EVALUATION OF OZONE AS A DISINFECTING AGENT TO ENHANCE THE QUALITY AND EXTEND THE SHELF LIFE OF RAW, VACUUM-PACKED FISH

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ABSTRACT

The primary purpose of this project was to evaluate the effect of ozone on the shelf life quality of vacuum-packed, refrigerated fish fillets. Another objective was to gather data justifying the utility of ozone as an alternative to chlorine sanitation in a production environment. Since many food quality and safety problems can be affected by sanitation, ozone was used to disinfect food contact surfaces and ambient air to explore its potential commercial opportunity as a broad-spectrum disinfecting agent.

The impact of ozone on the bacterial load of whole salmon was positive. Fish were held for 5 minutes in water chilled to 10^0 C (50^0 F) having a dissolved ozone concentration of 5 ppm. On average a two log cycle reduction in the natural surface microflora of fish was observed. In addition, the level of ozone did not negatively affect the appearance, color, or aroma of the treated samples. A shelf life evaluation showed that the keeping quality of ozone-treated salmon steaks was better than untreated salmon steaks during the first four days of storage.

The environmental applications of ozone are promising. Air quality was notably improved and the bacterial loads on food contact surfaces were greatly reduced. Wash-water and waste water quality were also markedly improved indicating cross-contamination problems could be mitigated with ozone.

Review of the Literature

Ozone is the second most powerful oxidant readily available and is an excellent sterilizing agent (Rice, Farquhar, and Bollyky, 1982). The use of ozone in water is well established (Rice and Browning, 1980). In Europe, especially France and Germany, ozone has been the primary sanitizer for public water systems, and some U.S. cities also sanitize their public water with ozone. In the United States, the Food and Drug Administration granted ozone GRAS status for the treatment of bottled water (FDA, 1995), and the FDA recently approved ozone for direct contact with food.

Ozone has been demonstrated to be more effective as a bactericide than chlorine without affecting the taste, odor, and color of food (Venosa, 1972; Graham, 1997). Its mode of action against bacteria is generally accepted to be through the formation of hydrogen peroxide and hydroperoxyl, hydroxyl, and superoxide radicals (Foegeding, 1985). Though the effect of temperature and pH on ozone efficacy are less pronounced than with chlorine (O'Donovan, 1965), low pH and temperature seem to favor the stability of ozone and therefore enhance efficacy. (Foegeding, 1985 and Yang and Chen, 1979).

Fetner and Ingols (1959) examined the effect of ozone on *E. coli* at 1^0 C (33.8^0 F) and found the lethal dose to be between 0.4 to 0.5 ppm at a cell concentration of approximately 10^4/ml. The researchers also discovered that ozone had a critical concentration beyond which there was no lethal additive effect. This is in contrast to other sterilants, such as chlorine and hydrogen peroxide, which exhibit a linear kill rate. So the effect of ozone seemed "all-or-none." Burleson, Murray, and Pollard (1975) did not observe this all-or none-effect, but they did inactivate *Staphylococcus aureus, Pseudomonas fluorescens,* and *Salmonella typhimurium* at ozone concentrations of up to 2 mg/l in culture concentrations of approximately 10^2/ml. The authors conducted their experiments in phosphate-buffered saline and in secondary effluent from a wastewater treatment plant. Bacteria suspended in the latter required a longer contact time to be inactivated. Broadwater, Hoehn, and King (1973) reported the all-or-none phenomena of ozone with *Escherichia coli, Bacillus cereus,* and *B. megaterium.* The threshold concentration of ozone required to kill these vegetative cells was between 0.04 and 0.71 ppm. Bacillus spores were inactivated at 2.29 ppm ozone at cell concentrations of approximately 10^6/ml. Restaino et al. (1995) did not observe the all-or-none kill effect in their studies of *S. typhimurium, E. coli, P. aeruginosa, L. monocytogenes, S. aureus,* or *B. cereus.* With the exception of *B. cereus,* the death curves were biphasic. The lethality of ozone seemed to be influenced by the type of organic material present. Soluble starch had no effect, but bovine serum albumin had a marked effect.

A number of studies have been conducted on spore-forming bacteria. Spores of *B. cereus* and *B. megaterium* could be completely inactivated by 2.29 ppm aqueous ozone within 5 minutes (Broadwater, Hoehn, and King, 1973). An ozone concentration of 5 ppm and a contact time of 15 minutes was necessary to reduce *B. cereus* and *B. subtilis* cultures by 3 logs (Haufele and Sprockoff, 1973). Another study showed that spores of *B. cereus, C. botulinum,* and *C. perfringens* were inactivated by ozone in 15 minutes at concentrations of 0.4 to 1.9 ppm.

B. stearothermophilus cultures were more resistant and could only be reduced by 60% under the previously mentioned experimental conditions (Foegeding, 1985). Similar results were obtained for

B. stearothermophilus and for *B. coagulans* using 0.5 ppm ozone. Up to 2 hours of exposure were necessary to reduce the numbers of these organisms by 4 logs, whereas other *Bacillus* species could be inactivated in less than 30 minutes (Naito and Shiga, 1982). A National Food Processors Association study showed a 3 log reduction in the counts of B. *stearothermophilus, C. sporogenes*, and *C. botulinum* after exposure to ozone concentrations of 3.5 to 6 ppm for 2 to 9 minutes (Ito and Seeger, 1980).

Little data exists comparing the effect of ozone to the effects of other sterilants on the same bacteria. However, research has demonstrated that an inactivation time of 10 minutes for *B. stearothermophilus* could be achieved by 3.5 ppm ozone, 2.5 ppm chlorine dioxide, and 200 ppm chlorine. On the other hand, the same study showed that chlorine was more effective than ozone in killing *C. sporogenes* (Seeger, 1978). By comparing published results from unconnected studies, it seems the bactericidal nature of ozone generally requires short exposure time and/or low concentrations to achieve optimal lethality (Ito and Seeger, 1980; Venosa, 1972).

Ozone has been shown to be an effective bactericidal agent in poultry processing and is now being used in chiller water. Studies by Yang and Chen (1978) demonstrated that ice water-soaked broiler parts exposed to 3.88 ppm ozone for 20 minutes and stored for 28 days in polyethylene bags had consistently lower microbial counts than untreated meat and the shelf life increased by 2.4 days. A separate study on the microbial suspensions from spoiled, ground, poultry meat showed a 7 log reduction at an ozone concentration of 19 ppm for 4 min. Sheldon and Brown (1986) also studied whole broiler carcasses. Birds washed in 3.0 to 4.5 ppm ozone had a greater than 75% average reduction in aerobic counts over untreated hot carcasses. Lipid oxidation values were lower in the ozone-treated carcasses, color values were indistinguishable between treated and untreated samples, and a sensory panel was unable to detect differences in flavor or aroma between the control and experimental products. Chang and Sheldon (1989) examined the use of ozone in combination with screening and diatomaceous earth filtration to recondition poultry process waters. The total microbial load was reduced by 3 logs, and the treatment significantly reduced chemical oxygen demand and total solids.

Applying ozone to beef briskets (Gorman et al., 1995) and beef carcasses (Reagan et al., 1996) did not seem to produce a strong antimicrobial effect. Gorman et al. achieved an aerobic count reduction of 2.7 to 2.9 log cfu/cm^2 using a water wash followed by ozone at 500 ppm. Reagan et al. only achieved a 1.3 log cfu/cm^2 reduction when using 0.3 to 2.3 ppm ozone. The work of Kolodyaznaya and Suponia (1975) showed that the storage period for frozen beef kept at 0.4^0 C (32.7^0 F) and 85 to 90% relative humidity could be extended by 30 to 40% with atmospheric ozone levels of 10 to 20 mg/m^3 provided the original microbial count did not exceed 10^3/cm^2. A French study showed little positive effect on the surface microflora of raw or frozen beef even at 500 ppm ozone (Fournaud and Lauret, 1972). In addition, the color and aroma of the beef were negatively affected by the ozone. To achieve optimal results with beef, a German supplier of ozone generators recommends releasing ozone concentrations of 3 mg/m^3 into the air 3 to 4 times daily at the start of storage. This approach, however, will only partially arrest microbial growth. To totally arrest microbial growth, higher ozone concentrations would be needed, but the sensory quality of the beef would significantly suffer (Rice et al., 1982).

Ozone has been suggested as an antimicrobial agent for bulk food items such as eggs, bacon, bananas, butter, mushrooms, cheese, and fruits. Research has shown that Gram negative pathogens and *L. monocytogenes* are so sensitive to ozone that ozone-treated water could easily inactivate these organisms on the surface of fruits and vegetables provided reactive organic interference is minimal (Restaino et al., 1995).

The application of ozone to seafood has been explored to a limited extent. In 1936, Salmon and LeGall prepared ozonated sea water for icing down fish and found the shelf life was extended by 5 days. The effect of ozone, however, was attributed to its lethality on the microflora of the sea water rather than on the microflora of the fish itself. More recently, the study of Lee and Kramer (1984) demonstrated that ozonated ice enhanced the keeping quality of sockeye salmon without promoting oxidative rancidity. Research by Haraguchi et al. (1969) has shown that soaking fresh jack mackerel and shimaaji in 30% NaCl containing 0.6 ppm ozone decreased viable bacterial counts on the skin surface by 2 to 3 logs compared to the controls. Rice et al. (1982) mentions a study by the Alaskan Ocean Products Company concerning ozonated ice made from fresh water, which extended the storage life of Coho, red and silver salmon by 50%. The level of ozone trapped in the ice was 0.5 ppm. Evaluations on taste, texture, odor, bacterial counts, and rancidity showed product quality was enhanced with ozonated ice versus non-ozonated ice.

Now that the regulatory status of ozone has evolved to include food, the food industry is beginning to examine the efficacy of ozone and its utility as an alternative to chlorine sanitation. The seafood industry is also justified in assessing the benefits of ozone as a sanitizing agent. This research was conducted to evaluate the shelf life benefits of ozone on vacuum-packed, fish steaks stored under refrigeration.

Materials and Methods

Research Location

This research project was conducted at Hanover Packing Company, now Hanover Sea Products LLC, located at 6824 Market Street, Wilmington, NC 28405.

Processing Area

Figure 1 illustrates the layout of the Hanover production facility. The size of the process area was 60'x18'x9' (LxWxH), and was maintained at approximately 14.4^0 C (58^0 F). The air was circulated by two air conditioning units mounted on the ceiling; however, there was no fresh air exchange. The source of the process water was from a private well that had a temperature of 20^0 C (68^0 F). The chemical composition of the water is shown in Table 1.

Ozone Generation

The ozone generator was designed and built by Del Industries (San Luis Obispo, CA), which is shown in Figure 2 . UHP-grade oxygen was used as the feed gas to produce ozone. A 100-gal capacity Rubbermaid® tub was used as a wash tank, which is shown in Figure 3. The dissolved ozone concentration was measured by a Rosemount Dissolved Ozone Sensor 1054B (Rosemount Analytical, Irvine, CA). An Eco Sensor™ Ozone Sensor A-20ZX (Santa Fe, NM) and a Cosmos Ozone Hunter Plus Model AET-030P (IN USA Inc., Needham, MA) were used to monitor the ozone

content of the air. The wash water temperature was maintained at approximately 10°C (50°F) using the following methods:

1. Mixing crushed ice and water at a 1:2 ratio in a 32-gal plastic bucket and transferring the cold water to the wash tub with a sump pump;
2. Recirculating the wash tub water through a copper coil embedded in an ice slurry contained in a 32-gal plastic bucket.

Sample Preparation

The original protocol called for tuna and bluefish; however, at the time our research was conducted, local boats were not catching either species. Therefore we chose to test fresh, Canadian farm-raised salmon which was 24 hours old and had been shipped by overnight air cargo. The carcasses had been packed in ice gutted but not headed. We also opted to ozone-treat the entire carcass rather than exposed muscle. The muscle of an intact carcass is practically sterile. Bacterial loads are located on the skin. So if surface bacteria were reduced, microbial contamination of the meat would also be minimized when the carcasses were processed. While ozone can, theoretically, penetrate fish skin, oxidative rancidity and pigmentation changes in the meat would be negligible. Therefore ozonation would not negatively impact the visual or the sensory characteristics of the raw meat.

The treatment parameter for the experimental salmon was a 5 minute residence time in 5 ppm dissolved ozone. Once the dissolved ozone reached the target concentration, a salmon was swabbed on one side with a sterile sponge (Nasco, Fort Atkinson, WI). The swab area was 100 cm^2 (5 cm x 20 cm) and was marked to avoid sampling from the same area after treatment with ozone. One salmon at a time was placed in a mesh basket and immersed in the ozone-treated water. Each fish was gently agitated periodically during the immersion cycle (Figure 4). After treatment, each fish was swabbed on the unmarked side in a 100 cm^2 (5 cm x 20 cm) area. A total of six salmon carcasses were washed per batch. Untreated fish served as the controls. Five raw meat samples, skin on and bone in, of similar size were excised between the pectoral fin and the tail fin in sequential cross-sections from the same side of each salmon. Salmon steaks from each of 3 fish were bagged together (3/bag for a total of 5 bags for the shelf life study), vacuum-packed (MultiVac Inc., Kansas City, MO), and held at $< 4.4^0$ C (40^0 F) until they were packed in ice and taken by car to the North Carolina State University Seafood Laboratory in Morehead City. The temperature during transit was maintained at approximately 0^0 C (32^0 F) for 2 hours with crushed ice.

Sanitation

The effect of ozone on the microbiology of the air and food contact surfaces was also examined. The Hanover Packing sanitation regimen dictates a cold water wash, a quaternary ammonium spray, followed by a final hot water rinse. Control salmon were processed after food contact surfaces were cleaned according to the Hanover regimen. Food contact surfaces, including rubber gloves and aprons, were sprayed with ozone-treated water prior to handling the experimental fish.

An audit to evaluate the sanitation efficacy of ozonated water was conducted using bioluminescence testing (Uni-Lite® Xcel, Biotrace Inc., Plainsboro, NJ). This test is based on the measurement of adenosine triphosphate (ATP), which naturally occurs in all bacteria, fungi, animals, and plants

including food residues. When ATP is brought into contact with the reagents luciferin and luciferase, chemical reactions occur that result in the production of light. The light output is proportional to the amount of ATP present. The light output is measured and converted by the Biotrace unit into relative light units (RLU). Fourteen sites on the preparation tables and on the cutting knives were selected for bioluminescence testing. The surfaces were swabbed before and after the application of ozonated water. The swabs were then transferred to the Biotrace unit and RLU measurements were obtained for each site.

Environmental samples were collected from the air and the run-off water:

Air sampling:

1. A Millipore M Air T™ Air Tester (Bedford, MA) was used to draw air through a micro-perforated sieve and impact air-borne microorganisms onto an agar surface. The tester was placed on a 7-foot high shelf in the middle of the processing room, as shown in Figure 5. The growth medium was pre-filled tripticase soy agar (TSA) cassettes. The airflow rate was set at 140 L/min for the first 500 liters and then 180 L/min for a total of 1000 liters of air. The plates were incubated at 35^0 C (95^0 F) for 48 hours in an tabletop Isotemp Incubator (Fisher Scientific Company, Pittsburgh, PA) brought to the trial site from the NCSU Seafood Laboratory.
2. Petrifilm™ disposable aerobic count plates (3M Microbiology Products Company, St. Paul, MN) were hydrated with 1 mL of 0.1% peptone water and were allowed to remain covered for at least 1 hour prior to use. The plates were then situated in different locations in the processing area and, with the top film lifted, were exposed to air for 15 minutes. The petrifilms were incubated at 35^0 C for 48 hours at the trial site.

Run-off water sampling:

A disposable 10-mL pipette was used to collect run-off water close to the floor drain in the center of the processing area. The water sample was kept in a Whirl-Pak® bag (Nasco, Fort Atkinson, WI) and analyzed within 1 hour at the trial site.

Microbiological analyses

Swab samples of the whole fish were placed in a Whirl-Pak® bag (Nasco, Fort Atkinson, WI) containing 90 mL of 0.1% peptone water. The swab sponges were pummeled in a Stomacher 400 Laboratory Blender for 2 minutes at normal speed. Dilutions were plated on Petrifilm™ and were incubated at 35°C for 48 hours.

For the shelf life evaluation, triplicates of controls and treated samples were analyzed the day of processing (Day 0). Remaining samples were held refrigerated at 5^0 C (41^0 F) for 12 days. Samples for the microbiological and chemical analyses were removed after 4, 8, and 12 days of storage. Between 19 and 23 g of skinless meat were mixed with 200 ml of Butterfield's Buffer (pH 7.2) for 2 minutes in a Stomacher 400 Laboratory Blender. Further dilutions of the salmon suspension were pour-plated on Plate Count Agar (PCA) and Tryptone Yeast Extract (TYE). PCA plates were incubated at 35^0 C. TYE plates were held under anaerobic conditions at 35^0 C. All plates were counted after 48 hours.

Chemical Analyses

Chemical analyses were conducted by Southern Testing & Research Laboratory in Wilson, NC. Approximately 150g of skinless meat was excised at the time the bacterial samples were prepared. The meat was vacuum-packed and was held frozen at - 68^0 C to arrest chemical and enzymatic activity until the chemical tests could be conducted. The degree of lipid oxidation was determined by 2-thiobarbituric acid (TBA) values; the pH of the meat was also measured. Because salmon is not a scombroid species, we did not conduct a histamine analysis.

Statistical analyses

The data were statistically evaluated using general linear models for Windows Version 7.00 (SAS Institute, Cary, NC).

Results and Discussion

Although the effect of ozone on environmental sanitation was not in the original protocol, we took this opportunity to collect additional information to advance our knowledge of ozone's disinfection capability.

Air quality

Controls were sampled on the days that ozone was not being generated. Ozone-treated air was produced by releasing ozone gas from the wash tub. The ozone concentration was measured at 0.02-0.08 ppm. Air sampling results from the Millipore Air Tester are shown in Figure 6. Plates on the top row represent the control set, and they were covered by air-borne bacteria, yeasts, and molds. The bottom row of plates in Figure 6 represents the ozone-treated air samples. Air quality gradually improved over time. Another notable improvement was the odor of the room. A faint smell of ammonia was perceptible prior to generating ozone, yet after completing the ozone trial, the ammonia smell was non-detectable. Figure 7 shows the Petrifilm results where different air numbers represent different sampling locations in the processing area. Control samples were prepared from February 14-pm to February 15-pm, and the ozone trials began on February 16-am. This data set also demonstrates that ozone improves air quality over time.

Food contact surfaces

Two methods were used to evaluate the cleanness of the food contact surfaces before and after ozone treatment: the bioluminescence (ATP) method and the traditional aerobic plate method (APC). The ATP and the APC results are shown in Table 2. The data show that some microbial numbers increased after spraying the plastic cutting tables with ozone-treated water. Figure 8 shows the tables were covered with deep grooves, such that as fish were filleted, microorganisms became embedded in the grooves. The sanitation crew did not hand-scrub the cutting boards during the day. Over time microorganisms in the grooves may have formed a protective biofilm. When swab samples were taken prior to the wash treatment, the tip of the swab only contacted the surface of the biofilm. After a 30 second spray, the pressurized, ozonated water probably dislodged the biofilm from the grooves and displaced bacteria from the biofilm. Therefore, when the plastic cutting tables were swabbed again, many more microorganisms were detected.

Figures 9 and 10 show that ozone not only reduced the microbial counts on the cutting knife and the

stainless steel cutting table, it also eliminated bacterial buildup. Knives and cutting boards at Hanover Packing are rinsed at the start of processing, but utensils are not typically rinsed between fish in the same batch. If one fish has a high bacterial load, microorganisms will likely contaminate other fish through contact with utensils and cutting boards. Furthermore, microorganisms from the fish surface will be transferred to knives and cutting boards and will build to a level that can contaminate incoming fish. This data demonstrates the importance of sanitizing food contact surfaces at frequent intervals to minimize cross-contamination.

Wash water quality

Since Hanover Packing draws its process water from a well, two issues were of concern: odor and microbial load. Odor was primarily due to the high level of sulfur in the water as indicated in Table 2. One change employees noticed when the ozone generator was activated was the aroma of the air improved as the ozone oxidized the sulfur. As Figure 11 shows, ozone reduced the total microbial load of the wash water by more than 3 logs. In addition, ozone maintained the bacterial counts to well below one log even after washing six fish.

Run-off water quality

A major source of environmental contamination in any processing facility is the water that pools on floors, particularly around the floor drains. Figure 12 shows that ozone reduced the microbial loads of the waste water. When facility workers cleaned food contact surfaces with potable water, bacteria were displaced but not destroyed. Should an employee splash through a pool of water, bacteria could become air-borne and contaminate food contact surfaces. This data shows that when ozonated water is used to clean and sanitize food contact surfaces, it displaces *and* destroys microorganisms, so cross-contamination is minimized.

Effect of ozone on fish quality

Figure 13 illustrates the effect of ozone on the microflora of treated, whole fish. The average decrease was approximately 2 logs, and the ozone did not impair the appearance, color, or aroma of the salmon.

The total aerobic and total anaerobic plate counts of the meat samples are shown in Figures 14 and 15, respectively. The control and ozone-treated samples differed significantly ($\alpha = .05$). At Time 0, a 0.5 to 1.5 log difference was observed between the controls and the ozone-treated steaks. This correlates with the results of the surface swabs. Between 0 and 4 days, the number of aerobic and anaerobic bacteria increased, but the population differences were not significant. The growth of bacteria did differ significantly ($\alpha = .05$) between 4 and 8 days and between 8 and 12 days. At the end of the shelf life, the bacterial counts between the control and ozone-treated samples were nearly identical. Had the vacuum-packed steaks been maintained on ice instead of holding them at 41^0 F, we may have observed a longer lag period in which microbial growth was slower, particularly among injured cells. A portion of the surviving bacteria were likely damaged after exposure to the ozone, and the higher storage temperature favored cell repair as well as growth. Considering that the average supermarket shelf life of ice-chilled seafood is 3-5 days, these results seem to indicate that the microbiological quality of ozone-treated fish could be enhanced during this short sales time period. Overall the keeping quality of fresh fish treated with ozone appears better than the untreated

product during the first four days of storage.

The TBA results for the control and ozone-treated steaks are shown in Figures 16 and 17, respectively. Although the TBA numbers at Day 4 significantly differed ($\alpha = .05$) from Days 0, 8 and 12 for both ozone-treated and untreated fish, there was no statistically significant difference in TBA values between the control and experimental samples. A discernable pattern cannot be seen in either sample set because of the large variability in values, particularly within a single fish. Ideally the level of malonaldehyde development in the TBA assay should be consistent among samples taken from a single meat sample; however, some research has shown that malonaldehyde can react with certain proteins to reduce the formation of TBA from aldehydes (Nawar, 1985). This could account for the fluctuating numbers seen in our TBA results. The pH of the samples remained relatively constant over time as shown in Figures 18 and 19.

Impact of Ozone on the Seafood Industry

Quality and safety are the highest priorities of the food industry. The FDA announced that all seafood marketed in the United States after mid-December 1997 had to comply with the Hazard Analysis & Critical Control Point (HACCP) food safety regulation. HACCP also played an important role in President Clinton's Food Safety Initiative. HACCP was initiated for the seafood industry to enhance international trade. While HACCP is concerned with only food safety, the program rests on a foundation of Good Manufacturing Practices, which address both sanitation and product quality. The control of spoilage organisms is a quality issue, while the control of pathogens, such *L. monocytogenes* and *Vibrio spp.* is a food safety issue. The FDA declared in Volume 66, No. 123, part 173.368 of the Federal Register that ozone is now approved as a food additive. The main goal of this project was to determine if ozone-treated water could extend the shelf life of processed seafood. The data collected from this research, which indicates that ozone can enhance the microbiological quality of raw fish, supports the efficacy of ozone in seafood processing.

Microbial cross-contamination in the workplace environment remains a leading cause of food-borne illness. To minimize the spread of harmful bacteria to finished products, better sanitation agents and enhanced sanitation regimens will be needed. Based on the results of this research, ozone seems a promising broad-spectrum disinfecting agent that should be considered as part of the any seafood processing sanitation protocol.

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Figure 1. Schematic of Processing Facility

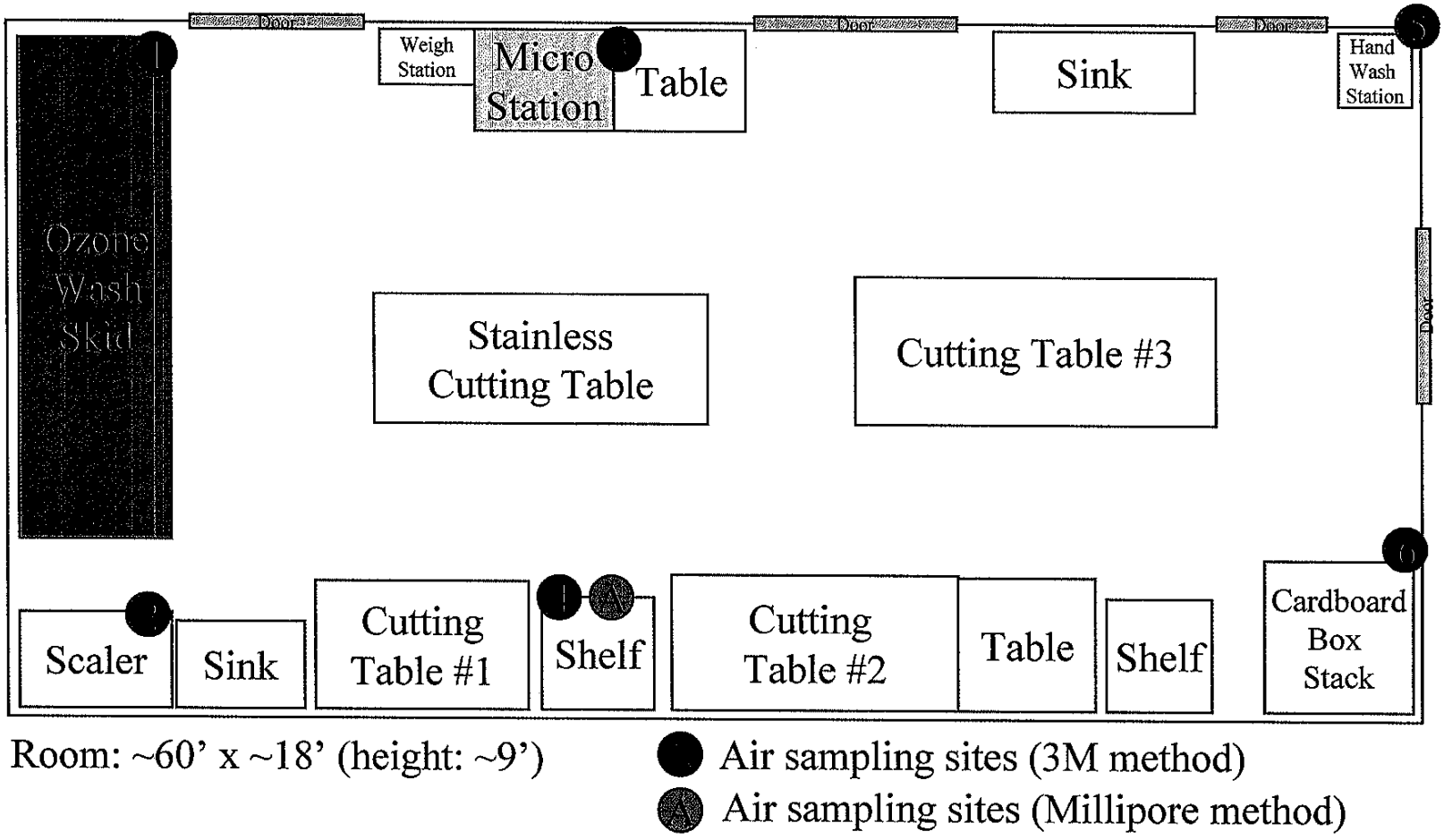


Table 1. Initial quality of process water

BOD (mg/L or ppm)	***COD*** (mg/L or ppm)	***Iron*** (mg/L or ppm)	***Mg*** (mg/L or ppm)	***Sulfur*** (mg/L or ppm)	***pH***
5.97	***34.6***	***1.45***	***2.00***	***4.03***	***6.4***

Figure 2. Ozone Generator

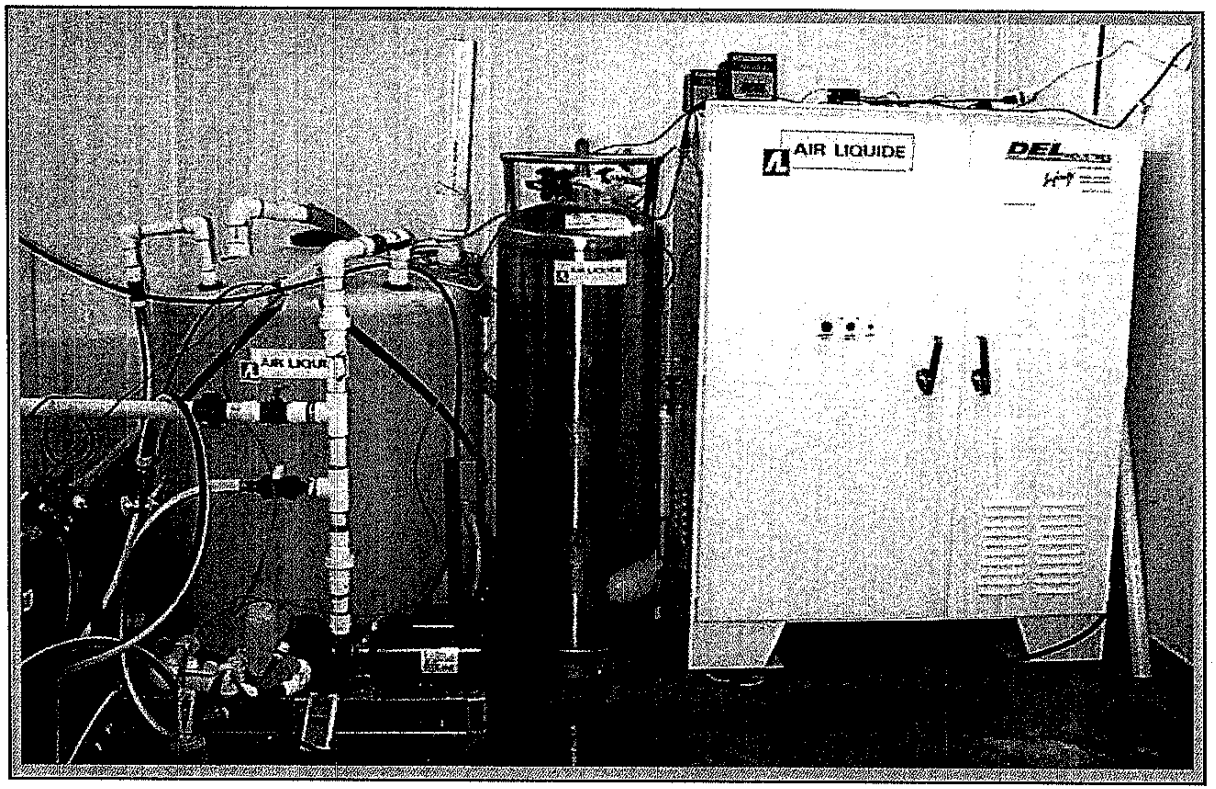


Figure 3. Ozone Wash Tank

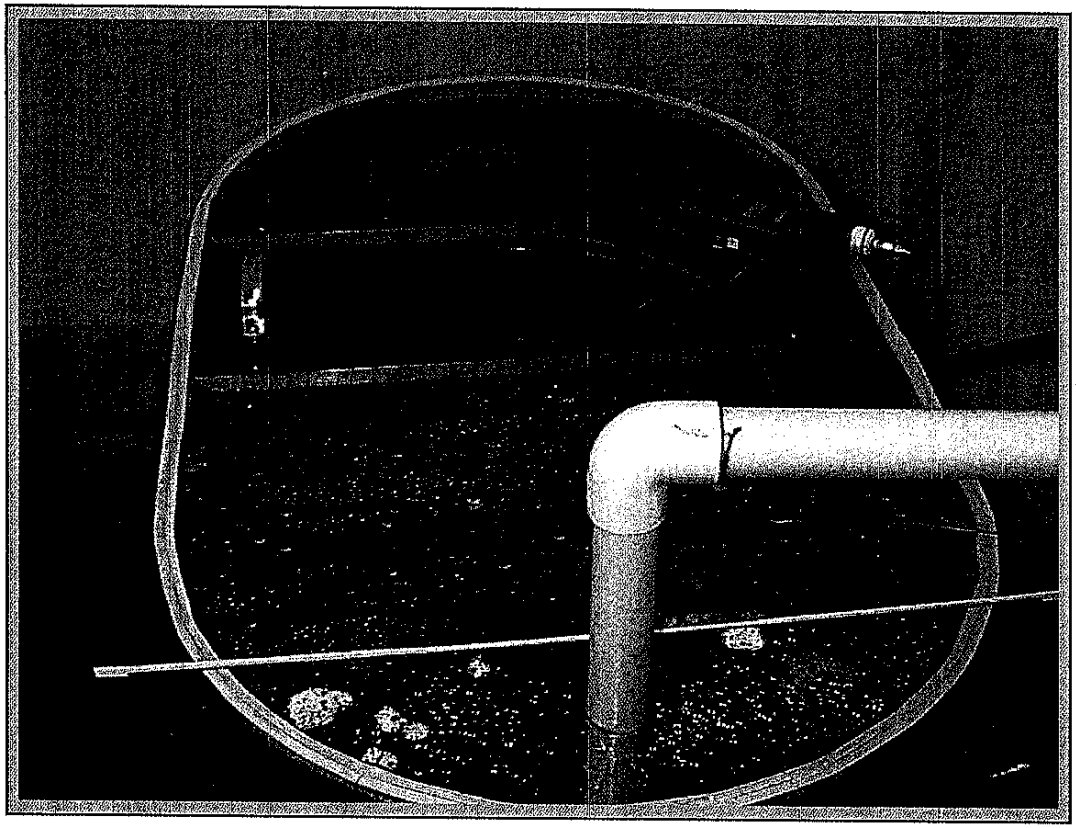
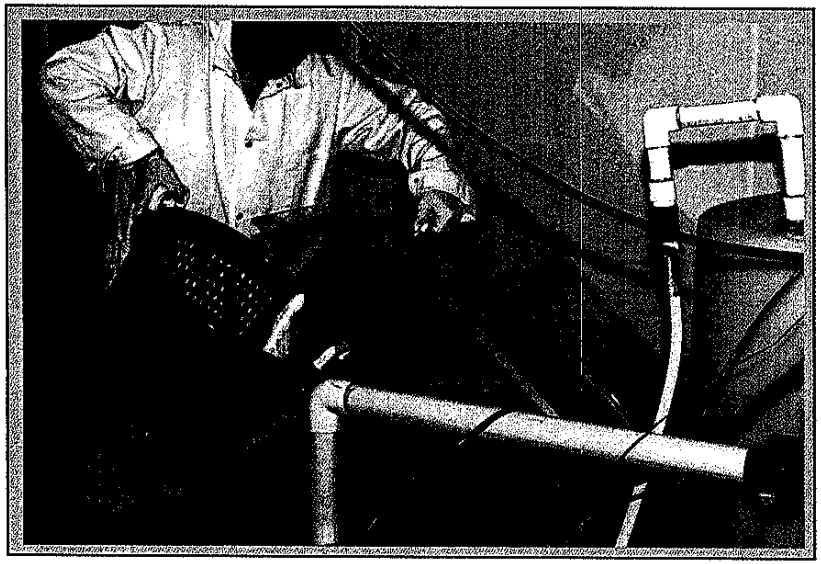


Figure 4.



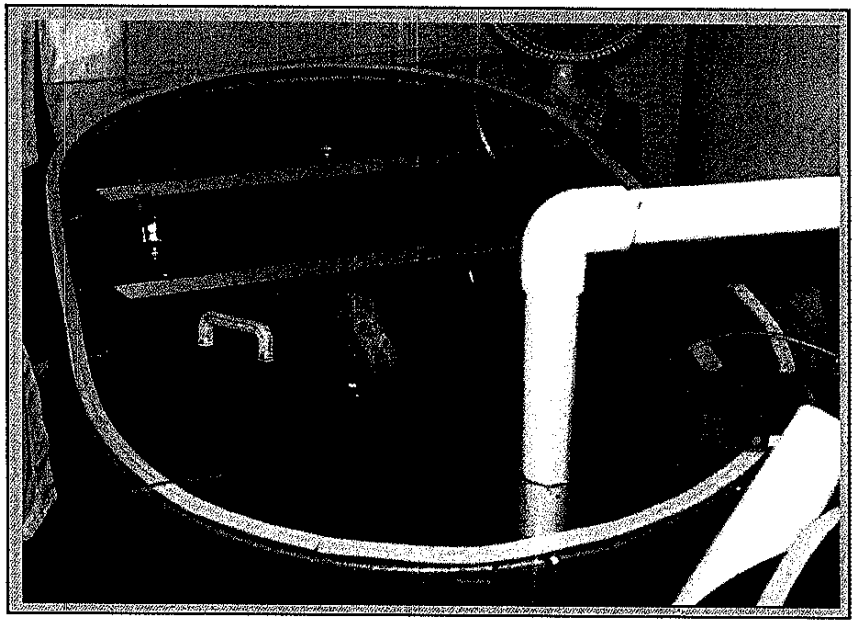


Figure 5. Millipore M Air T^{TM} Air Tester

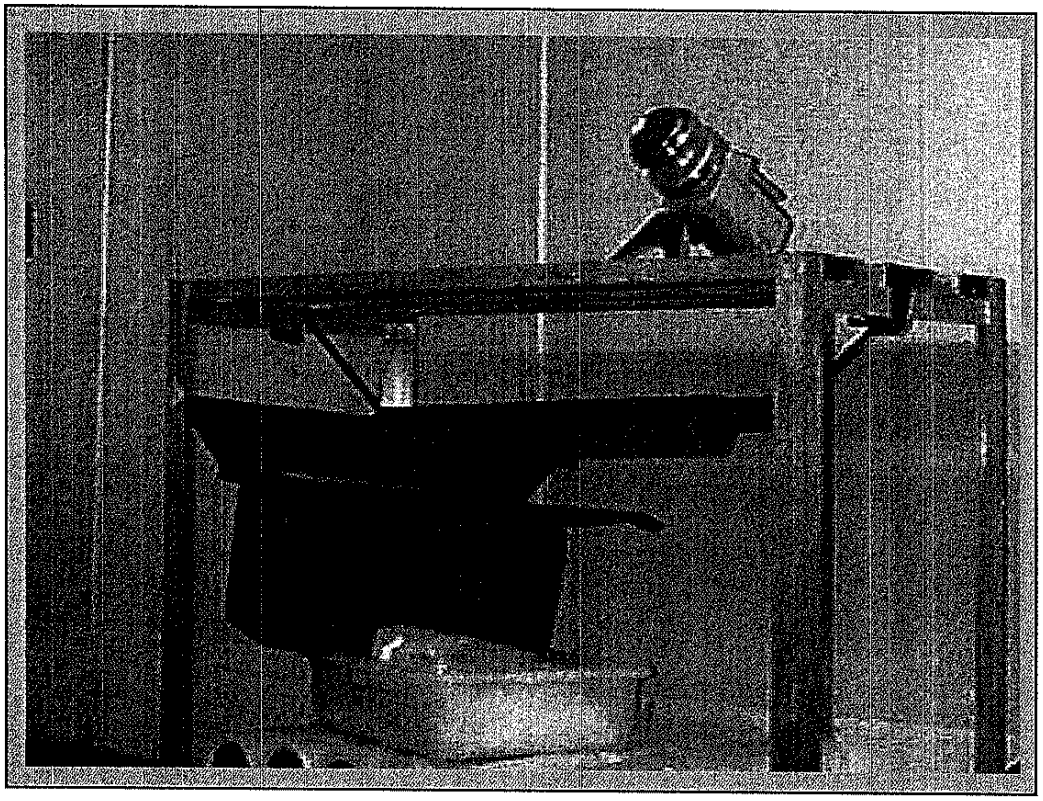


Figure 6. Millipore Air Tester Results

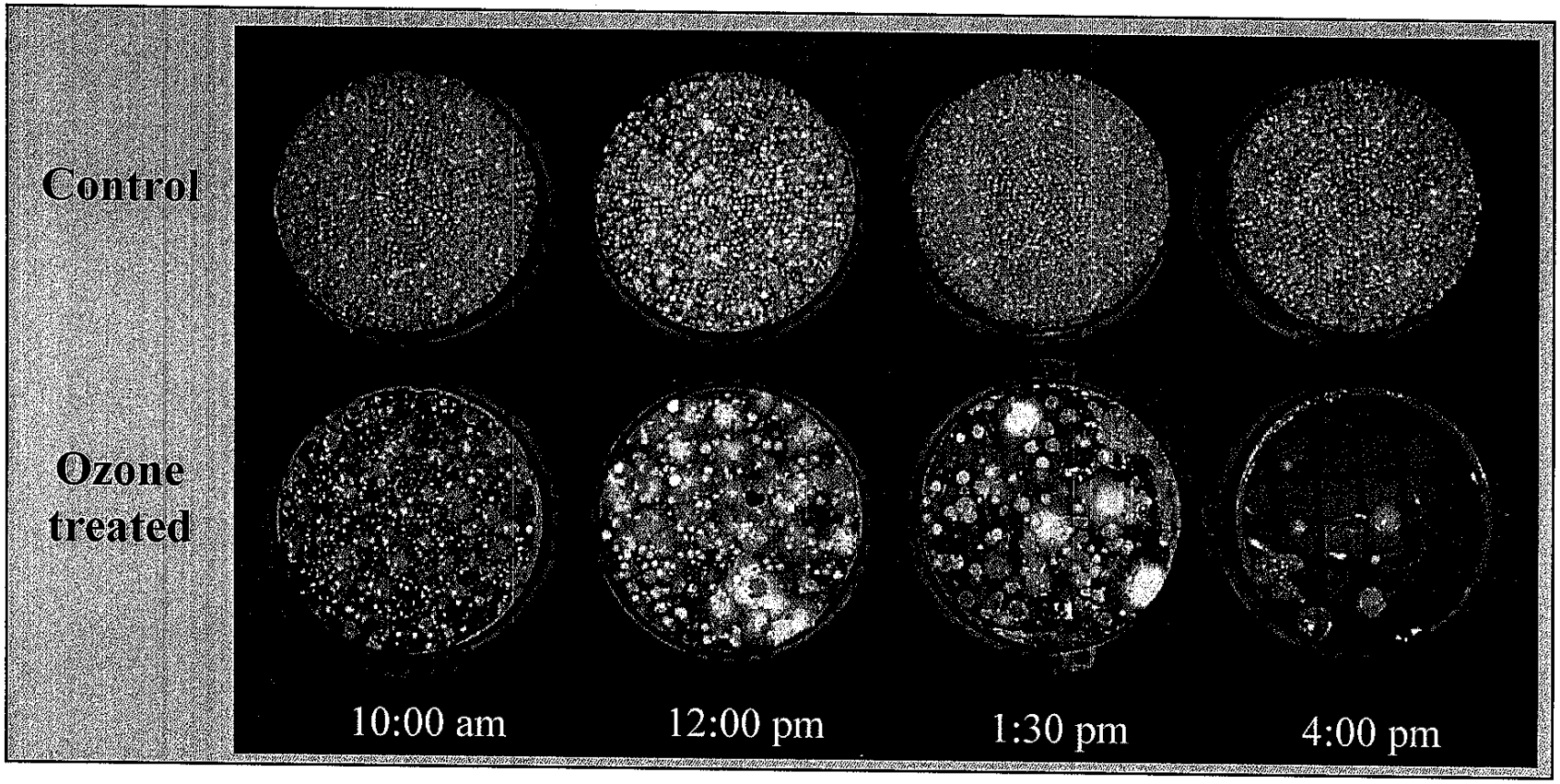


Figure 7. 3M Petrifilm Air Sampling Method
CFU = Colony Forming Units

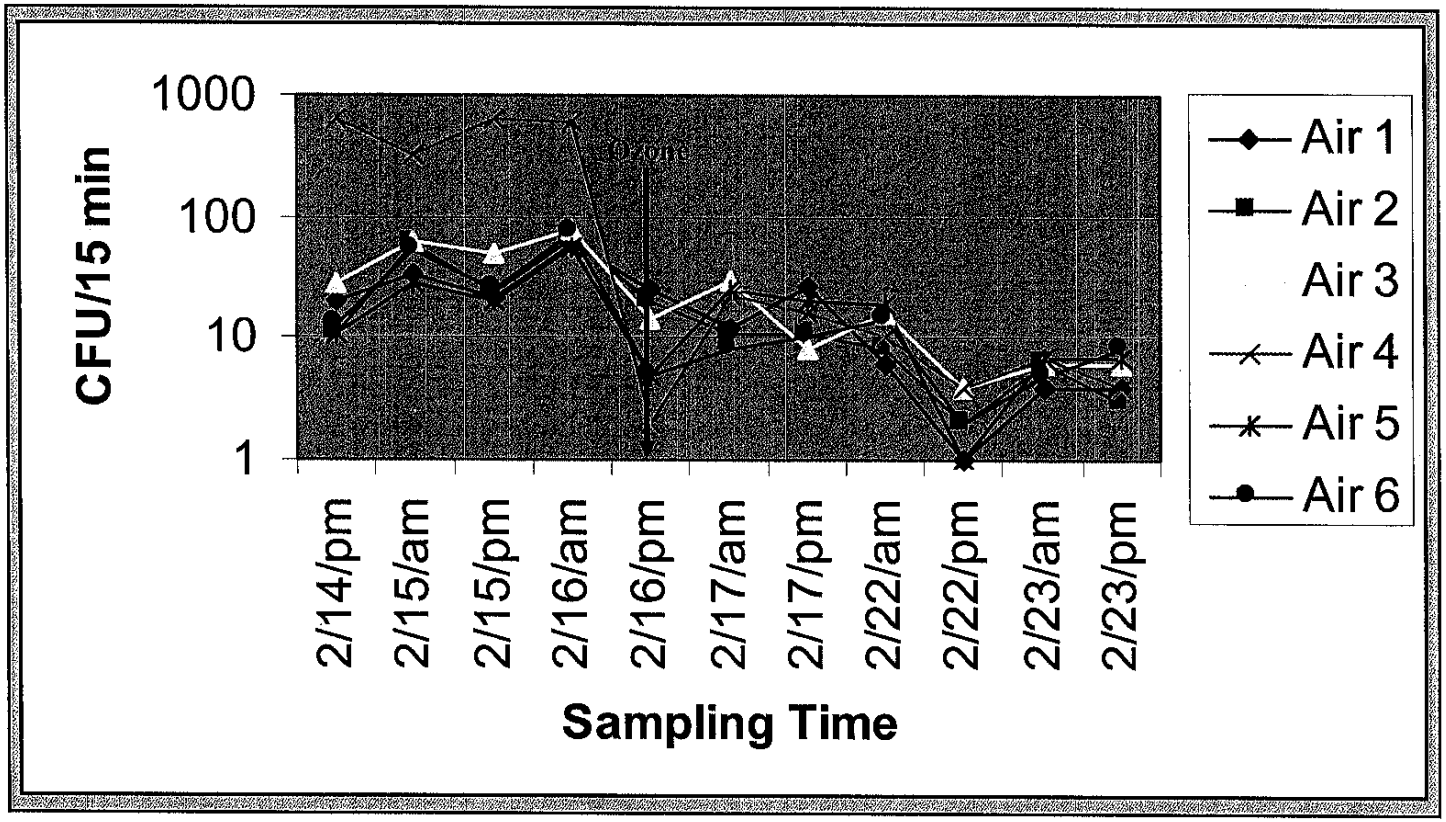


Table 2. Environmental Swab Results

	Control		Ozone	
Sampling Site	APC CFU/50 cm^2	RLU	APC CFU/50 cm^2	RLU
Plastic cutting table #1: Top	42000	4093	110000	15835
Plastic cutting table #1: Side	20000	3396	230000	15357
Plastic cutting table #2: Top	1800	465	690000	2395
Plastic cutting table #3: Top	>1000000	16548	<10	31416
Large knife blade	120000	119	11000	852
Small knife blade	200000	3815	7400	1278
Stainless cutting table	140000	931	<10	296

Environmental swab: pre-op swab
Control: before ozonated water rinse

Figure 8. Condition of Plastic Cutting Board

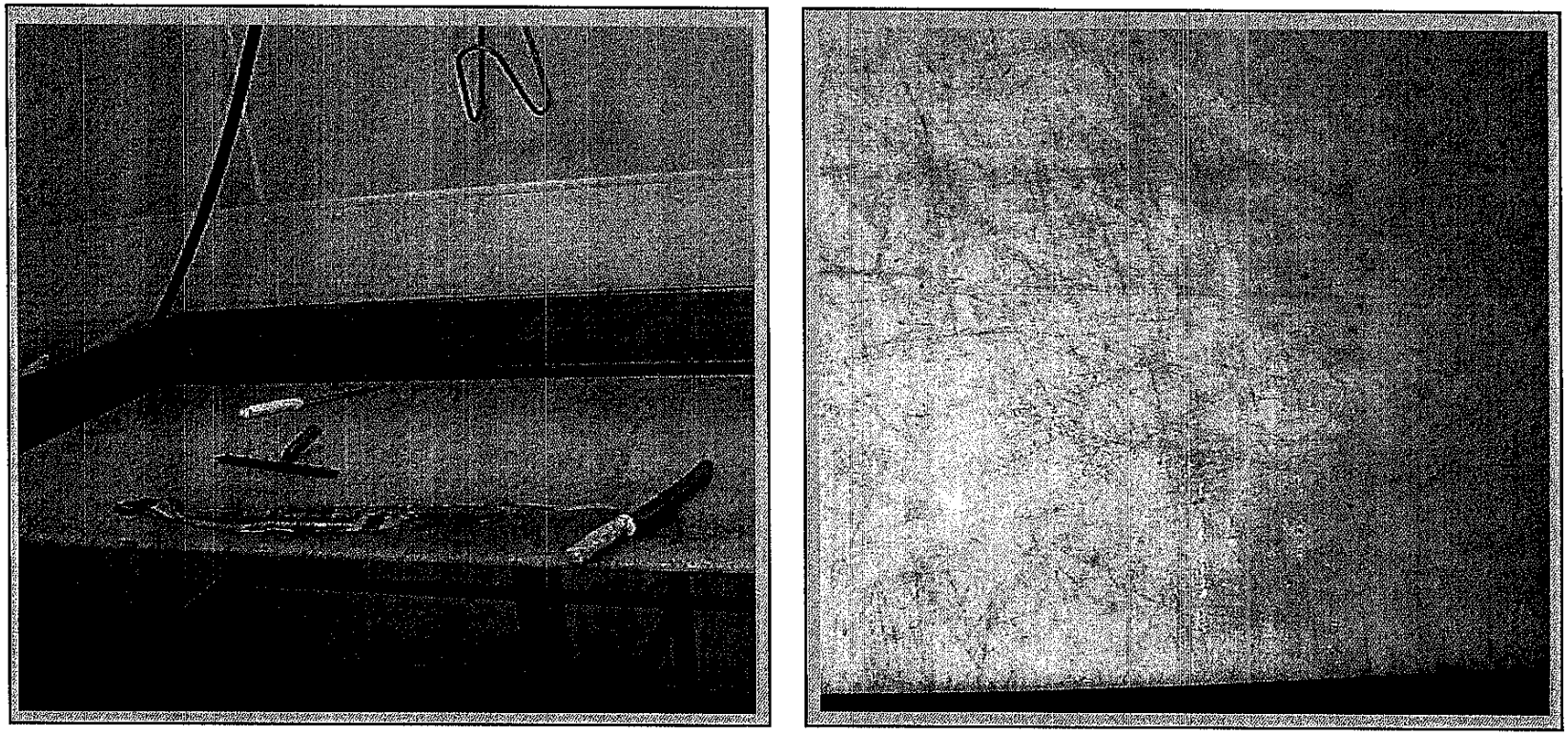
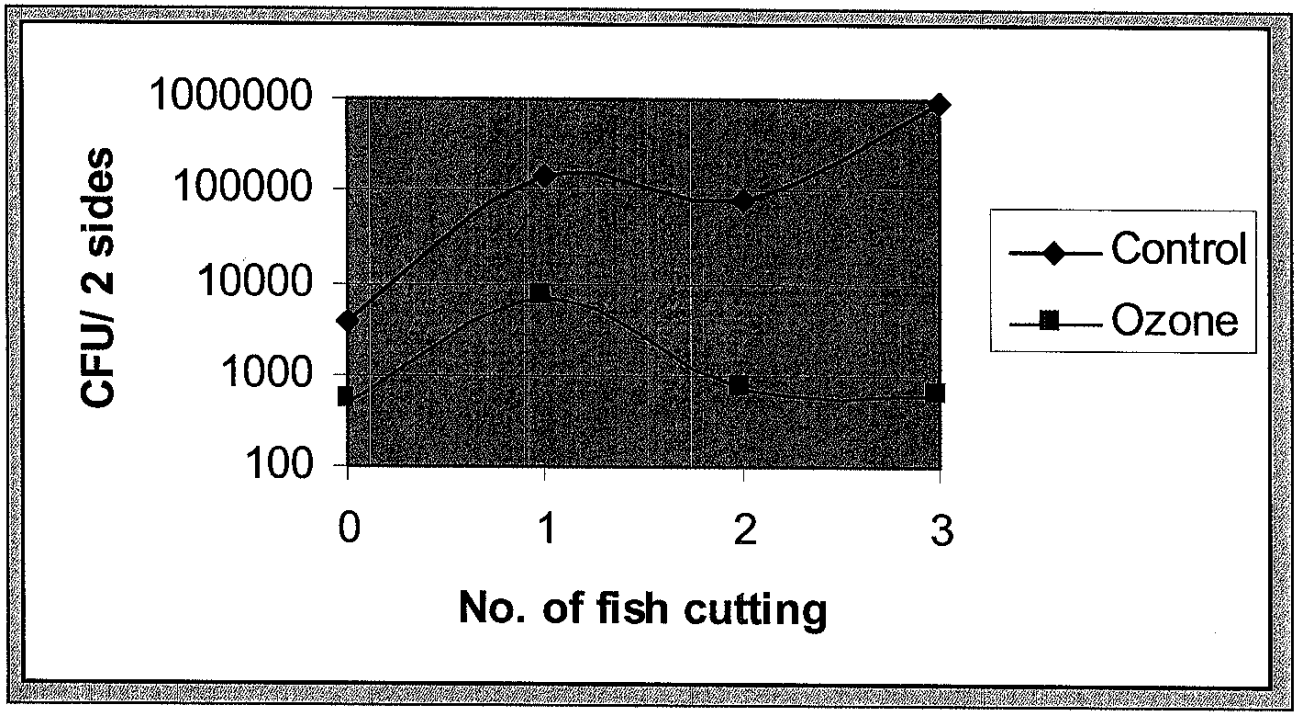


Figure 9. Knife Swab Results

CFU = Colony Forming Units

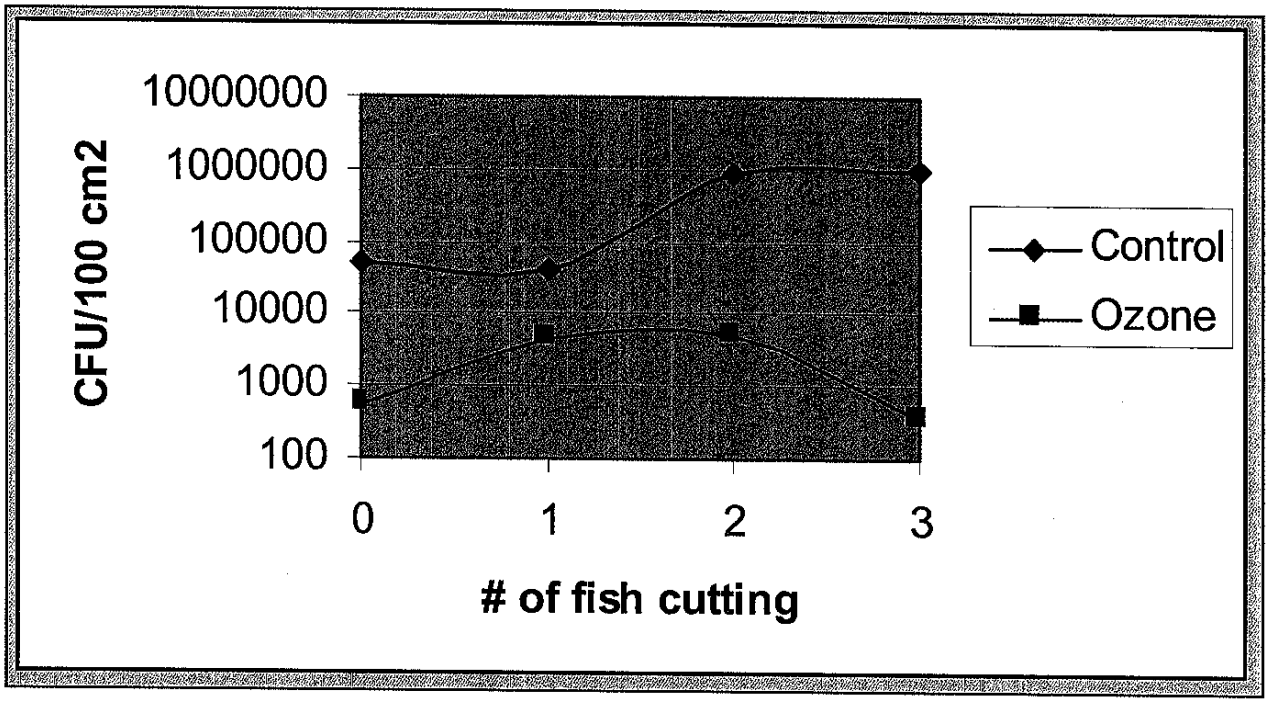


Control: current practice, only rinse knife in the beginning
Ozone: rinse each time before cutting fish

Figure 10. Table Surface Swab Results

CFU = Colony Forming Units;

cm2 = square centimeter



Control: current practice, only rinse table in the beginning

Ozone: rinse each time before loading fish

Figure 11. Fish Water Bacteria Results

CFU = Colony Forming Units;
ml = milliliter

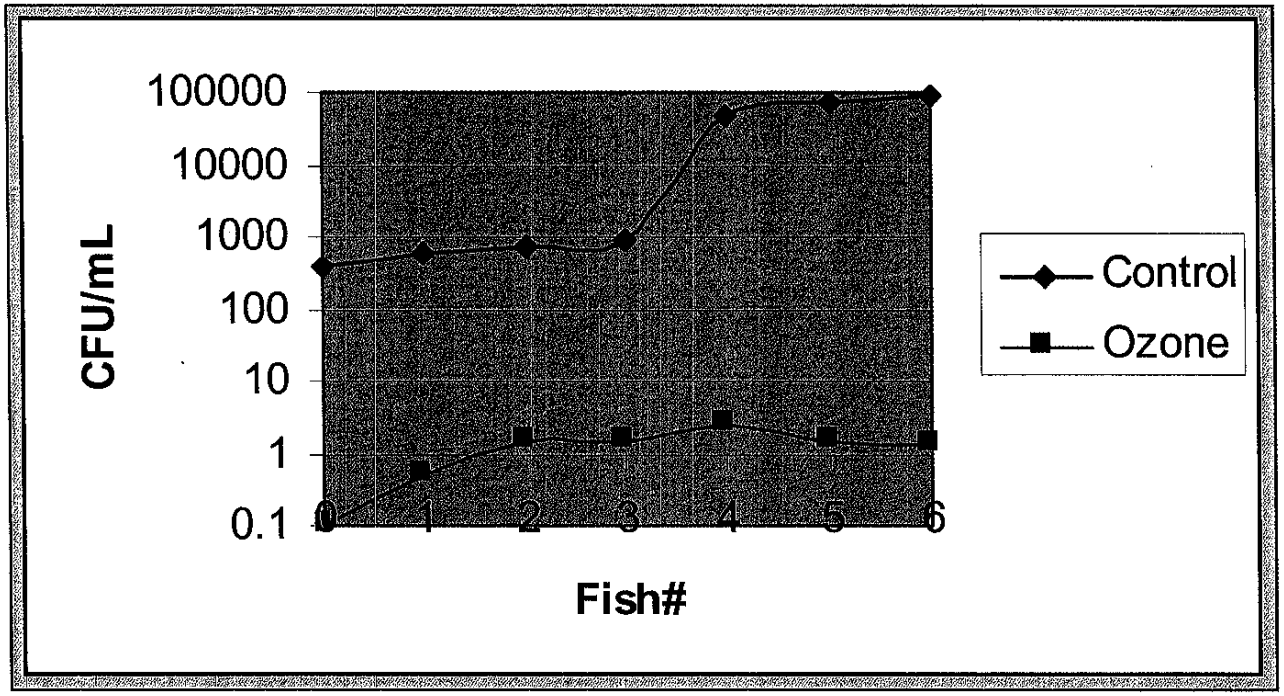
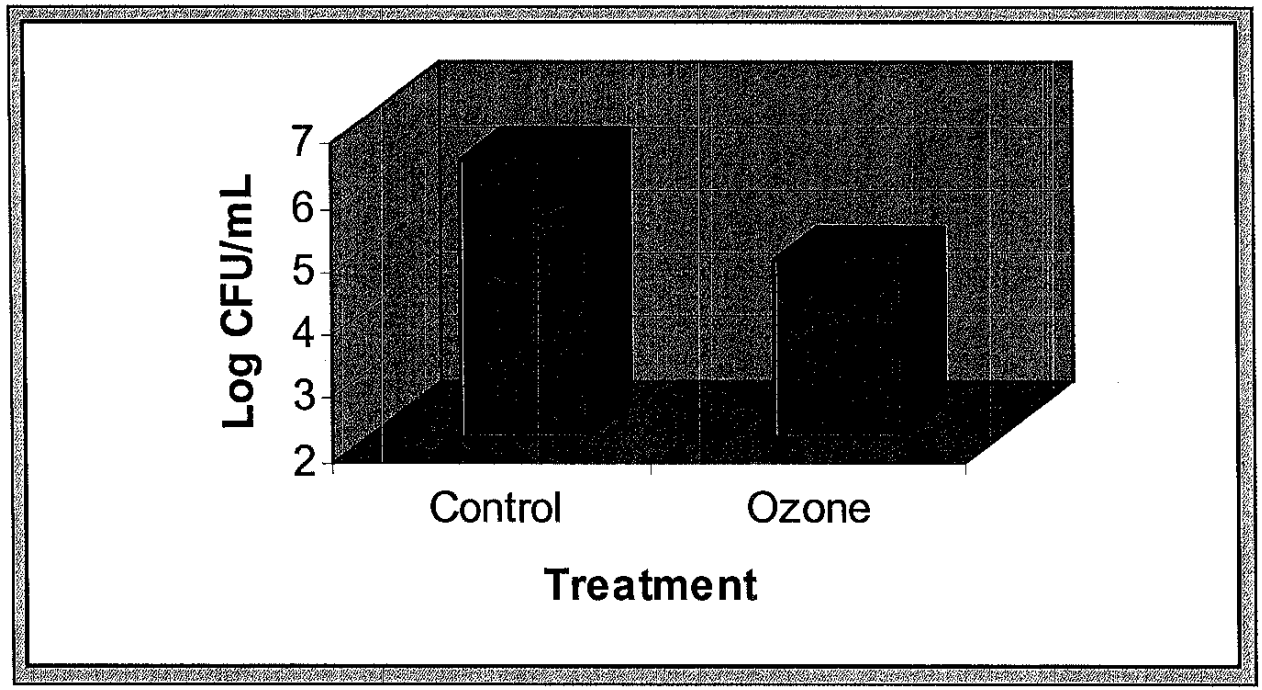


Figure 12. Runoff Water Micro Results

CFU = Colony Forming Units;
ml = milliliter



Runoff water samples were taken close to the drain in the middle of processing room.

Figure 13. Fish Surface Micro Results

CFU = Colony Forming Units;

cm2 = square centimeter

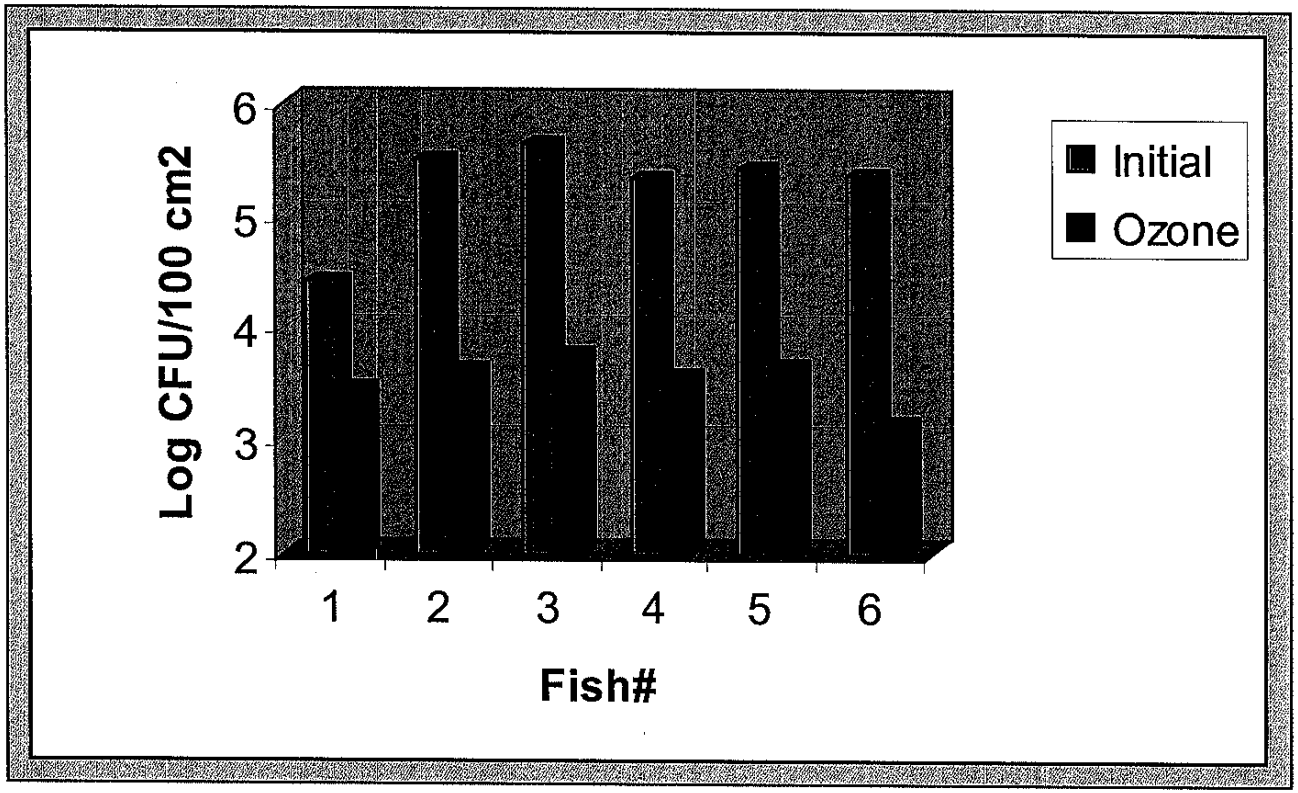


Figure 14. Aerobic Bacteria Counts

CFU = Colony Forming Units

g = gram

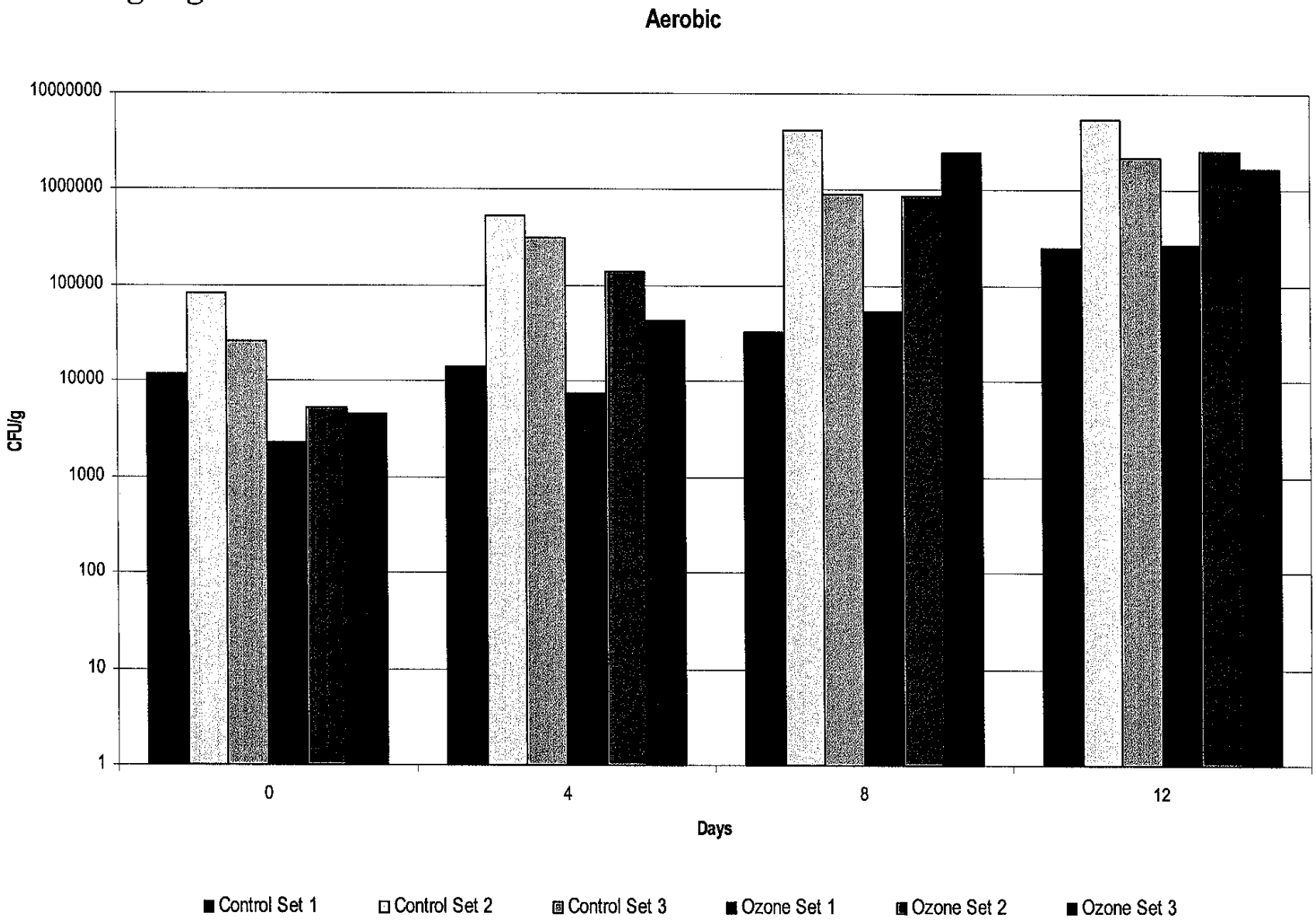


Figure 15. Anerobic Bacteria Counts

CFU/g = Colony Forming Units

g = gram