

# The Effects of Atmospheric Contact on the Rate of Acetylene Breakdown in *Azotobacter chroococcum*.

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## ABSTRACT

*Azotobacter chroococcum* is a bacterium known to break the triple bond that forms  $N_2$ . This is important for life that cannot devour other organisms that have already converted the  $N_2$  into usable forms. Nitrogen fixing bacteria break the triple bond and form compounds that plants and other bacteria can readily use. This same species of bacteria can also break the triple bond that appears in the compound acetylene. This experiment looked into the possibility of affecting the rate of breakdown by the amount of contact that the bacteria has to the surrounding environment. A gas chromatograph, equipped with a flame ionization diode, measures the amounts of acetylene present before and after a 24 hour period. This encompasses the idea of understanding the rates of breakdown in Nitrogen also. The outcome shows the atmospheric contact has a direct relation to the ability of the bacterium's breakdown of atmospheric air.

Keywords: *acetylene, Azotobacter chroococcum, nitrogen fixation, nitrogen fixing bacteria, gas chromatograph*

## INTRODUCTION

Soil microbiology is a well established perspective in the understanding of the microbiological world. Bacteria and other organisms in the soil influence the needs at the molecular level of plants and animals that live both above and below the surface of the soil. These microorganisms ensure that important cycles, such as the nitrogen cycle remain in continuous motion. These microorganisms do this by taking molecules that most plants and animals cannot use and create completely different molecules by either reshaping or completely modifying the original unusable molecule (Hardy, 1968).

Some plants do not have the means to harvest nitrogen from the soil, air, or have the ability to use it in the form that is naturally found. The existence of certain types of bacteria, *Azomonas agilis*, *Azospirillum brasilense*, *Azospirillum lipoferum*, *Azotobacter chroococcum*, *Azotobacter vinelandii*, and *Beijerinckia mobilis* (Chen, 1993), take those compounds and help make them more accessible for the surrounding organisms that control the nitrogen uptake. Some of these bacteria in the soil have the ability to convert the nitrogen compounds in the atmosphere. In most environments, these bacterium become naturally reoccurring, the free living bacteria take compounds such as  $N_2$  and break the bonds that form the compound to make it more accessible to the other living root systems in the rizosphere. This is very beneficial for the plants that need the nitrogen but cannot break the triple bond that forms the compound in its  $N_2$  form (Hardy, 1968).

The before mentioned bacteria break the triple bond of  $N_2$ . These are just a few of the model organisms used in labs to illustrate the properties of free living nitrogen fixing bacteria. I have chosen to use the variety known as *Azotobacter chroococcum*. The reasoning for this is the fact that the majority of

the research done includes the use of *Azotobacter chroococcum* bacteria so the culturing methods are well known. *Azotobacter chroococcum* is interesting by its location in nature; soils rich in clay like materials (Kisten, 2006).

*Azotobacter chroococcum*'s ability to break the triple bond in acetylene is well researched. This bacterium is known to break the triple bond in  $N_2$  as well as efficient in breaking the triple bond between the two carbon atoms in the acetylene compound (Revillas, 2000).

The measuring of the amounts of each gas present in the flask is done by a gas chromatograph. Due to the fact that the gas chromatograph that the science department owns cannot read the levels of  $N_2$  in the atmospheres chosen, the gas present in the flasks has been changed to acetylene. Although the primary difference is the fact that the nitrogen compound is broken into amine groups,  $NH_2+$ , the acetylene is in return converted into a completely different molecule in chemical properties. The amounts of acetylene present will be measured in the gas chromatograph.

This experiment is designed to investigate the contact with the atmosphere, and the effect the contact to the atmosphere has on the concentrations of acetylene breakdown.

## MATERIALS AND METHODS

An original uncontaminated colony of *Azotobacter chroococcum* will be grown and maintained throughout the whole experiment. *Azotobacter chroococcum* will be grown in a broth specifically designed for the bacteria. This broth consists of 2g of  $CaCO_3$ , 2g of glucose, .1g of  $K_2HPO_4$ , and .05g of  $MgSO_4 \cdot 7H_2O$ . Add this mixture to 0.1L of

distilled/deionized water. This broth was mixed thoroughly and distributed into test tubes. The tubes are then autoclaved at 15 psi. and 121 degrees Celsius. This colony will be maintained to ensure that all bacteria originate from the same genetics. The bacteria will be distributed among two different groups. These separate cultures of bacteria will be maintained in air tight vials with a rubber septum. The first step is to flush each of the vials with pure N<sub>2</sub> in order to maintain the same environment. Each one of these vials contains 2 mL of the broth.

The bacteria are introduced to the sterile broth filled vials at the exponential growth phase. The exponential growth phase occurs at approximately 24 hours after introduction to the environment. This is done by extracting 0.1 mL of broth from the original culture to insure equal distributions of the bacteria. The original culture was placed on an agitator to insure the distribution throughout the container during the extraction. These bacteria are then allowed 24 hours to reach their exponential growth phase.

After this period of 24 hours, the atmosphere in each one of the vials is injected with a gas mixture that contains N<sub>2</sub> and C<sub>2</sub>H<sub>2</sub>. The mixture is 1.25 mL of C<sub>2</sub>H<sub>2</sub> with 22 mL of N<sub>2</sub>. .01 mL of this mixture is injected into each one of the sterile vials.

The control group was placed in an incubator set at 25 degrees Celsius for a period of 24 hours in order to insure that the bacteria have a chance to go through their natural processes.

On the other hand, the experimental group was covered in aluminum foil and placed in an incubator shaker. The aluminum foil was to represent the darkness of the other incubator. The incubator shaker was set to 300 rpm and 25 degrees Celsius. These cultures were also allowed 24 hours to go through their natural processes.

After this, the atmosphere in each of the vials is analyzed using a gas chromatograph. The air in each of the vials is withdrawn to analyze. The peak area is then gathered and the analyzed using a distribution curve.

The amount of acetylene present in each of the vials was found by using the standard I created to inject into each of the vials. Taking ten withdrawals from the standard and measuring the acetylene in each sample allowed for a common average to be found. This average represents the amount used to compare as a difference in the original amount to the amount present after 24 hours.

## RESULTS

### Standard Curve

To convert the area under each of the curves on the results from the gas chromatograph, a standard curve is essential. This linear regression equation helped to establish a line of best fit to the data collected during the runs for the standard curve. The

slope of the line is equal to  $1.564e^{-6}$ . The R<sup>2</sup> value of this line of fit came out to be 0.999.

**Table 1.** Distribution of peak areas and acetylene volume for standard amount of acetylene injection. The average volume of acetylene for the group is 4.365 ng.

Peak Area	Acetylene Mass (ng)
2789567	4.3628
2864535	4.4801
2674893	4.1835
2890534	4.5207
2975865	4.6542
2765894	4.3258
2687950	4.2039
2859681	4.4725
2739485	4.2845
2659584	4.1595

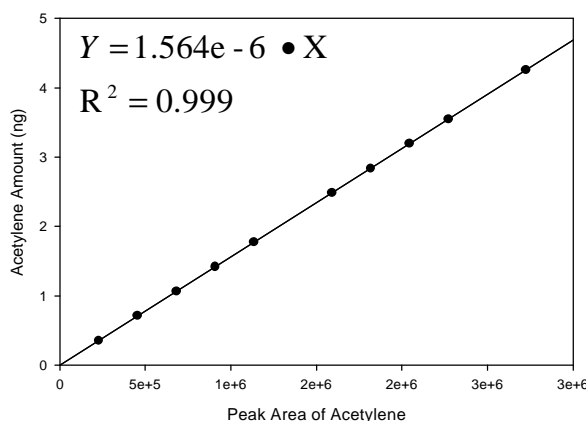


Figure 1. Standard curve for conversion of area to volume. The volume of acetylene appears on the y-axis while the area under the curve appears on the x-axis. The dots represent the measured amounts.

### Experimental Results

The first test was the control group. Two of the vials showed minimal change to the average found to represent the starting amounts. The remainder of the vials fell into a particular range of values. The average volume for the vials resulted in 2.130 ng.

The second test represents the treatment group. This group received the mixing which allowed for possible contact to the atmosphere in the vials. One more vial showed very minute differences in the acetylene before and after. The average for this test group resulted to be 1.689 ng.

The normality test between the two data sets failed. Using the Mann-Whitney Rank Test, the P-value was found to be  $P=.019$  where  $P < .05$  to show significance. This would conclude that the outcome

based on the medians would show a significant difference.

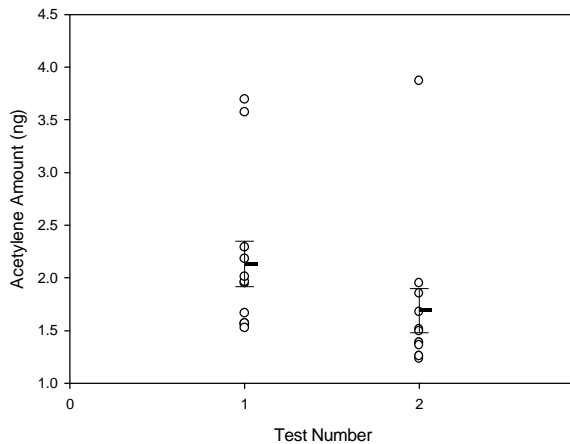


Figure 2. The effects of atmospheric contact on volume of acetylene broke down. Error bars represent 95% confidence interval. The circles represent the measured volumes. The solid bold line represents the mean of the samples. The brackets represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles.

## DISCUSSION

There was not nearly the sizable amount of difference in the outcomes between the control and the treatment groups. I believed that the chances that the bacteria had for coming in contact with the acetylene while being mixed would increase. The direct effect of this would cause a high difference in the amount of acetylene breakdown. This was not the case.

The amount of variance in each of the different vials also brought interest. The difference in the amounts used to create the average starting amount were not nearly the variance.

The outlying resultants could be due to many factors. For instance, the inability of the bacteria to reach the acetylene and bacteria may not have been able to sustain a culture in the environment.

There were was one main malfunction in the outcome. The first problem was to find a way to remedy the known concentrations of acetylene in the vials. This remedy was used by injecting the vials with a standard that I constructed myself. This standard consisted of 22mL of nitrogen and 1.25 mL of acetylene. This mixture then was injected into each of the cultured vials. I took ten injections to help in producing an average of the amounts of acetylene injected into the vials. To find a more efficient and reliable way to do this is a must if the experiment is duplicated.

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