Effects of Oxygen on Heliobacterial Photosynthesis

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ABSTRACT

Heliobacteria are a group of strictly anaerobic photosynthetic bacteria known for their unique production of bacteriochlorophyll g. Interestingly, bacteriochlorophyll g converts to chlorophyll a when exposed to continuous light and oxygen, although it is stable under short-term exposure. In this work, I studied the effects of oxygen on photosynthesis in the heliobacteria. In particular, I focused on P800 photooxidation, the first step of photosynthesis in heliobacteria. When exposed to continuous light under anoxic conditions, P800 photooxidation shows multi-phasic kinetics wherein a rapid initial bleaching reaches a maximum at 20-30 ms, followed by a slower recovery until a steady-state is reached. 50-60% of the initial bleaching is recovered, suggesting that a significant portion of P800 remains inactive during photosynthesis. When cells were suspended in oxic buffer, the recovery phase was eliminated, consistent with oxygen inhibiting secondary photosynthetic reactions. The oxic buffer effect allowed the estimation of the total amount of P800 in our samples. When combined with results under anoxic conditions that are reflective of the organism's natural state, we determined that a mere 6.7% of P800 is oxidized during normal photosynthetic conditions; this is in stark contrast to all other known photosynthetic systems where the entire primary donor pool is oxidized.

Methodology

In order to observe the effects of inhibitors on the light recovery of *Heliomicrobium modesticaldum,* the proper preparation was crucial. ATCC medium: 2061 PYE Medium was prepared with the addition of sodium sulfide, titrated to 7.0 pH within the anaerobic chamber for optimal cell growth. The cultures were inoculated through the use of the flame based portion of Hungate's technique to remove any possible contamination. Tests were performed 24 hours after inoculation in order to catch the end of the exponential growth phase. One milliliter of the culture was removed, spun down for 15 minutes in the centrifuge, then resuspended in one milliliter of MOPS buffer and added to another two milliliter of MOPS buffer in a glass cuvette. Each sample examined followed this protocol, performed anaerobically within the chamber. A UV-Spectra was collected utilizing the UV-Spectrometer to image the optical density, as well as to ensure that chlorophyll *a* was not present. Then the cells were observed employing the use of the JTS-10 spectrometer to view the bleaching effect and recovery to the steady state. When observing the effects of oxic buffer, oxic-MOPS was prepared outside the chamber in an aerobic setting and moved into the chamber. Cells were then resuspended in this buffer in place of the anaerobic MOPS buffer. These tests were performed in triplicate to receive the most reliable data. To evaluate the effects of actinic light intensity, this same procedure was followed while altering the intensity of the light on the JTS-10 spectrometer. A full range of intensities was taken for each sample, anoxic and oxic, using red and orange actinic light. Data was fit using the equation $B = (B_{max} I) / (K_{50} + I)$, where B is the max bleaching, B_{max} is the max bleaching under saturating light intensities, I is the light intensity, and K_{50} is the light intensity producing half-saturation.

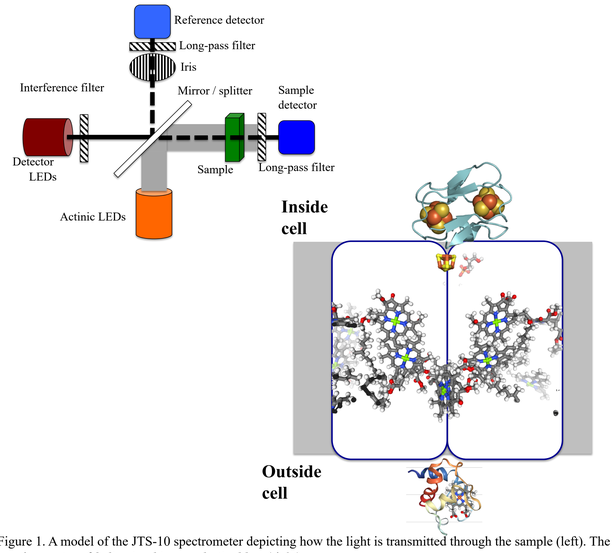

Figure 1. A model of the JTS-10 spectrometer depicting how the light is transmitted through the sample (left). The reaction center of *heliomicrobium modesticaldum* (right).

RESULTS

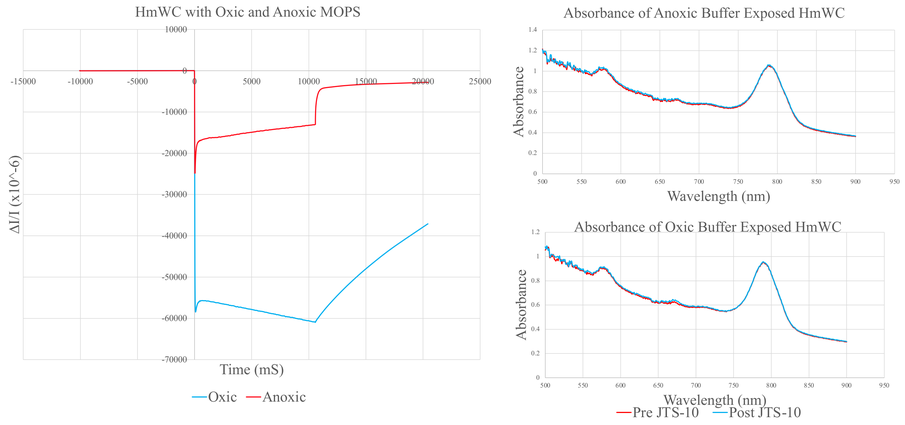

Figure 2. Light-induced absorbance changes of *H. modesticaldum* whole cells prepared with anoxic and oxic MOPS (left). Actinic illumination, provided by red LEDs centered at 720 nm, is indicated by the arrows. 10 µs detection pulses were emitted every 50 µs – 100 ms by near IR LEDs with an 800 nm interference filter. Spectra was taken pre and post JTS-10 readings for each sample (right).

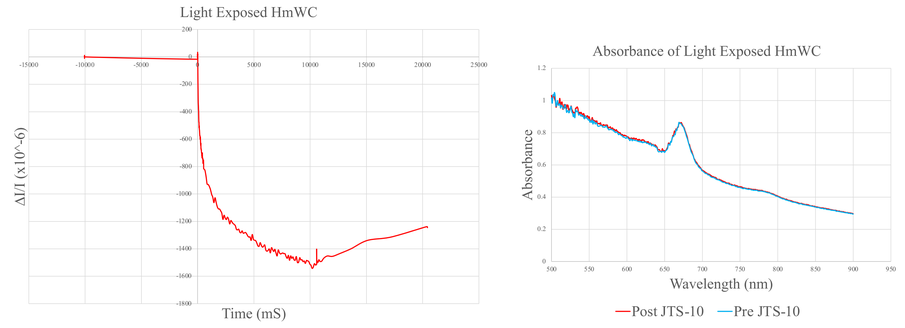

Figure 3. Light-induced absorbance changes of *H. modesticaldum* whole cells prepared with Oxic MOPS buffer and light for approx. 30 minutes (left). Actinic illumination, provided by red LEDs centered at 720 nm, is indicated by the arrows. 10 µs detection pulses were emitted every 50 µs – 100 ms by near IR LEDs centered at 810 nm. Spectra was taken pre and post JTS-10 readings for each sample (right).

Table 1. The average of the percent of P800 oxidized optimally (infinite) and the percent of P800 oxidized normally ± the standard deviation for red and orange light trials calculated using the Bmax's found using the Michaelis-Menton equation, while adjusting for minimum sum of squared residuals and the average of the collected steady-state values.

	Red Light	Orange Light
% of P800 Oxidized Under Steady-State Illumination	6.5 ± 2.5%	8.58 ± 0.69%
% of P800 Oxidized Initially at Light Saturation	68.7 ± 2.6%	28.3 ± 2.1%

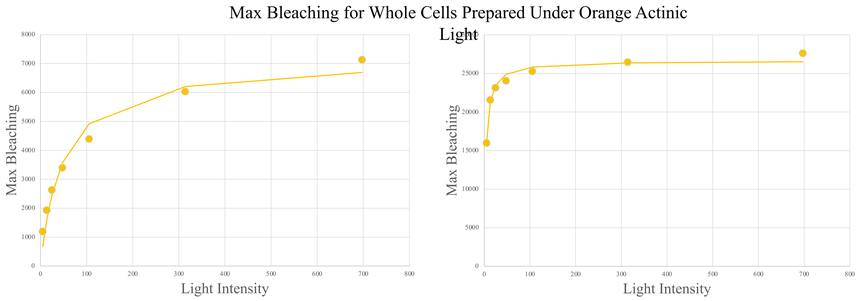

Figure 4. The effect of light intensity on the maximum observed bleaching for samples illuminated with orange prepared under anoxic (left) and oxic (right) conditions. Actinic illumination was provided by orange LEDs (left) centered at 630 nm. Data was fit using the equation $B = (B_{max} I) / (K_{50} + I)$, where B is the max bleaching, B_{max} is the max bleaching under saturating light intensities, I is the light intensity, and K_{50} is the light intensity producing half-saturation. In these samples, under orange light B_{max} = 7149.7 and K_{50} = 47.7 and under red light B_{max} = 8289.2 and K_{50} = 207.2.

Table 2. Average of the K_{50} values ± standard deviation calculated for data collected under anoxic and oxic conditions using orange light. Values were based off the Michaelis-Menton equation, while adjusting for the minimum sum of squared residuals.

	Orange Light / Anoxic	Orange Light / Oxic
K_{50} (µE m^{-2} s^{-1})	138 ± 30	4.5 ± 1.6

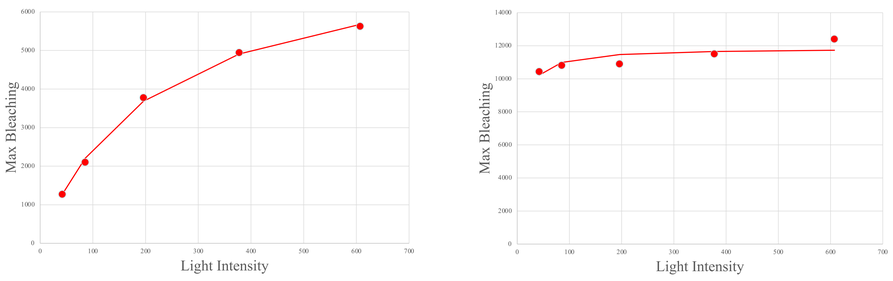

Figure 4. The effect of light intensity on the maximum observed bleaching for samples illuminated with red actinic prepared under anoxic (left) and oxic (right) conditions. Actinic illumination was provided by red LEDs (right) centered at 720 nm. Data was fit using the equation $B = (B_{max} I) / (K_{50} + I)$, where B is the max bleaching, B_{max} is the max bleaching under saturating light intensities, I is the light intensity, and K_{50} is the light intensity producing half-saturation. In these samples, under orange light B_{max} = 26654.04 and K_{50} = 3.4 and under red light B_{max} = 11859.0 and K_{50} = 6.8.

Table 3. Average of the K_{50} values ± standard deviation calculated for data collected under anoxic and oxic conditions using red light. Values were based off the Michaelis-Menton equation, while adjusting for the minimum sum of squared residuals.

	Red Light/ Anoxic	Red Light/ Oxic
K_{50} (µE m^{-2} s^{-1})	243 ± 35	10.1 ± 9.4

REFERENCES

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FUTURE DIRECTIONS

1. Explore the effects of other charged oxidants on the photo-oxidation of P800.
2. Repeat procedure to produce a light curve from red and orange actinic light sources on the same sample to compare and contrast the kinetics.
3. Oxygen curves to determine what affect different amounts of oxygen in the sample has.