The effect of acetate on the oxygen production of *Chlamydomonas reinhardtii*

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Abstract

Chlamydomonas reinhardtii is a unicellular green alga used for studying the processes of photosynthesis. *C. reinhardtii* can fix many forms of carbon, including acetate. The effect of acetate on the oxygen production of the wild type strain CC-1690 - wild type mt+ 21 gr was observed by measuring oxygen levels in culture media containing four different concentrations of sodium acetate: 0mM, 10mM, 20mM, and 30mM. The four treatments were exposed to a light intensity of approximately 2550 lux at 25^{o}C for 1 hour. Differences in initial and final oxygen concentrations and cell densities were measured for three replicates of each treatment. The 0mM medium was found to have the largest oxygen production at $5.6\times10^{-6}\pm 7.8\times10^{-7}$ mg/L/cell, while the 10mM, 20mM, and 30mM treatments were found to have similar oxygen productions at $2.66\times10^{-6}\pm 6.37\times10^{-7}$, $2.70\times10^{-6}\pm 6.91\times10^{-7}$, and $3.30\times10^{-6}\pm 4.99\times10^{-7}$ mg/L/cell respectively. A *p*-value of 2.1×10^{-4} was calculated through an analysis of variance and showed a statistical difference in the means of the four treatments. It is suspected that as *C. reinhardtii* used acetate as a carbon source instead of carbon dioxide, the photosynthetic rates in the treatments containing acetate were lowered, resulting in decreased oxygen production levels. We were able to conclude that the presence of acetate has an effect on the oxygen production of *C. reinhardtii*.

Introduction

C. reinhardtii is very abundant and widely distributed in not only every aquatic environment, but also in terrestrial soil (Allaby 2012). The cells are motile with a pair of flagella and are able to derive energy through different processes, including photosynthesis; hence the presence of chloroplasts found within cells (Allaby 2012). The complexity and interconnection of the metabolic network in *C. reinhardtii* suggest that the organism responds to a combination of environmental factors such as alterations to metabolites or nutrient sources available (Gérin *et al.* 2014). Therefore, we predicted that the metabolic process of the glyoxylate cycle, which metabolizes acetate, would have an effect on the processing of inorganic carbon in photosynthesis (Gérin *et al.* 2014).

Previous laboratory studies have revealed *C. reinhardtii's* ability to utilize acetate as a carbon source for growth (Heifetz *et al.* 2000). This suggests that *C. reinhardtii* can experience mixotrophic growth and use either acetate or CO_2 for growth. When these two sources are present however, *C. reinhardtii* cells primarily utilize acetate instead of CO_2 (Boyle and Morgan 2009).

To obtain a better understanding of the effects of acetate on photosynthetic rates of *C. reinhardtii*, we conducted an experiment to determine the amount of oxygen produced in varying concentrations of acetate. The chemical equation of the process of photosynthesis is: $6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$ (Figure 1). Since oxygen is the by-product of photosynthesis, measuring the concentration of oxygen in the medium that *C. reinhardtii* is grown in can provide us with insight on their photosynthetic rate. When acetate is used as an alternative carbon source for heterotrophic growth, sodium hydroxide is a produced as a by-product instead of oxygen (Heifetz *et al.* 2000). This chemical process is depicted in Figure 1.

Our proposed null hypothesis (H_O) was that the presence of acetate concentration will have no effect on the oxygen production of *C. reinhardtii*. We hypothesized (H_A) that the presence of acetate concentration will lead to a change in the oxygen production of *C. reinhardtii*. We predicted that as the concentration of acetate increases, there will be a steady decrease in the oxygen production by *C. reinhardtii.* We also expect the results from our experiment to be consistent with a similar study performed by Heifetz *et al*. (2000), who found that as the acetate concentrations increased, there was a decrease in O_2 production. In performing this study, we were able to explore *C. reinhardtii's* photosynthetic behaviour in sodium acetate specifically and whether the presence of sodium acetate in the natural environment could have a potential impact on marine life forms.

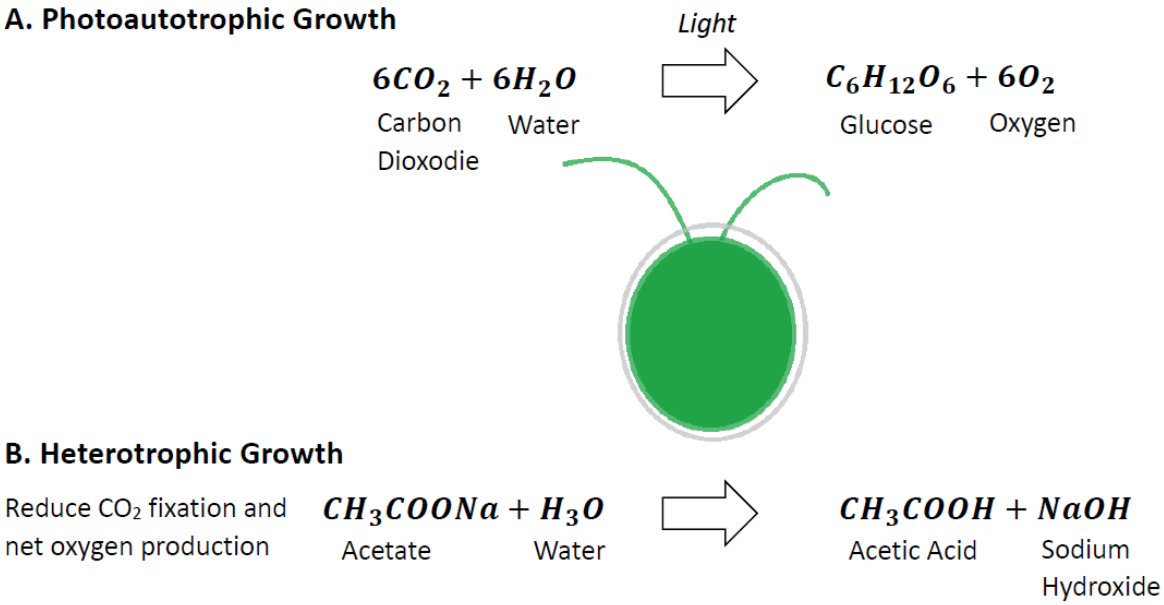

Figure 1. Two carbon-fixing pathways in *C. reinhardtii*. (A) *Photoautotrophic growth:* utilizes carbon dioxide to produce glucose and oxygen. (B) *Heterotrophic growth:* utilizes acetate to produce sodium hydroxide; presence of acetate will result in reduced carbon dioxide fixation and reduced net oxygen production.

Methods

Preparation of cell media

To determine the effects of acetate on photosynthetic rates of *C. reinhardtii*, sodium acetate was added to the culture media in which the *C. reinhardtii* cells were grown. The *C. reinhardtii* cells used for this experiment were the wild type strain CC-1690 - wild type mt+ 21 gr, grown in different concentrations of sodium acetate for a week. The amount of sodium acetate used for photosynthetic measurement and growth rates were discussed by Heifetz *et al.* (2000) and a similar range of concentrations of sodium acetate were used in this study. For this experiment, we tested four different concentrations of sodium acetate in the cell media: 0mM (control), 10mM, 20mM, and 30mM, as seen in Figure 2. We also used a procedural control that consisted of varying concentrations of sodium acetate in the culture media without any cells. For

this experiment, the concentrations of the procedural control that we tested were: 0mM, 10mM, 20mM, and 30mM of sodium acetate.

Figure 2. (From left to right) Cultured *Chlamydomonas reinhardtii* (CC-1690 (WT)) cells in sodium acetate concentrations of 0mM, 10mM, 20mM and 30mM.

Calculation of cell density

Initial cell density was obtained for the 0mM, 10mM, 20mM, and 30mM treatments. We used a haemocytometer to count the cells under a compound microscope and the cell density was calculated using the instructions provided. Three replicates were taken from each concentration and we calculated the average cell density of the three replicates for each concentration. The cell density for each treatment was then diluted to be equal to the cell density of the treatment containing no sodium acetate (0mM): 4.14×10^5 cells/mL.

Measurement of initial oxygen concentration

We separated the four treatments: 0mM, 10mM, 20mM, and 30mM sodium acetate medium into three 27 mL vials, providing three replicates for each treatment. We repeated this

step for the four procedural control treatments of 0mM, 10mM, 20mM, and 30mM sodium acetate. In total, 24 vials were used in this study. The oxygen concentrations were measured by inserting an oxygen probe into the medium of each vial.

Production of oxygen

In order to maintain consistent conditions for this experiment, the temperature was kept at 25 ^{o}C and the light intensity was approximately 2550 lux. A lamp was used as the source of light and the light intensity was measured by a light meter placed directly above the two water baths. Thermometers were also placed in each water bath to monitor the changes in temperature. To obtain the initial measurements of oxygen, we filled the vials with 27 mL of medium and then sealed them with lids. The vials had to be closed properly to prevent air bubbles from forming. This step was repeated as necessary until small air bubbles or no air bubbles were present. After measuring the oxygen concentration in each vial, we placed the vials into the water baths upside down to let the light penetrate through the vials evenly (Figure 3). We placed the vials in one of the water baths at staggered intervals until all 24 vials were in the water baths. We exposed each vial to light for one hour after which time we measured the final oxygen concentration.

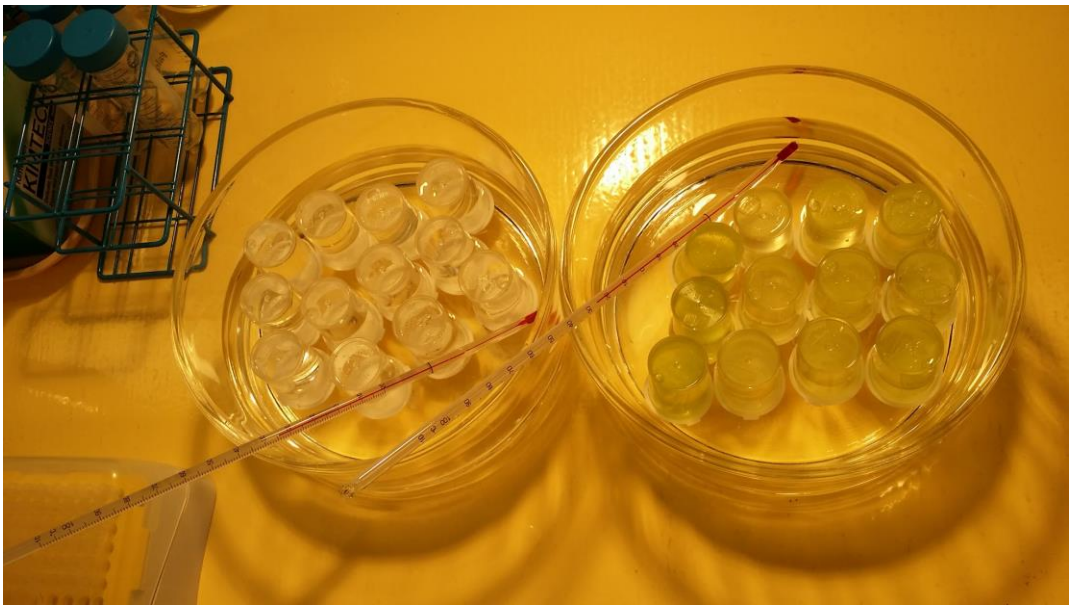

Figure 3. Procedural control (left) and acetate treatment (right) vials in water baths exposed to approximately 2550 lux and monitored at 25 ^{o}C.

Measurement of final cell density

We calculated the final cell density for the four concentrations of sodium acetate medium. We used a haemocytometer to measure cell density for three replicates for each treatment.

Statistical analysis

For our statistical analysis, we calculated means, standard deviations and 95% confidence intervals and used a one-way analysis of variance (ANOVA) test.

Results

The mean oxygen production was calculated per cell and is shown in Figure 4 for each treatment. In the 10mM and 20mM media, the average oxygen productions per cell were observed to be $2.7\times10^{-6}\pm 6.4\times10^{-7}$ and $2.7\times10^{-6}\pm 6.9\times10^{-7}$ mg/L/cell. The 10mM and 20mM treatments were also found to have the lowest oxygen yields among all the treatments. The

oxygen produced in the 30mM medium was slightly higher than the 10mM and 20mM treatments, with an increase of $3.3\times10^{-6}\pm 5.0\times10^{-7}$ mg/L/cell (Figure 4).

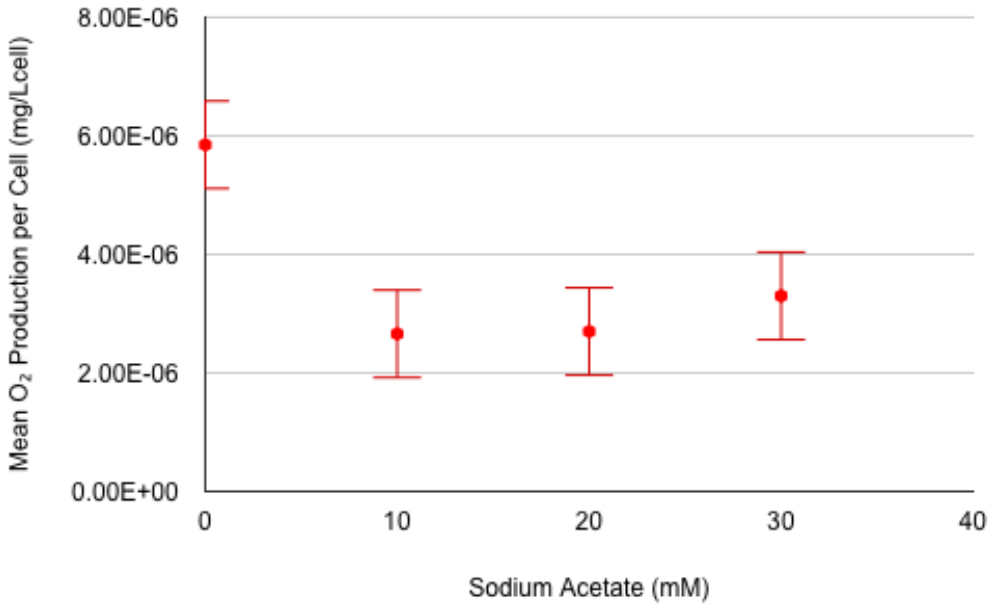

Figure 4. Mean oxygen production per cell (mg/L/cell) of *C. reinhardtii* in varying concentrations of acetate (0mM, 10mM, 20mM, 30mM) after exposure to approximately 2550 lux for 1 hour at 25 °C. Bars represent 95% confidence intervals, n=3.

The oxygen yield for the medium without any acetate (0mM treatment) was measured to be the highest at $5.6\times10^{-6}\pm 7.8\times10^{-7}$ mg/L/cell. Using a one-way ANOVA test, the calculated *p*-value was 2.1×10^{-4}, indicating that there is a significant difference between the mean oxygen production of our treatments (*p*-value < 0.05). The amount of variation in oxygen production in each treatment is also clearly shown in Figure 4, which indicates that there is no overlap among the confidence intervals of the 0mM treatment and the other three treatments. This suggests that the greater oxygen production in our 0mM treatment contributed to the significant difference observed among treatments. However, when comparing the treatments containing acetate (10mM, 20mM, and 30mM), Figure 4 shows that their confidence intervals do overlap,

indicating that the oxygen productions may not have been significantly different among these three treatments.

The smallest 95% confidence interval was calculated to be 5.6×10^{-7} for the 30mM treatment while the largest was 8.8×10^{-7} for the 0mM treatment., i.e., all four treatments have roughly the same 95% confidence interval range.

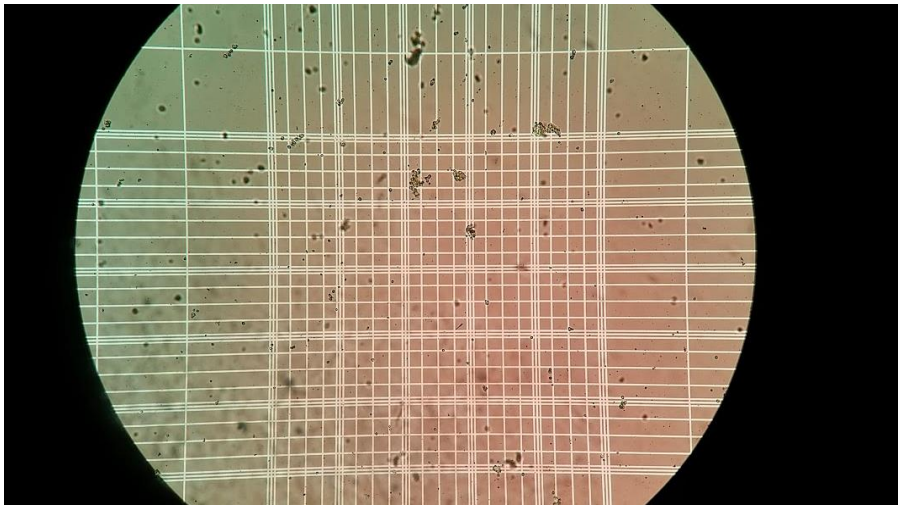

Figure 5. Fixed *C. reinhardtii* cells from the 0mM acetate treatment before dilution on the haemocytometer under 10x objective in an Axio compound microscope. Photo by Ivy Chang.

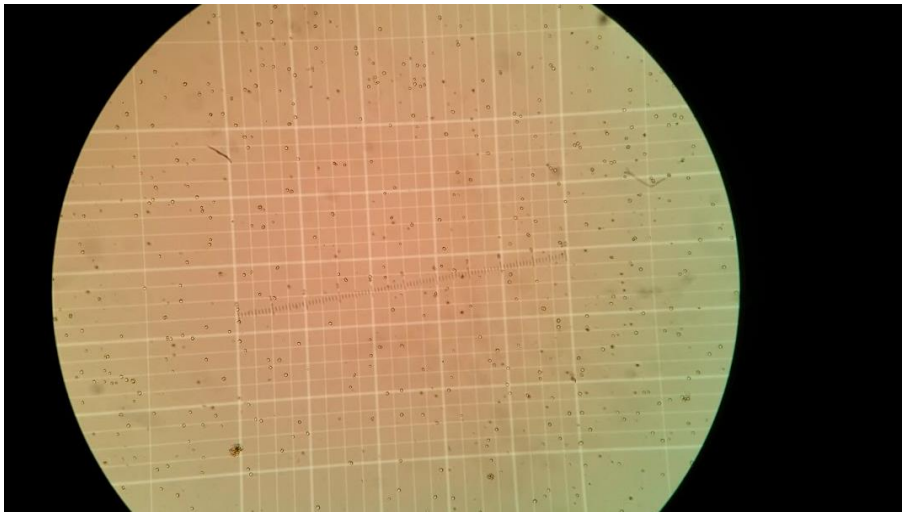

Figure 6. Fixed *C. reinhardtii* cells from the 20mM acetate treatment before dilution on the haemocytometer under 10x objective in an Axio compound microscope. Photo by Ivy Chang.

During the experiment, it was noted that there were fewer cells and less cell movement in the 0mM and 10mM acetate media when compared to the 20mM and 30mM treatments. The

differences in cell abundance can be seen in Figure 5 and Figure 6. These observations of the *C. reinhardtii* cells were made before the dilutions and therefore suggest that the abundance of cells increases with increasing acetate concentrations.

Discussion

The *p*-value obtained in our data analysis was less than 0.05, i.e., there is a significant difference in the oxygen production of our acetate treatments. Hence, we can reject the null hypothesis and provide support for the alternate hypothesis, indicating a correlation between acetate concentration and oxygen production. However, when we only compared the 10mM, 20mM, and 30mM treatments, there was overlap among the 95% confidence intervals, suggesting that there was no significant difference among the means for oxygen production. Thus, we are unable to provide support for our prediction that oxygen concentrations decrease with increasing concentrations of acetate. Unexpectedly, a slight trend of increasing oxygen concentration was observed as the acetate concentration increases.

The utilization of mixotrophic growth in *C. reinhardtii* enables it to assimilate both CO_2 (inorganic) and acetate (organic) carbon sources in the light (Gérin *et al.* 2014; Heifetz *et al.* 2000). In *C. reinhardtii's* metabolic pathway, acetate enters the glyoxylate cycle after being metabolized in the cytoplasm, whereas inorganic carbon is incorporated in the chloroplast (Gérin *et al.* 2014). In Figure 1, we predicted that the presence of acetate would increase acetate fixation and cause a decrease in CO_2 fixation and consequently, a decrease in net oxygen production as well. When comparing the 0mM concentration to any of the other treatments in our experiment, our results were consistent with Heifetz *et al.* (2000). The results showed that were was a significant decrease for both photosynthetic CO_2 fixation and oxygen evolution, supporting the effects of acetate on photosynthesis in *C. reinhardtii* (Heifetz *et al.* 2000).

Initially we had predicted that the oxygen levels would decrease with increasing levels of acetate. Our results showed that there was no significant trend of decreasing oxygen production among the three concentrations of acetate treatments. This was different from the results of Heifetz *et al.* (2000), who described decreasing maximum rate of net oxygen evolution with increasing acetate concentrations under high (saturating) light and high CO_2. We used same the concentration range of 3.7mM to 29.4mM of acetate as used by Heifetz *et al.* (2000) for our experiment; therefore it is not likely the results seen are due to the limited range of acetate concentrations. Possible reasons could be related to light intensity, concentrations of CO_2 present or experimental errors; all which could have also contributed to the increase in oxygen production per cell in the 30mM acetate treatment. However, the relatively similar confidence intervals between our four treatments indicated variation was constant for all treatments and there were no extraneous factors contributing to the observed oxygen production values. We can conclude that the presence of acetate does indeed have an effect on the oxygen production of *C. reinhardtii* by comparing the treatment with no acetate (0mM) to the treatments with acetate (10mM, 20mM, 30mM). In addition, the oxygen levels are known not to be a consequence of cell abundance because the oxygen production was calculated per cell, allowing a correlation to be made between the oxygen produced and the acetate present in each treatment.

There were a few limitations in our experiment that need to be taken into consideration. First, one of the major drawbacks was trying to eliminate the air bubbles that were formed in the process of transferring the different treatment media into the vials. The oxygen produced by *C. reinhardtii* in the vials could have escaped into the air bubbles, which would have further escaped into the atmosphere once the vial lid was opened. Therefore, it was crucial to eliminate the presence of air bubbles because this could have led to variation in oxygen production for

vials that had large air bubbles compared to vials with the absence of air bubbles, although there was a considerable amount of effort put into avoiding this source of error.

Another error that could have occurred was during the cell count process of *C. reinhardtii*. The medium had to be mixed well before transferring it to the haemocytometer, and during this procedure there could have been some inconsistency in swirling the media. Thus, this could have led to an underestimation of cell density. Having groups of cells clumped together could have resulted in an overestimation of cells. This could explain why a significant difference was not seen in the oxygen produced for the 10mM, 20mM, and 30mM treatments. However, we tried to minimize such error by ensuring to swirl the medium constantly in the flask prior to obtaining a sample.

Furthermore, since we performed a dilution, miscalculations and the ±0.1 uncertainty in the graduated cylinders we used could have resulted in some variability in our final results.

Conclusion

We rejected our null hypothesis and provided support for our alternative hypothesis. A significant difference was found among the mean oxygen production of our four treatments. Thus, we can conclude that oxygen production of *C. reinhardtii* is affected by the presence of acetate.

Acknowledgements

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