# EVALUATION OF DIFFERENT MEDIA IN STATIC BED BIOFILTERS FOR REMOVAL OF ENDOCRINE DISRUPTING COMPOUNDS IN DOMESTIC WASTEWATER

Lance E. Beecher, Sarah A. White, PhD

AUTHORS: Lance E. Beecher, Biosystems Eng / Institute for Environ Toxicology, Clemson University, Clemson, SC 29634-0312
Dr. Sarah A. White, Department of Environmental Horticulture, Clemson University, Clemson, SC 29634-0319

REFERENCE: Proceedings of the 2010 South Carolina Water Resources Conference, held October 13-14, 2010, at the Columbia Metropolitan Convention Center

Abstract. Treating endocrine disrupting compounds (EDC's) in domestic wastewater has become a major concern for the safety of human health and the environment. Human derived EDC's, such as 17β-estradiol, estrone and progesterone, are present in domestic wastewater effluent at levels high enough to jeopardize normal endocrine functions of aquatic organisms leading to decreased fecundity and feminization. For humans, there is limited understanding of chronic exposure but significant research findings demonstrate reproductive disorders in males along with elevated incidences of various forms of cancer. In wastewater treatment facilities, activated sludge along with extended sludge retention time appears to be the key process for EDC degradation. Additionally, adsorption onto specific media has demonstrated some success in physical removal of these compounds. This is especially true for more organic media. Combining these two processes can represent potential removal (as high as 99%) for some compounds; however, different studies show as little as 20% removal for specific compounds. Fixed bed biofilters are an alternative to these two separate components; they combine the process of mechanical (physical straining) and biological filtration into one unit. Initially, the media, in packed bed mode, acts as a strainer and is able to physically capture compounds. As bacteria are introduced into the filter, the media becomes coated with a thin film of bacteria that extract nutrients from the water as it passes through the bed. The introduced bacteria also have the capabilities of degrading or even adsorbing EDC's, further reducing concentration levels in wastewater effluent. This project involves the evaluation of different substrate media for potential removal of natural endocrine disrupting compounds that exist in domestic wastewater. Percent removal will be based on specific surface area of the media, porosity and adsorptive capabilities of the media.

## INTRODUCTION

Contamination of soil and water systems with natural and synthetic steroid hormones and their metabolites has become a major concern because of their endocrine-disrupting activity (Kolpin et al., 2002). Endocrine disrupting compounds (EDCs), are considered to be compounds of interest by both the scientific community and popular media because of their potential to decreased sperm counts, increased occurrence of cancer (testicular, prostate, and breast), and to reproductive disorders in human males (Peterson et al., 2000). Little is known about the adverse effects of these compounds on human health after low level exposure via drinking water or through dietary sources.

Hormones, from humans, are released into the environment on a constant basis. These steroid hormones, which are very stable, are excreted in the endogenous, active form or as conjugates that are easily bio-transformed into the free form (Baronti et al., 2000). Human females excrete about 5 µg/day each of 17β-Estradiol and Estrone (Hoffmann and Evers, 1986). However it has been documented that daily excretion rates can be as high as 10 and 100 µg by cycling woman, depending on the cycle phase (Tyler et al., 1998). It has been calculated that Estrone and Estradiol excreted in human urine is in the order of 4.4 kg/yr/one million inhabitants. This estimation could account for 50% of the observed estrogen in the influents to wastewater treatment plants (Johnson et al. 2000).

Now that the source has been identified, it then must be determined where and how the steroid hormones enter the environment. One main source of environmental exposure to estrogens is wastewater treatment plant effluent. As human population increases wastewater treatment necessarily expands to accommodate increased waste production; however, typical treatment methods do not effectively remove for pharmaceuticals and chemical metabolites that humans often excrete in urine or feces. Elevated concentrations of hormone metabolites and pharmaceuticals are then directly transported to surface waters that downstream wildlife including fish, amphibians, and other mammals are subsequently exposed to. Exposure to Estrone is primarily due to conjugates excreted into wastewater that then enters surface waters. The conjugates can be deconjugated by bacteria in the treatment plants or different aquatic environments and become biologically active again. Several studies have shown that estrogenic chemicals can be detected at high concentrations in fish bile (Legler et al. 2002; Etienne et al. 2005; Koerner et al 2005). Analysis of fish bile identifies elevated cytochrome P450 enzymes believed to be induced as a protective measure against the EDCs. Vitellogenin, a phospholipoprotein associated with egg yolk production, has been found to be produced in the liver hepatocytes under the control of estradiol in oviparous female fish, amphibians, reptiles, and birds (Walker et al. 2001). These effects are believed to lessen the fitness of these populations. Redirection of energy toward producing useless enzymes and elevated levels of inappropriate enzymes will affect the reproductive fitness of a population. Also, a significant number of the fish collected in sites believed to be effected by EDCs are seen to be infested with parasites and cancers. The indirect toxicity of such exposures is the potential for population feminization. Males and females become phenotypically indistinguishable as secondary male characteristics become diminished and energy sources are directed towards non-beneficial processes. This can lead to genetic bottlenecking that selects for individuals able to best handle the contaminant or stressor.

## STRATEGIES

For these reasons mentioned above, strategies must be developed to improve wastewater treatment technology and modify existing facilities to further treat water for these existing compounds before they enter the environment. Traditional wastewater treatment combines simplistic techniques to conquer difficult tasks. Traditional systems employ settling as a means for solids removal and activated sludge for nutrient degradation. These treatment schemes can also help in the elimination of EDCs by the same processes.

Adsorption of the EDCs is accomplished by the interaction of the compounds with settable solids. The target compounds readily absorb to solid surfaces and, because of their moderate hydrophobicity, sorption is the key removal mechanism for these compounds (Lai et al., 2002). Koh et al. (2009) examined adsorption rates in two biological wastewater processes and found that differences in removal was evident in relation to $LogK_{ow}$ values. There are some questions offered to try to distinguish between actual adsorption onto the surface of the media or interception by bacterial films present on the surface of the media. This may result in the type of media used or the chemical makeup of the media. It was also proposed that organic carbon content plays a major role in sorption of the EDC, 17β-estradiol (Jacobson et al., 2005).

Degradation, a biological process, is the primary reduction method for most nutrients contained in domestic wastewater. This process can also account for 70 to 80% removal of EDCs in wastewater. Degradation occurs in activated sludge section of the treatment plant and is mainly an aerobic process; however, the question that arises is if the process is rapid or slow. Kreuzinger et al. (2004) concluded that degradation of all three compounds was achieved at higher SRT's. SRT shorter than 1 day resulted in 16% removal, whereas SRT of 10 and 24 days resulted in 66% and 98% removal, respectively. Some have proposed that increasing sludge age would diversify bacterial groups in the treatment facility, allowing selection for specific organisms targeting EDCs (Langford et al., 2007). Others have hypothesized that it is not bacterial specific but simply native strains found in wastewater facilities foraging for carbon sources (Graham et al., 2003 and Gaulk et al., 2008). These studies have also suggested possible competition between nitrifying and heterotrophic bacteria. However, because of the better results with extended SRT's this is in favor of heterotrophic bacteria because they are associated with slow growth and aged sludge.

## EXPERIMENTAL DESIGN

Initial test were conducted to examine potential pathway for EDC removal. Static leaching tests were performed to calculate diffusion of EDCs from the water phase to adsorption onto the solid media material. Media was exposed to the compounds and water samples were taken to examine potential reduction in concentration levels in the water phase. Once potential adsorption values were determined then degradation part of project was started. A system was designed to evaluate media in a fixed (static) bed type of filtration. The system contained triplicate columns for each media and the system was designed to recirculate water from all columns to sump via small in-line water pump. Columns were constructed of clear PVC pipe and equipped with individual ball valves to control water flow into each column. Screen (500 micron) was placed on influent end to aid in retaining smaller media and supported with porous PVC plate (1.8" holes). Water was distributed equally to all columns, exposed to the media and then collected and returned to the sump. Media consisted of both sinking and floating media types. The sinking media was evaluated for use in constructed wetland systems and its natural ability to potentially adsorb the compounds based on its chemical composition. The floating media was evaluated mainly for bacterial growth and degradation processes. The plastic floating media is mainly used in commercially available filtration units used for water treatment. The system was inoculated with wastewater collected from a local wastewater treatment facility and bacterial mass was allowed to acclimate and attach to the media. Once the media is acclimated, wastewater was spiked to a known level with the EDCs. Water samples were extracted before and after the media beds to examine potential level reductions in EDC concentrations and to monitor background water quality parameters. Testing procedure for the EDCs consists of the Coat-A-Count procedure, which is based on antibody-coated tubes. For example, $^{125}$I-labeled estradiol competes with natural estradiol in the sample. After incubation, separation of bound from free is achieved by decanting. The tube is then counted in a gamma counter, the counts being inversely related to the amount of estradiol present in the sample. The quantity of estradiol in the sample is determined by comparing the counts to a calibration curve. This procedure will also be used for estrone and progesterone.

Once data is collected, results were used to determine efficiency of performance by determining percent removal for each media. In/out concentration levels, along with predetermined surface area dimensions and water flow, will be used to calculate daily conversion rates for each individual media type. The best sinking media will be further investigated in pilot scale constructed wetland systems. Constructed wetlands are very reliable in low cost, high efficiency removal of most nutrients and heavy metals contained in polluted water. They are large enough to have high water retention times which will increase removal rates; however, if space is not available for constructed wetland construction smaller more efficient systems must be developed for removal. Commercially viable filtration units will be evaluated with the best performing floating media.

## REFERENCES:


Baronti, C.; Curini, R.; D'Ascenzo, G.; Di Corcia, A.; Gentili, A.; Samperi, R; 2000. Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in receiving river water. *Environmental Science & Technology.* Vol. 34: 5059-5066.

Etienne, L.; Vermeirssen, M.; Koerner, O.; Shoenenberger, R.; Suter, M.J.F.; Burkhardt-Holm, P. 2005. Characterization of environmental estrogens in river water using a three pronged approach: Active and passive water sampling and analysis of accumulated estrogens in bile of caged fish. *Environmental Science and Technology* 39:8191-8198.

Gaulk, L. S.; Strand, S. E.; Kalhorn, T. F.; Stensel, H. D. 2008. 17β-ethinylestradiol transformation via abiotic nitration in the presence of ammonia oxidizing bacteria. *Environmental. Science and Technology.* 42: 7622–7627.

Graham, D. W. and Curtis, T. P. 2003. Ecological theory and bioremediation. *In Bioremediation: A Critical Review.* Head, I. M., Singleton, I., Milner, M. G., Eds.; Horizon Scientific Press: Norwich, UK. pp 61-80.

Hoffmann, B. and Evers, P. 1986. *In Drug Residues in Animals*, A. G. Rico (Ed.), pp. 111–146, Academic Press, New York.

Jacobson, A.; Lorenzen, A.; Chapmen, R.; Topp, E. 2005. Persistence of testosterone and EE2 in soils receiving swine manure or municipal biosolids. *Journal Environmental Quality.* 34: 861-871.

Johnson, A.C.; Belfroid, U.A.; Di Corcia, A. 2000. Estimating steroid estrogen inputs into activated sludge treatment works and observations on their removal from the effluent. *Science of Total Environment.* 256: 163 173.

Koerner, O.; Vermeirssen, E.L.M.; Burkhardt-Holm, P. 2005. Intersex in feral brown trout from Swiss midland rivers. *Fish Biology* 67:1734-1740.

Koh, Y. K. K.; Chiu, T.Y.; Boobis, A.R.; Scrimshaw, M.D.; Bagnall, J.; Soares, A.; Pollard, S.; Cartmell, E.; Lester, J. 2009 Influence of parameters on the biodegradation of steroid estrogens and nonylphenolic compounds during biological wastewater treatment processes. *Environmental Science Technology.* 43: 6646-6654.

Kreuzinger, N.; Clara, M.; Strenn, B.; Kroiss, H. 2004. Relevance of the sludge retention time (SRT) as design criteria for wastewater treatment plants for the removal of endocrine disruptors and pharmaceuticals from wastewater. *Water Science Technology.* 50: 149–156.

Lai, K. M.; Scrimshaw, M. D.; Lester, J. N. 2002. Prediction of the bioaccumulation factors and body burden of natural and synthetic estrogens in aquatic organisms in the river systems. *Science of Total Environment.* 289 : 159-168.

Langford, K. H.; Scrimshaw, M. D.; Lester, J. N. 2007. The impact of process variables on the removal of

PBDEs and NPEOs during biological sewage treatment. *Archives of Environmental Contaminant Toxicology.* 57: 1–7.

Legler, J.; Jonas, A.; Lahr, J.; Vethaak, A.D.; Brouwer, A.; Murk, A.J. 2002. Biological measurement of estrogenic activity in urine and bile conjugates with the in vitro ER-CALUX reporter gene assay. *Environmental Toxicology and Chemistry.* 21:473-479.

Tyler, C.R.; Jobling, S.; Sumpter, J.P. 1998. Endocrine disruption in wildlife: A critical review of the evidence. *Critical Review of Toxicology.* 28:319-328.

Walker, C.H.; Hopkin, S.P.; Sibly, R.M.; Peakall, D.B. 2001. *Principles of Ecotoxicology, 2nd Edition.* Taylor & Francis, New York, NY.