Methanotroph-Photoautotroph Coculture – A Flexible Platform for Efficient Biological CO_2-CH_4 Co-utilization

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Project Goals: In nature, microbial communities have developed a highly efficient way to recover the 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 of this project is to help address those gaps through developing experimental/computational tools to characterize a synthetic photoautotroph-methanotroph binary consortium, and to identify and validate interspecies interactions at both systems and cellular levels for a model methanotroph-photoautotroph coculture pair.

Abstract: Industrial, municipal, and agricultural waste streams contain stranded organic carbon, which can be converted into biogas through anaerobic digestion. It has been demonstrated that biogas has immense potential as a renewable feedstock for producing high-density fuels and commodity chemicals. However, the utilization of biogas presents a significant challenge due to its low pressure, high proportion of CO_2 and presence of contaminants such as H_2S, ammonia, and volatile organic carbon compounds. To tap into this immense potential, effective biotechnologies that co-utilize both CO_2 and CH_4 are needed. Using the basic metabolic coupling principles utilized by many natural consortia, we have demonstrated that photoautotroph- methanotroph co-cultures offers a flexible and highly promising platform for biological CO_2/CH_4 co-utilization. In this work, we focused on filling part of the knowledge gap, i.e., identifying the potential interactions at the systems level through a hypothesis-driven approach. Also, we present the very first effort to quantitatively model the growth dynamics of a photoautotroph-methanotroph coculture. These advancements will enable us to further examine the interspecies interactions within the coculture at molecular level.

Advantages of the proposed coculture platform Coupling methanotrophic metabolism to photosynthesis offers several advantages for engineering biogas conversion. That includes the exchange of *in situ* produced O_2 and CO_2 which could dramatically reduce mass transfer resistance of the two gas substrates. However, one very important question should be answered first: are there clear benefits of using the coculture than using single cultures sequentially for biogas conversion? This is a critical question applicable to any consortia-based biotechnologies, as the operation of the mixed culture can be more challenging than maintaining two single cultures sequentially. To answer the question, we have designed and conducted the comparison experiments for three cases, using *Methylotuvimicrobium buryatense* (methanotroph) - *Arthrosipira platensis* (cyanobacterium) as the model coculture system. Case A is the coculture; Case B is the sequential culture of cyanobacterium followed by methanotroph; Case C simulates the effect of the exchange of *in situ* produced O_2 between the coculture, where the amount of O_2 produced by the cyanobacterium in the coculture was injected into the methanotroph single culture. Our initial experimental results[1] clearly showed that the methanotroph-photoautotroph coculture offers significantly more benefit than sequentially operated single cultures. In addition, the model parameters of unstructured kinetic model, which were estimated using experimental data, further confirm that the synergy within the coculture is beyond the exchange of in situ produced O_2 and CO_2.

Kinetic Modeling of the Coculture The development of multi-organism platforms for commercial biogas conversion presents significant challenges which center around our ability to control function and composition of species in the coculture. An essential tool for the optimization, design and analysis of the coculture based biogas conversion is the development and validation of kinetic models that can accurately describe and predict the co-culture growth under different conditions. In this work, we have developed an unstructured dynamic model to capture the dynamics of the coculture growth under a variety of culture conditions. By considering the substrate exchange and self-shading effect, the unstructured kinetic model is able to capture the coculture growth dynamics accurately throughout the entire batch duration and can accurately predict the growth behaviour under a different light intensity [1], as shown in Figure 1A. Finally, both the model prediction and experimental results validated our hypothesis that the inoculum ratio of the two strains will not affect the final steady state ratio, as shown in Figure 1B.

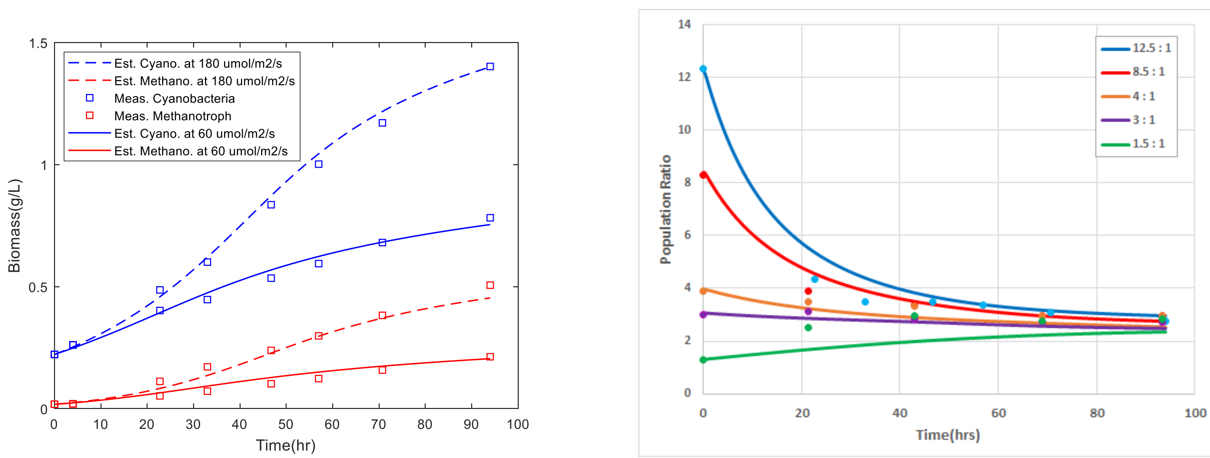

Fig. 1 A (Left). Comparison of the biomass concentration in the coculture predicted by the model (lines and dashed lines) with measurements (squares). B (Right). Both model simulation (lines) and experimental measurements (dots) confirm that despite different inoculum ratios (cyanobacterium : methanotroph), 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, Proceedings of 12th Dynamics and Control of Process Systems (DYCOPS 2019), Apr. 23-26, 2019, Florianópolis, BRAZIL, accepted;

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