MICROALGAE BIOMINERALIZATION TO VALORIZE STONE EXTRACTION LEFTOVERS

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Keywords: microalgae, biomineralization, stone leftovers, biodesign

1. Introduction

Biomineralization is a natural process performed by numerous and different organisms which produce mineralized structures. The most abundant biogenic minerals found in nature are calcium carbonate-based (Nudelman and Sommerdiijk, 2012) and microorganisms are well known to carry out a process called microbially induced calcium precipitation (MICP) in various environments (Chin et al.,2020). MICP can be performed by several microbial groups through different metabolic pathways like photosynthesis, urea hydrolysis, denitrification and sulphate reduction.
The most studied pathway for MICP is urea hydrolysis, which is also exploited to produce new materials. Between the microorganism capable of carrying out this process *Sporosarcina pasteuri* is the most renowned (Dhami et al., 2013). As an alternative to this pathway, induction of calcium carbonate precipitation by phototrophic microorganisms has also been studied. In particular, cyanobacteria have received most of the interest, especially those belonging to the genera *Synechococcus* and *Synechocystis*.
The aim of this work was to evaluate the capacity of selected microalgal strains to induce $CaCO_3$ precipitation through the photosynthetic pathway. Several microalgae, most of which belonging to cyanobacteria, were screened to identify the best strains in performing this process, with the objective to use them in the production of new biomaterials based on stone leftovers. This work was conducted with a multidisciplinary approach, involving microbiologists and designers.

1. Materials and methods

During the screening nine microalgal strains have been tested in order to verify their ability to induce calcium precipitation. The strains have been selected based on literature data on ability to perform MICP, ability for polysaccharide production and natural habitat of origin (e.g., selecting those known to host microorganisms with this metabolic capacity).

Table 1. Microalgae tested through the screening and standard culture medium

Genus/Species	Phylum	Culture medium
Synechococcus elongatus	Cyanobacteria	Freshwater
Synechococcus sp.	Cyanobacteria	Freshwater
Synechococcus sp.	Cyanobacteria	Marine
Synechocystis sp.	Cyanobacteria	Freshwater
Chroococcus minutus	Cyanobacteria	Freshwater
Chroococcus sp.	Cyanobacteria	Freshwater
Leptolyngbya sp.	Cyanobacteria	Freshwater
Nostoc sp.	Cyanobacteria	Freshwater
Porphyridium purpureum	Rhodophyta	Marine

The experiment was carried out in 100 ml flasks placed on an orbital shaker under continuous illumination. To maximize environmental condition uniformity, flask positions were interchanged daily. Microalgae were cultivated on a modified medium obtained from a standard medium base and enriched with Ca^{2+} and $NaHCO_3$ (test medium) to induce calcium carbonate precipitation. Standard medium was used as control. For each of these treatments, three replicates were set. Two flasks containing only enriched medium were used in the experiment to estimate precipitation in the absence of microalgae. Nutrients were added at need during the culture, while $NaHCO_3$ was added once during the trial.
Samples were collected periodically to follow the growth of the control and the test cultures. Growth of the cultures was evaluated through both dry weight determination and chlorophyll concentration analysis. At the end of the experiment samples of the culture medium were collected, after centrifugation to separate the cells, to analyze calcium content. Conductivity and pH in the culture medium were also measured periodically. The formation of crystals in the culture and the precipitation in the enriched medium without microalgae was observed by optical microscopy.
Besides measurement to evaluate biomineralization, polysaccharide production was also evaluated. Microalgae were cultivated in 100 ml flasks on standard medium in an orbital shaking incubator with CO_2 enriched atmosphere, at 28 °C under continuous illumination. After ten days the cultures were harvested and the medium was separated from the pellet by centrifugation. Polysaccharide released in the medium was estimated after extraction with ethanol and quantified by the phenol-sulphuric acid method.

2. Results

The screening showed that microalgae are able to grow on a Calcium and bicarbonate-enriched medium like. In most cases, culture in the enriched medium showed a higher growth with respect to the control cultures. The observations at the optical microscope showed that there was crystal formation, probably calcite, in the cultures grown in the enriched medium but not in the standard medium. Preliminary analysis showed that there was a significant reduction of Ca^{2+} concentration in the exhausted medium at the end of the trial for the test cultures.
Polysaccharide analysis showed that three out of the nine microalgae selected for the screening released a significant amount of polysaccharides in the medium.
Preliminary tests on stone extraction leftovers are ongoing.

3. Final remarks

Some of the screened microalgal strains showed a good potential for biomineralization through the photosynthetic pathway, although further research must be conducted to improve calcium carbonate precipitation and optimize the process.
Polysaccharides represent a nucleation site for calcium precipitation, it is worth looking more in depth into their characteristics and amount. Moreover, the ability to produce polysaccharides is also of interest for the realization of the new materials based on stone leftovers, as polysaccharides can work as a binding substance for the inert particles. Future work for new material realization will also evaluate this aspect.

4. Acknowledgments

Project INERTIAL is funded by EU-NextGenerationEU and by Cassa di Risparmio di Firenze.

5. References

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