Chemical Engineering Journal 510 (2025) 161383

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Enhancing dilute methane treatment through liquid phase alteration in a capillary bioreactor

Norbertus J.R. Kraakman [a,b], Luis Villarreal-Heras [a,b], Javier González-Martín [a,b], Sara Cantera [a,b], Raúl Muñoz [a,b], Raquel Lebrero [a,b,*]

[a] Institute of Sustainable Processes, University of Valladolid, Dr. Mergelina s/n., Valladolid 47011, Spain
[b] Department of Chemical Engineering and Environmental Technology, University of Valladolid, Dr. Mergelina s/n., Valladolid 47011, Spain

ARTICLE INFO

Keywords:
Capillary bioreactor
Dilute methane
Gas treatment
Mass transfer
Non-aqueous phase liquid
Surfactants

ABSTRACT

This study aimed to maximize the treatment of dilute methane emissions (<5% v/v) using a capillary bioreactor (CBR) to overcome the mass transfer limitations commonly encountered in biological technologies. Three universally used non-ionic surfactants (BRIJ 58, TWEEN 60 and SDBS) were tested for their ability to enhance methane bioavailability when combined with a non-aqueous liquid (silicone oil). The study evaluated each surfactant's effectiveness in increasing methane bioavailability, enhancing the cell hydrophobicity of a mixed culture of methane oxidizing bacteria, and improving the oil-in-water emulsion capacity at a concentration low enough to eliminate the risk of microbial inhibition. BRIJ 58 was selected and showed in combination with silicone oil potential to enhance gas–liquid mass transfer by >50 % in a capillary channel under segmented (Taylor) flow regime. The optimised liquid phase in the CBR supported stable removal of the methane (~4500 ppm_v = 0.45 % v/v) with elimination capacities over 200 g $m^{-3}h^{-1}$ at an empty capillary channel gas contact time of 23 s, which is one order of magnitude lower than the empty bed gas contact time of conventional biological gas treatment methods treating dilute methane. The improved emulsification of the oil-in-water emulsion combined with enhanced cell hydrophobicity appeared to be the main mechanism. Internal gas recirculation was applied to decouple the optimal gas–liquid turbulence conditions inside the capillary channel from the actual gas retention time. The study demonstrated that the addition of 20 % silicon oil and 160 mg L^{-1} BRIJ 58 significantly improved the overall methane abatement performance.

1. Introduction

Methane is the most abundant atmospheric organic gas released from major anthropogenic emission sources such as landfills, oil and natural gas systems, agricultural activities, coal mining, stationary and mobile combustion, and wastewater treatment processes. It is a significant greenhouse gas, responsible for around 30 % of the rise in global temperatures since the Industrial Revolution. The need for methane mitigation has increased dramatically as research indicates that this gas has greater climatic impact than previously thought [1]. In addition, as global efforts to mitigate CO_2 falter, aggressive methane mitigation is emerging as a lower cost strategy to curb climate change. Thus, reductions in methane emissions are imperative to controlling near-term global warming and improving air quality [2]. However, approximately 55 % of all the anthropogenic methane emissions have a concentration below the lower explosive limit of methane in air mixtures of 5 % v/v and are incompatible for energy recovery or for physical–chemical oxidation abatement processes. The cost of mitigating methane emissions strongly varies depending on the sources [3,4], but the economic viability of mitigation is especially challenging when the methane concentration is below 5 % v/v in air [5].

Microorganisms are capable of efficiently mineralising methane, but microbial activity is dependent on methane bioavailability in biological gas treatment systems. The treatment of methane-laden gaseous streams using biological methods presents challenges due to methane's poor solubility in water, high volatility, and chemical stability. Conventional biological gas treatment systems such as biofilters, biotrickling filters and bioscrubbers operate as laminar contactors. These configurations are limited in the removal of poorly water-soluble compounds and require extended gas residence times – often of several minutes – to achieve efficient removal due to the limited bioavailability of methane [6–9]. The laminar flow in conventional biological gas treatment

* Corresponding author at: Institute of Sustainable Processes, University of Valladolid, Dr. Mergelina s/n., Valladolid 47011, Spain.
E-mail address: raquel.lebrero@uva.es (R. Lebrero).

https://doi.org/10.1016/j.cej.2025.161383
Received 27 September 2024; Received in revised form 26 December 2024; Accepted 7 March 2025
Available online 10 March 2025

systems is a flow regime characterized by high diffusion and low advection and is the opposite of turbulent flow. Improved convection by advection (i.e., the transport by the larger-scale motion of currents in a medium, for example through mixing) would improve mass transfer. In this context, capillary reactors when operated under segmented (Taylor) flow regime provide an internal liquid recirculation that combines enhanced mass transfer with low pressure drops, two important factors affecting cost effectiveness for many industrial applications [10–12].

The addition of a non-aqueous phase to gas-treatment bioreactors has been shown in multiple studies to enhance the removal of hydrophobic compounds [13–16]. Silicone oil is generally considered the most suitable oil phase, with concentrations in the liquid media of the bioreactor of up to approximately 30 % (v/v) [13]. However, operational problems such as foaming and adhesion to reactor internals have been reported when using silicone oil. Moreover, oils have a different viscosity and surface tension than water and can therefore change the liquid physical characteristics and hydrodynamics, which can impact on the performance of many reactor types, including capillary reactors.

Surfactants have also shown to facilitate the removal of hydrophobic contaminants from contaminated soil and water, and to improve bioavailability through decreasing interfacial tension at the gas–liquid phase in gas treatment reactors [17,18]. Indeed, different studies have demonstrated that the use of surfactant can improve performance in gas treatment bioreactors. However, these studies have mainly focused on the abatement efficiency, without analysing the mechanisms behind the improvements [8,17]. In this sense, it is assumed that the addition of surfactants increases the solubility of the contaminant by reducing the surface tension at the immiscible phase and through micelles formation. However, other mechanisms have been also suggested that could enhance contaminant bioavailability, including increasing cell hydrophobicity, solubilization or emulsification of insoluble matter (such as biofilm material, grease, and oils), or promoting overall microbial metabolism if an easy-to-degrade surfactant is used [17]. Conversely, surfactants can lead to substrate competition (i.e., degradation of the surfactant versus contaminant), can alter essential bacterial proteins and inactivate enzymes on the bacterial outer membrane or can cause cell membrane disruption resulting in a negative effect in the biological gas treatment system [17,19].

Therefore, the addition of liquid phase additives in biological gas treatment reactors to improve overall performance in terms of removal efficiency and robustness requires a clear understanding of all the chemical, physical and biological processes involved. This study investigated the potential of dilute methane abatement using a capillary bioreactor and elucidated whether the liquid phase can be optimized to improve its overall performance. To this aim, synthetic surfactants were investigated to assess their potential to enhance bioavailability and mass transfer, both with and without the presence of silicone oil. Three non-ionic surfactants were selected for their widespread availability and common use in many households or industries as detergents, wetting agents, emulsifiers, foaming agents, or antistatic additives: BRIJ 58, TWEEN 60 and SDBS. To date, no studies have tested the effect of surfactant addition in a capillary bioreactor treating dilute methane in the presence of silicone oil as non-aqueous liquid phase.

2. Materials and methods

2.1. Overall approach

This study is divided into two parts: Part I focuses on the preliminary investigation of mass transfer under abiotic conditions, and Part II examines the biotic removal of dilute methane in a capillary bioreactor (CBR) containing multiple channels.

Part I – Mass transfer study

This section explores the impact of liquid modifications on the gas–liquid mass transfer rate in a single capillary channel operated with segmented (Taylor) gas–liquid flow under abiotic conditions. Different surfactants with and without a non-aqueous liquid phase (silicone oil) are studied. Key steps included:

1. Selection of universally used surfactants that are readily available at low cost and known to be biodegradable. Three non-ionic surfactants were chosen based on lower toxicity to bacteria compared to ionic surfactants.
2. Toxicity assessment: surfactants were tested at concentrations known to minimize microbial inhibition.
3. Methane Bioavailability Test: The maximum specific methane (CH_4) oxidation rate of a mixed methanotrophic consortium was determined in bottles containing dilute methane in the headspace at two concentrations of each surfactant after an adaptation period of 10 weeks.
4. Cell hydrophobicity analysis: The microbial cell hydrophobicity was measured to link observed methane oxidation rate differences to the cell hydrophobicity of the methanotrophic consortium.
5. Oil-in-water mixtures with increasing silicone oil concentrations and increasing surfactant concentrations were prepared to determine their Emulsion Capacity, Emulsion Stability and Foaming Potential.
6. Surfactant selection: The best-performing surfactant was chosen based on its ability to enhance emulsion stability and capacity, improve bacterial hydrophobicity, and boost CH_4 bioavailability without microbial inhibition.
7. Determination of the mass transfer rate of the selected surfactant in a single capillary channel configuration under abiotic conditions with and without the presence of silicone oil.

Part II – Biotic study in the CBR

This section investigates the effect of the addition of a selected surfactant (BRIJ 58) and silicone oil in a multi-channel CBR treating CH_4 using the methanotrophic consortium. Key steps included:

1. A CBR treating dilute methane using the methanotrophic consortium was operated to investigate the effect of the selected surfactant when combined with the silicone oil.
2. Process limitation assessment: Experiments were undertaken to elucidate whether the process was mass transfer or kinetically limited before and after liquid modifications.
3. Microbial community analysis: The structure of the methanotrophic consortium in the CBR was analyzed prior to liquid modifications and at the end of operation.

2.2. Chemicals and microorganisms

The medium used in this study consisted of a mineral salt medium containing KH_2PO_4 (0.7 g L^{-1}), $K_2HPO_4{\cdot}3\ H_2O$ (0.92 g L^{-1}), KNO_3 (3 g L^{-1}), NaCl (0.2 g L^{-1}), $MgSO_4{\cdot}7\ H_2O$ (0.35 g L^{-1}), $CaCl_2{\cdot}2\ H_2O$ (0.026 g L^{-1}) and 2 ml L^{-1} trace minerals solution containing EDTA (1 g L^{-1}), $FeSO_4{\cdot}7\ H_2O$ (0.08 g L^{-1}), $ZnSO_4{\cdot}7\ H_2O$ (0.005 g L^{-1}), $MnCl_2{\cdot}4\ H_2O$ (0.002 g L^{-1}), H_3BO_3 (0.001 g L^{-1}), $CoCl_2{\cdot}6\ H_2O$ (0.005 g L^{-1}), $CuCl_2{\cdot}2\ H_2O$ (0.001 g L^{-1}), $NiCl_2{\cdot}6\ H_2O$ (0.001 g L^{-1}) and $NaMoO_4{\cdot}2\ H_2O$ (0.002 g L^{-1}). The chemicals used for mineral salt medium preparation (PANREAC, Barcelona, Spain) had a purity of at least 99.0 %. The silicone oil (polydimethylsiloxanes) that was used as second liquid phase exhibited a viscosity of 20 cSt (Sigma-Aldrich, Madrid, Spain) or a viscosity of 220 cSt (Cogelsa, Spain). Surfactants used were TWEEN 60, BRIJ 58, and sodium dodecyl benzene sulfonate (SDBS) (all obtained from Sigma-Aldrich, Madrid, Spain).

The inoculum was obtained from two sources: fresh activated sludge from Valladolid wastewater treatment plant (Spain) and post-composted anaerobically digested sludge from Five Ford wastewater treatment plant (United Kingdom). Inocula were characterised as per methodology described in **Section 2.6**, before adding each ~50 % v/v in the final mixture.

Part I – Mass transfer study

2.3. Methane bioavailability test

From the mixed inoculum, methane oxidizing microorganisms were enriched in 2-L bottles containing 500 mL of medium while continuously stirred using a magnetic mixing plate at 400 rpm. An airflow of ~1 L min^{-1} containing methane at a concentration of ~500 ppm_v was continuously flowing through the headspace of the bottles. In total 10 bottles were used to which different additives (surfactant or oil) were periodically added to obtain the concentrations in the liquid shown in Table 1. The three selected surfactants TWEEN 80, BRIJ 58 and SDBS are all readily available at low cost and are biodegradable. In addition, these surfactants exhibit low potential for foaming and relatively low toxicity, and do not form salts with metallic ions when added to the nutrient solution to be dosed to a bioreactor due to their non-ionic character [20]. The concentration of the surfactants was slowly increased over time to minimise the risk of microbial inhibition. The final surfactant concentration was chosen either to obtain the critical micelle concentration (CMC) of the surfactant in water (TWEEN 60 and BRIJ 58) or to avoid microbial inhibition (SDBS) as observed in other biological gaseous filtration studies with SDBS and sodium dodecyl sulfate (SDS) [17,21].

The methane oxidation rate in the bottles was determined in Week 10, corresponding to 70 days after the initial addition of additives to the bottles. To measure the methane oxidation rate in each bottle, the continuous methane-containing air supply to the headspace was stopped, and the bottles were completely sealed with rubber stoppers and aluminium foil. The initial methane concentration in the headspace of each bottle was ~500 ppm_v (534 ± 12 ppm_v) and was measured in triplicate over time after 2, 5.5, 9, and 24 h. By the end of the test, the microbial cell hydrophobicity was determined as described next.

2.4. Microbial cell hydrophobicity

The hydrophobicity was determined in duplicate according to a slightly modified method as described by Wu and co-workers [21]. A sample of 40 mL was taken from each bottle after vigorously shaking. The harvested biomass was centrifuged for 5 min at 5000 rpm (Eppendorf, 5430 R) and then suspended in the original medium. Then the absorbance of the resuspended microorganisms (A1) was measured at 600 nm with UV–Vis spectrophotometer (Shimadzu UVmini-1240). The absorbance was kept at 0.5–0.6 with samples being diluted when necessary. Then 3 mL of the above suspended microorganisms solution was taken, and 0.75 mL of n-hexadecane was added, mixed using an advanced vortex mixer at 2400 rpm (ZX3, Velp Scientifica) while regularly shaking for exactly 2 min, and then stand for 30 min. Finally, the absorbance of the aqueous phase (A2) at 600 nm was measured and the cell surface hydrophobicity of microorganisms was calculated by the following Eq (1):

$$Cell\ Hydrophobicity(\%) = 100 \times (1 - A2/A1) \quad (1)$$

The measurement was repeated for each bottle the next day and the results were averaged.

Table 1
Bottles with different additives to grow methane oxidizing microorganisms.

Bottle	Additive type	Additive concentration (mg L^{-1})				
		Week 1	Week 2	Week 3	Week 4	Final concentration (CMC[a])
1	Control (medium only)					
2	Control (medium only)					
3	TWEEN	9	18	36	36	1.25 CMC
4	60[b]	9	18	36	72	2.5 CMC
5	BRIJ 58	28	56	112	112	1.25 CMC
6	[3)]	28	56	112	224	2.5 CMC
7	SDBS [4)]	3.1	6.3	12.5	12.5	0.013 CMC
8		3.1	6.3	12.5	25	0.026 CMC
9	Silicone oil only (5 % v/v)					
10	Silicone oil only (5 % v/v)					

[1)] CMC is the Critical Micelle Concentration in water with an increasing surface tension before reaching the CMC and a relatively constant surface tension after the CMC.
[2)] TWEEN 60 is a polyoxyethylenate sorbitol ester ($C_{64}H_{126}O_{26}$) with a HLB[5)] of 14.9.
[3)] BRIJ 58 is a polyethylene glycol hexadecyl ether ($C_{56}H_{114}O_{21}$) with a HLB of 15.7.
[4)] SDBS is sodium dodecylbenzenesulfonate ($C_{18}H_{30}NaO_3S$) with a HLB of 19.9.
[5)] HLB is the Hydrophilic Lipophilic Balance number which is an indicator of surfactant hydrophilic or lipophilic character and a dominating factor to improve solubility of hydrophobic compounds in water [22].

2.5. Emulsion activity and stability of oil-in-water liquid

Emulsions are mixtures of two or more liquids that are immiscible and are inherently unstable. Emulsions do not tend to form spontaneously and requires input of energy (e.g., stirring) to be formed and generally also to be maintained. The Emulsion Activity of oil-in-water mixtures containing surfactant was here determined in duplicate, which is a measure of the ability to form an emulsion, according to a methodology adapted from Kempka and co-workers [23]. In addition, the Emulsion Stability was determined in duplicate, which refers to the ability of an established emulsion to resist change in its properties over time. An appropriate surfactant can increase the stability of an emulsion so that the oil droplets dispersed in the dispersion medium do not change significantly with time as surfactants reduce the interfacial tension between the liquids.

The Emulsion Activity and Emulsion Stability were here determined for silicone oil in the medium solution (demineralised water containing a nutrient solution as defined in **Section 2.1**) to which different concentrations of surfactant were added. Silicone oil (20 cSt) was added to the medium in various ratios to obtain different concentrations of silicone oil. Surfactant was added in concentrations ranging from 0 mg L^{-1} to above its CMC. After that, the liquids were mixed using an advanced vortex mixer (VELP Scientific, ZX 3) for exactly 90 s at 3000 rpm. The Emulsion Activity was determined after 6 min by measuring the serum volume (A) and the total volume of the liquid after mixing (B) and calculated as per Eq. (2):

$$Emulsion\ Activity(\%) = (B - A)/B \quad (2)$$

The Emulsion Stability was determined after 60 min and 24 h by measuring the serum volume (A) and the total volume of the liquid (B) as per Eq. (3):

$$Emulsion\ Stability(\%) = (B - A)/B \quad (3)$$

In addition, the Foaming Potential was determined after 30 s by measuring the foam volume (C) and the total volume of the liquid before mixing (D) as per Eq. (4):

$$Foaming\ Potential(\%) = C/D \quad (4)$$

2.6. Abiotic mass-transfer rate in a single capillary channel

The gas-to-liquid mass-transfer rate of methane was measured in duplicate in a 0.54 m single capillary channel under segmented flow (Taylor flow) conditions. A glass capillary channel with an internal diameter of 2.4 mm was used through which the gas–liquid bubble train moved downwards. Air and water were introduced to the capillary channel via a simple T-connector at the top of the capillary channel and the air and liquid were disengaged at the bottom in a glass flask containing two overflows; one for the water in the bottom and one for air in the top (see set-up **Figure S-1 Supplementary Material**). A pump (Watson Marlow 323) was used to set the gas flow rate at 7.09 L h^{-1} and a second pump (Watson Marlow 323) was used to set the liquid flow rate

at 5.44 L h^{-1}. This resulted in a gas-to-liquid ratio of 1.3, an empty channel gas contact time of 0.7 s, and a superficial slug face velocity of approximately 0.77 m s^{-1} in the capillary channel. The airflow was recirculated through the capillary channel (internal loop), while the liquid was flowing only once through the capillary channel. A known amount of methane was injected in the recirculated air stream and its concentration was measured several times to confirm stable methane concentration in the recirculating airflow before starting the liquid pump. At t = 0 the liquid pump was started and the time course of methane concentration in the recirculating airflow was measured to determine the gas-to-liquid mass transfer of methane in the capillary channel. The gas-to-liquid mass transfer rate was determined for different liquid mixtures: (1) water only, (2) water and silicone oil (20 cSt at 10 % v/v), (3) water and BRIJ 58 (120 mg L^{-1}), and (4) mixtures of water, BRIJ 58 (120 mg L^{-1}) and silicone oil different in concentration (10 % and 25 % v/v) or viscosity (20 cSt and 200 cSt).

Part II – Methane treatment in capillary bioreactor

2.7. Capillary bioreactor set-up

The capillary bioreactor (CBR) consisted of 25 glass capillary tubes with an internal diameter of 2.4 mm, 1 mm wall thickness, and a length of 1.0 m. The liquid phase was recirculated using a pump (AquaForte Model DM-VARIO 22000S) and measured with a rotameter (Fisher&Porter 10A1197A). The total liquid volume in the bioreactor was 8.4 L. The gas phase was internally recirculated from the outlet of the capillary reactor using an EVO 10 compressor (EAD, Model H5P3 P 1, Spain) and the recycled gas stream was subsequently mixed with fresh inlet air containing methane. The mixture was introduced in the bottom reservoir of the CBR. Internal gas recirculation was shown to be beneficial for methane removal in a biotrickling filter [24] and was here adapted as a potential strategy to enhance methane removal in the CBR. The fresh inlet air was clean dry air from which all the CO_2 was removed before methane was introduced using a mass flow control meter (Aalborg, Model GFC 17). The clean dry supply airflow and the recirculating gas flow were measured with a rotameter (Aalborg, S/N 51588–2). The bottom reservoir of the CBR contained 6 mm scrubber packing (Kaldness K1 rings, Evolution Aqua Ltd., UK) to enhance its gas distribution before entering the capillary channels. A schematic representation of the set-up is shown in Fig. 1.

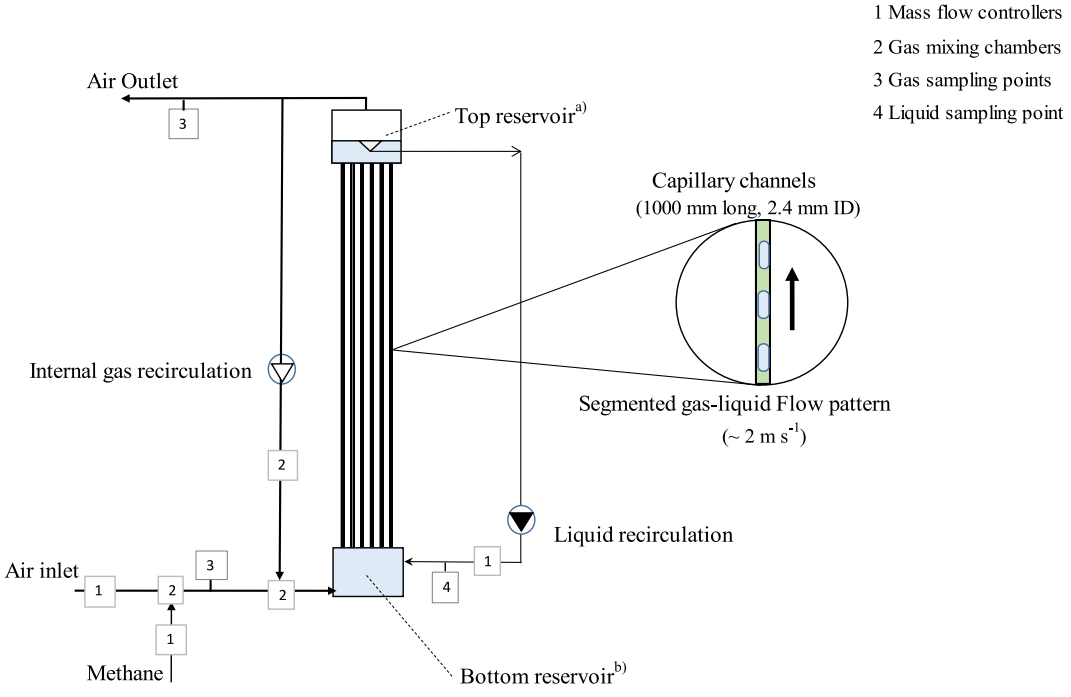
The temperature of the recirculation liquid of the CBR was controlled and constantly maintained at 24 °C. The pressure of the gas flows (inlet, outlet and recycled) was periodically measured with a pressure sensor (IFM PN7097). The capillary reactor was inoculated with the mixed inoculum (**section 2.1**) and acclimatized for several months in the CBR before starting the experiment to determine the effect of surfactant addition and silicone oil addition to the recirculating liquid in the capillary reactor on the removal of methane. Five days per week, 800 mL of recirculating liquid were removed from the CBR and replaced with fresh medium to avoid nutrient limitation and accumulation of inhibitory metabolites. All biomass and silicone oil were recovered and returned to the capillary reactor via centrifugation of the cultivation broth twice (5,000 rpm for 10 min) in a refrigerated centrifuge (Eppendorf, Model 5439 R).

The same operating conditions were maintained during the testing of the liquid additives: an up-flow segmented flow face velocity inside the capillary channels of 2.2 m s^{-1} and an internal gas recirculation (recycled gas to fresh inlet air) ratio of 15, which resulted in an empty channel gas residence time of 34 s. Internal gas recirculation was applied to decouple the optimal gas–liquid turbulence conditions inside the capillary channel from the empty channel gas residence time.

The empty channel residence time (*ECRT*) is defined as follows (Eq. (5)):

$$ECRT(s) = (V_c \times n_c)/(Q_g) \quad (5)$$

with V_c is the internal volume of a capillary channel (m^3), n_c the number of capillary channels in the CBR (−), Q_g the inlet air flow rate (m^3 h^{-1}).



a) The top reservoir (15 cm in diameter and 31 cm in height) served as gas-liquid disengagement zone.

b) The bottom reservoir (11 cm in diameter and 17.5 cm in height) served as gas-liquid mixing zone.

Fig. 1. Schematic representation of the experimental set-up of the capillary bioreactor.

The inlet methane load (IL) is defined as follows (Eq. (6):

$$IL\left(g\,day^{-1}\right) = Q_g \times C_i \times 24 \quad (6)$$

with C_i the inlet methane concentration (g m^{-3}).

The methane elimination capacity (EC) is defined as follows (Eq. (7):

$$EC\left(g\,m^{-3}h^{-1}\right) = (C_i - C_o) \times Q_g/(V_c \times n_c) \quad (7)$$

with C_o the outlet methane concentration (g m^{-3}).

The inlet methane concentration was maintained at a ~4500 ppm_v, and its removal was tested for the addition of surfactant and silicone oil according to the schedule shown in Table 2. The surfactant BRIJ 58 was selected based on results of the studies in PART I (Sections 3.1, 3.2 and 3.3). In addition, in order to elucidate whether the process was mass transfer or kinetically limited in Stage I and Stage V a step increase of the inlet methane load was applied and the methane removal rate monitored (IL).

2.8. Analytical methods

Methane concentrations in the inlet and outlet airstream of the CBR were measured daily in a Bruker 430 GC-TCD (Palo Alto, USA) equipped with a thermal conductivity detector, and a CP-Molsieve 5A and a CP-PoraBOND Q columns. The oven, injector and detector temperatures were maintained at 40 °C, 150 °C and 250 °C, respectively. Helium was used as the carrier gas at 3.9 mL min^{-1}.

The biomass, total nitrogen (TN), and total organic carbon (TOC) concentrations in the liquid phase were periodically quantified according to Standard Method 2540 D. The dissolved TN and TOC were determined after filtration of the sample through a 0.45 μm pore size filter in a TOC-VCSH analyser (Japan) with a TNM-1 chemiluminescence module. The pH (Crison BASIC-20+) and conductivity (Crison BASIC-30) in the liquid media were also monitored [25].

The concentration CO_2 of inlet and outlet gas stream of the CBR was measured using a GC-TCD (Agilent 8860, Santa Clara, USA) equipped with a CP-Molsieve 5A and a CP-PoraBOND Q columns. The oven temperature was maintained at 80 °C for 2.3 min after which it increased with 20 °C per minute to 150 °C. Helium was used as the carrier gas at XX mL min^{-1}.

The community structure of the two inocula (Activated Sludge Inoculum (InAS) and post-composted anaerobically digested sludge (InCS), as well as that of the CBR at the end of Phase I (prior to adding surfactant, BR) and at the end of Phase V (end of operation, TR) was analysed. Genomic DNA was extracted using FastDNA™ SPIN Kit for Soil (MP Biomedicals, USA). PCR amplification of regions 16S-V4-V5 was performed by using the primers GTGCCAGCMGCCGCGGTAA, CCGTCAATTCCTTTGAGTTT connecting with barcodes. PCR products of the appropriate size were selected by agarose gel electrophoresis. Equal amounts from each sample were pooled, end-repaired, A-tailed, and ligated with Illumina adapters to create sequencing libraries. These libraries were quantified using Qubit and real-time PCR, with size distribution checked by Bioanalyzer. Finally, the quantified libraries were pooled and sequenced on an Illumina platform to generate 250 bp paired-end reads at Novogene UK (Cambridge, UK). Paired-end reads were assigned to samples based on unique barcodes, and barcodes and primer sequences were trimmed using Python (V3.6.13) and Cutadapt (V3.3). FLASH (V1.2.11) was used to merge the paired-end reads, while fastp (V0.23.1) and the UCHIME algorithm handled data filtration and chimera removal [25,26]. Sequences were clustered into Operational Taxonomic Units (OTUs) using QIIME2 (202202) with the SILVA (V138.1) and RDP (V18) gene reference databases [27]. The top 35 taxa at genus level were selected to plot relative abundance histograms in Perl (V5.26.2) using SVG, and heatmaps in R (V4.0.3) using pheatmap [28]. Shannon alpha diversity index and Beta diversity were calculated using QIIME2. Functional predictions based on marker genes were performed with the R package PICRUSt2 (V2.3.0) [29]. The sequences obtained have been deposited in Genbank as Bioproject PRJNA1162485.

Table 2
Operational conditions for the capillary bioreactor.

Stage	Days of operation	Additive added	Phase concentration additives in CBR	Inlet methane concentration (ppm_v) ± SD[1)]	Inlet methane load (g/day) ± SD
I	10	None	0	4,692 ± 414	1.33 ± 0.12
II	9	Surfactant (2 x 40 mg L^{-1})	BRIJ 58 (80 mg L^{-1})	4,446 ± 237	1.26 ± 0.07
III	14	Silicone oil (400 mL)	BRIJ 58 (80 mg L^{-1}) + 5 % (v/v) silicone oil	4,581 ± 500	1.29 ± 0.14
IV	47	Surfactant (2 x 40 mg L^{-1})	BRIJ 58 (160 mg L^{-1}) + 5 % (v/v) silicone oil	4,310 ± 295	1.22 ± 0.09
V	35	Silicone oil (1,200 mL)	BRIJ 58 (160 mg L^{-1}) + 20 % (v/v) silicone oil	4,188 ± 200	± 0.06

[1)] SD is standard deviation

3. Results and discussion

Part I – Mass transfer study

3.1. Methane bioavailability test

The methane oxidation rate in the bottles containing methane oxidizing microorganisms was determined 10 weeks after the initial addition of additives to the bottles as per schedule shown in Table 1. To measure the methane oxidation rate in each bottle, the continuous air supply containing methane to the headspace was stopped and the methane concentration in the headspace of all bottles containing different liquid additives was measured over time as shown in Fig. 2. The biomass concentration in the liquid of the bottles ranged between 0.5 and 1 g L^{-1} to determine the maximum specific methane oxidation rate (MOR) which is defined as the mmol CH_4 removed per gram dry weight of biomass per day at the initial methane concentration of ~500 ppm_v. The maximum specific methane removal rate of the controls (Bottles 1 and 2 without any liquid additive) was the lowest, while being significantly higher in Bottles 7 and 8, reaching values up to 18 and 12 times higher when supplemented with SDBS at 12.5 and 25 mg L^{-1}, respectively, and in Bottle 5, achieving 15 times higher removal rates at the lower BRIJ 58 concentration of 112 mg L^{-1}. The specific methane removal rate of both bottles containing Tween 60 and the bottle with the higher BRIJ 58 concentration are only slightly higher when compared to the controls.

The results of the methane oxidation rate in the bottles containing SDBS in this study agrees with the observations done in the study of Wu and co-workers in a biotrickling filter treating n-alkane and methane. In their study, the methane removal efficiency (RE) increased from 35 % to 74 % with the addition of SDBS at a surfactant concentration of 15 mg L^{-1}, which was shown not to inhibit microbial growth [21].

Biological gas treatment studies testing surfactants from the BRIJ group have also shown an enhancement of removal performance consistent with the findings in our experiment with BRIJ 58. Ramirez and co-workers [30] observed an increase of methane removed between 6 % and 35 % when different surfactants of the BRIJ group were added to a conventional biofilter treating methane. In addition, Miller and co-workers [31]reported a nearly 20 % improvement of a biofilter treating toluene after the addition of BRIJ 35. Moreover, Dhamwichukorn and

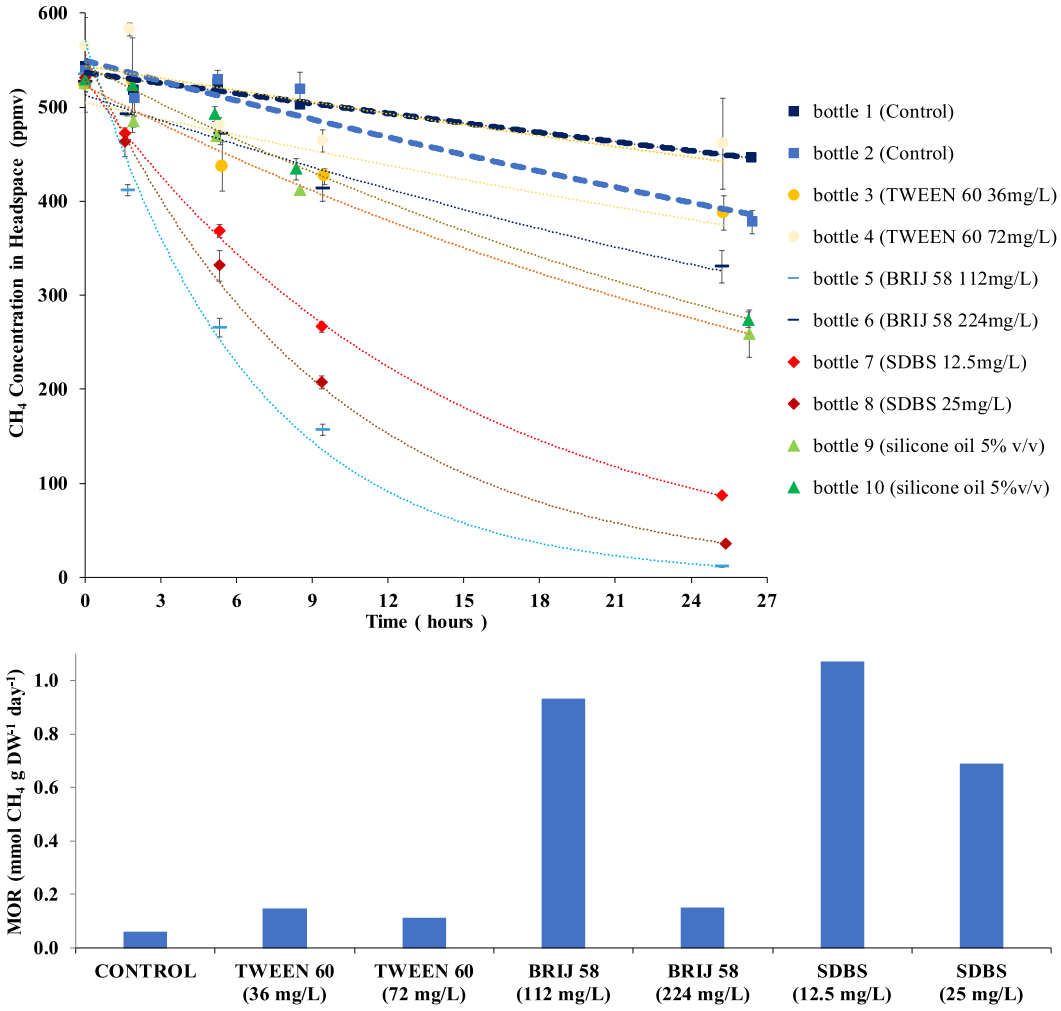


Fig. 2. Time course of the reduction of the methane concentration in the headspace of the bottles (upper graphic) and maximum specific methane oxidation rate (MOR) (lower graphic) after 10 weeks containing methane oxidizing microorganisms and different liquid phase additives. The controls (duplicate) in the upper graphic are shown in bold. The dotted lines were determined through exponential regression ($R^2 > 0.9$ in all cases).

co-workers [32] observed an increase of α-pinene removal from 26 % to 95 % when treating a mixture of α-pinene and methanol in a thermophilic biofilter after addition of a mixture of non-ionic surfactants, including BRIJ 35 and BRIJ 58. On the other hand, although bioavailability and biodegradation can be enhanced, the microbial growth rate can be inhibited at already relatively low BRIJ concentrations (< 1 CMC) as illustrated for BRIJ 30 [33].

The relatively small increase in methane oxidation rate observed in this study when TWEEN 60 was supplemented is consistent with findings from other studies, where the effect of a surfactant from the TWEEN group was neutral or only minor (either positive or negative). For example, TWEEN 20 neither affected the RE nor affected the development of the microbial community in a conventional biofilter treating toluene [34]. Similarly, it was observed that TWEEN 80 exerted an either neutral or a slightly positive effect on the mass transfer of conventional biofilters [35]. This may be explained by the observed lower influence of TWEEN surfactants on the apparent gas–water partitioning coefficient constant of contaminants compared to other type of surfactants [22]. Conversely, Ramirez and co-workers [30] showed an increase between 19 and 35 % in methane removal in a conventional biofilter when adding TWEEN surfactants intermittently to the system as part of the nutrient solution.

In addition, the toxicity of non-ionic surfactants has shown to be higher as their molecular weight (MW) increases, which makes the surfactant TWEEN 60 (MW 1312) potentially less suitable compared to SDBS (MW 348) and BRIJ 58 (MW 1122). Similarly, the effect in terms of removal efficiency (positive, neutral, negative) has shown to be strongly dependent on the concentration of the TWEEN surfactant added, as illustrated by Wang and co-workers [18]. Their study showed that while an increase in the TWEEN 20 concentration from 3.7 mg L^{-1} to 7.4 mg L^{-1} (~ 0.1 CMC) did enhance the RE of ethylbenzene in a biotrickling filter, a further increase to 37 mg L^{-1} and 74 mg L^{-1} (~ 1 CMC) resulted in a reduction of the RE. In a subsequent study, these authors observed an improvement in m-xylene removal in a biotrickling filter when TWEEN 80 was added at a relative high concentration of 100 mg L^{-1} (~ 6.7 CMC), but this was mainly attributed to the higher inlet m-xylene concentrations [36]. Deng and co-workers observed a 20 % increase of the hexane RE when treating hexane and dichloromethane in a biotrickling filter with TWEEN 20 at a relatively low concentration of 30 mg L^{-1} (= 0.5 CMC) in the recirculating liquid [37]. This increase in RE is comparable to the study of Amin and co-workers [39], who showed a slight (~14 %) improvement of xylene removal in a conventional biofilter when TWEEN 20 was applied daily as part of the nutrient solution at a concentration of 150 mg L^{-1} (= 2.5 CMC).

The highest specific methane removal rates for each surfactant in our study were observed at the lower rather than the higher surfactant concentration. This might also explain the large difference in specific methane removal rate in the bottles with the BRIJ 58 additive, with Bottle 5 containing half the concentration of Bottle 6. This observation supports reports from other studies regarding the risk of microbial

inhibition by surfactants at higher concentrations [19,21,31].

The methane removal rate in the headspace of both bottles containing only silicone oil (Bottle 9 and Bottle 10) only slightly increased when compared to the bottles containing no additive (Control Bottles 1 and 2). The specific methane removal rate in the bottles containing silicone oil could not be confirmed because the biomass concentration was not determined. Nevertheless, it can be inferred that silicone oil had a limited beneficial effect in our set-up, which was probably attributed to the low degree of emulsification of the oil–water mixture. A high degree of emulsification entails that tiny droplets of oil stay suspended within the water phase, while a low degree of emulsification progressively separates the oil from the water phase and forms an oil layer on top of the water phase. When properly emulsified, oil in tiny droplets would increase the methane liquid-oil and the gas-oil mass transfer due to its larger interfacial surface area.

This study confirms that surfactants can significantly improve bioavailability of dilute methane, with both BRIJ 58 and SDBS showing an increased bioavailability by one to two orders of magnitude at the concentrations tested. This improvement can be attributed to a reduction in the gas–liquid surface tension when the surfactant was added, and the formation of micelles that decrease the apparent Henry's law constant of the contaminants [21,22]. On the other hand, surfactants can adsorb onto the bacterial cell or may increase the bacterial cell membrane hydrophobicity, which enhances the bioavailability of hydrophobic contaminants [17,21].

Therefore, the day after measuring the bioavailability in the bottles, the cell hydrophobicity of the microorganisms was determined after having grown on methane for ten weeks (Fig. 3). The cell hydrophobicity of the microorganisms grown in the presence of the surfactant BRIJ 58 was ~87 % regardless of the concentration, and for those grown in the presence of SDBS was around 75 %, both significantly higher than the cell hydrophobicity of the methane oxidizing microorganisms in the controls (44 %). In contrast, the cell hydrophobicity of the microorganisms in the bottles containing TWEEN 60 was not enhanced, remaining at only 21 %. It was concluded that BRIJ 58 and SDBS in our experiment increased the cell hydrophobicity by nearly a factor of two when compared to the controls (without any additive), while TWEEN 60 did not increase the cell hydrophobicity at the concentrations tested, which was actually lower when compared to the control (no surfactant).

3.2. Emulsion activity and stability of oil-in-water mixtures

The Emulsion Capacity and Emulsion Stability as well as the Foaming Potential were determined for oil-in-water mixtures containing the three selected surfactants. The water was in this case demineralised water containing a nutrient solution (mineral salt medium, as described in **Section 2.1**) and the oil was silicone oil (20 cSt). Fig. 4 shows the results for the different oil-in-water mixtures for increasing silicone oil concentrations and increasing surfactant concentrations. Figure S-1 **(Supplementary Material)** shows photos of different oil-in-water mixtures.

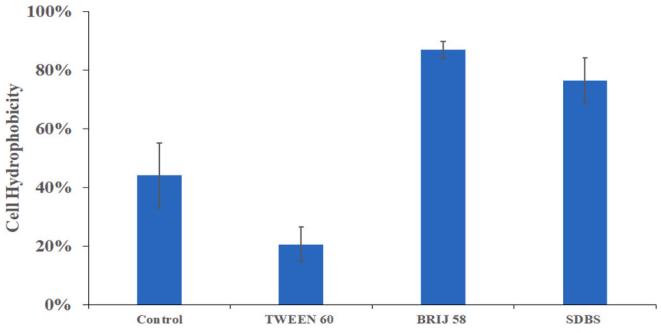


Fig. 3. The cell hydrophobicity of the microorganisms after growth on dilute methane in the presence of different surfactants.

The Emulsion Capacity and Emulsion Stability are 0 % when no surfactants are added, confirming that the oil-in-water mixture is not very stable when 20 cSt silicone oil and the medium solution are respectively used as oil and water phase. On the contrary, and as expected, the Emulsion Capacity increases with oil when surfactants are supplemented to the medium. In the particular case of BRIJ 58, the required surfactant concentration to form a stable emulsion does not need to exceed 80 mg L^{-1}. This can be explained by its CMC value (~90 mg L^{-1}), beyond which the surface tension between the liquids does not further decrease and remains relatively constant. Similarly, the higher CMC of SDBS (~960 mg L^{-1}) explains why the Emulsion Capacity increases at the higher surfactant concentrations tested, as the surface tension between liquids reduces with increasing surfactant concentration up till the CMC of the surfactant. Furthermore, the Emulsion Stability is highest for BRIJ 58, while the Foaming Potential is negligible for both BRIJ 58 and TWEEN 60 but significantly higher for SDBS.

3.3. Abiotic mass-transfer rate in a single capillary channel

The gas-to-liquid mass-transfer rate of methane in a single capillary channel was measured under segmented (Taylor) flow regime conditions at methane concentrations of ~5,000 ppm_v (= 0.5 % v/v) in ambient air for different liquids: (1) water, (2) water + silicon oil, (3) water + BRIJ 58 and (4) water + silicone oil + BRIJ 58, with the oil at two concentrations (10 % v/v and 25 % v/v) and two viscosities (20 cSt and 200 cSt). The results are summarized in Fig. 5 and show that the addition of only BRIJ 58 or only silicone oil did slightly (<10 %) increase the gas-to-liquid mass transfer of methane in the capillary channel. The combined addition of surfactant (BRIJ 58) and 10 % (v/v) silicone oil (both 20 cSt and 220 cSt) did significantly (~70 %) increase the gas-to-liquid CH_4 mass transfer in our set-up when compared to the control (water). The combined addition of the surfactant BRIJ 58 and 25 % (v/v) silicone oil (both 20 cSt and 220 cSt) did still increase the gas-to-liquid mass transfer, but less (~30 %) when compared with the control (water). Thus, it can be concluded that the addition of the surfactant BRIJ 58 can significantly enhance the gas–liquid mass transfer in the capillary channel but only when combined with silicone oil, which may be explained by the improved emulsion capacity as observed in the results obtained on emulsion capacity and stability **(**Fig. 4**)**. The enhancement of the methane gas–liquid mass transfer in the capillary channel was most noticeable when the amount of silicone oil was 10 %, rather than 25 %, while the viscosity (20 cSt or 200 cSt) did not made much of a difference.

The slight increase (12 %) in methane mass-transfer when only 10 % v/v 20 cSt silicone oil was added may be explained by the lower gas-oil partition coefficient of methane ($H_{Gas/Silicone\ Oil} = 0.82$) compared to the gas–water partition coefficient of methane ($H_{Gas/Water} = 28$). It can be calculated that an oil–water mixture containing 10 % (v/v) silicone oil could theoretically provide a partition coefficient ($H_{Gas/Water\text{-}Silicone\text{-}Oil\text{-}Mixture}$) for methane of 25.3, which is 11 % lower than the gas–water partition coefficient of methane ($H_{Gas/Water}$) of 28 and thus corresponding with the 12 % increase in methane mass-transfer measured in our study.

The observed 9 % increase in methane mass-transfer when only BRIJ 58 (120 mg L^{-1}) was added can only partly be explained by the ~4 % increase in methane solubility in water expected by adding the BRIJ surfactant, as discussed elsewhere [38]. The remaining increase may be attributed to experimental analysis error in our study, which is estimated to be a few percent.

A larger increase in methane mass-transfer was observed when the surfactant and the silicone oil were combined, an improvement seen in all silicone oil combinations tested, regardless the volume of the oil or the viscosity of the oil. The explanation is most likely related to the improved emulsification of the oil in the water where the surfactant stabilises the oil-in-water emulsion maintaining smaller oil droplets in the water phase. Smaller oil droplets enhance gas-to-oil mass transfer,

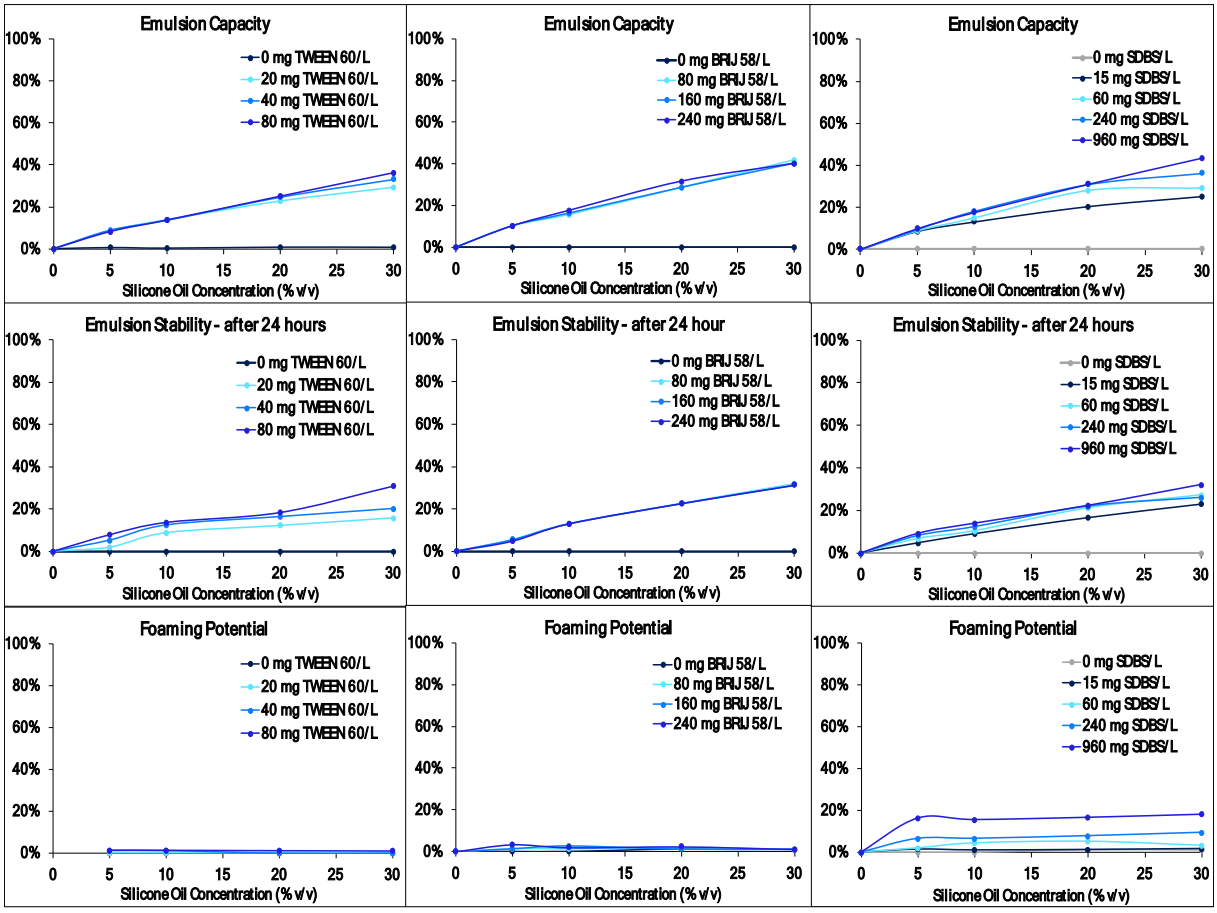


Fig. 4. The Emulsion Capacity (top), Emulsion Stability (middle), and Foaming Potential (bottom) of TWEEN 60 (left), BRIJ 58 (middle), and SDBS (right) at increasing silicone oil percentages.

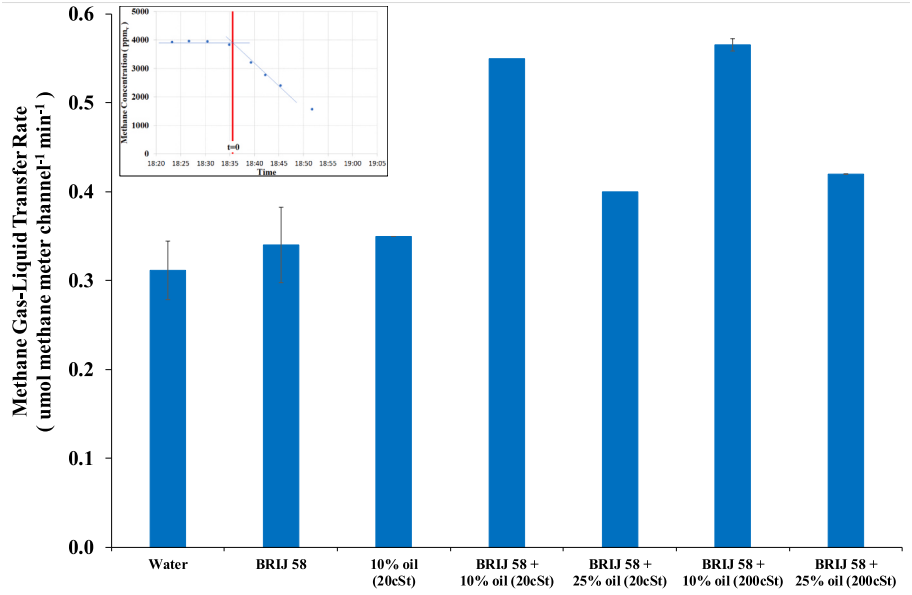


Fig. 5. Mass-transfer rate of methane in the single capillary channel with liquids containing different additives. Graphic in the top left corner showing an example of the gaseous methane concentration over time with t = 0 the start of the liquid recirculation.

which creates higher methane carrying capacity because the $H_{Gas/Silicone\ Oil}$ (= 0.82) is much smaller than the $H_{Gas/Water}$ (= 28). The oil-in-water emulsion is a mixture of two liquids that are immiscible and are inherently unstable and in which the oil tends to separate out from the water in the absence of the surfactant.

The enhancing effect of oil volume was larger than the effect of oil viscosity, as 10 % v/v volume provides better mass-transfer than 25 % v/v volume (regardless the viscosity of the oil) and 200 cSt viscosity

provides slightly better mass-transfer than 20 cSt (regardless the volume of the oil). The higher methane mass transfer observed with 10 % v/v silicone oil and surfactant compared to the 25 % v/v silicone oil with surfactant appears to be the result of a more optimal segmented flow patron. Increasing the silicone oil fraction from 10 to 25 % v/v made the length of the gas bubble (L_b) and the length of the liquid slug (L_s) combined become significantly longer ($L_b + L_s = L_u$ which is also called the one total unit length). In our study, the average total unit length (L_u) increased from about 1 cm to more than 3 cm (see **Figure S-3 Supplementary Material**). A shorter L_u may increase mass transfer as it reduces the risk that the liquid film surrounding the gas bubble get saturated, limiting further mass transfer as discussed elsewhere [10]. The higher viscosity of the oil provided slightly better mass-transfer (regardless the oil volume) and might be explained by the impact of viscosity on the liquid film thickness surrounding the gas bubbles. Increasing the liquid viscosity of the oil-in-water emulsion increases the liquid film thickness as explained elsewhere [39], which increases the methane carrying capacity of the liquid film each time a gas bubble passes it in the segmented flow in the capillary channel. The liquid film thickness flowing around gas bubbles is critical for gas–liquid mass transfer in a capillary channel under segmented (Taylor) flow regime. On the other hand, higher liquid viscosity can also hamper the gas–liquid mass transfer in a capillary channel under segmented (Taylor) flow regime, with the optimum viscosity dependent on the operating conditions and the liquid surface tension [10]. Moreover, higher viscosity increases the viscous drag forces relative to the surface tension forces, which may compromise capillarity. The Capillary number (*Ca*) represents this relation between viscous drag forces and capillary forces (Eq. (8):

$$Ca(-) = \mu v/\gamma \tag{8}$$

where μ is the viscosity (Pa s), v the liquid velocity (m s^{-1}), and γ the surface tension of the liquid in the gas phase (N m^{-1}). Increased viscous drag forces slows down the internal liquid recirculation, the vortex that enhances mass transfer through convection rather than diffusion. Thulasidas and co-workers [42] found that the liquid internal recirculation velocity reduces sharply and ultimately becomes zero with increasing the Ca number, with Ca > 0.6 being the theoretical value where the internal vortex becomes zero in a downward flow. In our experiments with the 20 cSt viscosity silicone oil the calculated Ca numbers were 0.06 and 0.12 for the 10 % v/v and 25 % v/v oil, respectively, while with the 200 cSt viscosity silicone oil 0.5 and 1.1 for the 10 % v/v and 25 % v/v oil, respectively. This assumes that the overall liquid viscosity is proportional to the oil-liquid fraction and the gas–liquid surface tension is 35.7 mN m^{-1} as per Peters and Arabali [43]. This assumption is a simplification but shows that adding more silicone oil, and especially adding silicone oil with a higher viscosity, may increase the Capillary number beyond the threshold where internal recirculation is reduced, and mass transfer is compromised.

Part II – Methane treatment in capillary bioreactor

3.4. Dilute-methane treatment in the capillary bioreactor

BRIJ 58 was selected as the surfactant to be tested in the CBR because of its potential to enhance CH_4 gas–liquid mass transfer in the presence of silicone oil (as illustrated in **Section 3.3**) as well as its ability to enhance the oil-in-water Emulsion Capacity and oil-in-water Emulsion Stability at a concentration low enough to eliminate the risk of microbial inhibition (as discussed in **Section 3.2**). Moreover, BRIJ 58 showed to enhance the cell hydrophobicity of methane oxidizing bacteria and to improve overall the bioavailability of dilute methane (as illustrated in **Section 3.1**).

Prior to the start of testing surfactant as an additive in the CBR, the microbiology had been exposed to methane as sole energy and carbon source for about six months in which optimal and stable process conditions of the CBR were established. At the start of the experiment the biomass concentration was measured multiple days and showed a total suspended solids concentration of 1.8 ± 0.3 g L^{-1}, of which 82 ± 11 % was volatile suspended solids. The pH and the conductivity of the recirculating medium at the start were 7.3 ± 0.0 and 5.1 ± 0.1 mS cm^{-1}, respectively, and stayed relatively constant during the entire test period of 120 days (7.4 ± 0.1 and 4.7 ± 0.2 mS cm^{-1}). The TN concentration was maintained between 40 and 90 mg N L^{-1} for the whole experiment and was on average 62 ± 15 mg N L^{-1}. At the beginning of Stage I, the TN concentration was ~90 mg N L^{-1}, slowly decreasing over time despite medium replenishment, reaching a concentration of 40 mg N L^{-1} by day 60 after the start of Stage I. Thus, 50 mg N L^{-1} as sodium nitrate was added to restore the initial nitrogen concentration of 90 mg N L^{-1}, steadily decreasing again to ~40 mg N L^{-1} by the end of Stage V. The TOC was measured once a week and was on average 189 ± 40 mg C L^{-1}. TOC contains all soluble organic carbon including any methane metabolites and may be an indication of metabolic product accumulation. The TOC slowly increased at the beginning of Stage I, then decreased gradually after the initial surfactant addition (Stage II). A slow increase of the TOC was again recorded, followed by another decrease after the second surfactant addition (Stage IV). This observation may be explained by previous studies indicating that surfactants can solubilise storage polymers, such extracellular polymetric substances (EPS) from biofilms, due to their detergent character, thereby limiting EPS accumulation [30,36]. A slower decrease of the TOC was recorded after additional silicone oil (Stage V).

During Stage I, the methane removal rate during a step increase (by a factor of 1.4) in inlet methane concentration was monitored to elucidate whether the process was mass transfer or kinetically limited before supplementing additives to its liquid phase (Fig. 6**a**). The elimination capacity directly increased from ~225 to ~290 g $m^{-3}h^{-1}$ (increase by a factor of 1.3) during this sudden methane load increase and decreased to previous steady state values when the inlet methane concentration was restored. This test confirmed that methane removal was mass transfer limited and not biologically limited. In addition, the determination of methane concentration in the liquid phase at the top reservoir of the CBR revealed a value of 0.0007 g m^{-3}, which is much lower than the theoretical equilibrium concentration of 0.0785 g m^{-3} calculated by the Henry's law, confirming that methane mass transfer from the gas to the liquid phase was the limiting mass transfer process.

The influence of the addition of surfactant and silicone oil as on methane removal was tested according to Table 2 (**Section 2.5**). The results of the methane removed in the CBR during the different phases are summarised in Fig. 7. During Stage I, when no additives were added, the operational conditions of the CBR resulted in a RE of 32.0 ± 4 %, corresponding to an EC of 156 ± 26 g $m^{-3}h^{-1}$. The addition of the surfactant in Stage II did not result in any significant change in the RE and the EC, remaining at 34.3 ± 2.5 % and 159 ± 18 g $m^{-3}h^{-1}$, respectively. In contrast, when the silicone oil was added in Stage III, both the RE and the EC increased by ~40 % up to 45.9 ± 4.4 % and 222 ± 45 g $m^{-3}h^{-1}$, respectively. BRIJ 58 showed to enhance the gas–liquid mass transfer in a capillary channel, but only when combined with silicone oil. The surfactant enhanced emulsification of the oil in the medium, which appears to be the main mechanism rather than altering the gas–liquid partial coefficient of methane.

No significant enhancement on the methane removal performance was observed after the increased surfactant addition in Stage IV, with average RE and EC values in this stage of 47.0 ± 4.2 % and 214 ± 27 g $m^{-3}h^{-1}$, respectively. However, increasing the silicone oil to 20 % (v/v) in Stage V did further increase, though slightly, the RE and the EC to 52.8 ± 6.1 % and 231 ± 30 g $m^{-3}h^{-1}$, respectively. This confirmed that the addition of silicone oil beyond 10 % v/v is beneficial in this case, especially since during this Stage V the inlet concentration was somewhat lower compared to the average concentration in earlier stages (4,195 ± 195 ppm_v during Stage V vs 4,404 ± 250 ppm_v on average during Stages I to IV).

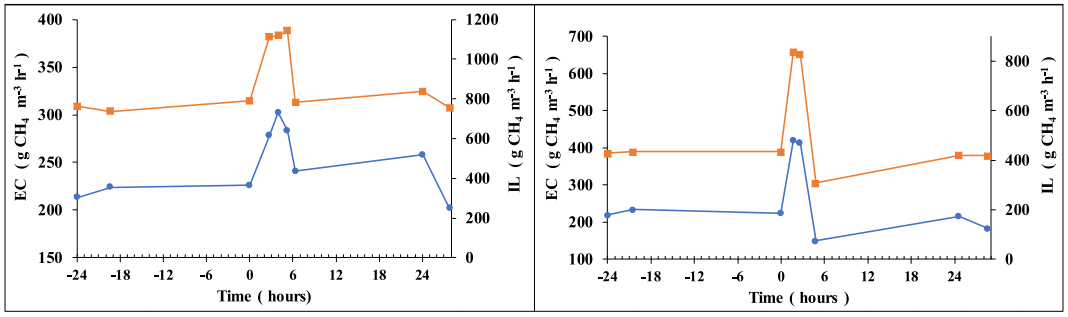


Fig. 6. Methane (●) elimination capacity (EC) and the methane (■) inlet load (IL) in the capillary bioreactor during the mass transfer limitation test before (Stage I, Fig. 6a, left) and after (Stage V, Fig. 6b, right) the supplementation of additives to the liquid phase.

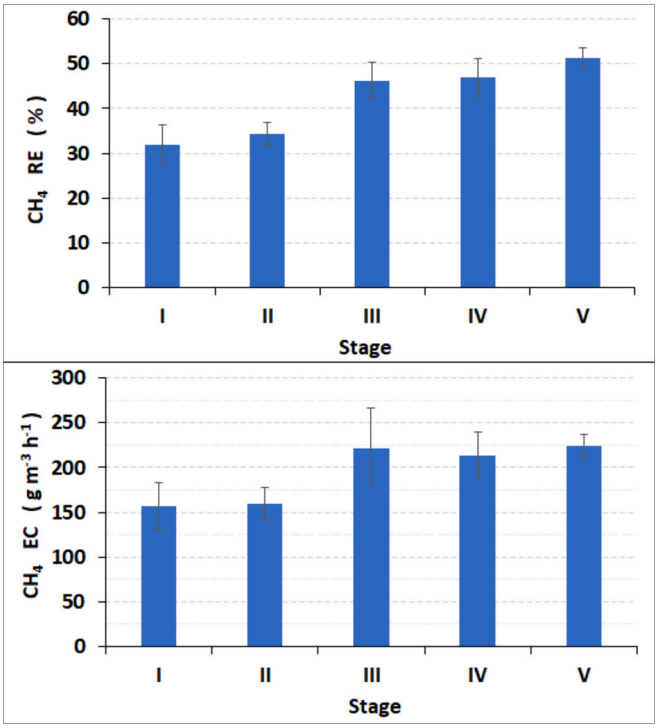


Fig. 7. Methane removal efficiency (upper) and methane elimination capacity (lower) in the capillary bioreactor during the different experimental stages.

The removal capacities reported herein are high compared to conventional biological gas treatment system treating dilute CH_4 emissions, especially when considering the extremely short gas contact time and relatively low inlet concentrations [40]. All studies with conventional biological systems treating dilute CH_4 required long gas contact times of several minutes and indicate that the bioavailability of the CH_4 hampers these bioprocesses. This observation done in study are in line with observations done by Kennelly and co-workers [41] where a horizontal flow bioreactor operated at an empty bed gas contact time of 45–55 min showed that a surfactant combined with silicone oil can improve the removal of dilute methane.

After Stage V, the methane removal rate under a sudden increase in CH_4 concentration by a factor of 1.93 was tested to elucidate the limiting mass transfer mechanisms (Fig. 6**b**). The elimination capacity directly increased from ~226 to ~417 g $m^{-3}h^{-1}$ (increase by a factor of 1.85) during this sudden methane load increase and decreased to previous steady state values when the inlet methane concentration was restored to its original concentration. The determination of methane concentration in the liquid phase at the top reservoir of the CBR also revealed a value of 0.0016 g m^{-3}, which is much lower than the theoretical equilibrium concentration of 0.0941 g m^{-3} calculated by the Henry's law, confirming that methane mass transfer from the gas to the liquid phase was the limiting mass transfer process.

The carbon dioxide (CO_2) production was measured at the end of Stage V. The results showed that about 76 ± 6.5 % of the methane degraded was recovered as CO_2, with the remaining carbon incorporated as biomass or accumulated as metabolic products in the recirculating liquid. Interestingly, the potential of silicone oil to act as a buffer for methane was confirmed in a test where the inlet methane load was interrupted for six days, while keeping the rest of the CBR operational without any changes. No deterioration in methane removal was observed following the methane supply interruption of six days, when measured 30 min after the restart of the methane supply to the CBR. The methane elimination before and directly after the six-day interruption were equal (the RE was 52 ± 2 % before vs 51 ± 1 % after and the EC was 219 ± 9 g $m^{-3}h^{-1}$ before vs 227 ± 4 g $m^{-3}h^{-1}$ after). This buffering capacity was further confirmed by the CO_2 produced during the first few days after the six-day interruption, which increased to 122 ± 11.4 % of the amount of methane removed from the air stream, indicating more methane converted by the methanotrophic bacteria in the CBR than methane removed from the air stream by the CBR.

During the last few days of the overall experiment the surfactant concentration was further increased from the 160 mg BRIJ 58 L^{-1} previously added to determine how a sudden increase of surfactant concentration would reduce methane removal in the CBR. Additional BRIJ 58 surfactant was added according to the following schedule: 80 mg L^{-1} on day 305, 160 mg L^{-1} on day 306, and 320 mg L^{-1} on day 307. The results showed that the methane RE dropped from 51 % to 44, 38 and 37 % after the addition of 80, 160 and 320 mg L^{-1}, respectively (see **Figure S-4 Supplementary Material**). Excessive foam formation was observed after the addition of the 160 mg L^{-1} and 320 mg L^{-1} dosages, which is indicative of major microbial cell lysis. These observations are in line with the observations done in the Methane Bioavailability Test (see **Section 3.1**), where BRIJ 58 at a concentration of 112 mg L^{-1} significantly enhanced methane bioavailability and methane oxidation rate, but it did not at the higher BRIJ 58 concentration of 224 mg L^{-1}, likely due to microbial inhibition.

3.5. *Microbial characterisation*

Metagenomic amplicon sequencing revealed that the use of surfactants and silicone oil, along with the high bioavailability of methane, promoted a strong specialization by the end of the operation of the Taylor flow bioreactor (**Figure S-5, Supplementary Materials**). This effect has been observed in previous studies that used silicone oil to enhance methane transfer in continuous stirred tank reactors [14]. This diversity is phylogenetically represented in Fig. 8 **and S-6**.

By the end of Phase I, during reactor operation without the addition of surfactants and silicone oil (BR), the most abundant aerobic methanotroph belonged to the genus *Methylosarcina*, a gammaproteobacterial (Type I) methanotroph, comprising 6 % of the total relative abundance.

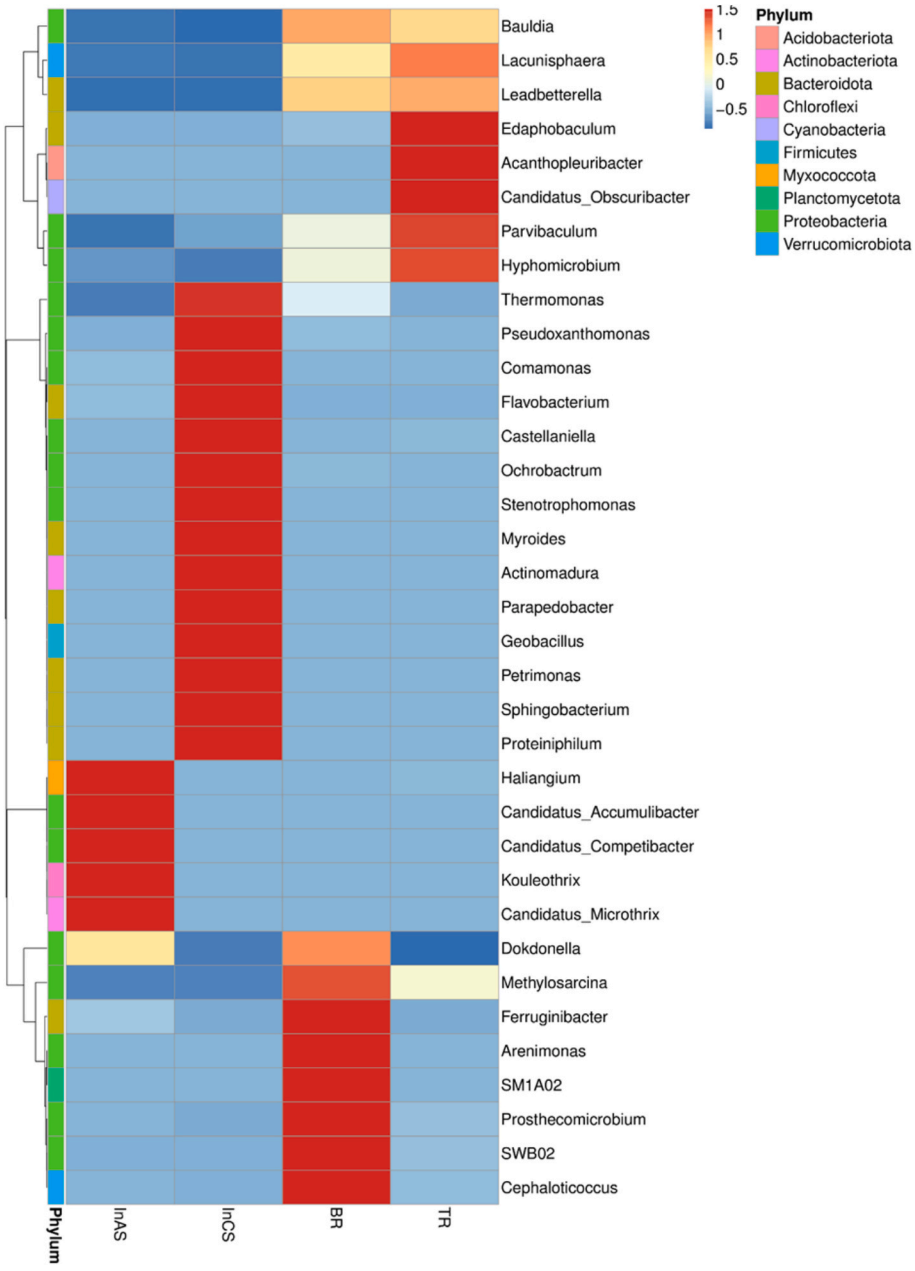


Fig. 8. Heatmap showing the comparison of each taxon in the inocula (InAS and InCS), at the end of Phase I (BR) and at the end of Phase V (TR). The rows show the Z value obtained by standardizing the relative abundance of each row of genera.

However, after transitioning to operations that included surfactants and silicone oil (TR), this population declined to 3 % relative abundance. Previous studies have demonstrated that the presence of silicone oil can significantly impact methanotrophic communities, often promoting the growth of certain genera, such as *Methylosarcina*, due to its capacity to form aggregates adhered to silicone oil [14]. However, the findings from this experiment indicate that the relative abundance of proteobacterial methanotrophs decreased when silicone oil and surfactants were introduced. Interestingly, in the later stages of operation, there was a marked increase in the relative abundance of *Lacunisphaera*. This genus is known to play a role in the dynamics of methanotrophic communities, and some species, such as *L. limnophila*—the species detected in our study— has been recently recognized as a potential verrucomicrobial methanotroph [42]. This increase may indicate a shift in the methanotrophic community dynamics driven by the addition of surfactants and silicone oil. In addition to these shifts, there was a notable rise in methylotrophic populations, particularly within the genus *Hyphomicrobium*. This genus is capable of oxidizing methanol and formaldehyde using unique dehydrogenases, enabling it to utilize these carbon sources without requiring NAD(P). The likely scenario here is that *Hyphomicrobium* cross-fed on methanol and formaldehyde, byproducts of the methane oxidation process, thereby contributing to the detoxification of the reactor environment. This cross-feeding likely had a synergistic effect, enhancing overall methane oxidation rates [43,44]. By the end of phase V, other microbial genera such as, *Edaphobaculum*, *Parvibaculum*, and *Obscuribacter* also showed increased abundance. These genera are known for their ability to degrade a wide array of complex carbon sources, suggesting they may have metabolized not only the byproducts of methane degradation but also the surfactant added during reactor operations [45]. In fact, the main predicted functions obtained by the end of the operation, outside of pathways necessary for basic metabolism, consisted of metabolic pathways related to fatty acid oxidation,

which could be related to the degradation of the surfactant (**Figure S-7**). However, further studies, including gene upregulation and multiomics, should be performed to corroborate the degradability of the surfactant under the conditions present in the CBR.

No accumulation of biomass on the walls of the capillary glass channels was observed during the entire period of more than 300-days operation of the CBR. This observation can be explained by the relatively high shear forces inside the channels and is consistent with the observations in other long-term studies where no biofilm attachment was observed inside capillaries [46,47].

4. Conclusions

In Part I of this study, different experiments were undertaken in which the liquid phase was altered for dilute methane treatment in a capillary bioreactor. The surfactants BRIJ 58 and SDBS, in contrast to TWEEN 60, both showed to be able to significantly enhance bioavailability of dilute methane at the concentrations tested. The lower apparent gas–liquid partition coefficient of methane and the enhanced cell hydrophobicity of the methane oxidizing consortium appear to be the main mechanism. The surfactant concentration required to obtain the maximum emulsion capacity of oil-in-water mixtures was low enough to prevent microbial inhibition for BRIJ 58 and TWEEN 60, but not for SDBS. This make SDBS less beneficial as additive in a bioreactor with silicone oil as non-aqueous phase, also because the foaming potential of SDBS is significantly higher than that of BRIJ 58 and TWEEN 60. BRIJ 58 was found to enhance the gas–liquid mass transfer in a capillary channel, but the effect was significant only when combined with silicone oil. The enhanced emulsification of the oil by the surfactant appeared to be the main mechanism for this enhancement, rather than the modification of the gas–liquid partial coefficient of methane.

In Part II, a capillary bioreactor containing silicone oil and BRIJ 58 successfully treated dilute methane (~4,500 ppm_v) at an elimination capacity of 231 ± 30 g methane per m^3 internal capillary channel per hour at an efficiency of 51 ± 2 % at an empty channel gas contact time of 23 s, which is one order of magnitude lower than the empty bed gas contact time of conventional biological gas treatment methods treating dilute methane. The improved emulsification capacity of the oil-in-water emulsion combined with enhanced cell hydrophobicity appeared to be the main mechanism. The optimised liquid phase consisted of water containing nutrients, silicone oil (20 % v/v, 20 cSt), and BRIJ 58 (160 mg L^{-1} = 1.8 CMC). The silicone oil acting as a buffer for methane was confirmed in a test that showed no deterioration in methane removal in the CBR following the methane supply interruption of six days. The use of surfactants and silicone oil, along with the improved bioavailability of methane, promoted a strong microbial specialization by the end of the operation of the CBR with the most abundant aerobic methanotroph belonging to the genus *Methylosarcina* with an increase in the relative abundance of *Lacunisphaera*. No accumulation of biomass on the walls of the capillary glass channels was observed during the entire period of more than 300-days operation of the CBR. It appears that a CBR with an optimized liquid phase, when operated with internal gas recirculation and thus decoupling optimal conditions for mass transfer from the gas contact time, may be a useful platform for further exploring the abatement of dilute methane.

CRediT authorship contribution statement

Norbertus J.R. Kraakman: Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Luis Villarreal-Heras:** Validation, Investigation. **Javier González-Martín:** Investigation. **Sara Cantera:** Writing – review & editing, Methodology, Data curation. **Raúl Muñoz:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Data curation. **Raquel Lebrero:** Writing – review & editing, Supervision, Resources, Project administration, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The Spanish Research Agency (PDC2022-133394-I00) is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2025.161383.

Data availability

Data will be made available on request.

References

[1] F.M. O'Connor, O. Boucher, N. Gedney, C.D. Jones, G.A. Folberth, R. Coppell, P. Friedlingstein, W.J. Collins, J. Chappellaz, J. Ridley, J. Ridley, C.E. Johnson, Possible role of wetlands, permafrost, and methane hydrates in the methane cycle under future climate change: a review, Rev. Geophys. 48 (2010), https://doi.org/10.1029/2010RG000326.
[2] IEA, 2023 Analysis, Methane Tracker (2023). https://www.iea.org/reports/global-methane-tracker-2023 (accessed August 6, 2024).
[3] WMO, World Meteorological Organization Greenhouse Gas Bulletin No. 19, (2023).
[4] J.H.M. Harmsen, D.P. van Vuuren, D.R. Nayak, A.F. Hof, L. Höglund-Isaksson, P. L. Lucas, J.B. Nielsen, P. Smith, E. Stehfest, Long-term marginal abatement cost curves of non-CO2 greenhouse gases, Environ. Sci. Policy 99 (2019) 136–149, https://doi.org/10.1016/j.envsci.2019.05.013.
[5] I. Pecorini, R. Iannelli, Landfill GHG reduction through different microbial methane oxidation biocovers, Processes 8 (2020), https://doi.org/10.3390/PR8050591.
[6] B. Khabiri, M. Ferdowsi, G. Buelna, J.P. Jones, M. Heitz, Methane biofiltration under different strategies of nutrient solution addition, Atmos. Pollut. Res. 11 (2020) 85–93, https://doi.org/10.1016/j.apr.2019.09.018.
[7] H. La, J.P.A. Hettiaratchi, G. Achari, P.F. Dunfield, Biofiltration of methane, Bioresour. Technol. 268 (2018) 759–772, https://doi.org/10.1016/j.biortech.2018.07.043.
[8] K.A. Stone, M.V. Hilliard, Q.P. He, J. Wang, A mini review on bioreactor configurations and gas transfer enhancements for biochemical methane conversion, Biochem. Eng. J. 128 (2017) 83–92, https://doi.org/10.1016/j.bej.2017.09.003.
[9] N.J.R. Kraakman, J. Rocha-Rios, M.C.M. Van Loosdrecht, Review of mass transfer aspects for biological gas treatment, Appl. Microbiol. Biotechnol. 91 (2011) 873–886, https://doi.org/10.1007/s00253-011-3365-5.
[10] S. Bordel, N.J.R. Kraakman, R. Muñoz, Theoretical analysis of gas–liquid mass transfer in Taylor flow capillary reactors, Chem. Eng. Sci. 292 (2024) 119949, https://doi.org/10.1016/J.CES.2024.119949.
[11] S. Haase, D.Y. Murzin, T. Salmi, Review on hydrodynamics and mass transfer in minichannel wall reactors with gas–liquid Taylor flow, Chem. Eng. Res. Des. 113 (2016) 304–329, https://doi.org/10.1016/j.cherd.2016.06.017.
[12] M.T. Kreutzer, F. Kapteijn, J.A. Moulijn, J.J. Heiszwolf, Multiphase monolith reactors: chemical reaction engineering of segmented flow in microchannels, Chem. Eng. Sci. 60 (22) (2005) 5895–5916, https://doi.org/10.1016/j.ces.2005.03.022.
[13] R. Lebrero, D.F. Osvaldo, V. Pérez, S. Cantera, J.M. Estrada, R. Muñoz, Biological treatment of gas pollutants in partitioning bioreactors, 2019. https://doi.org/10.1016/bs.ache.2018.12.003.
[14] S. Cantera, J.M. Estrada, R. Lebrero, P.A. García-Encina, R. Muñoz, Comparative performance evaluation of conventional and two-phase hydrophobic stirred tank reactors for methane abatement: mass transfer and biological considerations, Biotechnol. Bioeng. 113 (2016) 1203–1212, https://doi.org/10.1002/bit.25897.
[15] N.J.R. Kraakman, J. González-Martín, C.S. Garcia, S. Cantera, R. Lebrero, R. Muñoz, Multi-channel capillary bioreactor for hydrophobic VOC and CO2 abatement – process intensification through silicone oil addition, J. Environ. Chem. Eng. 12 (2024), https://doi.org/10.1016/j.jece:2024.113695.
[16] C. Pascual, S. Cantera, R. Muñoz, R. Lebrero, Comparative assessment of two biotrickling filters for siloxanes removal: effect of the addition of an organic phase, Chemosphere 251 (2020), https://doi.org/10.1016/j.chemosphere.2020.126359.
[17] P.A. Lamprea Pineda, K. Demeestere, M. Toledo, H. Van Langenhove, C. Walgraeve, Enhanced removal of hydrophobic volatile organic compounds in biofilters and biotrickling filters: A review on the use of surfactants and the

addition of hydrophilic compounds, Chemosphere 279 (2021), https://doi.org/10.1016/j.chemosphere.2021.130757.
[18] L. Wang, C. Yang, Y. Cheng, J. Huang, H. He, G. Zeng, L. Lu, Effects of surfactant and Zn (II) at various concentrations on microbial activity and ethylbenzene removal in biotricking filter, Chemosphere 93 (2013) 2909–2913, https://doi.org/10.1016/j.chemosphere.2013.09.109.
[19] D. Zhang, L. Zhu, F. Li, Influences and mechanisms of surfactants on pyrene biodegradation based on interactions of surfactant with a Klebsiella oxytoca strain, Bioresour. Technol. 142 (2013) 454–461, https://doi.org/10.1016/j.biortech.2013.05.077.
[20] I. Cattaneo, M.C. Astuto, Surfactants, anionic and nonionic, Encyclopedia Toxicol. (2024) 823–827, https://doi.org/10.1016/B978-0-12-824315-2.00357-2.
[21] X. Wu, Y. Lin, Y. Wang, S. Wu, X. Li, C. Yang, Enhanced removal of hydrophobic short-chain n-alkanes from gas streams in biotrickling filters in presence of surfactant, Environ. Sci Technol 56 (2022) 10349–10360, https://doi.org/10.1021/acs.est.2c02022.
[22] P.A. Lamprea Pineda, K. Demeestere, M. Sabbe, J. Bruneel, H. Van Langenhove, C. Walgraeve, Effect of (bio)surfactant type and concentration on the gas-liquid equilibrium partitioning of hydrophobic volatile organic compounds, J Hazard Mater 443 (2023), https://doi.org/10.1016/j.jhazmat.2022.130320.
[23] A.P. Kempka, F.J. Horvath, P. Fagundes, R.C. Prestes, Foaming and emulsification capacity, foam and emulsion stability of proteins of porcine blood, Rev. Brasil. Technol. Agroind. 9 (2015) 1797–1809.
[24] J.M. Estrada, R. Lebrero, G. Quijano, R. Pérez, I. Figueroa-González, P.A. García-Encina, R. Muñoz, Methane abatement in a gas-recycling biotrickling filter: evaluating innovative operational strategies to overcome mass transfer limitations, Chem. Eng. J. 253 (2014) 385–393, https://doi.org/10.1016/j.cej.2014.05.053.
[25] APHA, AWWA, WEF, Standard Methods for the Examination of Water and Wastewater, 23rd ed., 2017.
[26] R.C. Edgar, B.J. Haas, J.C. Clemente, C. Quince, R. Knight, UCHIME improves sensitivity and speed of chimera detection, Bioinformatics 27 (2011) 2194–2200, https://doi.org/10.1093/bioinformatics/btr381.
[27] T. Magoč, S.L. Salzberg, FLASH: Fast length adjustment of short reads to improve genome assemblies, Bioinformatics 27 (2011) 2957–2963, https://doi.org/10.1093/bioinformatics/btr507.
[28] C. Quast, E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, F. O. Glöckner, The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, Nucleic Acids Res. 41 (2013), https://doi.org/10.1093/nar/gks1219.
[29] R. Kolde, Pheatmaps: Pretty Heatmaps, (2019). https://cran.r-project.org/package=pheatmap (accessed August 6, 2024).
[30] G.M. Douglas, V.J. Maffei, J.R. Zaneveld, S.N. Yurgel, J.R. Brown, C.M. Taylor, C. Huttenhower, M.G.I. Langille, PICRUSt2 for prediction of metagenome functions, Nat. Biotechnol. 38 (2020) 685–688, https://doi.org/10.1038/s41587-020-0548-6.
[31] A. Avalos Ramirez, B.P. García-Aguilar, J.P. Jones, M. Heitz, Improvement of methane biofiltration by the addition of non-ionic surfactants to biofilters packed with inert materials, Process Biochem. 47 (2012) 76–82, https://doi.org/10.1016/j.procbio.2011.10.007.
[32] U. Miller, I. Sówka, W. Adamiak, The application of Brij 35 in biofiltration of the air polluted with toluene vapours, in: E3S Web of Conferences, 2018. https://doi.org/10.1051/e3sconf/20184400113.
[33] S. Dhamwichukorn, G.T. Kleinheinz, S.T. Bagley, Thermophilic biofiltration of methanol and α-pinene, J. Ind. Microbiol. Biotechnol. 26 (2001) 127–133, https://doi.org/10.1038/sj.jim.7000079.
[34] W.C. Chan, Y.H. You, The influence of non-ionic surfactant BRIJ 30 on biodegradation of toluene in a biofilter, Afr. J. Biotechnol. 9 (2010) 5914–5921.
[35] U. Miller, I. Sówka, W. Adamiak, The use of surfactant from the Tween group in toluene biofiltration, Arch. Environ. Prot. 46 (2020) 53–57, https://doi.org/10.24425/aep.2020.133474.
[36] P.A. Lamprea-Pineda, F.J. Carmona, K. Demeestere, J.J. González-Cortés, H. Van Langenhove, C. Walgraeve, R. Lebrero, Effect of surfactant type and concentration on the gas-liquid mass transfer in biotrickling filters used for air pollution control, J. Environ. Manage. 367 (2024), https://doi.org/10.1016/j.jenvman.2024.121968.
[37] L. Wang, C. Yang, Y. Cheng, J. Huang, H. Yang, G. Zeng, L. Lu, S. He, Enhanced removal of ethylbenzene from gas streams in biotrickling filters by Tween-20 and Zn(II), J Environ Sci (china) 26 (2014) 2500–2507, https://doi.org/10.1016/j.jes.2014.04.011.
[38] Y. Deng, G. Yang, P.N.L. Lens, Y. He, L. Qie, X. Shen, J. Chen, Z. Cheng, D. Chen, Enhanced removal of mixed VOCs with different hydrophobicities by Tween 20 in a biotrickling filter: Kinetic analysis and biofilm characteristics, J. Hazard. Mater. 450 (2023) 131063, https://doi.org/10.1016/J.JHAZMAT.2023.131063.
[39] M.M. Amin, A. Rahimi, B. Bina, F. Mohammadi Moghadam, H. Nourmoradi, M. Heidari, Effect of a non-ionic surfactant on xylene removal in a scoria-compost-based biofilter, Clean (Weinh) 44 (2016) 1759–1765, https://doi.org/10.1002/clen.201500415.
[40] N.J.R. Kraakman, S. Bordel, R. Lebrero, R. Muñoz, Dilute methane biofiltration through multi-channel taylor flow capillary bioreactors, Curr. Opin. Environ. Sci. Health (2025).
[41] C. Kennelly, S. Gerrity, G. Collins, E. Clifford, Liquid phase optimisation in a horizontal flow biofilm reactor (HFBR) technology for the removal of methane at low temperatures, Chem. Eng. J. 242 (2014) 144–154.
[42] B.P. García-Aguilar, A.A. Ramirez, J.P. Jones, M. Heitz, Solubility of methane in pure non-ionic surfactants and pure and mixtures of linear alcohols at 298 K and 101.3 kPa, Chem. Pap. 65 (2011) 373–379, https://doi.org/10.2478/s11696-011-0008-3.
[43] P. Aussillous, D. Quere, Quick deposition of a fluid on the wall of a tube, Phys. Fluids 12 (2000) 2367–2371, https://doi.org/10.1063/1.1289396.
[44] T.C. Thulasidas, M.A. Abraham, R.L. Cerro, Flow patterns in liquid slugs during bubble-train flow inside capillaries, Chem. Eng. Sci. 52 (1997) 2947–2962, https://doi.org/10.1016/S0009-2509(97)00114-0.
[45] F. Peters, D. Arabali, Interfacial tension between oil and water measured with a modified contour method, Colloids Surf. A Physicochem Eng Asp 426 (2013) 1–5, https://doi.org/10.1016/j.colsurfa.2013.03.010.
[46] Y. Zheng, H. Wang, Z. Yu, F. Haroon, M.E. Hernández, L. Chistoserdova, Metagenomic insight into environmentally challenged methane-fed microbial communities, Microorganisms 8 (2020) 1–17, https://doi.org/10.3390/microorganisms8101614.
[47] C. Martineau, F. Mauffrey, R. Villemur, Comparative analysis of denitrifying activities of Hyphomicrobium nitrativorans, Hyphomicrobium denitrificans, and Hyphomicrobium zavarzinii, Appl. Environ. Microbiol. 81 (2015) 5003–5014, https://doi.org/10.1128/AEM.00848-15.