Modelling of Polyhydroxyalkanoates Synthesis from Biogas by *Methylocystis hirsuta*

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ABSTRCT

Methylocystis hirsuta, a type II methanotroph, has been experimentally demonstrated to be able to efficiently synthesize polyhydroxyalkanoates (PHA) from biogas under nutrient-limited conditions. A mechanistic model capable of describing the relevant processes of *Methylocystis hirsuta*, which is currently not available, would therefore lay a solid foundation for future practical demonstration and optimization of the PHA synthesis technology using biogas. To this end, dedicated batch tests were designed and conducted to obtain experimental data for different mechanistic processes of *Methylocystis hirsuta*. Through utilizing the experimental data of well-designed batch tests and following a step-wise model calibration/validation protocol, the stoichiometrics and kinetics of *Methylocystis hirsuta* are reported for the first time, including the yields of growth and PHA synthesis on CH_4 (0.14±0.01 g COD g^{-1} COD and 0.25±0.02 g COD g^{-1} COD), the CH_4 and O_2 affinity constants (5.1±2.1 g COD m^{-3} and 4.1±1.7 g O_2 m^{-3}), the maximum PHA consumption rate (0.019±0.001 g COD g^{-1} COD d^{-1}) and the maximum PHA synthesis rate on CH_4 (0.39±0.05 g COD g^{-1}). Through applying the developed model, an optimal O_2:CH_4 molar ratio of 1.6 mol O_2 mol^{-1} CH_4 was found to maximize the PHA synthesis by *Methylocystis hirsuta*. Practically, the model and parameters obtained would not only benefit the design and operation of bioreactors performing PHA synthesis from biogas, but also enable specific research on selection for type II methanotrophs in diverse environments.

Keywords: biogas; modelling; polyhydroxyalkanoates (PHA); type II methanotrophs

INTRODUCTION

Polyhydroxyalkanoates (PHA) are biopolymers that could act as storage compounds for microorganisms under conditions of unbalanced growth [1, 2]. Some bacterial species are capable of producing PHA under conditions which restrict growth by nutrient limitation. Due to their biocompatibility, biodegradability, and thermal and mechanical properties similar to polyethylene and polypropylene, PHA have been regarded as a potential substitute for petrochemically-derived plastics [3, 4], the production of which, however, often entails environmental concerns such as greenhouse gas emissions [5]. Despite the currently small scale of industrial manufacturing of PHA worldwide [6, 7], the continuous development of the PHA market is hindered by the high costs associated with the production from carbon source and the downstream processing [5, 8], which are 4 – 9 times higher than those associated with the generation of conventional plastics [9-11].

Under such circumstances, the CH_4 present in biogas could serve as a low-cost feedstock for PHA synthesis [12, 13], especially considering its prevalent generation at wastewater treatment plants through waste activated sludge anaerobic digestion as well as the nature of methane itself as a greenhouse gas. As discussed by López, Arnáiz, Merchán, Lebrero and Muñoz [4], the potential of biogas as a renewable energy source for heat and electricity generation which usually necessitates high investment on site and incentives might weaken due to the huge reserves of shale gas detected worldwide and the decreasing prices of solar and wind energy. Therefore, the bioconversion of biogas into PHA with high added-value is a promising technology that could also assist in combatting climate change.

Capable of using methane as the sole carbon and energy source, methanotrophs are typically classified into two types based on their metabolic and physiological differences. Different from

type I methanotrophs, type II methanotrophs (e.g., *Methylocystis*, *Methylosinus* and *Methylocella* genera) are able to synthesize PHA from methane under nutrient-limited conditions (e.g., in the absence of nitrogen source needed for growth) [14, 15]. Among the type II methanotrophs identified, *Methylocystis hirsuta* has been found to possess a high PHA-accumulating capacity, as evidenced by its highest PHA production from methane recorded (i.e., PHA content of 43 – 45 % w/w) [4, 16]. Despite the reported experimental research, to the best of our knowledge, there is no specific mechanistic modelling work on *Methylocystis hirsuta*. This gap needs to be filled if optimizing PHA synthesis is desired. In particular, if the feast-famine regime is applied to achieve continuous PHA production (e.g., through manipulating the availability of nitrogen source), a clear understanding and reliable quantification of the stoichiometric and kinetic features of all processes involved in *Methylocystis hirsuta* (i.e., biomass growth/decay and PHA synthesis/utilization) would significantly benefit the design of the specific operational strategy.

This work therefore aims to develop a mechanistic model to describe the relevant processes of *Methylocystis hirsuta*, which might lay the foundation for future practical demonstration and optimization of the PHA synthesis technology using biogas. To this end, dedicated batch tests were firstly designed and conducted to obtain experimental data for different mechanistic process(es) of *Methylocystis hirsuta*. The experimental data obtained together with the batch test data reported by López, Arnáiz, Merchán, Lebrero and Muñoz [4] were then used to calibrate and validate the model. Finally, the developed model was applied in a case study to optimize the PHA production under the studied conditions in batch mode.

MATERIALS AND METHODS

Development of the Mechanistic Model

As presented in **Table 1**, the mechanistic model describes the relevant processes of *Methylocystis hirsuta* metabolism, including biomass growth/decay and PHA synthesis/utilization, through the relationships among 4 components, i.e., methane (S_{CH4}), oxygen (S_{O2}), PHA (X_{PHA}), and active biomass (i.e., *Methylocystis hirsuta*, X_B). Based on the findings of López, Arnáiz, Merchán, Lebrero and Muñoz [4], with the supply of methane and oxygen, the biomass growth process (r1) only takes place in the presence of nitrogen source (i.e., nitrate in this work), while the PHA synthesis process (r2) is merely activated in the absence of nitrogen source. Yield coefficients (Y) link substrates consumption to biomass growth and PHA synthesis, the rates of which are modelled using dual-substrate Monod equations. Similar to Chen, et al. [17] and Chen, et al. [18], a coefficient lower than 1 (k) accounts for the electrons diverted to the accompanying generation of products associated with biomass growth (i.e., not all the electrons released from methane oxidation are used for biomass growth), which was not specifically investigated in this work. Based on the results of relevant batch tests conducted in this work (detailed in the following section), the PHA utilization process in the presence of oxygen (r3) is depicted by a rate equation with the single Monod term of oxygen, while the biomass decay process (r4) is expressed by a zero-order rate equation.

Experimental Investigations

Inocula

The methanotrophic strain *Methylocystis hirsuta* (DSMZ no. 18500, Leibniz Institute, Germany) was inoculated (10% v/v) under sterile conditions in 125-mL crimp-sealed serum bottles containing 50 mL of nitrate mineral salt (NMS) medium with a pH of 6.8 prepared according to Bowman [19]. The 75-mL headspace of the bottles was filled with oxygen and methane supplied using gas cylinders of O_2 (≥ 99.5%) and CH_4 (≥ 99.995%) at an O_2:CH_4 ratio

of 66.7:33.3% (v/v), and was replaced upon the depletion of CH_4. The serum bottles were incubated at 30 °C and 200 rpm in an orbital shaker for approximately 7 days.

Batch tests

All batch tests described below were performed in duplicate in 2.2-L serum bottles with a liquid-phase working volume of 0.4 L. With an initial pH of around 7.0, the bottles were incubated at 25 °C and constantly mixed at 300 rpm. Gas and liquid samples were taken periodically for relevant analyses.

Batch tests for biomass growth were conducted at three different headspace compositions, with gas cylinders of O_2 (≥ 99.5%), He (≥ 99.5%) and synthetic biogas (70% CH_4, 30% CO_2) providing gas mixtures. The headspace CH_4:O_2:CO_2:He ratios of 29.2:29.2:12.5:29.2%, 29.2:43.8:12.5:14.6% and 29.2:58.3:12.5:0.0% corresponded to O_2:CH_4 molar ratios of 1:1, 1.5:1 and 2:1, respectively, which are termed Batch Test G1, G2 and G3 in this work. With 2.5% (v/v) of fresh *Methylocystis hirsuta* inocula in the 400-mL NMS medium, the bottles were incubated until the consumption of CH_4 and O_2 ceased.

Batch tests for biomass decay, termed Batch Test D, were performed in serum bottles with biomass previously grown at the O_2:CH_4 molar ratio of 2:1 for 2 weeks (i.e., Batch Test G3). The bottles were provided with an initial headspace O_2 concentration of 21% (v/v) by flushing air for 5 minutes through the bottle headspace with a gas compressor, thus ensuring a complete headspace replacement.

Batch tests for PHA synthesis, termed Batch Test S, were carried out in serum bottles supplied with 400 mL of nitrate-free mineral salt (NFMS) medium, which were inoculated with biomass

harvested from a culture broth grown as previously describes in Batch Test G3. The headspace of the bottles was supplied with a gas mixture containing an O_2:CH_4 molar ratio of 2:1. The bottles were incubated until the consumption of CH_4 and O_2 ceased.

Batch tests for PHA utilization, termed Batch Test U, were implemented as an extension of the previous PHA synthesis test (i.e., Batch Test S). Starting from the depletion of CH_4 in the bottle headspace, the bottles were incubated with the remaining O_2 for over 30 days.

Analytical methods

CH_4 and O_2 in the headspace of the bottles were measured by gas chromatography coupled with thermal conductivity detection according to Estrada, et al. [20] (the detailed method could be found in the supporting information (SI)). Total suspended solids (TSS) were analyzed according Lopez, et al. [21], whereas the optical density of the culture samples was determined at 600 nm by spectrophotometry. PHA extraction from *Methylocystis hirsuta* biomass was conducted referring to Lopez, Quijano, Perez and Munoz [21], while the determination of PHA concentration was carried out by gas chromatography coupled with mass spectrometry as detailed in the SI. The PHA content (%, in terms of weight) was referred to the total biomass concentration of the sample. For the convenience of model implementation, conversion factors of 1.67 and 1.42 (i.e., ratio between COD and TSS) were applied to determine PHA and biomass concentrations in terms of COD, respectively (by assuming the empirical formula of PHA and biomass as $C_4H_6O_2$ and $C_5H_7O_2N$, respectively).

Evaluations of the Mechanistic Model

The mass transfer of CH_4 and O_2 from the headspace to the liquid phase of the setup was described in the model using **Eq. 1**. To determine K_La_{O2}, dedicated duplicate batch tests were

conducted in the same serum bottles used in **Section 2.2** where only O_2 was supplied in the headspace and no biomass was provided in the liquid phase. K_La_{O2} was calculated by analysing the initial linear decline of gas-phase O_2 and assuming a correction factor of 0.95 due to the presence of biomass [22]. The calculated K_La_{O2} was then used to infer K_La_{CH4} according to **Eq. 2**.

$$R_x = K_La_x(\frac{S_{x,g}}{H_x} - S_{x,l}) \quad (1)$$

where R_x is the flux of gas x (i.e., CH_4 or O_2) from the headspace to the liquid phase (g m^{-3} d^{-1}), K_La_x is the mass transfer coefficient of gas x (d^{-1}), $S_{x,g}$ is the concentration of gas x in the headspace (g m^{-3}), $S_{x,l}$ is the concentration of gas x in the liquid phase (g m^{-3}), and H_x is the Henry's law constant [23].

$$\frac{K_La_{CH4}}{K_La_{O2}} = \sqrt{\frac{D_{CH4}}{D_{O2}}} \quad (2)$$

where K_La_{CH4} and K_La_{O2} are the mass transfer coefficients of CH_4 and O_2 (d^{-1}), and D_{CH4} and D_{O2} are the diffusion coefficients of CH_4 and O_2 in water (i.e., 1.84×10^{-9} m^2 s^{-1} and 2.42×10^{-9} m^2 s^{-1}, respectively) [24].

The following stepwise protocol was adopted to rigorously calibrate and validate the model (i.e., **Table 1**) in the modelling and simulation environment AQUASIM [25]. Through following the secant algorithm [26], AQUASIM was used to estimate constant variables (i.e., parameters of interest listed in **Table 2**) by minimizing the sum of the squares of the weighted deviations between measurements and calculated model results.

Step 1: The data of Batch Test D, which involved only process r4 in **Table 1**, were firstly used to estimate the biomass decay rate (i.e., k_{dec}).

Step 2: The data of Batch Test G1, G2 and G3, which involved both processes r1 and r4 in **Table 1**, were used to estimate the yield of growth on CH_4 (i.e., $Y_{g,CH4}$), CH_4 affinity constant (i.e., K_{CH4}) and O_2 affinity constant (i.e., K_{O2}). The value of the maximum growth rate on CH_4 (i.e., $\mu_{g,CH4}$) was directly taken from literature.

Step 3: The data of Batch Test U, which involved both processes r3 and r4 in **Table 1**, were used to estimate the maximum PHA consumption rate (i.e., $\mu_{PHA,O2}$).

Step 4: The data of Batch Test S, which involved processes r2, r3 and r4 in **Table 1**, were used to estimate the yield of PHA synthesis on CH_4 (i.e., $Y_{PHA,CH4}$) and maximum PHA synthesis rate on CH_4 (i.e., $\mu_{PHA,CH4}$).

Step 5: To further validate the results obtained in Step 4, the data of a reported, independent batch test [4] conducted in the same setup as Batch Test S but fed with different initial O_2 and CH_4 compositions, termed Batch Test E, were further tested using the developed model.

The developed model with parameters shown in **Table 2** was then used to optimize PHA production in batch mode. Referring to the conditions applied in the batch tests in **Section 2.2.2**, the initial biomass and CH_4 concentrations were set at 500 and 600 g COD m^{-3}, respectively. The initial O_2 concentration was adjusted between 300 and 900 g m^{-3}, thus forming simulation scenarios with an initial O_2:CH_4 molar ratio in the headspace from 1 to 3 mol O_2 mol^{-1} CH_4. The PHA content and utilization efficiencies of O_2 and CH_4 of different simulation scenarios were compared on the 20th day.

RESULTS AND DISCUSSION

Experimental Results

Figure 1A, B and **C** depicts the measured results of the batch tests for biomass growth, i.e., Batch Test G1, G2 and G3, respectively. With the simultaneous consumption of O_2 and CH_4, biomass was gradually formed but stagnated on the 9th day due to substrate depletion. O_2 was firstly depleted at the O_2:CH_4 molar ratio of 1:1 (see **Figure 1A**), while CH_4 was firstly exhausted at the O_2:CH_4 molar ratio of 1.5:1 (see **Figure 1B**). At the highest O_2:CH_4 molar ratio of 2:1 studied in this work, ~35% of the O_2 provided remained unconverted (see **Figure 1C**). This observation means that an O_2:CH_4 molar ratio between 1:1 and 1.5:1 would lead to the complete consumption of O_2 and CH_4 in the process of biomass growth. However, this ratio is slightly lower than the theoretically calculated value of 1.5:1 reported by Asenjo and Suk [27]. The discrepancy might be related to the assumptions made by Asenjo and Suk [27], e.g., using $C_4H_8O_2N$ to represent biomass and applying a hypothetical yield. The measured results of the batch test for biomass decay (i.e., Batch Test D) are shown in **Figure 2**. Due to aerobic decay in the absence of CH_4, both the concentrations of O_2 and biomass decreased gradually.

Figure 3A illustrates the empirical results of the batch test for PHA synthesis (i.e., Batch Test S). The simultaneous consumption of O_2 and CH_4 led to the production of PHA. After CH_4 was depleted by day 11, the PHA formed started to be consumed aerobically. Therefore, both the concentrations of PHA and O_2 decreased gradually till the end of the batch test. The measured results of the batch test for PHA utilization (i.e., Batch Test U) are presented in **Figure 4**. The simultaneous decline of O_2 and PHA clearly confirmed the capability of *Methylocystis hirsuta* to utilize PHA as an energy source.

Model Evaluations

With a high coefficient of determination (i.e., R^2=0.91) between the modelled and measured results of Batch Test D shown in **Figure 2**, k_{dec} of process r4 was estimated at 0.0033±0.0002

d^{-1} in the first step. **Figure 1A and B** illustrates the second-step calibration results of processes r1 and r4 using the results of Batch Test G1 and G2. With the good agreement between the modelled and measured profiles of Batch Test G1 and G2 (i.e., R^2=0.93 in **Figure 1A and B**), $Y_{g,CH4}$, K_{CH4} and K_{O2} were estimated at 0.14±0.01 g COD g^{-1} COD, 5.1±2.1 g COD m^{-3} and 4.1±1.7 g O_2 m^{-3}, respectively. The value of $Y_{g,CH4}$ is lower than that reported for *Methylocystis hirsuta* by López, Arnáiz, Merchán, Lebrero and Muñoz [4] (i.e., 0.21 g COD g^{-1} COD) as well as those reported for different type II methanotrophs by Rostkowski, et al. [28] (i.e., 0.23 g COD g^{-1} COD for *Methylosinus trichosporium* OB3b and 0.20 g COD g^{-1} COD for *Methylocystis parvus* OBBP), which could be due to the different environmental conditions applied or the various microbial strains studied. The estimated standard deviations of K_{CH4} and K_{O2} are quite significant, being 40% of the estimated parameter values. This is due to the fact that K_{CH4} and K_{O2} are highly negatively correlated in the model structure, with a calculated correlation factor of -0.94. In this case, a further validation process is usually needed. Therefore, the results of Batch Test G3 which involved processes r1 and r4 were used in the second-step validation process. As demonstrated in **Figure 1C**, the validity of the estimated K_{CH4}, K_{O2} and $Y_{g,CH4}$ of the developed model was verified by the good match between the modelled and measured trends (i.e., R^2 = 0.95).

As shown in **Figure 4** with R^2 of 0.92, $\mu_{PHA,O2}$ was estimated at 0.019±0.001 g COD g^{-1} COD d^{-1} in the third step using the data of Batch Test U which involved both processes r3 and r4. **Figure 3A** shows the fourth-step calibration results of processes r2, r3 and r4 using the results of Batch Test S. Through matching the modelled results to measured profiles of Batch Test S to a satisfactory level (i.e., R^2 of 0.88 in **Figure 3A**), $Y_{PHA,CH4}$ and $\mu_{PHA,CH4}$ were estimated at 0.25±0.02 g COD g^{-1} COD and 0.39±0.05 g COD g^{-1} COD d^{-1}. The value of $Y_{PHA,CH4}$ is higher than that reported for *Methylocystis hirsuta* by López, Arnáiz, Merchán, Lebrero and Muñoz [4]

(i.e., 0.19 g COD g^{-1} COD) but lower than those reported for different type II methanotrophs by Rostkowski, Pfluger and Criddle [28] (i.e., 0.47 g COD g^{-1} COD for *Methylosinus trichosporium* OB3b and 0.37 g COD g^{-1} COD for *Methylocystis parvus* OBBP), which could be ascribed to the difference in either environmental conditions applied or microbial strains studied. On top of the already acceptable uncertainty (i.e., with the estimated standard deviations being <15% of the estimated parameter values), the validity of the estimated $Y_{PHA,CH4}$ and $\mu_{PHA,CH4}$ of the developed model was further confirmed by the fifth-step validation results of processes r2, r3 and r4 using the additional results of Batch Test E. As demonstrated in **Figure 3B**, a high coefficient of determination (i.e., R^2=0.95) between the modelled and measured data was obtained.

Model-Based Optimization of PHA Production

To elucidate the O_2:CH_4 molar ratio leading to the complete consumption of O_2 and CH_4 in the process of PHA synthesis, a case study was performed using the developed model, the results of which are illustrated in **Figure 5**. When the O_2:CH_4 molar ratio in the headspace increased from the lowest level studied (i.e., 1 mol O_2 mol^{-1} CH_4), the amount of PHA produced increased firstly. Under such conditions, CH_4 was in excess while O_2 was limiting. Therefore, the CH_4 utilization efficiency kept rising while the O_2 utilization efficiency remained at ~100%. The PHA production peaked at the O_2:CH_4 molar ratio of 1.6 mol O_2 mol^{-1} CH_4, where nearly a complete utilization of O_2 (i.e., 97%) and CH_4 (i.e., 99%) was achieved. When the O_2:CH_4 molar ratio increased beyond 1.6 mol O_2 mol^{-1} CH_4, CH_4 became limited while O_2 was in excess. Consequently, the CH_4 utilization efficiency reached 100% while the O_2 utilization efficiency exhibited a declining trend, accompanied by a decreasing PHA production due to aerobic consumption. In summary, this case study showed that an O_2:CH_4 molar ratio of 1.6 mol O_2 mol^{-1} CH_4 would lead to the complete consumption of O_2 and CH_4 in the process of

PHA synthesis. This value is slightly higher than the theoretically calculated value of 1.5 mol O_2 mol^{-1} CH_4 reported by Asenjo and Suk [27]. The difference might be caused by the additional O_2-consuming processes considered in this work, i.e., processes r3 (i.e., PHA consumption) and r4 (i.e., biomass decay). Practically, in order to avoid the negative impact of limited O_2 availability on PHA production [29], an optimal O_2:CH_4 molar ratio of 1.6 mol O_2 mol^{-1} CH_4 is needed to maximize the PHA synthesis by *Methylocystis hirsuta*.

Implications of This Work

This work reports for the first time the stoichiometrics and kinetics of all mechanistic processes related to *Methylocystis hirsuta*, including biomass growth/decay and PHA synthesis/utilization, based on the experimental data of well-designed batch tests. Therefore, this work represents a valuable contribution to the current knowledge base of stoichiometrics and kinetics of methanotrophs which is more oriented on mixed cultures. This work would also significantly facilitate the design and operation of bioreactors devoted to PHA synthesis from biogas. For example, the model and parameters could be implemented in relevant setup to model and optimize PHA production in bioreactors (e.g., bubble column bioreactor [30, 31]) under feast-famine regime, which has been reported as an ideal operational strategy for PHA accumulation [32].

Moreover, the model and parameters obtained would also favor the practical selection for type II methanotrophs. For example, through applying a model integrating the stoichiometrics and kinetics of both type I and II methanotrophs in a fluidized bed reactor proposed by Pfluger, et al. [33], improvement of selection for type II methanotrophs and hence increased PHA production could be expected. As denitrifying anaerobic methane oxidation (DAMO) microorganisms and aerobic methane oxidation (AMO) microorganisms could thrive in environments suitable for

the growth and PHA synthesis of type II methanotrophs (i.e., with O_2 and CH_4 in the presence/absence of nitrate), the model and parameters obtained in this work could be coupled with those reported for DAMO and AMO (e.g., Daelman, et al. [34], Chen, et al. [35], Chen, et al. [36]) to assess the interactions between type II methanotrophs and potential competitors, especially in mixed culture environments. These aspects are subject to future specific investigations.

CONCLUSIONS

In this work, through utilizing the experimental data of well-designed batch tests and following a step-wise model calibration/validation protocol, the stoichiometrics and kinetics of growth/decay and PHA synthesis/utilization processes of *Methylocystis hirsuta* are reported for the first time. Through applying the developed model, an optimal O_2:CH_4 molar ratio of 1.6 mol O_2 mol^{-1} CH_4 was found to maximize PHA synthesis by *Methylocystis hirsuta*. Practically, the model and parameters obtained would not only benefit the design and operation of bioreactors performing PHA synthesis from biogas, but also enable specific research on selection for type II methanotrophs in diverse environments.

SUPPORTING INFORMATION

The Supporting Information is available free of charge on the ACS Publications website.

Method for CH_4 and O_2 concentrations determination;

Method for PHA concentration determination

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Table and Figure Legends

Table 1. Stoichiometric and kinetic matrix of the model

Table 2. Parameters of the model

Figure 1. Comparison between modelled and measured results of Batch Test (**A**) G1, (**B**) G2, and (**C**) G3.

Figure 2. Comparison between modelled and measured results of Batch Test D.

Figure 3. Comparison between modelled and measured results of Batch Test (**A**) S and (**B**) E.

Figure 4. Comparison between modelled and measured results of Batch Test U.

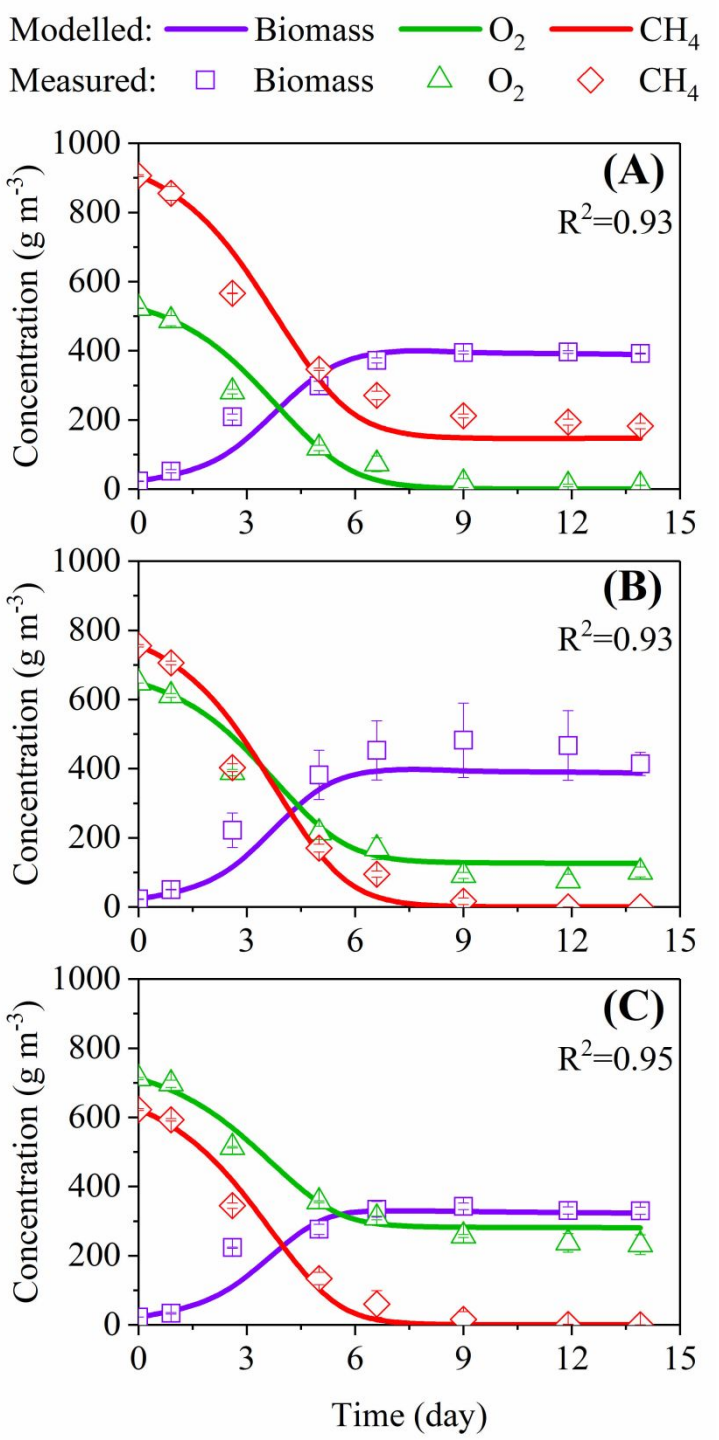
Figure 5. PHA content and utilization efficiencies of O_2 and CH_4 of simulation scenarios with an initial O_2:CH_4 molar ratio in the headspace ranging from 1 to 3 mol O_2 mol^{-1} CH_4.

Table 1. Stoichiometric and kinetic matrix of the model

	Component process	S_{CH4} g COD m^{-3}	S_{O2} g O_2 m^{-3}	X_B g COD m^{-3}	X_{PHA} g COD m^{-3}	Process rate equation
r1	Biomass growth in the presence of NO_3^-	$\frac{-1}{Y_{g,CH4}}$	$\frac{Y_{g,CH4}-1}{Y_{g,CH4}}k$	k		$\mu_{g,CH4}\frac{S_{CH4}}{K_{CH4}+S_{CH4}}\frac{S_{O2}}{K_{O2}+S_{O2}}X_B$
r2	PHA synthesis in the absence of NO_3^-	$\frac{-1}{Y_{PHA,CH4}}$	$\frac{Y_{PHA,CH4}-1}{Y_{PHA,CH4}}$		1	$\mu_{PHA,CH4}\frac{S_{CH4}}{K_{CH4}+S_{CH4}}\frac{S_{O2}}{K_{O2}+S_{O2}}X_B$
r3	PHA consumption		-1		-1	$\mu_{PHA,O2}\frac{S_{O2}}{K_{O2}+S_{O2}}X_B$
r4	Biomass decay		-1	-1		$k_{Dec}X_B$

Table 2. Parameters of the model

Parameter	Definition	Value	Unit	Source
Stoichiometric Parameters				
$Y_{g,CH4}$	Yield of growth on CH_4	0.14±0.01	g COD g^{-1} COD	This work
$Y_{PHA,CH4}$	Yield of PHA synthesis on CH_4	0.25±0.02	g COD g^{-1} COD	This work
i_{NXB}	Nitrogen content of biomass	0.07	g N g^{-1} COD	Henze, et al. [37]
k	Fraction of electrons used for biomass production	0.8	-	Chen, Liu, Peng and Ni [17], Chen, Lai, Fang, Zhao, Dai and Ni [18]
Kinetic Parameters				
$\mu_{g,CH4}$	Maximum growth rate on CH_4	1.17	g COD g^{-1} COD d^{-1}	Arcangeli and Arvin [38]
$\mu_{PHA,CH4}$	Maximum PHA synthesis rate on CH_4	0.39±0.05	g COD g^{-1} COD d^{-1}	This work
$\mu_{PHA,O2}$	Maximum PHA consumption rate	0.019±0.001	g COD g^{-1} COD d^{-1}	This work
k_{dec}	Biomass decay rate	0.0033±0.0002	d^{-1}	This work
K_{CH4}	CH_4 affinity constant	5.1±2.1	g COD m^{-3}	This work
K_{O2}	Oxygen affinity constant	4.1±1.7	g O_2 m^{-3}	This work



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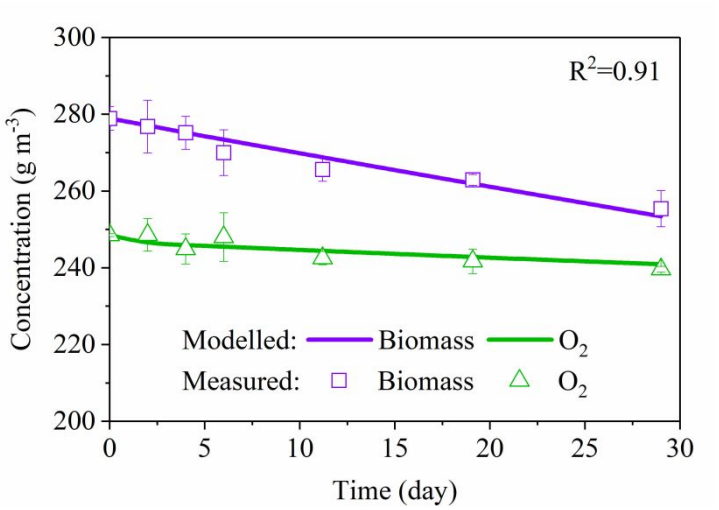


Figure 2. Comparison between modelled and measured results of Batch Test D.

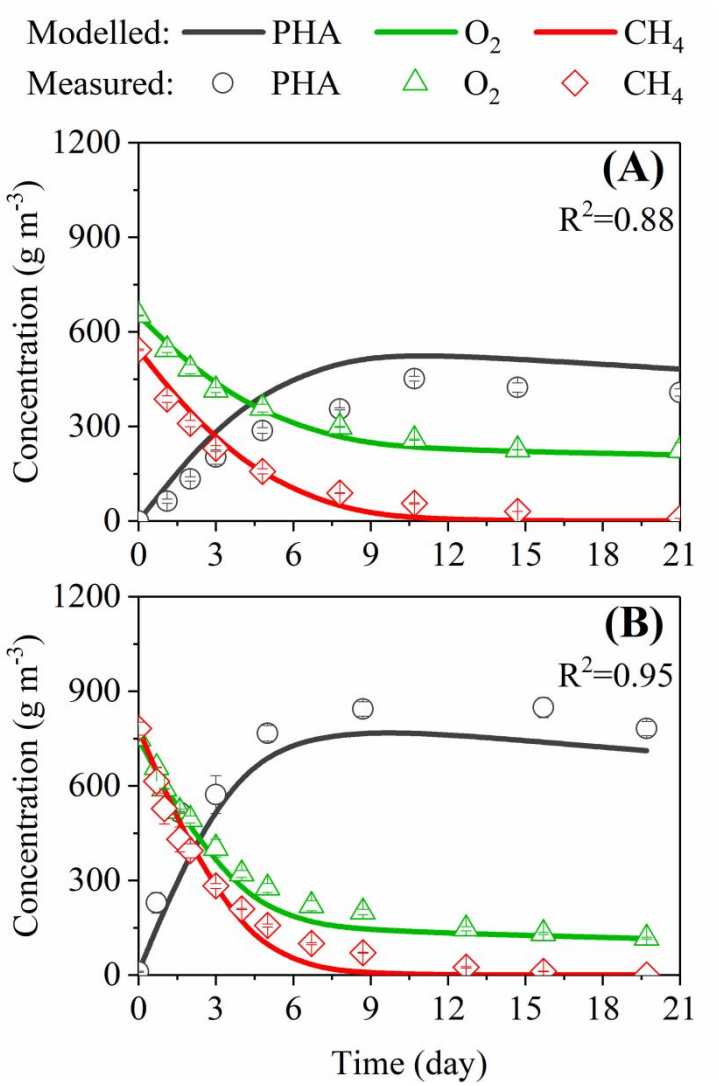


Figure 3. Comparison between modelled and measured results of Batch Test (**A**) S and (**B**) E.

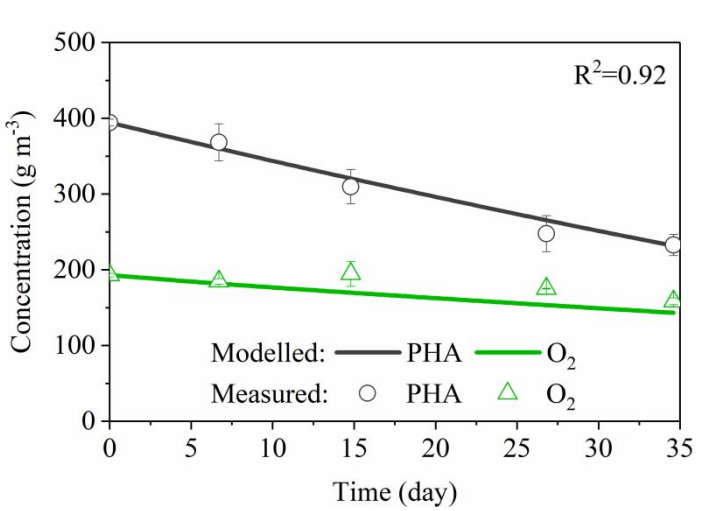
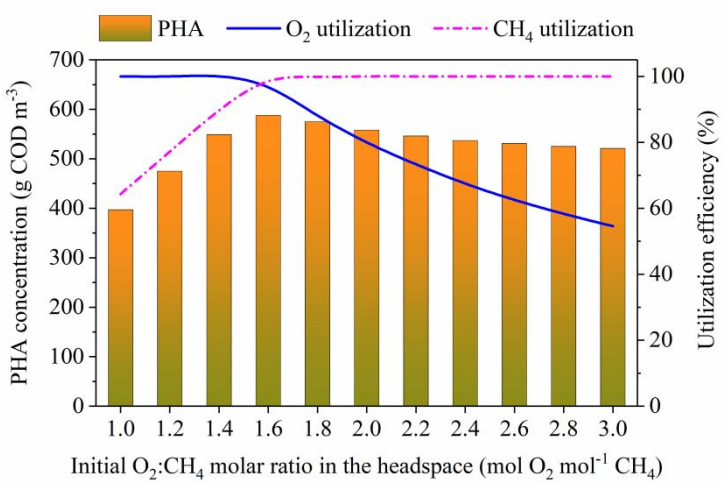


Figure 4. Comparison between modelled and measured results of Batch Test U.



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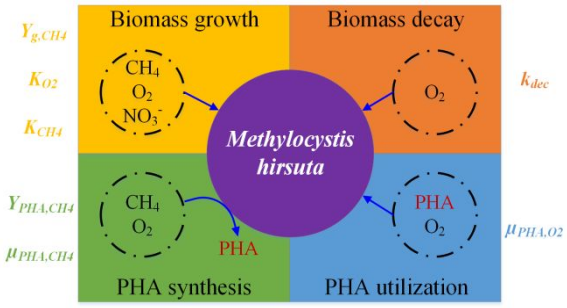
For Table of Contents used only



Synopsis: A mechanistic model describing the relevant processes of sustainable PHA synthesis from biogas by *Methylocystis hirsuta*.