A comparative assessment of the performance of fungal-bacterial and fungal biofilters for methane abatement

Alberto Vergara-Fernández[1]*, Felipe Scott[1], Felipe Carreño[1], Germán Aroca[2], Patricio Moreno-Casas[1], Armando González-Sánchez[3] and Raúl Muñoz[4]

[1] Green Technology Research Group, Facultad de Ingeniería y Ciencias Aplicadas, Universidad de los Andes, Chile.

[2] Escuela de Ingeniería Bioquímica, Facultad de Ingeniería, Pontificia Universidad Católica de Valparaíso. Chile

[3] Instituto de Ingeniería, Universidad Nacional Autónoma de México, Circuito Escolar, Ciudad Universitaria, 04510 Mexico City, Mexico

[4] Institute of Sustainable Processes, Universidad de Valladolid, Dr Mergelina s/n, 47011, Spain

*Corresponding author: A. Vergara-Fernández. Tel.+ 562 2618 1441. E-mail: aovergara@miuandes.cl

Abstract

Methane is an important contributor to global warming and especially for dilute emissions, its oxidation to carbon dioxide can be difficult and expensive. Biofiltration of streams carrying methane at low concentration in air have been treated with biofilters inoculated with methanotrophic bacteria. However, the role of fungi in methane is not well understood.

In this work, methane abatement was studied in a biofilter inoculated solely with the filamentous fungus *Fusarium solani* and compared to a biofilter inoculated with a consortium of methanotrophic bacteria (*Methylomicrobium album* and *Methylocystis sp*) and *F. solani.*

Results showed that F. solani degrade methane as the sole carbon source, achieving a maximum elimination capacity of 42.2 g m^{-3} h^{-1}, nearly half of the maximum elimination capacity of the fungal-bacterial consortium. Co-feeding o methane and *n*-pentane, a highly hydrophobic and easily degradable VOC, further improved the elimination capacity of both biofilters, with the elimination capacity of the fungal biofilter surpassing the one attained by the fungal-bacterial biofilter.

A concise mathematical model of the biofilter together with the evaluation of the second Damköhler number indicated that under the operational conditions here applied, the fungal biofilter performance was bioreaction limited meanwhile external mass transport limitation was found on the fungal/methanotrophic bacteria biofilter.

These results, and the estimated mass transfer coefficients, suggest that the beneficial effect of *F. solani* during CH$_4$ biofiltration was mediated by biomass hydrophobicity rather than to the formation of aerial hyphae structures increasing the mass transfer area.

Keywords: Methane abatement; Fusarium solani; Biofiltration; Mass transfer coefficient; Mathematical modeling.

1   Introduction

Methane (CH_4) is considered as a relevant greenhouse gas since its contribution to global warming in a 100 y horizon accounts for ~ 23%, while carbon dioxide (CO_2) contributes to 70%. Besides CH_4 has an average global warming potential 28 (in a 100 y horizon) times higher than CO_2 [1]. Atmospheric CH_4 concentration is increasing twice faster than CO_2 concentration [2,3].

In this context, biofiltration is a cost-effective alternative to control off-gas emissions of CH_4 below the flammability point (5% v/v)[2,4].   Indeed, methanotrophs-based biofilters have been extensively applied during the past two decades to reduce CH_4 emissions from landfills, livestock farms or even coal mines [5]. However, the low aqueous solubility of CH_4 (dimensionless gas-liquid Henry´s law constant of 29.4 at 1 atm and 25ºC) limits its cost-effective biological abatement [3]. Biofiltration systems typically contain mixed microbial populations (i.e. bacteria, fungi and yeasts) adapted to oxidize particular pollutants under ambient conditions (pH, temperature, moisture content, etc.). The use of fungi in biofilters may offer several advantages compared to conventional bacteria colonized-biofilters, such as a higher enzyme diversity and tolerance to extremophile conditions (low nutrient availability, low water activity and low pH values) [6]. In addition, several authors have claimed that the empty bed fraction of biofilters can be colonized by aerial hyphae. This can enhance the elimination capacity of hydrophobic pollutants such as CH_4 based on the increase in the specific mass transfer area mediated by hyphae growth, which are covered by lyophilic proteins able to easily solubilize hydrophobic gases [7–9]. In this context, the co-culture of filamentous fungi with methanotrophic bacteria can increase the CH_4 elimination capacity by increasing the partition coefficient of CH_4 in the biofilm established in the biofilter packed bed (bioavailability) [10,11].

The potential of fungi as a biological platform for enhancing the availability and biodegradation of methane has been reported by several authors [11–14]. Girard et al. [12] inoculated the fungi *Candida ingens*, *Sporotrichum pruinosum*, *Coprinus sp.* and *Cunninghamella elegans* in combination with methanotrophic bacteria in a biofilter treating methane, reaching a maximum methane elimination capacity (EC) of 18.8 g m^{-3} h^{-1} at an inlet load of 46.7 g m^{-3} h^{-1}. Lebrero et al. (2016) reported that *Graphium sp.* was able to degrade methane only when methanol was supplemented as a co-substrate. These authors evaluated also the performance and microbiology of a fungal-bacterial compost biofilter treating methane concentrations of 2% at empty bed residence times of 40 and 20 min under different irrigation rates, with daily mineral medium addition of 200 mL supporting EC of 37 g m^{-3} h^{-1}. Recently, Vergara-Fernández et al. [11] reported the ability of *Fusarium solani* to biodegrade CH_4 as the sole carbon source in microcosm experiments (0.3 g m^{-3} h^{-1} at 35°C and water activity of 0.95). To our understanding, this was the first report of methane consumption by fungi as the sole carbon and energy source. Moreover, *F. solani* could decrease the partition coefficient of methane up to two orders of magnitude compared with the partition coefficient of methane in water.

Mathematical modelling represents a powerful tool to optimize the design and operation of biological gas-treatment units. In this context, several reactive-internal transport/external transport based mathematical models have been reported to describe CH_4 oxidation in biofilters inoculated with methanotrophic bacteria under isothermal conditions [15,16] and under non-isothermal conditions [17]. However, to our knowledge, mathematical models describing fungi-based methane biofilters are scarce, which limits the developments of these high-performance biofilters.

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This work aims at characterizing for the first time the effects of CH_4 inlet load and residence time, as well as *n*-pentane co- feeding, on the performance of a biofilter treating methane using the fungi *Fusarium solani* alone and in a consortium with methanotrophic bacteria. Secondly, the role of the fungal biomass as an enhancer of methane bioavailability in the biofilms was explored, both experimentally and through a mathematical model.

2   Materials and methods

2.2   Microorganisms and inoculum

The methanotrophic bacteria used in this work were *Methylomicrobium album* ATCC 33003 and *Methylocystis* sp. ATCC 4924. Bacterial propagation was performed in NMS (Nitrate-mineral salt medium, ATCC 1306) without agar at pH 6.8 as previously reported by Cáceres et al. [18]. Propagation of the filamentous fungi *Fusarium solani* B1 (CBS 117476) was carried out as described by Morales et al. [19] in potato-dextrose agar. Fungal preservation, cultivation conditions and spore production were similar to those reported by García-Peña et al. [20]. Cultures were incubated in a rotary shaker (Incu-Shaker Mini, Benchmark) at 30°C and 80 or 200 rpm for fungi and bacteria cultivation, respectively.

2.3   Carbon source and mineral medium

The carbon sources used as model hydrophobic contaminants in the different experiments were methane (Indura Chile, 99.99%) and *n*-pentane (Merck, 99%). Glycerol (Merck, 99.9%) and methanol (Merck, 99.9%) were used as alternative carbon sources to support microbial growth during the start-up period of the biofilters. The mineral salt medium used for fungal growth in liquid cultures and biofiltration columns was previously reported by Arriaga and Revah (2005): $NaNO_3$ 18 g L^{-1}, KH_2PO_4 1.3 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 0.38 g L^{-1}, $CaSO_4 \cdot 2H_2O$ 0.25 g L^{-1}, $CaCl_2$ 0.055

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g L^{-1}, FeSO$_4$·7H$_2$O 0.015 g L^{-1}, MnSO$_4$·H$_2$O 0.012 g L^{-1}, ZnSO$_4$·7H$_2$O 0.013 g L^{-1}, CuSO$_4$·7H$_2$O

0.0023 g L^{-1}, CoCl$_2$·6H$_2$O 0.0015 g L^{-1}, H$_3$BO$_3$ 0.0015 g L^{-1} and glycerol 10 mL L^{-1}.

The composition of NMS medium used for bacterial growth was as follows [22]: MgSO$_4$·7H$_2$O

1.0 g L^{-1}, CaCl$_2$·6H$_2$O 0.2 g L^{-1}, KNO$_3$ 1.0 g L^{-1}, KH$_2$PO$_4$ 0.272 g L^{-1}, Na$_2$HPO$_4$·12H$_2$O 0.717 g

L^{-1}, 2.0 mL of chelated iron and 0.5 mL of a trace elements solution was additionally added to 1 L

of NMS solution. The chelated iron solution contains: 1.0 g L^{-1} ferric (III) ammonium citrate, 2.0

g L^{-1} EDTA sodium salt, 0.3 mL of HCl (concentrated), 100 mL of distilled deionized water. The

trace element solution contains per liter of distilled water: EDTA 0.5 g L^{-1}, FeSO$_4$·7H$_2$O 0.2 g L^{-1},

ZnSO$_4$·7H$_2$O 0.01 g L^{-1}, MnCl$_2$·4H$_2$O 0.003 g L^{-1}, H$_3$BO$_3$ 0.03 g L^{-1}, CoCl$_2$·6H$_2$O 0.02 g L^{-1},

CaCl$_2$·2H$_2$O 0.001 g L^{-1}, NiCl·6H$_2$O 0.002 g L^{-1}, Na$_2$MoO$_4$·2H$_2$O 0.003 g L^{-1}.

2.4 *Experimental set-up for methane biofiltration*

A diagram of the experimental system is shown in Figure 1. Two identical biofilters were set up

with PVC-clear columns (7.9 cm of diameter and 105 cm of height) divided into three equal length

modules. Each module was filled with 82 g of vermiculite (empty bed ϵ of 69%), reaching a total

packed bed volume (V_p) of 2.35 L. Each module was periodically sampled at the inlet and the outlet

of the gaseous stream from sampling ports. The moisture content of the solid support and the

pressure drop in each module of the biofilter were determined with a ProCheck sensor read-out

device (Decagon Devices, WA, USA) and a U-Tube manometer (using water as the manometric

fluid), respectively. The moisture content in the airstream at the inlet and the outlet of biofilter were

measured with a thermo-hygrometer (Testo 625, Testo, PA, USA). The biofilters were

continuously fed with different mixtures of pre-humidified air and pure CH$_4$ (99.99%, Indura

Chile). The humidifier consisted of a PVC-Clear column (diameter of 7.9 cm and 40 cm height)

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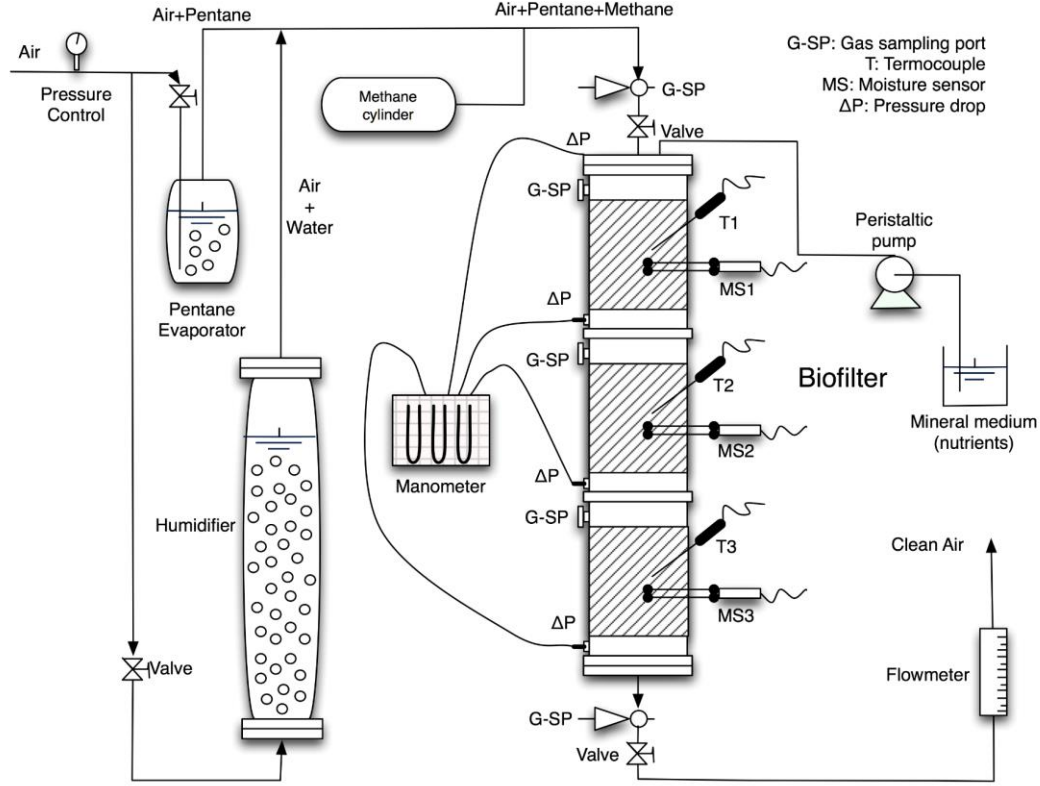
filled with 20 cm of water.

Figure 1. Schematic diagram of the laboratory-scale biofilter system

The performance of the methane biofilters was expressed in terms of methane elimination capacity (EC, g CH_4 m^{-3} h^{-1}), and methane and n-pentane inlet loading rate (IL, g m^{-3} h^{-1}), which were correlated with the empty bed residence time (EBRT):

$$EBRT = \frac{V_p}{Q} \tag{1}$$

$$EC = \frac{(C_{g,0} - C_{g,out})}{EBRT} \tag{2}$$

$$IL = \frac{(C_{g,0})}{EBRT} \tag{3}$$

Where Q and V_p represent the polluted air flow rate (m^3 h^{-1}) and packed bed volume (m^3), respectively, $C_{g,0}$ and $C_{g,out}$ the inlet and outlet methane concentrations (g m^{-3}) in the polluted

airflow, respectively.

The study of the influence of CH_4 inlet load on biofilters performance was conducted by setting different concentrations of CH_4 adjusting the flow rate of pure methane and humidified air. Both flow rates were controlled with mass flow controllers (Colepalmer, EW-32907-69, IL, USA). In the experiments where *n*-pentane or methanol were used, a stream of air controlled with a mass flow controller (Colepalmer, EW-32907-69, IL, USA) was bubbled in an evaporator with *n*-pentane or with methanol (depending on the experimental stage), and subsequently mixed with a humidied air stream containing methane

2.5   *Operational strategies during CH_4 biofiltration*

The two biofilters were operated in parallel. The fungal biofilter (BF) was inoculated with the fungus *F. solani*, while the Fungal-Bacterial Biofilter (FBB) was inoculated with a methanotrophic bacterial consortium composed of *Methylomicrobium album* and *Methylocystis* sp., and the fungus *F. solani*. All microorganisms used were previously grown in their respective mineral medium and glycerol at 4 g L^{-1}.

BF was inoculated with a mixture of 1000 mL of fungi (2.0 g L^{-1}) in mineral medium and 400 mL of fresh mineral medium, which was recirculated for 7 days through the packed column to favor the attachment of the microorganisms. FBB was inoculated with a mixture of 500 mL of methanotrophic bacterial culture (1.0 g L^{-1}) and 500 mL of fungal culture (2.0 g L^{-1}). The cell suspension containing both fungi and bacteria was mixed with 400 mL of mineral medium and recirculated for 7 days through the packed column to allow the attachment of the microorganisms. A methane laden airstream was fed to the columns for 80 days at an inlet concentration of 25 g CH_4 m^{-3} and a gas flow of 0.181 L min^{-1} (EBRT equal to 13 min), corresponding to an inlet load of 115.4 ±5.2 g m^{-3} h^{-1}. Methanol at a concentration of 1.0 g m^{-3} was supplemented to the CH_4-

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laden emission from day 33 to 40 according to Lebrero et al. [13] in order to foster microbial growth and CH_4 biodegradation in the biofilters. At day 55, 100 mL of mineral salt medium with glycerol at 4 g L^{-1} was added to the biofilters according to Vergara-Fernández et al. [23] while maintaining the methane inlet load.

2.5.1 *Influence of* CH_4 *loading rate and EBRT on the steady-state* CH_4 *elimination capacity*

The influence of the methane loading rate on the elimination capacity of FB and FBB was assessed by increasing the inlet CH_4 concentration from 6 to 94 g CH_4 m^{-3} at a constant *EBRT* of 12.2 ($\pm$0.9) min, which corresponded to loading rates between 31.6 and 437 g CH_4 m^{-3} h^{-1}. On the other hand, the influence of the *EBRT* on the methane elimination capacity was assessed by varying the *EBRT* between 6.0 and 23.3 min at a constant methane inlet load of 437($\pm$5.9) g CH_4 m^{-3} h^{-1}.

2.5.2 *Influence of n-pentane supplementation on the* CH_4 *elimination capacity*

The influence of *n*-pentane on the methane elimination capacity was evaluated by supplying *n*-pentane loading rates ranging from 48.1 ($\pm$2.6) to 238.1 ($\pm$6.8) g *n*-pentane m^{-3} h^{-1} at a constant methane loading rate of 131.1 ($\pm$5.0) g CH_4 m^{-3} h^{-1}.

2.6 *Estimation of* Fusarium solani *contribution to* CH_4 *biodegradation*

Methane biodegradation tests in microcosms were performed in order to quantify the contribution of *Fusarium solani* towards the methane elimination in the biofilter. Samples of 2.0 g of vermiculite with biomass were withdrawn from the biofilters FB and FBB by day 80. The samples were mixed with 10 mL of mineral medium containing the antifungal amphotericin B at a concentration of 32 µg mL^{-1}. Control experiments without the addition of amphotericin B were also performed. The consumption of methane as the sole carbon and energy source was assayed at an initial

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concentration of 27 ($\pm$1.5) g m^{-3} and 30°C for 15 days without stirring. The microcosms were established in 125 mL bottles hermetically sealed with 20 mm $\times$ 3 mm laminated silicone-PTFE (0.13 mm) septa, and aluminum seals.

2.7 K_Ba and Damköhler number estimation in biofilters treating CH_4 .

To determine the mechanism limiting CH$_4$ elimination in FBB and FB, the second Damköhler number (eq. 4) was calculated under the corresponding operational conditions, assuming that the fungal-bacterial biofilm and fungal biofilm followed first-order kinetics and Monod type kinetics, respectively (see supplementary section). This dimensionless number defines the ratio between the maximum methane biodegradation rate and the maximum methane mass transfer rate for a given condition. A second Damköhler number higher than one indicates the occurrence of external mass transfer limitation in the biofilter, while values lower than one are encountered in bioreaction limited scenarios [24].

$$Da_{II}^{j} = \begin{cases} \dfrac{kX_b^j V_b^j}{K_B a^j (1-\epsilon)V_p}, & \text{for } j = FBB \\[2ex] \dfrac{q_{max}X_b^j V_b^j H^j}{K_B a^j (1-\epsilon)V_p} \cdot \dfrac{1}{C_g^{in}}, & \text{for } j = FB \end{cases} \qquad \text{Eq. 4}$$

A series of experiments were carried out in order to estimate the overall mass transfer coefficient based on the biofilm phase (K_Ba) for both biofilters and the corresponding biokinetic parameters of the fungal or fungal-bacterial biofilm. The experiments were carried out at three different flows rates (0.3, 0.6 and 0.86 L min^{-1}). CH$_4$ inlet concentrations of 19, 16.5 and 15.5 g CH$_4$ m^{-3} were used in the FB tests, while CH$_4$ inlet concentrations of 28, 25 and 24 g CH$_4$ m^{-3} were used in FBB. All experiments were performed at 30 °C. The experimental procedure was as follows: Under

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steady-state at the target inlet CH_4 concentration, the flow of pure CH_4 was interrupted until the

outlet air stream reached a CH_4 concentration equal to zero. Then, CH_4 supply was restored.

CH_4 concentration was quantified off-line by gas chromatography (see Analytical methods) at the

outlet of the biofilters. The time course and profiles of CH_4 concentrations were adjusted to a

comprehensive biofiltration mathematical model adapted from [25] (see supplementary material).

2.8   Analytical methods

CH_4 gas concentration was determined from gas samples extracted using a 500 µL gas syringe

(Hamilton). The samples were injected in a gas chromatograph (Shimadzu 2014) equipped with a

TCD detector and a 60/80 Carboxen column (15 ft $\times$ 1/8 in $\times$ 2.1 mm). Injector temperature was

maintained at 150°C, while oven and detector temperatures were kept at 200°C and 220°C,

respectively.   *n*-pentane gas concentration was measured by FID-GC in a Shimadzu 2014

chromatograph (detection temperature 220 ℃, injection temperature 80 ℃ and column temperature

200 ℃) equipped with a capillary column RTX-5 Restex UE (30 m $\times$ 0.32 mm $\times$ 0.25 μm), using

helium as a gas carrier.

2.9   Scanning electron microscope

Samples of the vermiculite support with microbial consortium grown for 8 months were dried at

105 ℃ for 24 hours for scanning electron microscopy (SEM) imaging. Dried samples were

mounted onto stubs and gold-coated using JEOL fine-coat ion sputter JFC-1100. Samples were

visualized and micrographed using a scanning electron microscope (EVO MA 10 model, Zeiss),

with an EDS Penta FET Precision detector (Oxford Instruments X-act) at 20 kV accelerating

voltage.

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3   Results and discussion

3.1   *Biofilter start-up*

The elimination capacities achieved in the biofilters during the first 80 days of operation are shown in Figure 2. During the first 20 days, both biofilters showed comparable EC, with average values of 6.6($\pm$2.2) g m^{-3} h^{-1}. After 20 days of operation, an increase in the CH$_4$ EC of FBB was recorded, reaching a stable average EC of 17.5 g m^{-3} h^{-1}. Similar EC values were achieved in FB after 26 days of operation. This delay could be attributed to the slower growth rate of fungi compared to bacteria. Interestingly, both biofilters experienced a decrease in the EC after 30 days of operation, reaching values close to 8.7 g m^{-3} h^{-1}. The reason underlying the observed decrease in EC were not clear since temperature and humidity remained constant at the set points, and mineral medium was added once per week. Methanol was fed at a loading rate of 4.8 g m^{-3} h^{-1} from day 34 until day 55 in an attempt to increase the biomass content in the biofilters. This allowed increasing the CH$_4$-EC by 40% compared to the previous condition. 100 mL of mineral medium with 4 g L^{-1} glycerol were added at the end of day 55 to increase of biomass content in the biofilters. Figure 2 shows a rapid increase in EC in both biofilters following glycerol addition, reaching a maximum of 32.8 g m^{-3} h^{-1} by day 60 and stabilizing at 28.4($\pm$2.2) g m^{-3} h^{-1} in FBB. An average EC of 21.8 g m^{-3} h^{-1} was achieved in BF from days 63 to 80. The increase in CH$_4$ EC for both biofilters was likely due to the effectiveness of glycerol supporting a rapid growth of *F. solani* (*F. solani* was routinely grown in glycerol as sole carbon and energy source in our laboratory) in the biofilters. No growth enhancement was expected for the obligate methanotroph *Methylomicrobium album* ATCC 33003 [26] or the facultative methanotroph (able to grow on acetate and ethanol) *Methylocystis* sp. ATCC 4924 [27]. From day 80 onwards, the biofilters were operated with methane as the sole carbon and energy source (Figures 4, 5).
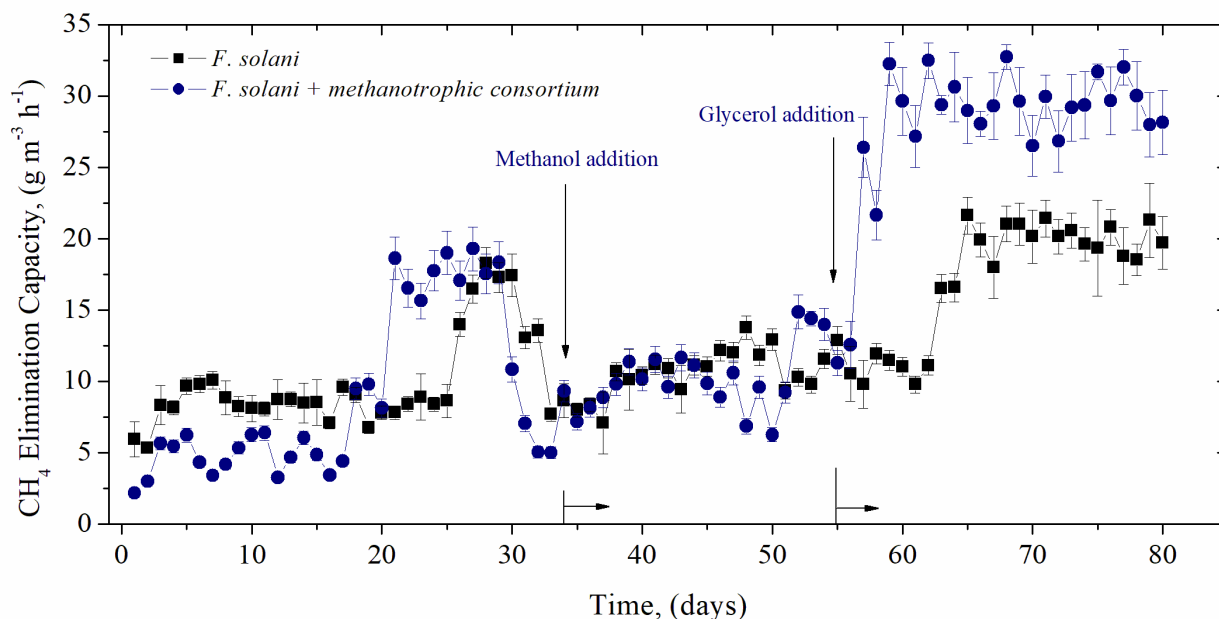
Figure 2. Time course of the methane elimination capacity of the biofilter inoculated solely with *Fusarium solani* and the biofilter inoculated with *Fusarium solani* and the methanotrophic consortium and operated at an inlet concentration of 25 g CH_4 m^{-3} and *EBRT* 13 min, corresponding to 115.4($\pm$5.2) g m^{-3} h^{-1}.

The maximum removal efficiencies recorded in FBB and FB were 26% and 17%, respectively, with EC comparable to those reported by Lebrero et al. [13] for a fungal-bacterial biofilter (35 g m^{-3} h^{-1}). The EC achieved in this work was higher than the 16 g m^{-3} h^{-1} attained by Pratt et al. [28] in a biofilter inoculated solely with a methanotrophic bacterial consortium.

3.2   Influence of CH_4 loading rate on the CH_4 elimination capacity

Figure 3 shows the CH_4 EC recorded at the different methane loading rates applied in both biofilters. The results obtained for FB showed a methane EC of 17 g m^{-3} h^{-1} for a critical methane loading rate of 125 g m^{-3} h^{-1}. On the other hand, it was not possible to obtain the critical methane-

loading rate for FBB. CH_4 EC of 75 and 37 g m^{-3} h^{-1} were recorded in FBB and FB, respectively, at the maximum methane loading rate applied 450 g m^{-3} h^{-1}. The ECs at a loading rate of 125 g m^{-3} h^{-1} in FB and FBB were more than two times higher than the values reported by López et al. [29] for a loading rate of 120 g m^{-3} h^{-1} (11.3 g m^{-3} h^{-1}). Lebrero et al. [13] reported an EC of 70 g m^{-3} h^{-1} for a methane loading rate of 120 g m^{-3} h^{-1} in a bacterial-fungal biofilter inoculated with *Graphium sp*. However, these authors reported that *Graphium sp*. was eventually displaced from the biofilter community and that the fungus was able to use methane only when methanol was also present. The higher EC obtained in the FBB operated in this study may be explained by the increase in the interfacial gas-biofilm contact area due to the presence of the filaments from fungi [30] and by the increase in the CH_4 concentration gradient mediated by the hydrophobic properties of the fungal filaments [11,31]. These fungal mediated mechanisms could increase the bioavailability of methane in the whole methanotrophic biofilm, which ultimately supported higher CH_4 degradation rates [14]. The later highlights the advantage of deploying methanotrophic fungal/bacterial consortia during CH_4 biofiltration[11].
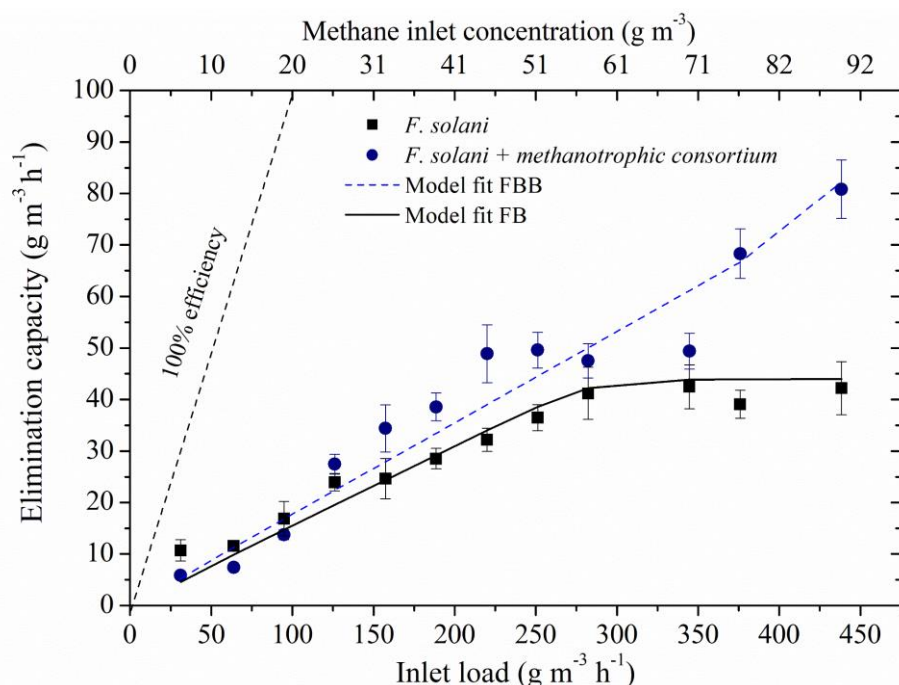
Figure 3. Methane elimination capacity *vs* loading rates for the biofilter inoculated with *F. solani* (FB) and biofilter inoculated with *F. solani* and a methanotrophic bacteria consortium (FBB). Model fits correspond to the solution of Eqs. S1-S3 with parameters shown in Table 1.

3.3   Influence of the EBRT at constant CH_4 loading rate on the elimination capacity

Figure 4 shows the effect of the *EBRT* at a CH_4 loading rate of $437(\pm 5.9)$ g m^{-3} h^{-1} in the EC of FB and FBB. A decrease in CH_4 EC was observed at increasing gas flow rates in both biofilters, which can be explained by the shorter contact time between methane and the methanotrophic biofilm at lower EBRT. The main decrease in CH_4 EC in both biofilters was observed when the EBRT was reduced from 23.3 to 6.0 min, the greatest reduction being observed in FBB (13.4 g m^{-3} h^{-1} decrease) in comparison with the FB (7.4 g m^{-3} h^{-1} decrease). When both biofilters were operated at EBRTs between 26 and 13 min, the EC stabilized between 26 and 16 g m^{-3} h^{-1} in FBB and FB, respectively. Despite FBB supported the highest EC, its performance was severely affected by shorter EBRTs. This may be related to either the microbial structure, the morphology of the methanotrophic biofilm, or a combination of both. Bacterial population in the biofilm exhibited a low methane mass transfer capacity, which might explain the mass transfer limitations recorded under low *EBRTs* in FBB. This lower methane mass transfer capacity of methanotrophic bacteria was recently reported by Vergara-Fernández et al. [11] during the assessment of the partition coefficients of methane in *Fusarium solani* and methanotrophic bacteria biomass ($C_g/C_{biofilm}$ of 0.2631 and 2.192, respectively) under similar operational conditions than those used in the present study. On the other hand, the decrease in *EBRT* in FB entailed a less severe effect on CH_4 EC, which may be related to the more homogenous hyphae biofilm growth. Thus, a decrease in EBRT would exert less impact on CH_4 EC in a scenario of enhanced CH_4 concentration gradients mediated by fungal hydrophobicity (Vergara-Fernández et al., 2016).
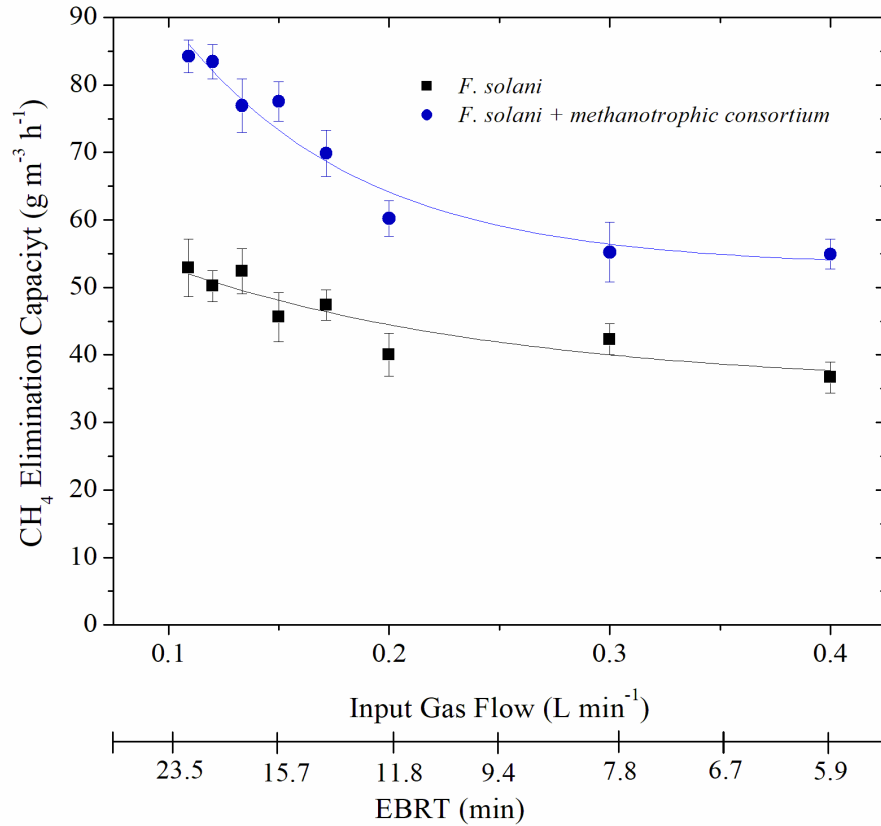
Figure 4. Influence of the *EBRT* on CH_4 elimination capacity at a constant methane loading rate $(437(\pm 5.9)\ \mathrm{g\ m^{-3}\ h^{-1}})$ in FBB and FB.

3.4 Influence of n-pentane on the CH_4 elimination capacity at a constant CH_4 loading rate

The addition of *n*-pentane aimed at providing an alternative carbon source to methane in order to foster the growth of *Fusarium solani* and to increase its surface hydrophobicity according to Vergara-Fernández et al. [8]. An increase in the CH_4 EC was observed in both biofilters when the *n*-pentane loading rate increased at a constant methane loading rate of 60 g m^{-3} h^{-1} (Figure 5). This increase was likely due to the higher hydrophobicity degree of the fungal cell wall, which mediated higher mass transfer rates and bioavailability of CH_4 for the fungal and fungal-bacterial biomass. While CH_4 EC in both biofilters was approximately 12 g m^{-3} h^{-1} at a methane loading rate of 60 g m^{-3} h^{-1}, the supplementation of *n*-pentane at 107 g m^{-3} h^{-1} at a similar methane loading rate increased the *EC* of FB by 75% (21±1.0 g m^{-3} h^{-1}) and by 34% in the FBB (16±1.1 g m^{-3} h^{-1}). The higher

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increase of *EC* in FB during *n*-pentane supplementation could be explained by the increase in the gas-biomass contact area mediated by the enhanced hyphae growth of the fungus in the presence of a hydrophobic carbon source such as *n*-pentane (Vergara-Fernández et al., 2011).
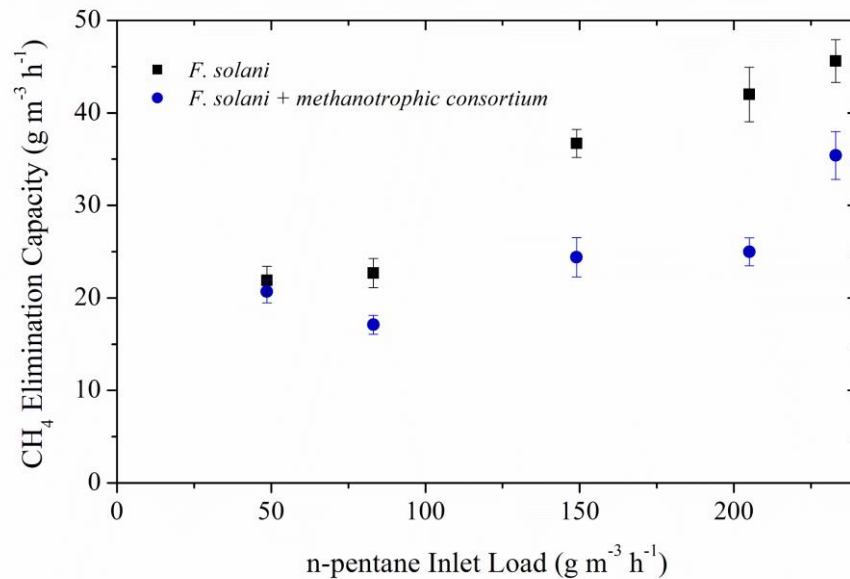

Figure 5. Influence of *n*-pentane loading rate on CH_4 elimination capacity in FBB and FB.

3.5   Estimation of Fusarium solani *contribution to CH_4 biodegradation*

Figure 6 shows the results obtained for the biodegradation of methane in microcosm assays. When amphotericin B was added to the packing material drawn from the FB (only *F. solani* was inoculated), no biodegradation of CH_4 was observed (Figure 6a). In the control microcosm (without amphotericin B) CH_4 degradation was observed at a rate of 0.9 g m^{-3} d^{-1}, indicating that *F. solani* not only promotes the mass transfer of CH_4 to the biofilm but contributes to CH_4 biodegradation. CH_4 biodegradation as the sole carbon and energy source by *Fusarium solani* was previously observed by Vergara-Fernández et al. [11]. On the other hand, when amphotericin B was added to the microcosms with *Fusarium solani* and methanotrophic bacteria, CH_4 biodegradation decreased

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from 1.3 g m^{-3} d^{-1} to 0.6 g m^{-3} d^{-1} (Figure 6b). This finding confirmed the beneficial effect of using a fungal/bacterial consortium for the biodegradation of CH$_4$. The methanotrophic bacterial consortium was able to biodegrade 46% of the initial CH$_4$, while fungi biodegraded 54%.
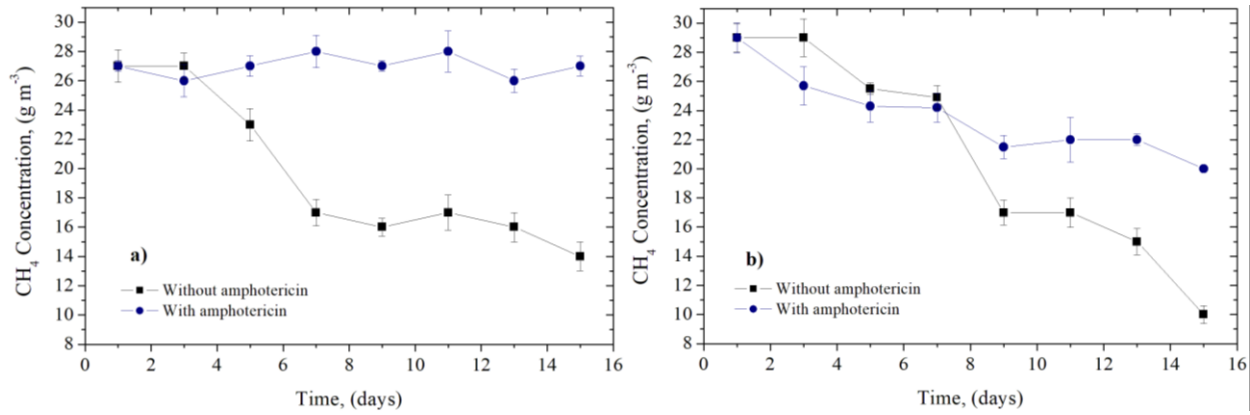

Figure 6. Effect of the addition of amphotericin B on the biodegradation of CH$_4$ in biomass obtained from a) the fungal biofilter, b) from the fungal/bacterial biofilter.

3.6   Characterization of the rate-limiting step: K$_B$a, biokinetics parameters and second Damköhler number estimation

Six dedicated experiments (three for the fungal and three for the fungal-bacterial biofilter) in duplicate were performed for the estimation of the global volumetric mass-transfer coefficient based on the biofilm phase ($K_B a$). The model coupling external mass transfer and bioreaction in the biofilter (Eqs. S1 to S3) was calibrated to the fungal and the fungal-bacterial biofilters CH$_4$ concentrations time-depended data presented in Figure 7 and the steady-state CH$_4$ EC data presented in Figure 3.
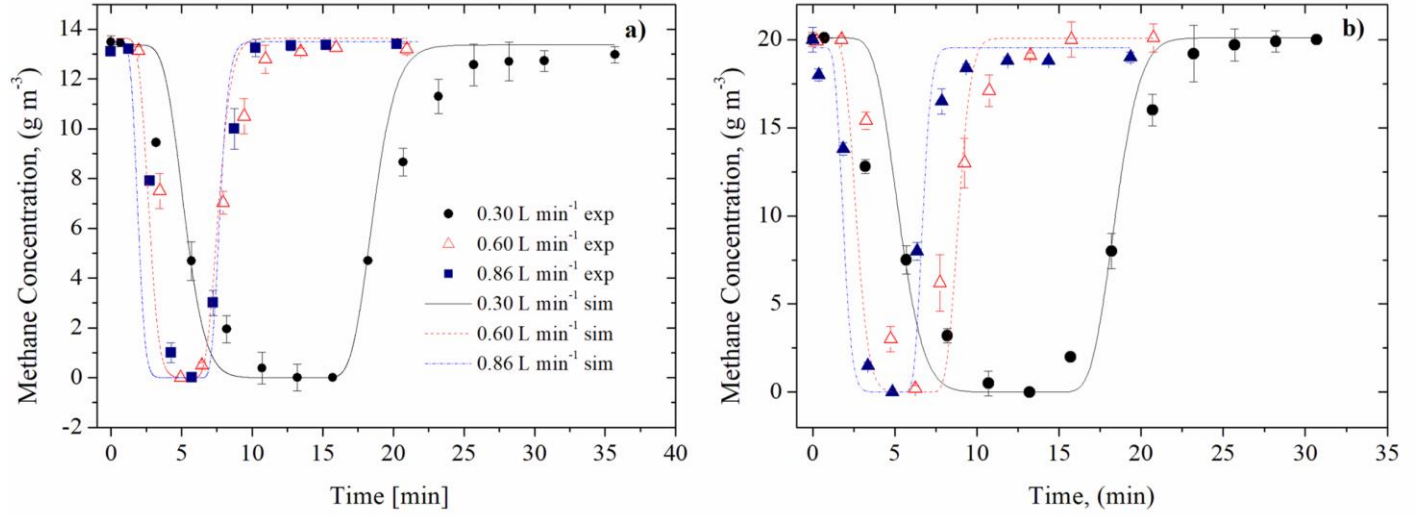
Figure 7. Dynamic CH4-in/CH4 out experiments for the estimation of $K_L a$ in a) FB and b) FBB.

The fitted parameters are shown in Table 1. The biofilm volume (V_b) in each biofilter was estimated based on the experimental biomass content and the dimensions of the packing material. The dry weight (ash-free) biomass content per mass of dry vermiculite was quantified as $102 \pm 15 \; mg_{biomass} g^{-1}$ in FB and $99 \pm 16 \; mg_{biomass} g^{-1}$ in FBB. Assuming a biofilm density of $1000 \; kg \; m^{-1}$, this is equivalent to a biofilm volume (V_b) of $8.4 \cdot 10^{-6} \; m^3$ and $8.1 \cdot 10^{-6} \; m^3$ for the FB and FBB, respectively. To ensure the feasibility of these values, biofilm depth was estimated by measuring the dimensions of 57 random vermiculite particles and estimating their volume as a parallelepiped. Assuming a biofilm density of $1000 \; kg \; m^{-1}$, the required biofilm depth to achieve the measured biomass contents per mass of vermiculite were 25.5 μm and 24.5 μm for FB and FBB, respectively. These biofilm thicknesses were comparable to the 40 μm measured in a bacterial biofilm of a biofilter degrading a mixture of benzene and toluene [33], but were smaller than the 240-280 μm reported by Cox *et al.* [34] for the biofilm formed in a biofilter degrading styrene (a less hydrophobic compound compared to CH4).

$K_B a$ values for each biofilter were directly proportional to the inlet air flow rate, with coefficients of determination of 0.997 and 0.954 for the FB and FBB, respectively. Despite data of global mass transfer coefficients for biofilters are scarce in the literature, the estimated $K_B a$ values compared well with the values found by Nielsen *et al.* [35] for toluene biofiltration using lightweight aggregates (Leca® pellets) as support with $K_L a$ values ranging from 9.7 to 38.2 h^{-1}. Considering the aerial morphology of fungal hyphae compared to planar biofilms formed by bacteria (see Figure S4 and S5), an increase in the specific area for mass transfer could be expected, an observation previously reported in literature [9,30]. However, the mass transfer coefficients calculated in this study revealed that the $K_B a$ values of FB are smaller than those estimated for FBB. On the other hand, *n*-pentane cofeeding experiments showed that CH$_4$ EC was improved by the presence of a VOC known to increase the hydrophobicity of *F. solani* [36]. Moreover, the sensitivity analysis of the values estimated for the model parameters (see Figures S2 and S3) indicated that, with the exception of the $K_B a$ values for steady state experiments, the sensitivity of the model towards the mass transfer coefficient values was low. In brief, these findings highlight the key role of hydrophobicity (decrease of the pollutant partition coefficient) over the potential enhancements of the morphological structure of aerial hyphae in fungal-based biofilters.

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Table 1. Parameters obtained after calibrating the model of mass-transfer and reaction (Eqs. 4-6) using the experimental data presented in Figures 4 and 7.

Parameters	Units	Fungal biofilter (FB)	Fungal bacterial biofilter (FBB)
$K_B a_{0.3\ \text{LPM}}$	h^{-1}	2.5	8.5
$K_B a_{0.6\ \text{LPM}}$	h^{-1}	6.9	12.3
$K_B a_{0.86\ \text{LPM}}$	h^{-1}	10.2	19.1
$K_B a_{SS-0.19LPM}$	h^{-1}	0.6	2.7
K_s	$g\ CH_4 m_{biomass}^{-3}$	0.3	-
q_{max}	$g\ CH_4 g_{biomass}^{-1} h^{-1}$	4200	-
k	$m^3 g_{biomass}^{-1} h^{-1}$	-	914

Figure 8 shows the effect of the gas flow rate over the Damköhler number on FB and FBB. In FBB, the Damköhler number was independent of the inlet CH_4 concentration as a result of the first-order kinetic assumption (see Eq. 7). However, the inlet CH_4 concentration influenced the Damköhler number in FB and this effect is also shown in Figure 8. Interestingly, our calculations suggest that FBB was mass transfer limited regardless of the inlet flow tested, which agreed with observations reported in section 3.3. On the other hand, FB seems to be bioreaction limited except at low CH_4 concentrations and low gas flow rates (0.19 L min^{-1} equivalent to an EBRT of 12.4 min), an observation supported by the influence of EBRT on CH_4 EC (see Section 3.3). Gomez- Borraz *et al.* [16] concluded that the performance of a compost bacteria-colonized biofilter was limited by the mass transport instead of by the bioreaction at a similar *EBRT* of 19 min and an inlet CH_4 concentration of 21 g m^{-3}.

The sensitivity analysis of the model parameters over a wide range (0.5 to 1.5 times the optimal value) indicates that for the FBB the parameter with the highest sensitivity was $K_B a_{SS-0.19LPM}$, while the mass transfer coefficients for the experiments performed at higher gas velocities have little impact on model fit (see supplementary material, Figures S2 and S3). Interestingly, the model

fit showed little sensitivity towards the first-order reaction rate constant. A similar trend was found in FB, albeit in this case the model was found to be sensitive to the maximum specific uptake rate (q_{max}) values. The sensitivity analysis confirmed the trends highlighted by the analysis of the Damköhler numbers.
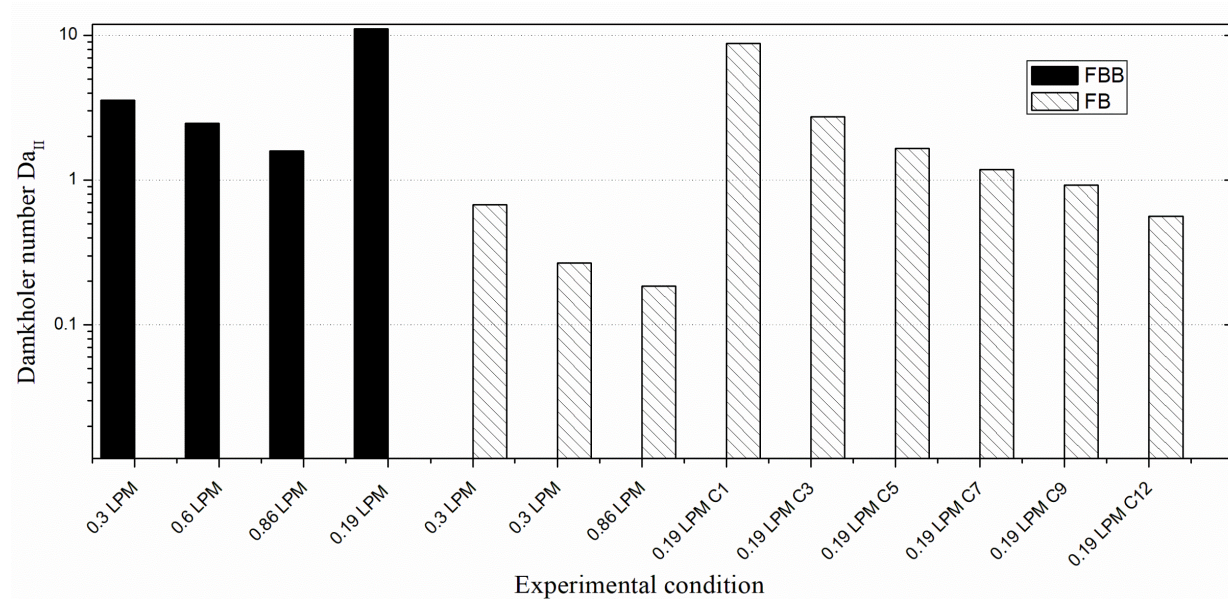

Figure 8. Estimated second Damköhler numbers at different inlet flow rates (logarithmic scale). For both biofilters, the flows 0.3, 0.6 and 0.86 L min^{-1} corresponded to the dynamic assays (Figure 7). Values shown for 0.19 L min^{-1} were obtained at steady-state under different inlet CH_4 concentrations (concentrations C1 to C12 in g m^{-3}: 6, 19.2, 31.9, 44.6, 57.2, and 94.0).

4   Conclusion

This work confirmed that the filamentous fungi *Fusarium solani* can degrade CH_4 as the sole carbon and energy source. The CH_4 elimination capacities of a biofilter inoculated with *F. solani* and bacterial methanotrophic consortium were higher than those recorded in a biofilter inoculated only with fungi regardless of the inlet loads and EBRTs tested, except when *n*-pentane was co-fed along with CH_4. The exposure of the fungal biomass to *n*-pentane, a highly hydrophobic and easily

degradable VOC, during CH4 biofiltration further improved the CH_4 EC likely due to an increase in the surface hydrophobicity and transport area of fungal hyphae. Overall, the fungal filter performance was bioreaction limited, while mass transport limitations were encountered in the fungal/bacterial biofilter. Finally, the estimated mass transfer coefficients and Damköhler numbers suggest that the beneficial effect of *F. solani* during CH_4 biofiltration was mediated by biomass hydrophobicity rather than to the formation of aerial hyphae structures increasing the mass transfer area.

5 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6 Acknowledgments

7 References

[1]   T. Skytt, S.N. Nielsen, B.-G. Jonsson, Global warming potential and absolute global temperature change potential from carbon dioxide and methane fluxes as indicators of regional sustainability – A case study of Jämtland, Sweden, Ecol. Indic. 110 (2020) 105831. doi:10.1016/j.ecolind.2019.105831.

[2]   J. Nikiema, R. Brzezinski, M. Heitz, Elimination of methane generated from landfills by biofiltration: a review, Rev. Environ. Sci. Bio/Technology. 6 (2007) 261–284. doi:10.1007/s11157-006-9114-z.

[3]   J.C. López, G. Quijano, T.S.O. Souza, J.M. Estrada, R. Lebrero, R. Muñoz, Biotechnologies for greenhouse gases (CH4, N2O, and CO2) abatement: state of the art and challenges, Appl. Microbiol. Biotechnol. 97 (2013) 2277–2303. doi:10.1007/s00253-013-4734-z.

[4]   C. Du Plessis, J. Strauss, E. Sebapalo, K. Riedel, Empirical model for methane oxidation using a composted pine bark biofilter⋆, Fuel. 82 (2003) 1359–1365. doi:10.1016/S0016-2361(03)00040-1.

[5]   M. Girard, A.A. Ramirez, G. Buelna, M. Heitz, Biofiltration of methane at low concentrations representative of the piggery industry—Influence of the methane and nitrogen concentrations, Chem. Eng. J. 168 (2011) 151–158. doi:10.1016/j.cej.2010.12.054.

[6]   S. Arriaga, S. Revah, Improving hexane removal by enhancing fungal development in a microbial consortium biofilter, Biotechnol. Bioeng. 90 (2005) 107–115. doi:10.1002/bit.20424.

[7]   J.W. Van Groenestijn, J.X. Liu, Removal of alpha-pinene from gases using biofilters containing fungi, Atmos. Environ. 36 (2002) 5501–5508. doi:10.1016/S1352-2310(02)00665-9.

[8]   A. Vergara-Fernández, B. Van Haaren, S. Revah, Phase partition of gaseous hexane and surface hydrophobicity of Fusarium solani when grown in liquid and solid media with hexanol and hexane, Biotechnol. Lett. 28 (2006). doi:10.1007/s10529-006-9186-4.

[9]   A. Vergara-Fernández, S. Hernández, S. Revah, Phenomenological model of fungal biofilters for the abatement of hydrophobic VOCs, Biotechnol. Bioeng. 101 (2008) 1182–1192. doi:10.1002/bit.21989.

24

[10] R. Muñoz, S. Villaverde, B. Guieysse, S. Revah, Two-phase partitioning bioreactors for treatment of volatile organic compounds, Biotechnol. Adv. 25 (2007) 410–422. doi:10.1016/j.biotechadv.2007.03.005.

[11] A. Vergara-Fernández, P. Morales, F. Scott, S. Guerrero, L. Yañez, S. Mau, G. Aroca, Methane biodegradation and enhanced methane solubilization by the filamentous fungi Fusarium solani., Chemosphere. 226 (2019) 24–35. doi:10.1016/j.chemosphere.2019.03.116.

[12] M. Girard, P. Viens, A.A. Ramirez, R. Brzezinski, G. Buelna, M. Heitz, Simultaneous treatment of methane and swine slurry by biofiltration, J. Chem. Technol. Biotechnol. 87 (2012) 697–704. doi:10.1002/jctb.3692.

[13] R. Lebrero, J.C. López, I. Lehtinen, R. Pérez, G. Quijano, R. Muñoz, Exploring the potential of fungi for methane abatement: Performance evaluation of a fungal-bacterial biofilter, Chemosphere. 144 (2016) 97–106. doi:10.1016/j.chemosphere.2015.08.017.

[14] J.P. Oliver, J.S. Schilling, Capture of methane by fungi: evidence from laboratory-scale biofilter and chromatographic isotherm studies, Trans. ASABE. 59 (2016) 1791–1801. doi:10.13031/trans.59.11595.

[15] V.B. Stein, J.P. Hettiaratchi, Methane oxidation in three Alberta soils: influence of soil parameters and methane flux rates., Environ. Technol. 22 (2001) 101–11. doi:10.1080/09593332208618315.

[16] T.L. Gómez-Borraz, A. González-Sánchez, W. Bonilla-Blancas, S. Revah, A. Noyola, Characterization of the biofiltration of methane emissions from municipal anaerobic effluents, Process Biochem. 63 (2017) 204–213. doi:10.1016/j.procbio.2017.08.011.

[17] V.C. Hettiarachchi, J.P.A. Hettiaratchi, A.K. Mehrotra, Comprehensive One-Dimensional Mathematical Model for Heat, Gas, and Moisture Transport in Methane Biofilters, Pract.

Period. Hazardous, Toxic, Radioact. Waste Manag. 11 (2007) 225–233. doi:10.1061/(ASCE)1090-025X(2007)11:4(225).

[18] M. Cáceres, J.C. Gentina, G. Aroca, Oxidation of methane by Methylomicrobium album and Methylocystis sp. in the presence of H2S and NH3, Biotechnol. Lett. 36 (2014) 69–74. doi:10.1007/s10529-013-1339-7.

[19] P. Morales, M. Cáceres, F. Scott, L. Díaz-Robles, G. Aroca, A. Vergara-Fernández, Biodegradation of benzo[α]pyrene, toluene, and formaldehyde from the gas phase by a consortium of Rhodococcus erythropolis and Fusarium solani, Appl. Microbiol. Biotechnol. 101 (2017) 6765–6777. doi:10.1007/s00253-017-8400-8.

[20] E.I. García-Peña, S. Hernández, E. Favela-Torres, R. Auria, S. Revah, Toluene biofiltration by the fungus Scedosporium apiospermum TB1, Biotechnol. Bioeng. 76 (2001) 61–69. doi:10.1002/bit.1026.

[21] S. Arriaga, S. Revah, Removal of n-hexane by Fusarium solani with a gas-phase biofilter, J. Ind. Microbiol. Biotechnol. 32 (2005) 548–553. doi:10.1007/s10295-005-0247-9.

[22] M. Cáceres, A.D. Dorado, J.C. Gentina, G. Aroca, Oxidation of methane in biotrickling filters inoculated with methanotrophic bacteria, Environ. Sci. Pollut. Res. 24 (2017) 25702–25712. doi:10.1007/s11356-016-7133-z.

[23] A. Vergara-Fernández, S. Hernández, S. Revah, Elimination of hydrophobic volatile organic compounds in fungal biofilters: Reducing start-up time using different carbon sources, Biotechnol. Bioeng. 108 (2011). doi:10.1002/bit.23003.

[24] T. Van Daele, D. Fernandes del Pozo, D. Van Hauwermeiren, K. V. Gernaey, R. Wohlgemuth, I. Nopens, A generic model-based methodology for quantification of mass transfer limitations in microreactors, Chem. Eng. J. 300 (2016) 193–208. doi:10.1016/j.cej.2016.04.117.

26

[25]   M.A. Deshusses, G. Hamer, I.J. Dunn, Behavior of Biofilters for Waste Air Biotreatment. 1. Dynamic Model Development, Environ. Sci. Technol. 29 (1995) 1048–1058. doi:10.1021/es00004a027.

[26]   K.D. Kits, M.G. Kalyuzhnaya, M.G. Klotz, M.S.M. Jetten, H.J.M. Op den Camp, S. Vuilleumier, F. Bringel, A.A. DiSpirito, J.C. Murrell, D. Bruce, J.-F. Cheng, A. Copeland, L. Goodwin, L. Hauser, A. Lajus, M.L. Land, A. Lapidus, S. Lucas, C. Medigue, S. Pitluck, T. Woyke, A. Zeytun, L.Y. Stein, Genome Sequence of the Obligate Gammaproteobacterial Methanotroph Methylomicrobium album Strain BG8, Genome Announc. 1 (2013). doi:10.1128/genomeA.00170-13.

[27]   A. Vorobev, S. Jagadevan, S. Jain, K. Anantharaman, G.J. Dick, S. Vuilleumier, J.D. Semrau, Genomic and Transcriptomic Analyses of the Facultative Methanotroph Methylocystis sp. Strain SB2 Grown on Methane or Ethanol, Appl. Environ. Microbiol. 80 (2014) 3044–3052. doi:10.1128/AEM.00218-14.

[28]   C. Pratt, A.S. Walcroft, K.R. Tate, D.J. Ross, R. Roy, M.H. Reid, P.W. Veiga, Biofiltration of methane emissions from a dairy farm effluent pond, Agric. Ecosyst. Environ. 152 (2012) 33–39. doi:10.1016/j.agee.2012.02.011.

[29]   J.C. López, L. Merchán, R. Lebrero, R. Muñoz, Feast-famine biofilter operation for methane mitigation, J. Clean. Prod. 170 (2018) 108–118. doi:10.1016/j.jclepro.2017.09.157.

[30]   A. Vergara-Fernández, F. Scott, P. Moreno-Casas, L. Díaz-Robles, R. Muñoz, L. Diaz-Robles, R. Muñoz, L. Díaz-Robles, R. Muñoz, Elucidating the key role of the fungal mycelium on the biodegradation of n-pentane as a model hydrophobic VOC, Chemosphere. 157 (2016) 89–96. doi:10.1016/j.chemosphere.2016.05.034.

[31]   A. Vergara-Fernández, B. Van Haaren, S. Revah, Phase partition of gaseous hexane and surface hydrophobicity of Fusarium solani when grown in liquid and solid media with

hexanol and hexane, Biotechnol. Lett. 28 (2006) 2011–2017. doi:10.1007/s10529-006-9186-4.

[32] A. Vergara-Fernández, S. Hernández, J. San Martín-Davison, S. Revah, Morphological characterization of aerial hyphae and simulation growth of Fusarium solani under different carbon source for application in the hydrophobic VOCs biofiltration, Rev. Mex. Ing. Química. 10 (2011) 225–233.

[33] S.M. Zarook, A.A. Shaikh, Z. Ansar, B.C. Baltzis, Biofiltration of volatile organic compound (VOC) mixtures under transient conditions, Chem. Eng. Sci. 52 (1997) 4135–4142. doi:10.1016/S0009-2509(97)00256-X.

[34] H.H.J. Cox, R.E. Moerman, S. van Baalen, W.N.M. van Heiningen, H.J. Doddema, W. Harder, Performance of a styrene-degrading biofilter containing the yeastExophiala jeanselmei, Biotechnol. Bioeng. 53 (1997) 259–266. doi:10.1002/(SICI)1097-0290(19970205)53:3<259::AID-BIT3>3.0.CO;2-H.

[35] A.M. Nielsen, L.P. Nielsen, A. Feilberg, K.V. Christensen, A Method for Estimating Mass-Transfer Coefficients in a Biofilter from Membrane Inlet Mass Spectrometer Data, J. Air Waste Manage. Assoc. 59 (2009) 155–162. doi:10.3155/1047-3289.59.2.155.

[36] A. Vergara-Fernández, O. Soto-Sánchez, J. Vásquez, A. Vergara-Fernandez, O. Soto-Sanchez, J. Vasquez, A. Vergara-Fernández, O. Soto-Sánchez, J. Vásquez, O. Sóto-Sanchez, J. Vásquez-Bestagno, A. Vergara-Fernandez, O. Soto-Sanchez, J. Vasquez, Effects of Packing Material Type on n-Pentane/Biomass Partition Coefficient for Use in Fungal Biofilters, Chem. Biochem. Eng. Q. 25 (2011) 439–444.

Myhre, G., D. Shindell, F.-M. Bréon, W. Collins, J. Fuglestvedt, J. Huang, D. Koch, J.-F. Lamarque, D. Lee, B. Mendoza, T. Nakajima, A. Robock, G. Stephens, T. Takemura and H. Zhang, 2013: Anthropogenic and Natural Radiative Forcing. In: Climate Change 2013: The

28

Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the

Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor,

S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge

University Press, Cambridge, United Kingdom and New York, NY, USA.

Stein, V.B., Hettiaratchi, J.P., 2001. Methane oxidation in three Alberta soils: influence of soil

parameters and methane flux rates. Environ. Technol. 22, 101–11.

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Supporting Information

A comparative assessement of the performance of fungal-bacterial and fungal biofilters for methane abatement

Alberto Vergara-Fernández[1]*, Felipe Scott[1], Felipe Carreño[1], Germán Aroca[2], Patricio Moreno-Casas[1], Armando González-Sánchez[4] and Raúl Muñoz[3]

A simplified version of the mathematical model of a biofilter proposed by Deshusses et al. [25] was applied for the estimation of the global volumetric mass transfer coefficient ($K_B a$). In the original work of Deshusses et al. [25], the gas phase in a biofilter was modeled as a cascade of stirred tank reactors (STRs). In the present work, each STR containing gas phase was connected not only to the previous and the following gas-phase STR, but also to the a STR representing the biofilm where bioreactions occur (see Figure S1). The super-script j stands for each biofilter.

Assumptions:

- Each subdivision of the gas-phase and the biofilm-phase is ideally mixed.

- The i-th gas phase STR has a volume equal to $\epsilon V_p / N$, where N is the number of reactors aling the height of biofilter.

- The i-th layer is fed from the i-1 stage at a rate QG_{i-1}^j and the methane mass flow exiting this stage is QG_i^j.

- Methane from the i-th gas-phase stage is transferred to the i-th biofilm phase at a rate $J_i = \frac{K_B a (1-\epsilon) V_p}{N}\left(\frac{G_i^j}{H} - B_i^j\right)$, where B_i^j is the dissolved methane concentration in the i-th layer of the j-th biofilter.

- Equilibrium and non-accumulation are assumed at the interface, hence the J_i is also the rate at which methane enters to the i-th biofilm section with volume V_b/N.

- In each section of the biofilm, no net growth of biomass is assumed during the experiments used for model calibration. Therefore, the biomass concentration X_b^j is constant and experimentally assessed. This assumption is justified since $K_L a$ estimation experiments and inlet load effect experiments (Figures 4 and 8) lasted three weeks.

- Moreover, it is assumed that the biocatalyst is homogeneously distributed throughout the biofilm.

- Finally, based on the results obtained in the experiments where inlet-load was changed, a first-order reaction kinetic was assumed to represent the activity of the biocatalyst in the biofilm for FBB and a Monod type kinetic for FB.

- $$Q_s^j(C_b) = \begin{cases} k X_b^j B_j, & \text{for } j = \text{FBB} \\ q_{max} X_b^j \dfrac{B^j}{K_s + B^j}, & \text{for } j = \text{FB} \end{cases} \qquad \text{Eq. S1}$$
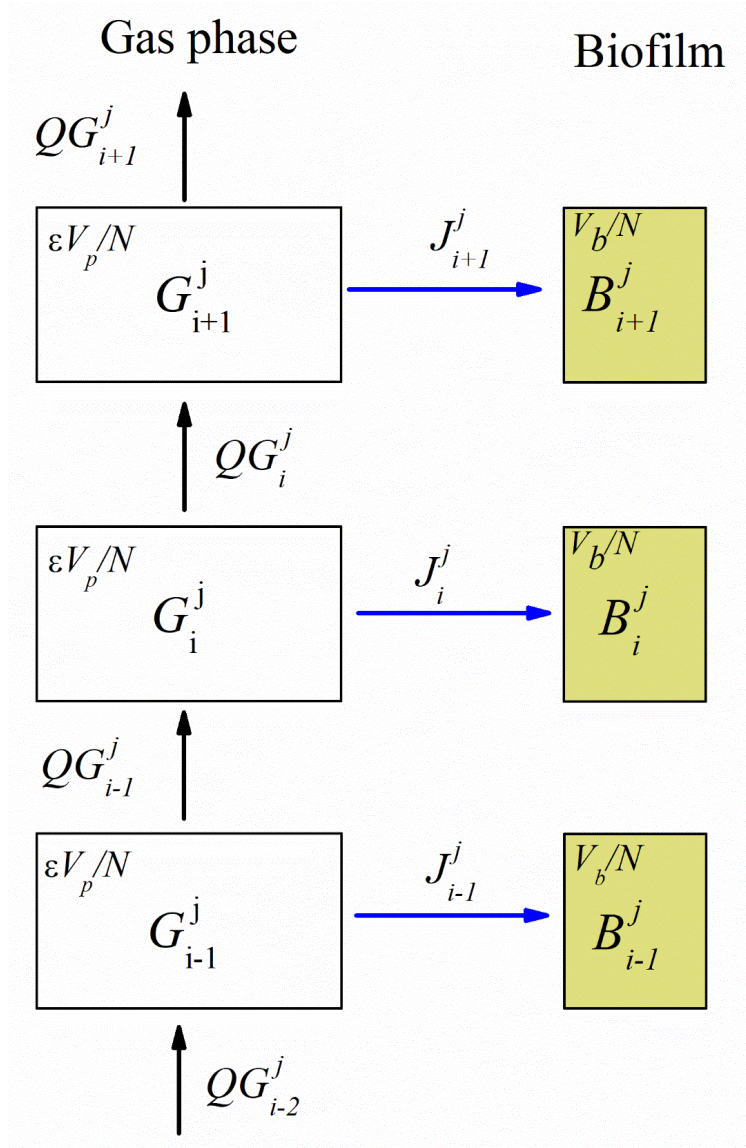
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Figure S1. Schematic description of the mathematical model for one section of the biofilter. The CH$_4$ laden air flows through the gas phase and CH$_4$ is transferred from each subdivision of the gas phase to its corresponding section of the biofilm.

The acronym ε represents the empty fraction of the bed, A$_T$ the cross-section of the reactor, Q the gas flow, K$_B$a^j the global volumetric mass transfer coefficient based on the volume of biofilm and H$_j$ the air-biofilm methane partition coefficient. The partition coefficient for the fungal biomass was

directly taken from a previous work by the authors [11], while the partition coefficient for the fungal-bacterial biofilms was estimated as 0.46 times the partition coefficient of the bacterial biomass and 0.64 times the partition coefficient of the fungal biomass. The factors 0.46 and 0.64 correspond to the contributions of bacteria and fungi towards methane degradation in the microcosms inoculated with biomass withdrawn from the FBB (see Section 2.6).

A non-steady state methane mass balance to the gas phase leads to (Eq. 5).

$$\frac{dG_i^j}{dt} = \frac{Q \cdot N}{\epsilon \cdot A_T \cdot L}\left[G_{i-1}^j(t) - G_i^j(t)\right] - K_B a^j \frac{(1-\epsilon)}{\epsilon}\left(\frac{G_i^j(t)}{H^j} - B_i^j(t)\right) \qquad \text{Eq. S2}$$

With boundary conditions:

$G_i^j(0) = 0$ for $i = \{1, \dots, N\}$ (empty initial reactor)

$$G_i^j(t) = \begin{cases} G^{in} \text{ (feed concentration during steady state)} \\ 0 \text{ (during kLa experiments when methane flow is off)} \end{cases}$$

A methane balance to the i-th section of the homogenous biomass phase yields:

$$\frac{dB_i^j}{dt} = K_B a^j (1 - \epsilon)\frac{V_R}{V_b^j}\left(\frac{G_i^j(t)}{H^j} - B_i^j(t)\right) - Q_s^j(B_i^j) \qquad \text{Eq. S3}$$

The initial condition for this equation is either $B_i(0) = 0$, for all sections (empty reactor), or the steady-state concentration per stage calculated using the operating conditions. The model parameters $K_B a^j$ value is dependent on the gas flow velocity. Therefore, a unique set of biokinetic parameters k, K_s and q_{max} was estimated for all the experiments, but eight values of $K_B a$ were fitted to account for the different gas flow rates (see Table 1). Parameters were estimated using non-linear fitting (patternsearch, MATLAB®). The steady-state simulations were obtained by dropping the differential terms in Eqs. (S2) and (S3).

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Sensitivity analysis

Model fit was sensibilized for each parameter in an interval where the minimum value was 50% of the best fit of a parameter and the maximum value was 1.5 fold. The sensitivity analysis was carried out by varying two parameters at a time, one of the parameters being the maximum substrate uptake rate (q_{max}) for the fungal biofilter or the first order coefficient for the fungal-bacterial biofilter (k). The second parameter was a mass transfer coefficient. A full factorial approach was used, thereby each response surface is formed by 100 combinations were the average distance between the predicted and experimental values was calculated. The average distance was defined as $\frac{\sqrt{SSE^j}}{N_m^j}$, where $SSE = (\epsilon^j)^T \epsilon^j$ was the sum of the squared error and was calculated as the product of the vector $\epsilon^j = G_m^j - G_{out}^{j,*}$, where G_m^j was the vector of the measured methane concentrations in the outlet of each biofilter j and $G_{out}^{j,*}$ the vector of methane concentration predicted by the model for the optimal set of estimated parameters. Finally, N_m^j denoted the number of experimental measurents used for each biofilter.
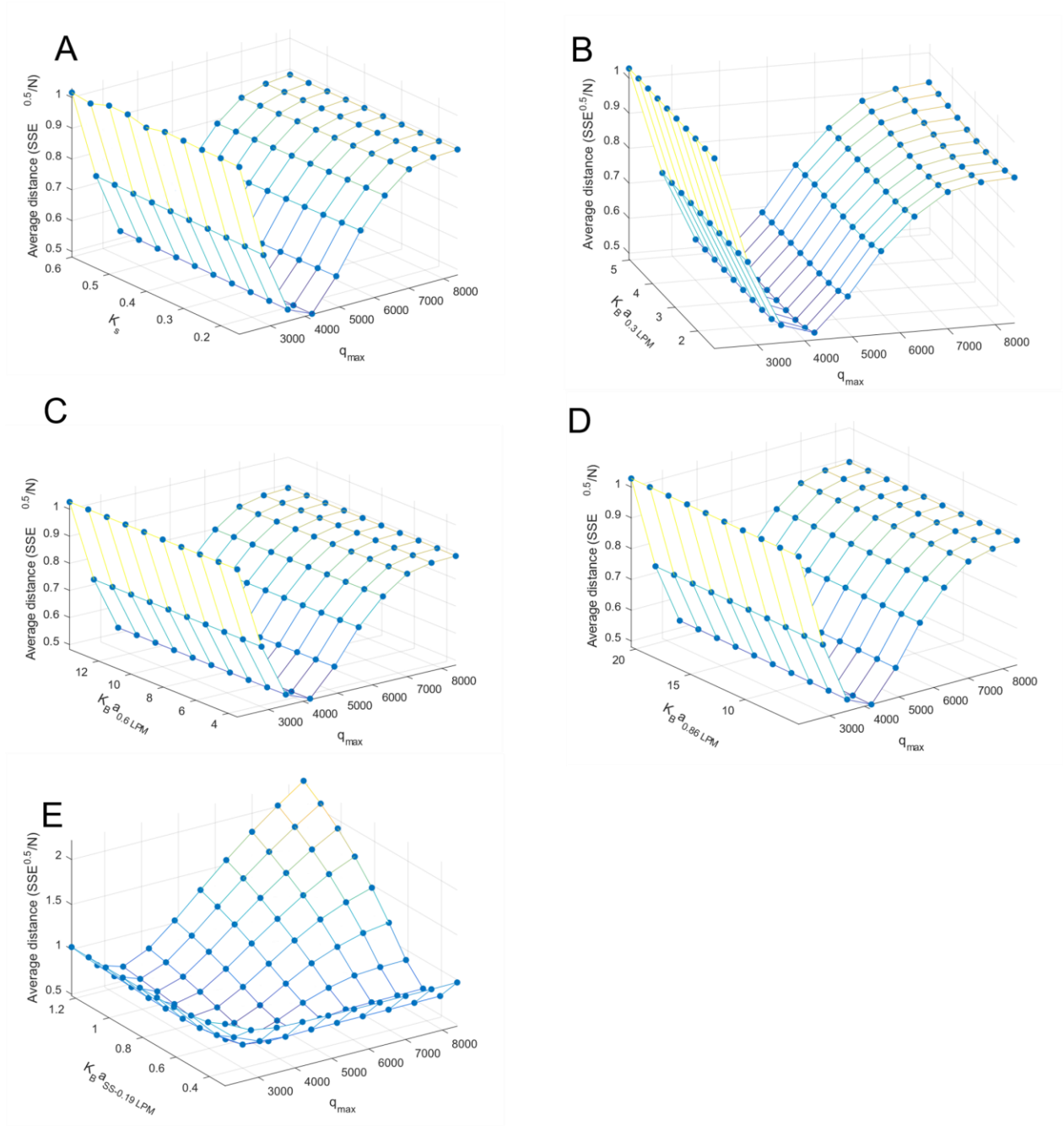
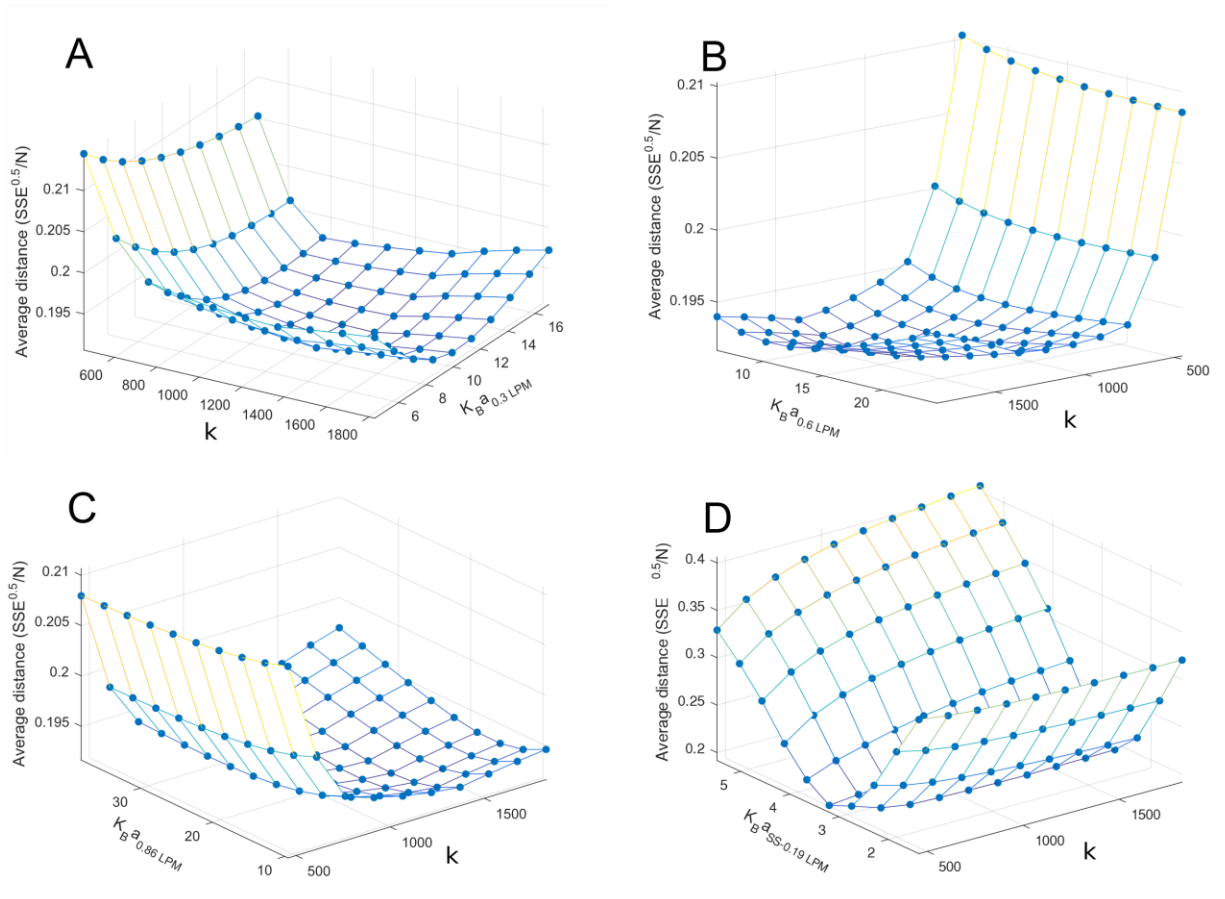**Figure S2**. Sensitivity analysis of the optimal parameter values estimated for the FB..

Figure S3. Sensitivity analysis of the optimal parameter values estimated for FBB.

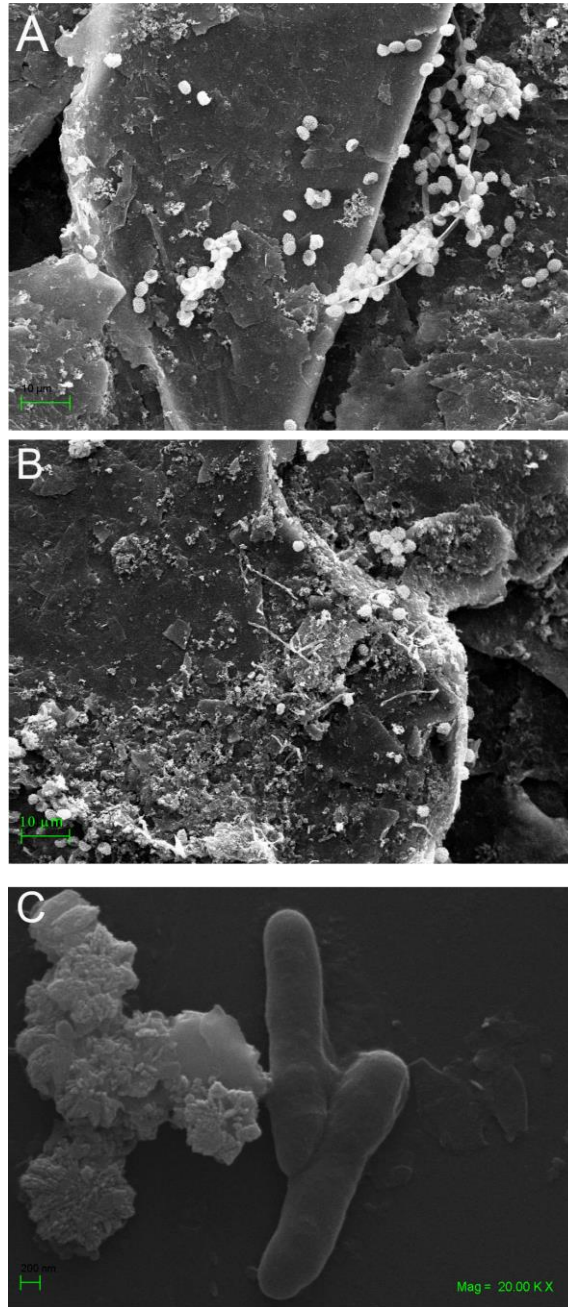## Scanning electron microscopy.

Figure S4. Scanning electron microscopy of the packing material withdrawn from the fungal-bacterial biofilter. The scale bar of A and B is 10 µm, C is 200 nm. Photographs A and B show the characteristic chlamydospores of *F. solani.* Photograph C shows two macroconidia next to what appears to be a crystal.
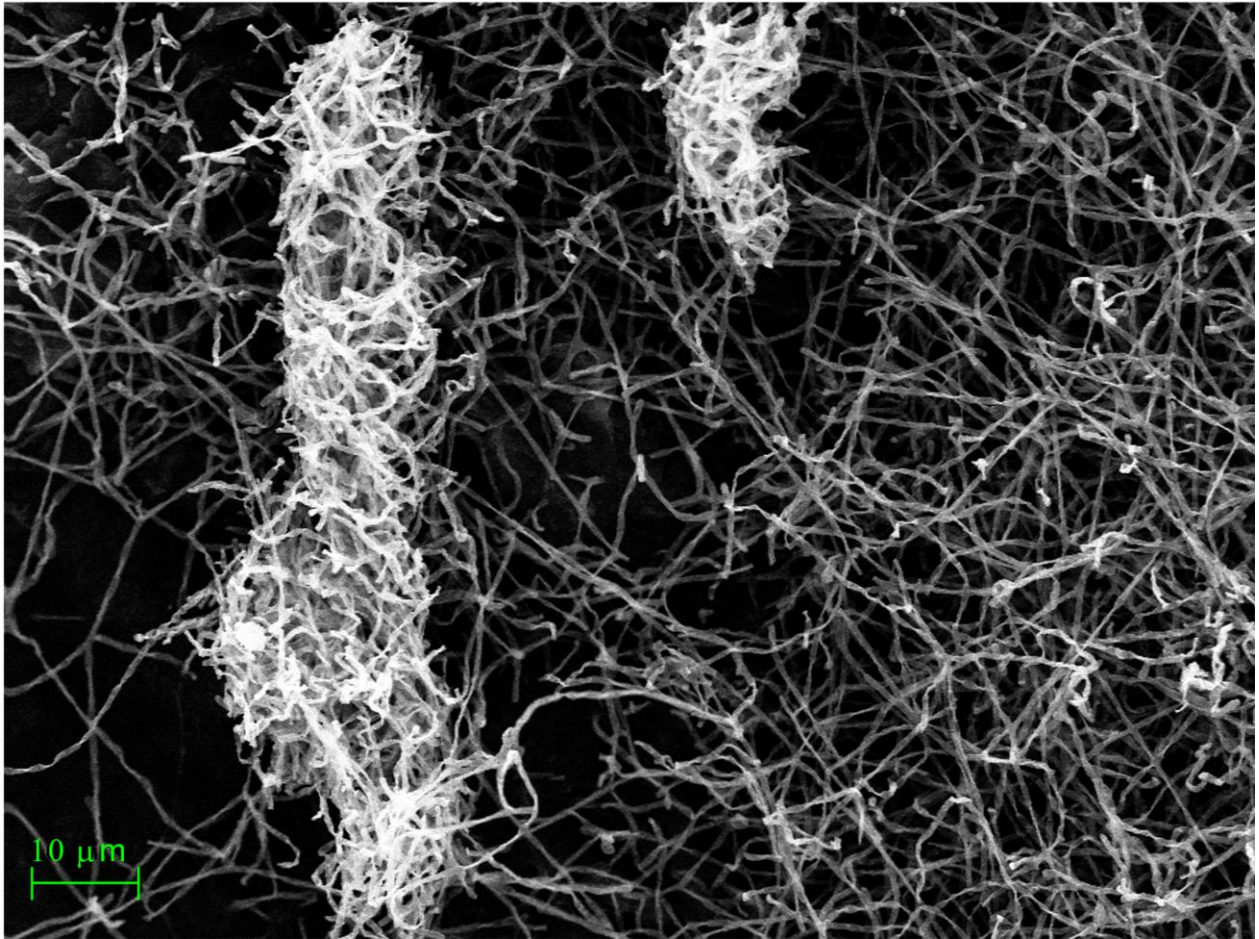
Figure S5. Scanning electron microscopy of the packing material withdrawn from the fungal biofilter. The scale bar is 10 μm. The image shows the characteristic hyphae and microconidia of *F. solani.*

Conflict of interest declaration for:

A comparative assessment of the performance of fungal-bacterial and fungal biofilters for methane abatement

Alberto Vergara-Fernández, Felipe Scott, Felipe Carreño, Germán Aroca, Patricio Moreno-Casas, Armando González-Sánchez and Raúl Muñoz.