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Country report

Biogas bioconversion into poly(3-hydroxybutyrate) by a mixed microbial culture in a novel Taylor flow bioreactor

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ABSTRACT

Biogas-based biopolymer production represents an alternative biogas valorization route with potential to cut down plastic pollution and greenhouse gas emissions. This study investigated for the first time the continuous bioconversion of methane, contained in biogas, into poly(3-hydroxybutyrate) (PHB) by a mixed methanotrophic culture using an innovative high mass-transfer Taylor flow bioreactor. Following a hydrodynamic flow regime mapping, the influence of the gas residence time and the internal gas recirculation on CH_4 abatement was assessed under non nutrient limiting conditions. Under optimal operational conditions (gas residence time of 60 min and internal gas recycling ratio of 17), the bioreactor was able to support a CH_4 removal efficiency of 63.3%, a robust CH_4 elimination capacity (17.2 g-CH_4 $m^{-3}h^{-1}$) and a stable biomass concentration (1.0 g L^{-1}). The simultaneous CH_4 abatement and PHB synthesis was investigated under 24-h:24-h nitrogen feast/famine continuous operation. The cyclic nitrogen starvation and the Taylor flow imposed in the bioreactor resulted in a relatively constant biomass concentration of 0.6 g L^{-1} with PHB contents ranging from 11 to 32% *w* w^{-1} (on a dry weight basis), entailing an average PHB productivity of 5.9 g-PHB m^{-3} d^{-1} with an associated PHB yield of 19.8 mg-PHB g-CH_4^{-1}. Finally, the molecular analysis of the microbial population structure indicated that type II methanotrophs outcompeted non-PHB accumulating type I methanotrophs, with a heterotrophic-methanotrophic consortium enriched in *Methylocystis*, *Hyphomicrobium*, *Rubinisphaeraceae* SH PL14 and *Pseudonocardia*.

1. Introduction

Biogas is one of the main by-products from the anaerobic digestion of organic waste and wastewaters. Biogas is typically composed of a mixture of CH_4 (≈50–70%), CO_2 (≈30–50%) and trace contaminants like H_2S, NH_3 and siloxanes (Muñoz et al., 2015). Methane (CH_4) is the main valuable component in biogas due to its high calorific value and therefore potential application as renewable energy vector (IEA, 2004). However, CH_4 generation may also represent an environmental burden if not properly managed due to its global warming potential (GWP) (≈25 folds the GWP of CO_2 in a time horizon of 100 years). For instance, the European Union 27 member countries (EU-27) emitted ≈385.4 Mt of CH_4 as CO_2eq in 2019, an amount that must be rapidly reduced as a result of the recent EU methane mitigation strategy (EEA (European Environmental Agency), 2021; European Commission, 2020). It is estimated that around 56% of methane emissions are diluted, which cannot be used for energy generation. These emissions mainly come from sewers, manure storage tanks, cattle operation and ventilated coal mines (Cantera et al., 2018), and severely contribute to global warming and climate change.

Despite the feasible use of biogas as fuel for heat and electricity generation, according to the EEA (2020), its 33 member countries still emitted 19.8 Mt of CH_4 as CO_2eq from landfills in 2017 as a result of the limited economic viability of combined heat and power plants due to their high investment, operational and maintenance costs (da Costa Gomez, 2013; Kaparaju and Rintala, 2013). Indeed, biogas exhibits a levelized cost of electricity (LCOE) similar to fossil fuels (0.05–0.19$ KWh^{-1}), but recently higher than the LCOE of solar and wind power (IRENA, 2021). In this context, there is an urgent need to develop innovative and cost-competitive valorization routes for biogas in order

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to foster the economic sustainability of anaerobic digestion and mitigate new potential CH_4 emissions.

The production of polyhydroxyalkanoates (PHAs) using biogas as the feedstock has been recently explored and might be competitive if properly optimized (Comesaña-Gándara et al., 2022; López et al., 2018; Pérez et al., 2020). These biopolymers are considered as a sustainable substitute of fossil-based plastics owing to their biodegradable nature and their low carbon footprint when produced from waste emissions such as biogas. Poly(3-hydroxybutyrate) (PHB) is the most representative type of PHA synthetized by type II methanotrophs under nutrient limiting conditions. In these organisms, CH_4 is firstly oxidized by a methane monooxygenase to then enter the serine pathway, where deprivation of nutrients such as nitrogen and phosphate induce the synthesis and intracellular accumulation of PHB (Cal et al., 2016; Pieja et al., 2011; Rodríguez et al., 2020a). To date, pure cultures have been mainly used as workhorse in continuous bioreactors converting CH_4 into PHB (García-Pérez et al., 2018; Rodríguez et al., 2020b), which represents an ecological and economical barrier to further scale up this process. Mixed cultures typically show a more robust performance against environmental fluctuations and process upsets than pure cultures, and show negligible differences on CH_4 abatement at different temperatures, O_2 concentrations and nutrients availability (Chidambarampadmavathy et al., 2015; Karthikeyan et al., 2015; Pérez et al., 2019a, 2019b).

Despite the significant advances carried out in this field over the past 10 years, a cost-effective PHB production using biogas as feedstock and type II methanotrophs as workhorse must overcome key technological barriers. Thus, high energy inputs and bioreactor volumes are needed to achieve an effective CH_4 mass transfer from the gas to the aqueous phase, which can jeopardize the economic feasibility of the process. To date, to the best of the authors' knowledge, only bubble column, fluidized bed and stirred tank bioreactors have been operated at lab and pilot scale for the simultaneous abatement of CH_4 and production of PHB (Chidambarampadmavathy et al., 2015; García-Pérez et al., 2018; Pfluger et al., 2011; Rodríguez et al., 2020b). Thus, there is an urgent need to develop and assess novel bioreactor configurations for the continuous CH_4 abatement and biopolymer production (Karthikeyan et al., 2015). In this regard, Taylor flow reactors are multi-capillary channels systems where sequences of gas bubbles and liquid slugs move in an upflow co-current mode (this is why the terms Taylor flow and slug flow can be used interchangeably), which can support gas–liquid volumetric mass transfer coefficients one order of magnitude higher than conventional reactors due to the internal recirculation within the liquid slug and gas bubble, the large specific surface area, and the small diffusion paths (Gupta et al., 2010; Rocha-Rios et al., 2013; Rodríguez et al., 2020c). This innovative reactor configuration has been tested in several different fields (e.g., biomedical, oil and gas industry, chemical processing, and so on (Gupta et al., 2010) with promising results but never tested in biogas-based biopolymer production applications.

This study assessed for the first time the performance of a novel Taylor flow bioreactor during biogas bioconversion into PHB using a mixed methanotrophic culture under continuous mode. The Taylor flow regime was initially mapped, and the influence of gas residence time and internal gas recycling on the CH_4 elimination capacity of the Taylor flow bioreactor was investigated. This study also evaluated the continuous production of PHB from biogas under optimal CH_4 mass transfer conditions using a mixed methanotrophic culture subjected to nitrogen feast/famine cycles. Finally, the structure of the microbial community supporting PHB synthesis from biogas under steady state was also investigated.

2. Materials and methods

2.1. Chemicals

The mineral salt medium (MSM) used during the entire experiment, unless otherwise specified, consisted of (mg L^{-1}): 409.5 KH_2PO_4; 534 $Na_2HPO_4{\cdot}12H_2O$; 2000 KNO_3; 200 $MgCl_2{\cdot}6H_2O$; 110 $CaCl_2{\cdot}2H_2O$; 2.76 $Na_2EDTA{\cdot}2H_2O$; 10 $CuSO_4{\cdot}5H_2O$; 5 $FeSO_4{\cdot}7H_2O$; 4 $ZnSO_4{\cdot}7H_2O$; 0.15 H_3BO_3; 0.27 $CoCl_2$; 0.2 $MnCl_2{\cdot}4H_2O$; 0.1 $NiCl_2{\cdot}6H_2O$ and vitamins (biotin, nicotinamide, p-aminobenzoic acid and panthotenic acid). Potassium nitrate was obtained from Cofarcas S.A. (Burgos, Spain), whereas the rest of the constituents required for the preparation of the MSM were acquired from PanReac AppliChem (Barcelona, Spain). Gas cylinders of CH_4 (purity $\geq$ 99.995%), O_2 ($\geq$ 99.5%) and synthetic biogas (70% CH_4, 30% CO_2) were purchased from Abelló Linde S.A. (Barcelona, Spain). The commercial Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV, with a PHV content of 12% mol) used for the preparation of standard biopolymer solutions in chloroform was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Inocula

An enriched methanotrophic culture obtained from *Sphagnum* (a peat moss rich in methanotrophs) and an enriched methanotrophic culture mixture obtained from *Sphagnum* + activated sludge, with the ability to synthesize PHB under nitrogen limitation (Pérez et al., 2019b), were mixed with a pure culture of the type II methanotroph *Methylocystis hirsuta* CSC1 (DSM 18500) (Rodríguez et al., 2020a). The three methanogenic cultures were independently inoculated in duplicate (under sterile conditions for *M. hirsuta*) at 5% ($v\,v^{-1}$) in 120 mL bottles (19 mL of MSM + 1 mL of inoculum) containing a 67%/33% O_2:CH_4 atmosphere to ensure oxygen non-limiting conditions, given that previous reports have identified a minimum of ~ 1.5 mol O_2 mol CH_4^{-1} for an effective CH_4 oxidation and PHB accumulation (Rodríguez et al., 2020a). The cultures were incubated at 30 °C and 250 rpm in an orbital shaker (MaxQ4000; Thermo Scientific, USA) for ~ 14 days. The headspace of the gas-tight bottles was daily replaced with a fresh 67%/33% ($v\,v^{-1}$) O_2:CH_4 atmosphere. The cultures were transferred to six 2.1-L gas-tight serum bottles (containing 380 mL of MSM + 20 mL of inoculum) and incubated at 25 °C and 300 rpm for 10 days using a similar 67%/33% ($v\,v^{-1}$) O_2:CH_4 headspace (the headspace of all the bottles was replaced with the initial O_2/CH_4 atmosphere on day 6). Prior inoculation, the cultures were centrifuged and resuspended in fresh MSM to a final volume of 600 mL and to a final biomass concentration of 7.5 g TSS L^{-1}.

2.3. Experimental setup

A 6-L (working volume) Taylor flow bioreactor with internal gas recirculation was used in this work (Fig. 1). The bioreactor consisted of two polyvinyl chloride (PVC) tanks, containing the methanotrophic culture in aqueous suspension, interconnected by 25 glass circular capillary tubes with an internal diameter of 3 mm and length of 1.5 m. The lower tank was constructed with a butyl perforated membrane to allow the sparging of a gas mixture composed of atmospheric air and synthetic biogas. The biogas stream was regulated by a mass flow controller (GFC17, AalborgTM, USA) to ensure a CH_4 concentration of 4% (26.2 g m^{-3}), whereas atmospheric air was supplied by an air compressor (PUSKA COMBA 3200 II). A water condenser (kept at 10 °C) was installed at the internal gas recirculation line in order to prevent operational problems derived from water condensation. A ESPA Tecno 052 M centrifugal pump was used to recycle the cultivation broth. Internal gas recirculation was carried out using a Watson-Marlow 520S® peristaltic pump (WMFTG, UK). The Taylor flow bioreactor was operated at 25 °C in a temperature-controlled room.

2.4. Taylor flow regime mapping

The mapping of flow regimes in the reactor was performed using air as the gas phase and MSM as the liquid phase by systematically varying the air flow rates (0.9–9.9 L min^{-1}) and MSM flow rates (3–10 L min^{-1}). In addition, the pressure in the inlet gas line was measured to later

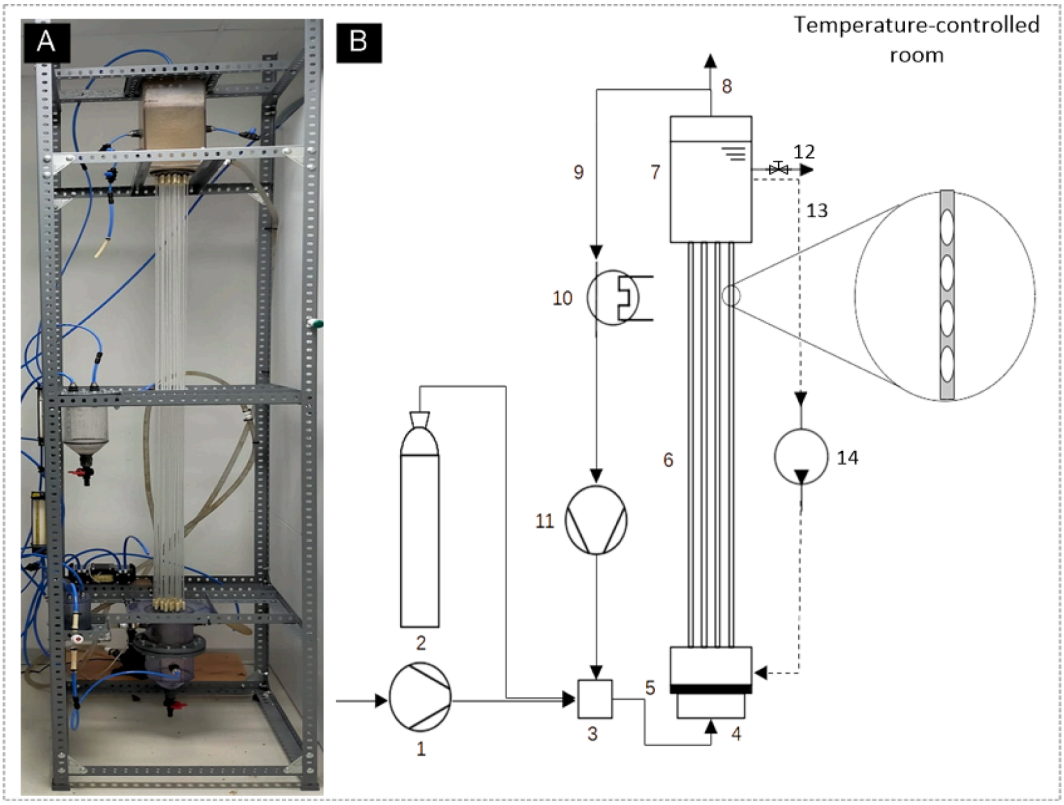

Fig. 1. Photograph (A) and sketch (B) of the innovative Taylor flow bioreactor used to produce PHB from synthetic biogas by a mixed methanotrophic culture. Slug flow regime is shown in a zoom-in. Air compressor (1), synthetic biogas tank (2), mixing chamber (3), gas inlet (4), butyl membrane (5), 3-mm capillary tubes (6), biomass containing chamber (7), gas outlet (8), gas recirculation line (9), condenser (10), gas compressor (11), liquid sampling port (12), liquid recirculation line (13), hydraulic pump (14).

calculate the gas flow rate and upflow gas velocities at atmospheric pressure. The hydrodynamic regime in the tubes was video-recorded and analysed afterwards to elucidate the occurrence of Taylor flow (Rocha-Rios et al., 2013).

2.5. Influence of gas flow rate and internal gas recycling on CH_4 abatement in a Taylor flow bioreactor

The influence of gas flow rates (0.9, 1.8 and 3.6 L min^{-1}) achieved without gas recirculation (inlet flow corresponding to the total gas flow) and with internal gas recirculation (inlet gas flow × recirculation ratio + inlet gas flow corresponding to the total gas flow) on CH_4 biodegradation was evaluated at a constant liquid recirculation flow rate of 7 L min^{-1} (which guaranteed Taylor flow regime according to the flow regime map performed). The duration of each operational stage was set by the achievement of stable CH_4 elimination capacities (ECs). Table 1 depicts the experimental design. Aliquots of 500 mL of MSM were daily exchanged to ensure nutrients availability, resulting in a dilution rate of 0.083 d^{-1}. An abiotic test was conducted to ensure the absence of any abiotic CH_4 degradation mechanism and the gas tightness of the bioreactor. CH_4, CO_2 and O_2 concentrations in the inlet and outlet gas streams were daily determined by GC-TCD using 100 μL samples drawn with a Hamilton® GASTIGHT® syringe (Hamilton Co., USA). Aliquots of 40 mL from the cultivation broth were daily taken to measure the pH, optical density (OD) and the concentration of total suspended solids (TSS) as a biomass concentration proxy, dissolved total organic carbon (TOC), dissolved total nitrogen (TN), and nitrate (NO_3^-) and nitrite (NO_2^-) concentrations.

Table 1
Experimental conditions tested during the optimization of CH_4 abatement.

Test no.	Total gas flow rate (L min^{-1})	Inlet gas flow rate (L min^{-1})	Recirculation ratio[a]	EBRT[b] (min)	Virtual EBRT[c] (min)	Inlet CH_4 loading rate[d] (g CH_4 $m^{-3}h^{-1}$)
1.1	0.9	0.9	0.0	6.7	6.7	236
1.2		0.1	8.0	60.0	6.7	26.2
1.3		0.2	3.5	30.0	6.7	52.4
2.1	1.8	1.8	0.0	3.3	3.3	471.6
2.2		0.1	17.0	60.0	3.3	26.2
2.3		0.2	8.0	30.0	3.3	52.4
3.1	3.6	3.6	0.0	1.7	1.7	943.2
3.2		0.1	35.0	60.0	1.7	26.2
3.3		0.2	17.0	30.0	1.7	52.4

a-Recirculation ratio [R]: the internal recirculation gas flow rate divided by the inlet gas flow rate.
b-Empty bioreactor residence time [EBRT]: the bioreactor volume divided by the inlet gas flow rate.
c-Virtual EBRT: the bioreactor volume divided by the total gas flow rate.
d-Inlet CH_4 loading rate: mass of methane supplied per unit of bioreactor volume and time.
All conditions were tested at a constant liquid recirculation flow rate of 7 L min^{-1}.

2.6. Simultaneous methane abatement and PHB production under cyclic nitrogen feast/famine operation

The assessment of the continuous production of PHB from biogas was divided into three operational stages. During stage I, the Taylor flow bioreactor was operated under nitrogen sufficient conditions at an

internal gas flow rate of 1.8 L min^{-1} (recirculation ratio = 17) and an inlet gas flow rate of 0.1 L min^{-1} for 11 days to ensure a stable methane elimination. Aliquots of 500 mL of cultivation broth were daily exchanged with fresh MSM. During stage II, the daily exchange of fresh MSM was supressed for the following 9 days to ensure nitrogen limiting conditions in the Taylor flow bioreactor, which was operated under similar gas operational conditions to stage I. During stage III, nitrogen feast famine cycles of 24-h:24-h were applied for 28 days to induce the biosynthesis of PHB under similar gas operational conditions to stage II. Thus, the supply of 0.5 L of MSM containing a nitrogen concentration of 165.3 mg L^{-1} (estimated to support biomass growth for 24 h based on the prevailing CH_4-EC) was only carried out every two days to induce nitrogen limiting conditions for 24 h (D = 0.042 d^{-1}). CH_4, CO_2 and O_2 concentrations in the inlet and outlet gas streams were daily determined by GC-TCD using 100 μL samples drawn with a Hamilton® GASTIGHT® syringe (Hamilton Co., USA). Aliquots of 40 mL from the cultivation broth were daily taken to measure the PHB content in the methanotrophic biomass, pH, OD, and the concentrations of TSS, TOC, TN, NO_2^- and NO_3^-.

2.7. Analytical procedures

CH_4, CO_2 and O_2 gas concentrations were measured in a gas chromatograph coupled with a thermal conductivity detector (Bruker 430, Bruker Corporation, USA) and equipped with CP-Molsieve 5A and CP-PoraBOND Q columns according to Carmona-Martínez et al. (2021). The outlet gas flow rate was measured by using the water volume displacement method. TSS concentration was quantified according to the 2540 method (APHA et al., 2017) using 0.45 μm pore size filters (Merck, Germany). Optical density was measured at 600 nm using a Spectrostar Nano (BMG Labtech, Germany). A Basic 20 pH meter (Crison, Spain) was used for the measurement of pH. TN and TOC concentrations were determined in a TOC-V analyzer equipped with a Shimadzu TNM-1 unit. NO_2^- and NO_3^- concentrations were measured by ion chromatography using a Waters 432 HPLC conductivity detector (Waters Corporation, USA) according to Guenka Scarcelli et al. (2021). Finally, PHB concentration was determined using gas chromatography-mass spectrometry (GC–MS) following digestion and extraction according to Rodríguez et al. (2020a). The structure of the microbial communities was analysed from two samples taken at the beginning of the bioreactor operation and at the end of the PHB production experiment. This analysis was carried out by following the 16S metagenomic sequencing library Illumina 15,044,223B protocol. The region V3-V4 of the 16S rRNA gene was amplified using the primer set 341F-805R (Klindworth et al., 2013). The sequencing data obtained were analysed into the QIIME2 platform (Bolyen et al., 2019). Clean amplicon sequencing variants (ASVs) were annotated against NCBI 16S rRNA database version 2021 at a 97% similarity, while SILVA database v.138 was used for those ASVs assigned with < 97% identity. Data was normalized using rarefaction technique from Phyloseq R package to perform alpha diversity analysis (Weiss et al., 2017). Shannon and Simpson indexes were calculated using vegan R package (Oksanen et al., 2020).

2.8. Performance indicators

The CH_4 elimination capacity (EC), CH_4 removal efficiency (RE), volumetric CO_2 production rate (RCO_2) and PHB productivity were calculated using equation (1), (2), (3) and (4), respectively:

$$EC = \frac{Q\cdot(CH_{4in} - CH_{4out})}{V}\left[\mathrm{g\,CH_4\,m^{-3}\,h^{-1}}\right] \quad (1)$$

$$RE = \frac{(CH_{4in} - CH_{4out})}{CH_{4in}} \times 100\ [\%] \quad (2)$$

$$RCO_2 = \frac{Q\cdot(C0_{2out} - CO_{2in})}{V}\left[\mathrm{g\,CO_2\,m^{-3}\,h^{-1}}\right] \quad (3)$$

$$PHB\ productivity = TSS\cdot PHB\cdot D\ \left[\mathrm{g\,PHB\,m^{-3}\,d^{-1}}\right] \quad (4)$$

where, $CH_{4\ in}$ and $CH_{4\ out}$ stand for the inlet and outlet CH_4 concentrations (g m^{-3}), $CO_{2\ in}$ and $CO_{2\ out}$ are the inlet and outlet CO_2 concentrations (g m^{-3}), Q is the inlet gas flow rate (m^3 h^{-1}), V is the bioreactor working volume (m^3), TSS is the biomass concentration (g m^{-3}), PHB is the content of biopolymer in the biomass (% $w\ w^{-1}$ of cell dry weight (CDW) expressed in the fraction form) and D is the dilution rate (d^{-1}).

3. Results and discussion

3.1. Taylor flow regime mapping

Three main flow regimes were identified from the flow regime mapping performed by varying the gas and liquid superficial flow rates, i.e., bubbly, churn and Taylor (or slug) patterns. The presence of gas–liquid segmented flow hydrodynamics (Taylor bubble flow) was clearly observed in the medium range of tested flow superficial rates of liquid and gas streams (Fig. 2). In contrast, bubbly and churn flow patterns were observed at a combination of low gas (<0.5 m s^{-1}) and liquid superficial velocities (<0.5 m s^{-1}), and high gas (1.28 – 1.5 m s^{-1}) and liquid superficial velocities (0.65 – 1.0 m s^{-1}), respectively. To ensure Taylor flow regime, the liquid recirculation flow rate was set at 7 L min^{-1} (0.66 m s^{-1}), while the total gas flow rate was varied in the range of 0.9 to 3.6 L min^{-1} (0.08 – 0.34 m s^{-1}). Comparatively, Rocha-Rios et al. (2013) found bubbly flow conditions in a single capillary system at low gas velocities ($1.7 \times 10^{-2} - 2.5 \times 10^{-2}$ m s^{-1}) in combination with liquid superficial velocities in the range of $6.0 \times 10^{-2} - 1.1 \times 10^{-1}$ m s^{-1}, and churn flow patterns when combining high gas superficial velocity ($4.0 \times 10^{-2} - 1.0 \times 10^{-1}$ m s^{-1}) with liquid superficial velocities in the range of $2.5 \times 10^{-2} - 4.5 \times 10^{-2}$ m s^{-1}. At this point it must be highlighted the inherent difficulty to compare the gas–liquid flow regime maps found in the literature since those are reactor specific (Kreutzer et al., 2005). Indeed, the prevailing behavior of two-phase gas–liquid flow in a given microchannel depends on several parameters such as the fluid properties, flow rates, ratio of phases, channel geometry, as well the material and roughness of the microchannels and pressure and temperature

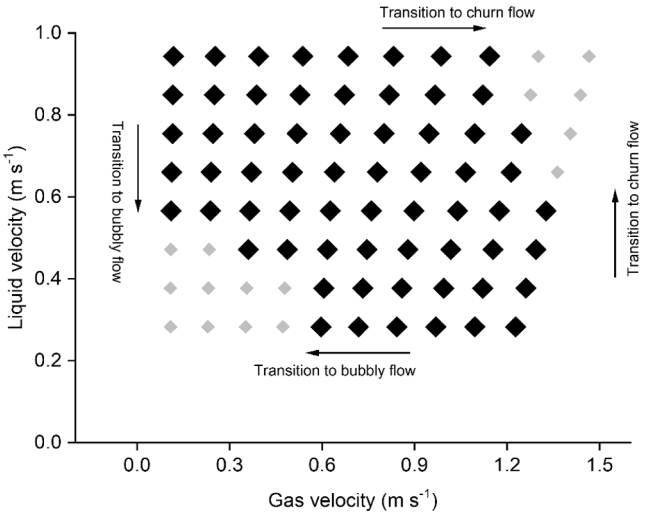

Fig. 2. Map of the gas–liquid flow regimes as a function of the gas and liquid velocities in the vertical, circular capillary microtubes. Black diamonds represent Taylor bubble flow while grey diamonds stand for bubbly and churn flow regimes.

(Gupta et al., 2010; Rocha-Rios et al., 2011).

3.2. Influence of gas flow rate and internal gas recycling on CH_4 abatement in a Taylor flow bioreactor

The continuous biodegradation of CH_4 in a Taylor flow reactor by a mixed methanotrophic culture was evaluated with and without gas recirculation under non-limiting nutrients conditions. In the absence of internal gas recycling, a gas flow rate of 0.9 L min^{-1} entailed a steady state CH_4-EC of 30.1 ± 4.5 g CH_4 $m^{-3}h^{-1}$, corresponding to a CH_4-RE of 13.0 ± 1.8%. A further increase in the gas flow rate to 1.8 L min^{-1} resulted in the highest EC observed of 49.5 ± 2.4 g CH_4 $m^{-3}h^{-1}$ with an associated CH_4-RE of 10.1 ± 0.6%, suggesting that mass transfer limitations constrained CH_4 abatement rather than biological activity. Indeed, RCO_2 increased from 38.2 ± 13.6 to 82.9 ± 6.1 g $m^{-3}h^{-1}$ when the gas flow rate was doubled from 0.9 to 1.8 L min^{-1}, the latter corresponding to a mineralization ratio (RCO_2/EC-CH_4) of 1.7, in accordance with those found by García-Pérez et al. (2018). This enhanced driving force caused a better CH_4 mass transfer performance, thus favouring CH_4 bioavailability and consequently its further utilization by the mixed methanotrophic culture (Pérez et al., 2019b). However, a higher total gas flow rate (3.6 L min^{-1}) led to a high turbulence within the bioreactor, which caused a rapid stripping of the biomass and a severe shear stress on the microbial community, thus impairing the CH_4-EC, which remained at 40.7 ± 4.0 g CH_4 $m^{-3}h^{-1}$ with an associated CH_4-REs of 3.9 ± 0.6%.

Under gas recirculation conditions, the CH_4-EC of the Taylor flow bioreactor increased when increasing both the inlet gas flow rate and the internal gas recirculation flow rate. Hence, steady state CH_4-ECs of 13.4 ± 1.2, 17.2 ± 1.9 and 21.1 ± 2.4 g CH_4 $m^{-3}h^{-1}$ (with associated CH_4-REs of 50.6 ± 2.5, 63.34 ± 0.9 and 71.31 ± 1.4 %, respectively) were recorded at total gas flow rates of 0.9, 1.8 and 3.6 L min^{-1}, respectively, operating at an inlet gas flow rate of 0.1 L min^{-1} with internal gas recirculation. Similarly, steady state CH_4-ECs of 20.1 ± 2.0, 26.7 ± 1.6, and 29.15 ± 3.8 g CH_4 $m^{-3}h^{-1}$ (corresponding to CH_4-REs of 35.5 ± 3.1, 46.2 ± 1.9 and 50.4 ± 6.6%) were observed at a constant inlet gas flow rate of 0.2 L min^{-1} under an internal gas recirculation flow rates supporting total gas flow rates of 0.9, 1.8, and 3.6 L min^{-1}, respectively (see Fig. 3). Thus, the increase in the inlet diluted biogas flow rate from 0.1 to 0.2 L min^{-1} supported a higher CH_4 concentration gradient between the gas and liquid phases at the expenses of lower CH_4-REs. It has been previously demonstrated that the volumetric mass transfer coefficient for CH_4 (K_La) can be enhanced by applying internal gas recycling, which decouples the real biogas residence time from the gas–liquid turbulence within the reactor (Estrada et al., 2014; Rocha-Rios et al., 2011). However, as previously mentioned, process operation at a total flow rate of 3.6 L min^{-1} caused biomass stripping and a severe shear stress inside the bioreactor due to the high turbulence created. This phenomenon was also observed at a lower extent when operating at an inlet flow rate of 0.2 L min^{-1} and a total gas flow rate of 3.6 L min^{-1} (recirculation ratio = 17), likely due to the acclimation of the microbial community to the gas shear stress (see Supplementary material). The CH_4-ECs observed in this study were endorsed by the recorded RCO_2 while working at gas flow rates of 0.9 and 1.8 L min^{-1} but this pattern was not observed when working at a gas flow rate of 3.6 L min^{-1} likely due to the decrease in biomass concentration (see Fig. 3 and Fig. S1). In this context, steady state CH_4-ECs were achieved at biomass concentrations of ≥ 1 g L^{-1}, except for the operational stages encountering the aforementioned biomass stripping and shear stress phenomena, thus confirming that CH_4-ECs were only affected by CH_4 transport and not by microbial activity.

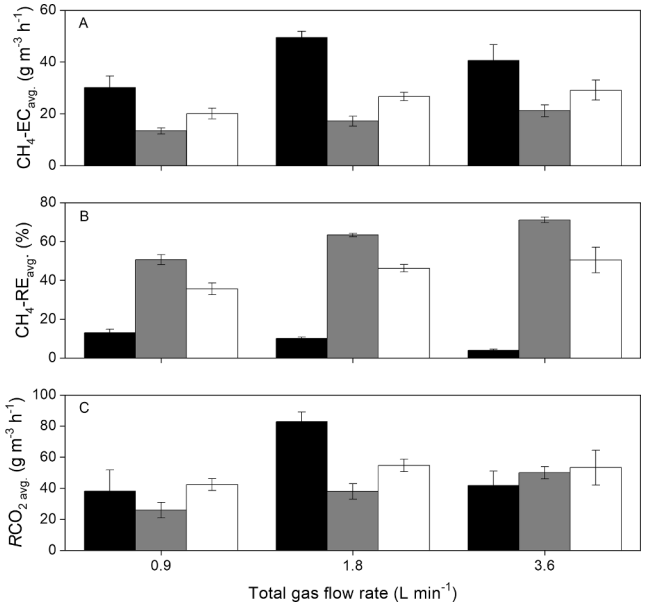

Fig. 3. Influence of total gas flow rate on steady state CH_4-EC (A), CH_4-RE (B) and RCO_2 (C) in tests conducted with no internal gas recirculation (blacks bars), and with internal gas recirculation at inlet gas flow rates of 0.1 L min^{-1} (gray bars) and 0.2 L min^{-1} (white bars).

Comparatively, the maximum CH_4-EC herein recorded (49.5 ± 2.4 g CH_4 $m^{-3}h^{-1}$) at an EBRT of 3.3 min was higher than those achieved in packed bed biofilm reactors devoted to the aerobic treatment of diluted CH_4 emissions. For instance, Nikiema et al. (2005) achieved a maximum CH_4-EC of 29.2 g CH_4 $m^{-3}h^{-1}$ in a biofilter (BF) operated at an inlet CH_4 concentration of 0.7% and an EBRT of 4.3 min with a mixed methanotrophic culture. Likewise, Avalos Ramirez et al. (2012) reported a maximum CH_4-EC of 21 g CH_4 $m^{-3}h^{-1}$ in a biotrickling filter (BTF) operated at a constant inlet CH_4 concentration of 4.8 g m^{-3} (inlet load of 61.8 g CH_4 $m^{-3}h^{-1}$) and an EBRT of 4.2 min with a mixed methanotrophic culture. However, the CH_4-ECs herein obtained were in agreement with the elimination capacities reported in suspended growth reactors. For instance, Rodríguez et al. (2020b) reported CH_4-ECs in a bubble column bioreactor (BCB) of 49–74 g CH_4 $m^{-3}h^{-1}$ at an EBRT of 30 min using a pure culture of *M. hirsuta* CSC1 at a CH_4 concentration of 14% ($v\ v^{-1}$). The significantly higher EBRTs (10 folds higher) along with the higher CH_4 gas–liquid concentration gradient likely explain the slightly higher CH_4-ECs obtained by Rodríguez et al. (2020b). García-Pérez et al. (2018) achieved a maximum CH_4-EC of 18.7 g CH_4 $m^{-3}h^{-1}$ in a BCB operated with a pure culture of *M. hirsuta* at an EBRT of 60 min and a virtual EBRT of 4 min, operational conditions similar to those supporting the maximum CH_4-EC in the present work. Rocha-Rios et al. (2009) recorded a higher maximum CH_4-EC of 106 g CH_4 $m^{-3}h^{-1}$ in a stirred-tank reactor (STR) working at an EBRT of 4.8 min, 800 rpm and a CH_4 concentration of 1% ($v\ v^{-1}$) using a mixed methanotrophic culture and 10% of silicone oil to improve CH_4 mass transfer to the methanotrophic broth. However, STRs typically exhibit significantly high energy demands caused by the need to maintain high stirring rates to promote CH_4 gas–liquid mass transfer (Rodríguez et al., 2020c).

On the other hand, higher CH_4-REs were supported when the inlet load of biogas was decreased and when the internal gas recirculation rate was increased. The maximum CH_4-RE recorded in this work, 71.1 ± 1.4% at an EBRT of 60 min, a virtual EBRT of 1.7 min and an inlet load of 26.2 g CH_4 $m^{-3}h^{-1}$, was in accordance with the RE values found in the literature. For instance, García-Pérez et al. (2018) achieved a maximum CH_4-RE of 75% at an EBRT of 60 min, a virtual EBRT of 4 min and an inlet load of 24 g CH_4 $m^{-3}h^{-1}$.Rodríguez et al. (2020b) recorded a maximum CH_4-RE of 70%, achieved at a ≈2-fold inlet methane load (59 g CH_4 $m^{-3}h^{-1}$) and an EBRT of 60 min. Finally, Rocha-Rios et al. (2010) obtained a maximum CH_4-RE of 59% using an inlet methane load of 65 g $m^{-3}h^{-1}$ and a 4.8 min EBRT in a STR.

3.3. Simultaneous methane abatement and PHB production under cyclic nitrogen feast/famine operation

Continuous PHB synthesis in a Taylor flow bioreactor under constant biogas supply and sequential N feast/famine cycles was investigated under optimal EBRT and internal recirculation ratio. A robust CH_4-EC, high CH_4-REs and stability in biomass concentration in the bioreactor were selected as the main criteria to elucidate the optimal operational conditions (i.e., an internal gas recirculation flow rate of 1.7 L min^{-1} and an inlet flow rate of 0.1 L min^{-1}). Interestingly, these operational conditions provided a more robust operation than an internal gas recirculation of 3.5 L min^{-1} and inlet gas flow rate of 0.1 L min^{-1} as a result of the lower shear stress and stripping of biomass. Approximately 11 days were needed to achieve steady CH_4-ECs (16.4 ± 2.9 g CH_4 $m^{-3}h^{-1}$) under nitrogen excess conditions in stage I (Fig. 4A). These constant CH_4-ECs (15.3 ± 0.7 g CH_4 $m^{-3}h^{-1}$) were maintained during stage II, despite the decreasing nitrogen concentrations (Fig. 4A and B). Process operation under feast/famine cycles resulted in fluctuating CH_4-ECs (12.5 ± 2.0 g CH_4 $m^{-3}h^{-1}$) with a corresponding average CH_4-RE of 46.3 ± 7.1%, which were correlated with N supply. Indeed, slightly higher CH_4-ECs were recorded after MSM replacement (and therefore N supply). The correlation between RCO_2 with MSM replacement during stage III was more marked than that of CH_4-EC. The CH_4-ECs recorded along the three operational stages were slightly lower than the values found during the mass transfer optimization assays described in section 3.2 (12.5–16.4 vs. 17.1 g CH_4 $m^{-3}h^{-1}$). This phenomenon was attributed to the gradual fouling of the membrane diffuser, which was similar to the observations of García-Pérez et al. (2018) using metallic fine bubble diffusers. Additionally, the lower biomass concentrations prevailing in the Taylor flow bioreactor (0.5–1.0 g L^{-1}), together with a tendency to form flocs were likely responsible of the low CH_4-EC herein recorded.

Biomass concentration experienced a gradual decrease during stage I from 1.1 to 0.6 g L^{-1} by the end of stage I (Fig. 4C). The absence of MSM replacement (and therefore culture withdrawal) during stage II resulted in an increase in biomass concentration from 0.5 to 0.98 g L^{-1}. During the 14 N feast/famine cycles implemented in stage III, biomass concentration slightly decreased to finally stabilize at 0.61 ± 0.01 g L^{-1} from day 43 onwards. These steady state concentrations were lower than the operational conditions set during biogas bioconversion into PHB in a BCB by Rodríguez et al. (2020b) (≥3 g TSS L^{-1}) and García-Pérez et al. (2018) (≥4.5 g L^{-1}).

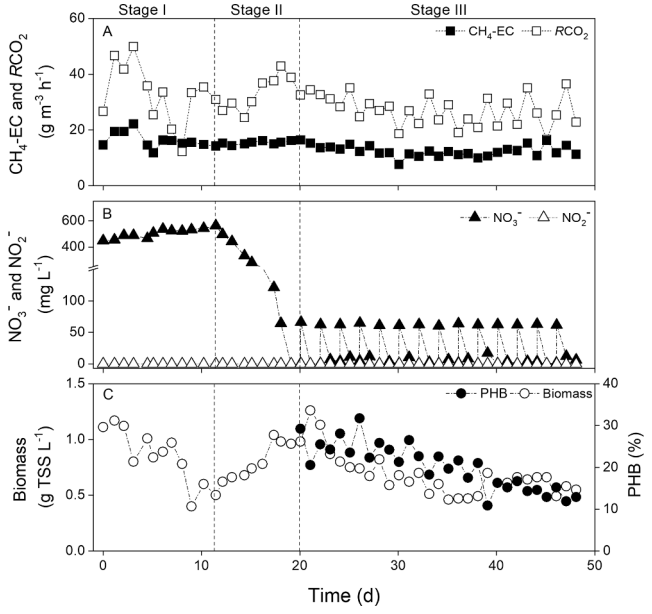

Fig. 4. Time course of (A) CH_4-EC and RCO_2; (B) NO_2^- and NO_3^- concentration in the culture broth; and (C) biomass and PHB concentrations.

Nitrate followed an expected pattern of stable concentrations in stage I, a decreasing concentration in stage II and a zigzagging concentration when implementing the feast/famine cycles in stage III (Fig. 4B). Contrarily, nitrite remained always below the detection limit, which prevented any inhibitory effect on methanotrophs, as previously found by Rodríguez et al. (2020a).

The N starvation periods imposed by the feast/famine cycles induced the synthesis and accumulation of bacterial PHB contents ranging from 11 to 32% (on a dry weight basis) (Fig. 4C). García-Pérez et al. (2018) observed a maximum PHB concentration of 40% in *M. hirsuta* fed with CH_4 (4%) under N feast/famine cycles of 24:48 h in a BCB with fluctuating CH_4-ECs. Rodríguez et al. (2020b) reported a maximum PHB concentration of 23% and an average concentration of ~ 15 ± 3% in a bubble column with *M. hirsuta* fed with CH_4 (9%). Overall, an average PHB productivity of 5.9 ± 2.8 g PHB m^{-3} d^{-1} (with a PHB yield of 19.8 ± 8.5 mg PHB g CH_4^{-1} and an associated specific CH_4-EC of 0.46 ± 0.1 g CH_4 g^{-1} biomass d^{-1}) was herein obtained, which was lower than those (40–60 g PHB m^{-3} d^{-1}) reported by Rodríguez et al. (2020b). These differences in PHB productivities might be explained by the lower dilution rates herein used (0.042 d^{-1} vs. 0.2 d^{-1}) and the lower biomass concentration that prevailed in our study (~3 vs ~ 0.6 g L^{-1}).

3.4. Bacterial diversity of the mixed methanotrophic culture

Molecular analyses were conducted at the beginning (inoculum) and at the end of the simultaneous methane abatement and PHB production experiment in order to elucidate the dynamics of the microbial communities governing the continuous CH_4 oxidation and PHB accumulation bioprocess. A relatively high species evenness and richness was observed with Shannon and Simpson (1 – λ) indexes of 2.86 and 3.22 and 0.88 and 0.88 for the sample harvested by day 0 and day 48, respectively. Proteobacteria (now Pseudomonadota) was the dominant phylum in the inoculum (75.1%), followed by Bacteroidetes (20.9%), Firmicutes (2.1%) and Actinobacteria (1.3%). At the end of the 48-day experimental period, the dominant phyla were Proteobacteria (53.5%), Planctomycetes (12.6%), Chloroflexi (11.6%), Bacteroidetes (9.1%), Actinobacteria (8.7%) and Verrucomicrobia (3.3%). A marked dominance of Proteobacteria and Bacteroidetes has been previously reported in obligate mixed methanotrophic consortia enriched from different environments such as landfill top cover and compost soils (Chidambarampadmavathy et al., 2017), *Sphagnum* mosses (Pérez et al., 2019b), marine sediments (Chidambarampadmavathy et al., 2017). At the genus level, as shown in Fig. 5, the dominant genera in the inoculum included *Methylocystis* (53.4%), *Chryseobacterium* (7.0%), *Sediminibacterium* (5.7%), *Achromobacter* (4.5%), *Sphingomonas* (3.5%), *Methylobacterium* (3.4%), *Pseudarcicella* (2.4%), *Brevundimonas* (2.2%), *Sphingosinicella* (1.6%), *Hyphomicrobium* (1.3%), *Lutispora* (1.0%). Comparatively, the dominant bacterial genera at the end of bioreactor operation were *Methylocystis* (31.9%), *Hyphomicrobium* (8.4%), *Rubinisphaeraceae* SH PL14 (7.5%), *Pseudonocardia* (5.3%), *Phycisphaeraceae* SM1A02 (3.4%), *Microscillaceae* OLB12 (3.4%), *Neochlamydia* (3.2%), *Fuscovulum* (2.7%), *Hydrogenophaga* (2.6%), *Taibaiella* (2.3%), *Aeromicrobium* (1.5%), *Xanthobacter* (1.3%), *Niabella* (1.3%), which together accounted for ≈75% of total bacterial community (Fig. 5).

It is noteworthy to highlight that type II methanotrophs outcompeted non-PHB accumulating type I methanotrophs despite the use of nitrate as the nitrogen source. *Methylocystis* was the only type II methanotroph and the most dominant bacteria detected throughout the Taylor flow bioreactor operation, which suggested that it was the main responsible of the simultaneous CH_4 abatement and PHB accumulation process. The presence of *Methylocystis* in the process can be well-explained by the type of microbial consortia used as the inoculum source, which were enriched in *Methylocystis* spp. when grown at 25 °C under an O_2– and CH_4-rich atmosphere and phosphorus/nitrogen limiting conditions (Pérez et al., 2019a, 2019b), and by the bioaugmentation strategy

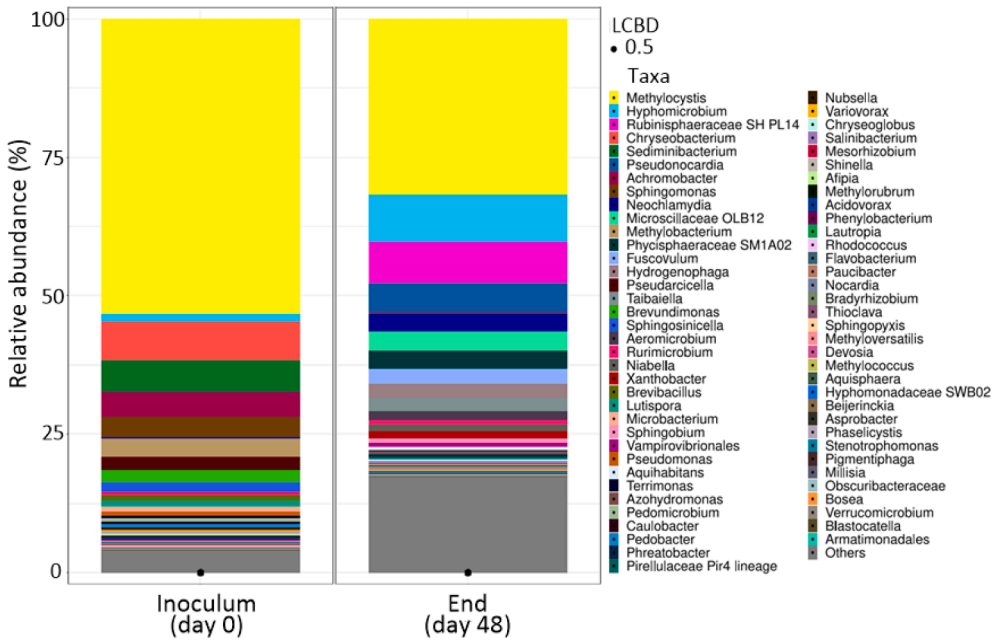

Fig. 5. Taxonomic diversity at the genus level of the mixed methanotrophic culture at time zero (inoculum) and at the end of the PHB accumulation experiment. Top 70 dominant bacteria were graphed while other bacterial taxa were grouped as "Others". LCDB: Local contribution to beta-diversity.

performed with *Methylocystis hirsuta* CSC1 (Rodríguez et al., 2020a). The use of nitrate as the nitrogen source may have also contributed to the dominance of *Methylocystis* in the mixed methanotrophic consortium (Kulkarni et al., 2022).

On the other hand, *Hyphomicrobium* is a heterotrophic denitrifier, facultative methylotroph able to grow on C_1 and C_2 compounds and accumulate PHA under nitrogen limitation (Cao et al., 2021; Fergala et al., 2018; Myung et al., 2015). It is well-known that co-occurring bacteria in methanotrophic consortia can grow on excreted CH_4 degradation metabolites (Carmona-Martínez et al., 2021; Salem et al., 2021), some of which might be toxic for methanotrophs. The co-occurrence of the type II methanotroph *Methylocystis* (ca. 55–80%) and the methylotroph *Hyphomicrobium* (ca. 5–10%) genera in heterotrophic-methanotrophic consortia has been previously reported (Chidambarampadmavathy et al., 2017; Fergala et al., 2018; Myung et al., 2015). Thus, Jeong and Kim (2019) argued that co-culturing of *Hyphomicrobium* and *Methylocystis* can produce an efficient and stable methane oxidation system, in which *Hyphomicrobium* improved the methane-oxidation rate and biomass growth due to cross-feeding of CH_4-derived carbon intermediates produced by *Methylocystis*. Thus, it was hypothesized that *Hyphomicrobium* might have played a synergistic action in the mixed methanotrophic culture not only by removing putative toxic by-products for *Methylocystis* but also by co-producing PHB under nitrogen limitation. In this context, a recent study by Salem et al.(2021) reported that the enrichment of type II methanotrophs with other PHB producers such as *Pseudomonas* may lead to a synergic PHB accumulation effect.

On the other hand, some *Pseudonocardia* spp. are known to degrade aliphatic hydrocarbons such as toluene (Juteau et al., 1999) and to growth on C_1 compounds such as formate, methanol and carbon monoxide as the sole carbon and energy source (Grostern and Alvarez-Cohen, 2013). Indeed, the actinobacterial genus *Pseudonocardia* has been detected in methanotrophic consortia (Burrows et al., 2021), but its role in the PHB-producing methanotrophic reactor remains unknown. Similarly, the role of *Rubinisphaeraceae* SH PL14 in the performance of the bioreactor is also unclear. To the best of the authors' knowledge, this is the first report of *Rubinisphaeraceae* SH PL14 in a methanotrophic accumulating PHB system. It should be noted that satellite bacteria (<1%) may exert a significant effect on the overall process, and they may even become dominant depending on the bioreactor operational conditions. Further studies are needed for a better understanding of the interactions and cooperation between methanotrophs and heterotrophs driving the simultaneous CH_4 abatement and PHAs production.

3.5. Implications and future perspectives

To the best of the authors' knowledge, this study was the first work performed in a high-mass-transfer Taylor flow bioreactor, which represents an important contribution to the state-of-the-art of PHB production using biogas as feedstock. However, further research must be conducted to optimize this innovative technology platform. First, the long-term operation and production of PHB using mixed cultures needs to be further assessed in terms of stability of the bioreactor performance and microbial population structure. In this regard, Helm et al. (2006) achieved a more stable community structure under a long-term operation (29 months and under phosphate limiting conditions) than the work herein presented. Secondly, new feast/famine cycling strategies must be tested to find out the optimal conditions that ensure simultaneously a high methane abatement and PHB productivities, and a stable biomass growth in high mass transfer bioreactors (Rodríguez et al., 2020c). In this context, it is imperative to assess the feasibility of continuous process operation in a single- or multi-stage reactors capable of supporting a simultaneous methanotrophic biomass growth, methane abatement and PHB synthesis (Rodríguez, 2022). Finally, the addition of valerate to trigger the synthesis of tailored PHAs (i.e. PHBV) should be considered in order to make this process more attractive from a biorefinery point of view (Cal et al., 2016; López et al., 2018).

4. Conclusions

The continuous biogas-based PHB production by a mixed methanotrophic culture grown under constant biogas supply, cyclic N starvation and Taylor flow regime was investigated. This is the first proof-of-concept study showing the feasibility of producing biopolymers from biogas in an engineered Taylor flow bioreactor, which is a reactor configuration characterized for sustaining relatively high mass transfer rates at a relative low energy consumption. The applied internal gas recycling strategy enabled the decoupling of the gas residence time from

the turbulence inside the reactor, leading to a better CH_4 abatement performance in terms of CH_4-RE. The Taylor flow regime and N-limiting conditions supported CH_4-REs of ≈50% and CH_4-ECs of ≈12.5 g CH_4 $m^{-3}h^{-1}$, while the PHB content ranged from 11 to 32% *w* w^{-1} of the CDW, which resulted in average PHB productivities of ≈6 g PHB m^{-3} d^{-1}. The molecular analyses revealed that type II methanotrophs outcompeted type I methanotrophs despite the high diversity found in the microbial community. *Methylocystis* was identified as the key PHB producer.

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

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2022.07.017.

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