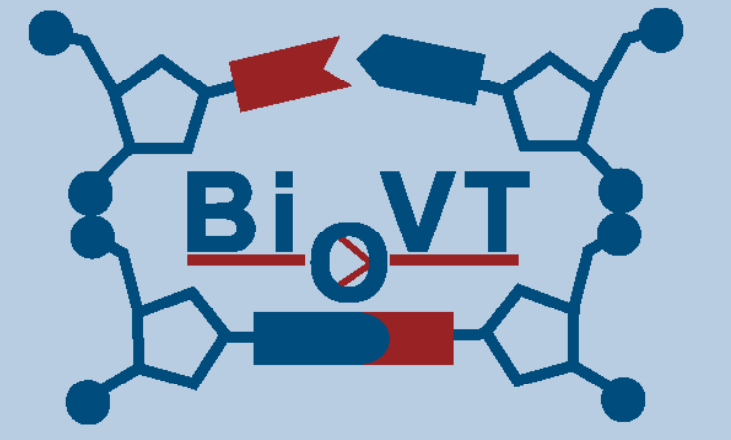


Optimization of the microbial induced calcium carbonate precipitation for the production of biocement



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Abstract

Microbial induced calcium carbonate precipitation (MICP) is a process that shows the potential to offer a more sustainable alternative to cement the main binder of concrete. During MICP calcium carbonate is produced through microbial activity. If the precipitation occurs in cavities between mineral particles, like silica sand, the calcium carbonate can form bridges between the particles and solidify them. Ureolytic microorganisms like *Sporosarcina pasteurii* provide an easy to control way to utilize MICP for biocement production. They metabolize Urea and thereby produce ammonia and carbonate. In the presence of soluble calcium ions, the latter is precipitated as calcium carbonate. The influence of key parameters on MICP on the strength of biocement was investigated in this study. To optimize the strength of silica sand treated with MICP a central composite design was employed to determine the influence of the concentration of urea and calcium chloride and the volume of cell suspension during treatment. Silica sand was treated sequential with cell suspension of *S. pasteurii* and a calcination solution containing urea and calcium chloride. The determined optimum conditions were urea concentration (1492 mM), calcium chloride concentration (1391 mM) and volume of cell suspension (7.47 mL). Specimen treated under these conditions showed a compressive strength of 1877 ± 240 kPa.

Microbial induced calcium carbonate precipitation

MICP

- (A) Urea is taken up by the ureolytic microorganisms and metabolized intracellularly to ammonia and dissolved inorganic carbon (DIC)
- (B) Local supersaturation and elevated pH cause precipitation of calcium carbonate
- (C) Limitations of nutrient supply due to encapsulation of the cell

Influence on MICP

- Concentration of Urea
- Concentration of Calcium-Ions
- pH
- Temperature
- Concentration of Cells

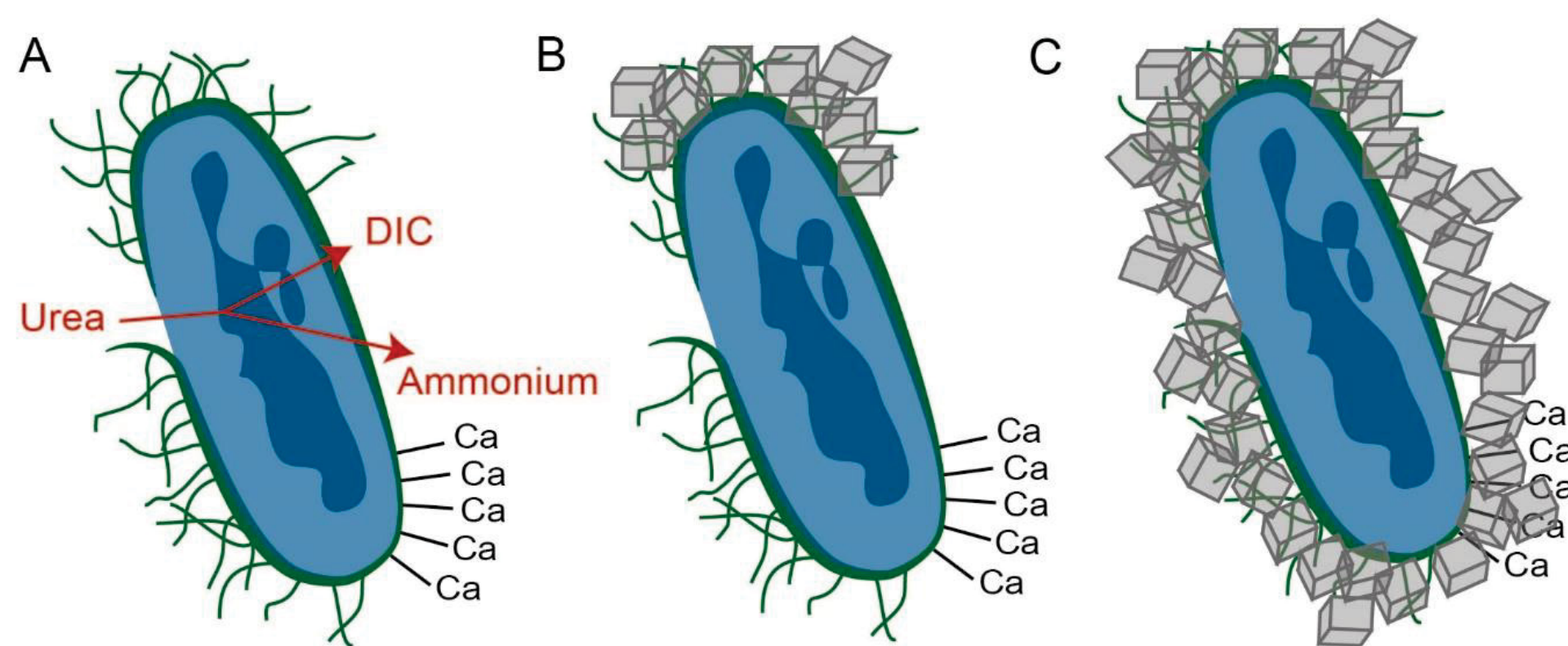


Fig. 1: Processes during ureolytic calcium carbonate precipitation.

Production of biosandstone

- *S. pasteurii* cells attach to the surface of silica sand
- Urea is hydrolysed intracellularly to Ammonia and Carbonate
- Negatively charged cell surface attracts positive charged calcium ions
- ➔ Cells act as nucleation sites for carbonate crystal formation

- Higher amounts of precipitated calcium carbonate result in higher compressive strength (see figure 3)
- Multiple cycles necessary for consolidation

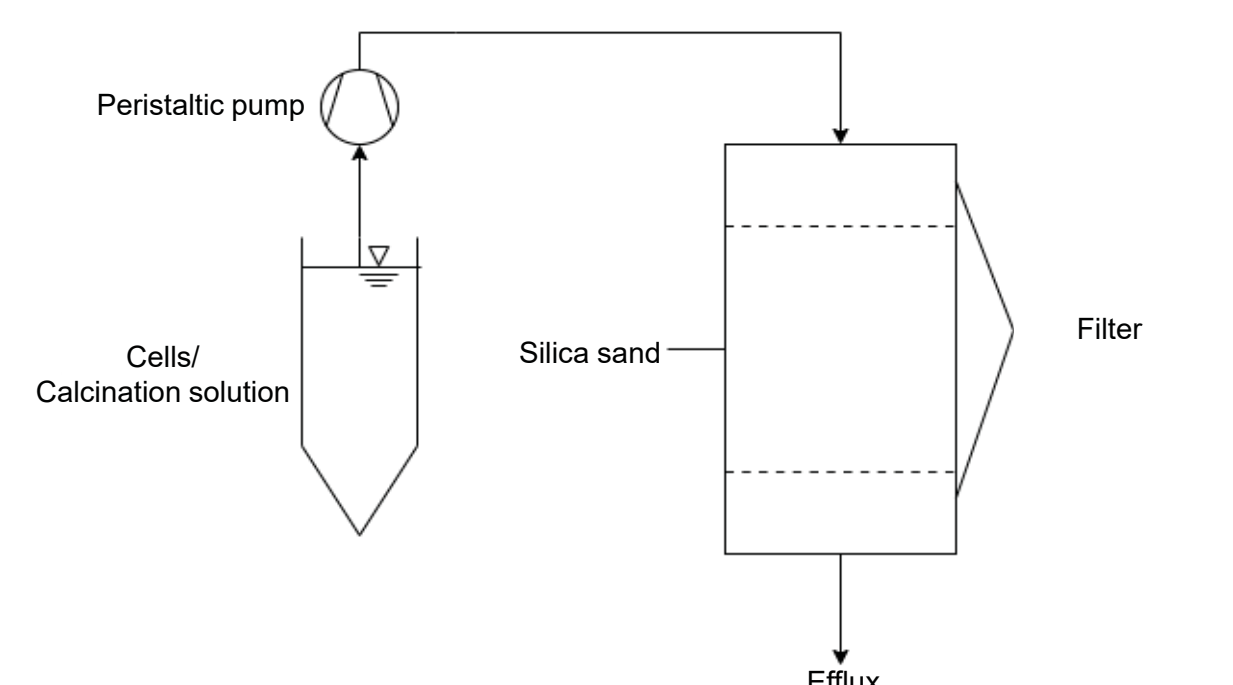


Fig. 2: Schematic of the experimental setup for calcination of silica sand

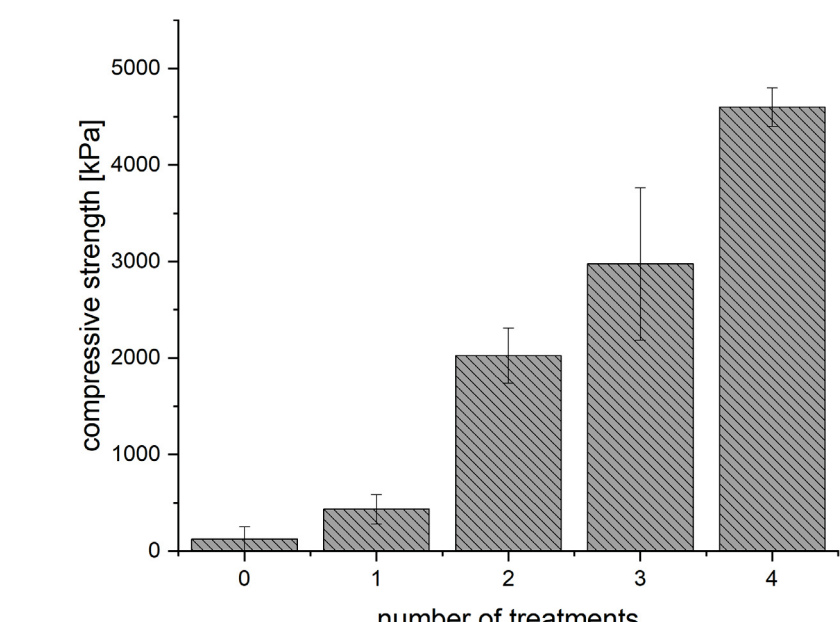


Fig. 3: Compressive strength after each treatment cycle of MICP. OD of cell suspension 1.74. Concentrations of Calcination Solution 3000 mM Urea, 1500 mM CaCl₂.

Conclusion and Outlook

The response surface methodology was used to optimize the compressive strength of silica sand treated with microbial induced calcium carbonate precipitation (MICP). Urea concentration, calcium chloride concentration and the volume of cell suspension were the independent variables tested. The results demonstrated that MICP is limited for high concentrations of calcium chloride. Furthermore, it was shown that there is an optimum amount of calcium chloride and urea for a certain number of cells in the specimen during MICP treatment. While comparing the optimized reagent with a protocol widely used in literature a higher efficiency of compressive strength regarding nutrient for MICP could be achieved. Further research is necessary to optimize the conditions for MICP regarding compressive strength of consolidated sand. Therefore the borders of the DoE will be extended. The results showed that high concentrations of calcium ions inhibit the production of calcium carbonate. Therefore different ureolytic organisms will be screened for their ability to produce calcium carbonate in presence of high concentrations of calcium ions.

Optimization of Biosandstone production with RSM

Tab. 1: Experimental range and levels of independent variables

Level	Urea concentration (mM)	Calcium chloride concentration (mM)	Volume of cell suspension (mL)
+α	2341	2341	11.7
1	2000	2000	10
0	1500	1500	7.5
-1	1000	1000	5
-α	659	659	3.3

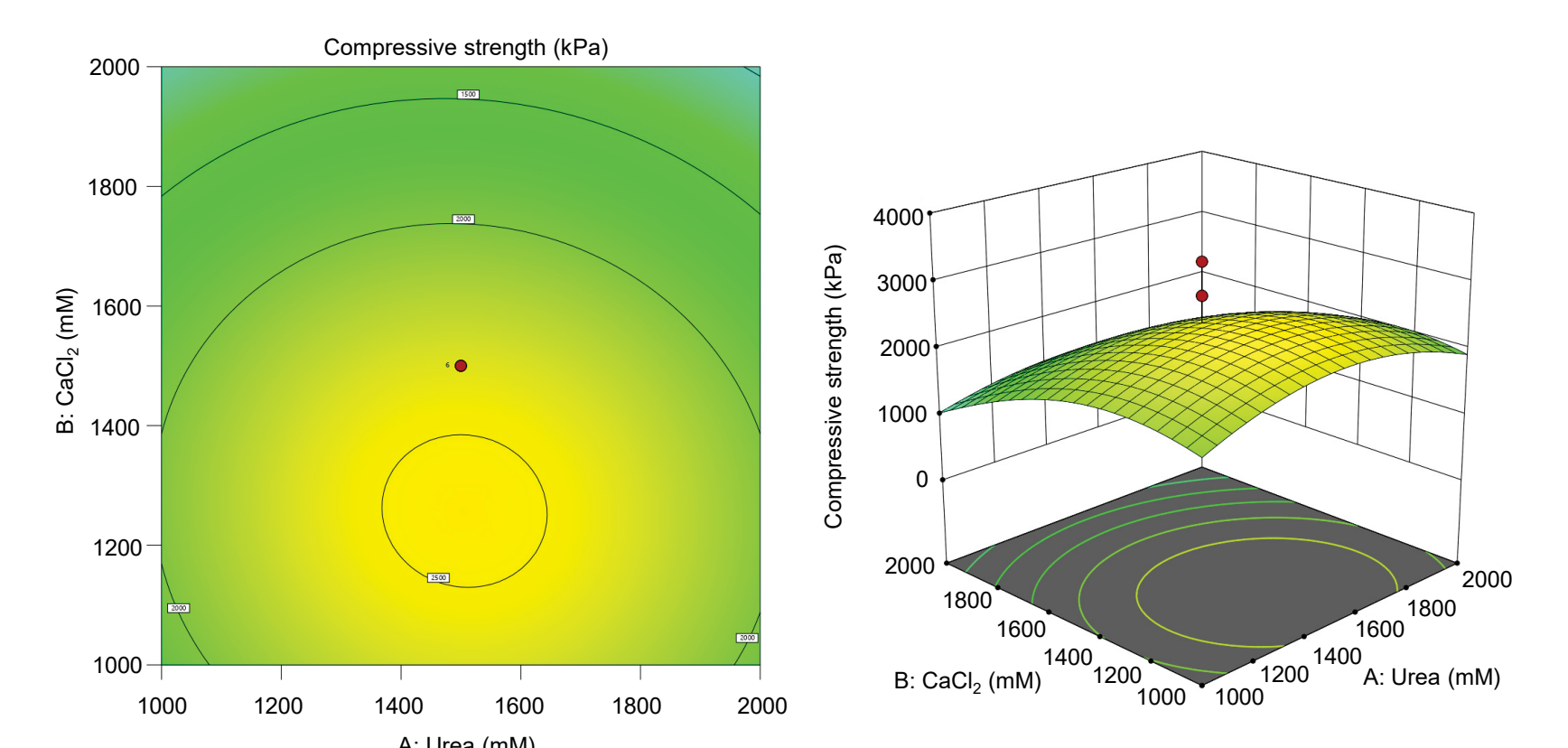


Fig. 4: Response surface plots of the interactive effects of urea concentration (A) and calcium chloride concentration (B) on the compressive strength at constant cell suspension volume of 7.5 mL

Optimization of MICP

- Optimization with Response Surface Methodology (RSM)
- Central Composite Design (CCD) was employed with 20 experiments
- Experimental data applied to quadratic model
- Model is significant (p-value 0.0018, R²=0.9815)
- High CaCl₂ concentrations inhibit Urease activity and lower MICP efficiency

➔ Predicted optimum at 1492 mM Urea and 1391 mM CaCl₂

Validation

- Prediction interval mean (PIMean) was used for validation
- Six Validation runs were conducted
- Data mean is compared with the prediction interval with a 95 % confidence interval
- 95% PI high 3467.55 kPa
- Data mean 1876.85 kPa
- 95% PI low 1603 kPa

➔ Model is valid

Efficiency of the optimized protocol

- Optimized protocol was compared with two protocols often used in literature
- Usage of higher concentrations of Urea and calcium beneficial for consolidation (See figure 5)
- Second highest absolute compressive strength with optimized protocol (1883 kPa)
- To assess the efficiency the compressive strength per mol of substrate was calculated
- Optimized protocol achieved the highest efficiency with 0.65 kPa per mol

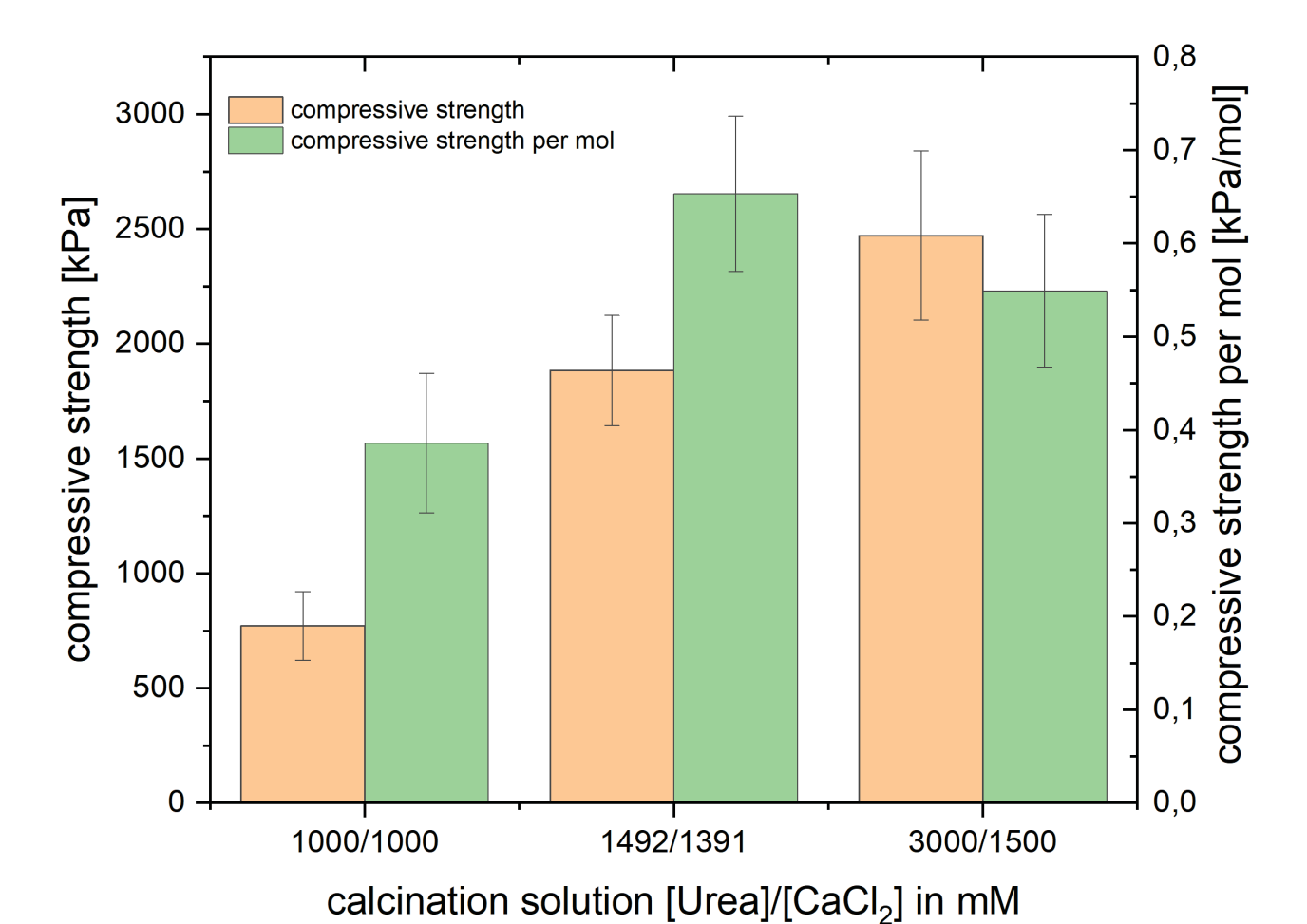


Fig. 5: Comparison of the optimized protocol with a protocol often used in literature. Number of treatments 3, temperature during treatment 30 °C, reaction time 24 h, drying (72 h, 60 °C), n=7