Effect of sub-zero temperatures and cement type on viability of *Bacillus pseudofirmus* in biological self-healing concrete

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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, are needed for this purpose. 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. Expanded clay (EC) was used as a carrier for two-component self-healing agent consisting of *Bacillus pseudofirmus* spores and calcium lactate. The concrete consists of EC, cement, sand and water. Viability of endospores was measured using a standard microbiological dilution-to-extinction method by CFU counting after plating on alkaline nutrient agar. The results showed that after 72 days of incubation at temperatures below 0 °C (-20 °C), the number of viable spores remained almost constant (~10^7 CFU/g of EC) compared to control specimens stored at the room temperature. That indicates that *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. Out of four cement types commonly used in Lithuania, the best survival rate was obtained in a concrete mix using the low alkali white CEM I cement. The number of viable spores after three days of concrete curing varied from 2.34×10^3 to 6.59×10^4 CFU/g of EC. Thus, an additional coating of EC aggregates is needed to improve the viability of bacteria in the concrete.