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Short communication

In situ-immobilization of two model cyanobacterial strains in ceramic structures: A new biohybrid material for photobioreactor applications

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ABSTRACT

Two cyanobacterial strains, *Synechocystis* sp. PCC 6803 and *Nostoc punctiforme* ATCC 29133 were immobilized within magnesium phosphate based cements, showing a viability and activity for at least 4 weeks. These biohybrids are considered as an alternative photobioreactor material for bioremediation or an improved yield of biotechnologically relevant molecules.

Immobilization of cyanobacteria is a promising method to simplify biotechnological processes or to achieve an increased photosynthetic efficiency for the production of target molecules. A sustained physical entrapment of photosynthetic microorganisms within chemically and mechanically stable inorganic host structures has been realized by silica sol-gels so far, but is still posing a challenge in terms of biocompatibility of the encapsulation process. The present study aimed to utilize mineral cements as highly resistant and freely shapeable host structure for the *in situ*-immobilization of two model cyanobacterial strains, *Synechocystis* sp. PCC 6803 and *Nostoc punctiforme* ATCC 29133. The viability of cyanobacteria was evaluated by fluorescence and scanning electron microscopy after immobilization within struvite forming cements using magnesium phosphate powder and either a 1 M or 2 M $NH_4H_2PO_4$ or a 1 M or 2 M $(NH_4)_2HPO_4$ binder solution. While *Synechocystis* showed good compatibility with each binder solution, appropriate immobilization conditions for *N. punctiforme* were found at 1 M $NH_4H_2PO_4$. Under these conditions cyanobacterial cells survived the cement setting process and the fixed state for at least four weeks. These biohybrids can be considered as an alternative photobioreactor material to silica sol-gel immobilized cyanobacteria for an improved yield of biotechnologically relevant molecules, environmental remediation, and for studies of metabolism and physiology.

Cyanobacteria are photosynthetic microorganisms present in many ecological niches in all types of environments on Earth. Their versatility and photoautotrophic lifestyle have attracted increasing interest regarding their potential for use in biotechnological applications. Many model cyanobacteria are easy to engineer genetically, and their use ranges from the production of potential biofuels and fine chemicals to the removal of contaminants such as heavy metals from the environment (Savakis and Hellingwerf, 2015; Micheletti et al., 2008).

While culturing cyanobacteria in liquid cultures is suitable for many applications, in some circumstances it may be desirable to immobilize the cells within or on a structure. Immobilization of the cells may facilitate handling of cyanobacteria and may also increase their desired activity by restricting growth and thereby diverting cellular resources towards production of target molecules. Up to now, several cyanobacterial strains have been successfully immobilized in non-toxic organic or inorganic carrier systems (de-Bashan and Bashan, 2010; Dickson and Ely, 2013). Organic carrier systems are based on natural or synthetic polymers. While these materials are excellently suited for cyanobacterial immobilization due to the mild solidification conditions and hydrophilic environment, the matrices show weaknesses concerning their low mechanical and thermal stability and fast degradation under humid conditions (Léonard et al., 2010). To circumvent these disadvantages,

Abbreviations: MgPC, magnesium phosphate cement; RT, room temperature; SEM, scanning electron microscopy; M, mol/l.

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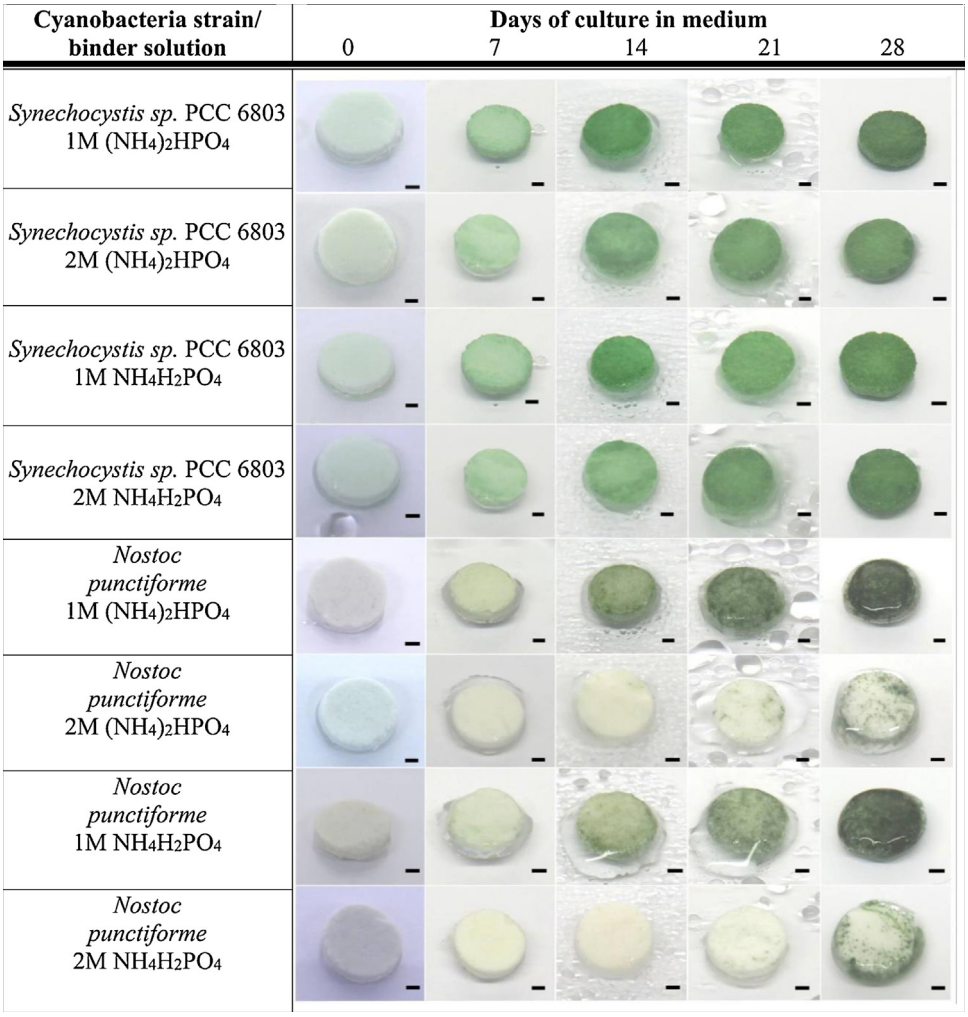

Fig. 1. *In situ*-immobilization of cyanobacteria within struvite forming cements using different binder compositions. Scale bar = 2 mm.

techniques are being developed that utilize inorganic materials based on chemically and mechanically stable silica sol-gels for entrapment or post-coating of polymer encapsulated cyanobacteria (Desmet et al., 2014; Ramachandran et al., 2009). Compared to the swellable and flexible network of polymers, silica provides a stiff hydrophilic and nanoporous matrix which allows nutrient and metabolite exchange as well as maintaining the physical fixation of the microorganisms. The successful encapsulation of photosynthetic microorganisms within silicagels with a sustained viability of several weeks is documented in current literature (Rooke et al., 2008; Dickson and Ely, 2011). Nevertheless, the synthesis of silica immobilized cyanobacteria is still challenging, particularly regarding the maintenance of microorganism viability over a prolonged time. The synthesis conditions of alkoxide-derived silicagels leading to reduced cell activity and cell lysis (Dickson and Ely, 2013). Gels produced from aqueous precursors poses problems as well, originating from nanoparticular silicon uptake or the tight confinement of the cyanobacteria within the silica-gel matrix (Rooke et al., 2008).

The current study investigates the suitability of porous magnesium phosphate cement (MgPC) structures for serving as host material for model cyanobacterial strains, with the focus on the biocompatibility of the synthesis route, the microstructure of the cement matrix, and the viability of cyanobacteria in the confined mineral environment.

Characteristics of MgPC are the rapid setting reaction (≤6min) (Kanter et al., 2014) and the high early strength, which provoked their utilization in civil engineering and has recently aroused interest of materials scientists as biomaterial for clinical applications (Mestres and Ginebra, 2011). With respect to the biocompatibility, mechanical properties with compressive strengths of 30–66 MPa and stiffness of about 20,000 MPa mm as well as the sub-micrometer pore structure (mean pore size <1 μm) with an overall porosity of (5–7) % combined with mild setting conditions (pH 5–8, RT) (Kanter et al., 2014; Mestres and Ginebra, 2011), MgPC seems to be a suitable host structure for biological entities. The cement solidification is based on struvite formation in an acid-base reaction between MgPC powder and an ammonium phosphate solution according to following equations:

$$2Mg_3(PO_4)_2 + 3(NH_4)_2HPO_4 + 36H_2O \rightarrow 6NH_4MgPO_4{\cdot}6H_2O + H_3PO_4 \quad (1)$$

$$Mg_3(PO_4)_2 + 3NH_4H_2PO_4 + 18H_2O \rightarrow 3NH_4MgPO_4{\cdot}6H_2O + 2H_3PO_4 \quad (2)$$

Discs of this material with a thickness of 2 mm were found to have a translucency of 12%, which increased to 20% in aqueous environment as a consequence of proceeding setting reaction (see Supplementary file). Accordingly, the incorporation of photoactive microorganisms was assumed to be feasible.

For silica sol-gel encapsulation several detrimental stressors on viability and photoactivity of cyanobacteria are known, comprising contact with alcohols or salts, and the sensitivity to these factors differs for various cyanobacterial strains (Dickson and Ely, 2013). In the present study, we tested two different cyanobacterial strains