Dynamic Genome-Scale Metabolic Network Modeling for a Novel Methanotroph-Cyanobacteria Coculture

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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 interaction feedbacks 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 research objective is to help address those gaps through developing experimental/computational tools to characterize a synthetic photoautotroph-methanotroph 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: Biogas derived from organic waste streams through anaerobic digestion has immense potential as a renewable feedstock for producing high-density fuels and commodity chemicals. However, utilization of biogas represents a significant challenge due to its low pressure and presence of contaminants. In our previous research, we have clearly demonstrated that methanotroph-photoautotroph cocultures offer a flexible platform for efficient biological CH_4-CO_2 coutilization. However, development of multi-organism platforms for commercial biogas conversion present significant challenges which center around our ability to control function and composition of species in the coculture. It has been well recognized that an essential tool for the optimization, design and analysis of the coculture based biogas conversion is the development and validation of kinetics models that can accurately describe and predict the co-culture growth under different conditions [1]. To this end, using *Methylomicrobium buryatense* - *Arthrosipira platensis* as the model coculture system, we have developed an unstructured model to capture the growth dynamics. Specifically, Monod-like models were developed to capture coculture growth. Two sources of substrate were considered in the model: gas transferred from gas phase and gas produced *in situ*. In addition, we rely on the fitted maximum cell growth rate for both strains to capture other potential interactions. Using designed experiments and the developed model, we clearly demonstrated that the synergistic effect within the coculture cannot be fully explained by the *in situ* substrate exchange, and there must be other "metabolic links" to explain the significantly enhanced cell growth of both strains in the coculture.

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In the era of omics big data, computational models are instrumental for turning different sources of data into valuable knowledge. Genome-scale metabolic models (GEMs) represent extensive knowledge bases that provide a platform for model simulations and integrative analysis of omics data. More importantly, it offers a convenient and powerful tool to test various hypothesis regarding "metabolic links" within the methanotroph-photoautotroph coculture. The GEMs,

especially the dynamic GEMs, offer a comprehensive picture of cellular metabolism and serve as a bridge that can better link the work of microbiologists and engineers in understanding and optimizing complex cellular metabolism. By integrating the available knowledge on each strain with data obtained in our own experiments, we use DFBAlab to implement the dynamic GEM for the model coculture. In the dynamic coculture GEM, besides the GEMs for each individual strain, the key inputs to the DFBA model are the uptake kinetics for different substrates. In this work, the substrate update rates are provided from the unstructured dynamic model we already developed.

Model validation was done through comparing model predicted product secretion rates and cell growth rates with experimental measurements over a course of batch coculture growth on biogas. It should be noted that obtaining accurate measurement of each species within the coculture is a highly challenging task. To address this challenges, we have developed an experimental-computational protocol for easy, fast and accurate quantitative characterization of the synthetic coculture [2]. Besides determining the individual biomass concentration of each organism in the coculture, the developed protocol also obtains the individual consumption and production rates of O2 and CO2 for the methanotroph and cyanobacteria. Besides these measurements, the overall dynamic trajectory over time, both measured low-frequency samples and unstructured model predicted high-frequency samples, offers significantly power in validating the model. Figure 1 compares the model predicted population ratio within the coculture for different inoculum ratios with measurements, with (a) from the unstructured kinetic model; (b) from the dynamic GEM.

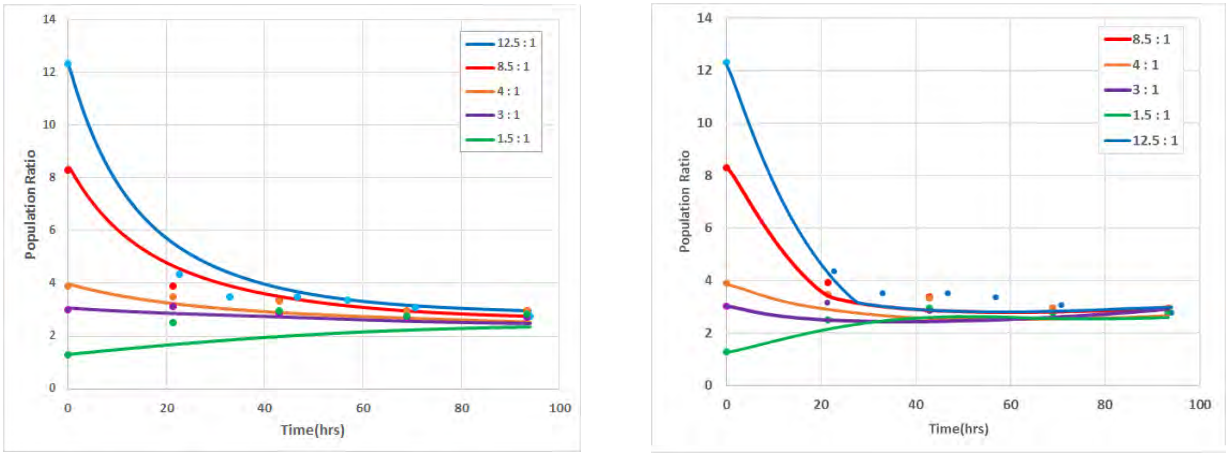

Fig. 1. Comparison of the population ratio within the coculture (cyanobacterium : methanotroph) predicted by the model (lines) with measurements (dots). A. (left) from unstructured kinetic model; B. (Right) from the structured dynamic GEM. Both models correctly predicted that despite different inoculum ratios, after reaching steady state, the population ratio of the two strains converge to the same value.

Publications:

1. Badr K., Hilliard M., Roberts N., He Q.P. and **Wang J.** (2019), Photoautotroph-Methanotroph Coculture – A Flexible Platform for Efficient Biological CO_2-CH_4 Co-utilization, *IFAC-PapersOnLine*, 52 (1), 916-921;

2. Stone K., He Q.P., & **Wang J.** (2019), Two Experimental Protocols for Accurate Measurement of Gas Component Uptake and Production Rates in Bioconversion Processes, *Nature - Scientific Report*, 9 (1), 5899

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