Prediction of required ozone dosage for pilot recirculating aquaculture systems based on laboratory studies - Study case

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Abstract In recirculating aquaculture systems (RAS), the water quality changes continuously. Organic and inorganic compounds accumulates creating toxic conditions for the farmed organisms. Ozone improves water quality diminishing significantly both bacteria load and dissolved organic matter. However, in a non-meticulously designed system, residual ozone might reach the culture tanks causing significant harm to cultured species or excess costs. The aim of the study was to predict the suitable ozone dosage in pilot RAS, for water treatment purposes, based on laboratory studies.

The ozone effect on water quality of freshwater RAS and system's ozone demand was investigated. Bench-scale ozonation experiments revealed the ozone demand of the system to be 180 mg O_3/h. Three different ozone dosages were applied to four replicated systems with fixed feed loading (1.56 kg feed/m^3 make up water). Results suggested that the optimal ozone dosage was 15g O_3/ kg feed. Selected water quality parameters were measured, assessing biofilters performance as well as nitrogen and carbon–based compound concentration change during ozonation. Overall, this study contributed to a better understanding of the challenges of an ozonated RAS leading to the optimal design of such systems.

Keywords: Ozonation, water quality, recirculating aquaculture systems, pilot-scale, laboratory study

1. Introduction

Land-based recirculating aquaculture systems (RASs) have become increasingly important as the available water is better utilized to achieve production continuity. In such systems, the water quality is impaired by the accumulation of pollutants allowing fish pathogens to grow. Although chemotherapeutants are widely used to control pathogens, they will be limited since they have been accused to affect biofilters performance and the nearby aquatic ecosystem upon discharge (Hohreiter and Rigg, 2001; Masters, 2004; Wooster *et al*., 2005).

Ozone has been implemented as a secondary water treatment technology (Langlais *et al*., 1991; Liltved *et al*., 2006) improving water quality. It oxidizes several deteriorating agents such as carbon and nitrogen based compounds, colour and suspended solids (Reid and Arnold, 1994; Summerfelt *et al*., 1997; Krumins *et al*., 2001; Summerfelt *et al*., 2008) reducing bacteria and fish pathogens (Bullock *et al*., 1997; Summerfelt *et al*., 2003; Tango *et al* 2003; Summerfelt *et al*., 2009).

In a non-meticulously designed system, excess of ozone, due to overdose, might reach the culture tanks causing significant harm to cultured species while huge amount even tiny excesses causes that electricity is wasted. Therefore, it is crucial to define the ozone demand of the specific system, ensuring a realistic "safety window" and improved water quality (Muller & Milton 2012).

Thus, this study aimed to develop a method to predict the ozone demand and to reveal a more direct approach to control the delivered ozone dosage in RASs. The required ozone dosage will be predicted solely based on water quality parameters analysed in the laboratory. To achieve this, water samples were collected from a pilot-scale system, simulating accurately an intensive full-scale system, and subjected to ozonation.

Overall, this project's objectives can be summarized thus: (I) to predict the effect of continuous ozonation in pilot-RAS on water quality, based on laboratory-scale experiments; (II) determine the optimal ozone dosage in fresh water pilot-RAS to ensure improved water quality without compromising fish health or welfare.

2. Material and Methods

2.1. Laboratory-scale experiments

Water samples were collected from a random rearing tank of one of the twelve identical pilot-scale RASs described in

Rojas-Tirado *et al*. (2016). The purpose of the batch-experiment was to define the ozone demand, to determine ozone's lifetime and the optimal ozone dosage, which ensures improved water quality, and to test the ozonation capacity of the system by indicating the critical range that ozonation can safely occur in such systems.

Several ozone doses ranging from 0 to 10 mg O_3/L, were spiked into 50 mL RAS water subsample as described in Hansen *et al*. (2016). To better control the ozone concentration in RAS sample, the same ozone dosage was added in acidified MilliQ water (50 mL), containing 5 mL phosphate buffer and sufficient amount of potassium indigotrisulphonate (Antoniou *et al*., 2013) and the ozone dose was measured by the loss of the colour of indigotrisulphonate as quantified by a fotometer.

To predict the optimal ozone dosage in pilot-RASs and to better engineer the water flows in recirculation, water samples were repeatedly ozonated after ozone depletion (Hansen *et al*., 2016). These samples used for ozone profiles utilizing a spectrophotometer. All samples, including the non-ozonated sample (control), were measured with a fluorimeter to define the ozone effect on natural fluorescence degradation (Spiliotopoulou *et al*., 2017).

2.2 Quantification

2.2.1 Ozone

A 20 g/h ozone generator from O3-Technology AB (Vellinge, Sweden), supplied with dry oxygen gas, generated ozone that was dispersed through a diffuser in a pressurised collection bottle containing ultra-pure water to create the ozone stock solution. Ozone concentration in the stock solution ranged between 70 and 110 mg/L.

2.2.2 Determination of ozone concentration

Ozone concentration was determined utilising the indigo method (Bader and Hoigné, 1981), modified as described in Antoniou *et al*., (2013). The absorbance of the unreacted indigotrisulphonate was measured at 600 nm with a spectrophotometer (Hach Lange).

2.2.2 Determination of ozone demand

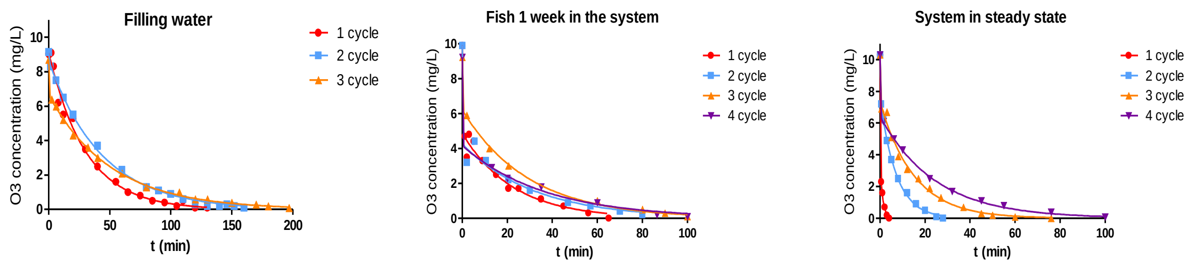
Fluorescence spectroscopy was used to determine indirectly the delivered ozone dosage in water as described in Spiliotopoulou *et al*. (2017), utilising a fluorimeter (Cary Eclipse, Varian). Ozone life-time was determined as described in Hansen *et al*., (2016) utilizing the indigo method.

2.2.3 Organic carbon determination

A Shimadzu ASI-V UVC/Persulphate analyser quantified the non-volatile organic carbon of raw samples. The injected sample volume was 3.00 mL and a calibration curve with potassium hydrogen phthalate standards from 50 to 2000 µg/L was determined (R^2=0.99) with quantification limit set to 50 µg/L.

3. Results and discussion

3.1 Ozone life-time



	1 cycle	2 cycle	3 cycle
K	0.03292	0.02304	0.01954

	1 cycle	2 cycle	3 cycle	4 cycle
K	0.04445	0.03123	0.03403	0.02746

	1 cycle	2 cycle	3 cycle	4 cycle
K	0.8544	0.1399	0.06670	0.04066

Figure 1. System's ozone demand based on kinetics; pollution build-up over time: a) filling water, b) sampling on day 7 and c) sampling on day 70.

To investigate the ozone demand of the system, seven different ozone dosages ranging from 0-10 mg O_3/L, were spiked in RAS water collected over time. Among the dosages, only one was selected to verify system's ozone demand, the dosage of 10 mg O_3/L. Initially, the filling water was subjected to ozonation to verify if the tap water (non-chlorinated ground water) had an initial ozone demand (day 0) – background pollution. Over time, two more sampling campaigns occurred, one week after the entrance of fish in the tanks and after 70 days (where system had reached steady-state in terms on biofilters performance). The samples were ozonated repeatedly upon depletion of ozone; expressed as cycles (Figure 1). When repeatedly ozonating the filling water (Figure 1a), it can be seen that the ozone life-time (K) is constant, suggesting that the filling water does not contain any carbon-based compound to react with ozone and thus, its decomposition in water is extended to more than 125 min. The water where fish lived in for approximately 1 week, responded differently than the filling water. In all the ozone cycles, an initial ozone demand of approximately 5 mg O_3/L was observed while the ozone life-time varied among repetitions (Figure 1b). The initial ozone demand and the shorter life-times in the second batch of samples can be explained by the pollution formed due to fish activity; either metabolic by-products or accumulation of uneaten feed. The pollution over days is expected to compile since fish are growing and the system is characterised by as intense (extremely low daily water exchange). Therefore, the significantly different ozone life-times in the last batch of water (day 70) compared to the previous samples were shorter, as expected. The first ozone dosage was consumed rapidly (<5min) since the water is heavily loaded by organic compounds (Figure 1c) and ozone reacts instantly with the easily degradable compounds. The more recalcitrant compounds were oxidized by the following ozone cycles.

3.2 System's ozone demand

These results can be also used to interpret the ozone demand of the system based on fish pollution in a period of 70 days. Base on the kinetics in the Figure 1b, we can conclude that the ozone demand for the 1- week RAS water ranges from 16 to 20 mg O_3/L. The 2nd, 3^{d} and 4th cycles are overlapping meaning that after the 2nd cycle there is no effect; so the demand is more than 10 mg O_3/L (1st cycle) and less than 20 mg O_3/L (2nd cycle); each cycle means addition of 10 mg O_3/L. Therefore, we speculated that the ozone demand could be roughly 18 mg O_3/L. Following the same line of thinking for the last batch of RAS water, we assumed that the ozone demand was between 30 to 40 mg O_3/L, thus 35 mg O_3/L.

Since the filling water had no background pollution, the ozone demand was defined exclusively based on fish pollution. Based on the results the daily ozone demand was 2.6 mg/l. Since the total volume of the system is 1700 L, 182 mg O_3/h is needed to purify the water. This amount of ozone will make the water as clean as drinking water, which is not the point in this kind of application. In RASs, water should be clean enough to ensure fish wellbeing but not sterile since in case of an accidental contamination the immune system of fish won't be able to protect them against pathogens. Therefore, the suggested optimal dosage for water treatment in this case study is approximately 130 mg O_3/h.

3.3 Upcoming experiments

Based on the predicted ozone demand of the system, a range of 4 different ozone dosages including a reference system, has been selected to be tested in pilot-RASs in order to investigate if the methodology to predict the ozone demand of the system remotely, is representative to the reality and the ozonation effect on water quality in such a system.

However, this experimental attempt is undergoing therefore, relevant results will be presented in the future. Nonetheless, we are confident to say that our theory to estimate the ozone demand of a system remotely is a promising approach with high industrial value, yet it is needed to be proven.

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