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Modeling of Polyhydroxyalkanoate Synthesis from Biogas by *Methylocystis hirsuta*

Xueming Chen,* Yadira Rodríguez, Juan C. López, Raúl Muñoz, Bing-Jie Ni, and Gürkan Sin

**ABSTRACT:** *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 *M. 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 *M. 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 *M. hirsuta* are reported for the first time, including the yields of growth and PHA synthesis on CH₄ (0.14 ± 0.01 g COD g⁻¹ COD and 0.25 ± 0.02 g COD g⁻³ COD), the CH₄ and O₂ affinity constants (5.1 ± 2.1 g COD m⁻³ and 4.1 ± 1.7 g O₂ m⁻³), the maximum PHA synthesis rate (0.019 ± 0.001 g COD g⁻¹ COD d⁻¹), and the maximum PHA synthesis rate on CH₄ (0.39 ± 0.05 g COD g⁻¹ COD d⁻¹). Through applying the developed model, an optimal O₂:CH₄ molar ratio of 1.6 mol O₂ mol⁻¹ CH₄ was found to maximize the PHA synthesis by *M. 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, modeling, polyhydroxyalkanoates (PHA), type II methanotrophs

1. **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. Because of 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,⁶⁻⁸ the continuous development of the PHA market is hindered by the high costs associated with the production from a carbon source and the downstream processing,⁵,⁶ which are 4–9 times higher than those associated with the generation of conventional plastics.⁹⁻¹¹

Under such circumstances, the CH₄ 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 et al.,³ 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 because of 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 a high added value is a promising technology that could also assist in combating 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 *Methylolcella* genera) are able to synthesize PHA from methane under nutrient-limited conditions (e.g., the absence of a 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 content of 43–45% w/w.⁴,¹⁶ Despite the reported experimental research, to the best of our knowledge, there is no specific mechanistic
expressed by a zero-order rate equation. Single Monod term of oxygen, while the biomass decay process \( r_4 \) is merely work. Based on the results of relevant batch tests conducted in this work, the PHA utilization process 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 \( M. hirsuta \), 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 first designed and conducted to obtain the experimental data for different mechanistic processes of \( M. hirsuta \). The experimental data obtained together with the batch test data reported by López et al.\(^1\) 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.

### 2. MATERIALS AND METHODS

#### 2.1. Development of the Mechanistic Model

As presented in Table 1, the mechanistic model describes the relevant processes of \( M. hirsuta \) metabolism, including biomass growth/decay and PHA synthesis/utilization, through the relationships among 4 components, that is, methane \( (S_{CH_4}) \), oxygen \( (S_O_2) \), PHA \( (X_{PHA}) \), and active biomass \( (i.e., M. hirsuta, X_B) \). Based on the findings of López et al.\(^4\), with the supply of methane and oxygen, the biomass growth process \( (r_1) \) only takes place in the presence of a nitrogen source \( (i.e., \text{nitrate in this work}) \), while the PHA synthesis process \( (r_2) \) is merely activated in the absence of a nitrogen source. Yield coefficients \( (Y) \) link substrate consumption to biomass growth and PHA synthesis, the rates of which are modeled using dual-substrate Monod equations. Similar to the reports by 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 \((i.e., \text{not all the electrons released from methane oxidation are used for biomass growth})\), which was not specifically investigated in this work. Based on the results of relevant batch tests conducted in this work \((\text{detailed in the following section})\), the PHA utilization process in the presence of oxygen \( (r_3) \) is depicted by a rate equation with the single Monod term of oxygen, while the biomass decay process \( (r_4) \) is expressed by a zero-order rate equation.

#### 2.2. Experimental Investigations

**2.2.1. Inocula.** The methanotrophic strain \( M. hirsuta \) (DSMZ no. 18500, Leibniz Institute, Germany) was inoculated \((10\% \text{ v/v})\) under sterile conditions in 125 mL crimp-sealed serum bottles containing 50 mL of nitrate mineral salt \((\text{NMS})\) medium with a pH of 6.8 prepared according to the reports by Bowman.\(^{19}\) The 75 mL headspace of the bottles was filled with oxygen and methane supplied using gas cylinders of \( \text{O}_2 \) \((\geq 99.95\%) \) and \( \text{CH}_4 \) \((\geq 99.995\%) \) at an \( \text{O}_2/\text{CH}_4 \) ratio of 66.7:33.3 \((\text{v/v})\) and was replaced upon the depletion of \( \text{CH}_4 \). The serum bottles were incubated at 30 °C and 200 rpm in an orbital shaker for approximately 7 days.

**2.2.2. 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 \( \text{O}_2 \) \((\geq 99.95\%) \), \( \text{He} \) \((\geq 99.9\%) \), and synthetic biogas \((70\% \text{ CH}_4, 30\% \text{ CO}_2)\) providing gas mixtures. The headspace \( \text{CH}_4/\text{O}_2/\text{CO}_2/\text{He} \) ratios of 29.2:2.9:12.5:29.2, 29.2:4.3:8.5:14.6, and 29.2:58.3:12.5:0.0 corresponded to \( \text{O}_2/\text{CH}_4 \) 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 \((\text{v/v})\) of fresh \( M. hirsuta \) inocula in the 400 mL NMS medium, the bottles were incubated until the consumption of \( \text{CH}_4 \) and \( \text{O}_2 \) ceased.

Batch tests for biomass decay, termed Batch Test D, were performed in serum bottles with biomass previously grown at a \( \text{O}_2/\text{CH}_4 \) molar ratio of 2:1 for 2 weeks \((i.e., \text{Batch Test G3})\). The bottles were provided with an initial headspace \( \text{O}_2 \) concentration of 21% \((\text{v/v})\) by flushing air for 5 min 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 medium, which were inoculated with biomass harvested from a culture broth grown as previously described in Batch Test G3. The headspace of the bottles was supplied with a gas mixture containing an \( \text{O}_2/\text{CH}_4 \) molar ratio of 2:1. The bottles were incubated until the consumption of \( \text{CH}_4 \) and \( \text{O}_2 \) ceased.

Batch tests for PHA utilization, termed Batch Test U, were implemented as an extension of the previous PHA synthesis test \((i.e., \text{Batch Test S})\). Starting from the depletion of \( \text{CH}_4 \) in the bottle headspace, the bottles were incubated with the remaining \( \text{O}_2 \) for over 30 days.

**2.2.3. Analytical Methods.** \( \text{CH}_4 \) and \( \text{O}_2 \) in the headspace of the bottles were measured by gas chromatography coupled with thermal conductivity detection according to Estrada et al.\(^{20}\) \((\text{the detailed method could be found in the Supporting Information})\). Total suspended solids \((\text{TSS})\) were analyzed according López et al.\(^{21}\) whereas the optical density of the culture samples was determined at 600 nm by spectrophotometry. PHA extraction from \( M. hirsuta \) biomass was conducted referring to the reports by López et al.\(^{21}\) while the determination of PHA concentration was carried out by gas chromatography coupled with mass spectrometry as detailed in the Supporting Information. The PHA content \((\% \text{ in terms of weight})\) was referred to the total biomass concentration of the sample. For the convenience of model implementation, conversion factors of 1.67 and 1.42 \((i.e., \text{ratio between COD and TSS})\) were applied to determine PHA and biomass concentrations in terms of COD, respectively.

### Table 1. Stoichiometric and Kinetic Matrix of the Model

<table>
<thead>
<tr>
<th>component process</th>
<th>( S_{CH_4} ) ((\text{g COD m}^{-3}))</th>
<th>( S_O_2 ) ((\text{g O}_2 \text{ m}^{-3}))</th>
<th>( X_B ) ((\text{g COD m}^{-3}))</th>
<th>( X_{PHA} ) ((\text{g COD m}^{-3}))</th>
<th>process rate equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_1 )</td>
<td>(-1) ( Y_{CH_4} ) ( k ) ( Y_{CH_4} ) ( k )</td>
<td>(-1) ( Y_{CH_4} ) ( k ) ( Y_{CH_4} ) ( k )</td>
<td>(-1) ( Y_{CH_4} ) ( k ) ( Y_{CH_4} ) ( k )</td>
<td>(-1) ( Y_{CH_4} ) ( k ) ( Y_{CH_4} ) ( k )</td>
<td>(-1) ( Y_{CH_4} ) ( k ) ( Y_{CH_4} ) ( k )</td>
</tr>
<tr>
<td>( r_2 )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
</tr>
<tr>
<td>( r_3 )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
</tr>
<tr>
<td>( r_4 )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
<td>(-1) ( Y_{PHA,CH_4} ) ( k ) ( Y_{PHA,CH_4} ) ( k )</td>
</tr>
</tbody>
</table>
Table 2. Parameters of the Model

<table>
<thead>
<tr>
<th>parameter</th>
<th>definition</th>
<th>value</th>
<th>unit</th>
<th>source</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Y_{g,CH_4} )</td>
<td>yield of growth on ( CH_4 )</td>
<td>0.14 ± 0.01</td>
<td>g COD g(^{-1}) COD</td>
<td>this work</td>
</tr>
<tr>
<td>( Y_{PHA,CH_4} )</td>
<td>yield of PHA synthesis on ( CH_4 )</td>
<td>0.25 ± 0.02</td>
<td>g COD g(^{-1}) COD</td>
<td>this work</td>
</tr>
<tr>
<td>( \ln_{cb} )</td>
<td>nitrogen content of biomass</td>
<td>0.07</td>
<td>g N g(^{-1}) COD</td>
<td>Henze et al.(^{37})</td>
</tr>
<tr>
<td>( k )</td>
<td>fraction of electrons used for biomass production</td>
<td>0.8</td>
<td>g COD g(^{-1}) COD</td>
<td>this work</td>
</tr>
<tr>
<td>( \mu_{g,CH_4} )</td>
<td>maximum growth rate on ( CH_4 )</td>
<td>1.17</td>
<td>g COD g(^{-1}) COD d(^{-1})</td>
<td>this work</td>
</tr>
<tr>
<td>( \mu_{PHA,CH_4} )</td>
<td>maximum PHA synthesis rate on ( CH_4 )</td>
<td>0.39 ± 0.05</td>
<td>g COD g(^{-1}) COD d(^{-1})</td>
<td>this work</td>
</tr>
<tr>
<td>( \mu_{PHA,O_2} )</td>
<td>maximum PHA consumption rate</td>
<td>0.019 ± 0.001</td>
<td>g COD g(^{-1}) COD d(^{-1})</td>
<td>this work</td>
</tr>
<tr>
<td>( \delta_{bc} )</td>
<td>biomass decay rate</td>
<td>0.0033 ± 0.0002</td>
<td>d(^{-1})</td>
<td>this work</td>
</tr>
<tr>
<td>( K_{CH_4} )</td>
<td>( CH_4 ) affinity constant</td>
<td>5.1 ± 2.1</td>
<td>g COD m(^{-3})</td>
<td>this work</td>
</tr>
<tr>
<td>( K_{O_2} )</td>
<td>( O_2 ) affinity constant</td>
<td>4.1 ± 1.7</td>
<td>g O(_2) m(^{-3})</td>
<td>this work</td>
</tr>
</tbody>
</table>

assuming the empirical formula of PHA and biomass as \( C_4H_6O_2 \) and \( C_2H_4O_2N \), respectively.

2.2.4. Evaluations of the Mechanistic Model. The mass transfer of \( CH_4 \) and \( O_2 \) from the headspace to the liquid phase was described in the model using eq 1. To determine \( K_{L,\alpha,CH_4} \) and \( K_{L,\alpha,O_2} \), 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. \( K_{L,\alpha,CH_4} \) was calculated by analyzing the initial linear decline of gas-phase \( O_2 \) and assuming a correction factor of 0.95 because of the presence of biomass.\(^{21}\) The calculated \( K_{L,\alpha,CH_4} \) was then used to infer \( K_{L,\alpha,CH_4} \) according to eq 2.

\[
R_x = K_{L,\alpha} \left( \frac{S_{x,g}}{H_x} - S_{x,l} \right) 
\]

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\(^{-1}\)), \( K_{L,\alpha} \) 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.\(^{21}\)

\[
\frac{K_{L,\alpha,CH_4}}{K_{L,\alpha,O_2}} = \frac{D_{CH_4}}{D_{O_2}} 
\]

where \( K_{L,\alpha,CH_4} \) and \( K_{L,\alpha,O_2} \) are the mass transfer coefficients of \( CH_4 \) \( (d^{-1}) \), and \( D_{CH_4} \) and \( D_{O_2} \) are the diffusion coefficients of \( CH_4 \) and \( O_2 \) in water (i.e., \( 1.84 \times 10^{-9} \) and \( 2.42 \times 10^{-9} \) 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 modeling 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 \( r_4 \) in Table 1, were first used to estimate the biomass decay rate (i.e., \( k_{bc} \)).

Step 2: The data of Batch Test G1, G2, and G3, which involved both processes \( r_1 \) and \( r_4 \) in Table 1, were used to estimate the yield of growth on \( CH_4 \) (i.e., \( Y_{g,CH_4} \)), the \( CH_4 \) affinity constant (i.e., \( K_{CH_4} \)), and the \( O_2 \) affinity constant (i.e., \( K_{O_2} \)). The value of the maximum growth rate on \( CH_4 \) (i.e., \( \mu_{g,CH_4} \)) was directly taken from the literature.

Step 3: The data of Batch Test U, which involved both processes \( r_3 \) and \( r_4 \) in Table 1, were used to estimate the maximum PHA consumption rate (i.e., \( \mu_{PHA,O_2} \)).

Step 4: The data of Batch Test S, which involved processes \( r_2 \), \( r_3 \), and \( r_4 \) in Table 1, were used to estimate the yield of PHA synthesis on \( CH_4 \) (i.e., \( Y_{PHA,CH_4} \)) and maximum PHA synthesis rate on \( CH_4 \) (i.e., \( \mu_{PHA,CH_4} \)).

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.

3. RESULTS AND DISCUSSION

3.1. Experimental Results. Figure 1A–C depicts the measured results of the batch tests for biomass growth, that is, Batch Test G1, G2, and G3, respectively. With the

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Comparison between modeled and measured results of Batch Test (A) G1, (B) G2, and (C) G3.
simultaneous consumption of O<sub>2</sub> and CH<sub>4</sub>, biomass was gradually formed but stagnated on the 9th day because of substrate depletion. O<sub>2</sub> was first depleted at a O<sub>2</sub>/CH<sub>4</sub> molar ratio of 1:1 (see Figure 1A), while CH<sub>4</sub> was first exhausted at a O<sub>2</sub>/CH<sub>4</sub> molar ratio of 1.5:1 (see Figure 1B). At the highest O<sub>2</sub>/CH<sub>4</sub> molar ratio of 2:1 studied in this work, ~35% of the O<sub>2</sub> provided remained unconverted (see Figure 1C). This observation means that an O<sub>2</sub>/CH<sub>4</sub> molar ratio between 1:1 and 1.5:1 would lead to the complete consumption of O<sub>2</sub> and CH<sub>4</sub> 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<sup>27</sup>. The discrepancy might be related to the assumptions made by Asenjo and Suk<sup>27</sup> for example, using C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>N 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. Because of aerobic decay in the absence of CH<sub>4</sub>, both the concentrations of O<sub>2</sub> and biomass decreased gradually.

![Figure 2](image-url)  
**Figure 2.** Comparison between modeled and measured results of Batch Test D.

**3.2. Model Evaluations.** With a high coefficient of determination (i.e., \( R^2 = 0.91 \)) between the modeled and measured results of Batch Test D shown in Figure 2, \( k_{sec} \) of process r4 was estimated at 0.0033 ± 0.0002 d<sup>−1</sup> in the first step. Figure 1A,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 modeled and measured profiles of Batch Test G1 and G2 (i.e., \( R^2 = 0.93 \) in Figure 1A,B), \( Y_{g(CH_4)} \), \( K_{CH_4} \), and \( K_{O_2} \) were estimated at 0.14 ± 0.01 g COD g<sup>−1</sup> COD, 5.1 ± 2.1 g COD m<sup>3</sup> and 4.1 ± 1.7 g O<sub>2</sub> m<sup>−3</sup>, respectively. The value of \( Y_{g(CH_4)} \) is lower than that reported for *M. hirsuta* by López et al.<sup>3</sup> (i.e., 0.21 g COD g<sup>−1</sup> COD) as well as those reported for different type II methanotrophs by Rostkowski et al.<sup>28</sup> (i.e., 0.23 g COD g<sup>−1</sup> COD for *Methylosinus trichosporium* OB3b and 0.20 g COD g<sup>−1</sup> COD for *Methylocystis parvus* OBBP), which could be because of the different environmental conditions applied or the various microbial strains studied. The estimated standard deviations of \( K_{CH_4} \) and \( K_{O_2} \) are quite significant, being 40% of the estimated parameter values. This is due to the fact that \( K_{CH_4} \) and \( K_{O_2} \) 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 \( K_{CH_4} \), \( K_{O_2} \) and \( Y_{g(CH_4)} \) of the developed model was verified by the good match between the modeled and measured trends (i.e., \( R^2 = 0.95 \)).

As shown in Figure 4 with \( R^2 = 0.92 \), \( \mu_{PHA,O_2} \) was estimated at 0.019 ± 0.001 g COD g<sup>−1</sup> COD d<sup>−1</sup> 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 modeled results to measured profiles of Batch Test S to a satisfactory level (i.e., \( R^2 = 0.88 \) in Figure 3A), \( Y_{PHA,CH_4} \) and \( \mu_{PHA,CH_4} \) were estimated at 0.25 ± 0.02 g COD g<sup>−1</sup> COD and 0.39 ± 0.05 g COD g<sup>−1</sup> COD d<sup>−1</sup>. The value of \( Y_{PHA,CH_4} \) is higher than that reported for *M. hirsuta* by López et al.<sup>3</sup> (i.e., 0.19 g COD g<sup>−1</sup> COD) but lower than those reported for different type II methanotrophs by Rostkowski et al.<sup>28</sup> (i.e., 0.47 g COD g<sup>−1</sup> COD for *M. trichosporium* OB3b and 0.37 g COD g<sup>−1</sup> COD for *M. parvus* OBBP), which could be ascribed to the difference in either the environmental

![Figure 3](image-url)  
**Figure 3.** Comparison between modeled and measured results of Batch Test (A) S and (B) E.

simultaneous decline of O<sub>2</sub> and PHA clearly confirmed the capability of *M. hirsuta* to utilize PHA as an energy source.
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 setups to model and optimize PHA production in bioreactors (e.g., bubble column bioreactor\cite{30,31}) under the feast-famine regime, which has been reported as an ideal operational strategy for PHA accumulation.\cite{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.,\cite{3} 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 O₂ and CH₄ 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.,\cite{29} Chen et al.,\cite{30} and Chen et al.\cite{31}) to assess the interactions between type II methanotrophs and potential competitors, especially in mixed culture environments. These aspects are subject to future specific investigations.

4. 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 M. hirsuta are reported for the first time. Through applying the developed model, an optimal O₂/CH₄ molar ratio of 1.6 mol O₂ mol⁻¹ CH₄ was found to maximize PHA synthesis by M. 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.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.9b07414.

Method for CH₄ and O₂ concentrations determination and method for PHA concentration determination (PDF)

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conditions applied or the 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 YᵢCH₄ and µᵢCH₄ 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 modeled and measured data was obtained.

3.3. Model-Based Optimization of PHA Production.

To elucidate the O₂/CH₄ molar ratio leading to the complete consumption of O₂ and CH₄ 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 O₂/CH₄ molar ratio in the headspace increased from 1 to 3 mol O₂ mol⁻¹ CH₄, the amount of PHA produced increased and the PHA utilization efficiency reached 100% while the O₂ utilization efficiency exhibited a declining trend, accompanied by a decreasing PHA production because of aerobic consumption. In summary, this case study showed that an O₂/CH₄ molar ratio of 1.6 mol O₂ mol⁻¹ CH₄ would lead to the complete consumption of O₂ and CH₄ in the process of PHA synthesis. This value is slightly higher than the theoretically calculated value of 1.5 mol O₂ mol⁻¹ CH₄ reported by Asenjo and Suk.\cite{27} The difference might be caused by the additional O₂-consuming processes considered in this work, that is, processes r₃ (i.e., PHA consumption) and r₄ (i.e., biomass decay). Practically, in order to avoid the negative impact of limited O₂ availability on PHA production,\cite{28} an optimal O₂/CH₄ molar ratio of 1.6 mol O₂ mol⁻¹ CH₄ is needed to maximize the PHA synthesis by M. hirsuta.

3.4. Implications of This Work.

This work reports for the first time the stoichiometrics and kinetics of all mechanistic processes related to M. 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

Figure 5. PHA content and utilization efficiencies of O₂ and CH₄ of simulation scenarios with an initial O₂/CH₄ molar ratio in the headspace ranging from 1 to 3 mol O₂ mol⁻¹ CH₄.
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Notes
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