ASSESSMENT OF DIFFERENT SUBSTRATES FOR REMOVAL OF ENDOCRINE DISRUPTING COMPOUNDS IN WASTEWATER AND THE POTENTIAL EFFECTS ON FOOD CHAIN PATHWAYS

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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 are present in domestic wastewater effluent at levels high enough to jeopardize normal endocrine functions of aquatic organisms leading to decreased fecundity and feminization. In wastewater treatment facilities, biologically active sludge along with extending both hydraulic and sludge retention times appear to be critical processes important for degradation of 17β-estradiol. By extending retention times, 17β-estradiol is able to stay in the system long enough to allow the biological process to prolong contact and maximize effectiveness. An additional biologically active transport approach that might show promise is the use of algae as a facilitator further reducing 17β-estradiol in tertiary wastewater. Historically, algal cultures have been used to polish contaminated water for discharge. Processes include further reducing nitrogen levels, removal of toxic heavy metals and concentrating and potentially transforming organic compounds to nonlethal concentrations. This study investigates using algae for the recovery and recycling of 17β-estradiol, via adsorption, from wastewater and possible transfer of toxicity to higher organisms in the food chain. Specifically, algal cells will be exposed to 17β-estradiol in solution to examine potential adsorption capabilities and also examine potential concentration effects of 17β-estradiol on algal cells. Additionally, contaminated algae will be fed to daphnia to investigate possible transfer of 17β-estradiol and its toxicological effects up the food chain. Survival and reproduction of daphnia will be considered to explain toxic effects.

INTRODUCTION

Organic micro-contaminants in the aquatic environment have been extensively reviewed (Auriol et al., 2006; Falconer et al., 2006; Ghasemi, 2011; Stangroom et al., 2000) however, limitations in awareness and scrutiny on the fate and behavior of endocrine disrupting chemicals (EDCs). Many EDCs are now conveyed as environmental toxins, even at low doses (Jones et al., 2001; Jones et al., 2007; Mills and Chichester, 2005). Contamination of soil and water systems with natural and synthetic steroid hormones and their metabolites has become a major concern for their endocrine-disrupting (ED) activity (Kolpin et al., 2002). These steroid hormones, also classified as EDCs, are considered 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 reproductive disorders in human males (Peterson et al., 2000; Racz & Goel, 2010). Little is known about the adverse effects of these compounds on human health after minimal exposure via drinking water or through dietary sources (Li et al , 2012; Stackelberg et al., 2007; Westerhoff et al, 2005).

Hormones, from humans, are released into the environment on a persistent basis (Shore & Shemesh, 2003). These natural 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; Duong et al., 2011). 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 17β-estradiol and estrone 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 in wastewater treatment plant effluent. Humans produce natural estrogens in the microgram range daily, concentrations of these hormones municipal wastewater is in the range of nanograms per liter. As human population increases wastewater treatment necessarily expands to accommodate increased waste production; however, typical treatment methods do not effectively remove 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 (Brian et al., 2005; Gunnarsson et al., 2009; Vajda et al., 2011). 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 (Almer, et al., 1998; Bjerregaard et al., 2008; Oucard & Eltra, 2006; 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. The indirect toxicity of such exposures is the potential for population feminization. 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 overcome 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.

Sorption of EDCs is accomplished by the interaction of compounds with living micro-organisms and inorganic solids. The target compounds readily sorb to inorganic 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 sorption rates in two biological wastewater processes and found that differences in removal was evident in relation to $LogK_{ow}$ values. There is question in trying to differentiate 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 selection or the chemical configuration of the media utilized in the unit. Conversely, living organisms (bacteria, fungi and algae) can be used and encourage involvement initially in sorption of these compounds then followed up with additional biological processes such as concentration, magnification, transformation and degradation (Lai, 2002). More specifically, algae play an important role in the fate of EDCs in the environment, given their substantial biomass, extensive range of habitat, and diversity (Lai, 2002). Occasionally, algae have been used to polish wastewater contaminated with other organic compounds and heavy metals by means of biosorption (Axelman, 1997). Finally, algae may degrade or take up EDCs, thereby acting as a medium for bioconcentration and later biomagnification in higher trophic levels leading to toxic or reduced fitness (Sijm, 1998).

EXPERIMENTAL DESIGN

Initial tests were conducted to examine potential 17β-estradiol (E2) removal by algal cells. Static sorption tests were performed to calculate diffusion of E2 from water phase onto suspended algal cells via sorption. Two different experiments were conducted: 1) constant E2 concentration with varying algal densities and 2) constant algal density with varying E2 concentrations. The selected algal species used in all experiments was *Pseudokirchneriella subcapitata*. In experiment 1, 50 mL aliquots of *P. subcapitata* were partitioned into Erlenmeyer flasks at densities ranging from 12 million to 60 million cells/mL.

Each density of algae included 5 replicates and a blank. Measured amounts of E2 were added to each flask. Nutrient water used to grow *P. subcapitata* was also examined to establish any possible sorption by the nutrients and minerals contained in the conditioned water. For experiment 2, the identical setup was employed however *P. subcapitata* density (13 million cells/mL) was constant and E2 concentrations ranged from 0 to 10000ng/L. Water samples were extracted at time 0, 2, 4

and 24 hours to examine potential E2 sorption over time. Testing procedure for E2 consists of the Coat-A-Count procedure, which is based on antibody-coated tubes. For example, I^{125}-labeled estradiol competes with natural estradiol in the sample. After incubation, separation of bound E2 from free E2 is achieved by decanting. The tube is then counted using 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 counts to a calibration curve computed internally within the computer program. Also, algal counts were recorded before and after to evaluate possible influence of E2 on algal cell density.

Once data was collected, results were used to determine efficiency of performance by determining percent removal for each density of algae. Algal densities were evaluated to determine potential toxicity of algae to E2. To further evaluate bioconcentration and biomagnification, contaminated algae were introduced to *Daphnia Magna* to examine survival and fecundity.

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