Optical Transmittance Characterization of Colloidal Silica Gels for Encapsulation of Oxygen Producing Cyanobacteria

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Introduction

Background

- Silica gel encapsulation provides a mechanical scaffold and protection to otherwise small and fragile bacteria, which makes the technology relevant to a wide range of engineering applications[1].
- Aeration is a common source for oxygen-requiring bacteria, but can be costly.

Hypothesis

- First hypothesis: The oxygen production rates of the encapsulated bacteria will increase with respect to optical transmittance of the gel.
- Second hypothesis: The cell loading density will limit oxygen production once the silica gel matrix becomes too crowded and the amount of light reaching the inner layer of the gel is negligible.

Methods

Approach

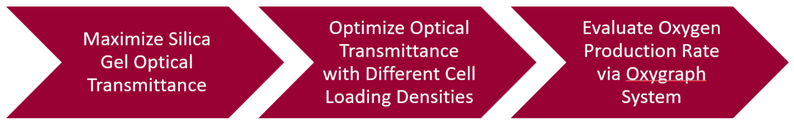

Figure 1. Experimental Approach.
The study was performed in a three-phase process. First, the optical transmittance of the silica gels was maximized by changing gel composition and characterization via UV-Vis spectrophotometry. The gel composition was changed by varying the Nanoparticle:Alkoxide crosslinker ratio [v/v] and the % Phosphate Buffered Saline (PBS) added. Two sizes of nanoparticles were tested: LUDOX HS-40, 12 nm in diameter, and LUDOX TM-40, 22 nm in diameter. Next, the optical transmittance was optimized while encapsulating different concentrations of cyanobacteria. Finally, the oxygen production rate of the optimized gels was evaluated with an Oxygraph System.

Experimental Setup & Procedure

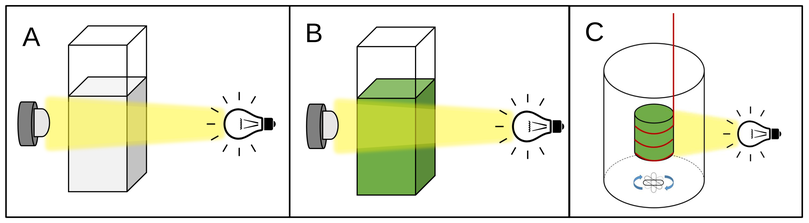

Figure 2. Setup illustrations for each of the three phases.
(A) Maximization of silica gel optical transmittance was performed using a UV-Vis Spectrophotometer. 2 ml gels were made in cuvettes then measured in the spectrophotometer after 1 hour of gelation time. Nanoparticle:Alkoxide ratios tested were 1:1, 1:3, 1:5, 1:7, and 1:9.
(B) Silica gel optical transmittance was optimized with different cell loading densities using a setup similar to the first phase. 2 ml gels were made in cuvettes then measured in the spectrophotometer after 1 hour of gelation time. The cyanobacteria concentrations tested were 0.01, 0.005, 0.001, 0.0005, and 0.0001 g/ml.
(C) Oxygen production rates for the optimized encapsulated cells were measured with an Oxygraph System. Silica gel encapsulated cyanobacteria "plugs" were made in a 96-well plate with a wire scaffold. After 1 hour of gelation time (in light), plugs were placed into BG-11 to initiate activity. After 1 hour storage in BG-11, plugs were inserted into the Oxygraph System where light induced oxygen production was measured.

Results

Experimental Setup (cont.)

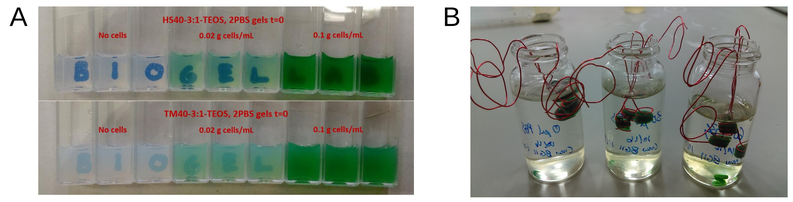

Figure 3. Images of silica gel encapsulated cyanobacteria.
(A) Example of silica gels (with and without cyanobacteria) made in cuvettes for optical transmittance measurement purpose. **Note: The samples shown do not constitute the data in the results.
(B) Silica gel encapsulated cyanobacteria "plugs." After 1 hour of gelation, plugs were stored in vials with BG-11 to initiate activity. Vials were held within a light chamber that followed a diurnal cycle.

Maximization of Silica Gel Optical Transmittance

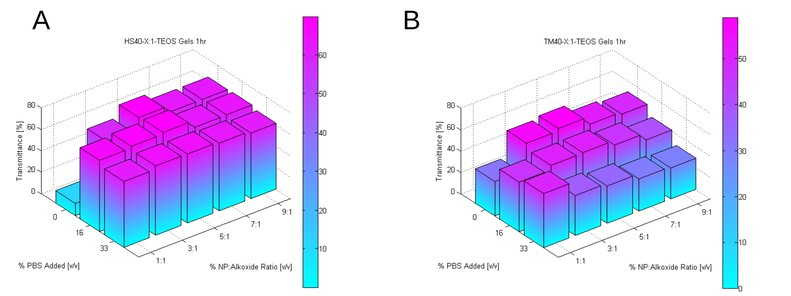

Figure 4. Optical Transmittance for Various Gel Compositions.
Optical transmittance characterization for silica gels (no cells) after 1 hour of gelation time. (A) Displays the HS40 gels and (B) displays the TM40 gels. The Nanoparticle:Alkoxide ratios [v/v] tested were 1:1, 3:1, 5:1, 7:1, and 9:1. For each ratio, gels made of 0%, 16%, and 33% added PBS [v/v] were tested. A colorbar illustrates the transmittance value for each sample.

Optimization with Different Cell Loading Densities

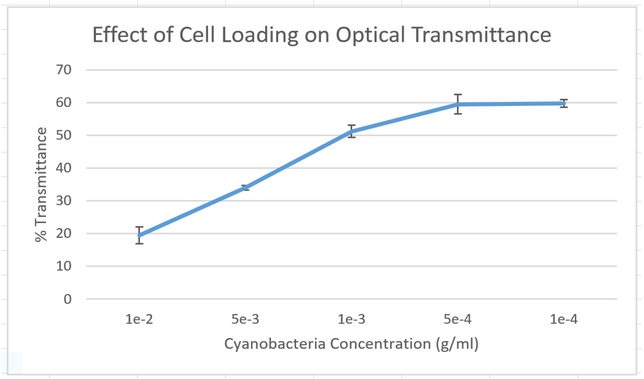

Figure 5. Optimization of silica gel optical transmittance for various cell loading densities. The selected gel was HS40-7:1-TEOS with 33% PBS. Cyanobacteria concentrations tested were 0.01, 0.005, 0.001, 0.0005, and 0.0001 g/ml. Bars illustrate standard deviation. For each concentrations, n = 3.

Results (cont.)

Evaluation of Oxygen Production Rates

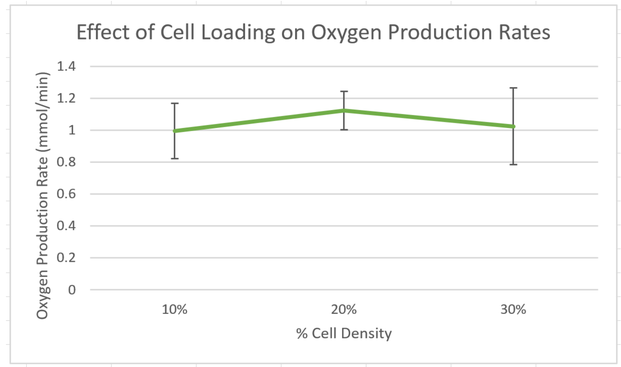

Figure 6. Evaluation of oxygen production rates for selected silica gels. The selected gel was HS40-3:1-TEOS with 16% PBS. Cell densities tested were 10%, 20%, and 30% cyanobacteria [v/v]. Bars illustrate standard deviation. For each of the cell densities, n = 3.

Conclusions

Major Findings

- A HS40-3:1-TEOS silica gel was fabricated to optimize oxygen production rates.
- As the first hypothesis suggests, the oxygen production rates of the encapsulated cyanobacteria increased with respect to the optical transmittance of the gel. HS-40 gels showed better transmittance than TM-40 gels.
- As the second hypothesis suggests, the cell loading density limits oxygen production once the matrix becomes too crowded at values greater than 20% (additional samples need for statistical verification).

Future Work

- Co-encapsulation of cyanobacteria with NCIB, a bacteria with enzymes useful in toxic wastewater remediation, would allow for a constant oxygen source, which is required for efficient treatment of hydrocarbon containing wastewater.
- A layered-system or core-shell design could be used to optimize oxygen and hydrocarbon transport between the two types of bacteria.

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References:
[1]Dickson DJ, Ely RL. "Silica sol-gel encapsulation of cyanobacteria: lessons for academic and applied research." Appl Microbiol Biotechnol 97 (2013):1809-1819