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Living emission abolish filters (LEAFs) for methane mitigation: design and operation

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Abstract

As one of the most potent greenhouse gases, methane is a critical target for the near-term mitigation of global warming. Efficient, scalable, easy-to-implement, and robust mitigation technologies are urgently needed to assist in reaching methane abolishment. The goal of this research was to test the applicability of active, extremophilic methanotrophic cells as a baseline concept for engineered systems aiming at methane capturing. The system, named living emission abolish filters (LEAFs), represents an array of immobilized biomaterials capable of capturing methane directly from vent streams. The biomaterials were made using cells of *Methylotuvimicrobium alcaliphilum* $20Z^R$, a robust halophilic methanotrophic bacterium with the ability to consume methane gas at low concentrations. Several critical parameters were tested, including (i) the composition of the matrix and optimal immobilization to increase catalyst load, (ii) the stability of methanotrophic cells, and (iii) the toxicity of trace gases (i.e. CO). We found that hydrogels coated with 2.3 mg cell dry weight/cm^3 methanotrophic cells represent the best-performing biomaterials. The methane reduction potential of LEAFs fluctuated from 20% to 95% and depended on the methane concentration in the gas stream and the stream flow rates. The potential for commercial-scale deployment and emissions reductions was also evaluated. Total greenhouse gas emissions (combined using the global warming potential GWP_{100}) from an example using a ventilation air methane source over a one-year period was shown to be reduced in two LEAF scenarios by 51% and 75%. Over longer time horizons, more significant reductions are possible as consistent methane consumption can be sustained. The study highlights the overall potential of the liquid-free bio-based composite methane mitigation system. Further improvements essential for system assembly and implementations should include (a) optimization of the cell immobilization protocols to improve cell load and the shelf-life of the system and (b) implementation of matrix moldings for cell immobilization to achieve optimal gas flow and increase the cell-gas interface.

1. Introduction

Greenhouse gases (GHGs), mostly CO_2, methane and nitrous oxide, are the main drivers of climate change. The emission of all GHGs needs to be targeted but reducing methane emissions would bring the biggest benefits in a short-time frame. Methane has a global warming potential (GWP_{100}) 27–30 times stronger than CO_2, meaning that 1 kg of emitted methane has 27–30 more climate effect than 1 kg of CO_2 in a 100 year time frame [1, 2]. Removing atmospheric methane and reducing anthropogenic methane emissions by 30% is one of the ambitious but urgently needed tasks for slowing climate-change progression. Methane emissions come from a variety of sources, ranging from small dairy farms to

large coal mines, challenging the concept of a universal methane mitigation system [3]. Over the last few decades, scientists have been developing various methane-capturing strategies, including several biological solutions [4].

Biology plays an important role in the global methane cycle, contributing to both methane production and methane oxidation [5]. Microbial methane utilization has been found in aerobic and anaerobic environments [5]. Aerobic methanotrophic bacteria, also known as methanotrophs, drive methane oxidation using oxygen-dependent enzymes [6]. Methanotrophs are the commonly used platforms for engineering bio-based systems that aim at methane capturing and oxidation. Most bio-based designs are centered on reinforcing activities of natural microbes toward higher methane fluxes [7]. Efficiency, simplicity in operation, and system cost are among the main challenges remaining.

Immobilization of methanotrophic microbes has been attempted over the last 20 years in order to identify a novel filtration system for methane [4]. Over these past few decades scientists have been trying to determine the most efficient matrix to immobilize methanotrophic bacteria by testing: natural soil, alginate, clay pellets, fly ash ceramsite, perlite compost, concrete, coal, compost, polyethylene rings, activated carbon, plastic bio-balls, gravel, sponge, wood chips, bark mulch, and glass fiber filters [8–17]. The design of most of these filtration systems typically includes immobilized cells on a specified substrate with mixed gas entering from one side and flowing through the system to exit through the opposite side. Methane-consuming bacteria in these filtration models are supplied with liquid mineral media continuously, typically using a nutrient recycling pump that has temperature regulation and the ability for sludge removal. The general idea for such biofiltration systems is to capture methane emissions and convert them into CO_2, with minimal biomass production to prevent the system from bio-clogging. However, keeping microbes in an active methane utilization stage with limited biomass production is challenging and often results in an unstable system.

Microbial methane oxidation provides cell carbon, energy, and water release by two pathways:

Q1. $CH_4 + 2O_2 = CO_2 + 2H_2O$ (oxidation—energy)

Q2. $CH_4 + O_2 = CH_2O + H_2O$ (assimilation—biomass).

The ratio between the pathways will differ in response to nutrient availability or environmental conditions (e.g. pH/salinity/temperature or oxidative stress). About 50% of the methane consumed is oxidized. Environmental stressors (high or low pH, salinity, and temperature) will shift the methane flux toward oxidation for energy generation. Theoretical predictions suggest that active methane consumption could supply up to 80% of the cellular water requirements in microbial species isolated from environments with low water availability. This research aimed to construct and assess a novel design, showcased in figure 1, named living emission abolish filters (LEAFs) for eliminating methane from coal mine ventilation without the need for outside intervention. The objective for the design of LEAFs was to eliminate the need for external nutrient supplementation, temperature regulation, and sludge removal which has been critical for previous models. The methanotrophic microbe used for testing LEAFs was *Methylotuvimicrobium alcaliphilum* (*M. alcaliphilum*) $20Z^R$, chosen for its robustness in thriving at high salinities and pH levels. These qualities were sought after due to the unique ability of LEAF's halophilic methanotrophs to sustain dryness, while preserving high methane oxidation rates. The ability of immobilized cells to stay active at solid-gas interphase enables construction of a simple cartridge-like system, which does not require nutrient recycling or water addition. The elimination of the aquatic phase has potential of improving gas exchange in the system because there is no gas-to-liquid transfer.

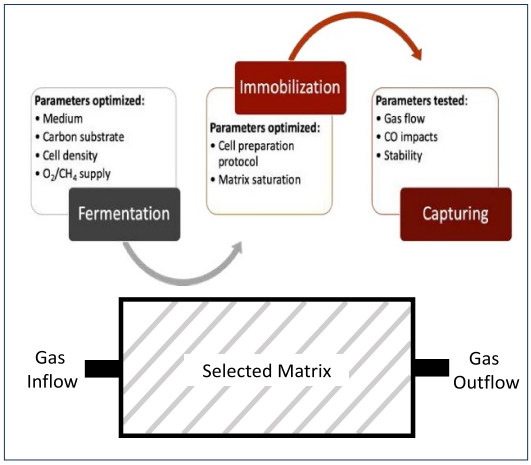

Figure 1. Parameters optimized for efficient LEAFs operation with the basic design of the system without any external manipulations needed.

2. Results

2.1. Immobilization matrix selection

For all immobilization tests, cell cultures of *M. alcaliphilum* $20Z^R$ were prepared using BioFlo/CelliGen 115 (3.0 l vessel) or BioFlo 120 (7.5 l vessel) fermenters following previously published protocols [18]. The following immobilization matrixes were tested: vegetable cellulose (sponge), premium glass, Whatman 17 CHR filter paper, alginate beads, super absorbent polymer (SAP, or hydrogels) (figure 2(A)).

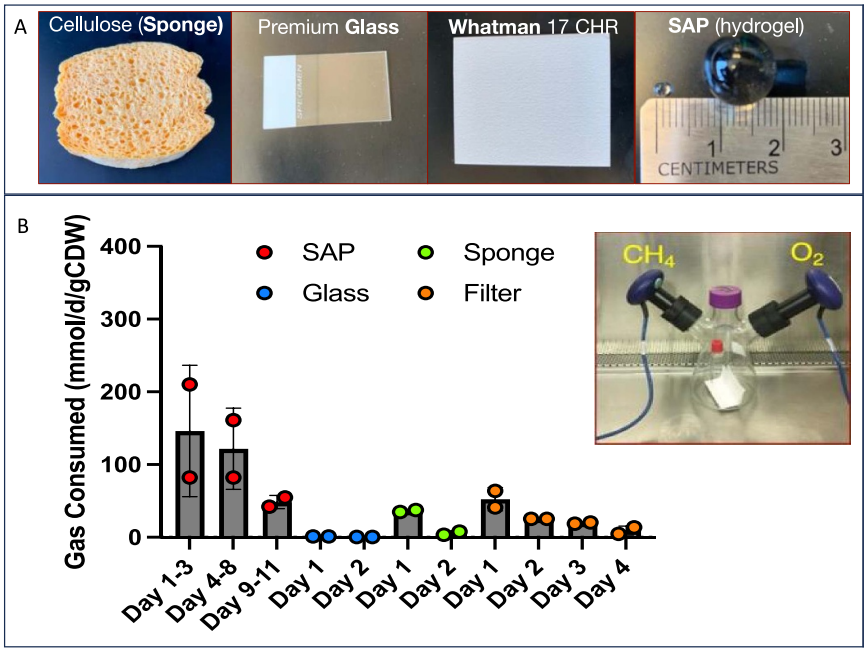

Figure 2. Immobilization matrix selection. (A) Matrixes tested for immobilization studies. (B) Gas consumption results from each matrix tested. (C) Scanning electron micrographs of cells immobilized on SAP.

After cell seeding, the matrices were placed into a sealed chamber connected to methane and oxygen blue-sense sensors to record the consumption rates. We collected gas consumption rates daily (glass, filter paper, and sponge) or every other day (SAP) and represented them as an average for each matrix. Methane consumption rates (i.e. activity) and duration of the active stage (i.e. stability) varied between matrices as shown in figure 2(B). All consumption data was normalized by gram cell dry weight (gCDW) which is a calculation previously identified to be 0.34 gCDW in 1 l of cells with an optical density (OD_{600}) of 1. Very poor activity (~0.5–1 mmol CH_4 $d^{-1}gCDW^{-1}$) and stability (up to 2 d) were observed for cells immobilized on glass. Cells immobilized on more absorbent substrates (sponge and filter paper) consumed methane for 2–4 d with the highest consumption at 63 mmol CH_4 $d^{-1}gCDW^{-1}$on day 1 and 25 mmol CH_4 $d^{-1}gCDW^{-1}$ on day 4. Cells imbedded onto the SAP made of hydrogel displayed the highest methane consumption rates, 31–155 mmol CH_4 $d^{-1}gCDW^{-1}$ for 7–11 d on average in a closed system. To further evaluate cell adhesion to the surface of the SAP matrix, several beads from the SAP matrix were removed from the experiment with immobilized *M. alcaliphilum* $20Z^R$ cells were removed from the experiment for use in scanning electron microscopy (SEM). After fixing and mounting the SAP matrix, micrographs were captured of its surface, gradually increasing magnification from top to bottom (figure 2(C)). These micrographs illustrated that cells were localized on the matrix's surface and possessed the characteristic morphology of rod-shaped *M. alcaliphilum* $20Z^R$ cells. When the SAP-beads were in the sealed chamber there was a decrease in CH_4 and O_2 consumption rates with every new gas addition and it was hypothesized this was due to cells becoming dormant once CH_4 and O_2 were limited in the system. Overall, these data suggested that hydrogels were the best matrix for immobilizing methanotrophic cells.

2.2. Mathematical model

The model used for the column in the LEAFs design employs a matrix of loosely packed hydrogel spheres seeded with *M. alcaliphilum* $20Z^R$ cells. Based on this matrix preparation, the simple mathematical model uses one basic building block: a 7 mm sphere (measured with a ruler) coated by a monolayer of methanotrophs. The predicted behavior of any assemblage of such spheres is then obtained by multiplying the expected uptake from a single sphere by the number of spheres.

At a diameter of 7 mm, each sphere has a surface area of $4\pi r^2 = 1.5$ cm^2. Estimating the cellular size to be about 2 μm^3 (1 μm width, 2 μm height, and 1 μm height), each cell (~8 μm^2 total surface area) covers about 4 μm^2 of surface area. It follows that a monolayer will need about 1.5 $cm^2/4$ $\mu m^2 = 38 \times 10^6$ cells /sphere

At a volume of about 8 μm^3/cell, and using a density of 1 g cm^{-3}, the calculated cell weight is 0.3 mg cell wet weight (CWW) per sphere or 0.06 mg CDW, assuming that CDW is 20%–30% of CWW. A model was then constructed to investigate correlation between cell load per sphere, sphere diameter and expected methane consumption for a 1 m^3 unit (figure 3). It was estimated that a 1 m^3 unit will require 0.7 kg cell biomass and can enable methane

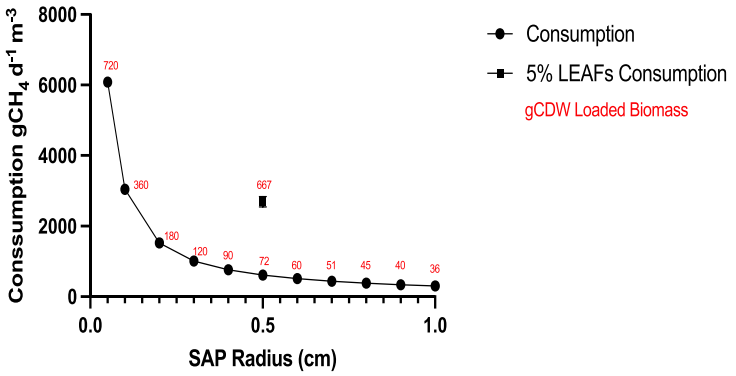

Figure 3. Expected biomass load (in red, g) and methane consumption (o) as a function of SAP bead size. LEAF Unit pilot size 1 m^3. A monolayer of cells was used to calculate cell biomass. The square indicates cell load applied to 0.5 cm SAP beads in this study (scaled to one m^3).

mitigation at the rate 6 kg CH_4 /d. Considering that methanotrophic cell biomass can be produced at the cost \$1.7–5/kg [19, 20], the system may offer an attractive mitigation platform and, thus, it was investigated further.

2.3. Gas consumption tests: flow system set-up

Once the immobilization matrix was defined, we transitioned from the sealed chamber to a continuous gas flow system to further validate feasibility of the dry fermentation concept for methane mitigation. The continuous flow system was set up with either a MCQ GB100 Series gas mixer to mix CH_4 and air, or a custom compressed gas mixture (Airgas) to provide gases into the unit at a given flow rate. Once the gas was mixed to a specified concentration, it was fed through BlueSens sensors and sent into the 120 cm^3 unit from the bottom. The gas would travel up through the matrix and exit through to top of the unit into tubing that fed into BlueSens gas sensors to measure gas consumption data.

The initial cell immobilization protocols were set up as described above. Typically, one gram of dry SAP was mixed with 10 ml of mineral media containing 80 mg of CDW. Once the SAP beads were coated with methanotrophic cells, they were transitioned to a continuous flow model, with both methane and oxygen consumption rates calculated over time.

2.3.1. Mini-units

The next step was to determine methane consumption rates at a range of gas flows and methane concentrations. The first model tested, shown in figure 4, is a 120 cm^3 benchtop unit. Methane consumption was tested at gas flow rates of 0.64–5.14 l hr^{-1} with methane concentrations at 1%, 3% or 5%, i.e. levels mimicking typical outputs of vented/emitted gases from coal mines, landfills, and husbandry (250–50 000 ppm) [13, 21–25]. Methane consumption was tested on units with 16 gCDW total of biomass supplied across 5 mini units in tandem. A variety of gas flow rates were tested starting with a flow rate of 0.64 l hr^{-1} and up to 5.14 l hr^{-1}. Units in this setup were run for 3 weeks testing each methane concentration and flow rate twice a week for replication of data.

The first methane concentration tested was 1% which was a 50/50 mixture from a gas tank of 2% CH_4 with air. At the flow rate 0.64 l hr^{-1}, more than half of the supplied methane (68 ± 18%) methane was consumed (figure 4(B)). When the flow rate was increased to 5.14 l hr^{-1}, 20 ± 8% methane was consumed. The methane consumption rate reached 14 ± 6.8 g hr m^{-3} which was estimated to be the maximum potential for the cell biomass in the system.

The next experiment conducted was with 3% methane which was a mixture of 3% pure methane and 97% air. At a flow rate of 0.64 l hr^{-1}, 89 ± 2% methane was consumed, with methane reduction potential of 24 ± 0.3 g hr m^{-3} (figure 4(C)). When the flow rate was increased to 5.14 l hr^{-1}, the methane consumption dropped to 34 ± 0.6%, however the methane reduction potential reached 73 ± 2.7 g hr m^{-3}.

The last methane concentration tested was at 5% methane which was a mixture of 5% pure methane with 95% air. At a flow rate of 0.64 l hr^{-1}, 95 ± 2% methane was consumed with methane reduction potential of 41 ± 3 g hr m^{-3} (figure 4(D)). When the flow was increased to 5.14 l hr^{-1}, the methane consumption dropped to 34 ± 4%, however the methane reduction potential reached 119 ± 15 g hr m^{-3}. These tests verified the potential for high methane consumption rates at high gas flow rates without the loss of efficiency.

2.3.2. System stability tests

The next task was to test the viability and activity of the immobilized cells over time without the addition of new nutrients, moisture, or temperature regulation. To evaluate the consumption of methane over time we left the units with mixed gas flowing through them and measured the rate of consumption every several days. BlueSens methane and oxygen sensors

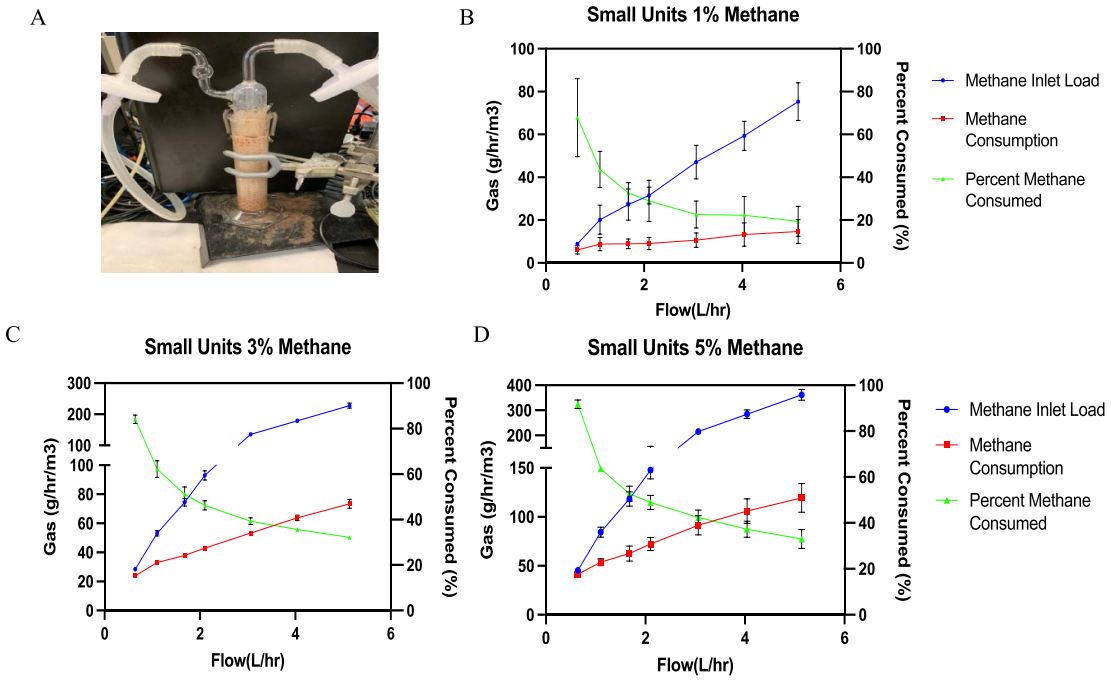

Figure 4. Testing methane consumption of 120 cm^3 at two different methane inlet concentrations with a variety of flow rates. (A) Methane was provided to the system at a concentration of 3% mixed with air. (B) Methane was provided to the system at a concentration of 5.5% mixed with air.

were used to measure the inflow and outflow concentrations of methane running through the system. The gas flow rate remained constant at a rate of 0.5 l hr^{-1} controlled by a mass flow controller. Methane consumption was tested in units with 24 gCDW total of biomass supplied across 5 mini units in tandem. The overall impact of the LEAFs technology depends on the system's stability over prolonged periods of time. Two duplicated units were set up at the beginning of the project for continuous data collection. After the initial set-up, the consumption increased by 25%; however, it dropped after 2 months of continuous operation. After four months one unit displayed 65% of its original consumption, while the second remained at 100%. On average, this represents 83% of the original consumption. The consumption fluctuated from 54% to 70% depending on the concentration of methane in the gas stream and represented on average 53 ± 19% with 5% CH_4 and 77± 9% with CH_4 supplied as 1% of the gas stream (flow rate of 100 SLPH/m^2) (figure 5).

2.3.3. Carbon monoxide toxicity tests

Methane emissions from coal mines or natural gas wells often come with <0.001% CO. While methanotrophs are capable of CO oxidation, CO can inhibit methane oxidation. To test its impact of CO on LEAFs, a gas mixture similar to coal mine gas was prepared and a series of tests were carried out in batch and gas flow experiments. Batch culture experiments were conducted on *M. alcaliphilum* $20Z^R$ cells grown in 25 ml NMS media with either just methane or with methane and carbon monoxide. A gas tank was purchased with carbon monoxide (0.009%), methane (0.5%), carbon dioxide (1.5%), oxygen (22.75%), and nitrogen (balance). Specified gases were injected into sealed bottles containing 25 ml of cultures through septum lids. The results from growing batch cultures in the presence of CO indicated no negative impacts, while there were positive impacts of CO on final optical density which went from 0.93 ± 0.04–1.30 ± 0.06 (figure 6). However, experiments with immobilized cells produced the opposite outcome, and in general CO spikes to the gas stream resulted in the reduction of CH_4 consumption. Furthermore, the impact of CO depended on the methane concentration in the gas stream (figure 5). In a gas stream with 5% methane, the consumption was reduced by 38%; while a 26% reduction was observed in a gas stream of 1% methane.

2.4. Scale up

To test the viability of scaling up the system to fit the parameters of the coal mine, a larger unit of 1840 cm^3 (shown in figure 7(A)) was tested at flow rates ranging from 0.6 l hr^{-1} to 28 l hr^{-1}. The system was tested at 3 methane inlet concentrations (3%, 1% and 0.1%). To mix each concentration either 3% pure methane was mixed with 97% air or a purchased 2% methane tank was mixed with air 50/50 for 1% methane and 5/95 for 0.1% methane. The concentrations were mixed with a MCQ GB100 Series gas mixer. Biomass was grown in a BioFlo 120 (7.5 l vessel) bioreactor to obtain sufficient biomass to fill the 1840 cm^3 unit. To

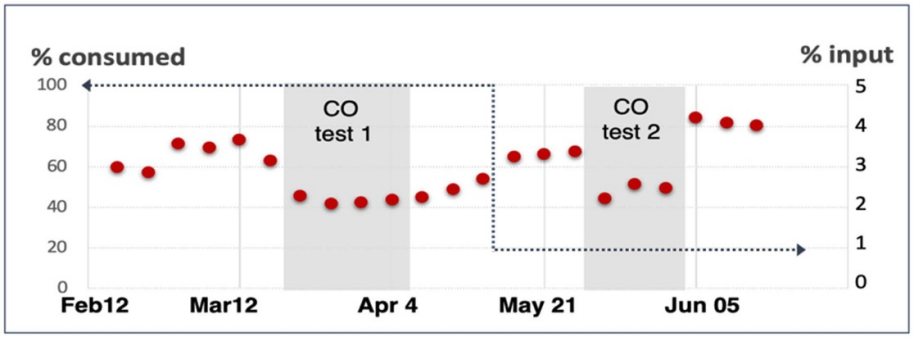

Figure 5. Consumption rates of 5 tandem mini-units over time. Consumption rates were taken every several days using blue sense sensors. The percent methane consumption was calculated by taking the inflow and outflow measurements, then subtracting the two.

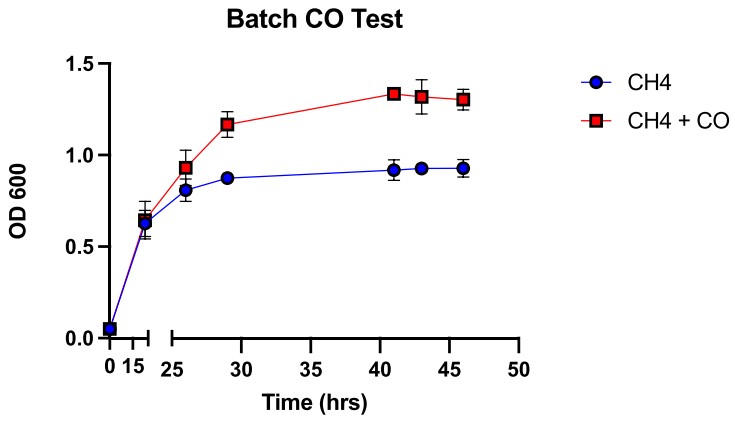

Figure 6. Growth curves of *M .alcaliphilum* $20Z^R$ grown in the presence of methane (0.5%) and carbon monoxide (0.009%).

fill the larger unit 100 g of SAP was added with 1.26 l of 2xNMS containing 6 gCDW of biomass. The scale up system was only tested once with standard deviation given by the LGR gas chromatography system.

The first test was with the methane concentration set at 0.1%, a low methane inlet load, comparable to typical mine-venting targets and landfill gas concentrations (demonstrated in figure 7(B)). At a flow rate of 0.6 l hr^{-1} there was 37.8 ± 1% of methane being consumed. The highest methane consumption at 0.1% was at a flow rate of 13 l hr^{-1} with a methane consumption rate of 0.61 ± 0.02 g hr m^{-3} which was 17.5 ± 0.6% of the inlet load.

The methane concentration was then increased to 1% resulting in higher methane inlet concentrations allowing for higher methane saturation throughout the system (figure 7(C)). The highest methane percent consumption at 1% was at a flow rate of 2.2 l hr^{-1} with 64.1 ± 0.1% being consumed at a rate of 8.5 ± 0.01 g hr m^{-3}. This consumption rate was improved when the flow was increased to 6.18 l hr^{-1} resulting in a consumption rate of 14.6 ± 0.02 g h rm^{-3} which was 36.2 ± 0.04% of the inlet load.

Lastly, we tested the system at a 3% inlet methane concentration. Figure 7(D) demonstrates that at a flow of 2.1 l hr^{-1}, methane was being consumed at a rate of 18.6 ± 0.1 g hr m^{-3} which was 57.7 ± 0.3% of the inlet load. When the flow was increased to 14.6 l hr^{-1} the unit consumed 18.2 ± 0.3% of the inlet load at a methane reduction rate of 53.3 ± 0.5 g hr m^{-3}. Overall, the larger unit consumed similar fractions of the inlet methane at lower gas flow rates similar to the data derived from the benchtop units. When the gas flow was increased the units consumed a lower fraction of the inlet load but at a higher rate per hour. These data exemplify that the unit can be scaled up from 120 cm^3 to 1840 cm^3 with comparable results.

2.5. LCA

In order to examine the potential for LEAF reactors to reduce overall greenhouse gas emissions from a ventilation air methane (VAM) stream, a life cycle assessment was carried out using VAM parameters. The business-as-usual (BAU) scenario for VAM release into the atmosphere resulted in GHG emissions of 7.92×10^8 kg CO_{2eq}, according to the GWP_{100} impact assessment method, as a large quantity of methane-containing gas was assumed to be released into the atmosphere with no methane removal. In Scenario 2, which represented a commercial-scale design based

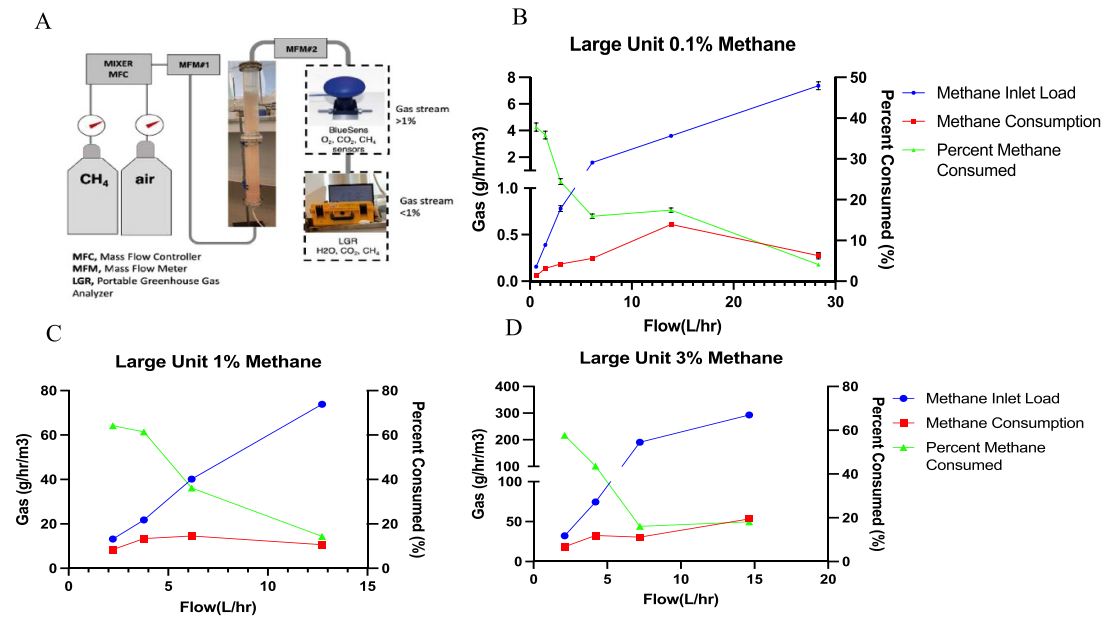

Figure 7. Methane consumption was tested using the larger 1840 cm^3 at 3 different methane inlet concentrations 0.1%, 1%, 3%. (A) Diagram of system setup. (B) Methane consumption at 0.1% inlet concentrations. (C) Methane consumption at 1% inlet concentrations. (D) Methane consumption at 3% inlet concentrations.

Table 1. Initial LCA results for coal mine VAM scenarios (kg CO_{2eq}) for 1 year of operation, for a model coal mine scenario with a VAM flow rate of 200 m^3 s^{-1}.

Scenario	Scenario 1	Scenario 2	Scenario 3
Scenario brief	BAU—uncontrolled emissions	1 large LEAFs reactor	5 smaller LEAFs reactors in tandem
Reactor media inputs	—	4.48×10^7	5.86×10^6
Supplemental power	—	3.82×10^6	3.82×10^6
Uncontrolled VAM emissions	7.92×10^8 [a]	—	—
Residual emissions from the reactor	—	3.32×10^8	1.88×10^8
Total GWP_{100}	$\mathbf{7.92 \times 10^8}$	$\mathbf{3.81 \times 10^8}$	$\mathbf{1.98 \times 10^8}$
% Reduction from BAU	—	**51.9%**	**75.0%**

[a] There is no reactor used in the BAU scenario with uncontrolled emissions, this is just what comes out of the uncontrolled VAM gas stream. A GWP_{100} value of 29.8 is used for methane, as suggested by the IPCC 2021 100 year method.

on the single large LEAF reactor, total GHG emissions reductions represented a 51% reduction from the BAU. The GWP_{100} impacts of providing reactor media and utilities was more than overcome by the benefits associated with methane consumption in the reactor, by roughly a factor of 10X (table 1). SAP solid support media was the most impactful input to this process, which was surprising to see. A fairly conservative assumption was made that SAP beads also needed to be completely re-supplied every 6 months for continued LEAF reactor operations, even though the SAP is not likely to degrade quickly in the ambient reactor conditions. If the solid SAP support material can be recovered and used in successive cycles, extending its useful life, emissions from this material should be proportionally lower than they are in this work. In Scenario 3, representing a series of 5 smaller LEAF reactors similar to the performance of the 120 cm^3 reactors, where methane conversion reached 84%, the overall greenhouse gas emission reduction over a year of operation compared to the BAU scenario was 75%, after the impacts of LEAF reactor inputs were included into the LCA study.

3. Discussion

To evaluate LEAF's efficiency, we compared them to previous biofiltration systems (supplementary materials, table S1). The table was integrated from a methane biofiltration review paper with our results transformed to the same units of gCH_4 m^{-3} d^{-1} [4]. The data from LEAFs can be compared to all other systems by size (120 cm^3, 5 tandem 120 cm^3, and 1840 cm^3), gas flow rates (0.64–28 l hr^{-1}), and methane inlet loads (3.7–8967 gCH_4 m^{-3} d^{-1}) [25–64]. Each previous filtration unit was tested for different applications and conditions with either new nutrients continually being added to decrease dryness, culture enrichment, temperature regulation, or cell debris removal. In contrast, LEAFs can operate for >5 months without

any additional supplementation and yet show the highest methane reduction potential.

Here we evaluate LEAFs as a prototype of a cheap, simple, scalable, cartridge-like systems for capturing methane. The system does not require water, eliminating challenges associated with the limitations of gas-to-liquid transfer, and thus, LEAFs could represent a transformative solution for microbial methane utilization. Operational parameters of LEAFs are expected to be similar to techniques applied for air purification technologies. LEAF units have potential to be applied to a variety of sources with high to low methane inlet concentration loads at a variety of flow rates. The system has yet to be tested at atmospheric methane concentrations of 1.9 ppm (∼0.0002%), but a recent study has emerged demonstrating that in liquid culture *Methylotuvimicrobium buryatense* 5GB1 was able to grow and consume methane at 200 ppm (∼0.02%) exhibiting the possibilities of using methanotrophic bacteria to mitigate atmospheric methane [65].

Key experimental parameters developed during the study were integrated into a life cycle assessment study to further evaluate the applicability of the technology for large scale deployment at methane-emission sources, such as coal mines or cattle barns. Significant greenhouse gas emission reductions are predicted to be possible, even with current versions of the technology, if deployed to oxidize methane from coal mine ventilation gas sources. A typical emissions scenario for coal mine ventilation air was used as a test case, with a measured methane concentration of 0.65% and a gas flow rate of 200 m^3 s^{-1}. Three scenarios were evaluated where the VAM was either allowed to be released into the atmosphere with no controls, or was routed through commercial scale LEAFs reflecting the design and performance of either the single large 1840 cm^3 reactor design or the series of 5 smaller 120 cm^3 reactors. Nutrient requirements were modeled after the media broth used in lab scale designs, and other inputs were developed in consultation with mining industry contacts. Greenhouse gas emissions from an example VAM source over a one-year period was shown to be reduced in two LEAF scenarios by 51% and 75%. Over longer time horizons, more significant reductions are possible, as consistent methane consumption can be sustained.

The study also highlights a set of additional optimizations, including cell preparations and immobilization. All data generated for this report are based on matrixes prepared immediately after fermentation and biomass separation. However, we recognize that an efficient procedure for catalyst preservation (alone or imbedded into matrix) remains to be solved for scale-up applications. Additional variables that still need to be examined are increasing the maximum cell load of the units, creating a protocol to print 3D constructs to decrease pressure build up, and increasing the porosity of the system to allow for an increase in the number of cells that come into contact with methane.

4. Materials and methods

4.1. Strain and growth media

M. alcalipilum $20Z^R$ is a methanotrophic strain isolated from the surface sediment of the highly alkaline soda lake Shara-Nur, Russia. Lab cultures were grown using the nitrate minimal salts (NMSs) 3% NaCl in batch or bioreactors as previously described [60]. The fermenter parameters were set to an agitation of 500 rpm, temperature of 30 °C, pH of 9.0, and a gas flow of 0.2 l hr^{-1} (3% methane and 97% air). To start the fermenter 100 ml (25 ml cultures) of inoculum in exponential phase of growth (∼0.4 OD) were added to the 7.5 l fermenter and samples were collected daily for optical density measurements. Once the optical density (600 nm) reached ∼4–5 (6.8–8.5 gCDW) to obtain adequate biomass for immobilization, 5 l of media was collected through the outflow port and centrifuged in 500 ml bottles at 4700 g for 30 mins.

4.2. Biomass immobilization

Biomass that was centrifuged from the fermenters was suspended in 2x NMS media in preparation for immobilization. This allowed for the biomass to be concentrated down to a lesser volume. For each unit a ratio of one gram of sterilized dry matrix (SAP) with a surface area of 1.5 cm^2 per sphere was mixed with 10 ml of 2x NMS media containing ∼0.08 gCDW of cells. Each unit would receive 6 g dry SAP with a total of 60 ml 2xNMS media containing ∼0.48 gCDW. The sponge matrix with surface area of 245 cm^2 received 60 ml of 2x NMS media containing ∼0.48 gCDW. The filter matrix with a surface area 63 cm^3 had to receive a higher concentration of cells due to having less absorption (10 ml of 2xNMS with ∼0.48 gCDW). The glass matrix had no absorptive properties with a surface area of 18.75 cm^2 so once the biomass was centrifuged the pellet was directly added to glass matrix at ∼0.10 gCDW per slide. All matrices and units for gas consumption were sterilized for 45 mins in an autoclave at 121 °C. Biomass was then added to each matrix in a biological hood and placed into each sealed chamber to keep sterile. The matrix set up phase is approximately 2 h for the filter paper, glass, and the sponge. Preparation of SAP takes about 6–12 h for mini and large units respectively. The moisture content was measured using an OHAUS MB120 Moisture analyzer in accordance with the standard protocol provided by the manufacturer. The initial moisture content of the SAP matrix was 88.2 ± 0.3%.

4.3. Scanning electron microscopy (SEM)

The images for SEM were taken following protocols previously described [66]. Cells immobilized on SAP were fixed in 2% glutaraldehyde and 0.5%–1% osmium tetroxide in 0.1 M cacodylate buffer for an

Table 2. Methane mitigation potential of LEAFs.

	Experimental set-up volume	Packing material	Inlet load ($gCH_4\ m^{-3}\ d^{-1}$)	Load consumed ($gCH_4\ m^{-3}\ d^{-1}$)	Maximum CH_4 removal	Reference
1	120 $cm^{3:}$ 3% CH_4	LEAFs	777 6548	376 648	48% 10%	This Study
2	120 $cm^{3:}$ 5.5% CH_4	LEAFs	1648 8967	1068 2588	64.8% 28.8%	This Study
3	600 $cm^{3:}$ 5 tandem units (1% CH_4)	LEAFs	788.4	663.1	84%	This Study
4	600 $cm^{3:}$ 5 tandem units (5% CH_4)	LEAFs	3942	2792.5	70.4%	This Study
5	1840 cm^3: 0.1% CH_4	LEAFs	3.7 176.7	1.38 6.6	37% 3%	This Study
6	1840 cm^3: 1% CH_4	LEAFs	316.2 1772.7	203.0 255.1	64% 14.4%	This Study
7	1840 cm^3: 3% CH_4	LEAFs	772.6 7032.6	445.7 1279.8	57.7% 18.2%	This Study
8[a]	166 897 cm^3	Compost; recycling paper pellets; mixture of compost and paper pellets, mixture of wood chips and compost	631	567	96%	[38]
9[a]	30 788 cm^3	Landfill cover soil and earth worm cast	6134	6134	100%	[25]

[a] Methane reduction potential of engineered systems. Only heights rates are presented. Additional comparisons are provided in supplementary materials (table S1).

hour on ice. The SAP was gently washed and then dehydrated through an ethanol series (50%, 70%, 95%, and 100%) for 5 mins in each concentration. Ethanol was removed through CO_2 critical point drying and SAPs were mounted and coated for SEM imaging.

4.4. Gas parameters

The gas flow and gas mixtures were controlled with a MCQ GB100 Series gas mixer. The mixer can be bought from MCQ instruments (MCQinst.com) that can provide low flow rates between 0.5–500 m min^{-1} per channel. The mixer was set up to either mix straight methane with air for the higher percent mixes (3% and 5% CH_4) or mix air with a methane tank with either a 2% CH_4 concentration or 0.5% CH_4 concentration for the 1% and 0.1% CH_4 concentrations, respectively. To measure gas input and output concentrations, BlueSens methane and oxygen sensors were attached before and after each unit. Three sets of BlueSens sensors- O_2, CO_2, and CH_4 (bluesens.com) detect corresponding gases with an accuracy less than 0.2% within each recorded reading. To measure gas consumption at 0.1% (1000 ppm) methane, a Los Gatos Research (LGR-ICOS GLA132-GGA) gas analyzer was attached to measure methane input and output. The LGR uses laser absorption spectroscopy and has accuracy to measure methane down to 0.2 ppb. The LGR system can be purchased at new.abb.com.

4.5. Life cycle assessment (LCA)

The LCA study was conducted in accordance with ISO guidelines [67]. A model VAM gas stream being emitted over a time period of 1 year was used to represent the methane control system boundary of interest, using parameters from Su *et al* [68] of a VAM stream examined in Australia, with a methane concentration of <0.65% (vol.) and a VAM flow rate of 200 $m^3\ s^{-1}$. In the business-as-usual (BAU) scenario, uncontrolled release of this VAM gas stream was assumed to occur for the one-year time horizon. In Scenario 2, a commercial-scale LEAF reactor was assumed to be used to control methane research with performance characteristics similar to those in Row 6 of table 2, the 1840 cm^3 unit capable of oxidizing 64% of input methane into carbon dioxide at 316.2 g m^{-3}day. These reactor performance metrics were scaled to size a reactor capable of handling the methane flow assumed in this study. Scenario 3 represented a different LEAF reactor configuration analogous to row 3 of table 2, where 5 smaller LEAFs reactors in series were capable of oxidizing 84% of a higher-volume methane stream at 1% CH_4, even though the total reactor volume was ∼3X less than the larger LEAF reactor used as a model for Scenario 2. Nutrient media was added with a composition identical to that described in section 4.1, with 800 kg water and 70 kg poly(methyl methacrylate) media for SAP beads used per m^3 of reactor volume. Reactor contents were assumed to be completely recharged every 6 months, to maintain the growth conditions

for the bacteria. In addition to reactor media, some supplemental utilities were provided to the system to account for continued VAM system performance. Based on consultations with a colleague in the coal mining industry, it was assumed that power requirements for maintaining equivalent VAM gas flow would be increased by 10% to account for the minor VAM gas obstruction presented by the LEAF reactor, adding 5.4 million kWh of electricity use per year in each scenario. Estimated power consumption was calculated by taking known VAM gas flow power consumption from a coal mine within the same region, and linearly scaling to the match the flow rate used in this study. No supplemental heating or cooling was assumed to be provided to the LEAF reactor, assuming that VAM gas temperature was sufficient to meet the needs of the reactor. In different parts of the world, using different methane gas sources, this assumption may need to be revisited with additional LCA work. LCA modeling was performed in SimaPro, version 9.4, using items from the ecoinvent V3 database [69] used to represent inputs to the study. The ecoinvent profile for medium-voltage Australian electricity was updated with the grid mix from 2022, with an emissions impact of 708 g CO_{2eq}/ kWh [70]. Global Warming Potential was assessed for each scenario using the IPCC 2021 100 year GWP method, where methane has a GWP value of 29.8.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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Conflict of interest

The authors declare that they have no known competing interest.

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