

# EFFECT OF SUB-ZERO TEMPERATURES AND CEMENT TYPE ON VIABILITY OF *Bacillus pseudofirmus* IN BIOLOGICAL SELF-HEALING CONCRETE

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## INTRODUCTION

Microbiologically induced calcite precipitation (MICP) can be used to fill cracks arising in concrete. Alkaliphilic spore-forming bacteria, which can survive extreme pH values and harsh conditions in concrete matrix and mineral precursor compounds such as calcium lactate are needed for this purpose. The bacterial metabolic conversion of calcium lactate results in the formation of calcium carbonate crystals that fill the cracks in concrete.

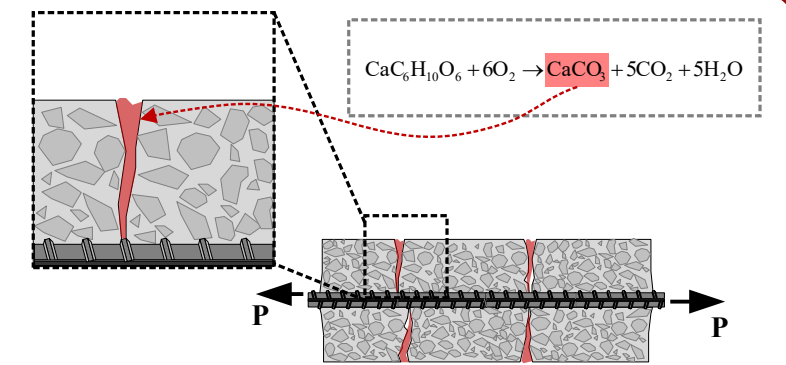


Fig. 1. Precipitation of calcium carbonate and crack healing

## OBJECTIVES

In this study, the viability of *Bacillus pseudofirmus* in sub-zero temperatures and in a concrete matrix containing several types of cement with different pH and metal ion concentrations was investigated.

## MATERIALS AND METHODS

- The sporulation of *Bacillus* bacteria was investigated using light microscopy.
- An expanded clay (EC) was used as a carrier for self-healing agent consisting of *Bacillus pseudofirmus* spores and calcium lactate.
- Concrete mix was obtained by mixing EC, cement, sand and water.
- Viability of spores was measured using standard microbiological dilution-to-extinction method by CFU counting after plating on alkaline nutrient agar.

## RESULTS

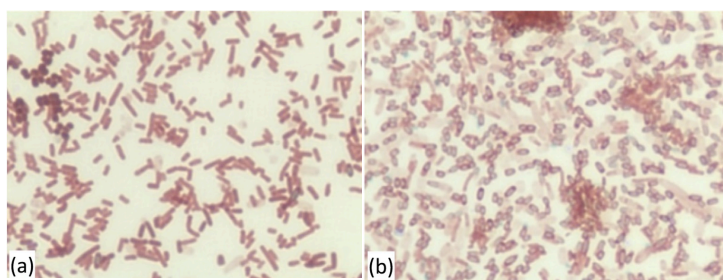


Fig. 2. *B. pseudofirmus* stained by Schaeffer-Fulton method before (a) and after (b) sporulation

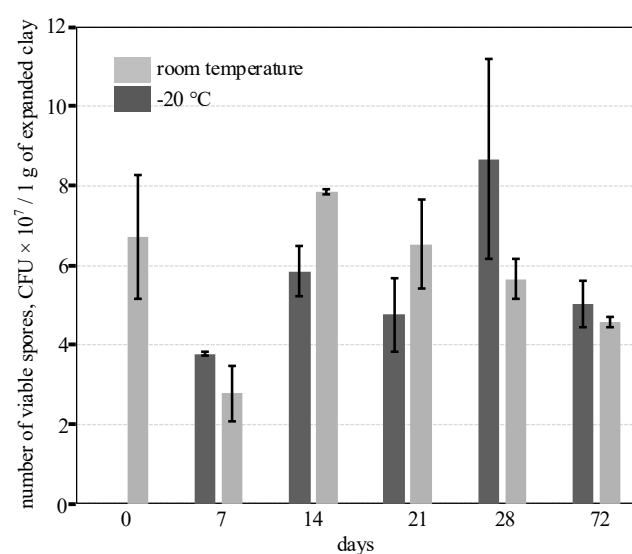


Fig. 3. Viability of *B. pseudofirmus* in sub-zero temperature

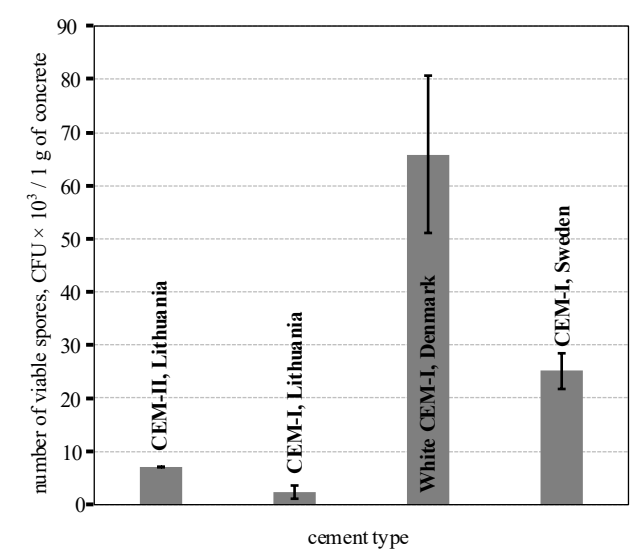


Fig. 4. Viability of *B. pseudofirmus* in concrete matrix depending on cement type

After 72 days of incubation of impregnated EC particles at -20 °C, the number of viable spores remained almost constant (~107 CFU/g of EC) compared to control EC particles stored at the room temperature. Four different brands of Portland cement were used for production of concrete specimens. The number of viable spores of *B. pseudofirmus* after three days of incubation in concrete with EC impregnation varied from 2.34 · 10<sup>3</sup> to 6.59 · 10<sup>4</sup> CFU/g of concrete. Out of four cement types commonly used in Lithuania, the best survival rate was obtained in a concrete mix using low alkali white CEM I cement.

## CONCLUSIONS

- *B. pseudofirmus* has a potential to be used in biological self-healing concrete for the Northern Europe region with high number of freeze-thaw cycles.
- Additional coating of EC aggregates is needed to improve the viability of bacteria in the concrete.

## REFERENCES

1. Jakubovskis, R.; Jankutė, A.; Urbonavičius, J.; Gribniak, V. 2020. Analysis of mechanical performance and durability of self-healing biological concrete. *Construction and Building Materials*, 260, 1-15.

## ACKNOWLEDGEMENT

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