Simulating Microbial Transformation of Pharmaceuticals in Subsurface Wetlands on the Bench-scale

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Introduction
The Living Levee is a constructed subsurface-flow wetland that receives effluent from the Oro Loma Sanitary District located in San Lorenzo, California. It was designed to improve coastal levees by replacing traditional grey infrastructure with natural sloped marshes (Figure 1). This new design aims to reduce costs for maintenance and energy and also provide key ecosystem services that would ultimately benefit the area.

With respect to water quality improvement, the original purpose of the wetland was to encourage nitrate removal through denitrification. However, researchers found the wetland was also able to reduce the concentrations of a number of trace organic contaminants (TrOCs), which are a diverse and expanding array of natural and anthropogenic substances including industrial chemicals, chemicals used in households, compounds and their metabolites excreted by people and by-products formed during wastewater and drinking-water treatment processes [1]. Past studies have suggested that the reduction in concentration of these compounds is mostly due to biotransformation, in which microbial activity results in structural alterations of TrOCs and formation of metabolites. However, the exact mechanism of biotransformation is difficult to study under field conditions. The objective of this study is to design a microcosm that can accurately simulate the conditions and pharmaceutical transformations seen in the wetland, ensuring that the most accurate bench-scale measurements can be made.

Materials and Methods
In order to simulate the conditions of the wetland, soil and porewater samples were taken directly from the field for use in the microcosms. The samples were taken near the inlet of the wetland, where removal of the pharmaceuticals is primarily observed. Approximately four gallons of soil and 1000 mL of porewater was collected. Half of the collected soil and porewater was autoclaved to attempt sterilization. 48, 20mL scintillation vials were filled with approximately 45 g of the untreated soil and another 48 vials were filled with the sterilized soil. Four types of porewater stock solution was created: untreated, untreated with nitrate, sterilized, and sterilized with nitrate. Each stock solution was spiked with a solution containing 13 different pharmaceuticals of interest for a final concentration of 1 ug/L of each compound. The two "nitrate" stock solutions were spiked with a potassium nitrate solution alongside the pharmaceutical spike for a final concentration of 3mg/L of nitrate. 2 mL of each porewater stock was added to the vials in order to make microcosms with the following characteristics: 24
untreated microcosms, 24 untreated + nitrate microcosms, 24 sterilized microcosms, and 24 sterilized + nitrate microcosms.

In order to understand how the concentrations of pharmaceuticals varied over time in the microcosms, water samples were taken from them at 0, 1, 3, 6, 8, 10, 13, and 15 days. The microcosm timeseries was then compared to wetland porewater samples collected along the flow-path. Once all samples were collected, pharmaceutical analysis was conducted using LC-MS [2]. In this study, the pharmaceuticals Acyclovir and Carboxy-Acyclovir were focused on due to their parent/metabolite compound relationship.

Results
The results show that for all four types of microcosms tested, a decreasing trend in C/C₀ occurred, as seen in Figures 2 and 3. This implies that either no biotransformation occurred in the microcosms or biotransformation occurred in all of the microcosms. Both of these implications lead to the idea that sterilization of soil and porewater via autoclave and the addition of nitrate spikes had no effect on the performance of transformation in the microcosms. For this reason, only data for untreated microcosms was analyzed further to determine if soil sorption or biotransformation was the main cause of the decreasing trend observed.

*Figure 2 (left) and Figure 3 (right). Graphs of Acyclovir and Carboxy-Acyclovir C/C₀ vs. time. The four different markers represent the four different types of microcosm tested.*

Figures 4 and 5 show graphs to help to confirm whether biological activity or sorption to soil is occurring; the red lines in each graph refer to the concentration of acyclovir and carboxy-acyclovir after the compounds have reached physical equilibrium with soil. If any data points fall below these lines, then that is indicative of biological activity. Only the data points for Carboxy-Acyclovir fell below the line, however this trend could be explained via kinetics. The initial concentration of Carboxy-Acyclovir is much higher than that of Acyclovir, and this high concentration can be a driving force for transformation.
Figure 4 (left) and Figure 5 (right). Graphs of Acyclovir and Carboxy-Acyclovir C vs. time for untreated microcosms.

Lastly, data collected from the microcosms was compared with the data from the wetland. Figures 6 and 7 show the $C/C_0$ vs hydraulic residence time (HRT) from the wetland and the $C/C_0$ vs days spent in the untreated microcosms. Although decreasing trends are seen in both graphs, the $C/C_0$ values do not match up with each other. From this finding, it can be concluded that the microcosms that were created were not such a good representation of the processes that actually occur in the wetlands.

![Figure 6](image1.png) ![Figure 7](image2.png)

Figure 6 (left) and Figure 7 (right). Graphs of the Wetland data and the Untreated microcosm data side-by-side comparison.

**Works Cited**
