Analyzing Microbial Populations in Woodchip Bioreactors

Author: Samayyah Williams Mentor: Dr. Monica Palomo

Introduction: Spadra Basin is the main source of drinking water for Cal Poly Pomona. Unfortunately , because of the high levels of nitrate NO_3^-, the water cannot be extracted at full capacity. The levels of NO_3^- are the result of an extensive agricultural history in the area by which NO_3^- leached into the ground water from fertilizer use. Consumption of excess NO_3^- 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 NO^-_3 should not exceed 45 mg/L (measured as NO_3^-). 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 woodchips bioreactors, have been studied because of its effectiveness to remove NO_3^- through denitrification. Denitrification is a naturally occurring process performed by microorganisms under anaerobic, NO^-_3 and carbon rich conditions, through which NO_3^- is reduced to nitrogen gas and released into the atmosphere. Several naturally produced enzymes, reductases, are used to facilitate the process. While the main goal is to reduce NO_3^- 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:

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.

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 ensure a long enough contact time between the influent and the media. The effluent water flowed out of the system, collected and analyzed. Figure 1 shows the general bioreactor setup while figure 2 show the bench scale system.

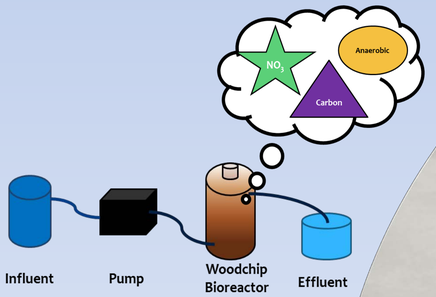


Figure 1: General Bioreactor set-up

Figure 2: Bioreactor set-up for the project. The columns (from right to left): 1-Woodchips; 2-Woodchips and Sawdust; and 3-Woodchips replicate

Methods and Materials

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 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 system to establish a constant flow rate through the columns. From day 8 to 24, groundwater was pumped through the system. From day 25 to 41, a 5 mM concentration of disodium succinate ($C_4H_4Na_2O_4$) was injected into each column through rubber ports to determine if the groundwater was carbon limited (figure 3). An Ion Chromatograph (IC) was used to analyze the nitrate, nitrite, and sulfate levels in the influent and effluent on a daily basis (figure 4). Influent nitrate levels ranged from 34 mg/L to 95 mg/L (as NO_3^-).

Figure 3: Injection of succinate through the use of rubber ports positioned 1inch apart along the length of the column

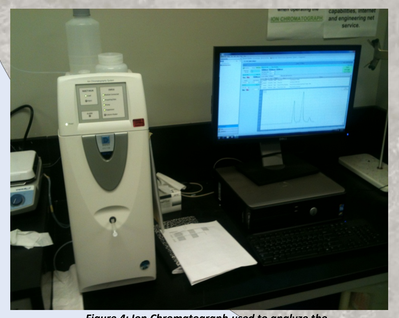


Figure 4: Ion Chromatograph used to analyze the concentration of nitrate in the influent and effluent.

Results: Phase I

All three columns displayed evidence of carbon limitation in the groundwater. Prior to the addition of succinate, systems with woodchips only, showed an average removal of 23 % of influent nitrate (see figure 5). The system with woodchips and sawdust removed an average of 39% (see figure 6). The addition of sawdust helped to confirm that the system was carbon limited. With the addition of succinate, nitrate removal increased significantly for in both systems, with woodchips and sawdust system showing the most dramatic increase. The woodchip 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.

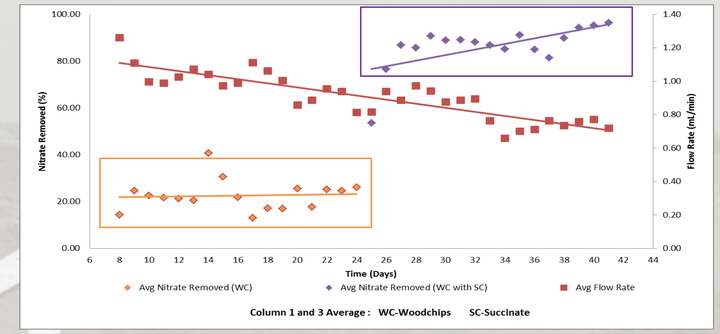
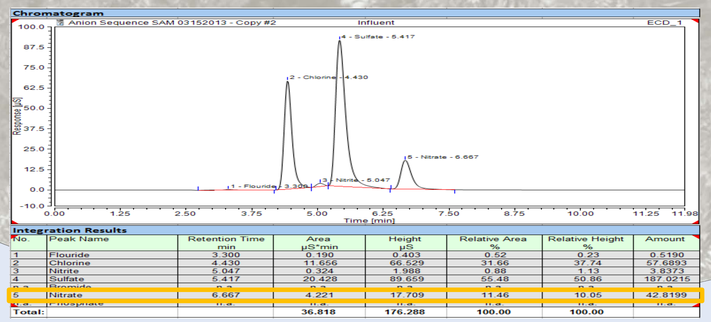


Figure 5: The average of the woodchip system results. Nitrate Removed (%) and Flow Rate (mL/min) vs Time (Days); The average flow rate of the two systems was maintained at a constant 1.0 mL/min. Days 8-24 (without succinate indicated by an orange square) show a nitrate removal of percent 23%; Days 25-41 show (with succinate indicated by a purple square) show a nitrate removal of 86%.

Figure 6: Woodchips and sawdust system 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 percent 38%; Days 25-41 show (with succinate indicated by the green square) show a nitrate removal of 93%.



Integration Results

No.	Peak Name	Retention Time min	Area µS*min	Height µS	Relative Area %	Relative Height %	Amount
1	Flouride	3.300	0.190	0.403	0.52	0.23	0.5190
2	Chlorine	4.430	11.656	66.529	31.66	37.74	57.6893
3	Nitrite	5.047	0.324	1.988	0.88	1.13	3.8373
4	Sulfate	5.417	20.428	89.659	55.48	50.86	187.0215
5	Nitrate	6.667	4.221	17.709	11.46	10.05	42.8199
Total:			36.818	176.288	100.00	100.00	

Figure 7:Sample Ion Chromatograph from March 15 2013 influent reading. The X Axis indicated the run time for analysis (12 minutes) and the Y Axis indicates the conductive response of each anion. The gold box indicates the influent results of nitrate, a concentration of 42.82 mg/L.

Results indicate that the groundwater has both excessively high levels of nitrate and low levels of carbon. The woodchip-sawdust system shows that the microbial population utilized that additional source of degradable carbon from the sawdust to increase nitrate removal. Further, the results show a significant increase in nitrate removal when a more readily source of carbon, the succinate, was added to all of the systems.

In addtion, sulfate levels in the influent and effluent remained at high averaging at 277 mg/L in the influent and 293 mg/L in the effluent, but showed no significant increase or decrease when run through the systems. Sulfate is an inhibitor in the denitrification process, specifically in the reduction of nitric oxide (NO) to nitrous oxide (N_2O), and will need to be removed for more effective denitrification. Future studies will analyze the effect sulfate has on the bench system.

Phase II: Microbial Analysis

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;
- 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 pyro-sequencing 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 agrose and 1x TAE and injected with the sample in the following order: DNA Ladder (1kb ladder); (1) Woodchip system no succinate; (1a) 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.

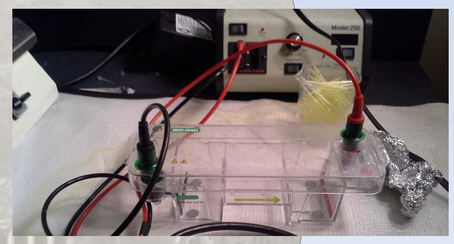


Figure 8 shows an electrophoresis set up used for the DNA gel.

Results: Phase II

Figure 9 confirms 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.

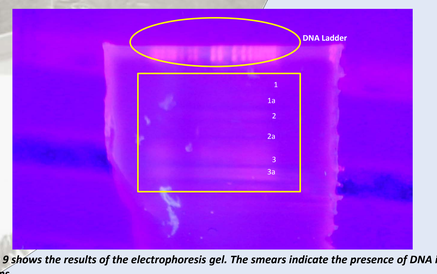


Figure 9 shows the results of the electrophoresis gel. The smears indicate the presence of DNA in each of the columns.

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.

Acknowledgements

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