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Advanced oxidation processes for treatment of 17β-Estradiol and its metabolites in aquaculture wastewater

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ABSTRACT

Recirculating aquaculture systems (RAS) allow food fish to be grown in a contained system with minimal water use (up to 99% recirculation) and a small land footprint. Currently, the use of natural estrogen (17β-estradiol; E2) as a feed additive is being studied to increase growth and feminization in American eels (*Anguilla rostrata*) being grown in a RAS. This study aimed to degrade concentrations of E2 and its metabolites, estrone (E1) and estriol (E3), in aquaculture effluent to below detectable levels to ensure the safe discharge of waste streams. Advanced oxidation processes (AOPs) have been previously used to degrade trace organic contaminants to great effect. This study tested two AOPs (UV and UV/H_2O_2) for the removal of E2 and its metabolites at low concentrations. Estrogen levels were analyzed using liquid chromatography-tandem mass spectrometry (LC–MS/MS). Increasing UV dose increased the degradation of estrogens and their removal was improved in aquaculture wastewater versus in pure water. The addition of H_2O_2 did not consistently improve the efficacy of the UV treatments. The results of this study indicate that these processes may be of future commercial use to remove estrogenic compounds from aquaculture wastewater.

1. Introduction

Recirculating aquaculture systems (RAS) have been used since the 1960s to culture aquatic organisms in a high-intensity setting (Masser et al., 1992). Although they can be biologically and technologically difficult to manage, RAS produce a higher product yield per amount of space (kg m^{-3}) and amount of water (kg L^{-1}) in comparison to extensive aquaculture farming (Masser et al., 1992; Piedrahita, 2003). Due to the substantial densities they support, total waste production is generally higher in a RAS than in lower yield operations. However, because these closed systems are extensively controlled, waste streams are more easily managed, and final waste discharge is often lower (per kg of production) than less intense aquaculture systems.

Rearing of *Anguilla* spp. in recirculation systems has been studied extensively (Dalsgaard et al., 2013; Heinsbroek, 1991; Heinsbroek and Kamstra, 1990; Liao et al., 2002). Freshwater eels are caught during the glass eel stage and are allowed to grow out in recirculation systems before being sold commercially. Though freshwater eels have been hatched and reared to the glass eel stage in captivity (Tanaka et al., 2003), this process has not yet been streamlined; As such, freshwater eel culturing currently relies on wild-caught fisheries. Recently, the American eel (*Anguilla rostrata*) industry has become of significant interest to international buyers, benefiting from the decline of Japanese eel (*Anguilla japonica*) population and the ban on the exportation of the European eel (*Anguilla anguilla*) (Cohen et al., 2017).

A. rostrata, along with other freshwater eel species, will differentiate its sex as a juvenile based on environmental conditions (Colombo and Grandidr, 1996). Freshwater eels are sexually dimorphic, with males maturing quickly to smaller sizes (200 g) while females will grow much larger (> 500 g) (Degani et al., 2003; Tzchori et al., 2004). Generally, freshwater eels are not as commercially productive as many other aquaculture species, as in high-density environments eels will become primarily male (Davey and Jellyman, 2005; Roncarati et al., 1997). Estrogen supplementation has been used successfully to encourage feminization (Degani and Kushnirov, 1992) and growth in freshwater eels (Anderson et al., 1996; Colombo and Grandidr, 1996; Degani et al., 2003; Satoh et al., 1992; Tzchori et al., 2004), and may be an effective solution to these production issues.

Recently, 17β-estradiol (E2) specifically has been used as a feed supplement to American eels with considerable success. There also seems to be no issues for human consumption; study animals fed a diet that included E2 as a feed additive showed a return to natural estrogen (i.e., E2) levels after a 5-day withdrawal period (Cohen et al., 2017). However, estrogens released into aquatic environments can have

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detrimental effects on freshwater organisms (Folmar et al., 1996; Harries et al., 1996; Jobling et al., 2006, 1998). As such, it is essential to minimize potential estrogen release from aquaculture waste streams. Advanced oxidation processes (AOPs), such as UV and UV/H_2O_2, have previously been used to remove estrogenic compounds from pure water and wastewater matrices (Cédat et al., 2016; Rosenfeldt et al., 2007; Ma et al., 2015; Zhang and Li, 2014).

This study aimed to determine the efficacy of UV and UV/H_2O_2 AOPs at degrading estrogens (E1, E2, E3) in two different water matrices (i.e., a pure or deionized (DI) water matrix and an aquaculture wastewater matrix). This research may enhance the understanding the application of AOPs in aquaculture industry and help to determine the most effective treatment techniques for pilot scale aquaculture wastewater treatment.

2. Materials and methods

2.1. Chemicals and reagents

E1, E2, E3, and internal standard stock solution of estrogen compounds, $^{13}C_6$-Estradiol, were purchased from Fisher Scientific (Ontario, Canada) and Cambridge Isotope Laboratories, Inc. (Massachusetts, USA) at a concentration of 1 mg mL^{-1} and 100 µg mL^{-1} in methanol and acetonitrile. Reference working stock solutions were prepared at a concentration of 1 mg L^{-1} in methanol and stored at −20 °C. Methanol and acetonitrile (LC–MS grade) were purchased from Fisher Scientific (Ontario, Canada). Pure water was generated by Milli-Q system (Reference A^+, Millipore) and had a resistivity of 18.2 mΩ cm^{-1} and total organic carbon (TOC) of < 5 µg L^{-1}. To test the efficacy of different treatments for the removal/degradation of E1, E2, and E3 in an aqueous solution, each analyte was prepared at a concentration of 10 µg L^{-1} in pure water and aquaculture effluent water collected from Dalhousie University's Aquatron Facility.

2.2. Collection of aquaculture effluent samples

Aquaculture effluent water was collected from a small RAS located at Dalhousie University's Aquatron Facility with a daily system volume of 3701 L. Daily water renewal was maintained between 10–20% of system volume at approximately 0.25–0.5 L min^{-1}. The RAS supported a biomass of ~65 kg of 2–3-year-old mature (majority female) eels at a density of 28 kg m^{-3}. Dissolved oxygen was maintained at 5–7 mg L^{-1}; system temperature was maintained at 25–28 °C. Eels were fed a maintenance diet of 165–250 g day^{-1} of Skretting Nutrafry XP feed, 3 mm pellet size. Eels had previously been fed an E2 supplement, but had been withdrawn from the drug for approximately 1 year. As such, no additional estrogen was being added to the RAS at the time of wastewater collection. E2 and its metabolites were added to wastewater samples just prior to treatment experiments, which are later described. Wastewater samples were collected in 4-L LDPE or 20-L HDPE bottles from the top of an effluent outflow and refrigerated at 4 °C until use.

2.3. Water quality analysis

Physical and chemical water quality parameters of collected aquaculture effluent water samples were measured as follows: pH and turbidity of water samples were measured using an Accumet® XL50 meter (Fisher Scientific) and a HACH® 2100 A N turbidimeter, respectively. Anions (i.e., fluoride, chloride, nitrate, nitrite, and phosphate) were measured by Ion chromatography (Dionex Aquion AS-AP, Thermo Scientific). Sulphate was not measured because it is not generally of concern in an aquaculture setting due to its low toxicity to aquatic species (Elphick et al., 2011) and was also expected to be extremely low. Ammonia and UV_{254} were analyzed using a HACH® DR5000 spectrophotometer. Total and dissolved organic carbon (TOC and DOC) were analyzed using a Shimadzu TOC-V Total Organic Carbon Analyser.

Table 1
Water quality parameters taken over the course of the study (n = 6).

Water Quality Parameters	
TOC (mg $L^{-1)}$)	2.4 ± 0.3
DOC (mg L^{-1})	2.5 ± 0.6
Turbidity (NTU)	0.41 ± 0.03
UV254 (abs cm^{-1})	0.033 ± 0.002
Fluoride (mg L^{-1})	0.55 ± 0.06
Chloride (mg L^{-1})	10 ± 2
Nitrate (mg L^{-1})	17 ± 3
Nitrite (mg L^{-1})	0.05 ± 0.04
Phosphate (mg L^{-1})	3.6 ± 0.9
Ammonia (mg L^{-1})	1.6 ± 0.5
pH	6.3 ± 0.9

Water quality data were recorded from December 19th, 2017 to February 2nd, 2018 and can be found in Table 1. Water quality is presented for the examination of the potential effects of these parameters on UV/AOP treatment of estrogens.

2.4. AOP batch experiments

A bench scale experiment of UV and UV/H_2O_2 photo-oxidation was carried out on 125 mL water samples containing 10 µg L^{-1} of 1) E2 only or 2) the mixture of E2 and its metabolites (E1, E3) in triplicate. The dose of 10 µg L^{-1} was chosen to give a concentration ten-fold higher than our method detection limits. Samples were treated using a bench-scale collimated beam unit (PS1-1-120, Calgon Carbon) equipped with a 1 kW medium-pressure mercury UV lamp. This lamp emittance is spectral in nature with 25% of the emitted light in the UVB + UVC range (200–300 nm region). The UV spectral graph for this lamp is presented in Figure S1 (in the Supporting Information). The average UV fluence of the collimated beam system was calculated as the product of the incident UV fluence rate at the center of the sample surface, a series of collimated correction factors (i.e, the petri dish factor and the water factor) provided by Calgon Carbon, and exposure time. Given the sensitivity of UV sensor by an International Light Model IL 1400 A radiometer with a model SUD240 UV sensor, the reaction time required to achieve the desired fluence in the range of 200 to 300 nm UV region was calculated. This UV fluence determination method follows the work suggested by Bolton and Linden (2003). The sample solutions were exposed to UV fluences of 100, 500, and 1000 mJ cm^{-2}. Hydrogen peroxide (H_2O_2) was dosed from a 3 mg mL^{-1} solution to achieve 1 or 10 mg L^{-1} concentrations, immediately spiked into the water samples as they were exposed to UV irradiation then quenched after UV exposure was complete with 2950 units mg^{-1} bovine catalase (Worthington Biochemical, USA). Previous studies using UV/H_2O_2 processes to treat estrogens have used higher H_2O_2 doses, from 5 to 90 mg L^{-1} (Cédat et al., 2016; Ma et al., 2015; Zhang et al., 2010), leading to the selection of 1 or 10 mg L^{-1} doses of H_2O_2. Additional testing was performed using a H_2O_2 dose of 20 mg L^{-1} on duplicate samples of a mixture of E1, E2, and E3 in pure water at UV fluences of 100, 500, and 1000 mJ cm^{-2}.

The hydrolysis of E2 and its metabolites and adsorption to the glassware and to other analytes present in the wastewater was also tested. Aquaculture wastewater was spiked with 10 µg L^{-1} of E2 and its metabolites and placed on a stir plate in the dark. Samples were taken over the course of three hours. A blank experiment was also conducted using 1 or 10 mg L^{-1} H_2O_2 without UV irradiance; these samples were mixed for 45 min prior to sampling. Both showed little to no degradation of E1, E2 or E3. To ensure that variations between testing performed in pure water and aquaculture wastewater were not due to differences in initial pH, additional tests were run in duplicate on pure water samples pH-adjusted to 6.3 to match aquaculture wastewater pH and spiked with a mixture of E1, E2, and E3. These samples were then