Utilization of *Sporosarcina pasteurii* for microbially induced calcite precipitation in recycling waste concrete fines

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Introduction

The excessive production of carbon dioxide in recent years and the related conclusion of the so-called Paris Climate Agreement of 2015 require the fulfilment of commitments necessary to reduce CO_2 emissions and achieve zero anthropogenic CO_2 emissions (known as carbon neutrality) by 2060-2080. The construction industry and especially cement production are significant contributors to CO_2 emissions not only during the production of building materials, but also during the storage and disposal of their waste products (rubble, grit, coarse aggregate, fine aggregate). Therefore, we explored the possibility of recycling waste concrete products (WCF) and thus fulfilling the strategy and concept of the circular economy. The recycling of WCF is accomplished through the utilization of a biomineralization proces known as microbially induced calcite precipitation (MICP). MICP involves the ability of microorganisms to synthesize minerals by precipitation using enzymatic activity (most commonly urease) in the presence of just waste products such as WCF. Two types of WCF differing in their physicochemical properties and age were investigated. We used an alkaliphilic bacterium *Sporosarcina pasteurii* DSM 33 capable of urease activity and thus precipitating calcium carbonate crystals. The experiment was carried out for 14, 30, 60 and 90 days.

Methods and results

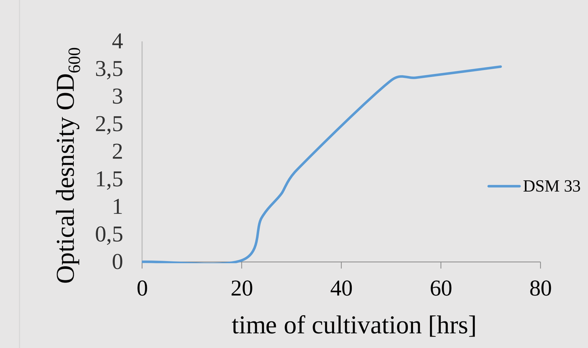
Optimization of experimental conditions

Tested conditions:

- Concentration of *S. pasteurii* DSM 33: OD_{600} of 2.5 or 5.0
- pH adjustment of WCF suspension
- Single/repeat addition of bacterial cells

→ Growth conditions of *S. pasteurii* DSM 33 (TSB+urea 20 g/L, 28 °C, 48-72 h, 120 rpm)

→ Determination of growth curve and urease activity (colorimetric phenol-hypochlorite method) *S. pasteurii* DSM 33



The optimal time for cultivation of DSM 33 with maximum urease activity was determined to be 48 hours (at the end of the exponential phase of the growth profile of DSM 33).

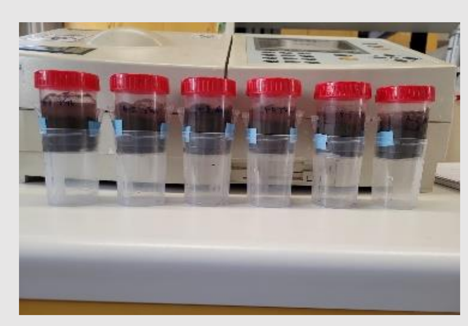
Experimental design

- Preparation of bacterial suspension of DSM 33 (OD_{600} = 5) = 10 ml
- Preparation of concrete samples = 7 g

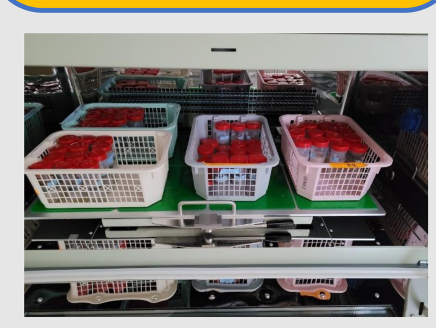
→ Mixing + adding 10 ml saline and 10 ml MICP (4 g/L Nutrient Broth, 20 g/L urea, 2.8 g/L $CaCl_2$)

→ Cultivation of samples at 28 °C, addition of 10 ml MICP solution (saline to control samples) every 48-72 hours

→ Cultivation of samples at 28 °C for defined period of time (14, 30, 60 and 90 days)



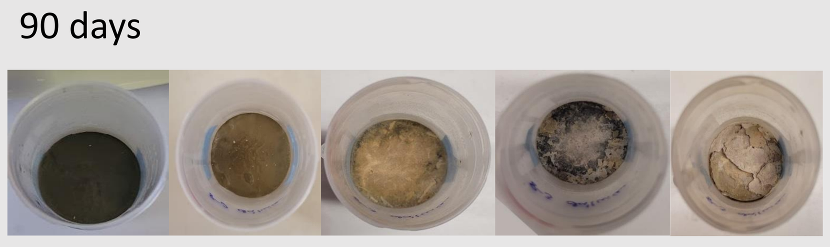
→ 30 days of drying (28 °C, without addition of MICP solution/physiological solution)



→ Composites analyses – SEM microscopy, XRD,EDS, mechanical properties

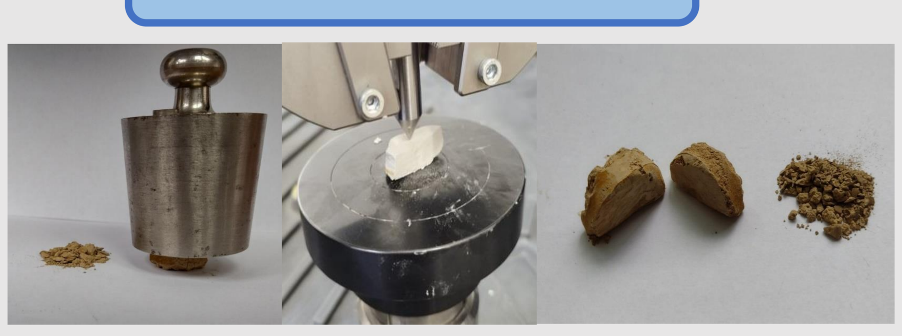
Effect of time of biocementation solution on the properties of composite samples

WCF – gutter: Composites after 7, 14, 30, 60 and 90 days



WCF – highway: Composites after 7, 14, 30, 60 and 90 days

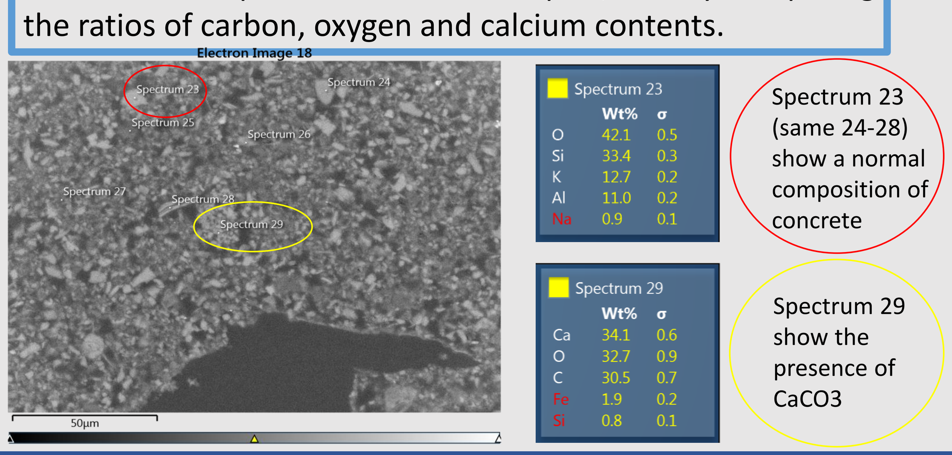
Mechanical properties



WCF – highway: Compostites prepared for testing mechanical properties (the testing is still in progress).

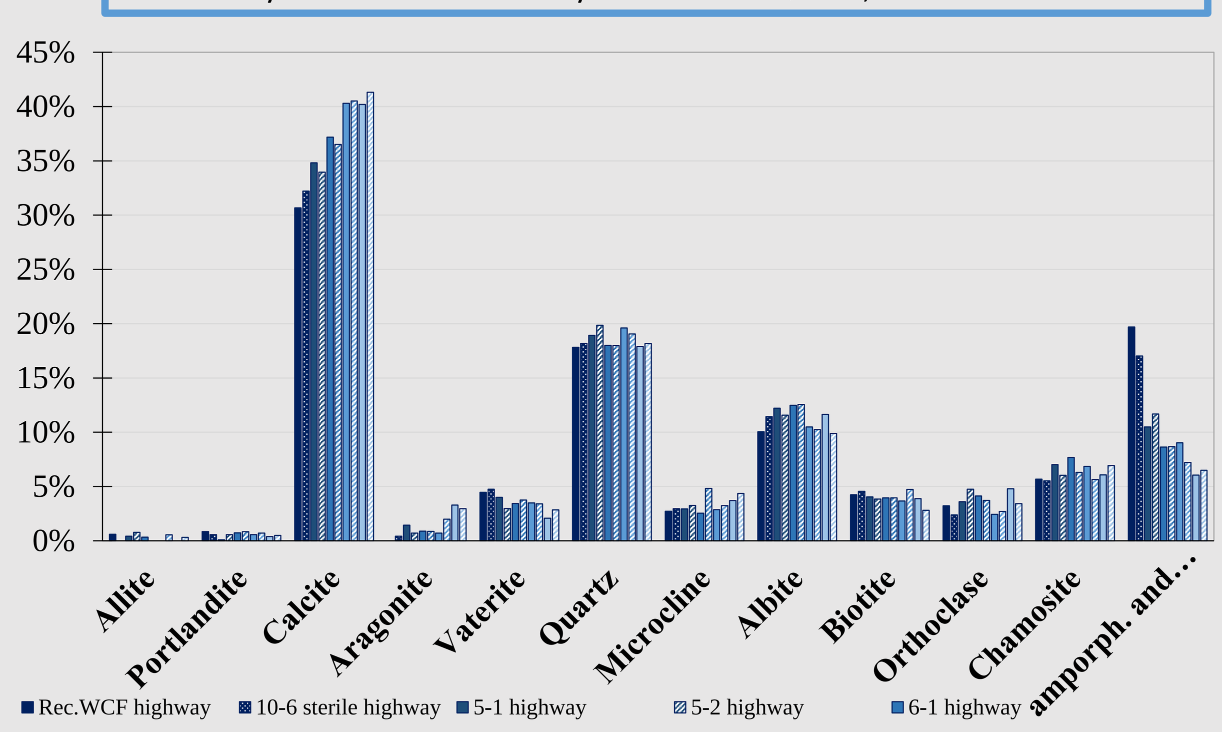
EDS analyses

The presence of $CaCO_3$ was confirmed both by EDS analysis, which was not performed on all samples, and by comparing the ratios of carbon, oxygen and calcium contents.



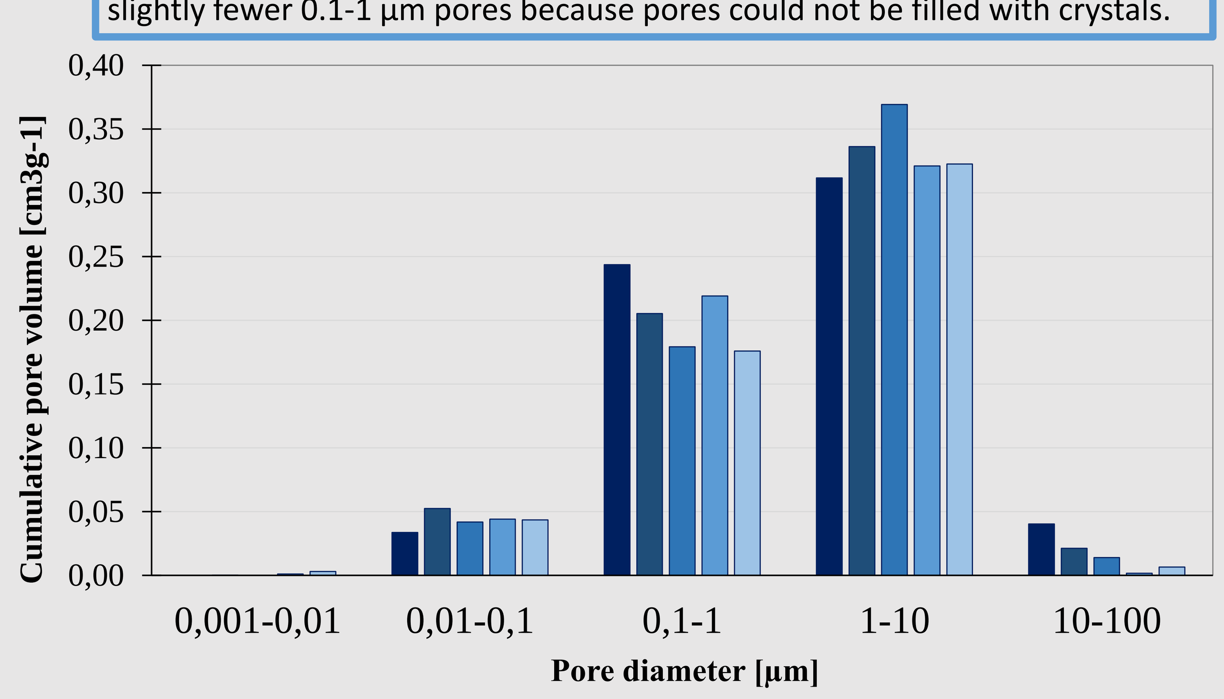
XRD analyses

An increase in $CaCO_3$ of approximately 10-12% was observed due to the effect of bacterial cells of *S. pasteurii* on WCF during the MICP process. The most frequently observed increase was in the crystalline form of calcite, followed by vaterite and other crystals such as Quartz, Albite or Muscovite.



Porosimetry

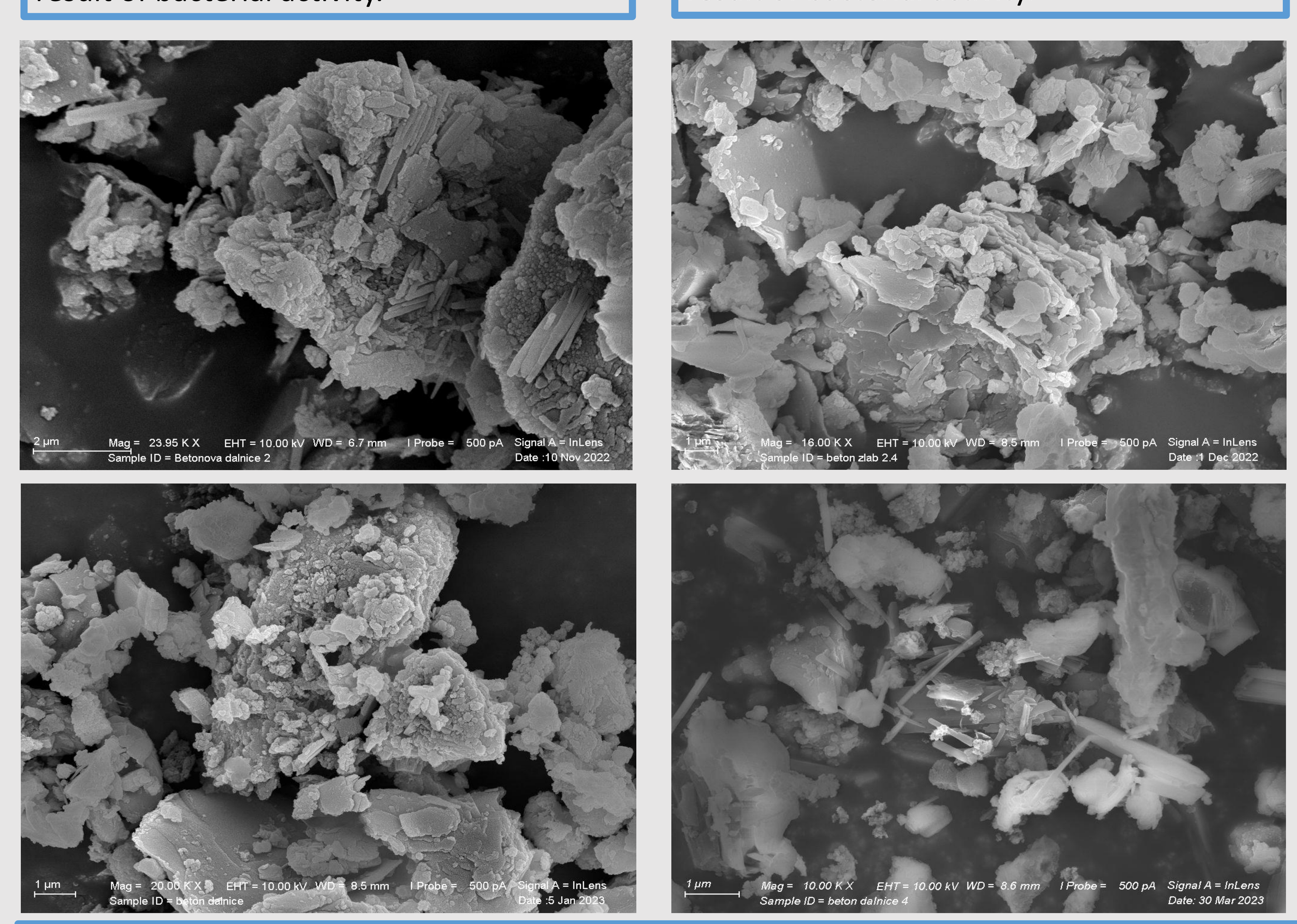
As expected, the effect of *S. pasteurii* bacterial cells on the porosity of the WCF product was observed. The formation of crystals (mainly calcite) results in the filling of gaps (10-100 µm) in the materiál, therefore these pores appeared minimal. In contrast, pores of 1-10 µm were most abundant, with slightly fewer 0.1-1 µm pores because pores could not be filled with crystals.



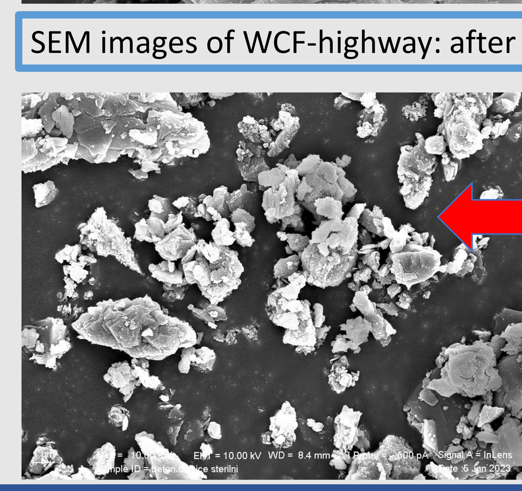
SEM microscopy

WCF-gutter grains covered with cluster of $CaCO_3$ crystals, which are formed as a result of bacterial activity.

WCF-highway grains covered with cluster of $CaCO_3$ crystals, which are formed as a result of bacterial activity.

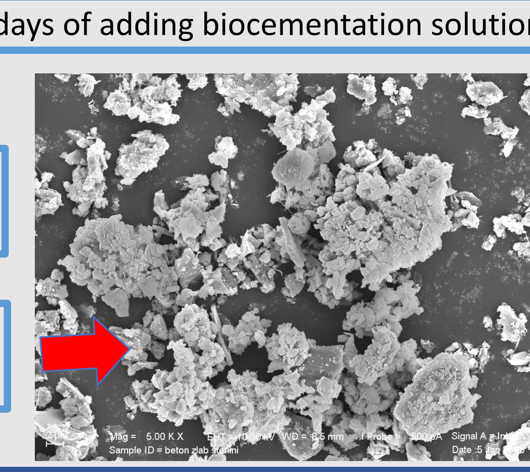


SEM images of WCF-highway: after 14, 30, 60 and 90 days of adding biocementation solution.



Sterile WCF-highway

Sterile WCF-gutter



Conclusions

Tested conditions for experiment and growth conditions for cultivation of *Sposarcina pasteurii* DSM 33:

- growth conditions: 28 °C, shaking 120 rpm, 48 hours; TSB medium supplemented with urea (final concentration 20 g/L) and $CaCl_2$ (final concentration 2.8 g/L)
- the effect of concentration of *S. pasteurii* DSM 33: more compact composites of WCF with OD_{600} of 5.0
- no significant effect on the strength, structure and quantity of the precipitated product was observed after repeated addition of bacteria
- the effect of pH adjustment on bacterial activity was observed – the optimum pH of the WCF suspension was set at 6.8±0.2

Effect of time of MICP process on the composite samples:

- the effect on the bonding of grains into a compact solid material was observed for WCF samples
- according to XRD: the amount of $CaCO_3$ polymorphs (calcite, vaterite, aragonite) increases with the length of biocementation solution addition
- porosimetry: the least pores was observed of size 10-100 µm due to the filling of the pores with the formed $CaCO_3$ crystals; smaller pores (from 0.01 up to 10 µm) are more common because they do not fill with the formed crystals
- based on SEM, EDS and XRD analyses - WCF-highway was selected as the more suitable WCF composite

Acknowledgement

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