Using Carbonic Anhydrase and Silica Nanomaterials in Carbon Dioxide Mitigation

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Abstract

As carbon dioxide levels in the atmosphere continue to rise, the necessity for efficient and effective carbon mitigation and sequestration techniques increases as well. However, attempts thus far have proven to be too expensive or speculative to be implemented on a global level. Our goal was therefore to devise a solution that is both efficient and cost effective. To do this, we utilized the catalytic properties of carbonic anhydrase, an enzyme contained in the lungs of mammals, to accelerate the reaction that converts carbon dioxide to bicarbonate, which can then be converted to useful products such as baking soda. Because enzymes are normally difficult to recover and can easily denature in suboptimal temperatures and pH levels, we attached them to silica nanospheres via enzyme immobilization in order to make them recoverable and more resilient to changes in environmental conditions. In testing this process, our results showed that a solution containing the immobilized enzymes was around 67 percent more efficient over the control, which contained no enzymes. Furthermore, we saw success in the silica's ability to immobilize the enzymes, as they were able to immobilize between 66.4 and 77.4 percent of the carbonic anhydrase in solution. Though we need to conduct further research to optimize this procedure, these positive preliminary results indicate that there is much promise in pursuing this method as means for carbon mitigation.

1 Introduction

Each year, millions of tons of carbon dioxide are released into the atmosphere, contributing to the greenhouse effect, which largely accounts for climate change and the disruption of global ecosystems. This gas, while innocuous in low quantities, inhibits the dissipation of heat from Earth's surface by trapping it in the upper atmosphere. This effect can lead to increased global temperatures, rising sea levels, shifting climates, increased ocean acidity, and many other environmental problems [1]. Potential longterm solutions involve curbing carbon emissions at the source by eliminating the use of fossil fuels in industry and everyday life. However, in order to slow the rate at which climate change occurs and allow for more time to implement a more permanent solution, we must also mitigate the level of carbon dioxide currently in the atmosphere.

To accomplish this goal, the procedure for carbon dioxide mitigation must fit several criteria. Because the scope of the issue is global, a solution that is both efficient and cost effective is vital. A procedure that takes decades or centuries to produce measurable effects would not suffice, as by that time, global climate shift may have already effected irrevocable changes to the climate and civilization. In addition, the solution must not be more harmful than the problem it seeks to resolve. Carbon dioxide must therefore either be safely stored or be converted into harmless, preferably useful, compounds. Ideally, such a solution would efficently utilize Earth's resources, to prevent further ecological destruction and reduce costs. Currently, no means of carbon sequestration fulfills these prerequisites.

However, the resolution to this issue may already exist within every mammal on Earth, in the form of enzymes that naturally convert carbon dioxide into absorbable compounds. Such enzymes play an integral role in the maintainence of a pH balance within the blood of mammals and assist in the removal of excess carbon dioxide from tissues [4]. Applied to the issue of climate change, these processes can be replicated outside of biological systems to convert raw carbon dioxide in the atmosphere to harmless, useful compounds. However, alone, the effective-

ness of enzymes in carbon mitigation is impeded by relatively high costs, an inability to recover the enzymes from solution, and the environmental limitations of enzymes.

The use of nanomaterials can mediate these issues, making the use of enzymes in carbon mitigation physically efficient and economically feasible. Through the process of enzyme immobilization, we can attach enzymes to nanomaterials, particles roughly one-thousandth the width of a human hair. In general, this procedure, which can be done with larger particles, allows for the enzymes to be easily recovered and reused, so long as they are prevented from denaturing [2]. The merit of using particles measured on the nano-scale, however, is that they provide a tremendous surface area on which the enzymes can be immobilized, significantly decreasing the amount of material needed and ultimately lowering the cost as well. Consequently, the combination of enzymes and nanomaterials has the potential to provide a low-cost method for effectively sequestering carbon dioxide.

2 Background

2.1 Current Carbon Mitigation Techniques

Carbon capture and sequestration, CCS, refers to any method of capturing CO_2 before it enters the atmosphere and storing it away [3]. There are currently a number of techniques in development and practice that attempt to mitigate carbon dioxide in the atmosphere with mixed results. One method of storing the carbon dioxide involves sequestering the gas in underground, empty oil reserves. Such a process involves pumping concentrated CO_2 gas via pipeline into the reserve where it is then absorbed in the porous rock within the well. Though this method is efficient and relatively cheap, the inherent danger of explosive leakage negates much of its apparent practicality. [1] Another method involves reacting CO_2 gas with ocean water to form carbonic acid. The carbonic acid then reacts with limestone to form calcium ions and bicarbonate ions, which can then be diluted in the ocean with no environmental impacts. [1] While this may be a better solution, it is unknown how well it can work on a large scale. As with the majority of other current carbon mitigation techniques, its speculative nature and low cost effectiveness prevent it from being a viable solution to this issue. Rather, there is more security in utilizing natural enzymatic processes that have been working for billions of years.

2.2 Science Behind Experiment

Our experiment primarily tests the efficiency of the enzyme Carbonic anhydrase in the conversion of carbon dioxide to bicarbonate. In general, enzymes are used in nature as catalysts, agents that lower the activation energy needed to carry out a chemical reaction, for biochemical processes that would otherwise be much slower and less efficient. Moreover, enzymes are not consumed in such processes and can therefore be reused indefinately [4]. This characteristic makes them not only biochemically efficient but also economically practical; one enzyme molecule can catalyze many reactions. We chose to use carbonic anhydrase in our experiment because it is one of the most efficient known enzymes and its function is specific to the reaction we desired to precipitate, the conversion of carbon dioxide to bicarbonate [4]. Although this process occurs naturally via the hydration and subsequent protonization of CO_2:

$$CO_2 + H_2O \longrightarrow H_2CO_3 \tag{1}$$

$$H_2CO_3 \longrightarrow HCO_3^- + H^+ \tag{2}$$

the reactions are not favored by equilibrium and sequesters only small quantities of carbon dioxide. With the use of carbonic anhydrase, the same result can be achieved much faster.

$$CO_2 + H_2O \xrightarrow{\text{Carbonic Anhydrase}} HCO_3^- + H^+ \tag{3}$$

Here, a single carbonic anhydrase enzyme can catalyze six million reactions per second, making the process tremendously efficient.

In chemistry, surface area is also a crucial factor of the rate at which reactions occur; specifically, more surface area directly relates to higher efficiency. With recent technological advancements, we can create silica nanospheres roughly 450 nanometers in diameter. We can then "etch" the surface of each sphere with a base, making them rough and thus further increasing surface area. This has much merit in chemistry because it allows for significant use with only modest amounts of material. We chose silica for this material because is it is highly abundant and very inexpensive. [1]

By combining carbonic anhydrase with silica nanospheres via immobilization, we optimize the overall effectiveness of the enzymes in the process that converts carbon dioxide to bicarbonate. The

process of immobilization is relatively simple and inexpensive, as the enzymes and silica nanoparticles naturally attract and attach to each other due to their opposite charges. Once immobilized, the enzymes have higher tolerances to changes in pH levels and temperature, preventing accidental denaturing, and they allow for easy recovery after usage, making them more economically practical. Even though immobilization slightly decreases the area of the enzymes' active sites, making them less effective, on a large scale, the long term benefits of resistance and reusability make this procedure a viable solution for carbon mitigation.

3 Methods and Experimental Design

3.1 Fabrication of Silica Nanoparticles

To create the silica nanomaterials needed for the immobilization of carbonic anhydrase, we employed a base hydrolysis of the compound tetraethyl orthosilicate (TEOS). In this process, we react TEOS with water to yield silica nanoparticles and ethanol. To accomplish the reaction, we made a solution containing 20 ml of ammonia, 200 ml of ethanol, 7.2 ml of water, and 12.0 g of TEOS. We stirred the solution for 1.5 hours, during which its color changed from clear to milky white, indicating the formation of suspended particles of silica. After centrifuging the solution and decanting the supernatant, we were left with the precipitated silica powder. Then we rinsed the silica with a solution containing 20 ml of ethanol and 20 ml of water before being centrifuging it again to ensure that the silica particles did not clump together. We completed this process twice to create two separate batches of silica nanospheres approximately 450 nm in diameter.

Following the creation of the silica, we etched the nanoparticles with potassium hydroxide in order to further increase their surface area. To etch the nanoparticles, we combined 1 g of silica, 20 ml of water, and 500 ml of .01 M KOH in solution. We created three separate batches, stirred for either 2 hours, 2.5 hours, or 3 hours, to test various degrees of etching and their effect on enzyme functionality and efficiency. Once we completed the etching process, we again centrifuged the solution to separate the supernatant from the precipitated silica powder. We then mixed the powder with water, sonicated it to ensure that the silica was completely dissolved, centrifuged again, and repeated this process three more times. The purpose of the repeated washing and centrifugation was to fully break up the silica particles, ensuring that only the silica and the attached OH- molecules remained in the precipitated powder as we prepared for enzyme immobilization.

3.2 Immobilization of Carbonic Anhydrase on Silica Nanospheres

To begin immobilization, we dissolved the solid silica into solution by adding 100 micrograms of silica to 4.9 ml of a phosphate buffer solution. The purpose of the phosphate buffer was to maintain a suitable pH level in the solution throughout the immobilization process.[1] We then sonicated the dissolved silica to ensure that the silica was completely solvated. Next, we added 50 micrograms of carbonic anhydrase and .1 ml of water to the solution. We then stirred the mixture for 2 hours at 4°C, so as to prevent the enzyme from denaturing. After stirring, we centrifuged the solution and separated the supernatant from the silica powder.

3.3 Testing the Effectiveness of Immobilization

To test the effectiveness of our immobilization procedure, we employed a BCA Assay, a process that indicates the concentration of a protein in solution. It relies on the reaction that occurs between peptides and Cu^{+2} ions, reducing the ions to Cu^{+1} in concentrations proportional to the original amount of protein present in solution. Then bicinchoninic acid chelate binds with the reduced ions, giving them a purple hue that is also proportional to the amount of protein present. Because of Beer's law, which directly relates the absorption of light in a solution to the solution's concentration, we can use UV spectroscopy to measure the amount of 560 nm light absorbed by the BCA complex and thus the amount of protein, or in our experiment, enzyme, in solution. To measure the concentration of our immobilized enzymes, we compared the results of the BCA Assay and UV spectroscopy against several control samples of known concentrations. The control samples consisted of a measured amount of standard protein in 5.0 ml of phosphate buffers such that the six control samples ranged in protein content from 0 micrograms through 100 micrograms. Next, we added the BCA assay solution to both the control samples and the supernatant from the 2 hour, 2.5 hour, and 3 hour silica. We incubated all samples in a hot water bath at 60°C for 2 hours, which allowed the copper reduction and the corresponding formation of the BCA chelate complex to occur.[1] At the end of this time, the color of both the control and experimental samples had changed

from clear to varying shades of purple indicating the concentrations of proteins in the solutions.

We then analyzed the absorption of each sample using a UV spectrometer. The results of this test showed how much UV light was absorbed at a corresponding wavelength. As BCA complexes absorb the most light at a wavelength of approximately 560 nanometers, the corresponding absorption level of control samples at that wavelength can be plotted with respect to known concentration, due to Beer's law.[1] From the linear relationship that results, the enzyme concentration of the unknown samples could be determined by simply plotting the absorption levels and reading the corresponding concentration. With the result of this procedure, we were able to determine how effectively the silica was able to immobilize a sample of carbonic anhydrase.

3.4 Measuring of Efficiency of Immobilized Carbonic Anhydrase

After immobilizing the enzymes and testing the effectiveness with which we did so, we conducted the experiment to test the efficiency of the immobilized enzyme in converting carbon dioxide to bicarbonate. In this experiment, we compared the time, T, it took for a saturated CO2 solution containing the enzymes to lower the pH of a .012 M Tris HCL buffer from 8.3 to 6.3 with the time, T_0, it took for the same solution without enzymes. The following equation relates the two times to give us the amount of enzyme activity present in the solution.

$$Unitofactivity = \frac{2 * (T_0 - T)}{T} \tag{4}$$

To collect the data for T and T_0, we first prepared a CO_2 saturated solution by bubbling CO_2 through 4 °C water for 30 minutes. Then, we prepared a solution of 6 ml of 0.02 M Tris HCl buffer, pH 8.0 in a 15 ml beaker, and added 4 ml of the chilled CO_2 saturated water. We started timing the reaction as soon as we mixed the solutions and stopped it when the pH meter dropped from 8.3 to roughly 6.3 to obtain T_0. We then repeated the timed process with unaltered enzymes present to obtain T . To compare the efficiencies of both unaltered and immobilized enzymes, we repeated this experiment with the immobilized enzymes and compared times. The following equation gave us the unit of activity per mg of enzyme in solution and was ultimately what we used as our basis for comparison.

$$Units/mg = \frac{2 * (T_0 - T)}{T * mg} \tag{5}$$

4 Results and Discussion

We measured the efficiency of the immobilized carbonic anhydrase to be 67 percent over the control sample in the conversion of carbon dioxide to bicarbonate. However, the unaltered carbonic anhydrase performed 8.8 percent better over the immobilized carbonic anhydrase. This discrepancy is expected, as the immobilization process inhibits some of the catalytic functionality of the enzyme. However, because the immobilized enzyme still performed much better than the control reaction, which operated without the use of any catalyst, we can conclude that the benefits gained in immobilization significantly outweigh the efficiency lost. The results from various tests conducted at different stages in the procedure were favorable as well. Results from the BCA Assay and UV spectroscopy, indicating the amount of enzymes successfully immobilized on our silica, show that the etched silica were able to immobilize much more of the enzymes than normal silica. While the normal silica nanospheres immobilized 64.4 percent of a 50 microgram enzyme per 100 milligram silica solution, the etched silica immobilized 77.4 percent the enztyme in a solution of the same concentrations. Because time spent etching is proportional to amount of surface area, we can conclude that higher surface area yields higher enzyme immobilization. Still, we acknowledge that these procedures are far from an optimized process, meaning that the efficiency of both the immobilization and reaction can be greatly improved.

5 Conclusion

Our goal was to mitigate carbon dioxide in the atmosphere using efficient and cost effective techniques. We also wanted to avoid producing byproducts that could be potentially harmful. By using the enzyme carbonic anhydrase, which rapidly converts carbon dioxide to bicarbonate, we were able to efficiently remove carbon dioxide from solution and thus accomplish our primary objective. The product of this reaction, bicarbonate, can then be reacted with sodium to form $NaHCO_2$, baking soda. Furthermore, we employed the process of enzyme immobilization, attaching carbonic anhydrase to silica nanospheres, to allow for the recovery and reuse of the enzymes, making our solution more economically feasible. Though this process hinders

enzymatic activity, the benefits of reusability and higher resilience to changes in environmental conditions greatly outweigh the costs. In testing this solution, we concluded that on a small scale, the enzymes can be effectively immobilized on silica nanospheres while maintaining a high level of efficiency. Further experimentation needs to be conducted in order to find the etching times and surface area that maximize both immobilization and the performance of the enzyme. Also, efficiency tests must be run on larger scales to show the economic feasibility and practicality of implementing this process on a global scale. However, these preliminary small-scale tests and results show tremendous promise.

References

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