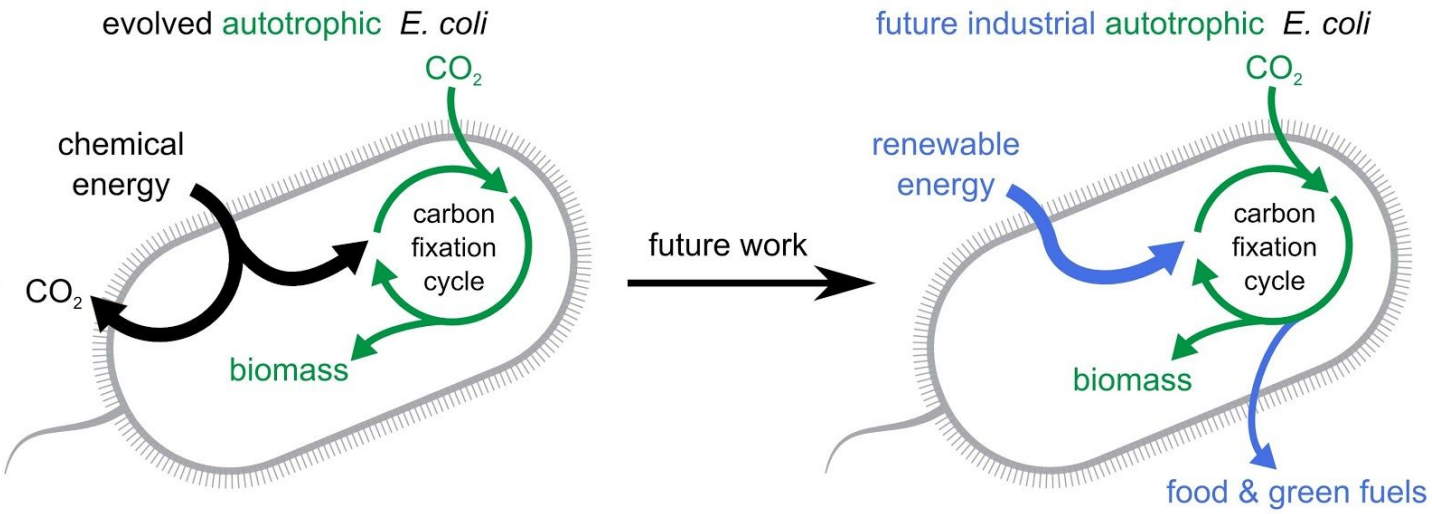
Carbon Sequestration through E. Coli

iCons 1 - Independent Case Study



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I. Project Summary

Our project focuses on answering the question of how effective *E. Coli* is in removing carbon from the air, and what are its possible applications for mitigating carbon emissions and carbon sequestration technology. We believe that *E. Coli* will be effective at sequestering carbon dioxide from the air at higher concentrations, which will suggest possible applications for carbon filtration systems.

Given the current state of the planet where every year there is an increase in the amount of carbon levels present in the atmosphere, many studies are looking for ways to reduce the excessive amount of carbon dioxide in the atmosphere; using microbes is one possible method that could be effective. We hope that our simple study can create a base for further discoveries, that could answer questions like is *E. Coli* an effective application for sequestering CO2, or which other species are more efficient in carbon sequestration, or what should be done to increase the productivity of these microbes?

II. Background

In recent times, the amount of carbon dioxide in the atmosphere has been increasing exponentially, which has led to the coining of the term “Anthropocene”, a new geologic epoch incited by manmade climate change. It was reported that for the past 200 years, approximately 405 gigatons (about 10^{15} grams) of carbon dioxide has been emitted into our atmosphere. Although the carbon cycle effectively works to reduce the amount of carbon in the air, as it can be absorbed through the soil, then transferred into the ocean, and the microbes under the sea through carbon fixation can return carbon to the food web or release oxygen as a byproduct, it is not enough to compensate of the sheer amount of carbon dioxide and methane being produced. The current amount of CO_2 has already exceeded the capability of the carbon cycle, leading to many consequences: global warming, climate change, the decrease in ocean pH, which then impacts the whole ecosystem. Scientists have long been finding out a way to sequester carbon in order to: first, reduce the amount of carbon dioxide in the air, and second, depending on the sequestering technique, to see if the carbon can be stored and be used for other purposes. Among many approaches, using microbes to support carbon sequestration is widely studied, some of those studies have found the following:

Some scientists have found some microbes that can produce ethanol, one of the most common biofuels.

David Johnson of New Mexico University argued that if we increase the ratio of fungi to bacteria in agriculture, the soil biome can be more effective in utilizing carbon and release less CO_2 into the atmosphere (but this result was questioned by other scientists).

Researchers from the University of Dundee found out that when they let *E. Coli* bacteria grow in a condition with no oxygen, they can produce an enzyme called FHL, which can interconvert carbon dioxide with liquid formic acid, a useful substance for several industrial applications. This process can be slow, but when they put *E. Coli* under high pressure of CO_2 and H_2 mixture, there was a complete conversion from carbon dioxide to formic acid.

These articles and studies discuss the methods of carbon removal, however, it is hard to find one that shows the efficiency of microbes in decreasing carbon dioxide. Therefore, we want to try to measure the amount of CO_2 that *E. Coli*, can remove in a given amount of time.

III. Methods

We are proposing an initial laboratory study whose data can then be used in a predictive model to extrapolate possible applications of the information we learn.

a. Overview of Study Design:

We will focus specifically on the effectiveness of *E. Coli* in removing CO_2 from the local atmosphere. There are certainly other microbes that can take CO_2 out of the surrounding air, however, too many samples would complicate the experimental process. Within experimentation with *E. Coli*, we will use different initial concentrations of CO_2 within the experimental system to see the relationship between the removal of CO_2 and the time taken within different conditions. CO_2 emissions are currently projected to increase by 2.3 parts per million per year. We will therefore not only test the efficiency of *E. Coli* CO_2 absorption in current atmospheric conditions but at projected future atmospheric concentrations, in 5-year increments. Also, using different initial conditions will allow us to see if the relationship between CFU and CO_2 removal is linear or a lot more dependent on the initial condition. CFU stands for colony-forming units, a standard measurement for the number of bacteria in a sample. Using this unit will allow us to standardize the data later on. By finding this relationship, we can predict how effective *E. Coli* would be in an atmospheric filter. Industrial processes would have much higher concentrations of CO_2 when they release into the atmosphere, so we could also scale up these results and apply them to a system for filtering carbon dioxide right at the source rather than once it has dispersed in the air.

b. Data Collection Procedures

E.Coli preparation: To prepare the *E. Coli* colonies, we will inoculate agar plates, incubate them for 16 hours, and then quantify the amount of bacteria in each using CFU, colony-forming units as a standard unit. Per each CO_2 initial concentration group, 5 plates will be prepared. This number will allow us to create a standard deviation so that our data is more sound.

System preparation: We will need 25 1 Liter containers to run the experiment for five different initial concentrations of CO_2 and 5 trials per concentration. The control trial will use atmospheric air as this is the concentration bacteria would face outside. The trials will be run at standard conditions, room temperature and 1atm, to prevent confounding variables. We will acquire CO_2 from a lab at different concentrations mirroring the projected atmospheric concentrations at 5 year intervals. The current concentration is 414 ppm CO_2, with a growth rate of 2.3 ppm/year (Lindsey). Thus, we will set up the initial conditions so that the second tank is 425.5 ppm for 5 years from now, the third tank at 437, and so on. After inserting the bacteria and CO_2, the lids of the 1 L container will be sealed air-tight.

CO_2 measurement: To measure how much CO_2 is consumed with time, NDIR, non-dispersive infrared, will be used. This device uses a form of absorption spectroscopy to analyze gases with infrared light. It will allow us to measure the carbon content of the containers in parts per million. We will insert a remote NDIR within each tank to prevent any change in concentrations from opening it up during the experiment phase. An initial concentration will be taken and then once sealed, the CO_2 content of each tank will be recorded every day for one month, about as long as *E. Coli* can survive at room temperature on a surface. Even if CO_2 is removed in quantities too minute to be registered by our device within our timeframe, we are running this experiment over the lifetime of the *E. coli* colony, so this should provide plenty of time for enough changes to occur. If not, we can increase the size of the colony in a later trial to account for this.

Example data table for t_0. There will be 31 of these tables to cover the month-long trial period.

	414 ppm	425.5 ppm	437 ppm	448.5 ppm	460 ppm
Trial 1					
Trial 2					
Trial 3					
Trial 4					
Trial 5					

c. Sources of Error.

Bacteria death may occur throughout this process. By running multiple trials at the same conditions, the exact effect of this will not become muddled with several others, however, it will be hard to prevent. Error can propagate from the CO_2 sensors themselves depending on how effective they are at different ppm. We can run an initial trial to measure how much our proposed *E. coli* colony size changes the concentration—if this change is measurable with our equipment, then we will use this size. If not, we can adjust the colony size to account for this. The data will be collected directly from the containers to limit system disturbances. The data will then be analyzed in Microsoft Excel daily to evaluate the progress of the experiment and see if any adjustments need to be made in the procedure. For example, if we see that the data is not behaving in one trial as other groups we can inspect that trial individually and assess the cause. We may find that the higher CO_2 concentrations will affect the growth or death of the populations of E. Coli and this may influence the change in CO_2 conversion throughout the process. Analyzing data daily will help us see when this is happening and account for it.

d. Assumptions in Study Design

We are assuming that the bacterial colony sizes do not change significantly between different samples. This may affect the rate of CO_2 absorption if so. We are also assuming that this is an effective mode of CO_2 capture or at least mirrors how CO_2 would be captured by the bacteria in a large scale setting. We are assuming that CFUs will be an applicable unit for further research as well. We are also assuming that the colonies will be a large enough size to create measurable changes in the CO_2 concentration of the tanks.

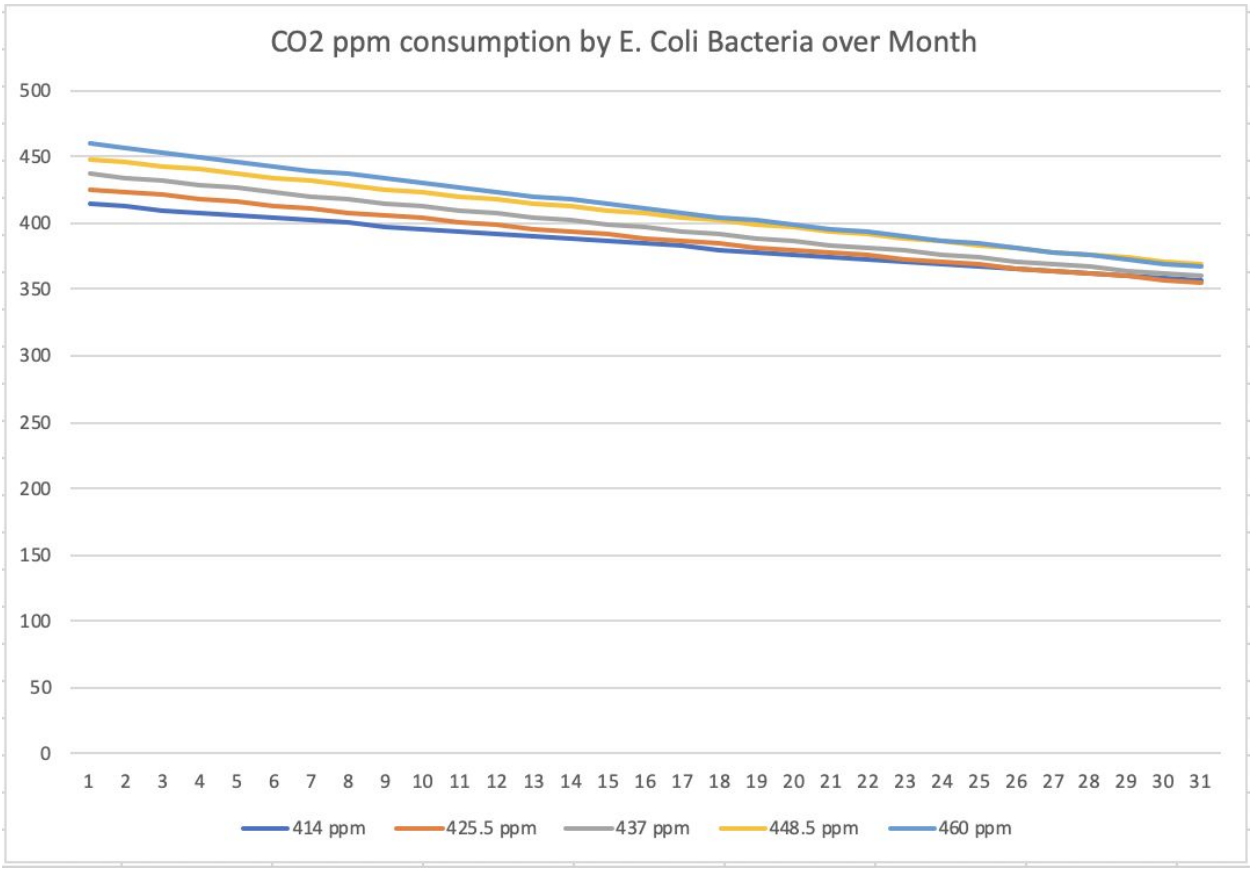
e. Data Analysis

In analyzing the data, we are looking for the effectiveness of E. Coli in processing CO_2 at different concentrations in the air. Thus, we will be looking at CO_2 concentration, $[CO_2]$, versus time graphs and use the slopes of their lines to quantify the effectiveness of the E. Coli in different conditions. An important step in processing the data is making sure that the difference in bacteria population size is accounted for. Thus, the $[CO_2]$ of each measurement will be divided by the initial CFU of that trial's plate. This value will be normalized to all of the other trials, making the data usable. From here, the rate of change in $[CO_2]$ can be measured per individual trial as well as the average change across a whole trial set. Even if a trend does arise, it is imperative to show that the data is significant. Thus, we will conduct an Anova to evaluate significant differences between the treatments.

IV. Anticipated Results

We anticipate that trials with higher initial carbon concentrations will sequester carbon at a faster rate. This assumption is based on a fundamental chemical interpretation of the consumption of

carbon, where a higher concentration will result in a higher rate. The graph below shows an example of what our results could resemble given our hypothesis. It is a hypothetical graph of five trials for the control group. Lines of best fit are added and are used to see the various slopes of the data. With this graph in particular, the slopes of the higher initial concentration samples are greater to reflect our prediction. Besides the control data, there will be four more graphs to analyze each concentration level. Each graph will have five lines representing the five trials at each concentration. The slopes of these lines are expected to decrease steadily. Standard deviation error bars will be added to the data points to signify the margin of other possible values while lines of best fit will show the overall trend of the data. Then there will be one separate graph with just the averages of all the trials for each of the five graphs. This graph will allow us to compare and contrast the effectiveness of the *E.coli* at removing CO_2 from the different concentration levels.



It is important to note that this graph is very ideal. The concentration change is linear and has no inconsistencies along the whole modeled trial. In reality, the data will likely appear much messier which is why the line of best fit is so important for visualizing the data. Also, the relationship we assumed is linear, but it may be that the CO_2 consumption will follow a more exponential pattern—it all depends on the reaction mechanics.

V. Timeline

Weeks One through Four - Data collection

For one month we will measure the amount of CO_2 consumed by the *E. Coli* bacteria in each of the 25 containers by comparing this amount to the initial amount recorded, using NDIR. The data collection will be recorded in parts per million. Throughout the process, we will input the data into a spreadsheet and ensure that something does not go awry with a trial. This means we will actively adjust any setups in case there is an error in carrying out the experiment.

Week Five - Analysis and Interpretation of Results

We will analyze the effects of the bacteria on each of the five concentrations. Thus, we will graph the CO_2 level versus time in hours to determine whether the *E. Coli* is having a significant effect on $[CO_2]$. Five graphs will be interpreted for the five different concentrations. Each of the five graphs will have the five trials of data recorded, so we can analyze whether or not a trend appears. After reviewing the data on each of the five graphs for five trials, we will see whether the $[CO_2]$ has changed significantly or not. A slight change in concentrations may mean that the *E.Coli* was consuming the CO_2, however, this would be hard to interpret whether or not the bacteria was solely responsible for this and not an example of human error. For this reason, the interpretation of trends among the data is vital. An Anova will be run to check the significance of the data and where data collection can be improved.

Phase	Week 1	Week 2	Week 3	Week 4	Week 5
Data Collection					
Analysis					
Interpretation of Results					

VI. Materials and Budget

Item	**Purpose**	**Cost ($)**
Agar Plates– 25 needed + few spare in case of accident.	To plate the *E. Coli* bacteria and grow them in a nutrient	20 per kit → ~60

Would order 3 "Essential Bacteria Growing Kits" from Amazon that come with→ - 10 Pre-Poured 100mm agar plates - 10 Cotton Swabs	broth	
25 Air Tight containers from the container store → 38 fl oz approx 1 liter	To place bacteria and CO_2 into	4 each → ~100
75 lb cylinder of CO_2 which holds approx 30 L CO_2	To put into containers	~300
E coli bacteria	Needed to measure growth and consumption of CO_2	One plate ~$15
NDIR would be accessed at a lab		
Total		**~ $475**

VII. Key Personnel

When researching the effects of *E.Coli* microbes on the mitigation of carbon emissions, background knowledge in climate change, carbon sequestration, and molecular biology is necessary. We also need to have a firm grasp of statistical modeling and data interpretation. Our team has taken this into account; with each of our members' specific background, we believe that we are an ideal group to carry out this project.

Lauren Gustafson majors in Earth systems with a focus on climatology, which will prove useful when analyzing the quality of the air before and after E.Coli has been introduced.

Liam Murphy is majoring in chemistry and mathematics so he has experience with chemical systems as well as being able to interpret data and make sense of models. These skills are important with this research because we will need to monitor CO_2 concentration throughout the experiment as well to make sense of what is occurring at the molecular

level to better improve experimental design. His math background will be useful with data interpretation especially because there will be 600 data points to work with.

Quynh Pham's major is Computer Science. She has knowledge in technical issues which can also help store and analyze data on the computer. This will be very important for the analysis of the data in the second and third phases of research.

Mehak Kang is a biochemistry and molecular biology major which means she will be able to make meaningful connections as to what the results of this experiment would mean when implementing it to a "real world" scenario. Mehak will also have the biological expertise to help lead the group in a deeper understanding of the *E. Coli* and the effects it would have on reducing the amount of carbon dioxide in the air.

VIII. Relevance of Proposed Study and Broader Impacts

We hope that our experiment will help to develop a path for atmospheric carbon sequestration technology through a replicable biological method. Removing CO_2 from the atmosphere has large social implications because of the negative side-effects of climate change. It is already known that *E. coli* can remove CO_2 from the air so there is not much new science going on, although the relationship between CO_2 concentration and conversion rates will be known. If a significant relationship is found between these, the results can be applied to carbon dioxide filter systems that are designed using *E. coli*. CO_2 would still be released into the atmosphere as this type of system could likely not be directly taken out of the atmosphere from the source of emissions. Reducing CO_2 emissions has great societal value because it will dampen drastic climate change in the coming years as less CO_2 will linger in the atmosphere. This will be important for large amounts of the human population as formerly arable land becomes unusable and all of the other economic damage that comes with climate change. Also, if the relationships we extract from the data can be scaled up to higher carbon dioxide concentrations, we could possibly apply our findings to systems that work on carbon dioxide emission sources which would have a much more direct impact on carbon emissions and reduction. This research will only make a dent into this issue, but researching the capabilities of *E. Coli* as a candidate for CO_2 removal will guide further studies and point to more refined experiments that could ultimately have a great impact.

When saying "great impact", we meant that the general public, not only scientists who are studying this area, but also other people around the world. Those living in densely populated areas, where air pollution is particularly bad, would benefit from a positive outcome in our work. A positive outcome would be that the *E. Coli* bacteria consume some of the carbon emissions in

the air, driving the overall CO_2 concentrations in the atmosphere down. Other scientists doing research on this may take this study and analyze its outcomes. If the *E. Coli* in the atmosphere shows no effect on CO_2 concentrations, other groups may wish to perform more studies on this to confirm these findings, which would disprove the hypothesis that *E. Coli* lowers carbon emissions. Scientists could then begin other experiments while ruling out the possibility of *E.Coli* bacteria as a solution.

Additionally, we can use social media and some science forums to show our research to a broad audience, and help raise more attention and awareness to the excess of CO_2 in our atmosphere and a technique to reduce it. Global warming leaves an impact on the general public hence the more CO_2 we can reduce in our atmosphere, the better we can improve our environment and the quality of life for everyone.

IX. References

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