Potential production of methyl chloride in an in-stream denitrifying woodchip bioreactor

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Background, Purpose and Hypothesis

Nitrate (NO_3^-) is a major contaminant of rivers, lakes and drinking water sources. Denitrifying woodchip bioreactors are becoming more common for removing NO_3^- in agricultural areas (Shipper et al. 2010). Previous research has determined that the production of greenhouse gases, nitrous oxide (N_2O) and methane (CH_4), occur in an in-steam denitrifying bioreactor designed for NO_3^- removal in an agricultural stream (Elgood et al 2010). In a second study, a reactor addition proved to be an effective device to limit CH_4 production in a woodchip bioreactor (Elgood 2010). In both studies effluent chloride (Cl^-) concentrations were generally higher than in the influent. The combination of several factors, including, an organic carbon substrate, CH_4 production and Cl^- abundance suggest there may be the potential for production of methyl-chloride (CH_3Cl) in these bioreactors.

CH_3Cl is a major contributor to ozone depletion, and accounts for approximately 20-25% of the total stratospheric chlorine input (Crutzen and Gidel, 1983). One of the well-known producers of CH_3Cl is commonly known as white rot fungi (Anke and Weber, 2006). As management strategies for limiting NO_3^-, including bioreactors, become more prominent, studying the potential negative environmental side-effects such as the production of CH_3Cl will remain a research priority.

The purpose of this investigation is to determine if white-rot fungi is present in an in-stream woodchip bioreactor, and if CH_3Cl is produced during lignin degradation.

It is hypothesized that woodchip bioreactors have the potential to produce and emit CH_3Cl.

Previous research indicates that Cl^- concentrations are greater exiting the field reactor and a lab reactor simulation. Increasing Cl^- concentration indicates a Cl^- source from degradation of organic sources and/or potential groundwater input into the field reactor. One of the main natural producers of CH_3Cl, white rot fungus, is found in N limited environments and can exist in a wide range of conditions. Some white rot fungi are known to tolerate O_2 limited environments, and previous experiments have documented the growth of white rot fungi in aquatic environments (Le X et al.). A substrate known as S-Adenosyl methionine is produced during lignin decay, and is believed to act as a methyl donor for CH_3Cl production. Due to NO_3^- limited conditions and organic matter degradation (lignin and cellulose), growth of white rot fungi and the subsequent production of CH_3Cl may occur. Potential infiltration of external groundwater sources may create aerobic zones at the edges of the reactor, increasing the possibility of favorable growth conditions for white rot fungi.

Materials, Analytical Equipment and Methodology

Materials: 60 ml glass serum bottles, 40 ml glass vials with open top cap and Teflon coated silicon septa, 30 ml plastic bottles, tape, syringes, syringe filters, needles, thermometer, field book, graduated cylinder, sulfuric acid (20%), woodchips, beet juice, nutrient agar, malic acid, tweezers, razor blades, kettle, acetylene torch.

Equipment: Balance, orbital shaker, HACH dissolved O_2 meter, Petri dishes, pressure cooker. Varian 3800 gas chromatograph, Dionex ICS90 ion chromatograph, Agilent 6890 GC with Agilent 5973 Mass Spectometer with Tekmar 3100 purge and trap concentrator and autosampler.

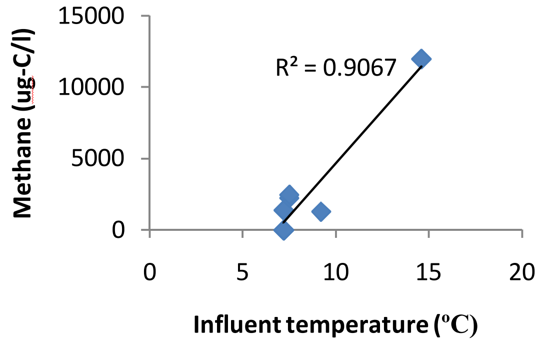
Field Methodology: Dissolved gas samples were collected from the reactor influent and effluent using 60 ml serum bottles. 30 ml anion samples were field filtered (0.45 µm). 40 ml CH_3Cl samples were preserved at pH 2 using 20% sulfuric acid. Dissolved O_2, water temperature, conductivity and pH were measured in the field.

Chemical Analyses: Anions and dissolved gases were analyzed at the University of Waterloo by ion chromatography and gas chromatography respectively. CH_3Cl samples were analyzed by gas chromatography-mass spectrometry at Environment Canada's, National Water Research institute, Burlington, Ontario.

Microbiology: 400 ml beakers were filled with 250 ml of a beet juice/agar solution.and autoclaved at 120 ^{o}C and 15 psi for 15 minutes to sterilize. Malic acid was used to lower the pH of the agar to 4 to discourage bacterial growth. Sterilized equipment was used to cut splinters from the interior of woodchips. Ten wood fragments were placed on each agar plate. Ten plates were used for column woodchips, 20 for reactor woodchips and 4 as controls.

Results and Discussion

During the sampling period, a strong correlation existed between methane production and reactor influent temperature (Figure1). During the same period, Cl^- concentrations were always greater in the effluent compared to the influent. Changes in concentration ranged from 7.5 - 15.0 mg Cl/L, with lowest changes associated with highest influent temperature (Figure 2). As illustrated in Figure 3, no significant relationship appears to exist between reactor discharge and the change in Cl^- concentration. This suggests the change in concentration is likely not due to groundwater input alone, but perhaps may be due to organic degradation.



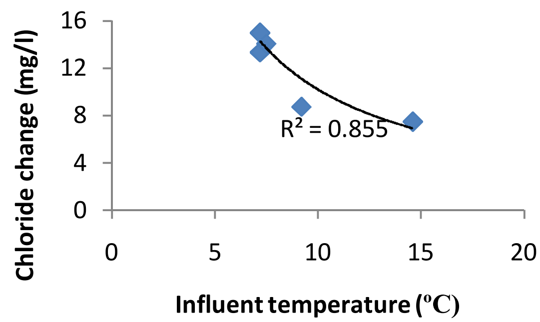


Figure 1: CH_4 production vs temperature

Figure 2: Cl^- change vs temperature

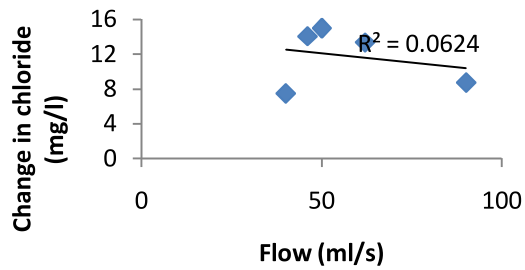


Figure 3: Cl^- change vs flow

(Error bars are not shown in figures as symbol size exceeds the error bars. Cl^- values were accurate to ±0.05 mg/L, and CH_4-C to 5%).

During the sampling period, no CH_3Cl production was observed. As sampling was limited to the inflow and effluent it is possible that production may still occur in the uppermost aerobic section of the reactor. In an attempt to determine if white rot fungi were present in this zone, nutrient agar was used to plate splinters with bioreactor woodchip fragments. After 24-72 hours, growth was observed on almost all plates. The colourized agar was totally bleached an amber or beige colour within 72 hrs, and growths appeared white and fibrous or as slimy pools. These characteristics are more indicative of bacterial than fungal growth. It is therefore unlikely, that white rot fungi are present in the bioreactors.

Conclusion

No CH_3Cl or white rot fungi were detected in this study. Additional sampling within the reactor, particularly in more aerated areas during periods of higher temperatures, and more sophisticated attempts to identify fungi may yet yield evidence of the production of CH_3Cl. At this time however, it appears that it is unlikely that woodchip bioreactors and in particular stream-bed bioreactors will have the undesirable side-effect of contributing to CH_3Cl production and subsequent ozone depletion.

Earlier Work

The hypothesis for this project was developed using data collected for ISEF and ISWEEP projects (2009 and 2010). All field sampling, lab work and analyses for the current project were conducted after October 1, 2011.

Acknowledgments

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