**Proposal: Modeling the effect of manganese peroxidase on the biodegradation of tetracycline in wastewater treatment plants in the presence of acetate, an inhibitor**

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Introduction and Background

Across the globe 100,000-200,000 tons of antibiotics are produced and used in a year1. In many places they are used to treat human and animal disease, as well as used to prevent disease and encourage growth in livestock. Of the many antibiotics in use, tetracycline and several of its derivatives are among the most commonly produced and used2. Tetracyclines are broad spectrum antibiotics and are used to treat gram-negative and gram-postive bacteria in both humans and animals2. In addition, they are commonly used in the United states to promote growth of cattle, swine, and poultry4. Besides tetracycline, the other common prescribed naturally occurring tetracyclines are oxytetracycline and chlortetracycline, both of which are approved as growth promoters in cattle in the United States1. Many other forms also exist which were synthetically derived. The structure of the three described compounds can be seen Figure 1.

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| Figure 1: Structure of Tetracycline and two natural other forms of it. Figure adapted from Jeong et al. (2009) |

When found in the environment, antibiotics are usually observed at very low levels in the nanograms per liter to micrograms per liter range, and are considered micropollutants1. They can also be characterized as emerging organic contaminants (EOC) as many of their effects in the environment are still unknown and only recently has their presence in natural systems started to cause concern. Tetracyclines are usually introduced into the environment after being excreted by a human or animal who consumed them. A substantial fraction, 25-90%, of the drug is not taken up by the consumer and so is released through waste4,5. From human consumption this usually leads to a path through a wastewater treatment system. Current wastewater treatment systems are not designed for nor are capable of removing such micropollutants. Some studies have shown the removal rate of antibiotics/tetracycline to be as low as 12% in wastewater treatment plants, which can lead to accumulation in the sludge which can then bring the antibiotic into natural systems when applied to fields2. There is an enormous range of measured concentrations depending on location vary from undetectable to hundreds of ug/L5. In livestock, a similar percentage of the pharmaceutical is released as waste, but it ends up leaching into soils and surface water rather than being run through a treatment system. In soil, tetracycline binds easily due to its high sorption coefficient which can cause levels in manure may be high while levels in adjacent groundwater systems are low6. Tetracyclines are also highly hydrophilic and have low volatility, which also contributes to their persistence in natural systems2.

In the environment, antibiotics have been measured in surface water, ground water, soils, and sediments at varying levels depending on proximity to sources as well as routes of contamination, and tend to be found more often than other pharmaceuticals and personal care products7. Some accumulation in plants has also been seen, which has unknown effects in humans when consumed8.

The major concern surrounding the presence of antibiotics in the environment is that it can lead to an increase in the number of antibiotic resistance genes and organisms, which then have the possibility to be transferred to humans through exposure to contaminated sources, such as through water sources or possibly through ingesting crops which were grown using fertilizer with a high number of resistant genes present when bioaccumulation has occurred8. The possible effects of this are unknown. Some known effects of tetracyclines on environmental systems are disruption of aquatic ecosystems through endocrine disruption through change in the steroidogenic pathways2. The levels of antibiotics necessary for these effects to occur vary, but as tetracyclines are slow to degrade in the environment it is likely that accumulation could occur and then the necessary levels of contamination would start to have an effect. It also remains unknown what risks there might be with chronic exposure to low levels of antibiotics, though levels currently found in groundwater wells are well below the levels necessary to cause acute toxicity in humans9. It is known however than exposure and prevalence of resistance genes are related, as seen in wastewater sludge, which can act as a major point source2. It has also been seen that the presence of tetracycline in soil can reduce the effects of nitrification in soil, which can limit the growth of legumes, while possibly benefitting other plants indicating more information should be collected6.

Current methods of treatment for antibiotics and other organic micropollutants tend to be physical and chemical methods such as membrane contractor processes, activated carbon and adsorption, ultraviolet technology, and ozone technology, among others, which are somewhat effective but not very feasible2,7. Another, yet-to-be-thoroughly-explored technology is biodegradation using enzymes obtained from several species of white rot fungi to assist in advanced oxidation processes (AOP) which can be more effective than just conventional biological processes in wastewater treatment7, 10, 11. In this proposal, the use of the enzyme manganese peroxidase (EC 1.11.1.13) as part of the biodegradation process will be explored in relation to wastewater treatment plants.

Overview and Objectives

The objective of this project is to determine the effectiveness of manganese peroxidase in a system with varying levels of acetate, an inhibitor, in the degradation of tetracycline using a modeling approach with an experiment to determine model parameters. This will also give information on the functioning of manganese peroxidase as a catalyst in systems more similar to wastewater treatment plants than in other experiments.

Several studies have been done to evaluate the effectiveness of manganese peroxidase in the removal of EOC’s in wastewater, but most have focused on an influent containing only the compound of interest, tetracycline or phenolic compounds. Wen et al. (2010) found a removal rate of 72.5% of tetracycline after 4 hours with 40 U/L of enzyme. The other parameters identified in that study were pH, temperature, concentration of MnP, concentration of H­2O2, concentration of Mn2+, and length of time to allow the reaction to occur. The results of this study are shown in Figure 2. This removal percentage is much greater than the removal in conventional activated sludge processes commonly in use. In practice, influent containing only tetracycline and necessary reactive compounds would be unlikely to occur and so determining if enzyme mediated degradation is remotely feasible in more complex systems would be an important next step, and is the step this model aims to address.

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| Figure 2: Results of the study done by Wen et al. (2010) showing the effect of various parameters on the degradation of tetracycline and oxytetracycline |

Model Development and Theoretical Considerations

A simple CSTR model will be used as the base for this model system. The system will be assumed to be at constant temperature and flow rate in addition to having only one enzyme acting in the system. There are two steps in the transformation of tetracycline, the first being the enzyme mediated reaction:

In a natural setting the Mn(III) would then be used to oxidize lignin, but in a treatment system the oxidation would be occurring on other organics such as some dyes or some other polycyclic aromatic hydrocarbons10.

In the model system, this oxidation will occur on tetracycline, the second step in the transformation, making this process a biologically catalyzed advanced oxidation process12. The manganese peroxidase, depending on the organism that synthesized it is inhibited by many different compounds to varying extents. Some of these inhibitors include acetate, ascorbic acid, and even H­2O2 at high concentration, despite its involvement in the reaction. The effect of acetate on this system will be modeled for conditions more similar to a real wastewater treatment plant. Non-competitive inhibition is assumed as acetate is not a possible reactive substrate to the enzyme.

From a mass balance, assuming the non-biological degradation rate is significant, non-competitive inhibition, and none of the substrates for the MnP are in excess:

Where Vmax  is the maximum reaction for the enzyme at the specified condition, b is the non-biological decay rate, KMn2+, K­H2O2, and KH+ (mg/L)are the half-saturation constants for each component in the enzyme mediated reaction, Q (mL/s) is the reactor flow, TCi (mg/L)is the initial concentration of tetracycline, TC (mg/L) is the concentration of tetracycline in the reactor and effluent, A (mg/L) is the concentration of acetate, KA (mg/L) is the half-saturation constant of the acetate, and T (mg TC/mg MnP) is the transformation yield that connects the enzyme activity the amount of tetracycline transformed and is the function of the reactor condition and chemical composition more than biological constraints of the reactor. This term is similar to those in co-metabolism models, as are sometimes used for the biodegradation of tetracycline as the enzyme is not ever reacting with the substrate of interest, only its products are13. Given the experiments done by Wen et al., it seems that Mn2+, H2O2, and H+ are in excess as the reaction was run in a way to determine optimal conditions. If it is assumed that these concentrations are well above the K-values for each the model simplifies to:

From this, only four parameters: b, T, Vmax, and KA will need to be determined.

The non-biologically mediated decay rate for tetracycline varies greatly depending on the environment from a few days to more than hundred days5. In this model that value will be taken as significant though it is also possible that the b term could be low enough that it can be taken as negligible in which case the model would simplify even further to:

In which only three terms would be needed to be determine, and the actual change in concentration of the tetracycline would be only a function of kinetic activity of the enzyme,

Work Plan and Experimental Set-Up

For model validation, an experiment using synthetic wastewater will also be completed. The experiment will include varying influent acetate levels to validate the various kinetic parameters of the model. The HRT will be kept constant, at around 4 hours, based on experiments by Wen et al. (2010) which found a stable removal rate for about 4 hours and longer. Temperature will be maintained at a value of 25° C to better represent real systems, which is a change from many previous studies. The concentrations of Mn2+, H2O2, and tetracycline will be maintained at values of 0.1mM, 0.2mM, and 50 mg/L respectively as based on the work of Wen et al. The acetate concentration will vary from 0 to 20 mg/L given common acetate concentrations in wastewater14. The influent will consist of the synthetic wastewater combined with the tetracycline and acetate. At other influent points, the MnP, Mn2+, and hydrogen peroxide will be added to the reactor. Other studies with manganese peroxidase have shown only negligible difference in effectiveness when crude vs purified enzymes are used, so to simplify the experiment crude enzyme will be used. A schematic of the reactor set-up is shown in Figure 3. To validate the model, regular sampling will be done on the effluent water to determine the amount of effluent tetracycline. These measurements can be used to determine the effectiveness of the system.

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| Figure 3: Reactor schematic for the experiment to determine the model parameters in the degradation of tetracycline. The concentration of Mn2+, H2O2, and amount of enzyme added will be kept constant throughout the experiment. |

Expected Outcomes

Success will be determined if the model results fit the experimental data and the enzyme is found to be effective over the range of acetate concentrations used. These results will indicate that there is more feasibility in using manganese peroxidase to treat tetracycline in wastewater, which will further inform future research in the removal of antibiotics from wastewater.

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