Building Tomorrow's Society
Bâtir la Société de Demain

Fredericton, Canada
June 13 – June 16, 2018/ *Juin 13 – Juin 16, 2018*

TREATMENT OF 17B-ESTRADIOL AND ITS METABOLITES IN WASTEWATER

Bennett, Jessica L.[1,2], Mackie, Allison L.[1], Park, Yuri[1] and Gagnon, Graham A.[1]
[1] Department of Civil and Resource Engineering, Dalhousie University
[2] js344308@dal.ca

Abstract: Recently, estrogens have been used to feminize and increase growth in American eels (*Anguilla rostrata)* being grown in a recirculating aquaculture system (RAS). The main objective of this study was to reduce concentrations of estrogens in aquaculture wastewater (17β-estradiol (E2) and its metabolites estrone (E1) and estriol (E3)) to below detection limits in order to assure stakeholders that no detrimental environmental effects will come from discharging this waste stream. Advanced oxidation processes (AOPs), including UV and UV with addition of hydrogen peroxide (H_2O_2), are known to effectively degrade trace organic contaminants. This study tested these AOPs for the removal of low concentrations (~10 µg/L) of E2 and its metabolites. Estrogen concentrations were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Increasing UV dose from 100 to 1000mJ/cm^2 increased the degradation of estrogens to near or below detection limits. Analytes were more effectively degraded by UV in aquaculture wastewater than in a pure water matrix. Adding H_2O_2 did not considerably or consistently improve the efficacy of the UV treatments. The results of this study indicate that these AOP water treatment processes are effective at degrading E1, E2, and E3 in aquaculture wastewater.

1 INTRODUCTION

The use of RAS (recirculating aquaculture systems) recirculation systems in the rearing of *Anguilla* species has been studied extensively (Dalsgaard 2013) *A. rostrata,* along with other freshwater eels, do not differentiate their sex until late in life, and will develop based on their environmental conditions (Colombo and Grandidr 1996). Estrogens have been used to encourage female sex differentiation (Degani and Kushnirov 1992) and increase growth in freshwater eels to great success (Degani et al. 2003); However, estrogens distributed into the aquatic environment are known to cause health concerns for freshwater organisms (Folmar et al. 1996). As such, it is crucial to control estrogen release into the environment from aquaculture settings.

Advanced oxidation processes (AOPs), specifically UV and UV/H_2O_2, have been used to remove estrogens from pure water and wastewater matrices. UV AOPs have been largely successful in degrading estrogenic compounds from pure water and wastewater (Cédat et al. 2016) but have not been tested for this purpose in an aquaculture wastewater matrix.

The aim of this study was to determine the efficacy of two AOPs (UV and UV/H_2O_2) at degrading E2 and its metabolites (E1, E3) in both pure water and aquaculture wastewater from American eel production. The results of this study will help to determine the best treatment techniques to be further studied for aquaculture wastewater treatment at pilot-scale.

2 MATERIAL AND METHODS

2.1 Chemicals Reagents

E1, E2, E3, and $^{13}C_6$-Estradiol (internal standard stock solution of estrogen compounds), were purchased from Fisher Scientific (Ontario, Canada) and Cambridge Isotope Laboratories, Inc. (Massachusetts, USA) at a concentration of 1mg/mL in methanol and acetonitrile. Reference working stock solutions were prepared at a concentration of 1mg/L in methanol and stored at -20 °C. Methanol and acetonitrile (LC-MS grade) were purchased from Fisher Scientific (Ontario, Canada). Deionised water was generated by Milli-Q system (Reference A^+, Millipore). 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 10µg/L in ultrapure water and aquaculture effluent water.

2.2 Collection of Aquaculture Effluent Samples

Effluent was collected from a small RAS located at Dalhousie University's Aquatron Facility with a daily system volume of 7323L. Makeup flow was calculated to be approximately 10% of system volume and was maintained at approximately 0.5L/minute. Wastewater samples were collected from the top of a swirl separator effluent outflow and refrigerated at 4°C until use.

2.3 AOP Batch Experiments

A bench scale experiment of UV and UV/H_2O_2 photo-oxidation was carried out as follows: 125mL water samples containing 10µg/L of E2 or E2 and its metabolites were treated using a bench-scale collimated beam unit (PS1-1-120, Cargon Carbon) equipped with a 1kW medium-pressure mercury UV lamp in the UVB + UVC range (200 – 300nm). Samples were exposed to UV fluences of 100, 500, and 1000mJ/cm^2. Hydrogen peroxide (H_2O_2) was dosed from a 3mg/mL solution to achieve 1 or 10 mg/L concentrations and quenched with 2,950units/mg bovine catalase. For the UV/H_2O_2 experiment, 1 or 10mg/L of H_2O_2 was immediately spiked into the water samples as they were exposed to UV irradiation.

2.4 Analytical Methods

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was performed by a combination of high performance liquid chromatography (HPLC) with mass spectrometry (MS). The HPLC system (from Agilent 1260) binary pump was used for all estrone (E1), 17β-estradiol (E2), estriol (E3) and $^{13}C_6$ estradiol (ISTD) detections. An Agilent Poroshell EC C-18 (3.0 X 50 mm, 2.7 µm) column was used and was maintained at 30°C throughout the run. The mobile phase comprising of ultrapure water (A) and the mixture of acetonitrile and methanol, 30%:70%: v/v (B) at 0.6mL/min was used for LC condition run. 80µL of sample was injected through the column. The gradient at 30% organic solvent (B) was initially held and the proportion of organic solvent was programmed to linearly increase to 100% over 10 min. A post-time of 5-min was added to make sure all the analytes were eluted within the run time. An additional 10 minutes of post time was allowed the column to re-equilibrate before next injection. This resulted in a total cycle time of 26 minutes. Mass spectrometry was performed on an Agilent 6460 Triple quadrupole mass spectrometry (QqQ). Source parameter settings were optimized and two transitions: a quantifier (most abundant product) and qualifier for target analyte were used for most of the compounds to increase specificity for method. Data acquisition and analysis was performed using Agilent MassHunter software (Version Rev B.08.00).

3 RESULTS

3.1 The Effect of UV and UV + H2O2 on 10µg/L of E2 Alone

[redacted]Pure Water Matrix

UV fluence had the greatest impact on E2 removal, with a high dose of UV (1000mJ/cm^2) resulting in greatest removal using UV alone and UV + 1mg/L H_2O_2. The addition of H_2O_2 did not seem to markedly improve analyte removal in comparison to UV alone. E2 was more readily removed alone than in a

mixture of its metabolites over all fluences and treatments, with the exception of a high dose of UV in concurrence with UV + 10mg/L H_2O_2 treatment, which showed similar removal of E2 both alone and in a mixture.

[redacted]Aquaculture Wastewater Matrix

UV fluence had the greatest impact on E2 removal in an aquaculture wastewater matrix E2 was removed to below the detection limit (BDL) of our LCMS-MS (1µg/L in aquaculture wastewater) at medium and high UV doses. We achieved consistently better removal of E2 in an aquaculture wastewater matrix in comparison to the pure water matrix. E2 removal was not considerably different alone or in a mixture of metabolites at any treatment or UV dose.

3.2 The Effect of UV and UV + H2O2 on 10µg/L of E1, E2, and E3 in a Mixture

[redacted]Pure Water Matrix

UV fluence had the greatest impact on the removal of E1, E2, and E3 in a solution. E1 was readily removed in every instance, reaching complete degradation to BDL of the LCMS-MS (0.4ug/L in a pure water matrix) after a medium dose of UV over all three treatments. E2 and E3 were removed at a similar rate. The addition of H_2O_2 did not seem to considerably improve the removal of E1, E2, or E3 in comparison to UV alone.

[redacted]Aquaculture Wastewater Matrix

UV fluence had the greatest impact on removal of E1, E2, and E3 in an aquaculture wastewater matrix (Figure 2). All metabolites were removed to BDLs at both medium and high doses, with the exception of E2 treated with a medium dose of UV and 10mg/L H_2O_2, where E2 was removed to just above the detection limit. Analytes were removed considerably better in an aquaculture wastewater matrix in comparison to a pure water matrix

4 DISCUSSION

Our study has shown that AOPs (i.e., UV, and UV/H_2O_2) are effective at degrading E2 and its metabolites (E1, E3) in both pure water and an aquaculture wastewater matrix. UV was more effective at degrading analytes in a wastewater matrix than in a pure water matrix, and the addition of H_2O_2 did not considerably improve analyte removal. In some instances, H_2O_2 addition did result in higher degradation, but not consistently (i.e., a higher dose did not always result in more degradation).

Other studies have discerned that UV radiation alone is not largely successful at degrading estrogens (Li et al. 2016) but is much more effective in combination with H_2O_2 (Rosenfeldt and Linden 2004). We did not find that UV alone, or in tandem with H_2O_2, degraded E2 and its metabolites to BDLs (below detection limits) up to a dose of 1000mJ/cm^2 (with the exception of E1, which was readily degraded to BDL at a dose of 500mJ/cm^2) in pure water.

We did not find that the addition of H_2O_2 improved analyte removal. Theoretically, H_2O_2 should increase removal efficiency of most compounds, as the interaction between H_2O_2 and UV light creates strong oxidant hydroxyl radicals (OH·), which effectively break down chemical bonds. The literature leads us to conduct more research would need to be completed to observe more definitive results.

The results of this research are important both economically and environmentally for the aquaculture industry. These preliminary results indicate that UV is effective at removing these analytes from wastewater.

Acknowledgements

We would like to thank the Nova Scotia Department of Fisheries and Aquaculture Research and Development program for providing the funding for this research, and NovaEel Inc. for providing the wastewater to carry out this work. We would also like to thank the staff and students at the Centre for Water Resource Studies at Dalhousie University for their help and support throughout this project.

References

Cédat, B., de Brauer, C., Métivier, H., Dumont, N. and Tutundjan, R. 2016. Are UV photolysis and UV/H2O2 process efficient to treat estrogens in waters: Chemical and biological assessment at pilot scale. Water research, 100: 357-366.

Colombo, G. and Grandidr, G. 1996. Histological study of the development and sex differentiation of the gonad in the European eel. Journal of Fish Biology, 48(3): 493-512.

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K. and Pedersen, P. B. 2013. Farming different species in RAS in Nordic countries: Current status and future perspectives. Aquacultural engineering, 53: 2-13.

Degani, G. and Kushnirov, D. 1992. Effects of 17β-estradiol and grouping on sex determination of European eels. The Progressive Fish-Culturist, 54(2): 88-91.

Degani, G., Tzchori, I., Yom-Din, S., Goldberg, D. and Jackson, K. 2003. Growth differences and growth hormone expression in male and female European eels [Anguilla anguilla (L.)]. General and comparative endocrinology, 134(1): 88-93.

Folmar, L. C., Denslow, N. D., Rao, V., Chow, M., Crain, D. A., Enblom, J., ... & Guillette Jr, L. J. (1996). Vitellogenin induction and reduced serum testosterone concentrations in feral male carp (Cyprinus carpio) captured near a major metropolitan sewage treatment plant. Environmental Health Perspectives, 104(10): 1096.

Li, M., Xu, B., Liungai, Z., Hu, H. Y., Chen, C., Qiao, J. and Lu, Y. 2016. The removal of estrogenic activity with UV/chlorine technology and identification of novel estrogenic disinfection by-products. Journal of Hazardous Materials, 307: 119-126.

Rosenfeldt, E. J. and Linden, K. G. 2004. Degradation of endocrine disrupting chemicals bisphenol A, ethinyl estradiol, and estradiol during UV photolysis and advanced oxidation processes. Environmental Science & Technology, 38(20): 5476-5483.