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Assessments of CO_2 biomineralization and its kinetics using indigenous microorganisms derived from landfill cover soil

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ABSTRACT

Biomineralization of carbon dioxide into geologically stable carbonate ($CaCO_3$) is associated with a wide range of microorganism in natural environment. Sewage sludge cake generated in municipal wastewater treatment plant is generally mixed with lime, before being disposed into landfill site anticipating effective biomineralization in the cover soil strata and solidification. Objective of the present study was to map the abundance and activity of indigenous microbial population for biomineralization, underneath the landfill cover soil (LFCS) in S landfill site, Korea. CO_2 biomineralization microcosm study was conducted with indigenous microorganism isolated from LFCS and was compared with other potential-pure culture strains. To characterize possible relationship between the rate of bacterial growth (biomass production) and $CaCO_3$ precipitation, batch kinetic experiments with live, dead, and inactivated bacteria either in nutrient solution or in inert electrolyte were also performed. Out of different microbial population, isolated from the LFCS, two predominant key species were *Bacillus megaterium* and *Alkaliphilus metalliredigens*. Significant CO_2 mineralization and increased carbonate precipitates (about 30% higher) were observed with the indigenous microorganism, than its abiotic control. Results suggest that the presence of solidified sewage sludge cake in LFCS can naturally and efficiently mitigate CO_2 produced from the landfill.

Keywords: Biomineralization; 16S rDNA; Landfill cover soil; Carbon capture; Sequestration

1. Introduction

The amount of anthropogenic CO_2 emitted into the atmosphere, primarily because of expanding use of fossil fuels for energy, has risen from preindustrial levels of 280 ppm to present levels of over 365 ppm [1] and anthropogenically-driven increases in atmospheric CO_2 could possibly be one of the most significant factor for climate change and global warming in coming centuries [1,2]. As a measure to control and even counteract the ever increasing CO_2 burden, apart from large array of conventional physical and chemical methods, researchers are considering biological technology for a benign solution, especially using a microalgae and microbial species such as soil bacteria, as major part of developing technology [3–6]. Biomineralization is known to be a mimic technology

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which converts or captures carbon dioxide into carbonate minerals ($CaCO_3$) mediated with a wide range of microorganisms in specific biological or natural environment [7]. Various microbial species are known to be capable of biomineralization of CO_2 into carbonate. Each polymorphic form of mineral carbonates precipitated by microorganisms is inherently different [8]. Various materials, which are bivalve shell, fly ash, and solidified sewage sludge can support as biomineralization media [2,8–10]. Among these materials, solidified sewage sludge has a high concentration of Ca^{2+} ion that makes it a preferred choice for biomineralization [11]. In this study, we would investigate biomineralization activity of territorial microorganisms to have the basic information for implementing solidified sewage sludge to biomineralization.

2. Materials and methods

2.1. Biomineralization support medium

In this study, solidified sewage sludge collected from sewage sludge solidification plants in S landfill (e.g. Incheon, Korea) was selected as the target medium for biomineralization. Measurement of pH and other proximate analysis (moisture, combustible, and ash) for the solidified sludge was performed according to the standard methods for solid wastes analysis, and other elemental properties and mineralogical properties were assessed through energy dispersive X-ray spectroscopy (EDX) coupled with scanning electron microscopy (SEM, S-4300SE).

2.2. Microorganism and culture condition

Bacillus megaterium ATCC® 10778 and *Bacillus (Sporosarcina) pasteurii* ATCC® 6453, known to have biomineralization potential, were used throughout the study. Optimal growth condition for *B. megaterium* are 30–35˚C, pH 7.0–7.5 but for *B. pasteurii* optimal growth conditions are 30˚C, pH 9–11, and also aerobic. Microbial growth media have been detailed in Table 1. Briefly, in 530 mL serum bottle (Wheaton) 200 mL medium was used in cultivation. Residual oxygen in head space of serum bottle supports the aerobic growth and the serum bottles were incubated at 200 rpm, 30˚C.

2.3. Batch test for CO_2 biomineralization

Mid-log phase growing culture inoculum was harvested through centrifugation (Hanil science, HA-1000-3, 3,000 rpm, 15 min) and the pellet was transferred into the support medium contained in experimental setup as depicted in Fig. 1. All experimental setup was autoclaved to prevent unwanted contamination (Sanyo, MLS-3780, 121˚C, 15 min). Prepared sample was loaded into reacting chamber, and filled up with mixed artificial air gas (Yusung gas, $N_2 + O_2$, 79%: 21%, mol/mol) with tedlar bag filled with CO_2 gas (99% purity). Using mini-pump (Sibata, MP-Σ30N), CO_2 gas was circulated at flow rate of 0.5 L/min (Table 2).

Circulated gas was sampled (0.5 mL) using gas syringe (HAMILTON, GASTIGHT® # 1002) through the gas sampling port sealing with rubber stopcock. Sampled gas was analyzed by GC/TCD (HP6890 series GC system, USA) with packed column (Alltech 403412–1417). After operation was completed, the support media sample was analyzed pH and SEM–EDX.

2.4. Identify the indigenous microorganisms derived from landfill cover soil

For characterization of the bacterial diversity, 10–100 mL of sample suspension was centrifuged for 15 min at 3,000 rpm (Hanil science HA-1000-3, centrifuge) [12].

From this, the total genomic DNA was extracted by bead beating using a FastPrep® Instrument (Q-Bio gene) and a FastDNA® SPIN Kit (Bio101 system, Q-Bio gene). PCR amplification (PCR Machine, Techgene) was performed with 16S universal primer (27F-5´´AGA GTT TGA TCM TGG CTC AG 3´´ and 1492R-5´´TAC GGY TAC CTT GTT ACG ACT T 3´´)that

Table 1
Growth and cultivation condition

Microorganism	*Bacillus megaterium* ATCC® 10778™	*Bacillus(Sporosarcina) pasteurii* ATCC® 6453™
Nutrient component	Nutrient Broth (BD 234000) Peptone: 5 g/L Meat extract: 3 g/L pH 7.0 (Autoclaved or filter sterilized)	NH_4-YE Medium Yeast extract, 20 g/L, $(NH_4)_2SO_4$, 10 g/L, in 0.13 M Tris buffer (pH 9.0), Agar(if needed), 20 g/L all ingredient autoclaved separately and then mixed

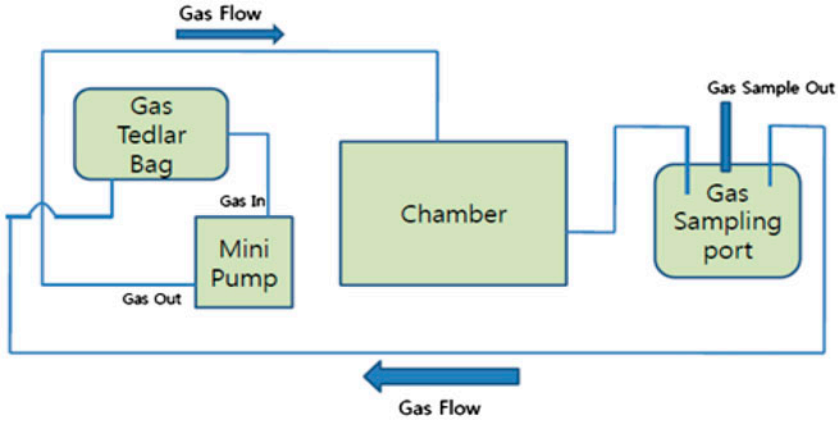

Fig. 1. Schematic diagram of CO_2 biomineralization batch test.

Table 2
Experimental conditions of batch test for CO_2 biomineralization

Index	Experimental conditions			
	Set 1–1	Set 1–2	Set 2	Set 3
Microorganism	*Bacillus megaterium*	*Bacillus pasteurii*		Indigenous microorganisms
Injected air (N_2 : O_2, 79 : 21, % v/v)	12.6 L			
Injected CO_2 (purity 99%)	5 L	5 L	10 L	10 L
Gas circulation rate	0.5 L/min			
Operation time, temperature, moisture content	48 h, 25℃, 63% w/w			
Sample ①	Solidified sludge (30 g) + nutrient medium (20 mL) + bacteria			Solidified sludge (40 g) + nutrient medium (20 mL) + bacteria
②	Solidified sludge (30 g) + nutrient medium (20 mL)			Solidified sludge (40 g) + nutrient medium (20 mL)

includes initial denaturation (95℃, 5 min), 35 cycles of denaturation (95℃, 1 min), annealing (54℃, 1 min), extension (72℃, 1 min), and final extension (72℃ for 10 min). The PCR products were purified by Winzard® SV Gel and PCR Clean-Up System (Promega, USA). The purified 16S rDNA PCR products were ligated to the pGEM-T easy vector (Promega, USA) and then inserted to the host cell (*E. coli* XL1-Blue). The plasmids, after being screened by X-Gal/IPTG, were collected by using a Winzard® Plus Minipreps DNA Purification System (Promega, USA). 16S rRNA gene sequence analysis for each of the plasmids was conducted with a 3100 Capillary DNA sequencer (Applied Biosystems). Nucleotide sequences of alignments were performed with BLAST in the database of the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/).

3. Results and discussion

3.1. Characteristic of target medium

Selected solidified sewage sludge was strongly alkaline (pH 12.5), and proximate analysis reveals a moisture content of 43.4%, combustible material and ash content of 12.4 and 44.3%, respectively. Elemental analysis of solidified sewage is presented at Table 3. SEM-EDX analysis suggests abundance of calcium ions (10.6%) inside solidified sewage sludge which served for CO_2 biomineralization.

Table 3
Characteristics of solidified sewage sludge

	Proximate analysis (%, wet)			Elements (%, dry)									
pH	Moisture	Combustible	Ash	C	O	Ca	Mg	Al	Si	S	Cl	K	Fe
12.5	43.3	12.4	44.3	28.1	52.3	10.6	1.9	1.8	3.3	0.7	0.5	0.2	0.6

3.2. 16S rRNA analysis of microbial community present in landfill cover soil

Through 16S rRNA analysis, various microorganisms were found in the solidified sewage sludge, of which *B. megaterium* known aerobic soil bacteria involved in biomineralization and *Alkaliphilus metalliredigens* which can reduce the metal ions to bicarbonate complex (MCO_3). These results shows that various territorial bacteria reside in landfill cover soil, which are more adaptable than their pure culture counterpart.

3.3. Variation of CO_2 concentration according to biomineralization

In Set 1–1 containing *B. megaterium*, CO_2 concentration reduced from 15.5 to 7.9% during 48 h of incubation. However, interestingly, nearly equivalent extent of CO_2 reduction (from 16.6 to 9.3%) was also observed in abiotic control set during the same time period. This could possibly be explained by the sub-optimal performance of *B. megaterium* in target medium with pH value of around 12—much different from its known optimal growth pH of 7.

However in Set 1–2, which was inoculated with *B. pasteurii*, CO_2 concentration appears to be undetectable after 36 h, and reduction was significantly different from its abiotic counterpart (Fig. 2(b)). With increased CO_2 content (from 5 to 10 L) in Set 2, the mixed microbial population caused about 76% of CO_2 reduction (from 29.7% v/v to 7.2% v/v) (Fig. 2(c)). In its respective control the reduction was much lower as anticipated (from 29.9 to 15.6% v/v).

With increased mass of solidified sludge (40 g) and CO_2 volume (10 L) in Set 3, injected CO_2 was totally absorbed into the solidified sludge (from 28.1% v/v to not detected) when alkaliphlic *B. pasteurii* (optimal pH

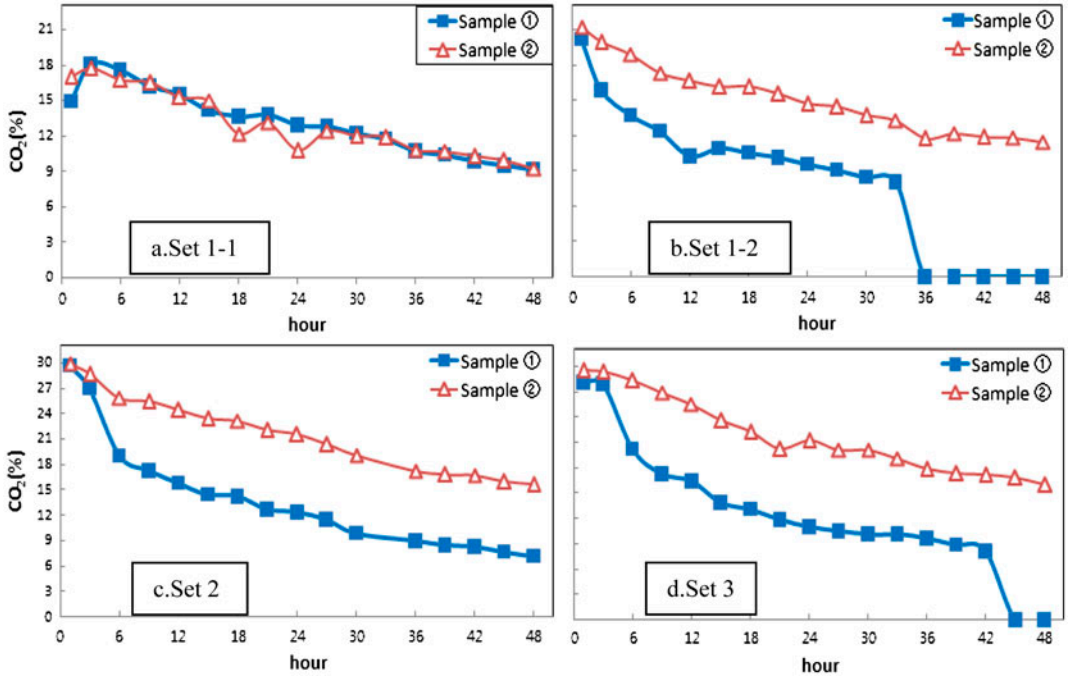

Fig. 2. Variation of CO_2 gas concentration (a. Set 1–1, b. Set 1–2, c. Set 2, d. Set 3). Abiotic control sets are referred as sample (2).

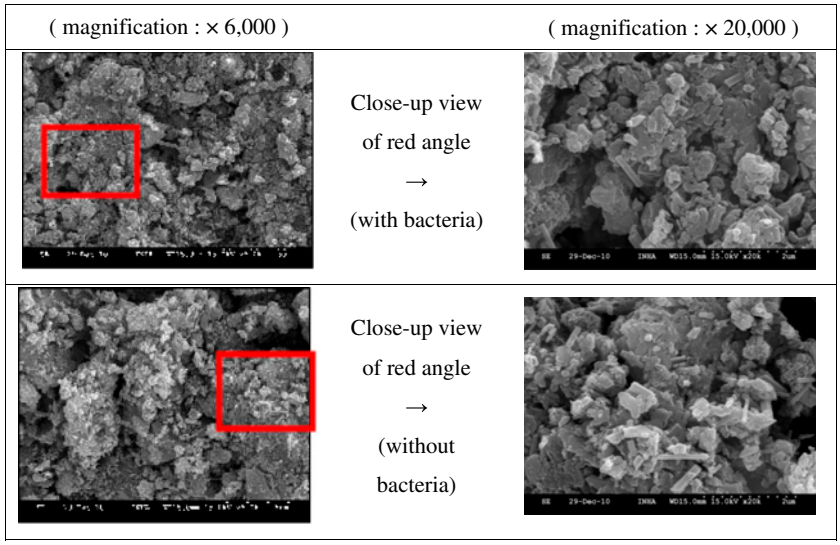

Fig. 3. SEM images of CO_2 biomineralization batch test products (Set 1–1).

9–11) was used (Fig. 2(d)). But in sample without bacteria, CO_2 reduction efficiency was just 46% (from 29.6 to 16.0% v/v).

3.4. Results of SEM-EDX

With *B. megaterium* (Fig. 3), there was no significant distinction between with bacteria and without bacteria. It considered that microbial activity (*B. megaterium*) was inhibited by pH of solidified sludge (pH around 12). SEM images in conformation to anticipation, suggest that, there are more calcium carbonate crystal granules formed in biomineralization sample (Figs. 4 and 5) than in control sets without bacteria. Hexagonal crystalline represent either calcite or aragonite polymorphs as the biomineralization by-products. The rhombohedral nuclei attached to the bacterial surfaces as seen in SEM images, at earlier stages of crystallization experiments, suggest that calcite may indeed be the predominant minerals on the cell surfaces of *Bacillus* sp. When the bacteria are absent, calcite nucleation may be retarded or compromised. In contrast, the formation of vaterite

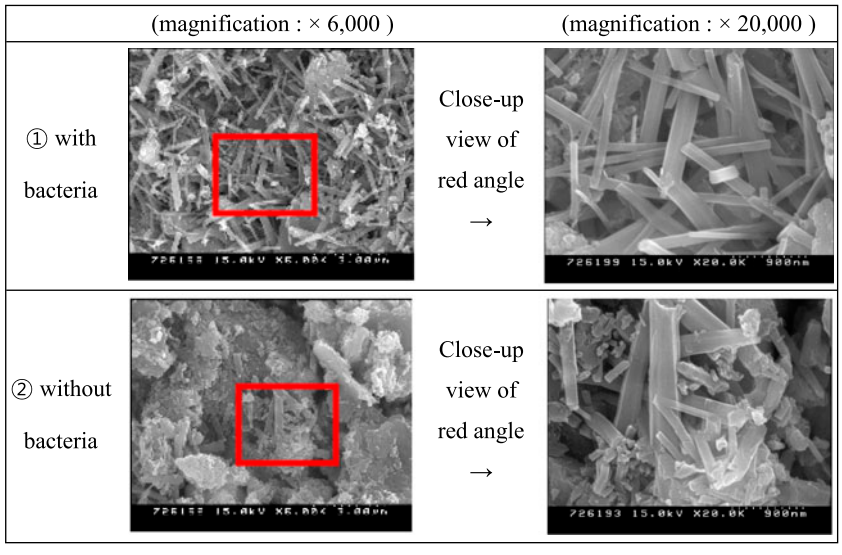

Fig. 4. SEM images of CO_2 biomineralization batch test products (Set 1–2).

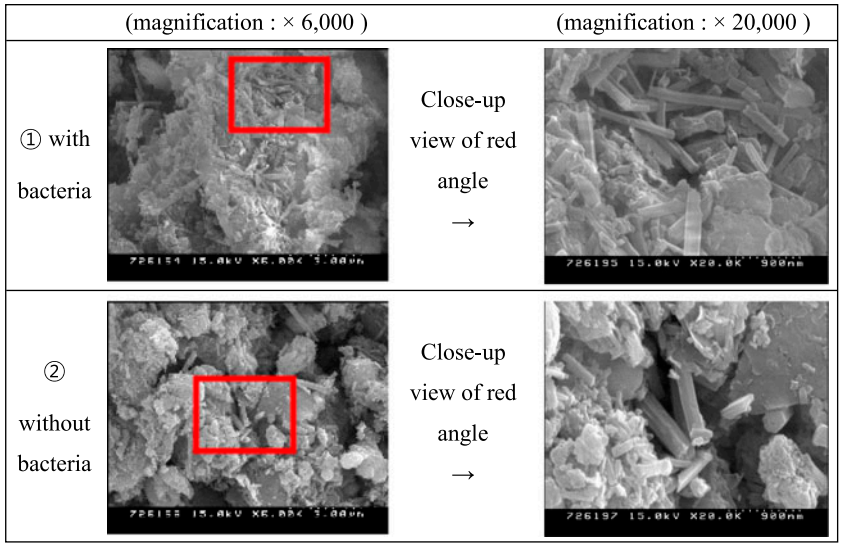

Fig. 5. SEM images of CO_2 biomineralization batch test products (Set 2).

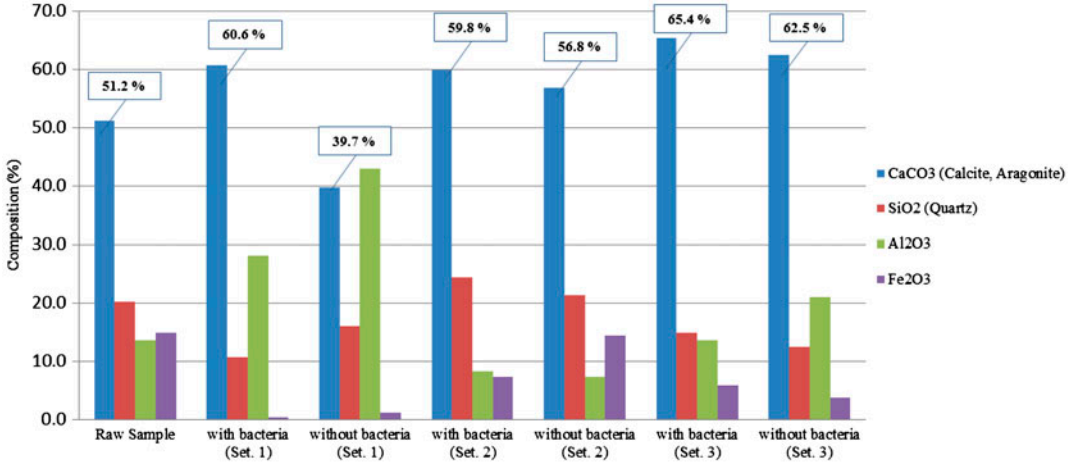

Fig. 6. EDX analysis result of batch test samples.

facilitated by the hydroxyl and carboxyl rich compounds may take place at a relatively faster pace. Such a reverse in nucleation kinetics between calcite and vaterite may ultimately lead to the formation of vaterite in the supernatant solutions [7].

EDX analysis (Fig. 6) showed that the biogeochemical processes induced precipitation of calcium carbonate using Ca-rich solidified sewage sludge under the CO_2 atmosphere. Carbonate minerals precipitated in all the samples but the extent of precipitation was always significantly higher in presence of potential microorganism when compared with their abiotic complement. No carbonate minerals formed using metal-rich fly ash without bacteria.

4. Conclusions

Through this study, we would confirm that solidified sewage sludge can support the biogeochemical process of CO_2 fixation through biomineralization. However, CO_2 fixation potential could be of different order of magnitude between laboratory scale and actual site condition depending on abundance of microorganism, nutrients, optimum temperature, and CO_2 concentration. Further research may corroborate the differences between laboratory and onsite implementation under field operation conditions. Territorial microorganisms are more adaptable on site than pure cultured microorganisms and should be considered seriously.

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