The Effect of Ibuprofen on Chlamydomonas Oxygen Production

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Abstract

Ibuprofen is one of many toxins that enter wastewater, polluting and affecting aquatic life. While another non-steroidal inflammatory drug, diclofenac, was found to negatively affect *Chalmydomonas reinhardtii's* photosynthetic pathway, the effect of ibuprofen has not been widely studied. Both being NSAIDs, the effect of ibuprofen on Chlamydomonas reinhardtii's oxygen production was explored. A one-way ANOVA test was used for data analysis and no statistical differences were found between the treatment levels and the O_2 production of each cell ($p = 0.823$). The findings also revealed comparable levels of variability, suggesting no effect on the treatment on *Chlamydomonas*. A more in-depth investigation is required such as analysis on something other than oxygen production, as the treatments did not yield any influence on it.

Introduction

Ibuprofen is a non-steroidal anti-inflammatory (NSAID) drug used for the treatment of inflammatory diseases and rheumatoid disorders (Ngo V, 2023). Amidst the global surge in pharmaceutical usage, waterborne residues of drugs like ibuprofen are increasingly detected, further driven by its accessibility over-the-counter, and its use for more common symptoms, such as headaches and mild pain. (Donk et, al, 2016 & Navrozidou et. al., 2019). Its high human consumption and low environmental degradability raise concerns about its effect on aquatic life, as the drug concentrations found in bodies of water and soils present adverse effects on aquatic organisms due to cytotoxic and genotoxic damage, high oxidative cell stress, and have detrimental effects on growth, reproduction, and behavior of microorganisms exposed (Jan-Roblero, 2023).

While the consequences mentioned above have not been extensively studied between ibuprofen use and *Chlamydonomas reinhardtii*, it is especially true in the case of another more well-studied, and more destructive NSAID in aquatic environments, diclofenac (Majewska,

2021). In fact, this NSAID was found to decrease the photosynthetic vitality of Chlamydomonas cells, leading to the possibility that there may be a similar occurrence with ibuprofen exposure. While they have different derivatives, diclofenac being derived from anthranilic acid, and ibuprofen from aryl/heteroaryl acetic acid, they have similar functions in the human body (Bindu, 2020). Thus, while not identical, there may be similar negative results among aquatic microorganisms to diclofenac as there are to ibuprofen. For these reasons, adverse changes in the oxygen production of our target organism, *Chlamydomonas reinhardtii,* are expected, which is not only a key oxygen producer through photosynthesis but also a fundamental component of aquatic food webs (Rochaix, J., 2013). This paper zeroes in on *Chlamydomonas*, a representative microalga found in diverse aquatic habitats, to shed light on how pharmaceutical pollutants might alter primary producers' roles in these ecosystems.

In this study, we assessed the changes in oxygen output by *Chlamydomonas* cells exposed to different levels of ibuprofen, monitoring the cellular oxygen production before and after treatment using an oxygen meter to infer possible disruptions in metabolic activities. Analyses of the oxygen production per cell will provide further insight into whether ibuprofen has negative impacts on *Chlamydomonas's* ability to function as a primary producer, and what further environmental implications this may have.

Methods

In order to test the effects on *Chlamydomonas* under different concentration of ibuprofen, we prepared three different treatments with series of concentrations of ibuprofen; Treatment 1: 14.7mg/250mL, Treatment 2: 5.88mg/250mL, Treatment 3: 2.35mg/250mL (Seoane et al., 2023). All treatment and applicable control groups in this experiment were made using 120 mL

of fresh media or treated media, and 70 mL of *Chlamydomonas* culture using disposable pipettes. First, we prepared a batch solution of Treatment 1 by grinding and weighing 24.6 mg of ibuprofen powder on a top-loading balance based on the initial concentration required, and the amount of ibuprofen in the pill. Pill coating weight was considered negligible. Then, we dissolved the pre-weighed powder in a 500 mL beaker with a magnetic stir bar using fresh media for *Chlamydomonas*. Once the highest concentration of ibuprofen concentration was achieved, we performed serial dilutions to obtain the desired Treatment 2 and Treatment 3 concentrations of ibuprofen.

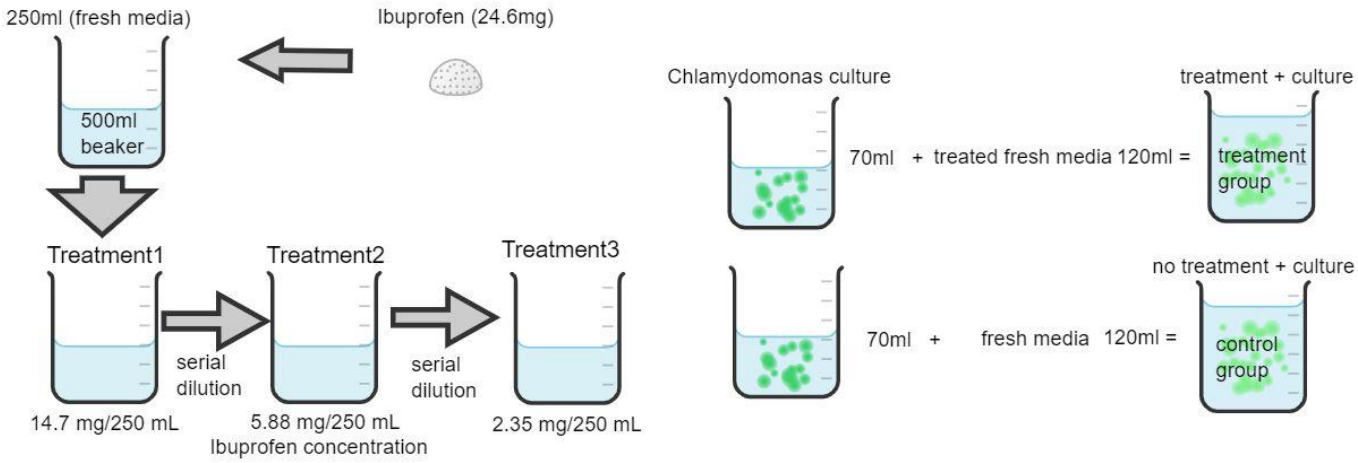

Figure 1: Schematic diagram depicting how treatment and control solutions were made, consisting of ground ibuprofen, fresh media, and *Chlamydomonas reinhardtii* cell culture. Mixing 120 mL of fresh media and 70 mL of culture ensured the desired ratio of 17mL of fresh media to 10 mL of culture was achieved in each 27mL vial.

To make our results more representable and reliable, we prepared each treatment vial in triplicates. Fresh media and the *Chlamydonomas* culture flasks were always swirled before mixing to ensure a homogenous solution was put into our treatment vials. We also prepared two different control groups for comparison, one of which only contained the regular ratio of 17 mL of fresh media and 10 mL of *Chalmydomonas* culture. The other control treatment of only fresh

media allowed us to track changes in the media over time. For both control and media control groups, we created them in triplicates in vials to match treatment groups and ensure more accurate data was measured.

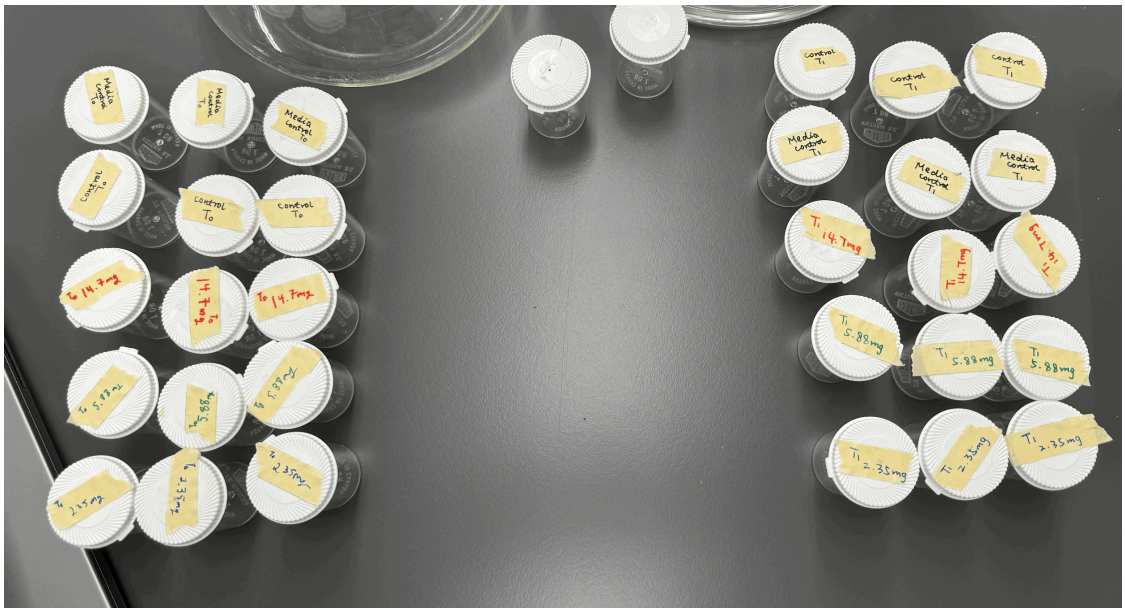

Figure 2: Image of all the vials created before they were filled with the corresponding solutions. Each treatment/control group was made of T_o and T_f groups, made in triplicates; vials on the left were measured before incubation, and the matching vials on the right were measured after 3 hours incubation at 30℃.

To measure oxygen production per cell, oxygen and cell count were taken before and after the 3 hour treatment and incubation time. However, to ensure accuracy of our results, we created two separate groups in each treatment: a triplicated set of vials for oxygen measurement and cell count before treatment, and another triplicated set of vials made at the same time to be measured after the treatment time. Treatments put in the incubator were immediately capped after the vial was filled. The light inside the incubator turned on and off periodically, and this was difficult to control. We measured the oxygen production using an Oxygen Meter immediately after the vial solution was made, swirling in the vial and taking the reading after approximately 10 seconds to ensure consistency across the vials. The *Chlamydomonas* cell

counting with a hemocytometer and compound microscope. In terms of counting *Chlamydomonas* cells number, we used hemocytometer. The number of alive and healthy cells per mL is equal to

$$\frac{number\ of\ alive\ and\ healthy\ cells}{number\ of\ counted\ square} \times dilution\ factor\ .$$

We are considering the amount of oxygen production instead of measuring cell growth so the healthiness of cells are more important to us. Since counting cells immediately before and after treatments was not feasible, 100ul of each vial were placed in a counting tube with 10 ul of setting solution using a micropipette, and placed in the refrigerator.

Results

Our data collected consisted of 3 different groups with four different treatment levels including a control group. Each group had roughly 2-3 measurements of the cell counts and 1 measurement of the oxygen levels. Each oxygen level's measurement was subtracted by the Media Control treatment and the result of that subtraction gives the oxygen produced in that timespan. An average of the cell count was taken and the O_2 production per cell was calculated via the O_2 divided by average cell count.

A one-way ANOVA test was performed to compare the four treatment levels and our significant value is set at 0.05.

	Df	SS	MS	F	Pr(>F)
Treatment	3	4.00E-16	1.33E-16	0.302	0.823

Residuals	8	3.53E-15	4.41E-15		

Figure 3: Df: Degrees of freedom; SS: Sum of squares; MS: Means squares; F: F ratio; Pr(>F): P values

Based on the ANOVA test, we can see a P-value of 0.823 which is much higher than our alpha level at 0.05 indicating that there is no statistical significant difference in the mean levels between the treatments and O_2 production per cell. The F value which measures variability is notably low which suggests that the variance in oxygen production levels is similar across all treatment groups.

The lack of significance revealed by both the F value and P value of the ANOVA test suggests that the treatment levels do not have a distinguishable impact on oxygen production.

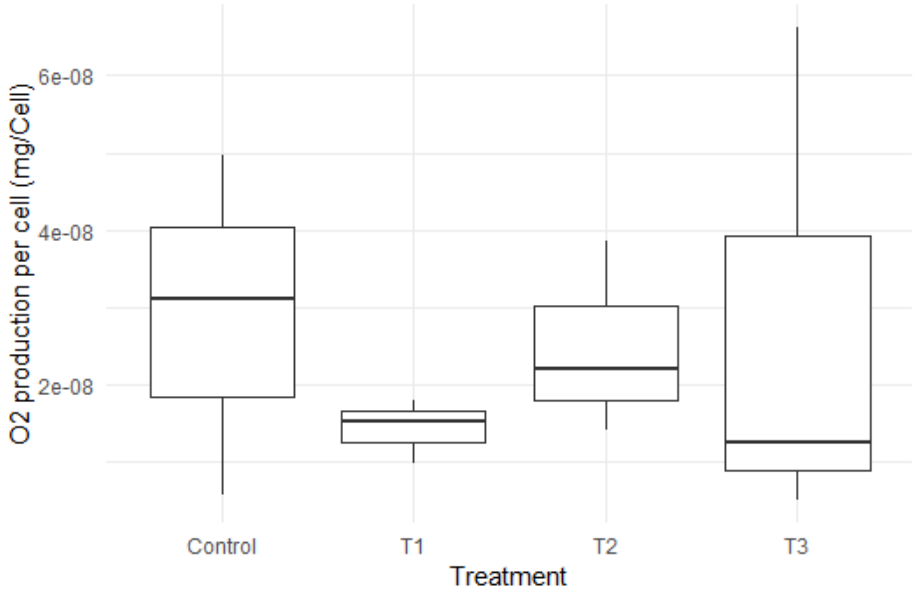

Figure 4: O_2 production per cell (mg/Cell) between 3 different treatment levels (n=3) and 1 control group (n=3). No significance difference between the 4 groups which was tested with a One-way ANOVA test ($p > 0.823$) .

Discussion

Upon investigating the effects of ibuprofen (IBU) on the oxygen production of *Chlamydonomas reintardtii* with a three hour incubation period at 30℃, the findings of this study suggest that there is no significant difference in oxygen production per cell across our treatment and control groups. Thus, we fail to reject the null hypothesis that the presence of ibuprofen affects the oxygen production of *Chalmydomonas reinhardtii* cells. To explain, a high p-value of 0.823 was obtained across our test groups, indicating that the presence of ibuprofen did not affect the cells' ability to produce oxygen at all three concentration levels. Researchers Moro et. al. (2021) note that while slight morphological differences were noted in *Chalmydomonas* cells exposed to IBU, its growth was not inhibited, but rather potentially stimulated.

The lack of significant results may be an indicator that there is no interaction between the *Chlamydomonas* culture and the ibuprofen for reasons such as not enough exposure time, different oxygen solubilities in the fresh media, mis-aligning chemical structures/receptors for cell-ibuprofen interaction, or possible degradation of ibuprofen in the fresh media. To explain, oxygen level was a difficult variable to measure and had to be limited to a shorter timeline than that done in Moro et. al.'s (2023) study, which was over 27 days. Since the vials were placed in an incubator at 30C, this may have impacted the solubility of oxygen, as solubility decreases with increased temperatures (Harvey, 2011). Furthermore, it is known that the oxygen production of *Chlamydomonas* is relatively highly affected by the absorbed light because the photobioreactor (PBR) of *Chlamydomonas* makes it experience light/dark (L/D) cycle to enhance the PBR efficiency so the oxygen production is high (Vejrazka et.al., 2013). It is possible that the light source in the incubator we were using for three-hour treatment of ibuprofen was not

suitable for *Chlamydomonas*, meaning the the efficiency of PBR is lower and this leads to less oxygen production.

Finally, the amount of ibuprofen we used may not be accurate since ibuprofen power was dissolved in fresh media of *Chlamydomonas* and the media is aqueous solution. The solubility of ibuprofen in organic media is higher than in aqueous solution, with the fresh media being an aqueous solution (Garzón & Martínez, 2004). The insoluble ibuprofen is highly influencing the amount of ibuprofen that was able to react with *Chlamydomonas*. However, a more plausible reason for less ibuprofen present may be due to its degradation in aqueous solutions over time. To explain, Iovino et. al., (2016) found that in pHs of 2.25 to 5.51 and finally to 8.25, the IBP concentration after an hour of treatment, decreases respectively to 45, 34, and 27 %. Since our treatment time was over 3 hours, it is very possible that most of the ibuprofen degraded in the solution, compounded by the fact that smaller amounts of ibuprofen degrade at a faster rate than if there was more in the solution (Iovino et. al., 2016). Taking into consideration all these factors, further research should better manage the variables to reduce the amount of factors that may be influencing our data. However, unforeseen chemical and biological interactions weren't the only factors that may have affected our results.

Errors relevant to our study include human error. Haemocytometer was used to count the cells for each sample. Based on the results we have, it is possible that the taken samples for cell counting had less cell amount because the solutions were not mixed well before taking samples. Another human error is coming from counting cells. It was not clear to distinguish cells from other impurities under the haemocytometer because some cells were relatively small and it is possible for us to miss counting them. Moreover, each sample was assigned at least two people to count and this allowed us to verify the results. But, there is a potential for subconscious bias in

our results because it is likely for one person who counted the cell would assume another person would have more accurate counting. To avoid this problem for future experiments, it will be helpful to separate the people who will count the same sample into different rooms while they are counting and not sharing the results until all the counts finish.

Conclusion

In conclusion, our study of effects of ibuprofen on the oxygen production capabilities of *Chalmydomonas reinhardtii*, done by a three-hour incubation process at 25°C, shows that there is no significant alteration in the oxygen production of the cells due to exposure to ibuprofen. This was concluded by the high p-value of 0.823 from the ANOVA test, indicating that there was minimal impact on the photosynthetic pathway across different levels of ibuprofen. Despite ibuprofen being a common pollutant in wastewater and its potential to affect aquatic life, this result indicates that ibuprofen does not significantly alter the oxygen production in *Chlamydomonas reinhardtii* in our concentrations tested. These results helps us understand NSAID pollutants in aquatic environments and highlight the need for more in-depth investigations to fully comprehend their ecological impacts.

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References

Bindu, S., Mazumder, S., & Bandyopadhyay, U. (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. Biochemical Pharmacology, 180, 114147. https://doi.org/10.1016/j.bcp.2020.114147

Donk, E. van, Peacor, S., Grosser, K., Domis, L. N. de S., & Lürling, M. F. L. L. W. (2016). Pharmaceuticals may disrupt natural chemical information flows and species interactions in aquatic systems: ideas and perspectives on a hidden global change. *Reviews of Environmental Contamination and Toxicology*, 91–105. https://doi.org/10.1007/398_2015_5002

Garzón, L. C., & Martínez, F. (2004). Temperature dependence of solubility for ibuprofen in some organic and aqueous solvents. *Journal of solution chemistry*, 33, 1379-1395.

Harvey, R., Lye, L., Khan, A., & Paterson, R. (2011). The Influence of Air Temperature on Water Temperature and the Concentration of Dissolved Oxygen in Newfoundland Rivers. Canadian Water Resources Journal, 36(2), 171–192. https://doi.org/10.4296/cwrj3602849

Iovino, P., Chianese, S., Canzano, S., Prisciandaro, M., & Musmarra, D. (2016). Degradation of Ibuprofen in Aqueous Solution with UV Light: the Effect of Reactor Volume and pH. Water, Air, & Soil Pollution, 227(6), 194. https://doi.org/10.1007/s11270-016-2890-3

Jan-Roblero, J., & Cruz-Maya, J. A. (2023). Ibuprofen: Toxicology and Biodegradation of an Emerging Contaminant. Molecules, 28(5), 2097. https://doi.org/10.3390/molecules28052097

Majewska, M., Harshkova, D., Pokora, W., Baścik-Remisiewicz, A., Tułodziecki, S., & Aksmann, A. (2021). Does diclofenac act like a photosynthetic herbicide on green algae? Chlamydomonas reinhardtii synchronous culture-based study with atrazine as reference. Ecotoxicology and Environmental Safety, 208, 111630. https://doi.org/10.1016/j.ecoenv.2020.111630

Navrozidou, E., Melidis, P., & Ntougias, S. (2019). Biodegradation aspects of ibuprofen and identification of ibuprofen-degrading microbiota in an immobilized cell bioreactor. Environmental Science and Pollution Research, 26(14), 14238–14249. https://doi.org/10.1007/s11356-019-04771-5

Ngo, V. T. H., & Bajaj, T. (2023). Ibuprofen.

Rochaix, J. (2013). Chlamydomonas reinhardtii. In Elsevier eBooks (pp. 521–524). https://doi.org/10.1016/b978-0-12-374984-0.00230-8

Seoane, M., Conde-Pérez, K., Esperanza, M., Cid, Á., & Rioboo, C. (2023). Unravelling joint cytotoxicity of ibuprofen and oxytetracycline on Chlamydomonas reinhardtii using a programmed cell death-related biomarkers panel. *Aquatic Toxicology*, 257, 106455.

Vejrazka, C., Janssen, M., Benvenuti, G., Streefland, M., & Wijffels, R. H. (2013). Photosynthetic efficiency and oxygen evolution of Chlamydomonas reinhardtii under continuous and flashing light. *Applied Microbiology and Biotechnology*, 97, 1523-1532.