

CAPTURE OF METHANE BY FUNGI: EVIDENCE FROM LABORATORY-SCALE BIOFILTER AND CHROMATOGRAPHIC ISOTHERM STUDIES

J. P. Oliver, J. S. Schilling



ABSTRACT. Livestock production accounts for a third of total U.S. anthropogenic methane (CH_4) emissions. Mitigating these greenhouse gas (GHG) emissions is central to efforts aimed at curbing near-term climate change, but low-cost, practical technologies are needed to reduce fugitive CH_4 from farms. Biofilters have mitigation potential, but current designs for odor are limited in their ability to capture CH_4 . Fungi have been shown to improve capture of hard-to-target gases in other biofilter applications and were investigated here for their ability to capture CH_4 . Using a lab-scale biofilter, several fungal species were shown to improve CH_4 capture compared to a bacterial system and sterile control. A subsequent experiment with *Pleurotus ostreatus* found capture to increase with increasing levels of fungal biomass. Using a chromatographic isotherm, the ability of fungal materials to sorb CH_4 was confirmed, and again greater sorption of CH_4 was possible when fungal biomass was increased. These results demonstrate the ability of fungi to capture CH_4 and warrant their investigation as a way to improve the CH_4 mitigation capacity of livestock emission biofilters.

Keywords. Biofilter, Chromatographic isotherm, Fungi, Methane, *Pleurotus ostreatus*, Sorption, Wood chips.

Livestock production is the largest global source of anthropogenic CH_4 (Jacobson et al., 2011; Steinfeld et al., 2006). In the U.S., animal farms and their wastes are also the primary source, responsible for a third of CH_4 releases with upward trends since 1990 (USEPA, 2015). Recent scientific (Montzka et al., 2011; Shindell et al., 2012) and government reports (UNEP, 2011; USDS, 2010; White House, 2014) all highlight the importance of mitigating agricultural CH_4 emissions. These documents target CH_4 for its relatively short atmospheric life (~10 years) and potency (~25 times the warming potential of CO_2), which make its reduction an effective strategy for mitigating near-term climate change. With this growing awareness and political interest, livestock production facilities in the U.S. and elsewhere will likely see increased regulation of their GHG emissions (Pratt et al., 2013). This creates an urgent need for cost-effective mitigation solutions that are practical for use on farms.

Biofiltration is a viable option for reducing CH_4 from livestock operations and is used already for farm odor (Chen and Hoff, 2009) and for CH_4 from landfills (Menard et al., 2012), petroleum systems (Venugopal et al., 2004), and coal mines (Limbri et al., 2014). Using diverse microbial biofilms to capture and degrade pollutants, biofilters can effectively treat effluents with mixed and low-concentration gases (Chen et al., 2009), including CH_4 levels too dilute to recover for energy generation (Limbri et al., 2014; Menard et al., 2012; Venugopal et al., 2004). This makes biofilters uniquely suitable for treating fugitive farm CH_4 that cannot be reduced by improved animal diet or controlled by manure digestion and biogas systems.

The main challenge limiting biofiltration of the CH_4 emitted from livestock facilities is the mass transfer of CH_4 from the gas phase to the active biofilm. This rate-limiting adsorption of CH_4 by the extracellular matrix of wet biofilms and direct adsorption of CH_4 to cells is subsequently referred to as capture. Once captured, CH_4 can follow biofilm concentration gradients to cells capable of oxidizing the compound. While CH_4 is successfully captured from long-residence, passive-flow effluents, such as landfills, typical barn and manure storage exhausts are mechanically emitted at high velocity. The short residence times sufficient for capturing and oxidizing soluble odors (i.e., NH_3 , H_2S) (Chen and Hoff, 2009) are too short for capturing hydrophobic CH_4 (Melse and Van der Werf, 2005; Nikiema and Heitz, 2009). As large-volume biofilters are not cost-effective for use on farms (Melse and Van der Werf, 2005; Montes et al., 2013; Streese and Stegmann, 2005), or the use of specialized liquid phase sorbents (Estrada et al., 2014; Kennelly et al., 2014; Ramirez et al., 2012), other

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CH₄ capture improvements must be developed.

Capture inefficiencies in landfill and mine biofilters have been improved by optimizing aerobic CH₄-oxidizing bacterial communities (methanotrophs) (Gebert et al., 2004; Kallistova et al., 2007; Lee et al., 2009; Murrell and Radajewski, 2000; Yoon et al., 2009; Yuan et al., 2009) based on their responses to media conditions (Nikiema et al., 2005; Rose et al., 2012), CH₄, and other pollutants (Kim et al., 2013; Menard et al., 2014; Ni et al., 2012). Non-methanotrophic biofilm community members have received less attention, as well as the ecological feedbacks that dictate CH₄ capture and methanotroph performance in livestock emission biofilters. Regarding methanotrophs, it is known that unlike passive-flow biofilters, where methanotrophs colonize thin bands between counter-gradients of O₂ and CH₄, methanotroph communities in forced-air biofilters are dispersed by unidirectional flows of O₂, CH₄ (Gebert et al., 2004; Yuan et al., 2009), and other volatiles (Ni et al., 2012). This dispersal alters the ratio of type I and II methanotrophs (separated by ultrastructure and methane monooxygenase (MMO) differences; Chen et al., 2007), which impacts CH₄ oxidation. It is also known that NH₃ and H₂S can suppress MMO and reduce CH₄ biofiltration performance (Caceres et al., 2014; Veillette et al., 2011) and may need to be pre-filtered. What is not well understood, however, is how microbial communities other than methanotrophs may impact CH₄ capture.

Microbial populations can enhance biofilter capture of select emissions. Although not yet harnessed for livestock emissions, fungal-dominant biofilters have been shown to improve capture of various industrial emitted hydrophobic gases (Cox et al., 1997; Estevez et al., 2005; Jorio et al., 2009; Kennes and Veiga, 2004; Prenafeta-Boldú et al., 2012; Rene et al., 2012; Spigno et al., 2003; van Groenestijn, 2001). The ability of fungi to improve capture of these gases relates in part to their unique filamentous growths (hyphae), which increase the effective surface area of the biofilter media. Secreted hydrophobic proteins (hydrophobins) coat these fungal hyphae, lowering surface tension at the aqueous-gas interphase of the biofilm and enabling the hyphae to extend into the air stream (Wosten, 2001; Wosten et al., 1999). When cultivated in the presence of hydrophobic vol-

atile organic compounds, fungal biomass increases in hydrophobicity as well as surface area, further increasing gas capture potential (Vergara-Fernandez et al., 2006). Not only this response but also the hydrophobin sheath of the fungal hyphae itself may be involved in the sorption of hydrophobic gases (Rene et al., 2012).

Some fungi have been screened for their ability to enhance the biofiltration of CH₄ (Girard et al., 2011), and recent efforts have shown that fungi can co-metabolically degrade CH₄ and methanol in a biofilter environment (Lebrero et al., 2016). However, the ability of fungi to capture CH₄ from fast-moving effluents has not been explored, although mass transfer of CH₄ from the gas phase to the biofilm is considered rate-limiting in biofiltration systems used on farms. If fungi can be harnessed to enhance capture of CH₄ from livestock facilities (as adsorptions on fungal hyphae and absorption into the biofilm, not as oxidation or elimination), then biofilters may be developed at low-cost to meet the anticipated GHG mitigation needs of farmers. The goal of this research was to test the potential ability of fungal biomass to improve CH₄ capture (sorption). We hypothesized that fungal-inoculated biofilter media treating manure storage emissions would capture more CH₄ than sterile media or media inoculated with a bacterial consortium. We also hypothesized that by increasing the amount of fungal biomass (in a biofilter and isotherm sorption column), more CH₄ could be captured from a CH₄ spiked effluent. Specifically, our objectives were to (1) use a lab-scale biofilter operated at short residence time to test the ability of fungal inocula to improve capture of CH₄ from a manure storage effluent, (2) use the multi-stage design of this lab-scale biofilter to test the effect of increased fungal inoculum on CH₄ capture, and (3) develop and use a chromatographic (flow-through) isotherm to evaluate the CH₄ sorption capacity of fungal cells.

METHODS

LAB-SCALE BIOFILTER

To investigate the influence of fungi on CH₄ capture under typical biofilter operational conditions, a lab-scale biofilter system was constructed (fig. 1). This system consisted

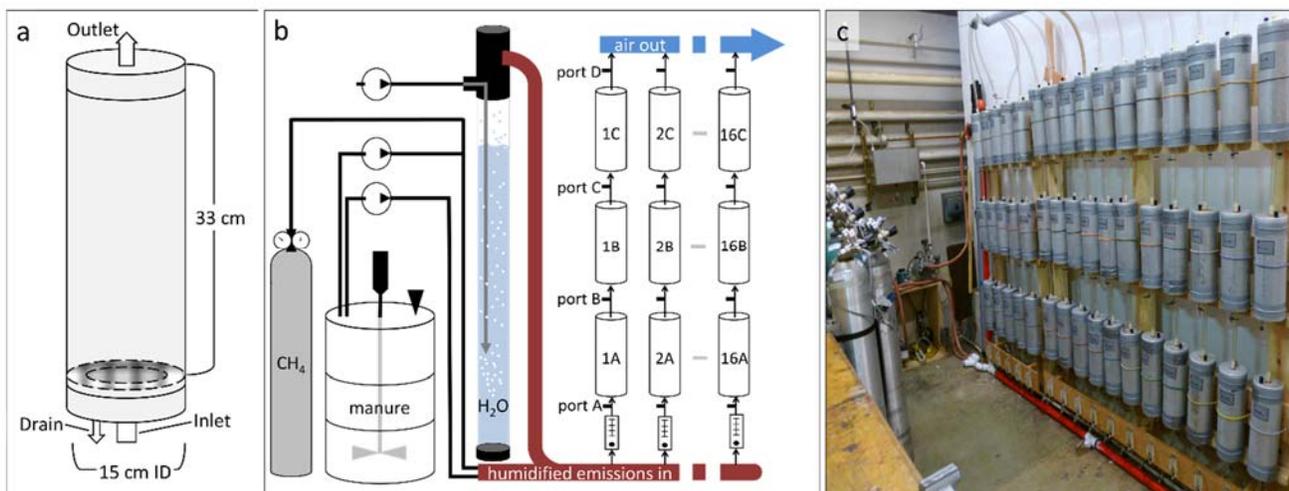


Figure 1. Schematics of (a) single biofilter column and (b) lab-scale biofilter assembly, and (c) a photograph of the installed system.

of 16 triplicate aluminum columns (0.33 m × 0.12 m ID) fitted tightly with aluminum pipe caps and connected in series with 13 mm ID tubing that fit inlet and outlet fittings on the caps. An aluminum ring (12 cm OD, 7 cm ID, 2 mm thick) was installed in the bottom of each column to prevent bypass flow and to position the media support screen. The columns were designed to be large enough for realistic bulk media but small enough for replication and autoclaving. Assembling the columns in triplicate enabled staging of different microbial treatments.

The biofilter columns were fed emissions from either the headspace of a 200 L anaerobic swine manure storage or CH₄ cylinder gas diluted with air. Vacuum pumps moved these emissions and a humidified air stream into a mixing chamber packed with glass wool and then into the inlet manifold. This mixing ensured that consistent effluent emissions were delivered to all columns. Flowmeters regulated flow to achieve a 25 s empty bed contact time (calculated by the methods of Schmidt et al., 2004). While longer residence times are used for CH₄ biofilters on passive-flow landfill covers (Sadasivam and Reddy, 2014), this rate is representative of the biofilter exhaust requirements of livestock facilities (Schmidt et al., 2004) in which fungi may help overcome CH₄ capture rate limits. Exhaust emissions were plumbed into a fume hood. A peristaltic pump was used to aseptically add 100 mL of sterile water to each column daily to maintain ~50% (wet basis) media moisture content. To assess treatment performance, inlet and outlet CH₄ emissions were measured using a multi-gas monitor (Innova model 1412, LumaSense Technologies, Ballerup, Denmark), and calibration cylinder gas was used to set measurement correction factors.

SCREENING THE POTENTIAL OF FUNGI TO IMPROVE CH₄ BIOFILTRATION

The first experiment compared three fungal inocula against a bacterial consortium and a sterile control. For this, all triplicate columns were packed with either: (1) *Pleurotus ostreatus* (Grey Dove, Field and Forest Products, Peshtigo, Wisc.), (2) *Phanerochaete chrysosporium* (No. 24725, American Type Culture Collection, Manassas, Va.), (3) *Dichostereum effuscatum* (field isolate), (4) a bacterial consortium (mix of field isolates), or (5) sterile chips ($n = 3$). All selected fungi use the white rot decay mechanism, which has demonstrated utility in other bioremediation systems (Pointing, 2001). Fungi, including those using the white rot decay mechanism, have shown some potential to improve the biofiltration of hydrophobic volatiles (Zamir et al. 2011) and methane (Girard et al., 2011; Lebrero et al., 2016). Additionally, *P. ostreatus* was selected because its spawn is widely available, *P. chrysosporium* was selected because it has unique thermophilic and asexual spore production capabilities that make it well-suited for growth in mulch, and *D. effuscatum* was selected because it dominated a large area of a full-scale biofilter treating swine emissions.

The fungal culture, *D. effuscatum*, was directly isolated from heavily colonized biofilter media (SE biofilter, Morris, Minn.; Janni et al., 2014) using Basidiomycete selective agar, with its identity verified using molecular methods. In brief, DNA was extracted from pure culture fungal hyphae

using the Extract-N-Amp Plant PCR Kit (Sigma-Aldrich, St. Louis, Mo.) with reagent volumes scaled down 10-fold. ITS-1 and ITS-4 primers were used to amplify fungal DNA. The DNA template was Sanger sequenced at the University of Minnesota Genomics Center (St. Paul, Minn.), and the sequence was compared to the Basic Local Alignment Search Tool (BLAST) database (National Center for Biotechnology Information, Bethesda, Md.). The isolate shared 99% sequence similarity with other *D. effuscatum* submissions.

The bacterial consortium was a mix of bacterial cultures isolated from biofilter media (SE and NE biofilters, Morris, Minn.; Janni et al., 2014) and purified from fungi using BBL Trypticase Soy Agar (Becton, Dickinson and Co., Sparks, Md.) amended with the antifungal cycloheximide (50 µg mL⁻¹, Sigma-Aldrich, St. Louis, Mo.). The isolates that comprised the bacterial consortium were not identified to species, but the presence of methanotrophs in the consortium was confirmed by PCR using methanotroph-specific primer sets (data not shown).

To prepare the treatments, birch (*Betula papyrifera*) wood chips (0.5 to 2.5 cm) were soaked for 24 h in tap water, drained, packed into autoclavable polypropylene culture bags (UG-080519-A, Unicorn Bags, Plano, Tex.), and sterilized (110 kPa, 121°C, 2 h, twice, 24 h apart). Cooled bags were inoculated with cultures, sealed, and incubated for four weeks. Colonized media was homogenized and aseptically packed into sterilized biofilter columns, and the columns were plumbed to the inlet and outlet manifolds. The anaerobic manure storage headspace was used as the emission source, with target CH₄ emissions between 25 and 75 ppm; emissions averaged 39.2 ± 21.9 ppm as measured by the multi-gas monitor (Innova model 1412, LumaSense Technologies, Ballerup, Denmark). The CH₄ emissions from this system and their fluctuations are similar to those observed in the field for a swine manure storage in Minnesota (Janni et al. 2014). The experiment was run for seven weeks (weeks 1 to 4: normal emissions; week 5: simulated shutdown period; and weeks 6 and 7: normal emissions) at room temperature (~22°C).

EFFECT OF FUNGAL BIOMASS ON CH₄ BIOFILTRATION

The second experiment tested the effects of fungal inoculum ratios on CH₄ capture using *Pleurotus ostreatus* (Grey Dove). Four treatments were tested: (1) all three biofilter stages were packed with sterilized media, (2) the top stage was packed with *P. ostreatus* and the bottom two stages were sterilized media, (3) the top two stages were packed with *P. ostreatus* and the bottom stage was sterile chips, and (4) all four stages were packed with *P. ostreatus* ($n = 4$). Inocula were prepared as above. For the emissions source, purified CH₄ was diluted with air to achieve an inlet gas concentrations of ~100 ppm; emissions averaged 105.2 ± 9.6 ppm as measured by the multi-gas monitor (Innova model 1412, LumaSense Technologies, Ballerup, Denmark). The experiment was run for four weeks at room temperature (~22°C).

CHROMATOGRAPHIC ISOTHERM TEST OF CH₄ SORPTION BY FUNGAL MATERIALS

To test the influence of microbial materials on CH₄ sorp-

tion, a flow-through chromatographic isotherm approach was used (fig. 2), similar to the experimental setup used by Goss (1992) to study vapor partitioning in soils. The isotherm test column consisted of a 10 cm × 1.6 cm ID stainless steel tube capped at both ends with 2 mm thick, 0.5 μm pore stainless steel fritted metal disks. Stainless steel compression fittings secured the disks in place, enabled quick exchanges of test media, and were fitted with denatured capillary gas chromatography (GC) columns (0.320 mm × 60 cm, Agilent Technologies, Santa Clara, Cal.). In an up-flow orientation, the test column was plumbed to the injection port and FID detector in the oven of a gas chromatograph (GC-2010 Plus, Shimadzu Corp., Kyoto, Japan). An inert carrier gas was used (He), and CH₄ injections (10 μL, 100 ppm) were made with a gas-tight syringe fitted with a Chaney adapter (Hamilton Co., Reno, Nev.). Tests were run under dry conditions, the GC oven was maintained near ambient (25°C), and the carrier gas flow rate was set to achieve a 25 s EBCT.

Ultrapure sand (40 to 100 mesh, muffle furnace to remove any residual organics) was used as inert control media, while fungal materials were homogenized with sand in various ratios for the treatments. Fungal spores were used for their uniformity and, like fungal hyphae, are known to have hydrophobic surface properties (Sulc et al., 2009; Tucker and Talbot, 2001). Spores representing both major fungal phyla (Basidiomycota and Ascomycota) were tested. *Puccinia triticina* (mixed wild type, USDA Cereal Rust Laboratory, St. Paul, Minn.) urediniospores were used as test Basidiomycota spores, and *Aspergillus niger* (No. 16888, American Type Culture Collection, Manassas, Va.) conidiospores were used as test Ascomycota spores. Homogenization of fungal materials was done gravimetrically and visualized using scanning electron microscopy (SEM). For SEM, sand and spore samples were mounted onto an SEM stub, sputter coated, and imaged on a variable-pressure SEM (S3500N, Hitachi Ltd., Chiyoda, Tokyo, Japan) at the University of Minnesota Imaging Center.

Unlike batch isotherm studies, the flow-through design of this system better models actual biofilter dynamics. Further-

more, by retrofitting a GC, abiotic conditions were tightly controllable, and the detection was real-time and highly sensitive. Under isothermic conditions, the observed CH₄ retention is a measure of its sorption patterns. By comparing the sorption patterns of a treatment to the sorption patterns of the control, the ability of fungal materials to sorb CH₄ can be tested (i.e., smaller peak areas with treatments compared to controls suggest that CH₄ is sorbed to the fungal material being tested).

STATISTICAL ANALYSES

All statistical analyses were conducted with R 3.1.3 (R Core Team, 2015). For the lab-scale biofilter tests, normality was verified by Shapiro-Wilk testing, and ANOVA tests were used to compare differences in CH₄ capture for each time point independently. For isotherm tests, due to outlier points, normal distribution was not verified by a Shapiro-Wilk test, and Kruskal-Wallis was used to test for differences in mean peak areas. Tukey HSD was used post-hoc for all tests to explore differences in CH₄ capture and peak area means. The alpha level was set to 0.05 for all analyses.

RESULTS

ABILITY OF FUNGAL BIOFILTERS TO MITIGATE CH₄

Greater capture of CH₄ was achieved by fungal inoculated columns than by the bacterial or sterile control treatments, which closely tracked one another (fig. 3 and table 1). *Pleurotus ostreatus* was the top-performing fungal treatment, capable of capturing >50 ppm CH₄ early in the experiment. Performances of the fungal treatments were typically higher early in the experiment, although significant CH₄ (~30 ppm) was captured by both *P. ostreatus* and *P. chrysosporium* at the end of seven weeks.

The ability of fungal treatments to capture CH₄ was greatest during anaerobic manure storage CH₄ shock emissions (rising significantly from an earlier inlet measurement) and following the shutdown period (fig. 3 and table 1). When the performances of the various treatments were compared dur-

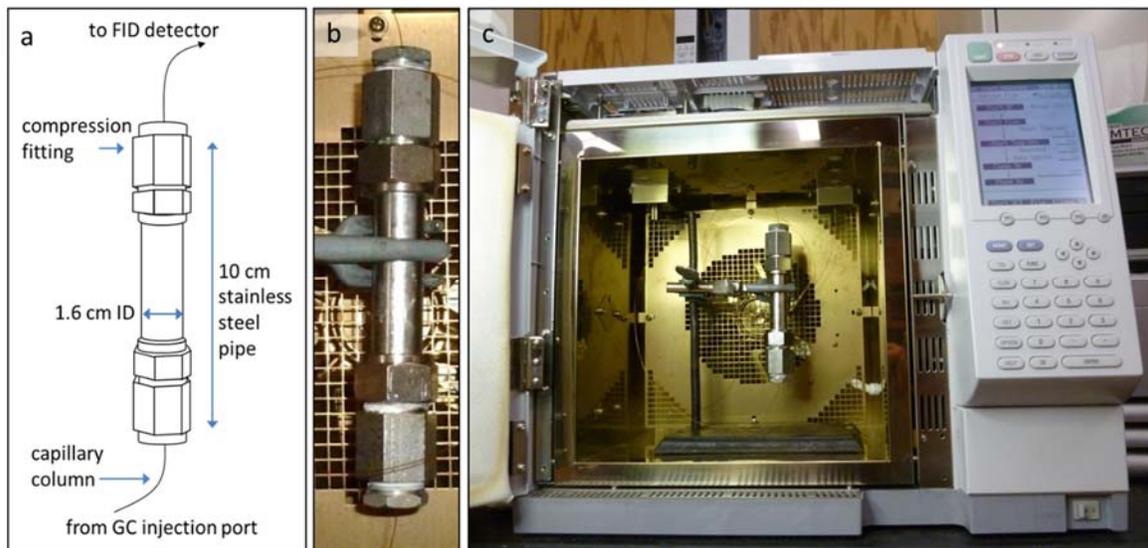


Figure 2. (a) Schematic of the isotherm column and photographs of (b) the column and (c) the column installed into the GC.

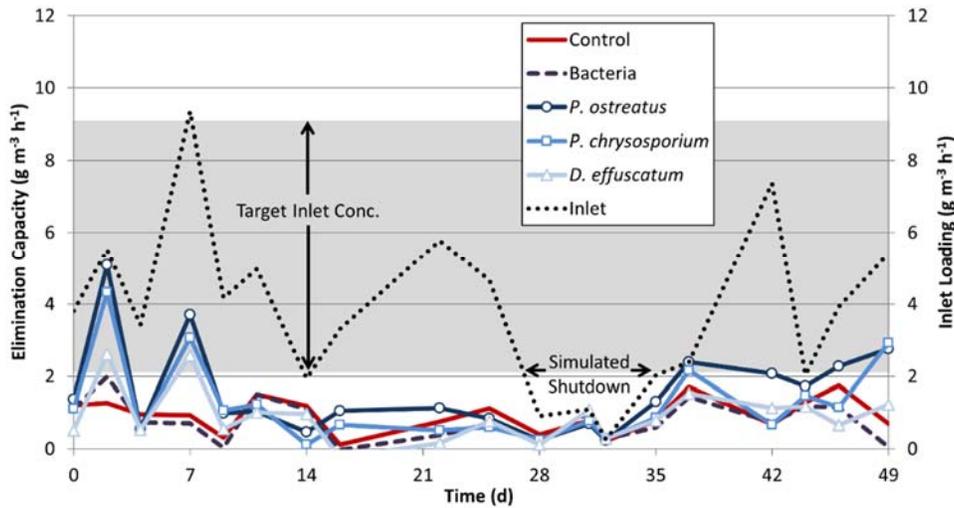


Figure 3. Capture of manure storage CH₄ by biofilter columns with various microbial inocula. Elimination capacity is the mass of CH₄ captured per biofilter media volume per unit of time. Inlet loading is the mass of CH₄ entering the biofilter media volume per unit of time. Inlet concentrations were 20 to 100 ppm CH₄. The flow rate was 1.46 m³ h⁻¹.

Table 1. Methane elimination capacity of treatments (three fungal inocula, a bacterial consortium inoculum, and a sterile control). For each treatment, the mean elimination capacity (and standard deviation) are presented. ANOVA was used to test for treatment differences, and Tukey HSD was used post-hoc to test for differences in mean elimination capacity between treatments. Different letters denote statistically significant differences in the mean elimination capacities ($n = 3$).

Time (d)	Inlet Loading (g m ⁻³ h ⁻¹)	Elimination Capacity (g m ⁻³ h ⁻¹)				
		<i>P. ostreatus</i>	<i>P. chrysosporium</i>	<i>D. effuscatum</i>	Bacteria	Control
0	3.81	1.37 (0.03) a	1.11 (0.16) a	0.51 (0.12) b	1.11 (0.28) a	1.2 (0.1) a
2	5.52	5.09 (0.84) a	4.33 (0.96) ab	2.61 (0.53) bc	1.98 (0.37) bc	1.3 (0.3) c
4	3.43	0.55 (0.09) ab	0.51 (0.25) a	0.53 (0.13) a	0.74 (0.14) ab	1.0 (0.1) b
7	9.35	3.70 (0.28) a	3.06 (0.72) a	2.56 (0.14) ab	0.71 (1.41) b	0.9 (0.4) b
9	4.18	0.99 (0.31) a	1.06 (0.28) a	0.55 (0.01) b	0.03 (0.63) b	0.3 (0.1) ab
11	4.97	1.03 (0.17) a	1.23 (0.55) a	1.01 (0.56) a	1.49 (0.19) a	1.5 (0.3) a
14	1.94	0.47 (0.25) a	0.12 (0.28) ab	0.96 (0.06) ac	1.10 (0.06) c	1.2 (0.2) c
16	3.31	1.05 (0.23) a	0.67 (0.29) ab	-0.24 (0.17) c	-0.03 (0.19) bc	0.1 (0.4) bc
22	5.77	1.12 (0.08) a	0.51 (0.14) bc	0.16 (0.08) c	0.37 (0.31) bc	0.7 (0.3) ab
25	4.66	0.84 (0.08) ab	0.60 (0.13) bc	0.77 (0.17) ab	0.70 (0.21) b	1.1 (0.1) a
28	0.90	0.24 (0.14) ab	0.24 (0.06) ab	0.12 (0.07) b	0.18 (0.05) ab	0.4 (0.1) a
31	1.09	0.70 (0.02) a	0.77 (0.28) a	1.08 (0.05) a	1.03 (0.13) a	0.8 (0.0) a
32	0.26	0.23 (0.05) ab	0.28 (0.01) ab	0.29 (0.00) a	0.28 (0.00) ab	0.2 (0.0) b
35	2.02	1.30 (0.30) a	0.86 (0.11) ab	0.79 (0.03) b	0.59 (0.12) b	0.8 (0.1) b
37	2.39	2.39 (0.00) a	2.16 (0.19) ab	1.52 (0.45) bc	1.44 (0.25) c	1.7 (0.2) abc
42	7.37	2.08 (0.46) a	0.67 (0.48) b	1.13 (0.49) ab	0.73 (0.36) b	0.7 (0.3) b
44	2.03	1.73 (0.12) a	1.47 (0.12) a	1.17 (0.35) a	1.17 (0.13) a	1.3 (0.2) a
46	3.92	2.27 (0.31) a	1.14 (0.49) bc	0.66 (0.16) c	1.15 (0.16) bc	1.7 (0.3) ab
49	5.41	2.75 (0.44) ac	2.92 (0.43) a	1.23 (1.40) ab	0.05 (0.79) b	0.7 (0.3) bc

ing these shock emission events, *P. ostreatus* and often the other fungal treatments had the greatest ability to capture CH₄ (table 2). Only at a few shock emission events was the ability to capture CH₄ shared with the bacterial treatment or sterile control.

INCREASED *P. OSTREATUS* BIOMASS IMPROVED CH₄ BIOFILTRATION

Generally, with increased fungal biomass (inclusion of more fungal inoculated stages) CH₄ capture increased (fig. 4 and table 3). While capture for sterile chips stayed below 6 ppm and averaged 3.3 ±2.0 ppm, capture when all three

Table 2. Statistical differences in CH₄ capture of microbial treatments during shock emissions. Different letters denote statistically different means.

Time (d)	Inlet Concentration (ppm)	Shock Amplitude ^[a] (ppm)	Treatment				
			<i>P. ostreatus</i>	<i>P. chrysosporium</i>	<i>D. effuscatum</i>	Bacteria	Control
2	58.39	18.12	a	ab	bc	bc	c
7	98.86	62.64	a	a	ab	b	b
16	34.99	14.47	a	ab	c	bc	bc
22	60.99	40.47	a	bc	c	bc	ab
35	21.40	18.68	a	ab	b	b	b
37	25.26	22.54	a	ab	bc	c	abc
42	77.94	75.22	a	b	ab	b	b
46	41.45	19.94	a	bc	c	bc	ab
49	57.17	15.72	ac	a	ab	b	bc

^[a] Shock amplitude is the maximum intensity of the inlet CH₄ concentration measured from the position of the previous lowest inlet CH₄ concentration. Only shocks greater than 10 ppm are included.

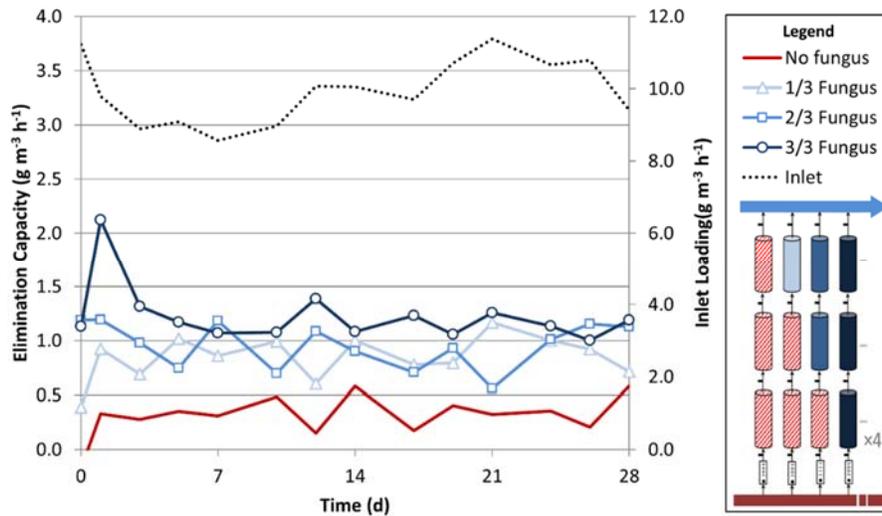


Figure 4. CH₄ capture by lab-scale biofilter columns packed with increasing amounts of *P. ostreatus* fungal biomass. Elimination capacity is the mass of CH₄ captured per biofilter media volume per unit of time. Inlet loading is the mass of CH₄ entering the biofilter media volume per unit of time. Inlet concentrations were 90 to 120 ppm CH₄. The flowrate was 1.46 m³ h⁻¹.

Table 3. Methane elimination capacity of treatments (different numbers of biofilter stages inoculated with *P. ostreatus* and biofilter stages with sterilized media). For each treatment, the mean elimination capacity (and standard deviation) are presented. ANOVA was used to test for treatment differences, and Tukey HSD was used post-hoc to test for differences in mean elimination capacity between treatments. Different letters denote statistically significant differences in the mean elimination capacities ($n = 4$).

Time (d)	Inlet Loading (g m ⁻³ h ⁻¹)	Elimination Capacity (g m ⁻³ h ⁻¹)			
		Three Stages	Two Stages	One Stage	Control
0	11.23	1.13 (0.58) a	1.19 (0.45) a	0.39 (0.61) ab	-0.17 (0.33) b
1	9.79	2.12 (0.28) a	1.19 (0.75) ab	0.92 (0.54) b	0.33 (0.39) b
3	8.89	1.32 (0.12) a	0.98 (0.45) ab	0.69 (0.40) ab	0.28 (0.28) b
5	9.08	1.17 (0.19) a	0.75 (0.57) a	1.02 (0.54) a	0.35 (0.18) a
7	8.57	1.07 (0.21) a	1.18 (0.29) a	0.86 (0.25) a	0.31 (0.27) b
10	8.96	1.08 (0.37) a	0.70 (0.15) a	0.99 (0.63) a	0.49 (0.21) a
12	10.07	1.38 (0.38) a	1.09 (0.22) ab	0.60 (0.33) bc	0.15 (0.40) c
14	10.05	1.08 (0.06) a	0.90 (0.64) a	1.00 (0.46) a	0.58 (0.40) a
17	9.70	1.23 (0.39) a	0.71 (0.35) ab	0.78 (0.18) ab	0.17 (0.26) b
19	10.71	1.06 (0.73) a	0.93 (0.23) a	0.80 (0.61) a	0.40 (0.16) a
21	11.38	1.26 (0.16) a	0.56 (0.38) b	1.17 (0.17) a	0.32 (0.29) b
24	10.66	1.13 (0.57) a	1.01 (0.35) a	1.00 (0.35) a	0.35 (0.41) a
26	10.79	1.00 (0.18) a	1.16 (0.73) a	0.92 (0.11) ab	0.21 (0.32) b
28	9.41	1.19 (0.84) a	1.13 (0.09) a	0.71 (0.18) a	0.58 (0.57) a

stages were inoculated with *P. ostreatus* reached 22.5 ppm initially and averaged 13.0 ± 2.9 ppm. Greater CH₄ capture by the three-stage fungal systems than by sterile controls was significant in eight of the 14 time points. Most of these were with the earlier time points (fig. 5). When fewer fungal stages were used, CH₄ capture declined, with capture by the two-stage *P. ostreatus* system averaging 10.2 ± 2.2 ppm and the one-stage *P. ostreatus* system averaging 9.0 ± 2.1 ppm.

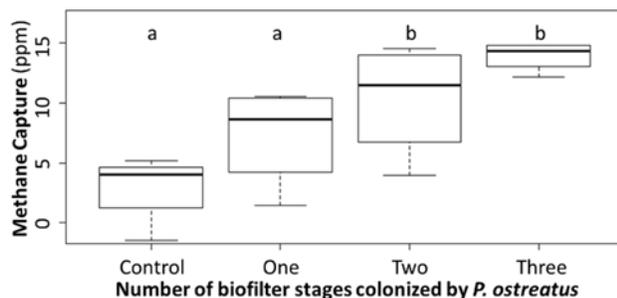


Figure 5. Boxplot of CH₄ capture by *P. ostreatus* at day 3 ($n = 4$). Different letters denote statistically different means ($\alpha = 0.05$).

FUNGAL SORPTION OF CH₄ IN A CHROMATOGRAPHIC ISOTHERM

Fungal spores coating sand grains were capable of sorbing CH₄. The surface dynamics of the test sorbent spores and their homogenized, even coating of sand particles were verified with SEM (fig. 6). When the inert sand control chromatographs were compared to chromatographs from a sand and spore mixture, significant sorption of CH₄ was observed (fig. 7). Evident by a consistent peak area of the treatments (i.e., the area did not increase with subsequent injections), the sorption sites appear to be unsaturated even when small fungal levels were present. Peak tailing would have indicated that CH₄ sorbed by the fungi quickly desorbed back to the gas stream (reversible sorption). As this was not observed, it is likely that the sorption of CH₄ onto the fungal cells is long-lasting, or perhaps with lack of CH₄ oxidizing organisms present, permanent (irreversible sorption).

Fungal materials from both major phyla (Ascomycota and Basidiomycota) showed an ability to sorb CH₄ (fig. 8). Increasing the proportion of fungal material relative to the inert sand further increased the ability of the isotherm column to sorb injected CH₄ (i.e., the peak area was negatively

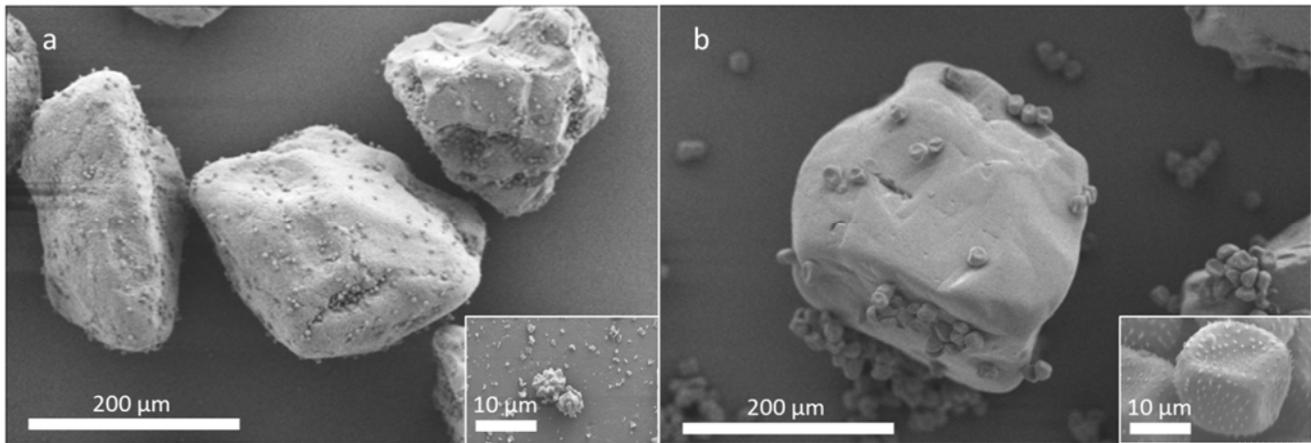


Figure 6. SEM images of sand mixed with (a) ascomycete and (b) basidiomycete fungal spores. Insets show spores at higher magnification.

correlated to the fungal ratio). While this pattern was not significant for the Ascomycota spores for the ratios tested, these material apparently sorbed more of the spiked CH_4 than the Basidiomycota test materials.

DISCUSSION

FUNGI IMPROVED CH_4 BIOFILTRATION AT LABORATORY SCALE

Fungal biofilms showed an enhanced ability to capture CH_4 compared to the bacterial biofilm. *Pleurotus ostreatus* was the top-performing fungal treatment in the initial experimental screen, sometimes demonstrating near-complete CH_4 capture. In the second experiment, CH_4 capture was increased by adding biofilter stages colonized by *P. ostreatus*. This suggests that fungi, such as *Pleurotus*, have biofiltration capacities for CH_4 and may offer a low-cost (~\$1,500 to inoculate a biofilter) means to improve CH_4 mitigation by biofilters designed currently for odor.

Pleurotus ostreatus is the second most cultivated fungal species globally, with spawn widely available without regulatory permit for biotechnology applications. This fungus is fast growing, able to colonize a range of woody and agricultural residue substrates even without sterilization (Sanchez, 2010),

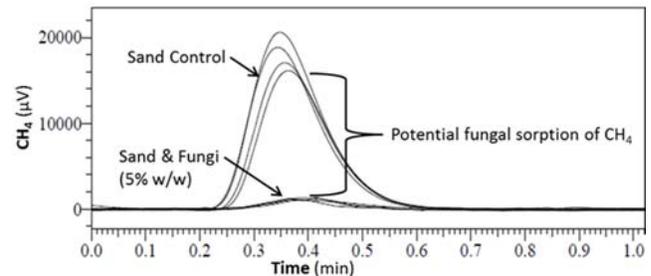


Figure 7. Overlay of chromatographic outputs of a sand control and a test sorbent of sand and Basidiomycota spores. The flame ionized detector of the GC combusts CH_4 in a hydrogen flame, emitting ions that are proportional to the gas concentration and can be detected as a voltage by an electrometer.

and can compete well in mulch and soil environments despite wood being its natural substrate (Baldrian, 2008). The ability to grow fast on assorted, non-sterile substrates in concert with the capacities of *Pleurotus* ligninolytic enzymes to degrade organopollutants has led to extensive investigation and use of this fungus in bioremediation systems (Cohen et al., 2002). *Pleurotus* and other white rot fungi have also been used to capture and accumulate inorganic pollutants (Baldrian, 2003), including the metals Ni(II), Cu(II), Zn(II), and Cr(IV) (Javaid et al., 2011). This ability to capture metals is likely related to their

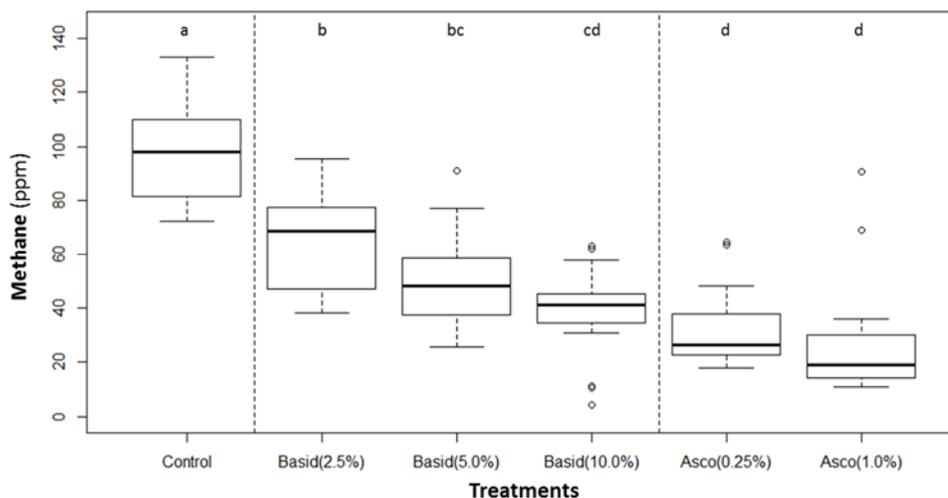


Figure 8. Detected CH_4 concentrations for control and test sorbents for various levels (w/w) of Basidiomycota (Basid) or Ascomycota (Asco) fungal spores (GC injection = 10 μL of 100 ppm CH_4 , $n = 20$). Different letters denote significantly different means ($\alpha = 0.5$).

regulation of metal-dependent ligninolytic enzymes and to functional groups in the hyphal sheath; proteins and carbohydrates of fungal hyphae have been identified as sites of this biosorption. Interestingly, there is some potential to reuse these hyphal growths as biosorbent (Javaid et al., 2011) as up to 90% of the metal sorption capacity has demonstrated reversibility when a dilute acid eluent has been used (Hu et al., 2014). The biosorption capacity of *P. ostreatus* can be quite significant, as hyphal surface area can reach $51.16 \text{ m}^2 \text{ g}^{-1}$ (Hu et al., 2014). Although fungal growths may clog a biofilter, overgrowth can be managed easily with backwashing or naturally mitigated by fungivorous mites (Kennens and Veiga, 2004).

Since fungi are not known to exclusively oxidize CH_4 (although some may be capable of co-metabolizing the gas; Lebrero et al., 2016), it is likely that similar biosorption capacities explain their ability to capture CH_4 . The predominant capture of CH_4 by fungal treatments early in the experiment and following increases in inlet emissions, along with the lack of sustained high performance, indicate that hyphal sorption of CH_4 is rapid and desorption is slow. This is similar to the sorption of metals by fungal hyphae, which without use of an eluent, can become difficult to desorb (Hu et al., 2014). However, in the case of CH_4 capture, slow desorption may be preferred, as it permits time for methanotrophic bacteria to oxidize the gas. The ability of fungal treatments to capture CH_4 effectively during early peak emissions and following a shutdown period for slow release is also advantageous, as it would control for the stochastic spikes in emissions that are typical of full-scale emissions patterns, thus stabilizing the CH_4 levels reaching methanotrophs. Longer experiments are needed to ensure establishment of a mature fungal-methanotroph biofilter community, better suited for studying capture and oxidation feedbacks and their responses to stochastic CH_4 loading.

FUNGI CAN SORB CH_4

The chromatographic isotherm studies also indicate that fungal materials can sorb CH_4 . Like the lab-scale system, increasing fungal biomass (i.e., increasing Ascomycota and Basidiomycota spore masses) improved CH_4 capture. The use of inactive resting spores supports the notion that this is not an active biodegradation process but rather a biosorption process. Like hyphae, spores are known to possess hydrophobic protein sheaths (Sulc et al., 2009; Tucker and Talbot, 2001). Used by spores for attachment to plant hosts, these hydrophobin layers also appear capable of capturing a poorly soluble GHG. The more significant capture of CH_4 by the Ascomycota fungal materials can be explained in part by the smaller size and higher surface area to volume ratio of the conidiospores, although differences in surface properties cannot be ruled out. Assessed visually here, Brunauer-Emmett-Teller (BET) testing could be used to quantitatively assess the differences in spore surface area and normalize isotherm responses for a clearer comparison of spore types.

As with the lab-scale biofilter trials, there was little evidence in the chromatograms for rapid reversibility of CH_4 sorption. Reversibility would have been observed in the peak overlay, which found no significant peak tailing or shift in detection time. There was also no indication that the CH_4 sorption capacity of the spore materials had been reached based on over 30 min of repeated CH_4 injections. If sorption

capacity had become saturated, the peak area would have slowly increased to the area of sand-only controls. Further work will thus be needed to identify the sorption capacity of these materials. Additionally, follow-up trials will be needed to investigate active fungal hyphae and test the effects of field-relevant moisture on CH_4 sorption.

INTERACTIONS OF FUNGI AND METHANOTROPHS

Fungi and bacteria closely compete and cooperate in natural substrates (Baldrian, 2008) and must often act jointly in biofilters for optimal mitigation to occur (Prenafeta-Boldú et al., 2012). Just as CH_4 generation is typically a combined bacterial and fungal effort (Beckmann et al., 2011), it is likely that capture and oxidation of CH_4 is also a community dynamic. Currently, we have a limited knowledge of the interactions between fungi and methanotrophs or their co-localization. While fungal degradation of secondary turnover C from methanotrophs does not always occur, fungal growth is stimulated by CH_4 -rich landfill gas similarly to methanotrophs (Watzinger et al., 2008). Based on this demonstration of CH_4 capture by fungi, perhaps fungi play an active, upstream role in CH_4 biofiltration and gain some unresolved benefit from methanotrophs. Answering these questions will be central to our understanding of CH_4 biofilter ecology and the role of fungi in CH_4 capture.

CONCLUSIONS

To our knowledge, this is the first work demonstrating the ability of biofilter fungi to capture the greenhouse gas CH_4 from high ventilation-rate effluents. Specifically, using a multi-stage, lab-scale biofilter, we were able to show that certain fungal inoculations routinely had higher CH_4 elimination capacities than bacterial consortium and sterile control treatments. By inoculating different stages of the biofilter with or without the fungus *Pleurotus ostreatus*, we were able to demonstrate that higher CH_4 elimination capacities could be achieved by increasing fungal biomass. Using a chromatographic isotherm approach, we more clearly demonstrated the ability of fungal cells to sorb CH_4 gas from a passing effluent. Despite the preliminary nature of the work, and the undoubted need for more testing, these findings suggest that microbes other than CH_4 oxidizing methanotrophs (i.e., fungi) may govern rate limits that affect the ability of biofilters to mitigate CH_4 . If fungi can improve CH_4 capture in a biofilter, thus facilitating subsequent oxidation, it is possible that by inoculating or selecting for specific fungi or microbial communities in a biofilter, a potent GHG can be mitigated simultaneously with odorous livestock production emissions.

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