

The Effect of Concentration of CO₂ on the Average Rate of Photosynthesis in Spinach Leaf Disks

Amelia Robinson-Brown

Heathwood Hall Episcopal School, Columbia, SC 29205

The purpose of this experiment was to determine the effect of carbon dioxide concentration on the rate of photosynthesis in spinach leaves. Small circular disks were cut out of the spinach leaves using a standard hole puncher. Then solutions of differing concentrations of carbon dioxide, 0.2%, 0.4%, 0.6%, 0.8%, and 1.0%, were all prepared, and each solution was spread equally among five cups. There was also a control solution that contained only water. Any gases within the leaves were then sucked with a syringe, using a specific technique to create a vacuum. For each solution of differing carbon dioxide concentration, there were five cups of solution with ten leaf disks per cup, so there was a total of 50 leaf disks per concentration. The cups were then exposed to light for 20 minutes, and the number of disks floating in each cup was measured every minute. Results were calculating by finding the ET₅₀ for each concentration. The ET₅₀ is the time it takes for 50% of the leaf disks to float and is a good indicator of the rate of photosynthesis. It was hypothesized that if the concentration of carbon dioxide was increased, then the rate at which photosynthesis occurs will also increase. The null hypothesis was that the concentration of carbon dioxide will have no effect on the rate of photosynthesis. The results of the experiment supported the hypothesis. So, the experiment suggests that there is a direct relationship between CO₂ concentration and the rate of photosynthesis.

Introduction

While photosynthesis may be considered to be a rather basic process, it is actually a complex and essential process to life on Earth. So, what has an effect on this process? The purpose of this study was to answer this question by studying the effect of carbon dioxide concentration on the rate of photosynthesis. Since photosynthesis is the primary source of the oxygen which humans require to survive, it is pertinent that the process is understood to its full extent. The first step of this study was to gather background information pertaining to the experiment in order to perform the most effective study as possible. Determining a method to measure the rate of photosynthesis was an outstanding question, but it was discovered that the rate can be determined either by measuring the consumption of carbon dioxide or the accumulation of oxygen (College Board 1997). It was determined that the rate would be determined by the accumulation of oxygen, which was represented by how long it took leaf disks, that were devoid of any gases, to float. According to another study, "Rising [CO₂] will impact plants and ecosystems through two processes, reduced *g_s* and increased *A*. Our understanding of the mechanism by which Rubisco responds to short-term increases in [CO₂] is well advanced, and our understanding of the different components of the guard cell-signaling pathway is advancing" (Ainsworth 266). This means that increased levels of carbon dioxide do have an effect on the rate of photosynthesis, but that particular investigation went into a deeper search of the process that is beyond the scope of this experiment. With this background information in mind, it was then clear how to proceed with the experiment.

The study consisted of testing four solutions with differing concentrations of carbon dioxide, along with one solution containing no carbon dioxide, which served as a control. Each solution was distributed equally into five labeled cups, and ten leaf disks were later inserted into these cups. The concentrations tested were 0.2%, 0.4%, 0.6%, 0.8%, and 1.0%. The cups were exposed to a light source for twenty minutes and the number of leaf disks floating was measured every minute. Once the data collection period was over, the ET₅₀ was calculated for each cup. This was done by calculating the amount of time required for 50% of the leaves to float. An average time was then calculated in order to find the average rate of photosynthesis for each concentration. It was hypothesized that if the CO₂ concentration were increased, then the rate of photosynthesis would also increase. The null hypothesis was that the concentration of carbon dioxide would have no effect on the rate of photosynthesis. All of the articles that were used for background research were in support of this hypothesis.

Methods

Experimental Design Matrix

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|---|---|
| Title: The Effect of Concentration of CO ₂ on the Average Rate of Photosynthesis in Spinach Leaf Disks | Hypothesis: If the concentration of CO ₂ is increased, then the rate of photosynthesis will increase, causing the leaf disks to float faster. |
| Dependent Variable: The Average Rate of Photosynthesis | Independent variable: The Concentration of CO ₂ |
| Levels of Independent variable: 0.2% CO ₂ 0.4% CO ₂ 0.6% CO ₂ 0.8% CO ₂ 1.0% CO ₂ | Number of Leaf Disks Per Trial: 50 50 50 50 50 |
| Constants: The same brand and type of spinach will be used, sodium bicarbonate only from the same bottle will be used, and the length of exposure to the light source and the amount of light emitted will be kept constant. | Control Group: No sodium bicarbonate will be used in the solution, so a solution of water. |

The first step of the experiment was to prepare the bicarbonate solutions. This was done by mixing baking soda into water to create a solution of the appropriate concentration, but in the case of the control no baking soda was added. The purpose of making this solution is that the bicarbonate serves as a source of carbon dioxide, which the leaf disks require, along with water and light energy, to undergo photosynthesis. The solution was then evenly distributed into five clear plastic cups, which were labeled with the concentration of the solution inside. Then a few drops of liquid soap were inserted into the solution, making sure to not make the solution sudsy. If the solution did form suds, more water was added to the solution. This is done because the soap wets the hydrophobic surface of the leaf, which then allows the solution to be drawn into the leaf disks. Then, using the whole punch, ten leaf disks are punched out for each cup, so fifty leaf disks for each concentration. When punching out the disks, it was imperative to make sure that one was not using major veins, but the fleshy, smooth part of the leaf.

The next step was the most difficult and also the most important. All of the gases within the leaf were drawn out by creating a vacuum with the syringe. This was done by first removing the plunger from the syringe and inserting ten leaf disks into the syringe. Then the plunger was re-inserted and pushed down until only a small amount of air was left in the syringe. Next, the syringe was inserted into the cup of solution, and a small amount of liquid (approximately 3 mL) was drawn out. After tapping the syringe to ensure that the leaf disks were suspended in the solution, the syringe was inverted, and the plunger was pushed in until no air remained in the syringe. Then a vacuum was created by covering the opening of the syringe with a finger and slowly pulling the plunger back, making sure that the leaves were still suspended in the solution. This position was held for ten seconds and then the plunger was gently released, allowing it to return to a neutral position. The leaf disks should then sink in the solution because all air has been drawn out of them. If the disks do not all sink, then this process of creating a vacuum can be repeated, but it should not be done more than three times, as to not damage the leaves. Then each syringe should be emptied into the appropriate clear plastic cup. Finally, each cup was exposed to the light source and the number of leaf disks floating was recorded every minute for twenty minutes.

Throughout this entire procedure, the first concern was safety. Safety was ensured by following proper lab procedure while preparing the bicarbonate solution. There was certainly no horseplay and focus remained on the experiment at all times. Safety goggles were worn at all times and caution was used while operating the hole punch. There were no significant hazards in this experiment, and is one that could easily be done at home to aid the explanation of photosynthesis. However, calculating the result does get slightly complicated.

After collecting this data, the ET_{50} was calculated. The ET_{50} is the amount of time it took 50% of the leaf disks to float, so in this case the time at which 5 disks floated. The purpose of calculating the ET_{50} was that the reciprocal of the ET_{50} ($1/ET_{50}$) is the rate of photosynthesis. The average ET_{50} was then calculated for each concentration, as well as the standard deviation. The standard deviation denotes how far apart the numbers were from each other, demonstrating the consistency of the results.

Results

Figure 1 shows the average rate of photosynthesis for each concentration tested. Based on the graph, it appears that there is a direct relationship between the average rate of photosynthesis and the carbon dioxide concentration. In other words, as the carbon dioxide increases, so does the rate of photosynthesis. The high correlation of the best fit line shows that it fits very closely to the actual points. In addition, the standard deviation value was used to calculate the error, and the variance in the data points for each concentration is shown on the graph. Unfortunately, no inferential statistics could be done on the data since the way of measuring the data was through the ET_{50} and since there were only five trials per concentration, there were not enough data points to run a statistical test such as an ANOVA. But the data shown in Figure 1 does support the hypothesis that an increased CO_2 concentration will cause the rate of photosynthesis to also increase.

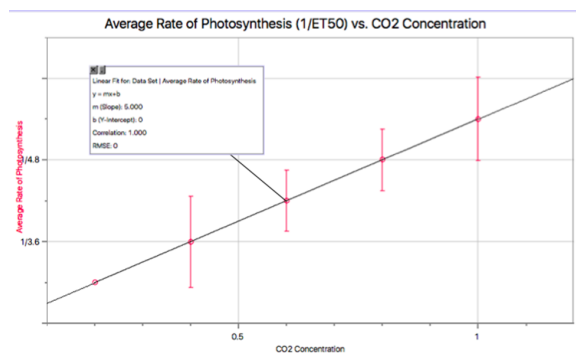


Figure 1

Conclusion

So, the purpose of this study was to discover the connection between the rate of photosynthesis and the concentration of carbon dioxide by measuring how long it took spinach leaf disks to float. The experiment found that there was a connection between the two and that the rate of photosynthesis is affected by an increased concentration of carbon dioxide. This also supports the findings of other studies that tested similar variables. But, errors could have occurred within the experiment when creating the vacuum with the syringe, choosing disks from the same part of the leaves, and with the amount of light exposure. With more time, these errors could be limited, and more data could be collected. Perhaps, future studies could be done comparing the rates of photosynthesis in different types of plants, particularly those that bloom perennially vs. annually.

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Notes and References

Ainsworth, E. A. and Rogers, A. "The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions". *Plant, Cell & Environment*, 30: 258–270. 2007.

College Board. "Lab 5: Photosynthesis." *AP Biology Lab Manual*: S61-S69. 1997.

Amane Makino, and Mae, T. "Photosynthesis and Plant Growth at Elevated Levels of CO₂." *Plant Cell Physiol* 40 (10): 999-1006. 1999.