Oct 21st, 12:00 AM

Experimental Measurement of Oxygen Dynamics in a Two-Dimensional Microbial Community

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Introduction

In natural ecosystems, oxygen-consuming and oxygen-producing organisms form nutrient cycles. The dynamics of these nutrient cycles and their associated metabolic activity are poorly studied.

Previous research has shown that under constant illumination, oxygen consumption of a microbial community stabilizes after a transient period [1]. We have set up an experiment to measure oxygen dynamics in a quasi-two-dimensional microbial community exposed to cycles of light and a constant nutrient flow.

Building the Chamber

A quasi-two-dimensional chamber is used. The chamber consists of an acrylic base, fluorescent dye-coated mylar with optical insulation, a rubber gasket (2mm thickness), and an acrylic top piece. The components are cut into shape using a laser cutting machine.

The dye used for the system is a ruthenium dye, RuDPP; the fluorescence of the dye is quenched by O_2 molecules. We use titanium (v) oxide to provide a layer of optical insulation between the dye-coated mylar and the rest of the chamber.

A thin layer of diluted dye, combined with polystyrene beads, is bound to a mylar sheet via spin coating to make the oxygen detector. We use an elastomer, PDMS (polydimethylsiloxane), for adhesion. 1.5mL of 2:1 hexane to PDMS solution with 4% titanium(v) oxide is pipetted onto the mylar, which is attached to a rubber mold. The detector is baked at 70^0C for an hour.

An approximately 25mm x 15mm rectangle is cut out of the oxygen detector sheet. The laser-cut rubber gasket laid onto the acrylic backing. A thin layer of PDMS is used around the edges of the detector cut-out to fix it onto the acrylic backing. The chamber is baked again at 70^0C for an hour.

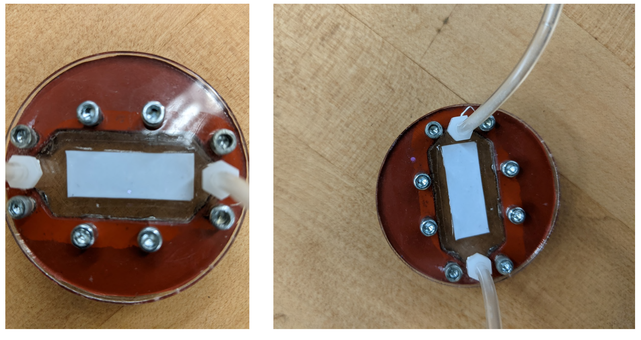

Fig 1: the chamber

Experimental Procedure

We obtain samples from a salt marsh in Woods Hole, Massachusetts. Samples are deposited in a tank and allowed to stratify, with cyclic exposure to light to mimic natural photosynthesis.

The chamber is sterilized between uses by filling with 5% bleach solution for 30 minutes, followed by thorough rinsing. The chamber is initially calibrated by exposing the chamber to atmospheric, pure nitrogen, and pure oxygen gases (Fig 2).

100µm glass beads are used as a homogenized substrate for the community. The substrate is inoculated with 1.5mL of pore water sampled from the tank, and the chamber is screwed shut.

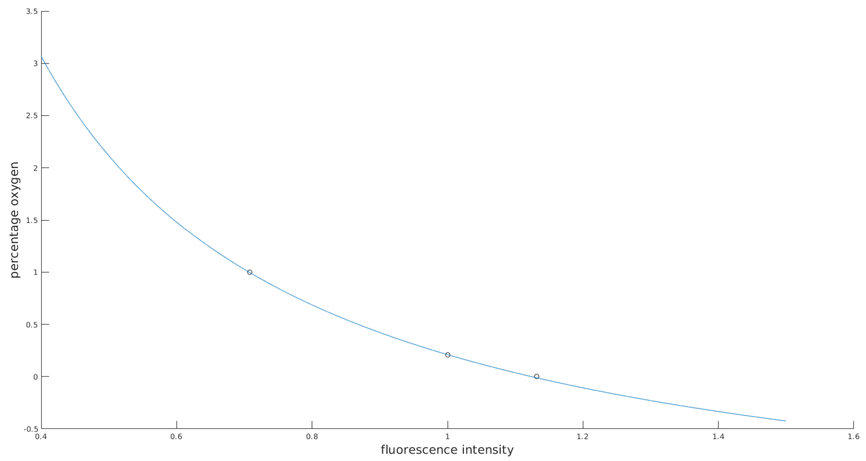

Fig 2: known relative oxygen concentration vs fluorescence intensity

Filtered pore water is flowed through the chamber at a rate of 0.8 hr^{-1} using a syringe pump, continuously replenishing nutrients in the chamber for 100 hours. The chamber is set up vertically so that effluents leave the chamber at the top inlet and pore water is replenished at the bottom outlet, allowing us to precisely control the flow of liquid.

The chamber is exposed to 90-minute cycles of light and dark. Two-dimensional images are taken at 5-minute intervals using a digital camera with a red filter. The intensity of each pixel relative to a background image is measured (Fig 3).

Results

After an initial transient period, relative oxygen concentration over time stabilizes (Fig 4). The relative oxygen available in the system reflects the metabolic activity of oxygen-consuming and oxygen-producing bacteria, which relaxes to a steady state over time in a system with constant nutrient flow.

Preliminary analysis shows that the metabolic activity of the community relaxes to a steady state along a low-dimensional trajectory.

Future Work

We plan to thoroughly characterize the trajectory of the community as it relaxes to a steady state. We also plan to constantly sample the effluent and extract DNA to characterize the composition of the microbial community as it approaches its relaxation point.

Reference

[1] Tejera, F., Libchaber, A., & Petroff, A. P. (2018). Oxygen dynamics in a two-dimensional microbial ecosystem. Physical Review E, 98(4), 1–7. https://doi.org/10.1103/PhysRevE.98.042409

Acknowledgements

With thanks to Brynna Moran, Aandishah Samara, and Ben Roque for assistance on this project, and to Prof. Alexander Petroff for the support and guidance.

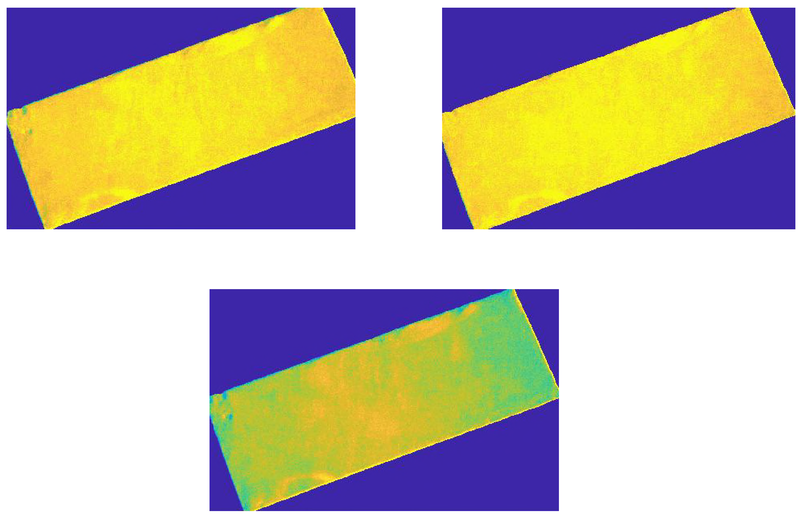

Fig 3: fluorescence intensity with light on, with light off, and with no nutrient flow (clockwise starting top left)

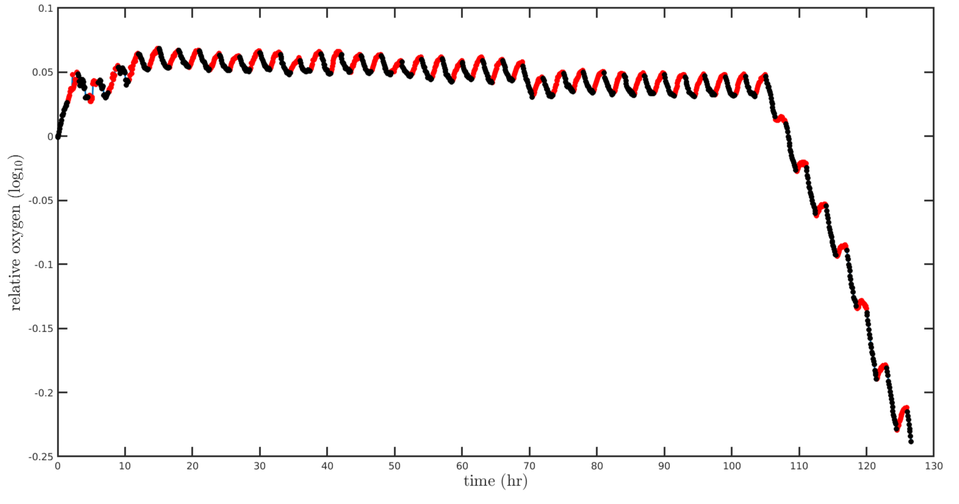

Fig 4: relative oxygen concentration (logarithmic scale) over time; syringe was replenished at 50 hours, and nutrient flow stopped at 100 hours