# The Effects of Temperature on the Oxygen Production of *Chlamydomonas reinhardtii*

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## Abstract

As a keystone species in British Columbia, salmon play a large role in the ecosystem as changes in their abundance can greatly affect the numbers of other species nearby (Hilderbrand et al., 2004). *Chlamydomonas reinhardtii (C. reinhardtii)* are single-celled green algae that are a major food source for salmon (Norambuena et al., 2015). Due to their abundance in streams, their oxygen ($O_2$) production contributes to the overall $O_2$ level of streams and can indirectly affect the health of salmon (Carter, 2005). This experiment tests the effects of simulated seasonal temperatures in Vancouver stream waters on the $O_2$ production in *C. reinhardtii.* Our goal is to better understand when the most ideal time for salmon to spawn is and the impacts of changing water temperatures due to climate change may have on *C. reinhardtii,* and consequently on salmon populations. This was conducted by incubating live cultures for 75 minutes in 7ºC, 17ºC and 27ºC waterbaths, and measuring the change in $O_2$ in the different treatments both pre- and post-incubation. Cell counts were performed in order to determine the $O_2$ produced per cell. Our null hypothesis was that temperature has no effect on $O_2$ production. By conducting a One-way ANOVA we obtained a $p$-value of 0.08.. Since $p > 0.05$, the differences between the treatments were not significant. The small sample size (n=3) limited the statistical analysis and the power. Had the sample size been larger, the $p$-value may have been less than 0.05. Overall, our results suggest that temperature does not have a significant effect on the $O_2$ production of *C. reinhardtii.*

## Introduction

Salmon are an important species that are responsible for bringing nutrients from the ocean up to rivers distributed inland and regulating the populations of other species they share an ecosystem with (Yanai & Kochi, 2005). Any changes in the population of salmon can greatly affect the terrestrial wildlife population and the ecosystem as a whole (Hilderbrand et al., 2004). This is especially true in British Columbia (BC) as they are large contributors to our temperate rainforests and economy. Thus, it is essential to understand the intrinsic properties of a healthy salmon life cycle and the factors that may influence salmon abundance in BC. *Chlamydomonas reinhardtii* (*C. reinhardtii*) is a common single-cell green algae that lives in freshwater around the world (Merchant et al. 2007). Since it is considered a major food source for salmon, their

abundance has a positive effect on salmon growth (Norambuena et al., 2015). Increasing water temperatures have been found to increase the photosynthetic rate of *C. reinhardtii,* resulting in increased $O_2$ production (Bui et al., 2014). As *C. reinhardtii* is found in abundance in the aquatic ecosystem, large changes in its $O_2$ production can significantly modify the overall $O_2$ levels in their microenvironments. A study has shown that reduced $O_2$ levels in water can increase the mortality and decrease the growth rate of salmon (Carter, 2005), suggesting that *C. reinhardtii*'s contribution of $O_2$ to the environment can directly influence the overall health of salmon. By studying the $O_2$ production rates of *C. reinhardtii* during the varying seasonal temperatures in Vancouver, we can better understand when *C. reinhardtii* is most beneficial to salmon, in terms of maintaining $O_2$ concentrations in streams. Moreover, climate change is creating rising water temperatures which will inevitably affect the environment and behaviours of many species inhabiting these waters (Mantua et al., 2010). Understanding how *C. reinhardtii's* $O_2$ contribution to the environment varies based on temperature can help us predict future consequences on salmon that are due to climate change. Our project aimed to describe the changes in $O_2$ production of *C. reinhardtii* at different temperatures, representing the different seasons *in vitro*. The seasons were represented by a treatment of 7°C waters for winter and a treatment of 17°C waters for summer (Damborg et al., 2015). The 27°C water treatment was within *C. reinhardtii's* optimal temperature range and was used as a control (Belsher et al., 2017). Although the water temperature can be lower than 7°C in the winter, 7°C was selected because the tolerance range of *C. reinhardtii* was found to be between 6°C to 35°C (McCombie, 1960). Temperatures lower than 7°C may lead to death which would have affected our results. Our null hypothesis was that temperature has no effect on the $O_2$ production of *C. reinhardtii*. The alternate hypothesis was that temperature does have an effect on the $O_2$ production of *C.*

*reinhardtii*. We predicted that *C. reinhardtii* would produce less $O_2$ as temperature was lowered, since water temperature and photosynthetic rate are positively correlated (Norambuena et al., 2015).

## Methods

The organism used in this project was *Chlamydomonas reinhardtii,* strain number cc-1690, wild-type MLT 21g. It was cultured by Mindy Chow in Wesbrook Building at the University of British Columbia located in Vancouver, BC. The culture was prepared in accordance with the 'Chlamydomonas Maintenance' document to a final pH of 6.5. A culture of 1000 mL of *C. Reinhardtii* and 800 mL of excess media were obtained. The excess media was used to create a 2:1 dilution of *C. Reinhardtii*, as demonstrated in **Figure 1**.

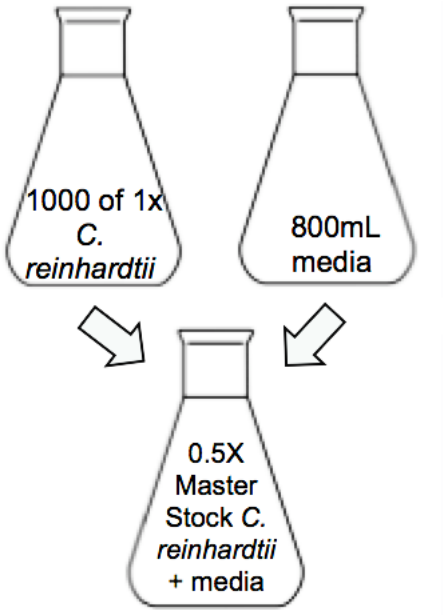


**Figure 1. The Creation of the 0.5X Master Stock**. A diagram showing the volumes and proportions used to create the 0.5X Master Stock of *C. reinhardtii* for the experiment. This 0.5X Master Stock would be used for all the various treatment vials.

After the dilution, the $O_2$ concentration of the Master Stock and stock media was measured to obtain the 'Initial $O_2$ Concentration' for all control and treatment vials. $O_2$ was measured using a dissolved-oxygen probe linked to a TI-84 graphing calculator via an Easy Pro-Link connector. We combined 100 μL of Master Stock and another 100 μL of media with 10 μL of Iodine Potassium Iodine (IKI) fixative such that the initial cell count could be counted at a later time.

Before incubation, three treatments of waterbaths were created at temperatures of 7°C, 17°C, and 27°C (**Figure 2**). We used a hot plate and ice to maintain the temperatures of all baths, and monitored them throughout the duration of incubation. Flood lamps were placed above the waterbaths to provide light needed by *C. reinhardtii* to photosynthesize. Light intensity was measured using the Lutron Brand LX-101 Lux Meter to ensure the light intensity was the same for all treatments - between 8000-8200 lux. The distance between the lamp and the waterbaths were adjusted to ensure similar lux values among treatments. The experimental setup can be observed in **Figure 3**.

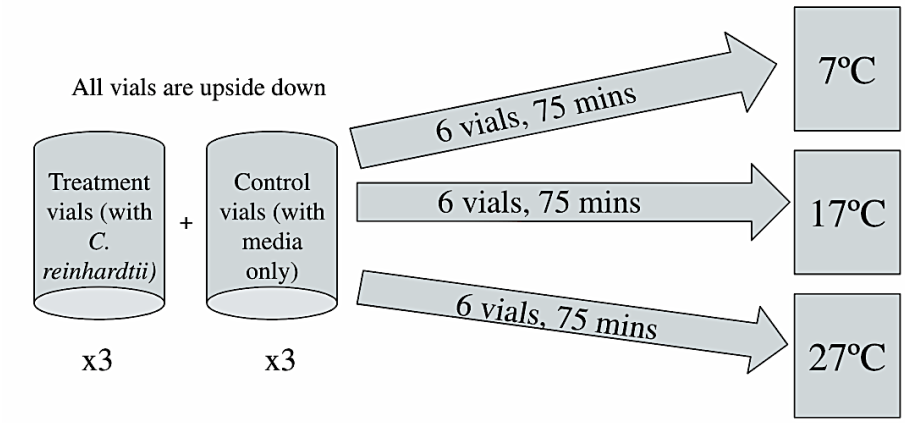


**Figure 2. The Experimental Conditions Used for both the Treatment and Control Vials (n=3).** This diagram shows the different temperature waterbath treatments that were used in the experiment: 7ºC, 17ºC and 27ºC. There were 3 replicates per treatment, as well as 3 controls per treatment, for a total of 6 vials in each of the waterbaths. The incubation period for all vials was 75 minutes. All vials were incubated upside down to ensure no bubbles could enter the vials while incubated.

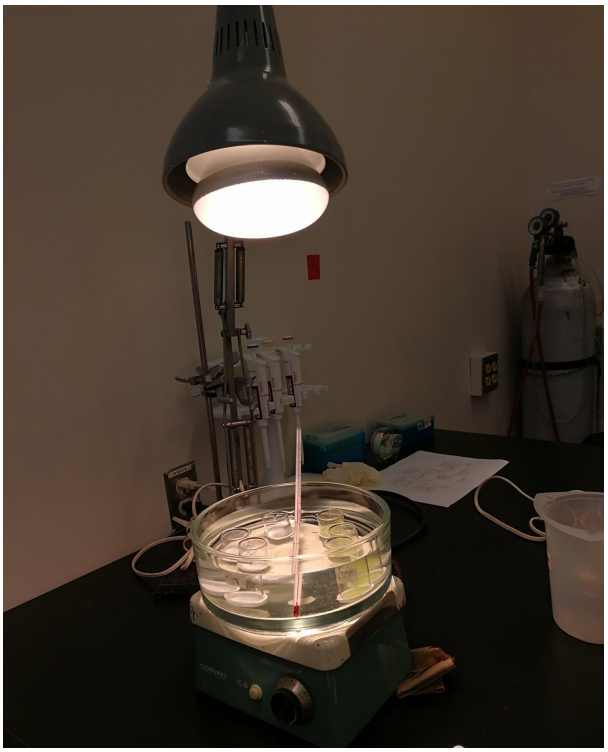

**Figure 3. The Experimental Set-Up of the Treatment and Control Vials in their Respective Waterbaths.** This figure displays the 6 vials (3 treatment and 3 control) in the 27ºC waterbath. The hotplate below was used to heat the water to ensure the water remained warmer than room temperature, while the thermometer helped monitor and ensure the temperature stayed constant throughout the duration of the experiment. The flood lamp delivered light to the photosynthetic *C. reinhardtii.* Ice was added if the waterbath became too warm.

After the waterbaths were created, the treatment and control vials were filled. The 27mL-capacity vials were submerged in either a beaker filled with the 0.5X Master Stock (treatment vials) or media (control vials). While wearing gloves, the vials were filled and capped while submerged in liquid, to ensure that the maximum volume possible entered the vials and to prevent formation of air bubbles inside. After all the vials were filled and labelled they were added in 1-minute increments to their respective waterbaths to ensure that $O_2$ could be measured immediately after removal from the water bath.

After an incubation time of 75 minutes, each vial was removed in the order in which they were incubated. The $O_2$ concentration was immediately recorded while simultaneously another lab member pipetted 100 µL from the vial and added it to 10 µL of IKI fixative, as can be seen in **Figure 4**. The IKI and sample were mixed well with a pipet to fix the cells so that they could be counted at a later time.

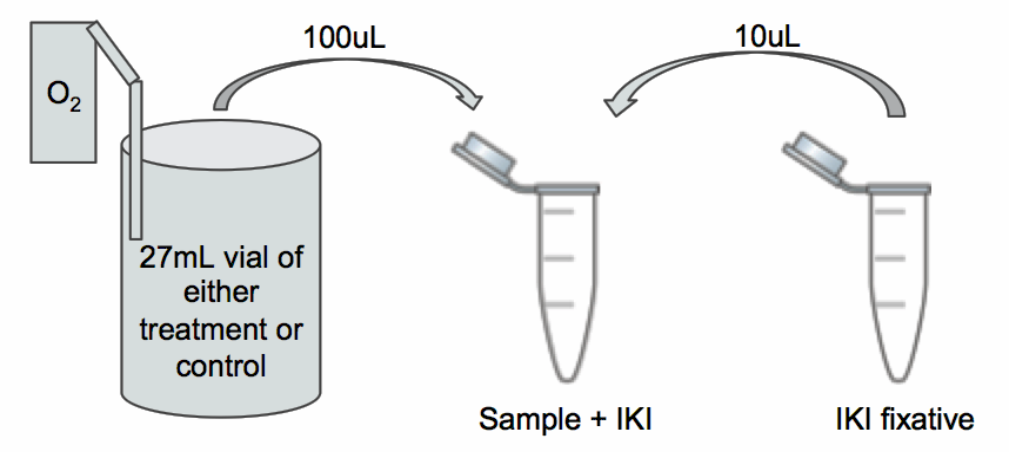


**Figure 4. The Post-Incubation Procedure to Measure $O_2$ and Fix Cells.** This diagram shows how $O_2$ was measured using the $O_2$ probe, while simultaneously 100 µL of the sample was combined with 10 µL of IKI fixative in an Eppendorf tube.

We counted the fixed cells using a BRAND 0.100 mm haemocytometer, following the instructions provided in the "Haemocytometer Instructions" for the Biology 342 lab. A tally clicker was used to ensure accurate counting of cells

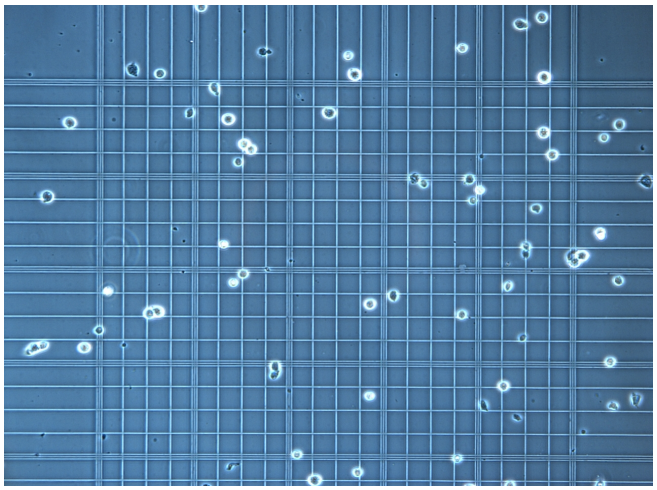

**Figure 5. Haemocytometer Showing Cells Under a Compound Microscope.** The image shows diagram shows an example of what our cells looked like under a compound microscope in the haemocytometer. The grid and squares assisted us to count the cells accurately. From 'Photography of a phase contrast microscopic view of CHO-Cells on a Neubauer improved counting chamber', by Wikimedia User:Alcibiades, 2006, https://commons.wikimedia.org/wiki/File:Neubauer_improved_with_cells.jpg. Reprinted with permission.

After we had obtained data for '$O_2$ produced' and our final cell counts, we were able to conduct our statistical analysis. The following example calculation was done to obtain the values on which the statistical analysis was conducted.

Example:

*Normalized $O_2$ Produced per cell (x°C treatment)* =

$$\frac{(Mean\ O2\ Produced\ by\ x\ Degree\ Treatment\ Vials) - (Mean\ O2\ Produced\ by\ x\ Degree\ Control\ Vials)}{(Mean\ number\ of\ cells\ in\ x\ degree\ treatments)}$$

We used the statistical software SigmaPlot v.11 (Systat Software, San Jose, CA, USA) to conduct our statistical analysis that would determine if the 'Normalized $O_2$ Produced Per Cell' was different between the three treatments.

Since the data displayed normality and equal variances, we were able to conduct a One-way ANOVA. A One-way ANOVA was conducted opposed to a t-test since we wanted to compare the means of three different treatments, while a t-test could only be used for two treatments. After the One-way ANOVA was conducted we obtained a *p*-value, which allowed us to either reject, or fail to reject the null-hypothesis.

## Results

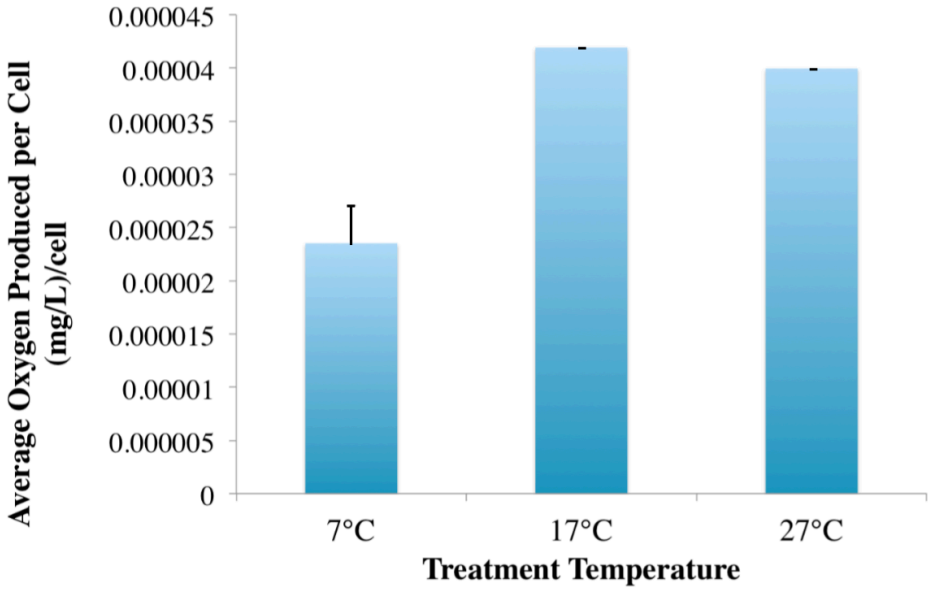


**Figure 7. The Average Oxygen Produced per Organism of *C. reinhardtii* at Various Treatment Temperatures (n=3).** Data is represented as mean $O_2$ produced per organism of *C. reinhardtii* ± standard error (SE) of the means at 7ºC, 17ºC and 27ºC. The mean $O_2$ produced per organism was $2.35 \times 10^{-5} \pm 3.52 \times 10^{-6}$ (mg/L)/cell for the 7ºC treatment, $4.19 \times 10^{-5} \pm 5.89 \times 10^{-7}$(mg/L)/cell for the 17ºC treatment and $3.99 \times 10^{-5} \pm 8.19 \times 10^{-6}$ (mg/L)/cell for the 27ºC treatment. Each treatment had n=3. Error bars represent standard error. After conducting a One-way ANOVA it was determined that $p = 0.08$.

After conducting a One-way ANOVA it was determined that $p = 0.08$. Since $p > 0.05$, we failed to reject the null hypothesis that the mean $O_2$ production in *C. reinhardtii* is the same between treatments. While graphically there are visible differences in the mean $O_2$ production between treatments, the differences between the treatments are not statistically significant. In **Figure 7**, the mean $O_2$ produced by organism ± the SE of the means in (mg/L)/cell is plotted for both the 7ºC, 17ºC and 27ºC treatments. The error bars in **Figure 7** represent the standard error. At the end of the 75 minute incubation time, there were no visible differences between the vials, before the incubation time. According to **Figure 7**, both 17ºC and 27ºC treatments had similar $O_2$

produced per organism, however the 7ºC treatment does appear to be less compared to the other two treatments.

## Discussion

The objective of this experiment was to determine the effects of the environmental temperature on the $O_2$ production of *C. reinhardtii*. We obtained a *p*-value of 0.08, and since $p > 0.05$ we failed to reject the null hypothesis that temperature has no effect on $O_2$ production of *C. reinhardtii.*

We predicted that when *C. reinhardtii* was subjected to lower temperatures less $O_2$ would be produced, and that more $O_2$ would be produced at higher temperatures due to higher photosynthetic rates (Bui et al., 2014). According to our data, this prediction is not supported since the treatments were not shown to be significantly different. However, the power of our performed statistical test was below the desired power of 0.800. While we are able to make observations about trends and what significance our results may have, we must interpret the results cautiously.

Our data suggests that changing water temperatures due to seasonal variation or climate change will not impact the $O_2$ produced by *C. reinhardtii* within temperature ranges of 7ºC - 27ºC. This suggests that salmon spawning in streams in the winter will be subjected to the same $O_2$ conditions as those spawning in the summer. Pacific Salmon return to spawn at fixed seasons (Cushing, 1969), and our data suggests that salmon will not need to adapt nor disrupt this pattern of spawning if seasonal temperatures do not cause $O_2$ levels to vary.

It has been observed that salmon swimming performance decreases and growth decreases in low $O_2$ environments, and salmon actively avoid areas of low $O_2$ concentrations (Carter, 2005; Davis, 1975). Since the trend in our data suggests that *C. reinhardtii* produce the same $O_2$ levels in the 7ºC - 27ºC range, swimming performance or growth will not be affected. Furthermore, it implies that salmon will not actively avoid areas due to temperature changes within the specified range since $O_2$ concentrations will remain unchanged in these areas.

In **Figure 7**, we see a trend of lower $O_2$ production at 7ºC compared to the higher temperatures. With climate change affecting the variability of the seasons by creating more extreme weather patterns (Meehl et al., 2000), this could mean that *C. reinhardtii* may exhibit lower $O_2$ production at cooler temperatures. A lower $O_2$ concentration in the salmon's environment during the winter seasons could have detrimental effects on salmon populations. The trends for 17ºC and 27ºC seem to show that during the warmer seasons climate change will not affect the $O_2$ production by C. *reinhardtii*. Future studies should therefore focus on the $O_2$ content of streams carrying salmon during the winter seasons, to ensure that $O_2$ levels do not reach dangerously low levels.

In addition to *C. reinhardtii,* salmon also consume other photosynthetic algae (Norambuena et al., 2015). Diatoms such as *Licmophora abbreviata* are major contributors of dissolved $O_2$ produced in oceans and waterways. For optimal growth and $O_2$ production, the environmental temperature for *L. abbreviata* should be 15ºC - 25ºC (Ohgai et al., 1984). If the climate becomes colder during the winter seasons, not only will *C. reinhardtii*'s $O_2$ production be affected, but other diatoms and photosynthetic organisms will be as well. While *C.*

*reinhardtii*'s $O_2$ production may be unaffected by temperature changes, other diatoms in the salmon ecosystems may be at risk and could have disastrous consequences for native salmon populations. Further studies investigating the impact of climate change and seasonal temperatures on $O_2$ production by other organisms is necessary for a better understanding of the impact of temperature on salmon health.

Light intensity can affect the $O_2$ output of *C. reinhardtii* (McCombie, 1960). A lamp was kept above each treatment with its intensity calibrated to ensure that all of the *C. reinhardtii* were receiving the same light intensity to reduce error. Additionally, the dilutions were created from a single Master Stock rather than creating individual dilutions in the treatment vials to further reduce error.

There were also some experimental limitations. Firstly, the oxygen probe sensor was just below the surface of the *C. reinhardtii* solution, which could have caused measurement errors in all samples as the sensor could have been influenced by atmospheric $O_2$ dissolving into the solution (Kester, 1968). Consequently, the measurement taken near the surface of the solution may not have accurately reflected the amount of $O_2$ dissolved in each vial. Therefore, deeper vials or a different oxygen probe should be used in the future so that the sensor is well below the surface to minimize the effects of atmospheric $O_2$ diffusion.

The *p*-value obtained was 0.08, a value that is quite small considering a sample size of only n=3. This suggests that with a greater sample size within each treatment group we may have been able to obtain a significant difference. In the future, it would be necessary to repeat this

experiment with more samples in order to truly see whether temperature has a significant effect on the $O_2$ production by *C. reinhardtii.*

## Conclusion

To conclude, our *p*-value of 0.08 > 0.05 prevented us from rejecting the null hypothesis that $O_2$ production by *C. reinhardtii* was the same in different treatment temperatures. This tells us that *C. reinhardtii* is not likely to be contributing to any significant change in stream $O_2$ contents that vary on a seasonal basis, and that any changes in $O_2$ that may be hurting or helping salmon may be attributed to other photosynthetic organisms , abiotic and/or biotic factors.

## Acknowledgements

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## Appendix 1: Raw Data from Experiment and Calculations for $O_2$ Production Per Cell

| Replicate # | O2 Initial | O2 Final | O2 Produced | Number of Cells | O2 Produced (Normalized) | O2 Produced per Cell |
|---|---|---|---|---|---|---|
| T1 | 10 | 8.8 | -1.2 | 25080 | 0.766666667 | 3.05688E-05 |
| T2 | 10 | 8.6 | -1.4 | 28050 | 0.566666667 | 2.0202E-05 |
| T3 | 10 | 8.5 | -1.5 | 23540 | 0.466666667 | 1.98244E-05 |
| C1 | 11.2 | 9.8 | -1.4 | 0 | | |
| C2 | 11.2 | 8.7 | -2.5 | 0 | | |
| C3 | 11.2 | 9.2 | -2 | 0 | | |
| | | Mean O2 Control | -1.966666667 | | | |

| Replicate # | O2 Produced per Cell |
|---|---|
| T1 | 3.05688E-05 |
| T2 | 2.0202E-05 |
| T3 | 1.98244E-05 |

| Replicate # | O2 Initial | O2 Final | O2 Produced | Number of Cells | O2 Produced (Normalized) | O2 Produced per Cell |
|---|---|---|---|---|---|---|
| T1 | 10 | 9.5 | -0.5 | 37125 | 1.6 | 4.30976E-05 |
| T2 | 10 | 9.5 | -0.5 | 38866.66667 | 1.6 | 4.11664E-05 |
| T3 | 10 | 9.5 | -0.5 | 38500 | 1.6 | 4.15584E-05 |
| C1 | 11.2 | 9.3 | -1.9 | 0 | | |
| C2 | 11.2 | 9.1 | -2.1 | 0 | | |
| C3 | 11.2 | 8.9 | -2.3 | 0 | | |
| | | Mean O2 Control | -2.1 | | | |

| Replicate # | O2 Produced per Cell |
|---|---|
| T1 | 4.30976E-05 |
| T2 | 4.11664E-05 |
| T3 | 4.15584E-05 |

| Replicate # | O2 Initial | O2 Final | O2 Produced | Number of Cells | O2 Produced (Normalized) | O2 Produced per Cell |
|---|---|---|---|---|---|---|
| T1 | 10 | 9.7 | -0.3 | 32450 | 0.766666667 | 2.36261E-05 |
| T2 | 10 | 10 | 0 | 22660 | 1.066666667 | 4.70727E-05 |
| T3 | 10 | 10.3 | 0.3 | 27775 | 1.366666667 | 4.92049E-05 |
| C1 | 11.2 | 10.1 | -1.1 | 0 | | |
| C2 | 11.2 | 10.1 | -1.1 | 0 | | |
| C3 | 11.2 | 10.2 | -1 | 0 | | |
| | | Mean O2 Control | -1.066666667 | | | |

| Replicate # | O2 Produced per Cell |
|---|---|
| T1 | 2.36261E-05 |
| T2 | 4.70727E-05 |
| T3 | 4.92049E-05 |