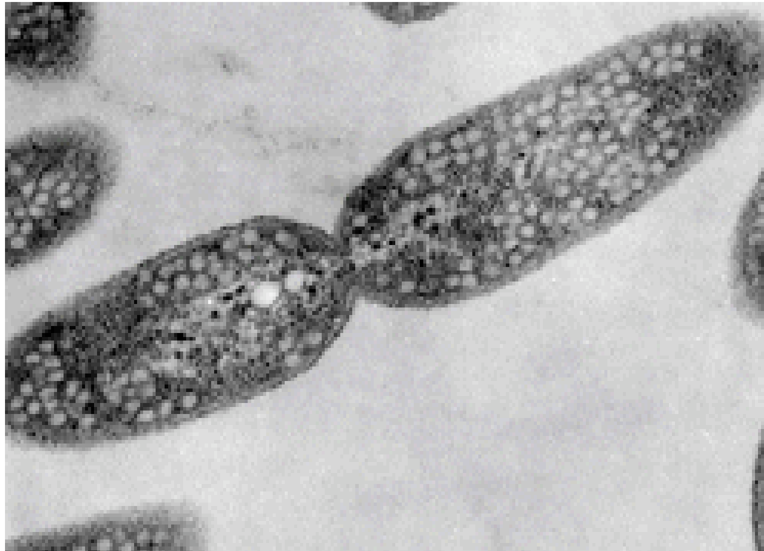
What Is The Most Successful Method To Culture *Rhodobacter Sphaeroides* To Yield The Greatest Amount Of Polyhistidine-Tagged Proteins?



Rhodobacter Sphaeroides undergoing cell division
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Abstract

Rhodobacter sphaeroides (commonly abbreviated as *Rb. sphaeroides*) are a species of bacteria capable of performing photosynthesis. Various methods of culturing *Rb. sphaeroides* to yield the greatest amount of photosynthetic reaction centers were investigated in this experiment. *Rb. sphaeroides* were cultured under different conditions (amount of light, temperature, oxygen, and time). After the culturing process, the bacteria were separated from the nutrient media through the process of centrifugation. The French Press Machine was used to break the bacteria's cell membrane. After breaking the cell membrane, the photosynthetic reaction centers were separated from the membrane particles by using the centrifuge machine. Ultraviolet spectrophotometry was performed to determine the photosynthetic reaction center concentration. The results of this investigation determined that culturing *Rb. sphaeroides* without light, in a 34° Celsius environment with oxygen for four days will yield the greatest amount of photosynthetic reaction centers.

Introduction

Photosynthesis is the most important biological process on Earth. Virtually all forms of life on Earth depend on photosynthesis for energy and oxygen. Photosynthesis is the process in which green plants and certain bacteria use carbon dioxide, along with water to convert solar energy into chemical energy and oxygen (Vermaas, 2004). Living things that are able to produce their own energy are known as autotrophs; organisms capable of absorbing solar radiation to create energy are called photoautotrophs (California Polytechnic State University, n.d.).

Rhodobacter sphaeroides, also known as *Rb. sphaeroides* are photoautotrophic bacteria frequently used by biophysicists and scientists to study photosynthesis. Photosynthesis occurs in the photosynthetic reaction center. Photosynthetic reaction centers from *Rb. sphaeroides* are protein-pigment complexes called polyhistidine-tagged proteins, which are located in the phospholipid bilayer – a two-layered structure that consists of phosphate and lipid molecules that form the cell membrane of *Rb. sphaeroides* (Gregory, 1989). A diagram showing the structure of the phospholipid bilayer is shown below:

Structure of the Phospholipid Bilayer

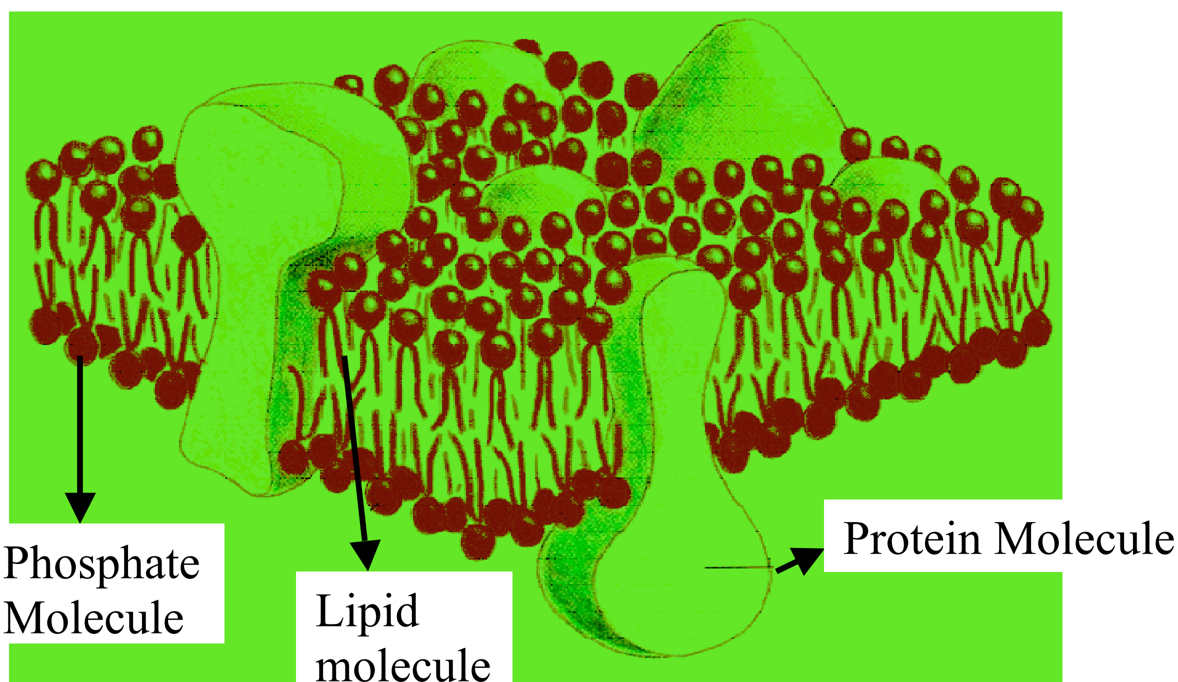

Figure 1. This diagram shows the structure of the phospholipid bilayer. The large structures are protein molecules. In the phospholipid bilayer of Rb. sphaeroides, the protein molecules are called polyhistidine-tagged proteins. The round and tail-like structures are phospholipids; the round "heads" are phosphate molecules and the "tails" are lipid molecules.

The four major variables that can affect the amount of polyhistidine-tagged proteins in *Rb. sphaeroides* are: amount of light, temperature, oxygen, and time. Scientists consider *Rb. sphaeroides* to be valuable when large amounts of polyhistidine-tagged proteins are present in the phospholipid bilayer of the bacteria because polyhistidine-tagged proteins are used as the basis of photosynthetic research.

Scientists frequently use *Rb. sphaeroides* to study photosynthesis because it is a simple bacterium that is easy to culture and work with. *Rb. sphaeroides* is also inexpensive to grow; in addition, it can survive for a long period of time. Virtually all nutrition and energy on Earth is the product of photosynthesis, therefore, understanding this process and its applications to crop and food production, the environment, electronics, and medicine is significant to human health. Thus, simple photosynthetic model systems like the photosynthetic reaction centers (polyhistidine-tagged proteins) from *Rb. sphaeroides* provide us the tools needed to study photosynthesis (Kaplan, 2005).

Studies indicate that the photosynthetic process is relatively inefficient. Of all the solar energy absorbed by plants, only 1 to 2 % of it is converted into chemical energy. Scientists believe sugar cane is the most efficient plant; sugar cane converts 8% of the solar energy it absorbs into chemical energy. If scientists can fully understand how photosynthetic reaction centers function, there is a great chance that the photosynthetic process can be sped up. Speeding up the photosynthetic process will greatly benefit crop and food production because an abundant amount of plants and vegetables can be grown in a short amount of time. This will not only benefit agriculture, but will also be a positive contribution to the economy (Gust, 1996).

Increasing the efficiency of photosynthesis can also be a huge advantage to the environment. Global warming is a topic of worldwide concern. Carbon dioxide in the atmosphere helps keep Earth warm by preventing heat from escaping back out into space. Due to the increase in the burning of fossil fuels, the amount of carbon dioxide in the atmosphere has been increasing drastically in the last 200 years (Hallman, 2000). With more carbon dioxide, more heat is trapped in the atmosphere, which will increase Earth's temperature, causing global warming. Photosynthesis helps remove carbon dioxide in the atmosphere and replaces it with oxygen, thus, reducing the effects of global warming. Therefore, increasing the rate in which plants perform photosynthesis can be a potential solution to global warming.

Another application of photosynthesis is to electronics, especially nanotechnology. At first glance, photosynthesis may seem to have nothing to do with electronics; however, researchers at the Massachusetts Institute of Technology are trying to power electronic devices, such as laptops and cell phones with photosynthetic reaction centers. The group of researchers at the Massachusetts Institute of Technology isolated photosynthetic reaction centers from spinach and placed it on top of a layer of organic semiconductors. On top of the photosynthetic reaction centers were a layer of glass lined with conductive material and a thin layer of gold. This set-up was structured like a sandwich; the first layer is the organic semiconductors, second is the reaction centers, and third is the glass with conductive material and gold. The researchers shined the "sandwich" with a laser light and the "sandwich" generated a tiny electrical current. Although the "sandwich" cannot produce sufficient amounts of energy, billions of them working together can generate enough energy to power a laptop and other electronics

(Zhang, 2004). The "sandwich" is extremely small in size; therefore, it can be used as a power source for small gadgets, such as iPods and MP3 players.

Scientists are also using concepts of photosynthesis to benefit the medical industry. Studies indicate that chlorophyll relatives (a type of photosynthetic reaction center) naturally localize in cancerous tumors. Once the chlorophyll relatives enter the human body, they naturally bond with cancerous tissues, and thus act as dyes that clearly show the boundary between cancerous and healthy cells. Since chlorophyll relatives are photosynthetic reaction centers, they absorb light; when the cancerous tissue (which are bonded with reaction centers) are shined with light, they will absorb as much light as they can. Excessive light absorption leads to tissue damage, which will destroy the cancerous cells, but leaving the healthy cells unharmed because the healthy cells are not tagged with reaction centers, they will not absorb as much light as the cancerous cells. This medical application of photosynthesis is still at its early stages of study, therefore, more research and studies needs to be performed (Gust, 1996).

A series of experiments to determine the most successful method to culture *Rb. sphaeroides* to yield the greatest amount of polyhistidine-tagged proteins was conducted. This investigation consists of four experiments. In the first experiment, *Rb. sphaeroides* were cultured using different amounts of light. The second experiment involved culturing *Rb. sphaeroides* in different temperatures. The third experiment involved culturing the bacteria with and without oxygen. The last experiment used time as a variable; *Rb. sphaeroides* were grown under different time periods.

The hypothesis for this experiment is that culturing *Rb. sphaeroides* with light and oxygen for four days in a 32° C. environment will yield the greatest amount of polyhistidine-tagged proteins.

Methods

One hundred milligrams of YCC media was made using 0.5 grams of yeast extract, 0.6 grams of Casamino acids, and 0.5 milliliters of solution C [for a complete procedure on making YCC media, refer to Appendix C]. The pH of the media was adjusted to 7.2 and separated into four glass media bottles; 25 milligrams in each bottle. The media was sterilized using the autoclave procedure [refer to Appendix D for the complete autoclave procedure]. After sterilization, the media was left alone for three hours, in order to allow it to cool down. In order to eliminate all other unwanted bacteria in the media, 25 microliters of tetracycline (an antibiotic; molecular formula: $C_{22}H_{24}N_2O_8$,) was added into each media bottle using a sterilized pipette [refer to Appendix E for the procedure on adding tetracycline]. The media bottle's opening was held over fire before and after the addition of tetracycline to preserve sterilization. After the addition of tetracycline, a sterilized pipette was used to add 25 microliters of *Rb. sphaeroides* into each media bottle [for the procedure on adding *Rb. sphaeroides*, refer to Appendix F]. A total of 20 bottles of media were prepared following this procedure.

The first experiment, using light as a variable was started. Three bottles of bacteria were used for this experiment. The first bottle, labeled "dark" was completely wrapped with aluminum foil; this bottle received no light for the duration of this experiment. The second bottle was labeled "half dark, half light"; the last bottle was

labeled “light”. All of the bottles were placed in the incubator-shaker. The incubator-shaker was set to rotate at a speed of 125 rotations per minute and a temperature of 34° C. A 25 watt light was placed in front of the incubator-shaker, shining directly at the bottles. After 48 hours, the bottle labeled “half dark, half light” was covered with aluminum foil. The bacteria (media bottles) were kept in the incubator-shaker until they turned into a red color. After the bacteria and media turned red, the bacteria and media were transferred to big flasks filled with two liters of sterilized media (each bottle of bacteria was transferred to a separate flask). The opening of the flasks were plugged with cotton and covered with aluminum foil. The bacteria were kept in the big flasks until all of the contents in the flask turned red. After turning red, the bacteria were separated from the nutrient media through the process of centrifugation [instructions on using a centrifuge machine is available at Appendix G]. After the media and bacteria were separated, the bacteria was collected and put inside a test tube bottle. Each test tube bottle was labeled according to the condition it received (“light”, “dark”, and “half dark-half light”). The test tube bottles with the bacteria were stored in the freezer.

The second experiment, using temperature as a variable was started. A bottle of bacteria was placed in the incubator-shaker set at a temperature of 30° C. The bacteria and media were transferred to a big flask filled with two liters of sterilized media after the bacteria turned red. The flask’s opening was plugged with cotton and covered with aluminum foil. After this, the flask of bacteria and media were kept in the incubator-shaker until the bacteria and media turned red. After turning red, the bacteria and media underwent centrifugation. After centrifugation, the bacteria were put inside a test tube bottles labeled “temperature experiment, 30° C” and stored in the freezer. This procedure

was repeated two times, but the incubator-shaker was set at a different temperature each time, 32° C, and 34° C.

The third experiment, using oxygen as a variable was started. Two bottles of bacteria were used for this experiment. The two bottles were covered with five layers of parafilm. After this, a tube connecting to a nitrogen tank was inserted into the bottles of bacteria. An output hole was made on the parafilm using a needle simultaneously. The nitrogen tank was turned on, and the tube connecting to the nitrogen tank was left in the bacteria bottles for three minutes. This will force all of the air in the bacteria bottles out because the pressure inside the bottle is much greater than the pressure outside. After three minutes, the nitrogen tank was turned off and the tube connecting to the nitrogen tank was removed. The bottles were covered with another layer of parafilm. The bottles were capped and placed into the incubator shaker, set at a temperature of 34° C. A 25 watt light was placed in front of the incubator shaker, and one bottle was covered with aluminum foil, in order to prevent light from entering. The bottles were left in the incubator-shaker until they turned into a red color. After turning red, each bottle was transferred into big flasks filled with two liters of sterilized media. The big flasks were covered with five layers of parafilm. The nitrogen tube was inserted into the flasks; an output hole was made with a needle simultaneously. The nitrogen tank was turned on and the nitrogen tube was left in the flasks for ten minutes, in order to force all of the air out. After ten minutes, the nitrogen tank was turned off; the tube was removed from the flasks, and another layer of parafilm was used to seal up the flasks. The flask that contains the bacteria that received no light initially was covered with aluminum foil. After this, both of the big flasks were placed in the incubator-shaker until the bacteria and

media turned into a red color. After turning red, the bacteria and media underwent centrifugation. The bacteria were collected and put into test tubes. One test tube was labeled “no oxygen” and another was labeled “no oxygen, no light”. Both of them were stored in the freezer.

The last experiment, using time as a variable was started. Three bottles of bacteria were used for this experiment. One bottle was labeled “two days”; another was labeled “three days, control”, and the last was labeled “four days (the three-day bottle will be used as the control)”. All of the bottles were put in the incubator-shaker until they turned into a red color. After turning red, the bacteria were transferred to big flasks filled with two liters of sterilized media. The flasks were all plugged with cotton and covered with aluminum foil. They were all placed back into the incubator-shaker. The flasks were kept in the incubator-shaker according to the amount of time (two days, three days, and four days) labeled on the bottles. After the time labeled on the flasks/bottles were up, the bacteria underwent centrifugation. Then they were placed in test tube bottles labeled “two days/three days/four days” and stored in the freezer.

All of the test tube bottles containing the bacteria were retrieved from the freezer. The French Press Machine was used to break the bacteria’s cell membrane [refer to appendix I for the French Press procedure]. After breaking the cell membrane, the bacteria were put in the centrifuge machine, to separate the membrane particles from the photosynthetic reaction centers. After this, the reaction center concentrations were determined by using the ultraviolet spectrophotometer [refer to appendix H for the ultraviolet spectrophotometer procedure). The results were compared afterwards.

For a concept map that provides an overview of the methods and procedure, refer to appendix B.

Identification of Variables in this Experiment

Independent Variable: the variable that is purposely manipulated or changed. *In this experiment, the independent variables are the amount of light, oxygen, temperature and time that the Rb. sphaeroides receives during the culturing process.*

Dependent Variable: the variable that is being observed, which changes in response to the independent variable. *In this experiment, the dependent variable is the amount of polyhistidine-tagged proteins yielded.*

Control: subjects or procedures that permits comparison with the experimental results. *In this experiment, the control is the bacteria cultured without light in a 34° C. environment, with oxygen for three days.*

Constants: conditions or things in the experiment that remain the same. *In this experiment, the constants are the amount of tetracycline added into the media, amount of Rb. sphaeroides added into the media, and the amount of time spent in the culturing process.*

Results

After the French Press process, an ultraviolet spectrophotometer was used to determine the photosynthetic reaction center (polyhistidine-tagged protein) concentrations.

In the experiment that used light as a variable, the amount of polyhistidine-tagged proteins yielded by the flask of bacteria that received light was undetermined. The flask of bacteria that received no light yielded 4.85 micromoles of polyhistidine-tagged proteins. The flask that received some light yielded 1.42 micromoles of polyhistidine-tagged proteins.

In the experiment that used temperature as a variable, the flask of bacteria cultured with a temperature of 30° C. yielded 3.07 micromoles of polyhistidine-tagged proteins. The flask cultured with a temperature of 32° C. yielded 3.61 micromoles of polyhistidine-tagged proteins. The flask of bacteria cultured with a temperature of 34° C. yielded 4.85 micromoles of polyhistidine-tagged proteins.

In the oxygen experiment, the flask of bacteria that received no light and no oxygen died; therefore, no polyhistidine-tagged proteins were yielded. The flask of the amount of polyhistidine-tagged proteins yielded by the bacteria that received light and no oxygen was undetermined.

In the time experiment, the flask of bacteria cultured for two days yielded 0.44 micromoles of polyhistidine-tagged proteins. The flask cultured for three days, which was the control in this experiment yielded 0.819 micromoles of polyhistidine-tagged proteins. The flask cultured for four days yielded 0.831 micromoles of polyhistidine-tagged proteins.

The graphs shown on the next few pages were produced by the ultraviolet spectrophotometer, which helps determine the reaction center (polyhistidine-tagged protein) concentration.

Comparison of Light and Dark Experiment Results

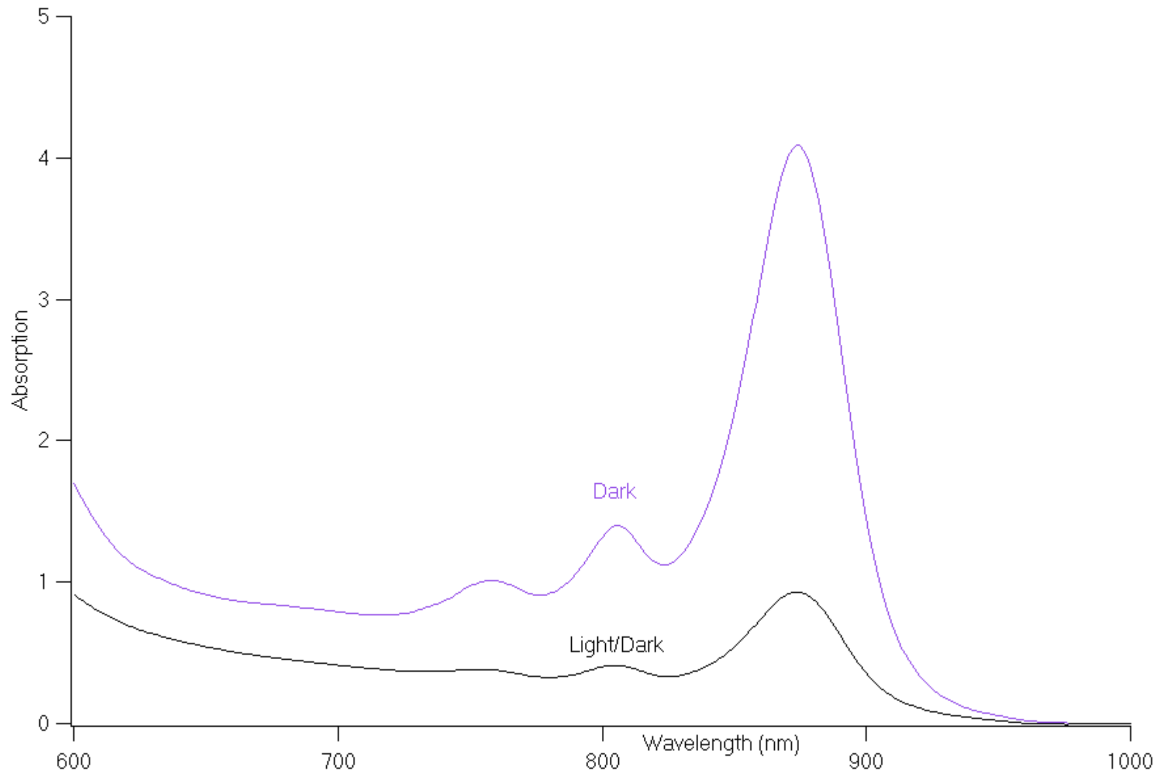

Figure 1. This is the graph produced by the ultraviolet spectrophotometer. The peaks on the graph represent the reaction center (polyhistidine-tagged protein) concentrations. At 806 nanometers the line labeled "dark" has a higher peak than the line labeled "light/dark"; therefore, the bacteria that was cultured in the dark has a higher reaction center concentration than the bacteria cultured with some light.

Finding the Reaction Center Concentrations

Absorption at 806 nanometers ÷ 0.288 = Reaction Center Concentration in micromoles (µm)

Dark: Absorption peak = 1.39737 → 1.39737 ÷ 0.288 = **4.85 µm per gram**

Light/Dark: Absorption Peak = 0.40944 → 0.40944 ÷ 0.288 = **1.42 µm per gram**

Comparison of Temperature Experiment Results

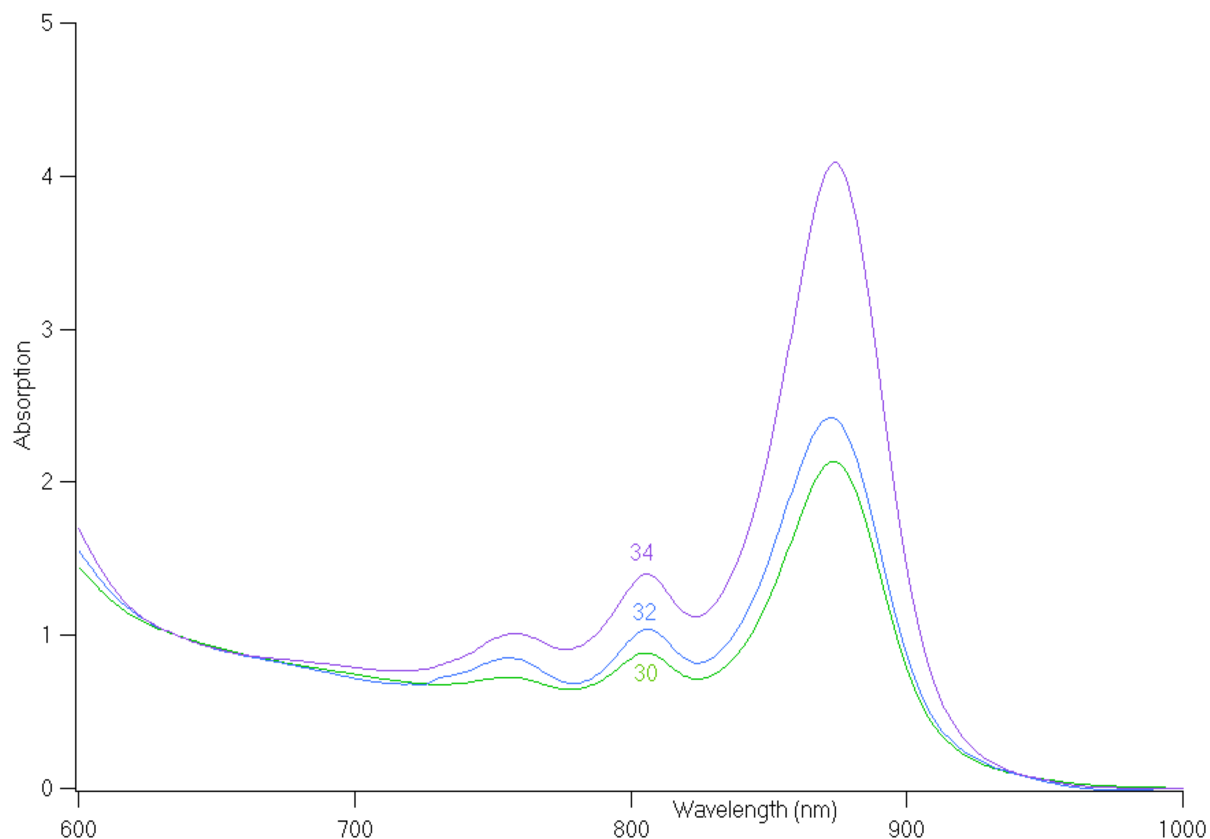

Figure 2. At 806 nanometers, the line labeled "34" has the highest peak; the line labeled "30" has the lowest peak. Therefore, the bacteria cultured in a 34° Celsius environment yielded the greatest amount of reaction centers. The bacteria cultured in a 30° Celsius environment yielded the least amount of reaction centers.

Finding the Reaction Center Concentrations

Absorption at 806 nanometers ÷ 0.288 = Reaction Center Concentration in micromoles (µm)

30° C. : Absorption peak = 0.88423 → 0.88423 ÷ 0.288 = **3.07 µm per gram**

32° C. : Absorption Peak = 1.0389 → 1.0389 ÷ 0.288 = **3.61 µm per gram**

34° C. : Absorption Peak = 1.39737 → 1.39737 ÷ 0.288 = **4.85 µm per gram**

Comparison of Time Experiment Results

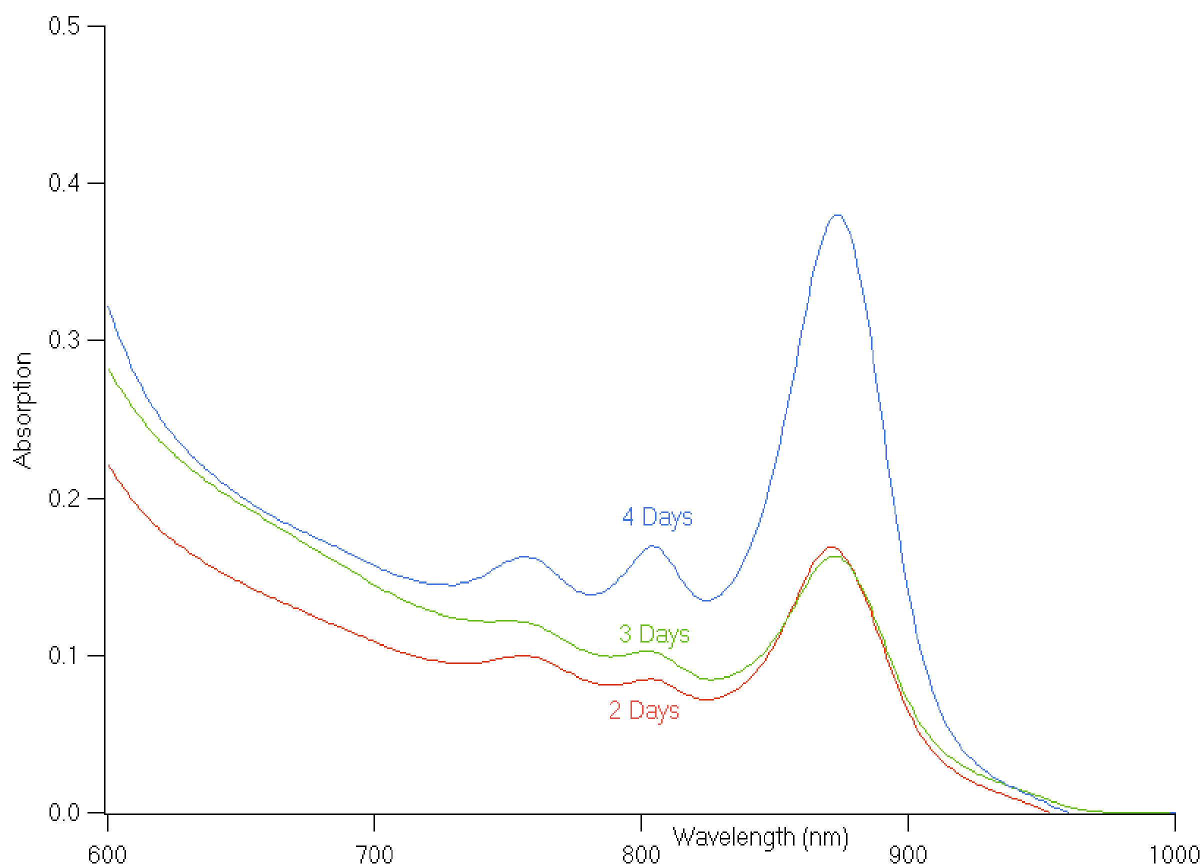

Figure 3. At 806 nanometers, the line labeled "4 days" has the highest peak and the line labeled "2 days" has the lowest peak. Therefore, the bacteria cultured for four days yielded the greatest amount of reaction centers. The bacteria cultured for two days yielded the least amount of reaction centers.

Finding the Reaction Center Concentrations

Absorption at 806 nanometers ÷ 0.288 = Reaction Center Concentration in micromoles (µm)

2 Days: Absorption peak = 0.12648 → 0.12648 ÷ 0.288 = **0.44 µm per gram**

3 Days: Absorption Peak = 0.2358 → 0.2358 ÷ 0.288 = **0.819 µm per gram**

4 Days: Absorption Peak = 0.23931 → 0.23931 ÷ 0.288 = **0.831 µm per gram**

The table below summarizes the amount of polyhistidine-tagged proteins yielded.

Summary of Results

Conditions Received by *Rb. sphaeroides*	**Amount of Polyhistidine-tagged Proteins Yielded**
Light	Amount of polyhistidine-tagged protein is undetermined
No light	4.85 micromoles per gram/0.73 grams per milliliter
Some light	1.42 micromoles per gram/0.213 grams per milliliter
30° Celsius, no light	3.07 micromoles per gram/0.4605 grams per milliliter
32° Celsius, no light	3.61 micromoles per gram/0.5415 grams per milliliter
34° Celsius, no light	4.85 micromoles per gram/0.73 grams per milliliter
No light, no oxygen	Died, no polyhistidine-tagged proteins yielded.
Light and no oxygen	Amount of polyhistidine-tagged protein is undetermined
Two days, no light	0.44 micromoles per gram/0.066 grams per milliliter
Three days, no light (Control)	0.819 micromoles per gram/0.123 grams per milliliter
Four days, no light	0.831 micromoles per gram/0.125 grams per milliliter

Figure 4 . This table shows the amount of polyhistidine-tagged proteins yielded. The bacteria that received "no oxygen with no light" died. The bacteria that were cultured for three days with no light is the control in this experiment. The bacteria cultured with no light in a 34 degrees Celsius environment yielded the greatest amount of polyhistidine-tagged proteins (4.85 micromoles per gram). The bacteria cultured without light for two days yielded the least amount of polyhistidine-tagged proteins (0.44 micromoles per gram).

Comparison of Data

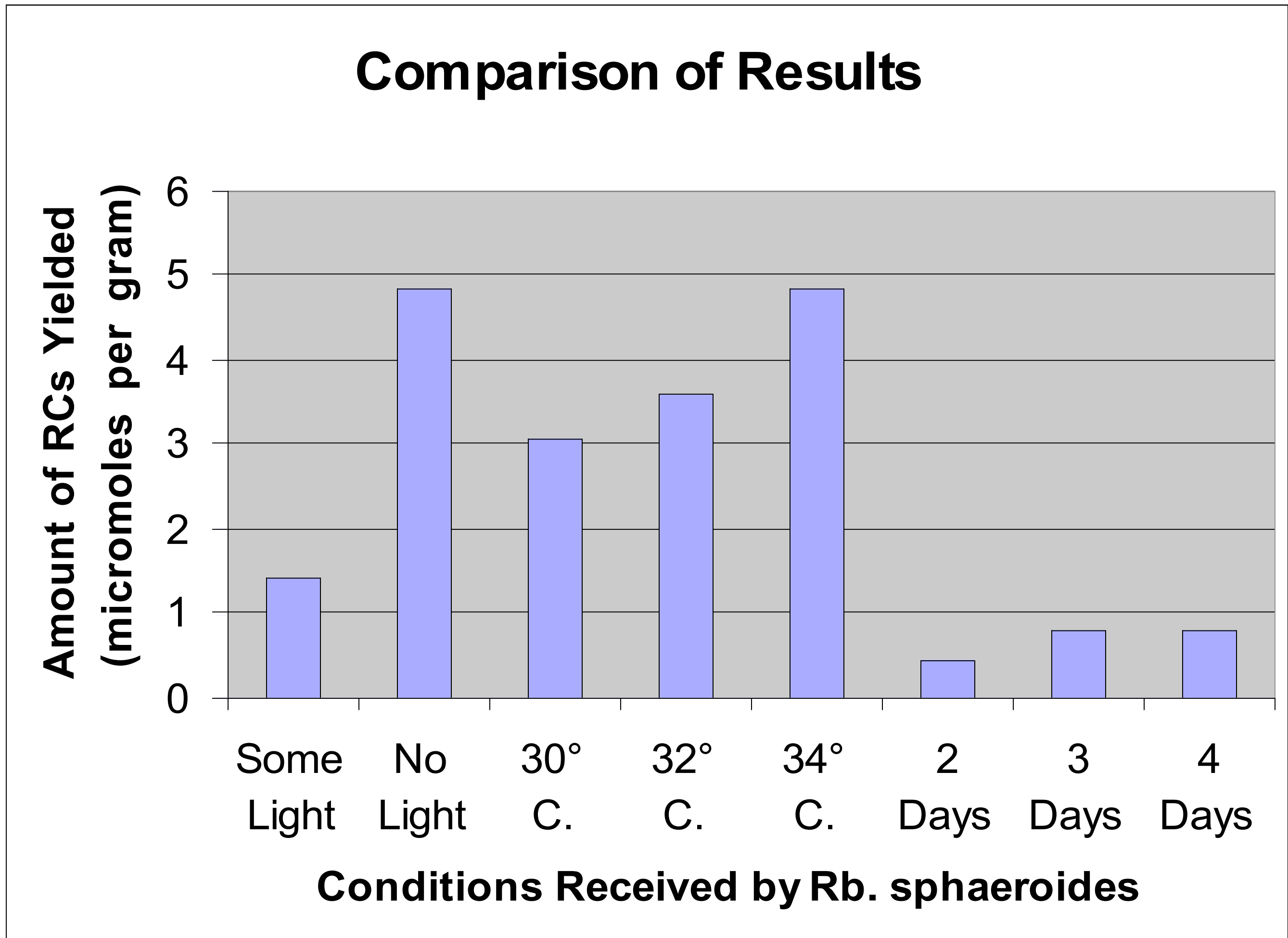

Figure 5. This graph is a visual representation of the data. The condition that yielded the greatest amount of reaction centers (polyhistidine-tagged proteins) can clearly be seen. The bacteria that were cultured with no light and 34° Celsius yielded the same amount of reaction centers, as well as the greatest amount of reaction centers. The bacteria cultured for two days yielded the least amount of reaction centers.

Conclusion

The hypothesis for this experiment was that culturing *Rb. sphaeroides* with light and oxygen for four days in a 32° Celsius environment will yield the greatest amount of polyhistidine-tagged proteins. This hypothesis was refuted by this investigation because the reaction center concentration of the bacteria cultured with light was undetermined. It was unable to be determined because no polyhistidine-tagged proteins were yielded; instead, another type of protein was yielded.

The bacteria cultured in a 30° Celsius environment yielded the least amount of polyhistidine-tagged proteins, while the bacteria cultured in a 34° Celsius environment yielded the greatest amount. The bacteria cultured in a 32° Celsius environment yielded more polyhistidine-tagged proteins that the 30° Celsius one, but less than the 34° Celsius. Therefore, the lower the temperature is, the fewer polyhistidine-tagged proteins will be yielded. 34° Celsius was determined to be the best temperature to culture *Rb. sphaeroides.*

In the time experiment, the bacteria cultured for four days yielded the greatest amount of reaction centers, while the bacteria cultured for two days yielded the least amount. The bacteria that were cultured for three days yielded more polyhistidine-tagged proteins than the bacteria cultured for two days, but less than the four days bacteria. Therefore, culturing the bacteria for four days will yield high amounts of polyhistidine-tagged proteins.

In the oxygen experiment, the bacteria cultured without light and oxygen died. The amount of polyhistidine-tagged proteins yielded by the bacteria cultured with light

but no oxygen is undetermined. Therefore, depriving *Rb. sphaeroides* of oxygen does not help in yielding more polyhistidine-tagged proteins.

As a conclusion, culturing *Rb. sphaeroides* without light for four days in a 34° Celsius environment with oxygen will yield the greatest amount of polyhistidine-tagged proteins.

Discussion

The *Rb. sphaeroides* cultured in a 34° Celsius environment yielded high amounts of polyhistidine-tagged proteins because bacteria usually thrive in warm temperatures. Warmth is an essential element for bacterial growth because heat speeds up chemical reactions, and thus speeds up the reproductive rate of bacteria. The *Rb. sphaeroides* cultured with 30° Celsius yielded the least amount of polyhistidine-tagged proteins because the low temperature slows down chemical reactions; therefore, it also slows down the reproductive rate of bacteria.

The *Rb. sphaeroides* cultured for two days yielded the least amount of polyhistidine-tagged proteins because the bacteria did not get enough time to mature; it is still in its early stages of life, therefore, it cannot produce high amounts of proteins. The bacteria cultured for four days had enough time to mature and absorb nutrients; as a result, it was able to yield high amounts of polyhistidine-tagged proteins. However, leaving the bacteria in the nutrient media for too long will lead to overgrowth, and eventually death, because the bacteria will absorb all of the nutrients until the population exceeds the carrying capacity allowed by the media, resulting in death.

In the light experiment, the *Rb. sphaeroides* that received light turned into a brown color, instead of the usual red color. The polyhistidine-tagged proteins from *Rb. sphaeroides* are red; therefore, if polyhistidine-tagged proteins were yielded, the contents in the flask should be red. However, the contents in the flask that received light was brown, therefore, polyhistidine-tagged proteins were not yielded. A possible explanation for this might be that the bacteria that received light yielded another type of protein. A 25 watt light bulb was placed in front of the incubator-shaker for about three days; the bacteria received an abundance of light. Excessive light absorption might have damaged the reaction centers and caused the bacteria to synthesize another type of protein (Zhang, 2005). The presence of light in the culturing process causes the formation of another type of protein.

In the oxygen experiment, the bacteria cultured without light and oxygen died because *Rb. sphaeroides* require light or oxygen to survive. With light, *Rb. sphaeroides* can perform photosynthesis and make its own food. With oxygen, *Rb. sphaeroides* can absorb nutrients from the media and break it down into energy by performing aerobic respiration – the process in which cells use oxygen to break down food molecules and nutrients to generate energy (Hunter, 2002). Therefore, *Rb. sphaeroides* can survive in the presence of either light or oxygen. This explains why the *Rb. sphaeroides* cultured without light and oxygen died; it was unable to perform photosynthesis because it had no light and it was unable to utilize the nutrients from the media through aerobic respiration because there was no oxygen. The flask of bacteria that received light but no oxygen turned into a brown color. The results were similar to the flask of bacteria discussed in

the light experiment; both of them turned brown because another type of protein was synthesized.

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All of the bacteria yielded more polyhistidine-tagged proteins than the control, expect for the bacteria cultured for two days with no light. The control (three days, no light), yielded 0.819 micromoles of polyhistidine-tagged proteins per gram. The bacteria cultured for two days with no light, yielded 0.44 micromoles of polyhistidine-tagged proteins per gram, which is less than the control.

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There weren't many errors in this experiment; however, there were certain aspects of the experiment that were supposed to be kept constant. The amount of *Rb. sphaeroides* added into each media bottle was supposed to be 25 microliters throughout the experiment. However, 25 microliters of *Rb. sphaeroides* took too long to culture; it took about one week for it to be ready for harvesting. Therefore, after the light experiment, the amount of *Rb. sphaeroides* added into each media bottle was changed to 200 microliters, in order to speed up the culturing process. The amount of bacteria added into each media bottle was supposed to be a constant; it should not have been changed, but due to time constraints, it had to be altered. Altering this constant made this investigation a little less scientific; it might have affected the results and made them not as accurate.

The amount of time spent on the culturing process should also be kept constant – three days. Three days is the average amount of time it takes for the bacteria to turn red, or ready for harvesting. However, the bacteria were harvested according to the amount of

time it takes to turn red, which varied from flask to flask. Altering the amount of time spent on the culturing process can greatly affect the results because allowing the bacteria to stay one more day in the nutrient media can result in more polyhistidine-tagged proteins being yield.

If this experiment were to be performed again, the amount of Rb. sphaeroides added into the media should be kept constant (25 microliters) and the time spent on the culturing process should also be kept constant (three days).

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The conclusion established by this investigation is not totally accurate or reliable because this experiment was only performed once. Due to the amount of time allowed, this experiment was unable to be performed a second time because this whole experiment took about five months to complete; it would take another five months to perform this experiment again. If the results of this experiment can be replicated, then the conclusion established is accurate and reliable. Unfortunately, this cannot be done because of time restraints.

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Polyhistidine-tagged proteins are used as the basis of photosynthetic research; scientists who conduct experiments on photosynthesis frequently use polyhistidine-tagged proteins as photosynthetic models. Therefore, many experiments and investigations can benefit from the conclusion established by this experiment. Scientists should follow this method – culture *Rb. sphaeroides* without light in a 34° Celsius environment with oxygen for four days if they want to perform photosynthetic research with polyhistidine-tagged proteins.

After this experiment, some questions that were aroused include: How does pH affect the growth of *Rb. sphaeroides*? Is there a catalyst that can be added to the media and bacteria during the culturing process to speed up the reproductive rate of *Rb. sphaeroides*?

Since photosynthesis requires light, why weren't any photosynthetic reaction center yielded in the flask of bacteria that received light? Why was another type of protein synthesized instead?

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Appendix A

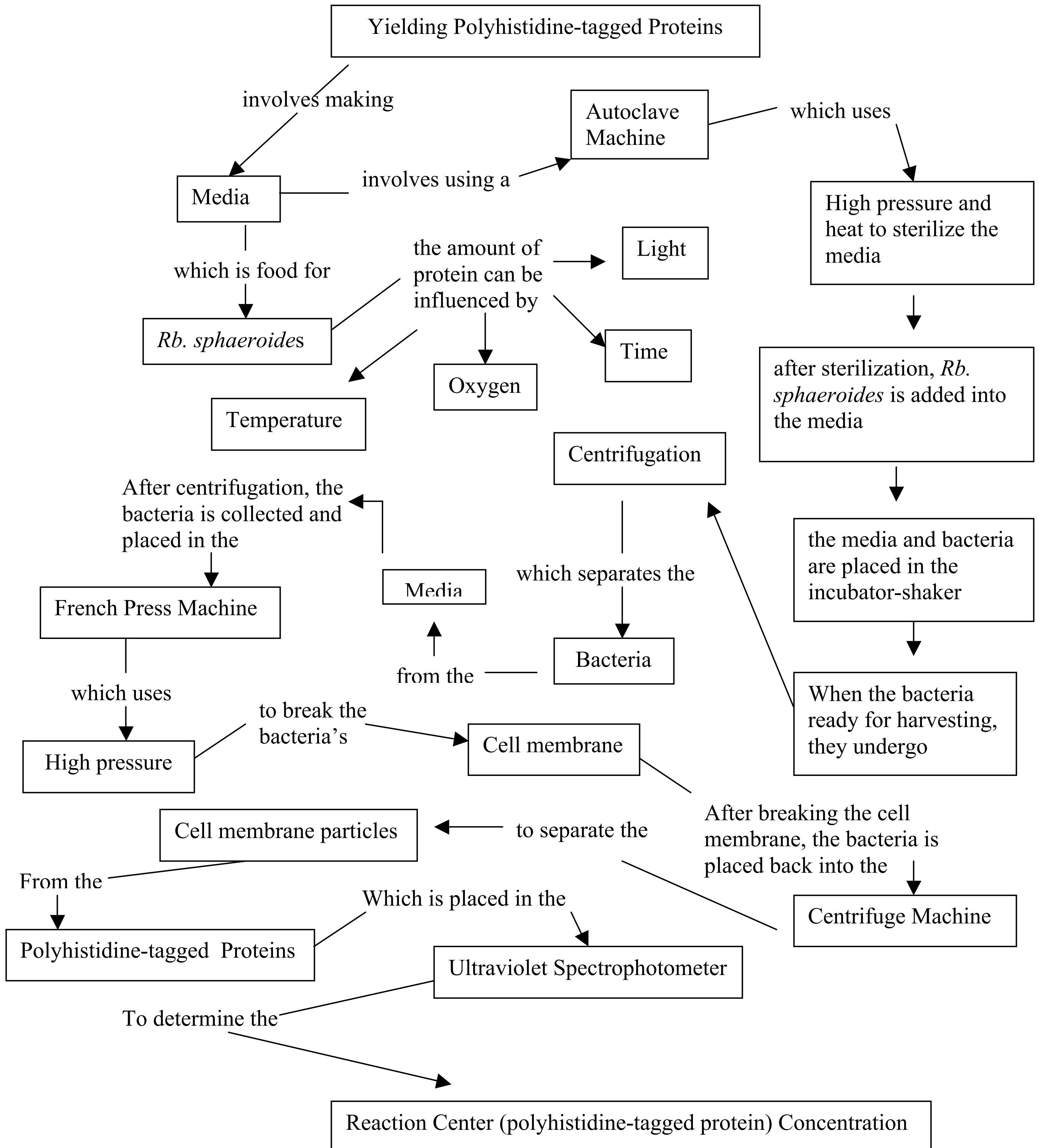
Complete List of Materials (with unfamiliar equipment explained)

1) Yeast extract
2) Casamino acids
3) Solution C (a mixture of various irons and metals)
4) *Rhodobacter sphaeroides*
5) Distilled water
6) pH meter (apparatus used to measure the pH of substances)
7) Hydrochloric acid (one molar concentration)
8) Sodium hydroxide (one molar concentration)
9) Autoclave machine (apparatus used to sterilize solutions and substances by using high temperature)
10) Centrifuge (apparatus used to separate substance of different densities)
11) French Press Machine (apparatus that uses high pressure to break cell membranes)
12) Ultraviolet spectrophotometer (apparatus used to measure protein/ reaction center concentration)
13) Flash spectrophotometer (apparatus used to measure the wavelength and absorbance of a substance in its excited state)
14) Digital balance (apparatus used to measure the mass of substances)
15) Double beam balance (in this experiment, the double beam balance was used to ensure that the media bottles had the same amount of media and bacteria in it)
16) Pipettes (professional eye droppers used to transfer solutions from one place to another)
17) Sterilized disposable pipette tips
18) Non-sterilized disposable pipette tips
19) Eye droppers
20) Graduated cylinders

21) Glass media bottles
22) Self-adhesive labels
23) Permanent marker
24) Distilled water
25) Parafilm
26) Spatula
27) Weighing paper (paper used to hold substances on the digital balance)
28) Scissors
29) Tetracycline (an antibiotic that eliminates all unwanted bacteria in this experiment)
30) Tris (chemical added to reaction centers for using the ultraviolet spectrophotometer; molecular formula: $C_4H_{11}NO_3$)
31) Incubator-shaker (an apparatus used to keep substances in motion and at a certain temperature)
32) -20° C. freezer
33) -80° C. freezer
34) Refrigerator
35) 25 watts light bulb
36) Aluminum foil
37) Cotton
38) Ethyl alcohol
39) Bunsen Burner (the Bunsen burner was used as a source of fire in this experiment)
40) Test tube bottles (plastic bottles in the shape of a test tube)
41) Disposable latex gloves
42) Safety goggles
43) Lab coat
44) Plastic cuvettes (very small plastic transparent containers used to hold liquid samples to put into the spectrophotometer)
45) Flint sparker (tool used to start a fire on the Bunsen burner)

Appendix B

Concept Map of Methods



Appendix C

YCC Media Procedure

Note: The amount of ingredients in this procedure is intended for making 100 milliliters of YCC media. To make more than 100 milliliters, determine the correct amount of ingredients to use by using ratios.

1) Put a piece of weighing paper on the digital balance.
2) "Zero" the digital balance.
3) Use a speculum to transfer 0.5 grams of yeast extract onto the weighing paper.
4) Gently pour the 0.5 grams of yeast extract into a graduated cylinder by folding the weighing paper into a spout.
5) Discard the weighing paper.
6) Put a new piece of weighing paper on the digital balance.
7) "Zero" the digital balance.
8) Use a new speculum to transfer 0.6 grams of Casamino acids onto the weighing paper.
9) Gently pour the 0.6 grams of Casamino acids into the graduated cylinder with the yeast extract.
10) Use a pipette with a non-sterilized tip to obtain 0.5 milliliters of solution C.
11) Instill the 0.5 milliliters of solution C into the graduated cylinder.
12) Fill the graduated cylinder up to 98 milliliters with distilled water.
13) Cut a piece of parafilm.

14) Put the piece of parafilm over the graduated cylinder (wrap it tightly, make sure the contents inside the graduated cylinder does not leak out).

15) Shake the graduated cylinder until all of the yeast extra, Casamino acids, and Solution C are completely dissolved.

16) Calibrate the pH meter.

17) Adjust the pH of the contents inside of the graduated cylinder to 7.2 by using eye droppers to add hydrochloric acid (to decrease the pH) or sodium hydroxide (to increase the pH). (Note: use a separate eye dropper for the hydrochloric acid and sodium hydroxide so the solutions don't get contaminated).

18) After adjusting the pH of the contents in the graduated cylinder to 7.2, add enough distilled water into the graduated cylinder to make the volume up to 100 milliliters.

19) Sterilize the media by following the autoclave procedure.

Appendix D

Autoclave Procedure

1) Loosen the caps on the glass media bottles.
2) Put the glass media bottles in the autoclave plastic bin.
3) Put on autoclave gloves.
4) Open the autoclave machine door turning the knob counter clockwise.
5) Put the autoclave plastic bin with the glass media bottles into the autoclave machine.
6) Close the door by turning the knob clockwise.
7) Set the time (45 minutes).
8) Press the “LIQUID” button.
9) After 45 minutes, the media is sterilized, but wait an additional 10 minutes to allow the pressure to stabilize; after the pressure arrow reaches 0, open the door.
10) Take the media bottles out of the autoclave plastic bins.

Appendix E

Procedure For Adding Tetracycline

1) Wipe the table with ethyl alcohol.
2) Get tetracycline out of the freezer.
3) Light-up the Bunsen burner.
4) Open the cap of one media bottle.
5) Hold the media bottle's opening over the flame for 10 seconds.
6) Use a sterilized pipette tip to put 25 microliters of tetracycline into the media bottle.
7) Hold the media bottle over the flame for 10 seconds.
8) Cap the media bottle back.

Appendix F

Procedure For Adding *Rb. Sphaeroides*

1) Wipe the table with ethyl alcohol.
2) Get *Rb. sphaeroides* out of the freezer.
3) Light-up the Bunsen burner.
4) Open the cap of one media bottle.
5) Hold the media bottle's opening over the flame for 10 seconds.
6) Use a sterilized pipette tip to put 25 microliters of *Rb. sphaeroides* into the media bottle.
7) Hold the media bottle over the flame for 10 seconds.
8) Cap the media bottle back.

Appendix G

Procedure For Using Centrifuge Machine

1) Distribute the flask of media and bacteria into 8 centrifuge bottles (only fill the bottles up to 2/3 full).
2) Make sure the amount of media and bacteria in each centrifuge bottle are equal by using the double beam balance.
3) Wipe the centrifuge machine and GSA rotor with alcohol.
4) Set the temperature to 4°C.
5) Set the speed to 9000 RPM (rotations per minute).
6) Set the rotor code to 10.
7) Set the time to 15 minutes.
8) Put the centrifuge bottles into the bottle compartments in the GSA rotor.
9) Lock the GSA rotor with the rotor top.
10) Wait until the centrifuge machine indicates that the temperature has lowered to 4°C.
11) After the temperature is down to 4°C, put the GSA rotor into the rotor compartment of the centrifuge machine.
12) Close the door.
13) Press the “Start” button.
14) After 15 minutes is up, wait for another 5 minutes before opening the door.
15) Take the centrifuge bottles out of the GSA rotor.

Appendix H

Ultraviolet Spectrophotometry Procedure

1) Prepare one milliliter of Tris with a pH of 8.
2) Prepare two ultraviolet cuvettes; put one milliliter of Tris in one, and one milliliter of reaction centers in the other.
3) Cover the cuvette tops with parafilm.
4) Insert the cuvette with Tris (the will be the “baseline”) in the cuvette compartment of the ultraviolet spectrophotometer.
5) Press “start” to determine the absorbance of the Tris.
6) After the absorbance is determined, take out the cuvette with Tris and put the cuvette with the reaction centers in the cuvette compartment.
7) Press “auto-zero”.
8) Press “start”.

Appendix I

French Press Procedure

1) Clean French Press Column with alcohol.
2) Uncap the French Press Column.
3) Pour sample into the French Press Column.
4) Cap the Column back.
5) Place the column into the French Press Machine.
6) Turn machine on.
7) Turn the Pressure Knob to “Medium”.
8) Wait 10 seconds.
9) Turn the Pressure Knob to “High”.
10) Place a centrifuge bottle in front of the output tube to collect bacteria.
11) After all of the bacteria have been collected, turn the Pressure knob to “Medium”.
12) Turn the Pressure Knob to “Low”.
13) Turn machine off.