# The Effect of the Presence of Urea and Different Concentrations of HEPES Buffer on Microbial-Induced Calcium Precipitation (MICP) by *Bacillus subtilis.*

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## Q1: Research Question

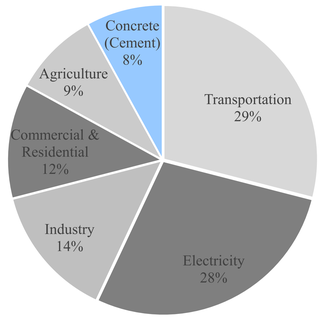


**Figure 1. Global carbon dioxide emissions by category.** Data from World Economic Forum (2024). Chart created by Seoyoon Lee using Excel.

1) Gaseous $CO_2$ dissolves in water to form a hydrated $CO_2$ state:

$CO_2\ (g) \leftrightarrow CO_2\ (aq)$

2) The hydration $CO_2$ reacts with water to generate $H_2CO_3$:

$CO_2\ (aq) + H_2O \leftrightarrow H_2CO_3$

3) The ionization of $H_2CO_3$ in the water generates $H^+$ and $HCO_3^-$:

$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$

4) Under alkaline conditions, $HCO_3^-$ is further ionized to form $CO_3^{2-}$ and $H_2O$:

$HCO_3^- + OH^- \leftrightarrow CO_3^{2-} + H_2O$

5) In the presence of $Ca^{2+}$, it reacts with $CO_3^{2-}$ to form $CaCO_3$ precipitation:

$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO3\downarrow$

**Figure 2. Process of MICP.** Reproduced from Qian et al., 2022, *Journal of $CO_2$ Utilization*, 55, 101849. https://doi.org/10.1016/j.jcou.2021.101849

How do the presence of urea and different concentrations of HEPES buffer (0 mM, 50 mM, 100 mM) affect the MICP efficiency of *Bacillus subtilis*?

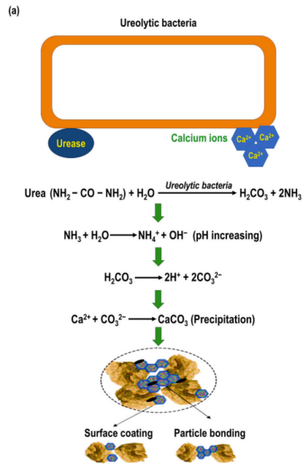


**Figure 3. Ureolytic pathway.** Reproduced from Hussain et al., 2025, Environmental Research, 270, 121006. https://doi.org/10.1016/j.envres.2025.121006

## Q2: Methodology

### Preparation of Standardized Overnight Culture of *B. subtilis* (OD600 ≈ 0.9)


**Figure 4. bacterial cultures in test tubes placed in the incubator shaker at 37 °C and 120 rpm.** Picture taken by Seoyoon Lee.

### Formulation of MICP Media with Controlled Urea and HEPES Concentrations

**Table 1. Components in Flasks 1 to 6.** Table created by Seoyoon Lee using Excel.

| Flask # | 1-3 | 4-6 |
|---|---|---|
| Calcium nitrate (g) | 2.1 | 2.1 |
| LB broth (g) | 3.8 | 3.8 |
| Distilled water (mL) | 150 | 150 |
| Urea (g) | 3.0 | X |

**Table 2. Components in Flasks A to F.** Table created by Seoyoon Lee using Excel.

| Flask # | A-C | D-F |
|---|---|---|
| Calcium nitrate (g) | 2.1 | 2.1 |
| LB broth (g) | 3.8 | 3.8 |
| Distilled water (mL) | 150 | 150 |
| Urea (g) | 3.0 | 3.0 |
| HEPES buffer (g) | 1.8 | 3.6 |

### 1 mL Inoculation of *B. subtilis* into Prepared MICP Media and 2-Week Incubation (10X LB Replenishment at Day 8)

**Figure 5. Flasks A to F in the incubator shaker at about 25°C & about 80 rpm.** Picture taken by Seoyoon Lee.

**Figure 6. Flasks A to F. (Day 4 of Culturing)** Picture taken by Seoyoon Lee.

### Vacuum Filtration and Drying of $CaCO_3$ Precipitates

**Figure 7. The set up for water vacuum filtration.** Picture taken by Seoyoon Lee.

**Figure 8. Filter papers being dried in the incubator oven at 100°C.** Picture taken by Seoyoon Lee.

### Weigh and Normalization of $CaCO_3$ Production

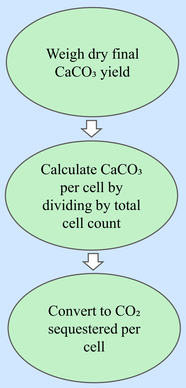


**Figure 9. Process of weighing and normalization.** Figure created by Seoyoon Lee using Power Point.

## Q3: Data Analysis & Results

**Figure 10. Images of final $CaCO_3$ precipitation on filter papers for Flasks 1 to 6. The top row is Flasks 1 to 3, with urea, from left to right. The bottom row is Flasks 4 to 6, without urea, from left to right.** Pictures taken by Seoyoon Lee.

**Figure 11. Images of final $CaCO_3$ precipitation on filter papers for Flasks A to F. The top row is Flasks A to C, with urea and 50 mM HEPES buffer, from left to right. The bottom row is Flasks D to F, with urea and 50 mM HEPES buffer, from left to right.** Picture taken by Seoyoon Lee.

**Table 3. $CaCO_3$ production and $CO_2$ sequestration efficiency under different treatment conditions.** Table created by Seoyoon Lee using Excel.

| Conditions | Flask # | $CaCO_3$ (g) | $CaCO_3$ per cell (g/cell) | $CO_2$ per cell (g/cell) |
|---|---|---|---|---|
| With urea | 1 | 0.2264 | $3.14 \times 10^{-10}$ | $1.38 \times 10^{-10}$ |
| | 2 | 0.0091 | $1.26 \times 10^{-11}$ | $5.54 \times 10^{-12}$ |
| | 3 | 0.0137 | $1.90 \times 10^{-11}$ | $8.36 \times 10^{-12}$ |
| Without urea | 4 | 0.2878 | $4.00 \times 10^{-10}$ | $1.76 \times 10^{-10}$ |
| | 5 | 0.169 | $2.35 \times 10^{-10}$ | $1.03 \times 10^{-10}$ |
| | 6 | 0.2352 | $3.27 \times 10^{-10}$ | $1.44 \times 10^{-10}$ |
| With urea & 50 mM HEPES buffer | A | 0.308 | $3.84 \times 10^{-10}$ | $1.69 \times 10^{-10}$ |
| | B | 0.6672 | $9.02 \times 10^{-10}$ | $3.97 \times 10^{-10}$ |
| | C | 0.4969 | $6.71 \times 10^{-10}$ | $2.95 \times 10^{-10}$ |
| With urea & 100 mM HEPES buffer | D | 0.145 | $1.81 \times 10^{-10}$ | $7.96 \times 10^{-11}$ |
| | E | 0.4865 | $6.06 \times 10^{-10}$ | $2.66 \times 10^{-10}$ |
| | F | 0.5944 | $7.40 \times 10^{-10}$ | $3.25 \times 10^{-10}$ |

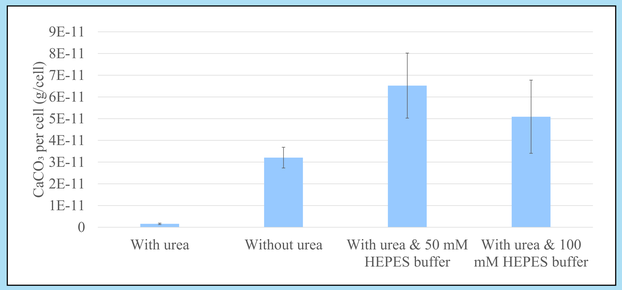


**Figure 12. The effect of the presence of urea and different HEPES buffer concentration on MICP efficiency. To reflect the variability within each set of replicates, error bars were included, representing the standard error of the three (or two) measurements.** Figure created by Seoyoon Lee using Excel.

## Q4: Interpretation & Conclusions

### Conclusion

- The presence of urea by itself decreases the MICP efficiency, but with the presence of 50 mM and 100 mM HEPES buffer, the MICP efficiency rises significantly.
- Ureolytic pathway is critical for MICP .

### Broader Impact

- Anywhere concrete infrastructure is widespread and cement manufacturing is a significant emitter, this approach could supplement existing carbon-capture strategies with a biologically driven option.
- These results also demonstrate that significant improvement in mineralization can be achieved through chemical condition management, specifically urea availability and HEPES buffer, without requiring genetically engineered strains, increasing the scalability and regulatory feasibility of industrial adoption.

### Future Steps

- Different $CO_2$ concentrations
- More replicates
- A wider range of urea/ HEPES buffer concentrations
- Different types of buffers
- Genetically modified *B. subtilis*