

## **Tools for Easy, Fast and Accurate Quantitative Characterization of the Methanotroph-Photoautotroph Coculture**

Kiumars Badr<sup>1\*</sup> (kzb0054@auburn.edu), Kyle A. Stone<sup>1</sup>, Q. Peter He<sup>1</sup>, Alexander S Beliaev<sup>2</sup>, Marina Kalyuzhnaya<sup>3</sup> and **Jin Wang**<sup>1</sup>

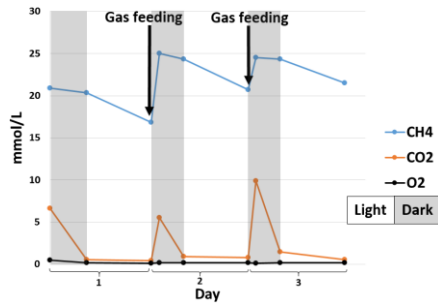
<sup>1</sup>Auburn University, Auburn, AL; <sup>2</sup>Pacific Northwest National Laboratory, Richland, WA; <sup>3</sup>San Diego State University, San Diego, CA

**Project Goals:** In nature, microbial communities have developed a highly efficient way to recover the energy and capture carbon from both CH<sub>4</sub> and CO<sub>2</sub> 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.

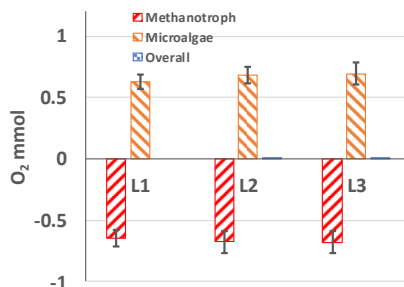
**Abstract:** Multispecies associations are ubiquitous in nature as they provide key ecosystem services such as carbon, nutrient, and metal cycling. It has been recognized that a mixed culture could offer a number of advantages over a conventional single-culture, such as complete utilization of substrate, better stability and robustness, higher product yield, higher growth rate, as well as the capability to carry out multistep transformation that would be impossible for a single organism. Despite these potential significant advantages, utilization of mixed cultures for biotechnological applications in bioenergy and related areas have been limited, partially due to the lack of effective tools to characterize the mixed culture accurately and frequently. In this project, we have developed experimental and computational protocols to quantitatively characterize the photoautotroph-methanotroph coculture. Specifically, we have developed experimental protocols to obtain accurate measurements of overall consumption and production rates for gas components CH<sub>4</sub>, O<sub>2</sub> and CO<sub>2</sub><sup>[1]</sup>. We have also developed computational procedures to estimate individual gas consumption and production rates by each organism<sup>[2]</sup>. Such quantitative characterizations laid the foundation for the proposed modelling work.

### **Accurate measurement of overall consumption and production rate for CH<sub>4</sub>, O<sub>2</sub> and CO<sub>2</sub>**

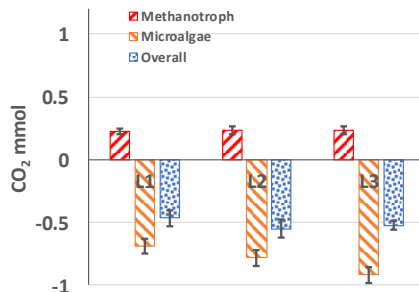
Due to gas phase volume/pressure (for batch experiments) or flow rate (for continuous experiments) change, and the pH dependent solubility of CO<sub>2</sub>, using the direct GC measurements of the headspace or off-gas composition to calculate the gas consumption/production rates can cause large errors. To address this challenge, we have developed two easy-to-implement experimental protocols and associated calculation procedures to obtain accurate measurements of gas component consumption and production rates, one for batch operation and one for continuous operation. For batch operations, we use nitrogen (or other inert gases) to re-pressurize the system to atmosphere pressure before taking samples; while for continuous operations, we use helium (or other inert gases) as an internal tracer to accurately measure off-gas flow rate. In addition, we use total inorganic carbon (TIC) to track dissolved CO<sub>2</sub>. The effectiveness and accuracy of the two



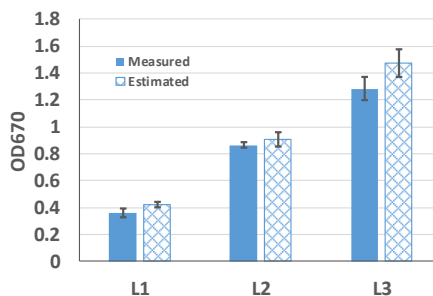
(a) Gas phase measurements



(b) O<sub>2</sub> consumption/production



(c) CO<sub>2</sub> consumption/production



(d) Total biomass

Fig. 1. Gas phase measurement over time (a), individual consumption/production (estimated) and overall change (measured) of O<sub>2</sub> (b) and CO<sub>2</sub> (c) for photoautotroph-methanotroph coculture during three light cycles. The results are validated by the good agreement between the estimated and measured total biomass during the same cycles (d).

protocols and associated calculation procedures are demonstrated using several case studies with both abiotic and biotic systems (both methanotroph and the coculture) <sup>[1, 2]</sup>.

### **Estimate individual gas consumption and production rates by each organism**

Estimating individual consumption/production rate of CH<sub>4</sub>, O<sub>2</sub>, CO<sub>2</sub> and other metabolites in co-culture is challenging, but is absolutely necessary for understanding the dynamics of the system. The main challenges are: 1. individual consumption/production rates of CO<sub>2</sub> and O<sub>2</sub> cannot be measured directly because of the coupling between photoautotroph and methanotroph; (2) the amount of dissolved CO<sub>2</sub> in the liquid medium must be estimated to complete the carbon balance. To address these challenges, we have developed a computational procedure based on mass balances and growth stoichiometric information (such as biomass yield) to compute the amount of O<sub>2</sub> and CO<sub>2</sub> consumed or produced by each organism <sup>[2]</sup>. One example is shown in Fig. 1. The accuracy of the developed protocol was validated by the good agreement between the estimated and measured total biomass.

### **Publications:**

1. Stone K., He Q.P., & Wang J. (2018), Two Experimental Protocols for Accurate Measurement of Gas Component Uptake and Production Rates in Bioconversion Processes, Nature Scientific Report, under review.
2. Bahr K., Hilliard M., Roberts N., He Q.P. and Wang J. (2019), Photoautotroph-Methanotroph Coculture – A Flexible Platform for Efficient Biological CO<sub>2</sub>-CH<sub>4</sub> Co-utilization, Proceedings of 12<sup>th</sup> Dynamics and Control of Process Systems (DYCOPS 2019), Apr. 23-26, 2019, Florianópolis, BRAZIL, accepted;

**Funding statement:** This material is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomic Science Program under Award Number DE-SC0019181; and by the U.S. Department of Education, Graduate Assistance in Areas of National Need Program under Award Number P200A150074.