University of Nevada, Reno

Utilization of Methane by Sulfate Reducing Bacteria:

Implications for Hydraulic Fracturing

A thesis submitted in partial fulfillment of the
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By

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Abstract

Leakage of natural gas, particularly methane, from hydraulically fractured oil and gas wells is being implicated as a factor for degradation of groundwater. Utilization of methane by sulfate reducing bacteria in drinking water aquifers has been hypothesized, but no direct evidence has been established to link the bacteria to the reaction. A series of column experiments were performed to examine this hypothesis and to monitor the changes that occurred in the water in each column when methane is purged through the columns. Changes that were monitored include pH and oxidation reduction potential, and sulfate, sulfide, iron, and manganese concentrations. The changes were compared to changes that occurred in a drinking water well in Dimock, PA after hydraulic fracturing, and the results had similar changes, including reduction in the oxidation reduction potential, and were coupled to the loss of sulfate to form hydrogen sulfide as well as increased metal concentrations. These column experiments provide supporting evidence that sulfate reducing bacteria can utilize methane and other natural gas components to degrade the quality of drinking water wells.

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Introduction

Natural gas, primarily methane, at hydraulic fracturing sites is being detected at a growing number of wells and raises concerns about the potential impact to drinking water wells (Vengosh et al., 2014). At several sites in Canada, the released methane enters the domestic aquifers by migrating upward from deep sedimentary natural gas reservoirs along fracture zones and exploration holes or defective production wells (Van Stempvoort et al., 2005). This migration results in causing several changes to the water chemistry, many of which are considered negative for drinking water for the communities that rely on groundwater. Migration of methane into shallow soils, groundwater, or surface water bodies has been studied in the past (Rose and Alexander, 1945; Preston, 1980; Kelly et al., 1985; Chafin et al., 1993; Chafin, 1994). The following study seeks to determine if the change can be attributed to increased activity of sulfate reducing bacteria (SRB) that utilize the methane as a carbon source to reduce sulfate into hydrogen sulfide. This microbial process can also impact the pH and oxidative reduction potential (ORP) of the water. Changes in the ORP of the groundwater can also result in the release of metals, particularly iron and manganese (Miller and Maest, 2013).

Hydraulic Fracturing

Hydraulic fracturing is a method used for extracting oil and natural gas from shale and other formations where the hydrocarbons are found in geological formations with the oil and gas tightly bound to substances such as shale and sandstone (Vengosh et al., 2014). The process involves drilling vertically to reach the shale formation and then drilling horizontally to increase the surface area of the shale that is exposed in the well. After the wells have been drilled, a mixture of water, sand, and other chemicals is

pumped into the well at very high pressures, causing the shale to fracture and release the oil and gas that was previously trapped. Because of the high pressures created during hydraulic fracturing, the fluid then flows back to the surface while the sand remains in the newly created fractures, allowing the oil and natural gas to continue flowing into the well where it is ultimately recovered as natural gas and/or crude oil.

Fugitive Methane

As hydraulic fracturing becomes more prevalent in the United States, there is an increasing concern about the release of natural gas, which is primarily composed of the potent greenhouse gas, methane. Methane that escapes a well without being flared or leaks from faulty well construction is defined as fugitive methane. A study by Brandt *et al* (2014) found that inventories consistently underestimate actual methane emissions at all scales. Regional variability makes estimating large scale emissions difficult because there is a very large difference between device-level studies and continent-scale studies when examined over the course of a year. Methane is released at the surface during hydraulic fracturing, and equipment leaks are also potential sources for methane release. Forty separate types of equipment are known to be potential sources of methane emissions including loose pipe flanges, leaky storage tanks, outdated compressors, inefficient pneumatic systems, and corroded pipes (Drouin, 2014). For wells that are in routine production, equipment leaks measured averaged 1.23 ± 0.44 g methane per minute per well (Allen et al., 2013), about 1-2 L methane per minute. If these leaks were to occur near a groundwater aquifer, methane could potentially become saturated in the water, leading to potential changes in water quality and water chemistry.

Fugitive methane is certainly not limited to hydraulic fracturing sites, and studies have examined the impact of topography on methane concentrations in water wells. A study by Heisig and Scott (2013) sampled 66 wells that were more than one mile from any known gas well and their results indicated "strong positive and negative associations between hydrogeologic settings and methane occurrence" based on whether a well was located in a valley or upland. Fifty-seven percent of wells located in valleys had methane levels greater than 0.1 mg/L while only 10 percent of upland wells exceeded that concentration (Heisig and Scott 2013). While important to note that methane can naturally migrate through geologic formations and dissolved in groundwater, from either biological or geologic deposits, the scope of the experiment described below focused on changes that occur when methane is introduced to groundwater systems that have not previously been exposed. The changes that occur in naturally affected groundwater that have methane present are likely to be similar to the conditions examined.

Biogenic and Thermogenic Methane

In addition to naturally high levels of methane in groundwater, another important aspect to consider is the source of the methane. Biogenic methane is created from biodegradation of organic material in the shallow subsurface, including alluvial and glacial drift deposits (Molofsky *et al*. 2011). Biogenic gas formation typically occurs at temperatures $<50^{\circ}C$ (Stopler *et al*. 2014), which is consistent with probable location in shallow deposits.

Thermogenic methane forms under high temperatures and pressures in sedimentary rocks such as shales, siltstones, and sandstones that have been deposited in marine and fluvial environments (Molofsky et al., 2011). As depth below the surface

increases, the increase in temperature yields abiotic gas formation when temperatures are between 157°C and 221°C (Stolper et al., 2014). Thermogenic gas formation occurs at depths and in geologic formations and is generally the target for hydraulically fractured wells, making any potential gas leaks from these wells likely to be thermogenic in origin. Isotopic analysis and gas-formation models based on temperature can usually determine the origin of gas to distinguish between thermogenic and biogenic gas, which is an important and defining characteristic when attempting to determine if hydraulic fracturing wells have introduced methane into groundwater. Whether the natural gas is primarily thermogenic or biogenic, its impact on a groundwater aquifer is likely to be very similar.

Background Study:

As the number of hydraulically fractured wells increases across the United States, concern is growing about potential contamination of groundwater that is used as drinking water supplies for communities. Although concerns exist about the chemicals used in hydraulic fracturing, the purpose of this study focused on concerns that methane is migrating to groundwater aquifers. Utilization by a consortium of bacteria can potentially consume the available oxygen in the groundwater, followed by the utilization of the fugitive methane by sulfate reducing bacteria to reduce sulfate to sulfide, as well as potentially cause other unfavorable changes to the groundwater. Methane is a known carbon source for sulfate reduction in marine systems, although the exact mode of utilization is not completely elucidated (Alperin and Hoehler, 2009; Barton and Fauque, 2009; Girguis et al., 2005). Whether the methane is introduced into groundwater through a leak in a well casing or another pathway, eventually the groundwater can become

saturated with methane. Once the saturated water supports sulfate reducing bacteria, it can show increased alkalinity, the presence of sulfide, increased soluble aluminum concentrations, because of increased pH, negative ORP, and soluble iron (Fe^{2+})and manganese oxides (Mn^{2+}) (Miller and Maest, 2013). Miller and Maest (2013) noted a drinking water well in Dimock, Pennsylvania that had been substantially affected following drilling of a Marcellus formation gas well within 200 meters of the drinking water well. In this case, the water quality in the domestic water well failed secondary drinking water standards within six months after the installation of a hydraulically fractured well. Iron, manganese, pH, and turbidity increased and rendered the water effectively undrinkable. Water sampling over time produced the following data (Table 1).

Table 1. A series of water-quality results from a Pennsylvania domestic well located within 1,000 feet of a hydraulically fractured well. The 7/8/2008 results are a pre-fracturing sample. These data were supplied to the authors by National Resource Defense Council and performed by a certified laboratory. Units are mg/L, except for ORP (mV) and pH (standard units).

Date	ORP	pH	Cl	Sulfide	TDS	Al	Fe	Mn	Methane
7/8/2008	--*	6.9	4.6	<1	151	--	0.12	--	--
1/6/2009	--	9.38	6.3	<1	290	1.03	0.354	<0.025	19
2/12/2009	--	9.39	33	<1	320	0.95	0.487	<0.01	24
4/14/2009	-73.7	9.39	11.9	<1	--	<0.1	0.318	<0.025	29
6/14/2009	-239	9.68	--	--	--	--	--	--	--
9/29/2009	1.7	9.62	5.79	5	347	0.76	7.49	0.23	25
12/3/2009	-80.6	9.59	7.88	6	308	1.06	8.88	0.23	29
2/4/2010	43.1	8.61	6.15	4	100	0.18	0.8	0.043	24
3/11/2010	-25.9	9.28	8.74	<5	270	2.36	14.5	0.63	22
4/15/2010	-116	9.42	14.5	<1	315	2.78	4.33	0.26	28
5/27/2010	-101.4	9.03	--	--	--	--	--	--	0.018
6/7/2010	-78.3	9.81	--	<1	--	--	--	--	25
1/2/2013	-23.9	9.73	8.7	0.27	303	0.438	0.381	0.0073	34

-- analyte not determined

Data Obtained from the Pennsylvania Dept. of Environmental Protection

The pre-fracture water sample, collected on 7/8/2008 had generally good quality water, within six months, the drinking water was degraded and largely undrinkable. The pH increased dramatically (pre-fracture pH 6.9 to over 9) after hydraulic fracturing occurred; ORP values shifted and became negative for most samples, total dissolved solids increased, as did the concentrations of aluminum, iron, manganese, and methane. While methane concentration in the initial sample was not determined, the concentration after six months was at effective saturation of 28 mg/L. The data obtained from the samples suggests that methane was responsible for the water-quality changes, and that microbial activity can potentially degrade the odor, taste, and increase turbidity in groundwater when methane is introduced as a carbon source. The changes in water-quality cannot definitely prove a cause and effect relationship; the goal of this study was to demonstrate that the changes that occurred in the water well in Dimock could have been caused by introduction of natural gas/methane, which could serve as a carbon source for the bacteria.

Hypothesis

When sulfate reducing bacteria are supplied with methane as a carbon source, are the changes in water quality in column experiments consistent with what has been observed in drinking water wells that are contaminated with natural gas? To establish a link between microbial activity and groundwater changes, the analytes that were measured during the column experiments included pH, ORP, sulfate, methane, iron, manganese, and sulfide.

Natural Gas/Methane Effects of Water

Hydraulic fracturing to obtain natural gas and other hydrocarbons is not a new process, but has expanded dramatically over the past 15 years, and now produces 30-40 percent of natural gas and 40 percent of oil in the United States (U.S. EIA, 2014). Recently there have been several questions raised about the safety and sustainability of the practice, particularly as it relates to protection of groundwater. Unintentional vertical movement of natural gas is a concern in Canada (Erno and Schmitz, 1994; Van Stempvoort et al., 1996, 2000; Rowe and Muehlenbachs, 1999). A study by Dyck and Dunn (1986) found elevated concentrations of methane in groundwater were associated with higher densities of exploration holes. Leakage from wells has been documented in numerous studies in Canada (Erno and Schmitz, 2004; Van Stempvoort and Jaworski, 1995; Van Stempvoort et al., 1996, 2000). Despite the documented leakage problems, very few studies have been conducted examining how migration of fugitive natural gas is affected by natural attenuation processes of the gas in the subsurface (Van Stempvoort et al., 2005). The experiment in this study was built upon Van Stempvoort's work of examining bacterial sulfate reduction acting as a terminal electron accepting process in the natural attenuation of fugitive methane in groundwater (Van Stempvoort et al., 2005).

The relationship between methane and sulfate concentrations has been studied previously (Maathius and Jaworski, 1997) with an inverse relationship between methane and sulfate concentrations in groundwater. Van Stempvoort et al. (2000) hypothesized that the presence of sulfate and methane may result in bacterial sulfate reduction, potentially causing depletion of methane in the subsurface using a process similar to the

inverse relationship between sulfate and methane observed in marine sediment profiles
(Iversen and Blackburn, 1981; Devol and Ahmed, 1981; Iversen and Jorgensen, 1985;
Niewöhner et al., 1998; Aharon and Fu, 2000; Boetius et al., 2000; Delong, 2000;
D'Hondt et al., 2002; Nauhaus et al., 2002). The chemical equation for the reaction that
occurs is:

$$CH_4 + SO_4^{2-} \rightarrow HS^- + HCO_3^- + H_2O \quad (1)$$
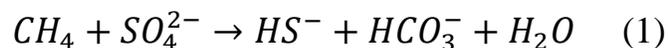
The reaction involves various enzymes, intermediate valence sulfur species, such as
sulfite (Trudinger, 1969), and intermediate valence carbon species, such as methanol
(Hanson and Hanson, 1996). This reaction assumes that methane is completely oxidized
to carbonate, and that the products are not released from the solution. In this case, the pH
of the aqueous systems is predicted to increase, as well as the alkalinity and the total
dissolved solids (as bicarbonate/carbonate species), which was observed in the Dimock,
Pennsylvania well.

Marine environments have been extensively studied, and the redox process
described earlier appears to involve a symbiotic consortium of sulfate reducing bacteria
and methane oxidizing Archaea (Boetius et al., 2000; Orphan et al., 2001). Creating a
link between the anaerobic oxidative that occurs for methane in marine sediments with a
similar process in fresh groundwater systems has not been studied in any detail. Davis
and Yarbrough (1966) reported slow oxidation of methane by sulfate reducing bacteria
isolated from groundwater sampled from an oil field aquifer. More recently, Zhang et al.
(1998) found a significant positive correlation between $\delta^{13}C$ values of methane and
sulfate concentrations, suggesting that methane oxidation was associated with bacterial
sulfate reduction. The experiment in this study provided bacteria with only natural gas as

a carbon source, and measured the sulfate reduction that occurred in the presence of the natural gas as compared to a control column and created conditions similar to those that would exist in aquifers near hydraulic fracturing sites.

Materials and Methods

Anaerobic bottom sediments were collected from Manzanita Lake on the campus of the University of Nevada, Reno to establish colonies of SRB. The anaerobic bacterial cultures from Manzanita Lake were placed in 1.5 L mason jars filled with pea-sized gravel and one liter of Postgate B solution prepared using 3.22 g $FeSO_4*7H_2O$, 0.58 g $Fe_2(SO_4)_3*2H_2O$, 0.07 g $(NH_4)_2SO_4$, 0.5 g KH_2PO_4, 4.5 g Na_2SO_4, 0.06 g $CaCl_2*2H_2O$, 0.06 g $MgSO_4*7H2O$, 5 mL sodium lactate @ 70% w/v per liter of solution and adjusting the pH to 6.7 using NaOH. All chemicals used for the Postgate B solution were obtained from Fisher Scientific. The jars were capped and had a glass tube fitted in the cap to deliver natural gas. These jars served as enrichment cultures for the column experiments.

Four columns were used in the experiment. Two large columns measuring 10 cm inner diameter by 1.5 m tall and tapered at the top and bottom to 4 cm, were used for the primary columns for samples. Two smaller columns measuring 5 cm inner diameter by 1.5 m tall were used for replicate columns. The bottom of each column was stoppered with a rubber stopper with a hole drilled in the center of it to accommodate a hose running through the hole and a valve attached to the bottom to obtain samples. A hard plastic hose with a 1 cm diameter was placed at the bottom of each column and was connected to a peristaltic pump for gas to be delivered directly to the bottom of each column. The hose was held at the center of the column as the pea-sized gravel was added to the column to ensure that the hose did not move as the gravel was added. An

equivalent volume of gravel was added to each of the columns, completely filling the smaller columns, leaving as little head space as possible. The gravel was then washed repeatedly (four volumes of water) to remove any excess dirt or organic matter on the gravel. The gravel was also washed prior to being put into the columns as an attempt to minimize the organic matter introduced.

Following the washing process, a modified Postgate B solution was added to fill the columns. The replicate columns were filled completely with Postgate B solution and an extra 500 mL of the solution was added to larger columns to maintain a fluid level above the gravel. The Postgate B solution was prepared by adding 0.08 g $MgSO_4*7H_20$, 0.02 g $FeSO_4(NH_4)SO_4*6H_20$, 0.02 g $KHPO_4$, 0.04 g NH_4Cl, 0.04 g Na_2SO_4, 0.005 g $CaCl*6H_20$, and 0.04 g yeast extract to one liter of tap water. The void volume of the small columns was approximately 1.5 L, which was the volume added to each of the four columns. After adding the Postgate B solution to each column, 50 mL of enrichment culture was injected into the bottom of each column. The entire volume of solution in each column was then drained into a large flask and poured back into the top of the column to promote equilibration.

Two columns, one large and one small, were then purged with house air that was filtered by a hydrocarbon trap filled with XAD4, and two columns were purged with house natural gas that had an added odorant, at a flow rate of 10 mL/min. The flow rate was delivered via peristaltic pumps that were connected to the plastic hoses inside each column. Prior to collecting each sample for measurements, 25 mL of liquid was purged from the bottom of the column and poured back into the top of the column. Samples were collected twice a week for seven weeks from each column and measured for pH,

ORP, and sulfate concentration. The pH of each column was measured with a Fisher

Scientific Accumet® pH Meter 915 that was calibrated using a one-point calibration with

a pH 7 buffer from BDH. The ORP was measured with an EcoSense® ORP 15A, and

calibrated using a Zobell Solution from YSI. Following the pH and ORP measurements,

samples were filtered through a Minisart NY 25 0.45 µm filter before being analyzed for

sulfate concentration by ion chromatography on an IonPac® AS 18 column on a Dionex

ICS-3000 using a five-point calibration curve of sulfate standards with a standard from

Ultra Scientific. The data obtained from these samples represents only the bottom of the

columns, since mixing of the water through the entire column was inefficient. The setup

of the columns is illustrated in Figure 1.

Figure 1: Column setup with pumps.

Problems with Experimental Setup

While the final column experiments used four glass columns and two different

gases, several variables of the experiments were changed to achieve those conditions that

yielded the best results. Aspects of the experiments that were changed include nutrients

provided to the bacteria, temperature of the lab, evaporation of solution in the columns, column material, and using bacteria cultures not obtained near hydraulic fracturing sites.

For the initial column experiments, only nitrate, phosphate, and sulfate were added to a solution containing bottom sediment from Manzanita Lake. Bacterial growth occurred at the beginning of the experiment because the organic matter provided an abundant supply of nutrients, but the sediment proved to be limiting, and bacterial growth soon stopped. After several failed attempts trying to provide as few nutrients to bacteria as was possible for them to grow, consultation with Dr. Ruth Gault, a microbiologist, provided a list of buffer recipes that supplied the necessary nutrients for the bacteria. The Postgate B solution used in the enrichment jars was found to be the recipe that produced a functioning consortium of bacteria, and after modifying the ingredients to limit the carbon present in the solution, this solution was selected for the columns.

The temperature of the lab was not constant, and in the winter saw temperatures in the columns drop below 15°C, which limited the growth potential of the bacteria. When temperatures were above 19°C in the columns during the summer, loss of sulfate was observed in the methane columns. In colder temperatures, sulfate loss was not observed in any of the columns. For ideal experimental conditions to model in vivo conditions, temperature would need to remain constant, as there is little change in temperature underground where sulfate reducing bacteria inhabit.

While purging air through the columns, increase in pressure was not desired, so gas was vented through the top of the columns. The hoses that pumped gas into the columns made it impossible place a stopper in the top of the columns, so parafilm was used to cover the columns in an attempt to reduce evaporation while still allowing air to

pass through. The parafilm also prevented a pressure buildup, but even with the film covering the columns, an increase in the sulfate concentration of columns that were purged with nitrogen was noticed. Evaporation from the nitrogen columns caused an increase in the sulfate concentration, and data from these columns is not included.

The material of the columns was initially glass, however at the end of the trial run, there was a noticeable green film inside all of the columns. Algal growth inside the columns is problematic because as the methane purged column should be anaerobic. Using glass as a column material allowed sunlight to enter, and because the columns were located by the window, algae grew in the columns. The first attempt at controlling algae was to design new columns made of black PVC pipe that would prevent sunlight from entering, and allow capping the columns to control evaporation. After a period of two weeks, both the nitrogen purged columns and methane purged columns had a strong odor of hydrogen sulfide, despite the nitrogen purged columns having no apparent carbon source for growth. After examining samples, black particulate was found floating in the samples. The PVC had been degraded during in the experiment, serving as a potential carbon source and also releasing hydrochloric acid into the columns, causing a drop in the pH of the nitrogen purged columns. The final design of our columns returned to glass columns. In an attempt to control the amount of sunlight entering, aluminum foil was wrapped around each.

Bacteria from Manzanita Lake in Reno was selected because of the proximity to campus and ease of obtaining the sediment. For an ideal experiment, sediment from an aquifer located near a hydraulic fracturing site would provide a better representation of bacteria type that would be present near underground hydrocarbon sources. Finding a

strain of bacteria that has evolved in hydrocarbon rich settings would maximize the ability of the bacteria to utilize methane for reducing sulfate. Tap water samples were acquired from Odessa, Texas in hopes of culturing sulfate reducing bacteria from an area where hydraulic fracturing occurs, but no cultures were produced from these samples.

Results and Discussion

Three different measurements were conducted for each column during the experiment, pH, ORP, and sulfate concentration. The sulfate concentration was initially 77 ppm in the columns and although all four samples showed an initial decrease, the control columns purged with air stabilized while the methane purged columns continued to decrease. The final sulfate concentrations of the control column and the control replicate were 68 ppm and 75 ppm respectively. The methane columns both underwent large losses of sulfate; the methane column (M) ended with a sulfate concentration of 36 ppm for a 53% loss while the methane replicate (MR) column ended at 14 ppm, a loss of 82% (Figure 2). The characteristic odor of hydrogen sulfide was detected in the methane columns within two weeks of the initiation of the column experiments, providing evidence for its formation as sulfate was lost.
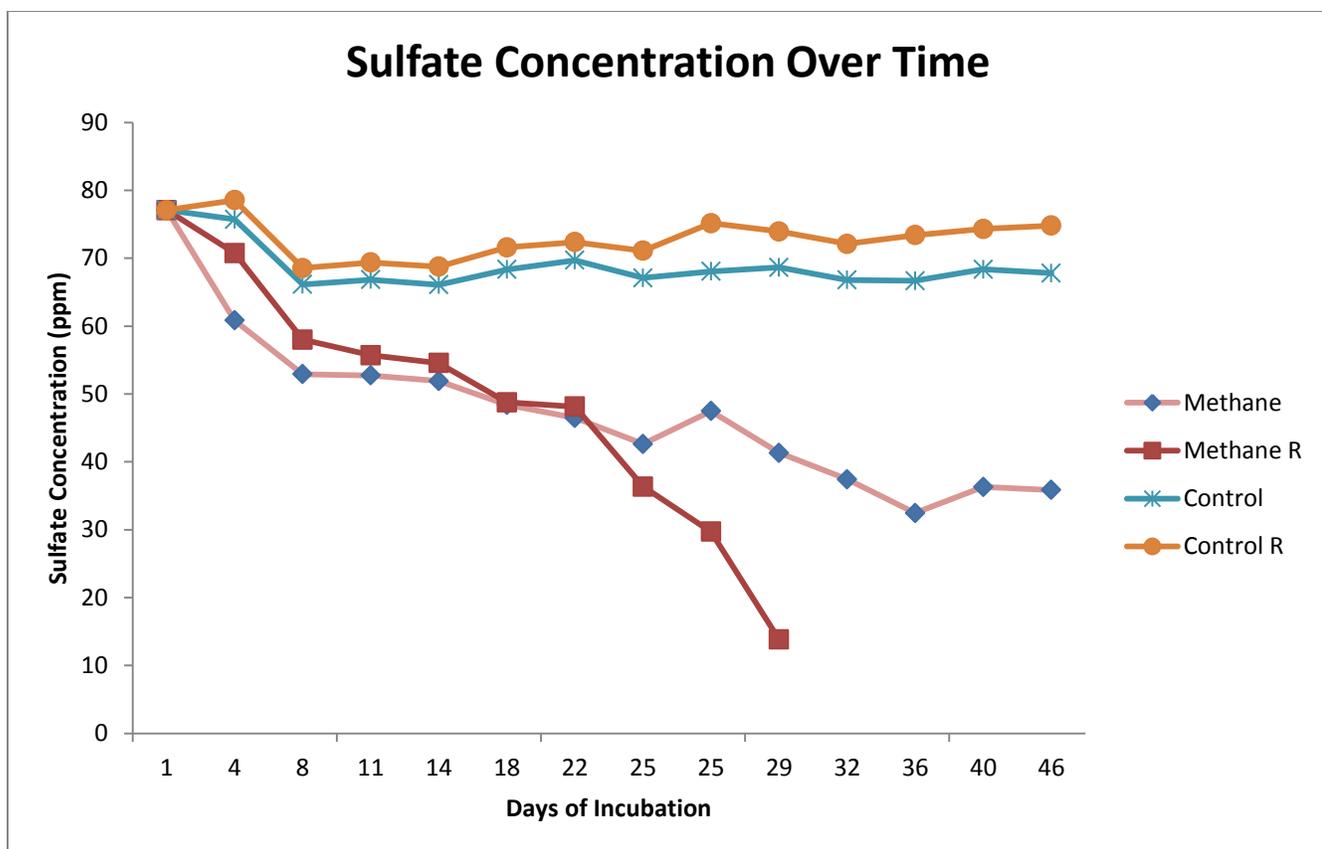
Figure 2. Changes in sulfate concentration over time in two control columns and two methane columns. Methane or air, respectively, was passed through the columns at a rate of 10 mL/min. The R columns are the smaller columns.

Oxidative Reductive Potential

The ORP of the columns provided an additional line of evidence for sulfate reduction. The majority of measurements for the methane columns were negative, indicating sulfate reduction. The ORP meter was calibrated using a Zobell solution, but even with the calibration, the ORP measurement was variable, and was dependent upon the concentration of the redox couples in the sample. Both of the control columns had ORP values that were consistently positive, while the methane columns had predominantly negative values. As the ORP value decreases, the likelihood of sulfate reduction increases (Figure 3).
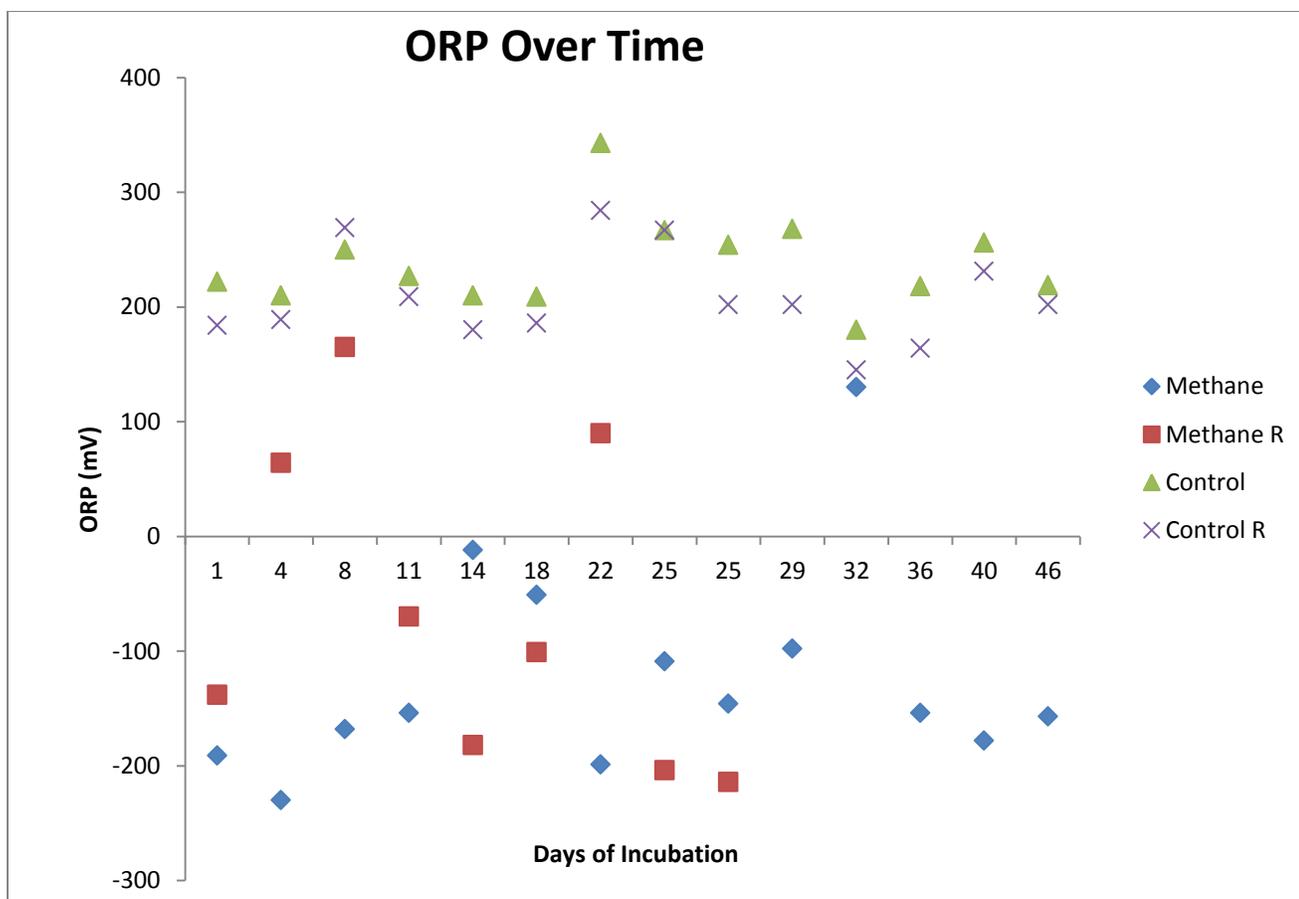
Figure 3. Change in ORP versus time of incubation for two control samples and two methane samples. ORP for Methane R column had fewer data points because the sulfate concentration dropped below detectable levels, at which times all measurements stopped.

pH

The pH of the solution in the methane columns was expected to increase as the sulfate was reduced to hydrogen sulfide, causing protons to be consumed in the reaction, as discussed previously. However, while the pH of the two methane columns was a bit higher than the large control column, there was no substantial increase in pH of the methane columns, as was expected, based on the Dimock well samples. The pH for all columns remained close to neutral throughout the experiment (Figure 4). While initially perplexing, the differences in the change in pH between the column experiments and the

Dimock samples can at least be partially rationalized by the differences in the substrate material. The Dimock aquifer surfaces may have had high iron oxides on the surfaces, and when ferric iron is reduced to ferrous iron, two protons are consumed for each iron atom reduced. Evidence for this chemistry is provided by the larger iron concentrations observed in the well water samples. The crushed granite surfaces used for the columns experiments are expected to contain much lower iron oxide concentrations which is suggested by the lack of iron in the water at the end of the experiment. A similar substantial increase in pH was observed with microbial reduction of ferric iron using glucose as the carbon source in acidic media (Kusel et al., 1999). In that study, the pH of the media increased from pH 3.2 to 5.8 during the course of the experiment, with large increases in soluble ferrous iron.

Additionally, lack of a large change in pH in the column experiments can be rationalized by the incomplete oxidation of methane. The anaerobic metabolism of methane by a consortium of bacteria is not well characterized. Both lactate and ethanol substrates are metabolized to acetic acid, which also depresses the pH of the solution (Barton and Tomei, 1995), and if the oxidation of methane is similarly incomplete, and formic acid is formed, then the pH would also be stabilized at a lower pH than if the anaerobic oxidation was complete and produced carbon dioxide. Other partially reduced sulfur compounds may also potentially be involved, and may affect the pH that is observed with the particular assortment of bacteria present in the inoculum. For example, production of sulfur as a terminal product reduces the consumption of protons by a factor of two for each sulfate reduced. During these experiments, formate production, was unfortunately not measured, but should be in future experiments.
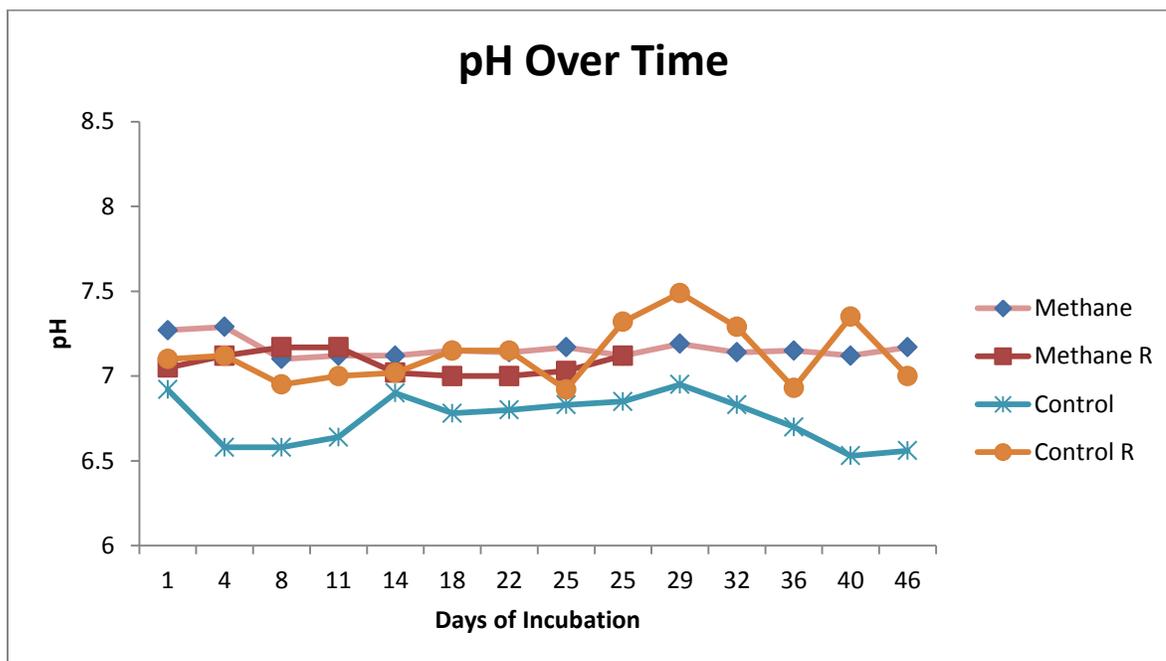
Figure 4. Change in pH versus time of incubation for two control columns and two methane columns. Methane R column measurements stopped once sulfate concentration dropped below detectable levels.

Methane, Sulfide, Iron, and Manganese

Following operation for seven weeks, final samples were collected from the two larger columns, filtered through 0.45 µm filters, and sent to Alpha Analytical (EPA certified laboratory, Sparks, NV) for methane, methanol, sulfide, iron, and manganese analysis. The control sample was easily filtered, while the methane sample rapidly plugged the filter, and use of a large volume 0.45 µm filter was required.

Table 2. Results from Alpha Analytical for control and methane column. Because of large volume sample requirements, duplicate samples were not analyzed.

	Control (mg/L)	Methane (mg/L)
Methane	0.14	25
Methanol	< 5 mg/L	< 5 mg/L
Sulfide	< 0.10 mg/L	0.17 mg/L
Iron	< 0.30 mg/L	< 0.30 mg/L
Manganese	0.031	1.2

The methane concentration for the methane column was near the saturation point of methane in water, (28 mg/L), consistent with what would be expected in an aquifer that had excess methane flowing through it. The reason for detectable methane in the control column is unclear, but may be a result of methane in the laboratory air. Methanol was not detected, although it is often observed in groundwater samples near wells that have been affected by methane. The detection limits of the analytical method for methanol were relatively high, and the lack of detection may reflect that relatively high detection limit. Hydrogen sulfide is the terminal product of reduction of sulfate, and sulfide was detected in the natural gas samples, and was also qualitatively detected by the characteristic odor. Neither the odor nor the chemical detection of sulfide was observed in the control columns. Iron was not detected in the methane columns, and the lack of iron present, despite the Postgate solution containing an iron compound, suggest that the iron was likely oxidized to ferric iron and precipitated in the control columns and likely precipitated as FeS in the methane columns. The elevated level of manganese detected in the methane column as compared to the control is consistent with provided evidence that under anaerobic conditions, manganese is reduced into a soluble form. In the presence of oxygen, manganese is also oxidized to the very low solubility, MnO_2. Manganese levels nearly 40 times higher in natural gas columns indicate that the conditions created by fugitive methane make it possible for manganese to be released from mineral surfaces beneath an aquifer. No manganese was added to the system, and it is believed that the pea gravel was the source of the detected manganese. Increased levels of manganese

have been measured in some water wells near hydraulic fracturing sites, which could potentially be caused by anaerobic conditions created by methane introduction.

In addition to sampling chemical species, information about bacteria present in the columns can provide information about metabolism and interactions that occur in the columns. Pictures of samples from the control column as well as the methane column were taken, with only the methane column showing significant bacterial growth. The anaerobic bacteria formed tetrads, and there was also evidence of biofilm formation. There were numerous rods (rod shaped) and cocci (spherical shaped) present, including endospore forming rods. A separate sample was sent to the University of Wisconsin for DNA analysis, but the results have not been received. Information about the bacteria and the micrograph below (Figures 5 and 6) were provided by Dr. Ruth Gault.
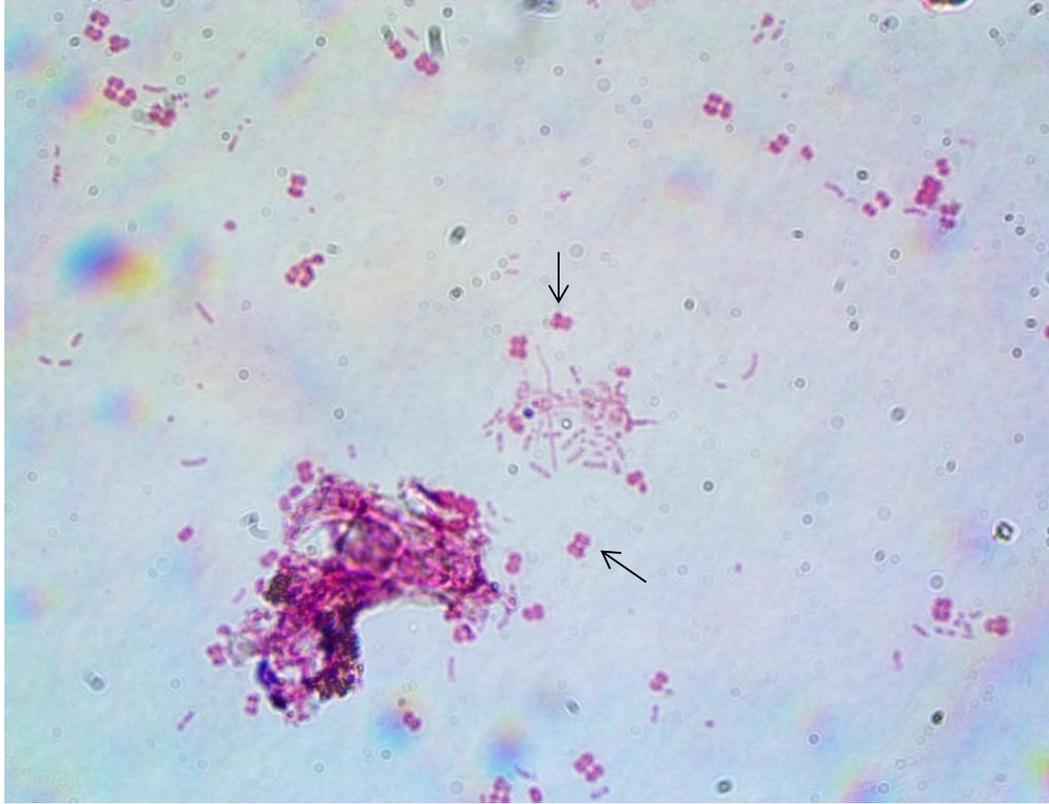
Figure 5. Bacterial growth in the methane column. Oil immersion was used for 1000X total magnification. A Gram stain was used on the bacteria. Tetrads are indicated by the arrow pointing to them. Photo courtesy of Dr. Ruth Gault
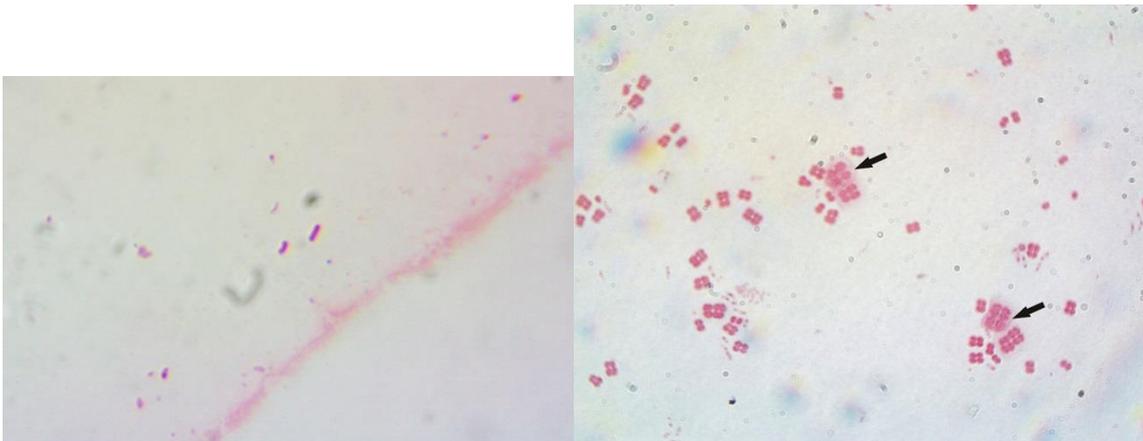

Figure 6: Control column (left) compared side by side with Methane Column (right). Arrows indicate the formation of a biofilm around the tetrads. The tetrads only appeared in the methane column. Both pictures were prepared using a Gram stain with oil immersion to give 1000X total magnification. Photo courtesy of Dr. Ruth Gault

Conclusion

These experiments suggest that sulfate reducing bacteria can utilize the carbon present natural gas, and cause reduction in redox potential in aquifer water, reduce sulfate to sulfide, and reduce and solubilize manganese oxides. While no primary drinking water standard exists for sulfide and only secondary standards exist for iron and manganese, the water quality can be severely degraded on a taste and odor basis. As hydraulic fracturing continues to increase oil and natural gas production in the United States, natural gas leakage is increasingly a concern. It is apparent from previous studies as well as the current study, that sulfate reducing bacteria can utilize methane while degrading the clarity and odor of groundwater. More studies such as these column experiments need to be performed and test various conditions that may influence the quality of groundwater. The column experiments were a first step in creating a linkage between anaerobic conditions leading to negative ORP values that allow sulfate reduction to be thermodynamically favorable. Although previous studies have made the assumption that sulfate reduction utilizing methane is occurring, the column experiments are the first direct evidence to support the process for fresh water. Examining the loss of sulfate in the column experiments with no carbon source available other than natural gas provides strong evidence to support sulfate reduction utilizing natural gas, which is primarily composed of methane. The loss of sulfate also indicates low redox conditions that are necessary to convert sulfate into hydrogen sulfide.

When comparing the column experiments to the results from the background study in Dimock, Pennsylvania, changes in the concentrations of sulfide, iron, manganese, and methane show similarities. The Dimock well had an increase in all four

of these measured species after fracturing had occurred, while the column experiments showed measurable sulfide, manganese, and methane in the natural gas columns. The lack of iron detected in the natural gas column can possibly be attributed to relatively high detection limits, microbial uptake, and iron precipitation as FeS.

More experiments are necessary to provide additional evidence that sulfate reducing bacteria can potentially have a negative effect on groundwater chemistry. The similarities present between the Dimock well and the column experiments show there is likely a connection between methane utilization by SRB and decreased water quality in drinking water wells. Expanding upon these initial column experiments will allow other analytes such as formate, iron, manganese, and sulfide to determined and help to evaluate whether or not they are affected by the anaerobic and low redox conditions that are created when SRB utilize natural gas to reduce sulfate. Ultimately, the importance of measuring and fully characterizing the natural gas wells prior to drilling for any kind of petroleum is clear. The source (biogenic vs. thermogenic) and concentration of methane should be clearly established prior to drilling, to determine whether the well drilling is responsible for degradation of odor, clarity, and taste that can occur in groundwater used as a domestic drinking source.

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