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Ozonation followed by ultraviolet irradiation provides effective bacteria inactivation in a freshwater recirculating system

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Abstract

Recirculating aquaculture systems may require an internal disinfection process to control population growth of pathogens and heterotrophic bacteria. Ozonation and ultraviolet (UV) irradiation are two technologies that have been used to treat relatively large aquaculture flows, including flows within freshwater systems that recirculate water. The objective of the present study was to evaluate the effectiveness of ozone application alone or ozone application followed by UV irradiation to reduce abundance of heterotrophic and total coliform bacteria in a water reuse system. Results indicate that when only ozone was applied at dosages – defined by the product of the ozone concentration times the mean hydraulic residence time (*Ct*) – that ranged from 0.10 to 3.65 min mg/L, the total heterotrophic bacteria counts and total coliform bacteria counts in the water exiting the contact basin were reduced to, respectively, 3–12 cfu/mL (1.1–1.6 LOG_{10} reduction) and 2–18 cfu/100 mL (1.9–3.1 LOG_{10} reduction). Bacteria inactivation appeared to be just as effective at the lowest ozone *ct* dosage (i.e., 0.1 mg/L ozone after a 1 min contact time) as at the highest ozone *ct* dosage (i.e., 0.2 mg/L ozone after a 16.6 min contact time). As with our previous research on UV inactivation of bacteria, we hypothesize that the recirculating system provided a selection process that favors bacteria that embed within particulate matter or that form bacterial aggregates that provides shielding from oxidation. However, when ozonation was followed by UV irradiation, the total heterotrophic bacteria counts and total coliform bacteria counts in the water exiting the UV irradiation unit were reduced to, respectively, 0–4 cfu/mL (1.6–2.7 LOG_{10} reduction) and 0–3 cfu/100 mL (2.5–4.3 LOG_{10} reduction). Thus, combining ozone dosages of only 0.1–0.2 min mg/L with a UV irradiation dosage of approximately 50 mJ/cm^2 would consistently reduce bacteria counts to near zero. These findings were orders of magnitude lower than the bacteria counts measured in the system when it was operated without disinfection or with UV irradiation alone. These findings indicate that combining ozonation and UV irradiation can effectively disinfect recirculating water before it returns to the fish culture tank(s).

Keywords: Ozonation; Ultraviolet irradiation; Advanced oxidation; Bacteria inactivation; Disinfection; Recirculating system; Water reuse; Aquaculture

1. Introduction

Fish feed is carbonaceous matter that is high in protein nitrogen, and it has a balance of minerals and vitamins. Therefore, nutrients and dissolved organics from uneaten feed, fish feces, and excretion create an environment favorable to a diversity for bacteria, protozoa, micrometazoa and fungi that have a major water quality impact in high density, water reuse aquaculture (Colberg and Lingg, 1978; Lohr and Gratzek, 1984; Bullock et al., 1993, 1997; Blancheton and Canaguier, 1995; Liltved et al., 1995; Hocheimer and Wheaton, 1995; Macphee et al., 1995; Blancheton, 2000; Leonard et al., 2000, 2002; Sugita et al., 2005;

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Michaud et al., 2006). Indeed, a reuse aquaculture system (RAS) with a nitrification reactor requires growth of autotrophic nitrifying bacteria, while heterotrophic bacteria are required to prevent accumulation of soluble biochemical oxygen demand (sBOD) or to provide denitrification.

Inadequate solids removal in culture systems can rapidly deteriorate water quality (Rakelmann and Hilge, 1983) and augment microbial growth (Blancheton and Canaguier, 1995; Blancheton, 2000; Leonard et al., 2000, 2002). Removal of suspended solids enhances fish health by improving water quality and removing potential pathogens associated with those solids. Husbandry practices that include regular tank cleaning and the flushing of sumps and pipes may reduce pathogen reservoirs and thereby decrease potential epizootic outbreaks (Bebak-Williams et al., 2002).

Virulent as well as opportunistic fish pathogens may buildup in reuse culture system – in the water column, in the biofilm, or on the fish – due to the prolonged water retention times, increased substrate concentrations, high fish densities, and continuous production techniques. As the pathogen concentration is amplified in the recirculating water, the risk of disease and epidemic loss increases. Obviously, strict biosecurity practices should be implemented to prevent introduction of fish pathogens from contaminated feed, water supply, fish and eggs from suppliers, and microbes carried into the fish culture facility by staff and visitors (Bebak-Williams et al., 2002). If biosecurity barriers are breached and fish pathogens enter a fish farm, then the disease problem must be addressed through disinfection techniques that are costly, time consuming, and do not necessarily lead to the elimination of the pathogen once introduced. Control of epidemics can be challenging when chemotherapeutants recirculate – returning to the fish culture tank or passing through the biofilter when opportunities for flushing these compounds are reduced due to makeup water limitations – or if the entire system requires sterilization (Heinen et al., 1995; Noble and Summerfelt, 1996; Schwartz et al., 2000; Bebak-Williams et al., 2002).

Fish health in recirculating systems would be more secure if an internal disinfection process were used to prevent the accumulation of fish pathogens. Although disinfection of recycled process water adds to the fixed and variable costs of these systems, mitigation of potential disease occurrence has been reported with ozonation by itself (Bullock et al., 1997; Ritar et al., 2006) and with ultraviolet (UV) irradiation by itself (Sharrer et al., 2005).

1.1. Ozonation

Ozone has a rapid reaction rate, produces few harmful by-products (bromine and bromate can be formed when bromide is present, e.g., in seawater), and forms dissolved oxygen as a reaction end product in freshwater (Summerfelt and Hochheimer, 1997; Summerfelt, 2003). Dissolved ozone is effective for color elimination, nitrite reduction, algae control, turbidity removal, improved micro-flocculation of fine particulates, and enhanced biological processing of dissolved organic molecules (Otte and Rosenthal, 1979; Rosenthal and Otte, 1980; Rice et al., 1981; Rosenthal and Kruner, 1985; Paller and Lewis, 1988; Reid and Arnold, 1992; Kaplan et al., 1994; Hozalski et al., 1995; Rueter and Johnson, 1995; Summerfelt and Hochheimer, 1997; Summerfelt et al., 1997; Summerfelt, 2003). In North America, we know of a number of large-scale commercial fish farms (rearing species such as tilapia, hybrid striped bass, Arctic char, Atlantic salmon, sturgeon, barramundi, and others) that add ozone to improve water quality and fish health. However, probably few of these commercial fish farms are ozonating at levels sufficient to achieve significant micro-biological disinfection.

In general, ozone is an effective bactericide, parasiticide, and virucide (Lohr and Gratzek, 1984; Colberg and Lingg, 1978; Liltved et al., 1995; Bullock et al., 1997; Liltved, 2002), however, some viruses have shown high resistance to ozonated seawater (Liltved et al., 2006). Ozone kills microbes by oxidation of the lipid bi-layer of microbial organisms; this action is a function of the dose (*Ct*), i.e., the product of the dissolved ozone concentration (mg/L) times the mean hydraulic residence time (min) in the contact chamber. In addition, degree of water quality can affect the ability to maintain a residual ozone concentration and, therefore, the dose required for microbial reduction. Colberg and Lingg (1978) achieved a 99.9% reduction of four bacterial fish pathogens (*Aeromonas liquifaciens*, *A. salmonicida*, *Pseudomonas fluorescens*, and *Yersinia ruckerii*) cultured in a phosphate buffered saline solution applying an ozone *Ct* of 0.12–0.50 mg/L min. Wedemeyer et al. (1978) disinfected water containing the fish viruses IHNV (infectious hematopoietic necrosis virus) and IPNV (infectious pancreatic necrosis virus) with an ozone exposure (*Ct*) of 0.005–0.010 mg/L min. In bench-top studies, Liltved et al. (1995) reported 99.99% inactivation (four log reductions in viable count) of four bacteria (*Aeromonas salmonicida salmonicida*, *Vibrio anguillarum*, *V. salmonicida*, and *Yersinia ruckeri*) and the IPNV within

180 s at residual ozone concentrations of 0.15–0.20 mg/L within fresh, brackish, and seawater. Ozone *Ct* doses of approximately 2 mg/L min have been used to control specific fish pathogens in the surface water supply at the US Fish and Wildlife Service's Dworshak National Fish Hatchery in Ahsahka, Idaho (Owsley, 1991) and the Northeast Fishery Center in Lamar, Pennsylvania (Summerfelt et al., in press). Ozone *Ct* doses reported were approximately 50% higher at the Cowlitz Salmon Hatchery in Tacoma, Washington, and the Merwin State Hatchery in Ariel, Washington (Cryer, 1992).

1.2. Ultraviolet irradiation

UV irradiation is also a technology used in aquaculture applications to inactivate microorganisms (Liltved et al., 1995, 2006; Liltved, 2002; Sharrer et al., 2005). UV irradiation has been applied in European hatcheries and grow out facilities using recirculating systems to produce turbot and sea bass (Blancheton, 2000). In North America, UV irradiation is often used to treat recirculating flows in salmon egg incubation, fry, and smolt recirculating systems.

UV irradiation inactivates microorganisms by destructive effect on nucleic acids. Under laboratory conditions, a UV dose of 2.7 mJ/cm^2 results in a 5-LOG_{10} reduction in *Vibrio salmonicida*, *Vibirio anguillarum*, and *Yersinia ruckerii*, and a 3-LOG_{10} reduction in IPNV at a UV dose of 122 mJ/cm^2 (Liltved et al., 1995). However, actual fish culture conditions may require longer exposure or higher dose (Liltved, 2002), because factors such as total suspended solids can affect UV transmittance (Loge et al., 1996) and bacteria may be protected by envelop of particulate matter (Emerick et al., 1999; Liltved and Cripps, 1999). For example, in a recirculating aquaculture system it was observed that a UV intensity greater than 1800 mJ/cm^2 was required to achieve a not quite 2-LOG_{10} reduction in heterotrophic bacteria (Sharrer et al., 2005). Farkas et al. (1986) found that UV irradiation within a recirculating system produced inconsistent inactivation or no inactivation of heterotrophic bacteria, *Aeromonas* [*hydrophila* and *punctata*], and *Flexibacter columnaris*. Sharrer et al. (2005) presented a hypothesis that recirculating systems that treat with UV irradiation provide selection pressure for bacteria that embed within particulate matter or that form bacterial aggregates, because this provides shading from some of the UV irradiation. Even if this hypothesis is invalidated, achieving total inactivation of bacteria in recirculating waters using only UV irradiation appears to be difficult.

UV irradiation is also effective at dissolved ozone destruction. In a recirculating system used for salmonid production, a UV irradiation dose of 49 $\pm$ 1 mW s/cm^2 removed 100% of the dissolved ozone when the inlet ozone concentration was $\leq$0.10 mg/L (Summerfelt et al., 2004). UV irradiation can be used to prevent dissolved ozone residuals from reaching the fish in recirculating systems that use ozonation for disinfection, i.e., when a dissolved ozone residual is maintained at the outlet of ozone disinfection chambers.

1.3. Advanced oxidation process: ozonation followed by UV irradiation

Advanced oxidation processes combine two of three processes: ozonation, UV irradiation, or hydrogen peroxide, to achieve synergistic oxidation effects for achieving enhanced microbial reductions or destruction of dissolved organic carbon compounds (Langlais et al., 1991). Use of ozonation followed by UV irradiation has been used in drinking water and wastewater applications to improve the efficiency of micro-biological inactivation (White, 1992; Amirsardi et al., 2001; Oh et al., 2003). In recirculating aquaculture systems, use of ozone at disinfecting levels will likely reduce the accumulation of fine particles in the recycled water, which could potentially improve the disinfection efficiency of subsequent UV irradiation.

The objective of this research was to assess the degree of total heterotrophic and total coliform bacteria inactivation using ozone alone (at several ozone dosages) and to determine if a synergistic effect is seen in the disinfection of microorganisms from process water in a fully recirculating fish culture system when UV irradiation is applied directly after ozonation.

2. Material and methods

The combined effect of dissolved ozone and UV irradiation on bacterial disinfection was conducted utilizing the 4800 L/min recirculating system (Fig. 1) at the Conservation Fund Freshwater Institute (Shepherdstown, WV). The recirculated system is described elsewhere (Davidson and Summerfelt, 2005; Sharrer et al., 2005). During the time of the study, the recirculating system was operated for the grow out of Arctic Char (*Salvelinus aplinus*). Fish were raised under a 24-h photoperiod, fed approximately 120-kg/day distributed in equal portions during eight feeding events (i.e., every 3 h), and maintained at culture densities ranging from 100 to 130 kg/m^3 through selective harvesting events.

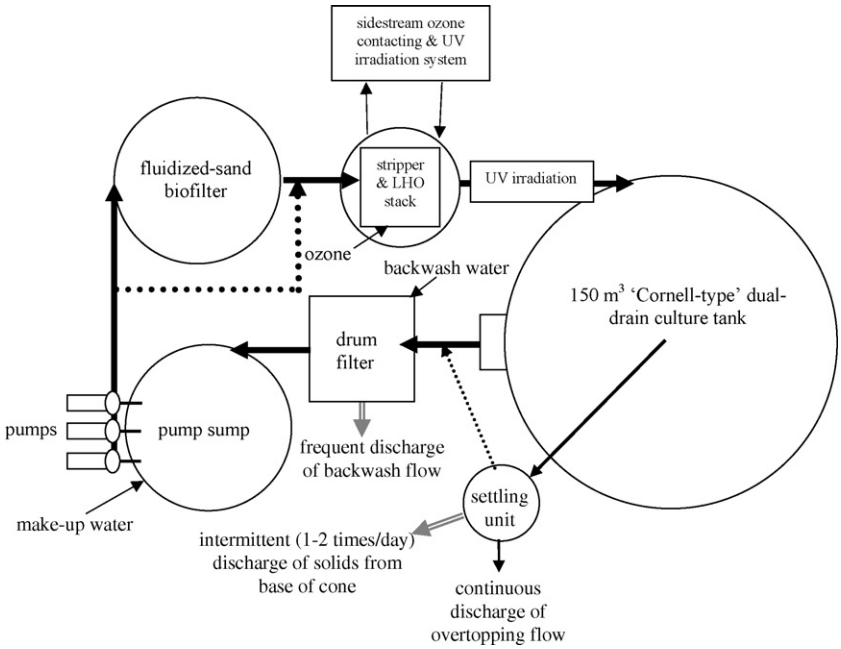

Fig. 1. Process flow drawing of the 4800 L/min recirculating grow out system for Arctic char at the Freshwater Institute, Shepherdstown, WV (from Davidson and Summerfelt, 2005).

To determine the presence of a synergistic effect of ozone and UV irradiation application a side-stream closed loop was operated utilizing water pumped from the recirculation system's low head oxygenation unit (LHO) sump (Fig. 2). Ozone feed gas was supplied using a PCI-Wedeco Model GSO40 (West Caldwell, NJ) and entrained into solution using the suction side of a 5-cm diameter venturi injector (Mazzei Injector

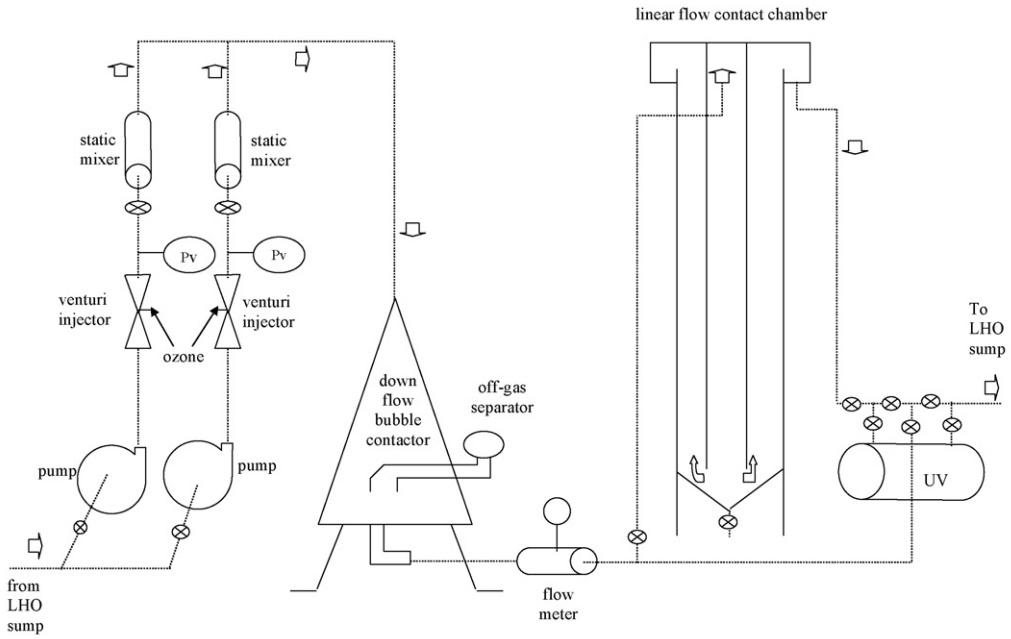

Fig. 2. Process flow drawing of water treated across the ozone contacting and UV irradiation side-stream loop at the low head oxygenation unit (LHO) sump.

Corporation, Bakersfield, CA). Ozone gas dissolution into process water was enhanced by using an inline static mixer followed by a down flow bubble contactor (Marine Biotech Inc., Beverly, MA) to capture and vent any off-gas out of the building. The side-loop system was operated to produce flow rates, measured with a Krohne Inc. (Peabody, MA) model IFS/020F magnetic flow meter, of approximately 150 and 300 L/min (3.1 and 6.2% of total system flow) resulting in hydraulic residence times (HRT) of 8.3 and 16.6 min within a plug-flow contact chamber, i.e., a U-tube contactor. Application of UV irradiation was then supplied by a UV Logic model 02AM15 (Trojan Technologies Inc., London, Ontario, Canada). UV doses (mJ/cm^2) at the two flow rates were determined utilizing a proprietary spreadsheet supplied by the UV unit manufacturer and is described by Summerfelt et al. (2004).

Four side-loop system sampling ports were used to determine dissolved ozone (O_3) concentration across the contact chamber (initial O_3 concentration, O_3 entering contact chamber, O_3 at midpoint, and O_3 exiting contact chamber) using Hach Chemical Company (Loveland, CO) Ozone AccuVac Reagent Ampuls (low, medium, and high range) and a DR/4000U spectrophotometer (Hach Chemical Company). Three residual ozone concentrations of 0.20, 0.10, and 0.05 mg/L (exiting contact chamber) were achieved by adjusting the ozone output produced at the ozone generator.

Total heterotrophic bacteria and total coliform bacteria were used as indicator organisms to determine the relative effectiveness of a given disinfection process; justification for the use of indicator organisms has been provided by Zhu et al. (2002). Three sampling sites were used to assess for bacteria counts (before ozone, after ozone, and after UV) from 6 to 17 times. Samples were colleted from the before O_3 site by placing the sterile sample bottle (upside down) into the LHO sump water and inverting the bottle approximately 0.5 m below the water surface. The after O_3 and after UV samples were taken from 1.3 cm valves located within 1 m (before and after) of the UV unit. The after O_3 port was opened and allowed to drain onto the floor at 2–4 L/min for approximately 3 min before the sterile sample bottle was placed under the water flow. The process was then repeated for the after UV sample. Heterotrophic bacteria counts were assessed using Hach Membrane Filtration Method 8242-TGE broth with TTC indicator. Colonies were counted, after the 48-h incubation period, using a low-power microscope and reported in colony forming units (cfu) per 1-mL sample. Total coliform counts were evaluated using Hach Membrane Filtration 8074 (m-Endo Broth) and counted with a low-power microscope. Colonies were reported in cfu per 100-mL sample. Removal efficiency of bacteria was calculated utilizing the following equation:

$$\text{bacteria removal}\ (\%) = \frac{(\text{count}_{\text{inlet}} - \text{count}_{\text{outlet}})}{\text{count}_{\text{inlet}}} \times 100$$

LOG_{10} reduction in bacteria across the treatment system was then calculated using the equation:

$$\text{LOG}_{10}\,\text{reduction} = -\log_{10}\left(1 - \frac{\%\,\text{removal}}{100}\right)$$

After noting that ozone inactivation of bacteria was not strongly dependent on ozone *Ct* over a range of 0.4–3.7 min mg/L, we conducted an additional study to determine bacteria inactivation at ozone concentrations of 0.1 and 0.2 mg/L after only a 1.0 min HRT, which was achieved by by-passing the ozone contact tank.

Statistical analyses were performed to assess if significant differences exist in mean bacterial counts before ozone, after ozone, and after UV. Specifically, a non-parametric analysis of variance (Friedman test) was conducted to evaluate statistical differences in all three means. Further, post hoc analysis utilizing a Wilcoxson signed-rank test was performed to assess statistical differences in mean bacterial counts in the after ozone and after UV treatments.

Water quality samples were also analyzed to characterize background water quality conditions within the side-loop system. Total ammonia nitrogen (TAN) was assessed utilizing the Hach Chemical Company Nessler method and a DR4000/U spectrophotometer. Total suspended solids (TSS) and total dissolved solids (TDS) concentrations were determined according to standard methods procedures (APHA, 1998) 2540D and 2540C, respectively.

Alkalinity was determined by titration according to standard methods (APHA, 1998). Measurement of pH was determined utilizing a Fisher Scientific Accumet pH Meter 915 (Pittsburg, PA). UV transmittance (%UVT) was assessed by placing a cleaned cuvette (with a 1 cm path length) of sample water into a DR4000/U spectrophotometer set to display transmittance at a wavelength of 254 nm.

3. Results and discussion

Table 1 describes water quality in the side-loop system. The UV transmittance was relatively high ($90 \pm 1\%$), while total suspended solids concentration was low (3.4 ± 0.4), which both benefit disinfection

Table 1
Water quality in the side-loop system during the study

Temperature (°C)	14.3 ± 0.04
pH	7.53 ± 0.02
Alkalinity (mg/L as $CaCO_3$)	219 ± 3
Total suspended solids (mg/L)	3.4 ± 0.4
Total dissolved solids (mg/L)	410 ± 10
UV transmittance (%)	90 ± 1
Total ammonia nitrogen (mg/L as nitrogen)	0.44 ± 0.06

using UV irradiation. Temperature in the fish culture system averaged 14.3 ± 0.04 °C.

3.1. Bacteria inactivation

3.1.1. Ozonation

At an ozone dose of 0.1–3.65 min mg/L, the total heterotrophic bacteria counts in the water exiting the ozone contact chamber averaged 3–12 cfu/mL, which was a mean LOG_{10} reduction of 1.15–1.62 (Table 2). There was not a significant correlation ($P = 0.386$) between mean total heterotrophic bacteria count remaining in the water at the end of the ozone contact tank versus the ozone *Ct* dose (Fig. 3). These results were counter-intuitive, because an increase in ozone *Ct* is typically expected to correlate with an increase in bacteria inactivation. However, there was a stronger, nearly significant correlation ($P = 0.071$) between ozone concentration and mean total heterotrophic bacteria count remaining in the water at the ozone contact tank outlet (Fig. 4). In this study, inactivating heterotrophic bacteria with ozone in a water recirculation system was more dependent upon ozone concentration exiting the contact tank than the hydraulic contact time.

For comparison purposes, Bullock et al. (1997) found that ozonating at levels of 0.025 kg ozone/kg feed fed, which was sufficient for both fish health and water quality improvements, did not produce even a 1 LOG_{10} reduction in heterotrophic bacteria in the water of a recirculating system used to produce rainbow trout. In the Bullock et al. (1997) study, heterotrophic bacteria counts remained in the range of 10^3 to 10^4 cfu/mL, both with and without system ozonation. However, Bullock et al. (1997) did not ozonate sufficiently to produce a measurable dissolved ozone residual under most conditions.

Total coliform bacteria counts in the water exiting the ozone contact chamber averaged just 2–18 cfu/100 mL over the ozone *Ct* range of 0.1–3.65 min mg/L (Table 3), which was a mean LOG_{10} reduction in total coliform bacteria counts of 1.9–3.1. A plot of the mean total coliform bacteria count remaining in the water at

Table 2
Mean (±S.E.) ozone concentration measured at outlet of contact chamber, water flow rate, hydraulic retention time, ozone *Ct*, UV dose, number of sampling events, total heterotrophic bacteria counts before ozone, after ozone, and after UV, and reduction in total heterotrophic bacteria counts using ozone alone and using ozone and UV irradiation

Ozone concentration measured at contact chamber outlet (mg/L)	Flow rate (L/min)	Ozone *Ct* (min mg/L)	UV dose (mJ/cm^2)	# of sampling events	Total heterotroph counts before ozone (cfu/mL)	Total heterotroph counts after ozone (cfu/mL)	Total heterotroph counts after ozone and UV (cfu/mL)	Reduction in heterotroph counts using ozone alone (LOG_{10})	Reduction in heterotroph counts using ozone and UV (LOG_{10})
Ozone contacting with 1 min hydraulic retention time									
0.10 ± 0.01	150 ± 0.1	0.10	91.1 ± 3.2	6	120 ± 34	3 ± 0.3	3 ± 1	1.60	1.60
0.21 ± 0.01	150 ± 1.1	0.20	86.1 ± 2.4	11	244 ± 146	6 ± 3	4 ± 2	1.61	1.79
Ozone contacting with 8.3 min hydraulic retention time									
0.05 ± 0.00	301 ± 1.0	0.42	47.5 ± 2.0	13	177 ± 48	12.5 ± 3.0	3.0 ± 1.0	1.15	1.77
0.11 ± 0.01	301 ± 0.6	0.91	42.5 ± 1.8	10	130 ± 60	3.4 ± 1.7	0.5 ± 0.2	1.62	2.41
0.21 ± 0.01	302 ± 2.0	1.74	54.7 ± 2.2	10	116 ± 25	5.2 ± 2.8	1.8 ± 0.8	1.35	1.81
Ozone contacting with 16.6 min hydraulic retention time									
0.04 ± 0.01	152 ± 1.7	0.66	105.1 ± 2.7	13	181 ± 40	11.6 ± 4.1	1.9 ± 0.9	1.19	1.98
0.10 ± 0.01	150 ± 1.0	1.66	112.7 ± 0.6	13	138 ± 6.4	8.1 ± 4.8	0.0 ± 0.0	1.23	[a]
0.22 ± 0.01	150 ± 1.3	3.65	107.7 ± 2.1	12	53 ± 10.4	2.7 ± 2.7	0.1 ± 0.1	1.29	2.72

[a] LOG_{10} removal cannot be calculated when the after ozone and UV total heterotroph counts were zero during all sampling events.

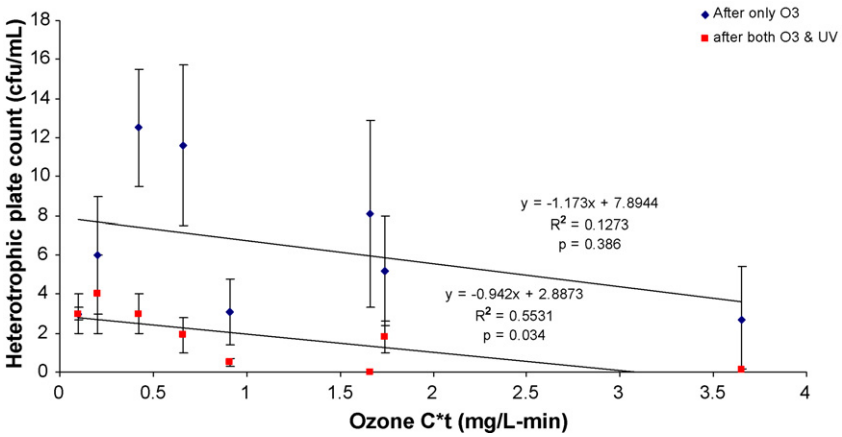

Fig. 3. Mean heterotrophic bacteria counts (with standard error bars) for each ozone *Ct* tested, where water samples were collected immediately following the ozone contact chamber (■) or following ozone and the UV irradiation unit (◆).

the end of the ozone contact tank versus the ozone *Ct* dose (Fig. 5) suggests a strong, nearly significant correlation ($P = 0.06$) between ozone *Ct* dose and the remaining total coliform bacteria count. However, the correlation between mean total coliform bacteria count remaining in the water post ozonation and the outlet ozone concentration (Fig. 6) was not significant ($P = 0.227$).

3.1.2. Ozonation followed by UV irradiation

UV irradiation dosages of 42.5–112.7 mJ/cm^2 that were applied post ozonation reduced total heterotrophic bacteria and total coliform bacteria counts in the water exiting the UV irradiation unit to means of only 0–4 cfu/mL and 0.1–3 cfu/100 mL, respectively (Tables 3 and 4). There was a significant correlation ($P = 0.034$) between ozone *Ct* dose and the total heterotrophic bacteria remaining in the water post ozone and UV irradiation, with total heterotrophic counts declining with increasing ozone *Ct* (Fig. 3). However, no correlation was suggested when the mean total coliform bacteria count remaining in the water exiting the UV irradiation unit was correlated against the ozone *Ct* ($P = 0.249$, Fig. 5) or the ozone concentration ($P = 0.363$, Fig. 6).

As was the case with ozone *Ct*, little difference was evident for bacterial inactivation at the different UV doses. Statistical analyses (Friedman test) indicated a highly significant ($P < 0.001$, $\alpha = 0.05$) difference among the three mean bacterial counts (before ozonation, after ozonation only, and after ozonation and UV irradiation) for both total heterotrophs and total coliform. Post hoc analysis applying the Wilcoxson signed rank test to further elucidate statistical differ-

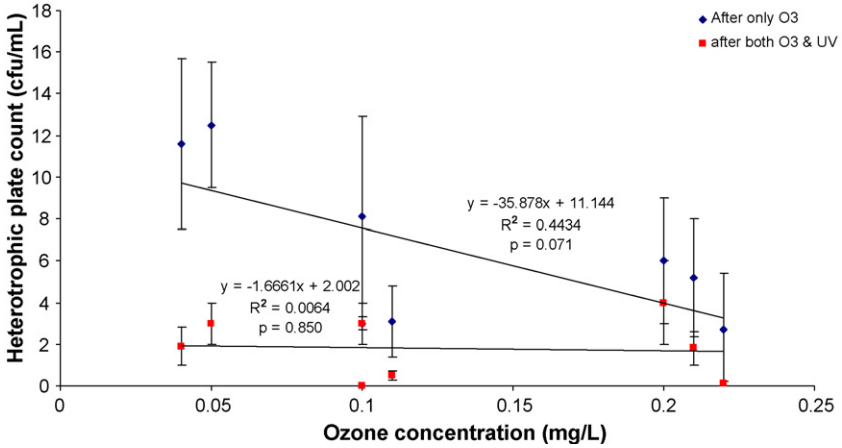

Fig. 4. Mean heterotrophic bacteria counts (with standard error bars) remaining in water samples collected immediately following the ozone contact chamber (■) or following ozone and the UV irradiation unit (◆) as a function of the ozone concentration maintained at the end of the ozone contact chamber.

Table 3
Mean (±S.E.) ozone concentration measured at outlet of contact chamber, water flow rate, hydraulic retention time, ozone *Ct*, UV dose, number of sampling events, total coliform bacteria counts before ozone, after ozone, and after UV, and reduction in total coliform bacteria counts using ozone alone and using ozone and UV irradiation

Ozone concentration measured at contact chamber outlet (mg/L)	Flow rate (L/min)	Ozone *Ct* (min mg/L)	UV dose (mJ/cm^2)	# of sampling events	Total coliform counts before ozone (cfu/100 mL)	Total coliform counts after ozone (cfu/100 mL)	Total coliform counts after ozone and UV (cfu/100 mL)	Reduction in total coliform counts using ozone alone (LOG_{10})	Reduction in total coliform counts using ozone and UV (LOG_{10})
Ozone contacting with 1 min hydraulic retention time									
0.10 ± 0.01	150 ± 0.1	0.10	91.1 ± 3.2	6	1995 ± 746	12 ± 4	0.2 ± 0.2	2.22	4.00
0.21 ± 0.01	150 ± 1.1	0.20	86.1 ± 2.4	11	1521 ± 586	18 ± 12	1 ± 1	1.93	3.18
Ozone contacting with 8.3 min hydraulic retention time									
0.05 ± 0.00	301 ± 1.0	0.42	47.5 ± 2.0	12	814 ± 239	4.3 ± 1.7	0.2 ± 0.1	2.64	4.02
0.11 ± 0.01	301 ± 0.6	0.91	42.5 ± 1.8	12	989 ± 439	4.0 ± 0.8	3.0 ± 2.0	2.39	2.52
0.21 ± 0.01	302 ± 2.0	1.74	54.7 ± 2.2	17	1047 ± 275	2.4 ± 0.6	0.1 ± 0.1	2.28	3.61
Ozone contacting with 16.6 min hydraulic retention time									
0.04 ± 0.01	152 ± 1.7	0.66	105.1 ± 2.7	13	2127 ± 975	12 ± 5.6	0.8 ± 0.4	3.11	4.31
0.10 ± 0.01	150 ± 1.0	1.66	112.7 ± 0.6	13	2122 ± 752	2.2 ± 0.6	0.1 ± 0.0	2.98	4.33
0.22 ± 0.01	150 ± 1.3	3.65	107.7 ± 2.1	14	2065 ± 718	1.6 ± 0.6	0.1 ± 0.1	2.25	3.42

ences in the after ozone and after UV bacterial counts indicated statistical differences of total coliform at all UV doses except for the lowest dose (42.7 mJ/cm^2). Post hoc analysis of mean total heterotrophs after ozone and after UV indicated statistical differences at UV doses 47.5, 54.7, and 112.7 mJ/cm^2.

Ozonation was effective in the reduction of microbial populations within the recirculating fish culture system and greater effectiveness was obtained with the use of UV irradiation post ozonation. An ozone *Ct* dose-dependent response was noted in the heterotrophic bacteria counts measured post ozone-UV. However, no ozone *Ct* dose-dependent response was detected in total coliform bacteria counts remaining after ozone/UV or in total heterotrophic counts post ozone alone. We are uncertain why a clear dose-dependent response was not detected in all cases. Lack of a clear dose response may have been due to the presence of colloidal particulate matter that exerted an ozone demand and at least partially shielded embedded bacteria from dissolved ozone. However, large variations detected in bacteria counts, as indicated by the standard error bars in Figs. 3–6, may also have contributed to the inability to detect an ozone dose-dependent response in all cases.

Treatment using ozonation alone or ozonation followed by UV irradiation produced many lower total heterotrophic bacteria counts than previous research in the same system using UV irradiation alone (Fig. 7; Sharrer et al., 2005). Sharrer et al. (2005) found that UV dosages of 78, 150, 303, 493, and 980 mJ/cm^2 could only achieve 0.4–0.9 LOG_{10} reduction in heterotrophic bacteria, while a UV dose of 1800 mJ/cm^2 could only achieve a 1.7 LOG_{10} reduction in total heterotrophic bacteria, leaving 181 ± 71 cfu/mL in the water exiting the UV irradiation unit. In comparison, the combined use of ozone and UV irradiation was effective at maintaining bacteria counts of 0.1–3 cfu/mL after treatment (Table 2; Fig. 7). With total coliform bacteria, however, Sharrer et al. (2005) found that UV irradiation alone resulted in complete inactivation (<1 cfu/100 mL) of total coliform at a UV dose of 77 mJ/cm^2. Amirsardi et al. (2001) showed that complete inactivation of total coliform was achieved with the application of ozone alone to wastewater. Further, the researchers found that total heterotrophic bacteria were reduced from 960 to 680 cfu/mL with ozone application, but reduction was augmented with UV irradiation to 120 cfu/mL. In contrast, Oh et al. (2003) found that little difference was evident with regard to *Escherichia coli* disinfection when applying UV alone, ozone alone, or a combined UV/ozone process. As with the UV inactivation (Sharrer et al., 2005), we hypothesize that

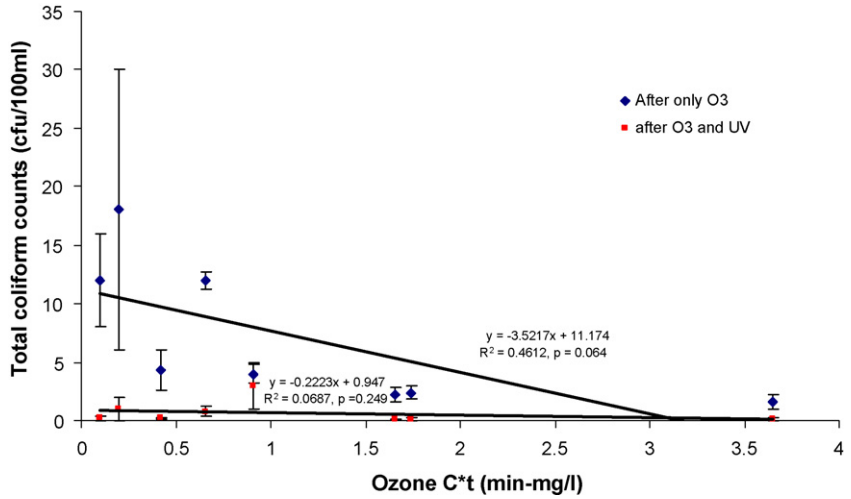

Fig. 5. Mean total coliform bacteria counts (with standard error bars) for each ozone *Ct* tested, where water samples were collected immediately following the ozone contact chamber (■) or following ozone and the UV irradiation unit (◆).

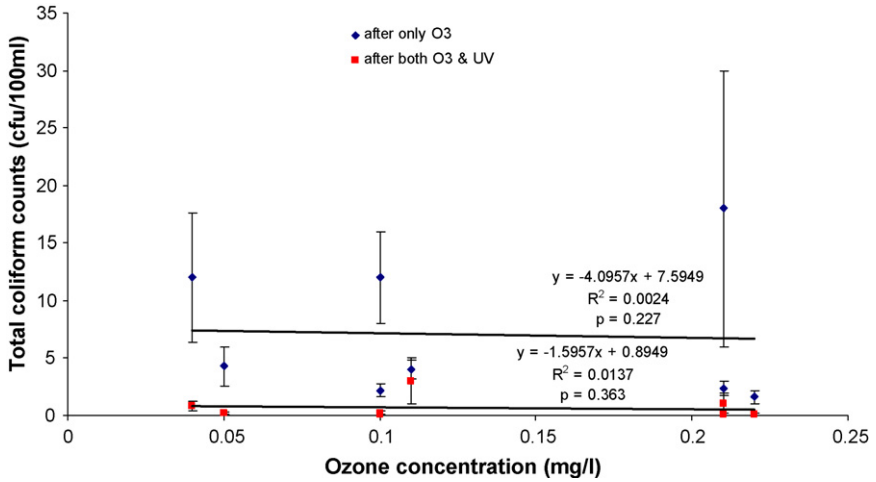

Fig. 6. Mean total coliform bacteria counts (with standard error bars) remaining in water samples collected immediately following the ozone contact chamber (■) or following ozone and the UV irradiation unit (◆) as a function of the ozone concentration maintained at the end of the ozone contact chamber.

Table 4
Mean (±S.E.) concentrations of ozone dosed (calculated[a]), as well as that measured at chamber inlet, middle, and outlet

Ozone concentration dosed[a] (mg/L)	Ozone concentration measured at contact chamber inlet (mg/L)	Ozone concentration measured at contact chamber midpoint (mg/L)	Ozone concentration measured at contact chamber outlet (mg/L)	Flow rate (L/min)
Ozone contacting with 8.3 min hydraulic retention time				
0.85 ± 0.04	0.75 ± 0.02	0.41 ± 0.01	0.21 ± 0.01	302 ± 2.0
0.78 ± 0.06	0.62 ± 0.03	0.27 ± 0.01	0.11 ± 0.01	301 ± 0.6
0.75 ± 0.07	0.51 ± 0.02	0.20 ± 0.01	0.05 ± 0.00	301 ± 1.0
Ozone contacting with 16.6 min hydraulic retention time				
1.2 ± 0.1	0.96 ± 0.04	0.44 ± 0.02	0.22 ± 0.01	150 ± 1.3
1.0 ± 0.2	0.55 ± 0.07	0.24 ± 0.02	0.10 ± 0.01	150 ± 1.0
0.9 ± 0.1	0.43 ± 0.04	0.15 ± 0.02	0.04 ± 0.01	152 ± 1.7

[a]Calculated using a mass balance that accounts for the mass flow rate of ozone in the gas phase that was transferred into the water flow.

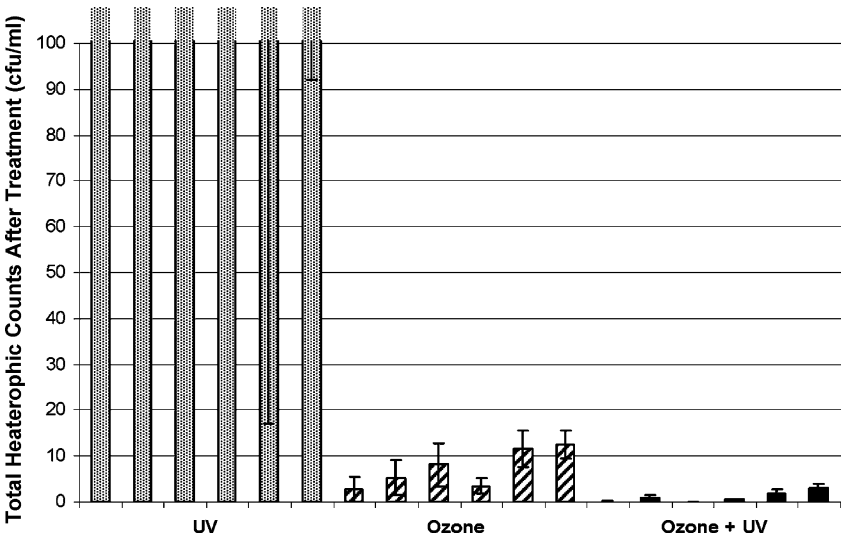

Fig. 7. Total heterotrophic bacteria counts after UV irradiation (1820, 990, 524, 303, 150, and 77 mW s/cm^2) alone all exceeded 100 cfu/mL (Sharrer et al., 2005), after ozonation (3.65, 1.74, 1.66, 0.91, 0.66, and 0.42 min mg/L) alone ranged from 3 to 13 cfu/mL (present study), and after combined ozonation and UV irradiation (3.65, 1.74, 1.66, 0.91, 0.66, and 0.42 min mg/L ozone dose at UV doses of 107.7, 54.7, 112.7, 42.5, 105.1, and 47.5 mW s/cm^2, respectively) ranged from 0 to 2 cfu/mL (present study).

because the water in the recirculating system passed through the UV irradiation unit approximately once every 30 min, that this provided a selection process that favors bacteria that embed within particulate matter or that form bacterial aggregates. This data suggests that continuous ozonation of the water may reduce the concentration of fine particulates or otherwise make the bacteria in the water more susceptible to UV irradiation. Fig. 7 compares total heterotrophic bacteria counts that remain in the recirculating water post disinfection when utilizing UV alone (Sharrer et al., 2005), ozone alone (present study), and the combined ozone/UV process (present study), which illustrates the effectiveness of sequential ozone/UV administration. Particle counts were not collected in the present research, but will be collected during our follow-up studies assessing ozonation and UV irradiation on bacterial reduction in a full-scale recirculating fish culture system.

3.2. Ozone dose and decay kinetics

The ozone demand of water in the recirculating system was low, because a calculated ozone dose of only 0.75–1.2 mg/L had to be transferred into the water flow to maintain 0.05, 0.1, and 0.2 mg/L of ozone at the outlet of the contact chamber at HRT's of 8–16 min (Table 4). In addition to the side-stream ozonation, during the present study all of the recirculating water was continuously ozonated within the low head oxygenation unit. Thus, approximately once every 30 min, all of the water in the recycle system was exposed to ozone, which tended to reduce the ozone demand of the water. In addition, the makeup water flow to the recirculating system was also relatively high (at 7–8% on a flow basis) to reduce heat gain in the system, which flushed the system almost once every 12–18 h and reduced the accumulation of dissolved organic carbon.

From an engineering standpoint, adding 0.75–1.2 mg/L of ozone into the recycle flow is not difficult, because it can be readily transferred into the system along with its oxygen carrier gas within the same gas transfer device that is used to provide super-saturation of dissolved oxygen to the fish culture tank. Also, dosing 0.75–1.2 mg/L of ozone to the recirculating flow is relatively little ozone compared to the 3–5 mg/L ozone demand encountered in many surface water disinfection applications (Summerfelt et al., in press).

4. Conclusions

Combining ozone dosages of only 0.1–0.2 min mg/L with a UV irradiation dosage of $\geq$50 mJ/cm^2 provides an advanced oxidation process that could consistently produce a post-treatment water nearly free from total coliform and total heterotrophic bacteria colony forming units. However, increasing ozone *Ct* did correlate with even lower counts of total heterotrophic bacteria post UV irradiation. In comparison, bacteria counts post ozone and UV irradiation were orders of

magnitude lower than the bacteria counts measured in the system when it was operated with UV irradiation alone or without any disinfection (Sharrer et al., 2005). Note, however, that the commercial growth media used to assess for indicator organisms may not reflect total numbers of viable organisms in the water column that could be identified through direct microscopy. Although the present research was conducted on a small side-stream flow, these findings indicate that combining ozonation and UV irradiation could be used to disinfect an entire recirculating flow before it returns to the fish culture tank(s). The authors think that the combined ozone and UV process will be readily scalable and technically achievable for commercial producers. We are presently evaluating ozonation and UV irradiation of the entire recirculating flow to determine if applying relatively low dosages of ozone prior to UV irradiation will produce similar reduction in bacteria and total particle counts. If the full-flow application of ozone and UV irradiation achieves nearly complete bacteria inactivation, its use could reduce the risk of epidemic loss in commercial-scale recirculating systems.

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