Photosynthetica navigatio: Exploring the relation between chloroplast translocation and oxygen evolution in the Chlorophyte *Eremosphaera viridis*[1].

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OBJECTIVE

To determine whether there is a correlation between oxygen evolution and chloroplast translocation at different light intensities in the acidophilic algae *Eremosphaera viridis*. In addition, we explored the effect of pH on the initial rates of oxygen evolution.

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INTRODUCTION

Photosynthesis is a unique biochemical process performed by algae, plants, and some species of bacteria (Lawlor, 2001). The thylakoid vesicles, found in the inner membrane of the chloroplast, are responsible for converting light energy into chemical energy. The chemical energy is used to synthesize carbohydrates and other organic compounds, which heterotrophic organisms use for energy and other nutritional requirements. The phenomenon of photosynthesis can be subdivided into light dependent and light independent reactions. The basic chemical reactions are illustrated below (Figure 1):

For the light dependent reactions:

$$12\ H_2O + 12\ NADP^+ + 18\ ADP + 18\ P_i + \text{Light Energy} \rightarrow 6\ O_2 + 12\ NADPH + 18\ ATP$$

For the light independent reactions:

$$12\ NADPH + 18\ ATP + 6\ CO_2 \rightarrow (CH_2O)_6 + 12\ NADP^+ + 18\ ADP + 18\ P_i + 6\ H_2O$$

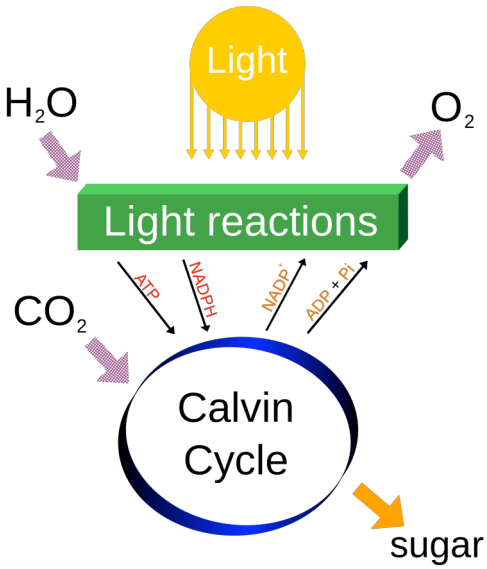


Figure 1. A diagrammatic representation of the major events in oxygenic photosynthesis.
Light is harnessed to produce both ATP and the reduced form of NADP. The electrons required for reduction are obtained from water, creating oxygen (O_2) as a byproduct. The NADPH and ATP are used to regenerate Ribulose 1,5 – bisphosphate (RuBP) in the Calvin cycle as it is used in carbon dioxide (CO_2) fixation to produce carbohydrate sugar $(CH_2O)_6$.

The diagram (by Daniel Mayer) was obtained from http://en.wikipedia.org/wiki/Photosynthesis (Accessed 22 august 2012)

Light travels in packets of energy, photons, which are an integral part in the process of photosynthesis. The sun emits electromagnetic radiation that enters the earth atmosphere. Solar irradiance varies as a function of wavelength with peak irradiance occurring between 400 nm and 500 nm (Figure 2). For photosynthetic purposes, organisms absorb light in the wavelength range of 400 to 800 nm (Nobel, 1991).

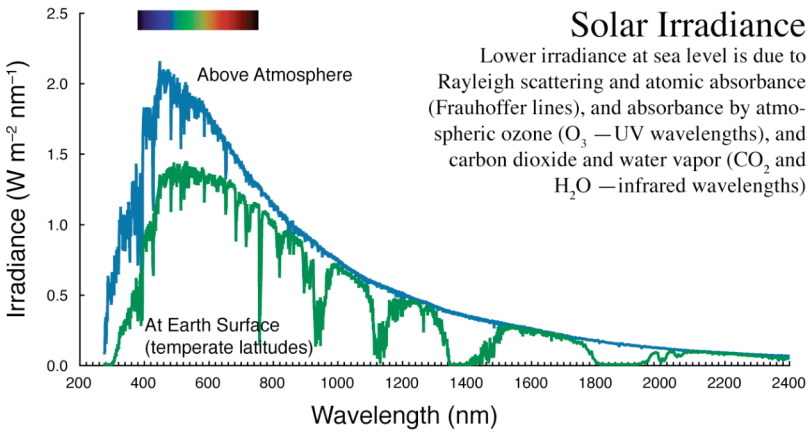


Figure 2. Solar irradiance *versus* wavelength. The data were obtained from http://rredc.nrel.gov/solar/spectra/am1.5/. Solar irradiation above the atmosphere and at sea level are shown.

The process of photosynthesis is initiated when a photosynthetic pigment, found in the chloroplast, absorbs a photon of light. Chlorophyll molecules, the most common photosynthetic pigment, are spread out around a specialized protein complex called the reaction center. As soon as a chlorophyll molecule absorbs light, the energy is transferred to nearby chlorophyll molecules. The energy is passed among chlorophyll molecules that collectively comprise the antenna pigments until it arrives at the reaction center. Once the energy arrives at the reaction center, in the form of an excited electron, ATP is produced and NADP is reduced to NADPH as the excited electron travels through the electron transport chain. To continue photosynthesis, electrons are supplied from water, releasing oxygen as a byproduct.

As the light intensity increases, the generation of excited state chlorophylls increases. This will cause more frequent downstream electron transport, which could lead to the formation of harmful photo-oxidative products. Some examples of these damaging products include: triplet state chlorophyll molecules and various reactive oxygen species (through direct reduction of O_2 to O_2^-, and subsequent formation of H_2O_2). It is believed that chloroplast translocation is used by cells to protect themselves from photo-oxidative damage.

Our research objective was to determine if there exists a correlation between light intensity and organelle translocation. To do so, we explored whether systrophe and the rate of oxygen evolution were linked to each other.

We also tested whether pH had an effect on the initial rate of oxygen evolution to compare with the growth dependence on pH determined by Khine and Lew (2010).

MATERIALS AND METHODS

Strains. The alga *Eremosphaera viridis* de Bary (CPCC 127) was obtained from the Canadian Phycological Culture Centre (CPCC, University of Waterloo, Waterloo, Canada; formerly UTCC [Acreman, 2004]). The CPCC 127 strain was isolated in 1987 from Plastic Lake, Ontario; a small lake that had a pH of 5.8 in 1981 and continued to acidify in subsequent years (at a rate of 0.035 pH units yr^{-1}) (Dillon et al., 1987). The species is commonly considered to be an acidophile —optimal growth of the CPCC 127 strain occurs at pH 5 to 6 (Khine and Lew, 2010). The cells are spherical, and large (100–120 μm in diameter).

The cultures were grown in 250 ml Ehrlenmeyer flasks mounted on an orbital shaker (125 rpm) under 50 μmol m^{-2} s^{-1} photon flux from T8 fluorescent lamps (6500 K color temperature) at room temperature (20–24 ºC) with serial transfer every 5–10 days. The culture media was Bold's Basal Medium (BBM, http://www.phycol.ca/media [Nichols and Bold, 1965]) supplemented with vitamins (Thiamine-HCl, 0.5 μg/ml; vitamin B_{12} 0.01 μg/ ml; and biotin 0.005 μg/ml). The major ions in Bold's are (in meq l^{-1}): Na^+ (5.96), Cl^- (3.17), NO_3^- (2.94), K^+ (2.26), P_i (1.72), SO_4^{2-} (0.32), Mg^{2+} (0.30), and Ca^{2+} (0.17); pH is *ca* 5.8 (Table I).

Table I: Bold's basal medium composition.

Salt (stock)	MW	Dilution	Final concentration
KH_2PO_4 (1.75 g/100 ml)	136.09	0.01	1.29 mM
$CaCl_2 \cdot 2H_2O$ (0.25 g/100 ml)	147	0.01	0.17
$MgSO_4 \cdot 7H_2O$ (0.75 g/100 ml)	246.5	0.01	0.30
$NaNO_3$ (2.5 g/100 ml)	84.99	0.01	2.94
K_2HPO_4 (0.75 g/100 ml)	174.2	0.01	0.43
NaCl (1.75 g/100 ml)	58.44	0.01	2.99
$Na_2EDTA \cdot 2H_2O$ (1.0 g/100 ml) KOH (0.62 g/100 ml)	372.24 56.11	0.001	0.027 0.11
$FeSO_4 \cdot 7H_2O$ (0.498 g/100 ml) H_2SO_4 (conc. 18 M) (0.1 ml/100 ml)	278.0 98.08	0.001	0.018 0.00018
Trace Metal Solution	*see below*	0.001	*see below*
H_3BO_3 (1.15 g/100 ml)	68.13	0.0007	0.012
Vitamins		0.000025	
Trace Metals (g/100 ml, *MW*, final concentration [**µM**]): H_3BO_4 (0.286, *68.13*, **42.0**), $MnCl_2 \cdot 4H_2O$ (0.181, *197.92*, **9.1**), $ZnSO_4 \cdot 7H_2O$ (0.0222, *287.54*, **0.77**), $Na_2MoO_4 \cdot 2H_2O$ (0.039, *241.95*, **1.6**), $CuSO_4 \cdot 5H_2O$ (0.0079, *249.7*, **0.31**), $Co(NO_3)_2 \cdot 6H_2O$ (0.00494, *291.03*, **0.17**)			
Vitamins (g/10 ml): Thiamine•HCl (0.1), Vitamin B_{12} (0.002), Biotin (0.001)			

Culture Preparation for Oxygen Electrode Systrophe Experiments. Cell suspensions (5 mL) from 1–2 week old cultures were transferred to a 15 ml plastic tube and bubbled with N_2 (to deplete oxygen in preparation for oxygen evolution measurements) for 1 minute. An aliquot (3 mL) of the cells was transferred to the oxygen electrode chamber, where they were well-stirred with a magnetic flea.

Oxygen Electrode Systrophe Experiments. The oxygen electrode apparatus was from Hansatech (DW1 oxygen electrode chamber and CB1-D3 electrode control unit; Hansatech Instruments Ltd., Norfolk, England). The output was calibrated twice before and twice after each experimental run with either dH_2O or Bold's medium at 'zero' and air-saturated oxygen levels (270 μM at 23 ºC). During the calibrations, air-saturated oxygen was depleted from the oxygen electrode chamber solution using sodium dithionate ($Na_2O_4S_2$). All measurements were performed at 23ºC. The temperature was maintained constant using a circulating water bath. Output was recorded on a Kipp and Zonen chart recorder (model BD 11E; Kipp & Zonen, Delft, Netherlands).

Light was provided from a 50 W tungsten-halogen lamp powered by a variable DC power supply to control light intensity. The lamp output was focused onto the oxygen electrode chamber with a collimating lens. A 3.5% (w/v) cupric sulfate ($CuSO_4•5H_2O$) filter (1.4 cm path-length) was placed between the collector lens and chamber (Figure 1). The filter was used to selectively irradiate the oxygen electrode chamber with photosynthetically active light, blocking UV and infrared light. Photon flux was between about 450 and 630 nm (maximal flux at 560 nm). The light intensity was measured with a radiometric probe (Model 268R with an internal radiometric filter [maximal light responsivity between 400 and 1000 nm] attached to a Model S471 portable optometer; UDT Instruments, San Diego, California, USA). The probe was placed on the side of the oxygen electrode chamber opposite from the tungsten-halogen lamp. The light intensity of the lamp was measured a few seconds after the lamp was turned on.

Figure 3. Illumination set-up for oxygen electrode experiments. The cell suspension was placed in the oxygen electrode chamber, stirred vigorously at 23 degrees Celsius. The light was focused on the cell suspension with a collimating lense and filtered with a cupric sulfate solution to remove ultraviolet and infrared light.

A few minutes after the lamp was turned on, 50 to 450 μL of 100 mM $NaHCO_3$ in Bold's medium were added to the cell suspension in the oxygen electrode chamber in order to stimulate oxygen production. During light treatments, four aliquots (2.5, 5, or 10 µl) of the cell suspension were removed using a positive displacement micropipette to obtain the percentage of cells that experienced systrophe (chloroplast translocation to the center of the cells) (Lew, 2010) relative to the total number of cells in the aliquot. This procedure was repeated four to seven times throughout each experimental run and the cells in each aliquot were counted once or twice. The oxygen electrode chamber was draped with a light-tight cloth for dark treatments.

Culture Preparation for pH Dependence Experiments. Cell suspensions (5 mL) from 1–2 week old cultures were transferred to a 15 ml plastic tube. The test tube was placed

vertically in a stationary position. Due to the size and high specific density of the cells, they settled to the bottom of the plastic test tube after a few minutes. The supernatant was removed and 6 mL of a buffer solution (0.1 mM KNO_3, 0.1 mM $MgSO_4 \cdot 7H_2O$, 1.0 mM $CaCl_2 \cdot 2H_2O$, 2.0 mM NaCl and 10 mM Buffer, Table II) was added to the cells. Buffers were selected on the basis of having a pK_a near the desired pH for the solution. The pH of the buffer solutions were adjusted using 5 N NaOH. After addition of the buffer solution, the cells were allowed to settle to the bottom, the supernatant decanted, and 6 mL of the same buffer solution were added to the cells and when the cells settled to the bottom, the supernatant was removed. Then, 6 mL of the buffer solution were added to the cells and the solution was bubbled with N_2 (to deplete oxygen in preparation for oxygen evolution measurements) for 1 minute. An aliquot (3 mL) of the cells was transferred to the oxygen electrode chamber, and stirred with a magnetic flea.

Table II: Buffer Solutions for pH Dependence Experiments.

Buffer	pH	pKa	Molecular Weight (g/mole)	100 mM (g/100 mL)	Volume of NaOH added to adjust pH (µL)
Phthalic acid	3	2.95	166.13	1.66	10
Phthalic acid	4	2.95	166.13	1.66	50
MES	5	6.15	195.23	1.95	50
MES	6	6.15	195.23	1.95	150
MOPS	7	7.2	209.26	2.09	299
TES	8	7.5	229.25	2.29	337
TAPS	9	8.4	243.20	2.43	387

Oxygen Electrode pH Dependence Experiments. The set up and procedure performed for these experiments were the same as the protocol followed for the systrophe experiments. However, there were minor adjustments. A few minutes after the lamp was turned on, 150 μL of 100 mM $NaHCO_3$ in Bold's medium were added to the cell suspension in the oxygen electrode chamber in order to stimulate oxygen production. Light treatments were performed at a photon flux of 1000 µmol m^{-1} s^{-1}. Four aliquots (5 µl) of the cells suspension were removed using a positive displacement micropipette to obtain cell densities. This procedure was repeated three times throughout each experimental run and the cells in each aliquot were counted once or twice. The oxygen electrode chamber was draped with a light-tight cloth for dark treatments. After each experiments, the pH of the cell suspension was determined (since the addition of $NaHCO_3$ caused the pH to become more alkaline, especially at acid pH). Results are plotted versus the final pH.

RESULTS

Light dependence of oxygen evolution and systrophe at various light intensities. The cells were irradiated at three different photon fluxes to determine the effect that light intensity has on chloroplast translocation and oxygen evolution. As the photon flux increased, the percentage of cells exhibiting systrophe increased, the same trend was seen for oxygen evolution (increasing with increased light intensity), however not as pronounced (Figure 4).

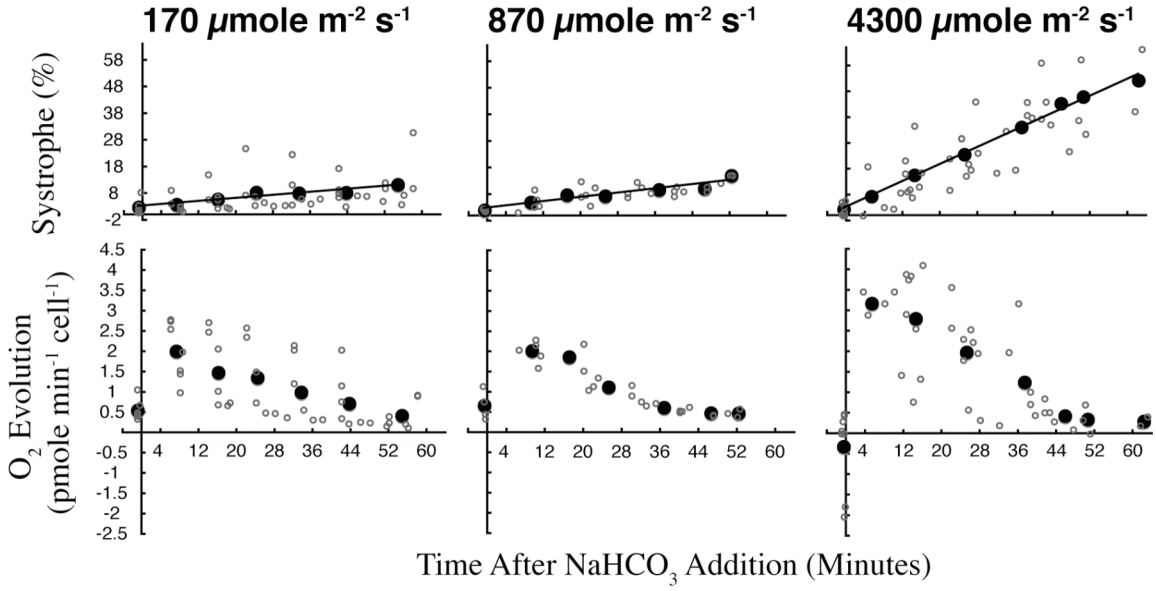


Figure 4: Percent systrophe (chloroplast translocation to the center of the cell) and rate of oxygen evolution as a function of time at various light intensities in *Eremosphaera viridis*. The O_2 evolution rates are similar to those reported by Lew (2011), which we calculated to be 1.1 pmole min^{-1} $cell^{-1}$ based on O_2 evolution rates reported by Lew (2011) at a photon flux of 400 nmole m^{-2} s^{-1} and assuming a cell diameter of 120 μm. The cells were grown at about 50 μmole m^{-2} s^{-1} irradiance in Bold's medium and transferred to the oxygen electrode chamber after bubbling with N_2 gas. Systrophe and oxygen evolution rates were measured at the times shown, after the addition of $NaHCO_3$. Small circles are individual measurement; large filled circles are means. Note that even at a very high light intensity, oxygen rates are similar to those at lower light intensities, even though systrophe is quite significant.

The Relation Between Oxygen Evolution and Bicarbonate Addition. The ratio of total amount of oxygen produced to the quantity of sodium bicarbonate added (to supply CO_2 for photosynthesis) was independent of light intensity (Figure 5). A two-tailed t-test was performed among each photon flux and the p-value was found to be greater than 0.05 demonstrating a non-significant variation between the three different light intensities.

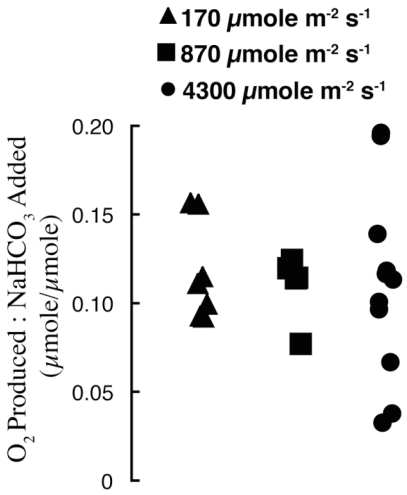


Figure 5: Ratio of total oxygen produced to the amount of added $NaHCO_3$. Data were collected at three different light intensities as described in the materials and methods section. Statistically, the data for the three light intensities does not show a significant variation ($p>0.05$).

The Relation Between Initial Rate of Oxygen Evolution and pH. There is a parabolic relationship between the rate of oxygen evolution and the pH of the cell suspension (Figure 6). The cells experienced the highest rate at a pH of 6.8 (Figure 6) declining at more acid (pH 5.8) and alkaline (pH 8.4) conditions. Even though results were collected The results for pH 4.9 and 5.3 are not shown on Figure 6 due to unusual results (Figure 7).

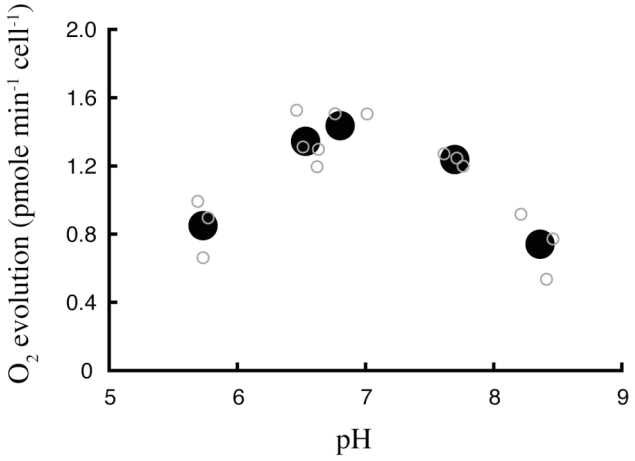
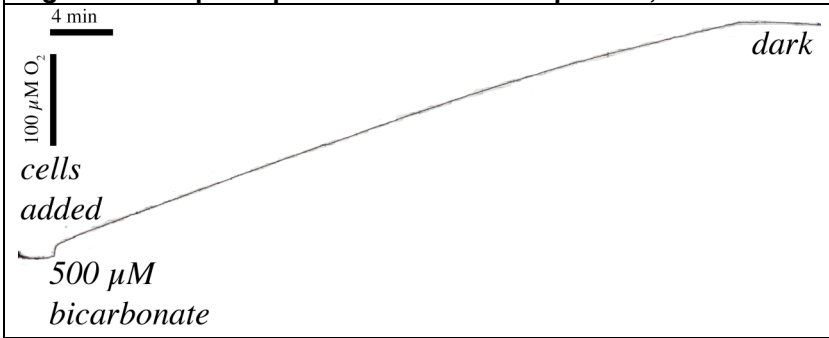


Figure 6: Initial rate of oxygen evolution as a function of pH. The relationship between these two variables appears to be parabolic between the pHs of 5.8 and 8.4. The highest rate of oxygen evolution occurs at a pH of 6.8. All pH measurements were performed after 150 μL of sodium bicarbonate was added to the buffer solution containing the cell culture. The data collected for pHs 4.9 and 5.3 was omitted from the figure due to unexpected experimental results (Figure 7). Small circles are individual measurement; large filled circles are means.

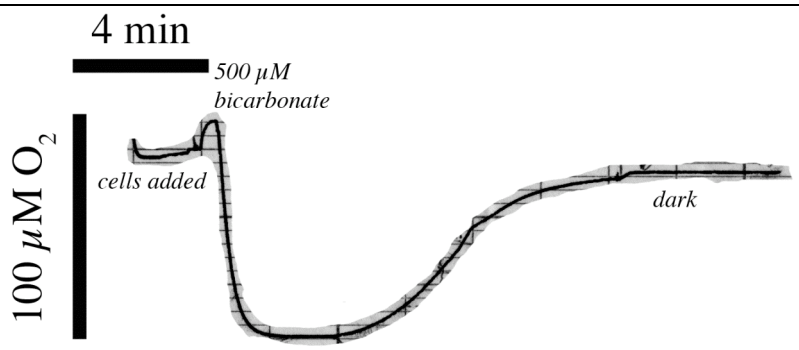
Rate of oxygen evolution at pH 5.3 and 4.9. At a pH of 6.6, a constant initial rate of oxygen evolution is observed (Figure 7a) after the addition of 150 μL of $NaHCO_3$. This type of response was observed in other replicate experiments at this pH, and at 5.8, 6.8, 7.7 and 8.4. At a pH of 5.3, oxygen was apparently consumed immediately after the addition of 500 μM of $NaHCO_3$ (Figure 7b), and then increased rapidly before quickly leveling off. Similar results were seen in all three replicates. We decided to omit this result from Figure 6 due to the inconsistency. The magnitude of the initial rate of oxygen production also fluctuated significantly during the three experiments performed at a pH of 5.3. Oxygen consumption after the addition of $NaHCO_3$ appeared inaccurate as the cell cultures had all the necessary components to initiate the process of photosynthesis, and thus produce O_2. At a pH of 4.9 the initial rate of oxygen evolution was constant but was

very steep and quickly leveled off (Figure 7c). The three replicates at this pH also showed a lot of variability and therefore they are not included in the data shown in Figure 6. Both of these results occurred when Phthalic acid was used as the buffer, which may be the cause of the anomaly.

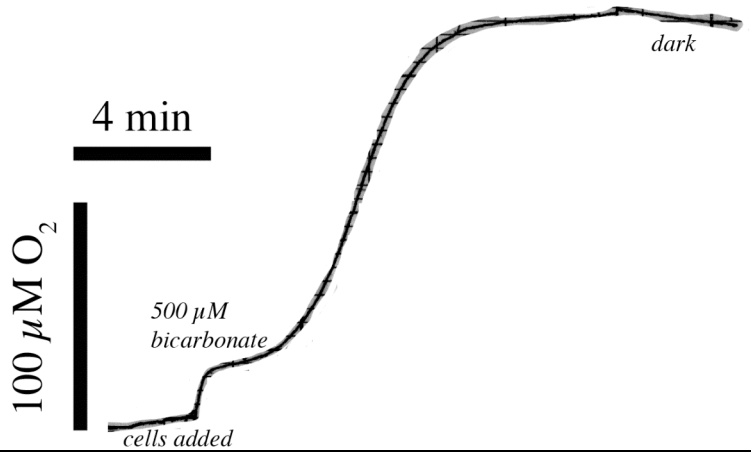
Figure 7: Sample experimental results for pHs 6.6, 5.3 and 4.9.



a) At pH 6.6, after addition of the bicarbonate, there was a long and steady evolution of oxygen. Similar results were obtained at pH 5.7, 6.8, 7.7 and 8.4.



b) At a pH of 5.3 (with phthalic acid buffer), the addition of bicarbonate caused an apparent consumption of oxygen, followed by some oxygen evolution, which rapidly ceased.



c) At a pH of 4.9 (with phthalic acid buffer), the addition of bicarbonate caused a rapid increase in oxygen evolution, which ceased after a short period of time.

DISCUSSION

The research described in this report is an extension of past research on the relationship between light intensity and chloroplast translocation towards the center of the cell (systrophe) (Gasumova, Moscaritolo and Lew, 2009). The work by Gasumova et al. confirmed previous reports on systrophe in *Eremosphaera viridis* (Weidinger, 1980, 1982, Weidinger and Ruppel, 1985). To explore the question whether systrophe is responsible for a decline in oxygen evolution, because of a decrease in the cross-sectional area available for light absorption, we examined the relationship between systrophe and the rate of oxygen evolution using the Clark electrode and cell suspensions. Moreover, we explored whether pH had an affect on the initial rate of O_2 evolution, to follow up on pH dependence of growth experiments performed by Khine and Lew (2010).

We found that as the light intensity increased, the percentage of cells that exhibited chloroplast translocation increased significantly (Figure 4). The photosynthetic rate also increased slightly at higher photon fluxes. However, the changes in O_2 evolution at different light intensities were not as pronounced as the systrophe percentages (Figure 4). The two phenomena appear to be unrelated. In *Eremosphaera viridis*, when the chloroplasts translocate to the center of the cell, the efficiency of light absorption should remain virtually unchanged, based on theoretical calculations (Lew, 2010). Therefore, systrophe may play only a small role in alleviating the effect of elevated light intensities on photosynthetic activity (Lew, 2010). We confirmed this experimentally with cell suspensions. It has been hypothesized that systrophe occurs at high light intensities in order to protect the nucleus, by shading it, from UV light that would be present at elevated light intensity from the sun (Lew, 2010). This would be difficult to test, but we could explore it using higher UV intensities by removing the protective cupric sulfate filter in Clark electrode experiments.

The pH dependence experiments showed that the highest rate of photosynthesis occurred at a pH of 6.8 (Figure 6). However, it has been well documented that *E. viridis* inhabits acidic lakes (pH=5.8), and previous work by Khine and Lew (2010) showed that maximal growth occurred at a pH of 5. Therefore, maximal photosynthetic rate and growth are not directly related. We also performed experiments at pHs 4.9 and 5.3 but the results were very different from the oxygen evolution experiments at more alkaline pH. The experiments at both of this pHs were performed using phthalic acid buffer. We believe that the buffer could be the reason for the variability that was seen in the initial rate of O_2 evolution at pHs 4.9 and 5.3.

In summary, we found that the alga *Eremosphaera viridis* experienced an increase in systrophe at higher light intensities but photosynthesis remained relatively constant rate, suggesting that the two phenomena are unrelated. By examining the effect of pH on photosynthesis, we demonstrated that optimal photosynthesis occurs at a pH that is more alkaline than the optimal pH for growth.

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