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Effects of two different ozone doses on total residual oxidants, nitrogen compounds and nitrification rate in seawater recirculating systems for black seabream *Acanthopagrus schlegelii* (Bleeker)

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ABSTRACT

Ozone was applied to seawater recirculating aquaculture systems (RAS) to measure the effects on water quality and biofilter efficiency. Three replicate experimental systems were used in this study. Each system consisted of four rectangular culture tanks, a sump, two settling chambers, a foam fractionator and a trickling biofilter. The control system (CS) was not ozonated, but Treatment 1 (T 20) and Treatment 2 (T 40) were ozonated with 20 and 40 g O_3/kg feed day^{-1}, respectively. Three hundred twenty black seabream (mean weight ± S.D. of 334.5 ± 1.9 g) were stocked into each system, and cultured for the entire 44-day study period. During this period, total residual oxidants (TRO), nitrogen compounds (Total-ammonia–N, TAN; nitrite–N, NO_2–N and nitrate–N, NO_3–N) were measured, and nitrification efficiencies of the trickling biofilters were calculated for each system.

Generally, the application of ozone to the seawater systems reduced TAN and NO_2–N concentrations in the culture tanks of both treatments. However, the nitrification rate of the biofilter in the T 40 and the CS systems was about 50% lower than the rate of the biofilter in the T 20 system. Therefore, the use of a moderate dose of ozone (20 g O_3/kg feed day^{-1}) appears to enhance nitrification, possibly through the reduction of dissolved organic matter that may interfere with bacterial activity.

However, application of ozone at 40 g O_3/kg feed day^{-1} appears to depress microbial activity associated with biofiltration and nitrogen removal efficiency, perhaps through toxicity of associated compounds or directly through residual oxidants. Based on the results of this study, continuous ozonation should not exceed 20 g ozone/kg feed (mean TRO 0.15 mg/L) in a seawater RAS to avoid negatively affecting the nitrification efficiency of the bio-filters.

1. Introduction

High levels of dissolved nutrients in land-based aquaculture effluents have led to concerns about eutrophication of receiving waters (De Pauw and Joyce, 1991; Barg, 1992; Stewart, 1997). Recirculating aquaculture systems (RAS) are a promising means of reducing the amount of effluents, thereby minimizing the environmental impact of intensive production facilities. Two major objectives of the RAS treatment processes are to physically and/or chemically remove organic matter and to optimize bacterial activity to accelerate nitrification rates in the biofilter. One means of achieving both of these goals is through the application of ozone to the culture water.

Ozone has been used in aquaculture to disinfect incoming water, or to reduce the organic material in effluents. Recently, ozone has been used to control ammonia–N, nitrite–N, nitrate–N, turbidity and water color (Park et al., 2013).

An allotrope of oxygen, ozone (O_3) consists of three oxygen atoms and is much less stable than the diatomic O_2. Ozone has strong oxidation potential through the release of an oxygen atom loosely bonded with the others to oxidize most organic and inorganic molecules in water.

The major nitrogen metabolic by-product released by fish in a RAS is the total ammonia–N (TAN), which is either directly excreted

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by fish or released through decomposition of uneaten food and feces.

Nitrite–nitrogen (NO_2^-–N) and nitrate–N (NO_3^-–N) are oxidized from TAN by the bacterial nitrification process. Ozone reacts with un-ionized ammonia (Eq. (1); this reaction occurs only in freshwater) and nitrite–N (Eq. (2)) to directly oxidize nitrate–nitrogen.

$$4O_3 + NH_3 \Rightarrow NO_3^- + 4O_2 + H_3O^- \quad (1)$$

$$NO_2^- + O_3 \Rightarrow NO_3^- + O_2 \quad (2)$$

The effect of ozone application on the chemistry of seawater is complex. Principal concerns to marine organisms are the reactions of ozone to the naturally occurring bromide and the TAN originating from feeds. In general, when ozone is injected into a seawater aquaculture system, the ozone reacts with bromide ion in seawater to generate a by-product, hypobromous acid (HOBr; Eq. (3)). However when TAN also is present, hypobromous acid (HOBr) reacts with TAN to produce bromamines; eventually, break-point bromination will restore bromide to its ionic state (Br^-). During this process, nitrogen gas and three hydrogen ions are produced, which consumes alkalinity, and decreases pH as follows (Haag and Hoigné, 1983; Tanaka and Matsumura, 2002).

$$3HOBr^- + 2NH_3 \rightarrow N_2 + 3Br^- + 3H^+ + 3H_2O \quad (3)$$

In natural seawater, ozone can combine with naturally occurring bromine to form residual oxidants that are toxic to fish (Hofmann, 2000). In artificial seawater where bromine is removed, however, chloramines will arise after ozone reacts with chloride and nitrogen compounds. Thus application rates of ozone in seawater must be carefully monitored to minimize the production of toxic residuals, and objectively evaluated to understand its effect on other water quality parameters.

Although there have been many studies about the application of ozonation in a RAS for hatcheries, these studies were mostly conducted either in freshwater or for seawater under very low organic loading or at very low salinities (Rosenthal and Kruner, 1985; Bullock et al., 1997; Summerfelt et al., 1997, 2009; Singh et al., 1999; Krumins et al., 2001; Ritar et al., 2006; Hofmann, 2000; Reiser et al., 2011). Recent studies on the use of ozone in seawater have been conducted on white shrimp (*Litopenaeus vannamei*), Atlantic halibut (*Hippoglossus hippoglossus*) and turbot (*Psetta maxima*) and have provided a better understanding of water quality dynamics concerning ozonation in seawater RAS (Tango and Gagnon, 2003; Reiser et al., 2010, 2011; Davidson et al., 2011; Schroeder et al., 2011). However, practical information is still needed to more fully understand the effect of different ozonation rates on water quality and fish production in a seawater RAS for grow-out. In addition, research on the research on the effects of ozonation on the nitrification rate of bio-filters has not been reported. Although changes in the concentrations of the different nitrogen compounds in a RAS are an indication of the activity of the nitrifying bacteria in the biofilter, the activity and overall number of nitrifying bacteria within the filter are likely affected by oxidants produced from ozonation. Therefore, the purpose of this study was to measure the changes of residual oxidant and nitrogenous compounds resulting from different ozonation doses and to calculate the effects on bio-filter efficiency as determined by nitrogen removal rates.

2. Materials and methods

2.1. Experimental recirculating system and ozone generation

This experiment was done over a 44-day period at the Fisheries Science Research Station, Gangwon Provincial College, South Korea. Three independent replicate systems (4.5 m^3 total volume per system) were used in this study. Each system consisted of four rectangular culture tanks (1.0 m L × 1.0 mW × 1.0 mH), a sump (0.5 m L × 0.5 mW × 1.0 mH), two radial solid settlers (0.6 mD × 1.0 mH), a foam fractionator (0.3 mD × 2.5 mH) driven by a 0.75 kW venturi pump, trickling bio-filter (1.2 mD × 2.0 mH) with Styrofoam bead media (2 mm diameter) and a 0.75 kW centrifugal pump (Fig. 1). Water volume turnover rate of the system was about 36 times a day.

Natural seawater was pumped from the northeast coast of South Korea, passed through a sand filter (40 μm), a mechanical screen (10 μm), then distributed to the culture tanks. Water from a side drain in the culture tank was pumped to a trickling biofilter through a foam fractionator, and then returned to the culture tank by gravity (water flow rate of 120 L/min). Settleable solids were removed by a radial flow settler through a center drain in the tank bottom.

Ozone was generated (corona discharged method, Model LAB-I, Ozonetech Inc., Dajeon, Korea) with pure oxygen as a feed gas (Model 21L, Oxus Inc. Gapyong, Korea) and was injected through a Venturi pipe into the by-pass line between the centrifugal pump and foam fractionator. The by-pass was extended to 15 m length and contact time was set at 4 min. The treated water with ozone in the by-pass line returned to the sump (water flow rate of 25 L/min) before the centrifugal pump to prevent direct contact between the fish and the ozonated water. The control system (CS) was not ozonated, while the two treatment systems were ozonated at the rates of 20 g ozone/day (T 20) and 40 g ozone day^{-1} (T 40) per 1 kg feed, respectively.

2.2. Experimental fish and system management

A total of 107 kg of black sea bream, *Acanthopagrus schlegelii* (Bleeker), with an average weight (mean ± S.D.) of 334.5 ± 1.9 g were stocked randomly into each system and equally distributed into four tanks (80 fish/tank). Feed (50% crude protein) was applied three times a day at 9:00, 13:00 and18:00 for a total of 1% of body weight. The same amount of feed was given to the fish in each system for the entire 44-day study period. Solids were removed twice daily by draining from the radial flow settler. Approximately 10% of the system water was replaced daily to compensate for losses from solids removal and evaporation. The water temperature was maintained from 22 to 23 °C and the average salinity was 33.6 ppt.

Prior to the start of the study, the trickling biofilters were operated for 6 months with a different population of black sea bream, to ensure that the media was fully seeded with nitrifying bacteria. The three systems were completely drained then refilled with newly filtered seawater before the start of the experiment.

2.3. Measurements

Table 1 shows the water quality analysis methods and measuring instruments used in this study. Water temperature, dissolved oxygen, pH and salinity were measured once daily (model 556MPS Yellow Springs Instruments Inc., Ohio, USA). Total ammonia–N (TAN), nitrite–N (NO_2–N) and nitrate–N (NO_3–N) were analyzed twice a week following initial setup, then once weekly thereafter. All three nitrogen analyses were also done during one 6-day period beginning on day 25, and one 24-h sampling period on day 33. The 24-h measurements were done every 4 h from 6:00 to 18:00. The 6-day samples were taken and analyzed daily at 9:00 starting on day 25. Water samples were taken from each tank at 13:00 and analyzed immediately. Meanwhile, diel changes of total residual oxidants (TRO), TAN, NO_2–N, and NO_3–N were checked every 4 h on the 33th day. Total residual oxidant (TRO; DPD method, APHA et al., 1995) was measured on the same schedule as the nitrogen compounds. Oxidation–reduction potential (ORP; model ORP-6000, DIK Co., Bucheon, KR) was monitored in real time.