UNIVERSITY OF CALGARY

MEMORIAL UNIVERSITY

CESAR CANADIAN ENERGY SYSTEMS ANALYSIS RESEARCH

CCEMC Project # B150173

Milestone 2 Report

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Eco-mimicry approach to methane capture in tailing ponds: Design of a multi bioreactor and gas sampling system for testing the performance of known methane oxidizing materials

Executive Summary

The government of Alberta has called for a 45% reduction in methane emissions from oil and gas operations by 2025. High methane emissions of up to 26 t/ha/yr have been measured in oilsands tailings ponds. This project was an initial proof-of-concept step towards designing a floating methanotrophic biofilter that could be used to limit methane emissions from these tailings ponds. We first designed and tested a laboratory microcosm to mimic the methane cycle in a natural peat bog, in which methane produced in deep anoxic sediment is efficiently oxidised in a floating organic mat instead of being released to the atmosphere. We then created a floating biochar mat on oilsands tailings water samples to demonstrate a similar biofiltration capacity on a tailings pond.

A system was designed where methane fed into the bottom of a water-filled column flowed upward through a floating mat of porous peat or biochar. This material slowed the diffusion of methane and supported the growth of methanotrophic bacteria. The system was designed to be able to constantly monitor gas flux rates, methane oxidation kinetics, and the development of microbial communities. The design of the floating biochar mat was optimized to achieve a removal of 84% of the methane supplied to it at a rate comparable to that observed in oilsands tailings ponds. The capacity of biochar as a substrate for microbial growth and an effective physical material to control diffusion of methane and O_2 was therefore demonstrated.

This project achieved its goal of designing and testing a meso-scale bioreactor system to mimic a field-scale floating for oilsands tailings ponds. The system can be used in future experiments to optimize mat design parameters and monitor long term effectiveness. This research was a promising initial step towards a long-term goal of engineering a floating biofilter to eliminate methane emissions from oil sands tailings ponds.

Introduction: Project Description and Goals

This project will design and test a laboratory microcosm to mimic the methane cycle in a natural peat bog. Methane fed into the bottom of a water-filled column will diffuse upward through a floating mat of porous material such as peat or biochar. This material will slow the diffusion of methane and support the growth of methanotrophic bacteria. The microcosm will be designed to allow long-term monitoring of gas fluxes and microbial growth, and therefore can later be used in multi-factorial experiments to optimize mat design parameters. This optimization research is an initial step towards a long-term goal of engineering a floating mat to eliminate methane emissions from oil sands tailings ponds.

This report addresses the tasks that were under the first and second milestones of the project.

Task 1.1. Order component parts and and/or have them made to design drawings. [April 10, 2015 to Sept 30, 2015]

From the start of the project (April 10, 2015) until August 2015, when the project accounts were finally set up, Drs. Layzell and Helleur designed the gas analysis system and placed orders for many of the component parts. There were no salary costs for this work.

Figure 1 provides a schematic drawing of the design that was prepared at that time.

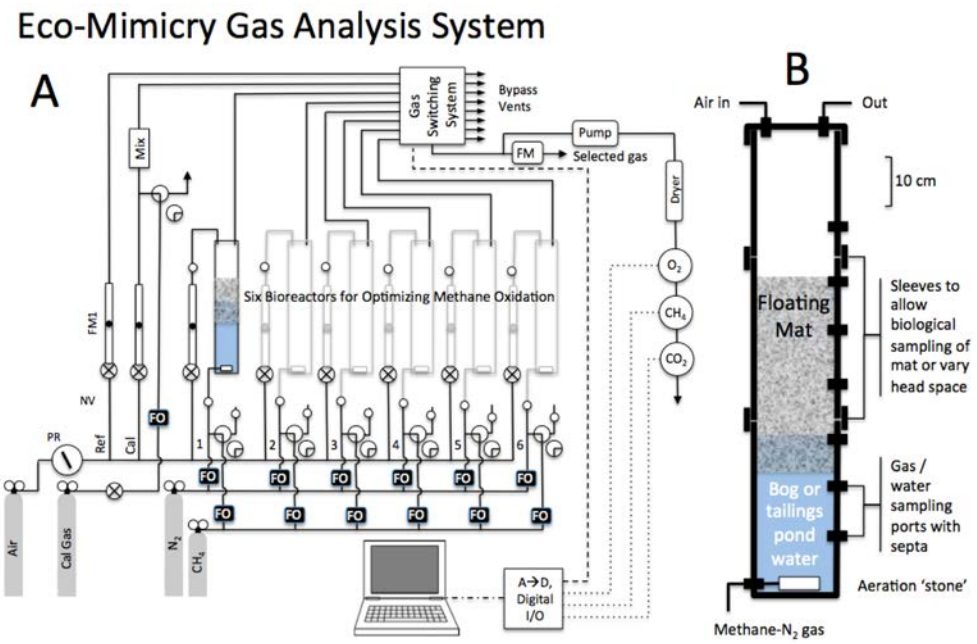

Figure 1. Schematic of the gas exchange system (Left) and the bioreactor (right)

In putting the design together, we were able to repurpose a number of instruments from earlier studies to reduce the materials costs, however, an increase in the labour costs was realized. This tradeoff was considered well worthwhile. These repurposed components included:

- A gas switching system (This $10000 instrument needed to be updated with a computer interface which was done for under $2000)
- Electronic Flow meter
- Infra-red CO_2 analyzer and O_2 and CH_4 detection systems (replacement sensors were purchased)
- Four channel Analog to Digital convertor and Digital I/O controller
- Compressed gas regulators and pressure regulators

A technical support position for a research Engineer was advertised and Dr. Parissa Mirjafari was hired, beginning on 17 August 2015. She not only ordered additional materials and supplies but has worked closely with the team to build, calibrate and test the gas analysis system and bioreactor.

Task 1.2. Assemble the Gas Analysis / bioreactor system and the sample gas analysis system. [August 17, 2015 to December 31, 2015]

The gas analysis system and bioreactor includes a large number of component parts that all must work together. Details on some of the more significant components will be summarized here.

A. Lab-Scale Bioreactor.

A Plexiglas column with an internal diameter of 4 inches (~10 cm) and height of 60 cm was built with sampling ports distributed every 10-20 cm up the side of the chamber (Figure 2). With this design, extra dry compressed air (Referred to as 'Air' in the text) flows into the top of the column through a ¼ inch OD Bev-A-Line tubing. This gas stream mixes with the headspace of the chamber.

In addition, a mixture of calibration gas (**CAL gas**: 20.79% CH_4, 20.95% CO_2, balance of N_2) was delivered through the lid to the bottom of the chamber using 1/8 inch PFTE tubing. [The gas exchange system also makes it possible to mix the CAL gas with pure N_2 gas before delivering it to the bioreactor]. The tube containing O_2-free gases can be delivered to an air stone attached to the end of the PFTE tubing.

A 150-mL Mason jar was used as a blank and is referred to as the 'CAL gas jar' in the text. Like the larger bioreactor, it had ports for air flow in and out, and for CAL gas flow in. The smaller volume ensured rapid mixing and response time for the two gas streams. In subsequent testing of the system, we have used a number of different sized chambers to hold the sample materials and create the desired conditions.

Figure 2. Bioreactor

B. Gas Exchange System.

The compressed gases were delivered to the bioreactor through a custom-made gas distributing system. This system was built on a laboratory cart with two hinged aluminum panels installed on the front and side.

Compressed gases first pass through a two-stage pressure regulator to deliver about 40 psi to a single-stage pressure regulator (right side of Figure 3). The outlet pressures from these regulators could be adjusted in the range from 1 to 10 psi and they were delivered to one of three supply manifolds (air, methane and nitrogen) that were installed behind the front panel. The air manifold had eight gas outlets, while the nitrogen and methane manifolds were each equipped six gas outlets.

In the case of the air outlets, the gas was delivered to 8 variable area flow meters (0-400 mL/min range) that could be adjusted from the front panel. The outflow from these flow meters were as follows:

Channel	Purpose
1	Reference
2	Calibration
3	Sample 1
4	Sample 2
5	Sample 3
6	Sample 4
7	Sample 5
8	Sample 6

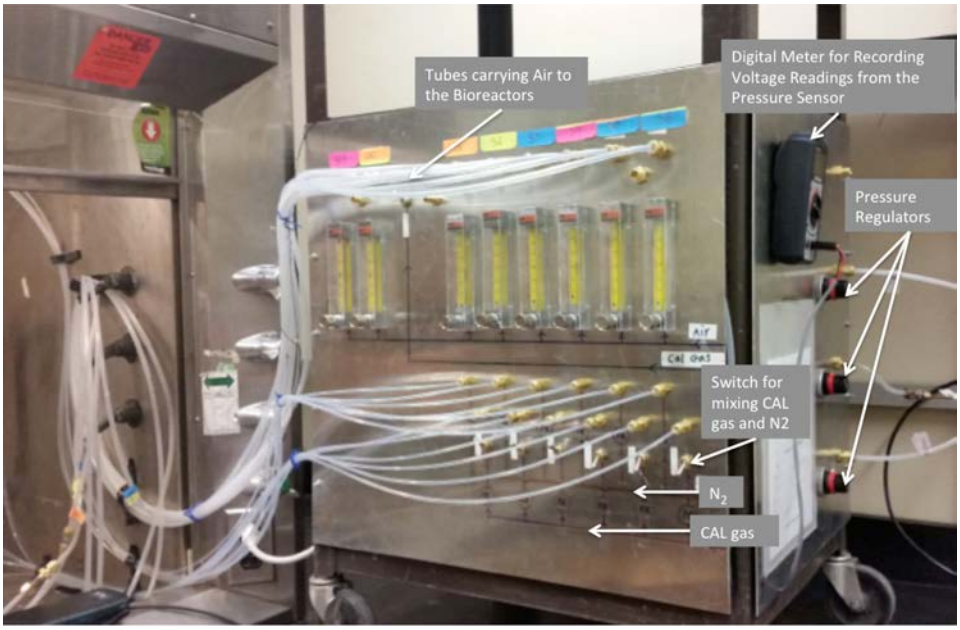

Figure 3. Photograph of the Gas Exchange System

The manifold outlets for CAL and N_2 gas streams were delivered to fixed lengths of PEEK (Polyether ether ketone) tubing (5 cm length for CAL gas, 2 cm length for N_2 gas). The PEEK tubing has a 1/16 inch OD but a very narrow bore ID (0.0025"), thereby creating a fixed orifice flow restriction. This makes it possible to achieve very low flow rates (<0.1 to 4 mL/min) of these gases by regulating the pressure within the respective manifolds. The pressure in the CAL and N_2 manifolds were typically greater than 2.5 psi (equivalent to 1.75 m of water column) meaning that the flow rate through the peek tubing to the sample chambers should be relatively little affected by being placed under 20 cm of water.

Before mixing with the N_2 gas stream and being delivered to each chamber, the CAL stream passed through a gas switching toggle valve (Clippard model FTV-3P), which allowed the user to divert and measure the flow rate using an electronic flow meter (Agilent Technologies-ADM 1000) or a bubble flow meter.

C. Differential Pressure Sensor:

The pressure within each of the three manifolds is monitored using a custom-made set of four differential pressure sensors (Figure 4). Four transducers (Digi-Key Corporation, model MPXV5100DP-CASE 1351-01) were purchased and incorporated onto circuit boards. The output from the transducers were delivered to a rotary switch that selected one signal to be passed to a digital multimeter (Figure 3). By measuring voltage, the multi-meter display could be used to calibrate the pressure sensors.

This calibration was done against a 2-m high, water-filled manometer that was made using tygon tubing and meter rulers purchased at a local building supply store (not shown).

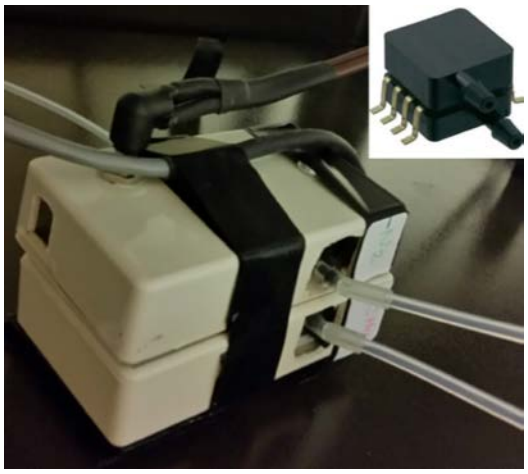

Figure 4. Four-channel, Custom-made differential pressure sensor and its transducer (inset)

D. Gas Switching System

The effluent gas from the bioreactor/mason jars is directed to an eight-channel gas switching system (Qubit Systems Inc, Model 244, Figure 5) that was donated to the project by Layzell. A Digital Control Unit (Model C200,

Figure 5. Gas Switching System (Left) & Digital Control Unit (Right)

Figure 6. Lab Pro Interface

Qubit Systems Inc) was built to connect this switching system to a Lappro interface (Vernier Software Inc).

Using this system, a computer running Logger Pro software can be programmed to work with the Labpro interface to send instructions though the digital control unit that controls the 8 Channel switcher. In so doing, one of the 8 channels of gas that are continuously flowing through the gas switching system is provided to the 'Analyzer out' port. That port delivers the selected gas to the 'T' piece, with one side being the inlet port for an analytical bench containing the O_2, CH_4 and CO_2 detectors and the other side venting to the atmosphere through a digital flow meter.

The digital flow meter was custom made and incorporated a Honeywell AWM3100V sensor. It provided a measure of the flow rate of the selected sample gas venting to atmosphere, thereby providing an indication as to whether there might be leaks in the system. Typically, a flow rate of about 200 mL/min is provided to the gas switching system for each channel. The Analytical bench draws 30-60 mL/min from this gas stream, leaving about 140 to 170 mL/min to vent past the flow meter. If the flow rate measured in the bypass drops below this range, the operator knows to look for leaks in the system, and not trust the sensor results.

E. The Analytical Bench

The 'Analyzer out' gas stream exiting the 8 channel gas exchange system was subsampled (about 30 mL/min) using a 12V DC pump (model WT7n, Worldtechon Inc., China) connect to a custom-built pump control circuit (Model QuMotor-3, Qubit systems Inc). The gas stream was dried by passing it through a Mg perchlorate column and then delivered it to an O_2 sensor (CiTiceL, model 40XV), then a CH_4 sensor (Figaro, model TGS 813), and finally to a Infrared CO_2 analyzer (Qubit Systems model S151) (Figures 7 and 8).

The CO_2, CH_4 and O_2 sensors all produce analog voltage outputs (0 to 5VDC) that are delivered to a Vernier Lab Pro interface (Figure 6), which digitizes them (12 bit) and provides the information to a computer (PC with Microsoft windows version 7 operating system) running Logger pro software (Vernier Software).

In addition to the three voltages representing gas concentrations, the Lab pro Interface was also used to monitor the bypass flow sensor. The labPro interface was also connected digitally to the Digital control unit (Figure 5) that manages the gas switching system.

Figure 8. Infrared CO_2 Analyzer

Figure 7. Flow through O_2 (top) and CH_4 (bottom) sensors used for these studies

Task 1.3. Calibrate the pressure and flow control systems and the sensors under a variety of abiotic conditions [November 1, 2015 to January 31, 2016].

After assembling the gas switching system, numerous calibrations needed to be carried out. These will be described below.

A. Differential Pressure Sensor Calibration

A set of four differential pressure sensors were built to monitor the manifold pressures and thereby control the flow rates for CAL and N_2 gases flowing through the PEEK tubing.

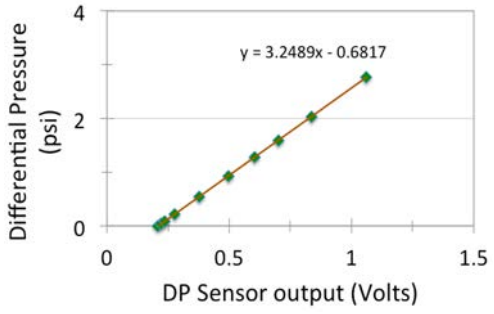

Figure 9: Calibration of differential pressure sensor

To convert the pressure sensor output voltages into pressure units of pounds per square inch (psi), a 2-m-high water-filled manometer was used. Different pressures were generated for both the electronic sensor and the manometer using an air-filled 60-mL syringe. The change in the pressure in cm of water column resulted in a change in the pressure in terms of voltage. The cm of water column differential values was used to calculate psi and plotted against the differential pressure sensor output to generate a calibration (Figure 9).

B. CO_2 and O_2 Sensor Calibrations.

Since the CO_2 analyzer has a linearizing circuit a two point calibration was carried out to convert the voltage output into ppm CO_2. A CO_2-free air was introduced into the sensor and the corresponding voltage was recorded. Then a CO_2 mixing ratio of 496 ppm (v/v) was provided. The following calibration line was then calculated:

$$CO2(ppm) = 491.58\, CO2(Volt) - 401.13$$

The O_2 sensor is also known to have a linear output with O_2 so again, a two-point calibration was carried out, one at 0% O_2 and the other with air (20.9% O_2). The readings resulted in a calibration line relating mixing ratio of oxygen to volts:

$$O2(\%) = 6.97O2\ (Volt) - 7.04$$

C. Digital Flow Meter Calibration.

This calibration method provides a flow rate measurement in volumetric units based on the local atmospheric pressure. Since Calgary is over 1000 m above sea level, the flow rates are about 10% higher than what they would be if converted to values at sea level. This information is included in the calculation gas exchange rates. A Honeywell (AWM3100V) digital flow meter is used for flow measurements. This flow meter measures the flow in terms of volts.

To calibrate the flow meter, a bubble flow meter was used with air flow. The voltage corresponding to each set flow rate was measured resulting in a calibration curve as shown in Figure 10.

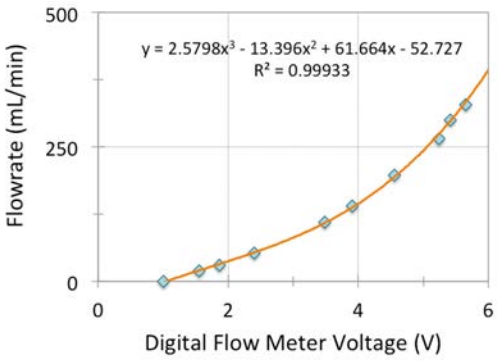

Figure 10. Calibration of flow meter

D. Calibration of Gas Chromatograph for CH_4 and CO_2

A gas chromatograph-flame ionization detector (GC-FID: Mandel SRI 8610C, equipped with a methanizer for CO_2 detection; 100°C column T; 6' Hayeseep D column) was used for measurement of CH_4 and CO_2 so it could be used to calibrate the CH_4 sensor, check the calibration of the CO_2 sensor, and calibrate the PEEK tubing for the flow of both gases (see below).

Since the area underneath each peak was proportional to the concentration of CH_4 or CO_2, a certified standard gas mixture of 0.997% CO_2 and 0.494% CH_4 (Praxair) was injected into the GC and the peak area recorded to create a calibration line that was set to pass through zero (The peak area is zero when the gas mixing ratio is zero). Figure 11 shows the calibration lines for CO_2 and CH_4.

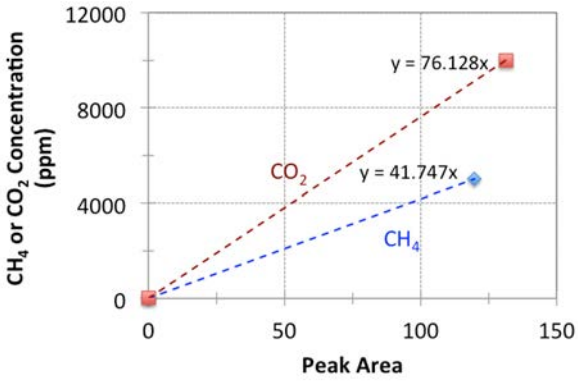

Figure 11. Calibration of the gas chromatograph for CH_4 and CO_2.

E. Calibration of Flowrate versus Pressure in PEEK Tubing.

In the Milestone 1 submission, we reported on the calibration of various lengths of the PEEK tubing in response to changes in the atmospheric pressure in the manifold upstream to the Peek tubing. However, the flow rate through each PEEK tube was carried out using the Agilent ADM-1000 flow meter, which did not recommend its use at flow rates below about 5 mL/min. Since we were measuring flow rates as low as 0.5 mL/min, a new and more reliable method was needed to quantify the flow rates of the gases through the PEEK tube as the manifold pressure was varied.

Figure 12 provides a schematic of the experimental design for calibration of the PEEK tube flow rates for a range of manifold pressures. First, we calibrated the influent air flow rate (measured by ADM-1000 flow meter) in each channel versus the pressure in the air manifold. This allowed us to calculate air flow rate as pressure changes in the air manifold (there was a slight pressure drop over time in the air manifold).

In the next step we started the gas-switching system and manually switched between channels 1 and 8, by connecting the effluent of each channel to a three-way polycarbonate stopcocks valve that was connected to the influent line of the Honeywell flow meter (Figure 11). Channel 1 was the reference air and the importance of switching to this channel was to monitor the background CO_2, CH_4 and O_2 mixing ratios continuously. There was no PEEK tube connected to this channel. Channel 8 was a 3 x 1/4" T-Swagelok fitting (Figure 12). Air was fed into the fitting from top and CAL gas from one side. The mixed gas exited the fitting from the other side. This design allowed us to perform the calibration with more accuracy. To calibrate each PEEK tube, the corresponding 1/8" PTFE tube carrying CAL gas from the toggle valve corresponding to that PEEK tube (Please refer to Task 1.2-B) was connected to the T-fitting.

Each time we switched to Channel 1 or 8 the pressure in the air manifold, methane manifold and nitrogen manifold were recorded. We also took a sample from the corresponding channel using a 3-mL syringe connected to the stopcock valve. The syringe was flushed a few times with the effluent gas in that channel and then a sample was taken. The syringe was air tightened immediately using a luer lock cap. All samples were analyzed for CH_4 and CO_2 using a GC-FID.

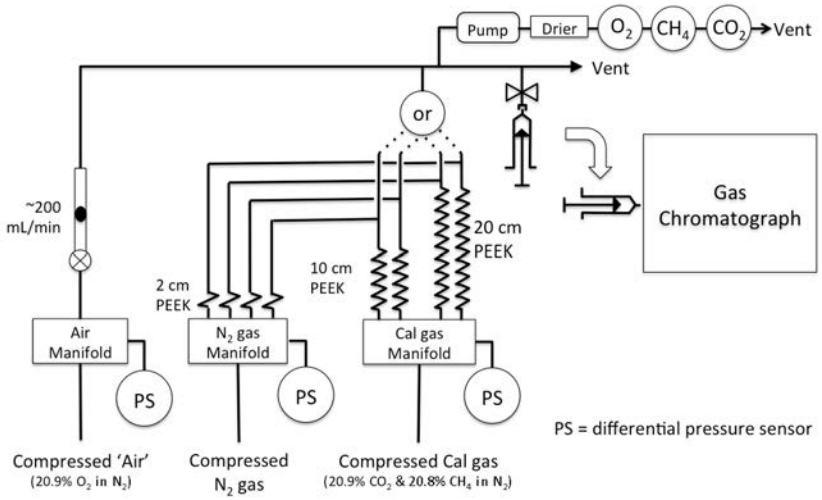

Figure 12. Schematic of the new design for calibration of PEEK tubes

The pressure in each manifold was calculated using the pressure sensor calibration equation (Figure 9). The mixing ratios (ppm) of CO_2 and CH_4 in each channel were calculated using the calibration equation developed for GC (Figure 10). The net mixing ratios of CO_2 and CH_4 in samples from Channel 8 were calculated by deducting the mixing ratios of these gases in the reference air. Figure 13 is an illustration of the steps taken for calculation of CAL gas flow rate in each PEEK tube. Initially CAL gas flow rate was calculated based on the calculated CH_4 flow rate and CH_4 mixing ratio in the CAL gas mix. Then we used this calculated CAL gas flow rate as the initial guess and used an iteration loop to come up with the CAL gas flow rates at which all other calculations and numbers emerged. The calculated flow rate was used as one point in our calibration. The calibration line was passing through zero, since flow rate of CAL gas is zero when there is no pressure on the methane manifold.

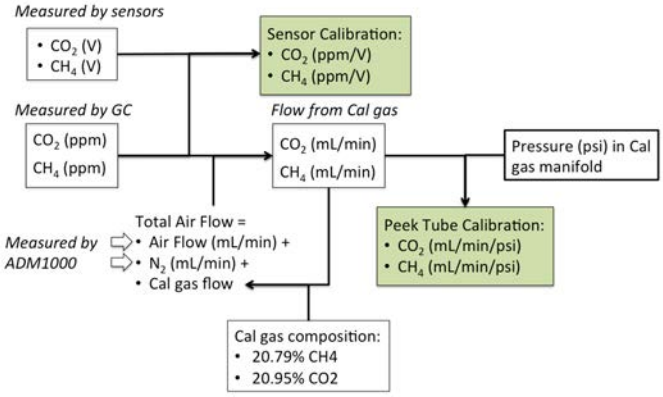

Figure 13. Steps taken for calculation of CAL gas flow rate in PEEK tubes

Table 1 is the summary of the calibration slopes for different gases. The slopes are presented as a range since each peek tube has a different slope.

Table 1: The flow versus pressure relationship for various gases through 10-cm and 20-cm PEEK tubing.

	20-cm PEEK	10-cm PEEK
Gas	Slope (mL/min/psi)	Slope (mL/min/psi)
CO_2	0.0134-0.0188	0.0236-0.0304
CH_4	0.016-0.0212	0.0232-0.0308
CAL Gas	0.064-0.0897	0.1107-0.1468

F. Calibration of the Methane Sensor.

Calibration of methane sensor that was reported in milestone 1 report was based on the calibration of CO_2 sensor. However we needed a more accurate calibration. Therefore we calibrated the methane sensor using the GC-FID. The experimental set up was the same as explained above (Figure 12). For the calibration, the gas exchange system was started and we manually switched between different channels as shown in Figure 14.

CO_2 and CH_4 readings on the sensors were both monitored with the Logger Pro software. At the same time, when we were switching to each channel, samples were taken from that channel. These samples were injected into the GC and the peak areas for CH_4 and CO_2 were recorded. The peak areas were converted to mixing ratio in ppm using the calibration graph in Figure 11. Then these mixing ratios were plotted against the voltage readings recorded for each sensor. In the new calibration, CO_2 mixing ratio had a linear relationship with the sensor's voltage (Figure 15), whereas CH_4 mixing ratio had a second order polynomial relationship with the sensor's voltage

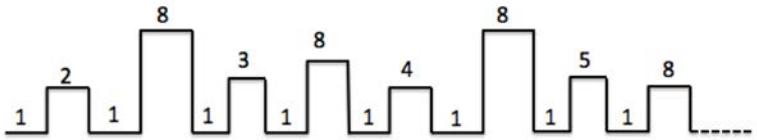

Figure 14.Order of switching channels in the calibration experiment

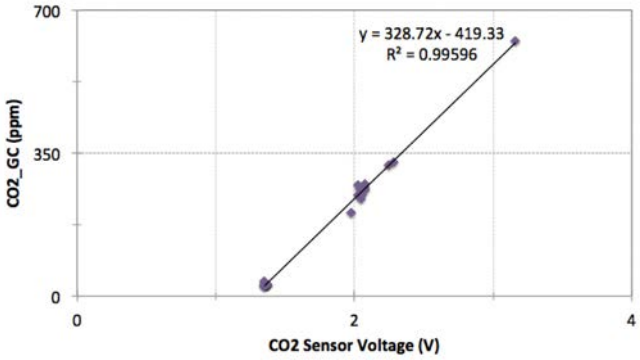

Figure 15. Calibration of CO_2 sensor vs GC

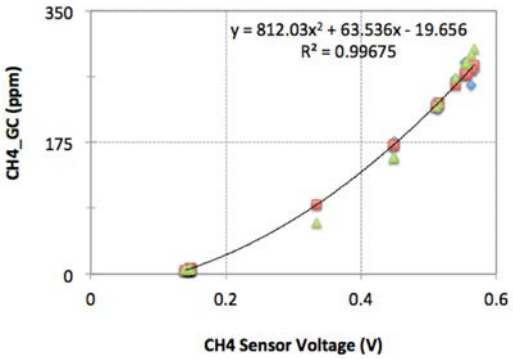

Figure 16. Calibration of CH_4 sensor vs GC

Task 1.4. Test the ability to reproducibly create and measure the O_2 and CH_4 concentration gradients within the bioreactor. [February 1, 2016 to March 30, 2016]

After assembling the gas exchange system and calibrating the gas flow and sensors a series of preliminary experiments were conducted to test the system and the Logger Pro software, to measure O_2 and CH_4 gradients in bioreactors.

A. Preliminary Experimental Set up to test the gas exchange system:

Multiple chambers were connected to the gas exchange system and the effluent gas from each channel was sequentially provided to the analytical bench. The sensor outputs were provided to the Lab Pro interface which digitized the signals and provided them to the Logger Pro software on the PC which provided a continuous plot of the data. The PC software (Logger Pro) was also programmed to control the gas switching system so a new gas channel would be provide to the analytical bench every 3 minutes.

Table 2 identifies the nature of each of the 8 channels. Note that in the channels that were provided with CAL gas, it was delivered to the bottom of each chamber through a 1/8-inch PFTE tubing while the air inlet and outlets were provided at the top of the jar. The effluents were directed to the gas switching system.

Table 2: Description of Samples used in the Preliminary Experiments

Sample	Vessel Type	CAL gas (mL/min)	Air (mL/min)	Liquid phase	Floating mat
CH1 (Ref)	none	0	210	none	None
CH2 (CAL)	Sm Mason	~0.8	195	none	None
CH3 (S1)	Lrg Mason	~0.8	197	Tailings	Biochar
CH4 (S2)	Lrg Mason	~0.8	195	Tailings	Biochar
CH5 (S3)	Lrg Mason	~0.8	195	Tailings	None
CH6 (S4)	Lrg Mason	~0.8	210	Tailings	None
CH7 (S5)	Lrg Mason	0	210	Tap	None
CH8 (S6)	Bioreactor	~0.8	215	Tailings	None

Figure 17 provides an example of the CO_2 mixing ratios tracing clearly showing how the sample number affects the amount of CO_2 in the effluent gas stream. Figure 18 shows a more complete record of multiple cycles including CO_2, CH_4, O_2 and Flow rate measurements. The system proved to be highly reproducible with time, and it was possible to obtain a measure of the gas exchanges from all 8 channels every 24 minutes (3 minutes per sample).

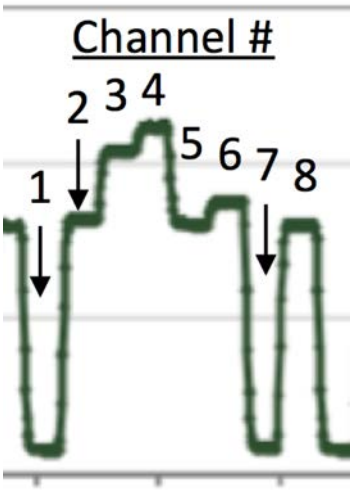

Figure 17. A Typical tracing of the CO_2 concentration over a 24-minute period during which time the gas switching system sampled all 8 channels.

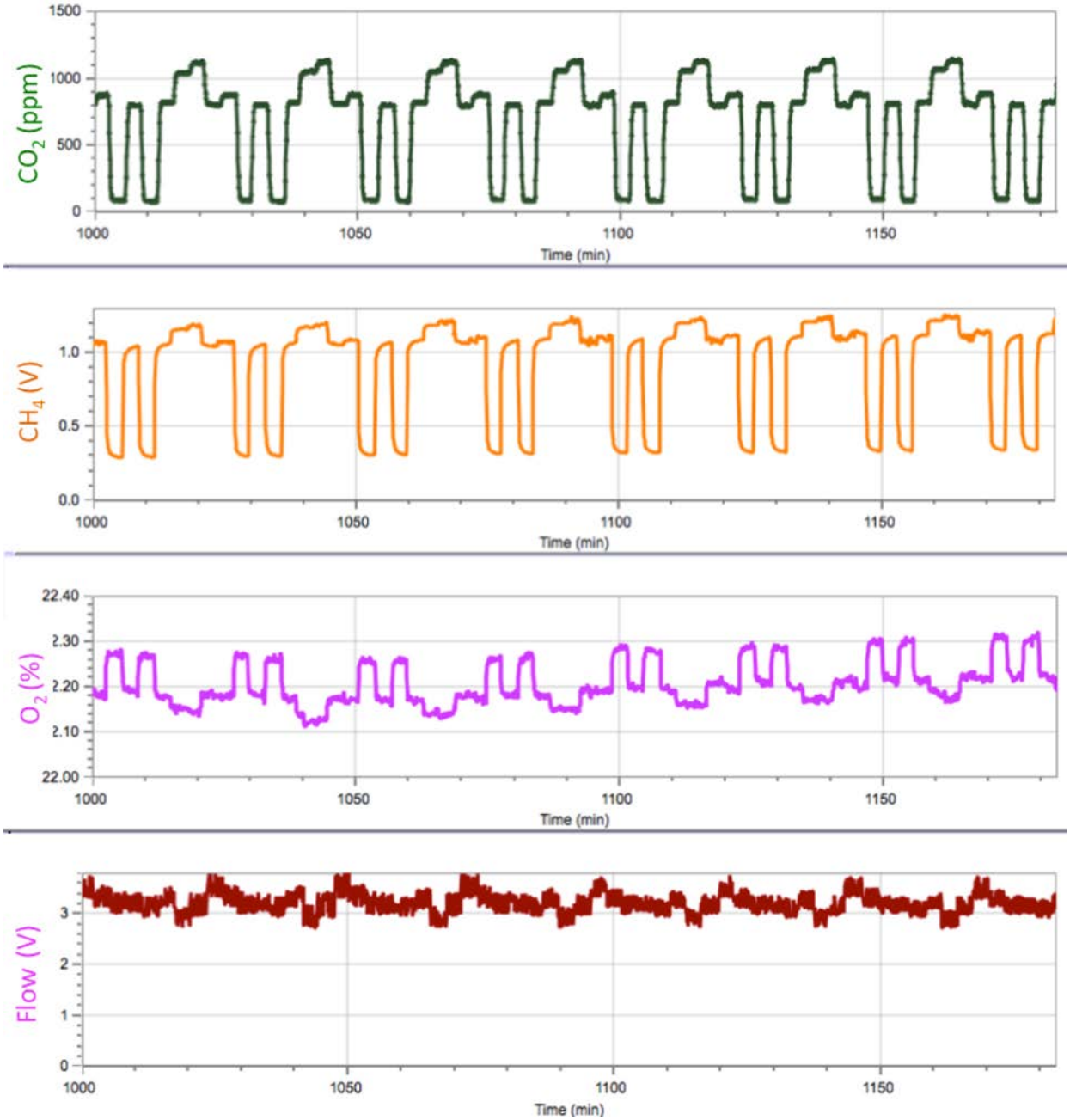

Figure 18. Concentrations of CO_2, CH_4 and O_2 plus bypass flow measurements over a 180-min (3-h) period of monitoring the gases coming from the 8 Channels of the gas exchange system. See Text and other figures and tables for details.

Notes:

- Channels 1 and 7 have low CO_2 mixing ratios since no CAL gas was provided to those vessels.
- Channel 2 is the CAL gas only
- Channels 3 & 4 contained tailings water and biochar, whereas Channel 5 and 6 contained only tailings water.
- In this system, which had only just been set up, there was no noticeable oxidation of CH_4 to CO_2 by methanotrophs contained in the tailings water or on the biochar.
- The relatively constant Flow rate voltage provided assurance that there were no significant leaks in the system.

B. Oxygen and Methane Gradients in the Bioreactor and Mason Jars

The oxygen gradients in the gas and liquid phases were measured using a Clarke style O_2 electrode (Vernier Software Inc.) above and below a tailings water solution that was continuously (for 1 week) bubbled with O_2-free CAL gas at a rate of about 1 mL/min using 1/8" PFTE tubing. After a week, a dissolved oxygen gradient was measured using a calibrated oxygen selective electrode (Vernier).

The electrode was connected to the LabPro interface and the output voltage monitored on the computer. As the sensor was lowered into the container, the voltage / O_2 concentration was monitored. Fortunately, the O_2 electrode can measure the O_2 in both air and water. For consistency, dissolved O_2 is not converted to a dissolved concentration measure (like mM or mg/L), but reported as the amount in equilibrium with a given gas mixing ratio- where air is 21% O_2 (v/v). (Figure 19)

Figure 19A shows the results in a chamber with no floating biochar while Figure 19B shows the results for a separate experiment in which a biochar mat was present but gently pushed aside so the electrode could reach into the tailings water.

Note that without a biochar mat, the gas phase oxygen mixing ratio only decreased to about 18.8% just above the water, but declined to about 14% at about 2 cm below the water. Interestingly the oxygen then increased at 4 cm depth and thereafter it decreased. These O_2 mixing ratios should be appropriate to support methanotrophic microbes, which are known to have a half-saturation constant for O_2 of below 0.1%.

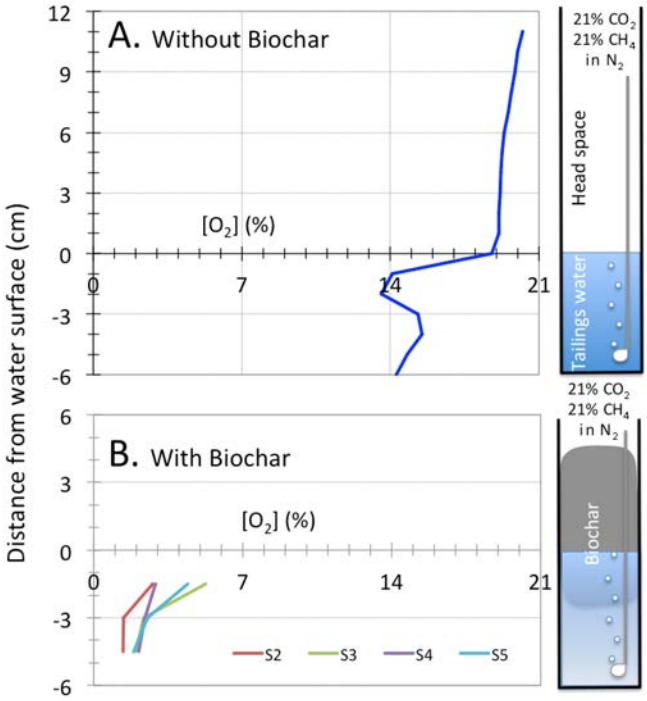

Figure 19: O_2 profiles measured by a Clark electrode in tailings water bubbled with CAL gas without (A) or with (B) a floating biochar/hemp 'mat'. S2, Tailings and Fine Biochar with CAL Gas; S3, Tailings and Medium Biochar With CAL Gas; S4, Tailings and Coarse Biochar with CAL Gas; S5, Tailings and Hemp straw with CAL Gas

To investigate the impact of a floating mat on the oxygen profile we repeated the measurement with a series of 1-L Mason jars (S2 to S5) filled with 500 mL of tailings water and a bag of floating fine biochar (S2), medium biochar (S3), coarse biochar (S4) and finally hemp straw (S5). CAL gas was introduced to all the jars. A flow of 200 mL/min of air was used to sweep the headspace in the jars to simulate the normal operation of the gas exchange system. The O_2 sensor was lowered into the tailings water at various depths and O_2 concentrations were recorded.

Figure 19B shows the depth profile of O_2 in the tailing water covered with biochar. Note that the O_2 at 1.5 cm depth varied between 2.8% to 5.3% depending on the type of the mat and size of the biochar. Oxygen at a depth of 4.5 cm was lowest for the fine biochar (the red line S2 in Fig 19), suggesting that the fine biochar creates a greater barrier to O_2 diffusion from the bulk air than does coarser biochar.

The low measured O_2 concentration in water is consistent with the fact that O_2 diffusion in water is at least four orders of magnitude slower than O_2 diffusion in air (when one considers solubility as well, the difference is about 350,000 times). With the diffusion barrier created by the biochar, and the fact that a CAL gas was continuously bubbling under the biochar, the O_2 concentration in the water may have been too low to support a high level of CH_4 oxidation. However, above the water level, the O_2 concentration should be much higher and the concept was that the interface zone created by the biochar should keep the CH_4 level high enough to facilitate CH_4 oxidation by methanotrophs.

To test this hypothesis, a 10-mL syringe fitted with a 1/16 inch OD sampling tube was used to sample the gas at various depths into the fine Biochar sample (S2), and then analyze the methane in each sample using the gas exchange system developed here. However, instead of using a pump to sample the gas and send it to the analyzer bench, the syringe itself was used to push the gas through the Mg perchlorate filter, O_2 sensor and CH_4 sensor. The system was tested against standards and found to work well in providing reproducible measurements.

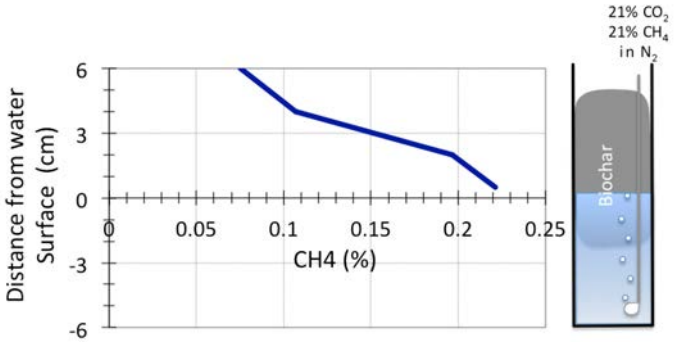

Figure 20: CH_4 profile measured in: S2, Tailings and Fine Biochar with CAL Gas;

As shown in Figure 20, the CH_4 mixing ratio close to the water surface was 0.22% (2200 ppm), or about 1/100th the mixing ratio delivered to the water in the CAL gas (20.79% CH_4). Within 6 cm of the water surface, the mixing ratio was only about 750 ppm (0.075%). This gradient was much shallower than that observed above the water in the absence of biochar (essentially below detection (50-100 ppm), data not shown).

These results show that the biochar is acting as a barrier to gas diffusion, but is the CH_4 concentration high enough to stimulate the growth of methanotrophs? According to Knief and Dunfield (2005) [1] in low affinity methanotrophs (the type of methanotrophs that are found in tailings ponds and peat bogs), K_m ranges from 1 to 20 **µ**M, or about 700-14000 ppm (0.07-1.4%). The low end of this range is similar the concentration we have measured in the biochar. If we are to achieve the maximum methane oxidation rate that will adsorb most, if not all, of the CH_4 provided to each chamber, we will need a concentration that is many times greater than the Km(CH_4) and yet also have a high concentration of O_2. The semi-porous part of the system containing gas pores that facilitate rapid O_2 and CH_4 transport to methanotrophs in water films (i.e. in the biochar above the water interface) will need a better supply of methane for the system to operate efficiently. It is likely that the system was not efficiently supplying methane to this part of the biofilter (see section C).

Michaelis-Menten Kinetics

The Michaelis-Menten equation is a well-known enzyme kinetics model that describes the relationship between the rate of an enzymatic reaction and concentration of the substrate as follows:

$$\vartheta = \frac{V_{max}.S}{K_m + S}$$

Where ϑ is rate of reaction, V_{max} is the maximum reaction rate and S is substrate concentration (i.e. methane concentration for oxidation of methane). The K_m is called Michaelis-Menten constant and is the concentration of substrate at which the reaction rate is half its maximum (V_{max}).

C. New Experimental Design

In Milestone 1 report we suggested to introduce a mixture of air and CAL gas into the jars. We tested this idea; however for various reasons we did not get the results we were hoping for. One reason was that in the new experimental design we were wrapping biochar in mesh bags and were floating the bag in water. The mesh bag was acting as a hindrance to the release of gas bubbles. The bubbles were accumulating underneath the bag and increased in size over time until they would burst all of a sudden. This made it extremely hard to monitor any changes that were happening in the jars. We tried other methods for floating the biochar in the water, but in most cases we had the problem of bubbles channeling through the jar's walls, which made the designs inefficient. On the other hand introduction of air as a mixture from the bottom of the jars is not something that naturally happens in the tailings ponds. Therefore we decided to change the design completely.

1. Claudia Knief and Peter Dunfield. 2005. Response and adaptation of different methanotrophic bacteria to low methane mixing ratios. Environmental Microbiology 7:1307-1317.

In the new design we used glass jars (i.d :10 cm, height: 21 cm) and we left a small air space between the mat (biochar/peat bog) and the aqueous phase. This was done by using glass stands and a metal mesh wire that were designed and built by Dr. Bob Helleur. The mesh screen was made from the steel-zinc plated mesh screen from Fisher Scientific. They were bent and molded and then the edges were cut so that they fit into the jars (Figure 21). A 1/8" hole was cut in the center of the mesh to pass the 1/8" PTFE CAL gas delivery tube through it. Also a 1'2" square was cut on the edge of the mesh in order to allow the use of a wick as outlined below. The glass stands were made using a glass tube (i.d: 9.5 cm, o.d. 10 cm). The tube was cut into 6-cm sections and then each section was cut into three equal pieces and the edges were smoothed out.

Figure 21. Picture of the glass stand and the wire mesh

Since the organic mat was not in touch with water we had to find a way to keep them moist to create the suitable environment for the microbial activity. A polyethylene rope was used as a wicking system to deliver water to the organic mats. The gas that was bleeding into the aqueous phase was a mixture of CAL gas and N_2. The flow rate of CAL gas was reduced to about 0.1 mL/min. The gas was bleeding into the water and then traveled up to the water surface and diffused into the gaseous space. This is a more efficient design with less channeling problem. It also gives methane a better opportunity to become into contact with methanotrophs by overcoming the diffusion barrier through controlling the moisture content. To set up the jars the three glass stands were inserted into the jars and the mesh wire was placed on top of them. Then the wicking rope was inserted into the jar with one end in the water/tailings water. In the next step the biochar/peat bog were transferred into the jar while we were wrapping the wicking rope in between layers. Figure 22 shows a schematic (plan and cross section view) of the jars, as well as a picture of a jar filled with biochar. As shown in Figure 22, the water level is just below the mesh wire allowing an air space between the floating mat and the aqueous phase.

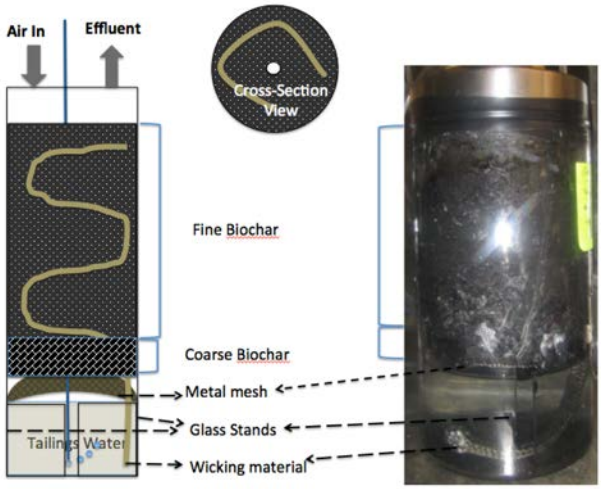

Figure 21. Schematic of the new design (Left: plan view,, Top: Cross section view, Right: picture of a jar filled with biochar)

Task 2.1. Collect samples of bog water and a core sample material from a northern bog or fen

Peat samples were collected in the wetlands of Blaketown pond situated north of town of Whitbourne in Newfoundland. A stainless steel tube with one end sharpened was used for the core sampling. The tube was cored into the frozen bog using an axe while rotating the tube periodically. Once the tube cut through the bog to the water table it was carefully lifted up placing one hand over the bottom of the tube. Then the core was removed using a ¼" plugger. The sample was kept frozen and couriered to Calgary in an instillation container via an overnight delivery. The sample was kept in a -20°C freezer until used. The samples were taken out of freezer the night before the start of the experiments and were left in the room temperature to thaw. Then they were transferred into the chamber. Figure 23 is a picture of thawed peat bog sample and a chamber with the peat bog in it.

Figure 23. Right: Peat bog; Left side: Peat bog in the chamber

Task 2.2. Make sufficient quantities of at least one form of biochar, treat with bacterial inoculants known to oxidize methane in tailings water, and set up as a floating mat in the bioreactors

Experiments were conducted to examine the influence of the type and size of biochar suitable as a substrate. The biochar used came from three different sources. Airterra, a biochar company based in Calgary kindly provided us with a batch of biochar that was produced by a Champion 2010 version of the Top Lift UpDraft (TLUD) model of cooking stove that is designed by Dr. Paul Anderson[1]. This gasifier cookstove (Figure 24) consists of two concentric chambers with a separate air intake for each. In this design the gasification (pyrolysis/gasification) step is separated from the combustion step, which results in the clean burning of wood and production of biochar. The biochar provided to us was produced from spruce wood boards (sources from RONA).

Figure 24. Pictures of the TLUD gasifier cookstove [1]

The second source were two sizes of biochar (coarse, and medium) produced in the Chemistry Dept of Memorial University. The biochars were produced batch-wise via slow pyrolysis method. Half a liter of woodchips were heated in a special glass vessel (Figure 25) housed in a small muffle furnace (Figure 25), with a 200 mL flow of N_2. The woodchips were heated from 80°C to 480°C at the rate of 25°C/min. They were hold at 480°C for 5 min and then were cooled down using a fan.

Figure 25. A. Coarse biochar, B: Medium Biochar, C: Glass Vessle, D. Muffle Furnace

The coarse biochar was made of 1-4 cm black spruce chips that were destined for Newfoundland's pulp mill. They were dried in oven overnight at 65° before being pyrolysed.

The medium biochar was prepared of 0.4-1 cm aspen woodchips from Alberta Pacific Forest Products.

The third type of biochar (small size) was prepared by the Abritech Inc in Quebec. It was prepared from small pieces of ash wood that were dried in ambient temperature. Four kilograms of the dried wood was loaded into a steel kiln (Figure 26) that was equipped with a mechanical paddle, which periodically stirred the chips. The temperature of the kiln slowly rose from 60°C to 490°C over 2 h. No inert gas was used. At the end the kiln was left to cool down overnight.

Figure 26. Left: Steel Kiln, B: Small Biochar

As mentioned in section C of task 1.4 a new bioreactor was constructed to be more efficient in terms of bringing methane into contact with biochar. To test these biochars suitability, five reactor jars were set up. The first jar was used as a control whereby bottom part of the jar (6 cm) was filled with distilled water. There was no wicking rope in this jar. On top of the mesh we added a 2-cm-deep layer of coarse dry biochar followed by an 8-cm layer of medium size dry biochar, and finally a 2-cm layer of small biochar on the top. For the next three jars the biochar (coarse, medium and small) was first soaked into tailings water that was inoculated with 250-ml of a methanotrophic enrichment cultures grown in the lab on tailings pond water plus a nutrient solution suitable for the growth of methanotrophic bacteria, that was pre-incubated under 10% CH_4 for 2 weeks [2]. Then the tailings water/enrichment culture mixture was separated from biochar and 450 mL of it was transferred to each jar. Above the mesh was filled with the tailings water-treated biochars in the following order; 2 cm of coarse biochar, 8 cm of medium biochar and 2 cm of small biochar was placed on top of the mat. A peristaltic pump was connected to one of these jars and the tailings water circulated inside the jar to keep the biochar wet. The fifth jar was filled with 450 mL of distilled water, then filled with peat on top of the mesh. Figure 27 shows the chamber jars. The experiments were performed in two parts. Changes in experimental conditions were designed to improve methane oxidation rates. In phase II we replaced the medium biochar with the fine biochar that was prepared by crushing the biochar provided by Airterra in a blender. Table 3 summarizes the contents and conditions in each chamber.

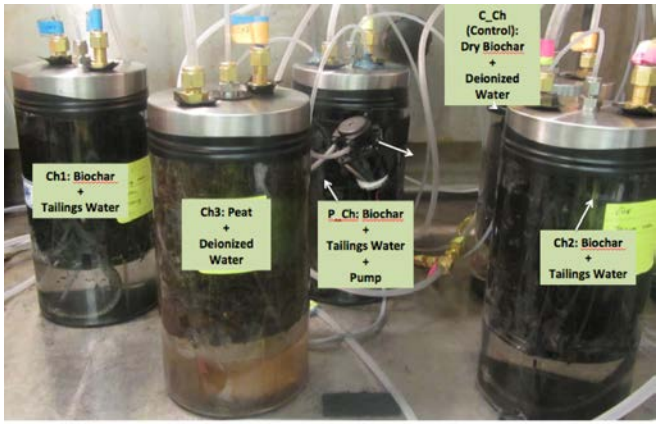

Figure 27. Picture of the chambers set up for experiments

2. Jürgen Heyer, Valery F. Galchenko, Peter F. Dunfield. 2002. Molecular Phylogeny of Type II Methane-Oxidizing Bacteria Isolated from Various Environments. Microbiology 148:2831-2846.

Table 3: Summary of the experimental setup for Task 2.3.

Chamber	Wicking/pumping	Phase	Floating mat	Aqueous phase
Ctr	No/No	I	Coarse, Medium and small Biochar	Distilled water
		II	Coarse and fine Biochar	
BC1P	Yes/Yes	I	Coarse, Medium and small Biochar	Tailings Water+Nutrient Solution+Inoculation
		II	Coarse and fine Biochar	
BC2	Yes/No	I	Coarse, Medium and small Biochar	Tailings Water+Nutrient Solution+Inoculation
		II	Coarse and fine Biochar	
BC3	Yes/No	I	Coarse, Medium and small Biochar	Tailings Water+Nutrient Solution+Inoculation
		II	Coarse and fine Biochar	
BC4	Yes/No		Peat Bog	Distilled water

Task 2.3. Quantify the rate of CO_2 and CH_4 exchange of the bog/fen and biochar mats over time and under a range of O_2 and CH_4 gradients created by varying the flows of N_2 and CH_4.

The improved experimental setup described above was used to study the effect of different factors, namely type of the floating mat, size of biochar, and methane and oxygen gradients on bio-reduction of methane.

In the first part of the experiments we measured methane, CO_2 and O_2 gradients at two different molar fluxes of CAL gas. We first adjusted the CAL gas flux at about 1000 μmol/m^2/min (about 240 μmol/m^2/min of CH_4) and waited 24 hours for the system to stabilize. The air flow to the upper part of the chambers was about 200 mL/min. After stabilization the top effluent port was opened and a 24 cm steel tube (OD: 1 mm) attached to a 30-mL syringe was inserted into the headspace above the mat and a sample was taken for oxygen measurement. The syringe was made airtight by putting a rubber luer lock cap on the tip. A 3-mL headspace sample was used for CH_4 and CO_2 measurements. The same procedure was repeated for sampling the interface between the mesh and the water. This was done by lowering the tube slowly into the floating mat until it hit the wire mesh. The samples were used for oxygen measurement as well as CO_2 and CH_4 measurements. Effluent port was kept closed in between samplings to avoid any leaks from the jar.

To analyze samples for O_2, samples were injected into the perchlorite column that was attached to the oxygen sensor at a rate that was close to the pump rate and the oxygen concentration was monitored on Logger Pro software. Once the level of O_2 stabilized the number was recorded. In order to properly measure CO_2 and CH_4 in the samples at the mat/ water interface and in the headspace just above the mat these gases were more accurately measured by GC-FID (Section 1.4 D).

In the next step the molar flux rate of the CAL gas was increased from 1000 μmol/m^2/ min to about 2000 μmol/m^2/ min (about 530 μmol of CH_4/m^2/min). A 24-h period for stabilization was required before measurements were taken. It was discovered that chamber 4 was leaking during phase I experiments and therefore the data from this chamber are not presented here. Figure 28 shows the amount of CH_4 in the headspace and interface of BC2, BC3 and Peat. In all biochar chambers mixing ratios of methane in the interface of the biochar were about 2-2.5 times larger than the mixing ratios in the headspace gas. Methane mixing ratios in the interface of the peat chamber were always higher than at the interface of biochar

chambers, and at 530 μmol $CH_4/m^2/min$ the methane mixing ratio in gas pores at the peat interface was about 14 times larger than in the headspace (Figure 28). This is due to the compact nature of the peat bog that does not allow CAL gas to escape to the headspace and therefore it builds up in the interface pores, where it is oxidised before reaching the headspace. Figure 29 shows that there was a CH_4 drop of about 500 ppm and 1200 ppm in BC2 and 2900 ppm and 3800 ppm in BC3 in low and high flux rates respectively. In the Peat chamber at 530 μmol $CH_4/m^2/min$ (high flux rate) there was a CH4 drop of 16500 ppm. Figure 30 demonstrates the change in O_2 concentration in each chamber at the two different flux rates. In the chambers filled with biochar (BC2 and BC3) there was only a small O_2 gradient at the two different CAL gas fluxes, with O_2 dropping only 2% in BC2 and BC3 (from 20.3 % to 18.3 % at 1000 μmol/m^2/min of CAL gas and from 19.21% to 17.62% at 2000 μmol/m^2/min of CAL gas in BC2, and in BC3 from 19.9% to 17.6 % at 1000 μmol/m^2/min of CAL gas and from 19.2% to 16.4% at 2000 μmol/m^2/min of CAL gas), whereas in peat there was a larger gradient with oxygen dropping about 12-14% (from 20.1% to 8.63% at 1000 μmol/m^2/min of CAL gas and from 19.82% to 5.9% at 2000 μmol/m^2/min of CAL gas). At higher flux rate there was a larger O_2 gradient in all chambers.

The data show that at 1000 μmol/m^2/min of CAL gas (190 or 200 μmol $CH_4/m^2/min$) the concentration of methane in the interface of biochar chambers near the lower limit of Km values for methanotrophs (Michealis-Menten constant), which shows that methane oxidation is methane limited rather than O_2 limited. The data also show that methane oxidation is more efficient at higher flux rates. In a study by Reddy et al. (2014)[3] they were able to achieve up to 60% methane reduction using a mixture of soil and biochar with 78% of particles smaller than 0.075 mm. Therefore we hypothesized that reducing the size of the biochar could help slow methane diffusion and increase methane concentrations at the interface. This hypothesis was tested in the second part of the experiments.

In part 2 of the experiments the medium biochar in the chambers was removed and the tailings water in the chambers was pumped out. Then the fine powder biochar was transferred to the chambers and the tailings water was pumped back to the jars, wetting the biochar as it was travelling down. In the Control chamber the water was not pumped out but the medium biochar was replaced with dry fine biochar.

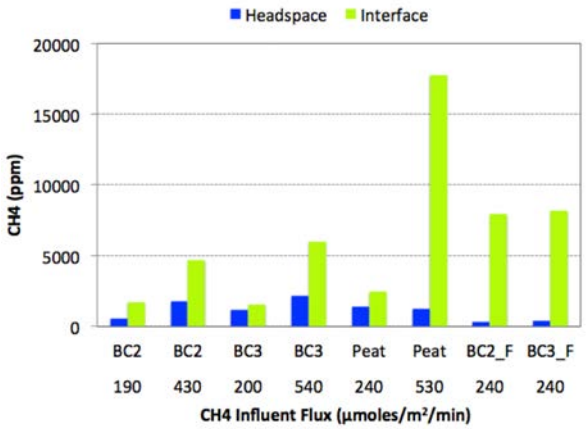

Figure 28. CH_4 in the headspace and the interface of chambers at two different CH_4 flux rates

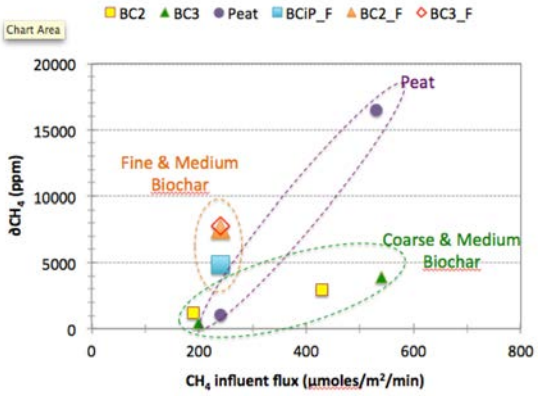

Figure 29. CH_4 gradient in chambers with different sizes of biochar and at different methane flux rate

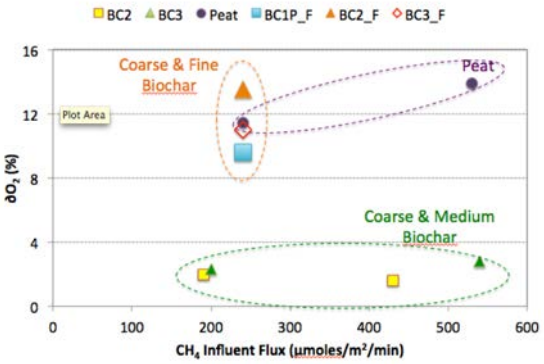

Figure 30. O_2 gradient in chambers with different sizes of biochar and at different methane flux rates

[3] KR Reddy, EN Yargicoglu, D Yue, and P Yaghoubi. 2014. Enhanced Microbial Oxidation in Landfill Cover Soil Amended with Biochar. J. Geotech. Geoenviron. Eng. 140:04014047

CAL gas flux was adjusted to about 1000 μmol/m^2/min. After 24 hours gas samples were taken from the headspace and interface of all the jars and were analyzed for O_2, CH_4 and CO_2. The same sampling and analysis procedure in part one was repeated in this phase. Methane in the interface of chambers BC2_F and BC3_F was 7913.56 and 8184.18 ppm respectively (Figure 28), which are considerably higher than the interface concentration of BC2 and BC3 at either low or high CH4 flux rates. In addition the interface concentrations in BC2_F and BC3_F are in the middle of the range reported for methanotrophs half saturation values (1290 to 20680 ppm). As Figure 29 shows, the magnitude in methane concentration change in these chambers is also considerably higher than the change in chambers with medium biochar. This shows that in chambers with fine biochar methane oxidation is substantially more efficient. Figure 30 shows the O_2 gradient in these chambers. For the same flux rate O_2 gradient was larger in the chambers with the fine biochar compared with the chambers with medium biochar. For instance in BC2_F and BC3_F with fine biochar oxygen dropped from about 19.5% to 6% and 8% respectively, which is a much larger gradient than we had in the same chambers with medium biochar (BC2 and BC3) and the same flux rate. This shows that reducing biochar size from medium to fine improves the concentration of methane at the interface, as well as the oxygen gradient in the chambers (Figure 30).

In the third part of the experiments gas switching setup was used as described in milestone 1 report to test and methane reduction in the chambers with fine biochar was measured. CAL gas flux was set at about 1000 μmol/m^2/min and the air flow in all chambers was about 200 mL/min. The same gas exchange procedure reported in Task 1.4-F was followed here and the effluent of chambers were connected to the pump in the same order shown in Figure 14. They were pumped into the O_2, methane and CO_2 sensors. The O_2 concentration, as well as voltages of methane and CO_2 sensors was monitored on the Logger Pro software. The concentrations and flux rates of methane were calculated using the calibration lines and the ideal gas law. The CH_4, CO_2 and O_2 measured in channels 2 through 7 represented the amount leaving the chambers, whereas channel 8 represented the CAL gas and N_2 mix flowing into the chambers. Figure 31 shows methane uptake in terms of μmol/m^2/min and percentage. Methane oxidation was 84.4% and 83.0% efficient in BC4_F and peat respectively, these were and the highest removal rates among all the chambers. In BC1P_F methane removal was 54.6%. We expected a higher methane removal in this chamber since the biochar was kept wet all the time. However it is possible that the recycling of water in the chambers had a negative effect on the activity of methanotrophs. There needs to be more tests done at different pump rates accompanied by measurements of total porosity of the biochar. Although BC2_F was a replicate of BC3_F methane removal in this chamber was substantially lower than in BC3_F. The previous measurements in these two chambers (Figure 28) showed a comparable CH_4 concentration in their interface and headspace. Therefore it is possible that something went wrong in BC2_F that led to the substantial drop in its performance.

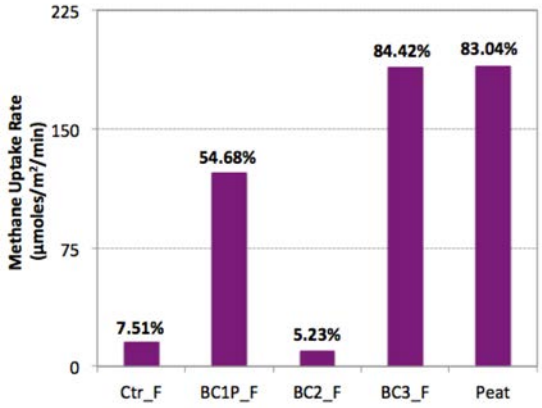

Figure 31. Methane uptake rate in each chamber with fine biochar. The numbers on top of each bar shows the percentage of methane removed.

In summary, the use of peat bog demonstrated that a high methane removal efficiency was possible in our model system. Based on our understanding of the peat chamber system, the biochar biofilter was optimized by using finer biochar rather than coarse biochar intially adopted. The denser biochar mat reduced the gas diffusion rates holding the methane in the mat filter for a longer time, allowing for more efficient methane bio-oxidation.

Task 2.4. Establish an O_2 Gradient that is near optimal for CH_4 oxidation, and then quantify the apparent CH_4 oxidation kinetics and calculate its apparent Km and Vmax.

Enzymatic activities are described with Michealis-Menten equation (Task 1.4_B and Figure 32) and Km and Vmax are two important characteristics of any enzymatic reaction. In this section we design an experiment to measure Km and Vmax for CH_4 oxidation kinetics in the biofilter system.

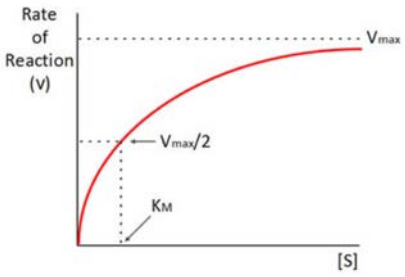

Figure 32. Michealis-Menten Enzymatic Model

Since BC4_F and Peat chambers had the highest methane oxidation activities, they were selected for measurement of oxidation kinetics. Enzyme kinetics are usually measured in a batch system by measuring methane oxidation rates at varying methane concentrations. However these chambers are operating in a continuous flow mode. Therefore the kinetics that are measured in them are apparent kinetics, and do not necessarily reflect the enzyme properties, but rather are emergent properties of the entire system integrating gas diffusion rates and methanotroph activity. They reflect the overall efficiency of the physical-biological system rather than the efficiency of the enzyme (which is essentially constant). We therefore express the kinetics as rate of methane oxidation versus the methane supply flow rates (rather than absolute methane concentrations), as this will give us a better idea of the efficiency of our system. Different methane supply rates were achieved by changing the flux rate of CAL gas through changing the pressure on the methane manifold, as well as by changing the length of the PEEK tubes.

In our experimental design, the CAL gas pressure was initially adjusted to 0.89 psi, then increased to 2.16, 4.35, and 5.75 psi at four stages. The air flow in all chambers was about 200 mL/min. However in the last stage with the highest CAL gas pressure, we expected methane concentrations higher than the sensor's upper limit. Therefore we increased the air flow rate to 500 mL/min. Before each pressure increase, the CAL gas delivery tubes (1/16" PTFE tubings) were connected to the 20-cm PEEK tubes. After each pressure increase the chambers were left to stabilize for 30 minutes. The gas exchange system was turned on and concentrations of O_2, CH_4 and CO_2 were monitored in the chambers. Figure 33 demonstrates the switching order between chambers. After the measurements with the 20 cm PEEK tubes were done, the CAL gas delivery tubes in each chamber were connected to the 10-cm PEEK tubes. The change in the length of the PEEK tube increased the CAL gas flux rate by about 1.5 times. At the end of the measurements with the 10-cm PEEK tubes, the CAL gas lines were re-connected to the 20-cm PEEK tubes and CAL gas pressure was increased. Figure 34 is a picture of the data collected for the first stage with CAL gas pressure of 0.89 psi. As can be seen, methane concentration in chambers BC4_F and peat is always lower than in channel 8, (connected to the same PEEK tube, as BC4_F or peat), which is a proof of methane oxidation in the chambers; However we expected a lower oxygen concentration in the chambers than in channel 8, since O_2 is consumed during methane oxidation. But this is not the case in figure 34. This was also observed for stage at 2.16 psi. However at 4.35 psi and 5.75 psi the oxygen in the chambers was lower than in channel 8.

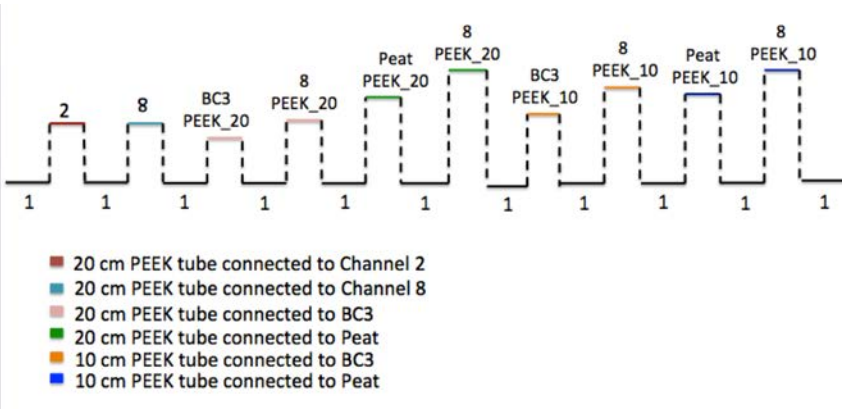

Figure 33. Schematic of the channel switching

All the data were converted to concentrations and molar fluxes using the calibration lines and the ideal gas law. The rate of methane uptake was calculated in each chamber with two sizes of PEEK tubes and was plotted against the influent molar flux of methane (Figures 35 and 36). As seen in this figure, the rate of methane oxidation increased with methane influent flux rate in each chamber, which is in agreement with the first order kinetics of Michealis-Menten equation (The linear part). The fact that we did not measure a slow down in the rate of methane oxidation and consequently a zero order kinetics could show that the system could function at higher CAL gas pressures and flux rates before getting saturated.

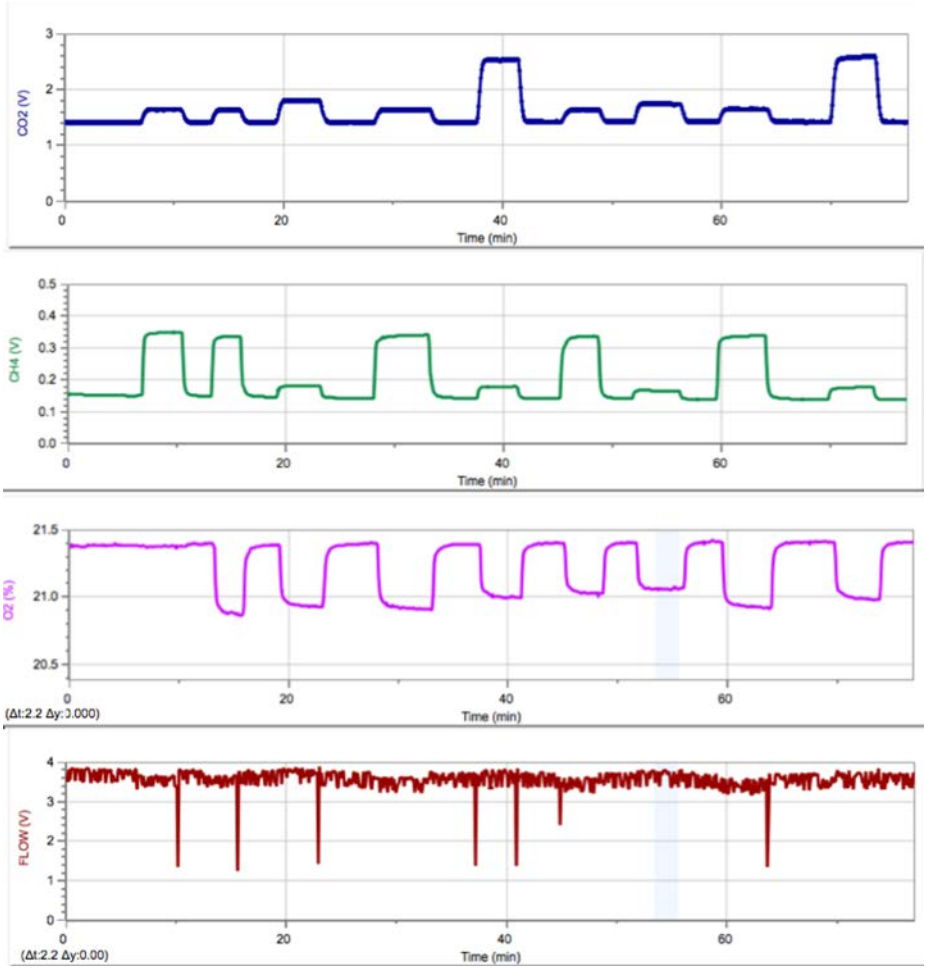

Figure 34. Data collected at 0.89 psi of Cal gas

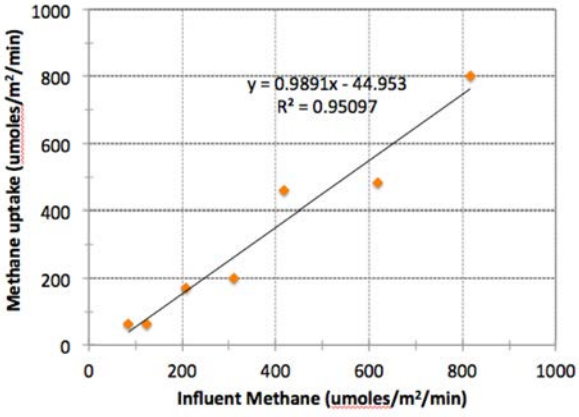

Figure 35. Change in rate of methane oxidation with influent methane flux rate

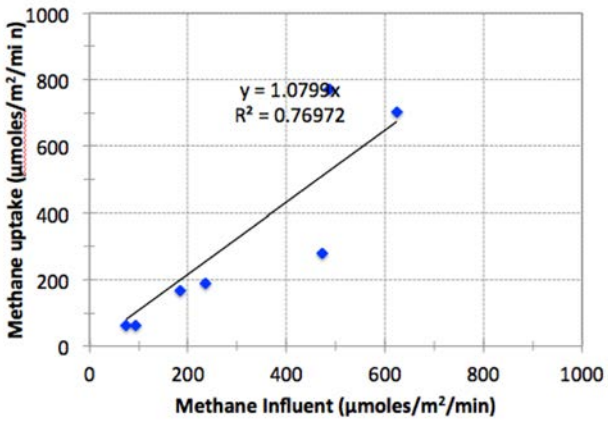

Figure 36. Change in rate of methane oxidation with influent methane flux rate

Task 2.5. Using the Results from 2.3 and 2.4 above, calculate the ability of a mat to oxidize the CH_4 found in an oil sands tailings pond.

Our best mats achieved a removal of 84% of the methane supplied, at an oxidation rate of near 170 $\mu mol/m^2/min$. (Figure 31). This converts to about 14 t/ha/yr of CH_4. This amount was chosen to be representative of oilsands tailings ponds in Alberta. Methane fluxes measured in tailings ponds vary from 0.004 to 26 t/ha/y [4]. Our biofilter efficiently removes a methane efflux representing 50% of that from the most strongly emitting pond in Alberta (Mildred Lake Settling Basin, at 26 t/ha/yr), and greater than the methane efflux measured in any other pond. This is a strong proof-of-concept for the system. Because our system kinetics are first order (Figures 35-36), methanotrophy in our system should respond positively to remove even higher fluxes if exposed to these. Further experiments will be able to test this.

Task 2.6. Retrieve mat samples for analysis of microbial communities over depth.

Since we have a biological system that uses the ability of microorganisms to oxidize methane, therefore characterization of the microbial community helps to understand the system better. Samples were taken from organic mat (biochar/peat) of each chamber at different depths, by inserting a 25 mL pipette into the chambers slowly, while twirling it until it hit the wire mesh. Then the pipette was removed. This created a deep hole in the mat. Then using a spatula some sample from various depths was scraped out and was transferred to eppendorf tubes. The spatula was rinsed with ethanol after each sampling. We also took samples from the liquid part of the chambers by attaching a 1/8" tube to a 30 mL syringe. The tube was inserted into different depths of the liquid and samples were taken from each depth. A new syringe and tube was used for each sampling depth.

Although the community analysis itself was not a specific deliverable of the project, a student is presently extracting DNA and prepared this for DNA sequencing. The biochar does seem to select for a different genus of methanotroph (*Methylobacter*) as opposed to the tailings alone (*Methylomonas*), indicating that the chemical conditions and gas gradients in the biochar biofilters are different from those in the tailings water alone.

[4] Christina C. Small, Sunny Cho, Zaher Hashisho, Ania C. Ulrich. 2015. Emissions from oil sands tailings ponds: Review of tailings pond parameters and emission estimates, Journal of Petroleum Science and Engineering 127: 490-501.

Financial Report.

Table 3 summarizes the expenses incurred under Milestones 1 and 2. Note that:

- Overall the lab of Dr. Helleur invested 24% more then originally committed, well the lab of Dr. Layzell invested 11% more than originally committed.
- The expenses charged to the CCEMC account the right on budget

Table 3. Financial report

	CCEMC				Memorial University (Bob Helleur)				University of Calgary (David Layzell)			
	Original	**Actual**	**Difference**	**%**	**Original**	**Actual**	**Difference**	**%**	**Original**	**Actual**	**Difference**	**%**
	Milestone 1											
Supplies	$15,425	$6,984	-$8,441	-55%	$4,575	$5,483	$908	20%	$-	$-	$-	
Travel	$0	$64	$0		$-	$-			$-	$-	$-	
Salaries & Benefits	$20,000	$29,145	$9,145	46%	$-	$-			$-	$-	$-	
Delivery Costs	$5,314	$5,429	$115	2%	$-	$-			$-	$-	$-	
TOTAL	**$40,739**	**$41,622**	**$884**	**2%**	**$4,575**	**$5,483**	**$908**	**20%**	**$0**	**$0**	**$0**	**$0**
	Milestone 2											
Supplies		$960	$960							$3,000	$3,000	
Travel	$750	$0	-$750	-100%	$1,550	$2,100	$550	35%				
Salaries & Benefits	$13,825	$12,847	-$978	-7%					$6,125	$3,826	-$2,299	-38%
Delivery Costs	$2,186	$2,071	-$115	-5%								
TOTAL	**$16,761**	**$15,878**	**-$883**	**-5%**	**$1,550**	**$2,100**	**$550**	**35%**	**$6,125**	**$6,826**	**$701**	**11%**
Overall	**$57,500**	**$57,500**	**$0**	**0%**	**$6,125**	**$7,583**	**$1,458**	**24%**	**$6,125**	**$6,826**	**$701**	**11%**

Travel by Helleur

Feb 23-25 2016	$1,300
June 27 2016	$800

DBL contributions to project:

- Two computers (1 gift, 1 loan)
- IRGA (gift)
- O2 sensor block (gift)
- CH4 sensor block (gift)
- 8 channel switching system (gift)
- A-D 4 channel system (gift)
- Drill press (loan)