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Method Article

Matlab implementation of a novel semi-structured kinetic model for methanotroph-photoautotroph cocultures

Kiumars Badr, Q. Peter He, Jin Wang*

Department of Chemical Engineering, Auburn University, Auburn, AL, USA

A B S T R A C T

This paper presents the matlab implementation details of a novel semi-structured kinetic model for methanotroph-photoautotroph cocultures. This includes the parameterization of the modeling equations, and the initialization of the simulation based on experimental conditions. More importantly, it provides details on how the differential equations governing mass balances in both gas and liquid phases are integrated together to simulate the system dynamics over time. The semi-structured kinetic model for methanotroph-photoautotroph coculture is validated using a wide range of experimental conditions. The model:

- Accurately predicts both the coculture growth in liquid phase and the gas composition changes in head space over time.
- Explicitly models the exchange of *in situ* produced O_2 and CO_2 within the coculture.
- Considers the self-shading effect on the growth of photoautotroph.

A R T I C L E I N F O

Method name: Semi-structured kinetic model for methanotroph-photoautotroph cocultures
Keywords: Methanotroph-photoautotroph cocultures, Kinetic model, Biogas conversion, Interspecies metabolic exchanges
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* Corresponding author.
E-mail address: wang@auburn.edu (J. Wang).

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Specifications table

Subject Area:	Chemical Engineering
More specific subject area:	Biochemical Engineering (kinetic modeling of coculture growth)
Method name:	Semi-structured kinetic model for methanotroph-photoautotroph cocultures
Name and reference of original method:	Not applicable (no such model exists before this work)
Resource availability:	Matlab codes to run in Matlab 2020a: https://github.com/AU-Wang-He-Group/Semi-structured-KineticModel.git

Introduction

Through metabolic coupling of methane oxidation and oxygenic photosynthesis, methanotroph-photoautotroph (M-P) cocultures offer a highly promising technology platform for biogas conversion [1–4]. For the development of various biotechnologies, it is essential to obtain kinetic models that can accurately predict microbial growth under different conditions. Specifically, a high-quality kinetic model provides a foundation to guide the optimal design and scale up of the bioreactors, as well as the optimization and control of the bioreactor operations. In the co-submitted work, "a novel semi-structure kinetic model for methanotroph-photoautotroph cocultures for biogas conversion", we presented the very first kinetic model for M-P cocultures, and demonstrated its superior performance using coculture growth experiments under a wide range of cultivation conditions. In this MethodsX paper, we present the mathematical details on the implementation of the semi-structured kinetic model.

*Method details

Fig. 1 provides an overview of different components involved in the semi-structured kinetic model and their interdependencies: growth of the photoautotroph; growth of the methanotroph; mass balance in the liquid phase; and mass balance in the gas phase.

Fig. 2 presents the flow chart of the semi-structured model implemented in Matlab and the associated model equations. In the following, we present the necessary detail of how the function blocks in the flow chart were implemented. Detailed instructions and example codes can be found at: https://github.com/AU-Wang-He-Group/Semi-structured-KineticModel.git. Table 1 lists the cultivation conditions of wet lab experiments corresponding to each simulation codes.

Parameterization

The parameters involved in the semi-structured kinetic model can be categorized into three groups: parameters needed for the Monod equation for individual biomass growth (maximum cell growth rate μ_{max}, and half saturation constant of substrates K_S), yield coefficients (Y), and parameters related to mass transfer between the gas and liquid phase (volumetric mass transfer coefficient k_La and effective Henry's constant H^e). Table 2 lists the model parameters for the coculture *Methylomicrobium buryatense* 5GB1 - *Arthrospira platensis*. It is worth noting that the semi-structured kinetic model is generally applicable to any M-P cocultures. If a different pair of M-P coculture is to be examined, the parameters for Monod equations and yields coefficients need to be modify according to the available data from literatures and/or designed experiments.

Initialization

The semi-structured kinetic model requires coculture growth condition to start the simulation, which includes gas composition (volume percentage of CH_4, CO_2 and O_2), light intensity (I_0, $\mu mol\ m^{-2}s^{-1}$), volume of liquid and gas phase (L), initial individual biomass concentration (gDCW/L), duration of growth and the initial total inorganic carbon in liquid (mmol/L) [5].

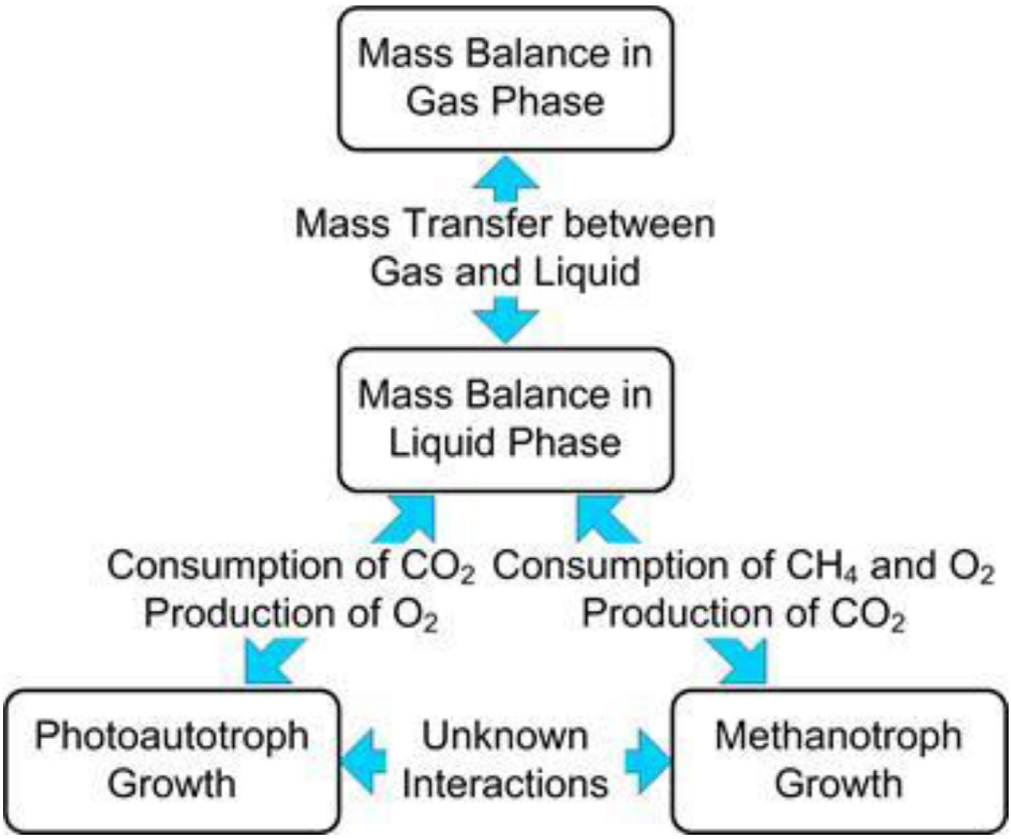

Fig. 1. An overview of the semi-structured kinetic modeling framework.

Growth of the photoautotroph and methanotroph

The growth of the photoautotroph is described using Monod model, with CO_2 and light intensity (I_a) as the two substrates. The growth of the methanotroph is also described using Monod model, with CH_4 and O_2 as the substrates.

As the available light energy to the cells in the culture broth depends on the biomass concentration due to the "self-shading" effect, we use the Beer-Lambert law for light distribution to estimate the attenuated light intensity (I_a) [6]:

$$I_a = I_0 \exp\left[-m\left(X^M + X^P\right)\right] \tag{1}$$

where I_0 is the direct measurement of incident light intensity ($\mu mol\ m^{-2}s^{-1}$), $(X^M + X^P)$ is the total biomass concentration of both methanotroph and photoautotroph; m is the absorption coefficient, which is modeled as linearly dependent on the incident light intensity in this work:

$$m = aI_0 + b \tag{2}$$

To determine the value of model parameter a and b, we first determined the value of m corresponding to the highest and lowest incident light intensities tested in this work by fitting the model predicted biomass concentrations with experimental measurements. The values of m were determined to be 3.2 and 5.3 for light intensity of 180 and 60 $\mu mol\, m^{-2}s^{-1}$, respectively. Then the model parameters a and b were determined by the straight line that passes through the two points corresponding to the two light intensities, as shown in Fig. 3.

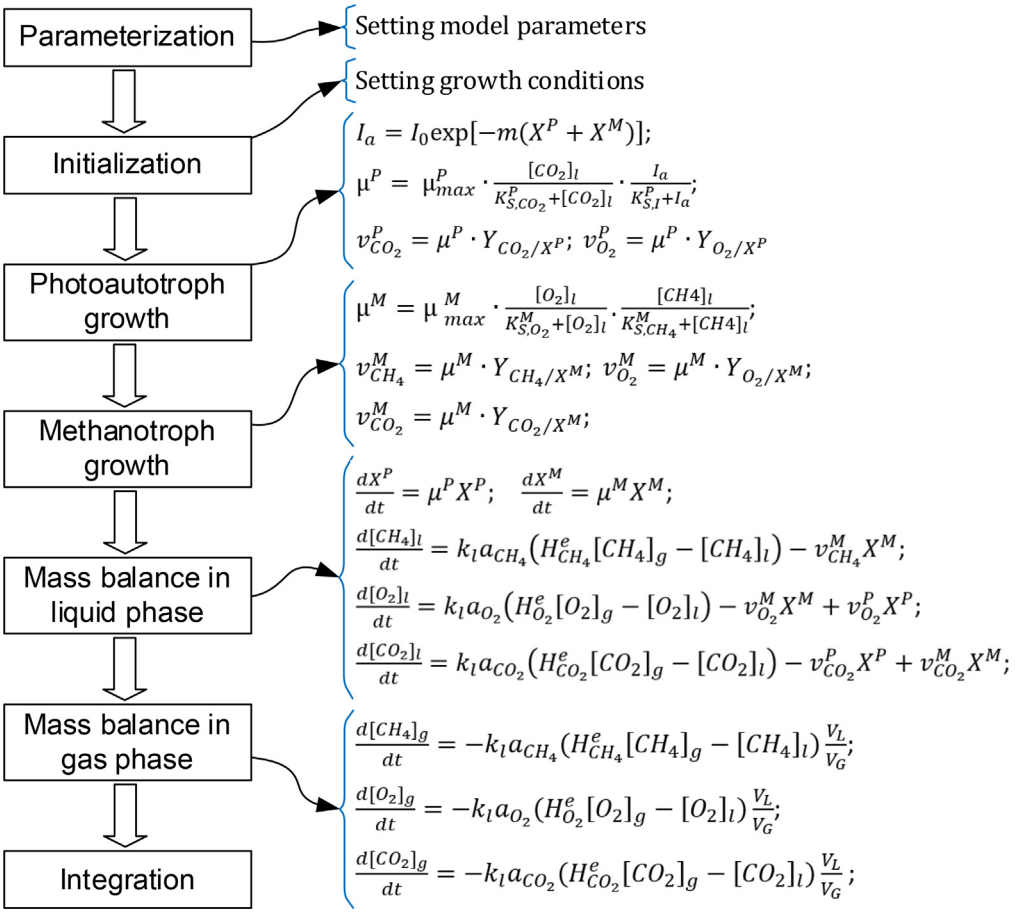

Fig. 2. The flow chart of the semi-structured model implemented in Matlab and the associated model equations.

Mass balance of the liquid and gas phases

The system dynamics were captured by the differential equations that describe the mass balance in the liquid phase and the gas phase, correspondingly. The exchange of *in situ* produced O_2 and CO_2 were explicitly captured in the mass balance equations for the liquid phase. For the mass transfer between the gas and liquid phase, we assume the distributions of various gas components between the gas and liquid phase are at equilibrium all the time.

In order to capture the effect of the biomass and culture medium on the solubility of different gas components, we use effective Henry's constant to determine the solubility of different gas components in the coculture broth. Such simplification works well for CH_4 and O_2, due to their small solubility and stable molecular structure when dissolved in aqueous solution. However, it is very challenging to determine the partition of CO_2 between the gas phase and liquid culture broth, mainly due to the dissociation of dissolved CO_2 into HCO_3^- and CO_3^{2-} ($CO_2 \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$). In addition, photoautotrophs can uptake both dissolved CO_2 into HCO_3^- as carbon supply, so it is not necessary to differentiate different dissolved/dissociated form of CO_2. In this work, we lump various forms of dissolved CO_2 together and term it "the total dissolved CO_2" that can be tracked

Table 1
Various conditions for each set of designed coculture experiments.

Experiment (Case)	System	Condition	Gas (CH_4:CO_2:O_2)*	Inoculum ratio (P:M)	Light intensity (μmol/m^2 s)
A	Coculture	a	70:30:0	12.5: 1	180
		b			60
B	Light intensities	a	80:20:0	12.5: 1	180
		b			140
		c			100
		d			60
C	Gas compositions	a	20:10:0	12.5: 1	180
		b	60:30:0		
		c	60:30:10		
		d	80:20:0		
D	Inoculum ratios	a	80:20:0	12.5: 1	180
		b		8.5: 1	
		c		4: 1	
		d		1.5: 1	
E	Coculture vs Sequential single culture	coculture	70:30:0	12.5: 1	180
		A. platensis	70:30:0	Inoculum conc. (0.218 gDCW/L)	
		M. buryatense	70:30:0**	Inoculum conc. (0.018 gDCW/L)	

* Volume/mole percentage. N_2 is the inert gas to make up to 100% when needed.
** The oxygen produced by the single photoautotroph was injected to the single methanotroph.

Table 2
Parameters and the obtained values used in the kinetic model.

Parameter	Obtained value	Unit	Parameter	Obtained value	Unit
μ^{P}_{max}single	0.024	hr^{-1}	$Y^{P}{}_{\frac{CO_2}{X^P}}$	31.85	$mmol/gDCW$
μ^{M}_{max}single	0.098	hr^{-1}	$Y^{P}{}_{\frac{CO_2}{X^P}}$	40.82	$mmol/gDCW$
μ^{P}_{max}coculture	0.034	hr^{-1}	$Y^{M}{}_{\frac{CH_4}{X^M}}$	85.47	$mmol/gDCW$
μ^{M}_{max}coculture	0.145	hr^{-1}	$Y^{M}{}_{\frac{CO_2}{X^M}}$	40.98	$mmol/gDCW$
K^{P}_{S,CO_2}	0.240	$mmol\ L^{-1}$	$Y^{M}{}_{\frac{O_2}{X^M}}$	114.94	$mmol/gDCW$
K^{M}_{S,O_2}	0.005	$mmol\ L^{-1}$	H_{CH_4}	0.0014	$mol\ L^{-1}\ atm^{-1}$
K^{M}_{S,CH_4}	0.028	$mmol\ L^{-1}$	H_{O_2}	0.0013	$mol\ L^{-1}\ atm^{-1}$
$K^{P}_{S,I}$	4.33	$\mu mol\ m^{-2}s^{-1}$	H_{CO_2}	0.035	$mol\ L^{-1}\ atm^{-1}$
a	−0.0175	–	$H^{e}_{CH_4}$	0.0341	–
b	6.40	–	$H^{e}_{O_2}$	0.0317	–
$k_L a_{CH_4}$	100	h^{-1}	$H^{e}_{CO_2}$	1.6120	–
$k_L a_{O_2}$	$1.17 \times k_L a_{CH_4}$	h^{-1}			
$k_L a_{CO_2}$	$0.90 \times k_L a_{CH_4}$	h^{-1}			

experimentally by measuring total inorganic carbon (TIC) of the liquid sample. The background TIC contained in the culture medium was measured and provided as part of the initialization [5].

To determine the partition of CO_2 between the gas phase ($[CO_2]_g$) and liquid phase (TIC) under the pH of 8.7–9, we have performed a set of designed experiments. In these experiments, feeding gas with different CO_2 concentrations (volume%) was bubbled through 100 ml of medium (90% Zarrouk medium [7] and 10% NMS2 medium [8]) for 15 min; then pH of the medium was adjusted to 8.7–9 using NaOH. Afterwards, the gas and liquid samples were taken to measure $[CO_2]_g$ and liquid TIC, respectively. Triplicates were performed for each feeding gas composition. The obtained results are plotted in Fig. 4.

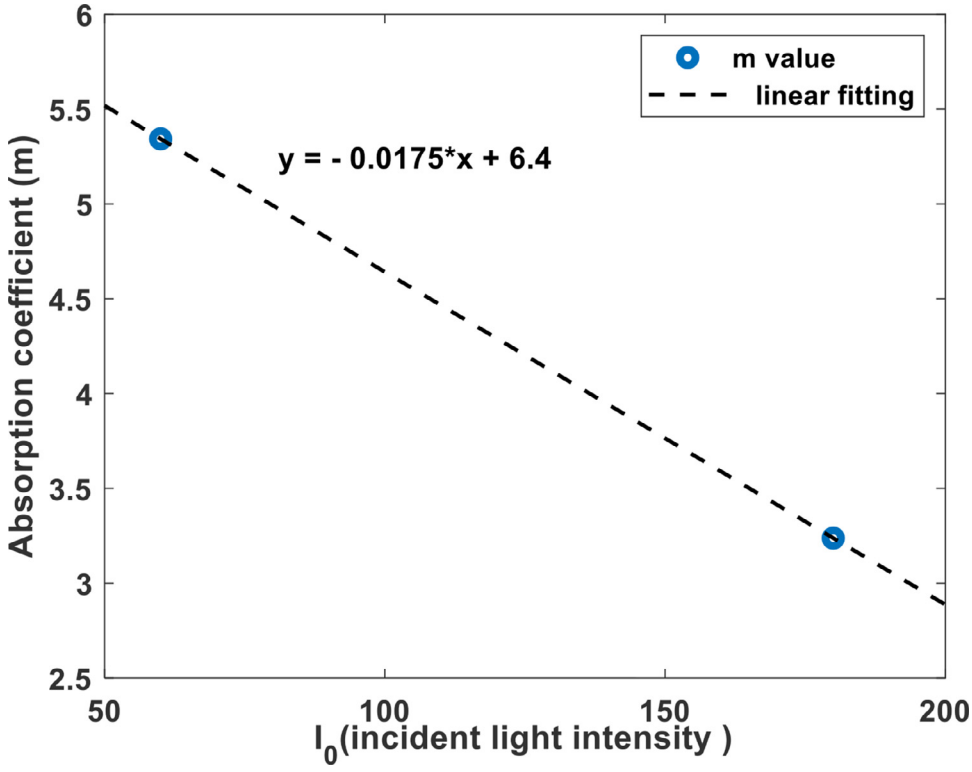

Fig. 3. Determination of the absorbent coefficient m for the Beer-Lambert law for light distribution with self-shading effect.

Clearly, there is a linear relationship between $[CO_2]_g$ and liquid TIC, and the empirical relationship is the following

$$TIC = 170.26 + 5.3595\,[CO_2]_g \tag{3}$$

In the coculture wet lab experiments, the gas and liquid samples were taken after each gas feeding event. The pH of the coculture broth was adjusted to 8.7–9 after the sampling. As the solubility of CO_2 depends heavily on the pH of the culture medium, after the pH adjustment, a significant amount of CO_2 became dissolved in the liquid phase. Therefore, it is necessary to determine the new partition between $[CO_2]_g$ and liquid TIC after pH adjustment, which can be done using Eq. (3). In the coculture experiment, the total amount of inorganic carbon (both in gas and liquid phase) in the system is fixed, and after adjusting pH, relationship between $[CO_2]_g$ and liquid TIC is described by Eq. (3), it is straightforward to derive

$$[CO_2]_g = \left(TIC_0.V_L + [CO_2]_{g,0}.V_G - \ 170.26V_L\right)/(5.3595V_L + V_G) \tag{4}$$

where $[CO_2]_{g,\,0}$ and TIC_0 are the gas and liquid measurement before pH adjustment. The liquid TIC after pH adjustment can be computed using Eq. (3).

Solving the ODEs and reinitialization

ODE45 (a Matlab function) was used to perform integration over each growth period. During the coculture experiment, the bottles were refed every 24 h, which resets the gas phase composition and dissolved gas concentrations in the liquid. Correspondingly, the integration process has to be reinitialized after each refeeding event. During the initialization, the biomass concentration at the end

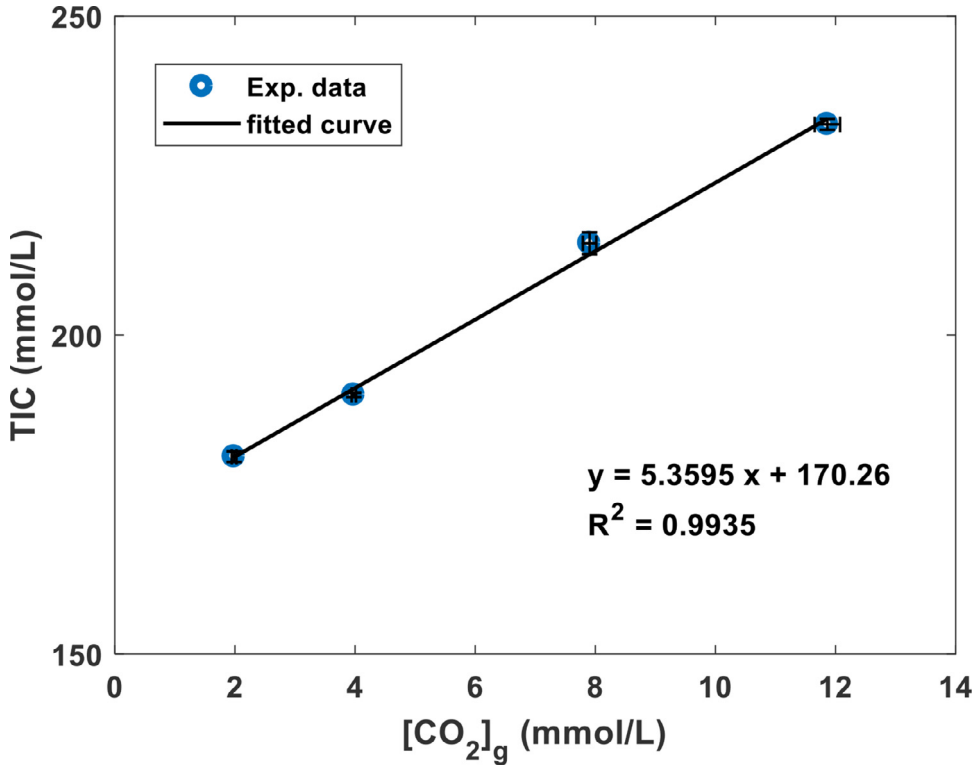

Fig. 4. The relationship between TIC and $[CO_2]_g$ after adjusting pH at different feed gas.

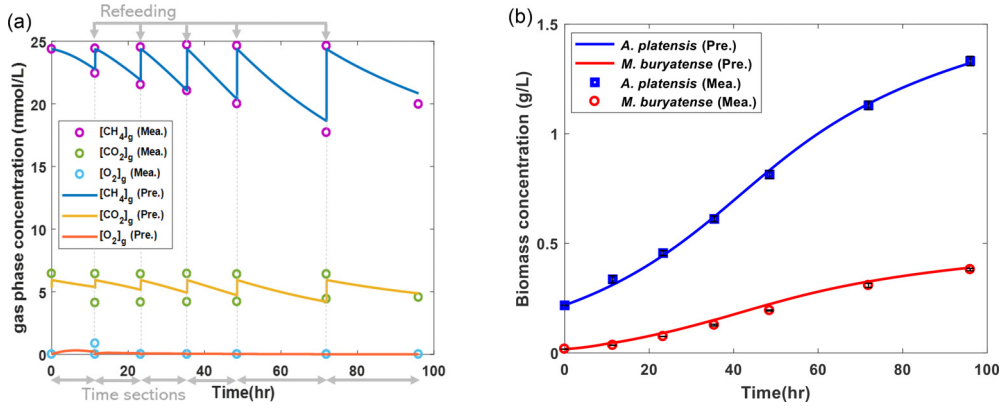

Fig. 5. Comparison of the semi-structured kinetic model prediction (Pre.) versus experimental measurement (Mea.) for the coculture system (*M. buryatense* 5GB1-*A. platensis*) at gas composition of 60%CH_4, 30%CO_2, 10%N_2; inoculum ratio of 12.5:1 (P:M); and light intensity of 180 μmol $m^{-2}s^{-1}$. (a) Gas phase concentration changes, the time that the system was refed and the time sections in the modeling is shown. (b) Biomass concentration of each individual strain in the coculture system, as the model predictions showed excellence agreement with experimental measurement. Source: https://github.com/AU-Wang-He-Group/Semi-structured-KineticModel.git.

of the previous growth period is set to be the initial biomass concentration for the following growth period; the gas phase composition is reset to be the same as the feeding gas, and the corresponding liquid phase concentrations were determined through the effective Henry's constant, following the same procedure of initialization.

Method validation

The kinetic model prediction and the corresponding experimental data for gas phase and biomass concentration in the coculture system are plotted in Fig. 5 to demonstrate the accuracy of the semi-structured kinetic model. The coculture growth condition for Fig. 5 was the following: gas composition of 60%CH_4, 30%CO_2, 10%N_2; inoculum ratio of 12.5:1 (P:M); and light intensity of 180 μmol $m^{-2}s^{-1}$. Addition examples can be found in https://github.com/AU-Wang-He-Group/Semi-structured-KineticModel.git.

Conclusion

By explicitly modeling the exchange of *in situ* produced O_2/CO_2 and coupling the individual biomass growth with gas phase composition changes, the semi-structured kinetic model allows predicting the dynamics of the M-P coculture system and behavior of each individual strain within the coculture under a wide range of growth conditions. Thus, this kinetic model is expected to the generally applicable to a wide range of M-P species and provides required information for development of the coculture-based biogas conversion technologies.

Acknowledgments

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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.

References

[1] E.A. Hill, W.B. Chrisler, A.S. Beliaev, H.C. Bernstein, A flexible microbial co-culture platform for simultaneous utilization of methane and carbon dioxide from gas feedstocks, Bioresour. Technol. 228 (2017) 250–256, doi:10.1016/j.biortech.2016.12.111.
[2] Z. Rasouli, B. Valverde-Pérez, M. D'Este, D. De Francisci, I. Angelidaki, Nutrient recovery from industrial wastewater as single cell protein by a co-culture of green microalgae and methanotrophs, Biochem. Eng. J. 134 (2018) 129–135, doi:10.1016/j.bej.2018.03.010.
[3] K. Badr, M. Hilliard, N. Roberts, Q.P. He, J. Wang, Photoautotroph-methanotroph coculture - a flexible platform for efficient biological CO_2-CH_4 Co-utilization, IFAC PapersOnLine 52 (2019) 916–921, doi:10.1016/j.ifacol.2019.06.179.
[4] N. Roberts, M. Hilliard, Q.P. He, J. Wang, A microalgae-methanotroph coculture is a promising platform for fuels and chemical production from wastewater, Front. Energy Res. 8 (2020) 230, doi:10.3389/fenrg.2020.563352.
[5] K. Badr, W. Whelan, Q.P. He, J. Wang, Fast and easy quantitative characterization of methanotroph–photoautotroph cocultures, Biotechnol. Bioeng. 118 (2021) 703–714, doi:10.1002/bit.27603.
[6] Q. Béchet, A. Shilton, B. Guieysse, Modeling the effects of light and temperature on algae growth: state of the art and critical assessment for productivity prediction during outdoor cultivation, Biotechnol. Adv. 31 (2013) 1648–1663, doi:10.1016/j.biotechadv.2013.08.014.
[7] C. Zarrouk, Contribution a l'etude d'une cyanophycee. influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de *Spirulina maxima* (Setchell et Gardner) Geitler. PhD thesis, University of Paris, France, (1966).
[8] A.W. Puri, S. Owen, F. Chu, T. Chavkin, D.A.C. Beck, M.G. Kalyuzhnaya, M.E. Lidstrom, Genetic tools for the industrially promising methanotroph methylomicrobium buryatense, Appl. Environ. Microbiol. 81 (2015) 1775–1781, doi:10.1128/AEM.03795-14.