Title: Elucidating the Evolution of Interspecies Metabolic Interactions within a Methanotroph-Cyanobacteria Coculture using Dynamic Genome-scale Metabolic Modeling

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Project Goals: In nature, microbial communities have developed a highly efficient way to recover energy and capture carbon from both CH_4 and CO_2 through interspecies coupling of methane oxidation to oxygenic photosynthesis. However, in order to successfully utilize mixed culture for biotechnology applications, both fundamental knowledge and technological gaps have to be addressed. The knowledge gap refers to the lack of systematic study for identifying and quantifying the interactions between community members and how the interactions affect system dynamics. The technological gap refers to the lack of effective methodology, and fast and low-cost analytical tools to characterize mixed culture systems frequently or in real-time. The overall objective of this research is to help address those gaps through developing experimental and computational tools to characterize a synthetic methanotroph-photoautotroph (M-P) binary consortium, to identify and validate interspecies interactions at both systems and cellular levels, and to engineer a model methanotroph-photoautotroph coculture pair for enhanced production of chemicals.

Abstract Text: Microbial communities are ubiquitous in nature and play a significant role in various biogeochemical cycles on earth. Microbial communities are dynamic in nature and exhibit different metabolic interactions under different environmental conditions. However, due the inherent complexity of microbial communities, their dynamic responses are not well understood. Recently, genome-scale metabolic modeling (GEM) emerged as an effective tool to study microbial communities. However, the representative dynamic GEM approaches cannot predict the interspecies metabolic interactions within a community, while the steady-state GEM approaches cannot capture the dynamic evolvement of the metabolic interactions. In this work, using a methanotroph-cyanobacteria (*Methylomicrobium buryatense* 5GB1- *Arthrospira platensis*) coculture as the model system, we present a dynamic GEM approach, termed DynamiCom that can predict the evolution of interspecies metabolic interactions within the coculture over time (Fig. 1). The proposed approach builds upon a semi-structured kinetic model (Fig. 2) we developed recently that can accurately predict the growth dynamics of the model coculture under a wide range of conditions. The cross-membrane fluxes, i.e., the individual substrate pickup rates and product excretion rates predicted by the kinetic model, are used as additional constraints for a steady-state coculture GEM (SteadyCom is used in this work). Besides consistently predicting the same set of top metabolic exchanges within the coculture under different *in silico* setups, the proposed approach
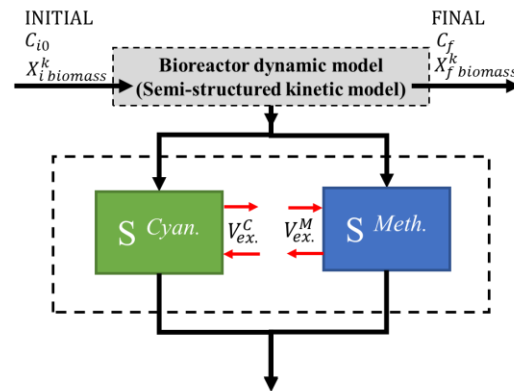
Fig. 1. Schematics of the proposed dynamic GEM model

predicted the establishment of the mutualistic relationship between the methanotroph and cyanobacteria (Fig. 3). Specifically, our results suggest the establishment of an emergent N-exchange within the coculture, where the cyanobacteria consume nitrate, while the methanotroph exclusively consumes ammonium excreted by the cyanobacteria after the mutualistic relationship is established.
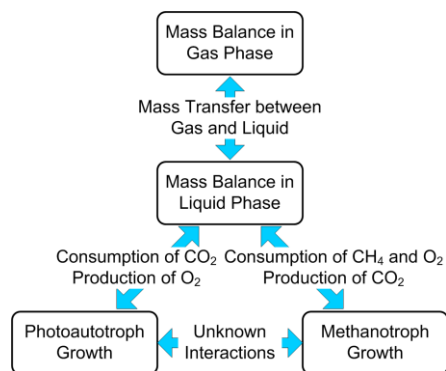

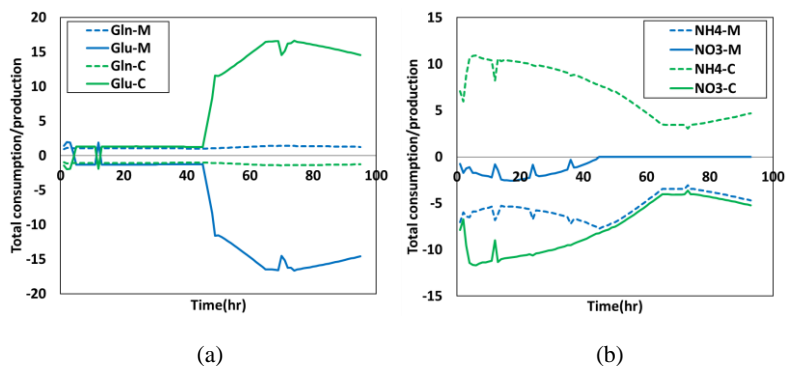Fig. 2. Overview of the semi-structured kinetic modeling framework

Figure 3. Dynamic evolution of (a) Glutamine (Gln) and Glutamate (Glu) as an example of top interspecies metabolic interactions; (b) ammonium (NH4) and Nitrate (NO3). M is produced(+)/consumed(-) by methanotroph and C produced(+)/consumed(-) by cyanobacteria. The irregularity in the prediction is the consequence of the change of CH_4/CO_2 uptake by the species during and after refeeding the system

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