The Impact of VigorOx® WWT II Wastewater Disinfection Technology on Endocrine Disruptors

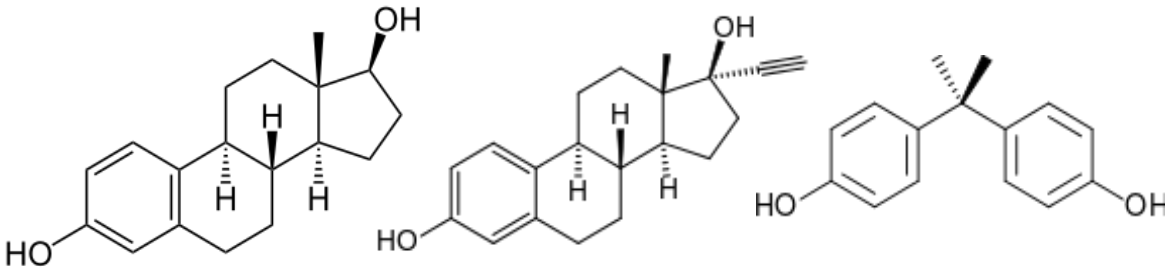
Endocrine disruptors (EDs) are a class of chemicals with the ability to interfere with the endocrine (hormonal) processes in mammals. Any hormonal process in the body can potentially be impacted by endocrine disruptors. As a result, EDs have been linked to many health related issues, such as learning disabilities, brain and cognitive development problems, breast cancer, deformations of limbs, feminizing of males and masculinizing of females, prostate and other cancers. Unlike Toxins, the impact of EDs on health is not typically short term, and long-term effects may be drastic.

EDs are found in many consumer and industrial products and various pharmaceuticals. Disposal of such products may lead to EDs migrating into the groundwater and drinking supplies, or by passing directly through the body, contaminating wastewater. Due to the potential of relatively low concentrations (< 1 ppb) of EDs having an impact on hormonal processes, and the relatively poor efficiency of treatment processes in removing EDs from the water supply, there is significant potential of EDs in wastewater having a negative impact on sensitive receiving bodies, such as wetlands, creeks and rivers.

This *Disinfection Forum* describes preliminary research that shows the potential for VigorOx® WWT II wastewater disinfection technology to deactivate EDs. Thus, application of VigorOx WWT II as a disinfectant for wastewaters may provide the additional benefit of deactivating EDs, reducing the potential impact of wastewaters on sensitive receiving bodies and on human health.

ED Deactivation Bench Test Procedure

The deactivation of EDs by peracetic acid (PAA) was investigated at the bench scale, utilizing stirred jars with spiked distilled (DI) water. Three endocrine disruptors, representing broad categories of EDs, were used as the target compounds for the study. These included: 17-beta-estradiol (**E2**, a human sex hormone and steroid; it is the most referenced compound in research and papers concerning EDs and is the primary female sex hormone), 17-alpha-ethinyl estradiol (**EE2**, a chemical derivative of estradiol; a major ingredient in hormonal contraceptive formulations; due to its prevalence, it substantially contributes to the ED concerns in municipal wastewater) and bisphenol-A (**BPA**, a synthetic chemical that structurally resembles ECs that is commonly found in plastics; it can block endocrine receptors and disrupt an organism's normal endocrine activities).



E2 EE2 BPA

Each jar was filled with one liter of DI water and spiked with concentrations of ED per Table 1.

ED	ED Spike (µg/L)
E2	5
EE2	9
BPA	21

Table 1: ED concentrations used during the bench test.

Each “spike” run was also dosed with VigorOx WWT II at one of three concentrations: 1, 5 and 10 ppm. Samples were pulled for analysis at 10 and 20 minutes of contact time. A blank containing distilled water was run for comparison.

For analysis, ED was extracted from the test solution using solid phase extraction (SPE) at Tulane[1]. Endocrine activity was assessed using a reporter gene assay method[2] and was performed at Xavier University. The purpose of this method was to evaluate the estrogen activity of the compounds before and after PAA treatment. The assay utilizes a T47D[3] derivative that has been stably transfected with the reporter gene. Thus, the T47D ERE luc cell line expresses the endogenous estrogen receptors of T47D and contains an exogenous estrogen responsive reporter gene (luciferase). Therefore, the estrogen specific transcription activity of a chemical is directly related to the luciferase measured in the lysate of treated T47D ERE luc cells. The T47D ERE luc assay procedure presented here is a modified version of published methods[3,4]. All tissue culture materials, such as media and sera, are commercially available.

The T47D ERE luc cells were seeded into 96 well plates and fed media containing treatment compounds. After two days of cultivation, the cell lysates were harvested and evaluated for luciferase activity. The assay monitors estrogen activity, which is quantified using a standard equivalent. The equivalent is a unit for reporting estrogenicity and it is based on the strength and activity of a standard chemical used in the assay. The ED assays applied in this project use estradiol (E2) as the standard chemical, meaning all recorded activity is based off an estradiol standard. This activity can be measured as a percentage of estrogen activity, with 10^{-10} M estradiol as 100%. This method does not measure the actual concentration of ED in the sample, but rather the endocrine disrupting capacity of the sample. As a result, a decrease in signal response corresponds to a decrease in the endocrine disruption of that particular compound compared to the reference estradiol.

ED Deactivation Bench Test Results

Results for the control samples demonstrated that there was neither a positive or negative change in ED activity when dosed with PAA. The PAA did not impact the assessment of ED reactivation at dosages from 1-ppm to 10-ppm, nor did it exhibit activity of its own. For the ED spiked samples, the following results were observed for E2, EE2 and BPA:

PAA Dosage (ppm)	E2 Activity				
	0 minutes	10 minutes		20 minutes	
	Control (ng/L as E2 Equivalent)	Results (ng/L as E2 Equivalent)	Reduction Percentage	Results (ng/L as E2 Equivalent)	Reduction Percentage
1	94.6	11.9	87%	4.2	96%
5	94.6	19.5	79%	14.5	85%
10	94.6	7.9	92%	9.8	90%

Table 2: PAA Impact on E2 Activity at 5 µg/L concentration

PAA Dosage (ppm)	EE2 Activity				
	0 minutes	10 minutes		20 minutes	
	Control (ng/L as E2 Equivalent)	Results (ng/L as E2 Equivalent)	Reduction Percentage	Results (ng/L as E2 Equivalent)	Reduction Percentage
1	89.0	11.1	88%	2.2	98%
5	89.0	19.0	79%	7.3	92%
10	89.0	2.7	97%	18.1	80%

Table 3: PAA Impact on EE2 Activity at 9 µg/L concentration

PAA Dosage (ppm)	Activity				
	0 minutes	10 minutes		20 minutes	
	Control (ng/L as E2 Equivalent)	Results (ng/L as E2 Equivalent)	Reduction Percentage	Results (ng/L as E2 Equivalent)	Reduction Percentage
1	0.72	0.05	94%	0.44	39%
5	0.72	0.57	21%	-0.02	99.9%
10	0.72	0.11	84%	-0.05	99.9%

Table 4: PAA Impact on BPA Activity at 21 µg/L concentration

As can be seen, peracetic acid significantly reduced the endocrine disrupting capabilities of these three EDs. Typically greater than a 90% reduction in endocrine activity was observed after 20 minutes of contact time. There is some scatter in the data for BPA, and this may be due to the fact that these BPA concentrations are near the detection limit of this methodology for BPA.

VigorOx WWT II Deactivation of EDs in an Actual Wastewater Treatment Facility

The effectiveness of VigorOx WWT II in de-activating EDs in actual wastewaters was investigated using the effluent from a wastewater treatment plant (WWTP) located in Louisiana. The outflow from this plant is being considered for discharge in the surrounding wetlands to help restore the wetlands following the impact of Hurricane Katrina. The plant currently uses hypochlorite as its disinfection technology and as a result cannot currently discharge its wastewater into the wetlands. The plant is investigating VigorOx WWT II as an alternative technology to aid in protecting sensitive species and to allow for discharge into the wetlands.

Testing was performed using PeroxyChem's disinfection pilot reactor that was located near the disinfection contact chamber of the WWTP. The primary goal of the addition of PAA is to meet the plant's regulatory bacterial requirement of less than 200 cfu / 100 mL of fecal coliforms. During the pilot testing period, influent fecal counts ranged from 330 to 4950 cfu / 100 mL. A PAA concentration of 2 mg/L was utilized, and was able to exceed the 200 cfu / 100 mL maximum after 7.5 minutes of contact time. 2 – 3 log reductions in total coliform were measured after 19 minutes of contact time.

Background ED activity was measured, as per the extraction procedure (performed at Tulane University) and receptor gene method (performed at Xavier University) as described above, for the secondary effluent wastewater prior to chlorine disinfection and for effluent wastewater that had undergone chorination. The results are shown below (N = number of samples measured).

Sample	**Activity (ng/L as E2 Equivalent)**
Average Background [Secondary Effluent] (Oct-8 to Oct-10)	11.7 (N=6) Range (1 – 40)
Chlorinated Effluent	29.1 (N=2)
Chlorination Effect	149% Increase in ED activity

Table 5: Average Estrogenicity of the Wastewater Before and After Chlorination

For assessment of the PAA impact on the EDs, samples were pulled from the pilot reactor through sampling taps prior to addition of PAA, and at locations representing 15 and 20 minutes of PAA contact time. Six total samples were assessed for ED activity (3 consecutive days on the first week, and 3 consecutive days on the second week). The average estrogenicity of the wastewater following PAA treatment for all samples is shown in Table 6.

Chlorination		PAA Treatment		Comparison of PAA Deactivation to Chlorination
Activity	%	Activity	%	

	Increase		Reduction	
29.1	149	8.2	30	179% reduction in EDC activity when PAA replaces chlorination disinfection

Table 6: Average Estrogenicity of PAA Treated Wastewater Compared to Chlorinated Wastewater

Application of PAA was shown to have an overall 30% reduction in the wastewater endocrine activity; whereas chlorination was shown to increase endocrine activity by 149% compared to the background values. This was achieved with a PAA dose designed to meet the bacterial reduction requirements.

Conclusions

VigorOx WWT II was shown to reduce the estrogenicity of three major endocrine disrupting compounds: E2, EE2 and BPA at both the bench scale and on actual wastewaters. ED activity reduction was achieved in the wastewater at concentrations designed to meet the plant's permit requirement for bacterial control. As a result, the application of PAA to control bacteria may provide the additional benefit of reducing the impact of EDs on the environment. Whereas chlorination potentially serves to increase the ED activity of wastewater, the inactivation of EDs by PAA makes it a suitable alternative disinfection technology for WWTPs with outflows into sensitive receiving bodies.

1. Method developed by R. Reimers, et al, Tulane University. Test performed by R. Reimers and Y. Xu.
2. Test performed by T. Wiese.
3. Pons, M., Gagne, D., Nicolas, J. C. & Mehtali, M. 1990. "A new cellular model of response to estrogens: A bioluminescent test to characterize (anti) estrogen molecules,"*Biotechniques*, 9, pp. 450-9.
4. Gagne, D., Balaguer, P., Demirpence, E. *et al.* 1994. "Stable luciferase transfected cells for studying steroid receptor biological activity," *Journal of Bioluminescence & Chemiluminescence*, 9, pp. 201-9.