DIFFERENT VOLUMES OF ACETIC ACID AFFECT THE OXYGEN PRODUCTION OF SPINACH LEAVES DURING PHOTOSYNTHESIS

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Abstract

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.

Motivation

Photosynthesis is the process of organisms synthesizing their own food by converting light energy to chemical energy. This is the chemical equation of the process of photosynthesis: $6CO2 + 6H2O \rightarrow C6H12O6 + 6O2$. The process converts sunlight and carbon dioxide into glucose, energy, water, and most importantly, oxygen gas.

Pollution, caused by burning fossil fuels, releases carbon dioxide, nitrogen oxides, and sulfur dioxide into the atmosphere. These greenhouse gasses then turn into carbonic, sulfuric, and nitric acid solutions, causing rainwater to become overly acidic. Acid rain decreases the pH of the leafs growth environment, affecting the nutrition and overall growth of plants negatively. Within the last decade, acid rain has become a problem as 11 percent more polluting gasses are emitted from factories, automobiles, and power plants around the globe. The acidity of acid rain ranges from 4.2 to 4.4, where our experiment applies and tests out the extremes of acid rain, showing the effects on a plant's oxygen production if pollution worsens.

Research Question

How does soaking spinach leaf in different concentrations of acetic acid (0ml, 20ml, 40ml, 60ml, 100ml) affect the amount of oxygen produced during 2 hours of photosynthesis?

Background Information

In this experiment, we will be collecting the oxygen gas produced from detached spinach leaves that had been soaked into different concentrations of acetic acids. Although the spinach detaches from the plants itself, the spinach can still perform the reaction photosynthesis because of the following reasons. Photosynthesis uses light energy to produce ATP and NADPH by consuming carbon atoms, and produces oxygen and glucose as the product; these processes take place in the chloroplast[4]. From the figure 1 below, the depiction of the inner structure of a leaf. The outer layers are called epicuticular surface reflectance, serving for the ratio of incoming light absorbed or transmitted by leaf[5]. Then, along with both the upper and lower epidermis covering the stem, root, leaf of the plants, it provides a protective barrier against water loss, mechanical injury, and infection[6]. Thus, all of the chlorophyll is protected under the epidermis, cuticle, and the epicuticular surface reflectance[5]. Once the chlorophyll is functional and healthy, photosynthesis can still happen. Secondly, since the spinach leaf is only detached from the plant within an hour, it can still perform photosynthesis and produce oxygen at a slower rate[4]. It happens at a slower rate because when the veins and stems are cut or broken, it decreases the rate of transporting water and nutrition to the leaf and rest of the plant. In conclusion, our group believes the leaf can still conduct photosynthesis due to the protection of epidermis as well as the broken vein; yet, it photosynthesizes at a slower rate.

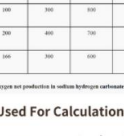


Figure 1: The Figure illustrates the inner structure of a leaf. (Reproduced from[4]).

Hypothesis

We hypothesized that when the concentration of acetic acid increases, then the amount of oxygen produced from spinach leaves will decrease because the acidity from acetic acid will denature the protein substances inside the spinach leaf, prohibiting the spinach leaves from performing photosynthesis[7]. There are various types of protein substances inside the stroma. For instance, the protein pumps in the electron transport chain such as P-type, V-type, and F-type. The P-type pump transports H+ (8); the V-type hydrolyzes ATP and pumps protons into the lumen of organelles(8), the F-type pump allows H+to move out of the concentration gradient, also known as the ATP synthase(8). All three types of pumps are made up of protein(8). In this case when protein substances are exposed to acidic environments and exceed its optimum pH value, the hydrogen bonds holding the protein together will kinetically start vibrating, breaking the bond eventually [7,8]. Thus, the structures would unfold, prohibiting the protein pumps to continue their function [7,8]. Without functional substances in the chloroplast, photosynthesis wouldn't happen, limiting the amount of oxygen it can produce.

Methodology

Experimental Variables

Independent Variable : Spinach leaves that are soaked in different concentrations of acetic acids
We manipulate the independent variable by mixing 20ml, 40ml, 60ml, and 100ml of acetic acid in 100ml of water separately, making different concentrations of acetic acids. Then, the spinach leaves will be soaked in the acidic solutions for 2 hours. The spinach leaves that are taken out of different concentrations will then be tested in the experiment.

Dependent Variable : Amount of oxygen produced
We will manipulate the dependent variable by using the water displacement method to find out the amount of oxygen produced. We designed to place the leaves inside a chamber. The leaves will be covered by a funnel with a sealed syringe at the top, collecting the oxygen produced from photosynthesis. Then, we will be looking at the scales on the syringe after 2 hours. The amount of oxygen will be measured in microliters as the syringe labels the volume with each gap of 100 microliters.

Control Variables :
Room temperature
The room temperature will be manipulated by conducting the experiment in the same environment. Also, we will use the AC to make sure the temperature is around 24 degree celsius

Type of leaves
We will be using spinach leaves for all of the experiments. The leaves will be freshly picked out from the same supermarket.

Size of leaves
We will be using a ruler to measure four equal size pieces, each piece will be 3cm x 3cm. Then, use a scissor to split one leaf of spinach into four equal pieces.

Amount of light intensity
We will place the samples under the same environment and use the same lamp to shine on it, making sure the light intensity is the same for all samples.

Method

To set up the additional 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 of 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 were each submerged into 5 different volumes of the acetic acid solution for 2 hours. After 2 hours 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 2 hours 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 lifted up and the spinach leaf was placed inside each funnel separately. The2 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 plants/objects/people are blocking the light source from the samples. The distance between the light source and the sample should be 20 cm apart.

Materials

1 pair Scissors
1 piece of clay
5 cups disposable cups
1 ruler 15cm ruler
1000ml of Distilled water
1 bag(six teaspoons) of Baking soda
1 stick mixing rod
1 spoon Wooden teaspoon
1 bag Fresh Spinach
5 syringe 5ml syringe
220ml of 99% acetic acid
5 slip of pH indicator paper
2 lamp desk lamp
5 funnels transparent funnels
800ml of Diluted Sodium Hydrogen Carbonate solution
2 lunch box 10x11x5 transparent lunch box
1 cylinder 100ml graduated cylinder
1 maker black permanent marker
1 timer Timer
1 roll tissue paper

Table 1: List of experimental materials and their quantities

Results

	0 ml. (+/- 0.5ml.)	20ml. (+/- 0.5ml.)	40ml. (+/- 0.5ml.)	60ml. (+/- 0.5ml.)	100ml. (+/- 0.5ml.)
Trial 1 (+/- 50µl)	200	200	300	600	50
Trial 2 (+/- 50µl)	100	300	800	1100	150
Trial 3 (+/- 50µl)	200	400	700	1100	100
Average µl for each volume	166	300	600	933	100

Table 1: Oxygen net production in sodium hydrogen carbonate solution chamber

	0ml. (+/- 0.5ml.)	20ml. (+/- 0.5ml.)	40ml. (+/- 0.5ml.)	60ml. (+/- 0.5ml.)	100ml. (+/- 0.5ml.)
Trial 1(+/- 50µl)	100	300	200	100	0
Trial 2(+/- 50µl)	200	200	300	100	0
Trial 3(+/- 50µl)	300	400	400	100	0
Average µl for each volume	200	300	300	100	0

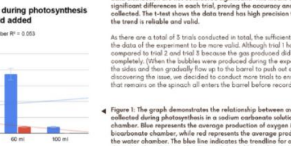
Table 2: Oxygen net production in sodium water chamber

Formula Used For Calculations

$pH = -\log[H+]$ $\quad M1V1 = M2V2$ $\quad C = n/V$ $\quad H^+ion = c \times \sqrt{\frac{Ka}{c}}$

Volume	0mL	20mL	40mL	60mL	100 mL
Concentration	0	0.17857g/mL	0.35714 g/mL	0.39286 g/mL	0.72214 g/mL
Molarity	0	2.98 M	4.97 M	6.55M	8.7M
H' ion concentration	x	0.00722 M	0.00905 M	0.0108 M	0.0625M
pH	?	2.14	2.05	1.97	1.93

Table 2: Calculated values of concentration, molarity, H+ ion volume, and pH value of different volume of acetic acid added



◀ 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.

T-test (between trials)

	Trial 1	Trial 2	Trial 3
Trial 1	x	0.1407	0.0766
Trial 2	0.1407	x	0.8149
Trial 3	0.0766	0.8149	x

	Trial 1	Trial 2	Trial 3
Trial 1	x	0.0213	0.0590
Trial 2	0.0213	x	0.0993
Trial 3	0.0590	0.0993	x

Table 4: T-values between each trial for the sodium bicarbonate chamber (above) and the water chamber (below). The T-values are all greater than 0.05 meaning the data distribution are not considered significant. This means the data doesn't have significant differences in each trial, proving the accuracy and precision of the data collected. The t-test shows the data trend has high precision throughout the trials that the trend is reliable and valid.

As there are a total of 3 trials conducted in total, the sufficient number of trials allows the data of the experiment to be more valid. Although trial 1 has less oxygen collected compared to trial 2 and trial 3 because the gas produced did not enter the syringe completely. (When the bubbles were produced during the experiment, it would form at the sides and then gradually flow up to the barrel to push out external water). After discovering the issue, we decided to conduct more trials to ensure the gas produced that remains on the spinach all enters the barrel before recording the results.

Discussion

The general trend of the sodium hydrogen carbonate chamber results shows that when the concentration of the acetic acid spinach leaves is soaked in increases, the oxygen it produces would increase accordingly until it reaches the concentration of 0.522 g/ml. When it reaches a certain limit, in this case, 8.7M, the leaf would turn into a brownish color and can produce very little photosynthesis, producing at most 100 microliters of oxygen. The moderate acidity of the 20,40, 60 ml acetic acid solution's spinach leaves promoted the chloroplast's activity, increasing the amount of oxygen produced in 2 hours. During the experiment, the bubbles would first form on the sides of the leaves, then flow up to the barrel to push out external water.

We have an additional water chamber trial because we suspect that the sodium bicarbonate solution reacts with the remaining acetic acid on the spinach surface. Acetic acid reacts with sodium bicarbonate solution, creating carbon dioxide gasses during the process, in our method of collecting oxygen, we couldn't differentiate the different gasses, whether it is oxygen or carbon dioxide. In order to increase the validity of our experiment, we tested a trial of the water chamber instead of the sodium bicarbonate solution. The results were similar, as the concentration of the acetic acid spinach leaves is soaked in increases, the oxygen it produces would increase accordingly until it reaches the 60ml concentration. The rise in oxygen production stopped at 6.55M of the acetic acid solution, which is different from the sodium carbonate chamber's 8.7M of acetic acid. The acidity denatured and destroyed the structure of the chloroplasts microorganisms inside the leaves, limiting the amount of photosynthesis it does. The data in the 60ml acetic acid solution section shows that the sodium hydrogen carbonate chamber collected 933 microliters and the water chamber only collected 100 microliters of oxygen gas. The significant difference in the amount of gas collected proved that the sodium bicarbonate and acetic acid reaction did make a contribution to the results, increasing the gas production in the data. The chemical reaction would produce excess carbon dioxide gasses, causing more gasses to be collected in the sodium hydrogen carbonate chamber. However, the general trend was mostly the same, stating that oxygen production would continue to increase until it reaches a pH level less than 2.

From the data above, Trial 1 had overall less oxygen gas collected than trial 2 and 3. One of the reasons is that the air bubbles created on the surface of the leaves weren't completely collected inside of the syringe's barrel. Therefore, it caused the overall gas collection to be less. As a result we made sure all the gas stuck on the side of the spinach leaves are collected into the barrel before recording the results. The better gas collection method made trial 2 and 3 more valid and accurate.

Figure 1 depicts the relationship between the average gas collected in sodium bicarbonate solution results is a positive correlation which when the acidity of the spinach leaves increases, the amount of oxygen produced during the photosynthesis process increases as well. However, when the concentration reaches 0.522g/ml, 8.7M the color of leaf will turn from green color into dark brown, producing only 100 microliters of oxygen. On the other hand, the moderate acidity of using 20ml, 40ml, 60ml of acetic acid mixed with 100ml of water, the spinach leaves accelerates the chloroplast's activity which also assists in the photosynthesis process thus creating more oxygen.

Our group also conducted an additional trial of using water only in the large container since the acetic acids that attach to the leaves' surface react 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 can not identify the two different gasses during the experiment. Our group decides to conduct another trial with the chamber filled with water solution, to increase the validity of the experiment. The results shown in table 5 demonstrate similar results in which the increase of acetic acid in the spinach soaked in the oxygen produced during the photosynthesis process increases as well. The increase of oxygen production ceased at 6.55 M of the acetic acid solution compared with the sodium carbonate chamber's 8.7M due to the high acidity of the solution that denatured and destroyed the structure of chloroplasts inside the leaves, decreasing the photosynthesis rate significantly. By using 60 ml of acetic acid, for example, the difference of 933µl and 100µl is relatively significant 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 highly remains the same, that the oxygen produced during photosynthesis will increase with higher acidity when the PH level is less than 2.

Conclusion

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. Our hypothesis was based on the fact that 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 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 5 [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 protatype, to avoid 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 grown 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 50 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 conclusion, we investigated the relationship between average gas collected during the photosynthesis process in a sodium bicarbonate chamber. We rejected our hypothesis that... 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[15]. The implementation also provides a setpoint to the government to interfere with ecosystem issues, preserving the environment [15]. In conclusion, the results of this experiment allow us to apply the concept to broader social development within the society.

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