1. Title of Proposal: Biological Treatment of Wastewater Contaminated with Estrogenic Compounds

2. Focus Category: Treatment, Wastewater, Water Quality

3. Keywords: Animal Feeding Operations, Bioremediation, Hormones

4. Duration: March 1, 2010 – February 28, 2011

5. Federal Funds Requested: \$5,000

6. Non-Federal Funds Pledged: \$10,000

7. Principal Investigator (graduate student):

Meghan Anne Gallagher 1st year Master of Science Student Department of Biological and Agricultural Engineering Texas A&M University <u>meghan.gallagher63@gmail.com</u> Cell: (210) 367-3905 325 Scoates Hall 2117 TAMU College Station, TX77843 – 2117

8. Co-Principal Investigator (faculty advisor):

Dr. R. Karthikeyan Assistant Professor Department of Biological and Agricultural Engineering Texas A&M University <u>karthi@tamu.edu</u> Office: (979) 845 - 7951 306 A Scoates Hall 2117 TAMU College Station, TX 77843-2117

9. Congressional District: Texas Congressional District 17

10. Abstract:

Currently there are no surface or groundwater regulations to address the concentrations of natural and synthetic hormones resulting from animal waste treatment processes and domestic wastewater treatment effluents. These compounds are considered as emerging contaminants. Estrogen concentrations as low as 10 ng/L in wastewater have been known to alter the sex of fish (Young and Borch, 2009). The current types of treatment available to treat hormone-

contaminated wastewater include photo-degradation, chemical oxidation, biological, and UV radiation. Biological treatment will be focused on in this research. Surface water and wastewater from various relevant sources will be collected and screened for bacteria that are capable of degrading hormones. Enrichment culture based methods will be used during the initial screening process. Potential bacterial species capable of degrading hormones will be isolated, genomic DNA from the isolates will be extracted, and sequenced. Then various environmental factors that control and govern the biodegradation of hormones will be studied to design biological treatment processes.

11. Statement of Critical Regional Water Problems:

The release of estrogen occurs through human and animal urine and feces. The most likely sources that would have high concentrations are waterbodies that receive wastewater effluents from animal feeding operations and domestic wastewater treatment plants (WWTPs). The different forms of estrogen are those that are naturally produced by human/animals, 17β -estradiol (E2) and estrone (E1), and the synthetically developed for the contraceptive pill, ethinylestradiol (EE2). The estrogen's molecular structure of four linked rings is highly stable and there are not many sites to have simple oxidation. E2 is able to oxidize and degrade easily but is converted to E1 which is more resistant to degradation (Mochet, 2009). The ethinyl group in EE2 causes it to be resistant against oxidation. The stability of EE2 can cause it to stay in the environment of longer, with a half-life of up to 17 days (Mochet, 2009).

Research was done 45 years ago to study the degradation of estrogenic compounds in the environment (Stumm-Zollinger and Fair, 1965). This study concluded that there would not be high enough concentration in the environment to cause a problem (Stumm-Zollinge and Fair, 1965). However, recent studies have shown that in streams that have concentrations as low as 10 ng/L can disrupt the endocrine system of fish, causing feminization of male fish in some cases (Young and Borch, 2009; Yu et al., 2007). Sources for high concentration of estrogens in streams can be resulting from WWTPs discharging into small creeks. This could be a major problem particularly in intermittent streams where there is only base flow or no flow. Base flow and no flow conditions do not allow for any dilution of estrogenic compounds. Most of the rural Texas streams are intermittent and have no flow or base flow during hot and dry summer months. WWTPs can discharge estrogen concentrations as high as 50 ng/L. This could be a potential concern since this is well above the threshold concentration that disrupts the endocrine system in aquatic biota.

Texas Commission for Environmental Quality (TCEQ) which closely follows the Environmental Protection Agency (EPA) guidelines do not currently regulate surface waters as to the levels of estrogenic compounds that are discharged into streams. Current treatment, sorption and

degradation, at WWTPs will lower the concentration but additional treatment is needed to get the concentration to a concentration level that does not have adverse affects. Some options for additional treatment include: ozone oxidation, inverse osmosis, ultrasound, nano-filtration, photo-catalytic degradation, and *in situ* native organisms as a biological treatment. All options except biological treatment are expensive and could have results in toxic byproducts. Biological treatment is a better option because it could be implemented with current treatment processes with relatively low costs in comparison to other treatment options.

12. Nature, Scope, and Objectives of the Research:

The major objective of my research is to design a biological treatment process for treating estrogen contaminated wastewaters and waters using *in situ* organisms. In order to achieve this overall objective, I will isolate an organism or enrich *in situ* microbial consortium that can use estrogen as a primary carbon source. I will screen different wastewater, water, sediment, and sludge for potential estrogen degrading bacteria. Screening sites will include: wastewater treatment plants, streams receiving wastewater, animal feedlots, and animal-waste treatment lagoons. Then, I will characterize the estrogen-degrading bacteria using molecular methods. I will conduct laboratory scale microcosm studies to elucidate biodegradation pathway and kinetics of E2. Based on the results from this research we will be able to provide insights into designing biological treatment process to treat wastewaters and waters contaminated with estrogenic compounds. I personally hope to gain experience in molecular biology techniques to characterize bacteria that degrades estrogen and have a better understanding of how estrogen is degraded in the environment.

13. Results Expected from this Project:

I expect to enrich in situ microbial consortium capable of degrading estrogenic compounds in water and wastewater. This consortium or isolate can be bio-augmented into already existing treatment processes to increase the treatment efficiency. I expect to describe the biodegradation pathway and kinetics of 17β -estradiol (E2). The research results from this study will be beneficial to address the emerging estrogenic contamination problem in Texas and National waters.

References:

Childress, H. (2009). Review of the Presence of Steroid Hormones in the Environment and Available Treatment Options. Unpublished term paper.

Fahrbach, M. (2006). Anaerobic degradation of steroid hormones. Unpublished dissertation.

Mochet, C. (2009). Microbial Degradation of Steroid Hormones in the Environment and Technical Systems. *Swiss Federal Institute of Aquatic Science and Technology*. Unpublished report.

Stumm-Zollinger, E. and G. M. Fair (1965). Biodegradation of Steroid Hormones. *Water Pollution Control Federation*. 37 (11), 1506-1510.

Young, R.B. and T. Borch. (2009). Sources, Presence, Analysis, and Fate of Steroid Sex Hormones in Freshwater Ecosystems – a Review. In *Aquatic Ecosystem Research Trends*, 103-164. G.H. Nairne, ed. Hauppauge, NY: Nova Science Publishers.

Yu, C-H., H. Roh, and K-H. Chu. (2007). 17β-Estradiol-Degrading Bacteria Isolated from Activated Sludge. *Environmental Science & Technology*. 41 (2), 486-492.