

Introduction

Calcium Carbonate Crystals

- Found in bones and teeth
- Found in the inner ear, called otoconia help detect gravity, balance, and movement.
- Contribute to calcium-based kidney stones when crystals accumulate and grow in the urinary system.
- Forms the shells of many mollusks
 - Clams, oysters, snails.
- Makes up much of the skeletal structure of coral reefs,
- Composes 95% of an eggshell
- Occurs naturally as the minerals calcite and aragonite
 - Found in rocks, sediments, caves, marine environments.
- Participates in the carbon cycle
- Large deposits of limestone
 - heat and pressure can make limestone into marble.
- Used in calcium supplements and antacids
- This matters because:
 - May improve our understanding of calcium-based crystal formation in the human body and bacteria interactions
 - Builds knowledge that could support future research in medicine and microbiology.

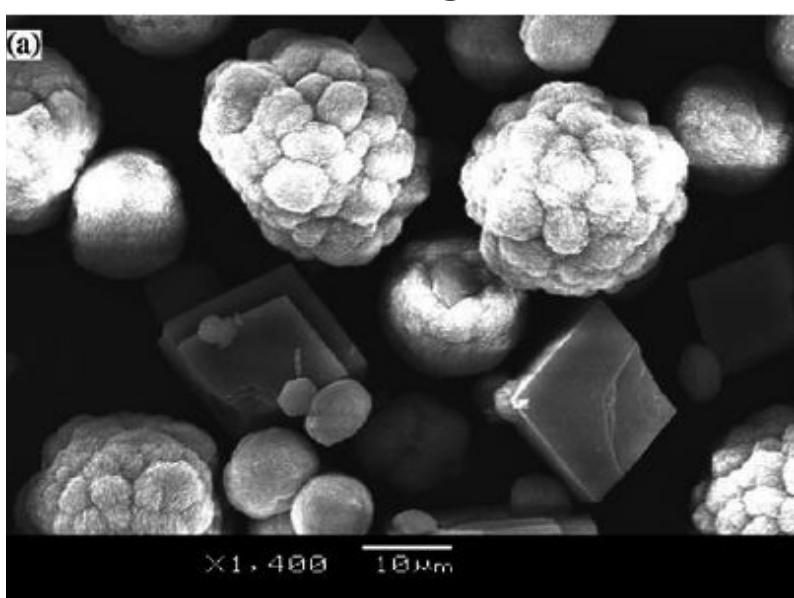


Figure 1. SEM photographs of calcium carbonate crystals. Kitamura (2002).

Methodology

This scientist hypothesized:

Calcium carbonate crystals will promote the most efficient growth for *Bacillus subtilis* and *Sporosarcina Ureae*, whereas they will inhibit the growth of *Escherichia coli* and *Aeromonas Sobria*.

She came up with this hypothesis based on information in her background research.

- Calcium ions are toxic to *E. coli* cells and cause growth to slow or stop (Tisa % Adler, 1992)
- Calcitic substrates “foster bacterial growth and metabolic activity (for *B. subtilis*)” (Rodríguez-Navarro, 2012).
- Bacterial cells serve as mineral scaffolds and possibly nucleation points for precipitation (Zambare, 2020).
- The results showed that the presence of calcium was the driving force of MICP (Wang, 2022).

Independent Variable:

4 different bacteria: *B. subtilis*, *E. coli*, *S. ureae*, and *A. sobria*.

Dependent Variable:

Percent of bacterial coverage on each Petri dish.

Control Groups: Growth of bacteria without calcium carbonate crystals.

Procedure:

1. Crack 3 eggs and collect then crush the shells.
2. Add 5% acetic acid and let it sit for 45 minutes.
3. Filter the mixture into another beaker and get a hot plate.
4. Boil off about half the mixture and pour it into a Petri dish.
5. Allow crystals to grow.
6. Prepare agar and pour it into Petri dishes.
7. Following aseptic technique, transfer bacterial cultures
8. into their own labeled dishes.
9. There should be 2 of each bacterial culture (control)
10. Add calcium carbonate crystals into each dish (except control)
11. Incubate at 30 degrees celsius and record daily observations.



Finalist Measuring Acetic Acid
Photo Taken by Science Teacher, 2025

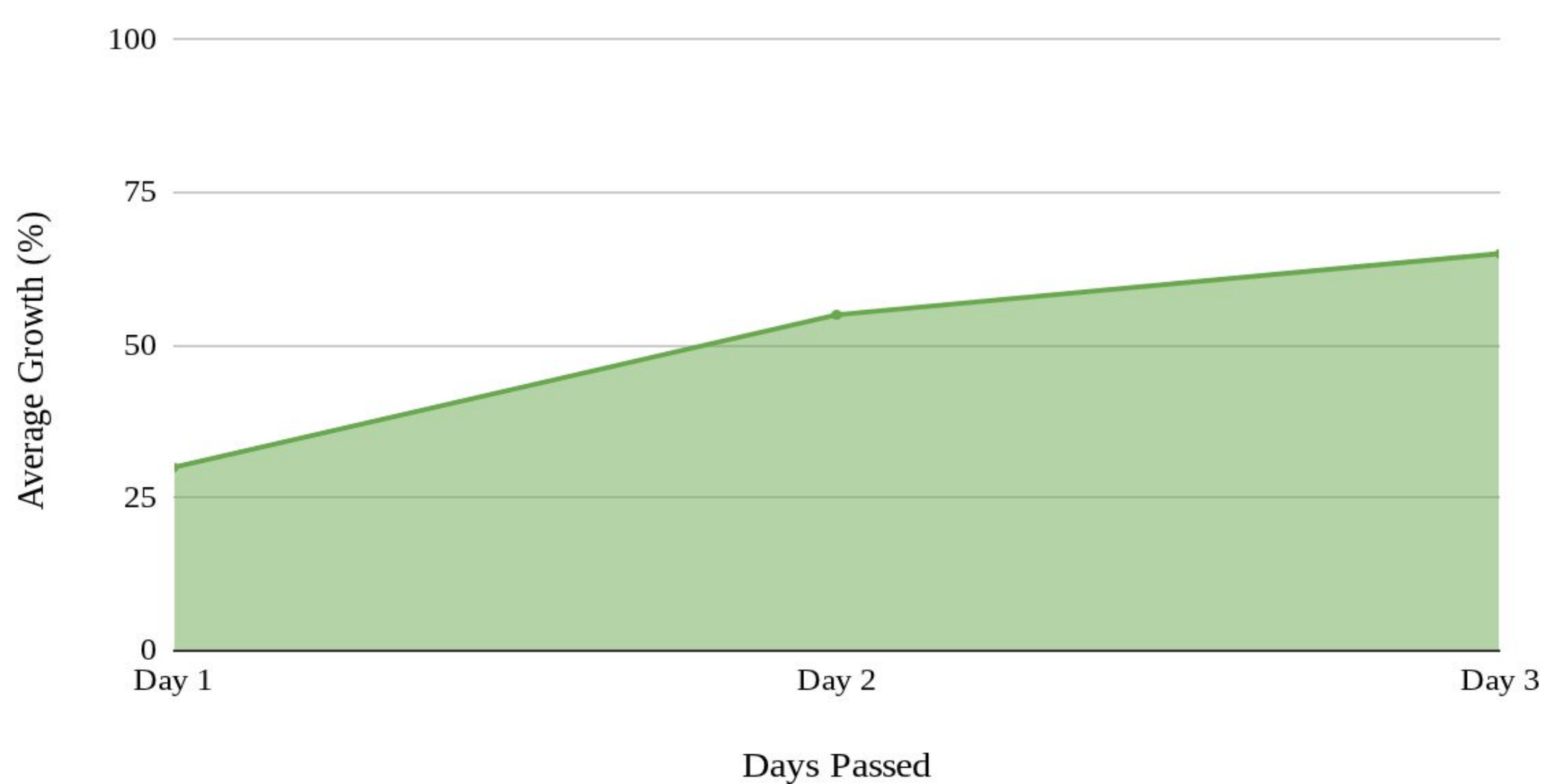


Finalist Marking Petri Dishes
Photo Taken by Science Teacher, 2025

How Do Calcium Carbonate Crystals Affect Bacterial Growth? CaCO₃ in Biology and Nature

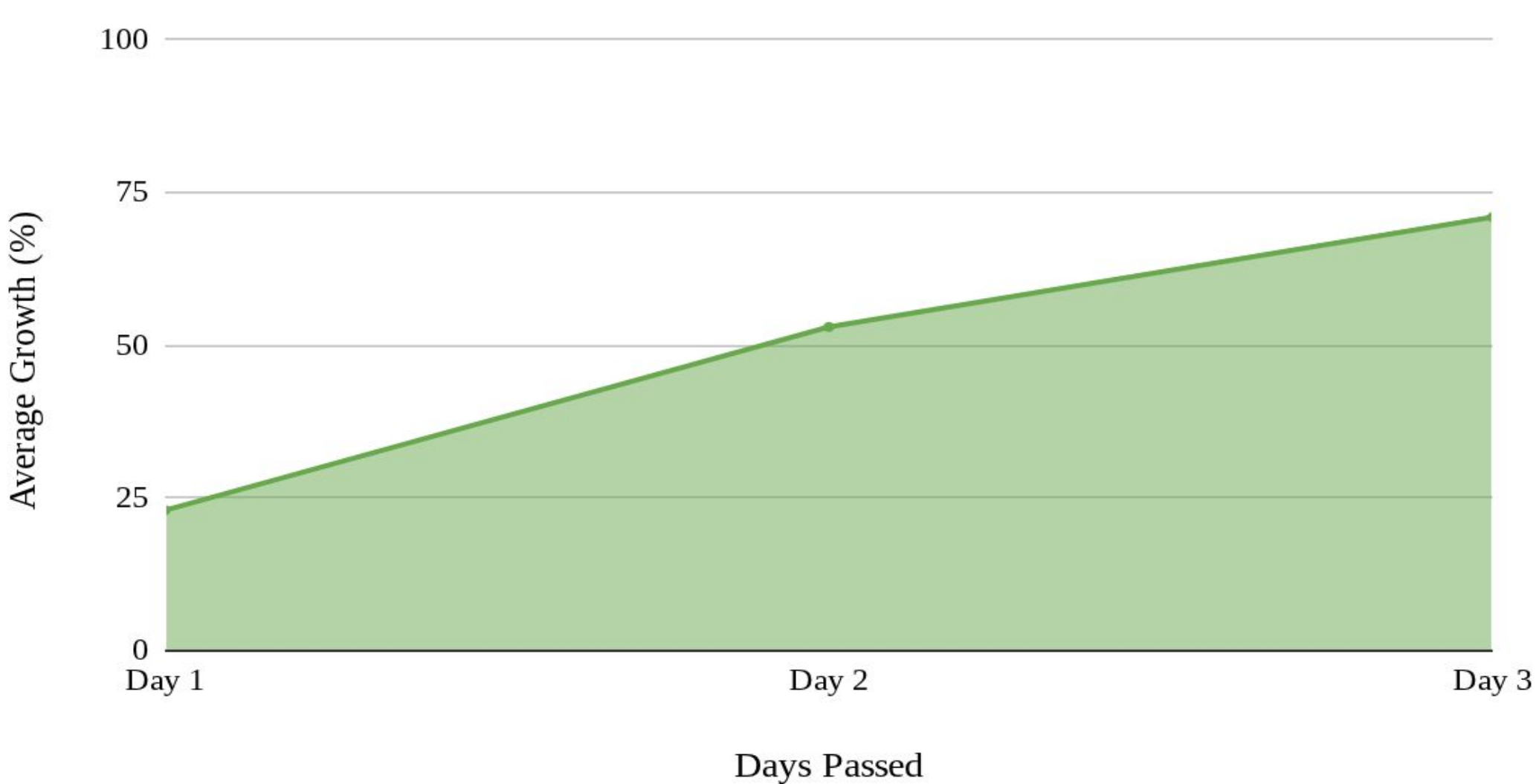
Data

Average Growth (%) for *S. ureae* (Experimental) over 3 days



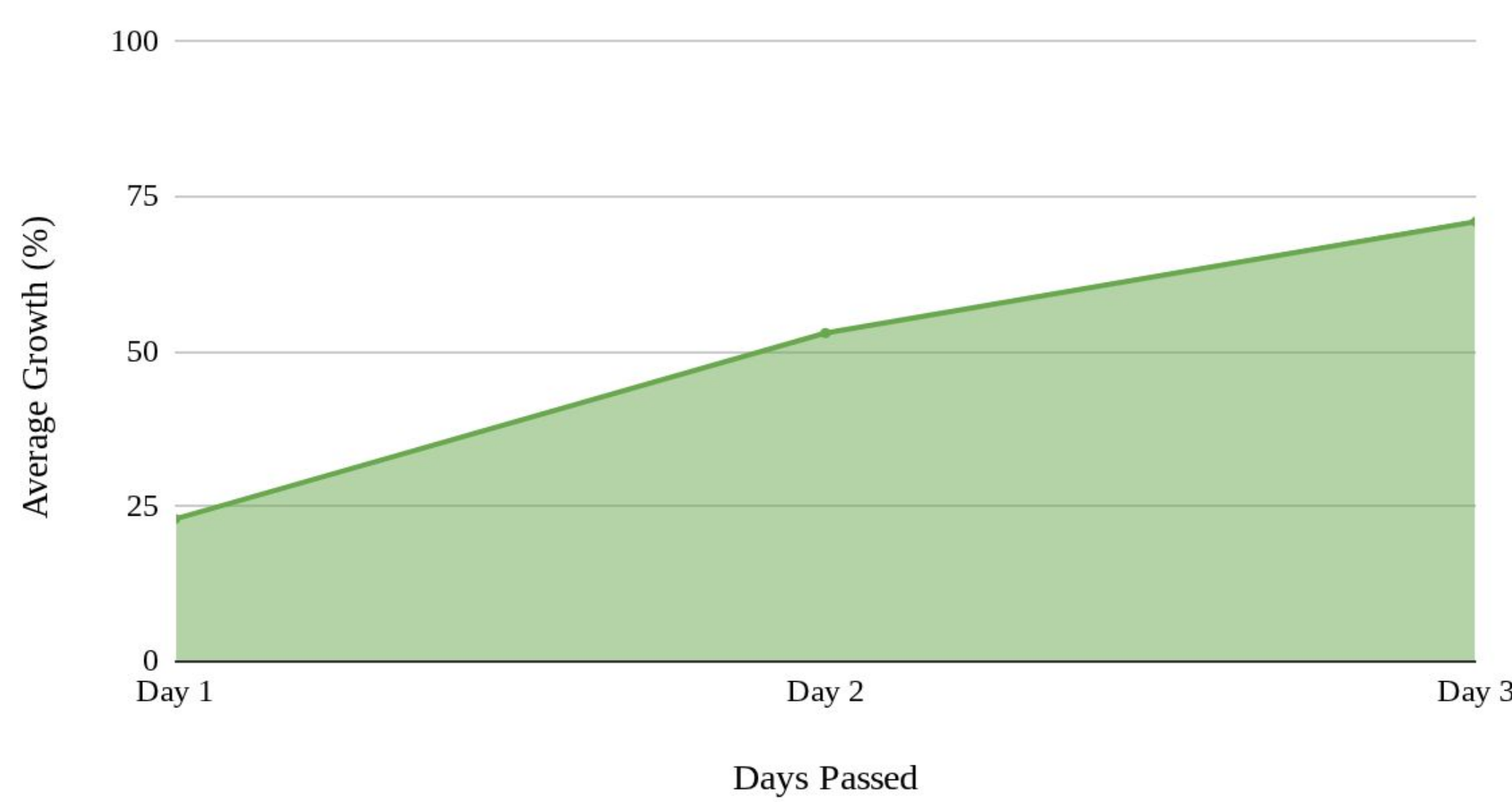
Graph created by Finalist using Google Sheets, 2026

Average Growth (%) of *B. subtilis* (Experimental) over 3 days



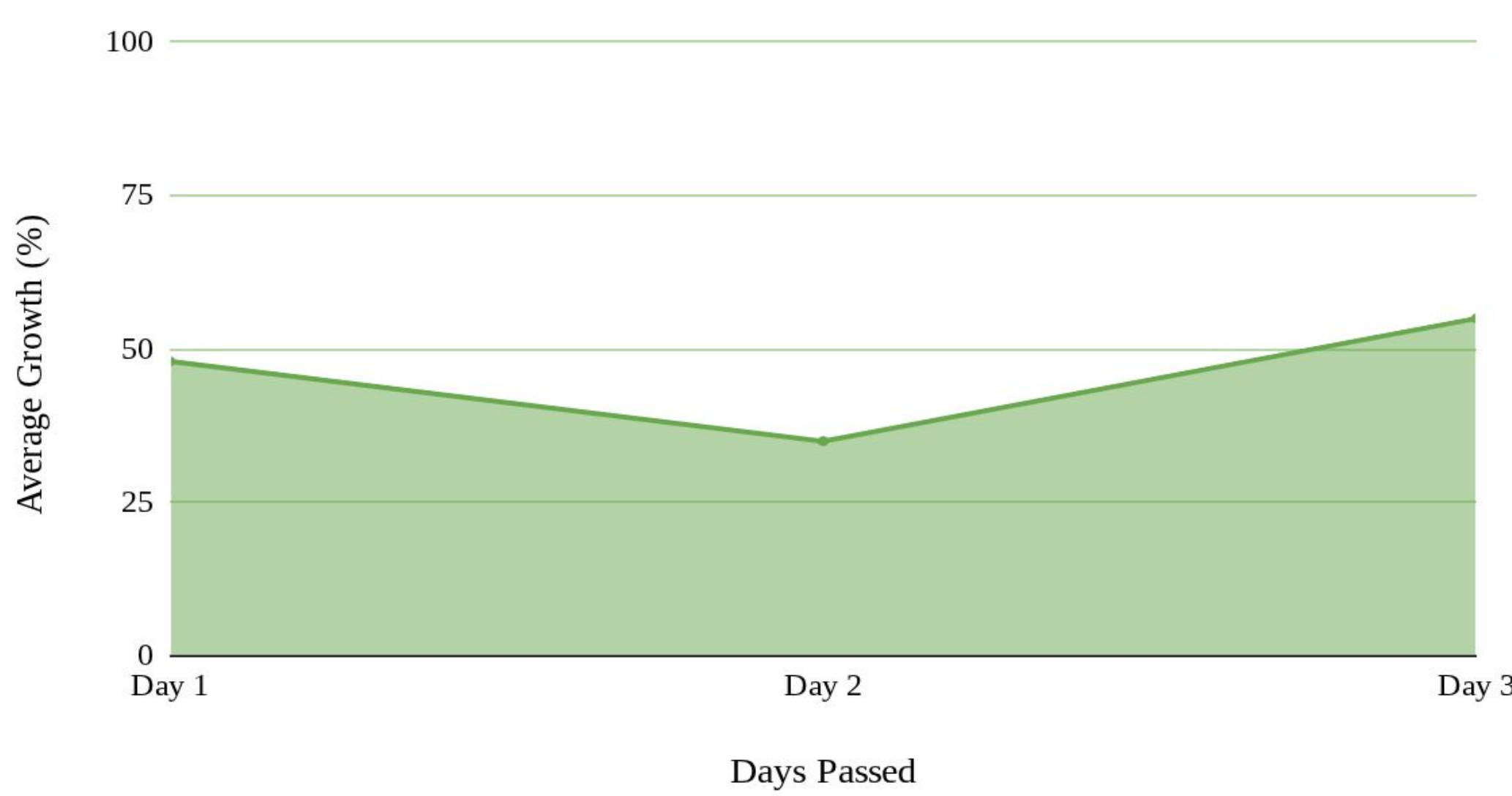
Graph created by Finalist using Google Sheets, 2026

Average Growth (%) of *B. subtilis* (Experimental) over 3 days



Graph created by Finalist using Google Sheets, 2026

Average Growth (%) of *A. sobria* (Experimental) over 3 days



Graph created by Finalist using Google Sheets, 2026

Statistical Analyses

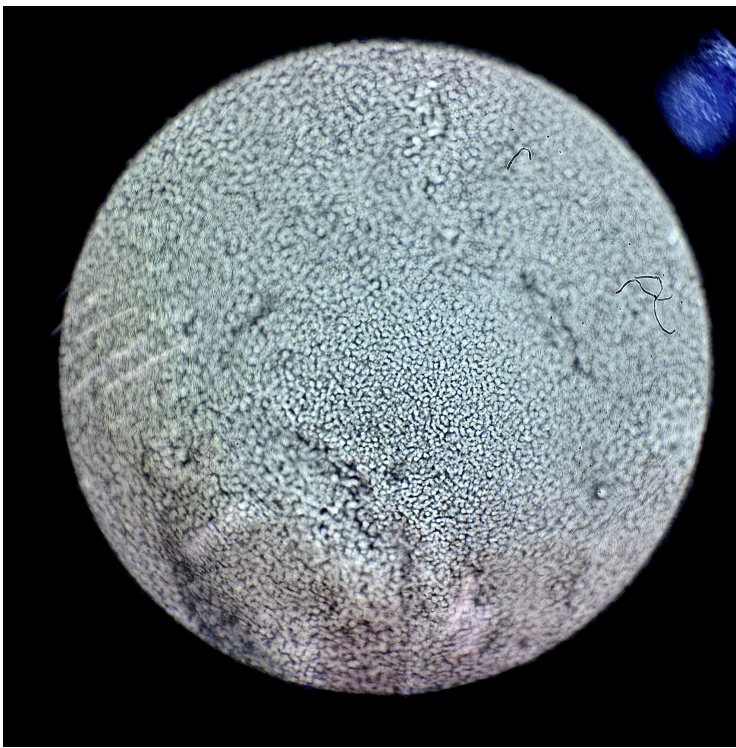
<i>E. coli</i>	Experimental	Control
Average	50%	94%
Standard Deviation	22%	3.13%
T-Test P value	0.0000186	

<i>B. subtilis</i>	Experimental	Control
Average	49%	91%
Standard Deviation	22%	3.74%
T-Test P value	0.004789	

<i>S. ureae</i>	Experimental	Control
Average	50%	90%
Standard Deviation	19.31%	3.22%
T-Test P value	0.000427	

<i>A. sobria</i>	Experimental	Control
Average	46%	81%
Standard Deviation	15%	1.88%
T-Test P value	0.004789	

Photographs



CaCO₃ Under 1600x Mag
Photo Taken through Microscope by Finalist, 2026



CaCO₃ Crystals
Photo Taken by Finalist, 2026



Finalist Holding Acetic Acid
Photo Taken by Science Teacher, 2026



B. Subtilis: Experimental
Photo Taken by Finalist, 2026



E.coli: Experimental
Photo Taken by Finalist, 2026



A.sobria: Experimental
Photo Taken by Finalist, 2026



S. ureae: Experimental
Photo Taken by Finalist, 2026

Results

The average bacterial coverage for *B. subtilis* with calcium carbonate crystals was 87.5%, and without CaCO₃ crystals (control group), it was about 90%.

The average bacterial coverage for the petri dishes with *S. ureae* and calcium carbonate crystals had an average of 75% coverage, and without calcium carbonate crystals (control group) had an average of 93%.

The average bacterial coverage for *E. coli* with CaCO₃ was 56.3%, and the average bacterial coverage for *E. coli* by itself (control group) was 95.5%

The average amount of bacterial coverage for *A. sobria* with CaCO₃ was 55%. Her control group showed much more growth than 55%.

Applications / Future Studies

My findings suggest that calcium carbonate (CaCO₃) may have potential antimicrobial applications. One finding was that CaCO₃ inhibited bacterial growth across all four species tested, suggesting that other species may also be inhibited. CaCO₃ or CaCO₃-based materials could be used as a broad-spectrum antibiotic.

Since CaCO₃ is also a major component of many animal shells, another potential is whether it contributes to some sort of natural defense against microorganisms. Comparing CaCO₃ with natural shells could help investigate this possibility.

One particularly interesting future direction involves kidney stones and UTIs. Because some kidney stones contain calcium-based minerals and *E. coli* is a common cause of UTIs, I wondered whether CaCO₃-rich environments could influence *E. coli* growth. My results show that CaCO₃ inhibits UTIs, but it does not mean that kidney stones do. They tell why to investigate this relationship further.

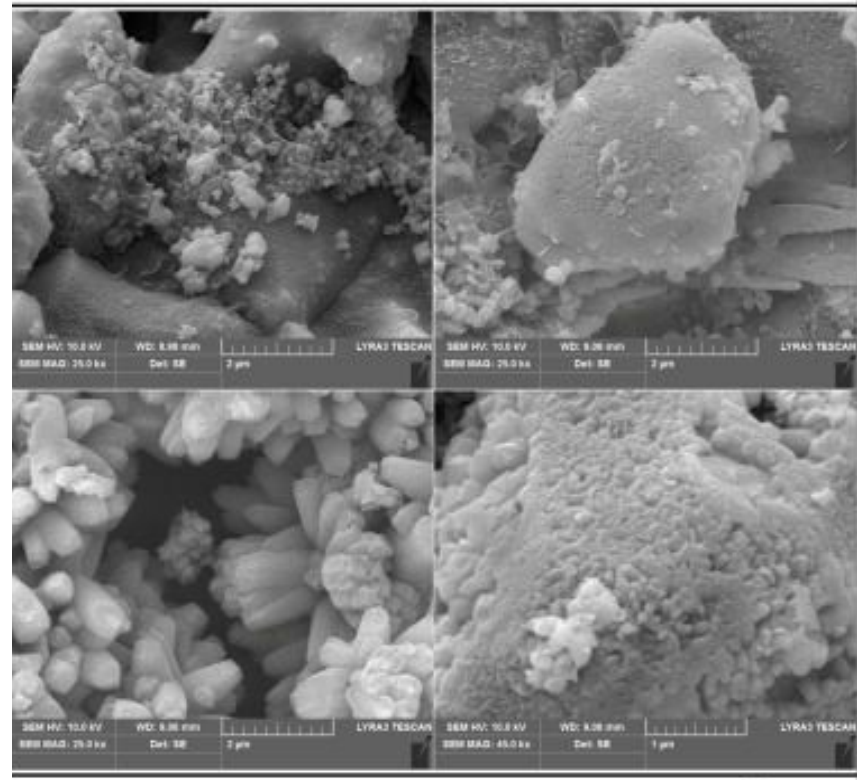


Figure 2. SEM-EDS analysis of thermally processed oyster shell powder showing CaCO₃ structures. Ahn et al. (2025).

Future studies could test additional bacteria, different CaCO₃ crystal forms and concentrations, and determine why CaCO₃ inhibited bacterial growth. Another question is whether the effect is specific to CaCO₃ or whether other calcium-containing compounds produce similar results.

This project also taught me that failure is part of scientific discovery. After several unsuccessful attempts to grow CaCO₃ crystals, additional research led me to develop a more consistent eggshell-based method. Each failed attempt helped improve my experimental design and generated new questions for future research.

References

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