Photosynthesis – AP Lab

SBI4UP

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Photosynthesis Rate

The general summary equation for photosynthesis:

$$2\ H_2O + CO_2 + light \rightarrow carbohydrate\ (CH_2O) + O_2 + H_2O$$

HOW CAN YOU MEASURE THE RATE OF PHOTOSYNTHESIS?

- Production of O_2

- Consumption of CO_2

* In this investigation you will be measuring the accumulation of O_2 to determine the rate of photosynthesis.

Designing the Lab

Factors to consider when designing your lab:

- What environmental variables might affect the net rate of photosynthesis? Why do you think they would affect it? How do you predict they would affect it?

- What features or variables of the plant leaves might affect the net rate of photosynthesis? How and why?

- Could the way you perform the procedure affect the outcome? If the outcome changes, does it mean the net rate of photosynthesis has changed?

Designing the Lab

WHAT IS THE DEPENDENT VARIABLE IN THIS EXPERIMENT?

- The time it takes for each disk to float *(the amount of O_2 produced in a certain period of time)*

WHAT IS THE INDEPENDENT VARIABLE IN THIS EXPERIMENT?

- This will vary with each experiment. What factors could affect the rate of photosynthesis?

Materials

General Materials required:

- Baking soda
- Liquid soap
- 2 plastic syringes
- Living leaves (spinach)
- Hole punch
- 2 clear plastic cups
- Timer
- Light source

Materials will vary depending on the parameters you are testing
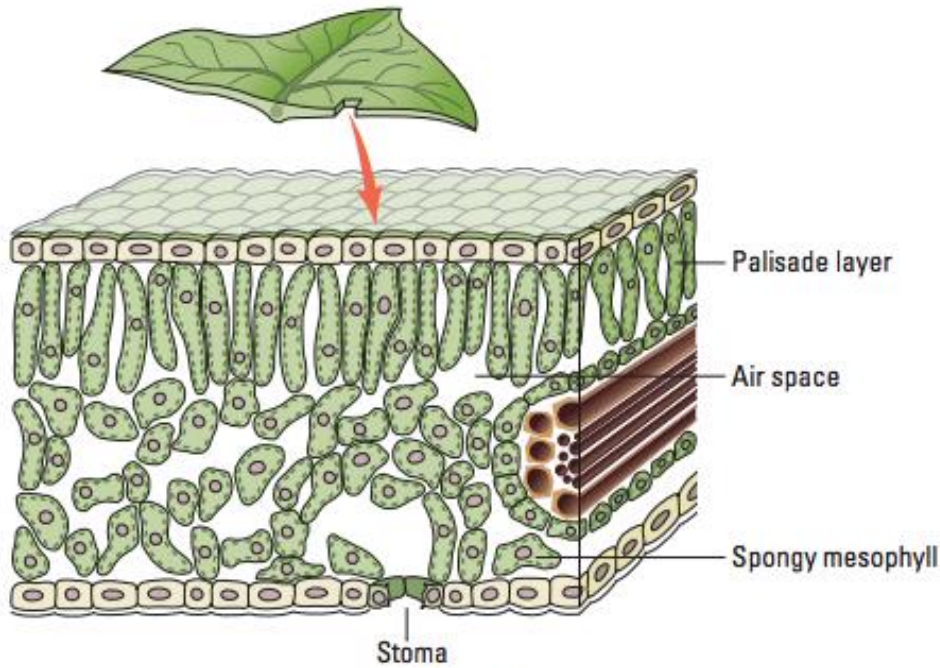
General Lab Procedure

Palisade layer

Air space

Spongy mesophyll

Stoma

Figure 1. Leaf Anatomy

Leaves float in water due to presence of air (air spaces) in the leaf.

HOW DOES ONE GET THEM TO SINK?

General Lab Procedure

Figure 6a. Creating a Vacuum in the Plunger

Figure 6b. Sinking Leaf Disks

Put leaf discs under vacuum by placing them in an aqueous solution inside a syringe, cover the hole of the syringe with your thumb, and then pull back on the syringe…negative pressure…sucks air out of leaf (fig 6a). Disks will sink (fig. 6b).

General Lab Procedure

We want leaf discs to still be able to do photosynthesis in the solution...but you just removed the air and replaced it with water, which has little dissolved CO_2...

Figure 6a. Creating a Vacuum in the Plunger

Figure 6b. Sinking Leaf Disks

What can we add to the water to put lots of dissolved CO_2?
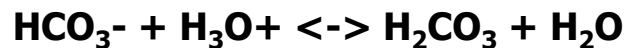
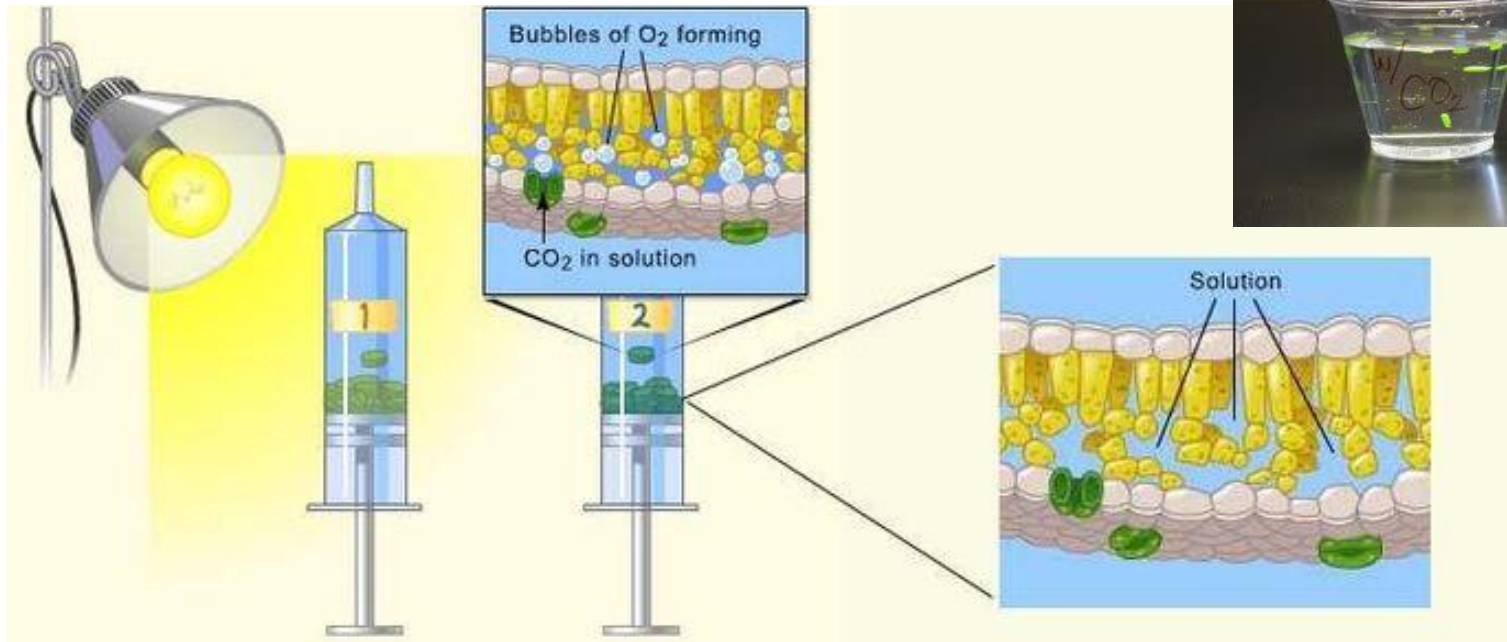Sodium bicarbonate (baking soda)

General Lab Procedure
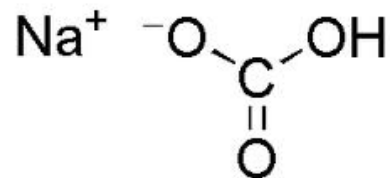
$Na^+HCO_3^-$ (sodium bicarbonate)

ADD BAKING SODA...

$Na^+HCO_3^-$ dissociates in aqueous solution to $Na^+ + HCO_3^-$

$HCO_3^- + H_3O^+ <-> H_2CO_3 + H_2O$

$H_2CO_3 <-> H_2O + CO_2$

Bubbles of O₂ forming

CO₂ in solution

Solution

When the disk leaves are exposed to light they float after a certain amount of time due to the O_2 being produced.

WHAT OTHER VARIABLE MUST WE ACCOUNT FOR WHEN USING THIS METHOD?

Cell Respiration is occurring, using some of the O_2 and producing CO_2 gas as so you are actually also observing cellular resp. though the amount of CO_2 = O_2 used...

a Using the straw, cut leaf disks from leaves of five or six day-old plants.

**b** Remove cap from the tip of the syringe. Pull the plunger out of the syringe. Blow the leaf disks out of the straw into the syringe. Replace the plunger.

straw

leaf disks

10cc syringe
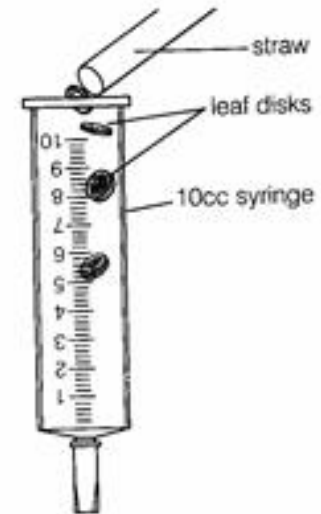
c Draw 8ml of NaHCO$_3$ solution into the syringe. Invert syringe as shown, tip-end up. Gently push the plunger to remove all the air.

d Put your finger over the syringe tip and pull the plunger. This will create a vacuum, which will pull the air and oxygen from the leaf disks.
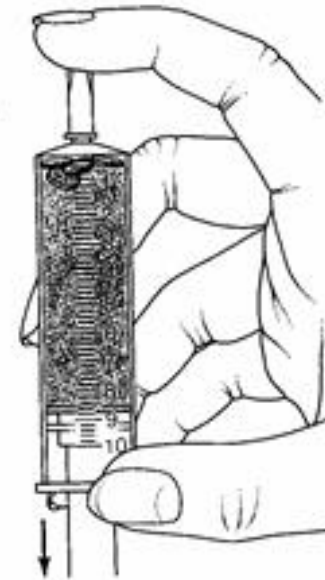
e Tip the end of the syringe down so that the leaf disks are in the solution. Release plunger; remove your finger. Turn syringe back up and tap the side repeatedly until all of the disks sink.

f Place the syringe narrow-end up about 5cm from the light-bank lights. Tap the syringe with your finger or on edge of desk to dislodge the floating disks.

light bank

General Lab Procedure

Step 1 Prepare 300 mL of 0.2% bicarbonate solution for each experiment. The bicarbonate will serve as a source of carbon dioxide for the leaf disks while they are in the solution.

Step 2 Pour the bicarbonate solution into a clear plastic cup to a depth of about 3 cm. Label this cup "With CO_2." Fill a second cup with only water to be used as a control group. Label this cup "Without CO_2." Throughout the rest of the procedure you will be preparing material for both cups, so do everything for both cups simultaneously.

Step 3 Using a pipette, add one drop of a dilute liquid soap solution to the solution in each cup. It is critical to avoid suds. If either solution generates suds, then dilute it with more bicarbonate or water solution. The soap acts as a surfactant or "wetting agent" — it wets the hydrophobic surface of the leaf, allowing the solution to be drawn into the leaf and enabling the leaf disks to sink in the fluid.

General Lab Procedure

**Why is dilute soap being added in step 3?**
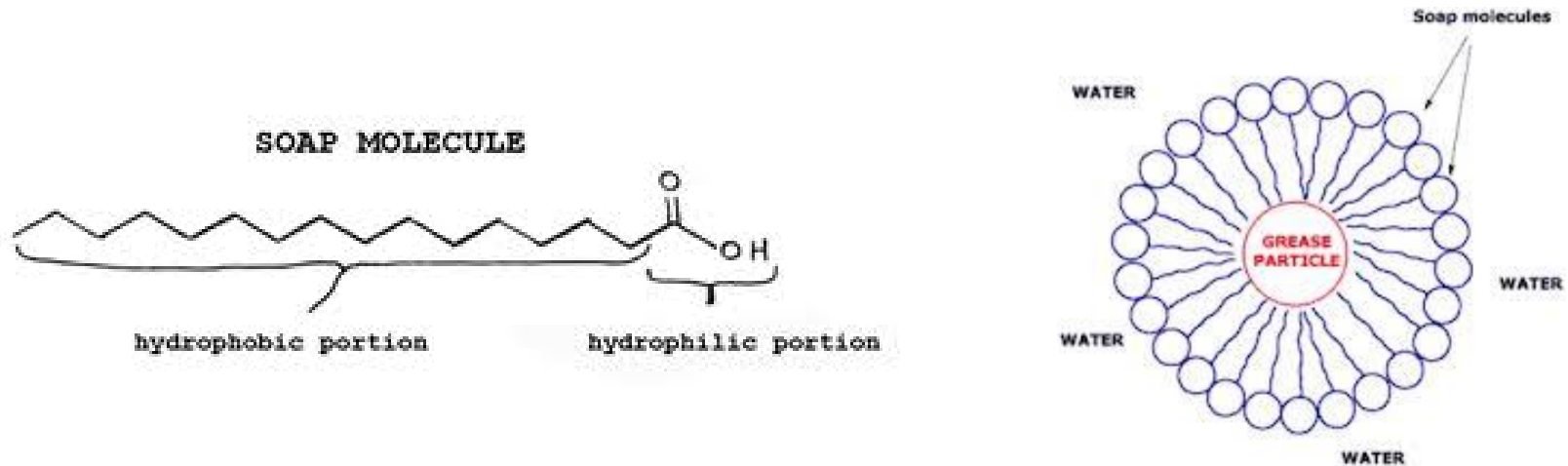
The cuticle of the leaf is hydrophobic and prevents the leaf discs from ever sinking. The soap, as you know, is amphiphilic (its essentially a fatty acid). It will coat the leaf discs (tails interacts with cuticle and carboxyl head interacts with water). This will allow the discs to sink after the gas is sucked out.

Analyzing Results

The ET_{50} (Estimated Time it takes for 50% of disks to float…median)

The ET_{50} is the estimated time it takes for 50% of the disks to float, which has been shown to be a reliable measure to compare between experiments.

% Floating Disks

%Floating Disk

ET_{50}

Time in minutes
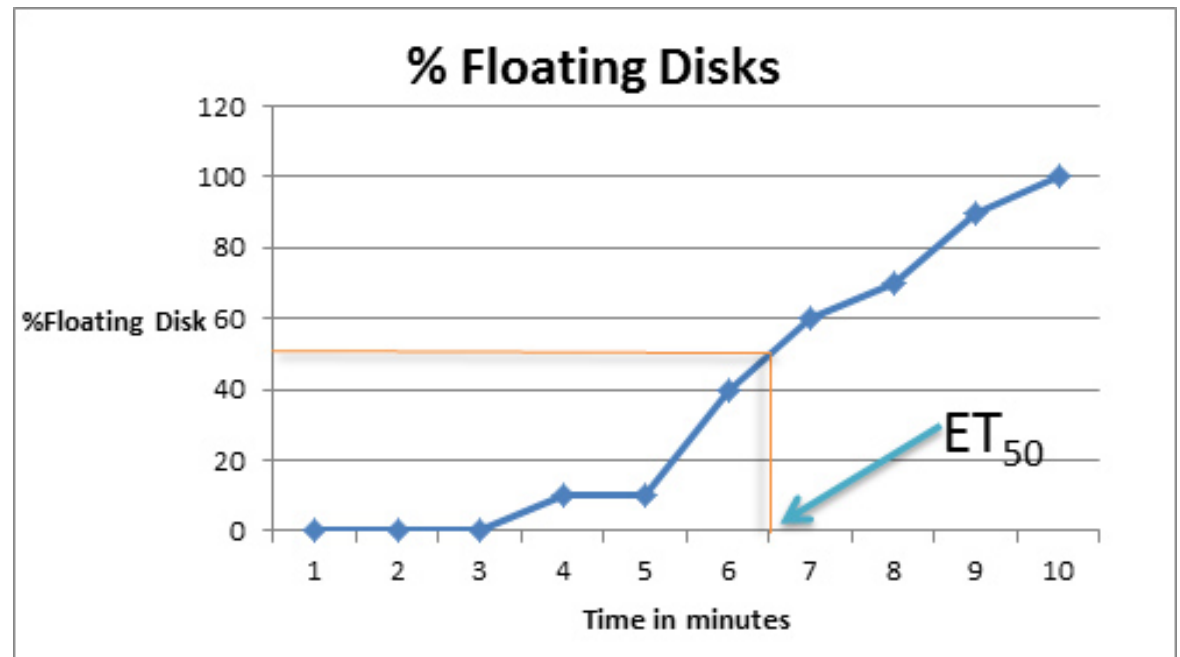
Analyzing Results

Trials	ET_{50} – Pure Water (s)_	ET_{50} – 0.2% bicarbonate (s)
1	415	388
2	420	364
3	408	371
4	395	395
5	424	410
6	398	381
7	416	379
8	410	405

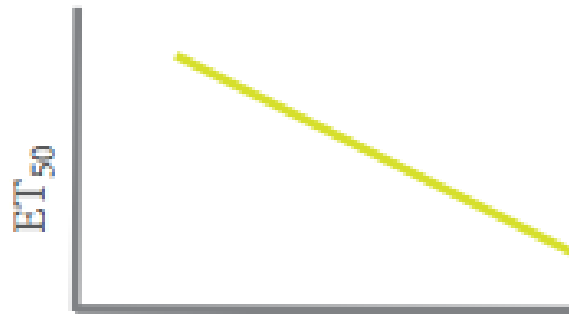Here is our data…now what do we do to analyze it and see if there if a difference between these two groups?
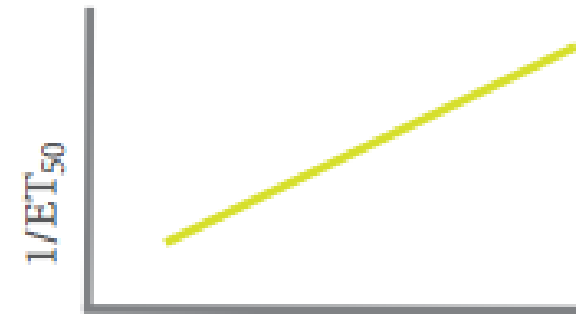
Analyzing Results

Photosynthesis vs Light Intensity
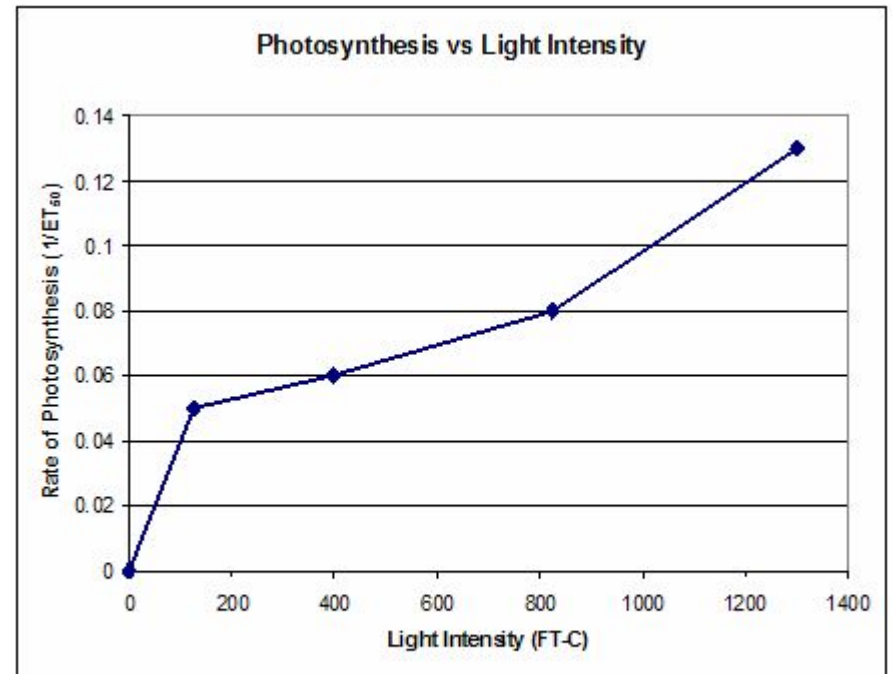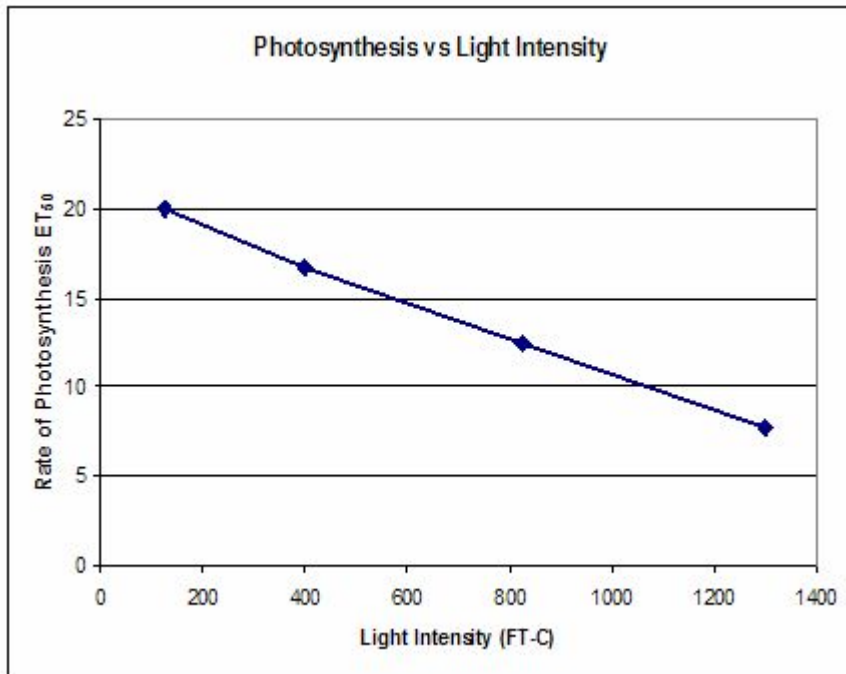
Analyzing Results

Figure 8a. Inverse Relationship
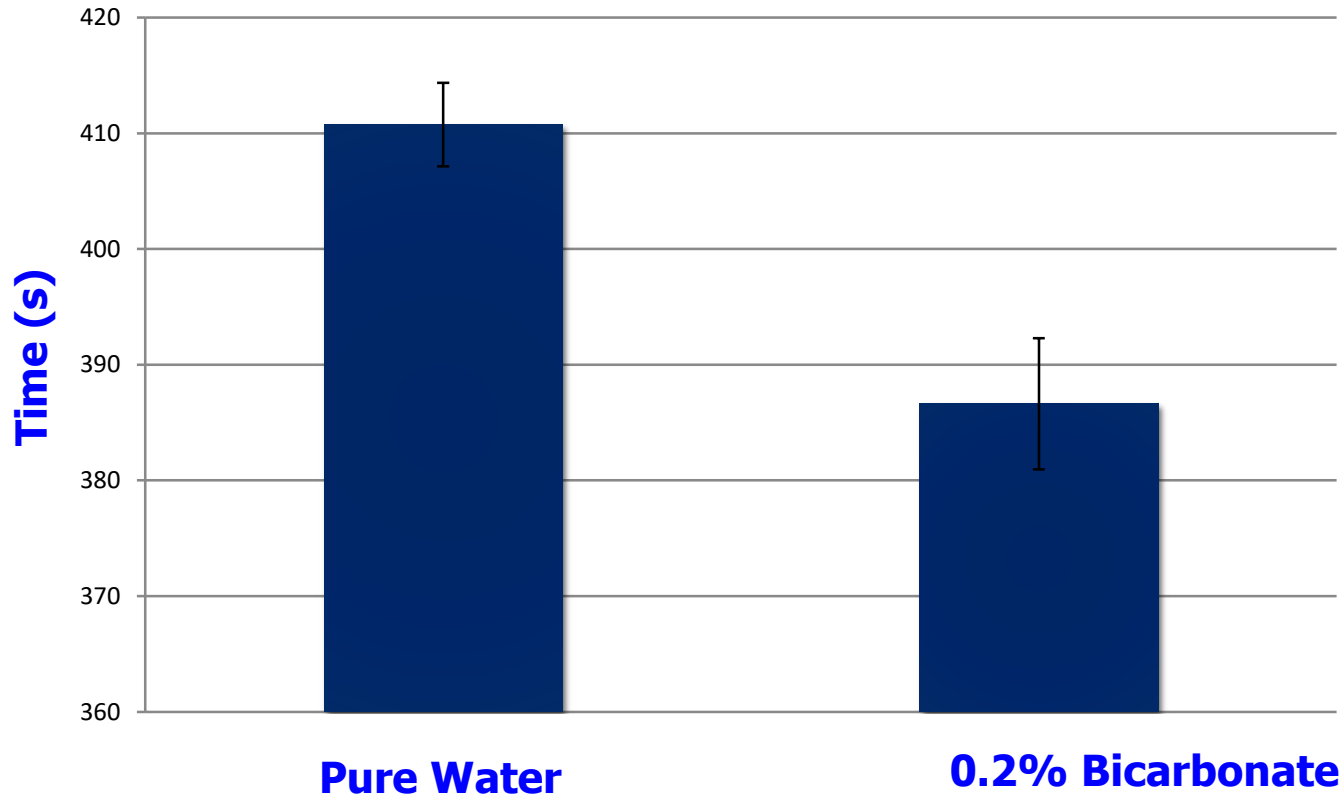

Figure 8b. Direct Relationship

Analyzing Results

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

$$\text{Standard error} = \frac{\sigma_x}{\sqrt{N}}$$

Trials	ET_{50} – Pure Water (s)_	ET_{50} – 0.2% bicarbonate (s)
1	415	388
2	420	364
3	408	371
4	395	395
5	424	410
6	398	381
7	416	379
8	410	405
MEAN	410	387
SD (σ)	10.2	16.0
SE	3.60	5.67

Analyzing Results

Figure X. Mean ET_{50} values for leaf disks evacuated and submerged in pure water and 0.2% bicarbonate solution. Error bars indicate standard error for eight trials (n=8).