Using pH and Cell Growth to Measure the Urease Activity of *Sporosarcina pasteurii* in Stuart's Urea Broth with Bromothymol Blue

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Abstract

Sporosarcina pasteurii can perform microbially-induced calcite precipitation (MICP)—hydrolyze urea and precipitate calcium carbonate crystals. This has potential applications in biocementation, though there are barriers to implementation. One way to overcome these barriers is to measure cell growth and urease activity of *S. pasteurii.* Because urea hydrolysis increases the surrounding pH, urease activity was measured using Stuart's Urea Broth with the pH indicator bromothymol blue. Additionally, a standard curve was generated to quantify pH change, and cell growth of *S. pasteurii* was measured before and after urea hydrolysis. It was found that that higher concentrations of bacteria resulted in higher pH values and a faster pH increase, indicating higher urease activity. It was also found that cell growth declines during urea hydrolysis, and the growth media seems to influence this decline. These findings suggest that higher cell concentrations should be used in MICP applications to produce the highest urease activity, and that the cell growth of *S. pasteurii* may not increase with urease activity.

Objectives

- Measure the urease activity of *S. pasteurii* as pH increase in Stuart's Urea Broth.
- Model pH change using standard curve
- Determine the cell growth of *S. pasteurii* during urease activity

Methods

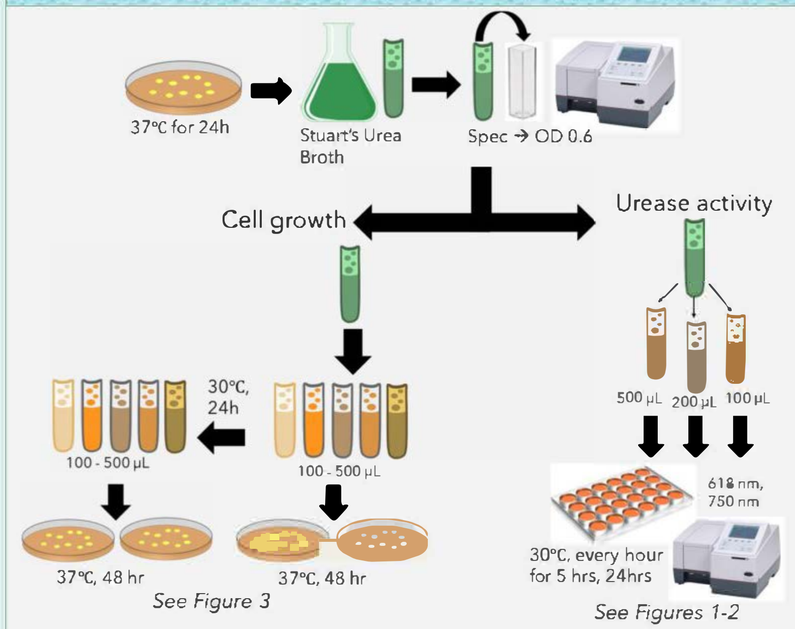

Figure 1. Graphical schematic for the methods and experimental design. *S. pasteurii* was resuspended in Stuart's Urea Broth, and urease activity and cell growth were measured over 24 hours.

Results

Highest increase in urease activity seen in higher bacterial concentrations

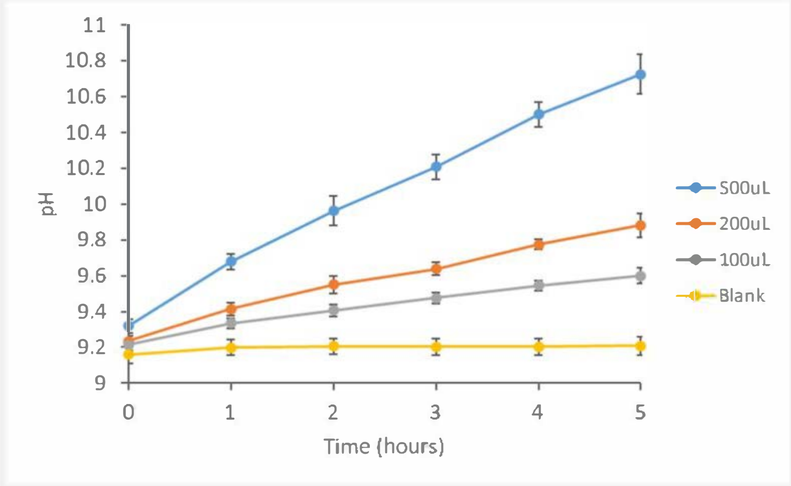

Figure 2. Increase in the mean pH of different concentrations of *S. pasteurii* in Stuart's Urea Broth over 5 hours. pH was determined from standard curve equation. Time is on the X axis, and pH is on the Y axis. Error bars represent standard deviation.

Highest bacterial concentration showed highest maximum urease activity

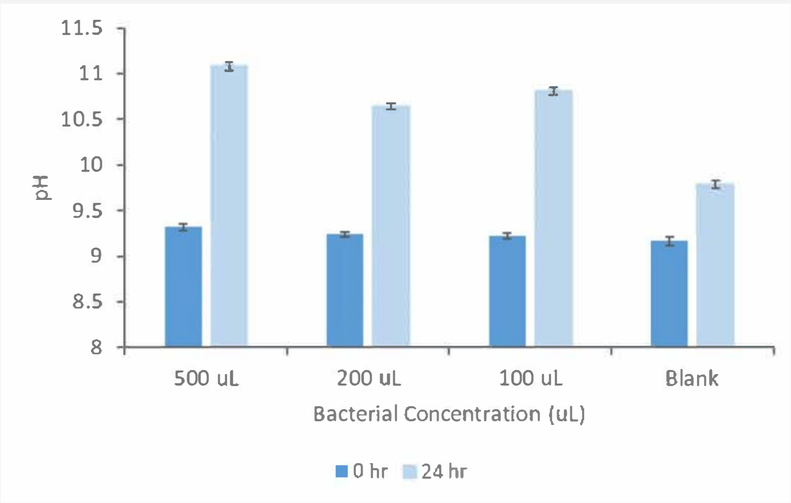

Figure 3. Initial and maximum pH value of different concentrations of S. pasteurii in Stuart's Urea Broth, at 0 hours and 24 hours later. Error bars represent standard deviation. Means were statistically different from each other.

Results (con't)

Differences in bacterial growth over 24 hours depending on media composition

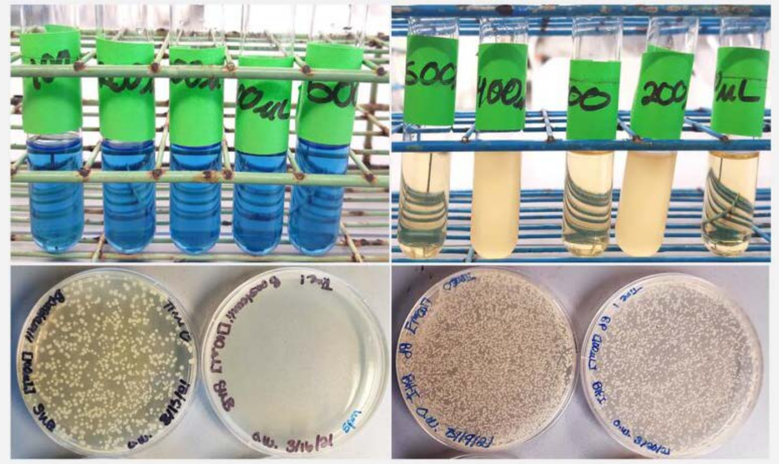

Figure 4. Turbidity of different concentrations of *S. pasteurii* after 24 hours of growth in Stuart's Urea Broth (top left) and BHI Broth with 2% urea (top right). Plating results of 100µL bacteria in Stuart's Urea Broth (bottom left) and BHI + 2% urea broth (bottom right) at 0 hours and 24 hours.

Conclusions & Future Directions

- Change in pH (urease activity) could be quantified using a standard curve.
- A higher bacterial concentration led a faster increase in urease activity and a higher maximum urease activity.
- Less bacterial growth was seen after 24 hours of urease activity. Bacterial growth declines more when using Stuart's Urea Broth, showing BHI broth is better for growth.
- Future directions can include:
  - Refining standard curve for more accurate pH measurement
  - Determining exact maximum urease activity of *S. pasteurii.*
  - Investigating cell growth using BHI + 2% urea broth
  - Quantifying bacterial growth over time.

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