Rates of respiration in peas experiment

Cellular respiration, aerobic, meaning occurring in the presence of oxygen occurs in the case of seeds. Respiration is the process by which cells breakdown or oxidize the organic molecule glucose into the lower energy state CO and H O. During this process, energy in the form of ATP is released. For aerobic respiration to occur the extremely electronegative diatomic element Oxygen is required in large amounts. So, when the organism is respirating, oxygen is consumed and CO and H O are created. This process occurs partially in the cytosol outside of the mitochondrion and partially in the mitochondria itself. The chemical equation for cellular respiration is: $C6H12O6 + 6O2 ——————-> 6CO2 + 6H2O + ~38 ATP$.[i]Respiration occurs in living organisms such as peas, which can be in a dormant state or in a state of germination. Seeds contain the embryo, the seeds' next generation and a supply of food enclosed in a seed coat. The seed is said to be dormant when they stay viable, although inactive metabolically as well as being incapable of germinating under normally favorable conditions fort the species, such as hydration and temperature. The seed is in essence sleeping and will awake only when the proper conditions are met or from some external stimulus, at which point the seed will begin to germinate. Germination, by definition, occurs when the dry seed sheds from its parent plant and takes up water, which is called imbibition, and this germination reaches completion when the root of the embryo visibly emerges through the seed's outer structures. Germination utilizes the food reserves within it. The germinating seeds undergo metabolic processes when introduced to water, thus they undergo respiration.[ii]

Simple respirometers, such as the ones used in this experiment, consist of a sealed container and the organism being tested as well as a substance, like KOH, potassium hydroxide, or soda lime pellets that absorb the carbon dioxide that is given off. The rate by which oxygen is taken in is calculated by finding the displacement of the fluid in the glass tube that is connected to the sealed container. Respirometers quantify the amount of respiration being undergone by the cell by measuring the amount of oxygen, water and/or carbon dioxide levels and mass flow rate.[iii]

To understand how the respirometer functions, it is helpful to understand the ideal gas law. The general gas law states that PV = nRT. P is the P is the pressure of the gas, V is the volume occupied by an n amount of moles of any gasses, R is the gas constant, and T is temperature in Kelvin. This law assumes that the volume of the molecules is negligible in comparison to the volume of the container the molecules were held in. It is implied from this law that when the temperature and pressure are constant, the volume of the gas is directly proportional to the amount of molecules of gas, that if the temperature and volume were constant, then the gas pressure changes in direct proportion to the number of gas molecules there are, that if the number of gas molecules and the temperature remain constant, then the pressure and volume are inversely proportional to one another, and that if the temperature is changed but the number of gas molecules remains the same, then the pressure or the volume, or both, will change in direct proportion to the temperature.[iv]

The CO produced during respiration is removed with potassium hydroxide (KOH), and according to the following reaction: , will form potassium

carbonate (K CO). Because of the removal of carbon dioxide, the volume of the gas in the respirometer is directly related to the amount of oxygen consumed by seeds. The glass beads, which are not organic, are used as a control group.

The purpose of this experiment is to compare the rate of respiration of the germinating peas with the rate of respiration by the dried, dormant peas. Based on the fact that when dormant, seeds do not undergo much of any metabolic pathways, and when germinating the seeds are undergoing metabolic processes such as respiration to create energy, it is hypothesized that the germinating peas will have the greatest rate of respiration, followed by the dried peas and glass beads, and the glass beads, as the inorganic control group, should show no respiration.

Materials and Methods

- 6 Respirometers (6 vials and 6 rubber stoppers)
- 50 Germinating Peas
- 50 Dried Peas
- Glass Beads
- A thermometer
- Absorbent Cotton (Cotton Balls)
- Non-absorbent Cotton (Cotton Sheets)
- 15 % KOH solution
- Stopwatch or timer of some kind
- Water
- Ice
- 2 Shallow baths

- A graduated Cylinder (50 mL)
- Pipette
- Masking Tape

To begin this experiment on respiration, two baths were filled with water. In one of the baths, the water temperature was adjusted, by adding ice, to approximately 10 C, while the other was allowed to settle at room temperature (about 25 C). After a 50 mL graduated cylinder was filled halfway (to 25 mL) with water, 25 germinating peas were placed in the graduated cylinder. When the peas were placed in the cylinder, some of its water was displaced, a measurement that is equal to the volume of the peas. The volume of the 25 germinating peas was then recorded. Once recorded, the peas were removed from the graduated cylinder and placed on a paper towel to dry. These germinating peas were used later in the experiment in respirometer one. Next, the same graduated cylinder was refilled again with 25 mL of water. Twenty-five dried peas (not germinating) were then placed into the water and then glass beads, the number of which was determined, were then added to the water until the volume of the beads and dried peas was equivalent to the volume of the germinating peas. These glass beads were then removed from the graduated cylinder and placed on another paper towel to dry. These dried peas and beads were later used in respirometer two. The graduated cylinder was refilled with 25 mL of water for a third time. Then, glass beads were added to the water in the cylinder until the volume, or the amount of water displaces, was equal to the volume of the germinating peas. Once the number of beads was determined, they were removed and placed on a paper towel where they remained until they

were used again in respirometer three. The same process with the germinating peas, dried peas and glass beads were repeated; the germinating peas were used in respirometer four, the dried peas and beads in respirometer 5, and the glass beads alone in respirometer 6.

Next, a small piece of nonabsorbent cotton was placed in each of the six respirometer, which were assembled by attaching a stopper and pipette to each of the six vials. These pieces of cotton were then moistened, using a pipette, with 15 % KOH. It was important to be sure that the insides of the respirometer vials were dry and that no KOH was remaining on the respirometers' sides and that each vial was the recipient of the same amount of cotton and KOH. A small amount of nonabsorbent cotton was then placed atop each of the six KOH-soaked pieces of absorbent cotton. Next, the initial set of germinating peas was placed in vial one, dry peas and beads in vial two and glass beads alone in vial three. The second set of germinating beads was then placed into vial 4, the dry peas and beads in vial five, and the beads in vial six. After filling each of the six vials with the necessary contents, a stopper that was fitted with a calibrated pipet was inserted into the open end of the tube.

Once the respirometes were set up, a sling made of masking tape was attached to each side of the water baths. Respirometers one, two and three were placed in the 10 C bath and the remaining three were placed in the room temperature bath. Then, after the respirometers were placed in the water, with the tips of the pipettes resting out of the water on the tape sling, there was a seven minute equilibration period. This seven minute period passed and all six respirometers were completely immersed in their baths.

Water entered the pipettes for a small distance after which it stopped. If the water had not stopped, the respirometers would have been checked for leaks. The respirometers were quickly places so that they were able to be read clearly thought he water. During the experiment, caution was taken not to jostle or move the respirometers once the experiment was underway. While this was occurring, the temperature was being monitored and maintained. The respirometers were then allowed to equilibrate for another three minutes, after which the water's initial position (at a time of 0), in each pipette was recorded to the nearest 0. 01 mL. The temperature was then checked and recorded again. Finally, in five minute intervals, for twenty minutes the readings of the water's position in all six pipettes was recorded.

It was hypothesized that the germinating peas would have the highest rates of respiration, the dry peas would have the second most, although not much, and that the glass beads would not show any signs of respiration because they are not organic or living. It was found that the result of this experiment support the initial hypothesis. Tables one and two show that in 10 C water and 25 C water, the germinating peas are consuming the most oxygen, and therefore it can be inferred that they are undergoing the most respiration. The dry beads were second, with variations in oxygen content, found by subtracting the water level from 1mL, most likely caused by pressure changes from changes in temperature. Because glass beads were the control group and are inorganic, they were not supposed to have any change in reading of the oxygen or water. They did, however, because of pressure changes resulting from the change in temperature in the bath, have a higher water level in the pipette in the end than they did in the beginning. Figure

three is showing that at higher temperatures, in the case of this experiment 25 C, the rate of respiration increases and is greater than at the lower 10 C. This is clearly seen when figures 4 and 5, as well as figures one and two, are compared. Figures four and five are the most accurate because the values used in the graphs are the corrected difference. The following formulas were used to find the difference and corrected differences: difference: (initial reading at time 0)- (reading at time X) and corrected difference: (initial pea seed reading at time 0 – pea seed reading at time X) – (initial glass bead reading at time 0 – glass bead reading at time X).. The corrected differences uses the control group, the glass beads, to find what the levels of water should have been. The slopes of figure five and two are showing respiration at 25 C, are much steeper for germinating peas than the same lines are in the graphs of the 10 C bath. Tables three and four are also showing that as the oxygen levels, oxygen being used, increases, the amount of water in the pipette is decreasing. This is indicative of their inversely proportional relationship. Table five and figure six sum up the experiment by showing that the rates, found by finding slope, of the respiration is highest in the respirometers with the germinating peas, and highest with at a temperature of 25 C. The lower respiration rates occurred in the respirometers with the dried peas; the lowest was in the respirometer in the 10 C bath.

Although the hypothesis was supported by the experiment, some data was inconsistent. For instance, in the first set of respirometers, the respirometers, one containing glass beads and another containing glass peas and dried peas, showed and increase in water level, meaning a decrease in oxygen consumption. In the second set of respirometers, however, oxygen

was consumed and water levels dropped in the respirometer with the dried peas and the glass beads were shown to not have any respiration taking place. This is consistent with the inconsistencies in temperature causing a change in pressure. In the room temperature baths, there were less changes in temperature and therefore less pressure changes and inaccuracies in the results. Overall, however, the experiment proved our hypothesis and it was found that the highest rates of respiration are in fact occurring in germinating peas at higher temperatures.