ULTRAFINE BUBBLE-ENHANCED OZONATION FOR WATER TREATMENT

by

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DEDICATION

To my father, mother, and siblings for their incredible love and support over my lifetime.

You will go out in joy
and be led forth in peace;
the mountains and hills
will burst into song before you,
and all the trees of the field
will clap their hands.

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3. Abstract

Ultrafine bubbles, often referred to as nanobubbles, have been used in various applications from environmental remediation to medicine. Even though the technology to generate ultrafine bubbles has been around for many years, the full potential of its applications has not been completely studied. This project seeks to study the use of ultrafine bubble technology for water treatment in combination with ozone gas. A factorial design experiment was chosen to test the effects of ultrafine bubbles on the concentration of an indicator organism, E. coli, in water as well as their effects on ozone gas being injected into water. Ozone gas or nitrogen gas was injected into water contaminated with E. coli as either ultrafine bubbles or fine bubbles as treatments for up to 60 minutes. Ultrafine bubbles were found to not have any significant effect on the concentration of E. coli in water. However, ultrafine bubbles did provide benefits when used in conjunction with ozone gas that regular, fine bubbles did not provide. The benefits included allowing the concentration of dissolved ozone in the water to decrease at a slower rate as well as allowing more ozone to dissolve into water at a higher rate than conventional methods of bubbling in ozone. While in this particular set of experiments the concentration of dissolved ozone in water didn't surpass 2 mg/L, which didn't allow for rapid disinfection and treatment of water, it is believed that with a more powerful ozone generator better results can be achieved. This project demonstrates the benefits and potential of injecting ozone gas as ultrafine bubbles into water as a way to effectively and efficiently disinfect and treat water.

4. Introduction

4.1. Background

GreenGate Fresh LLLP was founded in 2010 and provides fresh salad produce across America. The company operates in both Salinas, California and Yuma, Arizona, moving their machinery and operations by truck with the harvest seasons. In order to provide products with long shelf lives and fresh tastes, GreenGate Fresh uses a six step wash process to wash and process freshly harvested produce. Even with their current water conservation practices, GreenGate Fresh still uses just over 300,000 gallons of water per day. At their Yuma location, this large quantity of water is dumped into recharge basins while at their Salinas location it is dumped into the sewers which further incurs a sewage treatment fee. Furthermore, the water they purchase is delivered at about 70 degrees Fahrenheit which must be chilled to around 40 degrees Fahrenheit before use, which is an energy-intensive process. This project seeks ways to reduce the company's water, energy, and chlorine usage by introducing measures to allow part of the wash water to be recycled. By reusing the water that comes off of their washing cycles, a significant reduction in energy and water use could be achieved. The eventual goal is to develop a solution that would treat and recycle two-thirds of the water used to process fresh produce. Furthermore, exploring alternative methods of water treatment could reduce the levels of chlorine used and progress towards organic certification.

4.2. Problem Statement

The water rate in Yuma, Arizona for Commercial and Irrigation inside the city will average at $1.72 per hundred cubic feet of water over the next three years (Jan. 2017 to Jan 2019). Green Gate Fresh's operation uses approximately 40,232 cubic feet of water per day, amounting to roughly $20,760 per month in water costs alone the operation in Yuma, Arizona. Using California Water Service's water charge estimator as a gross estimate of water costs in Salinas, California, the cost of water is roughly $38,700 dollars per month and doesn't include the sewage treatment cost. The goal of the project is to introduce water recycling in order to reduce up to two-thirds of the water usage. Assuming that the project recycles half the water used to start off with, there is a projected cost savings of between $13,000 and $20,000 per month. These projections do not include anticipated cost savings from reducing the energy required to chill the water, as recycled water will be cooler than city water and will thus require less energy to cool to the desired temperature.

Currently, Green Gate Fresh is treating water used to process lettuce and other fresh produce with hypochlorite to disinfect the harvested produce and maintain a clean facility. There are significant benefits of using chlorination for disinfection; it is an industry standard treatment, it is low cost, and it is effective. Furthermore, treatment witch chlorine forms disinfectant residuals that remain in the water after treatment to continue to inactivate bacteria and suppress regrowth of pathogens. Organic standards set by the USDA allow for the use of chlorine materials used for disinfecting and sanitizing food contact surfaces with the exception that residual chlorine levels (Calcium hypochlorite, Chlorine dioxide, and Sodium hypochlorite) in the water must not exceed the maximum residual disinfectant limit under the Safe Drinking Water Act. The Safe Drinking Water Act sets the maximum residual disinfectant limit of chloramines and chlorine (Both as Cl_2) at 4 mg/L and chlorine dioxide (as ClO_2) at 0.8 mg/L (https://www.epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants#Byproducts).

The USDA allows ozone to be used on organic certified produce without limit, which makes it a possible treatment alternative to high doses of chlorine. Ozone is an unstable gas made up of three oxygen atoms. As such, it rapidly degrades into oxygen gas and a free radical oxygen atom. This highly reactive oxygen free radical has greater oxidative potential than the most common industry disinfectant, chlorine. As a result, ozone has been used in both industrial and private applications such as water treatment, disinfection, and odor removal. There are some other advantages to ozone treatment. It doesn't add chemicals to the water and it eliminates many taste and odor problems in treated water. The main disadvantages to using ozone, however, is that ozonation doesn't create germicidal residuals to prevent regrowth of pathogens in the water unlike chlorine-based treatments. Furthermore, ozone is less soluble in water than chlorine is in water, and ozone has a distinct and unpleasant odor that workers may complain about.

Previous studies have shown that when using dissolved ozone as a disinfectant, a contact time of 3 minutes at dissolved ozone concentrations of 4 mg/L can achieve a 3 to 5 log reduction in total coliforms that are naturally attached to leafy vegetables (Inatsu et al, 2011).

Ultraviolet light has germicidal and ozone-producing capabilities at wavelengths of 285 nm and 185 nm. UV has some distinct advantages including not requiring storage of hazardous materials, adding no smell or taste to water, requiring little contact time, and not having any potential for overdosing. UV radiation does have a few downsides. For one, UV radiation alone isn't effective when treating water with high levels of suspended solids, which reflects the condition of the water coming off the end of the line at Green Gate Fresh operations. Furthermore, UV disinfection does not leave disinfectant residuals in the water to continue suppressing bacterial regrowth after the UV treatment unlike chlorine and other chemical disinfectants.

Recent studies have explored the usage of fine bubbles in the micrometer range and ultrafine bubbles in the nanometer range to clean up water in both food processing and textile industries. Fine bubbles, also called microbubbles, are bubbles that have a diameter of 100 microns or less and have special properties due to their small size. Similarly, ultrafine bubbles, also called nanobubbles, are bubbles that have a diameter of 1 micron or less which gives the bubbles special properties due to the tiny size. Fine bubbles do not float to the surface but instead stay in the water, slowly diffusing gas into the liquid and shrinking in size until it implodes, which is believed to generate a small, localized shockwave that is thought to be able to break apart cell walls and other things in the water. Ultrafine bubbles on the other hand have been observed to remain stable in water for months. It's been observed in certain cases that water containing fine bubbles and ultrafine bubbles have been effective in removing biofilms and killing bacteria in water given a long enough treatment time. Since these treatment times need to range from several hours to a day, micro/nanobubbles by themselves would not be sufficient for the water treatment needs of Green Gate Fresh. However, the micro/nanobubble properties of not rising to the water surface and slowly diffusing gas into the water allows for oversaturation of gas in water and make it desirable to combine microbubble technology with ozone gas treatment. Using microbubbles in conjunction with ozone is expected to reduce the amount of ozone that is vented to the atmosphere thus reducing the odor of ozone in the facility, allow high dissolved ozone concentrations to ensure rapid disinfection of the water, and stabilizes the ozone in the water which will continue to disinfect the water for some time after the initial injection of ozone.

As another alternative for consideration, Exceptional Water Systems, a swimming pool company in Arizona, has been using air injected as microbubbles into swimming pool water then exposing this oxygenated water to high levels of UV radiation to maintain clean swimming pool water while using a minimal amount of chlorine. It is believed that doing so achieves disinfection on two levels; the UV light has germicidal effects while simultaneously energizing the oxygen in the microbubbles which generates free radicals which also have disinfecting effects. It should be noted that the water temperature is generally at or above room temperature. The effectiveness of UV irradiation has a correlation with water temperature, and as such warmer waters is usually required.

Water used to wash lettuce and other fresh produce is generally chilled before use. At Green Gate Fresh LLLP, the wash water is chilled to about 40 degrees Fahrenheit and chlorinated while the water they buy comes in at about 70 degrees Fahrenheit. As a result, a lot of energy is spent on chilling water alone. By recycling the water that comes off of their washing cycles and treating it with a system that doesn't add much heat to the water, a significant reduction in energy and water use could be achieved. Initial sampling of the water coming out of the facility shows that although there are no viable pathogens due to the high levels of chlorine used, there are about 0.18 g/L of plant particulates in the water.

4.3. Objectives

The purpose for performing this research is to determine whether ultrafine bubble technology can be beneficial for the treatment of captured produce wash water effectively. While the topic of micro/nanobubbles is not new, most of the research that have been conducted mostly concentrate on developing more efficient and more consistent methods of generating microbubbles and nanobubbles. Only recently has research on micro/nanobubble applications in water treatment, specifically in food processing, been undertaken. With this gap in the literature regarding fine bubble and ultrafine bubble applications, the major objective of this research is to validate the use of ozone nanobubbles to treat wastewater generated from washing fresh produce. This objective will be accomplished by addressing the following specific aims:

1: Compare the disinfecting effects of injecting ultrafine bubbles in the nanometer range with the effects of injecting fine bubbles into contaminated water.

2: Determine the effects injecting ozone gas as ultrafine bubbles in the nanometer range into water has on dissolved ozone concentration over time compared with injecting ozone gas as fine bubbles.

3: Determine if ozone gas injected into water as ultrafine bubbles is available in contaminated water for treating water.

4.4. Research Approach

A series of laboratory experiments were conducted at an indoor biosafety level one laboratory at the University of Arizona. The experiments followed a two factor factorial design to evaluate the capabilities of ultrafine bubbles and test different variables to gather and analyze results which influenced the design of the experiments. The main limitation of this work was an inability to directly measure the quantity and diameter of the gas bubbles that were being generated by the microbubble generators obtained by the lab. As such, an indirect method for quantifying microbubbles was used in the experiments. This indirect method involved measuring the dissolved oxygen and dissolved ozone concentrations in the water throughout the experiments and it was assumed that this measurement serves as a sufficient indication of the relative quantity and quality of the microbubbles in the water. Similarly, the lab did not have any protocol for measuring the amount of dissolved nitrogen in water so dissolved oxygen was used as an indirect method of measuring the amount of dissolved nitrogen in the water, as the amount of dissolved nitrogen in the water is inversely related to the amount of dissolved oxygen in the water.

5. Literature Review

5.1. History of Ozonation

5.1.1. Background

Ozone is an unstable gas made up of three oxygen atoms. As such, it rapidly degrades into oxygen gas and a free radical oxygen atom. This highly reactive oxygen free radical has greater oxidative potential than the most common industry disinfectant, chlorine. As a result, ozone has been used in both industrial and private applications such as water treatment, disinfection, and odor removal. There are some other advantages to ozone treatment. For instance, ozone doesn't add chemicals to the water and it eliminates many taste and odor problems in treated water. The main disadvantages to using ozone, however, is that ozonation doesn't create germicidal residuals to prevent regrowth of pathogens in the water unlike chlorine-based treatments, ozone is less soluble in water than chlorine is in water, and a system that uses ozone may require pretreatment of the water to reduce the water's hardness. Furthermore, most systems require large towers to maximize ozone dissolution and other equipment to capture and destroy off-gassed ozone.

There are a few ways ozone can be generated, including corona discharge, electrolysis, and UV irradiation (Gottschaulk, 2010). Ultraviolet lamps emitting light at 185 nanometers have been used for a long time to generate ozone. When oxygen passes over the lamp, the UV light splits O_2 molecules into single oxygen atoms, which bond with other O_2 molecules to form ozone. This method of ozone generation is not very efficient, and usually only produces a maximum ozone concentration of 0.5 percent and is usually only used for applications that only require small amounts of ozone (ozoneapplications.com). Electrolysis passes a current through water, splitting the oxygen atom in a water molecule from the hydrogen atoms. This creates ozone gas, oxygen gas, and hydrogen gas. This method requires pure water to be used and generates a lot of heat, although it produces ozone at a concentration of 20% to 30% by weight. A third method, corona discharge, is one of the most common methods of ozone generation. Corona Discharge utilizes an electrical charge diffused across a dielectric surface. When oxygen passes through the resulting electric field, oxygen molecules are split into individual oxygen atoms which then bond with other oxygen molecules, forming ozone at a concentration of between 3% and 5% by weight. There are some important considerations for producing ozone with this method. The first is that between 85% and 95% of the energy supplied ends up as heat energy, rather than producing ozone. Another consideration is that the input oxygen needs to be dry, as moisture in the gas can reduce the efficiency of ozone production and allow nitric acid to form. This can eventually lead to corrosion and equipment failure (http://www.lenntech.com/, http://www.ozonesolutions.com/). A fourth method of generating ozone is through passing pure oxygen through cold plasma, but this process is very expensive and thus rarely used.

At this time, the injection of ozone gas into wastewater is done by either pumping ozone gas through fine pore diffusers in water columns or by venturi injectors (Garcia-Morales 2012, Jaeger 2009). As the ozone gas bubbles rise through the water column in the first method, ozone is dissolved into the water where it then oxidizes the particles in the water. In a venturi injector, ozone is mixed into the water via venturi. All in all, the methods of ozonation all seek to inject small ozone bubbles into the water as smaller bubbles have more surface area relative to bubble volume, which increases the efficiency of ozone dissolution. One of the methods used to counter the buoyancy of ozone bubbles is to bubble in ozone along a vertical pipe or tower and having a downward flow of water to keep the ozone bubbles in the water as long as possible, allowing more ozone to dissolve into the water. When this method of injecting ozone into water is paired with a high frequency ozone generator, high levels of dissolved ozone between 10 and 12 mg/L can be achieved (Schulz et. al, 2000).

5.1.2. Ozone as a Disinfectant

As a disinfectant, ozone is very effective at destroying pathogens. Of note, ozone can be more effective than current standard treatment methods such as chlorine or UV irradiation for inactivating viruses, cryptosporidium, and other chemical-resistant pathogens (Mezzanotte 2007, Eriksson 2005, Korich 1990). Many studies have been conducted proving the efficacy of ozone treatment. One such study was conducted by Jyoti and colleagues in 2004, who compared ozonation treatment with acoustic cavitation and hydrodynamic cavitation. For the comparisons, Jyoti ran the following seven treatments on bore well water: 2 mg/L O3, ultrasonic horn, ultrasonic horn with 2 mg/L O3, ultrasonic bath, ultrasonic bath with 2 mg/L O3, hydrodynamic cavitation at 5.17 bars, and hydrodynamic cavitation at 5.17 bars with 2 mg/L O3. The group found that it took around 60 minutes to achieve a 93% kill rate of HPC bacteria when using an ozone concentration of 2 mg/L while it took only 15 minutes to achieve the same results with 4 mg/L of ozone (Jyoti, 2004). What the researchers found in this experiment was that while ozone does a good job disinfecting water, when paired with other physical methods of disinfection such as acoustic and hydrodynamic cavitation, the efficacy of the treatment was significantly increased and the amount of ozone needed to achieve the same level of potency also was significantly reduced. While large scale cavitation systems for industrial use currently are not very feasible, there is another method of introducing cavitation in water in order to supplement ozone treatment of water.

5.1.3. Shortfalls of Ozonation

One of the shortfalls of ozone treatment systems is that since ozone has a distinct odor and is an irritant to humans, especially to mucus membranes, such systems require vast setups that destroy or contain the ozone within the system. This is especially a problem with current ozonation methods which involve bubbling ozone gas through water, which is not very efficient as a majority of the ozone gas escapes from the water as the ozone gas bubbles rapidly rise to the surface of the water and burst. It is known that the mass transfer of ozone from bubbles into water is dependent on the contact time between the bubbles and water, as well as several other factors. While an easy way to increase the amount of time ozone bubbles stay in the water is to reduce the bubble size, injecting ozone as very small bubbles has been costly (Gong et. al, 2006). While there are methods to increase the efficiency of dissolving ozone into water, these usually involve building tall ozonation towers to give the ozone gas bubbles more time in the water. Similarly, ozone cannot be stored for long periods of time and thus requires on-site generation which when paired with the relatively higher costs of operating an ozone treatment system is more expensive to operate than other common treatment methods, such as using chlorine.

One of the reasons why ozone is a good treatment method also makes it less desirable for certain water treatment applications. Ozone does not leave residuals in solution that continue to disinfect and inhibit pathogenic growth in the water after the initial treatment event. Experiments have shown that ozone generally decays over the course of 15 to 20 minutes. One researcher found that "The DO_3 concentrations fell steadily in all non-continuous bubbling treatments. In the treatments with 0.2 ppm and 0.5 ppm starting concentrations fell to zero by 10 min, and in the 1.0 ppm and 2.0 ppm starting treatments fell to zero and 0.8 ppm, respectively after 15 min" (Ikeura, 2013). Another researcher looked at the decomposition of ozone at concentrations above 100 mg/L and observed that "ozone decomposes gradually over a period of 20 min. Fifty-five percent decomposition was observed at the end of 15 min" (Jyoti, 2004). For comparison, 1 ppm of ozone is nearly equivalent to 1 mg/L of dissolved ozone. There are many factors that influence the decomposition rate of dissolved ozone. Some of the more significant factors are the solution's pH, temperature, shear forces in the solution, and the presence of organic and inorganic compounds. The general trends are the rate of ozone decomposition increases as the pH increases, increases as temperature increases, and increases as the concentration of organic compounds increases. With inorganic compounds, the decomposition rate of ozone is more specific to individual compounds. For example, the rate of ozone decomposition increases with higher concentrations of hydrogen peroxide while surfactants like Dodecyl-Beta-D-Maltoside, Sodium dodecyl sulfate, and Dodecyl tri-methyl ammonium acetate all decrease the decomposition rate of ozone (Eriksson, 2005).

5.2. Ultrafine Bubbles and Fine Bubbles

5.2.1. Physical Properties

The existence of fine bubbles, also called microbubbles, has been known for a while now, and microbubble research has become more popular recently. Even more recently, ultrafine bubbles which are also referred to as nanobubbles have become a topic of great interest. A leading group in microbubble research defines microbubbles as "having a diameter < 50 μm and [they] have important technical applications due to their tendency to decrease in size and subsequently to collapse under water" (Takahashi, 2007). At this small size, microbubbles and even smaller nanobubbles have a different set of properties than larger bubbles in solution. For example, instead of floating to the surface and bursting, microbubbles in solution and under gravity tend to behave as solid colloid particles. Therefore, microbubbles stay in solution for extended periods of time, slowly decreasing in size due to a gradual dissolution of the gas within the microbubble into the surrounding solution until it eventually implodes, which is thought to create a localized, small shockwave similar to the effects of sonication. Generally, the time required for a microbubble to collapse is only several minutes although studies have shown microbubbles and nanobubbles to stay present in solutions for up to several months. Another result of the collapsing bubbles is the generation of hydroxyl radicals, which aid in disinfection of pathogens. (Takahashi 2006, Takahashi 2010). In an attempt to standardize terms relating to micro/nanobubbles, the Fine Bubble Industries Association recently classifies any bubble smaller than 100 microns in diameter to be a fine bubble and any bubble smaller than 1 micron to be an ultrafine bubble (www.fbia.or.jp/en).

There are several factors affecting the rate at which these micro/nanobubbles shrink and gradually implode. The primary factor is the composition of the gas, as different gas mixtures have different solubility and diffusion rates. Thus, other factors that influence the solubility and diffusivity of a gas in solution heavily influence the stability of these bubbles in solution. The most influential factors are associated with the physiochemical properties of the solution itself. For example, the solubility of a gas in a solution decreases as the temperature of the solution increases. Another way to both increase the stability of the micro/nanobubbles and reduce the average bubble diameter is to introduce a surfactant to the liquid (Xu, 2008). Furthermore, the stability of a bubble depends on the pressure difference between the gas inside the bubble and the water surrounding the bubble. When the pressure inside the bubble is greater than the pressure in the water, the gas in the bubble diffuses into the surrounding liquid. Furthermore, the gas pressure inside a bubble increases as the diameter of the bubble decreases. As a result, the rate at which the bubble shrinks and thus the rate at which gas dissolves from the bubble into the surrounding liquid increases over time (Xu, 2008). This is one of the advantages of injecting gas into liquids as microbubbles and nanobubbles, as the incredibly small size of micro/nanobubbles allows for much faster rates of ozone dissolution than injecting ozone as larger bubbles. Additionally, the half-life of ozone is longer when it is injected into water as micro/nanobubbles than when it is injected into water as larger bubbles (Sumikura 2007, Takahashi 2003).

5.2.2. Generation Methods

In general, there are three different methods of generating fine and ultrafine bubbles. The most popular method is through mechanical means. With this method, gas is injected into liquid as bubbles and then an impeller breaks up or shears the bubbles into fine and ultrafine bubbles. A similar method of generating micro/nanobubbles involves sonicating liquid with gas bubbles. The main downside to sonication is that it typically requires a surfactant to be present in the liquid in order to form the micro/nanobubbles. Compared to the mechanical methods of generating fine and ultrafine, sonication usually produces ultrafine bubbles with smaller diameters and at a higher concentration of ultrafine bubbles per volume of water (Xu, 2008). The third type of generating ozone is to use sharp pressure drops, called the pressure dissolution method. In this method, pressurized gas is dissolved into a solution that is then passed through a nozzle. When the mixture of dissolved gas and liquid passes through the nozzle, the mixture experiences rapid decompression and cavitation on the edges of the nozzle, which leads to the creation of microbubbles. Researchers in Kobe University, Japan looked into the pressurized dissolution method of generating micro/nanobubbles and found that this method of microbubble generation produced fine microbubbles at a high bubble count density. This improves upon the results of previous microbubble generation methods such as using shear to break up bubbles which creates bubbles with a wide range of diameters and a larger average bubble diameter. The ultrasonic method creates finer bubbles at a narrow range of bubble diameters but at lower densities (Maeda, 2015).

5.2.3. Measurement Methods

Measuring and quantifying fine and ultrafine bubbles in water has been somewhat difficult due to their physical properties. First, their small size makes it difficult to accurately measure their diameters due to limitations in optical imaging microscopes. One example of optically measuring the diameters of microbubbles was carried out using a microscope, a CCD video camera to capture images and video, and image processing software for bubble size and concentration determination. Secondly, microbubbles tend to shrink rapidly, which often results in smaller measured bubble diameters than the actual diameters. A third problem is that when micro/nanobubbles are not generated with uniform diameters, bubbles with different diameters tend to locate at different water depths due to the different rates at which bubbles with different sizes float. As a result, the sampling positions and timing may have an undesired yet significant effect on microbubble measurements. Furthermore, there is not yet a standard measuring technique for microbubbles so variations between the techniques of different research groups and even individuals may cause differences in results (Xu, 2008). Due to the difficulties and expense of the elaborate setups required to optically observe and measure microbubbles, another method of experimenting with such small bubbles involves presence validation rather than attempting to directly measure and quantify microbubbles. Several research groups have opted to indirectly measure microbubbles by measuring dissolved gas concentrations over time. Theoretically, the dissolved gas concentration in water where microbubbles are present will be higher while the gas is being injected and will decrease at a slower rate than if the gas was injected as larger bubbles. By comparing the dissolved gas concentration over time in different treatments, it is possible to determine whether micro/nanobubbles are present

(Sumikura, 2007). Among the current fine bubble industry, the most commonly used method of measuring fine bubble and ultrafine bubble size and concentration is done through Nanosight technology by Malvern. The Nanosight instruments can measure particles ranging from 10 nm to 2000 nm using light scattering properties and Brownian motion (malvern.com).

5.2.4. Biofilm/Surface Microbubble Treatment

Studies have been conducted over the potential for using microbubbles to break apart biofilms and treat surfaces covered with biofilms. In one such experiment, a solution of phosphate-buffered saline was recirculated through a microbubble generator producing microbubbles with diameters between 5 and 10 millimeters. Nylon membranes that with pre-cultured biofilms were then submerged vertically in the microbubble-laden PBS solution for 60 minutes. For comparison, the experiment was repeated with a solution of 0.5% NaOCl without microbubbles. It was observed that when treated with microbubbles for 60 minutes, the amount of fixed biomass, extra-cellular polysaccharides (PS), and extracellular proteins (PN) on the surface of the nylon membranes were reduced by between 85 to 88 percent. In contrast, nylon membranes treated with the 0.5% NaOCl solution for twice as long, 120 minutes, only saw a reduction in the amount of fixed biomass, PS, and PN by between 68 to 71 percent. These results were confirmed by confocal laser scanning microscopy (Agrawal, 2011).

5.3. Ozone Microbubbles

5.3.1. Water Treatment

A lab in Japan found that treating secondary effluent by injecting ozone gas at a concentration of 40 mg/L as microbubbles and a contact time of 10 minutes resulted in roughly a 25% inactivation of E. coli and a 44% inactivation of E. coli when the water was further ultrasonically irradiated (Sumikura, 2007). The microbubbles in the experiment had bubble diameters around 40 microns and were injected at a rate of 3 liters of ozone per minute. The study further suggests that air injected into water as microbubbles may lead to the generation of OH radicals. When paired with the hypothesis that collapsing microbubbles generate localized shockwaves powerful enough to break apart cell walls, it can be suggested that ozonation systems using microbubbles can even reduce the amount of ozone required to achieve the same level of bacterial inactivation.

In 2011, a different research group in Japan studied the effects of injecting ozone microbubbles into water for agricultural processes. The group found that when injecting roughly 5 mg/L of ozone into water as microbubbles, there was a 5 to 7 log reduction in viable bacteria count among free-floating bacteria in the water. When leafy vegetables such as lettuce, spinach, and cabbage were washed with water containing ozone microbubbles, an independent panel of taste testers did not find any significant difference in the taste and quality of the vegetables washed with ozone microbubbles versus other standard treatments used in the food industry (Inatsu, 2011).

5.3.2. Food Industry

When proposing treatment methods regarding food processing and food safety, it is important to consider any potential impact novel treatments may have on food quality and safety. A research group headed by Ikeura compared different vegetable washing methods to study how effectively different treatments could remove residual pesticides on the surface of vegetables. The treatments that were tested include ozone millibubbles, ozone microbubbles, dechlorinated water, and constant bubbling of ozone microbubbles. Throughout the experiments, the level of dissolved ozone was kept below 2.0 ppm. The pesticide that was tested was fenitrothion, which is a common pesticide used on crops. The experiments found that on lettuce and cherry tomatoes, treatments with ozone microbubbles removed more residual pesticide than ozone millibubble treatments and washing the vegetables with dechlorinated water. Additionally, the researchers observed that there were no discoloration or significant change in the pulling strength of lettuce leaves and cherry tomatoes, indicating that treatment with ozone microbubbles does not significantly affect produce quality (Ikeura, 2013). In a similar experiment, the group found that the same ozone microbubble treatment did not significantly affect the color and pulling strength of persimmon leaves (Ikeura, 2012).

5.3.3. Shortfalls

Despite the promising results, treatments of ozone microbubbles have shortfalls. When compared with using NaOCl solutions to wash leafy vegetables, ozone microbubble treatments seem to perform worse for reducing the amount of surface-attached bacteria on leafy vegetables. Injecting ozone gas into water as microbubbles did not seem to have any more germicidal effect on surface-attached bacteria than normally ozonated water has. The research group led by Inatsu found that the bactericidal activity of water containing ozone microbubbles was completely lost after washing the vegetables (Inatsu, 2011).

6. Materials and Methods

6.1. Materials and Equipment

The goals of this research were to compare the disinfecting effects of injecting ultrafine bubbles in the nanometer range with the effects of injecting fine bubbles into contaminated water, compare different methods of injecting ozone gas into water, and to determine if ozone gas injected into water as ultrafine bubbles is available in contaminated water for treating water.

The lab acquired two fine and ultrafine bubble generators and ran tests to compare the two. The first micro/nanobubble generator was an ASUPU Co., LTD ASK3M micro/nanobubble generator (shown in Figure 1). This micro/nanobubble generator uses a hybrid method of pressurizing the liquid and gas as they are mixed by an impeller and then releasing the pressure rapidly to form bubbles. According to experiments run by other labs, this method of bubble generation produces bubbles between 100 nm and 500 nm in diameter at a concentration of around 2.5E5 bubbles per mL. The ASK3M has a water flow-rate of 2 gallons per minute and a gas flow-rate of 100 mL per minute.

Figure 1. ASUPU ASK 3M Micro/Nanobubble Generator.

The second ultrafine bubble generator was a Gaia USA Inc. Model A49035 Ultrafine bubble generator with a 3/4 inch mixing valve (shown in Figure 2). This nanobubble generator uses a proprietary mixing method to inject gas into water as ultrafine bubbles in the nanometer range. Independent testing demonstrated that this generator produces bubbles that are on average 107 nm in diameter at a concentration of 1E8 bubbles per milliliter (Appendix I). The Gaia ultrafine bubble generator has an optimal water flow-rate of 30 gallons per minute and gas flow rate of 2 liters per minute.

Figure 2. Gaia USA Inc. Model A49035 Ultrafine Bubble Generator with 3/4" Mixing Valve.

As a control, air diffusing stones used in the beer brewing industry were used to compare any differences between micro/nanobubbles and bubbles (shown in Figure 3). The air diffusing stones used are 1 inch, stainless steel air stones with a pore size of 0.5 microns. Two of these air diffusing stones were connected in parallel during experiments. The gas flow rate through each air diffusing stone is around 0.75 liters per minute at 5 PSI.

Figure 3. Air diffuser, carbonating stone with 1/4" barb and 0.5 micron pores commonly used in the brewing industry.

Ozone for the experiments was generated onsite with an A2Z Ozone, Inc MP-1000 commercial ozone generator. The ozone generator is rated to produce 1 gram per hour of ozone with an oxygen feed flow rate of 4 L/min at a concentration of 0.98% by weight using Corona Discharge technology.

Figure 4. A2Z Ozone MP-1000 ozone generator.

Measurements of dissolved oxygen was conducted with a YSI Pro20 Dissolved Oxygen Instrument (shown in Figure 4). The dissolved oxygen instrument is a user calibrated instrument with a dissolved oxygen concentration measurement range from 0 to 50 mg/L that also measures water temperature, dissolved oxygen concentration, and atmospheric pressure.

Figure 5. YSI Pro20 Dissolved Oxygen Instrument.

The indicator organism chosen for these experiments is the K-12 strain of E. coli. E. coli K-12 is a model organism for many biological models as well as a commonly used indicator organism for water treatment tests. Of the major groups of coliforms, E. coli is typically not found growing and reproducing in the environment which combined with the other characteristics of E. coli makes it an excellent indicator organism for water treatment tests.

When water samples were drawn, the samples were diluted with phosphate-buffered saline solution through serial dilutions. The agar plates that were used to plate diluted samples during the experiments were made by adding 1.5 grams of agar powder per 100 mL of tryptic soy broth. After the mixture is autoclaved, 7 mL of the mixture was poured into each sterile petri dish then allowed to cool in a biosafety cabinet.

6.2. Ultrafine Bubble Effects on Ozone Dissolution and Dwell Time

In order to test if injecting ozone or ozone into water as ultrafine bubbles has any effect on the dwell time of ozone and dissolved oxygen in water, a series of tests were run to observe any effects. The test was repeated with the two micro/nanobubble generators. A 55-gallon barrel was filled with 50 L of tap water. Compressed oxygen was fed into the A2Z Ozone generator at 5 psi at a rate of 1.5 liters per minute. The ozone gas produced by the ozone generator was then fed into the Gaia ultrafine bubble generator. The microbubble generator was run until the dissolved oxygen and dissolved ozone concentration in the water stopped rising. The water was then allowed to sit while dissolved ozone concentrations were measured over time using a Hach dissolved ozone kit.

6.3. Main Experiments - Microbubble Disinfection of Coliforms

Previous literature suggested that the presence of microbubbles could disinfect water through the physical shockwaves generated by imploding microbubbles (Agrawal, 2012). The main set of experiments set out to determine if injecting gas into water as microbubbles had any effect on the bacteria population in water. The experiments followed a factorial design with two factors to compare the disinfecting effectiveness of different types of ozone injection methods as well to test for any effects ultrafine bubbles by themselves might have by injecting different gases as nanobubbles. In order to allow for reasonable comparisons between the different factors, the same experiment was run for every scenario, with the gas being injected and the injection method being the only two independent variables. The two gases used were ozone and nitrogen. Industrial-grade oxygen and nitrogen gas were obtained from the cryogenic services from the University of Arizona and ozone gas was generated on-site for experiments with an A2Z Ozone, Inc MP-1000 commercial ozone generator. The two methods of injecting gas into the water were the Gaia UFB generator and air diffusers with 0.5 micron pores. The purpose of using ozone and nitrogen is to observe whether ultrafine bubbles by themselves have any disinfecting ability as alluded to in literature, as nitrogen is an inert gas and should not be contributing to any effect on the E. coli. The experimental procedure (shown in Figure 5 and Figure 6) was as follows: Two liters of tryptic soy broth was inoculated with the k12 strain of E. coli and allowed to incubate at 37 Centigrade overnight. A food-grade, chemical resistant 55-gallon barrel was filled with 50 L of tap water and the temperature is regulated to 25 Centigrade. The gas being tested was injected into the water using the injection method being tested for 45 minutes. The dissolved oxygen or dissolved ozone concentration was measured. After 45 minutes, the 2 liters of overnight E. coli culture was rapidly mixed into the water. A water sample was drawn immediately to indicate the initial concentration of E. coli in the water. The gas was continually injected for an hour with water samples taken throughout the experiments, which were then diluted with phosphate buffered saline through serial dilutions, then plated on 7 mL agar plates made by mixing TSB media with agar. The plates were incubated at 37 Centigrade for 24 hours before the number of E. coli colonies were counted by hand. These counts allowed the concentration of E. coli in the water throughout the duration of the experiment to be calculated in colony-forming units (CFU).

To see whether increased levels of dissolved oxygen in the water while ozone gas was injected into the water had any effect on the indicator organism, oxygen was injected into water contaminated with E. coli and samples were taken over time. As a control for the main experiment, the experiment was also replicated without injecting any gas into the water. Instead, the E. coli culture was mixed into fresh tap water and left alone for 60 minutes, with water samples being drawn over time to determine the effects of tap water on E. coli in the water.

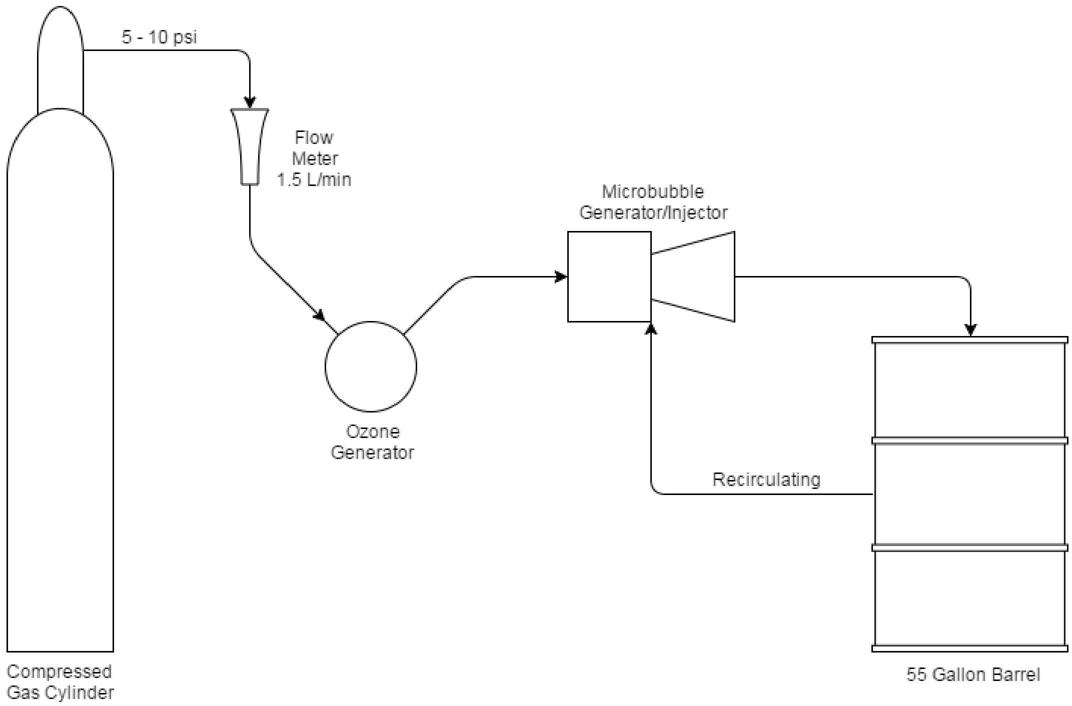


Figure 6. Diagram of the experimental setup. A compressed gas cylinder feeds compressed oxygen into an ozone generator which feeds ozone into the microbubble generator or air diffuser, which recirculates water in the experiment vessel.

Figure 7. A photograph of the experimental setup in the lab.

7. Results

7.1. Microbubble Effects on Ozone Dissolution and Dwell Time

The first set of experiments sought to observe differences in dissolved ozone dwell time between three methods of injecting ozone into the water. The three methods are injecting ozone with the Gaia Ultrafine Bubble Generator, which uses a proprietary mixing valve to shear bubbles into ultrafine bubbles, an ASUPU ASK3M micro/nanobubble generator which uses a two-step process involving pressurized gasses and an impeller to form microbubbles and nanobubbles (also called ultrafine bubbles), and just injecting gas into water using common air diffusers with 0.5 micron pores, commonly used in the brewing industry. The results of this series of tests are as follows and are displayed in Table 1.

7.1.1. Gaia Ultrafine Bubble Generator

When the ozone dwell time tests were run with a Gaia ultrafine bubble generator, the generator was run for 45 minutes before the dissolved ozone concentration stopped rising. The dissolved ozone concentration rose to a maximum of 1.4 mg/L at a water temperature of 28.6 Centigrade. It should be noted that the water temperature only rose by 0.5 Centigrade while the bubble generator was running and at lower temperatures, the solubility of ozone gas in water was higher. When the bubble generator was running, the concentration of dissolved ozone rose at a linear rate of approximately 0.045 mg/L per minute. On the other hand, the concentration of dissolved oxygen rose at a non-linear rate that could be approximated by the equation:

$DO_2 = -0.36t + 1.87t$ Equ. 1

After the bubble generator was turned off, the rate of dissolved oxygen steadily decreased at a rate of -0.0091 mg/L per minute while the concentration of dissolved ozone slowly decreased in a nearly linear rate that could be approximated by the equation:

$DO_3 = 0.00003t^2 - 0.015t$ Equ. 2

7.1.2. ASUPU ASK3M Microbubble Generator

When the previous test was run with the ASUPU ASK3M microbubble generator, the level of dissolved ozone and dissolved oxygen likewise stopped increasing at 45 minutes with a maximum dissolved ozone concentration of 0.94 mg/L and a dissolved oxygen concentration of 29.56 mg/L. After 45 minutes of running the bubble generator, the bubble generator was turned off and the water was allowed to rest. The water temperature initially started at 23.6 Centigrade and increased to 25 Centigrade after running the microbubble generator for 45 minutes. The level of dissolved oxygen in the water rose very quickly to around 29 mg/L in 10 minutes at a rate approximated by:

DO_2 = 0.066t^2 + 1.346t Equ. 3

However, the amount of dissolved oxygen did not increase to 30 mg/L despite the bubble generator continuing to run for some time afterwards. The concentration of dissolved ozone rose to a maximum of 0.94 mg/L at 40 minutes at a rate that can be approximated by the equation:

$DO_3 = -0.0008t^2 + 0.055t$ Equ. 4

When the bubble generator was turned off, the dissolved oxygen concentration in the water decreased at a rate approximated by:

$DO_2 = 0.0008t^2 - 0.225t$ mg/L Equ. 5

The dissolved ozone concentration in the water decreased at a rate approximated by:

$DO_3 = 0.00008t^2 - 0.023t$ mg/L Equ. 6

Compared to the Gaia UFB generator, the ASUPU ASK3M microbubble generator increased the concentration of dissolved oxygen to maximum levels much faster although it took about the same time to increase the concentration of dissolved ozone to maximum levels. This could probably be explained by the differing mechanisms that the two bubble generators utilize to form tiny bubbles. The Gaia UFB generator utilizes a mixing valve that shears the bubbles into ultrafine bubbles in the nanometer range, while the ASUPU microbubble generator injects air in a higher pressure and uses an impeller to violently mix the water, breaking the bubbles apart. The pressure then rapidly decreases which makes the bubbles even smaller. Since ozone decomposes rapidly when subjected to violent mixing in water but oxygen does not decompose as easily, the level of dissolved oxygen in the water rapidly rises as the gas is better mixed into the water, but the level of dissolved ozone increases slowly as most of the ozone prematurely decomposes into oxygen. The violent mechanical action also likely explains why the water temperature rose more when using the ASUPU microbubble generator instead of the Gaia UFB generator.

7.1.3. Air Diffuser (0.5 Micron)

As a control, the previous tests were run by bubbling ozone gas into the water using stainless steel air diffusers with a pore size of 0.5 microns. These air diffusers are usually used in the beer brewing industry to carbonate beer and produce fine bubbles rather than microbubbles or ultrafine bubbles. For this test, the water temperature remained at 25 Centigrade as there were no mechanical parts heating the water. The concentration of dissolved ozone seemed to increase at two distinct rates. For the first 15 minutes, the concentration of dissolved ozone increased at a rapid rate of 0.046 mg/L per minute. Afterwards, the concentration of dissolved ozone in the water steadily increased at a rate of 0.007 mg/L per minute until the concentration of dissolved ozone maxed out at 1.24 mg/L. After the ozonation was stopped and the water was allowed to rest, the concentration of dissolved ozone decreased at a rate that could be approximated by the equation:

$$DO_3 = 0.00005t^2 - 0.024t \qquad \text{Equ. 7}$$

Table 1. Data for Dissolved Ozone Dwell Time comparing the Gaia Ultrafine Bubble Generator, the ASUPU ASK3M Micro/nanobubble Generator, and an Air Diffusing Stone.

Ozone with Gaia UFB Generator			Ozone with ASUPU ASK3M MNB Generator			Ozone with 0.5 um Air Diffuser	
Time (min)	DO3 (mg/L)	DO2 (mg/L)	Time (min)	DO3 (mg/L)	DO2 (mg/L)	Time (Min)	DO3 (mg/L)
0	0	6.54	0	0	7	0	0
15	0.7	26.5	1	0.03	12.4	5	0.23
30	1.3	30.3	5	0.26	16.2	10	0.44
45	1.4	30.04	10	0.51	28.86	15	0.7
60	1.16	29.9	15	0.68	28.68	30	0.81
75	1.06	29.7	20	0.76	28.05	42	0.88
90	0.8	29.4	25	0.86	29.5	60	0.97
105	0.74	29.4	30	0.9	29.26	90	1.24
120	0.64	29.4	35	0.82	28.73	100	1.05
135	0.62	29.3	40	0.94	29.56	115	0.82
240	0.28	28.2	45	0.8	29	125	0.69
2640	0.04	16.06	50	0.77	29.22	165	0.4
			55	0.67	28.25	225	0.06
			65	0.52	24.3		
			80	0.33	23.4		
			95	0.22	24.8		
			110	0.12	22.4		
			125	0.04	22.4		
			135	0.02	22		
			165	0.01	22		

7.2. Main Experiments - Microbubble Disinfection of Coliforms

The purpose of the main set of experiments was to determine whether ozone gas injected into water as ultrafine bubbles is available in contaminated water for treating water as well as to compare the disinfecting effects of injecting different gasses into the water using different methods. The Gaia Ultrafine Bubble Generator represents the injection of gas into water as ultrafine bubbles in the nanometer range whereas the 0.5-micron air diffuser represents a more common method of injecting gas into water as fine bubbles. The results for the four different treatment combinations and two additional controls are discussed below and are shown in Table 2 and Figure 7.

7.2.1. Ozone with Gaia Ultrafine Bubble Generator

The first set of experiments ran looked at injecting ozone as ultrafine bubbles in the nanometer range using the Gaia UFB generator. The concentration of dissolved ozone ranged from 1 mg/L to 2.1 mg/L. As the units of mg/L and parts per million (PPM) have a one to one correspondence, the concentration of dissolved gasses are referred to in units of PPM. These series of experiments saw a 2 to 4 log reduction in the concentration of the indicator organism, E. coli, after 60 minutes of treatment. The 4 log reduction in E. coli was achieved when the initial dissolved ozone concentration was 2 PPM while the 2 log reduction was achieved when the initial dissolved ozone concentration was 1 PPM.

7.2.2. Ozone with 0.5-micron Air Diffuser

The next series of experiments injected ozone gas with air diffusers. The concentration of dissolved ozone could only reach between 1.05 PPM and 1.25 PPM which was reflected in a 1 to 2 log reduction in E. coli. Surprisingly, the higher dissolved ozone concentration of 1.25 did not result in a greater rate of E. coli reduction.

7.2.3. Nitrogen with Gaia Ultrafine Bubble Generator

The experiments injecting the inert nitrogen gas as ultrafine bubbles using the Gaia UFB generator showed negligible change in the concentration of E. coli even after 60 minutes of treatment.

At the time of the experiments, the lab did not have equipment to measure the concentration of dissolved nitrogen in the water so the concentration of dissolved nitrogen was indirectly measured by measuring the concentration of dissolved oxygen in the water with the assumption that the dissolved oxygen concentration in the water was inversely related to the concentration of dissolved nitrogen in the water.

7.2.4. Nitrogen with 0.5-micron Air Diffuser

When nitrogen gas was injected with air diffusers, the concentration of dissolved oxygen in the water only decreased to around 1.5 PPM. Like the previous experiments with nitrogen gas being injected as ultrafine bubbles, there was a negligible amount of change in the concentration of E. coli after 60 minutes of treatment.

7.2.5. Oxygen with Gaia Ultrafine Bubble Generator - Control

Looking at the experiments with ozone, it can be seen that after a reduction of E. coli in the first few minutes, the E. coli population seems to regrow before being reduced again. An experiment was run on the side to determine if high levels of oxygen in the water would affect the concentration of E. coli and perhaps explain the trend. The results of the experiment showed that when the water is oversaturated with oxygen, the concentration of E. coli in the water is no significantly affected.

7.2.6. Tap Water Only - Control

Another test was conducted to establish a control, where water spiked with E. coli was allowed to sit for 60 minutes without any kind of treatment. This test showed that there was no significant change in the concentration of E. coli in tap water without any kind of treatment.

Table 2. Collected data showing treatments and their respective E. coli log reduction.

	Ozone With Gaia UFB Generator			**Ozone With Air Diffuser 0.5 Micron**		
	1.0 PPM Ozone	***1.3 PPM Ozone***	***2.1 PPM Ozone***	***1.25 PPM Ozone***	***1.05 PPM Ozone***	***1.05 PPM Ozone***
Time (min)	***CFU/mL***	***CFU/mL***	***CFU/mL***	***CFU/mL***	***CFU/mL***	***CFU/mL***
0	2.6E+06	2.5E+06	1.5E+07	2.0E+07	1.5E+06	1.5E+06
5	7.2E+05	7.5E+05	7.7E+06	2.8E+07	5.0E+05	2.0E+06
10	1.6E+06	1.5E+06	9.2E+06	2.7E+07	-	-
20	2.7E+06	4.4E+05	-	2.0E+07	-	-
30	-	-	-	-	3.2E+05	2.6E+06
40	1.9E+05	2.3E+05	-	1.0E+07	-	-
60	3.0E+03	2.5E+04	2.1E+03	1.0E+06	5.0E+03	2.5E+04
Log kills:	**2.94**	**2.00**	**3.85**	**1.30**	**2.48**	**1.78**

	Nitrogen With Gaia UFB Generator				**Nitrogen With Air Diffuser 0.5 Micron**		
	0.7 PPM Oxygen	***0.7 PPM Oxygen***	***0.57 PPM Oxygen***	***0.6 PPM Oxygen***	***1.45 PPM Oxygen***	***1.5 PPM Oxygen***	***1.49 PPM Oxygen***
Time (min)	***CFU/mL***	***CFU/mL***	***CFU/mL***	***CFU/mL***	***CFU/mL***	***CFU/mL***	***CFU/mL***
0	4.5E+07	1.6E+07	2.5E+07	1.7E+07	1.3E+07	2.0E+07	1.5E+07
5	1.6E+07	2.9E+07	3.0E+07	9.0E+06	-	-	-
10	1.7E+07	3.6E+07	1.9E+07	1.1E+07	-	-	-
30	1.2E+07	1.0E+07	1.6E+07	1.6E+07	1.0E+07	1.4E+07	1.1E+07
60	9.2E+06	9.3E+06	1.2E+07	1.5E+07	1.1E+07	1.0E+07	9.9E+06
Log kills:	**0.69**	**0.24**	**0.32**	**0.05**	**0.07**	**0.30**	**0.18**

	Oxygen With Gaia UFB Generator			**Tap Water Only**
	33.5 mg/L Oxygen	***34.0 mg/L Oxygen***	***32.0 mg/L Oxygen***	***5.89 mg/L Oxygen***
Time (min)	***CFU/mL***	***CFU/mL***	***CFU/mL***	***CFU/mL***
0	1.5E+07	1.6E+07	1.5E+07	7.2E+07
5	1.5E+07	1.6E+07	1.6E+07	-
20	1.7E+07	1.8E+07	1.7E+07	-
30	-	-	-	2.8E+07
60	1.9E+07	1.9E+07	1.8E+07	5.5E+07
Log kills:	**-0.10**	**-0.07**	**-0.08**	**0.12**

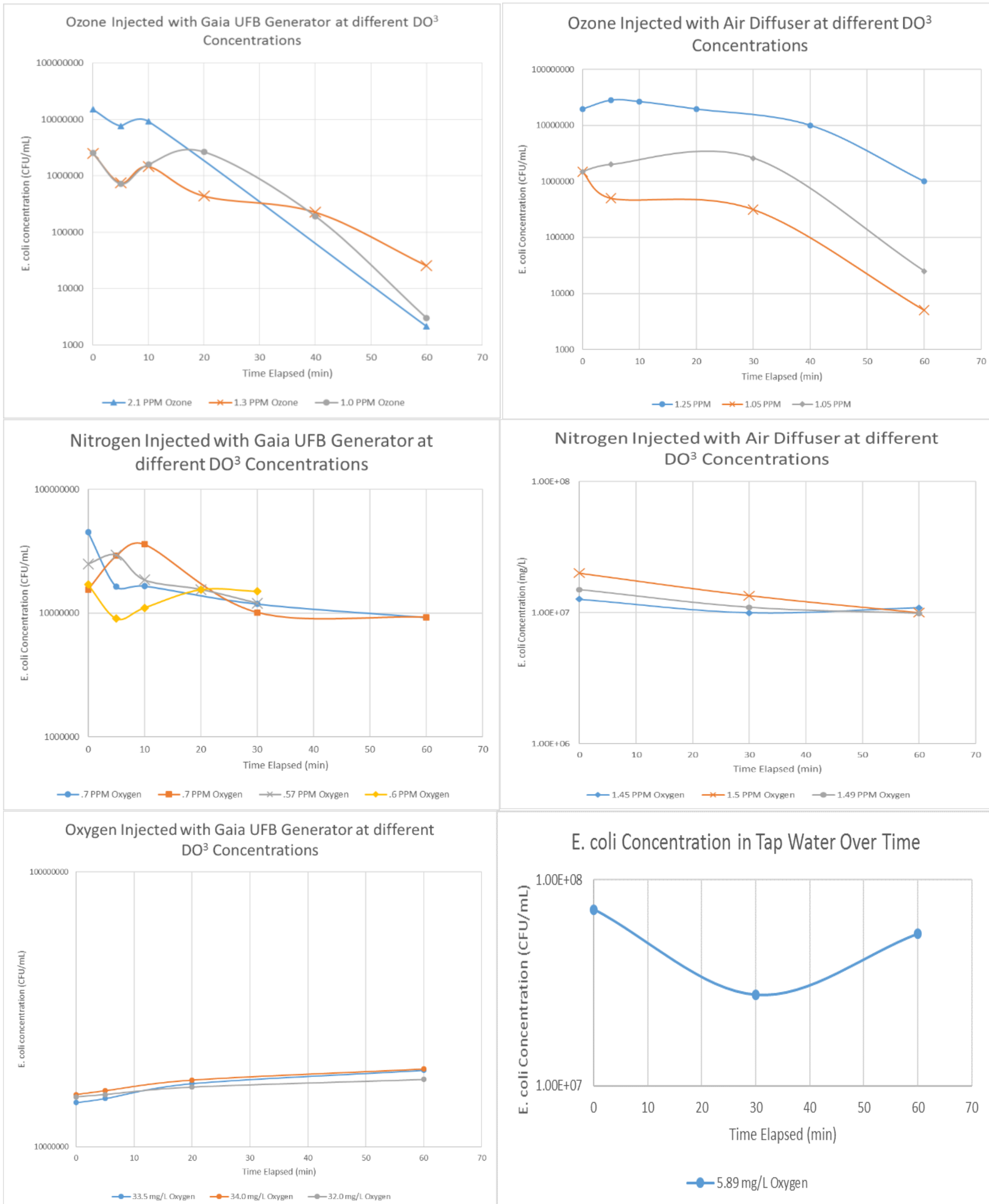


Figure 8. Graphs showing the E. coli concentration on a $\log_{10}$ scale in water over time for each treatment.

8. Analysis

8.1. Microbubble Effects on Ozone Dissolution and Dwell Time

To determine whether injecting ozone as ultrafine bubbles had any significant differences from injecting ozone normally through an air diffuser, ozone was injected into water through a Gaia ultrafine bubble generator, an ASUPU microbubble generator, and an air diffuser with 0.5 micron pores. The three tests were run for varying amounts of time up to different concentrations of dissolved ozone due to the different performances of the three methods of injecting ozone gas into water. The following chart (Figure 8) displays the data from the tests superimposed on top of each other.

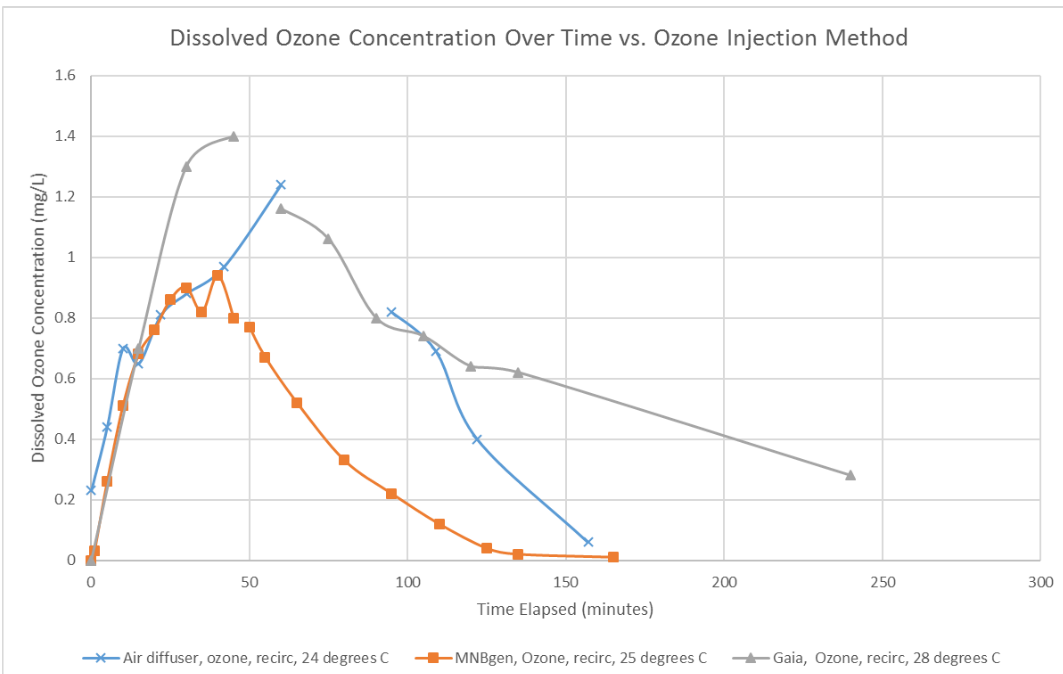


Figure 9. Comparison of ozone injection methods by observing dissolved ozone concentration over time.

Compared to the microbubble and ultrafine bubble generators, bubbling ozone into water resulted in about the same maximum dissolved ozone concentration in the water. However, injecting ozone as microbubbles and ultrafine bubbles allowed the dissolved ozone concentration to rise to the maximum concentration in about a third of the time it took for bubbling ozone into water with the air diffusers to reach the same maximum concentration. These difference between the three methods of ozone gas injection method can be seen when the dissolved ozone concentrations over time for each method are superimposed over each other in Figure 9.

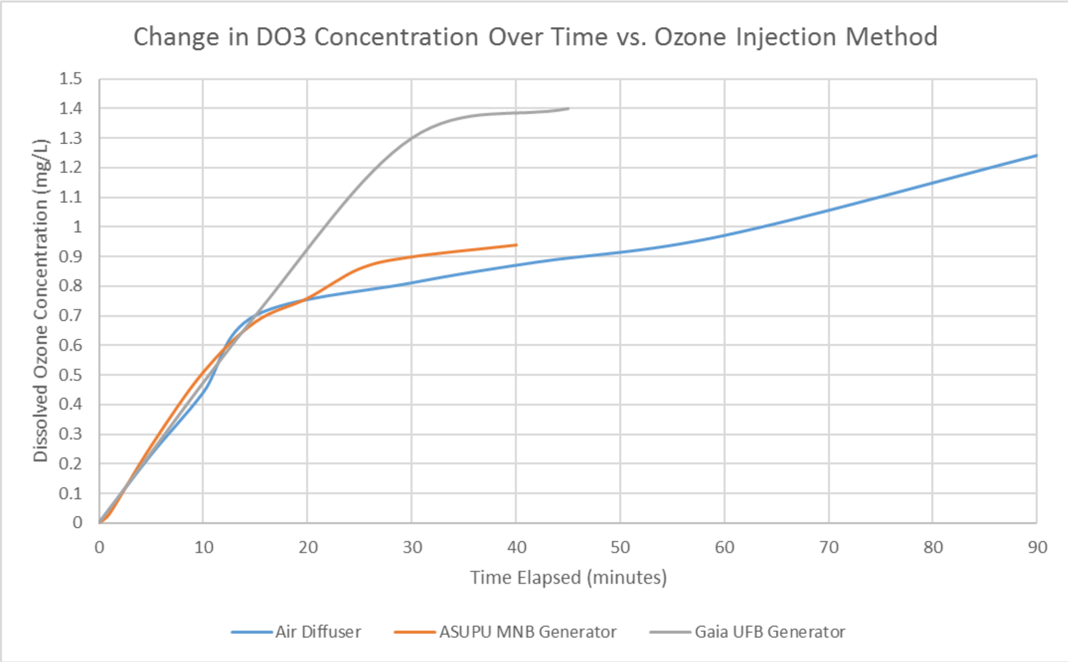


Figure 10. Comparison of the increase in dissolved ozone concentration over time with different ozone injection methods.

Furthermore, when ozone was injected into water using micro and ultrafine bubbles, the concentration of dissolved ozone in the water decreased at a lower rate than when ozone was bubbled into water using the air stones. Within about the first 20 minutes of ceasing ozonation, the concentration of ozone in water injected using air diffusers and the ASUPU microbubble generator both decrease at roughly the same rate, which is faster than the rate at which ozone injected by the Gaia UFB generator Beyond 20 minutes of ceasing ozonation, the concentration of dissolved ozone decreased at the fastest rate when ozone was injected by air diffusers, followed by the ASUPU microbubble generator, then finally the Gaia UFB microbubble generator, which had the slowest decrease of dissolved ozone. These results can be seen in Figure 10 which displays the change in dissolved ozone concentration in the water over time in order to normalize the results from the tests.

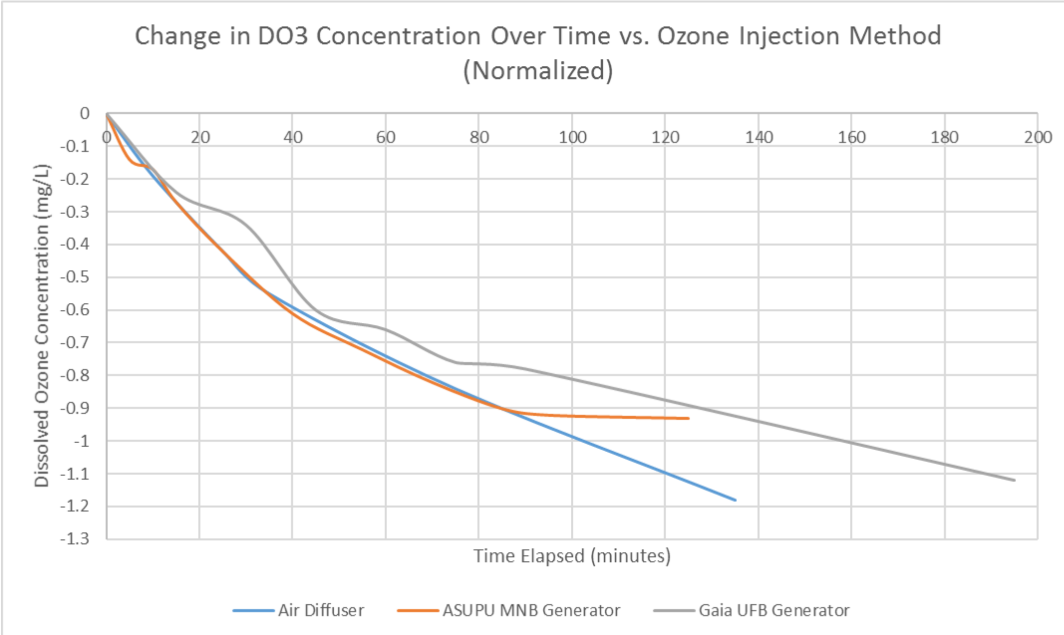


Figure 11. Comparison of the decrease in dissolved ozone concentration over time with different ozone injection methods after ozone injection was stopped.

8.2. Main Experiments - Microbubble Disinfection of Coliforms

The main set of experiments followed a two by two factorial design where the factors were the gas being injected into water spiked with E. coli and the method of gas injection. Since the objective of the project is to observe whether ozone gas injected as ultrafine bubbles can be used as an effective way to disinfect water, the disinfecting effects of ozone gas and ultrafine bubbles needed to be studied. For the gas factor, ozone was tested against nitrogen gas, which is inert and should not have any disinfecting effects on water containing E. coli. For the gas injection method, ultrafine bubbles were tested against fine bubbles injected by using an air diffuser representative of current ozonation equipment. Since it is known that ozone is a strong oxidizer, it is expected that ozone will reduce the concentration of E. coli more than nitrogen gas. By injecting the two gasses as either fine bubbles or ultrafine bubbles, the effects of ultrafine bubbles on both the gas being injected and on E. coli in the water can be observed.

At first glance, there seems to be a difference between the four treatments. Injecting ozone gas as ultrafine bubbles in the nanometer range seems to be the most effective disinfectant, followed by injecting ozone gas as fine bubbles, followed by injecting nitrogen gas as ultrafine bubbles, then finally injecting nitrogen gas as fine bubbles. In order to further analyze the results of the experiments, a two-way ANOVA test was run along with a Tukey HSD comparison (Table 3). The software used to analyze the results was “Two-Way ANOVA” written by Ian Holliday. The following figures show the results of the analysis.

Table 3. ANOVA Statistics, Tukey Honest Significant Difference Comparisons, and Levenes Test for Homogeneity of Variance for main experiments.

ANOVA Model				
Response ~ Treatment_A * Treatment_B				
means	0.182	1.602	0.229	0.911

ANOVA Statistics					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
	1				
Treatment_A	1	12.698	12.698	49.84	0
Treatment_B	1	1.406	1.406	5.52	0.047
Treatment_A:Treatment_B	1	0.623	0.623	2.445	0.157
Residuals	8	2.038	0.255		

Tukey Honest Significant Difference Comparisons				
	diff	lwr	upr	p adj
O-N	2.057	1.385	2.729	0
G-D	0.685	0.013	1.357	0.047
O:D-N:D	1.602	0.282	2.921	0.019
N:G-N:D	0.229	-1.091	1.549	0.942
O:G-N:D	2.742	1.422	4.062	0.001
N:G-O:D	-1.373	-2.692	-0.053	0.042
O:G-O:D	1.14	-0.179	2.46	0.092
O:G-N:G	2.513	1.193	3.833	0.001

Levenes Test for Homogeneity of Variance			
	Df	F value	Pr(>F)
Group	3	1.84	0.218
	8		

The results of the analysis shown in Table 3 indicate that there was a statistically significant difference between the injection of ozone and nitrogen into the water as a treatment, which was to be expected. When it came to injecting gas as an ultrafine bubble versus injecting gas normally through air diffusers, the p-value was 0.047, which indicates that the differences between the two methods of injecting gas is statistically significant. The interaction between the two factors was shown to have no statistically significant effect on the results, as the p-value was 0.157. A more in depth analysis using the Tukey HSD comparison indicated that while there is a statistically significant difference caused by the different methods of injecting gas, there was no statistically significant difference between the methods of injecting gas into the water as a treatment when a specific gas was injected using the two methods. This suggests that the disinfecting effects of the gas being injected has a far greater effect on the results than the method the gas is being injected, so long as the dissolved gas concentrations in the water are similar. Looking at the interaction between nitrogen gas injected as ultrafine bubbles in the nanometer range with the Gaia UFB generator and nitrogen gas injected as fine bubbles with the air diffuser, it is very clear that ultrafine bubbles in the nanometer range by themselves do not have any significant disinfecting effect in water. Even though there seems to be no difference in disinfection ability between ultrafine bubbles and fine bubbles, there still are benefits to injecting ozone as ultrafine bubbles in the nanometer range.

9. Conclusions

The research sought to evaluate the potential for using ultrafine bubbles in conjunction with ozone gas as a method of treating and disinfecting water. The experiments successfully demonstrated that while ultrafine bubbles by themselves do not appear to have any disinfecting of water treatment capabilities, there are benefits to pairing ultrafine bubble technology with ozone gas for water treatment. By injecting ozone gas into water in the form of ultrafine bubbles, it is possible to achieve higher levels of dissolved ozone in the water at the same water temperature or the same levels of dissolved ozone at higher water temperatures than if ozone gas was injected into water using conventional methods such as bubbling ozone gas through air diffusers. As ultrafine bubbles in the nanometer range do not float to the surface like normal bubbles do, significantly less ozone being injected into water escapes as bubbles popping at the water surface. Similarly, ultrafine bubbles tend to be more stable, allowing gaseous ozone to slowly dissolve into the water. Since ozone gas decomposes at a slower rate than ozone dissolved in water, injecting ozone gas into water as ultrafine bubbles allows the dissolved ozone to remain present in water longer than if ozone was bubbled into water.

10. Recommendations

While the research demonstrates the benefits and potential for using ultrafine bubble generators such as the Gaia UFB generator when injecting ozone into water for treatment, the high levels of dissolved ozone concentration in water described in other literature experimenting with ozone and microbubbles were not achieved. As a result, it was not possible to achieve any meaningful reduction in E. coli or destruction of cellulose particulate contaminants in the water with the shorter periods of treatment desired in industrial applications. It is believed that the unexpectedly low concentrations of dissolved ozone may be due to the relatively weak ozone generator used throughout these experiments. Furthermore, the experiments revealed that different mechanisms of generating ultrafine bubbles have drastic effects on the amount of ozone that can be successfully dissolved into water. More violent and energetic methods of generating microbubbles such as the mechanism used by the ASUPU ASK3 Micro-nanobubble generator tended to cause the ozone to decompose at a much greater rate than gentler mechanisms such as the one utilized by the Gaia UFB Generator. It is believed that with a more powerful ozone generator, a pressurized testing vessel, and a ultrafine bubble generator that utilizes a pressure dissolution mechanism, much greater dissolved ozone concentrations can be achieved with ultrafine bubble technologies. Further experiments could be conducted to test this hypothesis as well as to determine whether very high levels of dissolved ozone in water can be powerful enough to clear cellulose-based particulate contaminants from water.

11. Appendix

11.1. Appendix I: Gaia Ultrafine Bubble Generator Independent Testing

Gaia USA, Inc.
Scottsdale, Arizona

Gaia USA, Inc.
Measurement of Bubble Size Created With Gaia Mixing Technology
University of Osaka, Osaka, Japan
August 3 & 4, 2016

Description

Testing was conducted at University of Osaka, Photonics Department laboratory facility. IDEC Corporation, Fine Bubble Department owns and operates a lab at the University. Measurements were obtained using NanoSight 550 testing equipment manufactured by Malvern Instruments (http://www.malvern.com/). Bubbles generated by a Gaia Version 6 ¾"mixing valve with injected oxygen and carbon dioxide from a liquid gas bottle source were tested separately.

Summary Results

	Oxygen	Carbon Dioxide
Length of time system run	10 min	10 min
Starting Temp of DI Water	26.9 C	25.2 C
Ending Temp of DI Water	27.3 C	25.7 C
Volume of DI Water	33 L	33 L
Starting Concentration	6.69 ppm	2.4 ppm (pH 4.98)
Ending Concentration	29.96 ppm	1250 ppm (pH 3.33)
Concentration after 30 min of stopping system	29.46 ppm	1220 ppm (pH 3.39)
Gas Flow Rate and Pressure	2LPM @ 10 PSI	2LPM @ 10 PSI
Bubble Size	107 nm (0.107 um)	112 nm (0.112 um)
Bubble Concentration	100 million / mL	693.4 million / mL

Gaia technology produced ultrafine bubbles in the nanometer size range with bubble concentrations of 100 million/ml for injected oxygen and 693 million/ml for carbon dioxide.

 Page 1

Time Lapse Results

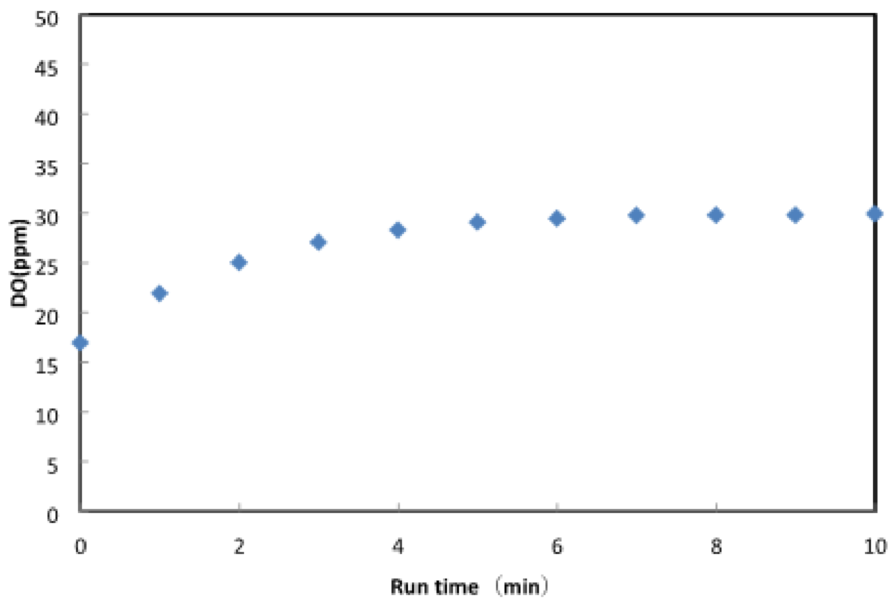
Oxygen Concentration Testing

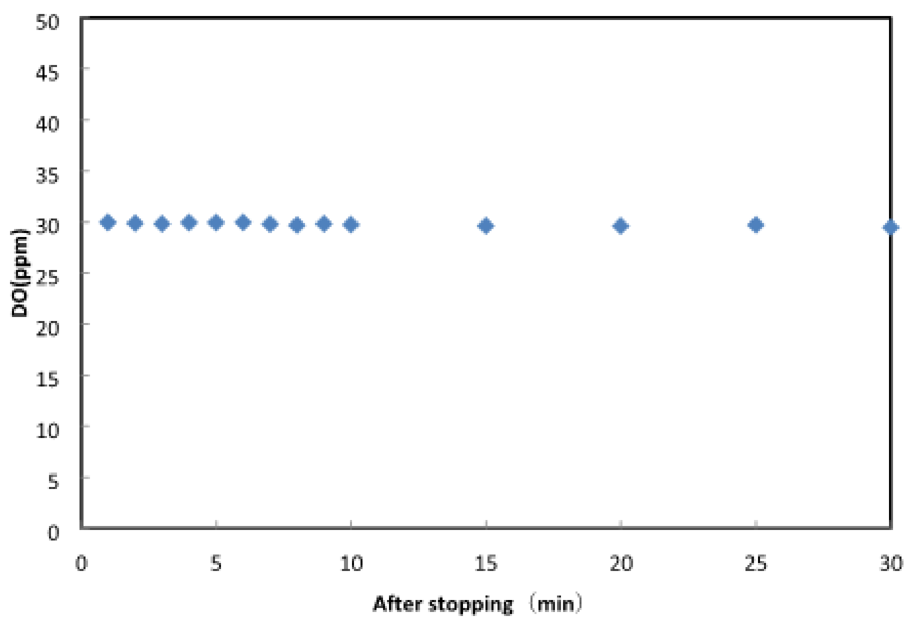
Parameters:

- Volume: 33 Liters, 8.72 gallons
- Gas Injection: 2LPM @10 PSI
- TDS: 2
- Starting DO: 6.69ppm
- Starting Temp: 27 C°/ 80.6 F°

Time (min)	Concentration (ppm)	Temperature (C°/ F°)
1	16.95	26.9 / 80.4
2	21.91	27 / 80.6
3	25.03	27 / 80.6
4	27.06	27 / 80.6
5	28.31	27.1 / 80.8
6	29.08	27.1 / 80.8
7	29.45	27.1 / 80.8
8	29.77	27.1 / 80.8
9	29.80	27.2 / 81
10	29.96	27.2 / 81
BUBBLE GENERATION STOPPED		
11	29.94	27.3 / 81.1
12	29.27	27.3 / 81.1
13	29.81	27.3 / 81.1
14	29.94	27.3 / 81.1
15	29.79	27.3 / 81.1
16	29.67	27.3 / 81.1
17	29.80	27.3 / 81.1
18	29.74	27.3 / 81.1
19	29.80	27.3 / 81.1
20	29.74	27.3 / 81.1
25	29.61	27.2 / 81
30	29.59	27.2 / 81

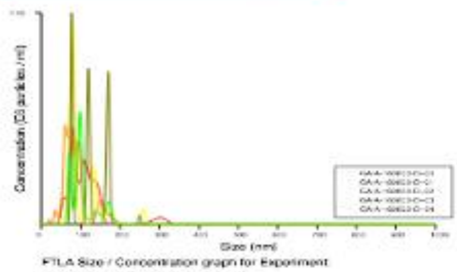
35	29.69	27.2 / 81
40	29.46	27.2 / 81



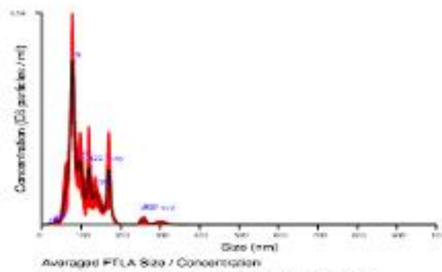


NANOSIGHT

GAIA-160803-O2 2L-30min_30min-



FTLA Size / Concentration graph for Experiment
GAIA-160803-O2 2L-30min_30min-



Averaged FTLA Size / Concentration
Red error bars indicate +/- 1 standard error of the mean

Included Files

GAIA-160803-O2 2L-30min_30min-00
GAIA-160803-O2 2L-30min_30min-01
GAIA-160803-O2 2L-30min_30min-02
GAIA-160803-O2 2L-30min_30min-03
GAIA-160803-O2 2L-30min_30min-04

Details

NTA Version:	NTA 3.0 0068
Script Used:	SOP Standard Measurement 11-58-50AM 03Aug2016.txt
Time Captured:	11:58:52 03/08/2016
Operator:	Kobayashi
Pre-treatment:	
Sample Name:	Water
Diluent:	
Remarks:	

Capture Settings

Camera Type:	SCMOS
Camera Level:	16
Slider Shutter:	1300
Slider Gain:	512
FPS	25.0
Number of Frames:	1498
Temperature:	26.9 - 27.0 °C
Viscosity:	(Water) 0.849 - 0.852 cP
Dilution factor:	Dilution not recorded

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 11.7 - 20.5 pix

Results

Stats: Merged Data	
Mean:	107.6 nm
Mode:	77.1 nm
SD:	44.8 nm
D10:	66.4 nm
D50:	93.7 nm
D90:	167.2 nm

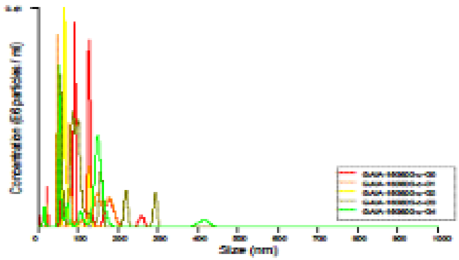
Stats: Mean +/- Standard Error	
Mean:	106.8 +/- 7.1 nm
Mode:	78.6 +/- 5.9 nm
SD:	40.2 +/- 4.6 nm
D10:	67.8 +/- 3.4 nm
D50:	95.9 +/- 7.9 nm
D90:	155.9 +/- 10.7 nm
Concentration:	1.61e+008 +/- 1.47e+007 particles/ml
	8.2 +/- 0.7 particles/frame
	13.4 +/- 1.5 centres/frame

Mixed Oxygen Bubble Size Test Results

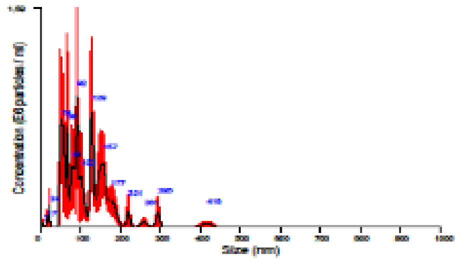
GAIA

NANOSIGHT

GAIA-160803-control 3-



FTLA Size / Concentration graph for Experiment:
GAIA-160803-control 3-



Averaged FTLA Size / Concentration
Red error bars indicate +/- 1 standard error of the mean

Included Files

GAIA-160803-control 3-00
GAIA-160803-control 3-01
GAIA-160803-control 3-02
GAIA-160803-control 3-03
GAIA-160803-control 3-04

Details

NTA Version:	NTA 3.0 0068
Script Used:	SOP Standard Measurement 10-51-01AM 03Aug2016.txt
Time Captured:	10:51:01 03/08/2016
Operator:	Tomioka
Pre-treatment:	
Sample Name:	Water
Diluent:	
Remarks:	

Capture Settings

Camera Type:	SCMOS
Camera Level:	16
Slider Shutter:	1300
Slider Gain:	512
FPS	25.0
Number of Frames:	1498
Temperature:	26.1 - 26.3 °C
Viscosity:	(Water) 0.863 - 0.866 cP
Dilution factor:	Dilution not recorded

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.7 - 32.2 pix

Results

Stats: Merged Data

Mean:	116.9 nm
Mode:	91.8 nm
SD:	65.4 nm
D10:	52.9 nm
D50:	103.3 nm
D90:	182.6 nm

Stats: Mean +/- Standard Error

Mean:	116.3 +/- 7.8 nm
Mode:	64.1 +/- 7.6 nm
SD:	59.7 +/- 8.1 nm
D10:	61.8 +/- 7.2 nm
D50:	103.4 +/- 13.7 nm
D90:	177.1 +/- 14.5 nm
Concentration:	5.11e+007 +/- 9.63e+006 particles/ml
	3.1 +/- 0.5 particles/frame
	6.9 +/- 1.4 centres/frame

Oxygen Bubble Size Test Control Before Mixing

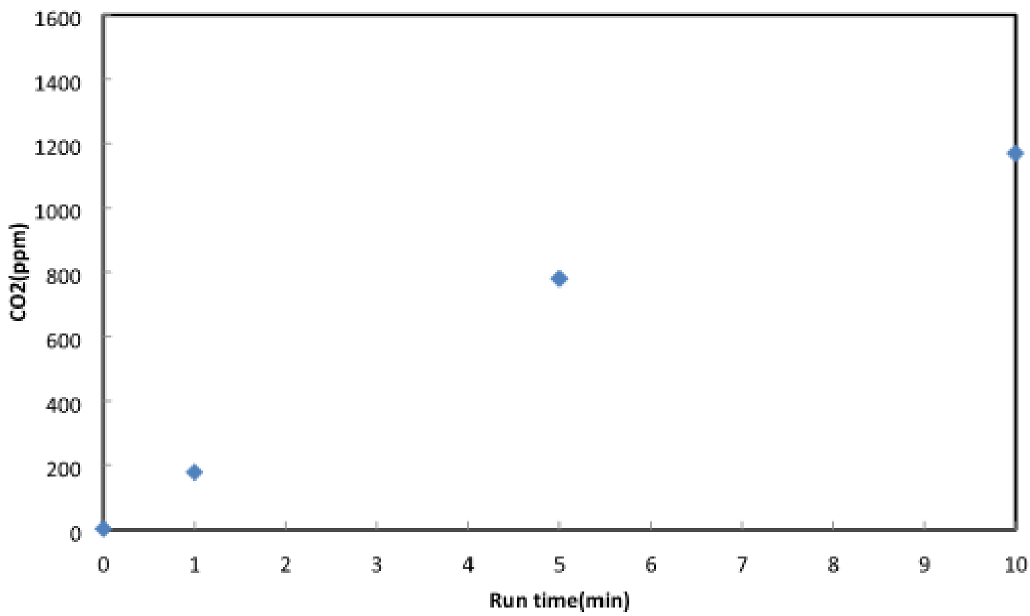
Carbon Dioxide Concentration Testing

Parameters;

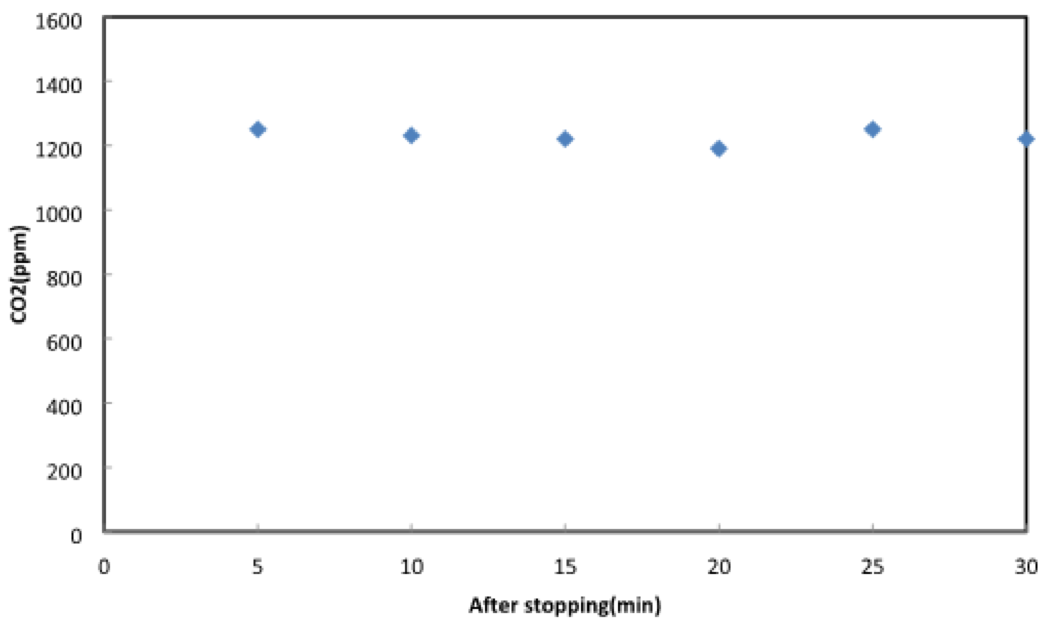
- Volume: 33 Liters, 8.72 gallons
- Gas Injection: 2LPM @10 PSI
- TDS : 2
- Starting CO2: 2.4 ppm
- Starting DO: 6.71 ppm

- Starting pH: 4.98
- Starting Temp: 25.2 C°/ 77.4 F°

Time (min)	Concentration CO2 (ppm)	Temperature (C°/ F°)	Concentration DO (ppm)	pH
1	178	25.2 / 77.4	6.05	3.45
2		25.2 / 77.4	5.24	3.33
		25.2 / 77.4	4.30	3.26
4		25.3 / 77.5	3.63	3.22
5	780	25.3 / 77.5	2.89	3.18
6		25.4 / 77.7	2.34	3.19
7		25.5 / 77.9	1.84	3.17
8		25.6 / 78.1	1.54	3.16
9		25.6 / 78.1	1.24	3.16
10	1170	25.7 / 78.3	0.94	3.15
Bubble Generation Stopped				
15	1250	25.7 / 78.3	0.88	3.33
20	1230	25.7 / 78.3	0.94	3.27
25	1220	25.7 / 78.3	0.88	3.32
30	1190	25.7 / 78.3	0.89	3.35
35	1250	25.7 / 78.3	0.90	3.38
40	1220	25.7 / 78.3	0.93	3.39



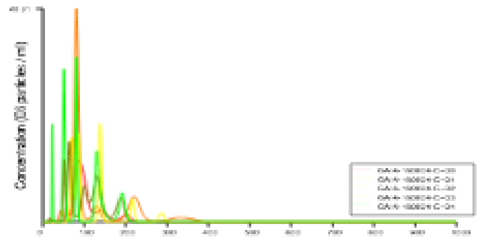
CO2(ppm)
1600
1400
1200
1000
800
600
400
200
0
0
1
2
3
4
5
6
7
8
9
10
Run time(min)



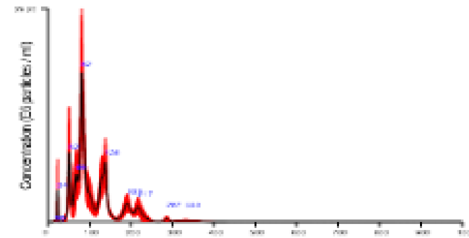
CO2(ppm)
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
After stopping(min)

NANOSIGHT

GAIA-160804-CO2 2L-10min_30min-



FTLA Size / Concentration graph for Experiment:
GAIA-160804-CO2 2L-10min_30min-



Averaged FTLA Size / Concentration
Red error bars indicate +/- 1 standard error of the mean

Included Files

GAIA-160804-CO2 2L-10min_30min-00
GAIA-160804-CO2 2L-10min_30min-01
GAIA-160804-CO2 2L-10min_30min-02
GAIA-160804-CO2 2L-10min_30min-03
GAIA-160804-CO2 2L-10min_30min-04

Details

NTA Version:	NTA 3.0 0068
Script Used:	SOP Standard Measurement 11-27-14AM 04Aug2016.txt
Time Captured:	11:27:14 04/08/2016
Operator:	Tomioka
Pre-treatment:	
Sample Name:	Water
Diluent:	
Remarks:	

Capture Settings

Camera Type:	SCMOS
Camera Level:	16
Slider Shutter:	1300
Slider Gain:	512
FPS	25.0
Number of Frames:	1498
Temperature:	26.4 - 26.6 °C
Viscosity:	(Water) 0.857 - 0.860 cP
Dilution factor:	1 x 10e1

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 13.1 - 24.8 pix

Results

Stats: Merged Data

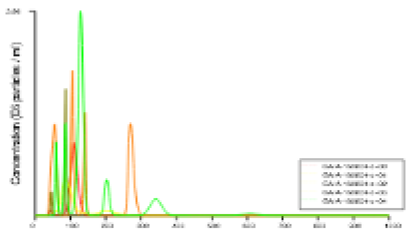
Mean:	112.0 nm
Mode:	81.5 nm
SD:	58.4 nm
D10:	50.3 nm
D50:	88.4 nm
D90:	197.4 nm

Stats: Mean +/- Standard Error

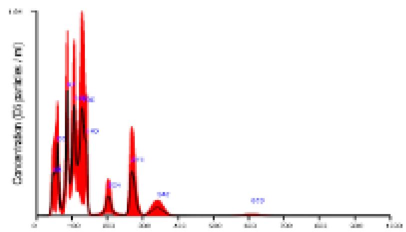
Mean:	111.4 +/- 5.9 nm
Mode:	80.4 +/- 14.8 nm
SD:	53.6 +/- 7.0 nm
D10:	60.5 +/- 5.0 nm
D50:	91.5 +/- 5.8 nm
D90:	190.0 +/- 14.5 nm
Concentration:	7.55e+008 +/- 7.12e+007 particles/ml
	3.8 +/- 0.4 particles/frame
	7.7 +/- 1.4 centres/frame

CO_2 Bubble Size Test Results

NANOSIGHT GAIA-160804-control-



FTLA Size / Concentration graph for Experiment:
GAIA-160804-control-



Averaged FTLA Size / Concentration
Red error bars indicate +/- 1 standard error of the mean

Included Files

GAIA-160804-control-00
GAIA-160804-control-01
GAIA-160804-control-02
GAIA-160804-control-03
GAIA-160804-control-04

Details

NTA Version:	NTA 3.0.0068
Script Used:	SOP Standard Measurement 09-47-57AM 04Aug2016.txt
Time Captured:	09:47:57 04/08/2016
Operator:	Tomioka
Pre-treatment:	
Sample Name:	Water
Diluent:	
Remarks:	

Capture Settings

Camera Type:	SCMOS
Camera Level:	16
Slider Shutter:	1300
Slider Gain:	512
FPS	25.0
Number of Frames:	1498
Temperature:	25.2 - 25.9 °C
Viscosity:	(Water) 0.882 - 0.885 cP
Dilution factor:	Dilution not recorded

Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 12.3 - 13.7 pix

Results

Stats: Merged Data

Mean:	133.3 nm
Mode:	89.6 nm
SD:	84.6 nm
D10:	57.9 nm
D50:	117.5 nm
D90:	271.1 nm

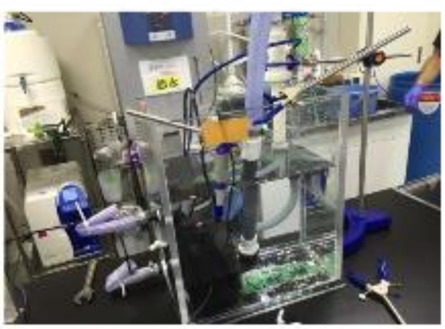
Stats: Mean +/- Standard Error

Mean:	120.7 +/- 14.5 nm
Mode:	103.6 +/- 0.0 nm
SD:	53.0 +/- 17.8 nm
D10:	68.6 +/- 3.2 nm
D50:	104.9 +/- 7.4 nm
D90:	203.1 +/- 45.2 nm
Concentration:	6.16e+007 +/- 2.11e+007 particles/ml
	3.1 +/- 1.1 particles/frame
	6.5 +/- 2.5 centres/frame

CO_2 Bubble Size Test Control Before Mixing

IDEC Corporation, Fine Bubble Department Lab at University of Osaka Photonics Department

Nanosight Testing Equipment



Generating Nano Bubbles with Gaia Technology

11.2. Appendix II: Dissolved Ozone Over Time Result Graphs

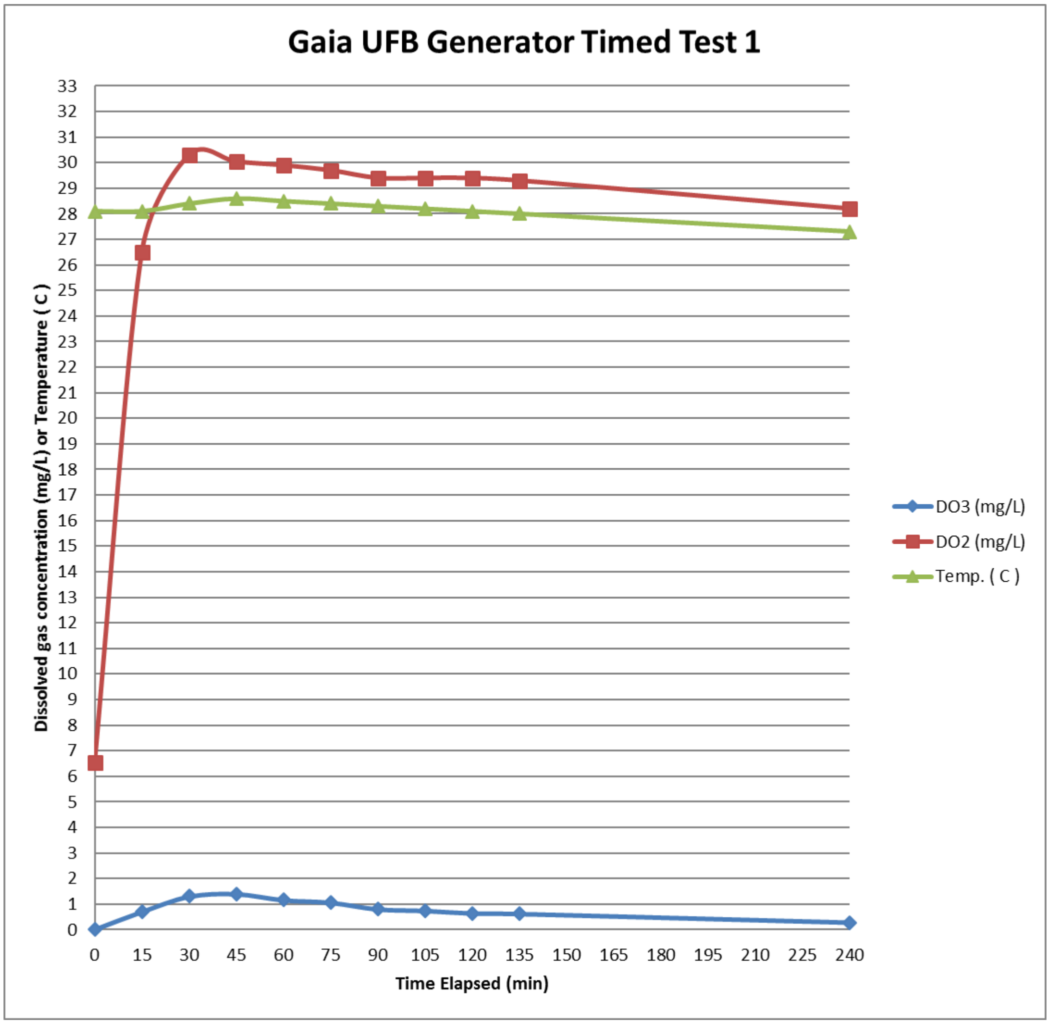


Figure 12. Dissolved ozone and dissolved oxygen concentration in water over time when injected as microbubbles with the Gaia UFB generator.

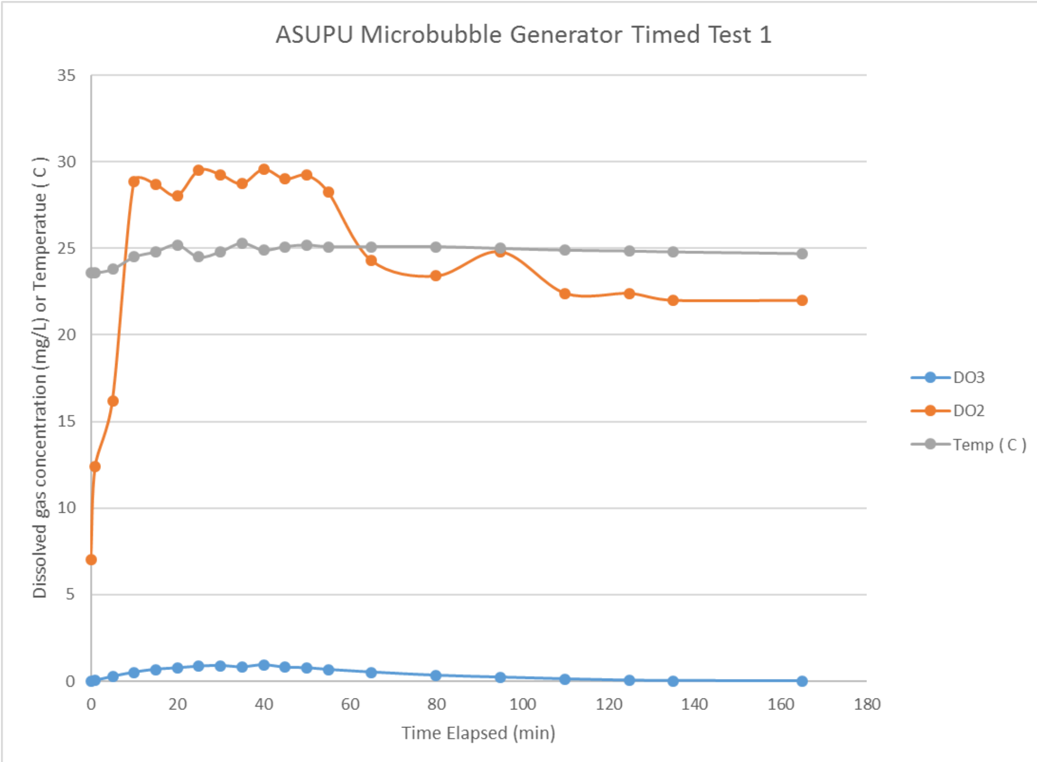


Figure 13. Dissolved ozone and dissolved oxygen concentration in water over time when injected as microbubbles with the ASUPU ASK3M micro/nanobubble generator.

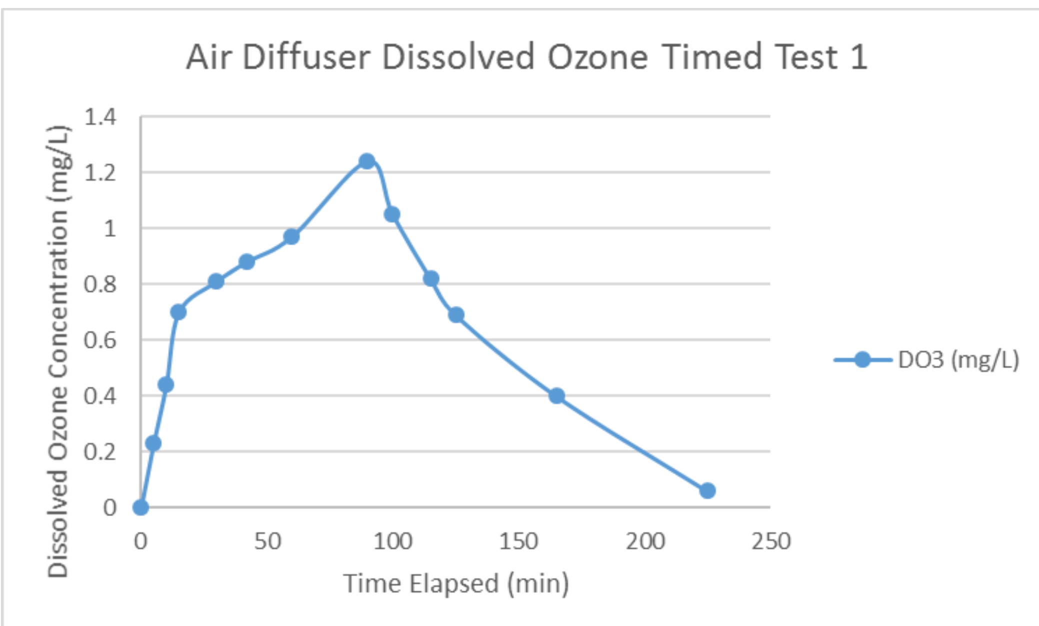


Figure 14. Dissolved ozone concentration in water over time when injected as fine bubbles with air diffusers.

11.3. Appendix III: Additional ANOVA Analysis Results

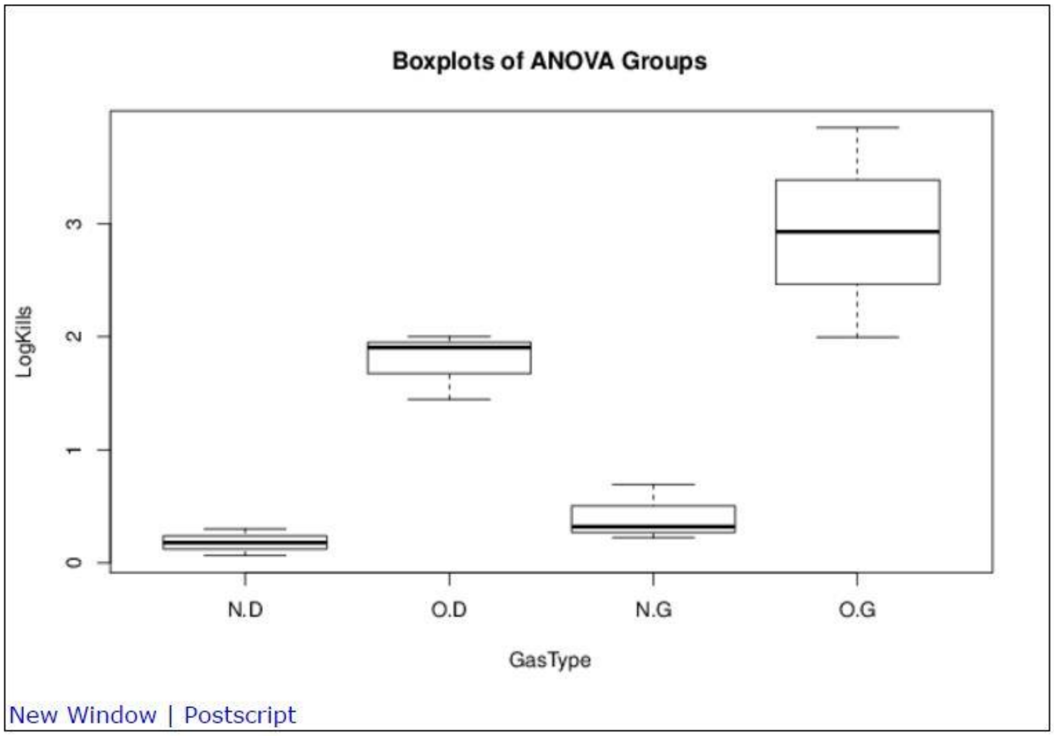


Figure 15. Boxplots of ANOVA Groups showing range of E. coli reduction for the different treatment combinations of gas and injection method. N = nitrogen gas, O = ozone gas, D = air diffuser, G = Gaia Ultrafine Bubble Generator.

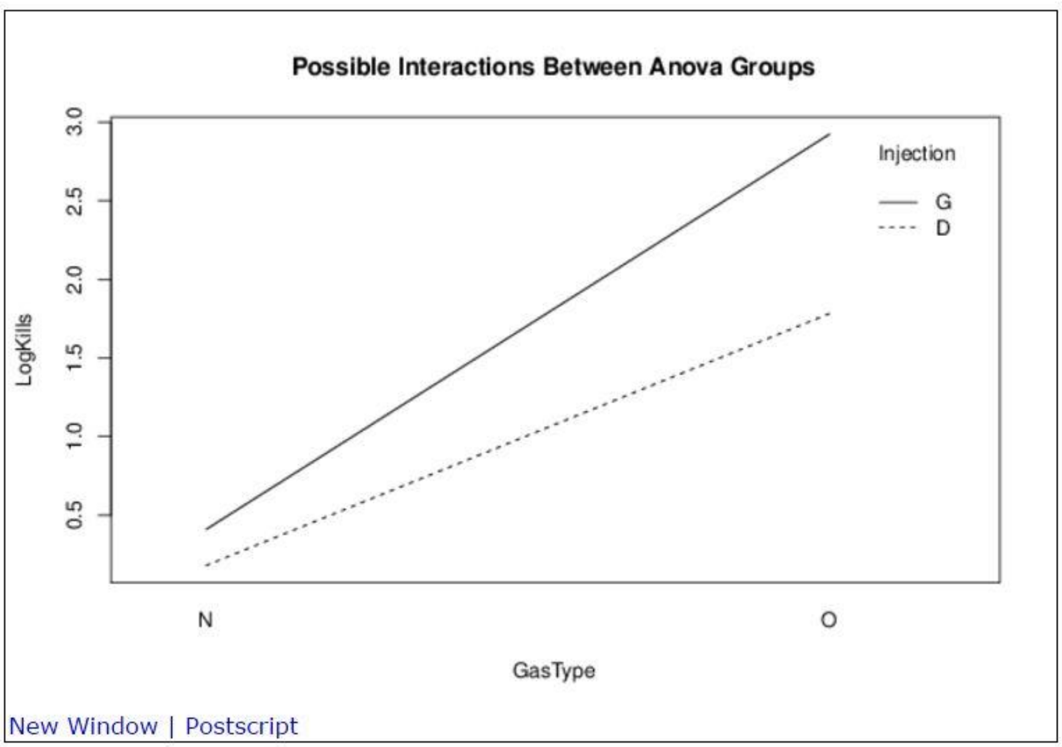


Figure 16. Possible interactions between ANOVA Groups. N = nitrogen gas, O = oxygen gas.

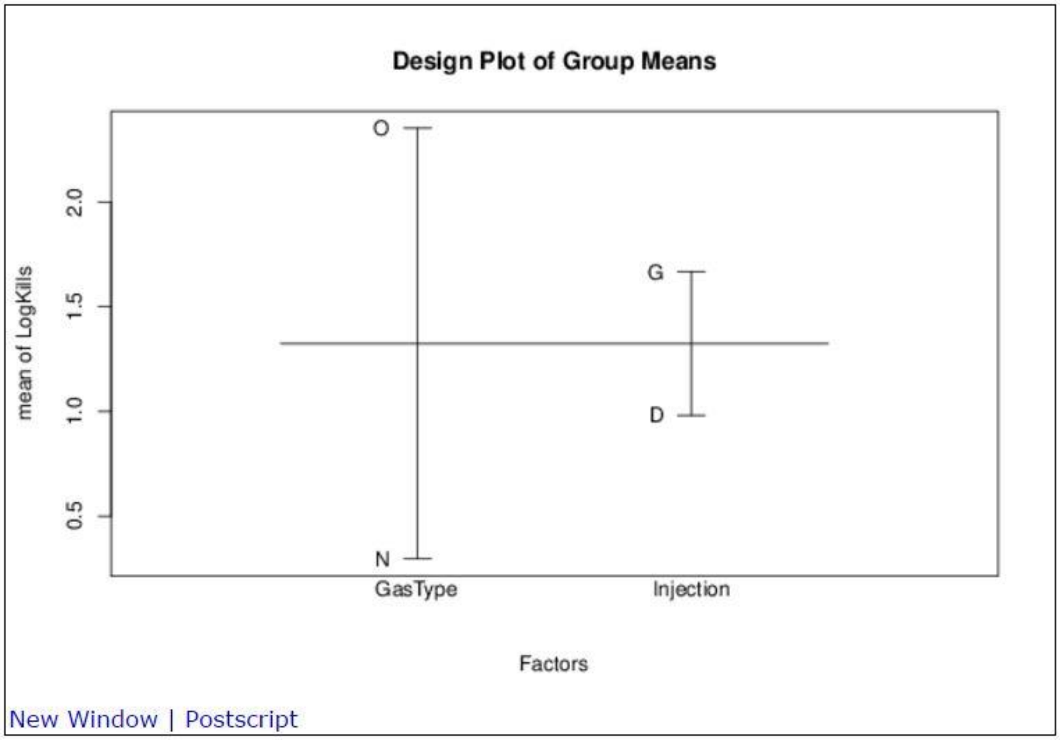


Figure 17. Group means of the treatment factors. O = ozone, N = nitrogen, G = Gaia Ultrafine Bubble Generator, D = air diffuser.

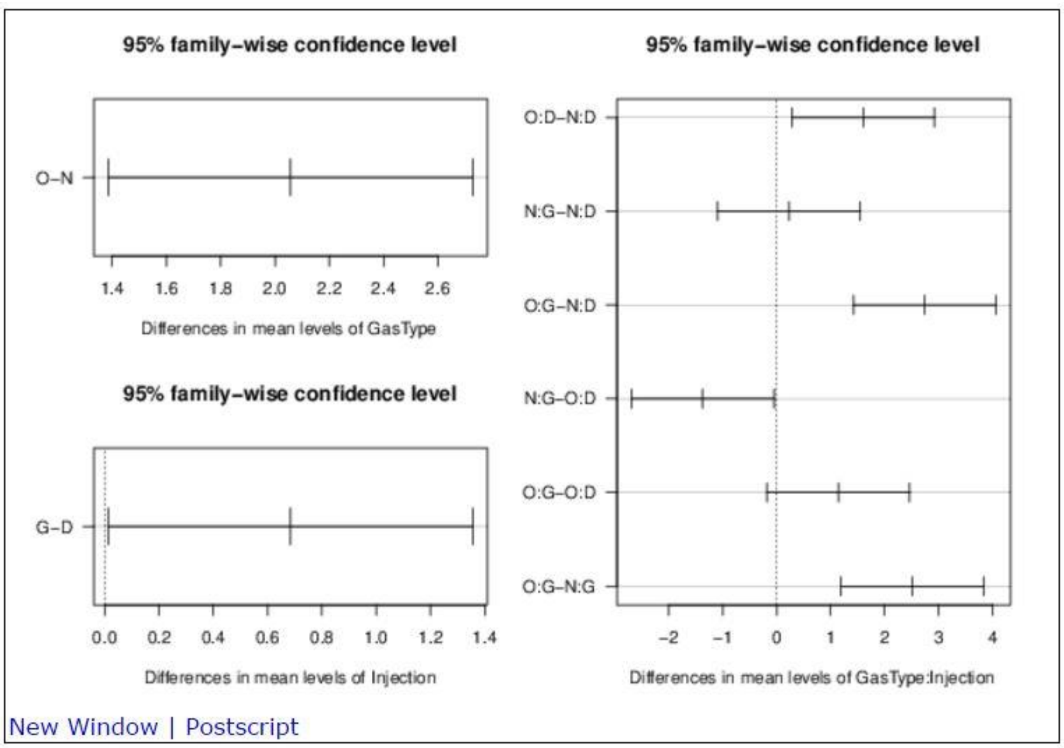


Figure 18. Differences in mean levels for each factor and interactions between factors.

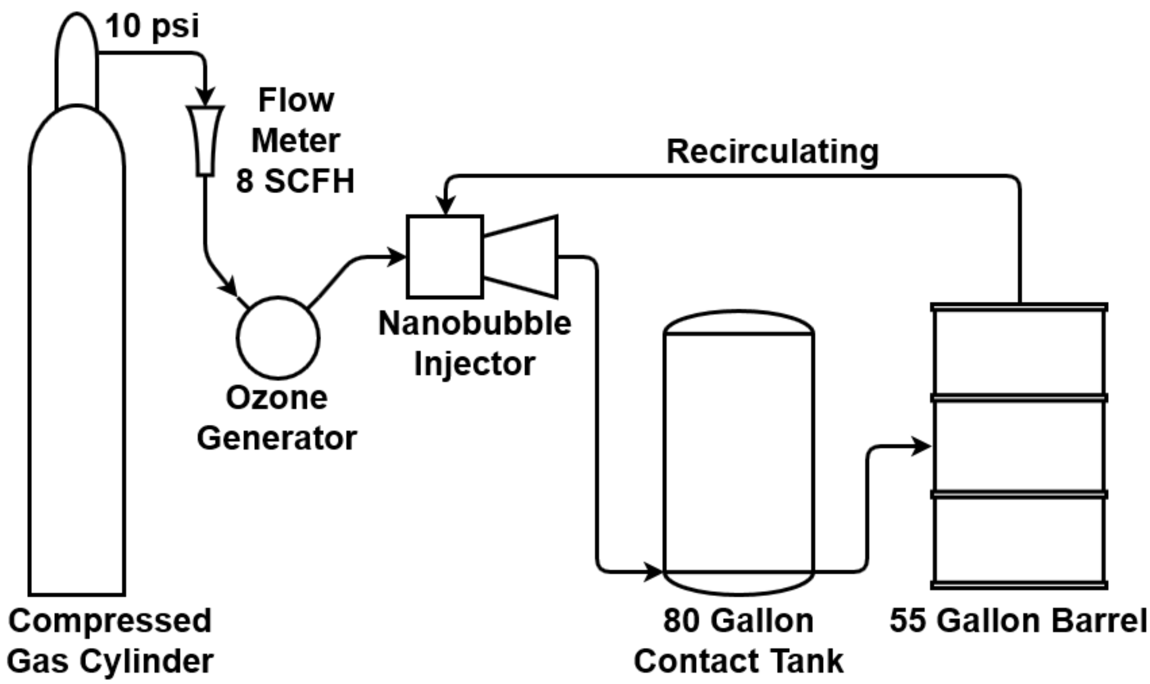
11.4. Appendix IV: Follow Up Experiment

After the thesis was written, a more powerful ozone generator was acquired and the ozone dwell time experiment was repeated with the new ozone generator.

Equipment:
The ClearWater Tech CD30 Ozone generator produces 30 grams of ozone per hour at a gas flow rate of 15 SCFH and a concentration of 6% ozone by weight. For the experiment, the gas flow rate was reduced to 8 SCFH with a projected ozone output of 20 grams per hour at 7% ozone by weight.

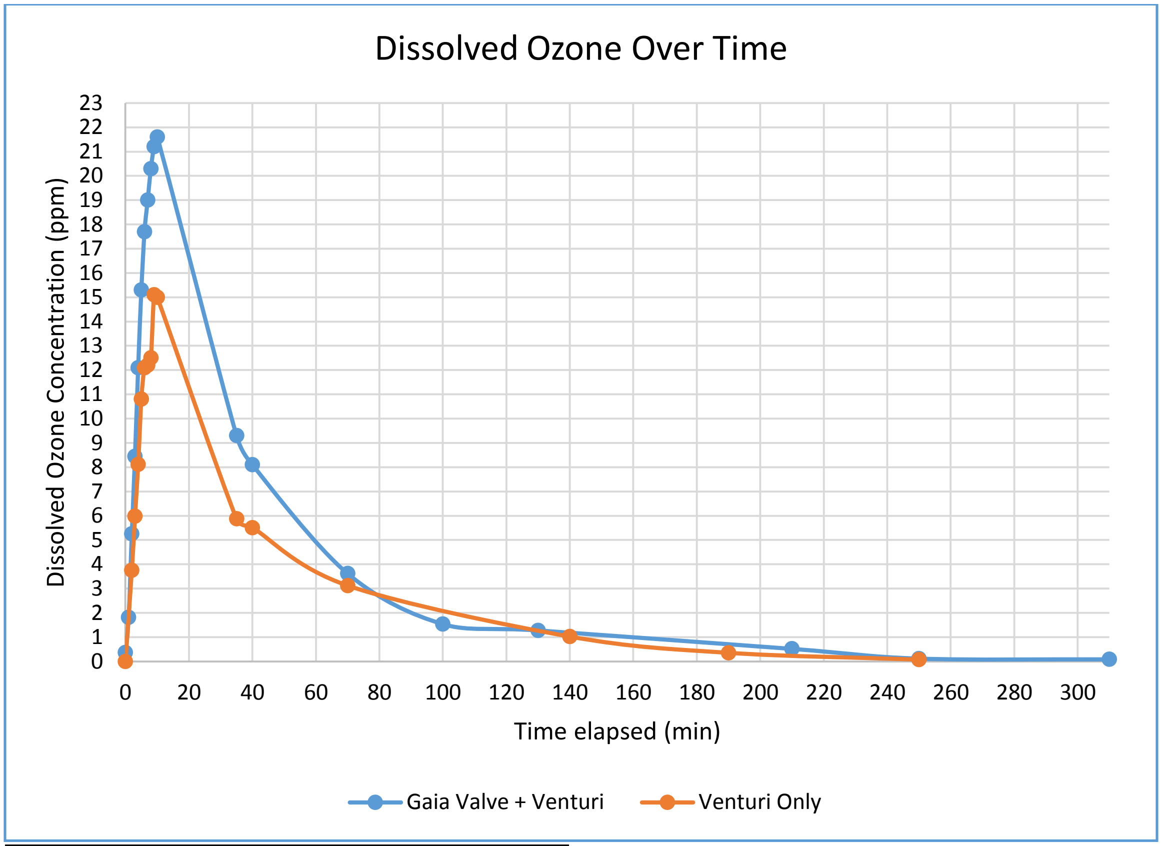
An 80-gallon contact tank was used in addition to the 55-gallon barrel drum to facilitate disposal of off-gassed ozone.

Methods:
The method for this experiment followed the method for the dwell time experiments discussed in the thesis with the exception that an 80-gallon contact tank was added in between the ozone nanobubbles injector and the 55-gallon barrel. Furthermore, the ozone generator and injectors were only run for 5 minute before the water was allowed to sit.



Results:

The results show that high levels of dissolved ozone are possible with a more powerful ozone generator and that the Gaia ultrafine bubble generator does significantly improve the dissolved ozone concentration in the water.



Venturi Only		Gaia Valve + Venturi	
Time (min)	DO3 (ppm)	Time (min)	DO3 (ppm)
0	0	0	0.36
2	3.75	2	5.25
3	5.98	3	8.45
4	8.11	4	12.1
5	10.8	5	15.3
35	5.87	35	9.3
40	5.51	40	8.1
70	3.12	70	3.62
140	1.02	100	1.54
190	0.35	130	1.28
250	0.07	210	0.52
		250	0.12
		310	0.09

12. References

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