Acidic Algae: Behavior of Nannochloropsis oculata under different acidity conditions

Environmental Issue:

Increased Carbon Dioxide levels in the atmosphere lead to increased Carbonic Acid levels in water. The rise in acidity affects the productivity of underwater microflora which are the Earth's primary producers of oxygen.

Background:

After the invention of the combustion engine, Atmospheric Carbon Dioxide presence has grown exponentially. Although, this is curbed by the ocean's massive uptake of gaseous Carbon Dioxide — as much as ⅓ (Sabine et al., 2004). According to Teem Earth and McGee (2015), the yearly average CO_2 ppm for 2020 is 412, while without the ocean it could've been 618 ppm. In addition to CO_2's greenhouse gas effect in the Atmosphere, it also acidifies the Aquasphere as Carbonic Acid when dissolved. Admittedly, Carbon Dioxide is often used in Photosynthesis by underwater plants and microorganisms, although the amount of photosynthetic activity may not nullify the Carbon Dioxide presence entirely. Moreover, the remaining CO_2 would simply remain as Carbonic Acid and negatively affect Photosynthesis and other biological processes.

CO_2 Time Series in the North Pacific Ocean

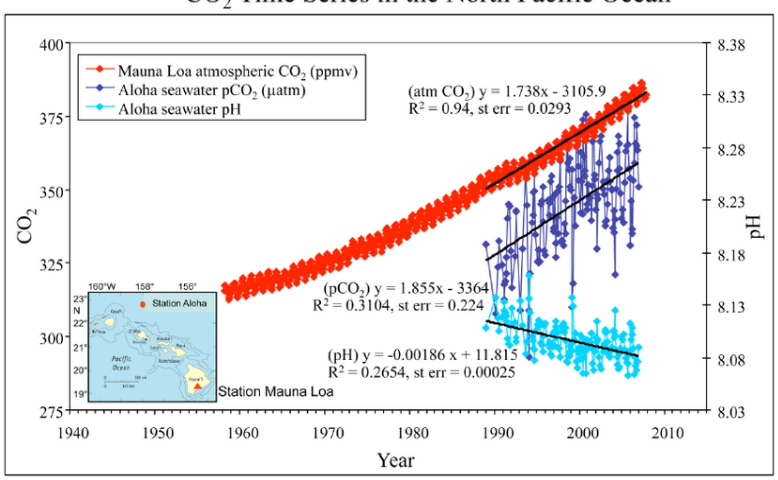

Figure 1. (Langdon, 2010). This graph shows the relationship between atmospheric CO_2 and oceanic pH. With reference to the equation $y = -0.00186x + 11.815$, the year (x) at which a certain pH (y) is reached can be deduced and utilized in prediction of time-to-pH.

Assuming a constant rate of acidification [even though the rate is expected to increase], the following pH levels will occur: pH range 7 [7.9 to 7] will occur at the year 2104 until 2588, when the pH will reach a range of 6. pH range 6 [6.99 to 6] will occur during the year 2588 until

3126. While these changes are over a very large period of time, it is important to consider that the effect on photosynthetic activity may be so great that even a minimal change in pH levels could impact the global concentration of Atmospheric Carbon Dioxide, and in turn Breathable Diatomic Oxygen.

The ocean exchanges about 65% of the world's Carbon Dioxide for Oxygen (National Ocean Service, 2021). As the largest producer of breathable Oxygen, out-performing even the most biologically active forests, the effects of pollution on the ocean are extremely important to analyze. Considering that in only 82 years' time, the ocean's concentration of $H+$ ions will increase an entire factor of 10, the analysis of pH's photosynthetic effects is environmentally pressing to determine.

Research Question:

To what extent does acidity of water affect the productivity of a specific underwater microflora, *Nannochloropsis oculata*?

Hypothesis:

The extent to which pH affects photosynthetic productivity will be that it will perform best at pH 10, as that is its natural environment, and worst at pH 6.

Rationale:

The pH of the North Pacific Ocean and the Atmospheric Carbon Dioxide present are correlated. This leads to the thought that the link between pH and Carbon Dioxide may be one with feedback. Specifically, when the Carbon Dioxide acidifies the water the photosynthetic organisms are less productive. Then less CO_2 is exchanged for Diatomic Oxygen and the positive feedback cycle continues. Similarly, some land plants are extremely sensitive to pH, such as roses. As organisms are sensitive to their niche's conditions, they may be sensitive to changes in pH and their photosynthetic processes may be interrupted.

Variables and Experimental Design:

Variables:

Independent	Dependent	Controlled
pH level	The amount of dissolved oxygen available after 24 hours **with regard to initial concentration.**	Amount of available light, amount of available nutrients, amount of algae, and **amount of time**.

Table 1. Variables acknowledged in this experiment.

Procedure:

Firstly, a sample of *Nannochloropsis oculata* was obtained. Then it was cultured to carrying capacity in 2500 mL of substrate. Then, the sample was evenly distributed across 25 beakers at 100 mL each. To each beaker, the amount of Acetic Acid necessary to reach the desired pH was applied, except the beakers in the pH 10 column, as the algae exist naturally at this pH — enough acetic acid was applied to reach pH 9, 8, 7 , and 6 respectively for each column. Then, the dissolved oxygen was measured and recorded for each beaker with an optical Dissolved Oxygen sensor from Pasco Scientific. After 24 hours, the Dissolved Oxygen Concentration in each beaker was measured again and recorded.

Safety and Ethical Concerns:

Given that Acetic Acid is a weak acid, is edible, and that the algae being tested are non-pathogenic, there are no Safety or Ethical concerns for this experiment.

Data:

pH's effect Algae Productivity before 24 hours					
Observed DO (mg/L)	pH				
	10	9	8	7	6
Trial 1	10.28	10.00	10.15	8.60	6.12
Trial 2	10.26	9.90	9.91	9.27	5.60
Trial 3	9.95	9.90	9.66	9.27	5.80

pH's effect Algae Productivity before 24 hours					
Observed DO (mg/L)	pH				
	10	9	8	7	6
Trial 4	9.86	9.67	9.18	9.05	5.55
Trial 5	9.50	9.20	8.94	8.82	6.33
Average	9.97	9.73	9.57	9.00	5.88

Table 2. Data collected on Mar. 14, experiment started on Mar. 14. This table lists the initial concentrations of DO in the beakers of algae. In the order of pH 6, trial 5 → pH 10, trial 1, the Acetic Acid was added to mimic Carbonic Acid. It took approximately 10 minutes to apply the correct amount of acid to every beaker, then the DO content was measured for each beaker with the photosynthetic light on in the same order acid was applied in.

pH's effect Algae Productivity after 24 hours					
Observed DO (mg/L)	pH				
	10	9	8	7	6
Trial 1	7.44	5.17	4.64	2.32	2.41
Trial 2	8.20	5.40	4.40	2.40	2.26
Trial 3	8.11	5.10	3.98	2.64	2.46
Trial 4	8.10	5.04	3.72	1.76	1.60
Trial 5	8.21	4.89	3.15	1.53	2.95
Average	8.01	5.12	3.98	2.13	2.34

Table 3. Data was collected 24 hours after initial pH was measured. The DO content was measured in the order pH 6 trial 5 → pH 10, trial 1.

Percent Change of pH's effect Algae Productivity after 24 hours					
Percent Change	pH				
	10	9	8	7	6
Trial 1	-27.6%	-48.3%	-54.3%	-73.0%	-60.6%
Trial 2	-20.1%	-45.5%	-55.6%	-74.1%	-59.6%
Trial 3	-18.5%	-48.5%	-58.8%	-71.5%	-57.6%
Trial 4	-17.8%	-47.9%	-59.5%	-80.6%	-71.2%
Trial 5	-13.6%	-46.8%	-64.8%	-82.7%	-53.4%
Statistical Data					
Average	-19.6%	-47.4%	-58.4%	-76.3%	-60.3%
Relative		-27.8%	-11.0%	-17.9%	16.1%
Standard Error of the Mean			0.1183152582		

Table 4. This table indicates the percent change from the original DO concentration to the DO concentration present after 24 hours. This table gives insight into how much the algae tend to change their photosynthetic production at each pH level in such a way that it is relative to their production before 24 hours.

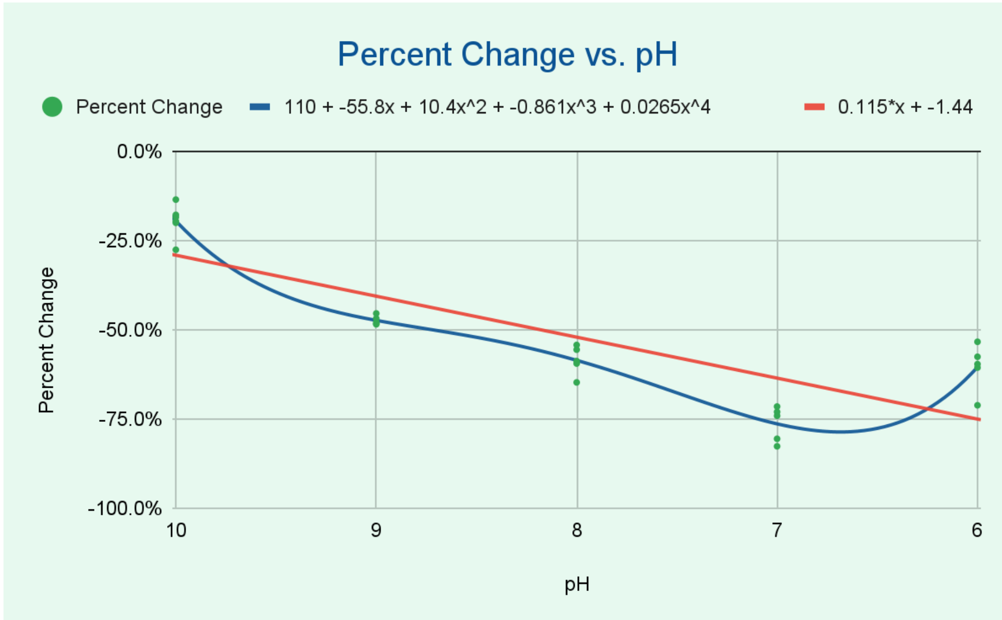

Figure 2. This graph shows two trendlines: one which shows the 4th level polynomial relationship of the two variables, and another which shows the linear relationship of the data. Data points for both are expressed in green.

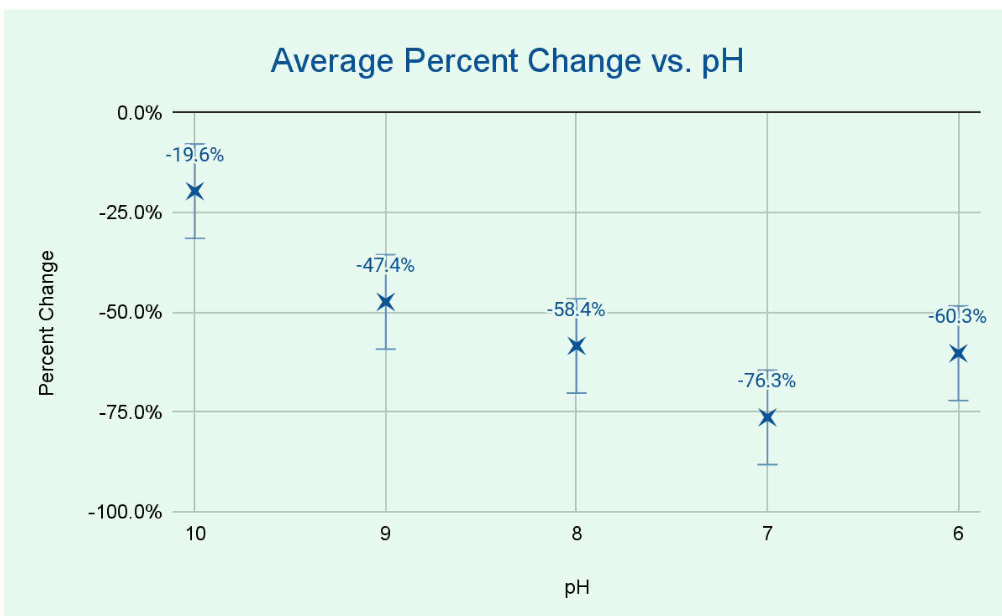

Figure 3. This graph shows the average percent change of the sampled population as X marks, as well as the Standard Error of the Mean as error bars.

Conclusion:

According to the data, pH affects the photosynthetic productivity of *Nannochloropsis oculata* to the extent that greater than 75% of the Dissolved Oxygen concentration (presumably related to the algae's presence and productivity) was reduced in the lowered pH environment. In relation to the aforementioned hypothesis, the algae were most productive at pH 10 but not least productive at pH 6, suggesting that it is partially correct.

According to Figure 2, it is possible that the change from initial oxygen concentration is bimodally distributed. With respect to Figure 3, one can determine that the red line (linear) does not match the data as its expected values for pH 7 and pH 6 do not fall within the error bars. The blue line (polynomial) suggests that the algae could become similarly productive to pH 10 at pH 5. The culture tank from which all algal samples used in this experiment come from exists naturally at pH 10. This suggests that the algae are adapted to function best at pH 10, although, with environmental modification the algae function similarly well at pH 6 or lower.

According to Figure 2's blue line, the data could have a bimodal distribution. Even with such a distribution, because the culture tank naturally exists at pH 10, it is reasonable to conclude that the algae's productivity at pH 6 or lower will not exceed that of itself at pH 10.

It is difficult to set a value at which one should consider the ocean as 'acidified'. While there are many reasons for this, primarily it is that there is no end to the extent at which humans can acidify the ocean. Quite often humans use substances near or at pH 1 or 2. This, while not bad, reduces one's awareness of the effect that it has on life and wellbeing. If the ocean were to reach a pH of 1 or 2, it is completely reasonable to assume that through some process, most life would be extinct. For the purposes of this experiment, pH range 10 to 6 was chosen to represent acidification because of the scope of time. For example, according to figure 1, it would take the ocean 3,254 years to reach pH 2. This scope of time is too broad, so a time window of at most 900 years was chosen, which led to pH as the lower boundary. As for pH 10, the algae naturally exist at this pH, so it was unimportant to basify them as that is the opposite of the subject of this paper.

According to the International Union for Conservation of Nature, the ocean has already begun to show signs of deoxygenation. By 2100, it is expected that ocean deoxygenation levels will fall 1-7% from their first recorded levels in 1960 which were . According to Figure 1, by the year 2100 the ocean will have a pH of 7.9. Interestingly, this observation of the IUCN fits the data observed here. As a decrease of 1 pH level will decrease 11 percent (Table 4), it is reasonable to deduce that a decrease of 0.1 pH levels will decrease the oxygen from 1-7%.

As discussed by Golda (2017), many other experiments and researchers have found that pH's effect on Algae extends deep into their physiology and activity. Some observations include effects on lipid composition, energy, and biomass, as well as cell health and rate of reproduction. Therefore, photosynthetic effects of pH on algae are acceptable to consider given the wide range of other influences pH has on algae.

Evaluation:

Sources of Error:

A source of error was the order in which the Acetic Acid was poured into the solutions. As previously mentioned, the beakers were acidified in the order of pH 6, trial 5 → pH 9, trial 1. The Algae's response to acidification was instant, therefore, waiting 10 minutes while the other samples were in the process of being acidified may have affected the results for those beakers which were acidified first [pH 6]. To correct this, the DO Concentration should be measured immediately for each beaker.

Another potential source of error is the amount of available nutrients present in each sample. As each beaker's levels of salt, potassium, Phosphorous, Nitrogen, etc was not tested, some change can be explained by difference in nutrient content.

Limitations:

Another limitation was the Dissolved Oxygen sensor. Ideally, there would have been a sensor for each beaker and the sensor would have been perfectly calibrated. However, the sensor registered a 'peak' on first insertion after stirring the solution, but then it would trend downward towards zero in all beakers. To standardize the data collection, the peak was taken as the DO concentration.

A major limitation was the replacement of Carbonic Acid with Acetic acid, which may have resulted in unintentional consequences. However, due to the difficulty of producing an environment which can accurately represent pH changes due to gaseous CO_2 concentrations it was unavoidable to use an alternative acidification method.

Improvements:

In addition to correcting all sources of error, any future experiments would be done with a sensor calibrated to salt water conditions and the mean after a set amount of time would be taken to record to standardize data collection. Finally, the beakers would not be stirred as to introduce Oxygen back into the sample.

Solution:

There are many solutions to ocean acidification like sequestration of atmospheric carbon dioxide, decreased industry in areas near the ocean, and ceasing the dumping of waste into the ocean. According to the IUCN (2019), the two most important and most effective solutions to ocean acidification would be the decrease of carbon dioxide emissions [through fossil fuels] and the reduction of ocean nutrient pollution.

Decreasing Fossil Fuel use will decrease the rate of acidification and it may even reverse the processes. For example, in 2020's lockdown seasons the global average CO_2 concentration fell an entire ppm because of a few month's inactivity. For a sustained period of time, the effect on the ocean's pH and oxygen levels would be realized. However, the Fossil fuel industry provides jobs for millions of people and would have large and unforeseen anthropogenic impacts. The economical risks associated with decreasing perceived supply of fossil fuels have been realized with "shortages" which has caused price gouging many times in the past. Thus, permanent restriction of their use would surely cause panic, although, the risk of money in comparison to clean and breathable air, drinkable water, and life are surely worthy of competition.

Introduction of human waste materials like plastic, biowaste [such as sewage and used medical products], and non-natural products into the ocean is another major contributor to the deoxygenation and acidification of the ocean. Specifically, effluent pipes of treated and untreated sewage from factories, water treatment centers, etc. introduce chemicals into the ocean which affect its wildlife and increase its pH. Illegalizing the dumping of human waste materials into the ocean would alleviate the stress it causes on ecosystems and the ocean itself greatly. However, the economical impact of forcing relocation of dump sites for materials of waste could be economically costly until a proper solution is found.

1,815 words

References:

Golda, R. (2017, August). *Assessing the influence of environmental pH on algal physiology using a novel culture system*. Sea Grant Oregon State University; Oregon State University. https://seagrant.oregonstate.edu/sites/seagrant.oregonstate.edu/files/y-17-003_assessing_the_influence_of_environmental_ph.pdf

How much oxygen comes from the ocean? (2021, February 26). NOAA's National Ocean Service. https://oceanservice.noaa.gov/facts/ocean-oxygen.html

International Union for Conservation of Nature. (2019, December 6). *Ocean deoxygenation*. IUCN. https://www.iucn.org/resources/issues-briefs/ocean-deoxygenation

Langdon, C. (2010, May). *Time series of atmospheric CO 2 at Mauna Loa (ppmv) and surface ocean pH and pCO 2 (µatm) at Ocean Station Aloha in the subtropical North Pacific Ocean*. ResearchGate; Scripps Institution of Oceanography. https://www.researchgate.net/figure/Time-series-of-atmospheric-CO-2-at-Mauna-Loa-ppmv-and-surface-ocean-pH-and-pCO-2-matm_fig1_253285582

Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., Bullister, J. L., Wanninkhof, R., Wong, C. S., Wallace, D. W. R., Tilbrook, B., Millero, F. J., Peng, T.-H., Kozyr, A., Ono, T., & Rios, A. F. (2004). The Oceanic Sink for Anthropogenic CO 2. *Science*, *5682*, 367–371. https://doi.org/10.1126/science.1097403

Teem Earth, & McGee, M. (2015, August 29). *Daily CO2*. Earth's CO2 Home Page; CO2.Earth. https://www.co2.earth/daily-co2