Effect of carbon supplementation on denitrification capabilities of bacterial communities in woodchip bioreactors

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Introduction

Agriculture is a driving force in California's economy, however the runoff from excess fertilizer use has led to environmentally disrupting consequences. 600,000 tons of fertilizer is used in California annually (Cheng and Thesing 2017). Monterey County ranks as the county with the fourth highest agricultural sales in the nation; the heavy use of fertilizer in the region has led to increased concentrations of nitrate (NO_3-) in water sources through agricultural runoff (USDA 2018). Environmental concerns revolving agricultural runoff have increased with higher frequencies of events such as toxic algal blooms. Algal blooms secrete poison into the water and deplete oxygen rendering the area unhealthy for marine life (Elinson 2016). Human health is also affected by this runoff. In a 2012 and 2013 United States Geological survey, 31% of domestic wells were reported as having nitrate concentrations above standard EPA limits (Graebner 2015). Exposure to high doses of nitrate may lead to methemoglobinemia, also known as blue baby syndrome in infants, which reduces oxygen in blood, and in some cases can result in death (Avery A, L'Hirondel J. 2003). Regardless of the negative consequences to the environment and human health, nitrate runoff in California's agricultural counties will continue to increase as agricultural production expands to meet the needs of a growing population.

Wetlands are a natural remedy for nutrient runoff, but are not the most efficient option. Wetlands possess bacteria and vegetation capable of uptaking any excess nutrients such as nitrate from the water. Incorporating a treatment wetland into an area with runoff however, requires land area and frequent maintenance which leads to expensive costs, and a loss of productive land (Vymazal 2006). One wetland situated on Molera Slough in Marina showed promise, but was limited by varying temperatures and lack of carbon, which led to further maintenance costs (Miller 2013). Woodchip bioreactors have been seen as a cheap, viable substitute for wetlands.

Purpose

Woodchip bioreactors are capable of higher levels of nitrate reduction through less acreage, less maintenance, and at shorter denitrification rates. Bioreactors house bacteria gathered from treatment wetlands in order to reduce nitrate in water. There is deficient research surrounding the limiting factors placed by carbon, temperature, and substrates on the denitrification in a woodchip bioreactor. Temperature in a bioreactor can be maintained within the required mesophilic range through a greenhouse setup, trapping in heat. Carbon is needed for the metabolic processes of the bacteria when breaking down nitrate. A substrate is needed for the bacteria to grow on. In a standard bioreactor, wood chips are used to provide both a carbon source and a substrate (Addy et al. 2016). A carbon supplement in the system other than wood chips may allow for significantly greater nitrate reduction than in systems with no carbon supplement. A supplement high in carbon that is cheap and easily accessible is corn starch. While wood chips supply carbon for the bacteria in the form of cellulose, a supplement such as corn starch could increase in the rate of denitrification of the bacteria. If a supplement proves to be beneficial, owners of bioreactors can increase the efficiency of their system with a simple implementation of corn starch, further reducing nitrate runoff levels. Landowners would save space by using a bioreactor as opposed to a less beneficial wetland, and the local environment would see greater reduction in excess nitrates.

Methods

Bioreactors used in these experiments consisted of an outer hoop house structure, with the inside containing a 550 gallon influent tank that pumped water through multiple channels draining out towards a 550 gallon effluent tank outside the bioreactor (Figure 1, Figure 2).

Significance of corn starch in the system was tested using two bioreactors and two methods: Single Pass and Recirculation. A single pass experiment involved dosing the water in the influent tank of a bioreactor to a known concentration of nitrate, then allowing the water to flow through two channels, one with woodchips and one without. The bioreactor acting as the experimental would be dosed with an excess 6:1 carbon to nitrate ratio of corn starch along with the initial nitrate dosage. Samples were collected at the channel ends from both bioreactors after one hydraulic residence time (HRT), meaning the time it took for a bolus of water to reach one end of the channel to the other. Samples were filtered through a vacuum using 0.45 µm pore size membrane filters, then tested for nitrate using a HACH D900 high range nitrate test. Recirculatory tests were conducted by having a tank recirculate with the channels in the bioreactor for a period of four days. Dosing and testing procedures followed suit with the single pass experiments, however samples were collected every 24 hours from the tank itself rather than sampling every HRT from the end of a channel.

Temperature for both experiment types was recorded through multiple NeuLog sensors, each tracking the temperature of the channel water, hoop house air temperature, and outside ambient temperature.

Nitrate readings collected were analyzed using R software, where multiple t-tests were conducted to see if carbon supplementation proved to give significantly different results.

Results

Following a t-test comparison of the nitrate reductions in the experimental bioreactor with corn starch and the control bioreactor with no corn starch, the test showed a significant difference with a p-value of 0.007. The difference in nitrate reduction between channels with woodchips and no woodchips were not significantly different. The comparison of the channels in the experimental bioreactor with corn starch gave a p-value of 0.961, whereas the comparison of the channels in the control bioreactor gave a p-value of 0.410.

Temperature was mitigated as a limiting factor throughout the experiments. The insulated structure and tarp cover for the channels helped maintain temperatures within the mesophilic range of 20 - 40 degrees Celsius. The average temperature remained around 22 degrees Celsius throughout the experimental summer months of June and July.

Discussion

The experimental bioreactor performed as expected due to the carbon supplement. The results suggest that the carbon supplement allows the microbial communities to break down nitrate at a faster rate than without, as seen in the control. There were periods of fluctuation in the results; times where nitrate levels increased . This could be attributed to any uneven flow through the woodchip channels. It is important that the concentration of water remain uniform as it travels through the system. Modification of the channel structure could possibly mitigate this effect. Nitrate reduction appeared to be greater during the first half of each experiment with corn starch. One possibility could be that the corn starch fell out of solution during the experiments and settled to the bottom of the channels. This could potentially lead to increased costs of having to keep the water in channels homogenous through the use of a mixing application. The methods involving corn starch dosage will need to be rectified in order to resolve this issue.

A t.test comparison between the experimental and control results from the recirculation was yet to be conducted. The results of this could be used to further support the significance of a carbon supplement for nitrate reduction. While the woodchips statistically did not show significantly different nitrate reduction , the benefits are noticeable compared to no woodchips.

Conclusion

From the data currently gathered, a carbon supplement provides a significantly greater nitrate reduction in woodchip bioreactors. As the experiments stand, further replication and data to ensure consistency among results is essential to provide stronger credibility of the results. If the data can validate the significance of using corn starch, those using a woodchip bioreactor will be able to confidently apply the supplement for greater efficiency.

References

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Appendix

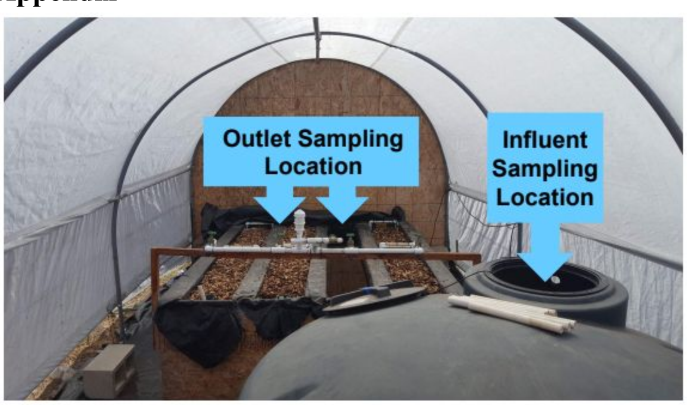

Figure 1. The inside of a bioreactor. Note the hoop house structure which helps maintain heat.

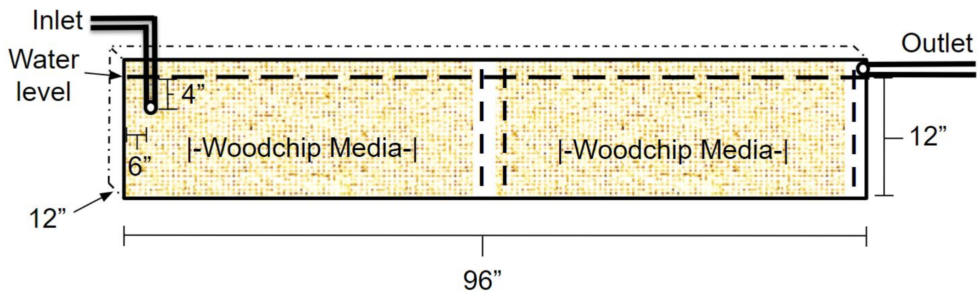

Figure 2. Cross section of a bioreactor channel with woodchips

Table 1. Nitrate reduction percentage between experimental and control bioreactors along three separate one hour HRTs gathered from one experiment..

	Experimental (NO3- Reduction)	Control (NO3- Reduction)
HRT 1	28%	-3%
HRT 2	52%	-16%
HRT 3	8%	-32%
AVG	29%	-17%
ST DEV	0.22	0.15

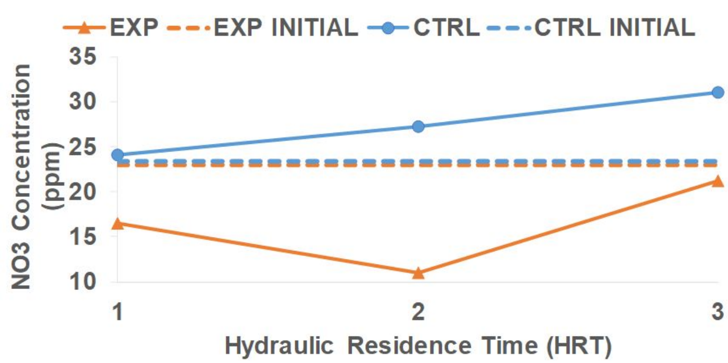

Figure 3. Nitrate concentrations of experimental and control bioreactors as samples were collected at different HRTs from one experiment run.

Table 3. Nitrate reduction percentage between experimental and control bioreactors gathered over a recirculatory experiment every 24 hours.

	Experimental			Control		
	Initial	Sample	% Reduction	Initial	Sample	% Reduction
24 HR	44.6	40.9	8%	35.4	38.9	-10%
48 HR	44.6	28.7	36%	35.4	30.4	14%
72 HR	44.6	37.3	16%	35.4	33.1	6%
96 HR	44.6	29.7	33%	35.4	32.1	9%