Carbon dioxide compensation by use of algae

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1. Abstract

Globally, the enhanced greenhouse effect is a large problem. The next generation is concerned about the future of our planet. If humanity does not slow down the emission of greenhouse gasses, the temperature on earth will keep rising at alarming rates. Planes produce a large amount of CO_2. At Maurick College in Vught lots of journeys are made abroad by plane.

The increase in CO_2 in the atmosphere has major consequences for the Netherlands: an increase of the sea level, the extinction of several species and even food and water shortages. That is the reason why it is really important to limit the contribution to global warming of our school. Global warming must be prevented by removing CO_2 from the atmosphere. Plants seem to be a good solution because of their ability to take CO_2 from the air and convert it into oxygen and sugars. Algae are single-celled plants that are easy to grow and use.

In our research the amount of CO_2 uptake of algae is investigated; how quickly is CO_2 absorbed by algae and what is the role of the temperature? With this data the amount of algae needed to compensate our flight to Florida will be calculated.

2. Keywords

Climate change, algae, carbon dioxide

3. The purpose of the research

The reason of doing this research is seeing the importance of doing something against the greenhouse effect and the global warming. 195 countries decided in "The Paris Climate Agreement" that in 2050 the rising of the average temperature on earth must have been decreased with 2 degrees Celsius. This decreasement can be reached by taking consequences for the agriculture, industry and for the citizens. Last year research has been done about the consequences from the greenhouse effect for the Netherlands and there has been concluded that, if the Netherlands does not take action soon there are terrible consequences for this country and the rest of the world. 3,93 million people in the Netherlands live below sea level and if the sea level will rise another one meter, 6,57 million people would live below sea level and would die.

This is terrible and something needs to be done against it. But the question is how? To start it is important that everyone is aware of the consequences and so try to reduce their CO_2 footprint. The school 'Maurick College' organises a lot of educational trips, often done by plane. Those trips are very good for the development of the students however it is not good for the global warming. It is important that the school finds a solution that those educational trips can be made and that it is CO_2-neutral. A great solution is using algae. Algae absorb CO_2 and convert it into oxygen and glucose because of the photosynthesis. The only thing the algae need to convert it, is water, and sunlight. This process shows that the algae absorb the CO_2 and so the amount of CO_2 in the atmosphere will decrease. In this research there is done investigation to find the best circumstances for the algae to absorb CO_2. With a CO_2 -sensor, the amount of CO_2 the algae absorb in a specific amount of time with the special circumstances, is measured. Also a pond is designed, that will be created in the school garden. The algae are in this pond and there is tried to realise the best circumstances for the algae. A goal is to make the flight to Florida CO_2 -neutral and so the amount of algae there is needed to realise that, is calculated. That amount of algae is put in the pond and so all the CO_2 emissions that are made with the plane, are compensated by the algae.

To conclude, the reason of this research is to prevent the Netherlands from a flood and the rest of the world from all these terrible consequences.

4. The method of research

First of all, research has been done by studying literature about the most effective and environmental friendly way to compensate CO_2 emissions. After concluding that algae are a potential way to compensate the CO_2 emissions, literature research has been done about the processes of photosynthesis that algae use. The best circumstances for the algae to grow were concluded and the ways to make sure that the algae could grow under these circumstances were sorted out. When the used circumstances were determined, the tests could be done at the three different temperatures. Finally, there has been calculated how many algae were needed to compensate the CO_2 emissions of the flight to Florida. Below, the literature research, the processes in and around algae and the components of the tests are further explained.

4.1 The need of algae

As many researches have showed, algae are capable of doing a process called photosynthesis (Vidyasgar, 2016). This means that they can take carbon dioxide out of the air and replace it with oxygen (Vidyasgar, 2016). This way algae can play a big role in reducing the high level of carbon dioxide in the air (Vidyasgar, 2016). Furthermore, algae are very easy to grow and you can grow them anywhere (Vidyasgar, 2016). By growing algae, you can get huge quantities of algae in a short period of time (Vidyasgar, 2016). And when you have more algae, this enhances the effect of the algae on the carbon dioxide levels even more (Vidyasgar, 2016). Algae are also very cheap, which makes such a project economically-feasible (Vidyasgar, 2016). The Rockefeller foundation already developed the technology to grow, harvest and process algae on large economically-feasible scale. (Levin, 2018)

4.2. The type of algae

The type of algae that has been used in the experiment is called *Chlorella Sorokiniana*, or in short, *C. Sorokiniana*. This sort is a freshwater microalgae with a characteristic emerald green color (Bashan, 2015). It was found by a Dutch microbiologist called Martinus W. Beijerinck in 1890 (Bashan, 2015). It belongs to the genus of *Chlorella* (Bashan, 2015). The name *Chlorella* is taken from the Greek "chloros", meaning green, and the Latin word "ella", meaning small (Bashan, 2015). *Chlorella* can, besides being used for reducing carbon dioxide in the air, also be used as a source for food and energy because of its high photosynthetic efficiency (Bashan, 2015). Research from the Russian CELSS experiment BIOS-3 determined that *Chlorella* could remove carbon dioxide and replace it with oxygen (Bashan, 2015). This species divides itself from the rest with its fast cell division rate of four new cells every 17 to 24 hours (Bashan, 2015). This specific sort is also used to research a way to improve biofuel efficiency (Bashan, 2015).

4.3. Photosynthesis

One of the properties of algae are chloroplasts, which make sure that the process of photosynthesis can take place. Photosynthesis is the process where plants, some bacteria and some protistans use the energy from sunlight to produce glucose from carbon dioxide and water. The general formula for the reaction that occurs when photosynthesis takes place is:

$$6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2$$

This reaction is an assimilative reaction what means that it takes energy to produce the glucose and the oxygen.

4.3.1. The light-dependent reactions

The process of photosynthesis contains two different stages. The first stage consists of the light dependent reactions. This reaction occurs in the grana which is located in the chloroplasts.

Chlorophyll is a complex molecule, needed for the process of photosynthesis. There are several modifications of chlorophyll, which occur among plants and other photosynthetic organisms. All of the photosynthetic organisms contain chlorophyll a. Chlorophyll a. is essential for photosynthesis because of its role as the primary electron donor in the electron transport chain, where the ATP is produced.

When chlorophyll a. absorbs sunlight, an electron gains energy. The electron is transferred to another molecule. The chlorophyll molecule is oxidized and has a positive charge.

Photoactivtation of chlorophyll a. results in splitting of water molecules by a condensation reaction, including phosphorylation.

$$2\ H_2O \rightarrow\ 4\ H^+\ + O_2 + 4\ e^-$$

Phosphorylation, the chemical addition of a phosphoryl group ($PO_3{}^-$) to an organic molecule, is carried out by enzymes. In addition, it is responsible for the transfer of energy to ATP and reduced nicotinamide adenine dinucleotide phosphate (NADP).

The electrons from the split molecules then react with a carrier molecule nicotinamide adenine dinucleotide phosphate (NADP), changing it from its oxidised state ($NADP^+$) to its reduced state (NADPH):

$$NADP^+ + 2e^- + \ 2\ H^+ \rightarrow\ NADPH + H^+$$

There is a proportional relationship between the light intensity and the speed of the reactions for photosynthesis. If you increase the light intensity, the process will become faster. Chlorophyll a. absorbs all of the colours of sunlight besides the greenlight. Chlorophyll a. reflects the green colour. The complementary colour of green is magenta. So the algae only use the magenta light, the violet-blue and orange-red wavelengths. To make sure there has been used as less as possible energy, there has been used a growth-stimulating lamp to increase the speed of the process of fotosynthesis and make the process more eficcient. By using a lamp only producing magenta light, the process is more sustainable.

4.3.1. The light-independent reactions

After the light-dependent reaction, the light-independent reaction takes place (Unknown author, R. S. 2004). The formed ATP and NADPH are used to make carbohydrates from carbon dioxide (Unknown author, R. S. 2004). Finally, glyceraldehyde 3-phosphate is formed (Unknown author, R. S. 2004).

The ATP is produced by the following process:

Electrons provide energy passing through the transport chain to pump H^+ ions from the stroma, across the thylakoid membrane into the thylakoid compartment (Unknown author, R. S. 2004). H^+ ions are more concentrated in the thylakoid compartment than in the stroma. H^+ ions diffuse from high to the low regions of concentration (Unknown author, R. S. 2004). This drives the production of ATP (Unknown author, R. S. 2004).

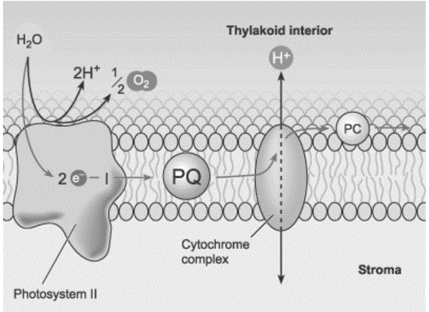

Figure 1: Light-independent reaction photosystem II. (Unknown author, R. S. 2004)

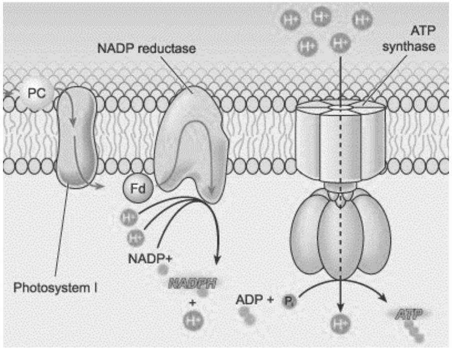

Figure 2: Light-independent reaction photosystem I. (Unknown author, R. S. 2004)

4.3.2. The light-dependent reactions

Another characteristic of algae is the stomata (Boris Börger, 2014). Those stomata are responsible for the carbon dioxide uptake (Boris Börger, 2014). Stomata are located on the underside of the algae (Boris Börger, 2014). Via the stomata flow the fabrics to the algae and away (Boris Börger, 2014). If there is enough light and the surrounding is wet enough, the stomata open up (Boris Börger, 2014). Carbon dioxide will flow into the algae and oxygen will flow out of the algae (Boris Börger, 2014). When the stomata are closed the process of photosynthesis will stop (Boris Börger, 2014). An increase in the concentration of carbon dioxide, increases the rate at which carbon is incorporated into carbohydrate and so the whole process of photosynthesis (Boris Börger, 2014).

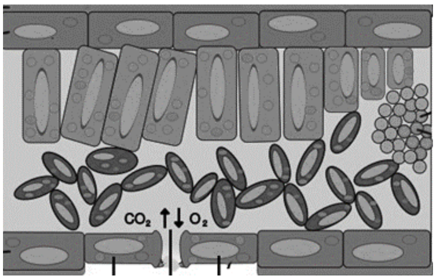

Figure 3: The working of the stomata (Boris Börger, 2014)

4.3.3. Temperature

Photosynthesis is dependent on temperature. It is a reaction catalysed by enzymes. As the enzymes approach their optimum temperatures the overall rate increases. Above the optimum temperature the rate begins to decrease until it stops. When these enzymes work at their optimum, the photosynthetic system produces the most chemical energy. This allows the algae to grow faster and reproduce more. What means that there is a bigger uptake of CO_2.

Three different temperatures are used to recreate the average water temperatures in the North Sea: 5.5 °C for winter, 20 °C for summer and 15 °C for autumn to see when the algae absorb the highest amount of CO_2. These values are averages of several years back in time.

Table 1: the average temperatures in the North Sea in degrees Celsius. (Edwin, 2012)

	2006	2007	2008	2009	2010	2011	2017	2018	Average
Winter	6	8	6	5	5	3	7	6	5.5
Autumn	14	16	15	15	14	15	15	16	15
Summer	23	19	20	20	20	18	19	21	20

To control a temperature during an experiment, a climate cabinet is used. This cabinet keeps the air temperature constant.

4.4. Nutrients

Furthermore need algae nutrients to grow. These anorganic elements will be converted by carbohydrates to organical elements like proteins and fats. The proteins and fats make sure that there will be released energy from ATP. ATP stimulates then the process of photosynthesis.

The optimum of grow for algae is in eutrophied water. This means water with a high level of ions and nutrients. The water on the surface in the agriculture contains eutrophied water. Tabel 1 shows the concentrations of nitrogen and phosporus in the agriculture in the Netherlands.

Table 1: nitrogen and phosphorus emissions to surface water from agriculture soils in relation to soil type for two periods ($kg\ ha^{-1}\ j^{-1}\ N\ or\ P$) (Schoumans, 2008)

		Sand			Clay			Peat
		Wet	Moderately dry	Dry	Wet	Moderately dry	Dry	Wet
1986-2000	N	65.1	58.7	31.4	36.2	24.6	22.0	28.5
2016-2030	N	33.9	23.6	14.5	25.2	16.9	15.0	19.1
1986-2000	P	2.60	0.93	0.42	2.86	1.97	1.26	3.10
2016-2030	P	2.60	0.85	0.38	2.62	1.93	1.20	2.94

According to the commodity card of 2006 (Berg, 2017), the biggest part of the Netherlands contains sand. So the values chosen are 65,1 N and 2,60 P. Par 150 mL of water, there has been 1,84 gram $K_3PO_4 \cdot 3H_2O$ and 19,7 gram KNO_3(see the appendix for the calculation)

To blend the nutrients and water, a magnetic stirrer has been used to create a flow in the water.

4.5. The pH range

Complete culture collapse due to the disruption of many cellular processes can result from a failure to maintain an acceptable pH. Most of the algae do well if the pH is between 6 and 8. The enzyme activity is higher between the 6 and 8. If the pH is divergent, there will take place denaturation. This means that the enzymes lose their spatial structure. The properties and the operation will change. According to the research from Wageningen University, the optimum for *C. sorokiniana* is 6,7 (Leerlingen van Het Streek uit Ede, 2013). pH fluctuations can be limited by using a chemical buffer. The water is containing nutrients. The nutrients solution was set with hydrochloric acid. The hydrochloric acid is added till a pH of 6,8.

Figure 5: the measurement of the pH

4.6. Grow bulb

Chlorophyll a. absorbs all of the colors of sunlight besides the green light. Chlorophyll a. reflects the green color. So the human eye only sees the green color. The complementary color of green is magenta. The algae only use the magenta light. A lamp uses energy. To make sure there is as less as possible energy, there has been used a lamp for magenta light.

Figure 6: the grow bulb and the rest of the test set up

4.7. Counting chamber

For the research it is important to know how many algae there are in the solution before and after the test. With this information it is possible to calculate precisely the amount of CO_2 that is absorbed by a specific amount of algae. First, 7 ml of the solution containing nutrients and buffer was measured with a volumetric pipette. This solution has been mixed with 7 ml of the algae. To determine how many algae 14 ml of the solution with algae contained, one drop (0,04 ml) of this solution with algae was put on a counting chamber and was studied with a microscope. This counting chamber has boxes with a certain surface. The original box consisted of 16 smaller boxes from each 0,25 mm by 0,25 mm and each of those boxes consisted of 25 smaller boxes from 0,05 mm by 0,05 mm. These different boxes were very beneficial for counting the algae. Because each box in which the algae had been counted could be checked off. Thus it became clear how many algae each box contained and how many algae could be found in 0,04 ml of the solution. Because of that, it was possible to calculate how many algae the complete solution (14 ml: 7 ml + 7 ml) contained. Because for instance, when in 0,04 ml of the solution are 100 algae, in 250 ml of the solution there are: $\frac{250}{0,04} \cdot 100 = 625\,000$ algae. This provided a significant view about the quantity of the algae there were in the solution before the test had been started.

4.7. The CO_2 meter

In the research, a CO_2 meter is used to measure the concentration of CO_2 in the air above the algae. The CO_2 meter used is the Grove – CO_2 & Temperature & Humidity Sensor (SCD30) from Seeed. The meter is calibrated to ensure it gives the right measurements. The calibration is done the way it was described in the user manual. This is added to the appendix. A program for the calibration was included with the meter. It was required for the program to keep the meter on for 7 days straight and it had to be outside for at least one hour per day. This way the calibration program could find the right settings for the meter.

The program we used to run the meter while taking measurements is written in the latest version of Arduino. The meter takes measurements every three seconds. In this measurement the concentration of CO_2, the temperature, the humidity and the time are concluded. The meter is connected to a Seeeduino V4.2 computer board. This computer board is connected to the computer via USB.

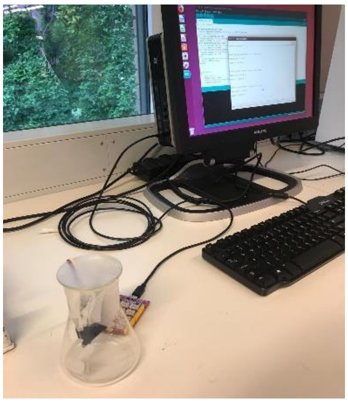

Figure 6: the meter in the erlenmeyer connected to the computer

The computer uses processing 3 to run the program. The program saves these values as a vsc file. This program used is written out in the appendix. Excel is used to open this file file and to depict a chart.

4.8. Dissolving CO_2 in water

The decrease of the amount of CO_2 is not only because of the absorption of the algae, there is also an amount of CO_2 that disappears in the water. The CO_2 is dissolved in the water, this process of dissolving takes place in 3 processes: (Riebeek, 2008)

(1) $CO_2 + H_2O \leftrightarrows H_2CO_3$

(2) $H_2CO_3 + H_2O \leftrightarrows HCO_3^- + H_3O^+$

(3) $HCO_3^- + H_2O \leftrightarrows CO_3^{2-} + H_3O^+$

(1) $K = \frac{[H_2CO_3]}{[CO_2]}$

(2) $K = \frac{[HCO_3^-]*[H_3O^+]}{[H_2CO_3]}$

(3) $K = \frac{[CO_3^{2-}]*[H_3O^+]}{[HCO_3^-]}$

The combination of hydrogen ions with carbonate form bicarbonate. Bicarbonate is a form of carbon that does not escape the water easily and determine the concentration of carbon dioxide in the water.

The concentration of carbon dioxide in the water depends on the temperature of the water. As water temperature increases, its abilitiy dissolve CO_2 decreases. This is because of the decrease of the pKw, when the temperature rises. The concentration of the bases and acids increases, the equilibrium constant decreases and the water dissolves less CO_2. Also the pH-value has an important influence of the ability of water to dissolve CO_2. If the pH-value increases the concentration of H_3O^+increases. The balance will shift to the left, because of the higher amount of H_3O^+. The amount of HCO_3_will decrease and so does the ability of dissolving the CO_2 in water.

5. Results of the research

In every experiment executed an increase in the PPM of CO2 in the first few hours can be seen. To find out which factor causes this increase a reference measurement has been executed for every temperature. In the reference measurement every factor has been kept the same except for the algae. In the reference measurement the algae are left out. This reference measurement gives the same increase at the beginning of the experiment. A connection has been found: The bigger the temperature difference, the longer it takes until the increase comes to a stop and levels out.

This increase can be declared by the temperature difference between the water inside the erlenmeyer and the air outside of the climate cabinet. Because the water temperature is still at the temperature of outside the climate cabinet, the water has to cool down to the right temperature. The sensor has to adjust to the temperature inside the erlenmeyer. Because of the temperature dropping to the right value, the sensor has to constantly adjust. This results in an increase of the PPM CO_2 in the first few hours. When the temperature drops to the right value, the increasement stops and comes to a constant value for a moment. This value will be used as our starting value.

Also there can be seen slight fluctuations in all of the graphs. These fluctuations appear because of the erlenmeyer not being completely airtight. when the school opens, students and other people make the CO_2 level inside of the building rise. These fluctuations mostly happen through the week. These fluctuations are similar in the experiments without algae what proves that they are not due to the algae.

5.1. Test at 5.5 degrees

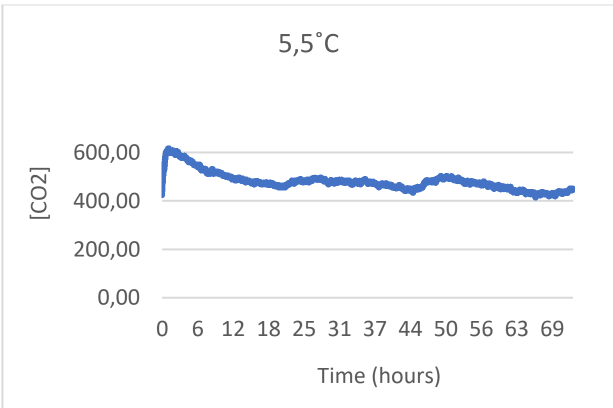

Figure 6: test at 5,5˚C with algae

In figure 6 the CO_2 concentration at 5,5˚C is plotted against the time. We can see that there is a small decrease in the concentration CO_2 from the starting value to end value. The starting concentration of CO_2 is at 2:09:01 with a value of 600,34 ppm. The end value is at 73:10:36nwith a value of 446,32 ppm. With this in mind the difference can be calculated: 154,02 ppm

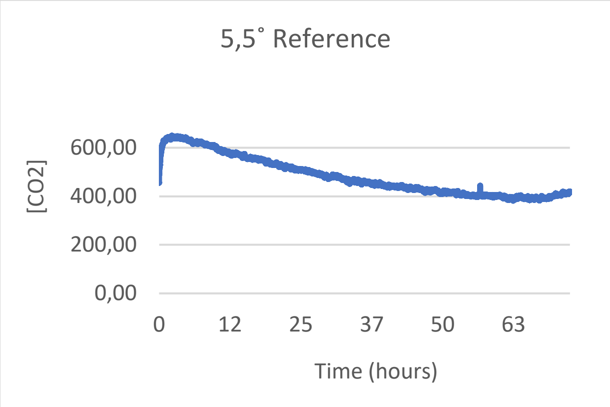

Figure 7: Reference test at 5,5˚C

Above the Reference experiment at 5,5˚C can be seen. At the beginning of the experiment the same peak can be seen as in the experiment with algae. The difference between the starting and end value is similar to the difference seen in the experiment with algae: 198,83 ppm. This similarity is the effect of the low temperature. At this temperature, algae are unable to take in a lot of CO_2 and thus are the results of the reference experiment and the experiment with algae practically the same.

5.1.2. Test at 15 degrees

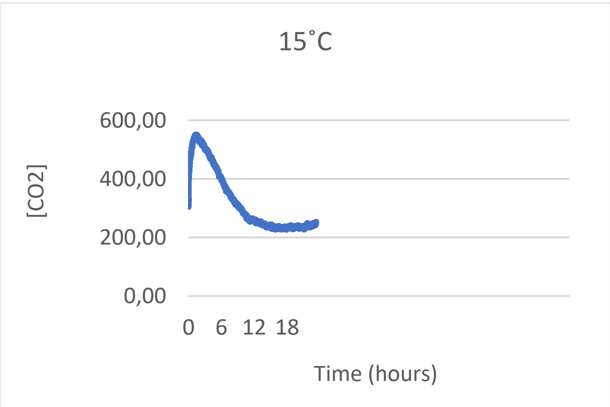

Figure 8: Test at 15˚C with algae

Figure 8 shows the reference experiment at a temperature of 15 degrees. As seen in the graph, the meter stops working after a little more than 24 hours. This time span is too short to see a clear drop in the concentration of CO_2. The decision is made to not conclude the experiments in the final conclusion.

5.1.3. Test at 20 degrees

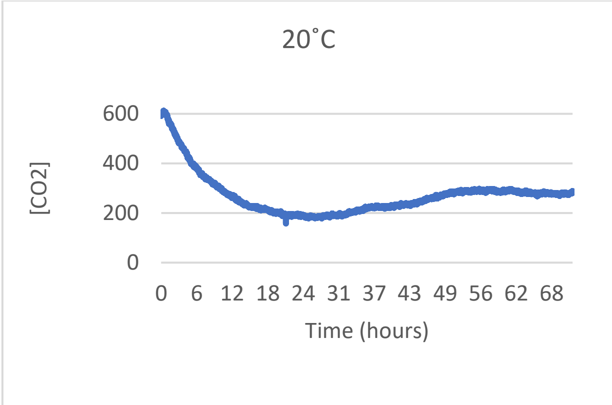

Figure 9: Test at 20˚C with algae

In this experiment we have a starting value of 612,01 ppm which occurs at 2:45:23. The end value is 284,59 ppm at 72:26:46. There can be seen a relatively large difference in concentration, namely 327,42 ppm.

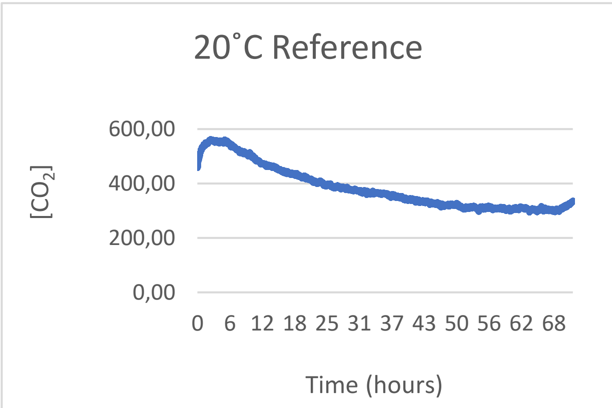

Figure 10: Reference test at 20˚C

The difference in concentration in the reference test is 225,5 ppm. The concentration difference in the experiment with and without algae at 20˚C is significantly further apart compared to the experiments with and without algae at 5,5˚C and a faster decline in the concentration of CO_2 can also be seen. These are the results of the much higher temperature. Higher temperatures cause the algae to grow and therefore process more CO_2, which results in a lower concentration of CO_2. This growth can also be seen in the pictures in the appendix.

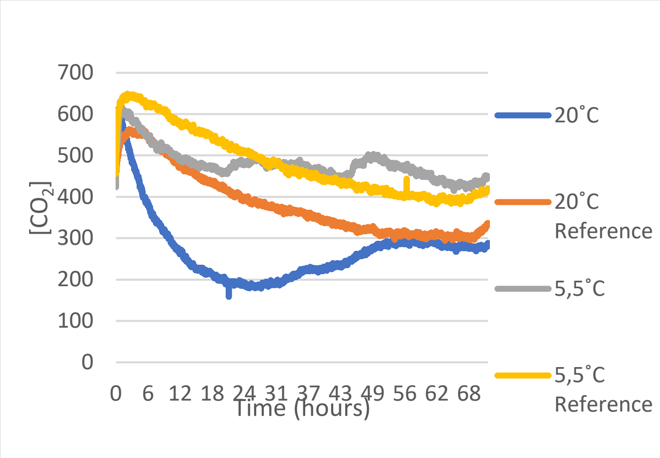

Figure 10: All the tests in one graph

In Figure 10, the results are shown in one graph. This clearly shows the difference between the results. There can be seen an immediate drop in the beginning of the experiments with algae.

5.2. The amount of algae needed for compensation of flight

The emission of CO_2 of our flight from Amsterdam to Bradenton has been calculated at the site www.flygrn.com . For 4 persons the emission of $CO_2$is $8{,}94 \cdot 10^3$kg.

The idea was that this would be compensated by a pond at the school premises. The amount of algae needed for this compensation depended on the four different seasons in the Netherlands. For each season there has been calculated how much CO_2 there has been absorbed. The spring and the autumn has about the same temperatures of the water. For the compensation there is needed $6{,}10 \cdot 10^7$ L of algae to compensate in one year $8{,}94 \cdot 10^3$ kg CO_2. (see the appendix for the calculation). This is equal to 24,4 Olympic sized swimming pools.

6. Conclusion

At the temperature of 20 degrees the algae has absorbed the highest amount of CO_2. In 72 hours and 26 minutes the algae absorbed 327,42 ppm CO_2. The emission of the flight from Amsterdam to Bradenton is $8{,}94 \cdot 10^3$kg CO_2. To compensate the flight in one year there will be needed a pond of $6{,}10 \cdot 10^7$ L algae. This is equal to 24,4 Olympic sized swimming pools.

7. Discussion

The fluctuations may be caused by the way the erlenmeyer and the climate cabinet are sealed. Because the amount of CO_2 in the school depends on the amount of people that are in the school at that time, the amount of CO_2 in the climate cabinet can be affected. A solution might be to seal the erlenmeyer with a cork instead of parafilm so that nothing but the meters cord can go through. This will make sure that the erlenmeyer is hermetically sealed.
Calibrating the CO_2-sensor before you start the test really is recommended. This way, experiment can be executed much faster and there won't be any retardments. When the sensor is not calibrated, the results are not reliable and there will be some fluctuations.

It is important to keep the algae in the right circumstances, meaning with enough light and the right temperature. Otherwise the algae will die.
Also, the amount of algae needed to compensate the flight to Florida can be higher, because the experiments performed were with 14 mL algae. This is very little in comparison to $6{,}10 \cdot 10^7$ L, which is needed. The processes of photosynthesis is partly dependent on the light. If you create a pond which is very deep but not that wide. The substance with algae below will have less light and the algae will absorb less CO_2.
Further research can been carried out how the algae grow. Now it is hard to say precisely how many algae have absorbed the amount of CO_2.This could maybe be researched by the way of procreate by algae.

8. Acknowledgements

First, we would like to especially thank Mr. van der Laag for guiding this research. He always supported us and our ideas and he gave useful advice. in addition, we would like to thank the rector of the school Mr. van de Linden for the great opportunity to take place in this conference and for helping financing this trip. We would like to thank Mrs. Boertjes and Mr. Vogels for making sure that we had access to the correct materials and that we knew how to use it. Than we would like to thank Mrs. Siebenhuener for giving her view on this report and for helping us with improving our english writing skills.

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