot Plant:Make Up Water Ozone Treatment

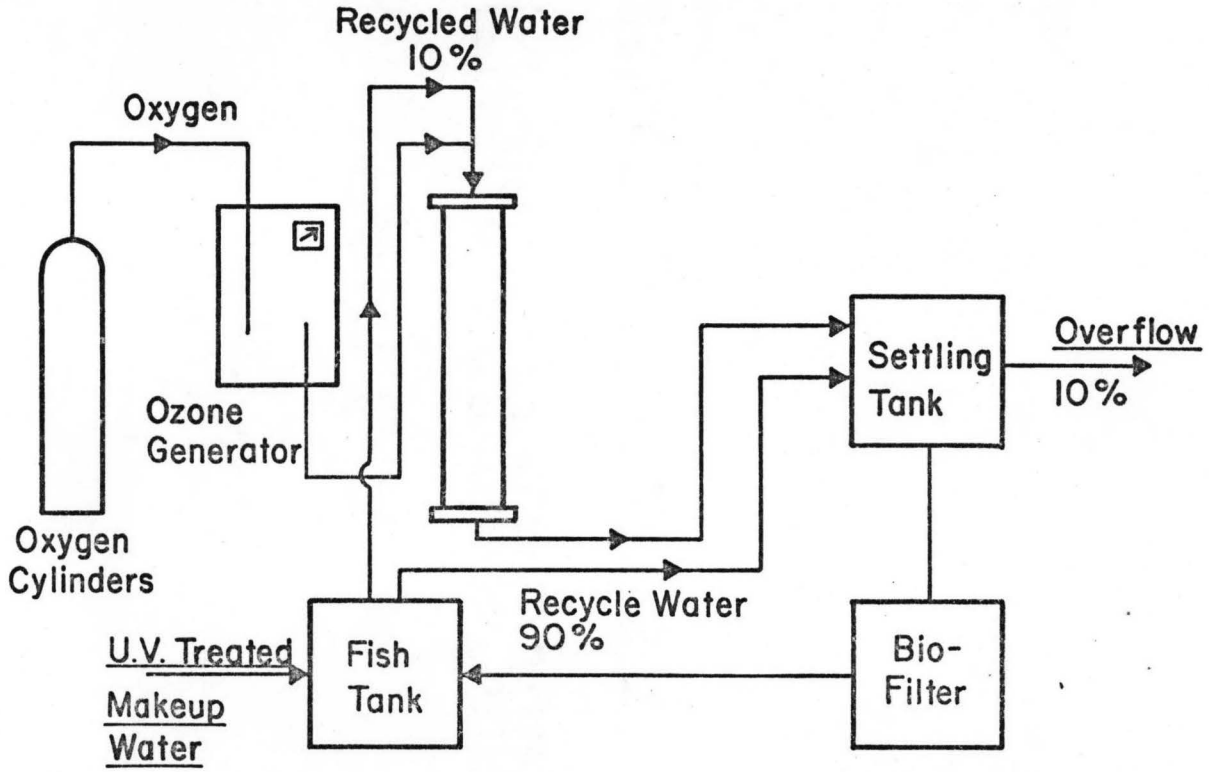

Figure 4. Flow Schematic – Integrated Pilot Plant:Recycle Water Ozone Treatment

LABORATORY STUDIES

Several laboratory studies were necessary before the integrated pilot plant run could begin. There were three objectives of these studies. First, to become familiar with the operation of the equipment and verify it was functional. Second, to find preliminary operating parameters. Third, to show that in a laboratory trial, the objectives of the integrated pilot plant run could be achieved. To this end, the laboratory studies included:

1. Verify and become familiar with a satisfactory method of ozone measurement.
2. Calibrate the equipment.
3. Show that sterilization can be achieved.
4. Show that algae growing in the fish hatchery biofilters is susceptible to destruction by ozone.

Ozone Measurement Techniques:

The first laboratory work was to verify and become familiar with methods for measuring ozone. These methods are discussed in the section on analytical methods, but are listed below for reference:

1. Iodometric - concentration in gas.
2. Iodometric - concentration in liquid.
3. Schechter analysis - concentration in liquid.
4. Orthotolidine Manganese-sulfate Method-concentration in liquid.

Unless otherwise stated, the Iodometric technique was used in the studies listed below.

Equipment Calibration:

After enough trial runs to become familiar with the analytical methods, calibration of the equipment was begun. It was felt that reproducibility of the methods, which have already been proven by other researchers, and equipment calibration could be carried out together.

The first calibration completed was the Grace ozone generator. At constant watts (150) gas flow through the generator was varied to produce a concentration in gas versus gas flow rate curve. This was accomplished by ozonating a known volume of potassium iodide and measuring the gas volume in the wet test meter. This was done for six different flow rates. A similar calibration curve was then developed for the Welsbach unit. Calibration details are in Reference 22.

The curves, as worked out, were much closer than one would have suspected. This was an unexpected benefit because it meant the Welsbach unit which was the backup unit (and actually ended up as the primary unit) could be interchanged with the Grace unit with little change of operating parameters.

Preliminary Sterilization Studies:

After calibration of the ozone generating units, trial studies were begun to see if sterilization could actually be achieved. This work was done in conjunction with the University of Idaho Department of Bacteriology. The analytical method for residual ozone used was the Shechter technique. The test bacteria chosen were Bacillus polymyxa spores, and the fish pathogens, Aeromonas salmonicida, Aeromonas liquifaciens, Pseudomonas fluorescens, and the causative agent of Hagerman Redmouth Disease. B. polymyxas spore was chosen because of its toughness. It was felt that if significant destruction of these could be achieved, other bacteria would be even more susceptible. References 21 and 22 and Appendix A contain the complete details of the laboratory sterilization studies.

The laboratory trial runs were all very encouraging. They indicated that a significant kill could be achieved within the contact time in the pilot plant contact column. No attempt was made to determine a minimum ozone to sterilization model. It was felt ozone demand of fish hatchery water would make any attempt to find minimum laboratory ozone requirements of little value.

Ozonation of Algae:

The ozonation of algae was a batch study with no attempt to find kill rates versus concentration of ozone. It was only desired to find whether or not ozone will destroy algae in a reasonable time. To do this, a trip was made to Dworshak and five gallons of biofilter water with a large amount of algae in it was obtained and transported immediately to the University. Approximately one and one half liters of this water was placed in a gas washing bottle. The ozone was then turned on and allowed to pass through the sample. Ozone flow rate and concentration was not determined, because it was necessary to change flow rates during the run, due to foaming. Immediate change was noted on the algae. Foaming and color change were noted within two minutes. At the end of fourteen minutes no green color remained in the algae. Observation under a microscope prior to ozonation identified one of the species as Spirogyra. The other observed was not identified. Inspection of the water under a microscope after ozonation showed the cell walls to be intact. However, all chlorophyll was bleached colorless. The majority of the cells had only the walls remaining, the insides being completely empty. Some cells in the centers of large algae clumps showed some chlorophyll remaining. This was, however, broken up into small clumps and not in a continuous chain as is typical of food storage in Spirogyra.

A control study was also done using oxygen. This was run to insure that it was not the physical action of gas bubbles passing rapidly through the sample that destroyed the algae. At the end of fourteen minutes, no change in the algae was noted with oxygen only. No foaming occurred during the oxygenation. It was concluded that the destruction of the algae was not due to physical mixing.

PILOT PLANT STUDIES

Prior to startup of the pilot plant for a continuous run, several one day runs were made to check for residual ozone levels. For these runs, raw water was ozonated in the contact column then passed to the sewer. Residual levels leaving the contact column were measured at various water flow rates, gas flow rates, and power levels. It should be noted that for 10 GPM water flow, residual ozone concentration decreases with increasing gas flow rate. However, at 20 GPM, residual first increases, then decreases with increasing gas flow rate. This phenomenon, along with the alternating slopes of the curves indicates a complex mechanism occurring in the column.

Prior to ozonation of the biofilter pilot plant, fifteen of the steelhead fry were dissected for gill studies. Photographs of the fish gills were taken. It was noted that some edema and "clubbing" of the lamellae was present. These fish were examined by the resident fish biologist at Dworshak who determined they were normal recycle hatchery fish.

A complete chronological account of the continuous pilot plant operation is detailed in Reference 22.

To summarize the study, a two week run of continuous ozonation of makeup water was successfully completed. This consisted of one week of oxygen as feed gas to the ozone generator and one week of air as the feed gas. The pilot plant was then realigned to treat 10 percent of the recycle water flow (see Figure 4). Difficulty in attaining a steady state condition of residual ozone out of the treatment column caused termination of this experiment after three days. The accidental ozone excursion experiment was then run for three days. At the conclusion of this experiment, the pilot plant was dismantled and returned to the University of Idaho.

PILOT PLANT RESULTS

Results of data collected during the continuous ozonation run are shown graphically in Figures 5 through 9. Additional details are given in Reference 22. Each figure, or group of figures is discussed separately below.

Figure 5: This shows ozone, UV and raw water bacteria levels. The ultraviolet sterilizers lower the bacteria level in the water. Ozone sterilization lowers the level of bacteria by a factor of three to ten more than the ultraviolet sterilization.

Figures 6, 7 and 8: These figures show nitrite, ammonia and nitrate levels in the fish tanks. During the continuous run, ammonia levels are lower than the run without ozone. Nitrate levels are higher during the ozone run. Nitrite levels are not significantly affected.

Figure 9: Biochemical oxygen demand shows no significant increase or decrease. There is a leveling affect during the ozone run.

Mortality Studies

The fish killed during the mortality study were histologically examined to determine if ozone caused other changes than on gill tissue. Tissues examined were heart, liver, gill, kidney and gastrointestinal tract.

The gills showed severe edema and desquamation of epithelium. This level of ozone in the fish tank (0.3 mg/l) was sufficient to attack and destroy the epithelium of the lamellae. Death was most probably from osmoregulatory failure.

Livers showed congestion and mild diffuse necrosis of liver cells. Kidneys showed mild necrosis of tubule epithelial cells and tubule lumens contained large quantities of proteinaceaus material. The pyloric caeca and intestine showed necrosis of the mucossa and submucossa. Heart muscle was stringy and pale staining with mild necrosis of muscle cells in the outer cortical layer.[17]

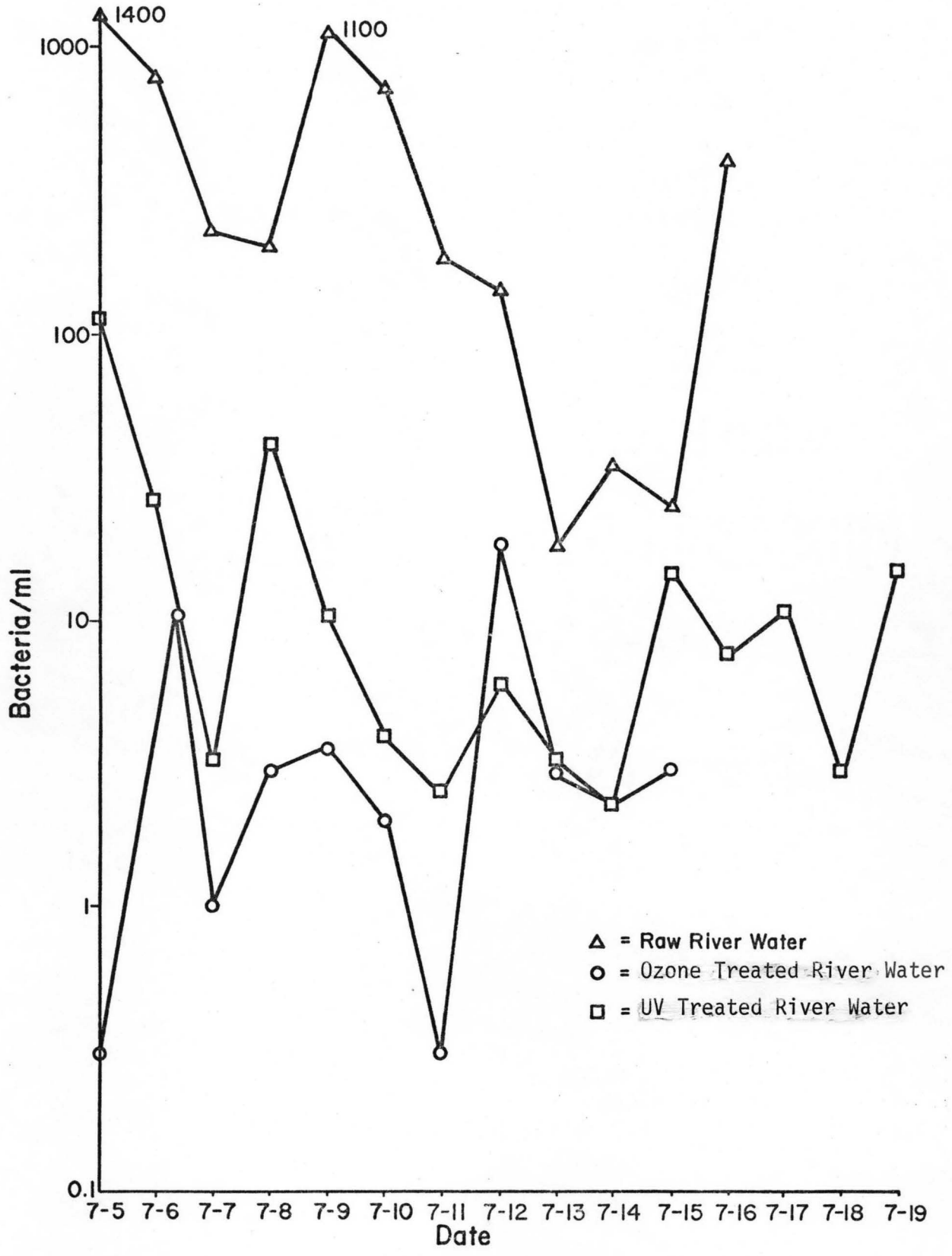

Figure 5. Total bacteria during continuous ozonation run.
Plate counts--average of three plates per sample.

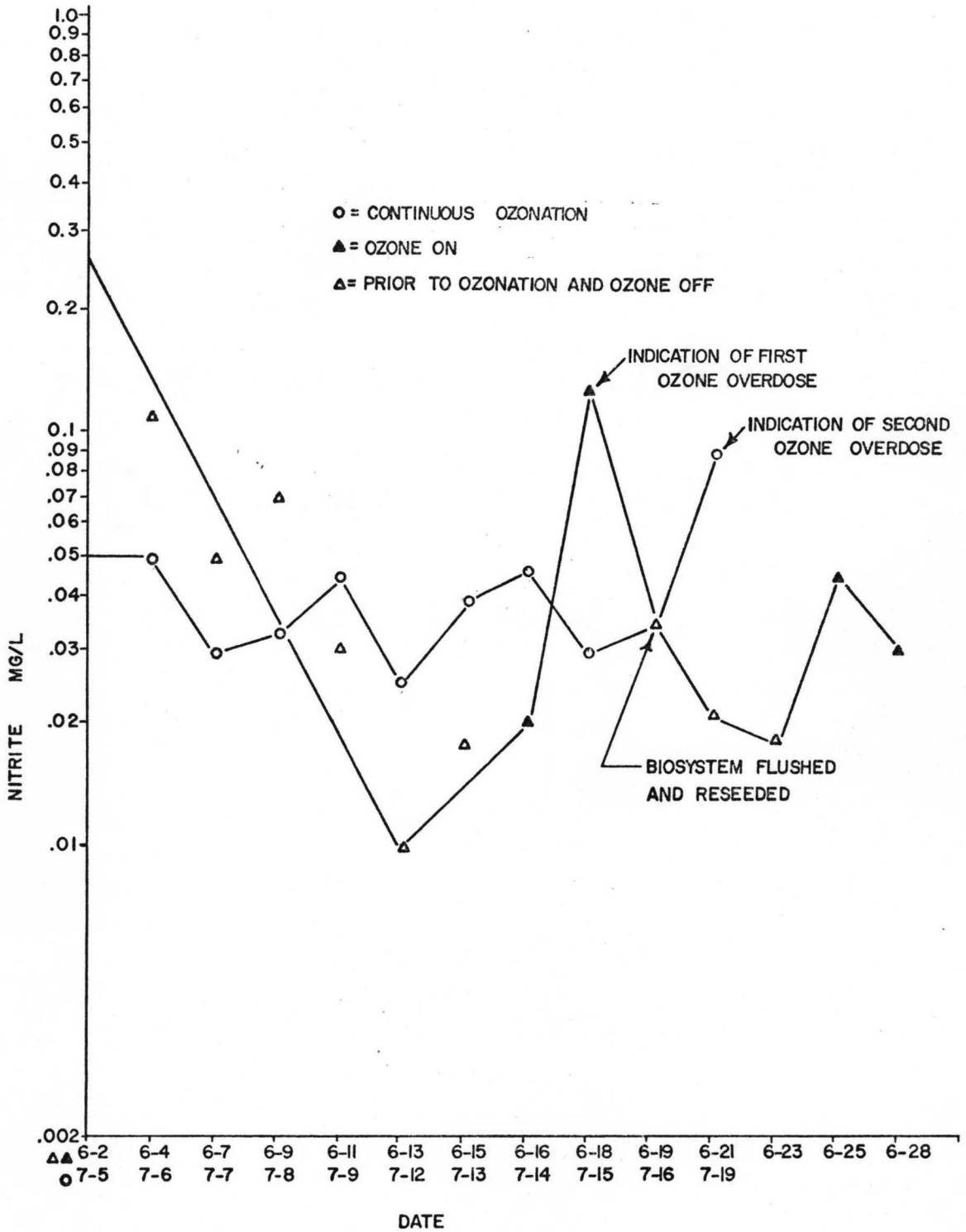

Figure 6. Biofilter pilot plant nitrite concentrations before and during continuous ozonation trial run.

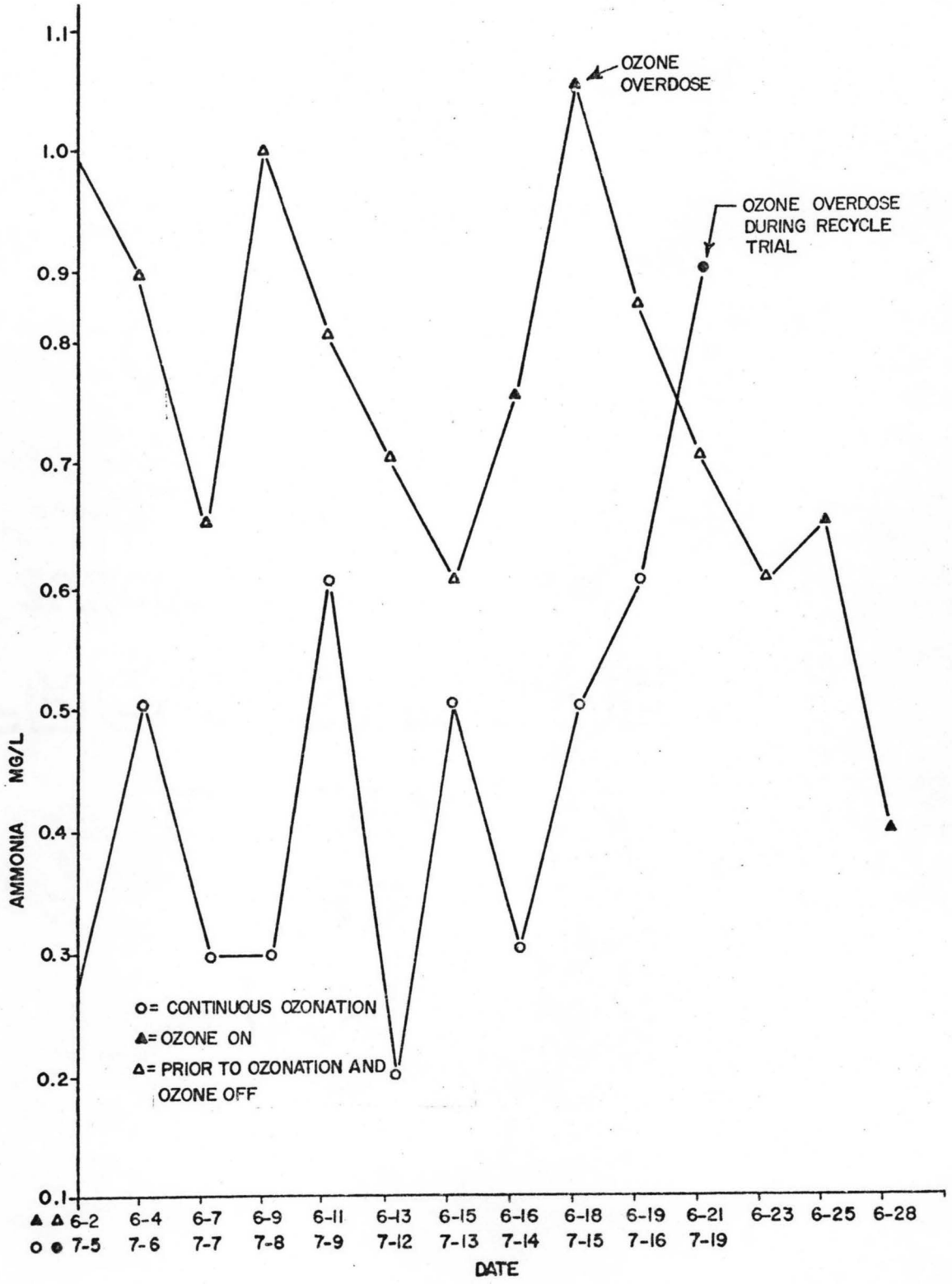

Figure 7. Biofilter pilot plant ammonia concentrations before and during continuous ozonation trial run.

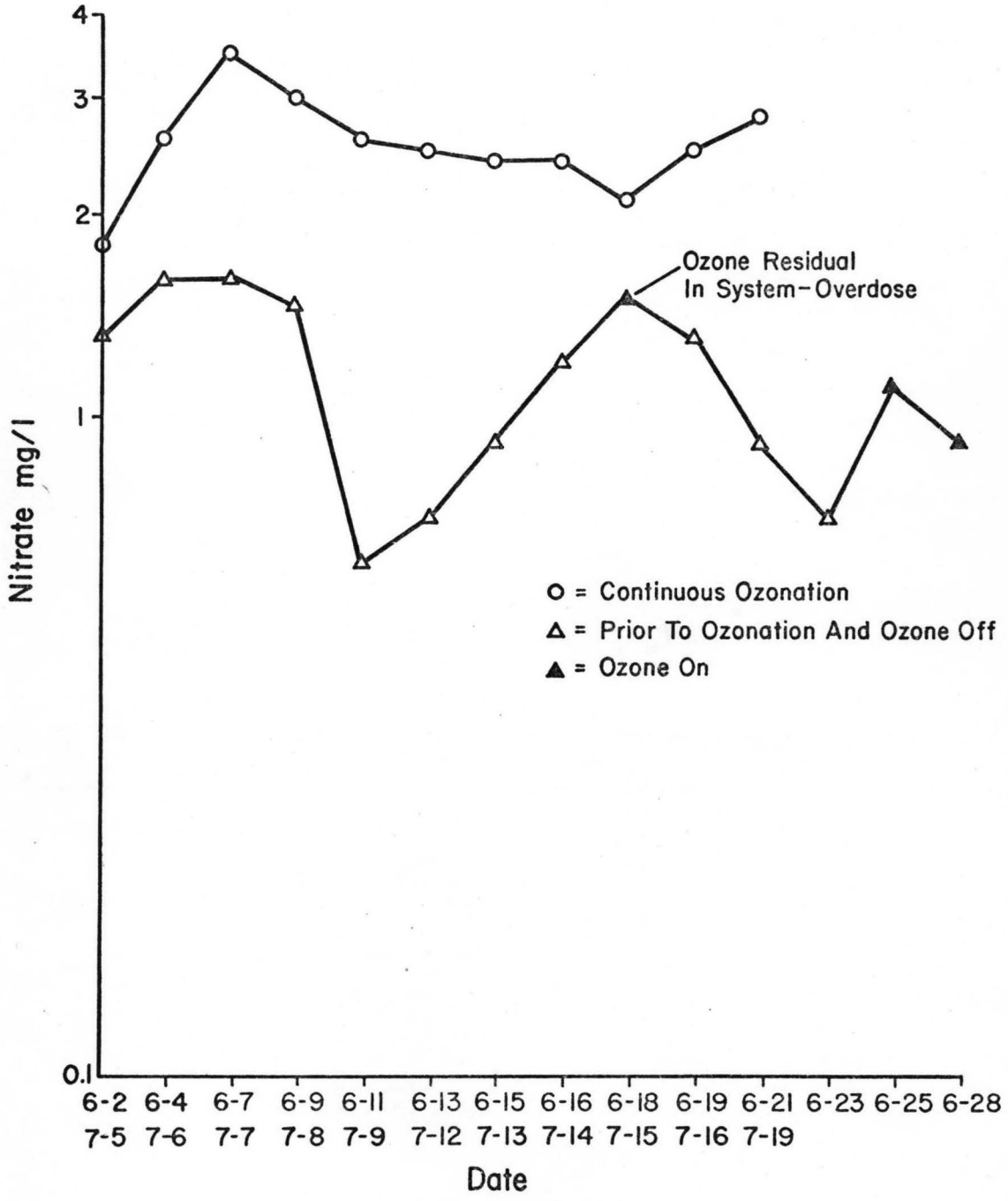

Figure 8. Biofilter pilot plant nitrate concentrations before and during continuous ozonation trial run.

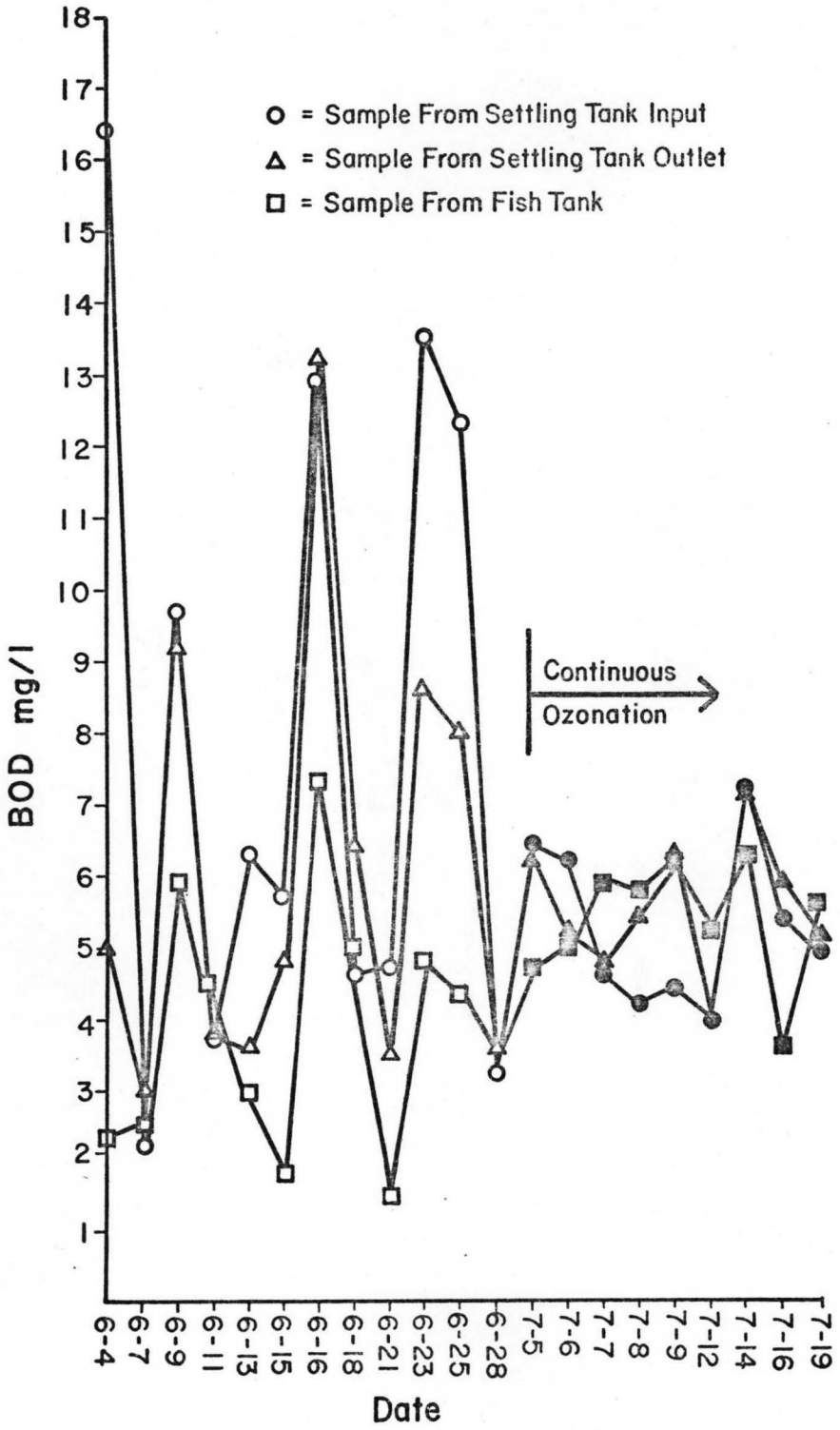

Figure 9. Biofilter pilot plant biochemical oxygen demand (BOD) concentrations before and during continuous ozonation trial run.

COST OF OZONE STERILIZATION

Analysis of an ozone system to treat 650 GPM of makeup water at Dworshak National Fish Hatchery is shown in Figure 10 and detailed in Appendix B. Estimated capital cost for the system is $164,000 and total annual cost is estimated at approximately $17,000/year. Capital cost for an ultraviolet system is approximately $90,000 and annual cost estimated at $12,000/year. Details are given in Appendix C.

Ozone does a significantly better job of makeup water sterilization than ultraviolet light. In doing so it may enhance the operation of other parts of the system. It is difficult to assign a dollar value to these indirect benefits, but they should be considered.

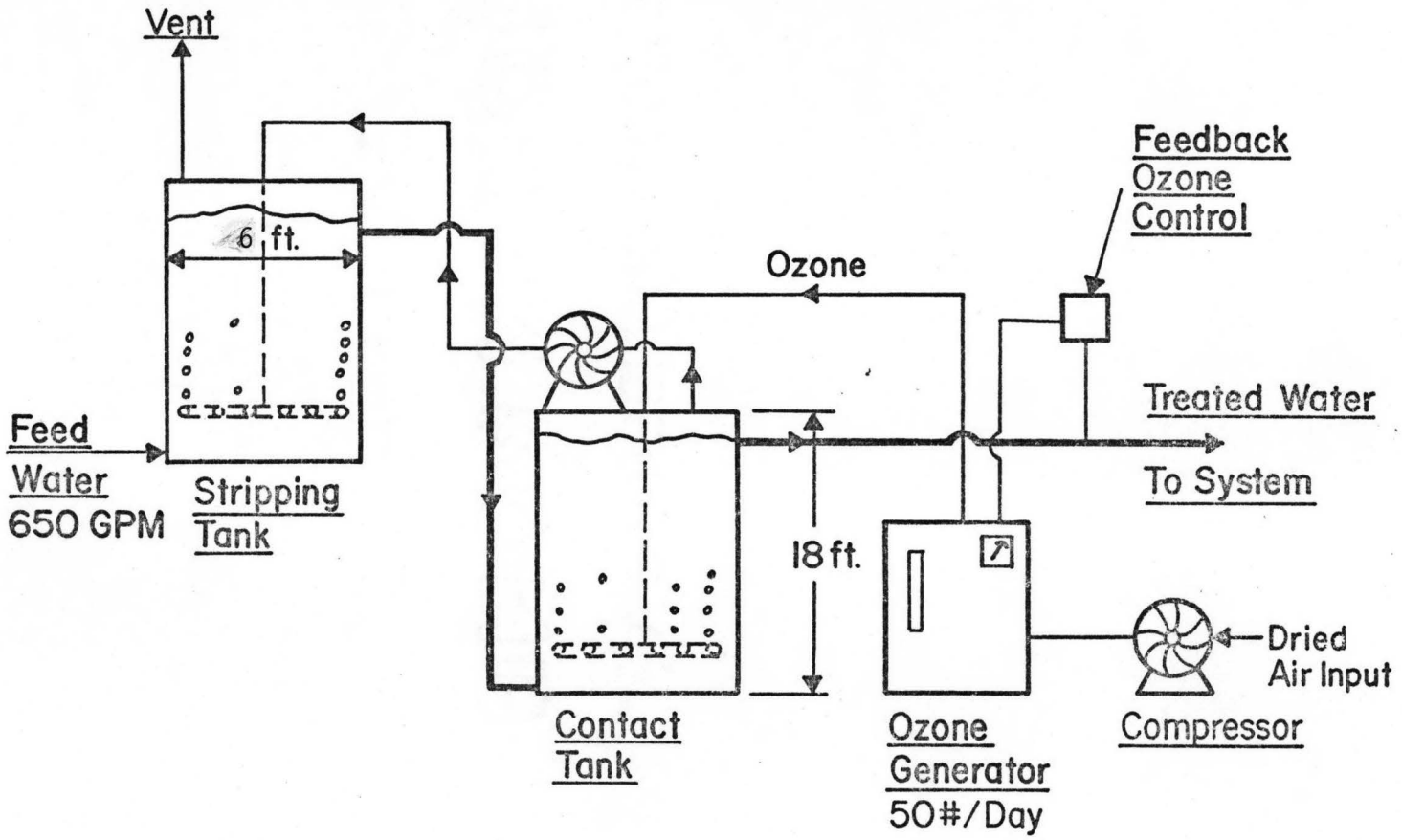

Figure 10. Preliminary Suggested Design:Ozone Treatment System 650 GPM Makeup

CONCLUSION

Ozone is a better sterilant for treatment of Dworshak River water than is the existing ultraviolet light system. In addition to destroying significantly more bacteria, ozone can destroy algae which ultraviolet sterilization does not. Destruction of incoming algae in the makeup water could have a profound effect on the system. Most important, biofilter action could be enhanced causing a general decrease of stress on the fish.

Other effects of ozone sterilization of the makeup on the water in the recycle loop are decreased ammonia levels, increased nitrates and increased total organic nitrogen. Biochemical oxygen demand (BOD_5) is also somewhat steadier during ozone sterilization of the makeup.

Decreased ammonia and increased nitrates indicate the activity of the nitrifying bacteria somehow enhanced. Increased total organic nitrogen may be caused by the action of residual ozone leaching organic solids. This leaching would prevent the continuous buildup, and sudden discharge of organic nitrogen into the system, a common occurrence in biological systems. In other words, the level of total organic nitrogen would be steadier, decreasing stress on fish. A steadier BOD_5 level would also lessen stress on the fish.

The above actions of ozone on the system may not be the direct result of ozone. Rather, it may be some indirect effects such as decreased algae causing enhanced nitrifier action.

Ozone treatment of the makeup water is a stable, easily operated method of sterilization. The ozone residual control generally requires little or no adjustment. Methods of determining residual ozone levels are simple, rapid and accurate. In addition, should an accidental overdose of ozone enter the system, several warnings occur before the levels become harmful to the fish. Ammonia and nitrate levels increase drastically. This is due to destruction of the nitrifying bacteria in the system by ozone. Ozone levels can be rapidly changed as soon as high ozone residual level is determined. This study also determined that steelhead fry and cutthroat can be exposed to 0.1 mg/l ozone for as long as two days and show no signs of stress. To introduce this level into a full size hatchery system would require a drastic accident.

As a treatment for recycle water, ozone can definitely sterilize the water. There are, however, several problems that indicate further study is required. A steady state concentration of ozone was never achieved during this part of the study. This was probably due to changing water quality, typical of a biological system. Further study is required to determine the control characteristics of applying ozone to the recycle flow.

During the two and one half weeks of continuous 24-hour per day operation

and in spite of two overdose accidents, no fish mortalities occurred. For approximately 20 hours per day the pilot plant was unattended. In addition, at the conclusion of the study the fish showed no signs of greater stress than at the beginning of the study.

During this study there was no detectable ozone odor in the incubator room. This indicates that at the levels required for sterilization, there is no hazard to operating personnel. During short term high ozone concentration trials, ozone odor was detectable in the room. For this reason it is recommended that an air monitor be used on any ozone system.

An ozone system is significantly higher in installation and annual cost but may be justified by the general improvement of the recycle system.

RECOMMENDATION

Based on the improved performance of ozone over ultraviolet light sterilization, it is recommended that an ozone system be installed to treat water for the proposed System IV at Dworshak. Using System IV, a long term study of the benefits or deficits of ozone treatment can be made. From this information it would be possible to determine if ozone treatment should replace all of the ultraviolet treatment.

BIBLIOGRAPHY

1. Evans, F. L., Ozone in Water and Wastewater Treatment, Ann Arbor Science Publishers, Inc., Ann Arbor, Mich., 1972.

2. Harding, H. L., Development of a Portable Ozone Test Unit, Masters Thesis, University of Idaho, 1971.

3. Rice, R. G., Browning, M. E., Ozone for Water and Wastewater Treatment, International Ozone Inst., Waterbury, Conn., 1975.

4. Rice, R. G., Blogoslawski, W. J., Aquatic Applications of Ozone, International Ozone Inst., Waterbury, Conn., 1975.

5. Giese, A. C., Christensen, E., "Effects of Ozone on Organisms," Physiological Zoology, Vol. XXVII, No. 2, April 1954.

6. Homan, C., "Effect of Ionized Air and Ozone on Plants," Plant Physiol., p. 975-78, Vol. 12, 1937.

7. Rosenlund, B. D., "Disinfection of Hatchery Water Influents by Ozonation and the Affects of Ozonated Water on Rainbow Trout," Aquatic Appications of Ozone, p. 59-66, International Ozone Institute, Waterbury, Conn., 1975.

8. Murphy, W. K., "The Use of Ozone in Recycled Oceanarium Water," Aquatic Applications of Ozone, p. 87-93, International Ozone Institute, Waterbury, Conn., 1975.

9. Sparrow, R. A., Fish and Wildlife Branch, Dept. of Recreation and Conservation, Victoria British Columbia, Personal Communications.

10. "Standard Methods for the Examination of Water and Waste Water," 13th ed., APHA, AWWA, WPCT, New York (1971).

11. Personal conversation with Patricia Colberg, Graduate Student, University of Idaho and Dr. A. J. Lingg, Associate Professor of Bacteriology, University of Idaho, Department of Bacteriology.

12. Wynn, Clayton S., et al., "Pilot Plant for Tertiary Treatment of Wastewater with Ozone," Superintendent of Documents, U.S. Government Printing Office, Washinton. D.C. Document No. EPA-22-73-146, 1973.

13. Shechter, Hana, "Spectrophotometric Method for Determination of Ozone in Aqueous Solutions," Water Research, p. 729-739, Pergamon Press, London, 1973.

14. Kilpatrick, M. O., Herrick, C. C., Kilpatrick, The Decomposition of Ozone in Aqueous Solution, American Chemical Society, p. 1784-1789, Vol. 78, May 5, 1956.

15. Rawson, A. E., "Studies in Ozonisation," Water and Engineering, March, 1953.

16. Veloz, Thomas, Aquafine Corp., Burbank, Calif., Personal Communication.

17. Smith, C. E., Hatchery Biologist, Fish Cultural Development Center, Bozeman, Montana, Personal Communication, September 3, 1976.

18. Rosen, H., Burhans and Sharpe Co., Seattle, Wash., Personal Communication.

19. McEthreon, L., Orbit Industries, Washougal, Wash., Personal Communication.

20. Hacker, R., Archer Blower and Pipe Co., Portland, Oregon, Personal Communication.

21. Colbert, P. J., Microbiological Considerations of Ozone Treatment of Reuse Water in Fish Hatcheries, M.S. Thesis, University of Idaho, 1977.

22. Morrison, T. J., A Pilot Plant Trial for Ozone Sterilization of Fish Hatchery Water, M.S. Thesis, University of Idaho, 1977.

APPENDIX A

Ozonation of Simulated Reuse Hatchery Water

ABSTRACT

The effectiveness of ozone as an alternative to current ultraviolet disinfection of makeup water and its potential for treatment of recycled water in commercial reuse hatcheries was considered in this study. Comparative survival rates in water were established for four bacterial fish pathogens (*Aeromonas salmonicida*, *Aeromonas liquefaciens*, *Pseudomonas fluorescens*, and the causative agent of Hagerman Redmouth Disease) and spores of *Bacillus polymyxa* during batch and continuous flow ozonation in the laboratory. A mixed bacterial-protozoan population isolated from soil was also subjected to ozonation, with protozoan survival monitored by hemacytometer. A specific microbial ozone demand was exerted during batch ozonation, while greater then 99% mortality of the fish pathogens was observed within 60 sec contact during continuous flow exposure at all concentrations of ozone applied. Spores of *B. polymyxa* were resistant at a concentration of 1.0 mg/l ozone. The oxidation rate for the combined bacterial-protozoan biomass closely approximated rates established in pure culture studies, with no significant difference in relative survival rates observed between bacteria and protozoa. Increased ozone concentrations yielded increased rates of mortality, while elevated carbon levels did not appear to exert a preferential ozone demand when added to suspensions of test organisms. Results of the survival studies refute the "all-or-none" phenomenon reportedly associated with ozone treatment. Oxidation of carbon and nitrite by ozone was rapid at low ozone concentrations, with carbon and ammonia oxidation rates exhibiting pH-dependence. The oxidation capacity of ozone in water was greatest at elevated pH even though the measurable ozone concentration was low. It is believed that the pH effect is due to free radical formation subsequent to ozone decomposition in aqueous systems.

LIST OF FIGURES

INTRODUCTION

The reduced availability of quality water for commercial aquaculture makes reuse hatchery design both economically and environmentally desirable. Yet recirculation of rearing water may enhance the potential for disease outbreak and accumulation of toxic metabolities. Since chemically treated intake and recycled water is unsuitable for fish culture, recycle hatcheries subject incoming water to sand filtration and ultraviolet radiation, while recycled water is passed to biological nitrification beds. Ultraviolet radiation is often incapable of adequately penetrating a stream of inflowing water (1) and biological oxidation of wastes is subject to fluctuations in efficiency (2).

One especially promising alternative treatment involves the use of ozone for disinfection of makeup and recycled water. Ozone is an allotropic form of oxygen with twice the oxidation potential of chlorine as hypochlorite ion. With a half-life in water of approximately 15 min, the only by-product of ozone treatment is molecular oxygen.

Only two reports have considered ozone use in reuse hatcheries. In a paper by Rosenlund (3), hatchery influent was subjected to ozone, but technical difficulties precluded completion of the study. Wedemeyer and Nelson (4) have determined inactivation curves for two bacterial fish pathogens during both batch ozonation and chlorination, and survival in untreated water. The purpose of this study was to assess the effectiveness of ozone as an alternative to current hatchery treatment by examining questions of microbiological concern.

MATERIALS AND METHODS

Ozone Production

Ozone was produced from oxygen in the laboratory by a reconstructed, water-cooled Welsbach model C1-D corona discharge generator (Welsbach Corp., Philadelphia, PA). A glass contact column (Pyrex No. 29/42) was modified for survival and oxidation studies allowing for syringe withdrawal of samples without interruption of the ozone flow. Ozone entered the column of water through a sintered glass dispersion tube suspended near the base of the contact chamber. Tygon tubing (Norton Plastics and Synthetic Div., Akron, OH) was threaded into the column and sealed in place to prevent escape of ozone into the laboratory. A 10 ml syringe was used to initiate sample flow with 1 ml or 2 ml samples withdrawn by sterile 1 ml or 5 ml glass syringes, respectively. Ozone concentrations in water were determined by the spectrophotometric method of Schechter (5).

Preparation and Enumeration of Cultures for Survival Studies

The four bacterial fish pathogens tested, Aeromonas liquefaciens, Pseudomonas fluorescens, HRM (recently designated Versinia ruckeri, also known as Hagerman Redmouth Disease, ERM, or Enteric Redmouth Disease), and Aeromonas salmonicida, were originally isolated from confirmed disease cases. The test organisms were propagated in shake flasks of Trypticase Soy Broth (TSB) at 25 C for 18 hr. Aliquots of the culture were centrifuged at 3000 x g for 10 min, washed once in sterile 0.02 M phosphate-buffered saline (PSB), and resuspended in sterile PBS.

Spore suspensions of Bacillus polymyxa were prepared following propagation on bottle-slants of Trypticase Soy Agar (TSA) for 5-7 days at 30 C. Cells were harvested, suspended in sterile PBS, heat-shocked in a boiling water bath at 80 C for 15 min, and sonicated for 30-60 sec with a Biosonik III unit (Bronwell Scientific, Rochester, N.Y.). The resulting suspension was centrifuged and washed three times in sterile PBS.

A mixed population of bacteria and biflagellated protozoans was isolated from soil and grown at room temperature on plates of 0.1% Tryptone and 1.5% agar, with a 9 ml surface layer of 0.1% Tryptone broth. The mixed culture was harvested after 48 hr, centrifuged, washed once, and resuspended in sterile PBS.

Survival of bacterial fish pathogens and B. polymyxa spores during exposure to ozone was monitored by surface spread plating in triplicate on TSA, while bacteria in the mixed soil culture were plated in triplicate on 0.5% Tryptone and 1.5% agar. Serial dilutions were made in 9 ml volumes of 0.1% Tryptone broth. Fish pathogen and soil culture plates were incubated at 25 C for 24-48 hr. Protozoan survival was followed by direct counts in a hemacytometer under a phase contrast microscope.

Glucose additions to organism survival systems were made at BOD levels of 5, 10, 25, 40, 50, and 100 mg/l. Test conditions at pH 6.0 and pH 7.2 were maintained by 0.02 M phosphate buffer, while 0.0125 M borate buffer allowed for oxidations at pH 8.2 and pH 9.3.

Carbon, Ammonia, and Nitrite Oxidations

A 5 ml glass syringe was used to withdraw 3 ml samples at 1 min. intervals, which in turn were dispensed as 1 ml replicates into 10 ml precombusted glass ampules containing 5 ml water, 0.2 g potassium persulfate ($K_2S_2O_8$), and 0.25 of 6% phosphoric acid. The ampules were sealed and autoclaved for 4 hrs. at 17 psi. Carbon as glucose was measured by infrared spectroscopy using the Model 0524B Total Carbon System (Oceanography International Corp., College Station, TX).

A glass syringe was used to withdraw samples at 1 min. intervals, which were dispensed as 2 ml volumes for duplicate nitrate or nitrite determinations. The samples were added directly to the respective reagents in order to facilitate inactivation of ozone. Oxidation of ammonia as ammonium chloride (NH_4Cl) was followed as nitrate formation by the chromotrophic acid method (6). Oxidation of nitrite as sodium nitrite ($NaNO_2$) was monitored as nitrite disappearance by the sulfanilic acid method (6).

Plotted values of experiments represent mean values of a minimum of three runs in the survival studies and two determinations in the case of carbon ammonia, and nitrite oxidations.

RESULTS AND DISCUSSION

Results of batch ozonation studies in pH 7.0 distilled water are plotted in Fig. 1. A specific microbial ozone demand was exerted by liquefaciens, (hydrophila), fluorescens, and HRM (ruckeri), while salmonicida appeared the most sensitive to ozone exposure at 1.0 mg/l ozone. These results are contrary to data reported in studies by Wedemeyer and Nelson (4) in that survival of A. salmonicida during ozone exposure was reportedly greater than that of HRM. Though the concentration of ozone in this study remained constant, these data may be used to explain the frequency of the so-called "all-or-none effect", a reportedly characteristic feature of ozone treatment (7, 8). The methodology by which all-or-none data have usually been gathered is by some type of batch exposure system (9, 10). The subsequent interpretation of such data has resulted in support of the all-or-none concept. In batch studies of poliovirus inactivation, Majumdar et al. (10) reported their findings as threshold concentrations, yet both methodology and results were similar to those for batch ozonation of bacteria reported in this research.

Figure 2 summarizes results of continuous flow ozonation. The rates of die-off in pH 7.0 adjusted distilled water for A. liquefaciens, P. fluorescens, and HRM (V. ruckeri) increased as the concentration of ozone in solution increased. Likewise, the death rate of A. salmonicida was dependent on ozone concentration yet, as in batch application, exhibited greater sensitivity to ozone. The more rapid die-off of A. solmonicida is clearly evident in Fig. 2, which compares the relative survival of the four bacterial fish pathogens during continuous flow at 0.1 mg/l ozone. The other organisms appear comparably sensitive to ozone treatment. Survival curves for continuous flow studies agree well with reports of other investigators (11, 12), who also failed to observe the all-or-none effect in inactivation studies of a variety of microorganisms. There are no reports in the literature to explain the consistently observed sensitivity of A. salmonicida to ozonation.

In addition to survival monitored in distilled water, carbon as glucose was introduced at concentrations equivalent to BOD levels of 5, 10, 25, 40, 50, and 100 mg/l. No differences in bacterial survival were detectable at elevated carbon levels under the same experimental conditions of continuous flow ozonation. The effect of dissolved organic matter on survival of organisms exposed to ozone-treated water has received some attention (9, 13, 14). Results of such studies confirm that organic matter in solution may compete with microorganisms by exerting an ozone demand, resulting in less efficient disinfection of water. These reports, however, considered much higher carbon levels than were added to test systems in this study.

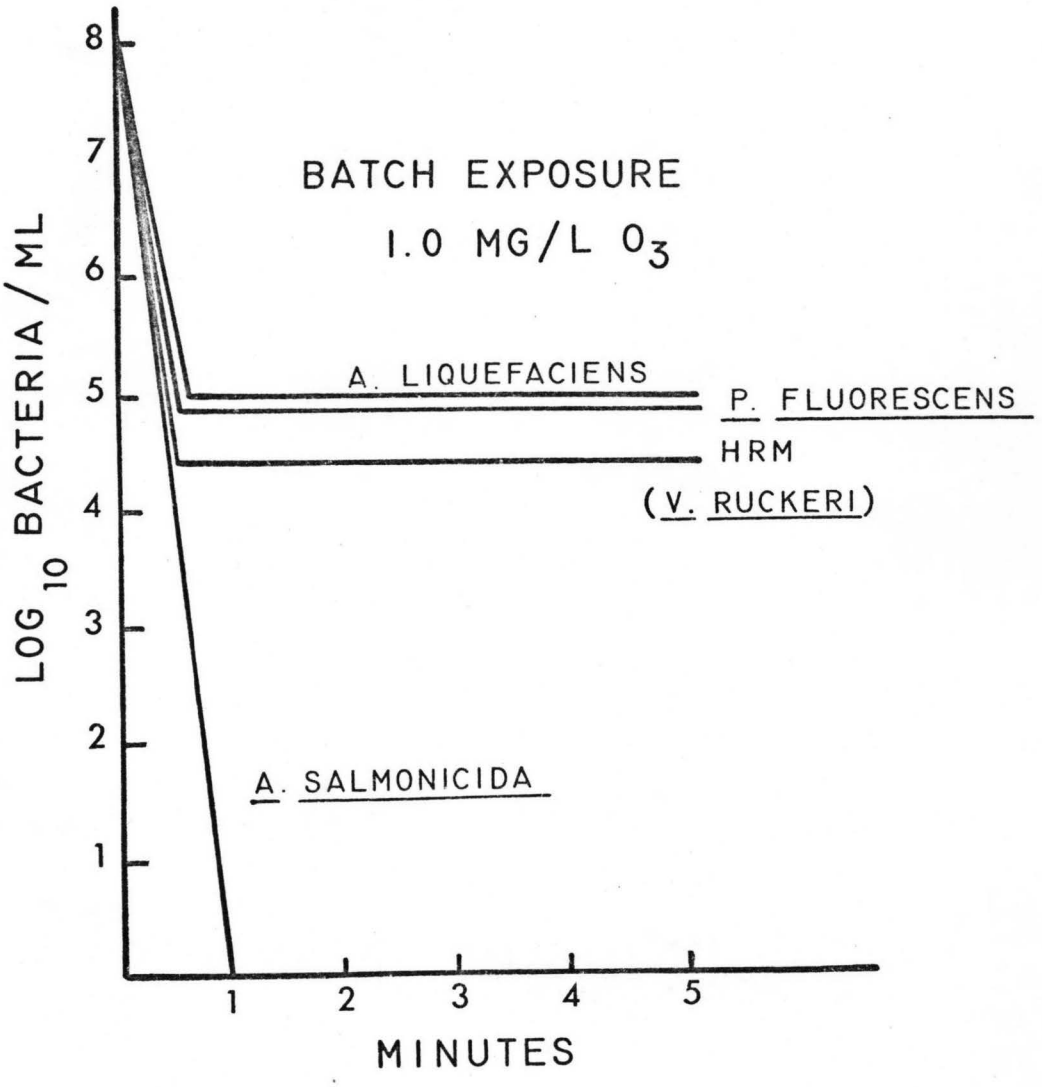

Figure 1. Survival of Aeromonas liquefaciens, Pseudomonas fluorescens, HRM (Versinia ruckeri), and Aeromonas salmonicida during batch ozonation at 1.0 mg/l ozone in pH 7.0 distilled water.

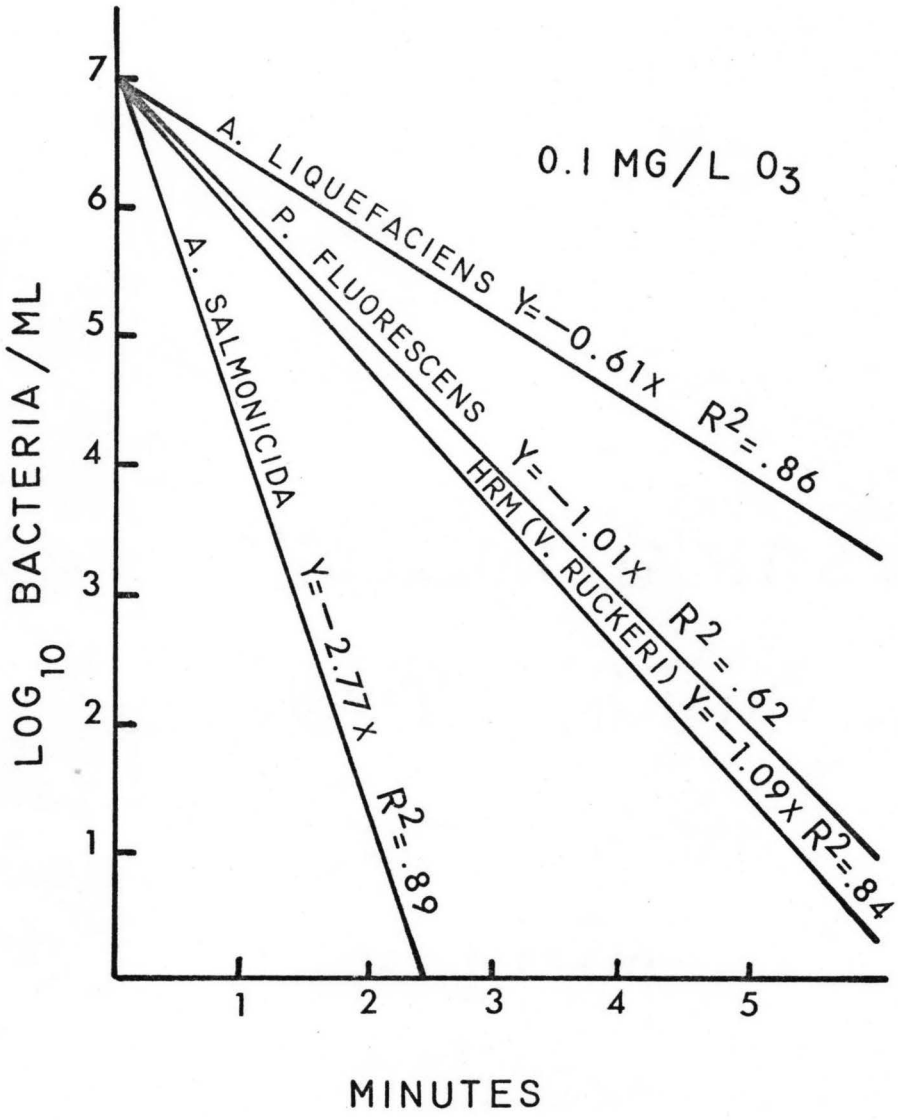

Figure 2. Relative survival rates of Aeromonas liquefaciens, Pseudomonas fluorescens, HRM (Versinia ruckeri), and Aeromonas salmonicida during continuous flow ozonation at 0.1 mg/l ozone in pH 7.0 distilled water.

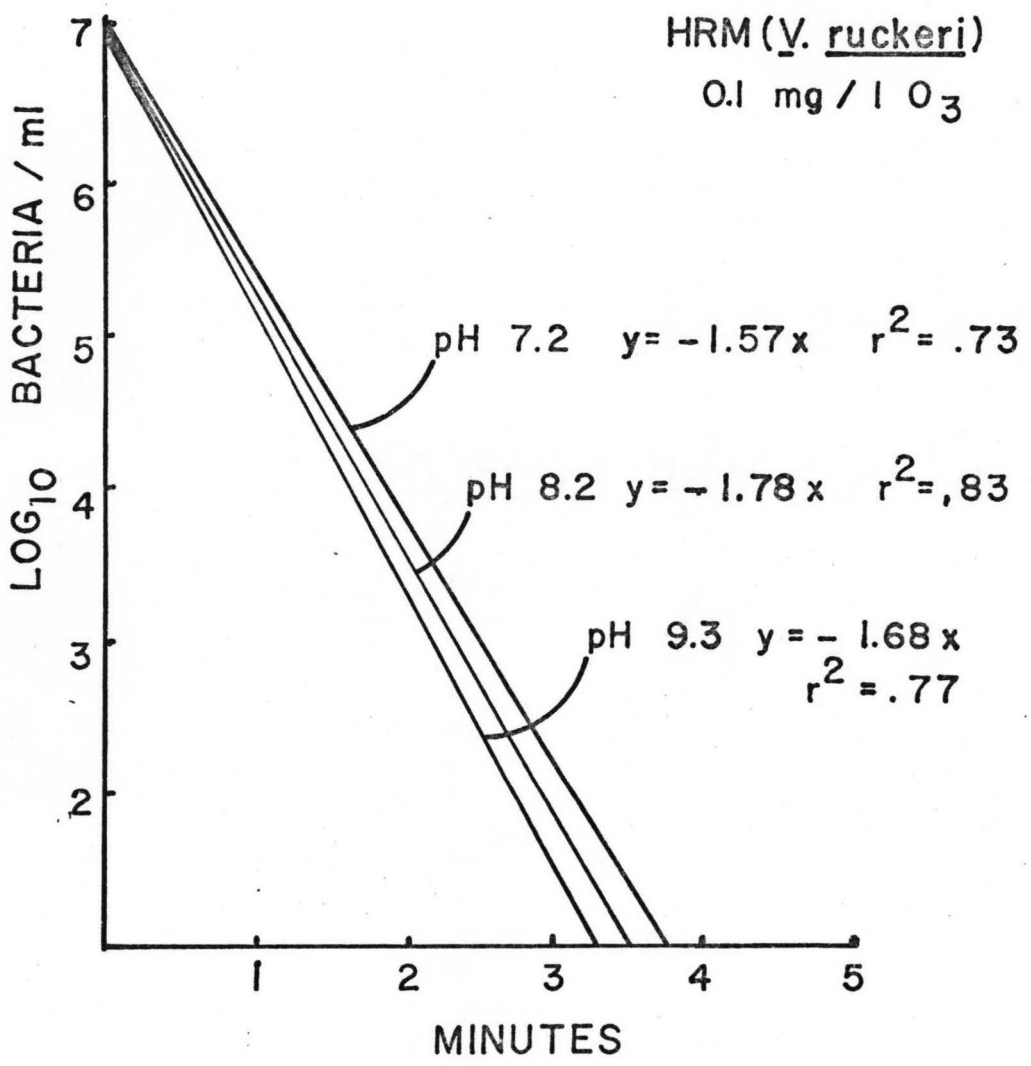

Figure 3. Influence of pH on survival of HRM (Versinia ruckeri) during continuous flow ozonation in buffer solutions at 0.1 mg/l ozone.

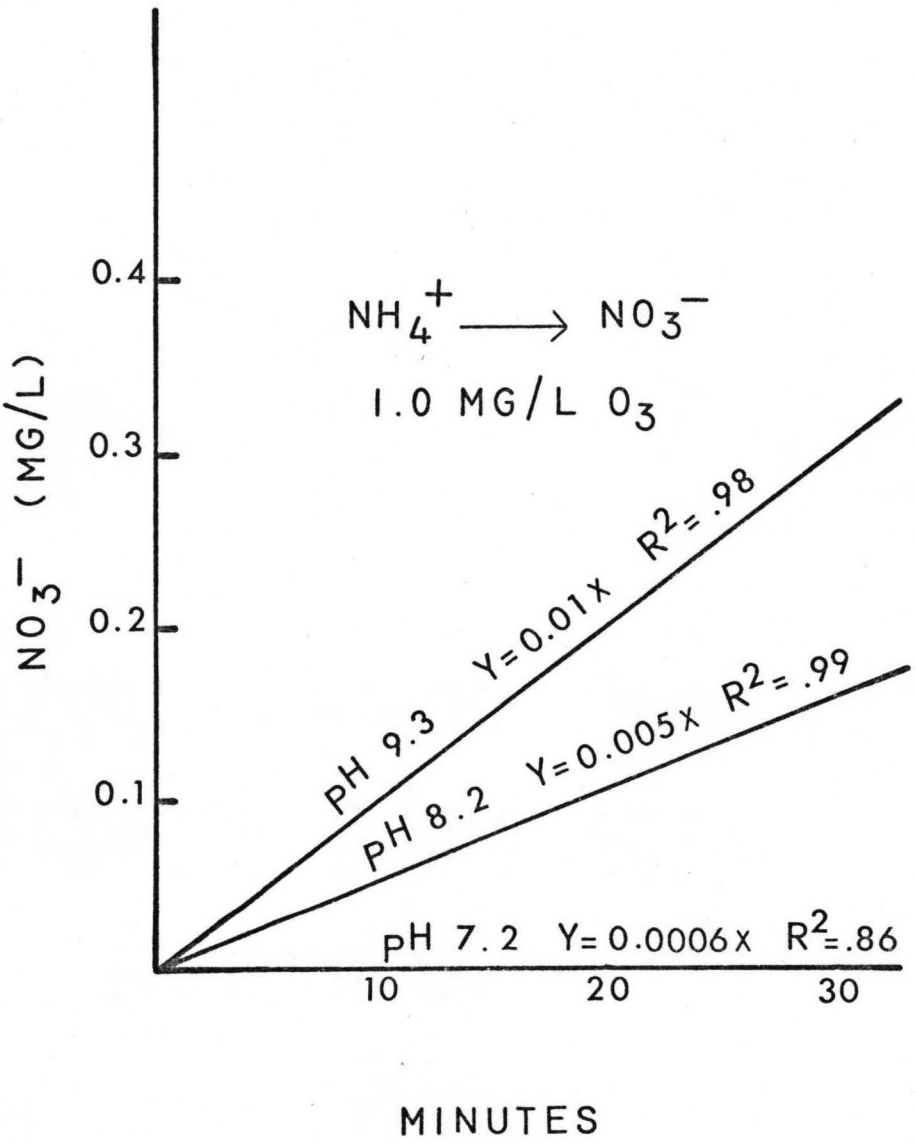

Figure 4. Influence of pH on oxidation of ammonia as NH_4Cl during continuous flow ozonation in buffer solutions at 1.0 mg/l ozone.

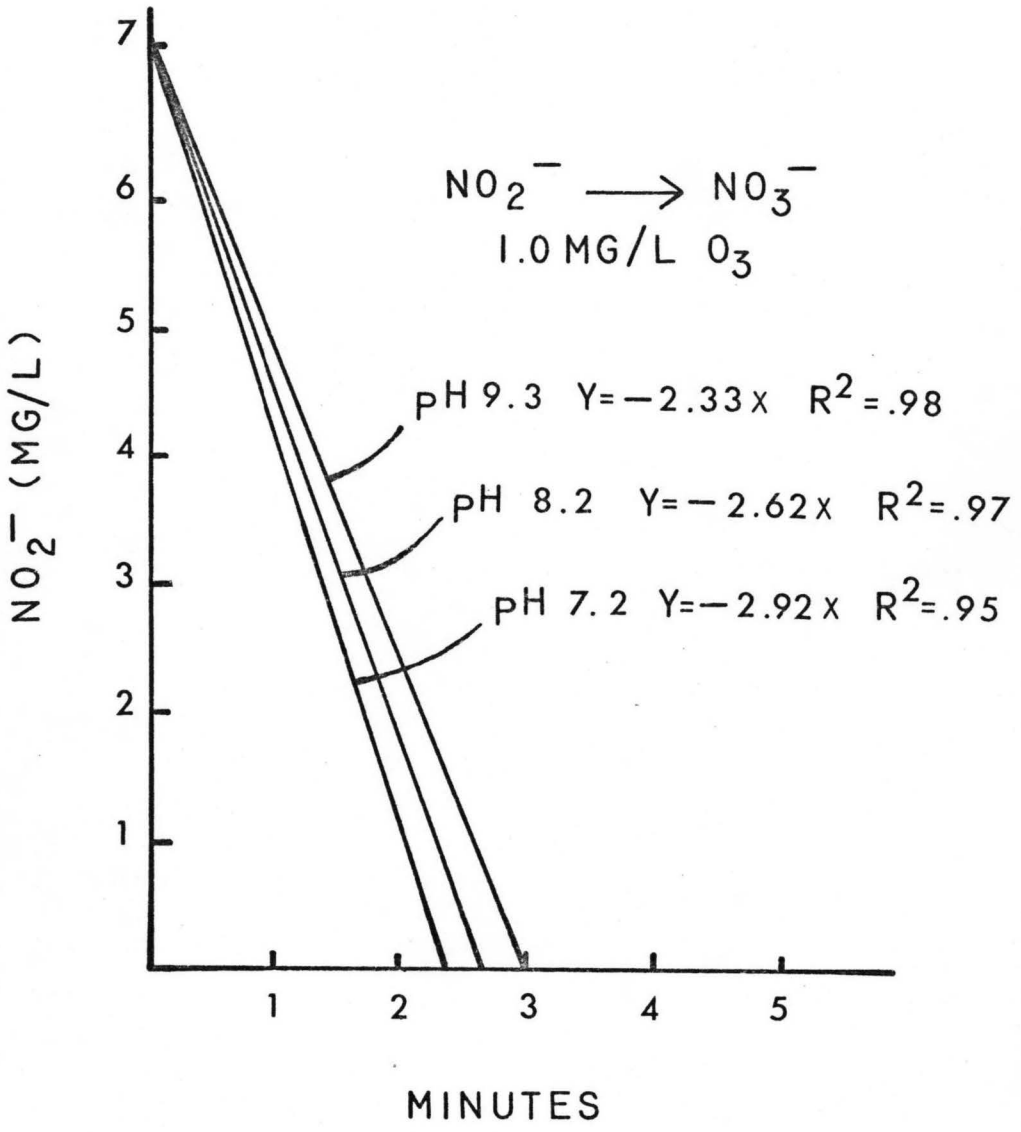

Figure 5. Oxidation of nitrite as $NaNO_2$ during continuous flow ozonation in buffer solutions at 0.1 mg/l ozone.

These data suggest that greater than 99% mortality of dense suspensions of pure cultures of the four fish pathogens tested can be achieved within 60 sec. of contact in water at low ozone concentrations. Increased ozone concentrations yielded increased rates of mortality, while elevated levels of soluble carbon did not appear to exert a preferential ozone demand when added to suspensions of test organisms.

Spore suspensions of Bacillus polymyxa were subjected to ozone as a model of the most resistant organisms. The maximum concentration of ozone that was consistently generated with the available equipment was approximately 1 mg/l ozone. At that level, spore viability was unaffected by continuous flow exposure to ozone as monitored for 10 minutes. Survival data for ozone inactivation of bacterial spores is restricted to a paper by Broadwater et al. (9). Their results subscribe to the all-or-none phenomenon with threshold concentrations established between 2.03 and 2.29 mg/l ozone for spores of Bacillus cereus and Bacillus megaterium during batch ozonation.

Since many protozoans pathogenic to fish are sporozoans (15) and difficult or impossible to culture by ordinary means, a mixed population of bacteria and biflagellated protozoans was isolated from soil. The oxidation of the combined biomass at pH 7.2 closely approximates oxidation rates established for the pure culture studies. Analysis of variance indicates no significant difference in the relative survival rates between bacteria and protozoa at 0.1 mg/l ozone. Research on ozone inactivation of protozoans is limited to some early microscopic observations by Giese and Christensen (16) and evaluations of the cysticidal potential of ozone on cysts of the waterborne agent of amoebiasis (17, 18).

The concentrations of ozone in aqueous systems appears to be pH-dependent. Buffer solutions at four pH levels were ozonated for 15 min. with subsequent measurement of the final ozone concentrations. As the pH increased, the concentration of ozone decreased.

This variance in oxidation potential with increased pH was examined more closely in a series of measurements of carbon oxidation. Carbon as glucose was subjected to ozonation at 1.0 mg/l ozone with oxidation rates measured in buffers of pH 6.0, 7.2, 8.2, and 9.3, as monitored by infrared carbon analysis. The oxidation activity of ozone was greater at elevated pH, even though the measurable ozone concentration was actually lower.

Since the influence of pH seemed substantial for carbon oxidation, one of the fish pathogens, HRM, was tested in buffer solutions at the same pH levels. No significant differences in survival at 0.1 mg/l ozone were evident (Fig. 3) as compared to survival in pH 7.0 distilled water. Likewise, no significant differences in survival of the mixed bacterial-protozoan population were observed as the hydrogen-ion concentration increased to pH 8.2 and pH 9.3. Though pH had no apparent effect on rate of kill in

either organism test system, carbon oxidation clearly exhibited pH-dependence. Consequently, the biocidal activity of ozone appears due to a mechanism more specific than random oxidation.

Since oxidation of ammonia in recycled water is essential for maintenance of aquatic animals (1, 19), ammonia as ammonium chloride (NH_4Cl) was exposed to 1.0 mg/l ozone with oxidation followed as nitrate formation. Oxidation of ammonia also appeared to be pH-dependent (Fig. 4). There was only negligible nitrate formation at pH 7.2. Nitrite oxidation, monitored as nitrite disappearance, was rapid even at 0.1 mg/l ozone (Fig. 5), with the pH effect statistically insignificant in this case.

Formation of free radical species due to ozone decomposition in aqueous systems (20, 21, 22, 23, 24) would account for the decreased ozone concentration in water at elevated pH. Carbon oxidation data reveals the oxidation potential of such free radicals in water.

No pH effect was evident on survival of either HRM or the mixed bacterial-protozoan population during ozonation. This suggests that the mode of action of ozone on biological matter is not necessarily influenced by free radical formation, but that oxidation readily occurs without regard for the pH of the system nor the available oxidizing species.

Data for ammonia oxidation by ozone agree with recent work by Singer and Zilli (25). The lack of ammonia oxidation at pH 7.2 could easily be alleviated in an aquaculture system by lime clarification (25).

Recent evidence suggests that nitrite levels in excess of 0.2 mg/l can be related to anoxia and heavy mortalities in hatchery operations (26). One of the persistent problems with the biological nitrification systems in reuse hatcheries has been incomplete ammonia oxidation, with subsequent nitrite accumulation. It is apparent from results of this study that this problem could be efficiently resolved by ozonation.

On the basis of these results, it is easy to visualize an on-line ozone treatment system effectively alleviating the disease potential associated with large-scale fish culture, while efficiently oxidizing potentially toxic metabolites such as nitrite and ammonia. However, successful installation and operation of such a facility is dependent on the economics of such an investment (27) we well as provision of a mechanism for ozone removal prior to recirculation. There is concern that even low ozone "residuals", though short-lived, may cause gill adhesions and mortality in fish exposed to freshly ozonated water (3). A short-term holding tank, step-ladder return flow to the raceways, or passage through an activated charcoal filter are potential methods of ozone stripping. If these problems are overcome, the potential of ozone treatment of water in reuse hatcheries is both warranted and promising.

LITERATURE CITED

1. Honn, K. V. and W. Chavin. 1976. Utility of ozone treatment in the maintenance of water quality in a closed marine system. Mar. Biol. 34: 201-209.

2. Johnson, P. W. and J. McN. Sieburth. 1974. Ammonia removal by selective ion exchange, a backup system for microbiological filters in closed-system aquaculture. Aquaculture 4: 61-68.

3. Rosenlund, B. D. 1975. Disinfection of hatchery influent by ozonation and the effects of ozonated water on rainbow trout. In: Aquatic Applications of Ozone, Blogoslawski, W. J. and R. G. Rice, Eds. International Ozone Institute, Inc., Syracuse, N.Y. p. 59-65.

4. Wedemeyer, G. A. and N. C. Nelson. 1977. Survival of two bacterial fish pathogens (Aeromonas salmonicida and the Enteric Redmouth Bacterium) in ozonated, chlorinated, and untreated waters. J. Fish. Res. Board Can. 34: 429-432.

5. Schechter, H. 1973. Spectrophotometric method for determination of ozone in aqueous solutions. Water Res. 7: 729-737.

6. APHA. 1976. Standard Methods for the Examination of Water and Wastewater. APHA Publications, Washington, D.C.

7. Fetner, R. H. and R. S. Ingols. 1956. A comparison of the bactericidal activity of ozone and chlorine against Escherichia coli at 1°. J. Gen. Microbiol. 15: 381-385.

8. Perlman, P. G. 1969. Ozone treatment of water inactivates the poliomyelitis virus. Wat. Wastes Eng. 6: 14.

9. Broadwater, W. T., R. C. Hoehn, and P. H. King. 1973. Sensitivity of three selected species to ozone. Appl. Microbiol. 26: 391-393.

10. Majumdar, S. B., W. H. Ceckler, and O. J. Sproul. 1973. Inactivation of poliovirus in water by ozonation. J. Water Poll. Control Fed. 45: 2433-2443.

11. McNair Scott, D. B. and E. C. Lesher. 1963. Effect of ozone on survival and permeability of Escherichia coli. J. Bacteriol. 85: 567-576.

12. Burleson, G. R., T. M. Murray, and M. Pollard. 1975. Inactivation of viruses and bacteria by ozone, with and without sonication. Appl. Microbiol. 29: 340-344.

13. Ingram, M. and R. B. Haines. 1949. Inhibition of bacterial growth by pure ozone in the presence of nutrients. J. Hyg. 47: 146-158.

14. Dickerman, J. M., A. O. Castraberti, and J. E. Fuller. 1954. Action of ozone on waterborne bacteria. J. New Eng. Water Wk. Assoc. 68: 11-15.

15. Amlacher, E. 1970. Textbook of Fish Diseases. T.F.H. Publications, Jersey City, N.J. 302 p.

16. Giese, A. C. and E. Christensen. 1954. Effects of ozone on organisms. Physiol. Zool. 27: 101-115.

17. Kessel, J. F., D. K. Allison, M. Kaime, M. Quiros, and A. Gloeckner. 1944. The cysticidal effects of chlorine and ozone on cysts of Endamoeba histolytica, together with a comparative study of several encystment media. Amer. J. Trop. Med. 24: 177-183.

18. Newton, W. L. and M. F. Jones. 1949. The effect of ozone in water on cysts of Endamoeba histolytica. Am. J. Trop. Med. 29: 669-681.

19. Spotte, S. H. 1970. Fish and Invertebrate Culture. Wiley-Interscience, New York. 145 p.

20. Gorbenko-Germanov. D. S. and I. V. Kozlova. 1973. Mechanism of ozone decomposition in alkaline aqueous media. Dokl. Akad. Nauk. SSSR 210: 851-854.

21. Gorbenko-Germanov, D. S. and I. V. Kozlova. 1974. Intermediate decomposition products of ozone in alkaline aqueous media investigated by electron spin resonance. Zhur. Fiz. Khim. 48: 166-168.

22. Hoigne, J. and H. Bader. 1975. The role of the hydroxyl radical in the ozonation of aqueous solutions. Chimica 29: 20.

23. Hoigne, J. and H. Bader. 1976. The role of hydroxyl radical reactions in ozonation processes in aqueous solutions. Water Res. 10: 377-386.

24. Peleg, M. 1976. The chemistry of ozone in the treatment of water. Water Res. 10: 361-365.

25. Singer, P. C. and W. B. Zilli. 1975. Ozonation of ammonia in wastewater, Water Res. 9: 127-134.

26. Liao, P. B. and R. D. Mayo. 1972. Salmonid hatchery water reuse systems. Aquaculture 1: 317-335.

27. Majumdar, S. B. and O. J. Sproul. 1974. Technical and economic aspects of water and wastewater ozonation: a critical review. Water Res. 8: 253-260.

APPENDIX B

OZONE SYSTEM
PRELIMINARY DESIGN
COST ANALYSIS

Basis

1. Makeup water flow rate = 650 GPM.
2. Maximum ozone required = 3 mg O_3/liter water.
3. Two contact chambers, five minutes retention time in each chamber. Counter-current contacting. First tank primarily used for stripping ozone out of vent gases (see Figure 10).
4. Feed gas to ozonator is air.

Calculations - Preliminary Design

1. 3 mg/l delivered to 650 GPM = 23 lbs/day ozone.
2. Total air flow rate to contact chambers: absorption approximately 60% efficient.

   $\frac{23 \text{ lbs/day}}{0.60}$ = 38 lbs/day ozone generated

   = 12 Ft^3/Hr. pure ozone at STP

   Generator produces 1% by volume ozone:

   $\frac{12 \text{ } Ft^3/Hr.}{0.01}$ = 1,200 Ft^3/Hr. at STP total air flow rate

3. Contact chambers (two each)
   650 GPM, 5 min. retention time (for liquid).
   1,200 Ft^3/Hr. air (STP)
   6 Ft. x 18 Ft. high x 3/16 wall stainless steel tank (includes three foot clearance at top)

Capital Equipment (1977 Costs)	Direct Costs $
1. Ozone generator, 50#/day-package unit with air dryer and compressor (FOB plus 15% installation cost)[18]	74,200
2. Two each 3,800 gallon stainless steel tanks, 6' x 18' x 3/16 wall, domed, one end [19]	9,500 (each)

3. 1,200 Ft^3/Ht. Blower (STP)[20]	2,200
4. Ozone/Liquid mixing Devices--Two Required	3,500
5. Special Ozone Instrumentation and Alarms	5,000
Total Direct Cost	103,900
Indirect Cost (34% of Direct Cost)	35,000
Total Direct and Indirect Cost	138,900
Contingency and Contractors' Fee (18% of D and I Cost)	25,000
Total Capital Investment	$163,900

Annual Cost (365 days/year)	$/year
Electricity 10 KW-HR/LB Ozone (at 2.2 cents /KW-HR)	4,015
Operating Labor	1,500
Maintenance and Repairs (3% of Direct Cost)	3,117
Depreciation (20 year life)	8,195
Total Cost:	$16,827/year

APPENDIX C

ULTRAVIOLET SYSTEM
PRELIMINARY DESIGN
COST ANALYSIS

Basis

1. Makeup water flow rate = 650 GPM
2. Ultraviolet contacting system identical to existing installations at Dworshak

   Serpentine contactors supplied by Aquafine Corporation. Supplied system includes all electrical controls and safety devices.

Capital Equipment (1977 Costs)	Direct Cost $
1. Ultraviolet contactor, complete with valves and instrumentation[17] (F.O.B. PLUS 50% installation cost)	57,000
Total Direct Cost	57,000
Indirect Cost (34% of Direct)	19,400
Total Direct and Indirect Cost	76,400
Contingency and Contractors Fee (18% of D and I Cost)	13,750
Total Capital Investment	$90,150

Annual Cost (365 days/year)	$/year
Electricity (4 KW at 2.2¢/KWHR)	770
Operating Labor	1,500
Maintenance	
Lamp Cleaning (4 times/year)	350
Relamping (2 times/year)	3,200
Miscellaneous (3% of direct cost)	1,710
Depreciation (20 year life)	4,500
Total Cost	$12,030/year