# CO<sub>2</sub> Reduction Rate of Photosynthetic Bacteria with Ceramics

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#### Abstract

To address global warming caused by increased  $CO_2$  emissions, many studies on  $CO_2$ reduction have been conducted worldwide. In carbon capture and storage (CCS) technology, the mineral carbonation process causes  $CO_2$  to react with particular metals or compounds to stably fix CO<sub>2</sub>. Many relevant studies have been performed on this capture process. Based on research describing photosynthetic bacteria that fix  $CO_2$  in the process of producing  $H_2$  under light irritation, this study combines ceramics with photosynthetic bacteria to develop a material capable of reducing environmental CO<sub>2</sub>. To investigate the possible combinations this study conducted experiments on ceramic materials of hardened cement pastes and lightweight aggregates made using waste from the Korean Y power plant. The photosynthetic bacteria Rhodopseudomonas pentothenatexigens AE8-5 was used and cultivated in a standard culturing medium. To examine the  $CO_2$  reduction rate, a 165 mL sealable glass bottle was used and  $CO_2$  was injected through the rubber lid using a svringe. Ceramic specimens were placed in the medium with the photosynthetic bacteria, and then were cultured in a shaking incubator at 25-30°C, a pH of 7, 6000 lux irradiation, and a shaking rate of 120 RPM. After the injection of the bacteria, gas chromatography-thermal conductivity detection analysis was performed on the gases in the bottles, and the presence of  $CO_2$  was confirmed. When 30 g of the ceramic aggregate with a low reaction rate with  $CO_2$  was used and the photosynthetic bacteria were irradiated, the  $CO_2$  concentration was reduced by ~40%. It is necessary to address the amount of ceramic material used and the shaking friction generated in order for uniform light irradiation when the ceramic is combined with photosynthetic bacteria. The results demonstrated that materials combining photosynthetic bacteria and ceramic are applicable for future studies.

Keywords: CO<sub>2</sub> reduction, Photosynthetic bacteria, Aggregate, Medium

### **1. Introduction**

To address global warming caused by increased  $CO_2$  emissions, many studies on reducing environmental  $CO_2$  have been conducted globally [1]. Carbon capture and storage (CCS) has become an active research field. In CCS technology, the mineral carbonation process induces  $CO_2$  to react with particular metals or compounds to fix  $CO_2$  in a thermodynamically stable condition. Many studies on the process have been performed [2-5].

In pastes hardened by pozzolanic reactions, a method was proposed that induced  $Ca(OH)_2$ , generated by the hydration reaction, to react with supercritical  $CO_2$  and thereby increase the reaction rate of conversion to  $CaCO_3$  and thus fix  $CO_2$  stably [6]. The method of using  $Ca(OH)_2$  to store  $CO_2$  in a stable state and increase the application of the species and methods of storing it stably have been researched [7, 8].

A study on photosynthetic bacteria that produce  $H_2$  under light irradiation revealed that when  $CO_2$  is fixed under light, the purple sulfur bacteria *Chromatium vinosum* uses molecular  $H_2$  as an electron donor. Microorganism-based  $H_2$  production technology could be applied beneficially to various fields, such as pure energy production,  $O_2$  generation, C fixation in air, and the disposal of organic wastes including food factory waste water and food waste [9-12].

Global research on the possibility of fixing  $CO_2$  in air using photosynthetic bacteria has been performed. As described earlier, research on CCS based on ceramic materials is being conducted worldwide. However, not many studies exist on constant  $CO_2$  reduction by the application of microorganisms. Therefore, to maximize the  $CO_2$  reduction rate, this study developed a ceramic material that serves as a host for bacteria to maximize the lifetime and efficiency of the photosynthetic bacteria; the bacterial applicability and efficiency change were observed over time. If bacteria can be successfully cultured and sustained in ceramic material, microorganism/ceramic mixed construction materials may be developed, which would aid in constant  $CO_2$  reduction

The container used in the experiment was a 165 mL sealable glass bottle in which the medium and ceramic specimens were placed. The bottle was sterilized in an autoclave at 120°C for 10 min. After sterilization, the container was sealed with a rubber lid and aluminum cap to block external gas. Bacteria and CO<sub>2</sub> were inserted through the rubber lid using a syringe, and then were cultured in a shaking incubator at 25–30°C, a pH of 7, 6000 lux illumination, and 120 RPM. At intervals during the culturing, 100 µL gas samples were collected from the culture using a syringe, and a GC-TCD was used to continuously monitor the concentration of CO<sub>2</sub> in the bottle.

# 2. Experiments

# 2.1. Materials

The ceramic specimens were an aggregate made with waste and a hardened cement paste. The aggregate comprised lightweight aggregate (LWA) produced by the Korean Y power plant sieved to particulate sizes of 5–10 mm. For the cement specimen, ordinary Portland cement (OPC) produced by the Korean A Firm was mixed with ISO standard sands and hardened. Regarding the dry material mix ratio of the cement specimen, binder and sand were mixed at the ratio of 1:0.2 by weight, and the water-to-binder ratio was 30 wt%. The specimen was cured at normal temperature and pressure, and then cut to  $5 \times 5 \times 10$  mm for use.

# 2.2. CO<sub>2</sub> Reduction Rate

The photosynthetic bacteria used in this experiment were *Rhodopseudomonas pentothenatexigens* AE8-5, cultured in the 27s medium. Table 1 presents the composition of the 27s medium.

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Yeast extract	1.0g
Trisodium citrate dehydrate	1.0g
Sodium Thiosulfate Anhydrous	1.0g
Absolute ethanol	0.5ml
$KH_2PO_4$	0.5g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.4g
NaCl	0.4g
NH <sub>4</sub> Cl	0.4g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.05g
Trace element solution SL-6	1ml
L-cysteine	0.035g
Distilled water	1 L
pH	7.0

## Table 1. Composition of 27s Medium

## **3. Results and Discussion**

#### 3.1. Cement-based Specimen

In this experiment, to combine the photosynthetic bacteria with the cement specimen, the cement specimen was placed in 27s medium. To investigate the CO<sub>2</sub> reduction rate according to inoculation, the basic experiment consisted of placing 100 g cement specimen and 50 mL medium into the bottle, setting the total height of the bottle contents based on that of the bottle containing 100 mL of medium, and in samples with bacteria inoculation, bacteria was inoculated at the concentration of 0.5 g/L medium. A syringe was used to inject 60 cm<sup>3</sup> CO<sub>2</sub> into the bottles. The samples were prepared as shown in Fig. 1. The cement specimens were carbonated in both ambient and supercritical CO<sub>2</sub> conditions (40°C, 80 Pa, 60 min). Each specimen, before and after carbonation. The cement specimens under ambient and supercritical conditions were marked as Cement (Am) and Cement (S.C.), respectively. The initial value of CO<sub>2</sub> was measured with the use of GC-TCD 4 h after CO<sub>2</sub> injection, but all CO<sub>2</sub> was absorbed in the bottle.

To investigate which factors removed  $CO_2$  in the previous experiment, the cases in which bottles containing 25 g specimen and those in which 50 mL of 27s medium was added to the specimen were compared before and after carbonation. After 60 cm<sup>3</sup> CO<sub>2</sub> was inserted, the change in CO<sub>2</sub> concentration over 4 h was measured. The results are illustrated in Fig. 2.

As shown in Fig. 2, almost no difference appears in  $CO_2$  reduction efficacy before and after the carbonation period. This unexpected result indicates that the cement specimen fails in performing the full carbonation reaction under supercritical conditions. Given that all cement-based specimens showed constant reductions in  $CO_2$  concentration to different degrees,  $CO_2$  is assumed to be reduced by cement carbonation [13, 14]. It was found that the specimen mixed with the medium had a faster  $CO_2$  reduction than the specimen alone.  $CO_2$  was reduced in the presence of the medium without the application of the photosynthetic bacteria occurred because  $CO_2$  was dissolved in the medium itself.



Figure 1. Photographs of Specimen Bottles using 27s Medium and Cement Specimen. (100 mL medium, 100 g cement sample, 0.5 g/L bacteria, 60 cm<sup>3</sup> CO<sub>2</sub>). (a) Medium only, (b) medium + bacteria, (c) medium + cement (S.C.), (d) medium + cement (S.C.) + bacteria, (e) medium + cement (Am) + bacteria



# Figure 2. Result of GC-TCD Analysis of Cement-based Specimens. This Figure Shows the Effect of the Presence of the Medium and Supercritical Carbonation Conditions. In the Legend, Am and S.C. Correspond to Ambient Conditions and Supercritical Conditions, Respectively.

For the cement specimens, regardless of carbonation and bacteria, the injected  $CO_2$  was completely absent after 4 h. To increase the  $CO_2$  concentration in the sample, 60 cm<sup>3</sup>  $CO_2$  was injected again after the first 60 cm<sup>3</sup>  $CO_2$  insertion. In this case,  $CO_2$  also disappeared completely in the bottle. The reduction effect of the carbonation of the cement specimen is both faster and greater than the reduction effect of the bacteria. Therefore, the additional  $CO_2$  reduction effect of the photosynthetic bacteria was difficult to observe. In the experiment in which the ceramic specimen was combined with photosynthetic bacteria, the cement specimen constantly reacted quickly with  $CO_2$ , regardless of carbonation. This limited the investigation of the role of photosynthetic bacteria in the CCS process. In the basic experiment conducted on the effect of photosynthetic bacteria when a ceramic material is used with photosynthetic bacteria, it was concluded that a ceramic material with a low  $CO_2$  reactivity should be used. Therefore, a ceramic material of

artificial LWA made with recycled waste material from a Korean power plant was tested.

### 3.2. Ceramic LWA Specimen

In this experiment, a sintered eco-material with expected low  $CO_2$  reactivity was used, in the form of the LWA made with waste from the Korean Y power plant. The medium was placed in bottles with the ceramic aggregate, and the bacteria were inoculated in the medium. The proliferation of the photosynthetic bacteria was observed to verify whether they could proliferate normally. To accomplish this, the height of the medium in the reactor bottle was maintained at a constant level regardless of the amount of ceramic aggregate contained in the bottle, which was intended to maintain a constant partial pressure of  $CO_2$ . In other words, 25 g aggregate was placed in the bottle first, and then the medium was added for the proliferation of bacteria. In this way, the height of the bottle contents was set to the same as that of the 100 mL medium used as a control in this study. Under these circumstances, the  $CO_2$  reduction effect was compared among different environments in the bottles. Fig. 3 shows photographs of the specimen bottles before bacteria inoculation and after 7 d of culturing.



## Figure 3. Photographs of Specimen Bottles using 27s Medium, Bacteria, and Ceramic Aggregate According to the Time. The Heights of the Bottle Contents were Fixed. From the First Bottle on the left: 100 mL medium only; 100 mL medium and 0.5 g/L bacteria; medium with 25 g LWA; and medium, 25 g LWA, and 0.5 g/L bacteria. Each bottle contained 60 cm3 CO2 at first. (a) Immediately after inoculation and (b) 7 d after inoculation

As shown in Fig. 3, after 7 d, the sample with the aggregate turns red, the color of the photosynthetic bacteria, just as the samples with the bacteria and medium do. This demonstrates that the ceramic aggregate does not greatly influence the proliferation of bacteria; thus, the experiment continued. To expose the ceramic aggregate to  $CO_2$  in the bottle, 95 g aggregate was placed based on the height of 100 mL medium in the control bottle, and then 45 mL medium was inserted for the proliferation of bacteria. This sample was compared to the control sample. Fig. 4 shows photographs taken throughout the culturing time.





# Figure 4. Photographs of Specimen Bottles using 27s Medium, Bacteria, and Ceramic Aggregates Acording to the Time. The Heights of Bottle Contents were Fixed. From the left: 100 mL medium only; 95 g ceramic aggregate and 45 mL medium; 100 mL medium and 0.5 g/L bacteria; 95 g ceramic aggregate, 45 mL medium, and 0.5 g/L bacteria. Each bottle initially contains 26 cm3 CO2. (a) Immediately after inoculation, (b) 3 d, (c) 5 d, and (d) 7 d after inoculation.

As shown in Fig. 4, the control sample clearly turns red with inoculation. Bacteria were tested to proliferate normally in the presence of aggregate; however, the sample with aggregate does not clearly change in color. This may result from the large amount of aggregate preventing irradiation when light is irradiated in one direction in the shaking incubator; thus, the sample is not evenly exposed to light and the photosynthetic bacteria fail to proliferate normally.

Fig. 5 depicts the results of GC-TCD analysis on the specimens according to the number of culture days after inoculation. As shown in Fig. 5, the sample with medium only experiences almost no change in concentration of CO<sub>2</sub>. The samples with the aggregate, photosynthetic bacteria, or both tend to reduce the  $CO_2$ concentration in general, though to different degrees. The sample with aggregate only reduces the CO<sub>2</sub> concentration unexpectedly, possibly because of carbonation by the effect of trace Ca in the aggregate. For the sample with both aggregate and photosynthetic bacteria, the  $CO_2$  reduction rate was expected to increase because the aggregate would serve as a host for the bacteria. However, the photosynthetic bacteria may have been insufficiently irradiated because of the aggregate, and consequently they performed aerobic respiration instead of photosynthesis. As a result, this sample has a higher CO<sub>2</sub> concentration than either the sample with medium and bacteria or that with medium and aggregate. As presented in Fig. 5, 5 d after inoculation, the sample with medium and bacteria has the greatest reduction in  $CO_2$  concentration, with a 40%  $CO_2$  reduction rate. Therefore, it is concluded to be necessary to change the amount of ceramic material in the process of combining the bacteria with the ceramic material, and to expose the photosynthetic bacteria uniformly to irradiation when the ceramic material is used.



### Figure 5. Results of GC-TCD Analyses of the Specimens Shown in Figure 4

For the cases with ceramic material, uniform irradiation was achieved by changing the method of shaking in the incubator. In the experiment, light irradiation occurred in the uniaxial direction; thus, the specimen bottle was laid horizontally and shaken. Fig. 6 shows the photographs of the samples shaken in this method after inoculation, according to the number of culture days.

As presented in Fig. 6, when the bottle is shaken while horizontal, the aggregate moves dramatically, and the friction caused by this motion breaks the aggregate into pieces. Therefore, with elapsed time, the color of the medium darkens. The changed shaking method is concluded to impede light irradiation, rather than to improve it. As a result, in the case of the experiment with the shaking incubator, the physical properties of the ceramic material and the shaking direction both influence light irradiation. Therefore, it is necessary to consider both these factors to maximize the light irradiation.



(a)

(b)



(c)

(d)

Fig. 6. Photographs of Specimen Bottles using 27s Medium, Bacteria, and Ceramic Aggregates According to the Time, with Bottles Aligned
Horizontally before Shaking. The Heights of the Bottle Contents were Fixed. From the left: 100 mL medium only; 95 g ceramic aggregate and 45 mL medium; 100 mL medium and 0.5 g/L bacteria; 95 g ceramic aggregate, 45 mL medium, and 0.5 g/L bacteria. Each bottle initially contained 26 cm3 CO2.
(a) Immediately after inoculation, (b) 3 d, (c) 5 d, and (d) 7 d after inoculation

Fig. 7 illustrates the measured  $CO_2$  concentration in the bottles after they were shaken horizontally. As shown in Fig. 7, the sample with medium only experiences only a slight change in  $CO_2$  concentration, just as in Fig. 5. However, the sample with both medium and bacteria experiences a constant reduction in  $CO_2$ concentration, and after 7 d, the  $CO_2$  concentration reduction rate approaches 50%. The sample with medium and ceramic aggregate also shows a reduction in  $CO_2$ concentration. This seems to be influenced by the specimen, as in the previous results (Fig. 5). The sample with medium, specimen, and inoculation experiences a gradual increase in  $CO_2$  concentration, and shows the highest final concentration. The destruction of the ceramic aggregate darkens and fogs the medium, preventing irradiation, and therefore the bacteria in the sample perform aerobic respiration instead of photosynthesis, thus increasing the  $CO_2$  concentration in the specimen bottle. Therefore, when the aggregate is used, the uniform irradiation of the photosynthetic bacteria is the most important factor in  $CO_2$  reduction.

Based on the previous experimental results, the amount of aggregate was decreased to 30 g, and the initial shaking method with bottles held vertically was applied in the experiment. The purpose of the experiment was to analyze the effect of changing the composition of the sample on the  $CO_2$  reduction rate by decreasing the amount of the aggregate specimen and increasing the irradiation of bacteria. Fig. 8 presents photographs of samples in the above condition after inoculation according to the number of culture days.



Figure 7. Results of GC-TCD analyses of the specimens shown in Figure 6

As shown in Fig. 8, in the experiment in which the amount of aggregate was decreased to encourage uniform irradiation, the sample containing aggregate, medium, and bacteria turns red, similar to the sample with medium and bacteria, and the bacteria are observed to culture normally. Unlike the sample bottles subjected to the horizontal shaking method, the aggregate destruction by the friction of shaking is not prevalent.

Fig. 9 depicts the GC-TCD analysis results for the samples with reduced aggregate amounts that were shaken without substantial breakage of the ceramic aggregate to promote uniform irradiation of photosynthetic bacteria, based on the results of the above experiment. As shown in Fig. 9, the sample with medium only and the sample with medium and aggregate show only slight changes in  $CO_2$ concentration. With the inoculation of the photosynthetic bacteria, the sample with medium only and the sample with both medium and aggregate experience reductions in CO<sub>2</sub> concentration. When both medium and bacteria are used, CO<sub>2</sub> is reduced by 50% at the highest reduction level. When the aggregate is used alone, the concentration falls by ~40%. The difference in  $CO_2$  reduction rate depending on the presence of aggregate is attributed either to the relative decrease in the amount of medium by the increased volume of aggregate, in order to maintain the bottle filling level at that in the case where aggregate was used, or that the presence of the aggregate impedes uniform irradiation. More detailed research to resolve this question will be conducted. This experiment demonstrated that the amount of ceramic aggregate influenced the CO<sub>2</sub> reduction rate.





Figure 8. Photographs of Specimen Bottles using 27s Medium, Bacteria, and Ceramic Aggregates According to the Elapsed Time. The Heights of the Bottle Contents were Fixed. From the left: 100 mL medium only; 100 mL medium and 0.5 g/L bacteria; Medium and 30 g Aggregate; medium with 30 g aggregate and 0.5 g/L bacteria. Each bottle initially contains 26 cm3 CO2.
(a) Immediately after inoculation, (b) 3 d, (c) 5 d, and (d) 7 d after inoculation.



Figure 9. Results of GC-TCD Analyses of the Specimens Shown in Figure 8

# 4. Conclusions

To achieve the constant reduction of  $CO_2$  by combining a ceramic material with photosynthetic bacteria, this study applied two ceramic materials to a medium promoting the proliferation of photosynthetic bacteria and analyzed the compatibility of the ceramic and culturing materials. When a cement specimen was used, the reaction of the cement specimen with  $CO_2$  occurred faster than the reaction of the photosynthetic bacteria with CO<sub>2</sub>, regardless of the pre-processing of carbonation or carbonation under supercritical conditions. As a result, the CO<sub>2</sub> reduction effect of photosynthetic bacteria was obscured. It was deemed necessary to plan research more carefully in the future. The factors influencing the  $CO_2$ reduction rate included the type of ceramic material, the amount of ceramic material, the shaking method, the amount of medium, and the degree of light irradiation. It was expected that the ceramic material would safely host the photosynthetic bacteria, thus aiding in the proliferation of bacteria and increasing the CO<sub>2</sub> reduction rate. However, while the ceramic material negatively affected the  $CO_2$  reduction rate, the presence of the ceramic material still led to a  $CO_2$  reduction rate approaching 40%. Therefore, this study is meaningful in proving that, if a construction material were manufactured to combine ceramic material with photosynthetic bacteria in everyday applications, the material would likely contribute to the constant and effective reduction of environmental CO<sub>2</sub>. In future studies, the correlation between the amount of medium and the  $CO_2$  reduction rate, the determination of the minimum amount of medium to maintain a certain CO<sub>2</sub> reduction rate, and the measurement of the  $CO_2$  reduction rate in the field as opposed to the laboratory will be researched, thus establishing the basic knowledge necessary for the actual application of such a construction material

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