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Wood Chips as a Carbon Source for Denitrification in Household Slow Sand Filters

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The objective of this study was to evaluate the suitability of using woodchips as a carbon source for denitrification in intermittently operated household slow sand filters. This was accomplished through a series of batch and column experiments where fishpond bacteria were used to inoculate the reactors, and the resulting nitrate and nitrite concentrations observed over 24 h. Results from batch experiments in 100 mL serum bottles loaded with 0.5, 3 and 5 g of woodchip material, and 200 mg/L nitrate feed achieved 78%, 100% and 90% nitrate removal respectively. Nitrite concentrations in the batch reactors were 55, 0 and 98 mg/L after 24 h. A second set of batch experiments where 3 g of column sand was used as an inoculum showed a drop in nitrate from 200 mg/L to 182 mg/L. Three columns were constructed and fed intermittently with 3 L of nitrate contaminated water daily. The filter columns consisted of a woodchip only column, a sand only column and a mixed sand and woodchip column. After allowing them to stand for 24 h after being inoculated with denitrifying bacteria, the three columns were able to achieve complete denitrification within the 24 h filter run for a 50 mg/L nitrate feed. Nitrite production peaked at 2.4mg/L during this filter run. For a 200 mg/L nitrate feed, complete denitrification was achieved in the woodchip only column, effluent from the mixed and sand columns measured at 45 and 156 mg/L of nitrate, and 8 and 4 mg/L of nitrite respectively. A heterotrophic plate count gave counts of $9x10^3$, $4.8x10^5$, $9.9x10^4$ and $3.3x10^5$ cfu/mL for the feed, woodchip, sand, and mixed effluent filter streams at 200 mg/L nitrate feed.

1. Introduction

Nitrates, because of their high solubility and difficulty to fix to the soil, are among the most widespread groundwater contaminants globally. When consumed, nitrates are converted to nitrites by intestinal bacteria which then can directly interfere with the oxygenation of blood and lead directly to oxygen deficiencies in various regions of the body. Infants are more likely to suffer from this condition than adults (Majumdar, 2003). While the threat to human health has always been the primary concern with nitrates, more regions are recognizing the threat also posed to aquatic environments through eutrophication (Gutiérrez et al., 2018).

To curb these threats, many countries have set regulatory standards that limit the concentration of nitrates in their drinking water supply. In the second edition of their global overview of national regulations and standards for drinking-water quality, 124 of the 125 countries and territories that were audited by the World Health Organization had a regulatory value set for their drinking water quality. And of these nations, 71 had a value set that was equal to or higher than the WHO limit of 50 mg/L as NO_3^- (Organization, 2021). In the natural environment, nitrates occur as a result of distinct non-point sources, including atmospheric sources, wildlife sources, as well as the degradation of soil organic matter, with different regions showing different background nitrate levels, based on the particular elements at play in that environment (Menció et al., 2016). While natural phenomena do contribute significantly towards the nitrate pollution in water bodies, anthropogenic activities have been flagged as the greatest contributors of nitrates. These sources include leachates from land fill sites, manure in animal feedlots, farmlands where nitrogen rich fertilizer is used, as well as direct discharge of poorly treated domestic and industrial wastewaters (Abascal et al., 2022). These instances occur worldwide and include examples such as in China where 7.83% of rivers exceed the 45 mg/L limit, with some even exceeding

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90 mg/L because of domestic waste and industrial water (Zhang et al., 2020), as well as seepage from septic tanks causing elevated nitrate levels in over 30% of wells in a study in Cote d'Ivoire (Eblin et al., 2019).
Globally around 2.5 billion people worldwide rely on groundwater for their drinking water needs (Grönwall and Danert, 2020), and this figure will only grow as the world population grows and climate change effects unfold. It is therefore vital that nitrate water contamination is limited in ensure a safe water supply for users. While some countries including the European countries of Sweden and Norway had imposed a tax on and later directly regulated the use of nitrogen rich fertilizers to limit pollution (Meyer-Aurich et al., 2020), prevention alone simply cannot suffice with many water bodies being contaminated and treatment of the supply necessary before the water is used. Some treatment approaches that can be employed include chemical precipitation, distillation, ion exchange, reverse osmosis and biological denitrification (Dahab, 1991). While each of these has a proven record in treating contaminated water, access to some of these technologies is still a major challenge for the substantial amount of people globally living without access to safe drinking water in many rural, remote, or developing regions. For these groups point of use filters are the most viable with slow sand filters and in particular household filters finding the most widespread application (Sobsey et al., 2008). These however are by default not optimized for nitrate removal but instead for pathogen removal. Research however has been performed into optimizing the slow sand filtration process for nitrate removal. For heterotrophic denitrification, a carbon source is necessary to act as an electron donor during the process. Examples of this research include work done by (Aslan and Cakici, 2007) as well as (Khanitchaidecha, 2010) where acetic acid, acetate, ethanol and hydrolyzed rice where used as carbon sources to facilitate the heterotrophic denitrification.
These choices of carbon sources however are not the most easily accessible in remote, rural, or emerging communities and in some instances in more developed communities for wastewater treatment. Additionally, because of their liquid state, some of them require a significant amount of work for dosing for the denitrification process. The emphasis then falls of finding carbon sources that are not toxic, readily and cheaply available, easy to dose, and can facilitate complete denitrification (Ahmed, 2022). Some work has already been performed at exploring other alternative sources out there including the work done by (Aslan and Türkman, 2004). In this study denitrification was studied using various natural organic substances in batch units, and then used in an up-flow laboratory reactor. The carbon sources explored included s poplar, hornbeam, pine shavings and wheat straw, with the wheat straw proving the most effective for facilitating denitrification.
In a similar fashion, this work aimed to explore the viability of using woodchips as a carbon source for denitrification in slow sand filters. Woodchips as a carbon source for denitrification already have a proven track record in recirculating aquaculture systems (Lindholm-Lehto et al., 2020) as well as in woodchip denitrification bio reactors for subsurface agricultural drainage water (Hoover et al., 2016).
In the work, intermittently fed household scale slow sand filters were built, loaded with woodchip and sand material, and fed with a synthetic wastewater stream. The denitrification rates, and contaminant profiles of nitrate and nitrite along the column lengths were evaluated in depth. Additionally, batch reactors were also setup and operated to evaluate how the ratios between the carbon and nitrogen loadings in the reactors affect denitrification rates. The cell count of the column reactor input and output was evaluated to see how time within the reactor affects the water microbiology.

2. Materials and method

2.1 Chemicals and reagents

Sodium Nitrate ($NaNO_3$) purchased from Glassworld South Africa, was used to create a synthetic nitrate feed for the column work. This was achieved by weighing out 1.37 g of dried sodium nitrate and dissolving it in deionized water to make a 1000 ppm solution. By diluting the stock solution with varying volumes of deionized water, working concentrations were obtained for the experimental work. Saline solution was prepared by dissolving 8.5 g of Sodium Chloride (NaCl) (Glassworld, South Africa) in 1 L of deionized water and autoclaved at 121 ˚C for 15 min. The saline solution was used to wash and harvest bacteria cultures. Woodchips for the denitrification work were sourced from Plant Land Garden Centre in South Africa. The woodchip mix consists of a mixture of locally sourced softwoods that were remnants of a woodwork shop. The packed woodchips ranged from 5 cm and below in diameter and had a packed porosity of 0.60. Washed sand purchased from EDCO Trading CC in South Africa was used for filling the filter columns. Washed sand is preferred for sand filters as large solid particles in the sand can clog the filter causing a significant reduction in filter efficiency (Mahlangu et al., 2011).

2.2 Bacteria harvesting

Inoculating bacteria for the studies was collected from sediment samples attained from a fishpond in Gauteng, South Africa (25° 35' 10.15" S; 28° 10' 27.289" E). The sediment was collected in a dark pail at a depth of 30 cm

below the pond bed and 1 m from the shore. After collection it was transported to a laboratory to be stored in a cold room at -5˚C. The bacteria in the sediment is believed to have become acclimatized to nitrate presence as nitrates are produced as a byproduct of ammonia resulting from fish waste, food and plant organics in the water. It has also proven effective in experiments by (Chen et al., 2017) denitrification experimental work.
To cultivate the bacteria, 25 g Luria-Bertani (LB) (Merck Germany) broth was first dissolved in 1 l of deionized water then autoclaved for 15 min at 121˚C. When cooled to room temperature, 1 g of sediment was inoculated in a serum bottle containing 100 mL of broth and wrapped with aluminum foil to prevent the ingress of light. Before sealing with a rubber stopper, the bottle was purged for 3 min using nitrogen gas to remove all oxygen from the mixture. The bottle was then stored on a shaker in a warm room for 24 h to allow for colony development. The cells were then harvested by washing with saline solution after centrifuging at 4000 rpm for 10 min.

2.3 Batch experiments setup

Harvested cells were first inoculated into sterile serum bottles. Dilutions of the nitrate stock solution and deionized water were then used to make up 100 mL of the desired nitrate concentration for the experiment. Depending on the nature of the experiment, a predetermined amount of woodchip substrate or sand was then fed to the serum bottle. The bottles were then purged with inert nitrogen gas for three minutes, sealed, and then stored on a rotary lab shaker in a warm room at a temperature of 30˚C. Samples were collected using sterile syringes and stored in a cold room at -5˚C until analysis. For the batch analysis work, sampling was spread out over a 24 h period with samples taken at 0, 6, 12, and 24 h respectively.

2.4 Column experiments setup

Three PVC filter columns were constructed for this work, they were built to mimic household slow sand filters in dimension and mode of operation. The columns were colored to protect ingress by light, and they were fed daily on an intermittent basis for the duration of the study. All the columns had the same build dimensions and the only difference between them was the internal packing. Column 1 was packed with sand only, column 2 a 1:1 mixture of sand and woodchips with the bottom and top 5 cm consisting of sand alone, and column 3 was packed with woodchips only. The column filters were built to the following specification, 1.2 m total height, 1 m standing water height, 0.85 m of packing, 1 L feed reservoir, and 3 sample ports spaced at equal 25 cm increments below the standing water height. The column diameter was 110 cm.
For column startup, nitrate reducing bacteria was harvested as described, diluted with deionized water, and fed to the filter until saturation. This was performed for all three columns. The columns were then allowed to stand for 24 h. and then fed with a synthetic nitrate contaminated feed of 3 L daily. The feed was fed to the columns over 1 h amounting to an average flow of 0.31 m/h over the feeding period. The feed flow rate was chosen in accordance with the feed flow rate for slow sand filters such that it was between 0.4 and 0.2 m/h (Verma et al., 2017). As with the batch experiments, samples were collected using a sterile syringe and stored at in a cold room at -5˚C until analysis.

2.5 Analytical method

The nitrate and nitrite concentrations were determined using a 940 Professional IC Vario ion chromatograph (Metrohm, Herisau, Switzerland) with separation column Metrosep C 6-250/4.0 (Metrohm, Switzerland) and eluent mix of 2,4 pyridine dicarboxylic acid (PDCA) and nitric acid (Metrohm, Herisau, Switzerland).

2.6 Heterotrophic plate count

A heterotrophic plate count was conducted for the feed, and effluent waters for the three columns. This was done during 200 mg/L nitrate feeding. The agar plates were prepared by pouring 100 µL of sample under flame onto agar plates that had been prepared using 500 mL of distilled water that had been autoclaved at 121˚C after the addition of 12.5 g of LB broth and 6 g of LB agar. Spread plate technique with serial dilutions was used for the plate count. After poring and spreading, the plates were stored for 24 h in a warm room at 30˚C.

3. Results and discussion

3.1 Batch experiments results

Two main sets of batch experiments were performed. In the first set of experiments the feed nitrate concentration was fixed at 200 mg/L and the woodchip substrate loading was varied, with woodchip mass of 0.5, 3 and 5 g chosen arbitrarily. The soluble organic compound content of woodchips varies significantly with age and time within the filter (Robertson, 2010). As such the dry mass of the woodchips was used as the basis for this experimental work. The experiment was performed to evaluate how the woodchip load affected the

denitrification. The results from this work are illustrated in figure 1a for the nitrate depletion and in figure 1b for the nitrite production and depletion during the first 24 h of the experiment runs. In the work, the lower woodchip load (0.5 g) results in a slower rate of nitrate depletion, while the higher concentrations initially supported faster denitrification, from 12 h onwards limited denitrification was observed with the 5 g woodchip batch reactor. A possible explanation for this is that bacteria immobilization had taken place. Immobilization occurs when the C:N ratio is too high and instead of releasing nitrogen, microbes instead utilize and tie up nitrogen (Brust, 2019). This would suggest that there is an optimum C:N ratio for the denitrification using woodchip material in the batch reactor. The batch reactor which was loaded with 3 g of woodchip material was also the only one to achieve complete nitrate and nitrogen removal over the 24 h period. Suggesting that this batch was operating closest to the optimum C:N ratio.

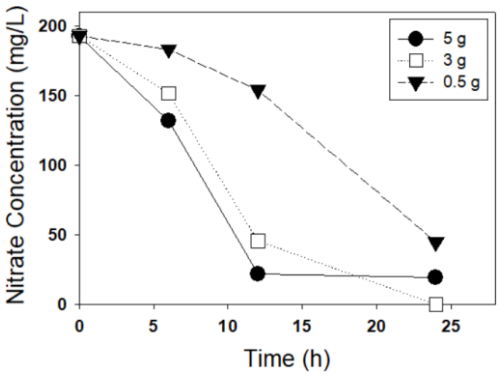

Figure 1a: Nitrate concentration over 24 h for batch reactors loaded with 5, 3, and 0.5 g of woodchip material

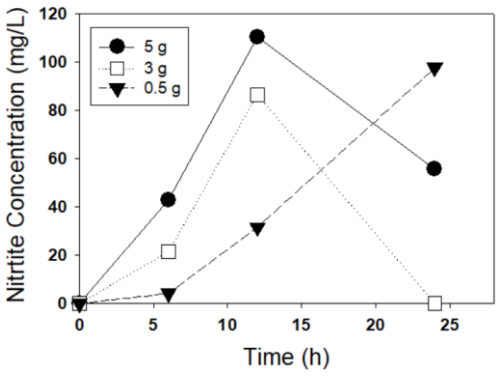

Figure 1b: Nitrite concentration over 24 h for batch reactors loaded with 5, 3, and 0.5 g of woodchip material

In the second set of experiments, using 3.0 g of woodchip and sand. Batch reactors were also inoculated so that the individual contributions of the two inoculums could be understood to help predict and understand column behavior. The results for the work are plotted in figure 2. For this experiment, both batches had been inoculated with harvested bacteria. While the woodchip batch reactor accomplished significant denitrification over the 24 h run, very little was seen with the sand batch. The behavior also translates for nitrite activity. This observation further cements the fact that the carbon source is essential for the denitrification activity.

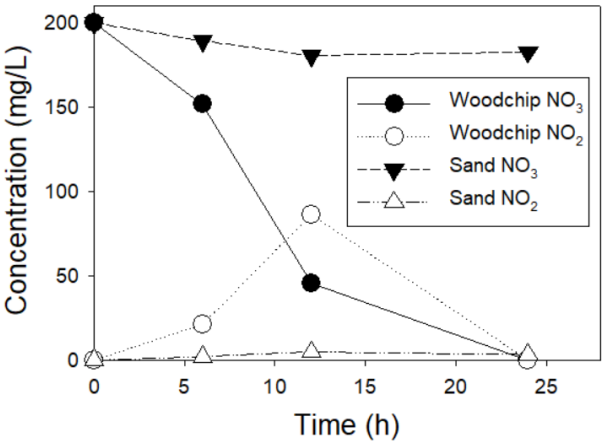

Figure 2: Nitrate and Nitrite concentration for batch systems inoculated with woodchip substrate vs sand only

3.2 Column experiments results

The results from the column experiment are listed in figure 3. These are the results obtained after all three columns were fed with 200 mg/L of nitrate contaminated water and sampled 24 h after column feeding. During earlier column runs they were fed with 50 mg/L of feed and complete denitrification was observed across all the sample ports of the mixed columns and with minor nitrate depletion in the sand column. This reduction in the

sand nitrate coupled with the 79 % reduction observed in the figure during the 200 mg/L feed runs could indicate the presence trace amounts of carbon substrate in the sand filter packing contributing to denitrification.

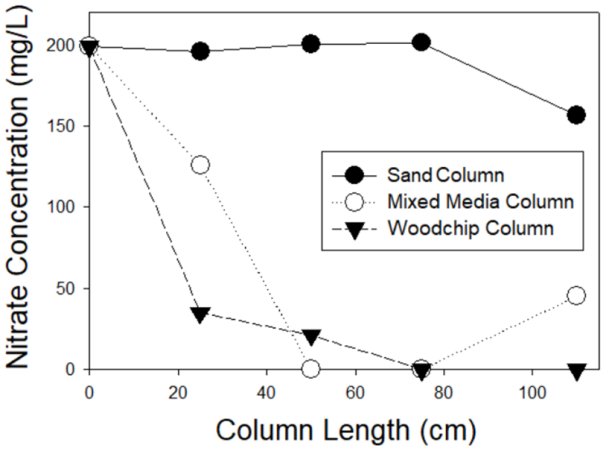

Figure 3: Nitrate concentration profile across the filter columns at 200 mg/L feed after 24 h

The mixed media filter as well as the woodchip only filter however did achieve greater denitrification rates with effluent outputs of 45 mg/L and 0 mg/L nitrate respectively. The woodchip filter column was the best performing of the three columns achieving complete denitrification early in the sample run. While the results from the mixed media column show complete denitrification early in the run, the jump in nitrate concentration coupled with the fact that in the sealed vessel nitrification is unlikely, points to the possibility of channeling within the filter. As a result of specific routes being used by the water within the filter, water around the sampling ports may be stationary without disruptions for longer allowing for greater denitrification to take place for the trapped water. Furthermore, the nitrification process occurs under aerobic conditions (Muck et al., 2019). Given the configuration of the filter, aerobic conditions would not be present during the filter run to facilitate nitrification, this phenomenon is confirmed by the conditions present at the end of the filter run where the nitrate concentration climbs again.

Another observation of note during the column studies was the presence of very little of the nitrite intermediary during the column runs. Almost no nitrite was observed across the sample ports with nitrites only showing up in low concentrations in three samples taken. This was in stark contrast to the batch reactions where prior to complete reduction of nitrate, significant nitrite accumulation was observed. It is presumed that because the filter columns follow plug flow dynamics, as soon as nitrate is converted to nitrite at the interface of the feed medium, it is driven further into the column where the nitrate concentration is lower. And because there is limited nitrate to act as a limiting reagent deeper in the column, Michaelis–Menten kinetics are followed, and the nitrite is depleted almost as soon as it emerges. It is also worth noting that water from the woodchip inoculated columns was much more turbid and colored than water from the sand column, indicating a high level of organic material that may need to be treated prior to use of the water.

3.3 Heterotrophic plate count results

A heterotrophic bacteria plate count was also obtained for the feed effluent from the reactor systems at 200 mg/L nitrate. Through serial dilution a plate count of 4.8×10^5, 3.3×10^5 and 9.9×10^4 cfu/mL were obtained for the woodchip, mixed, and sand column effluents. The feed count was 9×10^3 cfu/mL. The high ratio between the woodchip and mixed columns indicate that the columns provide habitable conditions for heterotrophic bacteria, and as such may need to be treated prior to use. As sand filters are built to reduce microbial contamination, the high colony count could point to poor filter maturation after inoculation.

4. Conclusion

This study illustrated that it is possible to achieve denitrification using fishpond bacteria coupled with woodchips as a carbon source for denitrification, in both batch and intermediately fed column reactors. Depending on the woodchip load and initial nitrate concentration, complete denitrification can be reached within a 24 h time frame. While the woodchip load does enhance denitrification, evidence from the batch experiments suggest that higher concentrations could have an inhibitory effect on the denitrification. Further research looking into the specific organic substrates leaching from woodchips and their effect on denitrification is recommended. The

configuration of the reactor also has a significant effect of the formation if the nitrite intermediary product, with the column reactors resulting in samples with less nitrite in them at any given point during the filter run. Due to the high heterotrophic bacteria colony count present in the effluent from the filter studies as well as discoloration of the water, additional treatment for organic and bacteria content is recommended depending on the water use.

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