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Geochemical Control of Methanogenesis in Cape Lookout Bight, North Carolina

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To the Graduate Council:

I am submitting herewith a thesis written by Richard Kevorkian entitled "Geochemical Control of Methanogenesis in Cape Lookout Bight, North Carolina." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Microbiology.

Karen G. Lloyd, Major Professor

We have read this thesis and recommend its acceptance:

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Geochemical Control of Methanogenesis in Cape Lookout Bight, North Carolina

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ABSTRACT

Hydrogen exerts thermodynamic control over the exclusion of methanogens by sulfate reducers in Cape Lookout Bight, NC, marine sediments. This has been demonstrated by previous in situ measurements, but has never been demonstrated in a batch incubation of unamended sediments and has never been combined with identification of the microorganisms involved in this process. We made triplicate anoxic incubations of sediments from the upper 3 cm of sediment over 122 days while taking weekly samples for DNA extraction, cell counts, and measurements of methane, sulfate, and hydrogen. The headspaces of the bottles were initially gassed with nitrogen and the third was subsequently gassed with methane, although the methane disappeared within the first two weeks and after that the incubation served as a third replicate. While sulfate was present, the hydrogen concentration was maintained below 2 nM. Hydrogen started to rise as sulfate concentrations fell below 3 mM, coinciding with a small increase in methane. Only when sulfate was depleted and the hydrogen concentration started to rise, was methane continuously produced. Quantitative PCR (qPCR) suggested that Methanosarcinales and Methanomicrobiales increased when sulfate was depleted in all three incubations. 16s rRNA gene Miseq tag libraries supported the increase of these methanogens as well as a novel archaeal group, Kazan 3A-21, and sulfur-oxidizing bacteria. qPCR and tag libraries showed that the methanogen-like archaea, ANME-1, increased during early methanogenesis, but the values were near detection limits and were therefore noisy. The tag libraries suggested that sulfate-reducing bacteria maintained similar population levels throughout the sulfate reduction phase, decreased as sulfate was depleted, and then rebounded during the methanogenic phase. This most likely signified a switch from sulfate reduction to syntrophic fermentation of organic matter with methanogens. Total cell counts a declined with the decrease of sulfate until a recovery corresponding with production of methane. Our results suggested that competition for hydrogen influences what metabolic processes can occur in marine sediments and that a diversity of sulfate reducers and methanogens are involved in this competition.

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CHAPTER I

INTRODUCTION AND GENERAL INFORMATION

Methane is a globally important greenhouse gas. It traps twenty-one times more heat per molecule than carbon dioxide (CO_2) (EPA). Several hundred teragrams of methane are produced annually in marine sediments of which only a small fraction reaches the atmosphere (Reeburgh 2007). The degradation of organic matter by microbes is indirectly responsible for methane's production and subsequent destruction, as explained below.

As organic matter is deposited on the sea floor, microbes break it down into smaller carbon-containing molecules and H_2. In marine sediments depleted of oxygen the next most energetically favorable terminal electron acceptor is utilized. This is usually nitrate, sulfate, or manganese and iron species (Froelich et al. 1979). When these are no longer available then CO_2 can be used resulting in the production of methane gas by the process of methanogenesis. The spatial distribution of these electron acceptors based on their thermodynamic energy yield results in vertical zonation seen in many marine sediments resulting with potential energetic yield declining with depth (D'Hondt 2004).

The methane produced in deeper sediment horizons accumulates and will diffuse upward, or in very organic-rich sediments will ebullate to the surface rapidly. In many circumstances the methane diffusing toward the surface will never reach it as it is oxidized back to CO_2 (Martens & Berner 1977). This anaerobic oxidation of methane (AOM) occurs in the presence of sulfate reduction in an area of the vertical sediment profile labeled the sulfate/methane transition zone (SMTZ) and is believed to be carried out by archaeal members of the *Euryarchaeota* called ANME's (Anaerobic Methanotrophs) acting in a consortium with sulfate reducing bacteria (Boetius et al. 2000; Hinrichs et al. 1999).

Important to both reactions of sulfate reduction and methanogenesis is the presence of molecular hydrogen. It acts as an electron donor; four molecules of which are required for either CO_2 or sulfate, depending on the reaction. Its partial pressure in large part will dictate the energy to be gained in the reactions and potential favorability of one reaction over another (Hoehler et al. 1998; Hoehler et al. 1994; Hoehler et al. 2001). H_2 also controls AOM, since it will only occur when H_2 concentrations are low enough to make reverse methanogenesis energetically favorable.

In order to determine both the phylotype of microbes responsible for the production of methane as well as the role of H_2 in the competition between sulfate reducers and methanogens we created a microcosm experiment with marine sediments from Cape Lookout Bight, North Carolina (CLB). CLB is a natural methane seep with very high organic matter deposition rate and has been well studied in the past (Martens et al. 1998, Hoehler et al., 1994, Hoehler et al., 1998).

By utilizing a temporal analog for depth and terminal electron succession we were able to remove the muddying feature of the SMTZ and separate the experiment into two zones, the sulfate reduction zone and the methane production zone. The other advantage of our approach is that we could be certain what processes occurred because we could observe them in real time. Over the course of 122 days we measured the concentrations of H_2, methane, and sulfate on a weekly basis. In addition to the geochemical measurements, biological samples were taken to be analyzed by direct microscopy, quantitative PCR, 16s rRNA analysis.

The geochemical profile of this site is well established, it is our goal to build upon this data and add the biological component to it. We tested the hypothesis' that H_2 is responsible for the exclusion of methanogenesis by sulfate reduction, and that a diverse range of microorganisms are responsible for these processes.

CHAPTER II

BACKGROUND

Sulfate Reduction

Sulfur is one of the most abundant elements on Earth and is linked to both the carbon and the nitrogen cycle. It has a wide range of oxidation states, ranging from +6 to -2, oxidized to reduced. Sulfate, in the absence of oxygen, can be reduced to hydrogen sulfide (Equation 1) with H_2 as an electron donor. It is hydrogen sulfide that gives anoxic sediments their characteristic rotten egg smell. Sulfate reduction yields -151.9 kJ/mole of energy at standard state, which is considerably less than the several thousand provided by aerobic respiration (Thauer et al. 1977). The hydrogen sulfide can react with natural metals giving the sediments a black appearance or in some cases contributing to problems with corrosion in sulfate containing waters (Beech 2007). This is an important link in the global sulfur cycle, where the breakdown of organic matter provides the reductants to convert sulfate, typically at a concentration of 28 mM in seawater, to sulfide by microbes where it is returned to its mineral state in the form of pyrite (FeS_2), the most abundant form of sulfur.

$$4\ H_2 + SO_4^{2-} + H+ \rightarrow HS^- + 4\ H_2O \quad \text{(Equation 1)}$$

Methane Production

Methane is an important greenhouse gas. The atmospheric concentration of methane gas has risen to 1800 ppb from less than 800 ppb prior to the era of industrialization (IPCC 2007). The relative amount is not nearly as high as the 400 ppm of CO_2 composing some 0.04% of the Earths atmosphere by volume, but methane's potency warrants concern. The biological production of methane gas or methanogenesis, by anaerobic microbes is a globally important process in the carbon cycle. Methane is the terminal result of biomass degradation only in anaerobic environments that are low in other electron acceptors such as sulfate, nitrate, or reduced iron species.

Organic matter deposited in the oceans is used as a carbon source by microbes for the production of biomass. The complex polymers are broken down into smaller pieces until eventually methane is the final product. This process accounts for 74% or several hundred teragrams of methane production globally(Liu & Whitman 2008). Almost 2% of the net global photosynthetically fixed carbon ends up as methane (Thauer et al. 2008).

Dissolved CO_2, a product of fermentation, results in the production of methane (Equation 2) yielding -135.6 kJ/mol of energy at standard state (Thauer et al. 2008). This process is called hydrogenotrophic methanogenesis because of the use of H_2 as the electron donor with the reduction of CO_2. The net reaction of methanogenesis combines molecular hydrogen with CO_2 to yield methane and water:

$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$ (Equation 2)

A second type of methanogen utilizes acetate, an important intermediate in many biological pathways, to produce methane. This occurs by splitting the acetate molecule (Equation 3) and oxidizing the carboxyl group to CO_2 and reducing the methyl end to methane (Liu & Whitman 2008). This process is known as acetoclastic methanogenesis and yields -31 kJ/mol, considerably less than that of methanogenesis (Muyzer & Stams 2008).

$CH_3COOH \rightarrow CH_4 + CO_2$ (Equation 3)

The third type of methane production is called methylotrophic methanogenesis. This process strips a methyl group off of C1 carbon compounds (Equation 4) such as methanol and methylamines to produce methane (Poulsen et al. 2013).

$CH_3OH + H_2 \rightarrow CH_4 + H_2O$ (Equation 4)

Sediment Biogeochemical Zonation

Marine sediments harbor an amazingly diverse and unique set of organisms that thrive beyond the reach of oxygen (Parkes et al. 2005; D'Hondt et al. 2004). In non-chemolithoautotrophic systems sedimentary microorganisms utilize photosynthetically-derived organic matter that reaches it from terrestrial or marine photic zones.

The vertical zonation of redox reactions used by microbes in marine sediments is determined by the abundance, availability, and energetic yield of different electron acceptors (Froelich et al. 1979). The absolute depth where sulfate reduction gives way to methanogenesis is determined by the rate of sedimentation at that particular site. The complex organic matter that is deposited on coastal marine seafloors is derived either from terrestrial sources or from primary production in associated nutrient rich waters. A portion of the polymers is broken down into shorter chains and monomers that are used by microbes in the production of biomass. Underneath places of bioturbation or other vertical mixing, oxygen is rapidly depleted very close to the sediment/water interface resulting in anoxic conditions beneath that area (Martens et al. 1998). The energy production per mole of organic matter decreases with depth with the availability of oxidants (Froelich et al. 1979). Following oxygen, nitrate is the next best electron acceptor and then metal oxides such as iron and manganese (Figure 1). Sulfate is utilized next. Finally, microbes utilize CO_2 as an electron acceptor in the last step in organic matter degradation, which is methanogenesis.

This zonation is not limited to marine sediments, Kirk et al. (2004) reported its presence in ground water wells where ferric iron reduction preceded sulfate reduction vertically, with methanogenesis being the final zone reported. The competitive exclusion for electron donors in anoxic sediments creates distinct zones that can vary in depth and size representing a varying age of sediment that is

determined by the rate of deposition.

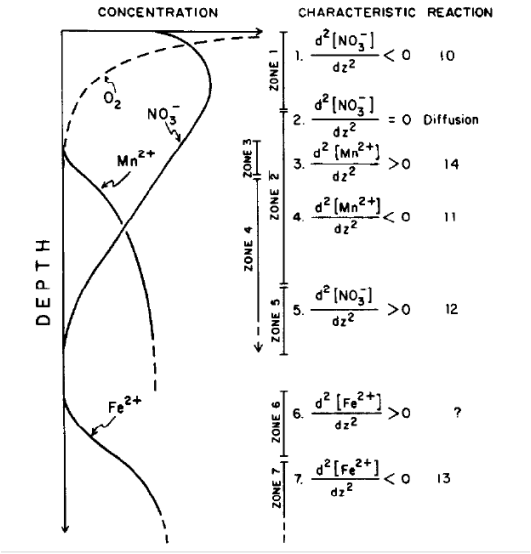


Figure 1: Hypothetical order of redox reactions expected with increasing depth in marine sediments. Reprinted from Froelich et al. 1979.

Exclusion of lower energy-yielding processes from each horizon may occur through one of two mechanisms. In the first, common substrates are brought to a concentration determined by the thermodynamic threshold of the higher energy yielding respiration. This makes the lower energy-yielding respiration unfavorable thermodynamically, when the relative concentrations of reactants and products are taken into account (Hoehler et al. 1998). The second mechanism is that kinetic barriers result in the observed zonation between sulfate reducers and methanogens (Bethke et al. 2008). In the kinetic barrier method methanogens are excluded by their slower growth yield relative to sulfate reducers.

H_2's role in driving redox zonation is not clearly understood. It is however a critical component of a large number of microbial processes. Hydrogenases are class of enzyme that can be found in all domains of life (Vignais & Billoud 2007). These enzymes separate H_2, yielding two charged protons and two electrons. H_2 is usually the limiting substrate in a reaction because it often has stoichiometries greater than one (see equations above) and therefore its relative concentrations play a key role. The metabolic process of fermenting large carbon structures creates large amounts of H_2 as a product. However, if H_2 concentrations were allowed to build up locally then it would make the very process of fermentation unfavorable by a buildup of products against the reactants disturbing the equilibrium. Therefore, hydrogen-consuming reactions operate to restore the balance and keep H_2 levels low with the caveat that too low would then make the redox reactions unfavorable for the respiring organisms.

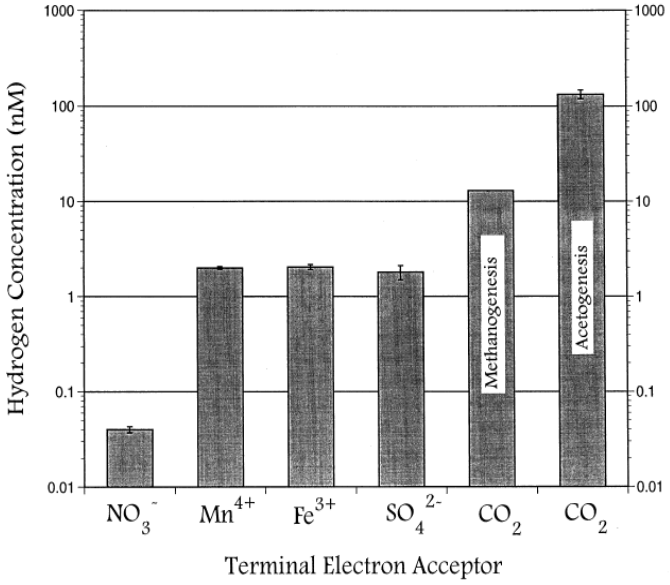


Figure 2: The theoretical effect of various electron acceptors on hydrogen concentration. Reprinted from Hoehler et al. 1998.

This gives rise to a balance between production and consumption of H_2 that is determined by the strength of the terminal electron accepting process this creates a thermodynamic exclusion of lower energy yielding redox reactions. Figure 2 illustrates the theoretical effect various electron acceptors have on hydrogen concentrations (Hoehler et al. 1998). For example, the H_2 concentration would be 1.6 nM for sulfate reduction and 13 nM for methane production in order to yield the same -20 kJ/mole for each reaction (Hoehler et al. 1998). This excludes methanogenesis from occurring in the area of sulfate reduction, as the hydrogen available is too low in concentration for it to be used by methanogens. *In situ* observations support the theory that only after sulfate has been depleted and the H_2 concentration rises to a level that becomes energetically feasible for methanogens to use it can methane production occur (Hoehler et al. 2001).

Sulfate Reducing Bacteria

Sulfate reducing bacteria (SRB) are a group of microbes that use dissolved sulfate as a terminal electron acceptor in their central metabolism. All microbes require sulfur compounds as components of cellular machinery and many acquire them from a process called assimilatory sulfate reduction (Muyzer & Stams 2008). However, only SRB and some thermophillic archaea perform dissimilatory sulfate reduction. In this process microbes use the redox potential of sulfate in their respiratory metabolism and do not incorporate this sulfur into their biomass. A key

way to identify sulfate reducers is by the identification of the functional gene *dsrAB* that encodes the dissimilatory sulfite reductase, a key enzyme in the pathway (Wagner 1998). Another way to identify them is that all cultured mesophilic sulfate reducers are members of the *Deltaproteobacteria* and *Firmicutes*, and relatives of these can be observed in marine sediments (Lloyd et al. 2006). SRB's have been shown to play a role in the global carbon cycle by utilizing a plethora of carbon sources from acetate and fumurate to long chain fatty acids, aromatic compounds, and short chain alkanes (Muyzer & Stams 2008; Kniemeyer et al. 2007). These carbon sources are broken down and made more bioavailable to other microbes or in some cases, are oxidized completely back to CO_2.

Many SRB can also utilized other electron acceptors such as Fe(III) and As (VI) (Muyzer & Stams 2008). In the absence of sufficient electron acceptors many SRB can also ferment organic acids and alcohols resulting in acetate, H_2, and CO_2; so they are not limited to sulfate reduction as their sole source of energy (Plugge et al. 2011). In the Black Sea, sulfate-reducing taxa of *Desulfobacteraceae* in sediment layers depleted of sulfate have been reported (Leloup et al. 2007). Immediately after sulfates depletion SRB could be utilizing the cryptic sulfur cycle (Holmkvist et al. 2011). After cryptic sulfur cycling has ceased SRB may participate in fermentation. Many of the fermentation products such as H_2, CO_2, and acetate can be channeled directly to a methanogen, removing waste products and keeping the redox potential high (Schink et al. 2006).

Methanogens

Methanogens are a phylogenetically diverse group found primarily in the *Euryarchaeota* branch of archaea (Thauer et al. 2008). There are seven orders of methanogens in the archaeal domain: *Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales, Methanopyrales, Methanoplasmatales,* and *Methanomassiliicoccus* (Dridi et al. 2012; Paul et al. 2012). Methanogens can be found in a wide variety of anaerobic environments. Anywhere rich in organic matter and low in oxygen and sulfate could potentially harbor methanogens. They can be found in the guts of ruminants and termites as well as waste treatment plants and landfills (Ferry 1999). The widest geographical distribution of methanogens is in wetlands, and ocean sediments. Methane is produced as part of their central metabolism as a result of many unique and complex enzymes and cofactors, with a critical final step mediated by a nickel-containing enzyme, methyl coenzyme M reductase (Hallam et al. 2003; Friedrich 2005; Ferry 1999).

Many of methanogen groups are hydrogenotrophs that reduce CO_2 with H_2 serving as the electron donor (Liu & Whitman 2008). Hydrogenotrophic methanogenesis is autotrophic and only require salts and trace metals in addition to CO_2 and H_2 to produce energy and biomass (Offre et al. 2013). There are several cultured hydrogenotrophic methanogens including *Methanosarcina barkeri, Methanospirillum hungatei, Methanobacterium bryantii,* and *Methanococcus voltaei* differing in cell shape and size, but usually between 1-2 μm in size (Demirel & Scherer 2008).

Acetoclastic methanogens gain energy by converting acetate into methane.

As much as two thirds of microbially-produced methane is generated from acetate (Liu & Whitman 2008). Only two genera, *Methanosarcina* and *Methanosaeta,* are known to contain acetoclastic methanogens within the metabolically diverse order of *Methanosarcinales*. Groups of cultured acetoclastic methanogens include *Methanosaeta thermophilia and Methanosaeta concilii* (Demirel & Scherer 2008).

The recently characterized *Thermoplasmata* have been implicated as methylotrophic methanogens by dismutating methylated compounds such as methanol into methane (Paul et al. 2012). *Thermoplasmata* in the gut of ruminates have been found to utilize methylamines as well in their methanogenic pathways (Poulsen et al. 2013).

Methanogen-like

Anaerobic Methanotrophs (ANMEs) are a non-monophyletic collection of Euryarchaeota, in the class of *Methanomicrobia*, which have been shown to oxidize methane aerobically (Figure 3) (Hinrichs et al. 1999). ANMEs are thought to perform AOM with by SRB by passing electron equivalents between the two. An example of a bacterial partner are members of *Deltaproteobacteria* either *Desulfosarcina* or *Desulfococcus* (Boetius et al. 2000; Knittel & Boetius 2009). ANMEs have been found to exist in sediments where AOM has been shown to occur, as well as methanogenic sediments (Knittel & Boetius 2009). ANMEs can be found widely distributed in locations lacking a well-defined SMTZ such as methanogenic zones, cold methane seeps in coastal sediments, methane hydrate zones, hydrothermal vents, and even potentially in the anoxic water column in the Black Sea (Knittel and Boetius 2009). This process is characterized as the reversal of the methanogenic pathway, with methane being oxidized back into CO_2.

AOM has been demonstrated to operate favorably under the low H_2 concentrations maintained by sulfate reducing bacteria (Knittel & Boetius 2009; Hoehler et al. 1994). However, H_2 as the electron shuttling molecule has not been universally accepted and the identity of the intermediate molecule is still under debate. Alternatively, electrons may be transferred directly to methanogens by way of conductive nanowires connecting the two microbes (Reguera et al. 2005). Understanding how this occurs and what microbes are responsible for it is important for determining the plasticity of this cap on natural methane emissions.

Site Selection

Cape Lookout Bight (CLB) is a shallow barrier island that traps organic matter from a lagoon and has very organic rich sediments (Figure 4). The average sediment deposition rate is about 10 cm per year, which is very high (Alperin et al. 1992; Canuel et al. 1990; Chanton et al. 1983). In comparison, the sedimentation rate of the Baltic Sea, another active depositional environment is 0.14 cm per year (Muller 1978). Below 2mm sediments are anoxic and free of bioturbation (Canuel & Martens 1993). Sulfate reduction occurs in sediments while sulfate is present, the

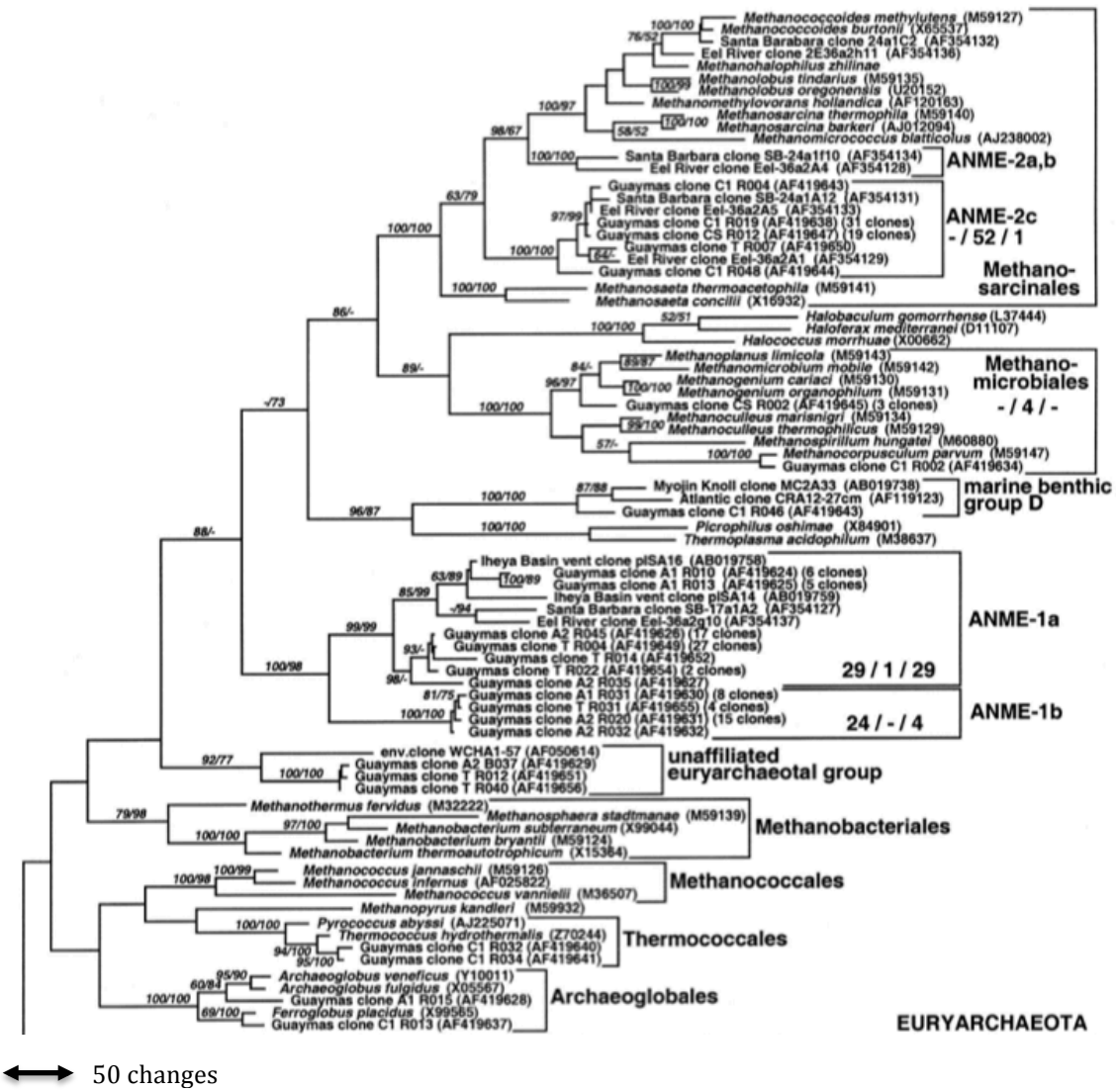


Figure 3: Distance tree of archaeal clones from Guaymas sediment cores based on 16S rDNA gene sequences. Bootstrap values (in percent) based on 1,000 replicates each (distance and parsimony) are shown in branches with more than 50% bootstrap support. (Reprinted from Teske et al. 2002)

penetration of which changes seasonally between 5 cm in the winter and 12 in the summer (Hoehler et al. 1994). Methane production only occurs at depths below that

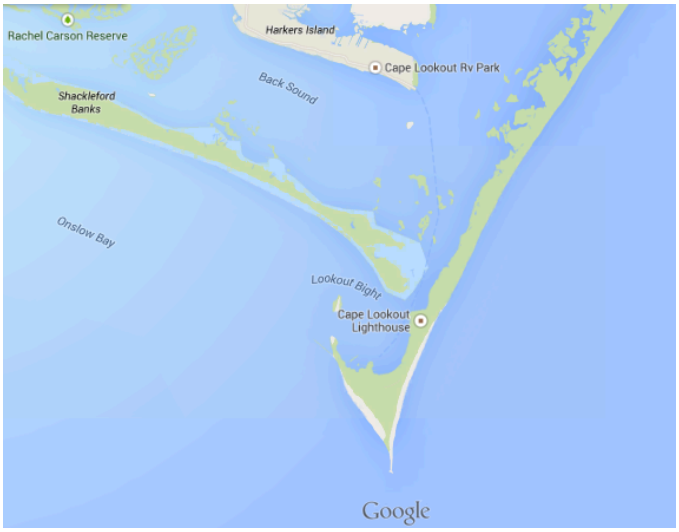


Figure 4: Location of Cape Lookout Bight, NC; including the lighthouse. Google Maps

of sulfate's presence. Production of methane occurs at a shallower depth in summer due to sulfate depletion occurring at a deeper depth in the winter than summer dependent on sediment temperature (Klump & Martens 1989). When methane concentrations in the porewater exceed methane's saturation point it can ebullate out forming a gas bubble. Rates of oxidation of methane at the site represent a fraction of the total methane production and no net AOM is observed (Hoehler et al. 1994). In a previous survey of CLB, sulfate reduction declined from an initial rate of 900 μM/d and methane production began at 10 cm beneath the sediment at a rate of about 300 μM/d (Alperin et al. 1992). Turnover rates of H_2 in the methanogenic zone of CLB were found to be between 3-5 nM/s, with partial pressures in the methanogenic zone below 13 nM (Hoehler et al. 2002). Figure 5 illustrates the correlation between sulfate and H_2 in the sediment column. While sulfate is present above 8 cm in depth, H_2 is kept to below 1 nM in both CLB profiles. Only after sulfate is depleted do H_2 concentrations rise to about 15 nM. This data correlates with what would be expected for H_2 while in association with either sulfate reduction or methanogenesis. As previously determined by the geochemistry measured from CLB sediments the energy available from methanogenesis was about -10 kJ/mol and no less than -19 kJ/mol for sulfate reduction (Alperin et al. 1992, Hoehler et al. 1993). Sediment Incubations from CLB have been performed in the past measuring geochemistry, but not H_2 or analysis of the microbial community. A temporal analog for depth was created by Alperin et al (1992) in a closed incubation. Very small amounts of AOM were detected, but were only a small fraction of the total methane

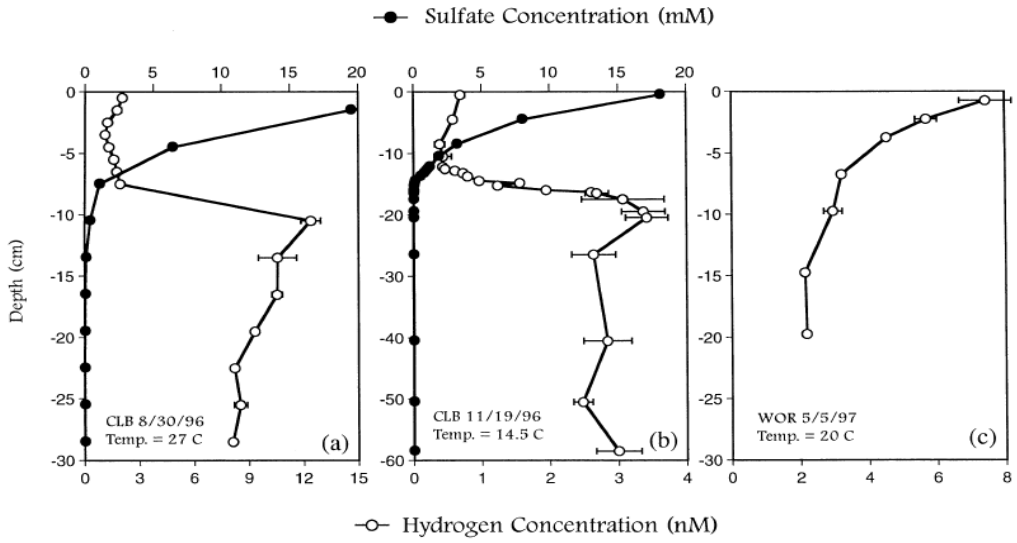


Figure 5: Hydrogen concentration vs. sediment depth. (a, b) Cape lookout Bight, August and November respectively. (c) White Oak River. Note the different scales used. Reprinted from Hoehler et al 1994.

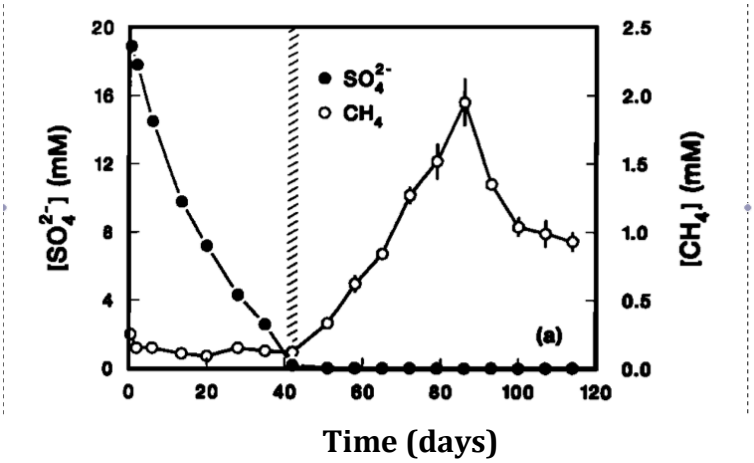


Figure 6: Geochemical data from an incubation of Cape Lookout Bight sediments with the concentrations of sulfate and methane measured over time. Methane is only being produced after sulfate is depleted after 40 days. Reprinted from Alperin et al. 1992

production. The benefits of this system are that samples can be taken in real time as opposed to using vertical spacing to infer rates over depositional periods, giving greater confidence in process measurements.

CHAPTER III

Hypothesis

Question 1: Are sulfate reduction and methane production zones separated vertically in Cape Lookout Bight as determined by thermodynamic competition for hydrogen?

- Hypothesis 1.1: Hydrogen concentrations can be measured in situ using an equilibration method.
- Hypothesis 1.2: Methane concentrations are low in the sulfate reduction zone.
- Hypothesis 1.3: Hydrogen concentrations will be held to the thermodynamic minimum as determined by zone.

Question 2: Does the separation of sulfate reduction and methanogenesis over time in a microcosm mimic diagenesis, and is it consistent with thermodynamic competition for hydrogen?

- Hypothesis 2.1: Methanogenesis will only occur after sulfate is depleted.
- Hypothesis 2.2: Hydrogen concentrations will be in equilibrium at the thermodynamic limit for sulfate reduction during sulfate reduction.
- Hypothesis 2.3: Hydrogen concentrations will be in equilibrium at the thermodynamic limit for methanogenesis during methanogenesis.

Question 3: What types of sulfate reducing bacteria participate in sulfate reduction, and are the able to switch to fermentation during methanogenesis?

- Hypothesis 3.1. Relative amounts of 16S rRNA gene sequences closely related to cultured SRB will decrease at the point of sulfate depletion.
- Hypothesis 3.2: Relative amounts of 16S rRNA gene sequences closely related to cultured SRB will increase during methanogenesis, as they form syntrophies with growing methanogen populations.

Question 4: What types of organisms increase in abundance during methanogenesis, indicating either a direct or indirect participation in the process?

- Hypothesis 4.1: Relative amounts of 16S rRNA gene sequences closely related to cultured methanogenic archaea will increase with methane concentrations.
- Hypothesis 4.2: Relative amounts of 16S rRNA gene sequences not closely related to cultured methanogens will also increase during methanogenesis. This is likely to include the ANME-1 and ANME-2 groups, which have been shown to contain the critical enzymes necessary for methanogenesis.

- Hypothesis 4.3**:** Relative abundance of 16S rRNA gene sequences from some groups of uncultured archaea and bacteria will change through the course of the incubation indicating their direct or indirect participation in methanogenesis.

CHAPTER IV

MATERIALS AND METHODS

Hydrogen Equilibration

In situ porewater hydrogen concentrations were measured by using an equilibration method adapted from Hoehler et al. (1998). Sediment cores were taken from the Tennessee River and White Oak River Estuary. Five milliliters of sediment collected using a cutoff syringe were inserted into a 10 ml glass vial and capped with a butyl stopper and the headspace gassed out with oxygen scrubbed nitrogen gas and a second had a small amount of hydrogen gas added. The depth segment of the WOR used was 21-30 cm and the Tennessee River was between 15 and 30cm. The concentration of hydrogen was measured hourly for the first five hours then daily over six days. 500 µl of gas from the headspace was removed using a glass syringe and measured on a hydrogen analyzer (Peak Performer).

Sampling site

Samples were collected with SCUBA divers at Cape Lookout Bight, North Carolina (34.6205° N, 76.5500° W) in October of 2013. Thirty PVC push cores of 8 inches in length as well as a one-meter core were collected, capped, refrigerated, and then returned to the lab in Tennessee within 48 hrs. Using a plunger inserted from the bottom the first three cm of sediment taken from each core was placed in a 2 L Erlenmeyer flask by way of a funnel. About ten core tubes were needed to fill each of the three flasks to 1.5 liters of sediment.

Cape Lookout Bight Depth Profile

The one-meter sediment core collected from CLB was used for describing the geochemistry of the site. The core was sectioned in three centimeter increments measured from the top down. For determination of methane concentrations in situ 4 ml of sediment was placed in a 26 ml glass vial using a cut off syringe. 1ml of 0.1 M KOH was added with a butyl rubber stopper to cap the vial and then shaken to mix the base with the sediment. 100 µl of the headspace was removed and injected into a gas chromatograph with a flame-ionized detector (Agilent) for measurement of methane. Total cell counts were determined by SYBRGold direct microscopy as described below.

Sulfate was measured by filling up a 15 ml falcon tube completely with sediment. The tube was then centrifuged at 5,000-x g for five minutes and using a syringe the supernatant was removed avoiding the uppermost layer that had been exposed to air. This was then filtered through a 0.2 µm syringe filter into an Eppendorf tube containing 100 µl of 10% HCl to drive off sulfide. The sample was then measured on an Ion Chromatograph.

Porosity was determined by adding 3ml of sediment to a pre-weighed vial allowing it to dehydrate over several weeks in an oven.

Incubation

Each of the three flasks was fitted with a custom butyl rubber stopper with a hole drilled through the center to accommodate a glass and Teflon stopcock (with a six mm bore stopcock) for the removal of samples. Two 18-gauge needles with steel stopcocks were inserted into the stopper as well. Using the luer-lock fitting on the needles, ultra high purity nitrogen gas that had been scrubbed of oxygen using heated copper fillings was flowed through the bottles and the second needle was the outflow. Once the system was determined to be anoxic, all the ports were closed and the flasks inverted and placed in a ring stand and kept at constant room temperature of 20°C in the dark.

Samples were taken every seven days using the glass stopcock; about 32 ml of sediment was removed using a 60 ml plastic catheter tip syringe. Using the syringe, two 15 ml conical centrifuge tubes were filled, one used for porewater analysis and the other frozen at -80°C for later molecular analysis. One ml of sediment was placed in a 2 ml screw cap tube to be fixed as described below and used for cell counts as described below. Hydrogen and methane gas samples were taken using the steel needle ports. Prior to gas sampling 2 ml of anoxic N_2 gas was used to blow the needle clear of sediment. After sampling, 30 ml of O_2 scrubbed N_2 was injected into the bottle to replace the lost volume.

Sulfate was determined in the same manner as described above, with the remaining porewater used to for determining the pH.

500 µl of headspace gas was injected into a hydrogen analyzer (Peak Performance) to determine the concentration of hydrogen in the headspace. Premixed standard hydrogen ppm lab bottles were purchased from Sigma-Aldrich. Methane was determined by using injected 500 µl of gas from the headspace into an evacuated glass bottle to be later analyzed on a gas chromatograph with a flame ionized detector (Agilent). A fresh dilution series of methane gas was created for use as standards every measurement cycle.

Microscopy

Fresh sediment of 1 ml in volume taken from the catheter syringe and 500 µl of 3% paraformaldehyde in PBS were vortexed together in a 2 ml O-ring cap tube, and then refrigerated overnight. Then the tube was centrifuged at 3000 xg for ten minutes, and supernatant was removed. The sample was washed with PBS and then stored in 1:1 PBS:EtOH at -20°C; these samples were to be used for total cell counts as well as CARDFISH microscopy. A working dilution of the samples was made by diluting to 1:40 and sonicating at 20% power for 40 seconds on ice to break apart disaggregate cells from sediment.

Total cell counts were determined using a Leica epifluorescence microscope with SYBRGold (Invitrogen) DNA stain. 20 µl of the sample were added to 5 ml of PBS with 500 µl of 5x concentration SYBRGold at room temperature in the dark for 10 minutes. The samples were then filtered onto a 0.2 µm polycarbonate filter (Fisher) and mounted onto a slide with Citiflour antifadent (Fisher).

Quantification of specific taxa from the samples was determined by direct microscopy using CARD-FISH analysis adapted from (Pernthaler et al. 2002).

Diluted samples were put onto 0.2 µm polycarbonate filters and placed face down in a 100 µl dot 0.1% warm low melting point agar on parafilm then incubated for 30 min at 46 °C. Each filter was then cut into pie shaped wedges with a scalpel and labeled with a pencil with the probe to be used.

The filters were washed in 0.01M HCl for 10 minutes to remove endogenous peroxidases and rinsed with sterile water. A permeabilization solution was made for archaeal probes using 10 mg/ml of Proteinase K and for bacterial probes a 10mg/ml solution of lysozyme was used. The filters were incubated in the permeabilization solution for 30 min and rinsed in water. The hybridization buffer was made for each probe by adding 1 µl of probe with 299 µl of 35% formamide buffer. Filter wedges were placed on respective probe solutions on a parafilm covered slide and the extra solution pipetted onto kimwipes and placed beneath the slides inside a horizontal 50 ml falcon tube to act as a hybridization chamber. The chambers were incubated at 46°C overnight.

A wash buffer was made fresh and heated to 2°C above the hybridization temperature. The filter wedges were removed from the chamber and placed in the wash buffer for five minutes. The samples were then incubated in the amplification solution containing a fluorescently labeled tyramide for 30 min in the dark at 37 °C. The samples were counterstained with DAPI DNA dye and mounted on class slides and stored in the dark at -20°C.

Table 1: HRP labeled probes utilized for CARDFISH analysis.

Probe	Sequence	Target	Reference
ARCH915	GTG CTC CCC CGC CAA TTC CT	Archaea	Stahl & Amann 1991
EubI-III	GCA GCC ACC CGT AGG TGT and GCT GCC ACC CGT AGG TGT and GCT GCC TCC CGT AGG AGT	Bacteria	Daims et al. 1999
ANME1-350	AGT TTT CGC GCC TGA TGC	ANME-1	Boetius et al. 2000
EelMS932	AGT TTT CGC GCC TGA TGC	ANME-2	Boetius et al. 2000
DSS658	TCC ACT TCC CTC TCC CAT	Desulfosarcina	Manz et al. 1998
MG1200	CGGATAATTCGGGGCATGCTG	Methanomicrobiales	Raskin et al. 1994
MCG493	CTTGCCCTCTCCTTATTCC	Miscellaneous Crenarchaeotal Group	Kubo et al. 2012
MCG410	TCCGCTGAGGATGGCTTTT	Miscellaneous Crenarchaeotal Group	Kubo et al. 2012

Quantitative PCR

Sediment samples frozen at -80°C were used for analysis by extracting DNA using the Fast DNA kit for Soil (MP Bio), which offered favorable yield over the MoBio Power Soil Kit. Quantitative PCR was used to determine copy numbers of several taxa by using 16s rRNA genes with Qiagen's Quantifast SYBRGreen kit on a BioRad IQ5 machine. DNA standards were prepared either from existing stocks (Lloyd et al. 2011) or from TOPO plasmids (Invitrogen) containing PCR amplified inserts of closely related relatives of the environmental sample. DNA standards

were quantified using Hoechst dye in a flourimeter (Hoefer). For quantification of archaea, primers A915f (DeLong, 1992) and A1059r (Yu et al., 2005) were chosen for their broad coverage. Bacteria primers used were B340r and B515f (Nadkarni et al. 2002). ANME-2 primers were ANME-2-240f and ANME-2-538r (Boetius et al. 2000). ANME-1-830r (Boetius et al. 2000) and ANME-1-628f (Lloyd et al. 2011). The archaeal order of *Methanomicrobiales* was represented by the primers A110f and MG1200r (Narihiro & Sekiguchi 2011), these primers have good coverage of the taxa, but exclude ANMEs and a standard was synthesized by Invitrogen for our use. *Methanosarcinales* were represented by MSMX860f and A1100r primers (Narihiro & Sekiguchi 2011) that broadly cover the taxa while excluding the ANMEs. For the Methnanosarcinales DNA standard the qPCR primers were used to amplify DNA extracted from the CLB incubation, and verified by sequence. The amplified fragments were then inserted in a TOPO plasmid (Invitrogen), which then was amplified using the ONESHOT Competent cells (Invitrogen). The amplified plasmid was extracted for use as a standard in a dilution series. All measurements were quantified with a simultaneous DNA standard.

Table 2: Real time PCR primers used for taxonomic quantification.

Primer Name	Sequence	Target
A915f	GTGCTCCCCCGCCAATTCCT	Archaea
A1059r	GCCATGCACCWCCTCT	Archaea
B340r	TCCTACGGGAGGCAGCAGT	Bacteria
B515f	CGTATTACCGCGGCTGCTGGCAC	Bacteria
ANME-2-240f	CTATCAGGTTGTAGTGGG	ANME-2
ANME-2-538r	CGGCTACCACTCGGGCCGC	ANME-2
ANME-1-830r	TCG CAG TAA TGC CAA CAC	ANME-1
ANME-1-628f	GCT TTC AGG GAA TAC TGC	ANME-1
A1100f	TGGGTCTCGCTCGTTG	Methanomicrobiales
MG1200r	CGGATAATTCGGGGCATGCTG	Methanomicrobiales
MSMX860f	AGGGAAGCCGTGAAGCGCC	Methanosarcinales
A1100r	TGGGTCTCGCTCGTTG	Methanosarcinales

16S Ribosomal RNA Gene Amplicons

Fresh DNA extract from every time point was extracted using FastDNA kit for soil (Mp Bio) from frozen stocks. Negative controls of sterilized sediment and a blank water extract were used as well. The samples were then transferred to the Center for Environmental Biotechnology at the University of Tennessee in Knoxville for library prep, clean up, and sequencing using an Illumina MiSeq. The V4 region primers used were the 806r (GGACTACHVGGGTWTCTAAT) and 515f (GTGCCAGCMGCCGCGGTAA) developed by Caporaso et al. (2012) as a universal primer pair for Bacteria and Archaea which were shown to have good coverage on the Silva database. The reads were analyzed using the mothur platform (Schloss et al. 2015) and normalized against the total reads per sample.

CHAPTER V

RESULTS AND DISCUSSION

Hydrogen Equilibration

The H_2 equilibration method is based on the theory that the microbes present in the subsample of sediment will return the headspace to their equilibrium concentration of H_2 by balancing the production of H_2 by fermenters and its consumption by respiratory organisms. When the headspace was gassed with very high H_2 concentrations stable equilibrium values were not observed with the exception of the TN River, which reached stable equilibrium within five hours (Figure 7-A). Figure 7-B shows that a 5-hour incubation was sufficient for reaching an equilibrium value that remained stable for many days afterward. In both the Tennessee River and the White Oak River estuary, adding H_2 increased the time to reach equilibrium values between 0.3 nM to 1nM. The controls remained constant below 0.1 nM for TN river 1, TN river 2 controls stayed at 29 nM, and the WOR controls at 1.5 nM. The use of non-H_2 amended gas provided better results as the measurements were within the range of detection.

In Cape Lookout Bight sediments, sulfate declined from 14 mM within the first 3 cm, is depleted and remains below 1 mM at about 9 cm beneath the seafloor (Figure 8). Methane was present in the whole column and began to increase after sulfate is depleted. It is likely that these measurements were underestimations since gas ebullition was visible during sampling. H_2 concentration declined from 2 nM to 0.5 nM by the first 3 cm. This depth profile did not match that described by Hoehler et al 1994. The H_2 concentrations in a previous study (Hoehler et al. 1994) with depth were also described in Figure 5 and did not match the data we collected. An initial H_2 concentration of 4 nM was seen in the sulfate reduction zone by and rose to 15 nM when transitioning to the methanogenic zone (Hoehler et al. 1998). The H_2 profile did match that by Hoehler et al (1998) for the WOR. A possible explanation for this discrepancy could be if the pH decreases with depth then this would result in the decrease of molecular hydrogen concentrations (Hoehler 1994).

Total cellular abundance generally declines with depth at Cape Lookout Bight (Figure 9). From an initial population of 1.3 $x10^9$ cells/gram wet sediment at the shallowest to 5.0 $x10^8$ cells/ gram at the deepest point. This is consistent with expectations as the availability of labile carbon sources and terminal electron acceptors declines with depth and by extension time.

Incubation Geochemistry

All three replicate incubations showed similar trends in sulfate with concentrations falling below 1 mM by about 68 days from the start of the incubation. For the incubations, the first 68 days will be referred to as the sulfate reduction zone, and the time following that the methane production zone. After 68 days the sulfate concentrations increased a small amount in all three incubations, and stayed at <1.5

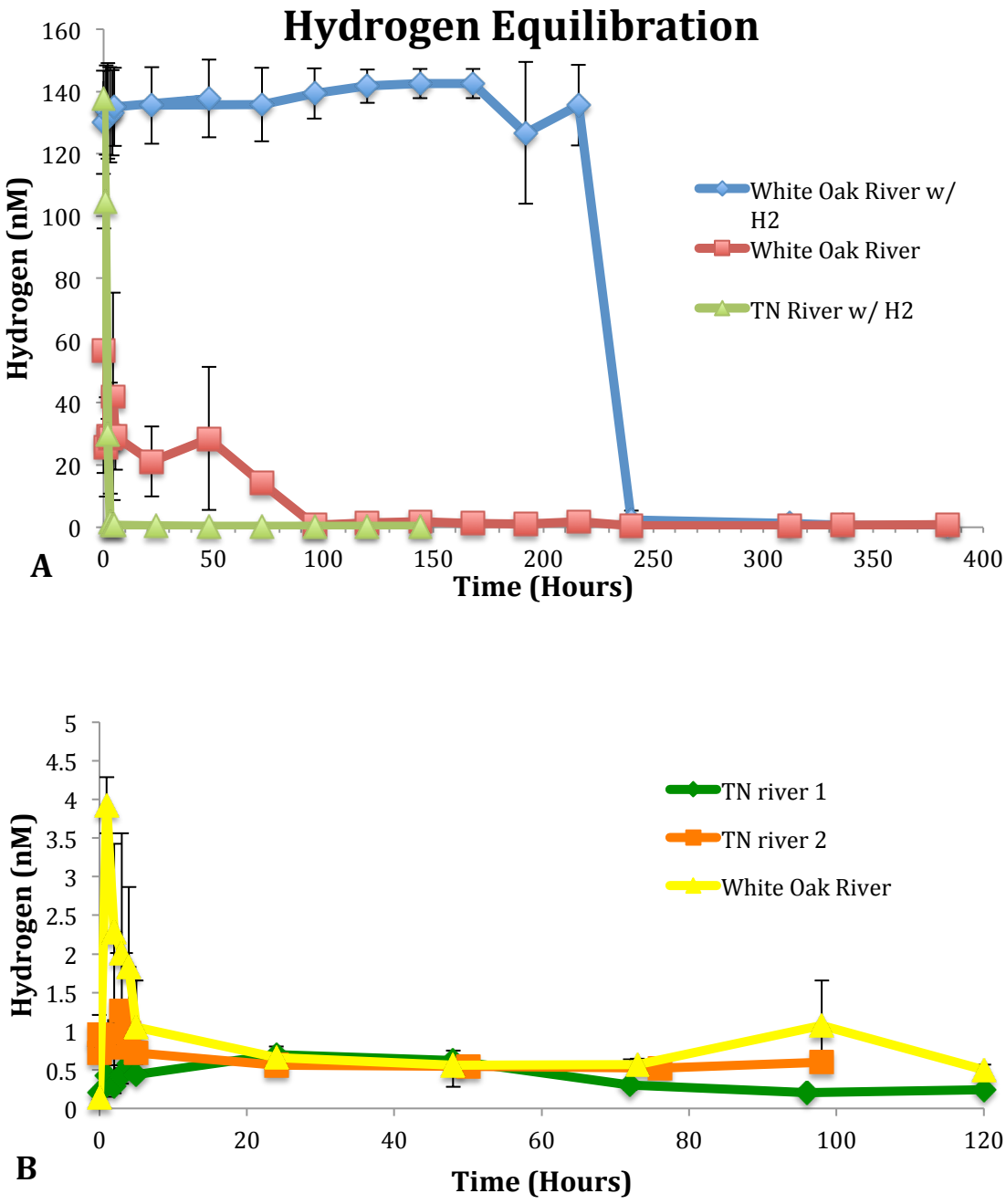


Figure 7(A, B): Equilibration trials of In Situ hydrogen concentrations in various aquatic sediments. Hydrogen equilibration is achieved within four hours at a value of 0.34 nM (A) for the Tennessee River sample and ten days for the White Oak River when Hydrogen is added. In the WOR where little hydrogen was added, within the measurable range of our equipment, the sample took less than 4 days to equilibrate. With hydrogen added the controls were consistently above detection limit. Samples from the Tennessee River (B) as well as a sample taken from the WOR, each gassed with pure N_2 reached equilibration at about 0.7 nM within 24 hours after resetting the headspace. Samples were measured in triplicate and error bars represent one standard deviation. Control for TN river 1 was constant at 0.09 nM, White Oak river at 1.5 nM, and the TN river 2 was 29 nM.

Sample Site Characterization

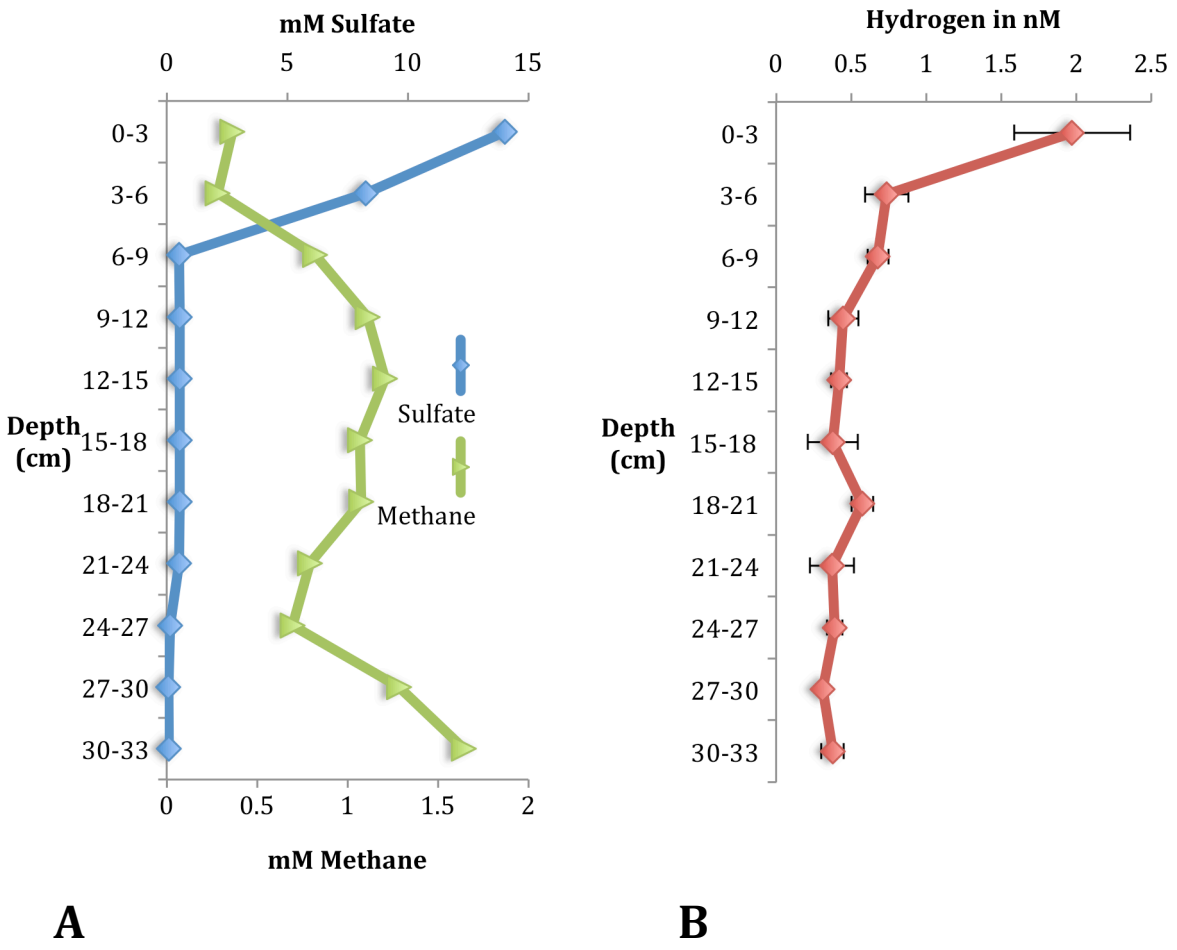


Figure 8 (A, B): Geochemical profile of sediment core from Cape Lookout Bight. Porewater analysis of sulfate and methane concentrations (A) as well as hydrogen (B); samples were taken in October 2013. Error bars represent triplicate equilibration measurements.

mM for the rest of the incubation. This could be a result of a few things. Oxygen leaking into the bottle during sampling could cause some of the hydrogen sulfide formed by sulfate reduction to be oxidized back to sulfate. But, that as the sulfate does not continue to increase it remains a mystery.

The methane for both of the anaerobic incubations started out about 0.1 mM in the headspace. This was possibly a remnant of dissolved methane in the porewater that came out of solution after the headspace was replaced with nitrogen gas. The concentration of gaseous methane remained below 0.05 mM during the time that

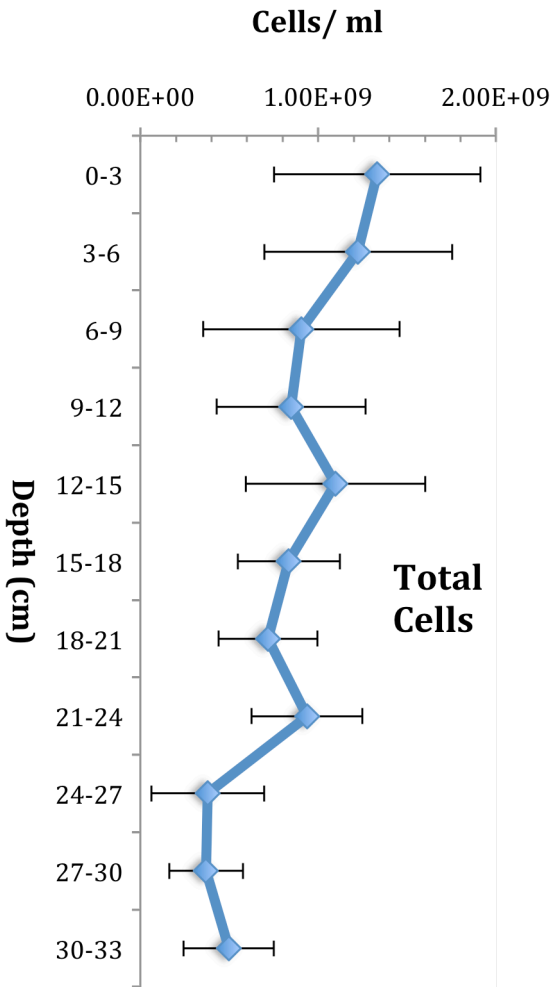
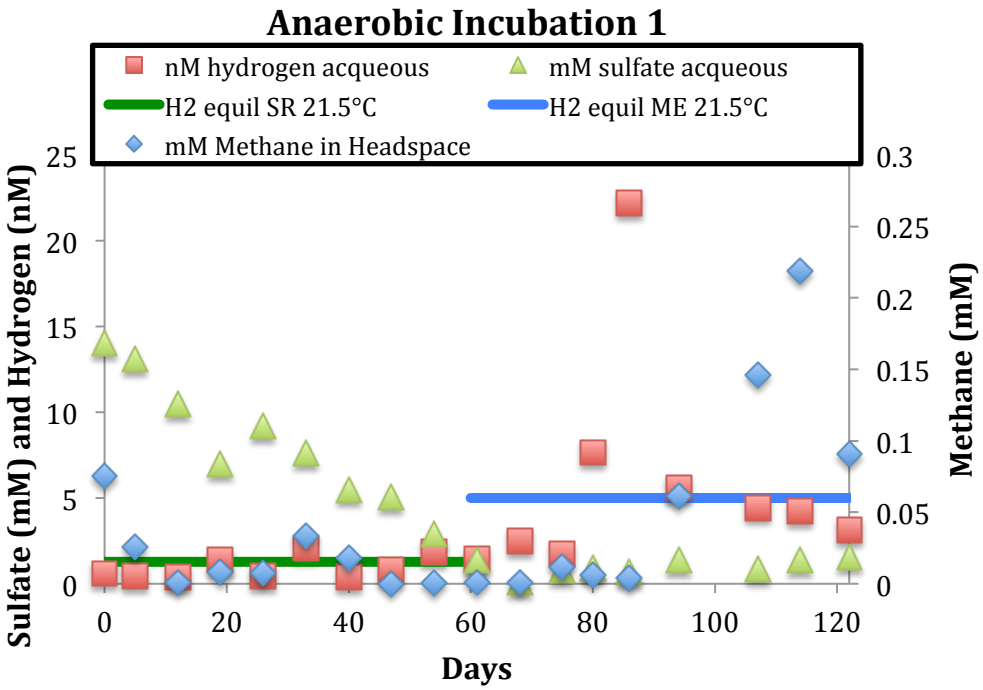
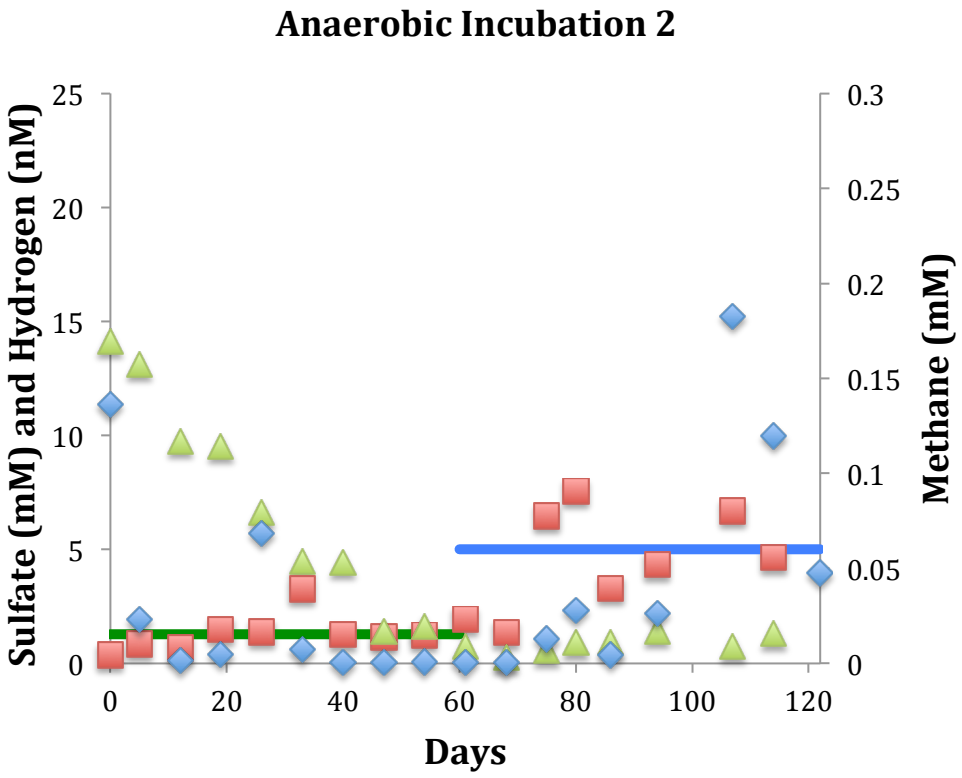


Figure 9: Total cells per ml of sediment at Cape Lookout Bight with depth. Concentration was determined by direct count fluorescence microscopy using SYBRGold DNA stain**.** Error bars represent standard deviation.

Figure 10: Sulfate, hydrogen, and methane data for the replicate incubations. Starting headspace for incubations A) and B) was oxygen-scrubbed nitrogen gas and for C) was methane gas. Note that for the methane spiked bottle the first two methane data points are not plotted (see discussion in text). The hydrogen equilibration values for sulfate reduction (SR) and methanogenesis (ME) were calculated using experimental results by Hoehler et al. 1998. Methane concentrations are in the gas phase; hydrogen and sulfate concentrations are reported per volume porewater in the aqueous phase. Data marker legend in panel A applies to all three panels.

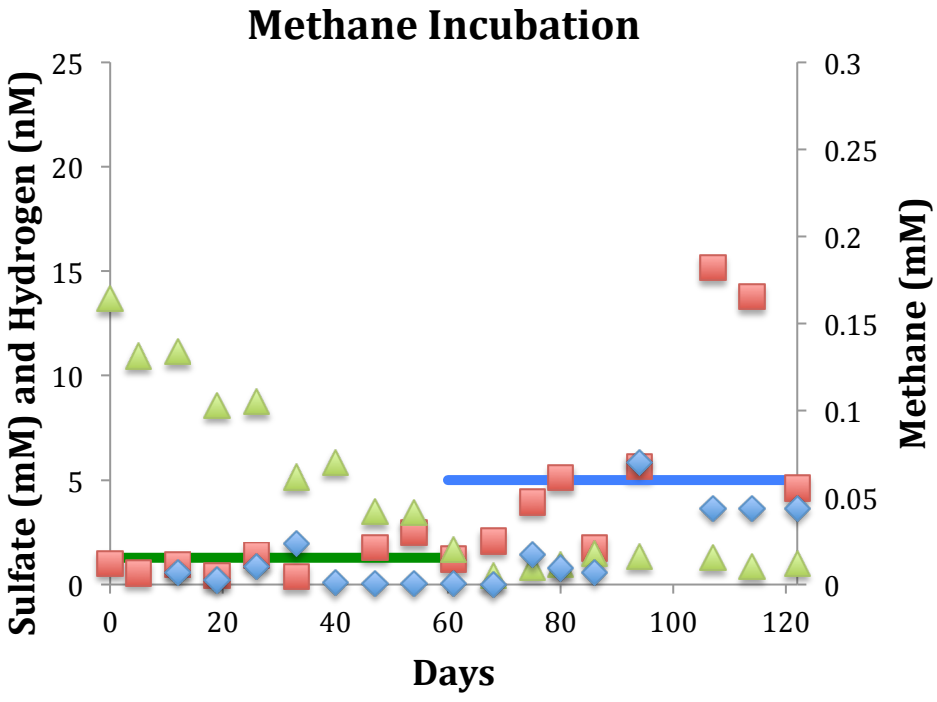


A.



B.

Figure 10. Continued



C.

Figure 10. Continued

sulfate is present with the exception of a few data points that may have been due to inhomogeneities in the system. Sustained methane production is not seen until after sulfate has been depleted around day 68. Upon reaching a peak of 0.1 to 0.2 mM the methane concentration then dropped again in both incubations. This was also seen in (Alperin et al. 1992) where the pressure built up by gas production caused a leak during sampling that caused the loss of methane from the system (Figure 6). Evidence that this also occurred in our incubation was the presence of a rotten egg smell indicative of hydrogen sulfide during subsampling. Therefore, we can interpret methane increases as biological in our incubation, but not methane decreases because they are indistinguishable from a leak.

H_2 in all three of the incubations was kept low at about 2nM during the sulfate reduction period. This agrees with what was expected based on Hoehler's previous calculations of H_2 equilibration points based on temperature for sulfate reduction performed on similar sediments. In Figure 10 A, B, and C, after sulfate was depleted, the H_2 concentrations began to rise to as high as 20 nM. This coincided with an observed rise in methane. The concentrations of H_2 quickly fell back down and settled at about 5 nM, which also agrees with the H_2 equilibration point for methane production (Hoehler et al. 1998).

16S rRNA gene Analysis

Unconstrained amplification bias removes the comparability of abundance of bacteria to that of archaea, however we can conclude that the relative abundance of bacteria and archaea was consistent and did not change (Figure 11). The phylum *Firmicutes*, known to contain the gram-positive sulfate reducers, was not well represented in this incubation. *Deltaproteobacteria*, however, were present and, with the exception of *Desulfobacteriales,* did not correlate with sulfate concentration (see Figure A-1).

Desulfobacteriales (Figure 12) markedly declined in relative abundance at 61 days from the start of the incubation, which is when sulfate is near depletion. Their abundance quickly recovered in the next few weeks and eventually rose above that seen during the sulfate reduction phase of the incubation. *Deltaproteobacteria* have the ability to ferment organic matter as an alternative metabolic pathway, often in syntrophy with methanogens (Muyzer & Stams 2008). The *Deltaproteobacteria* still only represent a tiny fraction of all the amplified sequences present in the microcosm accounting for generally only 3%. *Beggiatoa,* a white filamentous sulfur oxidizing bacteria that was observed on the sediment cores and detected initially but disappeared within the first ten days (Teske et al. 2002). Several bacterial taxa including *Halothiobacillus* and *Chromatiaceae* that have been known to contain the *soxB* gene, part of a sulfide oxidation pathway, increased in abundance after sulfate has been depleted (Headd & Engel 2013; Meyer et al. 2007). Of the *Deltaproteobacteria,* only about a third of the assignable reads were designated to Genera that had cultured members (Figure 13).

The most prevalent archaea in the amplicons were the *Thermoplasmata, Halobacteria*, and *Marine Benthic Group B*. None showed any consistent changes in relative abundance

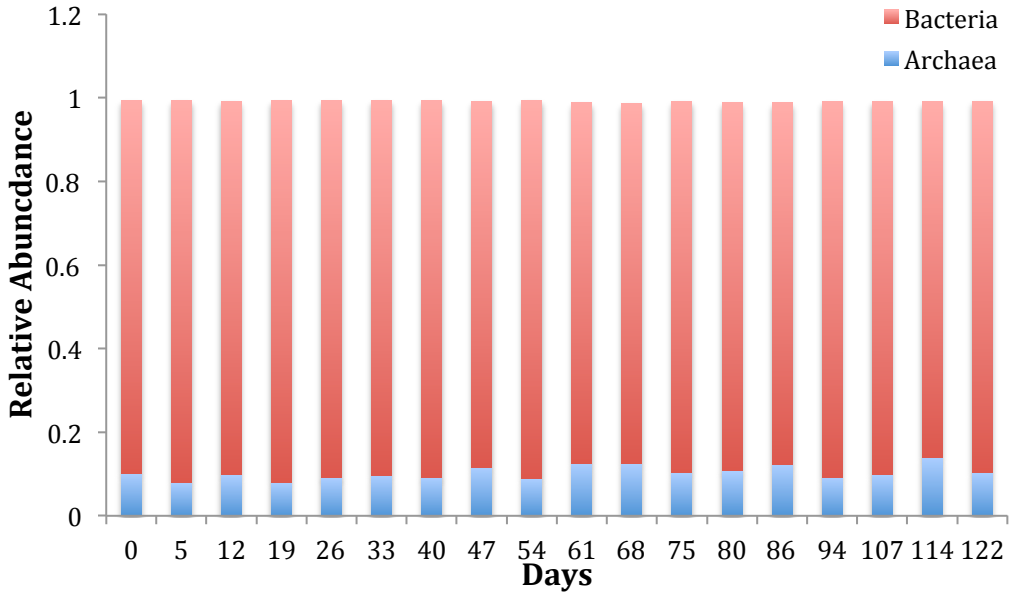


Figure 11: Relative abundance of Archaea and Bacteria for Anaerobic Incubation 1. Domains were compared to the total number of reads from each sample. Archaea (blue) consistently made up around 10% of the population while Bacteria (red) made up about 90%. The primers used excluded Eukaryotes.

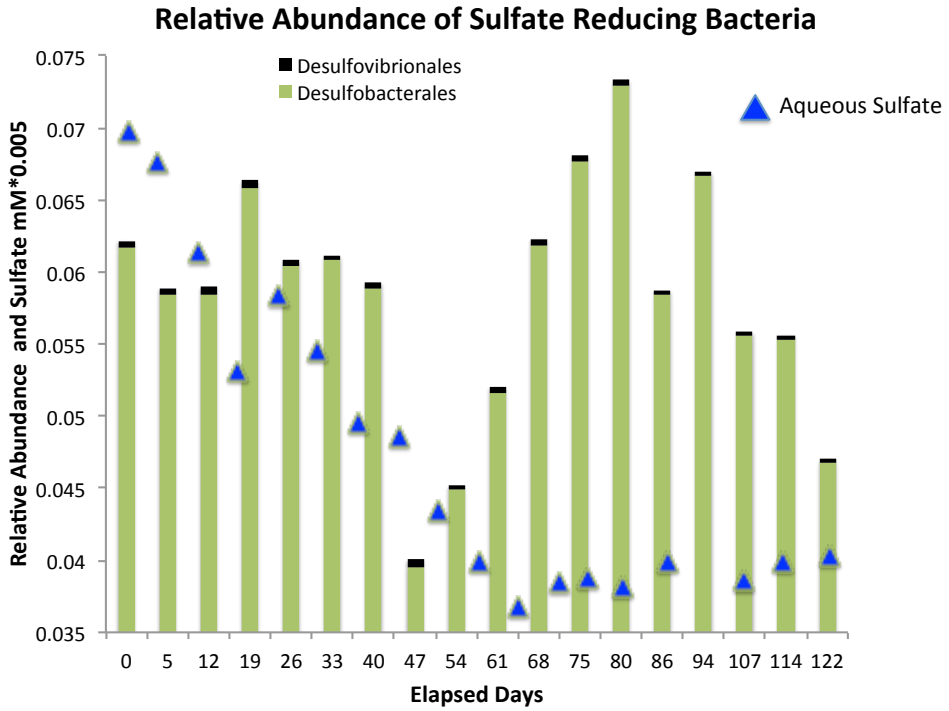


Figure 12: Relative abundance of *Desulfobacterales* in Anaerobic Incubation 1 with aqueous sulfate concentrations. The sulfate concentration (blue triangle) was transformed by multiplying by 0.005 in order to plot them on the same axis as the relative abundance and is meant to describe overall trends. The relative abundance of *Desulfobacterales* (green) declines with the depletion of sulfate at 61 days, but recovers quickly.

over the course of the incubation. The relative abundance of the methane-producing phylum *Methanomicrobia* increased six-fold within the methanogenic zone but still didn't even account for 1% of the amplicons. The *Methanosarcinales* and *Methanomicrobiales* began to show continuous increases in relative amplicon abundance after 80 days, which correlated to an increasing concentration of methane in the headspace (Figure 14). The growth continued until it leveled out on the last two days of the incubation. Notable also is the increase in relative abundance of ANME-1 in the methanogenic zone. Given that ANME-1 are believed to be obligate methanotrophs (Knittel & Boetius 2009), it is unexpected that they are present in these incubations where no methanotrophy was observed. ANME-2 within the *Methanosarcinales*, however, was in too low read abundance to observe a trend (Figure 15). This could be a result of true absence of ANME-2, mismatches between ANME-2 and the universal primers, or inability to taxonomically distinguish short reads of ANME-2 from total *Methanosarcinales*. While sulfate reducers were present in the methanogenic zone there was no apparent active sulfate reduction, so ANME-1 archaea may have also performed methanogenesis, a possibility that is supported by previous studies (Lloyd et al. 2013; Niemann et al. 2005).

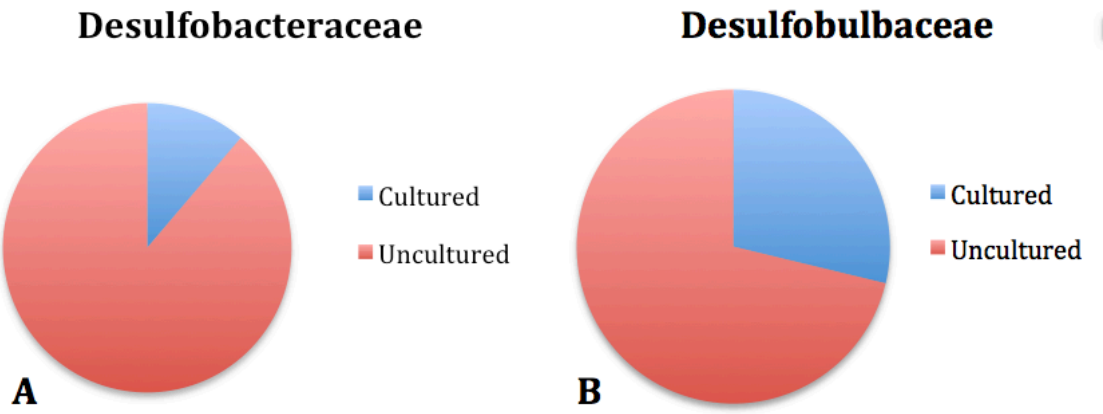


Figure 13 (A, B): The proportion of two families within the order *Desulfobacterales* detected in amplicon libraries that have cultured relatives, based on sequences in the SILVA database. The third family, *Desulfoarculaceae*, in the Order was excluded as it was in very low read abundance.

A novel member of the *Thermoplasmata*, Kazan 3A-21, also increased in relative abundance during methanogenesis (Figure 16). In contrast, the closely related *Thermoplasmatales*, did not change in relative abundance throughout the incubation, even though they contain methanogenic groups (Paul et al. 2012). Kazan 3A-21 showed a similar trend to the *Methanosarcinales* and had large increase in abundance with the production of methane suggesting that they might have a role in methanogenesis. There is no published data to our knowledge about their potential metabolism. Kazan 3A-21 is named after the Kazan mud volcano in

the eastern Mediterranean Sea, which is rich methane and hydrogen sulfide (Heijs et al. 2007). The 95 sequences submitted to the SILVA database for Kazan 3A-21 are from a wide variety of anoxic environments including sulfidic springs, shale bore holes, hydrothermal vents, and waste treatment plants (Quast et al. 2013).

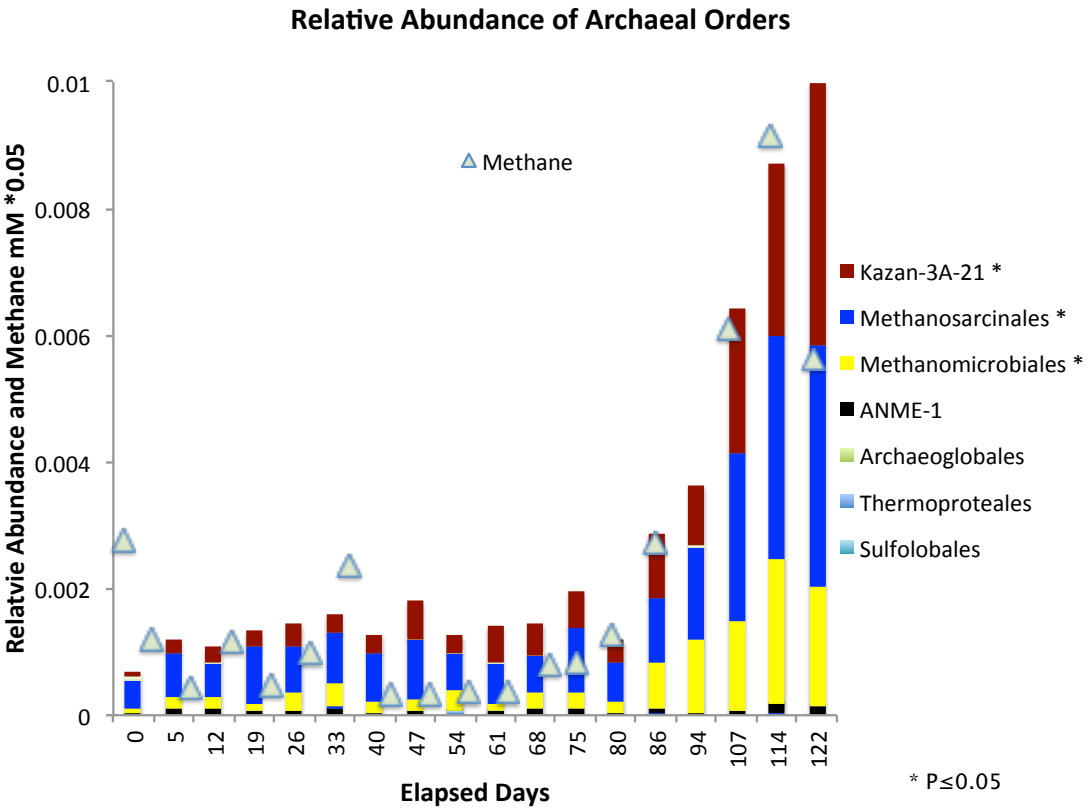


Figure 14: Relative abundance of archaeal orders with methane concentrations. Relative abundance was determined by using 16S rRNA gene analysis with Anaerobic Incubation 1 samples. Unclassified reads were removed. Methane concentrations were transformed to conform with the axis by multiplying by 0.05 from original concentrations. Asterisks indicate clades that significantly increase in average relative abundance after 80 days (paired t-test, $P \leq 0.05$), corresponding to the increase in methane concentrations.

Quantitative PCR

Quantitative (qPCR) of the sum of total archaea and bacteria demonstrated no clear systemic bias relative to SYBRGold microscopy cell counts, however they were not well-correlated (Figure 15), matching our previous results in other environments (Lloyd et al. 2013). Therefore we will examine only relative

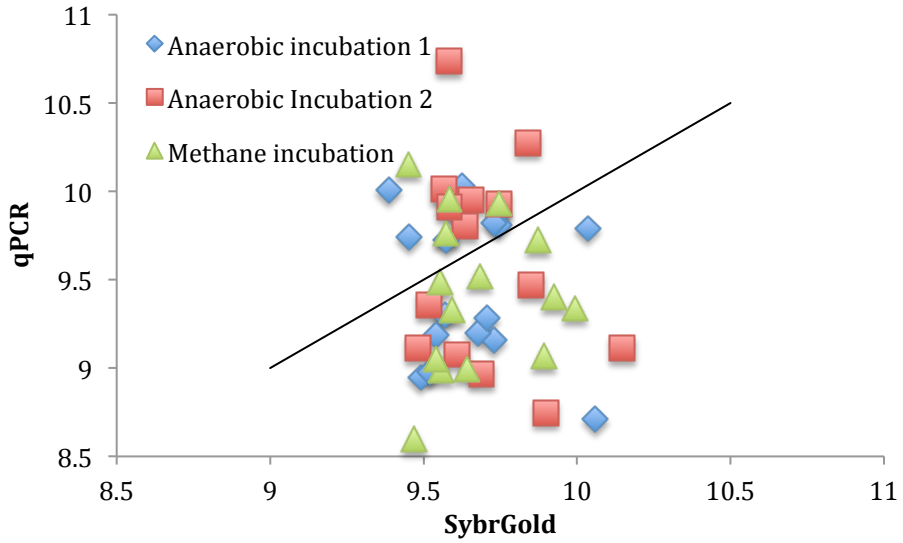


Figure 15: Log comparison of total prokaryotic cell counts with real time PCR against SYBRGold DNA staining fluorescence microscopy. The solid line is a 1:1 line.

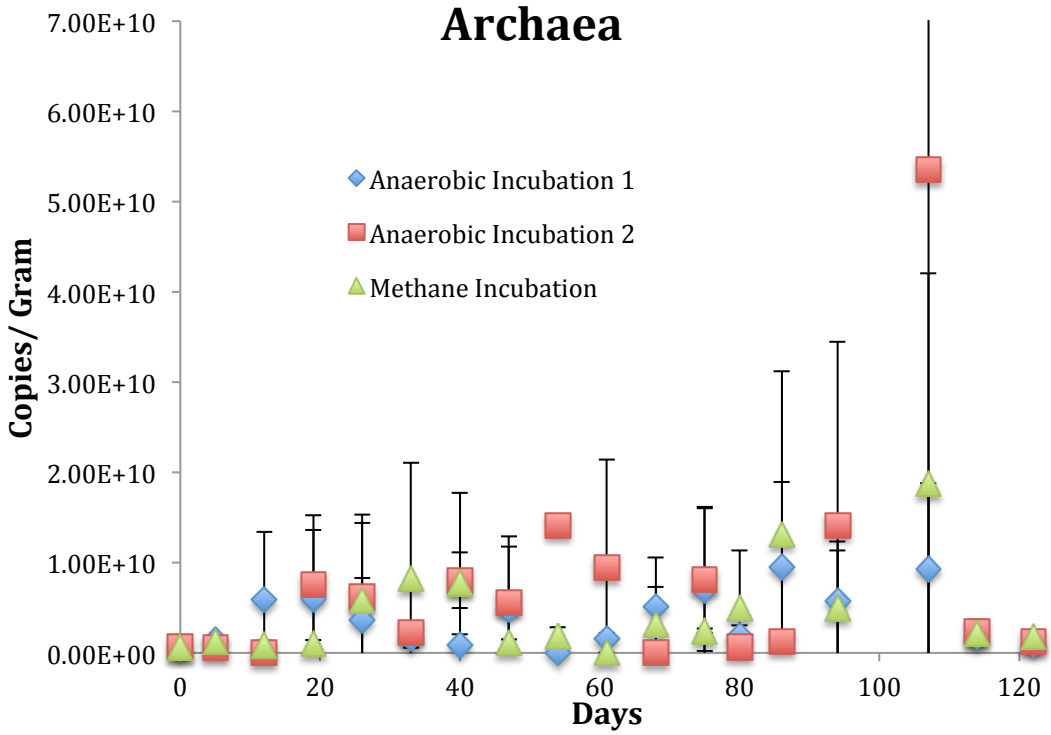


Figure 16: Archaeal abundance determined by qPCR. Error bars represent 1 standard deviation in duplicate measurements.

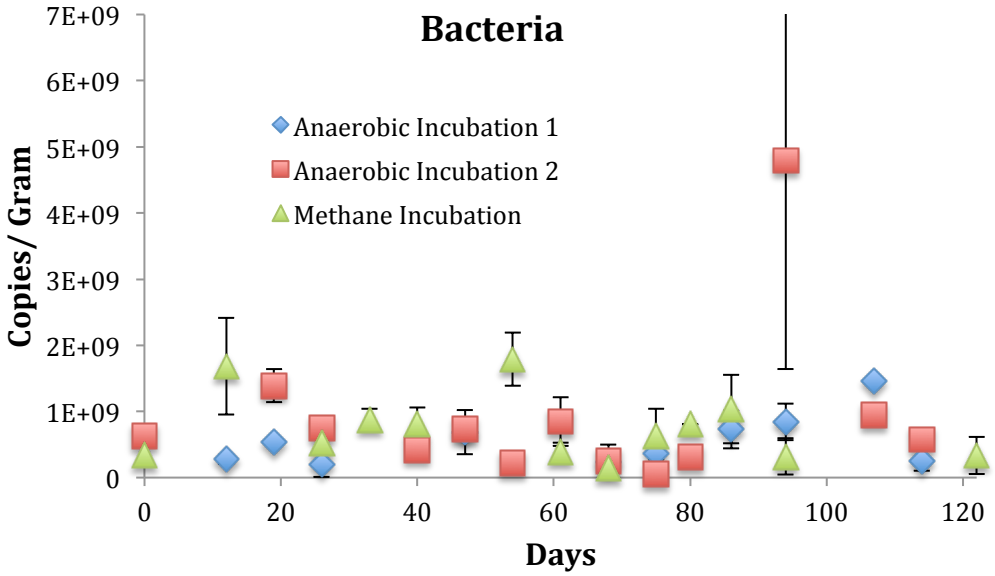


Figure 17: Bacterial abundance as determined by real time PCR. Error bars represent 1 standard deviation in duplicate measurements.

abundances, rather than absolute, with consistently higher archaea than bacteria qPCR values (Figures 16 and 17). *Methanosarcinales*, excluding ANME-2, and *Methanomicrobiales* showed an increase in abundance for all three incubations after sulfate was depleted at 68 days, correlating with with methane production (Figures 18 and 19). Both *Methanomicrobiales* and *Methanosarcinales* were present but not consistently increasing during the time of sulfate reduction. The first anaerobic incubation and the methane incubation had single spikes in *Methanomicrobiales* at day 33 that coincided with an increase in methane at that time (Figure 18). The initial measurements for *Methanomicrobiales* differed widely; from the order of 10^6 with second anaerobic incubation to nearly 10^8 with the first anaerobic incubation, even on the same plate run. Absolute quantification with real time PCR is difficult to achieve and can be better analyzed as relative measures, rather than absolute (Lloyd et al. 2013).

qPCR values for 16S rRNA gene copy numbers of ANME-1 increased during methanogenesis in all three incubations and, similarly to *Methanomicrobiales* and *Methanosarcinales* (Figure 19). However, this increase was transient and had high variability in replicates, possibly due to being near the detection limit. ANME-2 (Figure 21) was at higher 16S rRNA gene copy numbers than ANME-1, but like ANME-1, Methanosarcinales, and Methanomicrobiales, increased in abundance during methane production in incubations 1 and 2.

Epifluorescence Direct Microscopy

The total cell counts (Figure 22) for all three incubations have a similar trend of initial abundance declining for about 60 days during the sulfate reducing phase until the population recovers slightly during the methanogenic phase. The population was between 4-6 X 10^9 for most of the incubation with the initial point slightly above 10^{10} cells/gram.

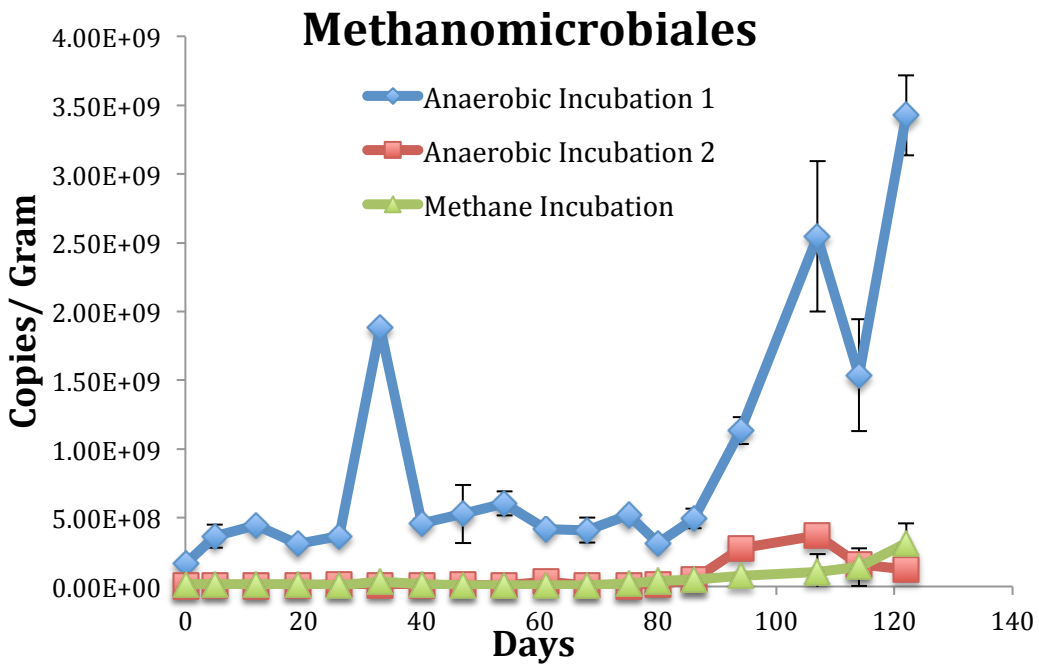


Figure 18: *Methanomicrobiales* abundance as determined by qPCR. Error bar represent 1 standard deviation in duplicate measurements.

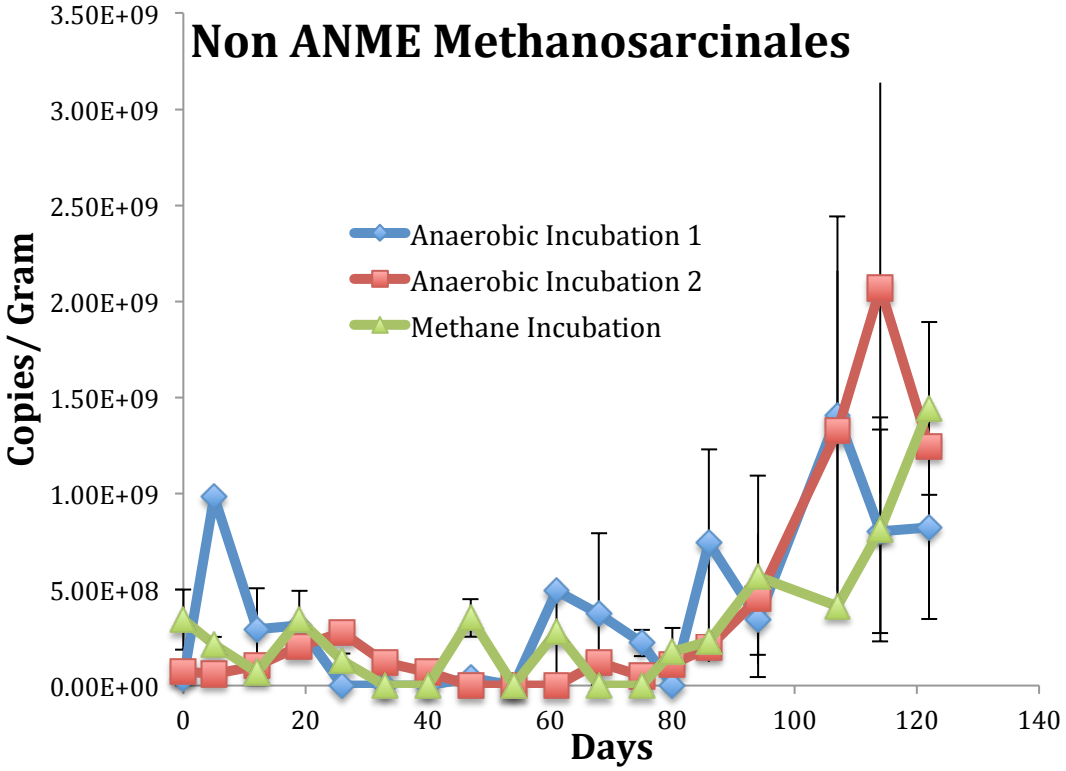


Figure 19: *Methanosarcinales* abundance as determined by qPCR. ANME-2, which is within this order, was excluded using specific primers. Error bars represent 1 standard deviation in duplicate measurements.

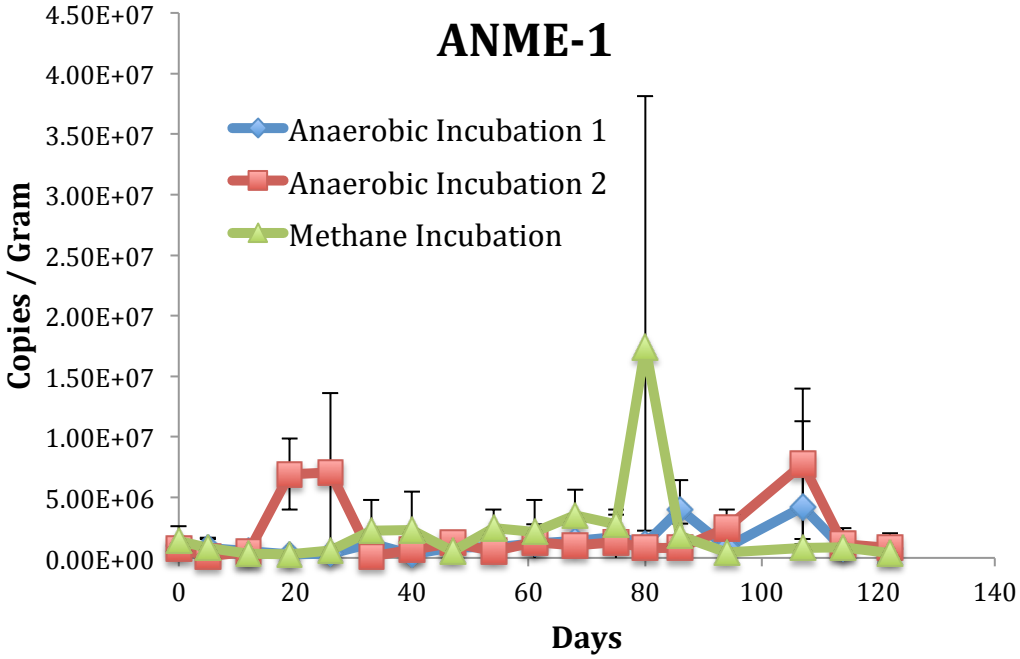


Figure 20: ANME-1 abundance as determined by real time PCR. Error bars represent 1 standard deviation in duplicate measurements.

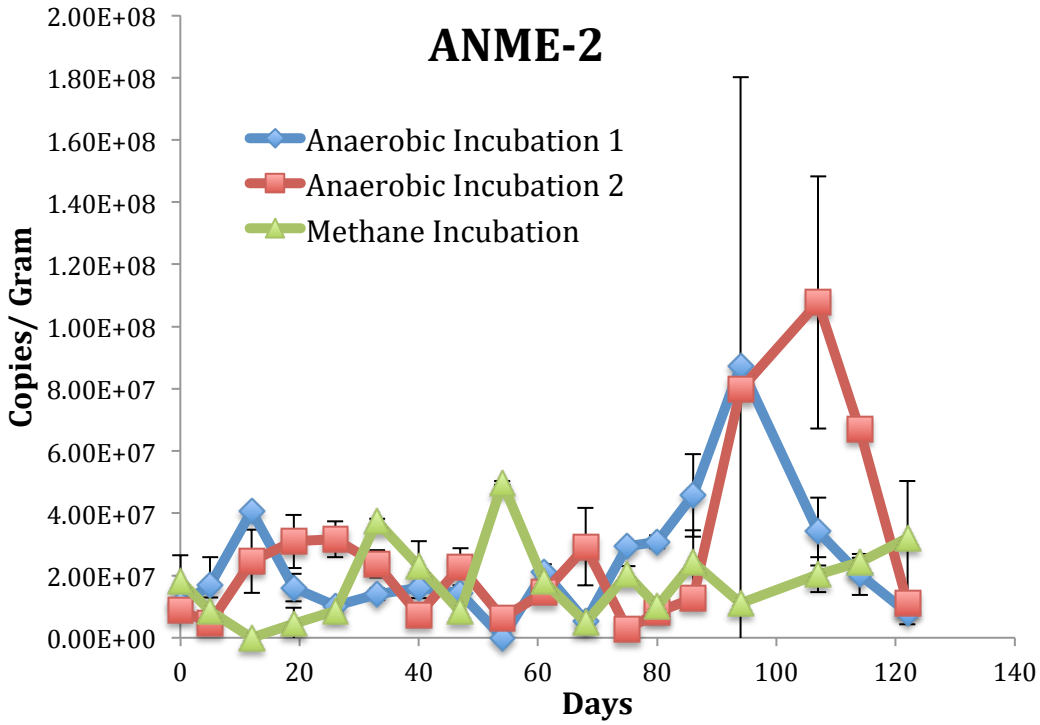


Figure 21: ANME-2 abundance as determined by real time PCR. Error bars represent 1 standard deviation in duplicate measurements.

In incubations 1 and 2, all CARD-FISH counted taxa increased in abundance around the point of sulfate depletion between 61 and 68 days (see Appendix). However, the data are otherwise so variable, that this spike should not be interpreted as meaningful. Cell counts ranged between 2.0 $X10^8$ up to nearly 1.0 $X10^9$. The deficiencies of our CARDFISH data can be explained in some part by the various physical and chemical manipulations done on the samples that could cause attrition. When the filters are washed between various steps it was not uncommon to see sediment granules left behind. Although not quantitative, CARD-FISH provided evidence that specific taxa, such as the sulfate reducing bacteria and ANME-1 were present and intact (Figure 23).

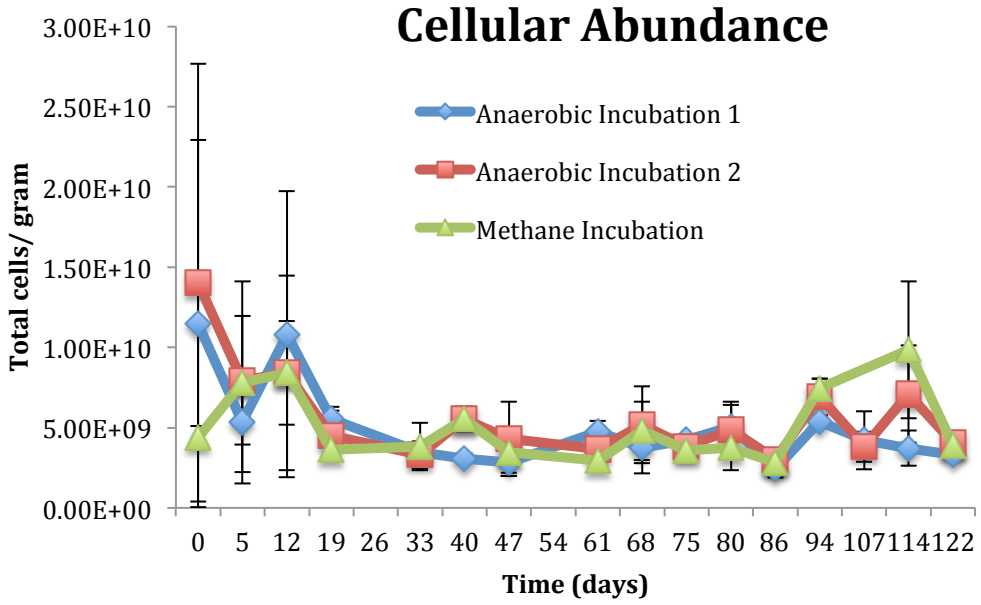


Figure 22. Total cellular abundance determined by direct epifluorescence microscopy. SYBRGold DNA stain was used for the three incubations to visualize cells. Results are the mean of thirty fields per sample, with error bars representing one standard deviation.

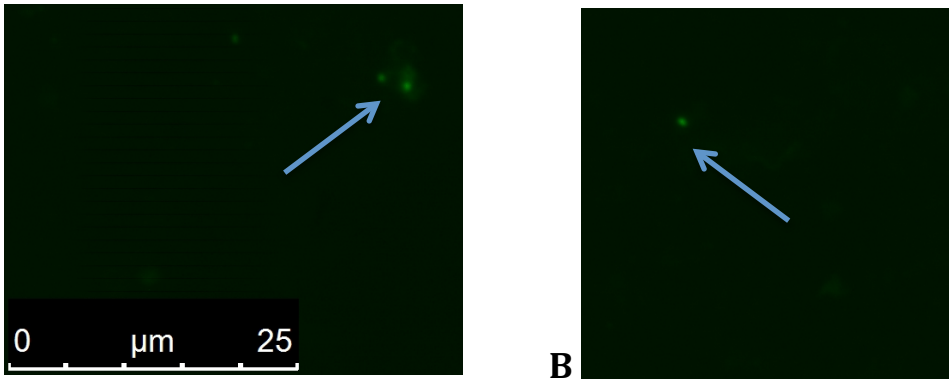


Figure 23: (A, B) Fluorescently labeled cells visualized using an epifluorescence microscope. Sample taken from the Anaerobic Incubation 1 after 12 days using CARDFISH epifluorescence microscopy. Sulfate reducing bacteria were targeted and visible in (A) and probes specific for ANME-1 were used in (B). Scale applies to both images.

CHAPTER VI

CONCLUSIONS

H_2 equilibrations in both freshwater and estuarine sediments confirm that we can accurately measure the H_2 concentrations in the sediment core *in situ* using the method of Hoehler et al. 1998 (Figure 7). Using this method we were able to measure the H_2 depth profile at CLB. The H_2 concentrations did not correspond to the thermodynamic minimum for sulfate reducers in the sulfate reduction zone and methanogens after sulfate depletion. The trend of H_2 concentration declining with depth did, however, agree with what Hoehler et al. 1998 reported from White Oak River estuarine sediments and was dissimilar to what has been seen at CLB in other seasons (Hoehler et al. 1998). We found that the CLB had abundant methane throughout the core, including the sulfate reduction zone suggesting high rates of methanogenesis leading to upward methane advection through upper sediments, although it may not be produced there. It is possible that because our samples were taken in October, they were not in steady state with respect to H_2 thermodynamics due to seasonal changes at that time. Our equilibration data showed that a few days of constant conditions were required to reach steady state H_2 concentrations. In October, the temperature fluctuated by many degrees from day to day, and possibly the microbial community had not yet reached thermodynamic equilibrium at the new temperature on the day we sampled. An alternative explanation for the hydrogen depth trend could be decreasing pH values with depth (Hoehler et al. 1998).

In the microcosm experiment, sulfate reduction and methanogenesis were separated in time, mimicking a diagenetic profile. Methane production in all three incubations began in a sustained way when sulfate concentrations were low. Sustained increases in methane only occurred after sulfate was depleted. During the time of sulfate reduction the H_2 concentration was at about 2 nM, which is the equilibration point for that metabolism in the conditions of CLB (Hoehler et al. 1994). After sulfate was depleted the H_2 rose quickly and once methane began to be produced in large amounts the H_2 came back down to 5nM which was the calculated equilibration point for methanogenesis (Hoehler et al 1998). This supports the theory that H_2 determines the succession of terminal electron acceptors, sulfate and CO_2, resulting the observed vertical zonation of sediments.

According to 16S rRNA gene libraries, the relative abundance of sulfate reducing bacteria declined for a two-week period with the depletion of sulfate. Their abundance rebounded to near or above their previous levels, relative to total 16S rRNA gene sequences following that period. This trend was evident in the Order *Desulfobacterales,* which was the only clade to show any response or change in abundance throughout the incubation. One way that their growth could be explained is that they switch to fermenting short chain fatty acids or aromatics and form a syntrophy with the methanogens (Zhou et al. 2011).

Using 16S rRNA gene libraries, the period of methane production saw a significant increase mainly in three archaeal orders; *Methanomicrobiales*,

Methanosarcinales, and Kazan 3A-21. The *Methanomicrobiales* and *Methanosarcinales* are closely related to cultured methanogens, and it is therefore expected that they increase with methane production. The Kazan 3A-21, however, trends very well with established methanogens and methanogenesis, but is unrelated to cultured microorganisms, so its physiology is unknown. We suggest a possible role for this group in Methanogenesis, supported by the fact closely related sequences are found in diverse environments containing natural gas. Based on qPCR (for ANME-1 and ANME-2) and 16S rRNA data (for ANME-1), ANME groups are present throughout the course of the incubation. ANME-1 even increases in the 16S rRNA gene libraries, and ANME-1 and ANME-2 increase in the qPCR data before other *Methanosarcinales* and *Methanomicrobiales* increase, despite the incubations demonstrating no net AOM. The presence of ANME archaea in methanogenic sediments has been observed previously (Lloyd et al. 2011). However, their presence and apparent growth in sediments that are not likely to have previously undergone net AOM suggests they may be methanogens. If the pathway for AOM is a reversal of the methanogenesis pathway and ANME archaea have all (ANME-2) or most (ANME-1) the molecular machinery for methanogenesis (Meyerdiercks et al. 2010, Hallam et al. 2004, and Pernthaler et al. 2008), then it is likely that ANME archaea act as methanogens in Cape Lookout Bight.

The simplest explanation for our data is that ANME-1 and ANME-2 produce methane during methanogenesis. However, we cannot rule out that ANME populations present in methanogenic sediments could be oxidizing methane in some fashion. They are believed to be obligate syntrophs with SRB's (Knittel and Boetius 2009), which are present throughout the incubation, although epifluorescence microscopy did not illuminate any microbial aggregates that would indicate such syntrophy. Too little is known about the Kazan 3A-21 to suggest at any probable primary metabolism, other than their apparent phylogenetic relation to *Thermoplasmata*; some members of which can use methylamines as a methanogenic substrate (Offre et al. 2013). Their increase in abundance alongside known methanogens correlating with the increase in methane concentrations suggest that they too may be producing methane.

Future Directions

There were several aspects of this study that need further study. The role of iron reduction in CLB and its effect on the geochemistry as a potential terminal electron acceptor needs to be studied in order to determine its interference with sulfate reduction. Next, the prevalence of acetogenesis in CLB has been studied by Hoehler et al (1999), both in the field and in lab incubations, but the microbial response to acetate dynamics needs to be studied. There is evidence to suggest that acetogens take advantage of the high H_2 concentration after the depletion of sulfate to fuel their metabolism until methanogens can pull the H_2 down enough to exclude them (Hoehler et al. 1999).

The increase in abundance of Kazan 3A-21 along with methane production mirrored that of other methanogens. This is a new and uncharacterized taxa that have been reported in a wide range of environments associated with methane.

Understanding their role in these niches could illuminate a new an interesting microbe.

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APPENDIX

A plot of the sum of bacteria and archaea with CARD-FISH vs. SYBRGold cell counts shows no correlation. Furthermore, there appears to be undercounting by CARDFISH. The numbers reported with qPCR of prokaryotes is more in line with the SYBRGold counts than the CARDFISH. The Miscellaneous Crenarchaeota Group (MCG's) was attempted to be quantified with two CARDFISH probes and was found to be persistent at low levels, this was confirmed using the 16S data and no evident trends in abundance were clear.

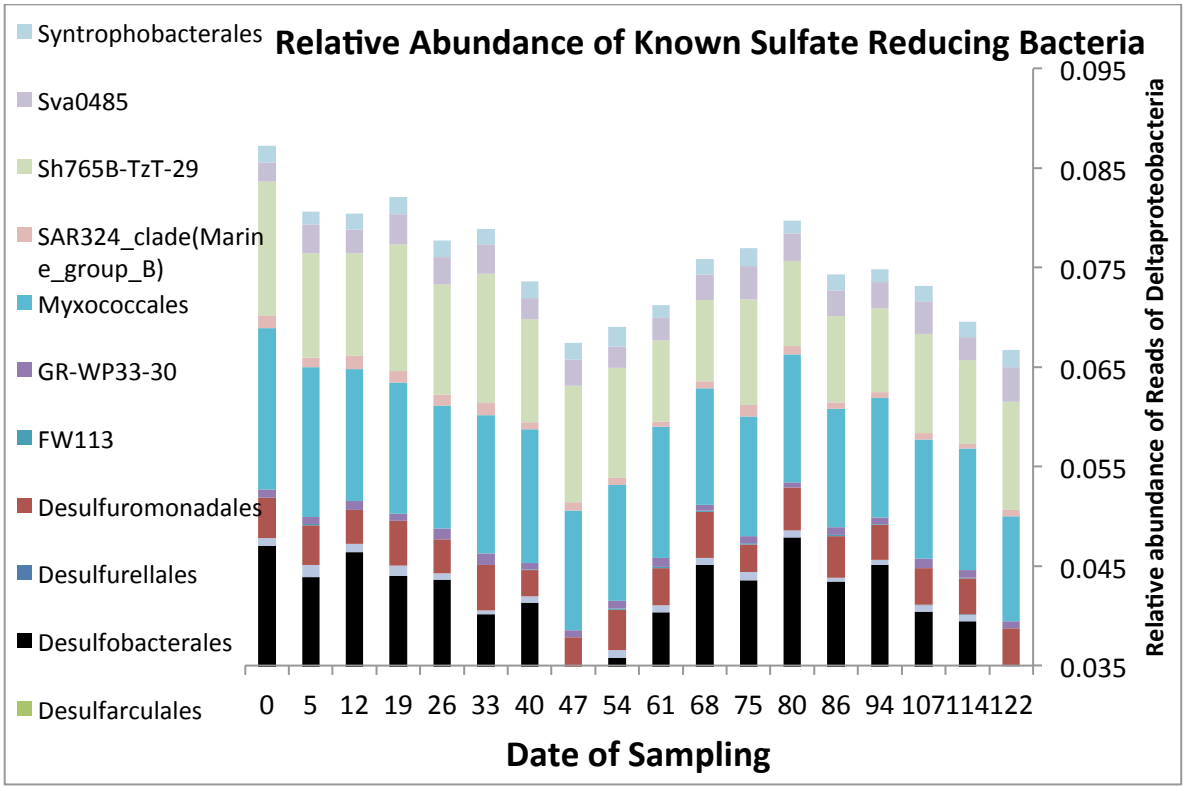
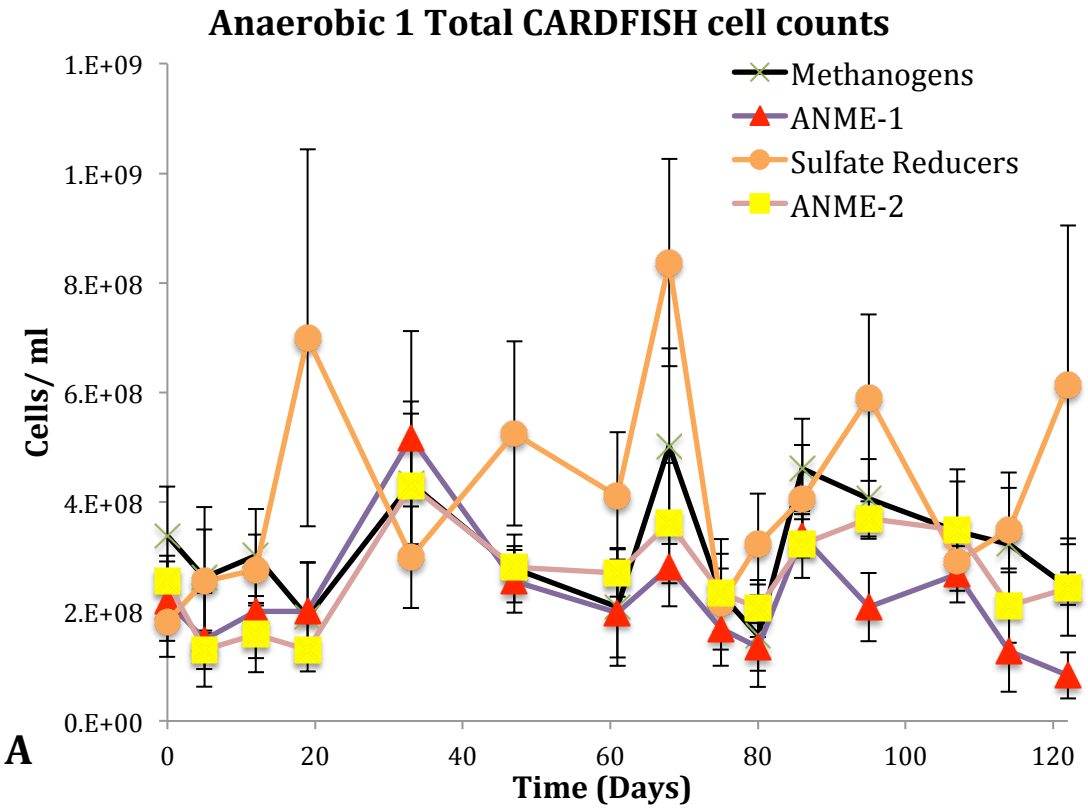


Figure A-1: Relative Abundance of 16S Amplicons for *Deltaproteobacteria*. Most taxa do not show any change with sulfates depletion except for *desulfobacterales.*

Cellular Abundance using CARDFISH analysis

Figure A-2: (A, B, and C) Microbial abundance using CARDFISH epifluorescence microscopy. (A,B) represent the two anaerobic incubations and (C) the methane incubation. Results are the mean of thirty fields.



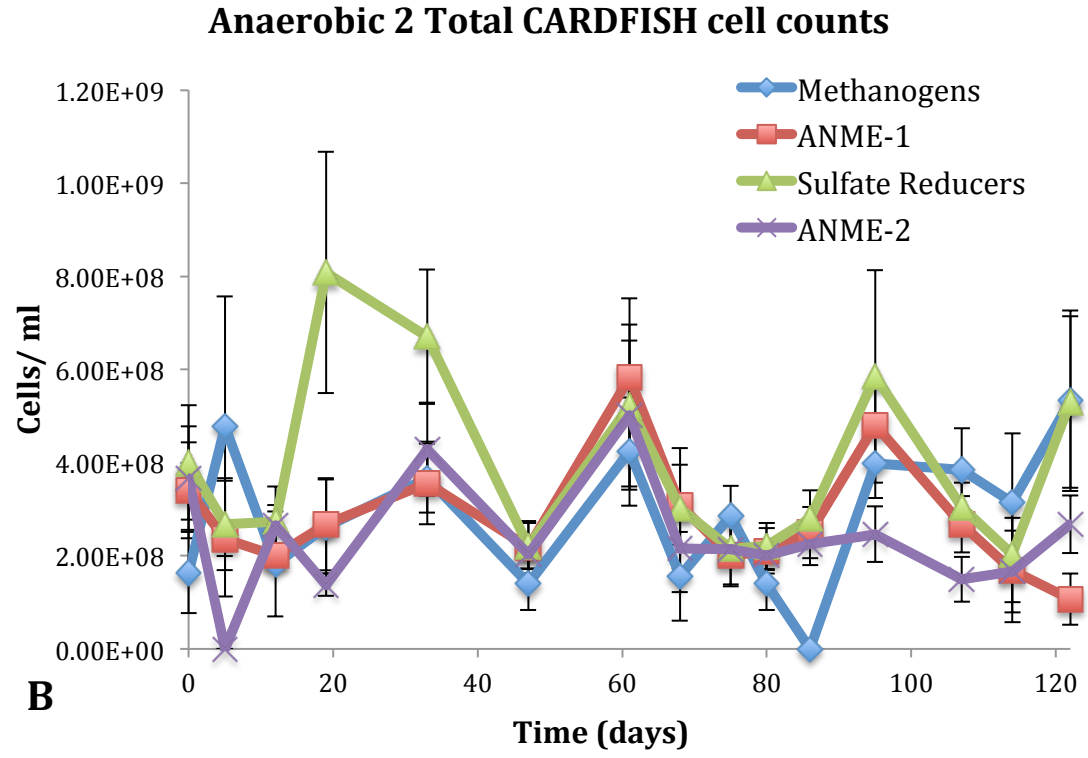


Figure A-2. Continued.

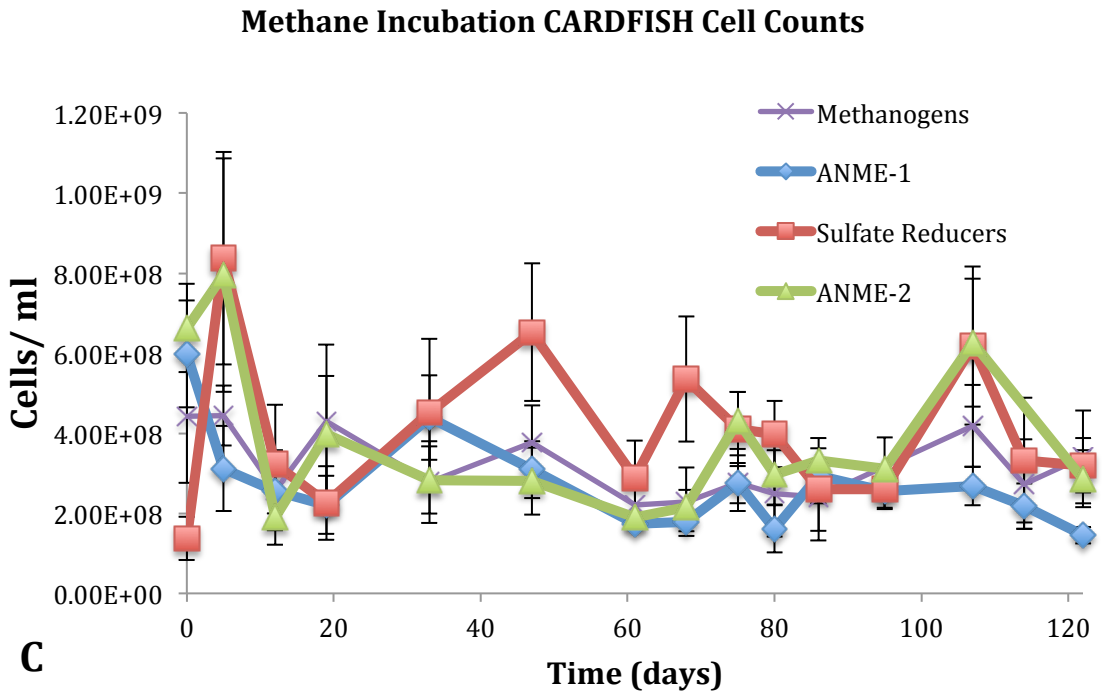


Figure A-2. Continued

Table A-1: Porewater Geochemistry of Cape Lookout Bight.

Depth Segment	Porosity	Cells/gram	Methane mM	mM Sulfate	nM Hydrogen
0-3	0.832606153	3.33E+09	0.073177074	14.01802151	1.97294561
3-6	0.823663974	3.07E+09	0.056647227	8.249685108	0.737274265
6-9	0.823890174	2.27E+09	0.164184094	0.507121403	0.678643829
9-12	0.781232226	2.13E+09	0.210987705	0.540543552	0.44761473
12-15	0.779093006	2.75E+09	0.228780506	0.540548396	0.418570171
15-18	0.765409729	2.09E+09	0.198729616	0.541129735	0.377364604
18-21	0.752424664	1.80E+09	0.19687233	0.541129735	0.574786302
21-24	0.82923368	2.35E+09	0.159726607	0.513031683	0.37164446
24-27	0.779756481	9.55E+08	0.131867315	0.147902335	0.390250482
27-30	0.784754473	9.29E+08	0.24516177	0.074120725	0.030989296
30-33	0.781525577	1.25E+09	0.31202407	0.076882085	0.375888939
Control	NA	6.50E+06	0.000223534	12.59567871	0.01547786

Sample taken by push core with a total length of 33 cm. Core was sectioned in three-centimeter increments. Within 36 hours of acquisition.

Table A-2: Geochemistry during Cape Lookout Bight sediment incubations.

Incubation Name	Total time elapsed (days)	mM sulfate aqueous	mM Methane in Headspace	nM Hydrogen aqueous
Anaerobic 1	0	14.04272842	0.075161857	0.585119063
Anaerobic 1	5	13.10967929	0.025490813	0.446612979
Anaerobic 1	12	10.50348803	0.00110094	0.38620119
Anaerobic 1	19	6.97606821	0.00815511	1.3973972
Anaerobic 1	26	9.184962697	0.007447865	0.448446765
Anaerobic 1	33	7.598100959	0.033442374	2.039766981
Anaerobic 1	40	5.474275748	0.017737365	0.367457037
Anaerobic 1	47	5.046022672	0.000126888	0.801433682
Anaerobic 1	54	2.910570681	0.000365091	1.819948726
Anaerobic 1	61	1.373413429	0.000817805	1.400272975
Anaerobic 1	68	0.090107548	0.000847012	2.49976716
Anaerobic 1	75	0.794496657	0.011819632	1.761377286
Anaerobic 1	80	0.949520395	0.00627918	7.634986081
Anaerobic 1	86	0.683073346	0.00423506	22.22065332
Anaerobic 1	94	1.400058134	0.061335358	5.573294261
Anaerobic 1	107	0.867164034	0.146036568	4.444388496
Anaerobic 1	114	1.395213642	0.219054851	4.260536728
Anaerobic 1	122	1.564770856	0.090937725	3.123547975
Anaerobic 2	0	14.13768046	0.13630049	0.387363448
Anaerobic 2	5	13.12711947	0.02286965	0.860062398
Anaerobic 2	12	9.744210832	0.001304818	0.695685065
Anaerobic 2	19	9.536866583	0.004893066	1.489298994
Anaerobic 2	26	6.639230695	0.068637187	1.398730862
Anaerobic 2	33	4.468074799	0.007301828	3.275397633
Anaerobic 2	40	4.437554501	0.000407756	1.282791091
Anaerobic 2	47	1.423311695	0.000423506	1.173153996
Anaerobic 2	54	1.627749249	0.000715579	1.255119921
Anaerobic 2	61	0.726673772	0.000350488	1.955970727
Anaerobic 2	68	0.252398023	0.00032128	1.364630786
Anaerobic 2	75	0.566805542	0.013001596	6.456924859
Anaerobic 2	80	0.896230985	0.028071627	7.556311448
Anaerobic 2	86	0.939831412	0.004527134	3.296675339
Anaerobic 2	94	1.400058134	0.026286582	4.336641011
Anaerobic 2	107	0.746051739	0.182545709	6.69137666
Anaerobic 2	114	1.293479314	0.119749985	4.63811519
Anaerobic 2	122	1.44365856	0.047755007	3.151731499
Methane	0	13.68181378	74.82110561	1.011936571
Methane	5	10.92626683	1.176247018	0.554982534

Table A-2. Continued

Incubation Name	Total time elapsed (days)	mM sulfate aqueous	mM Methane in Headspace	nM Hydrogen aqueous
Methane	12	11.15589575	0.006931844	0.956116026
Methane	19	8.587830637	0.00256886	0.432857065
Methane	26	8.755450053	0.010368596	1.398730862
Methane	33	5.127410135	0.023365851	0.366946619
Methane	40	5.832768143	0.001182491	59.15099146
Methane	47	3.501598682	0.000525732	1.774867281
Methane	54	3.412460033	0.000540335	2.483593215
Methane	61	1.692665439	0.00075939	1.206982642
Methane	68	0.435519814	0	2.069809344
Methane	75	0.8284081	0.017138467	3.933536381
Methane	80	0.939831412	0.00934634	5.103885223
Methane	86	1.492103478	0.006863719	1.751468487
Methane	94	1.356457708	0.070097552	5.633382672
Methane	107	1.278945839	0.04381097	15.16512778
Methane	114	0.867164034	0.04381097	13.772259
Methane	122	1.012498789	0.043690751	4.625542392
NEGATIVE	122	12.59567871	0.000223534	0.443178847

Table A-3. Total cell counts per gram of sediment from the Cape Lookout Bight incubations as determined by SYBRGold direct microscopy

Sample	Days elapsed	SYBR cells/gram
Anaerobic Incubation 1	0	1.15E+10
Anaerobic Incubation 1	5	5.36E+09
Anaerobic Incubation 1	12	1.08E+10
Anaerobic Incubation 1	19	5.55E+09
Anaerobic Incubation 1	26	
Anaerobic Incubation 1	33	3.47E+09
Anaerobic Incubation 1	40	3.09E+09
Anaerobic Incubation 1	47	2.84E+09
Anaerobic Incubation 1	54	
Anaerobic Incubation 1	61	4.73E+09
Anaerobic Incubation 1	68	3.75E+09
Anaerobic Incubation 1	75	4.21E+09
Anaerobic Incubation 1	80	5.08E+09
Anaerobic Incubation 1	86	2.43E+09
Anaerobic Incubation 1	94	5.37E+09
Anaerobic Incubation 1	107	4.22E+09
Anaerobic Incubation 1	114	3.72E+09

Table A-3. Continued

Sample	Days elapsed	SYBR cells/gram
Anaerobic Incubation 1	122	3.31E+09
Anaerobic Incubation 2	0	1.40E+10
Anaerobic Incubation 2	5	7.94E+09
Anaerobic Incubation 2	12	8.42E+09
Anaerobic Incubation 2	19	4.49E+09
Anaerobic Incubation 2	26	
Anaerobic Incubation 2	33	3.30E+09
Anaerobic Incubation 2	40	5.55E+09
Anaerobic Incubation 2	47	4.30E+09
Anaerobic Incubation 2	54	
Anaerobic Incubation 2	61	3.68E+09
Anaerobic Incubation 2	68	5.17E+09
Anaerobic Incubation 2	75	3.84E+09
Anaerobic Incubation 2	80	4.88E+09
Anaerobic Incubation 2	86	3.04E+09
Anaerobic Incubation 2	94	6.92E+09
Anaerobic Incubation 2	107	3.82E+09
Anaerobic Incubation 2	114	7.10E+09
Anaerobic Incubation 2	122	4.06E+09
Methane Incubation	0	4.38E+09
Methane Incubation	5	7.82E+09
Methane Incubation	12	8.42E+09
Methane Incubation	19	3.61E+09
Methane Incubation	26	
Methane Incubation	33	3.83E+09
Methane Incubation	40	5.55E+09
Methane Incubation	47	3.47E+09
Methane Incubation	54	
Methane Incubation	61	2.94E+09
Methane Incubation	68	4.80E+09
Methane Incubation	75	3.58E+09
Methane Incubation	80	3.75E+09
Methane Incubation	86	2.82E+09
Methane Incubation	94	7.45E+09
Methane Incubation	107	
Methane Incubation	114	9.85E+09
Methane Incubation	122	3.91E+09

Table A-4: Microbial abundance using CARDFISH direct microscopy.

Sample	Days Elapsed	Cells/ml	STD dev	Target Taxa	avg cells/grid	Total Cells
Anaerobic Incubation 1	0	1.61E+08	1.03E+08	MCG	2.13	64
Anaerobic Incubation 1	5	2.63E+08	1.29E+08	MCG	3.43	103
Anaerobic Incubation 1	12	2.52E+08	7.66E+07	MCG	3.30	99
Anaerobic Incubation 1	19	1.14E+08	8.21E+07	MCG	1.53	46
Anaerobic Incubation 1	33	3.25E+08	8.99E+07	MCG	4.23	127
Anaerobic Incubation 1	47	2.78E+08	8.36E+07	MCG	3.63	109
Anaerobic Incubation 1	61	1.35E+08	1.02E+08	MCG	1.80	54
Anaerobic Incubation 1	68	2.83E+08	1.18E+08	MCG	3.70	111
Anaerobic Incubation 1	75	1.53E+08	5.54E+07	MCG	2.03	61
Anaerobic Incubation 1	80	2.13E+08	7.04E+07	MCG	2.80	84
Anaerobic Incubation 1	86	3.33E+08	1.17E+08	MCG	4.33	130
Anaerobic Incubation 1	95	4.29E+08	1.01E+08	MCG	5.57	167
Anaerobic Incubation 1	107	3.43E+08	9.74E+07	MCG	4.47	134
Anaerobic Incubation 1	114	2.03E+08	1.30E+08	MCG	2.67	80
Anaerobic Incubation 1	122	2.16E+08	8.88E+07	MCG	2.83	85
Anaerobic Incubation 1	0	3.64E+07	2.17E+07	MCG	0.93	28
Anaerobic Incubation 1	5	1.20E+08	4.48E+07	MCG	2.00	60
Anaerobic Incubation 1	12	1.56E+08	5.28E+07	MCG	2.47	74
Anaerobic Incubation 1	19	1.14E+08	4.01E+07	MCG	1.93	58
Anaerobic Incubation 1	33	3.46E+08	1.20E+08	MCG	4.90	147
Anaerobic Incubation 1	47	1.27E+08	6.19E+07	MCG	2.10	63
Anaerobic Incubation 1	61	8.84E+07	3.44E+07	MCG	1.60	48
Anaerobic Incubation 1	68	2.03E+08	9.71E+07	MCG	3.07	92
Anaerobic Incubation 1	75			MCG		0
Anaerobic Incubation 1	80	2.21E+08	5.77E+07	MCG	3.30	99
Anaerobic Incubation 1	86	3.28E+08	4.99E+07	MCG	4.67	140
Anaerobic Incubation 1	95	2.34E+08	5.69E+07	MCG	3.47	104
Anaerobic Incubation 1	107	1.61E+08	5.07E+07	MCG	2.53	76
Anaerobic Incubation 1	114	8.58E+07	5.68E+07	MCG	1.57	47
Anaerobic Incubation 1	122	1.72E+08	4.10E+07	MCG	2.67	80
Anaerobic Incubation 1	0	3.38E+08	8.99E+07	Methanomicrobiales	4.50	135
Anaerobic Incubation 1	5	2.63E+08	1.28E+08	Methanomicrobiales	3.53	106
Anaerobic Incubation 1	12	3.02E+08	8.60E+07	Methanomicrobiales	4.03	121
Anaerobic Incubation 1	19	1.90E+08	9.93E+07	Methanomicrobiales	2.60	78

Table A-4. Continued

Sample	Days Elapsed	Cells/ml	STD dev	Target Taxa	avg cells/grid	Total Cells
Anaerobic Incubation 1	33	4.34E+08	1.49E+08	Methanomicrobiales	5.73	172
Anaerobic Incubation 1	47	2.78E+08	6.21E+07	Methanomicrobiales	3.73	112
Anaerobic Incubation 1	61	2.08E+08	1.08E+08	Methanomicrobiales	2.83	85
Anaerobic Incubation 1	68	5.02E+08	1.78E+08	Methanomicrobiales	6.60	198
Anaerobic Incubation 1	75	2.31E+08	1.01E+08	Methanomicrobiales	3.13	94
Anaerobic Incubation 1	80	1.56E+08	9.37E+07	Methanomicrobiales	2.17	65
Anaerobic Incubation 1	86	4.60E+08	9.18E+07	Methanomicrobiales	6.07	182
Anaerobic Incubation 1	95	4.06E+08	7.28E+07	Methanomicrobiales	5.80	174
Anaerobic Incubation 1	107	3.46E+08	9.13E+07	Methanomicrobiales	4.60	138
Anaerobic Incubation 1	114	3.22E+08	1.32E+08	Methanomicrobiales	4.30	129
Anaerobic Incubation 1	122	2.44E+08	8.89E+07	Methanomicrobiales	3.30	99
Anaerobic Incubation 1	0	2.18E+08	7.25E+07	ANME-1	3.37	101
Anaerobic Incubation 1	5	1.48E+08	8.56E+07	ANME-1	2.47	74
Anaerobic Incubation 1	12	2.00E+08	8.56E+07	ANME-1	3.13	94
Anaerobic Incubation 1	19	2.00E+08	9.00E+07	ANME-1	3.13	94
Anaerobic Incubation 1	33	5.17E+08	1.95E+08	ANME-1	7.20	216
Anaerobic Incubation 1	47	2.55E+08	5.75E+07	ANME-1	3.83	115
Anaerobic Incubation 1	61	1.98E+08	8.19E+07	ANME-1	3.10	93
Anaerobic Incubation 1	68	2.81E+08	7.18E+07	ANME-1	4.17	125
Anaerobic Incubation 1	75	1.69E+08	6.81E+07	ANME-1	2.73	82
Anaerobic Incubation 1	80	1.35E+08	4.34E+07	ANME-1	2.30	69
Anaerobic Incubation 1	86	3.38E+08	3.94E+07	ANME-1	4.90	147
Anaerobic Incubation 1	95	2.08E+08	6.23E+07	ANME-1	3.13	94
Anaerobic Incubation 1	107	2.68E+08	5.15E+07	ANME-1	4.00	120
Anaerobic Incubation 1	114	1.27E+08	7.40E+07	ANME-1	2.20	66
Anaerobic Incubation 1	122	8.32E+07	4.20E+07	ANME-1	1.63	49
Anaerobic Incubation 1	0	2.16E+08	8.62E+07	Archaea	3.23	97
Anaerobic Incubation 1	5	2.91E+08	1.25E+08	Archaea	4.20	126
Anaerobic Incubation 1	12	1.14E+08	6.67E+07	Archaea	1.93	58
Anaerobic Incubation 1	19			Archaea		
Anaerobic Incubation 1	33	3.12E+08	1.26E+08	Archaea	4.47	134
Anaerobic Incubation 1	47	3.38E+08	1.04E+08	Archaea	4.80	144
Anaerobic Incubation 1	61	2.33E+08	2.41E+08	Archaea	3.45	100

Table A-4. Continued

Sample	Days Elapsed	Cells/ml	STD dev	Target Taxa	avg cells/grid	Total Cells
Anaerobic Incubation 1	68	3.93E+08	9.59E+07	Archaea	5.50	165
Anaerobic Incubation 1	75			Archaea		0
Anaerobic Incubation 1	80	2.42E+08	9.27E+07	Archaea	3.57	107
Anaerobic Incubation 1	86	3.72E+08	1.65E+08	Archaea	5.23	157
Anaerobic Incubation 1	95	5.20E+08	2.27E+08	Archaea	7.13	214
Anaerobic Incubation 1	107	2.47E+08	9.55E+07	Archaea	3.63	109
Anaerobic Incubation 1	114			Archaea		
Anaerobic Incubation 1	122	2.91E+08	1.58E+08	Archaea	4.20	126
Anaerobic Incubation 1	0	1.89E+08	9.38E+07	Bacteria	2.82	79
Anaerobic Incubation 1	5	1.66E+08	1.17E+08	Bacteria	2.53	76
Anaerobic Incubation 1	12	1.64E+08	9.82E+07	Bacteria	2.50	75
Anaerobic Incubation 1	19			Bacteria		
Anaerobic Incubation 1	33	4.73E+08	1.93E+08	Bacteria	6.47	194
Anaerobic Incubation 1	47	5.77E+08	3.24E+08	Bacteria	7.80	234
Anaerobic Incubation 1	61	2.37E+08	1.22E+08	Bacteria	3.43	103
Anaerobic Incubation 1	68	3.93E+08	1.36E+08	Bacteria	5.43	163
Anaerobic Incubation 1	75	2.18E+08	1.58E+08	Bacteria	3.20	96
Anaerobic Incubation 1	80	2.29E+08	3.87E+07	Bacteria	3.33	100
Anaerobic Incubation 1	86	3.85E+08	1.04E+08	Bacteria	5.33	160
Anaerobic Incubation 1	95	3.38E+08	1.34E+08	Bacteria	4.73	142
Anaerobic Incubation 1	107	3.74E+08	1.08E+08	Bacteria	5.20	156
Anaerobic Incubation 1	114			Bacteria		
Anaerobic Incubation 1	122	2.37E+08	8.00E+07	Bacteria	3.43	103
Anaerobic Incubation 1	0	1.82E+08	6.50E+07	Sulfate Reducing Bacteria	2.60	78
Anaerobic Incubation 1	5	2.55E+08	9.48E+07	Sulfate Reducing Bacteria	3.53	106
Anaerobic Incubation 1	12	2.76E+08	6.46E+07	Sulfate Reducing Bacteria	3.80	114
Anaerobic Incubation 1	19	6.99E+08	3.44E+08	Sulfate Reducing Bacteria	9.23	277
Anaerobic Incubation 1	33	2.99E+08	9.27E+07	Sulfate Reducing Bacteria	4.10	123
Anaerobic Incubation 1	47	5.25E+08	1.68E+08	Sulfate Reducing Bacteria	7.00	210
Anaerobic Incubation 1	61	4.11E+08	1.16E+08	Sulfate Reducing Bacteria	5.53	166

Table A-4. Continued

Sample	Days Elapsed	Cells/ml	STD dev	Target Taxa	avg cells/grid	Total Cells
Anaerobic Incubation 1	68	8.37E+08	1.89E+08	Sulfate Reducing Bacteria	11.00	330
Anaerobic Incubation 1	75	2.16E+08	6.28E+07	Sulfate Reducing Bacteria	3.03	91
Anaerobic Incubation 1	80	3.22E+08	9.31E+07	Sulfate Reducing Bacteria	4.40	132
Anaerobic Incubation 1	86	4.06E+08	9.79E+07	Sulfate Reducing Bacteria	5.47	164
Anaerobic Incubation 1	95	5.90E+08	1.52E+08	Sulfate Reducing Bacteria	7.63	229
Anaerobic Incubation 1	107	2.91E+08	4.13E+07	Sulfate Reducing Bacteria	4.00	120
Anaerobic Incubation 1	114	3.48E+08	7.69E+07	Sulfate Reducing Bacteria	4.73	142
Anaerobic Incubation 1	122	6.14E+08	2.91E+08	Sulfate Reducing Bacteria	8.13	244
Anaerobic Incubation 1	0	2.55E+08	4.69E+07	ANME-2	3.87	116
Anaerobic Incubation 1	5	1.30E+08	3.56E+07	ANME-2	2.27	68
Anaerobic Incubation 1	12	1.59E+08	6.99E+07	ANME-2	2.63	79
Anaerobic Incubation 1	19	1.30E+08	1.25E+07	ANME-2	2.27	68
Anaerobic Incubation 1	33	4.29E+08	1.32E+08	ANME-2	6.10	183
Anaerobic Incubation 1	47	2.81E+08	3.86E+07	ANME-2	4.20	126
Anaerobic Incubation 1	61	2.70E+08	4.35E+07	ANME-2	4.07	122
Anaerobic Incubation 1	68	3.61E+08	1.10E+08	ANME-2	5.23	157
Anaerobic Incubation 1	75	2.34E+08	7.16E+07	ANME-2	3.60	108
Anaerobic Incubation 1	80	2.05E+08	5.22E+07	ANME-2	3.23	97
Anaerobic Incubation 1	86	3.22E+08	6.11E+07	ANME-2	4.73	142
Anaerobic Incubation 1	95	3.69E+08	3.23E+07	ANME-2	5.33	160
Anaerobic Incubation 1	107	3.48E+08	1.11E+08	ANME-2	5.07	152
Anaerobic Incubation 1	114	2.11E+08	6.79E+07	ANME-2	3.30	99
Anaerobic Incubation 1	122	2.42E+08	2.97E+07	ANME-2	3.70	111
Anaerobic Incubation 2	0	2.13E+08	1.02E+08	MCG	2.80	84
Anaerobic Incubation 2	5	2.03E+08	1.35E+08	MCG	2.67	80
Anaerobic Incubation 2	12	3.15E+08	1.04E+08	MCG	4.10	123
Anaerobic Incubation 2	19	1.40E+08	1.13E+08	MCG	1.87	56
Anaerobic Incubation 2	33	3.61E+08	1.52E+08	MCG	4.70	141

Table A-4. Continued

Sample	Days Elapsed	Cells/ml	STD dev	Target Taxa	avg cells/grid	Total Cells
Anaerobic Incubation 2	47	2.08E+08	8.25E+07	MCG	2.73	82
Anaerobic Incubation 2	61	4.21E+08	1.33E+08	MCG	5.47	164
Anaerobic Incubation 2	68	8.84E+07	6.81E+07	MCG	1.20	36
Anaerobic Incubation 2	75	2.57E+08	6.83E+07	MCG	3.37	101
Anaerobic Incubation 2	80	2.11E+08	8.20E+07	MCG	2.77	83
Anaerobic Incubation 2	86	2.68E+08	9.38E+07	MCG	3.50	105
Anaerobic Incubation 2	95	1.95E+08	7.11E+07	MCG	2.57	77
Anaerobic Incubation 2	107	2.39E+08	9.38E+07	MCG	3.13	94
Anaerobic Incubation 2	114	1.22E+08	8.36E+07	MCG	1.63	49
Anaerobic Incubation 2	122	2.11E+08	7.99E+07	MCG	2.77	83
Anaerobic Incubation 2	0	7.80E+07	4.86E+07	MCG	1.47	44
Anaerobic Incubation 2	5	1.51E+08	8.31E+07	MCG	2.40	72
Anaerobic Incubation 2	12	1.12E+08	4.11E+07	MCG	1.90	57
Anaerobic Incubation 2	19	1.69E+08	7.97E+07	MCG	2.63	79
Anaerobic Incubation 2	33	3.93E+08	1.01E+08	MCG	5.50	165
Anaerobic Incubation 2	47	1.40E+08	1.55E+07	MCG	2.27	68
Anaerobic Incubation 2	61	2.16E+08	4.41E+07	MCG	3.23	97
Anaerobic Incubation 2	68	1.95E+08	6.59E+07	MCG	2.97	89
Anaerobic Incubation 2	75	1.69E+08	8.91E+07	MCG	2.63	79
Anaerobic Incubation 2	80	1.40E+08	1.55E+07	MCG	2.27	68
Anaerobic Incubation 2	86	2.21E+08	4.06E+07	MCG	3.30	99
Anaerobic Incubation 2	95	2.65E+08	6.26E+07	MCG	3.87	116
Anaerobic Incubation 2	107	1.12E+08	5.20E+07	MCG	1.90	57
Anaerobic Incubation 2	114	1.64E+08	5.48E+07	MCG	2.57	77
Anaerobic Incubation 2	122	1.12E+08	3.63E+07	MCG	1.90	57
Anaerobic Incubation 2	0	1.64E+08	8.68E+07	Methanomicrobiales	2.27	68
Anaerobic Incubation 2	5	4.78E+08	2.79E+08	Methanomicrobiales	6.30	189
Anaerobic Incubation 2	12	1.82E+08	1.13E+08	Methanomicrobiales	2.50	75
Anaerobic Incubation 2	19	2.63E+08	1.01E+08	Methanomicrobiales	3.53	106
Anaerobic Incubation 2	33	3.67E+08	7.38E+07	Methanomicrobiales	4.87	146
Anaerobic Incubation 2	47	1.40E+08	5.67E+07	Methanomicrobiales	1.97	59
Anaerobic Incubation 2	61	4.24E+08	1.17E+08	Methanomicrobiales	5.60	168
Anaerobic Incubation 2	68	1.56E+08	9.53E+07	Methanomicrobiales	2.17	65
Anaerobic Incubation 2	75	2.86E+08	6.46E+07	Methanomicrobiales	3.83	115

Table A-4. Continued

Sample	Days Elapsed	Cells/ml	STD dev	Target Taxa	avg cells/grid	Total Cells
Anaerobic Incubation 2	80	1.40E+08	5.67E+07	Methanomicrobiales	1.97	59
Anaerobic Incubation 2	86			Methanomicrobiales		0
Anaerobic Incubation 2	95	3.98E+08	7.42E+07	Methanomicrobiales	5.27	158
Anaerobic Incubation 2	107	3.85E+08	8.92E+07	Methanomicrobiales	5.10	153
Anaerobic Incubation 2	114	3.15E+08	1.48E+08	Methanomicrobiales	4.20	126
Anaerobic Incubation 2	122	5.33E+08	1.94E+08	Methanomicrobiales	7.00	210
Anaerobic Incubation 2	0	3.41E+08	1.03E+08	ANME-1	4.93	148
Anaerobic Incubation 2	5	2.37E+08	1.24E+08	ANME-1	3.60	108
Anaerobic Incubation 2	12	2.00E+08	2.57E+07	ANME-1	3.13	94
Anaerobic Incubation 2	19	2.68E+08	9.90E+07	ANME-1	4.00	120
Anaerobic Incubation 2	33	3.56E+08	8.86E+07	ANME-1	5.13	154
Anaerobic Incubation 2	47	2.18E+08	5.64E+07	ANME-1	3.37	101
Anaerobic Incubation 2	61	5.82E+08	1.71E+08	ANME-1	8.03	241
Anaerobic Incubation 2	68	3.09E+08	8.56E+07	ANME-1	4.53	136
Anaerobic Incubation 2	75	2.00E+08	6.60E+07	ANME-1	3.13	94
Anaerobic Incubation 2	80	2.11E+08	4.93E+07	ANME-1	3.27	98
Anaerobic Incubation 2	86	2.50E+08	5.48E+07	ANME-1	3.77	113
Anaerobic Incubation 2	95	4.81E+08	8.28E+07	ANME-1	6.73	202
Anaerobic Incubation 2	107	2.68E+08	6.11E+07	ANME-1	4.00	120
Anaerobic Incubation 2	114	1.69E+08	1.12E+08	ANME-1	2.73	82
Anaerobic Incubation 2	122	1.07E+08	5.53E+07	ANME-1	1.93	58
Anaerobic Incubation 2	0	3.38E+08	7.34E+07	Archaea	4.80	144
Anaerobic Incubation 2	5	2.86E+08	7.40E+07	Archaea	4.13	124
Anaerobic Incubation 2	12			Archaea		0
Anaerobic Incubation 2	19			Archaea		0
Anaerobic Incubation 2	33	3.87E+08	1.09E+08	Archaea	5.43	163
Anaerobic Incubation 2	47	1.90E+08	1.31E+08	Archaea	2.90	87
Anaerobic Incubation 2	61	3.41E+08	1.41E+08	Archaea	4.83	145
Anaerobic Incubation 2	68	2.34E+08	8.79E+07	Archaea	3.47	104
Anaerobic Incubation 2	75	2.24E+08	5.28E+07	Archaea	3.33	100
Anaerobic Incubation 2	80	1.90E+08	1.31E+08	Archaea	2.90	87
Anaerobic Incubation 2	86			Archaea		0
Anaerobic Incubation 2	95	3.64E+08	8.29E+07	Archaea	5.13	154
Anaerobic Incubation 2	107	1.51E+08	4.39E+07	Archaea	2.40	72
Anaerobic Incubation 2	114	1.48E+08	8.24E+07	Archaea	2.37	71
Anaerobic Incubation 2	122	2.86E+08	1.02E+08	Archaea	4.13	124

Table A-4. Continued

Sample	Days Elapsed	Cells/ml	STD dev	Target Taxa	avg cells/grid	Total Cells
Anaerobic Incubation 2	0	2.37E+08	6.29E+07	Bacteria	3.43	103
Anaerobic Incubation 2	5	1.74E+08	6.65E+07	Bacteria	2.63	79
Anaerobic Incubation 2	12			Bacteria		0
Anaerobic Incubation 2	19			Bacteria		0
Anaerobic Incubation 2	33	3.48E+08	1.28E+08	Bacteria	4.87	146
Anaerobic Incubation 2	47	4.16E+08	1.84E+08	Bacteria	5.73	172
Anaerobic Incubation 2	61	2.63E+08	1.18E+08	Bacteria	3.77	113
Anaerobic Incubation 2	68	2.16E+08	6.97E+07	Bacteria	3.17	95
Anaerobic Incubation 2	75	2.11E+08	4.65E+07	Bacteria	3.10	93
Anaerobic Incubation 2	80	4.00E+08	1.98E+08	Bacteria	5.53	166
Anaerobic Incubation 2	86	3.20E+08	7.84E+07	Bacteria	4.50	135
Anaerobic Incubation 2	95	4.50E+08	8.75E+07	Bacteria	6.17	185
Anaerobic Incubation 2	107	2.21E+08	5.65E+07	Bacteria	3.23	97
Anaerobic Incubation 2	114	1.79E+08	6.59E+07	Bacteria	2.70	81
Anaerobic Incubation 2	122			Bacteria		0
Anaerobic Incubation 2	0	4.00E+08	1.23E+08	Sulfate Reducing Bacteria	5.40	162
Anaerobic Incubation 2	5	2.68E+08	9.85E+07	Sulfate Reducing Bacteria	3.70	111
Anaerobic Incubation 2	12	2.73E+08	7.65E+07	Sulfate Reducing Bacteria	3.77	113
Anaerobic Incubation 2	19	8.09E+08	2.59E+08	Sulfate Reducing Bacteria	10.63	319
Anaerobic Incubation 2	33	6.71E+08	1.45E+08	Sulfate Reducing Bacteria	8.87	266
Anaerobic Incubation 2	47	2.21E+08	4.95E+07	Sulfate Reducing Bacteria	3.10	93
Anaerobic Incubation 2	61	5.23E+08	1.74E+08	Sulfate Reducing Bacteria	6.97	209
Anaerobic Incubation 2	68	3.02E+08	1.29E+08	Sulfate Reducing Bacteria	4.13	124
Anaerobic Incubation 2	75	2.16E+08	7.79E+07	Sulfate Reducing Bacteria	3.03	91
Anaerobic Incubation 2	80	2.21E+08	4.95E+07	Sulfate Reducing Bacteria	3.10	93

Table A-4. Continued

Sample	Days Elapsed	Cells/ml	STD dev	Target Taxa	avg cells/grid	Total Cells
Anaerobic Incubation 2	86	2.81E+08	5.92E+07	Sulfate Reducing Bacteria	3.87	116
Anaerobic Incubation 2	95	5.85E+08	2.29E+08	Sulfate Reducing Bacteria	7.77	233
Anaerobic Incubation 2	107	3.04E+08	5.34E+07	Sulfate Reducing Bacteria	4.17	125
Anaerobic Incubation 2	114	2.03E+08	1.02E+08	Sulfate Reducing Bacteria	2.87	86
Anaerobic Incubation 2	122	5.30E+08	1.85E+08	Sulfate Reducing Bacteria	7.07	212
Anaerobic Incubation 2	0	3.67E+08	1.11E+08	ANME-2	5.30	159
Anaerobic Incubation 2	5			ANME-2		0
Anaerobic Incubation 2	12	2.65E+08	4.36E+07	ANME-2	4.00	120
Anaerobic Incubation 2	19	1.38E+08	2.37E+07	ANME-2	2.37	71
Anaerobic Incubation 2	33	4.29E+08	1.00E+08	ANME-2	6.10	183
Anaerobic Incubation 2	47	2.03E+08	3.03E+07	ANME-2	3.20	96
Anaerobic Incubation 2	61	5.02E+08	1.60E+08	ANME-2	7.03	211
Anaerobic Incubation 2	68	2.16E+08	9.39E+07	ANME-2	3.37	101
Anaerobic Incubation 2	75	2.14E+08	1.35E+05	ANME-2	3.34	97
Anaerobic Incubation 2	80	2.01E+08	3.12E+07	ANME-2	3.17	92
Anaerobic Incubation 2	86	2.25E+08	4.48E+07	ANME-2	3.48	101
Anaerobic Incubation 2	95	2.46E+08	5.95E+07	ANME-2	3.76	109
Anaerobic Incubation 2	107	1.50E+08	4.87E+07	ANME-2	2.52	73
Anaerobic Incubation 2	114	1.66E+08	8.79E+07	ANME-2	2.73	82
Anaerobic Incubation 2	122	2.68E+08	6.20E+07	ANME-2	4.03	121
Methane Incubation	0	5.28E+08	1.31E+08	MCG	6.83	205
Methane Incubation	5	3.64E+08	2.00E+08	MCG	4.73	142
Methane Incubation	12	1.30E+08	1.14E+08	MCG	2.27	68
Methane Incubation	19			MCG		0
Methane Incubation	33	4.89E+08	2.10E+08	MCG	6.87	206
Methane Incubation	47	1.87E+08	8.21E+07	MCG	2.87	86
Methane Incubation	61	1.59E+08	6.15E+07	MCG	2.50	75
Methane Incubation	68	1.38E+08	6.55E+07	MCG	2.33	70
Methane Incubation	75	2.60E+08	1.17E+08	MCG	3.90	117
Methane Incubation	80	1.43E+08	8.33E+07	MCG	2.10	63
Methane Incubation	86	2.70E+08	8.45E+07	MCG	3.73	112
Methane Incubation	95	1.98E+08	1.37E+08	MCG	3.10	93

Table A-4. Continued

Sample	Days Elapsed	Cells/ml	STD dev	Target Taxa	avg cells/grid	Total Cells
Methane Incubation	107	4.97E+08	1.56E+08	MCG	6.63	199
Methane Incubation	114	2.05E+08	1.34E+08	MCG	2.90	87
Methane Incubation	122	2.52E+08	8.38E+07	MCG	3.40	102
Methane Incubation	0	3.38E+08	7.79E+07	MCG	4.80	144
Methane Incubation	5	4.76E+08	2.05E+08	MCG	6.57	197
Methane Incubation	12	1.40E+08	6.40E+07	MCG	2.37	71
Methane Incubation	19	1.82E+08	7.10E+07	MCG	2.90	87
Methane Incubation	33			MCG		0
Methane Incubation	47	1.79E+08	4.85E+07	MCG	2.57	77
Methane Incubation	61	1.38E+08	4.37E+07	MCG	2.03	61
Methane Incubation	68	1.79E+08	4.19E+07	MCG	2.47	74
Methane Incubation	75	2.29E+08	7.27E+07	MCG	3.33	100
Methane Incubation	80	1.14E+08	7.51E+07	MCG	1.87	56
Methane Incubation	86	2.65E+08	7.95E+07	MCG	3.80	114
Methane Incubation	95			MCG		0
Methane Incubation	107	3.35E+08	6.15E+07	MCG	4.69	136
Methane Incubation	114	3.56E+08	7.12E+07	MCG	5.03	151
Methane Incubation	122	9.88E+07	7.03E+07	MCG	1.73	52
Methane Incubation	0	4.42E+08	1.66E+08	Methanomicrobiales	5.83	175
Methane Incubation	5	4.45E+08	7.50E+07	Methanomicrobiales	6.10	183
Methane Incubation	12	2.57E+08	1.00E+08	Methanomicrobiales	3.70	111
Methane Incubation	19	4.29E+08	1.93E+08	Methanomicrobiales	5.97	179
Methane Incubation	33	2.78E+08	1.02E+08	Methanomicrobiales	4.03	121
Methane Incubation	47	3.77E+08	9.22E+07	Methanomicrobiales	5.30	159
Methane Incubation	61	2.21E+08	4.69E+07	Methanomicrobiales	2.90	87
Methane Incubation	68	2.29E+08	8.52E+07	Methanomicrobiales	3.00	90
Methane Incubation	75	2.76E+08	5.03E+07	Methanomicrobiales	4.13	124
Methane Incubation	80	2.50E+08	1.08E+08	Methanomicrobiales	3.80	114
Methane Incubation	86	2.42E+08	8.55E+07	Methanomicrobiales	3.70	111
Methane Incubation	95			Methanomicrobiales		0
Methane Incubation	107	4.19E+08	1.02E+08	Methanomicrobiales	5.97	179

Table A-4. Continued

Sample	Days Elapsed	Cells/ml	STD dev	Target Taxa	avg cells/grid	Total Cells
Methane Incubation	114	2.73E+08	1.12E+08	Methanomicrobiales	3.97	119
Methane Incubation	122	3.41E+08	1.16E+08	Methanomicrobiales	4.83	145
Methane Incubation	0	5.98E+08	1.33E+08	ANME-1	8.23	247
Methane Incubation	5	3.12E+08	1.07E+08	ANME-1	4.57	137
Methane Incubation	12	2.55E+08	5.49E+07	ANME-1	3.53	106
Methane Incubation	19	2.21E+08	7.22E+07	ANME-1	3.10	93
Methane Incubation	33	4.39E+08	1.06E+08	ANME-1	5.90	177
Methane Incubation	47	3.09E+08	7.12E+07	ANME-1	4.13	124
Methane Incubation	61	1.74E+08	1.01E+07	ANME-1	2.63	79
Methane Incubation	68	1.79E+08	2.41E+07	ANME-1	2.70	81
Methane Incubation	75	2.76E+08	6.99E+07	ANME-1	4.00	120
Methane Incubation	80	1.61E+08	5.87E+07	ANME-1	2.53	76
Methane Incubation	86	2.94E+08	6.94E+07	ANME-1	4.23	127
Methane Incubation	95	2.55E+08	4.08E+07	ANME-1	3.73	112
Methane Incubation	107	2.68E+08	4.81E+07	ANME-1	3.90	117
Methane Incubation	114	2.18E+08	5.68E+07	ANME-1	2.87	86
Methane Incubation	122	1.46E+08	2.07E+07	ANME-1	1.93	58
Methane Incubation	0	3.74E+08	1.50E+08	Archaea	5.27	158
Methane Incubation	5	2.81E+08	1.27E+08	Archaea	4.07	122
Methane Incubation	12			Archaea		0
Methane Incubation	19			Archaea		0
Methane Incubation	33	5.02E+08	1.63E+08	Archaea	6.50	195
Methane Incubation	47	2.63E+08	5.75E+07	Archaea	3.97	119
Methane Incubation	61	1.27E+08	5.36E+07	Archaea	2.23	67
Methane Incubation	68	1.59E+08	7.76E+07	Archaea	2.50	75
Methane Incubation	75	1.46E+08	4.06E+07	Archaea	2.33	70
Methane Incubation	80	2.52E+08	8.40E+07	Archaea	3.80	114
Methane Incubation	86			Archaea		0
Methane Incubation	95			Archaea		0
Methane Incubation	107	3.02E+08	7.20E+07	Archaea	4.43	133
Methane Incubation	114	1.04E+08	8.05E+07	Archaea	1.90	57
Methane Incubation	122	1.38E+08	5.54E+07	Archaea	2.03	61
Methane Incubation	0	4.37E+08	1.61E+08	Bacteria	6.00	180
Methane Incubation	5	3.22E+08	1.66E+08	Bacteria	4.53	136
Methane Incubation	12			Bacteria		0
Methane Incubation	19			Bacteria		0
Methane Incubation	33	5.30E+08	2.03E+08	Bacteria	7.27	218
Methane Incubation	47	2.42E+08	9.40E+07	Bacteria	3.17	95

Table A-4. Continued

Sample	Days Elapsed	Cells/ml	STD dev	Target Taxa	avg cells/grid	Total Cells
Methane Incubation	61	2.47E+08	5.20E+07	Bacteria	3.23	97
Methane Incubation	68	4.68E+07	6.02E+07	Bacteria	1.20	36
Methane Incubation	75	1.77E+08	2.50E+07	Bacteria	2.87	86
Methane Incubation	80	3.30E+08	1.68E+08	Bacteria	4.70	141
Methane Incubation	86	2.94E+08	9.50E+07	Bacteria	4.23	127
Methane Incubation	95	3.85E+08	1.06E+08	Bacteria	5.53	166
Methane Incubation	107	5.51E+08	1.38E+08	Bacteria	7.53	226
Methane Incubation	114	1.46E+08	1.11E+08	Bacteria	2.33	70
Methane Incubation	122	1.61E+08	7.05E+07	Bacteria	2.63	79
Methane Incubation	0	1.38E+08	5.43E+07	Sulfate Reducing Bacteria	2.03	61
Methane Incubation	5	8.37E+08	2.65E+08	Sulfate Reducing Bacteria	11.00	330
Methane Incubation	12	3.22E+08	1.49E+08	Sulfate Reducing Bacteria	4.30	129
Methane Incubation	19	2.26E+08	9.24E+07	Sulfate Reducing Bacteria	3.30	99
Methane Incubation	33	4.52E+08	1.83E+08	Sulfate Reducing Bacteria	6.20	186
Methane Incubation	47	6.53E+08	1.72E+08	Sulfate Reducing Bacteria	8.77	263
Methane Incubation	61	2.89E+08	9.38E+07	Sulfate Reducing Bacteria	4.17	125
Methane Incubation	68	5.36E+08	1.56E+08	Sulfate Reducing Bacteria	7.33	220
Methane Incubation	75	4.11E+08	9.26E+07	Sulfate Reducing Bacteria	5.33	160
Methane Incubation	80	3.98E+08	8.24E+07	Sulfate Reducing Bacteria	5.17	155
Methane Incubation	86	2.60E+08	1.28E+08	Sulfate Reducing Bacteria	3.40	102
Methane Incubation	95	2.60E+08	5.01E+07	Sulfate Reducing Bacteria	3.40	102

Table A-4. Continued

Sample	Days Elapsed	Cells/ml	STD dev	Target Taxa	avg cells/grid	Total Cells
Methane Incubation	107	6.19E+08	1.97E+08	Sulfate Reducing Bacteria	8.00	240
Methane Incubation	114	3.33E+08	1.56E+08	Sulfate Reducing Bacteria	4.87	146
Methane Incubation	122	3.20E+08	6.81E+07	Sulfate Reducing Bacteria	4.70	141
Methane Incubation	0	6.63E+08	1.10E+08	ANME-2	9.10	273
Methane Incubation	5	7.96E+08	2.92E+08	ANME-2	10.80	324
Methane Incubation	12	1.90E+08	6.82E+07	ANME-2	2.90	87
Methane Incubation	19	3.98E+08	1.45E+08	ANME-2	5.57	167
Methane Incubation	33	2.83E+08	8.43E+07	ANME-2	4.10	123
Methane Incubation	47	2.81E+08	8.39E+07	ANME-2	4.17	125
Methane Incubation	61	1.90E+08	1.26E+07	ANME-2	3.00	90
Methane Incubation	68	2.16E+08	4.26E+07	ANME-2	3.03	91
Methane Incubation	75	4.32E+08	7.13E+07	ANME-2	5.80	174
Methane Incubation	80	2.96E+08	7.45E+07	ANME-2	3.97	119
Methane Incubation	86	3.33E+08	3.32E+06	ANME-2	4.43	133
Methane Incubation	95	3.12E+08	7.81E+07	ANME-2	4.27	128
Methane Incubation	107	6.27E+08	1.60E+08	ANME-2	8.20	246
Methane Incubation	114			ANME-2		0
Methane Incubation	122	2.86E+08	7.15E+07	ANME-2	4.07	118

Table A-5: Microbial abundance using real time PCR.

Incubation	Days	Primer Set	copies/ gram (1)	copies/ gram (2)	Avg copies/ gram	Standard Dev
Anaerobic Incubation 1	0	ANME-2	9.60E+06	1.17E+07	1.06E+07	1.46E+06
Anaerobic Incubation 1	5	ANME-2	2.32E+07	1.05E+07	1.68E+07	9.02E+06
Anaerobic Incubation 1	12	ANME-2	4.07E+07		4.07E+07	#DIV/0!
Anaerobic Incubation 1	19	ANME-2	1.29E+07	1.93E+07	1.61E+07	4.50E+06
Anaerobic Incubation 1	26	ANME-2	1.02E+07	1.05E+07	1.03E+07	1.76E+05
Anaerobic Incubation 1	33	ANME-2	1.26E+07	1.53E+07	1.39E+07	1.88E+06
Anaerobic Incubation 1	40	ANME-2	1.85E+07	1.37E+07	1.61E+07	3.41E+06
Anaerobic Incubation 1	47	ANME-2	1.35E+07	1.53E+07	1.44E+07	1.25E+06
Anaerobic Incubation 1	54	ANME-2			#DIV/0!	#DIV/0!
Anaerobic Incubation 1	61	ANME-2	2.30E+07	1.94E+07	2.12E+07	2.52E+06
Anaerobic Incubation 1	68	ANME-2	6.19E+06	4.42E+06	5.31E+06	1.25E+06
Anaerobic Incubation 1	75	ANME-2	2.93E+07		2.93E+07	#DIV/0!
Anaerobic Incubation 1	80	ANME-2	3.23E+07	2.93E+07	3.08E+07	2.12E+06
Anaerobic Incubation 1	86	ANME-2	3.59E+07	4.06E+07	4.58E+07	1.33E+07
Anaerobic Incubation 1	94	ANME-2	1.54E+07	9.78E+06	8.73E+07	9.28E+07
Anaerobic Incubation 1	107	ANME-2	2.63E+07	4.19E+07	3.41E+07	1.10E+07
Anaerobic Incubation 1	114	ANME-2	1.56E+07	2.49E+07	2.03E+07	6.59E+06
Anaerobic Incubation 1	122	ANME-2	5.43E+06	1.08E+07	8.09E+06	3.76E+06
Anaerobic Incubation 2	0	ANME-2	7.18E+06	1.06E+07	8.89E+06	2.42E+06
Anaerobic Incubation 2	5	ANME-2	2.86E+06	6.63E+06	4.74E+06	2.67E+06
Anaerobic Incubation 2	12	ANME-2	1.74E+07	3.17E+07	2.46E+07	1.01E+07
Anaerobic Incubation 2	19	ANME-2	3.70E+07	2.49E+07	3.10E+07	8.58E+06
Anaerobic Incubation 2	26	ANME-2	3.57E+07	2.76E+07	3.17E+07	5.76E+06
Anaerobic Incubation 2	33	ANME-2	2.06E+07	2.68E+07	2.37E+07	4.37E+06
Anaerobic Incubation 2	40	ANME-2	9.31E+06	5.66E+06	7.48E+06	2.58E+06
Anaerobic Incubation 2	47	ANME-2	1.86E+07	2.70E+07	2.28E+07	5.98E+06
Anaerobic Incubation 2	54	ANME-2	6.83E+06	6.04E+06	6.43E+06	5.54E+05
Anaerobic Incubation 2	61	ANME-2	1.41E+07	1.52E+07	1.47E+07	8.17E+05
Anaerobic Incubation 2	68	ANME-2	2.04E+07	3.81E+07	2.93E+07	1.25E+07
Anaerobic Incubation 2	75	ANME-2	1.88E+06	3.50E+06	2.69E+06	1.14E+06
Anaerobic Incubation 2	80	ANME-2	9.31E+06	6.97E+06	8.14E+06	1.66E+06
Anaerobic Incubation 2	86	ANME-2	1.29E+07	1.27E+07	1.28E+07	1.56E+05
Anaerobic Incubation 2	94	ANME-2	7.97E+07	8.00E+07	7.98E+07	2.25E+05
Anaerobic Incubation 2	107	ANME-2	1.36E+08	7.90E+07	1.08E+08	4.06E+07
Anaerobic Incubation 2	114	ANME-2	6.71E+07		6.71E+07	#DIV/0!
Anaerobic Incubation 2	122	ANME-2	1.21E+07	9.90E+06	1.10E+07	1.56E+06
Methane Incubation	0	ANME-2	2.42E+07	1.25E+07	1.83E+07	8.26E+06
Methane Incubation	5	ANME-2	1.16E+07	5.46E+06	8.53E+06	4.34E+06

Table A-5. Continued

Incubation	Days	Primer Set	copies/ gram (1)	copies/ gram (2)	Avg copies/ gram	Standard Dev
Methane Incubation	12	ANME-2			#DIV/0!	#DIV/0!
Methane Incubation	19	ANME-2	8.12E+06	8.00E+05	4.46E+06	5.18E+06
Methane Incubation	26	ANME-2	7.60E+06	9.55E+06	8.57E+06	1.38E+06
Methane Incubation	33	ANME-2	3.69E+07	3.80E+07	3.75E+07	8.21E+05
Methane Incubation	40	ANME-2	2.87E+07	1.73E+07	2.30E+07	8.05E+06
Methane Incubation	47	ANME-2	1.00E+07	6.66E+06	8.34E+06	2.37E+06
Methane Incubation	54	ANME-2	5.03E+07	4.93E+07	4.98E+07	6.47E+05
Methane Incubation	61	ANME-2	1.86E+07	1.74E+07	1.80E+07	8.38E+05
Methane Incubation	68	ANME-2	5.14E+06	4.59E+06	4.86E+06	3.90E+05
Methane Incubation	75	ANME-2	1.86E+07	2.23E+07	2.04E+07	2.57E+06
Methane Incubation	80	ANME-2	8.59E+06	1.17E+07	1.01E+07	2.18E+06
Methane Incubation	86	ANME-2	1.53E+07	1.79E+07	2.43E+07	1.02E+07
Methane Incubation	94	ANME-2	1.07E+07	1.17E+07	1.12E+07	6.95E+05
Methane Incubation	107	ANME-2	1.63E+07	2.43E+07	2.03E+07	5.65E+06
Methane Incubation	114	ANME-2	2.52E+07	2.31E+07	2.42E+07	1.47E+06
Methane Incubation	122	ANME-2	4.50E+07	1.93E+07	3.21E+07	1.82E+07
Anaerobic Incubation 1	0	ANME-1	1.33E+06	7.80E+05	1.06E+06	3.92E+05
Anaerobic Incubation 1	5	ANME-1	3.13E+05	1.33E+06	8.21E+05	7.18E+05
Anaerobic Incubation 1	12	ANME-1	5.41E+05	6.08E+05	5.74E+05	4.72E+04
Anaerobic Incubation 1	19	ANME-1	1.72E+05	3.86E+05	2.79E+05	1.51E+05
Anaerobic Incubation 1	26	ANME-1	4.28E+05	7.88E+05	3.52E+05	3.30E+05
Anaerobic Incubation 1	33	ANME-1	1.86E+06	5.26E+05	1.19E+06	9.44E+05
Anaerobic Incubation 1	40	ANME-1	3.05E+05	2.93E+05	2.99E+05	8.20E+03
Anaerobic Incubation 1	47	ANME-1	8.89E+05	8.59E+05	8.74E+05	2.16E+04
Anaerobic Incubation 1	54	ANME-1	9.90E+05	7.60E+05	8.75E+05	1.63E+05
Anaerobic Incubation 1	61	ANME-1	2.31E+06	1.16E+05	1.21E+06	1.55E+06
Anaerobic Incubation 1	68	ANME-1	2.39E+06	2.33E+06	1.38E+06	1.14E+06
Anaerobic Incubation 1	75	ANME-1	7.69E+05	5.50E+05	1.72E+06	1.84E+06
Anaerobic Incubation 1	80	ANME-1	1.96E+06	4.41E+05	1.20E+06	1.07E+06
Anaerobic Incubation 1	86	ANME-1	6.13E+06	4.49E+06	4.00E+06	2.42E+06
Anaerobic Incubation 1	94	ANME-1	8.91E+05	7.00E+05	8.52E+05	7.18E+05
Anaerobic Incubation 1	107	ANME-1	1.45E+05	3.37E+05	4.22E+06	7.04E+06
Anaerobic Incubation 1	114	ANME-1	1.13E+06	5.05E+05	7.73E+05	6.36E+05
Anaerobic Incubation 1	122	ANME-1	1.49E+06	7.19E+05	7.92E+05	6.66E+05
Anaerobic Incubation 2	0	ANME-1	1.19E+06	3.19E+05	7.55E+05	6.17E+05
Anaerobic Incubation 2	5	ANME-1	2.54E+05	6.39E+04	1.59E+05	1.35E+05
Anaerobic Incubation 2	12	ANME-1	5.43E+05	4.88E+05	5.15E+05	3.94E+04
Anaerobic Incubation 2	19	ANME-1	4.84E+06	8.99E+06	6.92E+06	2.93E+06
Anaerobic Incubation 2	26	ANME-1	2.40E+06	1.17E+07	7.03E+06	6.55E+06

Table A-5. Continued

Incubation	Days	Primer Set	copies/ gram (1)	copies/ gram (2)	Avg copies/ gram	Standard Dev
Anaerobic Incubation 2	33	ANME-1	1.78E+05	9.12E+04	1.34E+05	6.12E+04
Anaerobic Incubation 2	40	ANME-1	1.24E+05	2.11E+05	6.81E+05	7.05E+05
Anaerobic Incubation 2	47	ANME-1	1.99E+06	2.34E+05	1.23E+06	8.75E+05
Anaerobic Incubation 2	54	ANME-1	4.91E+05	5.85E+05	5.38E+05	6.59E+04
Anaerobic Incubation 2	61	ANME-1	1.33E+06	1.31E+06	1.32E+06	1.19E+04
Anaerobic Incubation 2	68	ANME-1	8.73E+05	1.21E+06	1.04E+06	2.37E+05
Anaerobic Incubation 2	75	ANME-1	1.48E+06	1.09E+06	1.29E+06	2.74E+05
Anaerobic Incubation 2	80	ANME-1	4.42E+05	2.33E+05	8.21E+05	6.31E+05
Anaerobic Incubation 2	86	ANME-1	7.51E+05	8.59E+05	8.05E+05	7.67E+04
Anaerobic Incubation 2	94	ANME-1	3.53E+06	1.44E+06	2.48E+06	1.48E+06
Anaerobic Incubation 2	107	ANME-1	2.58E+06	2.23E+06	7.77E+06	6.23E+06
Anaerobic Incubation 2	114	ANME-1	8.09E+05	3.62E+05	1.15E+06	1.33E+06
Anaerobic Incubation 2	122	ANME-1	2.65E+06	1.74E+05	8.23E+05	1.22E+06
Methane Incubation	0	ANME-1	5.32E+05	2.69E+05	1.41E+06	1.20E+06
Methane Incubation	5	ANME-1	1.41E+06	1.94E+05	8.00E+05	8.57E+05
Methane Incubation	12	ANME-1	3.41E+05	3.73E+05	3.57E+05	2.27E+04
Methane Incubation	19	ANME-1	9.01E+04	5.00E+05	2.95E+05	2.90E+05
Methane Incubation	26	ANME-1	5.55E+05	5.91E+05	6.35E+05	9.74E+04
Methane Incubation	33	ANME-1	2.75E+04	5.86E+06	2.26E+06	2.52E+06
Methane Incubation	40	ANME-1	7.92E+05	1.68E+05	2.30E+06	3.17E+06
Methane Incubation	47	ANME-1	3.81E+05	7.33E+05	5.57E+05	2.49E+05
Methane Incubation	54	ANME-1	1.16E+06	1.03E+06	2.45E+06	1.56E+06
Methane Incubation	61	ANME-1	5.92E+05	5.57E+05	2.12E+06	2.68E+06
Methane Incubation	68	ANME-1	5.02E+06	1.99E+06	3.51E+06	2.15E+06
Methane Incubation	75	ANME-1	1.93E+06	3.64E+06	2.78E+06	1.21E+06
Methane Incubation	80	ANME-1	2.76E+06	3.21E+07	1.74E+07	2.07E+07
Methane Incubation	86	ANME-1	1.84E+06	3.13E+06	1.84E+06	9.86E+05
Methane Incubation	94	ANME-1	5.03E+05	3.96E+05	4.50E+05	7.57E+04
Methane Incubation	107	ANME-1	1.05E+06	5.99E+05	8.27E+05	3.23E+05
Methane Incubation	114	ANME-1	7.65E+05	9.34E+05	8.49E+05	1.20E+05
Methane Incubation	122	ANME-1	5.03E+05	2.56E+05	4.07E+05	1.32E+05
Anaerobic Incubation 1	0	Methanomicrobiales	1.78E+08	1.50E+08	1.64E+08	2.04E+07
Anaerobic Incubation 1	5	Methanomicrobiales	4.26E+08	3.06E+08	3.66E+08	8.47E+07
Anaerobic Incubation 1	12	Methanomicrobiales	4.55E+08	4.32E+08	4.43E+08	1.64E+07
Anaerobic Incubation 1	19	Methanomicrobiales	3.15E+08	3.08E+08	3.12E+08	4.66E+06
Anaerobic Incubation 1	26	Methanomicrobiales	3.41E+08	3.88E+08	3.65E+08	3.27E+07
Anaerobic Incubation 1	33	Methanomicrobiales	1.89E+09	1.87E+09	1.88E+09	1.60E+07
Anaerobic Incubation 1	40	Methanomicrobiales	4.37E+08	4.82E+08	4.60E+08	3.19E+07
Anaerobic Incubation 1	47	Methanomicrobiales	3.78E+08	6.78E+08	5.28E+08	2.12E+08

Table A-5. Continued

Incubation	Days	Primer Set	copies/ gram (1)	copies/ gram (2)	Avg copies/ gram	Standard Dev
Anaerobic Incubation 1	54	Methanomicrobiales	5.43E+08	6.67E+08	6.05E+08	8.76E+07
Anaerobic Incubation 1	61	Methanomicrobiales	4.24E+08	4.07E+08	4.16E+08	1.21E+07
Anaerobic Incubation 1	68	Methanomicrobiales	4.74E+08	3.45E+08	4.09E+08	9.12E+07
Anaerobic Incubation 1	75	Methanomicrobiales	5.32E+08	5.08E+08	5.20E+08	1.70E+07
Anaerobic Incubation 1	80	Methanomicrobiales	3.41E+08	2.92E+08	3.16E+08	3.43E+07
Anaerobic Incubation 1	86	Methanomicrobiales	4.45E+08	5.44E+08	4.94E+08	7.01E+07
Anaerobic Incubation 1	94	Methanomicrobiales	1.20E+09	1.07E+09	1.13E+09	9.76E+07
Anaerobic Incubation 1	107	Methanomicrobiales	2.93E+09	2.16E+09	2.55E+09	5.46E+08
Anaerobic Incubation 1	114	Methanomicrobiales	1.25E+09	1.82E+09	1.54E+09	4.06E+08
Anaerobic Incubation 1	122	Methanomicrobiales	3.22E+09	3.63E+09	3.43E+09	2.91E+08
Anaerobic Incubation 2	0	Methanomicrobiales	1.59E+06	1.97E+06	1.78E+06	2.74E+05
Anaerobic Incubation 2	5	Methanomicrobiales	9.77E+06	2.47E+06	6.12E+06	5.16E+06
Anaerobic Incubation 2	12	Methanomicrobiales	4.97E+06	1.02E+06	2.99E+06	2.79E+06
Anaerobic Incubation 2	19	Methanomicrobiales	1.35E+07	2.15E+06	7.84E+06	8.05E+06
Anaerobic Incubation 2	26	Methanomicrobiales	1.56E+07	1.78E+07	1.67E+07	1.58E+06
Anaerobic Incubation 2	33	Methanomicrobiales	1.20E+07	1.51E+06	6.77E+06	7.45E+06
Anaerobic Incubation 2	40	Methanomicrobiales	9.58E+06	6.87E+06	8.23E+06	1.92E+06
Anaerobic Incubation 2	47	Methanomicrobiales	1.68E+07	6.24E+06	1.15E+07	7.47E+06
Anaerobic Incubation 2	54	Methanomicrobiales	1.25E+07	5.91E+06	9.22E+06	4.67E+06
Anaerobic Incubation 2	61	Methanomicrobiales	3.03E+07	3.85E+07	3.44E+07	5.81E+06
Anaerobic Incubation 2	68	Methanomicrobiales	1.29E+07	1.78E+06	7.33E+06	7.84E+06
Anaerobic Incubation 2	75	Methanomicrobiales	8.76E+06	1.50E+06	5.13E+06	5.13E+06
Anaerobic Incubation 2	80	Methanomicrobiales	2.40E+07	5.55E+06	1.48E+07	1.30E+07
Anaerobic Incubation 2	86	Methanomicrobiales	4.57E+07	5.47E+07	5.02E+07	6.31E+06
Anaerobic Incubation 2	94	Methanomicrobiales	2.87E+08	2.68E+08	2.78E+08	1.34E+07
Anaerobic Incubation 2	107	Methanomicrobiales	4.07E+08	3.36E+08	3.72E+08	4.99E+07
Anaerobic Incubation 2	114	Methanomicrobiales	1.61E+08	1.57E+08	1.59E+08	3.21E+06
Anaerobic Incubation 2	122	Methanomicrobiales	1.08E+08	1.38E+08	1.23E+08	2.16E+07
Methane Incubation	0	Methanomicrobiales	2.07E+07	1.63E+07	1.85E+07	3.12E+06
Methane Incubation	5	Methanomicrobiales	1.40E+07	1.93E+07	1.67E+07	3.78E+06
Methane Incubation	12	Methanomicrobiales	1.44E+07	1.95E+07	1.69E+07	3.54E+06
Methane Incubation	19	Methanomicrobiales	1.76E+07	1.63E+07	1.70E+07	8.99E+05
Methane Incubation	26	Methanomicrobiales	1.09E+07	6.68E+06	8.79E+06	2.98E+06
Methane Incubation	33	Methanomicrobiales	3.62E+07	3.96E+07	3.79E+07	2.40E+06
Methane Incubation	40	Methanomicrobiales	2.34E+07	1.27E+07	1.80E+07	7.52E+06
Methane Incubation	47	Methanomicrobiales	1.45E+07	5.28E+06	9.89E+06	6.51E+06
Methane Incubation	54	Methanomicrobiales	1.69E+07	8.58E+05	8.87E+06	1.13E+07
Methane Incubation	61	Methanomicrobiales	1.39E+07	5.84E+06	9.90E+06	5.73E+06
Methane Incubation	68	Methanomicrobiales	1.65E+07	3.19E+06	9.85E+06	9.42E+06

Table A-5. Continued

Incubation	Days	Primer Set	copies/ gram (1)	copies/ gram (2)	Avg copies/ gram	Standard Dev
Methane Incubation	75	Methanomicrobiales	2.90E+07	1.35E+07	2.13E+07	1.10E+07
Methane Incubation	80	Methanomicrobiales	5.28E+07	3.00E+07	4.14E+07	1.62E+07
Methane Incubation	86	Methanomicrobiales	6.24E+07	3.53E+07	4.88E+07	1.92E+07
Methane Incubation	94	Methanomicrobiales	8.57E+07	6.66E+07	7.62E+07	1.36E+07
Methane Incubation	107	Methanomicrobiales	1.98E+08	1.22E+07	1.05E+08	1.31E+08
Methane Incubation	114	Methanomicrobiales	2.39E+08	4.46E+07	1.42E+08	1.38E+08
Methane Incubation	122	Methanomicrobiales	4.16E+08	2.13E+08	3.14E+08	1.43E+08
Anaerobic Incubation 1	0	Methanosarcinales	3.83E+07		3.83E+07	#DIV/0!
Anaerobic Incubation 1	5	Methanosarcinales	9.88E+08	9.77E+08	9.82E+08	8.41E+06
Anaerobic Incubation 1	12	Methanosarcinales	1.44E+08	4.45E+08	2.95E+08	2.13E+08
Anaerobic Incubation 1	19	Methanosarcinales	3.14E+08		3.14E+08	#DIV/0!
Anaerobic Incubation 1	26	Methanosarcinales			#DIV/0!	#DIV/0!
Anaerobic Incubation 1	33	Methanosarcinales			#DIV/0!	#DIV/0!
Anaerobic Incubation 1	40	Methanosarcinales			#DIV/0!	#DIV/0!
Anaerobic Incubation 1	47	Methanosarcinales	3.85E+07		3.85E+07	#DIV/0!
Anaerobic Incubation 1	54	Methanosarcinales			#DIV/0!	#DIV/0!
Anaerobic Incubation 1	61	Methanosarcinales	4.94E+08		4.94E+08	#DIV/0!
Anaerobic Incubation 1	68	Methanosarcinales	2.29E+08	8.48E+08	3.73E+08	4.21E+08
Anaerobic Incubation 1	75	Methanosarcinales	2.71E+08	1.73E+08	2.22E+08	6.92E+07
Anaerobic Incubation 1	80	Methanosarcinales			#DIV/0!	#DIV/0!
Anaerobic Incubation 1	86	Methanosarcinales	3.98E+08	1.09E+09	7.42E+08	4.87E+08
Anaerobic Incubation 1	94	Methanosarcinales	2.13E+08	4.72E+08	3.43E+08	1.83E+08
Anaerobic Incubation 1	107	Methanosarcinales	6.75E+08	2.14E+09	1.41E+09	1.03E+09
Anaerobic Incubation 1	114	Methanosarcinales	4.27E+08	1.18E+09	8.03E+08	5.31E+08
Anaerobic Incubation 1	122	Methanosarcinales	4.86E+08	1.16E+09	8.24E+08	4.77E+08
Anaerobic Incubation 2	0	Methanosarcinales	7.51E+07	6.93E+07	7.22E+07	4.11E+06
Anaerobic Incubation 2	5	Methanosarcinales	5.74E+07	6.33E+07	6.04E+07	4.19E+06
Anaerobic Incubation 2	12	Methanosarcinales	9.91E+07	1.06E+08	1.03E+08	5.10E+06
Anaerobic Incubation 2	19	Methanosarcinales	2.04E+08	2.06E+08	2.05E+08	8.92E+05
Anaerobic Incubation 2	26	Methanosarcinales	2.57E+08	2.96E+08	2.77E+08	2.77E+07
Anaerobic Incubation 2	33	Methanosarcinales	1.61E+08	8.07E+07	1.21E+08	5.65E+07
Anaerobic Incubation 2	40	Methanosarcinales	6.79E+07	7.10E+07	6.95E+07	2.25E+06
Anaerobic Incubation 2	47	Methanosarcinales			#DIV/0!	#DIV/0!
Anaerobic Incubation 2	54	Methanosarcinales			#DIV/0!	#DIV/0!
Anaerobic Incubation 2	61	Methanosarcinales			#DIV/0!	#DIV/0!
Anaerobic Incubation 2	68	Methanosarcinales	1.05E+08	1.37E+08	1.21E+08	2.22E+07
Anaerobic Incubation 2	75	Methanosarcinales	4.01E+07	6.27E+07	5.14E+07	1.60E+07
Anaerobic Incubation 2	80	Methanosarcinales	6.51E+07	1.58E+08	1.11E+08	6.55E+07
Anaerobic Incubation 2	86	Methanosarcinales	1.46E+08	2.52E+08	1.99E+08	7.54E+07

Table A-5. Continued

Incubation	Days	Primer Set	copies/ gram (1)	copies/ gram (2)	Avg copies/ gram	Standard Dev
Anaerobic Incubation 2	94	Methanosarcinales	2.12E+08	6.91E+08	4.52E+08	3.39E+08
Anaerobic Incubation 2	107	Methanosarcinales	7.34E+08	1.92E+09	1.32E+09	8.35E+08
Anaerobic Incubation 2	114	Methanosarcinales	1.31E+09	2.82E+09	2.07E+09	1.07E+09
Anaerobic Incubation 2	122	Methanosarcinales	1.40E+09	1.09E+09	1.25E+09	2.19E+08
Methane Incubation	0	Methanosarcinales	4.54E+08	2.33E+08	3.43E+08	1.56E+08
Methane Incubation	5	Methanosarcinales	2.41E+08	1.83E+08	2.12E+08	4.05E+07
Methane Incubation	12	Methanosarcinales	1.06E+08	3.15E+07	6.89E+07	5.29E+07
Methane Incubation	19	Methanosarcinales	2.40E+08	4.49E+08	3.44E+08	1.48E+08
Methane Incubation	26	Methanosarcinales	1.01E+08	1.56E+08	1.29E+08	3.92E+07
Methane Incubation	33	Methanosarcinales			#DIV/0!	#DIV/0!
Methane Incubation	40	Methanosarcinales			#DIV/0!	#DIV/0!
Methane Incubation	47	Methanosarcinales	2.83E+08	4.21E+08	3.52E+08	9.79E+07
Methane Incubation	54	Methanosarcinales			#DIV/0!	#DIV/0!
Methane Incubation	61	Methanosarcinales	4.34E+08	1.23E+08	2.78E+08	2.19E+08
Methane Incubation	68	Methanosarcinales			#DIV/0!	#DIV/0!
Methane Incubation	75	Methanosarcinales			#DIV/0!	#DIV/0!
Methane Incubation	80	Methanosarcinales	2.64E+08	8.91E+07	1.77E+08	1.24E+08
Methane Incubation	86	Methanosarcinales	2.50E+08	2.08E+08	2.29E+08	2.91E+07
Methane Incubation	94	Methanosarcinales	1.98E+08	9.38E+08	5.68E+08	5.24E+08
Methane Incubation	107	Methanosarcinales	4.31E+08	3.96E+08	4.14E+08	2.49E+07
Methane Incubation	114	Methanosarcinales	4.00E+08	1.23E+09	8.13E+08	5.84E+08
Methane Incubation	122	Methanosarcinales	1.76E+09	1.12E+09	1.44E+09	4.50E+08

Table A-6. Further qPCR data for CLB incubation with Archaea and Bacteria specific primers.

Incubation Name	Days	Primer set	copies/ gram (1)	copies/ gram (2)	Avg copies/ gram	Std Dev
Anaerobic Incubation 1	0	Archaea	1.34E+08	6.56E+08	5.15E+08	3.33E+08
Anaerobic Incubation 1	5	Archaea	1.17E+09	1.71E+09	1.44E+09	3.84E+08
Anaerobic Incubation 1	12	Archaea	5.90E+08	1.12E+10	5.91E+09	7.52E+09
Anaerobic Incubation 1	19	Archaea	5.28E+08	1.14E+10	5.94E+09	7.66E+09
Anaerobic Incubation 1	26	Archaea	2.86E+08	6.94E+09	3.61E+09	4.70E+09
Anaerobic Incubation 1	33	Archaea	1.53E+09	2.83E+09	1.55E+09	9.95E+08
Anaerobic Incubation 1	40	Archaea	1.71E+09	5.28E+07	8.81E+08	1.17E+09
Anaerobic Incubation 1	47	Archaea	9.68E+08	9.68E+08	4.80E+09	8.09E+09
Anaerobic Incubation 1	54	Archaea			#DIV/0!	#DIV/0!
Anaerobic Incubation 1	61	Archaea	1.15E+09	2.02E+09	1.59E+09	6.15E+08
Anaerobic Incubation 1	68	Archaea	5.63E+09	1.26E+10	5.09E+09	5.46E+09
Anaerobic Incubation 1	75	Archaea	4.58E+08	1.35E+10	6.96E+09	9.20E+09
Anaerobic Incubation 1	80	Archaea	1.04E+09	1.55E+09	1.92E+09	1.11E+09
Anaerobic Incubation 1	86	Archaea	2.17E+10	1.20E+10	9.49E+09	9.49E+09
Anaerobic Incubation 1	94	Archaea	1.05E+09	1.04E+10	5.73E+09	6.63E+09
Anaerobic Incubation 1	107	Archaea	2.56E+09	1.60E+10	9.29E+09	9.51E+09
Anaerobic Incubation 1	114	Archaea	1.05E+09	2.02E+09	1.75E+09	6.11E+08
Anaerobic Incubation 1	122	Archaea	4.88E+08	1.20E+09	9.49E+08	4.00E+08
Anaerobic Incubation 2	0	Archaea	2.10E+08	9.90E+08	6.74E+08	4.10E+08
Anaerobic Incubation 2	5	Archaea	3.14E+08	8.04E+08	5.59E+08	3.46E+08
Anaerobic Incubation 2	12	Archaea			#DIV/0!	#DIV/0!
Anaerobic Incubation 2	19	Archaea	9.85E+08	1.24E+09	7.57E+09	7.67E+09
Anaerobic Incubation 2	26	Archaea	3.87E+08	1.20E+10	6.21E+09	8.23E+09
Anaerobic Incubation 2	33	Archaea	1.92E+09	2.60E+09	2.26E+09	4.84E+08
Anaerobic Incubation 2	40	Archaea	5.87E+09	1.02E+10	8.04E+09	3.08E+09
Anaerobic Incubation 2	47	Archaea	9.97E+09	1.29E+09	5.63E+09	6.14E+09
Anaerobic Incubation 2	54	Archaea	1.48E+10	1.34E+10	1.41E+10	1.01E+09
Anaerobic Incubation 2	61	Archaea	9.35E+08	1.79E+10	9.42E+09	1.20E+10
Anaerobic Incubation 2	68	Archaea			#DIV/0!	#DIV/0!
Anaerobic Incubation 2	75	Archaea	1.35E+08	4.46E+09	8.13E+09	7.94E+09
Anaerobic Incubation 2	80	Archaea	3.73E+08	8.57E+08	6.15E+08	3.43E+08
Anaerobic Incubation 2	86	Archaea	5.64E+08	1.03E+09	1.30E+09	8.94E+08
Anaerobic Incubation 2	94	Archaea	2.98E+09	6.06E+09	1.41E+10	2.04E+10
Anaerobic Incubation 2	107	Archaea	5.92E+09	7.41E+09	5.36E+10	6.70E+10
Anaerobic Incubation 2	114	Archaea	1.00E+09	3.64E+09	2.37E+09	1.32E+09
Anaerobic Incubation 2	122	Archaea	3.97E+08	1.39E+09	1.19E+09	7.18E+08
Methane Incubation	0	Archaea	4.30E+08	8.54E+08	6.42E+08	3.00E+08

Table A-6. Continued

Incubation Name	Days	Primer set	copies/ gram (1)	copies/ gram (2)	Avg copies/ gram	Std Dev
Methane Incubation	5	Archaea	7.33E+08	1.16E+09	1.18E+09	4.56E+08
Methane Incubation	12	Archaea	5.22E+08	5.16E+08	8.34E+08	5.46E+08
Methane Incubation	19	Archaea	5.37E+08	1.02E+09	9.78E+08	4.24E+08
Methane Incubation	26	Archaea	1.08E+09	2.04E+09	5.86E+09	9.44E+09
Methane Incubation	33	Archaea	1.58E+09	3.39E+09	8.21E+09	1.28E+10
Methane Incubation	40	Archaea	6.87E+08	1.48E+10	7.74E+09	9.98E+09
Methane Incubation	47	Archaea	8.66E+08	1.39E+09	1.13E+09	3.71E+08
Methane Incubation	54	Archaea	1.14E+09	2.56E+09	1.85E+09	1.01E+09
Methane Incubation	61	Archaea	6.21E+06	8.24E+06	7.23E+06	1.44E+06
Methane Incubation	68	Archaea	2.02E+08	6.11E+09	3.16E+09	4.18E+09
Methane Incubation	75	Archaea	2.25E+09	2.60E+09	2.42E+09	2.42E+08
Methane Incubation	80	Archaea	4.57E+08	9.49E+09	4.97E+09	6.39E+09
Methane Incubation	86	Archaea	6.88E+09	4.99E+09	1.31E+10	1.80E+10
Methane Incubation	94	Archaea	5.09E+08	9.47E+09	4.99E+09	6.34E+09
Methane Incubation	107	Archaea	2.26E+09	3.52E+10	1.87E+10	2.33E+10
Methane Incubation	114	Archaea	1.17E+09	3.03E+09	2.17E+09	9.37E+08
Methane Incubation	122	Archaea	2.46E+09	1.42E+09	1.80E+09	5.72E+08
Anaerobic Incubation 1	0	Bacteria				
Anaerobic Incubation 1	5	Bacteria				
Anaerobic Incubation 1	12	Bacteria	3.27E+08	2.28E+08	2.77E+08	6.99E+07
Anaerobic Incubation 1	19	Bacteria	5.38E+08	5.30E+08	5.34E+08	5.95E+06
Anaerobic Incubation 1	26	Bacteria	6.46E+07	3.27E+08	1.96E+08	1.86E+08
Anaerobic Incubation 1	33	Bacteria				
Anaerobic Incubation 1	40	Bacteria				
Anaerobic Incubation 1	47	Bacteria	3.45E+08	4.86E+08	6.87E+08	3.38E+08
Anaerobic Incubation 1	54	Bacteria				
Anaerobic Incubation 1	61	Bacteria				
Anaerobic Incubation 1	68	Bacteria	1.70E+08	2.95E+08	2.33E+08	8.83E+07
Anaerobic Incubation 1	75	Bacteria	4.82E+08	3.73E+08	3.63E+08	9.16E+07
Anaerobic Incubation 1	80	Bacteria				
Anaerobic Incubation 1	86	Bacteria	5.26E+08	9.30E+08	7.28E+08	2.86E+08
Anaerobic Incubation 1	94	Bacteria	1.04E+09	6.44E+08	8.42E+08	2.80E+08
Anaerobic Incubation 1	107	Bacteria	1.45E+09		1.45E+09	#DIV/0!
Anaerobic Incubation 1	114	Bacteria	3.56E+08	1.47E+08	2.52E+08	1.48E+08
Anaerobic Incubation 1	122	Bacteria				
Anaerobic Incubation 2	0	Bacteria	6.50E+08	5.95E+08	6.23E+08	3.88E+07
Anaerobic Incubation 2	5	Bacteria				
Anaerobic Incubation 2	12	Bacteria				
Anaerobic Incubation 2	19	Bacteria	1.57E+09	1.21E+09	1.39E+09	2.48E+08

Table A-6. Continued

Incubation Name	Days	Primer set	copies/ gram (1)	copies/ gram (2)	Avg copies/ gram	Std Dev
Anaerobic Incubation 2	26	Bacteria	8.40E+08	6.56E+08	7.48E+08	1.30E+08
Anaerobic Incubation 2	33	Bacteria				
Anaerobic Incubation 2	40	Bacteria	5.09E+08	4.34E+08	4.20E+08	8.04E+07
Anaerobic Incubation 2	47	Bacteria	7.77E+08	6.86E+08	7.31E+08	6.43E+07
Anaerobic Incubation 2	54	Bacteria	1.95E+08	2.48E+08	2.21E+08	3.74E+07
Anaerobic Incubation 2	61	Bacteria	1.11E+09	5.86E+08	8.46E+08	3.68E+08
Anaerobic Incubation 2	68	Bacteria	8.49E+07	4.24E+08	2.54E+08	2.40E+08
Anaerobic Incubation 2	75	Bacteria	4.22E+07	6.33E+07	5.28E+07	1.49E+07
Anaerobic Incubation 2	80	Bacteria	3.54E+08	2.66E+08	3.10E+08	6.21E+07
Anaerobic Incubation 2	86	Bacteria				
Anaerobic Incubation 2	94	Bacteria	1.97E+09	4.21E+09	4.80E+09	3.16E+09
Anaerobic Incubation 2	107	Bacteria	9.43E+08		9.43E+08	#DIV/0!
Anaerobic Incubation 2	114	Bacteria	6.71E+08	4.74E+08	5.73E+08	1.39E+08
Anaerobic Incubation 2	122	Bacteria				
Methane Incubation	0	Bacteria	2.79E+08	4.17E+08	3.48E+08	9.79E+07
Methane Incubation	5	Bacteria				
Methane Incubation	12	Bacteria	1.17E+09	2.20E+09	1.68E+09	7.33E+08
Methane Incubation	19	Bacteria				
Methane Incubation	26	Bacteria	8.54E+08	7.25E+08	5.16E+08	3.25E+08
Methane Incubation	33	Bacteria	9.94E+08	7.62E+08	8.78E+08	1.64E+08
Methane Incubation	40	Bacteria	6.23E+08	9.87E+08	8.05E+08	2.57E+08
Methane Incubation	47	Bacteria				
Methane Incubation	54	Bacteria	1.50E+09	2.07E+09	1.79E+09	4.01E+08
Methane Incubation	61	Bacteria	2.89E+08	4.88E+08	3.88E+08	1.41E+08
Methane Incubation	68	Bacteria	1.00E+08	1.79E+08	1.40E+08	5.58E+07
Methane Incubation	75	Bacteria	9.19E+08	3.30E+08	6.25E+08	4.16E+08
Methane Incubation	80	Bacteria	8.04E+08		8.04E+08	#DIV/0!
Methane Incubation	86	Bacteria	1.77E+09	9.91E+08	1.04E+09	5.17E+08
Methane Incubation	94	Bacteria	5.12E+08	1.26E+08	3.19E+08	2.73E+08
Methane Incubation	107	Bacteria				
Methane Incubation	114	Bacteria				
Methane Incubation	122	Bacteria	1.36E+08	5.29E+08	3.33E+08	2.78E+08

VITA

Richard Thomas Kevorkian was born in Roanoke, Virginia on March 24, 1990 to parents George and JoAnn Kevorkian. One of five children, he would graduate from William Byrd High School in 2008. Following graduation, Richard moved to Blacksburg, VA where he attended Virginia Polytechnic Institute and State University. In May of 2012 Richard would graduate with a Bachelor of Science in Biology. He next moved south to Knoxville, Tennessee where he began studying in the field of biogeochemistry under the leadership of Dr. Karen Lloyd in order to pursue a Master of Science degree in Microbiology at the University of Tennessee. Following his graduation at Tennessee he will be pursuing a PhD in Marine Biology at Texas A & M at Corpus Christi under Dr. Brandi Reese.