Methane Production and Removal in a Denitrifying Stream-bed Bioreactor

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Background, Purpose and Hypothesis

Nitrate (NO$_3$) is a major contaminant of rivers, lakes and potential drinking water sources$^{(1)}$. Denitrifying bioreactors are currently being tested for treating NO$_3$ in agricultural drainage$^{(2)}$. A bioreactor uses microbes to remove NO$_3$ in the influent, converting it to nitrogen gas (N$_2$). Previous research (October 2008-March 2009) at a stream-bed bioreactor indicated that incomplete denitrification results in nitrous oxide production. Data also indicated methane (CH$_4$) was produced in early fall but not during later winter months (Figure 1).

CH$_4$ is a potent greenhouse gas (GHG), with a current warming potential of 25 times that of carbon dioxide (CO$_2$)$^{(3)}$. If bioreactor use continues to increase, it is important to understand their CH$_4$ emissions and global warming potential.

The purpose of this project is twofold: to determine seasonal variation in CH$_4$ production in an operational stream-bed denitrifying bioreactor; and to determine if it is possible to reduce CH$_4$ emissions from such a woodchip reactor through the implementation of a secondary reactor.

It was hypothesized that CH$_4$ would be produced in the bioreactor when stream temperatures increased to above 15$^\circ$C$^{(4)}$. It was also hypothesized that CH$_4$ produced in the primary reactor could be oxidized through the mixing of reactor effluent and original source water in a secondary reactor. A variety of bacteria utilize CH$_4$ for energy, so it was thought that it would be possible to utilize naturally occurring bacteria to oxidize CH$_4$ to CO$_2$, which has a lower warming potential than CH$_4$. 
Materials, Analytical Equipment and Methodology

The materials required for field sampling included: 60 ml glass and 30 ml plastic sample bottles, tape, exetainers, syringes, needles, safety goggles, nitrile gloves, mercuric chloride solution, HACH 40Qd meter, graduated bucket, stopwatch, cooler and ice. Additional materials for the lab experiment included: three-way valves, woodchips, gravel, Plexiglas columns, tin foil, Peristaltic pumps, timers, tubing, tube clamps and graduated cylinders. Analytical equipment included: Balance, Orbital shaker, Varian CP-3800 gas chromatograph, Dionex ICS90 ion chromatograph and a Technicon Auto Analyzer.

Field Methodology: Dissolved gas and anion samples were collected approximately every two weeks from January to October 2009. Dissolved O₂ and temperature were measured in the field. Dissolved gas samples were collected in 60 ml serum bottles and preserved in the field using Mercuric Chloride. 30 ml Anion samples were field filtered to 0.45 um.

Lab Methodology: To simulate the reactor, woodchips were obtained from the field reactor and gravel was purchased for the secondary columns. Three Plexiglas columns were assembled and leak tested prior to the media being added. 60L of stream water was collected from above the field reactor, transferred to the lab and refrigerated (4°C) until use. Stream water was pumped into the woodchip column and left stagnant to allow the microbial population to cultivate. After a conditioning period (4 days), stream water was pumped through the woodchip and first gravel columns. NO₃, SO₄, temperature, dissolved CH₄ and O₂ were monitored over a period of 18 days. Anions and dissolved gases were sampled from the inflow, 2 ports in the first column
(simulating the bioreactor), and a port near the top of the second column until effluent concentrations from column one and column two remained fairly constant. Following this period, the outflow of column 2 was mixed with source water in a 2nd gravel column in the attempt to remove CH$_4$. NO$_3$, SO$_4$, Temperature, dissolved CH$_4$ and O$_2$ was monitored for an additional 16 days. Additional samples were collected from the stream water fed to column three and from ports at the bottom and top of the third column (Appendix 1). Anions and dissolved gases were analyzed using a Dionex ICS90 ion chromatograph and a Varian 3800 gas chromatograph respectively.

**Results and Discussion**

Results of the field study are presented in Figures 1-3. Seasonal variation in temperature was accompanied by variable NO$_3$ removal, SO$_4$ reduction and GHG. Effluent O$_2$ concentrations were <1mg/L, suggesting denitrification was occurring in all seasons. NO$_3$ effluent ranged between <0.01-3.9 mg/L, and removal between 18-100%. SO$_4$ reduction between January and October ranged between in 0.2-5.3 mg/L in the effluent (Figure 2). CH$_4$ was produced in warmer periods (June-October). Concentrations ranged from 100 - 7800 µg C/L (Figure 1). When CH$_4$ emissions were calculated as potential emissions per square meter, production ranged between -3 and 1237 mg C m$^{-2}$ d$^{-1}$ (Figure 3).

![Figure 1](image_url). Avon stream-bed reactor, comparison of stream water and reactor effluent; dissolved CH$_4$ concentrations.
In the lab woodchip column simulation, dissolved oxygen was consumed rapidly. Complete denitrification occurred in the first half of the column and partial denitrification of stream water added to the secondary reactor occurred in the bottom of the reactor (C3). SO$_4$ reduction occurred in all columns. These conditions were favourable for CH$_4$ production. (Figure 4b).

When effluent from the woodchip column and first gravel column were mixed in equal proportions with source water in the secondary reactor, O$_2$ was consumed almost immediately and ~60% of the additional NO$_3$ was denitrified (C3B). SO$_4$ reduction in the secondary reactor was coupled with up to 73% CH$_4$ removal(C2T).

Results of the column experiment suggest the hypothesis, that CH$_4$ could be removed in a secondary reactor, was correct. O$_2$ was consumed and CH$_4$ oxidized during rapid denitrification which occurred as soon as the source water entered the secondary reactor (Figure 4). No oxidation occurred in the top of the final column and concentrations actually increased, suggesting that methanogenesis resumed in that portion of the reactor.
As denitrification continued in the secondary reactor accompanied by CH₄ reduction, it is possible that in addition to SO₄ reduction, denitrification using CH₄ as the carbon source is also responsible for removal of CH₄.

**Conclusion**

Bioreactors are efficient methods of removing NO₃, providing the benefit of improved water quality. This study demonstrates that reactor design and environmental conditions play a role in determining CH₄ production. Careful consideration of flow dynamics and the use of secondary reactors may limit CH₄ production and minimize resulting emissions. With increasing concern over GHG emissions, all attempts must be made to minimize GHG production, including GHG produced by remedial technologies.

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