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Article

Methanotrophic bioremediation for the degradation of oceanic methane and chlorinated hydrocarbons

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SUMMARY

Methane (CH_4) has been underrepresented as a contributor to the global warming effect related to carbon dioxide (CO_2). Carbon dioxide is about 200 times as abundant in the atmosphere, however volume alone does not give a clear picture of its environmental impact. Methane is much more immediate in the way it damages the earth as a greenhouse gas, with most of its absorbed heat generated in the first 20 years after emission where carbon dioxide has an environmentally damaging "slow burn" over what typically lasts several hundred years. When methane is removed from the atmosphere, we will see the effect much more immediately, and have a chance to help generations in the much less distant future. The objective of this study was to develop a novel, low-cost biotrickling filter able to degrade methane as well as co-metabolize carcinogenic chlorinated hydrocarbons. While methanotrophic bioremediation has been adapted for *in situ* degradation in freshwater, there has never been an *ex situ* remediation system tailored for the degradation of methane in marine environments. The dynamic and modular biotrickling filter demonstrates the feasibility of methanotrophic bioremediation for the degradation of oceanic methane and chlorinated hydrocarbons in our waterways and oceans.

INTRODUCTION

The levels of chlorinated hydrocarbons have reached an all-time high over the past 20 years due to increased use of pesticides and epoxides (2). The resulting hydrocarbons that runoff is detrimental to life on this planet. Remnants of the chlorinated hydrocarbons have been found in soil, rivers, and coasts across the 50 states, and in fishes in the sea after a decade from the pesticide first use (2). The hydrocarbon that is most worrisome is the trichloroethylene (TCE).

TCE is a volatile chlorinated hydrocarbon that is used as a solvent in industrial manufacturing. TCE is often discharged from factories situated on the beach, where research suggests that exposure to elevated levels of TCE are directly correlated to cases of nervous system malfunction, liver and kidney cancer, non-Hodgkin lymphoma, as well as a myriad of carcinogenic effects also seen in marine life (3). Coral reefs and coastal ecosystems contain over 30% of marine biodiversity and are extremely sensitive to pollutants (4). Eliminating TCE is crucial to protecting biodiversity

Methanotrophs are phylogenetically diverse with two main divisions of bacteria: Gammaproteobacteria (Type I methanotrophs) and Alphaproteobacteria (Type II methanotrophs) that use the RuMp pathway and the serine pathway respectively. *Methylocystis Sp.* is a strain of Type II Methanotrophs that are typically 0.2μ and stain gram-negative. *Methylocystis Sp.* grows optimally in a range of 20-30 °C and 6.8 pH (5).

Through the enzyme Methane Monooxygenase (MMO), methanotrophs can break the C-H bond in methane. Specifically, methanotrophs' sMMO (solute) and pMMO (particulate) enzymes can break down this C-H bond. sMMO and pMMO are found across both Type I and Type II bacteria. Typically, Type I Methanotrophs possessing pMMO genes exhibit minimal TCE degradation in comparison to Type II organisms who possess sMMO (6). This ability gives methanotrophs the unique ability to co-metabolize methane and various other harmful chlorinated hydrocarbons with a C-H bond, including TCE. Applications using methanotrophs, including methanotrophic bioremediation, have been proven to be cost-effective alternative solutions to typical wastewater treatments (7).

Bioremediation is the applied use of microorganisms to consume or break down harmful pollutants to decontaminate a polluted site. There are many subcategories of bioremediation based on either type of degradation or the use of microorganisms. We are using bacterio-remediation through biotrickling in which pollutants trickle down a series of filters inoculated with bacteria. Various methanotrophic bioremediation techniques have been adopted to decontaminate methane contaminated sites. Still, there has never been any *in situ* methanotrophic bioremediation techniques adapted for the degradation of oceanic TCE (7).

The Plug Flow Reactor (PFR) model is a standard idealized system used to understand how chemical reactions occur within systems of cylindrical geometry and a continuous input of reactants. The biotrickling filter is modeled as an idealized PFR to describe pressure, temperature, and substrate concentrations over time. The PFR model assumes no axial dispersion such that substrate concentration is proportional to the amount of volume that the gas passed through (8). The PRF model is the optimal system used to describe gaseous biotrickling filters because no mixing along the column occurs and allows for implementation of a series of differential equations to understand how the geometry of the system

affects degradation rates (9).

This *ex situ* biotrickling system is envisioned to sit on the shore of polluted waterways. A continuous stream of water will flow through the system and allow for the degradation of chlorinated hydrocarbons and methane. Incoming water would be entered into the system as a mist in the same way the Ammonia Salts Medium (AMS) buffer is introduced into the system. Effluent sludge would be collected by the system while clean water would be discharged back into the waterway.

Methylocystis sp. growth-activation on the polyurethane bed of the biotrickling filter was done through methane injections. AMS was cycled throughout the system at a constant flow rate exiting from a humidifier at the top of the column.

When observing the growth of methanotrophs inside of the biotrickling filter, the independent variables were time in which the experiment was conducted and initial methane concentration, the control variables were the temperature, humidity, pH, and nutrient availability, and the dependent variable was the methane concentration read by the gas sensors.

Our research team hypothesized that if we introduced a healthy mixed culture of methanotrophic bacteria into a biotrickling filter of optimal conditions then we would be able to demonstrate methane degradation and suggest the feasibility of chlorinated hydrocarbon degradation. The results of this study showed how the system can yield high methane degradation rates while providing optimal conditions through temperature and humidity monitoring, thus indicating the feasibility of TCE degradation.

RESULTS

We constructed a biotrickling filter with multiple sensors running along the column to measure methane flow **(Figure 1)**. Our team successfully grew mixed cultures of *Methylocystis Sp.* in liquid culture. We characterized the growth of methanotrophic bacteria using various assays. Because our lab was shut down due to the COVID-19 outbreak before our methanotrophs were ready to use, we ran dry bed tests to quantify variables pertaining to gas flow throughout the system **(Table 1)** and solve for a hypothetical degradation rate using mathematical models with previously collected data on our bacteria growth kinematics (9). The goal of degrading methane preceded TCE because methane was easier to obtain, and we believe effective degradation of methane would indicate the feasibility of TCE degradation using the same biotrickling filter (7). Although only a dry test has been performed thus far, mathematical modeling demonstrates that both methane and TCE can be reliably degraded using the biotrickling filter.

Monod Kinetic Growth Equations affect the methane flow throughout the column due to a pulse injection **(Figure 2)**. Although the biotrickling filter was sealed with Flex Seal and healthy precautions were taken to ensure a closed system, the hypothetical degradation rate accounts for possible elements of the system in which gas can escape, an effect which would be amplified by pressure increases such as the one corresponding with the initial start of gas flow to the system. This can be simulated by tweaking the pressure variable to drop on a curve triggered by t=0 in accordance with the geometry of the filter, although a logical consequence of this is that the hypothetical methane concentration dips below zero during this initial period. This choice was made to account for the physical reality of mechanical wear and possible small inconsistencies in the filter, as our column radius was somewhat larger than that described by literature (5), and we felt confident in the benefit because this negative

Parameter	Symbol	Value	Units	Reference
Maximum Specific Growth Rate	$Y_{x/s}$	0.94	d^{-1}	Experimental
Semi-Saturation Constant	K_s	0.43	$g \cdot m^{-3}$	Fitted
Biomass-Substrate Yield	μ	0.23	$g \cdot g^{-1}$	Experimental
Partition Coefficient	H	29.4	-	(5)

Table 1: Plug Flow Reactor Parameters. The Maximum Specific Growth Rate and the Biomass-Substrate were derived from spectrophotometric data at OD_{600}.

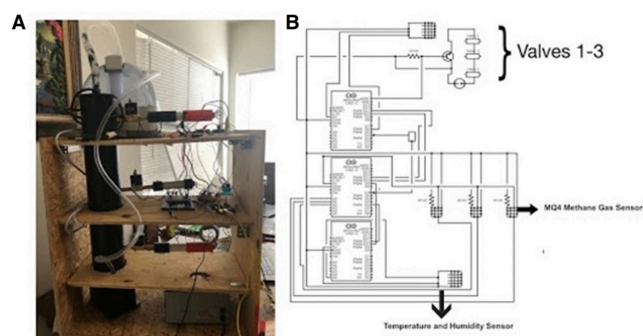

Figure 1: Biotrickling Filter Design. (A) All the microcontrollers are being read from serial ports, and temperature and humidity data can be read independently of methane concentrations. (B) Valves are wired in series to trigger simultaneously, and are rated at 12V DC. The trigger transistor is rated at 33V.

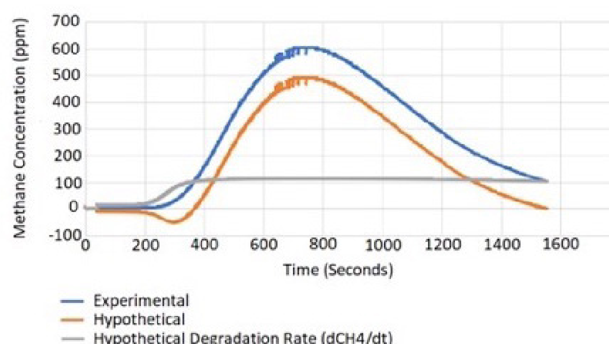

Figure 2: Hypothetical Degradation Results. Using the Monod Growth Kinetic set of ordinary differential equations (4) described in "Differential Equations and C-Curves", parameterized with our biological growth constants in **Table 1**, we solved for our total degraded methane using experimental data.

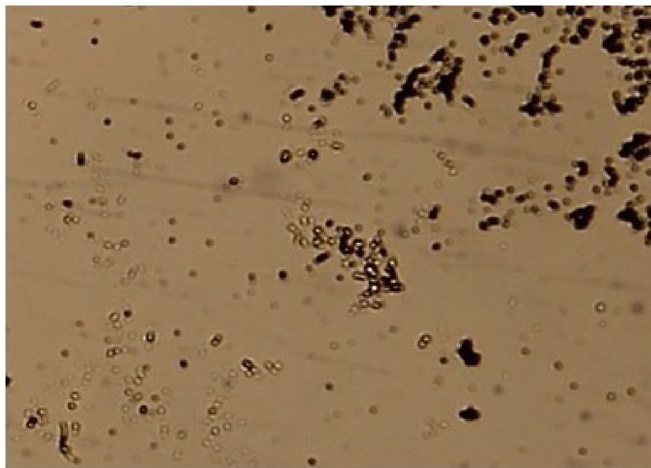

Figure 3: Gram Stain. After two weeks of incubation, a gram stain was performed on Liquid Culture 1. The red hue is indicative of a thin peptidoglycan layer (gram negative) within the bacterial membrane and further confirms our success in growing methanotrophs in mixed culture.

concentration effect would be impossible in an experimental C-Curve, yielding a more valuable comparison of data after the initial accounted pressure drop.

Microscopy Gram Stain

Qualitative data retrieved from the morphic characterizations showed evidence towards the presence of a methanotrophic community. The gram stains on the Liquid Culture 1 **(Figure 3)** and gram stain on pure culture of *Methylocystis sp.* (data not shown), showed an overwhelming similarity with most gram-negative bacteria within the culture. With no observable gram-positive bacteria, we concluded that our liquid culture bacterial populations were majority methanotrophic. Although methanotrophs do not have growth rates as high as many of the bacteria observed within this culture, our microscopic characterizations showed a healthy mixed population with significant indication of methanotrophic cultures, which is ideal since methanotrophs typically grow optimally in mixed culture.

PCR and Gel Electrophoresis

The presence of a healthy methanotrophic culture is confirmed by location of the PCR bands **(Figure 4)**. The length of the pMMO gene present within type II Methanotrophs is approximately 219 bp (10). When run simultaneously with a 1 kbp DNA ladder, the pMMO streak appeared directly below the 250 bp indicator on the ladder. Using densitometry, we calculated a DNA concentration of 120.95 ng/mL.

Sensor Accuracy

One minute of methane was injected into the top of the tank to calculate the tank-to-sensor proportionality constant. Assuming that the max concentration recorded by the sensors in this figure was the actual ppm value inside the tank, it can be concluded that the sensor values in **Figure 5**, needed to simply be multiplied by constant to calculate actual readings in the tank. It was calculated that methane concentrations inside the actual tank were, on average, 66 times larger than the methane concentrations measured by the sensor. The discrepancy between these two values means that much of the methane completed passed through the column before it reached the sensor. Because the sensors extended far outside the body of the biotrickling filter, it took an average of 500 seconds for methane values to reach max value. However, the ppm concentrations in the sensors were

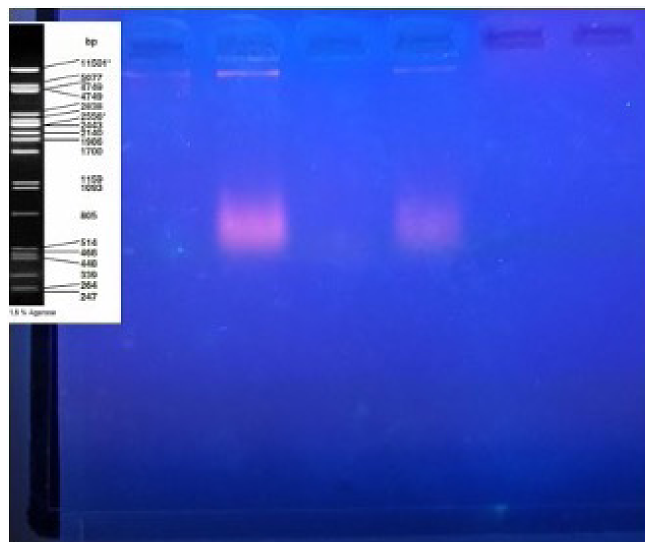

Figure 4: Spectroscopy. Wells from right to left. Well 1: 10kB ladder. Well 2: Pure Culture. Well 3: E-Coli. Well 4: Liquid Culture 1. Gel-Electrophoresis ran on 1.5% agarose gel for 15 minutes at 100v. Band length indicates that pMMO protein was present in both samples. Using densitometry, our Liquid Culture 1 had 70% as much protein concentration as the pure culture.

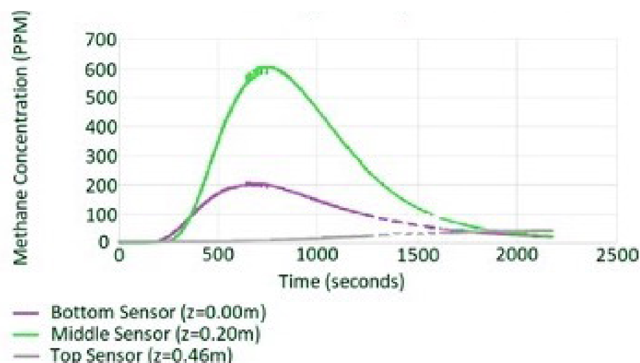

Figure 5: Dry Bed Trial Results. Data was collected from one drybed trail from every MQ4 sensor in the biotrickling filter over a 2200 second monitoring period during which Methane was pumped in from the bottom of the column. Z represents the height from the base of the column where the sensor was placed (Z = 0 is the bottom of the column). The middle sensor accumulated the most methane due to the thick polyurethane that restricted air flow through the column, and the top sensor began to plateau at around t=1000 as gas built the pressure to push through the layers.

directly proportional to the ppm concentrations in the column because as gas flowed through the column it reached the sensors at the same rate at which it travelled through all layers of polyurethane. This proportionality allows our model to also assume that such a rate occurred over the same time interval. Therefore, the time range of the sensors did not need to be interpolated with the actual time range of methane flowing through the column. The area under the C-Curves of sensor readings and actual ppm values were assumed to be directly proportional meaning that Residence Time Distribution (RTD) did not change.

The methane sensor was determined to be accurate under constant gas exposure in the biotrickling filter. Steady-state concentrations varied with a standard deviation of 129.59 ppm/m^3, less than one percent of actual values (data not shown). The sensor's accuracy was, therefore, determined to be within 0.006 % of the actual methane concentration. The MQ-4 sensor has shown precision while constantly exposed to methane showing that prolonged exposure does not increase or decrease voltage across the sensor. Because effective biotrickling filters can degrade methane at rates of 23.32 g CH_4/m^3 * hours (6), such small deviations do detract from overarching trends and are not significant at such high concentrations. Additionally, sensor accuracy is determined to not be affected by the accumulation of rising methane to the top of the column. The volumetric density of methane at one atmosphere did not change considerably because temperatures inside the biotrickling filter varied around 23-38 °C. While the rate at which methane flowed through the column was slowed down by the polyurethane bed when steady state concentrations of methane were reached methane density above and below the columns did not change and did not interfere with the accuracy of recorded data.

Biotrickling Filter Conditions

Our biotrickling filter provides Methanotrophic bacteria with optimal growth conditions. Because fresh ammonia salts medium was cycled into the system, the methanotrophs remained in the acceleration phase of growth, which was calculated using the Monod Growth Kinetic Model (9). In this way, we could ensure methane to be the only limiting substrate in the system, and **Figure 5** shows the readings collected by the middle sensor when we cycled methane throughout the system from an external tank. The PPM levels read by the sensor displayed in this C curve were then mapped onto google spreadsheets, and we used built-in algorithms to create a graph of the degradation rate of our filter based on ODEs (Ordinary Differential Equations) which outline degraded methane in variation to other factors, like methane level and gas flow (8). **Table 1** outlines the parameters of our plug flow reactor and bacteria to give a sense of the scale of the project, and **Table 2** displays the parameters used to solve for the hypothetical degradation rate in **Figure 2**.

Differential Equations and C-Curves

In our PFR, layers of polyurethane act as the bed of the biotrickling filter. As the inlet gas passes through the system, a film is observed with the methanotrophic bacteria on the surface of the foam to degrade the methane. Because TCE is metabolized with methane, the amount of methane degradation will be correlated with TCE degradation; our PFR is able to show the feasibility of degrading TCE through known methane degradation rates.

The Monod Growth Kinematic equation gives the methane degradation rate parameterized with our biological growth constants described in **Table 1** and is given as (4):

$d/(CH_4)/dt = -1/Y_{x/s} * u_{max} * [CH_4]/(K_s + ([CH_4]/H)) * X$.

Figure 2 takes the Monod Growth Kinetics and shows how this hypothetical degradation rate would have affected methane concentrations in the column over time given a set time pulse injection. These results are hypothetical and describe ideal conditions under the given assumptions of this equation.

The effectiveness of our biotrickling filter is confirmed in **Figure 6** which described our system as a set of three ODE's parameterized with the geometry of the filter in **Table 2**. As bacterial growth occurs on the biotrickling filter, air flow through the column is slowed down. As gas is recycled through the bottom of the system, a pressure drop should occur through the system. **Figure 6A** describes the pressure drop that occurs due to bacterial growth. In test trials of our system, our group used pulse injections. **Figure 6C** depicts

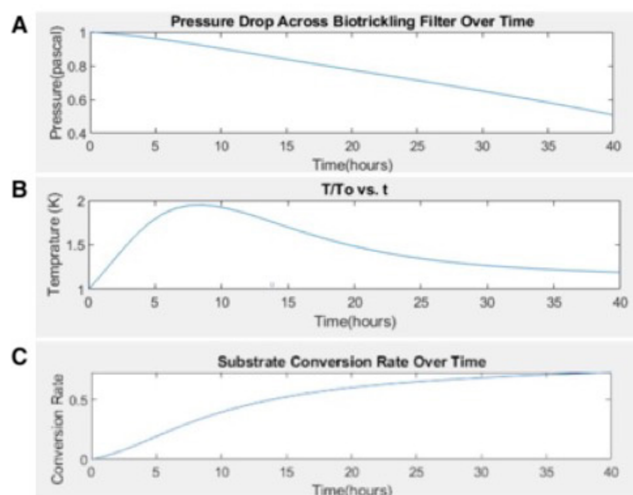

Figure 6: System Conditions over Time. (A) Pressure drop in the system over a 40 hours monitoring period. Biomass accumulation on the bed often restricts airflow and leads to pressure drops in the system. (B) T is the temperature inside the system and To is the inlet temperature; thus, T/T_o describes the temperature changes in the system as a factor of the initial inlet temperature. Temperature increases as the activation energy is overcome and reaction rate increases. As substrate conversion rate reaches an asymptote, the temperature decreases. (C) Under ideal conditions our biotrickling filter accumulates close to 60% of substrate. Substrate conversion rate reaches asymptote as gas flows and leaves the column. Other factors such as pressure drop inhibit growth.

how the total methane oxidized evolves over time with a singular pulse injection. The conversion rate (f(t)) is described as the following logistic regression:
$f(t) = 0.710/(1+6.986e^{(-0.175t)})$ where t is the hours since the pulse injection.

Methane Degradation

Oxidation rates were given by: $E(t) = c(t) / \int_0^t (c(t)\, dt$ (9). Using the idealized C-Curve from the middle sensor in **(Figure 4)**, our biotrickling was determined to have a RTD of 906.4 seconds or just over 15 mins. After integrating the Hypothetical Methane Degradation Rate **(Figure 5)**, over the average Residence Time Distribution RTD (906.4 seconds), it was calculated that our system had the potential to degrade 5.7 ppm/s ± .000342 ppm/s. Multiplying the Monod degradation rate with the sensor to tank proportionality constant derived from the C-Curve in **(Figure 4)**, our group calculated a hypothetical degradation rate of 20856 ppm/hour. The Hypothetical Total Methane Degraded Curve derived closely resembles a logistic regression **(Figure 4)**.

Methane degradation is the key in fighting climate change. Running continuously for one year, our system has the capability to degrade 25.2 kg CH_4. Over the next 100 years, this has the same impact on climate change as removing 705.6 kg (0.7 tons), of carbon dioxide. Over the next 20 years, the more immediate impact is equivalent to 1905.09 kg (2.1 tons) of carbon. Our dynamic and modular biotrickling filter suggests the feasibility of methanotrophic bioremediation for the degradation of oceanic methane and chlorinated hydrocarbons.

DISCUSSION

The ideally homogeneous density of gases flowing through a plug flow reactor will instead vary down the reactor if there is a change in total moles with reaction, or a change in temperature or significant change in pressure, and therefore the correct modelling of these factors is critical in the future of our biotrickling filter maintenance, especially when considering the small ranges of T, P, and pH that are considered optimal conditions for Methanotrophs. **Figure 6B** describes the temperature changes in the system. Because our system was assumed to be a non-isothermal, non-adiabatic plug flow reactor, this graph correctly describes temperature increases associated with exothermic methanotrophic reactions. In **Figure 6C**, the conversion rate (f(t)) is described as the following logistic regression: $f(t) = 0.710/(1+6.986e^{(-0.175t)})$ where t is the hours since the pulse injection. The asymptote of the curve was 0.701, meaning that, under ideal conditions, our biotrickling filter has the capacity to degrade 70.1% of initial methane that enters the system. The conversion rate reaches an asymptote because methane is not stagnant in the system. If the methanotrophic degradation rate was greater than equal to the flow of methane through the column then the conversion rate would be 100%. However, because the degradation rate given under the parameters of the PFR model is less than the flow rate, the conversion rate plateaus as methane exits the system unoxidized.

The Monod Kinetic Growth Equations that we solved were independent of the actual sensor reading yet were applied to the sensor reading data based on how the equation itself evolved with time. Thus, while the methane in the tank was reaching the sensors at a slow rate, the decrease in methane concentration at a given time period, given by the Monod equations, was greater than this increase. This error can be corrected by offsetting the time period in which we started to track the methane concentration in the column by yielding a larger initial ppm concentration at time t=0.

In further biostimulation trials, it is important to change the environmental conditions to favor the prominence of sMMO.

There is a possibility of instrumental error in the readings

Parameter (Plug Flow Reactor)	Symbol	Value	Units	Reference
Inlet Gas Flow Rate	V_0	1	m^3/s	Experimental
Total Reactor Volume	V	0.14	m^3	Experimental
Inlet Gas Density	Rho	0.665	kg/m^3	Experimental
Mass-Average Heat Capacity	Cpm	20	J/kg	(8)
Heat Transfer Coefficient	Ua	60	$J/K/s/m^3$	(5)
Inlet Gas Pressure	P_0	$200e^3$	Pa	(5)
Inlet Temperature	T_0	297	K	(8)
Jacket Temperature	T_j	330	K	Fitted
Dry Bed Residence Time	T_{avg}	906.4	S	Experimental
Heat of Reaction	ΔH	$-10e^3$	J/Mol	(5)
Pressure Drop Constant	Kdp	$1\ e^3$	Pa/m^3	Fitted
Activation Energy	E	$10\ e^3$	J/Mol	(5)
Initial Concentration	$[CH_4]$	0.022	Moles	Experimental

Table 2: Parameters used to solve idealized Plug Flow Reactor equations.

from the MQ4 gas sensors. Winsen electronics rates the MQ4 gas sensors at a minimum of 300 ppm, although it's notable that, experimentally, we observed a very different behavior. When the sensor was tested in a tupperware container flooded with methane and continually drained, readings only begin to amplify in noise and break a smooth decay curve pattern below about 10 ppm, in fact the MQ4 sensors could match known sub-300 ppm concentrations above the same boundary of 10 PPM. While the manufacturer likely increased the claimed minimum ppm to ensure proper functioning, we are confident that our readings above 10 ppm are accurate, as this range of functionality has been confirmed via multiple testing methods.

Our bioremediation using *Methylocystis sp.* via biotrickling system to reduce TCE levels is successful because our data demonstrates methane reduction. We are confident of our methanotrophic isolation since our gram stain and PCR banding pattern all represented correct biosignatures (10).

After the incubation period, gram-stains data indicate a mixed culture growing; however, we are confident that methanotrophs were not outcompeted. The polyurethane provides methanotrophs and other bacteria with a solid medium for growth. Less bacterial growth is seen on cultures with no polyurethane.

Although our system allows for methanotrophic bacteria to metabolize methane in the acceleration phase of growth, gas only remains in the system for an average of the RTD. Thus, the conversation rate of gas should reach a maximum as all the methane passes out of the column.

Methylocystis sp. growth-activation on the polyurethane bed of the biotrickling filter was done through measured methane injections and AMS nutrient cycling throughout the system resulting in the optimal rate of methane degradation. Temperature and humidity were constantly monitored and adjusted if they deviated outside optimal growth conditions described by the following source (12).

The degradation rates of methane are proportional to the substrate concentration within the column, and therefore, with larger injections (i.e.: 5 minutes; 10 minutes; constant), our group hypothesizes that this degradation rate can be significantly increased. Our parameterized differential equations effectively and dynamically model the methane degradation rates within the column and lend themselves to larger-scale systems.

This research can be continued by further examination of how different environmental factors affect methanotrophic growth and MMO expression. Certain trace metals, like copper, catalyze the MMO reaction, thus determining the trace metal concentration before bioremediation. Further additions to the biotrickling filter, such as filtering out effluent water streams, would increase the system's effectiveness.

MATERIALS AND METHODS

Pure Cultures

A pure culture of *Methylocystis sp.*, obtained from the biological culture repository ATCC (American Type Culture Collection), was used to grow liquid cultures (10).

Gram Stains

To characterize the liquid samples collected, our research team followed the standard gram stain protocol outlined by Animal Health Diagnostic Center at Cornell University. Briefly, bacteria smears were heat fixed onto slides, then stained stepwise with crystal violet, iodine, and safarin solutions (12).

Incubation

Cultures were grown as previously published (4). Liquid Cultures were grown in Ammonia Salts Medium (AMS) adjusted to 6.8 pH and were incubated at 20 °C under a 50 - 70 % methane atmosphere. Using an orbital incubator shaker, liquid cultures were shaken at 115 RPM and re-flushed every 3-5 days.

Biotrickling Filter Design

The novel, home-built biotrickling filter described in **Figure 1** is capable of providing optimal growth conditions to methanotrophic bacteria at a low construction cost. The biotrickling filter constructed in this study consists of a hollow 4" diameter PVC cylinder with 20 polyurethane foam layers, each 0.5 ft^2, in its center on which bacteria can grow. The cylinder is 18" tall (represented as the Z axis in **Figure 4**). A wooden shelf constructed around it houses 3 MQ4 gas sensors and an arduino uno microcontroller network which calculates live ppm readings based on a live feed of data, attained from three sampling ports throughout the column. The bottom and top sensors were below and above the layers of polyurethane while the middle sensor was situated halfway through the bed, at the same level as the polyurethane. Our system has three programmable solenoid valves that can be opened on specified time intervals and allow our system to reduce gas dissipation. Humidification of 500 mL of methanotrophic nutrient buffer, AMS (2), at the top of the column allowed greater methane contact throughout the bed and allowed methanotrophs to remain in the acceleration phase of growth. All edges were double flex-sealed. The one trial presented in this study was a pulse injection of methane 1 minute at a flow rate of 0.5 LPM (Liters Per Minute) and monitoring period of 2200 seconds. Protocol development from following sources (9).

Spectrophotometry

5 mL of the soil sample C polyurethane methane dilution (9 seconds) was added to a test tube with 5 mL of NMS medium to achieve a 1:1 dilution. OD_{600} was measured using a spectrophotometer.

PCR and Gel Electrophoresis

We used the previously published *pmo*-DNA primers to amplify DNA fragments from type II Methanotrophs and DNA fragments from all methanotrophs in general so that we could

confirm the existence of these organisms with a given sample and its abundance (10). PCR amplification was done using the following program: 1) 94 °C for 1 minute, 2) 94 °C for 1 minute, 3) OT for 1 minute, 4) 72 °C for 2 minutes, 5) Steps 2-5 were repeated for 50 cycles total, 6) 94 °C for 1 minute, 7) OT for 1 minute, 8) 72 °C for 10 min (4). PCR products were run out on a 1.5% agarose gel with ethidium bromide and run out at 100V for 15 min.

Sensor Network and Programming

The sensor system consists of three tubes spaced vertically on the side of the main chamber, each fed from ¼ inch 12V DC normally closed steel solenoid valves manufactured by US Solid. Each tube contains an MQ4 methane sensor obtained from manufacturer Winsen Electronics, and the top and bottom tubes each contain an AM2030 temperature and humidity sensor obtained from manufacturer Innovative Chemical Technologies. Because the MQ4 methane sensors have an offset in their readings with large changes in humidity or temperature, the AM2303s are used to compensate for the offset in MQ4 readings according to a linear regression outlined by the manufacturer. The AM2303s each have a dedicated microcontroller to avoid necessitating an I2C interface, which would call alternating sensor addresses and require the import of several additional libraries. Each AM2303's microcontroller transmits pulse-width modulation waves to the central controller in real time, which decodes the pulse lengths to receive readings and adjust the methane ppm levels to maintain accuracy. This system was also confirmed experimentally by ensuring the sensors could measure a correct ppm value in a sealed chamber of known methane concentration, which was read by a high-accuracy industrial gas sensor from our school's lab. ppm values were fluctuated with sealed tubes to flood and absorb methane, and we confirmed that our sensors' values fluctuated accordingly with the same readings.

Dry Bed Residence Times

The model equation of the initial breakthrough time with polyurethane (Figure 4) was calculated using the following equation: $c(t) = L / (1+67.663e^{(-.0001622t)})$ where t is time measured in seconds and f(t) represents the concentration of methane in ppm CH_4/m^3. Using this model equation, Residence Time Distribution (RTD), was calculated using equations from an ideal plug flow reactor with an injection time in a specific time increment. Using the model equation, (13), our team was able to determine the specific amount of time that the gas was in contact within the polyurethane.

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