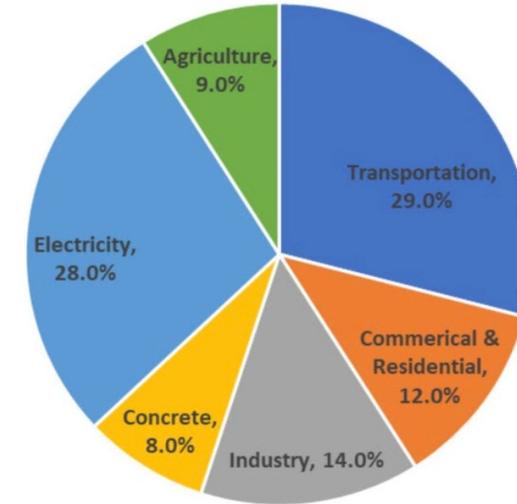


**The Effect of the Presence of Urea and Different Concentrations of HEPES Buffer on Microbial-Induced Calcium Precipitation (MICP) by *Bacillus subtilis*.**

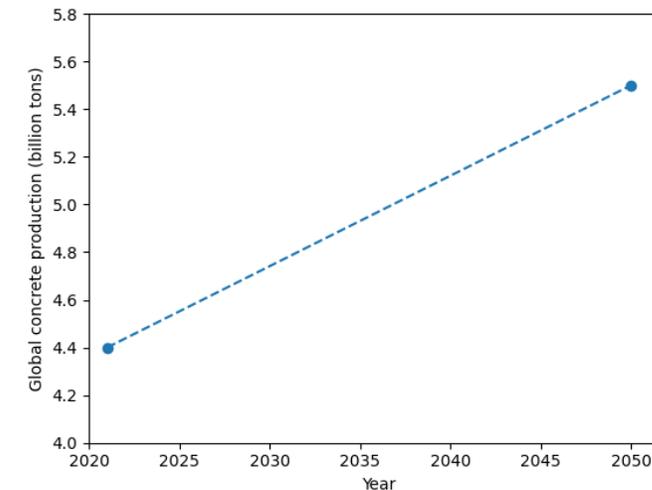
**Seoyoon (Yunnie) Lee '26**

# Motivation

- Cement production → Calcination
- Carbon Capture and Sequestration (CCS)
- Microbially Induced Calcite Precipitation (MICP)
- Industrial waste →  $\text{Ca}(\text{NO}_3)_2$  or  $\text{CaO}$



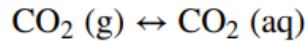
**Figure 1.** Global carbon dioxide emissions by category.



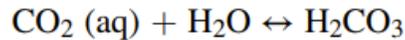
**Figure 2.** Projected Global Concrete Production (2021-2050)

# Process of MICP

1) Gaseous CO<sub>2</sub> dissolves in water to form a hydrated CO<sub>2</sub> state:



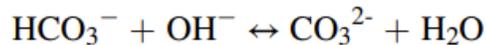
2) The hydration CO<sub>2</sub> reacts with water to generate H<sub>2</sub>CO<sub>3</sub>:



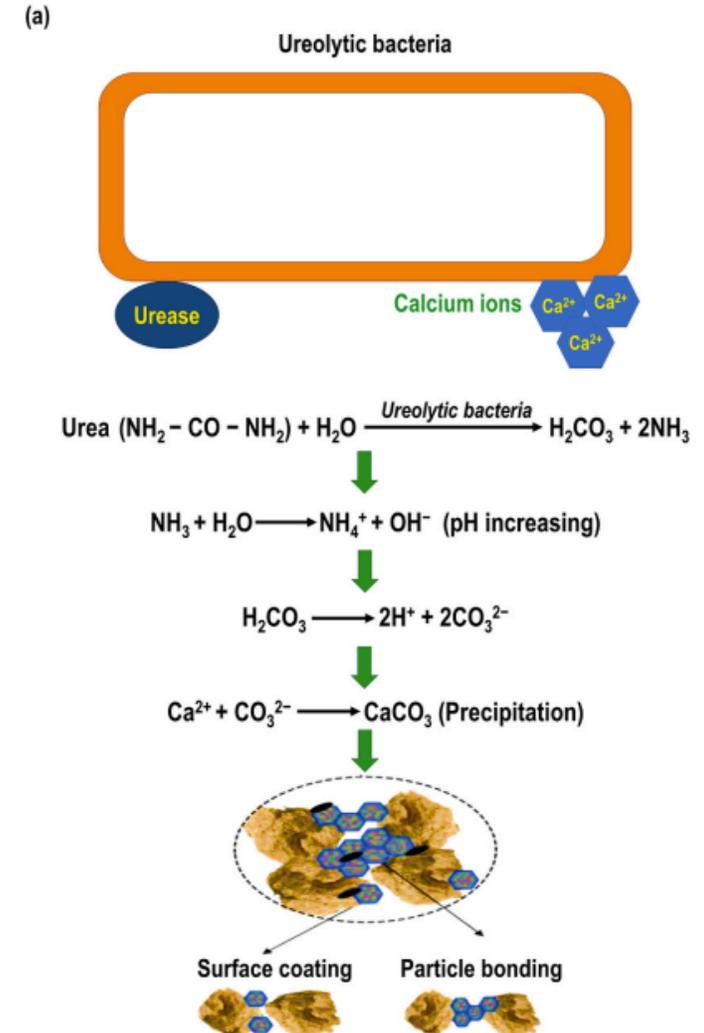
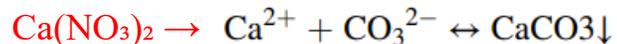
3) The ionization of H<sub>2</sub>CO<sub>3</sub> in the water generates H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup>:



4) Under alkaline conditions, HCO<sub>3</sub><sup>-</sup> is further ionized to form CO<sub>3</sub><sup>2-</sup> and H<sub>2</sub>O:



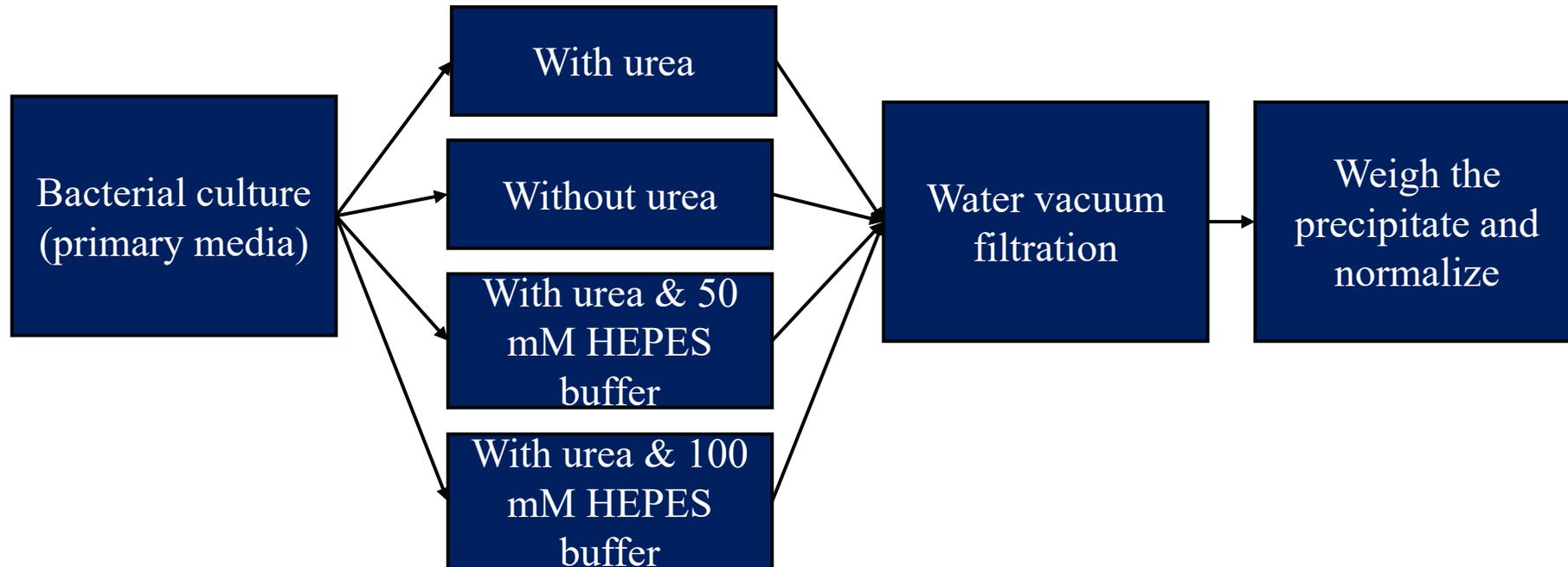
5) In the presence of Ca<sup>2+</sup>, it reacts with CO<sub>3</sub><sup>2-</sup> to form CaCO<sub>3</sub> precipitation:



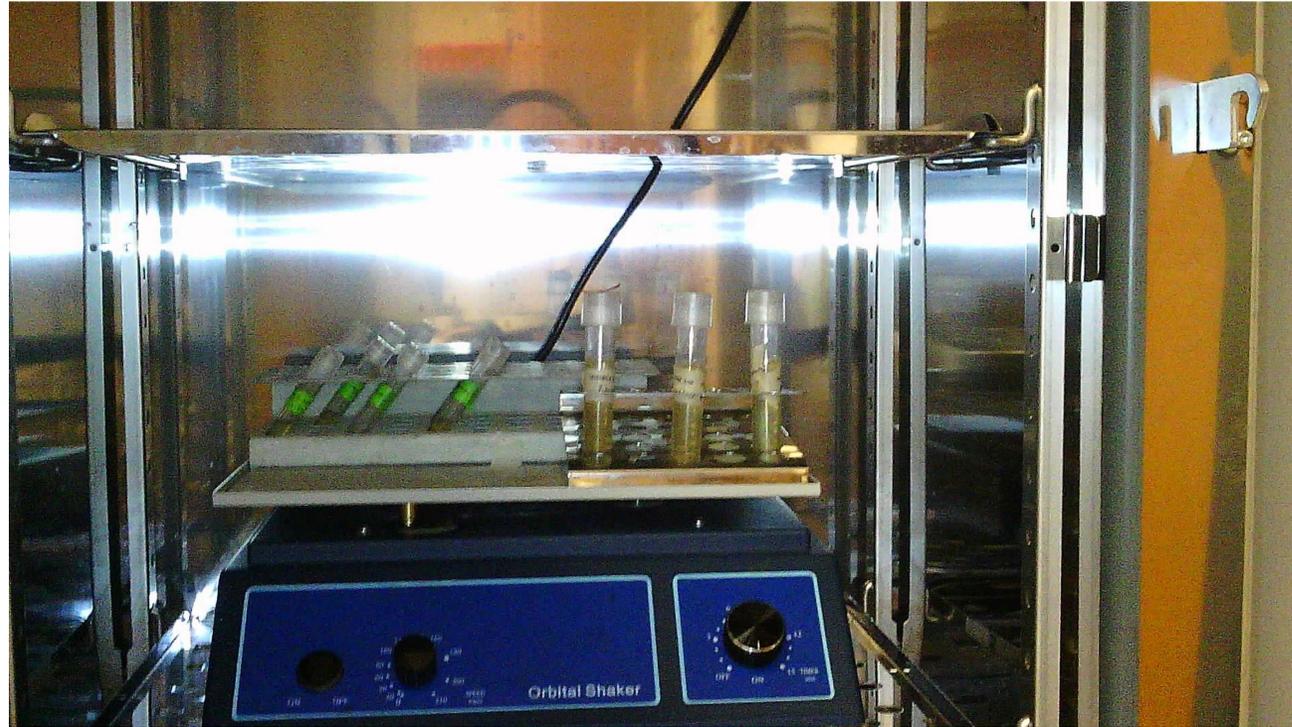
**Fig. 3** Conceptual diagram of bacterial mechanism for bio-concrete formation through urea hydrolysis.

# Methods

- Bacterial culture (primary media)
- Media preparation (secondary media)
- Precipitation filtration



# Bacterial culture (primary media)



**Figure 4.** Bacterial cultures in test tubes placed in the incubator shaker.

# Secondary media preparation

**Table 1.** Components in Flasks 1 to 6.

Flask #	1-3	4-6
Calcium nitrate (g)	2.1g	2.1g
LB broth (g)	3.8 g	3.8g
Distilled water (mL)	150 mL	150 mL
Urea (g)	3.0 g	0 g

**Table 2.** Components in Flasks A to F.

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

# Precipitation filtration



**Figure 5.** The set up for water vacuum filtration.

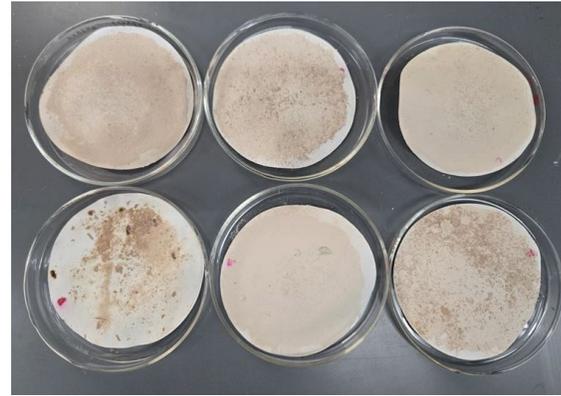


**Figure 6.** Filter papers being dried in the incubator oven.

# Results and discussion



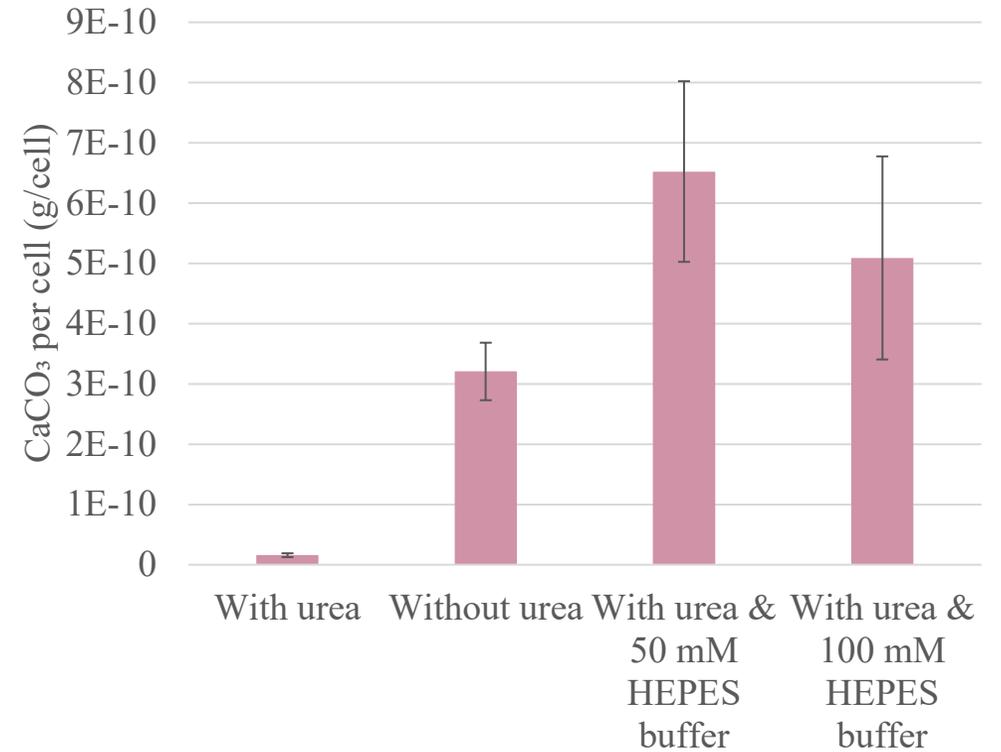
**Figure 7.** Flasks 1 to 6.



**Figure 8.** Flasks A to F.

**Table 3.** Total values.

Conditions	Flask #	CaCO <sub>3</sub> (g)	CaCO <sub>3</sub> per cell (g/cell)	CO <sub>2</sub> 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 9.** The effect of the presence of urea and different HEPES buffer concentrations on MICP efficiency.

# Conclusions

- 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 .

# Applications & Future works

- Anywhere concrete infrastructure is widespread
- chemical condition management  
-> increases the scalability and regulatory feasibility of industrial adoption
- Different CO<sub>2</sub> concentrations
- More replicates
- A wider range of urea/ HEPES buffer concentrations
- Different types of buffers
- Genetically modified *B. subtilis*

# Citations

- Nunes, L. J. R. (2023). The rising threat of atmospheric CO<sub>2</sub>: A review on the causes, impacts, and mitigation strategies. *Environments*, 10(4), 66. doi: 10.3390/environments10040066
- Isaac, G., Nicholas, P., Paul, G., Pietroni, N., Calleja, T. V., Xie, M., & Schork, T. (2024). Automated shotcrete: A more sustainable construction technology. In I. S. Dunmade, M. O. Daramola & S. A. Iwarere (Eds.), *Sustainable Engineering: Concepts and Practices (Green Energy and Technology)*, pp. 331–345). Springer. doi: 10.1007/978-3-031-47215-2\_19
- Qian, C., Yu, X., Zheng, T., & Chen, Y. (2022). Review on bacteria fixing CO<sub>2</sub> and bio-mineralization to enhance the performance of construction materials. *Journal of CO<sub>2</sub> Utilization*, 55, 101849. doi: 10.1016/j.jcou.2021.101849
- Hussain, A., Ali, D., Suprokash Koner, Zeng-Yei Hseu, & Hsu, B.-M. (2025). Microbial induce carbonate precipitation derive bio-concrete formation: A sustainable solution for carbon sequestration and eco-friendly construction. *Environmental Research*, 270, 121006. doi: 10.1016/j.envres.2025.121006
- Kadapure, A. J., Dalbanjan, N. P., & S K, P. K. (2025). Characterization of heat, salt, acid, alkaline, and antibiotic stress response in soil isolate *Bacillus subtilis* strain PSK.A2. *International Microbiology: The Official Journal of the Spanish Society for Microbiology*, 28(2), 315–332. doi: 10.1007/s10123-024-00549-z
- Kim, J. K., Mulrooney, S. B., & Hausinger, R. P. (2005). Biosynthesis of Active Bacillus subtilis Urease in the Absence of Known Urease Accessory Proteins. *Journal of Bacteriology*, 187(20), 7150–7154. doi: 10.1128/JB.187.20.7150–7154
- Prakash, O., Ujjwala Waghmare, Chauhan, A., & Patil, Y. (2025). Optimizing experimental conditions: the role of buffered environments in microbial isolation, physiological studies, and taxonomic characterization. *Applied and Environmental Microbiology*, 91(1), e01728-24. doi: 10.1128/aem.01728-24
- Pictures were taken by Seoyoon Lee.
- Flow chart was made by Seoyoon Lee using PowerPoint.