Analyzing Microbial Populations in Woodchip Bioreactors

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Introduction: Spadra Basin is the main source of drinking water for Cal Poly Pomona. Unfortunately, because of the high levels of nitrate NO3-, the water cannot be treated at full capacity. The levels of NO3- are the result of an extensive agricultural history in the area by which NO3- is leached into the ground water from fertilizer use. Consumption of excess NO3- is especially hazardous to infants in the fact that it inhibits the ability of blood to transport oxygen, resulting in Blue-Baby Syndrome or death. In order to comply with health standards, water from the basin is blended with imported water from the State Water Project and the Colorado River. EPA and California standards dictate that the level of NO3- should not exceed 45 mg/L (measured as NNO3-). However, the method of blending groundwater with imported water proves to be costly and non sustainable. As such, the use of natural treatment systems, like woodchip bioreactors, have been studied because of its effectiveness to remove NO3-, through denitrification. Denitrification is a naturally occurring process performed by microorganisms under anaerobic, NO3-, and carbon rich conditions, through which NO3- is reduced to nitrite and released into the atmosphere. Several natural produced enzymes, reductases, are used to facilitate the process. While the main aim is to reduce NO3- to nitrogen gas, the process may conclude at one of its intermediate phases depending on the conditions of the environment. Sulfate, which is also present in the groundwater, inhibits the reduction of nitric oxide to nitrous oxide. Additionally, carbon-limitation and the presence of oxygen will also inhibit the denitrification process. The process is shown below:

\[ \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{N}_2 \]

Phase I: Bioreactor Analysis

The bioreactor under anaerobic conditions, with nitrate and carbon rich conditions facilitate the denitrification process. The influent groundwater was pumped through the bioreactor from the bottom to the top to ensures a long enough contact time between the influent and the media. The influent flow was out of the system, collected and analyzed. Figure 1 shows the general bioreactor setup where figure 2 shows the bench scale system.

Methods and Materials

For this project, three (3), 1.0 Liter bioreactor columns were set up (1a) with woodchips as the media and one (1) with a mix of sawdust and woodchips as media. The use of sawdust provides an additional source of carbon for the microbial population in the columns. The porosity of each column was found prior to the start of the project. Influent groundwater was collected and replenished daily. Groundwater was run through each system at an average flow rate of 1.0 ml/min. Two pumps, with dual channels were used. For the first seven (7) days, DI water was pumped through the columns to allow the microbial population to colonize the media. For this project, three (3), 1.0 Liter bioreactor columns were set up: two (2) with woodchips as the media and one (1) with a mix of woodchips and sawdust as media. The use of sawdust provides an additional source of carbon for the microbial population in the columns. The porosity of each column was found prior to the start of the project. Influent groundwater was collected and replenished daily. Groundwater was run through each system at an average flow rate of 1.0 ml/min. Two pumps, with dual channels were used. For the first seven (7) days, DI water was pumped through the columns to allow the microbial population to colonize the media.

Results: Phase I

All three columns displayed evidence of carbon limitation in the groundwater. Prior to the addition of succinate, systems 1a and 2a achieved an average removal of 23% of influent nitrate (see figure 5). The system with woodchips and sawdust removed an average of 33% (see figure 6). In addition to succinate, systems 1a and 2a showed significant nitrate removal (see figure 7). The woodchips system had an average removal of 86% and the woodchips and sawdust system an average of 93% (see figures 5 and 6). Figure 7 displays sample results from the IC analysis.

Figure 5: The average of the woodchips results. Nitrate Removed (%) and Flow Rate (mL/min) vs Time (Days); The flow rate was maintained at a constant 1.0 mL/min throughout the project. Days 8-24 (without succinate indicated by the yellow square) show a nitrate removal of about 38%; Days 25-41 show (with succinate indicated by the green square) show a nitrate removal of about 93%. Days 25-41 show both systems impacted by a purple square show a nitrate removal of about 96%

Figure 6: Woodchips and sawdust system results. Nitrate Removed (%) and Flow Rate (mL/min) vs Time (Days); The average flow rate of the systems was maintained at a constant 1.0 mL/min. Figure 6 (without succinate indicated by a yellow square) shows a nitrate removal of about 38%; Days 25-41 show both systems impacted by a purple square show a nitrate removal of about 96%

Results: Phase II

Phase II consisted of analyzing the microbial population in the bioreactor to determine:
• the presence of bacteria in the system;
• the type of bacteria present; and
• the response of the bacterial community to succinate addition.

A PCR analysis using specific primers will be used to amplify DNA of the bacteria present in the reactor, while pyrosequencing will confirm the type of bacteria present.

Method and Materials

Woodchip samples were removed during each portion of the bioreactor phases (without succinate and with succinate). DNA was extracted through a bead beating method. An electrophoresis gel (see figure 8) was made using agarose and 1x TAE and injected with the samples in the following order: DNA Ladder (1kb ladder); (2) Woodchip system without succinate; (3a) Woodchip system with succinate; (2) Woodchip-sawdust system without succinate; (2a) Woodchip-sawdust system with succinate; (3) Redundant woodchip system without succinate; and (3a) redundant woodchip system with succinate.

Results: Phase II

Figure 9 shows the results of the electrophoresis gel. The smears confirm the presence of DNA within each of the three columns. Each of the DNA smears are roughly 1kb in size.

A PCR analysis will need to be performed to determine the type of denitrifying present.

Conclusions and Future Work

Based on the results of the column analysis, it is evident that the groundwater extracted from Spadra Basin is carbon limited. Low levels of carbon inhibit the denitrification process because the microbial population lacks the energy source required to reduce nitrate to nitrogen gas.

Despite this fact, a denitrifying microbial community was able to establish and adequately reduce nitrate with the addition of added sources of carbon.

For future studies, a PCR analysis will be conducted to determine the type of denitrifying bacteria present as well as study the effects of sulfate to the bioreactors.

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Purpose:

This study conducted the analysis of the bioreactor and its limitations for nitrate removal, as well as the investigation of the microbial population extracted from the woodchips used as bio media.