Method Article

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

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ABSTRACT
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 O₂ and CO₂ within the coculture.
- Considers the self-shading effect on the growth of photoautotroph.

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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 $\mu_{\text{max}}$, 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 $k_{1,a}$ and effective Henry’s constant $H^f$). Table 2 lists the model parameters for the coculture *Methylocrobium 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 $\text{CH}_4$, $\text{CO}_2$ and $\text{O}_2$), light intensity ($I_0$, $\mu\text{mol m}^{-2}\text{s}^{-1}$), 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].
Growth of the photoautotroph and methanotroph

The growth of the photoautotroph is described using Monod model, with CO₂ and light intensity \( I_a \) as the two substrates. The growth of the methanotroph is also described using Monod model, with CH₄ and O₂ 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]:

\[
I_a = I_0 \exp[-m(X^M + X^P)]
\]

where \( I_0 \) is the direct measurement of incident light intensity (\( \mu \text{mol m}^{-2}\text{s}^{-1} \)), \( (X^M + X^P) \) 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:

\[
m = aI_0 + b
\]

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 \( \mu \text{mol m}^{-2}\text{s}^{-1} \), 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. 1. An overview of the semi-structured kinetic modeling framework.
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 \textit{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$_3^{2-}$ ($\text{CO}_2 \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- \leftrightarrow \text{CO}_3^{2-} + \text{H}^+$). 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        |            |                               | 12.5: 1              | 180                           |
|                   | Sequential single   |            |                               |                      |
|                   | culture             |            |                               |                      |
|                   |                     |            |                               |                      |
|                   | M. buryatense       |            | 70:30:0                       |                      |

$^*$ 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 |
|-----------|----------------|------|-----------|----------------|------|
| $\mu_{\text{max single}}$ | 0.024 | hr$^{-1}$ | $Y_{\text{P CO}_2}$ | 31.85 | mmol/gDCW |
| $\mu_{\text{max single}}$ | 0.098 | hr$^{-1}$ | $Y_{\text{P CO}_2}$ | 40.82 | mmol/gDCW |
| $\mu_{\text{max coculture}}$ | 0.034 | hr$^{-1}$ | $Y_{\text{P CO}_2}$ | 85.47 | mmol/gDCW |
| $\mu_{\text{max coculture}}$ | 0.145 | hr$^{-1}$ | $Y_{\text{P CO}_2}$ | 40.98 | mmol/gDCW |
| $K_{\text{CO}_2}^C$ | 0.240 | mmol L$^{-1}$ | $Y_{\text{P CO}_2}$ | 114.94 | mmol/gDCW |
| $K_{\text{CO}_2}^D$ | 0.005 | mmol L$^{-1}$ | $H_{\text{CO}_2}$ | 0.0014 | mol L$^{-1}$ at m$^{-1}$ |
| $K_{\text{CO}_2}^H$ | 0.028 | mmol L$^{-1}$ | $H_{\text{CO}_2}$ | 0.0013 | mol L$^{-1}$ at m$^{-1}$ |
| $K_{\text{CO}_2}^T$ | 4.33 | μmol m$^{-2.5}$ s$^{-1}$ | $H_{\text{CO}_2}$ | 0.035 | mol L$^{-1}$ at m$^{-1}$ |
| a | –0.0175 | – | $H_{\text{CO}_2}$ | 0.0341 | – |
| b | 6.40 | – | $H_{\text{CO}_2}$ | 0.0317 | – |
| $k_1 a_{\text{CH}_4}$ | 100 | h$^{-1}$ | $H_{\text{CO}_2}$ | 1.6120 | – |
| $k_2 a_{\text{CO}_2}$ | 1.17 $\times$ $k_1 a_{\text{CH}_4}$ | h$^{-1}$ | $H_{\text{CO}_2}$ | – | – |
| $k_3 a_{\text{CO}_2}$ | 0.90 $\times$ $k_1 a_{\text{CH}_4}$ | h$^{-1}$ | $H_{\text{CO}_2}$ | – | – |

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% NMS 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 $[\text{CO}_2]_g$ and liquid TIC, and the empirical relationship is the following

$$\text{TIC} = 170.26 + 5.3595 [\text{CO}_2]_g$$  \hspace{1cm} (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 $[\text{CO}_2]_g$ 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 $[\text{CO}_2]_g$ and liquid TIC is described by Eq. (3), it is straightforward to derive

$$[\text{CO}_2]_g = \left( \text{TIC}_0 \cdot V_L + [\text{CO}_2]_{0_g} \cdot V_G \right) - 170.26 V_L \left/ \left( 5.3595 V_L + V_G \right) \right.$$ \hspace{1cm} (4)

where $[\text{CO}_2]_{0_g}$ 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 $[\text{CO}_2]_g$ 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 $\mu$mol 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%CH₄, 30%CO₂, 10%N₂; 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 O₂/CO₂ 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.

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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.

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