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Aqueous Mesocosm Techniques Enabling the Real-Time Measurement of the Chemical and Isotopic Kinetics of Dissolved Methane and Carbon Dioxide

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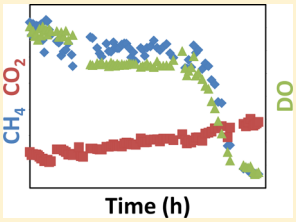
Supporting Information

ABSTRACT: Previous studies of microbially mediated methane oxidation in oceanic environments have examined the many different factors that control the rates of oxidation. However, there is debate on what factor(s) are limiting in these types of environments. These factors include the availability of methane, O_2, trace metals, nutrients, the density of cell population, and the influence that CO_2 production may have on pH. To look at this process in its entirety, we developed an automated mesocosm incubation system with a Dissolved Gas Analysis System (DGAS) coupled to a myriad of analytical tools to monitor chemical changes during methane oxidation. Here, we present new high temporal resolution techniques for investigating dissolved methane and carbon dioxide concentrations and stable isotopic dynamics during aqueous mesocosm and pure culture incubations. These techniques enable us to analyze the gases dissolved in solution and are nondestructive to both the liquid media and the analyzed gases enabling the investigation of a mesocosm or pure culture experiment in a completely closed system, if so desired.



1. INTRODUCTION

Blooms of microorganisms have been shown to be major removal mechanisms for chemical perturbations in aquatic systems, one of the more recent and notable examples being the Deepwater Horizon disaster in the Gulf of Mexico.[1−5] A mesocosm is an experimental technique that involves collecting and observing a natural environmental sample (e.g., seawater) under controlled conditions and is ideal for a detailed study of such microbially mediated events.[6−8] However, obtaining sufficient data during rapidly changing biogeochemical conditions, for example, during microbial blooms, is a common challenge that is pervasive even in more controlled laboratory experiments investigating pure cultures.[9−11] This problem is especially challenging when investigating the biogeochemistry of dissolved gases; diffusive exchange with the atmosphere during sample storage and analysis can cause changes in dissolved gas concentration and the biogeochemical reaction. Here, we present techniques that do not contaminate the biogeochemical state of the system during incubation as well as techniques for the rapid, automated, and nondestructive analysis of dissolved greenhouse gas concentrations and stable isotopes. These techniques were developed with a focus on the biogeochemical formation and destruction mechanisms of methane (CH_4), and thus, CO_2 was also of interest as it is both a potential reactant and product for methane production and oxidation. However, these techniques can also be adapted to investigate different biogeochemical systems consuming or producing either of these greenhouse gases and other accompanying constituents (e.g., trace metals, nutrients, dissolved oxygen, nitrogen species, other hydrocarbons, etc.).

Previous investigations of methane oxidation have been limited by a paucity of techniques available for the time-course analysis of chemical and isotopic changes during microbial blooms. In several experiments, seawater is collected in glass vials and oxidation rates are determined only at a specific time rather than over the entire oxidation event; any time-course changes in concentrations and isotopes have only been measured at the beginning and end of the incubation due to the methodological limitations.[12−14] One of the main challenges with incubating seawater in rigid vials is that the removal of an aliquot for analysis can often perturb the biogeochemical state by introducing a gaseous headspace, changing dissolved gas concentrations, and introduction of contaminants, thereby making the remainder of the sample useless for further analyses.[1−3,514−17] Thus, the method we present here opens a new avenue of research because it enables for the first time the measurement of concentration and isotope changes over the entire course of a biogeochemical reaction especially with respect to methane kinetics.

Prior studies into processes such as methane consumption (methanotrophy) and production (methanogenesis) have

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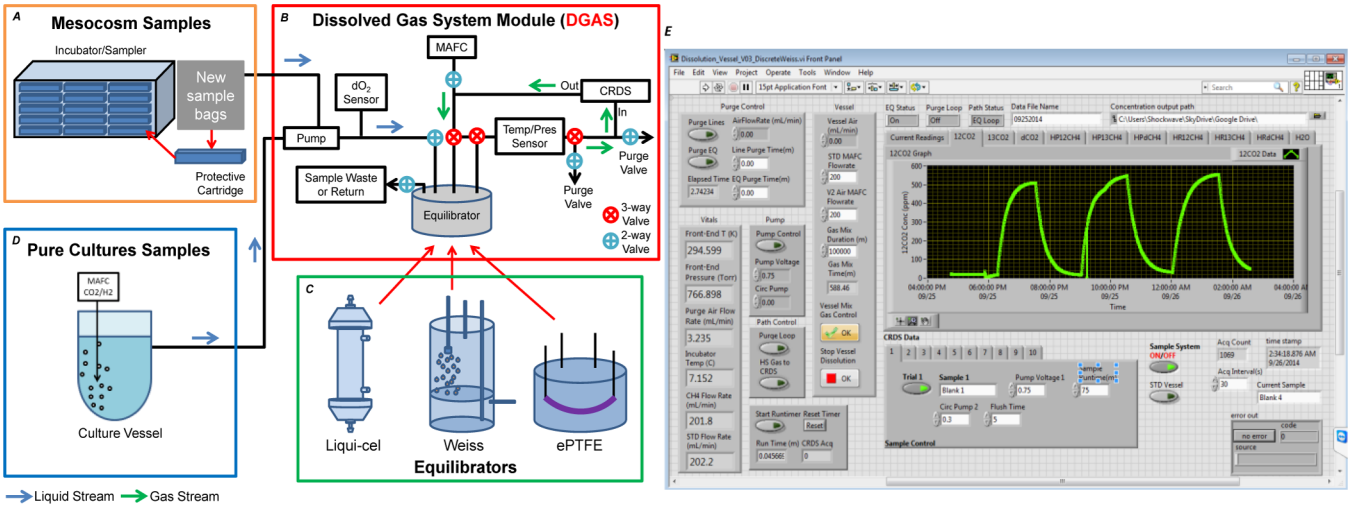

Figure 1. System setup with either mesocosm or pure culture configuration. (A) Mesocosm incubation system with samples bags inside protective cartridges. (B) Dissolved Gas Analysis System (DGAS) contains the Picarro G-2201i analyzer, valves to control liquid sample and gas flow, dissolved oxygen optode, temperature and pressure sensor, a mass air flow controller for purging the system. (C) The Weiss, ePTFE, and Liqui-cel equilibrators tested for use in the DGAS unit. (D) While our application presented is for discrete mesocosm samples, this system could be adapted for continuous analysis of a pure culture style experiment. (E) Control program written in National Instruments LabVIEW.

shown very interesting results with respect to chemical and isotopic kinetics during microbial blooms. For example, during the Deepwater Horizon disaster, several authors presented data suggesting a microbial bloom of methanotrophic bacteria completely oxidized the released methane within several months of the start of this disaster.[1,2,4,18,19] However, this conclusion remains contentious due to suspicions of chemical limitations.[5] A major uncertainty is how biogeochemical limitations affect the rates of aerobic methane oxidation during microbial blooms, highlighting the need for a more fundamental understanding of this biochemical process. Because the Deepwater Horizon methanotrophic response took place heterogeneously over an area of approximately 73 200 km^2 in the northern Gulf of Mexico,[2] such a fundamental understanding is difficult to obtain from the field data alone and more controlled mesocosm experiments are insightful.

Similarly, stable isotopic kinetics have been previously investigated as a function of changing microbial growth during pure cultures of methanogenic archaea and bacteria.[9−11] These studies observed changes in stable isotopes of methane and the isotopic fractionation factors as a function of microbial growth phase, varying as the cell growth continued through early phase, lag phase, and stationary phase growth. However, because the analytical techniques employed in these investigations were both manual and destructive, samples were collected at the gaseous exhaust of the bioreactor rather than the bioavailable methane dissolved in the media.

In both of these previous examples, high temporal resolution measurements of chemical and isotopic changes would help quantify the biogeochemical dynamics during rapidly changing processes such as microbial blooms. Here, we present new high temporal resolution techniques for investigating dissolved gas concentration and stable isotopic dynamics during aqueous mesocosm and pure culture incubations. These techniques analyze the gases dissolved in solution and are nondestructive to both the liquid media as well as the analyzed gases enabling the investigation of a mesocosm or pure culture experiment in a completely closed system, if so desired. The analyzed aliquot and dissolved gases analyzed can be returned back into the main sample thus providing a closed system setup that is nondestructive. This technique uses a laser spectroscopy analyzer that is capable of analysis at rates approaching 0.1 Hz, thus enabling very thorough investigations of dissolved gas chemical and isotopic dynamics during microbial blooms.

2. MATERIALS AND METHODS

The techniques developed here involve three main independent and interconnecting parts (Figure 1). First, an incubation vessel was developed with the characteristics of being chemically clean, impermeable to dissolved gases, flexible to allow the removal of liquid without causing a vacuum or needing to introduce a substance for displacement, and structurally sound to hold a mass of water greater than 10 kg (Figure 1A; S1, Supporting Information). Second, because high precision and sensitivity analysis of methane and carbon dioxide can only be accomplished in the gaseous phase (not directly on gases dissolved in solution), a method of equilibrating the dissolved gas with the interior of a laser spectroscopy cavity was needed (Figure 1C). The laser spectroscopy technique employed here is Cavity-Ringdown Spectroscopy (CRDS; Picarro G2201-i) because it is high precision and nondestructive to the analyzed gases. Third, an integrated and automated system was developed for the clean, rapid, and automated analysis of samples in either a continuous closed-system analysis mode or a discrete sample mode. The integration of the second and third parts is labeled the DGAS unit (Dissolved Gas Analysis System) (Figure 1B).

2.1. Materials. *2.1.1. Sample Storage.* Water samples are normally retrieved via precleaned Niskin or Go-flo bottles onboard a research vessel. Once these samples are brought back to the surface, they must be transferred to a storage vessel for the incubation experiment. This storage vessel must possess five characteristics: (1) The storage vessel must be chemically clean. While borosilicate glass vials are conventionally used for batch experiments,[20] trace metals leaching from the glass and stoppers are a pervasive problem[21,22] and could potentially influence the background metabolic activity of the microbial

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population.[23] For this reason, acid washed polyethylene or Teflon bottles are generally used for trace metal sampling.[21] (2) Because the biogeochemistry of dissolved gases is of interest, the vessel must have a low diffusivity for dissolved gases, a property that is true for glass but not for polyethylene and Teflon.[24−27] (3) The vessel must allow for the removal of an aliquot for analysis without influencing the biogeochemical state of the system. To remove an aqueous aliquot from a sealed rigid vial, prior studies have either used displacement with an aliquot of pure gas or water or over pressuring the vials with a gaseous headspace.[28] Unfortunately, these aliquot removal techniques perturb the biogeochemical state of the system, rendering further analyses of the dissolved gas dynamics useless. For this reason, a flexible vessel would be preferred so that the volume of the vessel would simply reduce as aliquots are removed. (4) Sample volumes of 10 L or more are not uncommon for mesocosm experiments,[6] so the vessel must be structurally sound to support a mass of greater than 10 kg. (5) The vessel must be able to maintain the in situ pressure.

Requirements 1−4 were satisfied using 15 L sample storage bags manufactured by Pactech Packaging LLC. The bags were custom-made from a laminated construction of five layers of film (outside to inside): polyester, low density polyethylene, metalized polyester, low density polyethylene, and linear low density polyethylene. The metalized polyester prevents diffusion of gas through the bag while the polyester and low density polyethylene provide a clean surface as well as structural integrity to the bags. These bags were held in protective cartridges constructed from 1/4 in. thick clear polycarbonate sheets. Each cartridge is fitted with Biochem pinch valves for controlling the flow of water from the bag without influencing the trace metal concentration (Figure 1A and Figure S1). While maintaining the in situ pressure would be preferred, this cannot be accomplished outside of the ocean without sacrificing requirements 1 and 3. Thus, requirement 5 was ignored.

Once samples are ready for incubation, they are placed into the custom incubator. The cartridges that house the sample bags act as an extra layer of protection for the bags as well as a storage solution inside the incubator (Figure 1A; Figures S1 and S2). The incubator consists of a 700 L Frigidaire freezer controlled to subambient temperatures above freezing with a custom proportional integral derivative (PID) controller (Omega CNi-3253). Temperature precisions of <0.1 °C are common with these custom incubators. This setup allowed for seawater samples to be incubated at in situ temperatures and maintain gas concentrations and chemical purity.

2.1.2. CH_4 Inoculation. To simulate an abrupt intrusion of methane into the natural oceanic environment, we used isotopically standardized CH_4 to inoculate the samples. Once the bags were filled with the water samples, a volume of standardized gas can be introduced which serves as the reactant. In the experiment described below, 150 mL of CH_4 with known stable isotope ratio was introduced via mass air flow controller (Aalborg) to the bag as a substrate for methane oxidation. The samples were allowed to equilibrate for 24 h before all the gaseous headspace was removed with a peristaltic pump (Cole Parmer). This ensured that the dissolved gas concentration in the seawater was not re-equilibrating with a gaseous headspace during an experiment. This provided a dissolved concentration of ca. 308−532 μM CH_4, 150 μM DO. The range in observed concentrations is due to different salinity (solubilities) of the water samples and potentially the injected CH_4 did not reach full equilibration before removal of the headspace.

2.1.3. Blanks and Standards. Blanks and standards were stored in the same fashion as the samples but were prepared before field campaigns. They were prepared using ultra clean DI water from a Milli-Q A10 water purification system with <2 ppb TOC and resistivity of 18.2 MΩ. The blank water was purged of dissolved gases using UHP N_2 inside a 5L culture vessel and then transferred to a sample bag. Standards were created by introducing metered amounts of the same standardized CH_4 used in the samples at sea. Standard bags were allowed to equilibrate over 24 h and then excess headspace was pumped out of the bags. The blank and standard bags were then stored in the same fashion as the sample bags inside the custom temperature controlled incubator (Figure S2).

2.1.4. Dissolved Gas Analyzer System (DGAS). The dissolved gas analyzer system (DGAS) is the core of the analytical capabilities of this method (Figure 1B and Figure S3). The DGAS unit contains the equilibrator, sample gas controls, water sample pump, Picarro CRDS unit (G2201-i), and purge gas control (Figure 1B). The sample is pumped into the dissolved gas equilibrator which contains one of three different equilibrators: Weiss method,[29] ePTFE membrane, and Liqui-cel membrane contractor (Figure 1C and Figure S4). The different equilibrators were investigated to determine the response time which could limit the number of samples that could be measured with the DGAS system.

While several sample bags may be incubating in parallel, each analysis must be conducted serially as there is only one CRDS analyzer. The procedures here describe the discrete analysis of samples, which is necessary when several samples are being incubated in parallel (Figure 1A). However, if only one sample is under investigation, a continuous analysis strategy can be employed with sampling rates approaching 0.1 Hz (Figure 1D and Figure S5). In discrete analysis mode, the analysis of each sample requires 10−75 min depending on user specified settings and choice of equilibrator configuration.

From the incubator, sample aliquots are plumbed to the DGAS unit through 1/8" FEP or C-flex tubing to the sample pump (KNF NF25). The control program (written in NI LabVIEW; Figure 1E) initiates a run by opening the pinch valve of the target sample bag and then pumps an aliquot out at a rate of 30 mL/min. This flushes a dissolved gas equilibrator of the prior liquid aliquot as well as introduces a new aliquot. Prior to entering the equilibrator, the liquid aliquot goes through a custom flow cell housing an Aandara dissolved oxygen optode 3830 (Figure S6). The custom flow cell has an internal volume of 2.8 mL. Once the pump has flushed the equilibrator with 5 volumes of liquid aliquot, the sample pump turns off and pinch valves on both sides of the equilibrator are closed to isolate the aliquot. During this equilibration time, the vacuum pump in the CRDS is constantly recirculating the gaseous headspace in the equilibrator and analyzing the gases while the control program is storing multiple parameters of data. The data parameters include $\delta^{13}C-CO_2$ and $\delta^{13}C-CH_4$, dissolved CO_2, CH_4, and oxygen concentrations, temperature of the incubator, temperature of the gas stream, sample ID, water vapor concentration, gas pressure, date, and time. Once the analysis has run for a user-determined time period, the sample run completes, a mass air flow controller (Aalborg) is set to purge the equilibrator with ultrazero N_2, and the process is repeated again with the next sample. This automation allows for multiple time points for each sample throughout the day with minimal user intervention.

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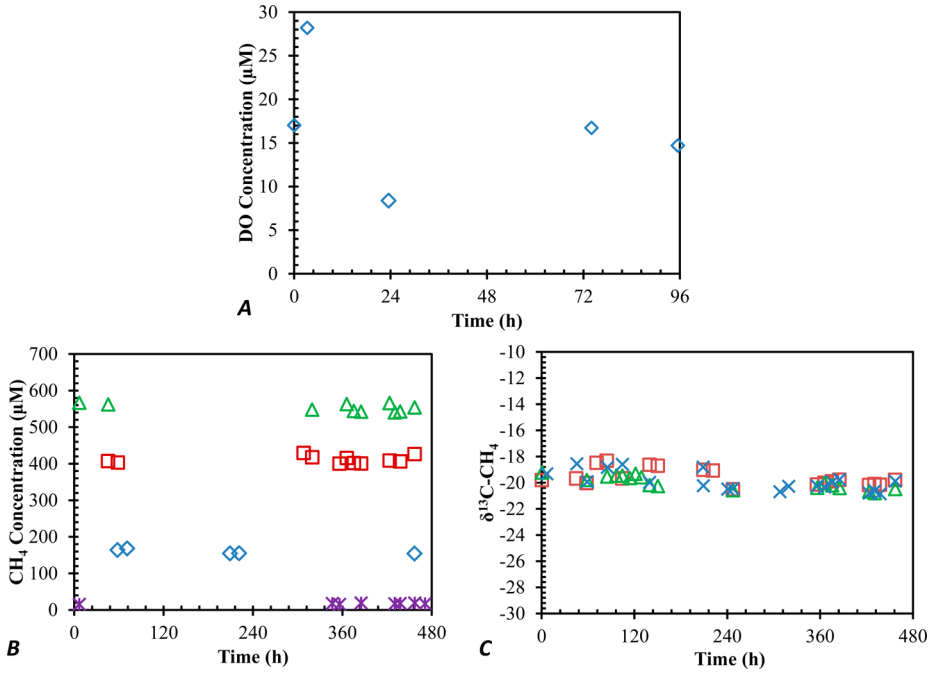

Figure 2. (A) Dissolved oxygen (blue ◇) results from sample bag test to determine oxygen permeability over a four day period. The average DO was 17.02 μM across 95.5 h with a standard deviation of 7.158 for 5 samples. Normal atmospheric equilibrated water is approximately ~280 μM. B.) Mesocosm CH_4 standards run during the mesocosm experiment showing the storage bag integrity. The blanks (purple *) had an average $^{12}CH_4$ value of 17.37 ± 1.40 (2 standard deviations) and %RSD of 8.07%. The 50 mL standard (blue ◇) had average of 159.3 μM ± 6.47 (2 standard deviations) and %RSD of 4.06%. The 100 mL standard (red □) had average of 410.7 μM ± 10.2 (2 standard deviations) and %RSD of 2.49%. The 250 mL standard (green △) had an average of 577.5 ± 10.6 (2 standard deviations) and %RSD of 1.92% C.) The $\delta^{13}CH_4$ of standard bags, CH_4 used to make the standards was standardized at −20 ‰. The 50 mL standard had an isotopic average of −19.57 ± 0.65 (2 standard deviations) and %RSD 3.36%. The 100 mL standard had an isotope average of −20.00 ± 0.50 (2 standard deviations) and %RSD of 2.52%. The 250 mL standard had an isotopic average of −19.93 ± 0.74 (2 standard deviations) and %RSD of 3.69%.

A point to note, the Picarro CRDS vacuum pump is not leak free from the factory. Therefore, substantial attempts were taken to make the pump as leak proof as possible. The CRDS pump was stripped down of all parts that are more likely to leak. The extraneous NPT adapters, barbed fittings, and vinyl tubing were replaced with all stainless steel parts: NPT to Swagelok 1/8 in. compression fittings and stainless steel 1/8 in. tubing. The NPT fittings and all seams on the pump were sealed with vacuum epoxy (TorrSeal) to decrease leaks. This process did improve the flow rate and gas impermeability. Unsatisfactory performance from the first sealing process resulted in multiple attempts to reseal the seams of the vacuum pump. Despite many attempts, the pump maintained small but noticeable leaks. However, for the purpose of these experiments, the gas leak in concentration is negligible compared to the concentrations of gases that are being measured. Picarro now sells an improved leak-resistant version of this vacuum pump.

2.1.5. Weiss Equilibrator. The "Weiss equilibrator"[29] was made from a 30 mL Teflon serum vial with holes drilled in the bottom for a water frit, an air inlet line, and an air sample line (Figure 1C; Figure S4A). The serum vial was inverted, and the neck of the bottle was capped and a hole drilled for the sample return line. One tube is used for water sample introduction with a frit at the end for showering the water inside, another tube for returning headspace analyzed back into the equilibrator and the last tube for removing gas from the headspace for analysis by the CRDS unit. The headspace-to-sample ratio is 2.0. (For conditions where the sample is pumped continually through the equilibrator, the final concentrations in the headspace will not be influenced by the headspace-to-sample ratio in the equilibrator. However, the headspace-to-sample ratio influences the final concentration in the headspace when discrete samples are analyzed due to the limited sample available to equilibrate with the headspace).

2.1.6. ePTFE Membrane Equilibrator. The ePTFE membrane was acquired as sample material from Bard Peripheral Vascular OEM Products. The Venaflow is tubular with a diameter of 6 mm, length of 700 mm, and wall thickness of 0.75 mm. This was fitted inside a custom bored PTFE cylinder with a top fitted with O-rings to seal off the chamber similar to as was described previously.[30,31] The top is fitted with 4−1/4" UNF O-ring Swagelok fittings, 2 of which are connected to the ePTFE tubing with a barbed 1/4" NPT fitting on the inside of the top (Figure S4B). The other two fittings are used to remove and return headspace gas into the analysis loop for gas analysis. The headspace-to-sample ratio is 6.19.

2.1.7. Liquicel G542. The Membrana Liqui-cel G542 unit (Figure 1C and Figure S4C) has an inlet and outlet for passing the liquid sample through the device. The other two connections are for the vacuum or "sweep gas" that will draw the dissolved gas through the membrane as it passes through the G542. This contactor was made for the general use of removing gases from liquids in large manufacturing processes. The G542 was tested in order to determine if it is valid to use as an equilibrator. The headspace in this equilibrator is the volume inside the G542 (78 mL) but exterior to the membrane tubes. The interior of the membrane tubes that liquid flows through is denoted as the lumen side that has a volume of 52 mL. The headspace-to-sample ratio is 1.47.

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Table 1. Trace Metal Analysis on Sample Bags[a]

procedure	sample	Cd	Pb	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As
no rinse	average (nM)	0.02	0.09	0.13	0.85	< DL	< DL	0.03	< DL	0.53	7.71	< DL
	std dev.	0.02	0.01	0.08	0.16	0.73	0.47	0.02	0.53	0.37	1.98	0.14
Milli-Q DI water rinse	average (nM)	0.01	0.08	0.13	0.76	2.15	1.21	0.03	< DL	0.50	4.40	< DL
	std dev	0.00	0.01	0.01	0.05	0.11	1.40	0.02	1.35	0.08	0.27	0.06
acid wash/Milli-Q DI water rinse	average (nM)	< DL	0.06	< DL	0.83	2.41	< DL	0.09	< DL	0.69	2.20	< DL
	std dev	0.00	0.00	0.02	0.06	0.20	1.65	0.13	0.43	0.17	0.81	0.14

[a]The first test was carried out on bags with no rinse procedure. The second test was carried out on bags after Milli-Q DI water rinse for 4−5 days. The third test was taken after an acid leach for 4−5 days and then subsequent Milli-Q DI water rinse. Detection limits (DL): Cd, 0.004 nM; Pb, 0.02 nM; V, 0.1 nM; Cr, 0.02 nM; Mn, 2 nM; Fe, 1 nM; Co, 0.02 nM; Ni, 2 nM; Cu, 0.4 nM; Zn, 1 nM; and As, 0.1 nM. Light rare earth elements (La, Ce, Pr, Nd) in the bag blanks were never higher than the detection limit of 2 pM.

2.2. Assessment Procedures. *2.2.1. Sample Storage.* During the sample storage development, several different materials and constructions were tested for the storage bags. The final material and bag construction were tested for oxygen permeability by removing dissolved gases in DI water with ultrazero N_2 as the purge stream. This was carried out in a 5L glass culture vessel and subsequently transferred to the sample bag (Figure S5). The bag was tested over several days by pumping the water past an oxygen optode with a custom flow cell (Figure S6).

The standard and blank measurements were carried out during a 10 day assessment of the long-term storage of sample bags (Figure 2B,C). In addition, the sample bags were cleaned and trace metal blanks were tested. This was done by introducing ∼4−5L of 1.2 M reagent grade HCl into the bag, allowing it to soak for 4−5 days, and then flushed five times with trace metal clean distilled/deionized water. Then ultra-clean distilled/deionized water was allowed to soak in the bag for 4−5 days until a sample was taken for measurement (SF-ICP-MS; Thermo-Fisher Element 2). Also, the trace metal purity of the bag material was tested without any acid precleaning.

2.2.2. Equilibrators. The equilibration times of the three equilibrators were tested using water with known concentrations of dissolved CH_4 and CO_2. A temperature controlled bioreactor vessel (Chemglass 5L vessel) was used to create a water sample with a known concentration of dissolved CH_4 and CO_2 by bubbling standard gases through Milli-Q water and mixing with an impeller (Figure 1D and Figure S5). A second bioreactor vessel (Chemglass 2 L vessel) was used as blank water source and was created by bubbling ultrazero N_2 gas through Milli-Q water and mixing with an impeller. The standard and zero waters were pumped sequentially into an equilibrator to simulate rapidly changing methane concentration conditions and quantify the equilibrator response times during both decreasing and increasing CH_4 and CO_2 concentration conditions. The LabVIEW program was set to switch between the two water streams after equilibration was reached. The standard concentrations of 500 ppm of CO_2 was used for one set of experiments, 1000 ppm of CH_4 for another, and 2000 ppm of CH_4 was used for the last set.

3. RESULTS AND DISCUSSION

3.1. Results of Technique Assessment. *3.1.1. Sample Bag Evaluation.* Sample bag integrity was determined by filling the bag with 15 L of DI water and subjecting it to physical stress tests such as dropping it from a height of 30−50 cm and bumping them to simulate rough handling in the field and at sea. The bag material and heat seals were able to withstand the physical tests with no noticeable damage.

Next, the results of the oxygen permeability experiment for the bag vessel displayed minimal permeability. The starting dissolved O_2 concentration was 17 μM and after 95.5 h the measured concentration was 14 μM. The average dissolved O_2 concentration was 17 μM with a standard deviation of 7.2. While a decrease in the dissolved O_2 concentration was not expected, it is viewed as the lower end of sensitivity for the optode. Nonetheless, the concentration of dissolved O_2 did not increase above this baseline condition over 96 h (Figure 2A). The blank, 50, 100, and 250 mL standards had an average CH_4 value of 17.37, 159.3, 410.7, and 552.9 μM respectively. Blank and standards did not show any significant trends over the 10 day testing period (Figure 2B,C).

Finally, trace metal testing indicated most of the trace metals of concern were below detection limit. Zn, however, was significantly higher than the analytes prior to the acid/DI wash. After the washing procedure Zn concentrations dropped 71% (7.71 to 2.2 nM), however, the detection limit for a bag after acid/DI wash is 1 nM for Zn (Table 1). This indicated that the Pactech bags were sufficiently trace metal clean after acid wash/DI water cleanings and thus suitable for use in mesocosm studies.

3.1.2. Equilibrator Evaluation. The Weiss, ePTFE membrane, and Liqui-cel membrane methods of dissolved gas equilibration all allowed for the successful measurement of the dissolved gas concentrations; however, variations in response time, accuracy, and precision were observed. The response time (τ) characterizes the amount of time it takes for the equilibration method to reach the standard concentration measured, more specifically, the time for the concentration to change by a value of 1/e (Table 2).[29] With the CO_2 standard as reference, the fastest equilibration method was the Liqui-cel membrane contactor, followed by the Weiss method, and last the ePTFE (Table 2 and Figure S7A,B). CH_4 equilibration showed slightly different results where the Liqui-cel membrane contactor was fastest, ePTFE second, and Weiss method the

Table 2. τ Values for Tested Equilibrators[a]

	high concentration		low concentration	
	τ-CO_2 (min)	τ-CH_4 (min)	τ-CO_2 (min)	τ-CH_4 (min)
G542	2.12	27.78	16.66	22.3
ePTFE	13.88	38.33	13.88	45.52
Weiss	12.82	67.45	5.95	92.75

[a]τ values for Liqui-cel G542, ePTFE, and Weiss equilibrators tested in high and low concentration CO_2 and CH_4 dissolved waters.

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last (Table 2and Figure S7C,D). The data indicates that the Liqui-cel membrane contractor should be used with respect to the response times. The next consideration is the accuracy of the equilibrator. Figure S7A,B indicates that Liqui-cel membrane contractor underestimates the true concentration by 31 ppm (7.3%), ePTFE membrane underestimates by 29 ppm (6.8%), and Weiss method overestimates by 29 ppm (6.8%). From the data, the Liqui-cel membrane contractor is the most advantageous to use in the DGAS unit; however, calibrations must be made using water with known concentrations of CH_4 and CO_2 to correct for this undersaturation.

Fractionation of isotopes is also of concern whenever equilibrating gases between dissolved and gaseous phases, especially when a membrane separates the two phases. Thus, the fractionation of each equilibrator was determined with reference gases. For $\delta^{13}C-CO_2$, all three equilibrators were within 3% difference of the isotopic standard gas (Table 3).

Table 3. Equilibrator Fractionation Statistics Using Standard Gas As Reference[a]

	average $\delta^{13}C-CO_2$	SD	%RSD	average $\delta^{13}C-CH_4$	SD	%RSD
Weiss	−41.07	0.26	0.63	−41.00	1.55	3.78
G542	−39.17	0.63	1.61	−37.41	0.23	0.61
ePTFE	−40.28	1.07	2.66	−29.49	0.17	0.58
standard	−40.21	0.40	0.98	−40.23	2.57	6.39

[a]Each equilibrator was used to analyze standard gas-equilibrated water to determine the amount of fractionation from the true $\delta^{13}C-CO_2$ and $\delta^{13}C-CH_4$ measured.

$\delta^{13}C-CH_4$, however, showed signs of isotopic fractionation where the Weiss equilibrator had a 1.91% difference, Liqui-cel 7.02% difference, and ePTFE with the largest difference at 26.7% (Table 3). While isotopic fractionation more readily occurs with methods of membrane equilibration, the methane oxidation studies presented below were more focused on the relative changes in isotopes rather than absolute values.

3.2. Application of Technique. *3.2.1. Mesocosm Results.* All the above tests were in preparation for a mesocosm study carried out aboard the R/V Endeavor during July 2014. Samples were collected in the Hudson Canyon along the northern U.S. Atlantic margin. Blank and standards (Figure 2B,C) were analyzed along with the samples collected. The DGAS unit was prepared to use the ePTFE membrane equilibrator for this mesocosm experiment. Figure 3A shows the concentration data points from a seawater samples between ~0 and 404 h. The concentration of CH_4 decreased over time correlating with the CO_2 concentration increase in Figure 3A. While CO_2 is the terminal product of aerobic methane oxidation, background levels of dissolved CO_2 are 3 orders of magnitude larger than CH_4 making it difficult to determine more precise CO_2 concentration increases due to methane oxidation. For this same reason, it would be difficult to determine the amount of carbon used for biomass versus the amount of carbon output as CO_2 during the oxidation process. $\delta^{13}C-CH_4$ and $\delta^{13}C-CO_2$ data were also obtained and are shown in Figure 3B. The $\delta^{13}C-CH_4$ values indicate that $^{12}CH_4$ is being consumed more readily, fractionating the remaining CH_4 to isotopically heavier $\delta^{13}C-CH_4$ values. While $\delta^{13}C-CO_2$ values started at 150 ‰ and dropped to −50 ‰ toward the beginning of experiment, it is uncertain what caused the

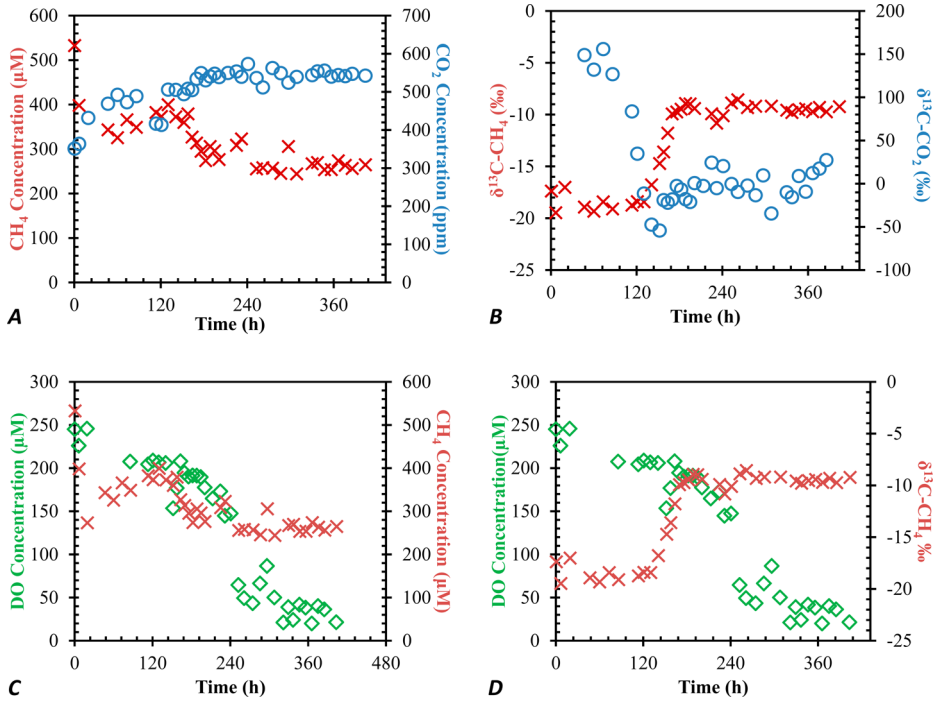

Figure 3. (A) Dissolved CH_4 (red ×) and CO_2 (blue ○) concentration versus time over the course of a mesocosm experiment where microbial oxidation of dissolved CH_4 correlates with the increase in dissolved CO_2 concentration. (B) The correlated increase in $\delta^{13}C$-CH_4 (red ×) values with the decrease in $\delta^{13}C$-CO_2 (blue ○) values as uptake of the lighter isotope of CH_4 is shifted into the CO_2 pool from oxidation. (C) 2 min averages of the dissolved O_2 (green ◇) concentration during mesocosm experiment paired with CH_4 (red ×) concentrations showing the decline of oxygen as CH_4 is oxidized. (D) The same sample DO concentration (green ◇) decline plotted with dissolved $\delta^{13}C$-CH_4 (red ×) increase also suggesting the lighter $^{12}CH_4$ being consumed more readily due to microbial isotope dynamics.

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issue. The CH_4 and CO_2 concentration data coupled with the $\delta^{13}C-CH_4$ and $\delta^{13}C-CO_2$ data suggests that methanotrophic species were growing throughout the ~17 days this specific mesocosm experiment was under analysis. To further support this data, the DGAS unit measured DO concentration continuously while each sample was pumped in for analysis (Figure 3C,D). The decreases in DO concentrations over time add to the evidence that aerobic methanotrophy is responsible for the methane concentration decrease. As part of methane oxidation, methane carbon is incorporated into the cellular biomass of methane oxidizing bacteria. If methane carbon is not incorporated into biomass during microbial oxidation, but is fully oxidized to CO_2, the stoichiometric ratio for DO:CH_4 is 2:1. A plot of DO and CH_4 concentrations displays a linear slope of 1.8 which is close to the expected 2:1 ratio (Figure 4).

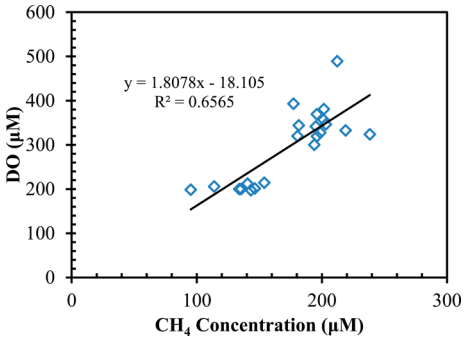

Figure 4. Plot of DO and CH_4 concentrations to show the consumption ratio. The slope of the linear regression is the consumption ratio and indicates that this is close to the expected 2:1 ratio for methane oxidation.

Because the mesocosms were a closed system, the Rayleigh equation can be used to model the data to determine the fraction reacted (Figure 5).[34] The fraction reacted (f),

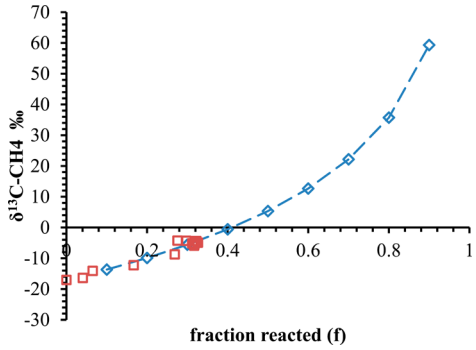

Figure 5. Rayleigh plot (blue ◇) that models the fraction (f) of methane reacted. Isotopic values from the measured sample (red □) indicate that the Hudson Canyon sample only oxidized ca. 35% of the methane leaving ca. 65% unreacted.

approaches 0.35, indicating that close to 65% of the initial methane pool remains unreacted. This suggests that a limiting factor in this sample prevented more methane from being consumed.

4. FURTHER CONSIDERATIONS

The DGAS unit was coupled with the ePTFE membrane equilibrator for the mesocosm study with success; however, further testing has shown the Liqui-cel G542 unit would be able to measure samples at a faster rate. During postmesocosm field trials, it was discovered that the ePTFE membrane biofouled if it was used and allowed to stagnate (Figure S7C,D). In addition, ePTFE membranes of this type are subjected to medical regulations because they are used as human implants. It has thus proved difficult to obtain more ePTFE membranes for use in our research application. In future mesocosm studies, the G542 unit will be used as the main equilibration vessel to collect concentration and isotope data at a higher rate as well as with a higher precision. While the DGAS unit was proven for use in a mesocosm experiment, there are numerous additional applications. For example, the DGAS can be used in pure culturing situations to continuously acquire data of the dissolved concentration of gases without significant perturbation to the culture. The functionality and analysis rates of this system for pure culture experiments in a large bioreactor will be similar to the response time tests we conducted of the DGAS unit and equilibrators (Tables 2 and 3; Figures 1D, Figures S5 and S7). Another potential application would be using the DGAS system for air–sea flux analyses similar to other equilibration style systems.[29,32] This DGAS system could also be utilized in other biogeochemical investigations beyond CO_2 and CH_4 studies so long as the appropriate detectors are incorporated. For example, a N_2O CRDS exists therefore nitrogen cycling could potentially be examined using a modified DGAS system.[33] To conclude, this automated, higher resolution, and less destructive method improves rates of experimental measurements of mesocosm and pure culture experiments, allowing the investigator a clearer and more comprehensive view of these biogeochemical systems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b04304.

Pictures of the DGAS unit and the components as well as additional information for the characterization of the equilibrators tested in the DGAS unit (PDF)

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Notes

The authors declare no competing financial interest.

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