JOURNAL OF
EMERGING INVESTIGATORS

Article

Different volumes of acetic acid affect the oxygen production of spinach leaves during photosynthesis

Jui-Ning Wang[1]*, Yun-Hsi Hou[1]*, Shiu-Mei Li[2]
[1]Kang Chiao International School, Taipei City, Taiwan
[2]Parent
*Authors contributed equally

SUMMARY

The burning of fossil fuels, leading to an increased amount of carbon emissions, is the main cause of acid rain. Acid rain is precipitation that has elevated levels of hydrogen ions due to the formation of carbonic acid. Acid rain affects the process of photosynthesis, which makes the topic valuable to investigate. In the course of chemistry, our group has learned the background information about acid and bases with its properties. With the passion of biology, our group determined to create a lab that consists of both knowledge of chemistry and biology.

In 1990, the implementation of the Clean Air Act aimed to limit the amount of carbon emission that causes acid rain with a total annual loss of a hundred 97 million dollars in commercial forest. With such findings, our group utilizes plants to further investigate the relationship between pH value and photosynthesis. In this experiment, our group hypothesized that rain with a lower pH will decrease the rate of photosynthesis, causing less oxygen to be produced in the reaction. Extreme acidity destroys chloroplast structure, decreases chloroplast ATP synthase activity, and reduces photosynthesis and plant growth. However, by conducting an experiment with spinach and utilizing a syringe to collect the amount of oxygen produced during the experiment, we discovered a breakthrough in which a slightly acidic environment can foster photosynthesis until it reaches a pH level less than two.

INTRODUCTION

Pollution, caused by burning fossil fuels, releases carbon dioxide, nitrogen oxides, and sulfur dioxide into the atmosphere (1). These greenhouse gasses then turn into carbonic, sulfuric, and nitric acid solutions, causing rainwater to become overly acidic (2). Acid rain decreases the pH of the leaf's growth environment, affecting the nutrition and overall growth of plants negatively (3). Within the last decade, acid rain has become a problem as 11% more polluting gasses are emitted from factories, automobiles, and power plants around the globe (1, 6).. The pH of acid rain ranges from 4.2 to 4.4(4). Our experiment applied and tested the extremes of acid rain, showing the effects on a plant's oxygen production if pollution worsens (5).

Photosynthesis is the process of organisms synthesizing their own fuel by converting light energy to chemical energy (6). The chemical equation of the process of photosynthesis is stated as: $6CO_2$ (g) + $6H_2O$ (l) → $C_6H_{12}O_6$ (s)+ $6O_2$ (g) (7).

We hypothesized that acid rain with a lower pH would decrease the oxygen production of leaves. To address this, we measured oxygen production from spinach leaves with increasing volumes of acetic acid. There are various types of proteins inside the stroma. Stroma is a fluid-filled internal space of the chloroplasts, encircling the grana and the thylakoids. For instance, the protein pumps in the electron transport chain such as P-type, V-type, and F-type (8). When proteins are exposed to acidic environments and exceed their optimal pH values, the hydrogen bonds holding the protein together will kinetically start vibrating, eventually breaking the bonds (7, 8). Thus, the structures would unfold, prohibiting the protein pumps to continue their function (7, 8). Without functional proteins in the chloroplast, photosynthesis would not happen, limiting the amount of oxygen it can produce.

The process converts sunlight and carbon dioxide into glucose, energy, water, and most importantly, oxygen gas (5). In this experiment, we collected the oxygen gas produced from spinach leaves that have been soaked into different volumes of acetic acids, with increasing volume of acetic acids decreasing the amount of oxygen production. We wanted to investigate how soaking a spinach leaf in different volumes of acetic acid would affect the amount of oxygen produced during 30 minutes of photosynthesis.

RESULTS

Our experiment utilized funnels, syringes, and different volumes of acidity to test how different volume of acidity affects the photosynthesis production during the reaction. By sealing the syringe and putting the spinach inside different volumes of acids, we would be able to test out oxygen production. We also used sodium bicarbonate as the experiment took place because it provides carbon dioxide for the plant to absorb as photosynthesis requires not only light but also carbon dioxide, in order to process.

To test how different volume of acetic acid affects the amount of production of oxygen, we place spinach leaves in a syringe with different volume of acetic acids (20ml, 40ml, 60ml, 80ml, 100ml, mixed with 100ml of distilled water), and measured the production of oxygen. By controlling the amount of water filled within the syringe, the size and types of leaves fixed ratio between the amount of water and baking soda, the amount of time it takes to soak the leaves, and the total duration of the photosynthesis process will be 30 minutes as well, starting when the LED lights turn on (**Figure 2**), allow

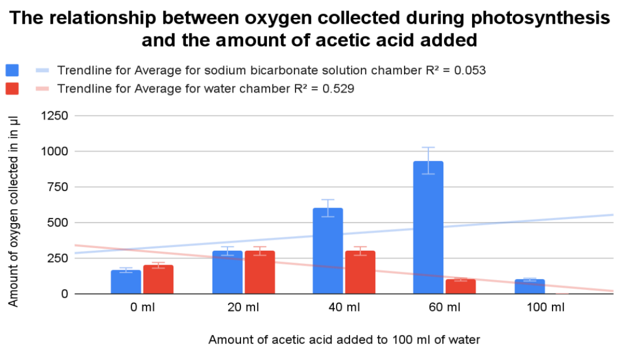

Figure 1: The graph demonstrates the relationship between average oxygen collected during photosynthesis in a sodium carbonate solution chamber or water chamber. Blue represents the average production of oxygen in the sodium bicarbonate chamber, while red represents the average production of oxygen in the water chamber. The blue line indicates the trendline for oxygen produced in the sodium bicarbonate chamber and the red line indicates the trendline for oxygen produced in the water chamber.

enhances the validity of the study that limits the influence of external factors.

Referring to **Figure 1**, we calculated the results that the photosynthesis process in lower pH value produced more oxygen until it reached pH level of 2 (**Figure 1**).

Moreover, from the t-test we calculated, all of the p values are greater than 0.05, which means that the differences between each trial are considered not significant (**Table 2**). This means the data doesn't have significant differences in each trial, proving the accuracy and precision of the data collected.

The relationship between the average gas collected in sodium bicarbonate solution results in a positive correlation which when the acidity of the spinach leaves increases, the amount of oxygen produced during the photosynthesis process increases as well (**Figure 1**). However, when the

Figure 2: The photo represents the experimental set up. We used the water displacement method to find out the amount of oxygen produced. We placed the leaves inside a chamber. The leaves were covered by a funnel with a sealed syringe at the top, collecting the oxygen produced from photosynthesis. The amount of oxygen was measured in microliters.

	0mL	20mL	40mL	60mL	100 mL
volume	0	0.17415g/mL	0.29854 g/mL	0.39184 g/mL	0.52245 g/mL
Molarity	0	2.90 M	4.97 M	6.53M	8.7M
H^+ ions volume	x	0.00722 M	0.00945 M	0.0108 M	0.0125M
pH	7	2.14	2.03	1.97	1.93

Table 1: The table shows the calculated volume, molarity, H+ ions volume, and pH value for each acetic acid solution

concentration reaches 0.522g/mol (8.7 M) the color of leaf will turn from fresh green color into dark brown, producing only 100 microliters of oxygen. On the other hand, the moderate acidity of using 20 mL, 40 mL, or 60 mL of acetic acid mixed with 100mL of water, the spinach leaves accelerated their chloroplasts' activity which also assisted in the photosynthesis process and thus created more oxygen.

We also conducted an additional trial of using water only since the acetic acid that remains on the leaves' surface reacts with the sodium bicarbonate solution which may form more bubbles than it should have. As a double replacement chemical reaction occurs when acetic acid and sodium bicarbonate are mixed, it produces carbon dioxide gasses which we cannot distinguish from oxygen in this experiment. The results shown in **Table 1** demonstrate similar results in each trial, with oxygen production increasing with increasing volumes of acetic acid used to soak the spinach (**Table 1**). The increase of oxygen production ceased at 60ml of acetic acid solution compared with sodium carbonate chamber's 100 ml is due to the high acidity of the solution that likely destroyed the structure of chloroplasts inside the leaves, decreasing the photosynthesis rate significantly.

Taking the average data for 60 mL of acetic acid as an example, the difference of 933uL and 100uL is relatively high which further proves that the use of sodium bicarbonate will contribute to the increased gas production in the results. However, observing the data by different solutions (sodium bicarbonate solution and water), the general trend still largely remains the same; the oxygen produced during photosynthesis increases with higher acidity when the pH level is less than 2.

DISCUSSION

After thorough analysis, we rejected the hypothesis that increased acidity would cause a decrease in oxygen produced during the experiment. Conversely, the amount of oxygen produced during the experiment increases until the pH reaches below 2. After this point, the amount of oxygen reduces when the pH is 1.97 and 1.93. However, pH 1.97 and pH 1.93 is an extremely acidic environment which denatures proteins, destroys chlorophyll structure, restrains the expression and activity of six chloroplast ATP synthase subunits, and decreases the rate of photosynthesis and as

	Trial 1	Trial 2	Trial 3
Trial 1	x	0.14068	0.07663
Trial 2	0.14068	x	0.81490
Trial 3	0.07663	0.81490	x

Table 2: The table shows the p value calculated from the t tests comparing results for the sodium bicarbonate chamber

oxygen production (9).

Between 0 mL to 60 mL of acetic acid, the oxygen produced during the experiment results in a positive correlation between the two variables. This could be because there is a layer of plant cuticle that protects the chloroplast from the outer environment so the chloroplast may still be functional after soaking into acid solution (10). Research has also shown that the layer of epidermis is most active in moderately acidic environments, around pH 3 (11). The scientific reason above could explain the rejection of our hypothesis, suggesting that some acids added to the plant will actually help increase its oxygen production.

We have evaluated the strengths and weaknesses of, and improvements for our experiment. By using the syringe to measure the amount of gas produced during the experiment, it allows us to record quantitative data which increases the reliability and accuracy of the results. Moreover, the use of sodium bicarbonate is because it provides carbon dioxide for the plant to absorb since photosynthesis requires not only light but also carbon dioxide to process. Thus, it also achieves higher efficiency for the whole experiment because of the excessive amount of carbon dioxide the sodium bicarbonate provides.

During the experiment, we observed that when bubbles would form during the process of photosynthesis, the bubbles would first form around on top of the leaf surface, then gradually increase in size until it ultimately flows upwards to the syringe. We would have to provide additional output, such as shaking the prototype, to assist the bubble collection. Furthermore, the leaves, which we needed to detach from their original plants, were slowly dying because of the loss of water and nutrition (12). This may negatively affect the rate of photosynthesis.

One improvement to be made could be the use of pond water algae instead of spinach. Algae already grows in the environment of water; it does not need the use of sodium bicarbonate to provide additional carbon dioxide for the plants to photosynthesize (13). This could significantly decrease the chances of collecting gasses other than oxygen. We also would not have to destroy or detach the plant itself; it could function as a proper plant, receiving nutrients from the pond water and carbon dioxide. Another improvement, in order to ensure all oxygen produced during the experiment is collected, could be to shake the device every five minutes to promote the movement of particles. Lastly, to improve the experiment, we can try to use an LED plant bulb because it produces strong output in both blue and red wavelengths with only little light in other regions. This could reduce heat build-up and reduce temperature change (14). LED plant bulbs are commonly used for indoor greenhouse planting (14).

In the future, we have decided to extend the project by using acetic acid to grow the plants, asking how different volumes of acetic acid affect the height of the plant. By directly using acetic acid as the main source to water the plants, we can explore more aspects of the effect of acid rain on plant health, such as whether the use of acetic acid causes any destruction to the structure of the leaf, or how it affects photosynthesis rate as well.

In conclusion, we investigated the relationship between average gas collected during the photosynthesis process in a sodium bicarbonate chamber. We rejected our hypothesis of the increased acidity would cause a decrease in oxygen produced during the experiment because the amount of oxygen produced from photosynthesis didn't decrease while the acetic acid volume increased. In fact, when the acetic acid volume increased, it produced more oxygen gas, until the pH value reached below 2. The average pH of acid rain lies around 4, which according to our conclusions, is beneficial for boosting the rate of photosynthesis. Yet, if the acidity of the rain continues to rise, the impact of a pH below 2 would then become lethal and detrimental to the environment. As such, the Clean Air Act of 1990 targeted acid rain, limiting the sulfur dioxide emissions for 88 percent, at the same time, the air-quality standards in U.S. emissions of nitrogen dioxide decreased 50% between 1990 and 2017(1). The implementation also provides a setpoint to the government to interfere with ecosystem issues, preserving the environment (1). In conclusion, the results of this experiment allow us to apply the concept to broader social development within the society.

MATERIALS AND METHODS

To set up the acidified spinach leaves

Label each disposable cup as 0mL, 20mL, 40mL, 60mL, and 100mL using the marker. The 100mL graduated cylinder was used to measure 100mL of distilled water for each cup. Cut a total 20 pieces of 5 cm x 5cm pieces of fresh spinach leaves using scissors. In order to separate the different volumes of acetic acid, measure then pour 0,20,40,60,100mL of acetic acid in each corresponding labeled disposable cup. 4 pieces of small spinach was each submerge into 5 different volumes of acetic acid solution for 15 minutes. After 15 minutes have elapsed, all the remaining acid or liquid in the disposable cups was poured out and the small spinach pieces were left inside the cups.

To set up the water displacement device

400mL of diluted sodium bicarbonate solution was poured into one transparent box. Both desk lamps on the table were set up, facing the light to the sodium bicarbonate solution chamber. The plunger of all five syringes was pulled out , leaving the barrel on the table. A piece of clay was used to seal the small opening at the top of the barrel. The funnel was attached to the barrel and the entire device was filled with diluted sodium bicarbonate without any air bubbles inside.

To measure the amount of oxygen collected from thirty minutes of photosynthesis

One piece of spinach was gently taken out of the 0mL, 20mL, 40mL, 60mL, 100mL cups. The device inside the chamber was carefully lift up and the spinach leaf was placed inside each funnel separately. The thirty minute timer started and the water level on the syringe barrel for each device was recorded on a table.

To set up the LED light source

The LED light source has a wavelength of approximately 580 nm. The LED lamp was set directly on top of the samples. Make sure no additional obstacles are blocking the light source from the samples. The distance between the light source and sample should be 20 cm apart.

Received: June 7, 2022
Accepted: August 24, 2022

Published: February 24, 2023

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