Cyanobacteria biomineralized material for offshore applications

Master thesis

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April 24th, 2024

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Master Thesis
MSc. Integrated Product Design

24-4-2024

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Executive Summary

Cyanobacteria are photosynthetic microorganisms found in a variety of environments, including marine water bodies. Some species are able to perform biomineralization, producing minerals such as calcium carbonate ($CaCO_3$) that may act as biocement. The biomineralization capability of cyanobacteria has already been explored in the development of Living Building Materials (LBMs), composed of an inert scaffold of sand and hydrogel, that contributes to CO_2 capture.

The offshore industry is a significant user of concrete, contributing to 11% of the global CO_2 emissions. Therefore, the application of cyanobacteria biomineralized materials is envisioned as a way to reduce CO_2 emissions in this sector.

The cyanobacteria biomineralized material has been studied in the fields of construction and is applied in 3D printing, but its potential for offshore applications still needs to be explored.

This study aims to explores the proprieties of cyanobacteria biomineralized materials to catter some requirements of offshore applications, thereby contributing to a more sustainable practice. This was done by first recreating the material from other studies, assessing its mechanical proprieties and testing it in underwater conditions.

This was followed by a tinkering process where material qualities (e.g., cyanobacteria optical density (OD), biomineralization/curing time, type of hydrogel, aditional coating) were adjusted to fullfill its purposes.

The impact of these changes were measured through submerging the material in seawater and assessing its mechanical proprieties.

The use of cyanobacteria at an OD of 2.4, resulted in the strongest material. Using agar as an hydrogel binder, countered the dissolvability of the material with a gelatine binder. On the other hand mechanical tests showed that the agar bonded material was significantly weaker than the gelatine bonded material. Adding a silicone rubber coating to the gelatine bonded material did not make the material resistant to seawater. A prolonged biomineralization time improved the strength of the material significantly but more exploration is required to determine if the biomineralization time is the result of this strength or the adjusted sand/medium ratio.

Overall, this study demonstrated that cyanobacteria biomineralized materials can be applied in offshore applications since the right cyanobacteria OD, binder, coating and biomineralization time are employed.

Glossary

LBM
Living Building Material. A material that contains a living organism that is used in the building industry

Biomineralization
The production of minerals with $CaCO_3$ as the most common one, that are deposited in the matrix of living organisms (Hu et al., 2011)

Synechococcus sp. PCC 7002
A strain of cyanobacteria that is used to make the material

Design space
A design process based on focussing on all the complex multi-dimensional different design solutions, on a certain topic (the space) (Westerlund, 2005)

Bacterial culture
Bacteria grown for scientific purposes, or the activity of breeding and keeping particular living things in order to get the substances they produce (Culture, 2024)

Demi water
Also known as demineralized water or distilled water, this is water of which the salts and minerals are removed (Demi Water - Lenntech, n.d.)

Reculturing
Adding more medium or making new flasks with medium to allow for more cyanobacterial growth

Inoculation
Adding the cyanobacteria to a suitable situation for growth

Table of contents

Chapter 1

Project Description

Content

Introduction

In this chapter the project will be introduced and the context of the subject will be explained. The knowledge gaps in this field will be explored and the goal of this project will be made clear.

1.1 Introduction

The building sector is responsible for 39% of global carbon dioxide emissions, with concrete use contributing to 11% of those emissions, being therefore a big contributor to climate change (Concrete needs to lose its colossal carbon footprint, 2021; Embodied Carbon Actions – Architecture 2030, 2022). Concrete is commonly used in the offshore industry, in the construction of oil rigs (e.g., for only 44 oil platforms, 2.966.357 m^3 concrete is used (Skinner, 2023; Fernandes et al., 2008)), bridges, and tunnels. Structures in the offshore industry often need to be attached to the seabed. Bridges for example have piers, they support the bridge in the seawater, the most common ways to place them are with caissons, cofferdams, or driven piles (Obinna, 2023). All these construction methods use, or allow for the use of, reinforced concrete, and are therefore very impactful on the environment.

It is estimated that the global demand for concrete will increase up to 23% by 2050 (Embodied Carbon Actions – Architecture 2030, 2022). To limit further global warming to +2^{o}C by 2050, a significant reduction of Co_2 emissions by 24% is required (Reinhardt et al., 2023). Therefore, there is a need for new innovative technologies that enable the reuse of existing materials (Tam, 2008; Noguchi et al., 2011), as well as materials that help reduce carbon emission (Technology Roadmap - Low-Carbon Transition in the Cement Industry – Analysis - IEA, 2018; Concrete needs to lose its colossal carbon footprint, 2021). In this way, the development of a new material for the offshore industry that could replace the impactful concrete could significantly contribute to reducing pollution in the offshore sector.

Cyanobacteria can help modernise the offshore industry. These bacteria can be found in several environments, including water bodies, and are considered one of the oldest organisms on earth with fossil records dating 2.8 billion years ago. Cyanobacteria perform photosynthesis, and as a byproduct they emit O_2, being therefore considered the initiators of life on earth (Tetsch, 2016).

Cyanobacteria can also biomineralize, this means that they can produce minerals such as calcium carbonate ($CaCO_3$). This capability has been used to design materials that capture CO_2 (Beatty et al., 2022). If a medium with nutrients, cyanobacteria such as *Synechococcus* sp. PCC 7002, sand and a hydrogel are combined, biomineralization reactions can create a cement-like material (Armaly et al., 2023; Delesky et al., 2023; Heveran et al., 2020; Qui et al., 2021; Reinhardt et al., 2023). This Living Building Material (LBM) can convert CO_2 to O_2 through photosynthesis, meanwhile it also captures CO_2 through biomineralization. Both these characteristics of the LBM can lower the CO_2 percentages in the air. If next to this the sand that is used is obtained from local areas and this material is applied for offshore purposes, the industry will be less impactful on the environment.

1.2 Problem definition

Cyanobacteria biomineralization has been studied in the fields of construction (Delesky et al., 2023; Heveran et al., 2020; Jang et al., 2023; Qui et al., 2021) and is applied in 3D printing (Reinhardt et al., 2023; Armaly et al., 2023), still the development of a cyanobacterial biomineralized living material for offshore applications needs to be explored.

For this thesis the main research questions will be:

- Can we design a cyanobacteria biomineralized material for offshore applications?
- Can the material stay alive when applied in simulated offshore conditions?
- What are the mechanical properties of the designed material?
- How can we tune material properties to fit offshore applications?

1.3 Goal

Cyanobacteria's biomineralizing capability has been explored for the development of a living building material (LBM) with possible applications within the construction environment (bioconcrete) (Heveran et al., 2020). This biomaterial, composed of an inert structural scaffold of sand and a hydrogel, structurally supports living cyanobacteria, that mineralizes and toughens the hydrogel. The current research has so far focused on the necessary conditions for the development of this material, still its possible applications and/or further development for offshore applications remains to be explored.

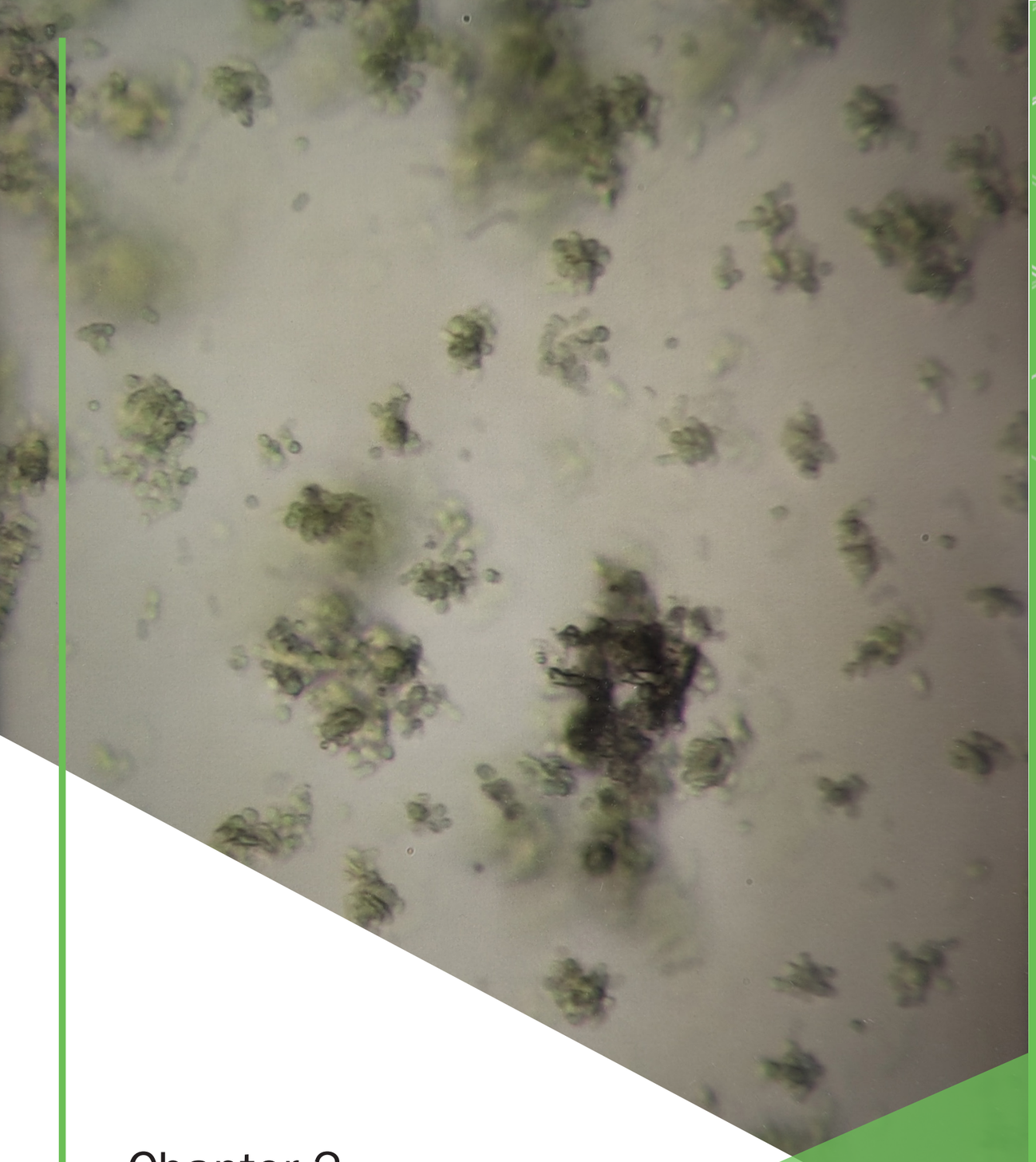
The goal of this thesis is to design a material that caters to the requirements of offshore applications, thereby contributing to a more sustainable practice.

1.4 Approach

The deliverables for this thesis are a report with a design space and showcase in the shape of a datasheet with ways on how to make the material for each intended application together with different variations of test cubes, and a poster.

In this thesis, the following stages were executed: "Research", "Development, experiment and lab work", "Demonstrating and documenting".

The "Research" stage provided the knowledge of the existing information on the topic through literature research to be able to make test setups in the lab. Then, the "Development, experiment and lab work" stage followed with first replicating the living building material, testing its water resistibility and later developing the material for offshore applications and processing of the data. This stage needed a lot of time for iteration, because experiments in the lab are never linear. The last stage was "Demonstrating and documenting", where the final datasheet and design space were made.



Chapter 2

Cyanobacteria and biomineralization

Content

Introduction

This chapter dives into the bacterium that is used for the making of the building material, it will explain the origin of the cyanobacteria, its habitat and its capabilities. The relation of those capabilities to the building material will also be elaborated on.

2.1 Introduction

Cyanobacteria, commonly known as blue-green algae, were selected for the development of the material (see image 2).

These organisms can be found in a variety of environments including water bodies like oceans, lakes or rivers. Fossil records of 2.8 billion years have been found containing cyanobacteria (Reinhardt et al., 2023). Cyanobacteria perform photosynthesis and as a byproduct they emit O_2, because of this they are considered the initiators of life on earth (Tetsch, 2016).

Cyanobacteria are part of the bacteria family (see image 1). There are many different types of cyanobacteria.

For this thesis the cyanobacterium *Synechococcus* sp. PCC 7002 was selected. This cyanobacterium is part of the Chroococcales order, see image 1 (Schaap, 2015). *Synechococcus* sp. PCC 7002 ranges from 0.4 µm to 6 µm and is a marine/euryhaline unicellular cyanobacterium, capable of thriving across a broad spectrum of NaCl concentrations, with exceptional tolerance to high-light irradiation (Schaap, 2015; Ludwig & Bryant, 2012). Its optimal growth temperature is 38^{O}C (Schaap, 2015).

This strain of cyanobacteria is proven to be able to biomineralize (Sidhu et al., 2022), leading to the production of minerals such as $CaCO_3$. *Synechococcus* sp. PCC 7002 does this with the help of photosynthesis (Zhu & Dittrich, 2016), contributing to the capture of CO_2 in materials (Beatty et al., 2022).

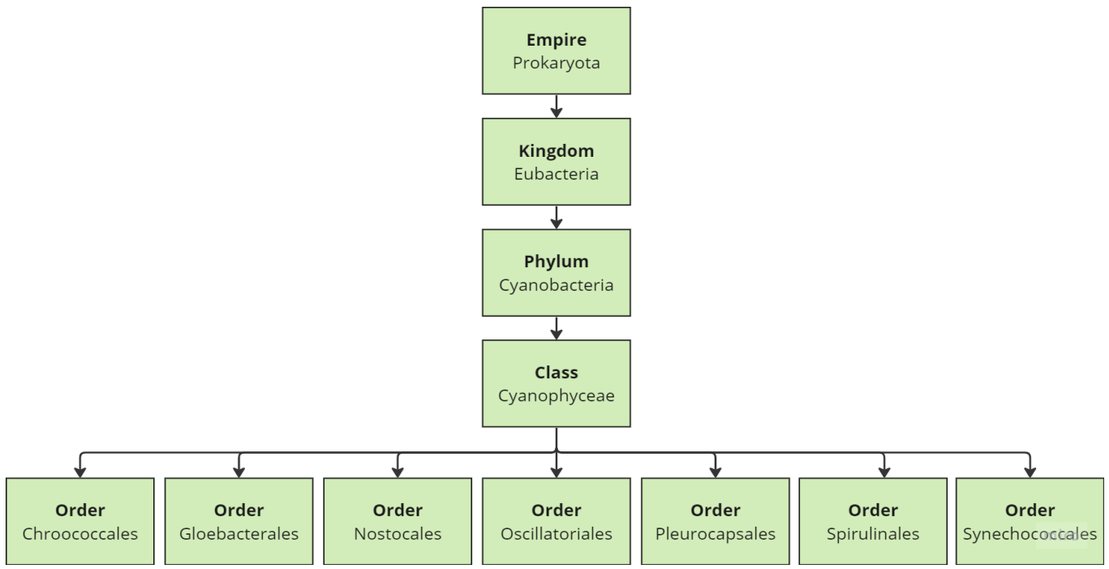


Image 1, Cyanobacteria classification (Allaf & Peerhossaini, 2022)

2.2 Advantages of cyanobacteria

The development of cyanobacterial biomineralized materials offers several advantages. Biomineralization through photosynthesis has the potential to sequester CO_2 during cell growth and the biomineralization process. As cells grow, assimilated carbon is used to produce energy and biomass, whereas additional CO_2 is permanently fixed in biomineral form. In addition, the application of the photosynthetic pathways for biomineralization, does not release environmental pollutants, as other pathways may do (Beatty et al., 2022).

The use of cyanobacteria also makes it possible to eventually make the material living, which could open doors to make it self-healing or reactive to environmental conditions.

2.3 Potential risks of cyanobacteria

The cyanobacterium *Synechococcus* sp. PCC 7002 lives in water bodies and is free living. It is described as non-pathogenic, being therefore considered biosafety level 1 (*Synechococcus* Sp., n.d.).

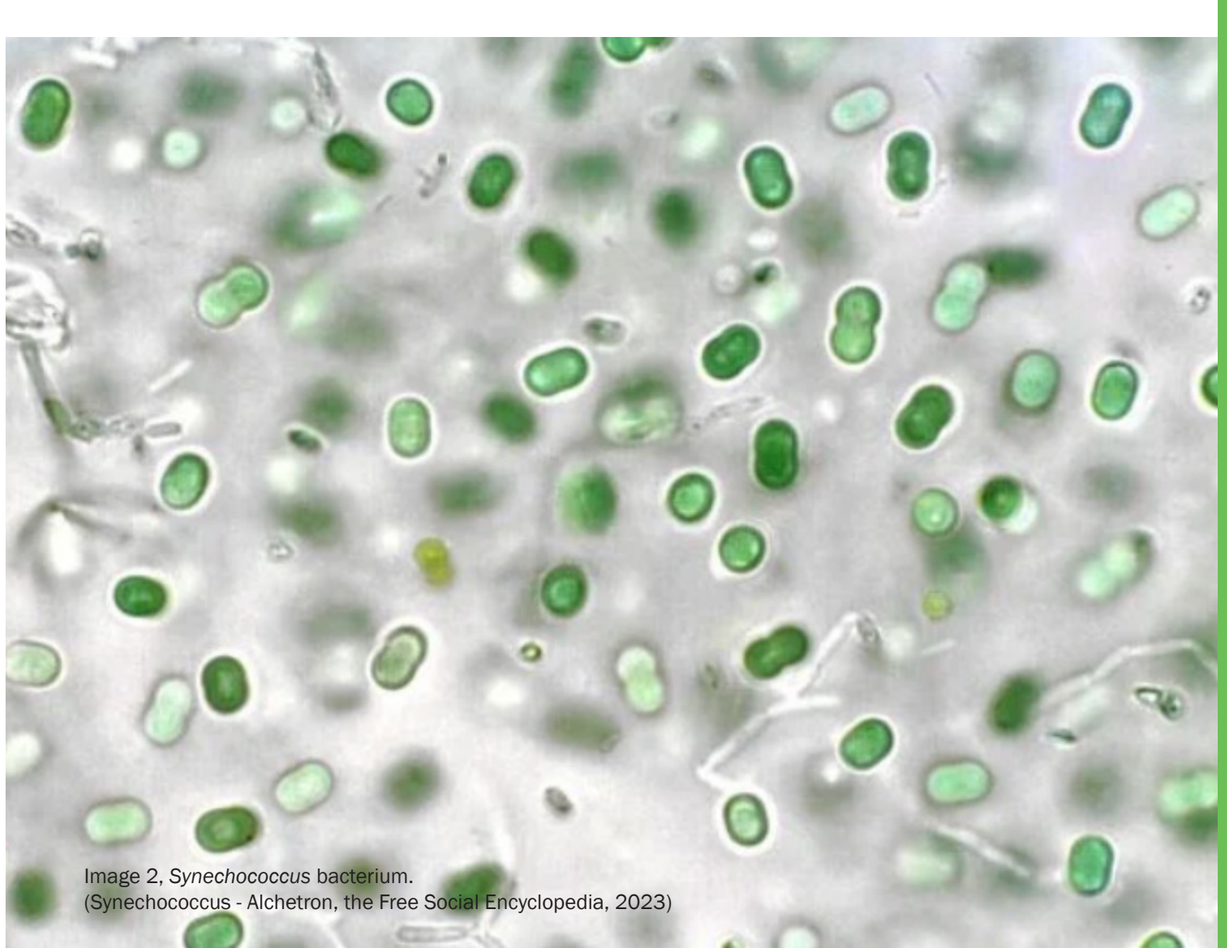


Image 2, *Synechococcus* bacterium.
(Synechococcus - Alchetron, the Free Social Encyclopedia, 2023)

2.4 Photosynthesis and biomineralization

Biomineralization is the process where minerals are formed from the environment through the natural process of living organisms. Several pathways can lead to biomineralization, photosynthesis being the one that occurs in cyanobacteria (Beatty et al., 2022).

Photosynthesis and associated CO_2 capture, leads to the formation of $CaCO_3$ crystals because of an exchange of HCO_3- and OH- across the cell membrane (Miller and Colman, 1980). Cyanobacteria use HCO_3- as a carbon source, that gets into the cell and follows a chain of reactions which leads to the release of OH- out of the cell (see image 3) (Zhu & Dittrich, 2016). Although this process is not yet very well understood, it leads to an alkalinization of the microenvironment of the cell. This higher pH leads to the increase of the speed of the calcification reaction (Jiang et al., 2013; Chegg, n.d.). Additionally, Ca^{2+} accumulates at the cell surface, leading to the eventual formation of $CaCO_3$ (see image 3) (Beatty et al., 2022).

When calcium chloride ($CaCl_2*2H_2O$) and sodium bicarbonate ($NaHCO_3$) are added to the environment of the cell, there is enough HCO_3- for the cyanobacteria to absorb and start the reactions to increase the pH. Because of the added $CaCl_2*2H_2O$ there is also enough Ca^{2+} in the environment for the calcification reaction to form $CaCO_3$ crystals.

2.5 Conclusion

For this thesis the cyanobacterium *Synechococcus* sp. PCC 7002 is used. This is a bacterium that can biomineralize with the help of photosynthesis. The biomineralization is essential to develop the material. To initiate biomineralization, the nutrients $CaCl_2*2H_2O$ and $NaHCO_3$ need to be accessible for the cyanobacteria.

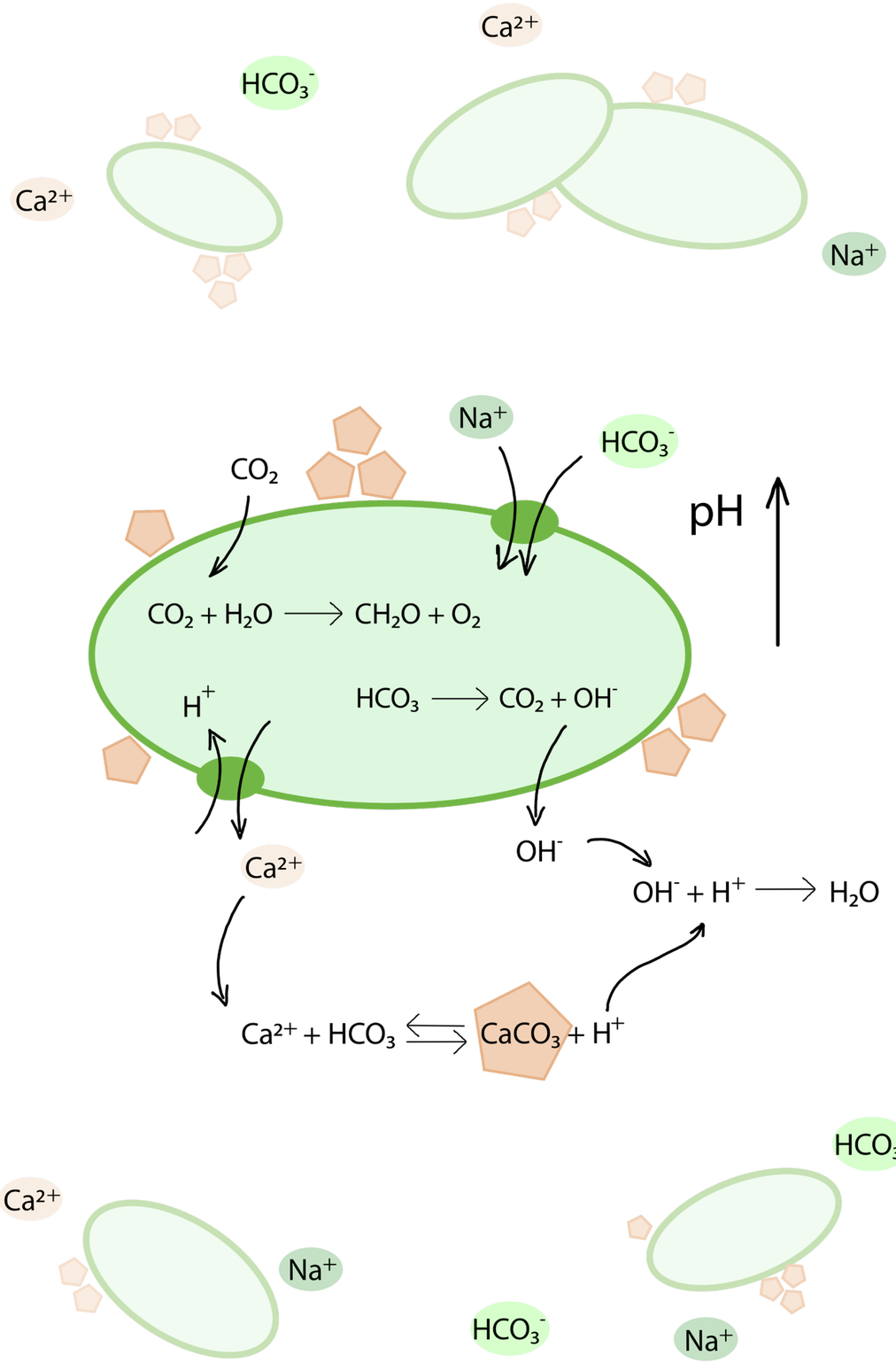


Image 3, Biomineralization through photosynthesis
Adapted from (Beatty et al., 2022; Chegg, n.d.; Zhu & Dittrich, 2016)

Chapter 3

Engineering with living materials

Content

Introduction

In this chapter Biodesign and engineering with living materials will be introduced, together with its challenges and examples.

3.1 Biodesign

Biodesign
Biodesign can be defined as the incorporation of living organisms or ecosystems as essential components in design, thereby enhancing the function of the end product (Myers, 2012). This means that Biodesign can create products that have living parts and designs that have features that are based on nature's processes or shapes. One of the goals of Biodesign is to change the linear life of products to a more sustainable and recyclable life (Parakul, 2021).

Living Building Materials
Living Building Materials, also called LBM's, are materials that are used for construction or industrial design and contain a living component. This living component adds beneficial qualities to the material, or it makes the material more sustainable. LBM's are considered environmentally friendly because of their minimal greenhouse gas emissions during manufacturing (Jang et al., 2023).

3.2 Product examples

An example of Biodesign and designing with living (building) materials is the project CyanoFabbrica by Cinzia Ferrari (Cyanobacteria by Cinzia Ferrari – Future Materials Bank, n.d.), who created glasses that have a frame made of a living building material, through the use of biomineralization by cyanobacteria (see image 4). The sunglasses sector has to do with greenwashing, this project was created to initiate new investigations and conversations about innovation in this sector (Cyanobacteria by Cinzia Ferrari – Future Materials Bank, n.d.). The used living material is sustainable and can be reused after its use.

Another example of using living (building) materials is the living concrete in the Artis zoo (Levend Beton Verduurzaamt ARTIS-Aquarium, 2023). The wall of the aquarium was seriously compromised because of the salt water in the aquarium. The zoo now replaced its concrete with bio concrete, that has the ability to heal itself and therefore doubling the lifespan of the concrete (see image 5) (Levend Beton Verduurzaamt ARTIS-Aquarium, 2023).

Image 4, CyanoFabbrica (Cyanobacteria by Cinzia Ferrari – Future Materials Bank, n.d.)

3.3 Challenges

Engineering with Biodesign and living building materials brings some challenges with it. One of the biggest challenges is to keep the material alive during its use phase, if that is the purpose. For some applications the material or product does not need to be kept alive, but for some others the used organism needs to be alive to give the material or product certain features. Because the organisms need specific conditions to be alive (e.g., water, nutrients, temperature, etc.), it is crucial that the final material facilitates those. For example, certain humidity levels may need to be maintained, or access to certain nutrients needs to be provided or there is the need to have access to light. Providing these conditions can be a challenge in some applications.

Next to this is, and in particular with cyanobacteria biomineralized materials, is that those are still in their early development, and therefore not much is known about it, meaning that many aspects still need to be discovered and/or tested. This slows down the process of applying the material in construction or products. However, extensive research is currently underway, and the field of Biodesign is rapidly rising.

3.4 Conclusion

Biodesign and engineering with living (building) materials is designing/engineering products or materials through the use of living components. This gives the material or product beneficial qualities like self-healing or being more sustainable. This thesis investigates the use of a cyanobacterial biomineralized material for offshore applications with the aim to contribute towards sustainability.

Image 5, Bio concrete in aquarium of Artis (Levend Beton Verduurzaamt ARTIS-Aquarium, 2023).

Chapter 4

Material introduction

Content

Introduction

This chapter dives into the development of the cyanobacterial biomineralized material, including its necessary components and preparation methods. The growing conditions of the cyanobacteria will be explained, together with the material components for its production.

4.1 Introduction to the cyanobacterial biomineralized material

Living Building Materials (LBM), made from cyanobacterial biomineralization have been previously developed (Heveran et al., 2020). These were able to successive regenerate itself up to three viable generations from one parent generation. For that cyanobacteria, specific nutrients, river sand, and gelatine (which acts as a binder) were applied (Heveran et al., 2020). The developed cyanobacterial material, at its mass equilibrium, was stronger than the control group which had no cyanobacteria. In addition, the material was able to maintain cyanobacteria alive up until 30 days, as long as sufficient humidity conditions were provided (Heveran et al., 2020).

4.2 Cyanobacteria culturing

Before the material can be made the cyanobacteria need to be grown. In this study the cyanobacterium *Synechococcus* sp. PCC 7002 was grown based on previously described protocols (e.g., Heveran et al., 2020). For its growth, a suitable medium, providing the required nutrients, was needed. In this research cyanobacteria were grown in liquid nutrient medium A+mod. All the components in table 1 were added to 800 ml of demi water. The medium was then filter sterilized with a 0.2 µm filter, to remove any possible contaminations and allow for cyanobacteria growth.

Once the growth medium A+mod was prepared, the cyanobacteria were added to the flasks and placed in an incubator with the following settings: day/night rhythm (16:8h), 22^{o}C, on a shaker ≈ 130 rpm. The cyanobacteria were re-cultured approximately every week.

Synechococcus sp. PCC 7002 grows by duplicating itself. The culture growth and “health” can be judged by the green colour. The darker green flasks with cyanobacteria and medium are the ones with healthier cultures, due to the presence of chlorophyl (see image 6). A colour change to a yellowish colour may be an indication of culture stress, indicating a less healthy state (see image 7).

Temperature
Biomineralization studies have been applying different temperatures for the growth and maintenance of cyanobacteria, from 22^{o}C to 37^{o}C (Heveran et al., 2020; Reinhardt et al., 2023; Jang et al., 2023; Armaly et al., 2023). In the biolab at IDE TU Delft, this strain has been maintained at 22^{o}C, and therefore this temperature was chosen for this study.

Shaker
It is ideal for *Synechococcus* sp. PCC 7002 to be placed on a shaker. This allows the cyanobacteria to get better access to all the nutrients in the medium. If they stand still, they also tend to move and stick to the bottom of the flask.

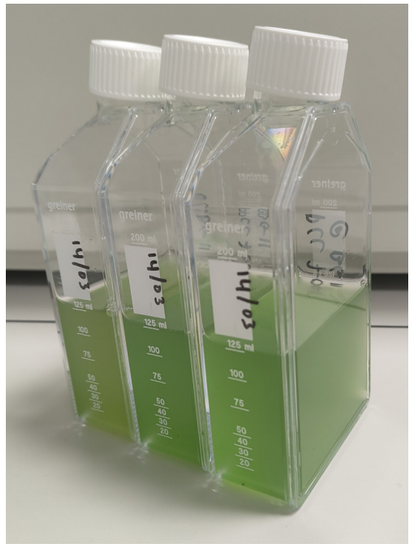


Image 6, Healthy cyanobacteria culture

Image 7, Unhealthy cyanobacteria culture

(filtersterilized 0.2 µm)	A+mod medium Gr/0.8L	ALS medium Gr/0.8L
Monopotassium phosphate	0.04	0.04
Magnesium sulfate	4	4
Sodium nitrate	0.8	0.8
Ferric ammonium citrate & EDTA disodium magnesium	0.005 & 0.001	0.005 & 0.001
Potassium chloride	0.48	0.48
Tric HCl	0.8	0.8
Sodium chloride	14.4	-
Biomineralization components		
Calcium chloride	0.296	11.76
Sodium bicarbonate	-	6.72

Table 1, Medium ingredients

4.3 Components of the cyanobacterial biomineralized material

To develop the current material three main components were needed: cyanobacteria in medium, sand and gelatine (see image 9).

Cyanobacteria
Once the cyanobacteria are grown in their growing medium A+mod, they need to be transferred to a medium where they can biomineralize. This medium is called A+ low salt (ALS) medium. It is different from the A+mod medium because it contains more calcium chloride ($CaCl_2$) and it contains Sodium bicarbonate ($NaHCO_3$) instead of sodium chloride (NaCl), see table 1. The cyanobacteria have to be in that medium for at least 10 hours to allow for enough biomineralization. After 10 hours they can be combined with the sand to develop the cyanobacterial biomineralized material.

Sand
Three sand types were considered for this study; Gamma ophoogzand, beach sand from Wemeldinge (Zeeland; 51.521251, 3.999638), and seabed sand. The particle size that is needed for the material is 0.25 – 1 mm, as previously described (Armaly et al., 2023). All sand types were observed under the microscope, see image 10. The Gamma sand did not have enough sand particles of the right size and was a completely different sand type than used in other studies, so it was not selected for further experiments. The beach and seabed sand looked roughly the same. The beach sand was selected to work with because it was easier to obtain and corresponds the most with the sand that was previously used in other studies (Heveran et al., 2020; Armaly et al., 2023). In addition, it contributes to a more sustainable approach, since it is local sand and this research aims the developing of a material envisioning offshore applications (see image 8). After collection, the sand was taken to the lab and dried in an oven at 60^{O}C for 19 hours because it needed to be fully dry to be able to be sieved.

Once the sand had the right particle size, it needed to be cleaned, since it still has organic material like bacteria, fungi, and shells. The cleaning was done with 4% hydrochloric acid (HCl) in distilled water divided over several flasks. Once the sand is mixed with the distilled water and HCL, it is left for 24 hours. After 24 hours the sand was washed with distilled water until the pH reached 7.

Gelatine
To maintain the material in a certain shape, and provide suitable conditions for cyanobacterial growth, a material that allows for crosslinking needs to be added (Persaud et al., 2022). Gelatine was selected, since it was previously used (Heveran et al., 2020). The gelatine works as a carrier and a binder, it will hold all the cyanobacteria and biomineralized crystals. It will also attach to all sand particles, (see image 9).

Image 8, Retrieved sand and sea water from Wemeldinge

4.4 Conclusion

The cyanobacterial biomineralized material consists of three main elements: cyanobacteria in medium, sand and gelatine. To be able to develop the cyanobacterial biomineralized material, materials need to be prepared, cyanobacteria need to be grown in conditions that are beneficial to them and beach sand needs to be cleaned.

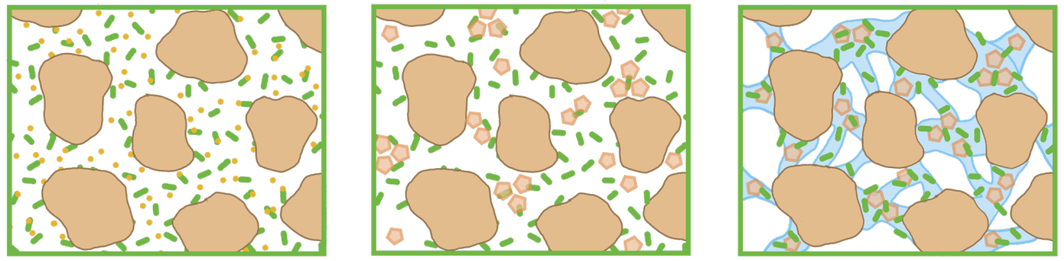


Image 9, Developments stages of the building material

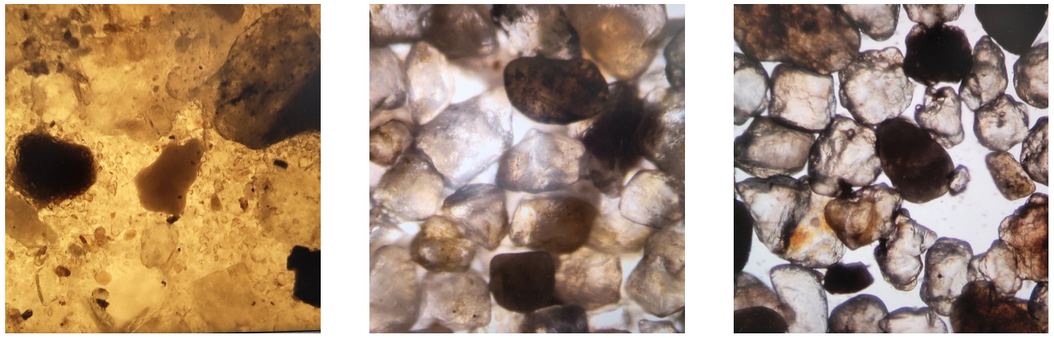
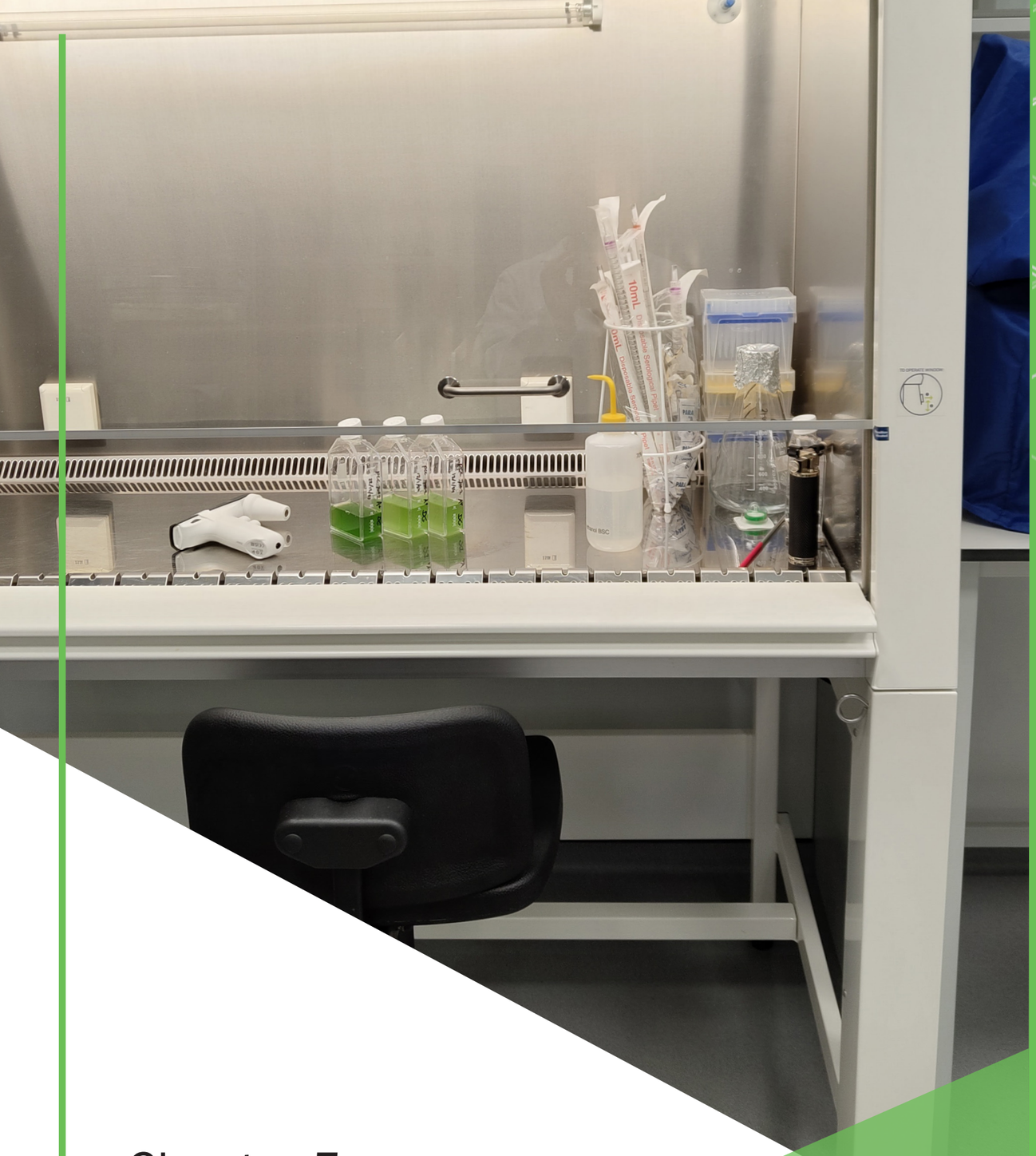


Image 10, Considered sand types under microscope 50x magnification



Chapter 5

Equipment

Content

Introduction

This chapter describes the equipment and materials that have been used for the experiments, and explains their function and settings. They are divided in three groups: equipment and materials needed for growing cyanobacteria, equipment and materials needed for material development, equipment and materials needed for the testing of the material. This chapter describes the equipment and materials that have been used for the experiments and explains their function and settings. They are divided in three groups: equipment and materials needed for growing cyanobacteria (5.1.), the development of the proposed biomineralized material (5.2.), and for its material's testing (5.3.).

5.1 Growing cyanobacteria

Laminar flow cabinet
A laminar flow cabinet is commonly used in Biolabs. This cabinet provides a sterile environment, avoiding the contamination of the bacteria's cultures by other organisms (see image 12). It operates by drawing in ambient air through HEPA filters to remove microorganisms and particles. The filtered air is then blown in a uniform flow over the workspace, creating a sterile environment. When cyanobacteria cultures needed to be regrown, new flasks are made inside this cabinet.

Incubator
An incubator is a cabinet that can provide special environmental conditions in which the microorganisms can grow optimally (see image 11). Two incubators were used, one for growing the cyanobacteria and one for storage of the LBM cubes. This incubator provides the adequate day/night rhythm (16:8h) that is needed for cyanobacteria growth.

Inside the incubator the flasks with cyanobacteria were placed on a shaker plate that moves in little circles to keep the cyanobacteria moving (see image 16).

Once the LBM is made, the cubes are stored in another incubator, see image 15. This incubator had a temperature of +/- 23^{O}C. The day/night rhythm in this incubator was (20:4h) with an ambient RH.

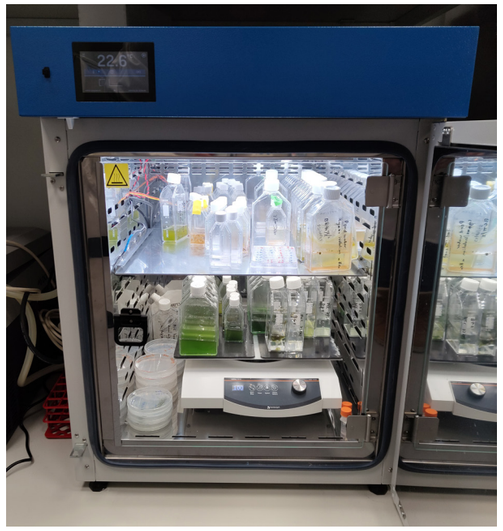


Image 11, Incubator used for bacteria growth

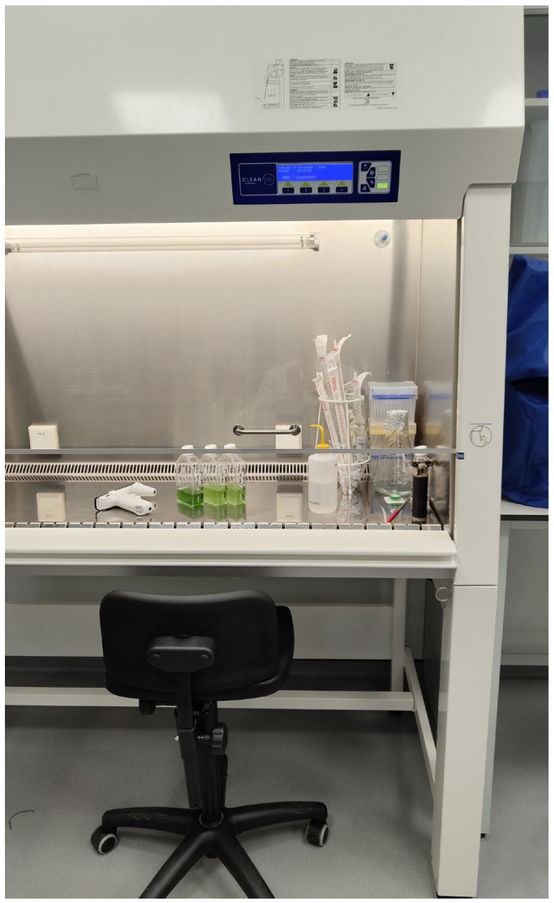


Image 12, Laminar flow cabinet

5.2 Development of the proposed biomineralized material

Centrifuge
The cyanobacteria are first grown in flasks with A+mod medium. To prepare the material, cyanobacteria need to be inoculated in ALS medium with gelatine at a defined optical density (OD). This was done by concentrating them. To do this, the cells are centrifuged in the VWR mega star 3.0 at 3000 rpm for 5 minutes (see image 13). The cyanobacteria will sink to the bottom of the flask, creating a green pellet (see image 14), and allowing the excess medium to be discarded.

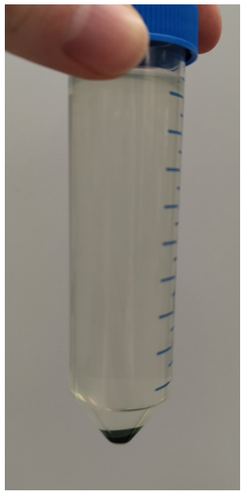


Image 14, Bacteria and medium after centrifuging

Spectrophotometer
To measure the cyanobacterial density used to develop the material, a spectrophotometer was used (see image 18). This machine emits light of a specific wavelength and measures the intensity of the light that passes through a sample. The amount of light absorbed by a sample is directly proportional to its optical density (OD), where higher values are indicative of a higher number of cells. The wavelength used was 730nm also written as OD_{730}, commonly applied for the measurement of cyanobacteria cell densities.

Sieve
The particles of the sand that was used for this material needed to be a certain size: 0.25 mm to 1.0 mm (Armaly et al., 2023). To obtain those particles a sieve was used. After drying, the sand was put in the Haver & Boecker EML 200 Premium sieve with the settings: Amplitude 1.5 mm, Time 4 min, Interval 15 sec (see image 19). The sieve sizes that were used were 1.4 mm, 1.0 mm and 250 µm.

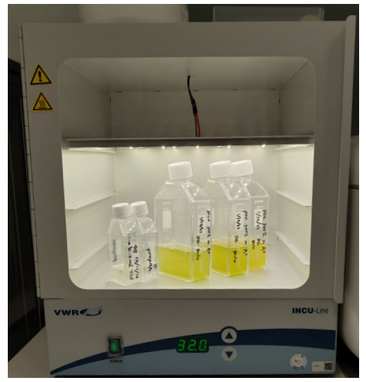


Image 15, Incubator used for storage of material cubes

Image 13, Centrifuge

Image 16, Shaker plate

5.3 Material testing

ZwickRoell Z010 machine
To determine the material properties, compression tests have been conducted on the ZwickRoell Z010 machine (see image 21). The compression components for 10kN tests were used. The settings were: Speed 200 mm/mm; Pre-load 2N; Speed, pre-load 20 mm/mm; Test speed Position controlled 1 mm/min; Upper force limit 9 kN; Maximum deformation 2 mm. The results have been analized by adding error bars representing standard deviation of the sampels in graphs and conducting a t-test to find a p-value (*<0.05,**<,0.01,***<0.001) and significant difference between sampels.

Light microscope with screen
A light microscope was used to look in more detail at the cyanobacteria, sand, medium and cyanobacterial biomineralized material. This microscope also has a screen that allows for taking pictures (see image 17).

Scanning electron microscope (SEM)
A Scanning Electron Microscope was used to look at the biomineralization crystals in more detail. The SEM used is JSM-IT700HR (see image 20). To be able to observe crystals, samples with and without cyanobacteria were put on a thin glass slide and air dried. The samples are coated with gold with the JEOL JFC-1300 auto fine coater prior to SEM observations (see image 22).

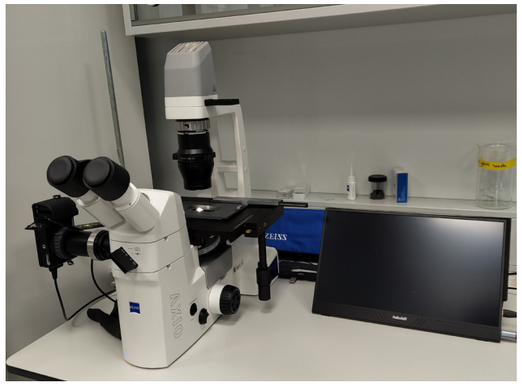


Image 17, Light microscope with screen

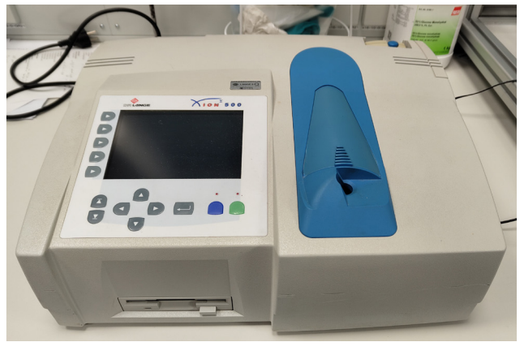


Image 18, Spectrophotometer

Image 19, Sieve

Image 20, Scanning electron microscope

Image 22, Auto fine coater

Image 21, ZwickRoell Z010 machine

Chapter 6

Approach

Content

Introduction

This chapter explains the approach that is taken to eventually be able to reach the goal of making a living building material, through cyanobacterial biomineralization, suitable for offshore applications. It dives into all the possible changes that will be made to the material and into what the application possibilities for this material can be considered.

6.1 Possible pathways for application

The first step consisted in validating the replication of cyanobacterial biomineralization for the development of LBM using the same strains/conditions previously applied (Heveran et al., 2020). Following this, the material could be tested for offshore applications, and further developments could be made to meet specific requirements and fulfil its intended purposes.

As the goal of this study was to develop a material suitable for offshore applications, the first envisioned potential application involves the material's deployment underwater, for example as an attachment to the seabed for bridges. But this is not necessarily needed, since offshore application can also mean that the material will be partly submerged or not submerged at all. An example are dikes, which have a big portion remaining above water. Another option for the material is to be implemented as a temporary material, this can be used for temporary construction.

This led to the exploration of three different pathways for the application of the material (see image 23):
- Application for under water use
- Application for above water use
- Temporary construction for under water use

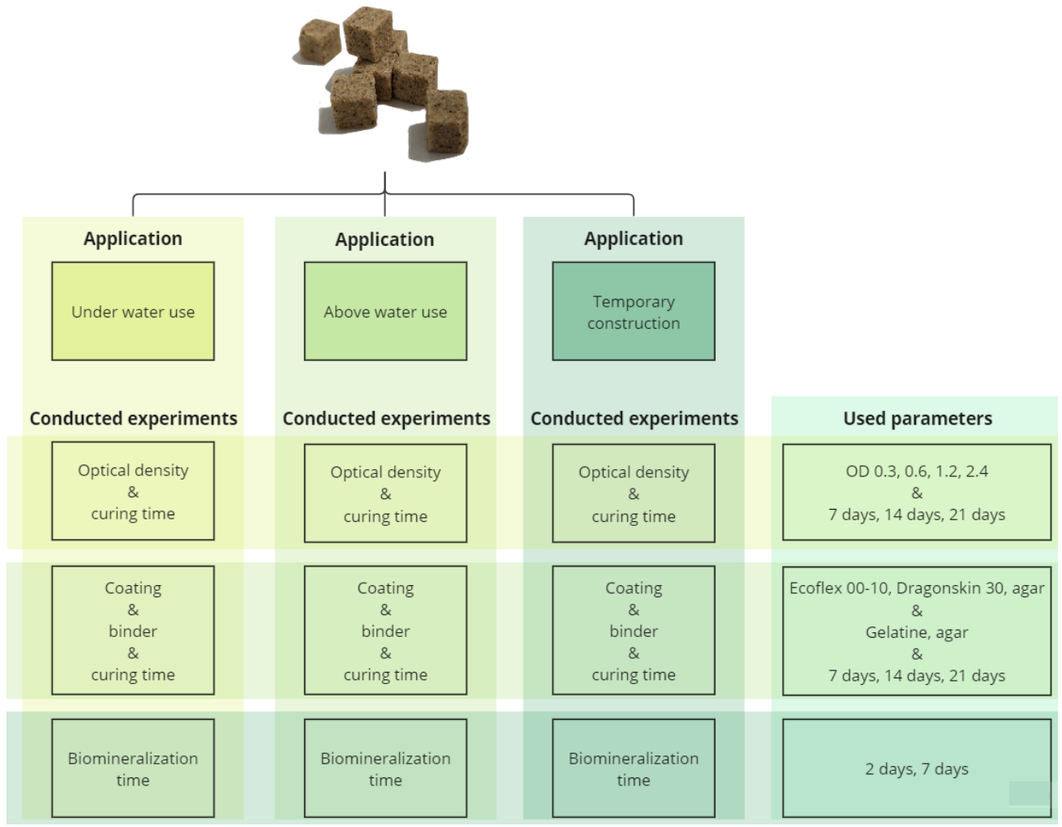


Image 23, Overview of all conducted tests for each application

6.2 Possible pathways for improvement

There is a lack of studies employing cyanobacterial biomineralized living materials for offshore applications, since most of them are only made and applied in environments where no seawater is involved, like 3D printing (Armaly et al., 2023) (Reinhardt et al., 2023). It is therefore important to consider that to sustain offshore conditions, particularly seawater submersion, the material probably needs to be adapted. Consequently, it is important to identify the components of the material that can be changed to possibly make it seawater resistant or more durable for temporary construction.

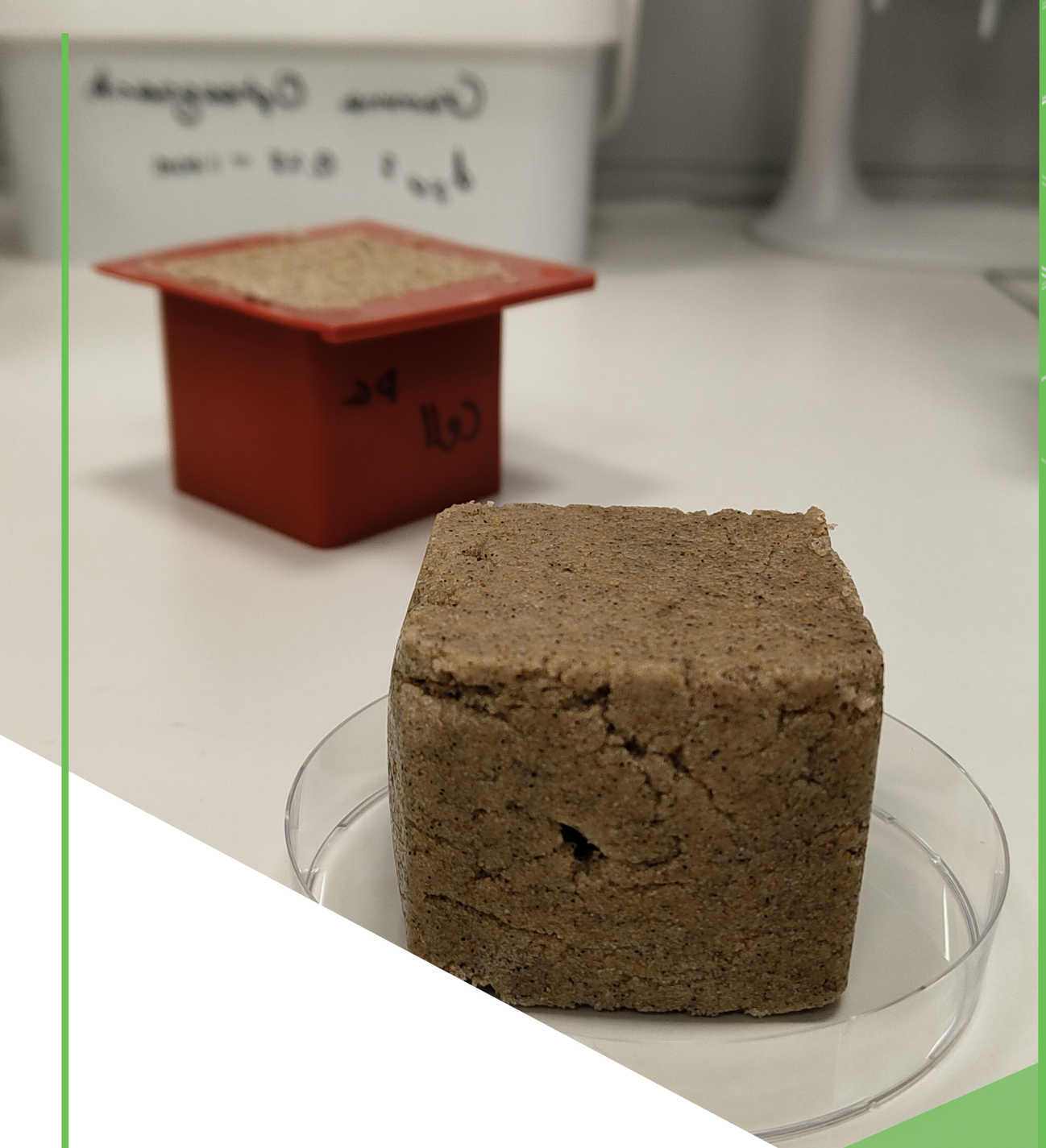
The previous developed LBM consists of three main components: sand, cyanobacteria in medium and gelatine (Heveran et al., 2020). Every small detail can change the material, leading to many openings for change. For example, changing: the binder, the amount of cyanobacteria, the ratio of sand and medium, the growing time, the curing time, adding a coating, etc. If one part of the material is changed, it should be tested individually and combined with all the other changes made to the material, to really know the influence of the change. This is why a selection was made on what parts of the material will be changed. In this study, it was decided to focus on changing the cyanobacteria density, the binder, adding coatings, and use different biomineralization and curing times, (see image 23).

Optical density
If you look at the process of biomineralization it could be that there are not enough cyanobacteria in the medium to make enough crystals to keep the material together and make it strong. That is why four different OD_{730} of cyanobacteria have been chosen to test with: 0.3, 0.6, 1.2, 2.4.

Different binder and adding coatings
The focus on the binder is because gelatine is dissolvable in water. Because the gelatine is the binder of all the components of the material it could be that when that binder falls away the complete material will fall apart. An agar water resistant binder has therefore been chosen to test with.
If the binder is not suitable another option is to add a coating over the material to not let it touch water. For this, three different water resistant coatings have been selected, silicone rubber Ecoflex 00-10, silicone rubber Dragon skin 30 and agar.

Biomineralization time
The changing of the biomineralization time is based on the biomineralization process. This process needs time to happen, it could be that the cyanobacteria present do not have enough time to biomineralize enough for the material to become strong and seawater resistant. If the biomineralization time is increased and sand is already added to the incubated mixture, this can result in better bonding of the crystals and sand particles. Therefore two different biomineralization times were chosen: 2 days and 7 days.

Different curing time
Because the material will be put in cube shapes and it is a wet substance at first, the material needs time to set and dry. It could be that the material dissolves in water or is not strong because it has not completely set yet. Therefore three different curing times have been selected, one week, two weeks and three weeks.



Chapter 7

Initial material validation

Content

Introduction

Before the material can be optimised for offshore applications, we first needed to validate the replication of cyanobacterial biomineralization for the development of the LBM using the same strains/conditions previously described (Heveran et al., 2020). The protocol followed in this study relies on already published methodologies with modifications. Once replication is proven, the LMB can be submitted to offshore conditions, particularly submersion in seawater, and improved for those.

7.1 Biomineralization validation

To ensure the biomineralization ability of *Synechococcus* sp. PCC 7002, a mineralization assay was first performed in liquid culture, and samples were observed by Scanning Electron Microscope (SEM) at the Faculty of Applied Sciences, TU Delft.

Preparing samples
For this observation two sample flasks were prepared. One flask with cyanobacteria in ALS biomineralization medium and one flask with ALS biomineralization medium without cyanobacteria, as a control group. Both the flasks were incubated at 22^{o}C with a day and night rhythm of (16;8h) on a shaker at 130 rpm.

Validation with Scanning Electron Microscope
After two days of incubation the samples were prepared for the SEM analysis. From each flask a droplet of 50 µl was taken and put on a separate glass plate. The samples were air dried in the laminar flow cabinet at room temperature for 11 days. The dried samples were then placed on the specimen holder on the specimen exchange rod of the SEM (see image 24), and moved to the specimen chamber, the samples were then analyzed by SEM (Yu, n.d.).

SEM analyses (see image 25) reveal that the crystals in the control group were bigger than the ones in the group with cyanobacteria. The control group had many salt crystals which were not present in the sample with the cyanobacteria.
Next to this, the sample with cyanobacteria had crystals that were all the same shape whilst the control group has crystals with varying shapes.

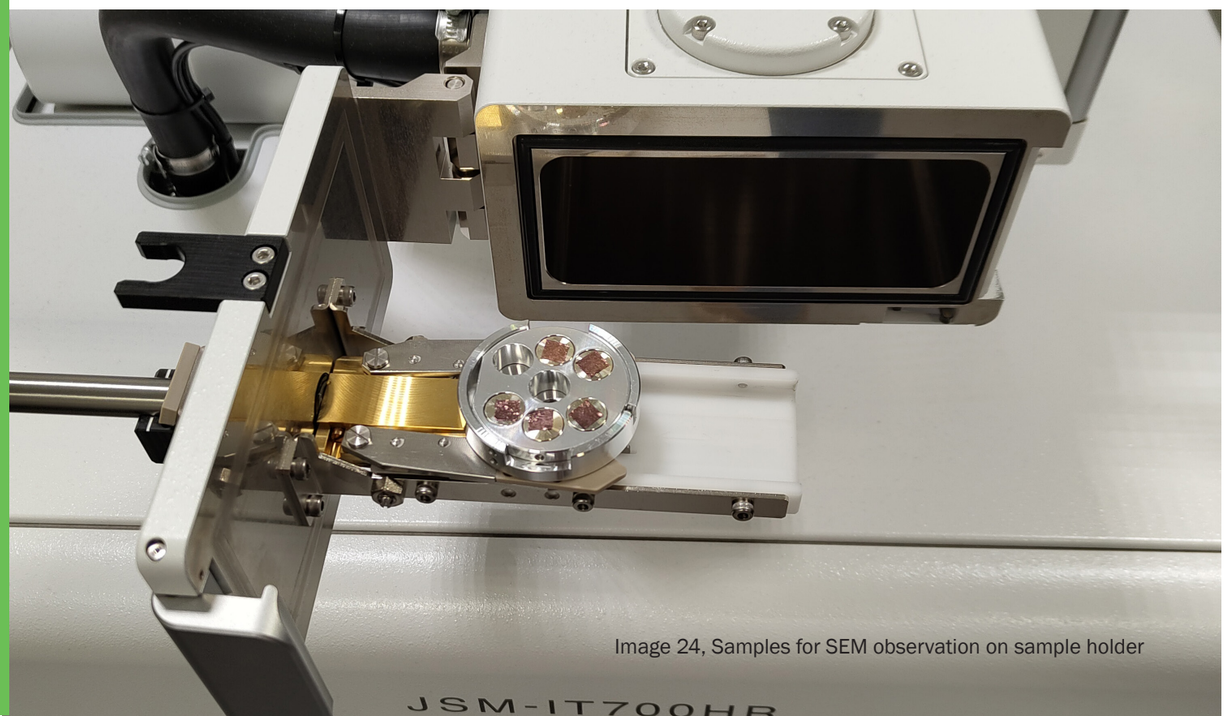


Image 24, Samples for SEM observation on sample holder

Conclusion
From the comparison between the control group and the cyanobacteria group it can be concluded that the crystals in the ALS medium with cyanobacteria are made because of bacterial biomineralization and the ones in the control group are not. The control group has clusters of crystals, but the cyanobacteria group is more evenly spread. This is good for the material because that means the crystals and cyanobacteria will be evenly spread in the LBM. This improves the material's properties.

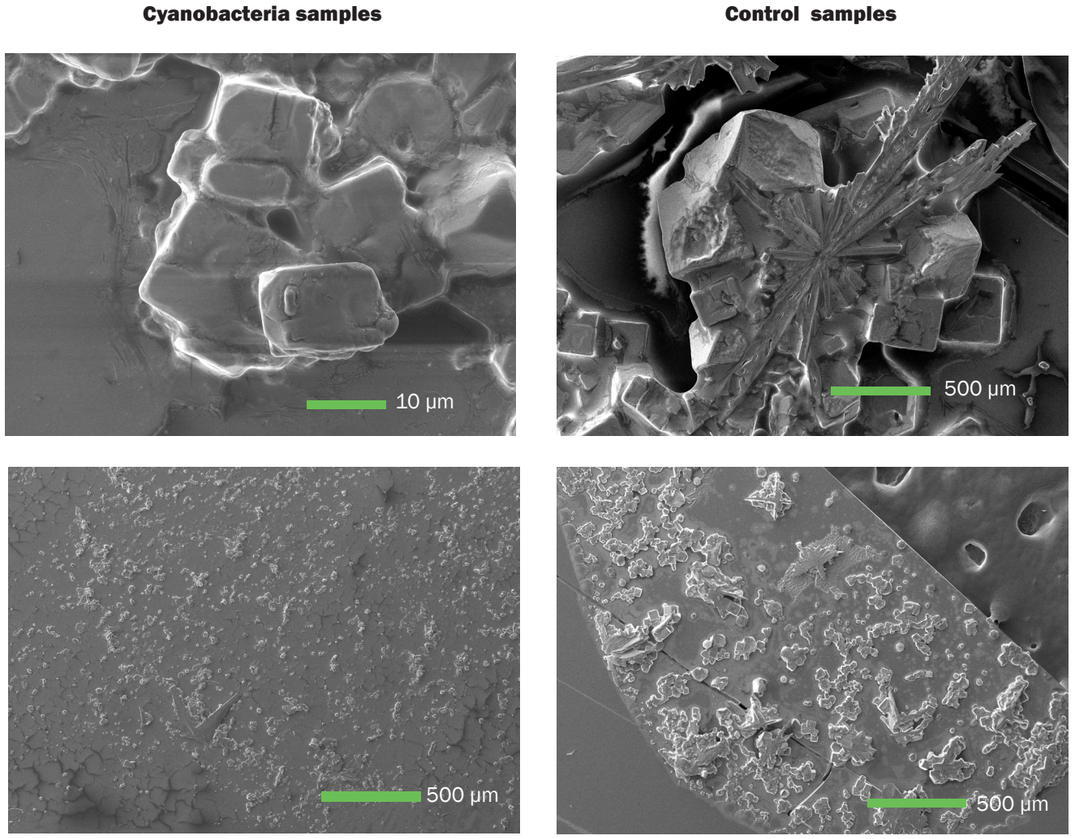


Image 25, SEM results

7.2 Material protocol validation

Now that the biomineralization capability of *Synechococcus* sp. PCC 7002 was confirmed, the material could be made following an adaptation of the previously used protocol (Heveral et al.,2020). A cube of 50mm with cyanobacteria and a control cube of 50mm without cyanobacteria were made.

Protocol for making the cyanobacterial biomineralized material
Once the cyanobacteria were grown and the sand was clean (see chapter 4.3), the following steps for developing the material were performed, in accordance to Heveran et al., 2020 with some modifications (see image 26).

The ALS medium without calcium chloride and sodium bicarbonate was prepared and heated to 50^{o}C. Gelatine was added slowly (100gr/1000ml). The temperature was reduced to 40^{o}C and sodium bicarbonate was added. After this, the pH was measured and adjusted to approximately 7.6. Then calcium chloride was added to the ALS solution and the temperature was reduced to 37^{o}C. The medium was divided into two flasks, one for the control group (no cyanobacteria) and one for the cyanobacteria group. Lastly, the cyanobacteria were added to the corresponding medium flask. The flasks were incubated at approximately 32^{o}C, for 18 hours with a day/night light rhythm (20:4h).

After 18 hours the medium was taken out of the incubator and added to the sand (300ml/1000gr). This mixture was stirred semi continuously by hand for one hour, to ensure complete mixing. After one hour of mixing, the mixture was put in cube moulds. These moulds were stored in a fridge at 4^{o}C for 20 hours. After 20 hours, the cubes were demoulded and stored in the fridge at 4^{o}C.

To reduce mistakes a calculation sheet was made to calculate all the amounts of the ingredients that are needed to develop the material (see appendix A).

Material appearance and behaviour
During the mixing of the sand with the medium differences could be observed between the mixture with cyanobacteria and the control mixture. The mixture with cyanobacteria was smoother and easier to stir, whilst the control mixture was stiff and heavy to stir.
There was also a colour difference between the mixtures, the mixture with cyanobacteria was lighter than the control mixture.
The control mixture also reduced in volume during mixing whilst the mixture with cyanobacteria expanded.
Once the mixture of the material is put in a mould and has been in the fridge for 8 hours the material stays in the desired shape and can be demoulded. After demoulding the control cube was very dense and the cube with cyanobacteria was spongy and could be teared easily. A month later they were both fully cured and felt like rocks.

Conclusion and discussion
Form this experiment it can be concluded that the material can be replicated following the previously described protocol, with small adjustments. The beach sand from Wemeldinge turned out to be no problem to develop the material. Also the changes in temperature compared to what was previously used (Heveran et al., 2020) still made it possible to develop the material. The different incubation time and setting time did also not hinder the making of the material.

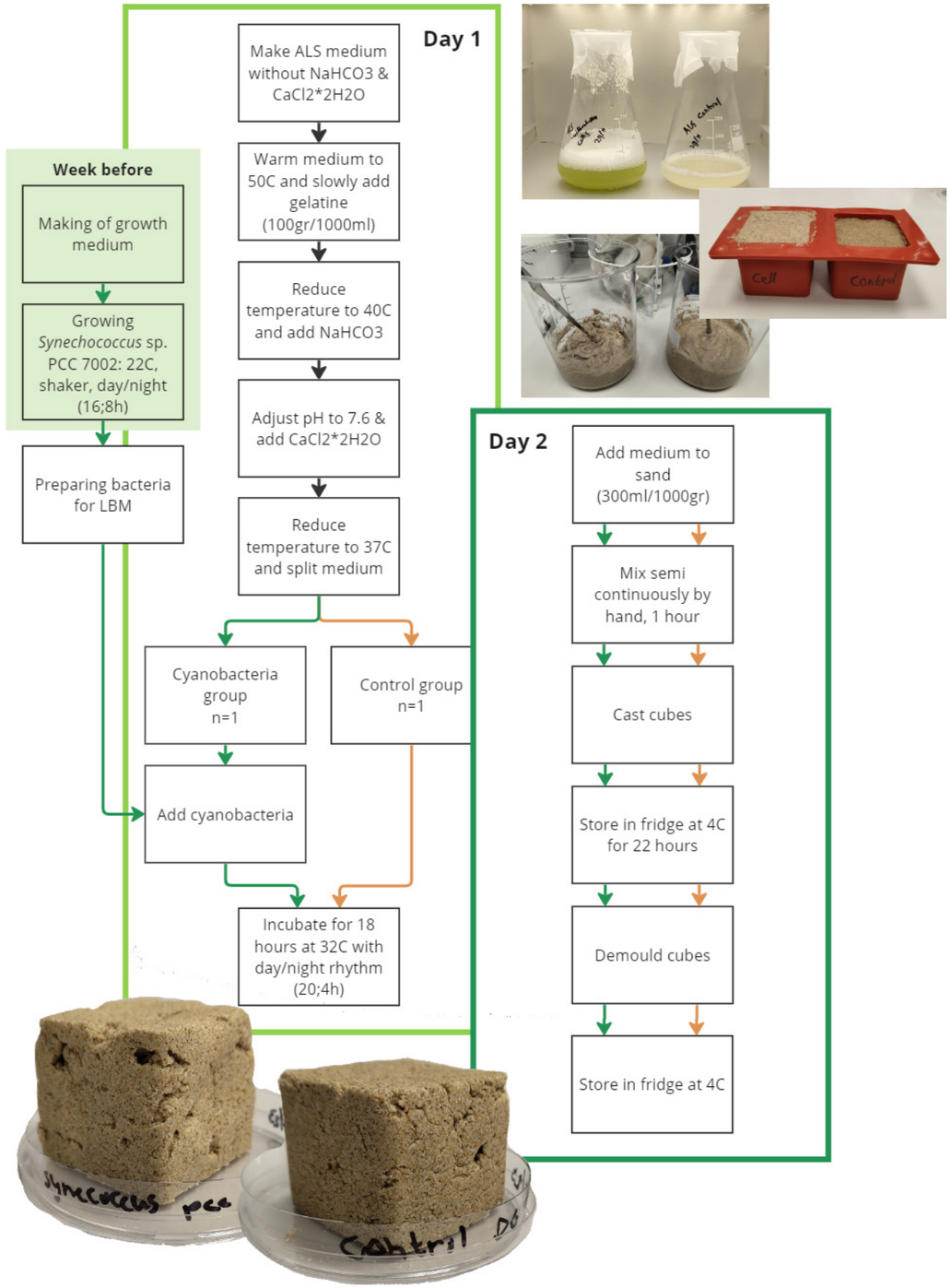


Image 26, Overview of preparation of samples, adapted from Heveran et al., 2020

7.3 Material performance in submerged conditions

It has been validated that the biomineralization is possible and the material can be recreated. The material could now be tested in seawater. For this experiment smaller cubes of 35 mm were made, in order to build more replicates and therefore perform more tests. Cubes were submerged in seawater obtained from the Oosterschelde, close to the beach in Wemeldinge where the sand was collected. In addition, compression tests were conducted, and the viability of the cyanobacterial cells was tested.

The cubes were made following the protocol in image 29 and appendix C. The cubes that were made are shown in image 28 and 29.

Material appearance and behaviour
During the development of the material similar observations were noted as those made during the development of the previous cubes (chapter 7.2): the mixture containing cyanobacteria was smoother, expands more, and displayed a noticeable difference in colour compared to the control group.

Additionally, other observations were noted: 1.) For the casting of this material a tamper was used so all the air bubbles could be pushed out. When the cubes were demoulded the ones with cyanobacteria had no holes in them whilst the control group still had many of gaps. 2.) The cubes with cyanobacteria whom were curing close to each other had sides that cured less fast, a dark spot was visible (see image 27). Those sides were the sides that were close to the other blocks.

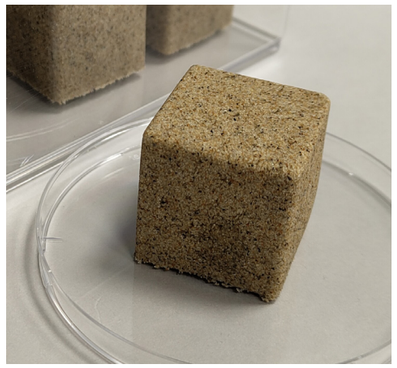


Image 27, Slow curing parts on cubes

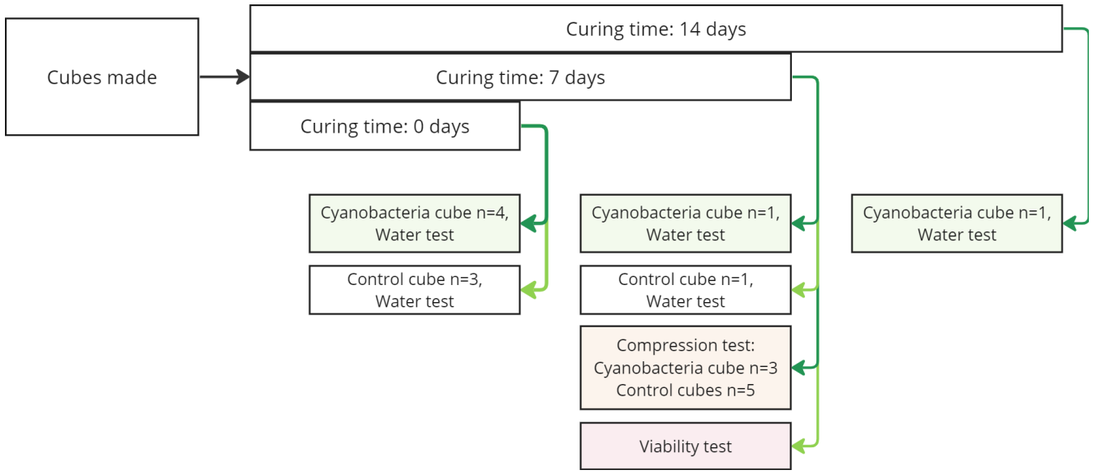


Image 28, Overview of developed cubes

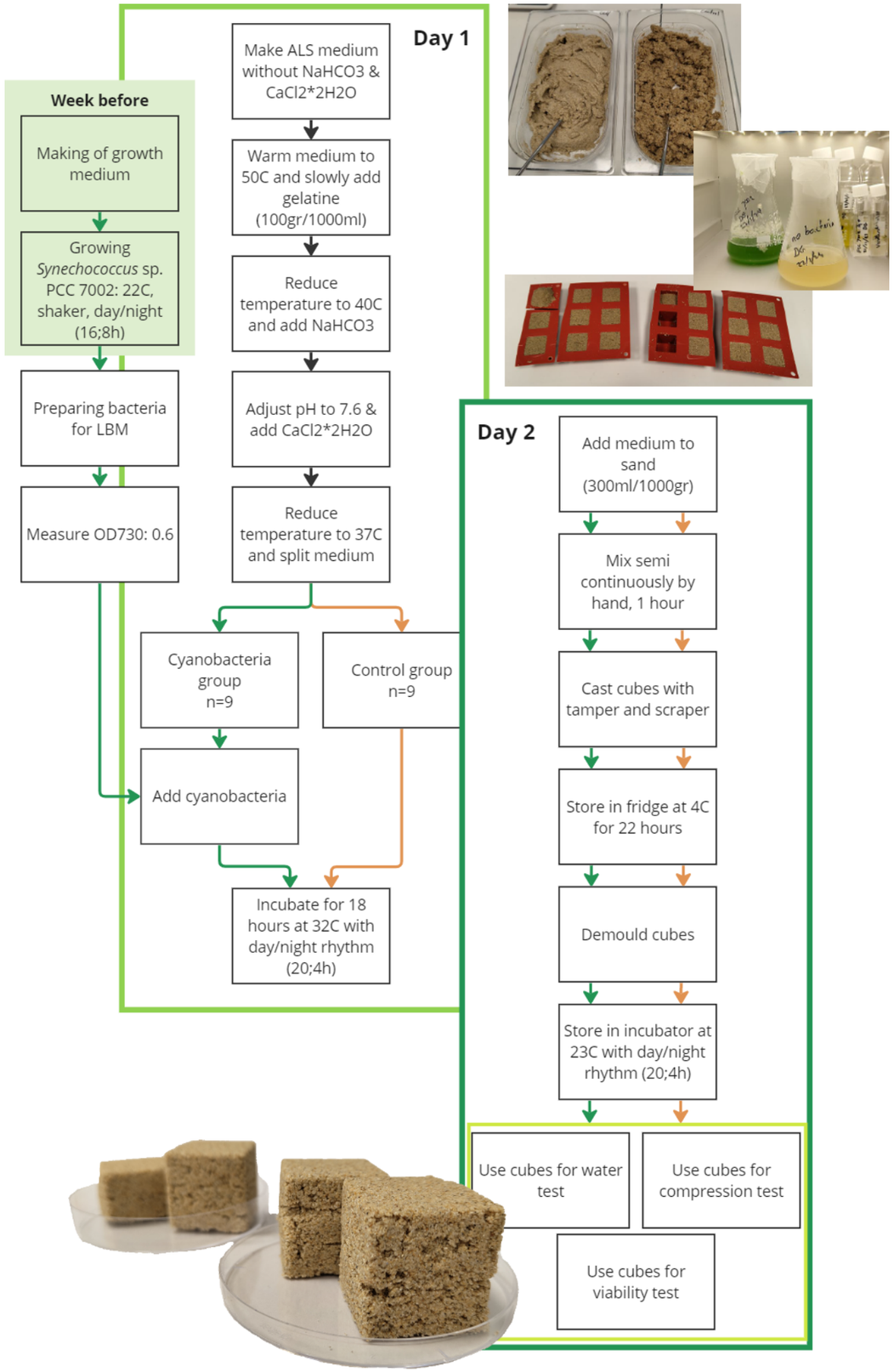


Image 29, Overview of preparation of samples, adapted from Heveran et al., 2020

7.4 Results and discussion

Water test
When the cubes were put in seawater, the following observations were made (see table 2 and image 32). With or without cyanobacteria the cubes first become soft and then fell apart, they are completely dissolved within 3 days. After 7 days they even become a black pile of sand (see image 32).

Compression test
At day 7 of the curing of the cubes at a room temperature of 23^{O}C with a light/dark rhythm (20;4h), compression tests were conducted. Cubes from the cyanobacteria group and the control group were tested together with cement (“snelbeton”) cubes that had cured for 5 days (see image 31 and appendix B). With this compression test the maximum force the blocks could withstand was measured.
The cubes are not compressed until they break, but are tested until the force that is needed to compress the cube lowers. The lowering of the force means that the cube has already formed little cracks and is plastically deformed, the force that is needed to reach that stage is called the F_{max}, and therefore can handle less force.

The average F_{max} (see image 31) that the control cubes could handle was 5817 N, whereas cubes with cyanobacteria could handle 2474 N, revealing that the control cubes were significantly stronger than the cyanobacteria cubes. Furthermore the cement cubes could withstand a force of 394 N, being therefore significantly weaker than the control and cyanobacteria material.

Viability test
Another important aspect of the material is that it can be alive. A viability test was conducted, with cubes that had been curing for 7 days at 23^{O}C with a day/night rhythm (20:4h) and ambient relative humidity, to determine if these cubes were still viable. Three cubes were cut and put in flasks with A+mod medium (2 flasks per cube), which provides nutrients for the cyanobacteria to grow, see image 30. The flasks were incubated at approximately 23^{O}C with a day/night rhythm (20:4h) for 2.5 weeks when the viability was observed under the microscope and by the presence of a green colour.

A droplet was taken from each flask and viewed under the light microscope. It was possible to visualise different living organisms but no cyanobacteria were observed. This could have been due to the fact that during a 2.5 week period, the other organisms could have been competing with the cyanobacteria, leading to its death. Their inability to survive corresponds with results of Heveran et al., when the material was maintained in an ambient relative humidity, the cyanobacteria lost their viability within 5 days (Heveran et al., 2020).

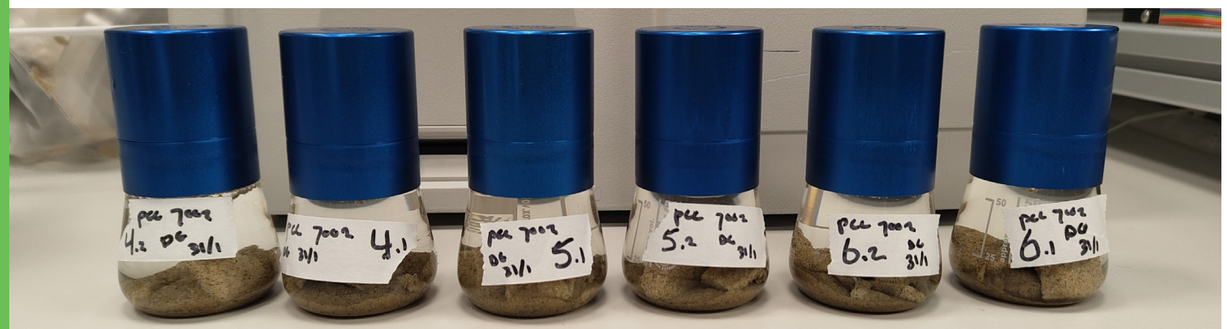


Image 30, Parts of cubes in A+mod medium for viability test

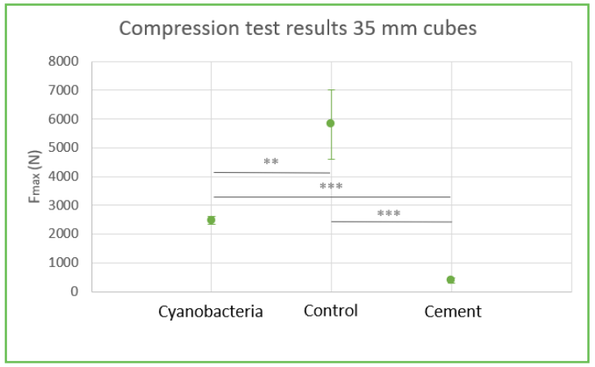


Image 31, Compression test results

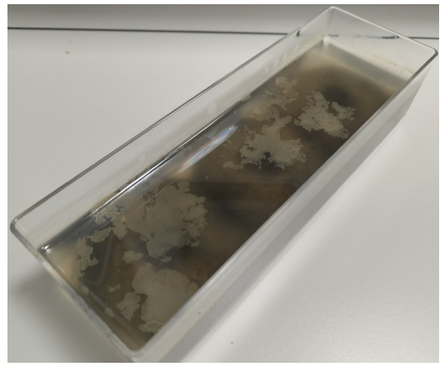


Image 32, The material after 7 days of submersion

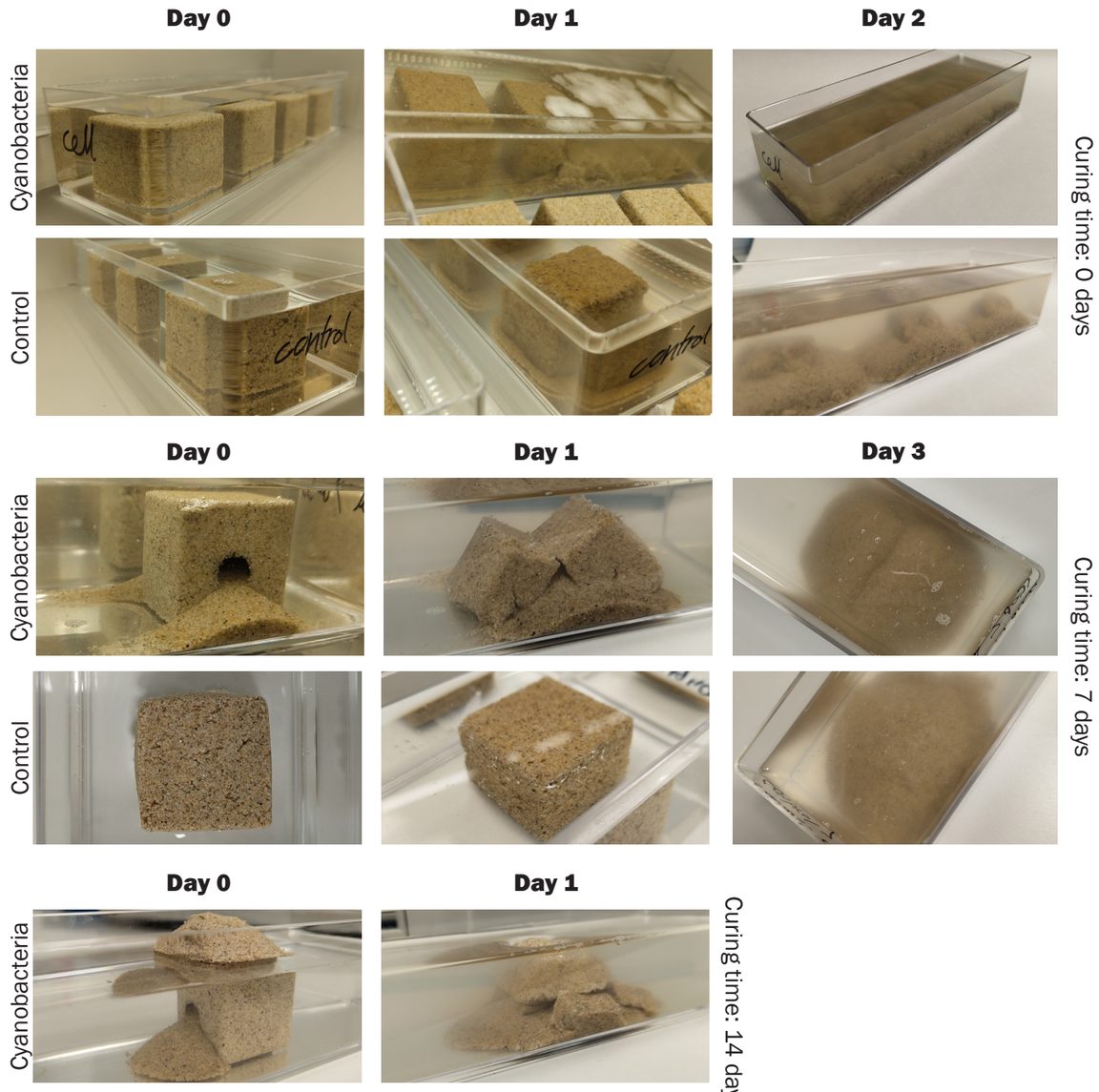


Table 2, Overview of observations made of water test

7.5 Conclusion

The conclusion that can be drawn from the seawater experiment is that this material is not suitable to be applied in seawater since it dissolves within 3 days when submitted in offshore conditions. The control material without cyanobacteria is also not suitable for under water use, because it also completely dissolves within 3 days.

The cubes that were put in the seawater right after demoulding dissolved. This could be due to the fact that, there was not enough time for biomineralization and curing to occur effectively. Still, after 7 days of curing at 23^{O}C the material was still not able to survive in seawater, opening the doors for experiments with changing the protocol and ingredients of the material.

The compression test (see image 31) showed that the cubes with the cyanobacteria are significantly less strong than the control cubes after 7 days of curing, which is the opposite result from previous studies (Heveran et al., 2020). However, our tests were performed at day 7, whereas in the other study the material test was performed after a mass equilibrium at 30 days, given more time to form strong connections through biomineralization (Heveran et al., 2020). It is therefore beneficial to look at the influence of the curing time on the cubes.

Additionally another finding that was noted was that the blocks that cured close to each other had a slower curing spot on the side that was close to another block. This caused the block to form a hole on that place when put in seawater. This is an interesting design opportunity. With this method of making wet spots, shapes can be made without additional construction materials. This could be interesting to be used in temporary construction. Still, more research would be needed to explore this possibility.

Note: In this experiment accidentally the sand-mixture ratio was not 300 ml mixture to 1000 gr of sand but 300 ml mixture to 1600 gr of sand. This could have influenced the results, it could have been that there was not enough medium with gelatine to hold all the sand particles together which lead to the collapse of the cubes. The cubes with the right sand ratio need to be recreated to ensure if the material will dissolve or not, they are incorporated in another experiment (see chapter 8.1).

Chapter 8

Thinkering with the material

Content

Introduction

Now that it is validated that the cyanobacteria are capable to lead to the production of a biomineralized material, that cannot withstand being submerged under sea-water, an explorative process of the material qualities was conducted, in order to optimise it for offshore applications. In this tinkering process, different cyanobacteria concentrations, curing times, hydrogels, coatings, and biomineralization times were tested. Since cubes of 50mm and 35mm take to long to dry and demand more sand, cubes of 10mm have been developed.

8.1 Cyanobacteria optical density and curing time

The validation experiment revealed that the developed material cannot be sustained when submerged in seawater, but when exposed to dry conditions the material maintains its integrity. To optimise the material, different cyanobacteria concentrations were tested, together with changes in the curing time. This is because it could have been that there were not enough cyanobacteria in the material to produce biomineralization crystals to give the necessary strength to the material. For that reason, the optical densities at 730nm of 0.3, 0.6, 1.2, and 2.4 were chosen. Previous studies have applied OD_{730} of 0.3 and 0.6 (Heveran et al., 2020; Qui et al., 2021; Delesky et al., 2023), OD_{730} of 0.6 has also been used in chapter 7.3. The higher densities 1.2 and 2.4 were selected to see its effect on the development of the material. A control group without cyanobacterial cells and a ("snelbeton") cement group were added to be able to compare the new material.

The curing time could also have influenced the strength and seawater resistance of the material, since it may have been too short to allow the material to completely strengthen and cure. Since the previous experiment demonstrated that the developed material does not withstand being submerged in seawater immediately after demoulding lead to the decision to cure the material for a period of 7, 14 and 21 days before testing it underwater. In addition, compression tests were performed.

The cubes are made following the steps in image 34 and appendix C, some adjustments have been made to the protocol based on observations during the development of the material in chapter 7. The incubation temperature of the medium is reduced to 23^{o}C instead of 32^{o}C to allow for better cyanobacterial growth. Also the setting time in the fridge is reduced to 8 hours instead of 22 hours, it is not an ideal situation for the cyanobacteria to be in dark and cold environments, but 8 hours at 4^{o}C is the minimum time and temperature that is needed for the gelatine to set in a firm texture (Owens, 2023).
The cubes that were made are shown in image 33 and 34.

Since the cubes are now 10mm instead of 35mm, it is not possible to compare the compression test results from the validation test with this compression test.

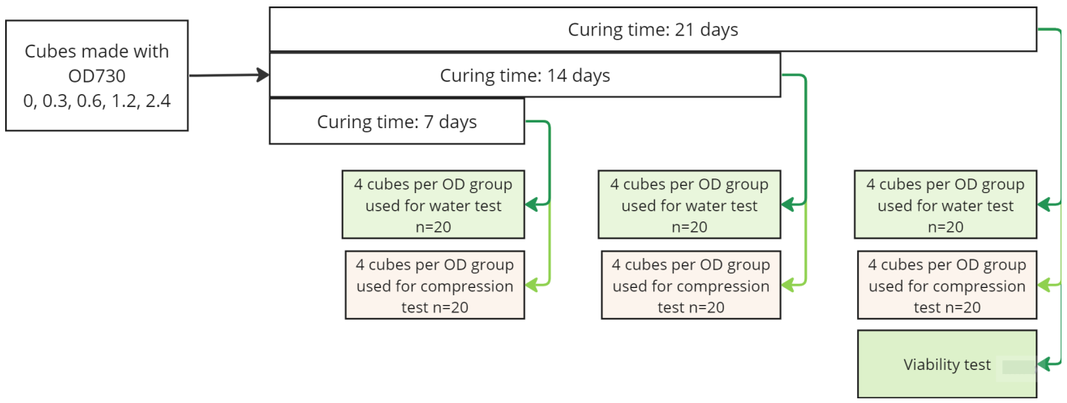


Image 33, Overview of developed cubes

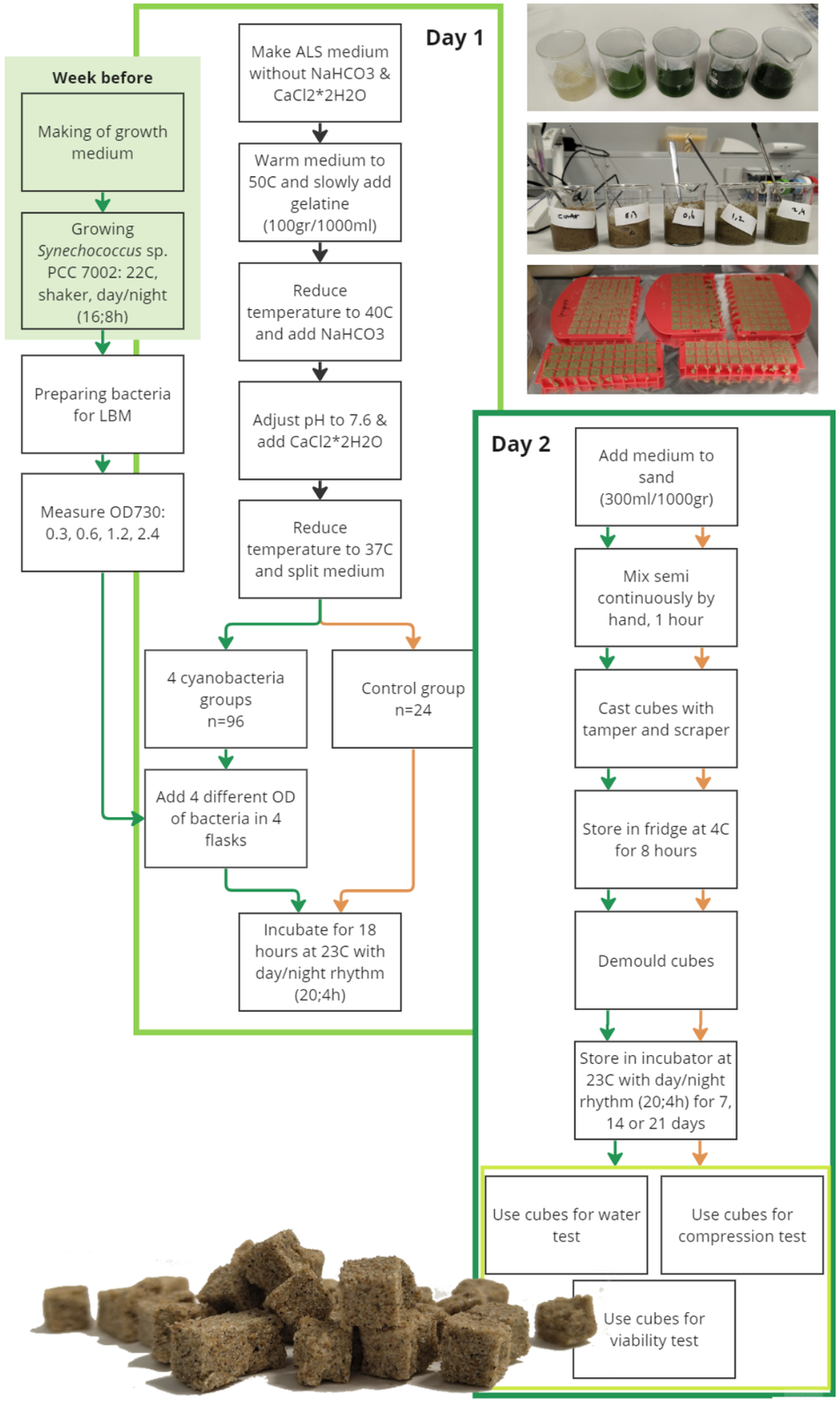


Image 34, Overview of preparation of samples, adapted from Heveran et al., 2020

Results and discussion

7 days
After 7 days of curing, 4 cubes from each of the five groups of material, OD_{730} 0.3, 0.6, 1.2, 2.4 and 0 (control), were separately submerged in seawater in a 20 wells plate. Since the shape of the cubes differs between replicas, it was decided to use 4 cubes from each group in order to be able to draw conclusions from the seawater and compression tests.

Once the cubes were submerged in seawater (see image 36), one of the cubes (OD_{730} 0.6) immediately lost some sand particles. All of the other cubes maintained their shape until the following day, where all the cubes were partly dissolved. Still, it was noticeable that the control and OD_{730} 1.2 cubes were the ones that were dissolving slower. On the second day all the cubes were completely dissolved.

The compression test conducted (see image 35.1) on the non-submerged material showed that the cubes with OD_{730} 2.4 were significantly stronger than the control cubes. The cubes with OD_{730} 0.3 also showed a significant difference, being stronger compared to the control group. All the other cyanobacteria densities showed no significant difference compared to the material without cyanobacteria. Compared to cement (“snelbeton”) cubes of 10mm (see image 35.1 and appendix B) all the cubes were significantly stronger.

14 days
To see if a longer curing time had influence on the integrity and strength of the material, after a curing time of 14 days 4 cubes of each material group were submerged in seawater (see image 36).
When submerged in seawater one OD_{730} 0.3 cube and two OD_{730} 0.6 cubes, immediately partly dissolved. On day one all blocks were partly dissolved, with only one small piece still standing. One cube with OD_{730} 0.3 and 2 from the control group still had a block shape but were also dissolving. On day two all cubes were dissolved.

The compression test conducted on the non-submerged material (see image 35.2), showed that the cubes with OD_{730} 2.4 were significantly stronger than the control cubes. The cubes with OD_{730} 0.3 and 0.6 also showed a significant difference, being stronger than the control material. But the cubes with OD_{730} 1.2 showed no significant difference compared to the material without cyanobacteria. When compared to the cement, all the cubes were still significantly stronger.

21 days
After 21 days of curing the next 4 cubes of each group were submerged in seawater (see image 36).

Again right after submersion, the material maintained its integrity, on day one all the cubes were partly dissolved and on day two all the cubes were completely dissolved.

The compression test conducted on the non-submerged material (see image 35.3 and 35.4) showed that the cubes with OD_{730} 2.4 compared to the control cubes, were not significantly stronger anymore. Only the cubes with OD_{730} 0.6 and 1.2 (see image 35.3) had a significant difference with the control groups, being significantly less strong.

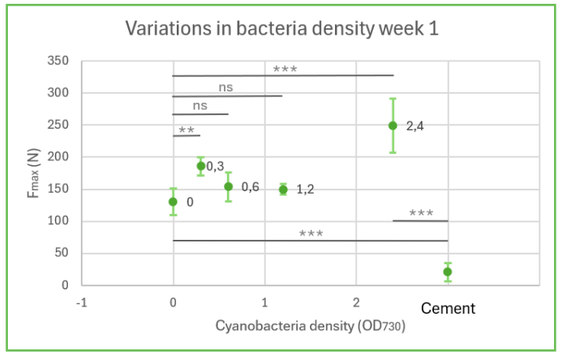


Image 35.1

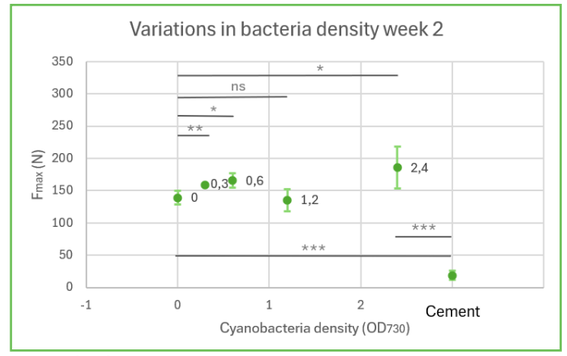


Image 35.2

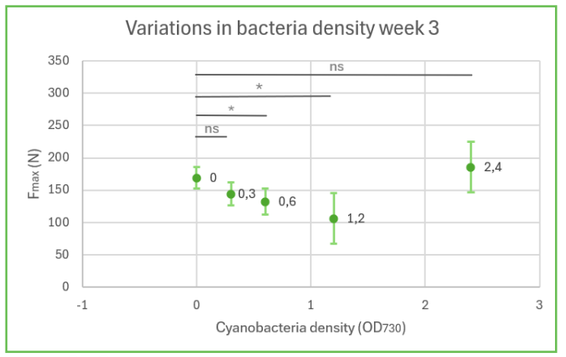


Image 35.3

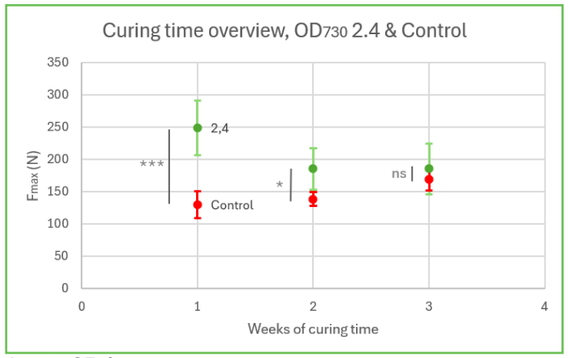


Image 35.4

Image 35, Compression results

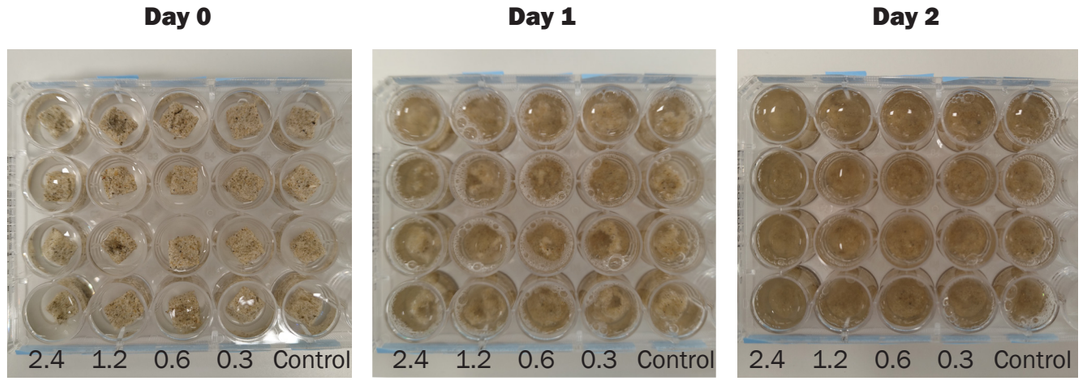


Image 36, Overview of cubes in water after 14 days of curing. It also represents the results after 7 and 21 days of curing.

If the maximum force that all the cubes can handle is compared over the curing time (see appendix B), it can be concluded that the strength of the material does not significantly change over time. The control cubes tend to become stronger over time, whilst the bacteria cubes tend to lose some strength.

Cyanobacteria influence the biomineralization process, they increase the pH in their microenvironment which leads to the increase in speed of the biomineralization reaction. When the material with OD_{730} 2.4 and the control material are compared (see image 35.4), it can be concluded that the material with OD_{730} 2.4 gains strength faster than the control material because of the cyanobacterial influence on the chemical reaction. Eventually the control material will increase to the level of strength of the material with OD_{730} 2.4, but it takes longer to get there.

Viability test
After three weeks of curing the viability of the five groups with different OD_{730} was tested. Sand particles of these cubes were taken and put in separate tubes in petri plates with A+mod medium (see image 37), after seven days a droplet of each tube was obtained and observed under the light microscope. Different living organisms were visualised but no cyanobacteria were observed. The material had been curing in ambient RH for three weeks, aligning with Heveran ‘s findings, where the material is not viable anymore within 5 days in ambient RH, providing 50% RH or more improves the viability (Heveran et al., 2020; Delesky et al., 2023). Qui et al. found that adding a desiccation protectant can improve viability in ambient RH, so adding this to the material would be an improvement (Qui et al., 2021).

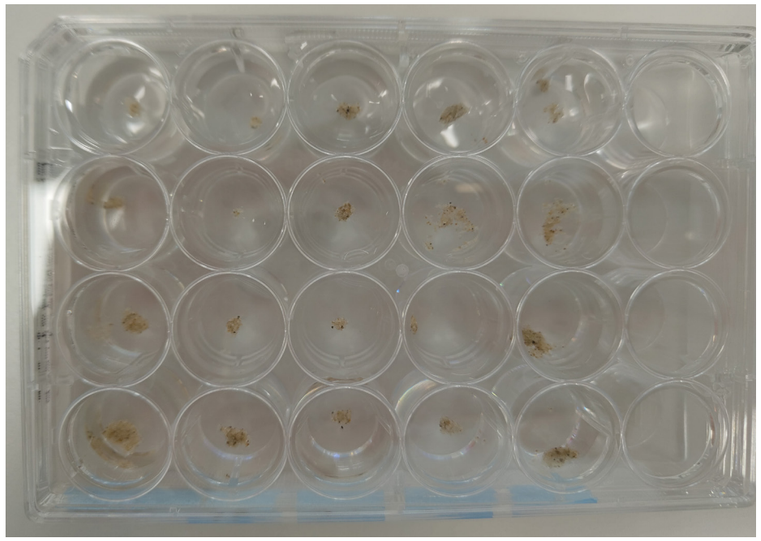


Image 37, Viability test

Conclusion

From the water test it can be concluded that the difference in cyanobacteria optical densities and curing time have no visible influence on the dissolvability of the material in seawater. So, another factor of the material needs to be improved to stop the dissolving of the cyanobacterial biomineralized material before it can be implemented in under water use.

The compression tests showed that the material, after curing for one and two weeks, either with or without bacteria is significantly stronger than cement that has been curing for one and two weeks. When the control and bacteria cubes are compared, it can be concluded that for the first two weeks bacteria with OD_{730} 0.3 and 2.4, significantly strengthen the material. After three weeks of curing this difference is not visible anymore. The material with OD_{730} 2.4 can handle the most force, this cyanobacteria optical density will therefore be used in follow-up experiments.

And after two weeks the OD_{730} 0.6 is also significantly stronger than the control group, where OD_{730} 0.6 is also stronger than OD_{730} 0.3, Delesky et al. used an OD_{730} of 0.6 and compared their compression test results to Heveran et al. who used OD 0.3 (Delesky et al., 2023). The OD 0.6 was stronger but Delesky put the reason for this difference on a difference in sand type between the studies (Delesky et al., 2023). In this study the sandtype between the OD_{730} 0.3 and OD_{730} 0.6 was the same and still a difference in compression strength was detected, which leads to suspect that the sand type was not the reason for the difference in the results of Heveran et al. and Delesky et al.

Because the strenght of the material does not change much over time, it can be concluded that the curing time therefore has little to no influence on the strength of the mateial. The third week of curing will be eliminated from follow-up experiments, because it added no extra information.

For temporary construction it can be concluded that the building material may be suitable, but only up to two days, which is not preferred. The material dissolves within two days, this is a shorter dissolving time than the material in chapter 7.3, probably because of the smaller sample size.

For above water use an optical density of 2.4 is most suitable together with a curing time of 7 days, since it strengthens the material the most.
For under water use none of the optical densities improve the material, since it dissolves.
For temporary construction above water the optical density of 2.4 is the most suitable because of its strength. But optical densities of 0.3 and 0.6 after two weeks of curing are also suitable. For temporary construction under water, the optical density does not matter because all the cubes dissolve within roughly the same timeframe, the material is therefore only usable for two days but enlarging the size of the material can prolong its survivability.

Note: In chapter 7.3, the wrong sand ratio was used, the cubes with the right sand ratio needed to be tested again. This has been done in this chapter, the cubes with OD_{730} 0.6 and the control group have the same ratios as the cubes used in the previous chapter. These also dissolved within two days, the middle part did not fall out this time, so that could have been a result of the wrong sand-mixture ratio.

8.2 Coating, binder and curing time

The previous experiments (chapter 8.1) revealed that neither the cyanobacteria density or curing time improves the material qualities for under water use. But the cubes with OD_{730} of 2.4 is significantly stronger than the control group, when the material is not exposed to underwater conditions, which improves the materials suitability for above water use.

To improve the material for under water use, a different binder and a coating were tested together with different curing times (see image 38). As can be seen in image 39, the binder binds all the sand particles together embedding the cyanobacteria and crystals. It seems like the crystals and cyanobacteria are not the ones connecting the sand particles, they only strengthen the binder that connects the sand particles. So it could be possible that the blocks dissolve because the binder dissolves in the seawater and this releases the sand particles. Another way to make the blocks seawater resistant is to never let them touch seawater, with a water resistant coating.

It was decided to substitute the gelatine binder for agar. In addition, the agar was also used as one of the coatings, together with silicone rubber Dragon skin 30 and silicone rubber Ecoflex 00-10.

Agar
Agar is a gelling agent, that is often used to grow microbial cultures in biolabs (Labshop, 2023). But it is also often used as a vegan replacement for gelatine (Pit&Pit, n.d.). Agar contains no inhibitors that can interfere with chemical reactions which is ideal for the development of micro-organisms (Biolab Diagnostics Laboratory Inc., 2016). This makes the component also appealing to use as a binder and/or as a coating. Armaly and collegues successfully used agar as a binder for their LBM for 3D printing (Armaly et al., 2023).

Agar is also highly water resistant (BillBall, 2021), which makes it suitable for a water resistant coating. Next to this is agar also biodegradable (BillBall, 2021), this makes it more interesting as a coating or binder than the silicone rubbers. But also more vulnerable because nature can break it down.

Dragon skin 30
Dragon skin silicone rubber is a high-performance platinum cure liquid silicone compound which is often used for medical prosthetics because of its physical properties and flexibility (Smooth-On, Inc., n.d.-a). It is a silicone rubber that can be stretched to 364% without tearing (Smooth-On, Inc., n.d.-a). It is also a translucent silicone which allows light to pass through which is needed to keep the cyanobacteria alive.

Ecoflex 00-10
Ecoflex is a platinum-catalyze silicone rubber. Once cured, the silicone is a very soft and very strong and stretchable material (Smooth-On, Inc., n.d.-b). It can stretch for 800% before it tears (Smooth-On, Inc., n.d.-b). The Ecoflex rubber is also used for prosthetic appliances. The Ecoflex 00-10 rubbers are less strong than the Dragon skin 00-30 rubbers, (the number indicates the strength). Just like the Dragon skin, Ecoflex is also translucent and ideal for the cyanobacteria growth that is light-dependent. Ecoflex 00-10 also has been used by Li Chenghai as a cover for a living product that needed to survive in seawater (Li et al., 2023).

For this experiment eight groups of cubes (1 cm^3) were made (see image 38), four groups with a gelatine binder, either coated with silicone rubber Dragon skin 30, silicone rubber Ecoflex 00-10, agar or non-coated. The other four groups are agar bonded cubes, either coated with silicone rubber Dragon skin 30, Ecoflex 00-10, agar or non-coated. After either 7 or 14 days of curing the cubes were submerged in seawater to test their suitability for under water use or compression tested to test the suitability of the material for above water use. No cubes were coated in agar after a curing time of 14 days because the agar was not suitable as a coating.

The cubes with gelatine as a binder were made following the protocol in image 41 and appendix C.
The cubes with agar as a binder were new and needed a new protocol, see image 40 and appendix C.

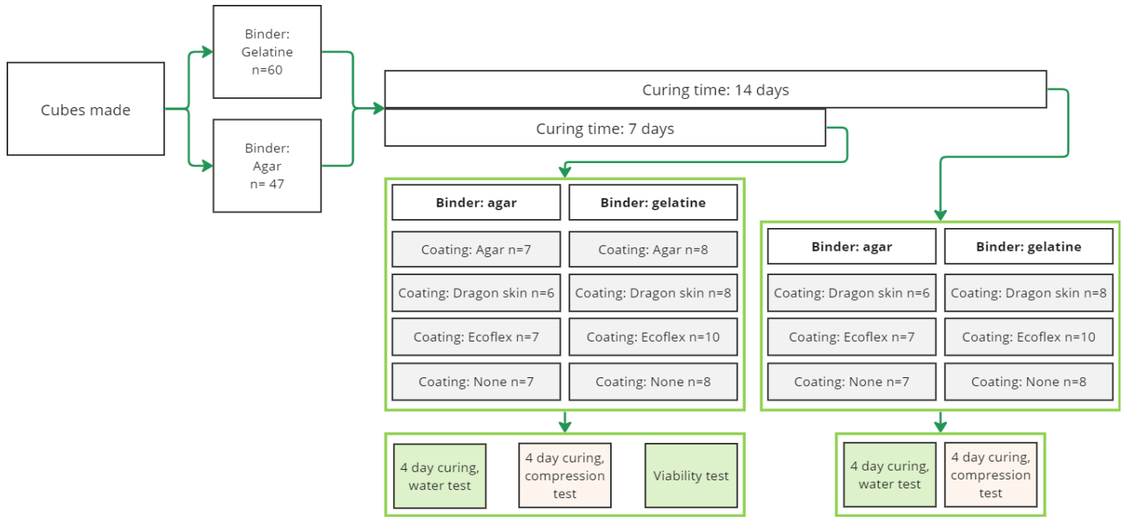


Image 38, Overview of developed cubes

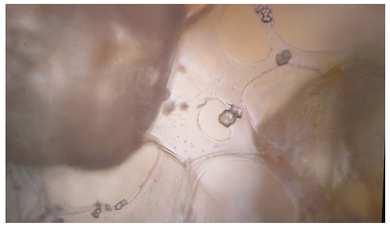


Image 39, Gelatine with crystals bonded to sand particles

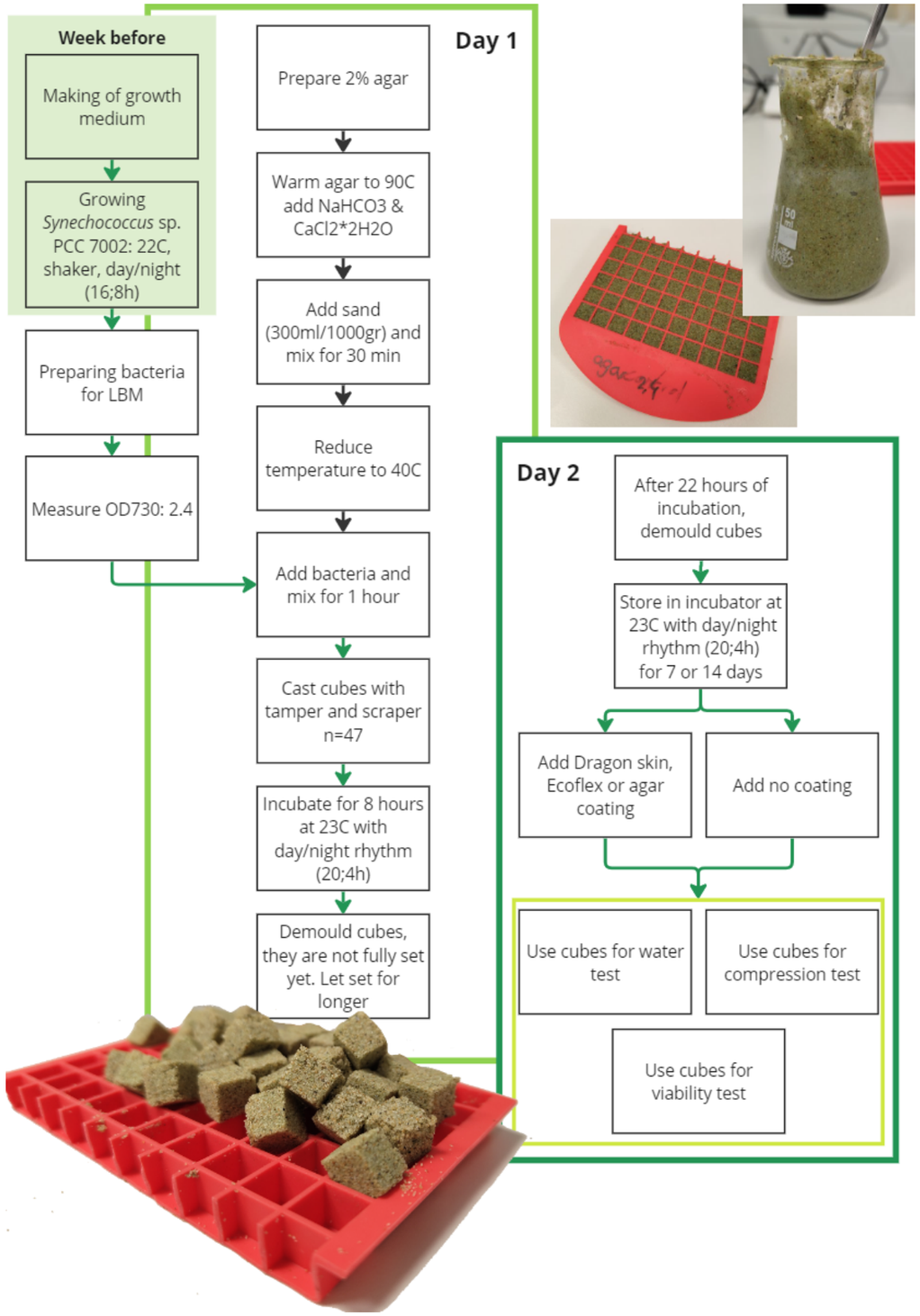


Image 40, Overview of preparation of agar bonded samples, adapted from Heveran et al., 2020 and Armaly et al. 2023

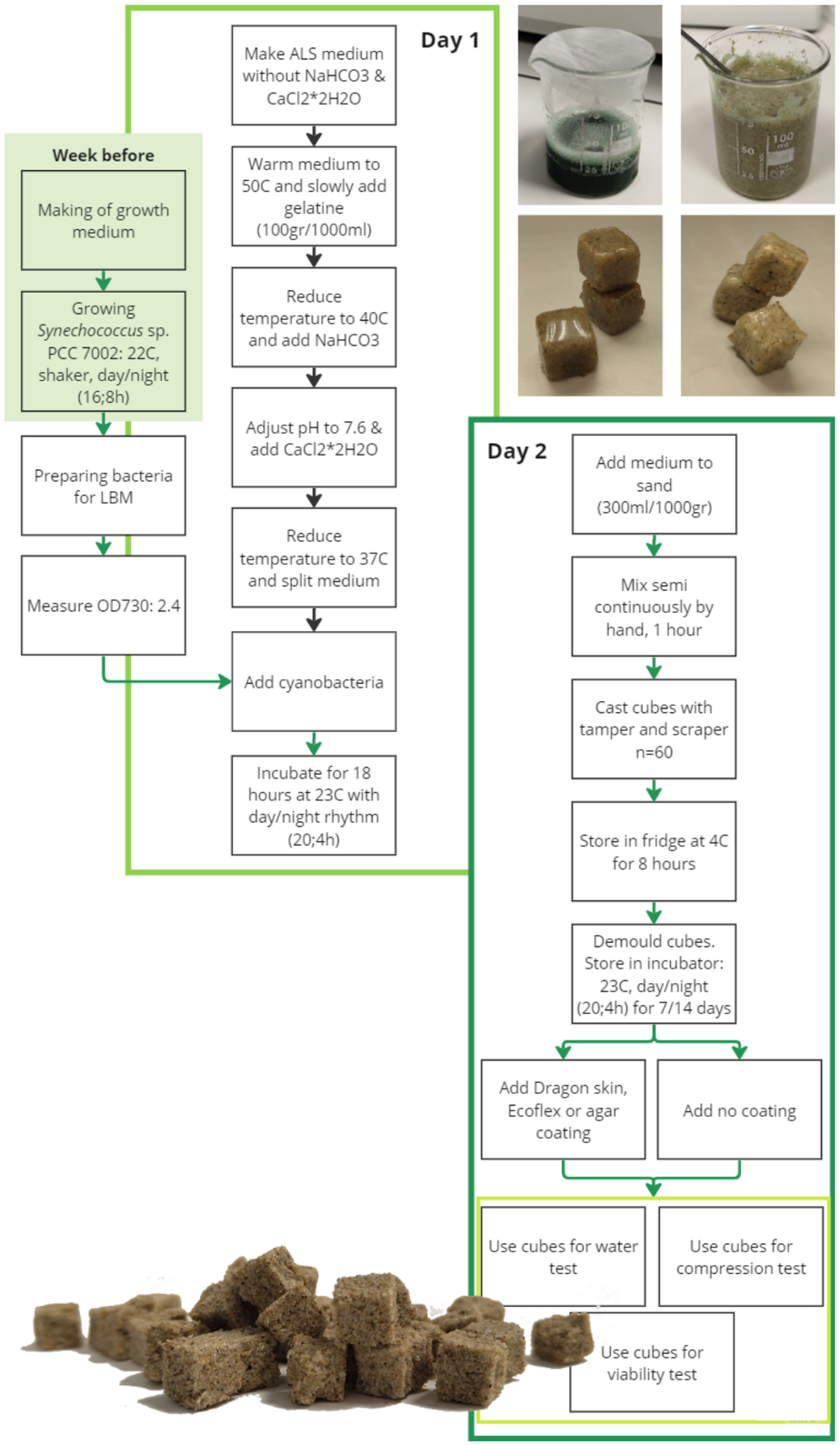


Image 41, Overview of preparation of gelatine binded samples, adapted from Heveran et al., 2020

Material appearance and behaviour

Agar has a melting point of 90^{O}C, which makes it hard to use in combination with cyanobacteria. These organisms cannot survive at that temperature but the agar needs to be melted to be able to add the cyanobacteria (Labshop, 2023). So first the medium is made with melted agar, the biomineralization nutrients and sand, the temperature is then reduced to 40^{O}C, right before agar will solidify. This is a temperature the cyanobacteria survival so they can be added to the mixture and casted in moulds.

After 8 hours of curing the agar cubes were not ready to be demoulded, they crumbled. This could have been the result of a shorter biomineralization time than 18 hours, because of the different protocol, which could have led to not enough biomineralization to hold the material together. After another 22 hours of curing the blocks were compact enough to be taken out of their moulds (see image 40).

During the dipping process in the different coatings, the agar cubes gained a dark colour, whilst the gelatine blocks kept their original light colour (see image 43). The cubes stayed solid whilst being dipped in the silicone rubbers, when the cubes were dipped in the agar coating (see image 42) the cubes started to fall apart. They gained a coating but they did not have their original cube shape anymore and the coating did not distribute homogeneously. For this reason no agar coating was added for the cubes that had been curing for 7 days.

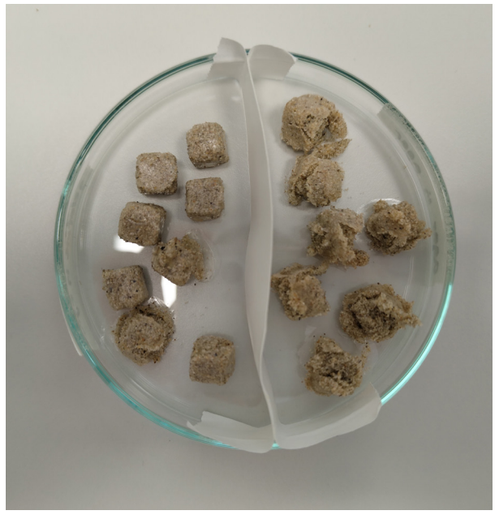


Image 42, Cubes with agar coating. Left gelatine bonded cubes, right agar bonded cubes

Image 43, Left, agar bonded cubes with silicone rubber coating, Right, gelatine bonded cubes with silicone rubber coating

Results and discussion

Water test

All eight groups of cubes were put in seawater after a total of 7 days of curing (see image 44). On day zero the cubes maintained their integrity. The next day the gelatine bonded cubes without a coating partially dissolved, whereas the agar bonded cubes without a coating maintained their shape as well as all the agar and gelatine bonded cubes with a silicone rubber or agar coating. On day 2 the gelatine bonded cubes were completely dissolved, no changes happened to all the other cubes. On day 5 the agar bonded cubes without a coating collapsed when touched, the gelatine and agar bonded cubes with an agar coating were completely dissolved, the agar bonded cubes with a silicone rubber coating are still sturdy whilst the gelatine bonded cubes with silicone rubber coating are soft. After being submerged in seawater for 7 days, the agar bonded cubes with no coating stayed together in seawater but were very fragile.

After a total of 14 days of curing, the second batch of cubes was put in seawater (see image 44). This test had the same results as the water test done after 7 days of curing.

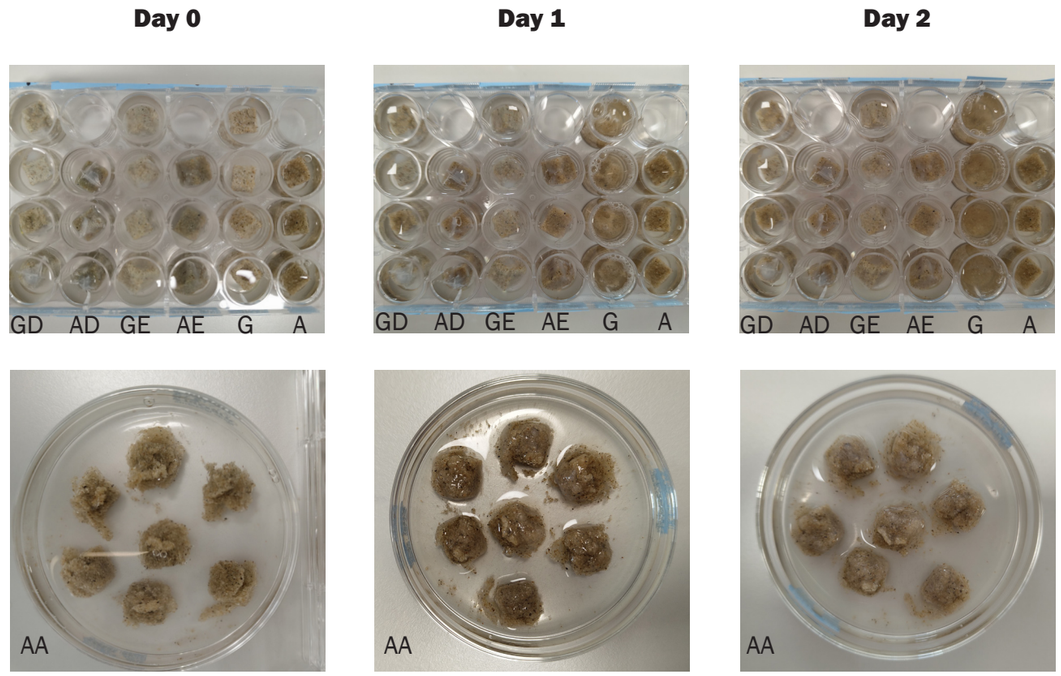


Image 44, Overview of cubes in water cured for 7 days, top: silicone rubber coating, bottom: agar coating. It also represents the results after 14 days of curing.

AE = Agar bonded cube with Ecoflex 00-10 coating
AD = Agar bonded cube with Dragon skin 30 coating
AA = Agar bonded cube with agar coating
A = Agar bonded cube without coating

GE= Gelatine bonded cube with Ecoflex 00-10 coating
GD = Gelatine bonded cube with Dragon skin 30 coating
GA = Gelatine bonded cube with agar coating (not shown)
G = Gelatine bonded cube without coating

Compression test
The compression test of the gelatine bonded cubes after 7 days of curing (see image 46.2 and appendix B) shows that the is no significant difference between the cubes with and without a coating, they can all handle approximately 230N, which is also around the same force that is measured in the material with an OD_{730} of 2.4 in chapter 8.2. After two weeks of curing (see image 46.4) there was still no significant difference detected between the gelatine bonded cubes with a coating and without a coating, the agar coated cubes were not tested because the coating was not suitable for underwater applications due to non-homogeneous distribution.

The compression test of the agar bonded cubes after 7 days of curing (see image 46.1) shows that also this binder has no significant difference between the coated and not coated cubes, they could all handle around 16N. Only in week 2 (see image 46.3) the agar cubes without a coating were significantly less strong than the coated ones.

The cubes that were used in the water test and had a silicone coating were taken out of the seawater after 7 days to be compression tested. The cubes with a gelatine binder were not suitable for testing, because the material in the coatings was dissolved. The agar bonded cubes with a silicone rubber coating had maintained their sturdiness after being exposed to seawater for 7 days. The agar bonded cubes in a silicone rubber coating after submertion (see image 46.6) could handle approximately 7N which is significantly less than before submertion when they could withstand 16N.

The compression tests show (see image 46.5) that all the cubes with agar as a binder are significantly weaker than the gelatine bonded cubes after one week of curing and after two weeks of curing. The average force the agar bonded cubes can handle (see image 46.1), after one week of curing, in compression is 16N. This is also no significant difference with the concrete cubes that have cured for one week. After two weeks of curing (see image 45.5) the agar bonded material can withstand an average load of 18N, whilst the gelatine bonded cubes can withstand 218N.

Viability test
Four types of cubes that have cured for 7 days were used for the viability test, the agar bonded cubes with an Ecoflex 00-10 coating and a Dragon skin 30 coating, and the gelatine bonded cubes with an Ecoflex 00-10 coating and a Dragon skin 30 coating. Sand particles of these cubes were taken and put in separate tubes in petri plates with A+mod medium, after seven days a droplet of each tube was obtained and observed under the light microscope, see image 45. Different living organisms were visualized but no cyanobacteria were observed.

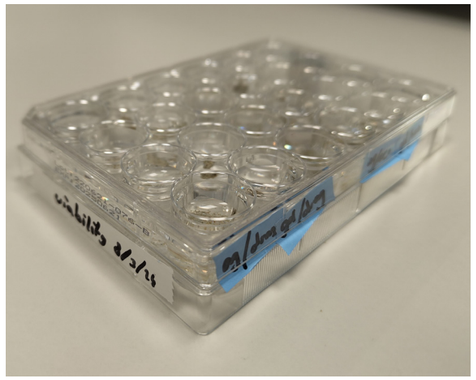


Image 45, Viability test

Compression tests for above water use

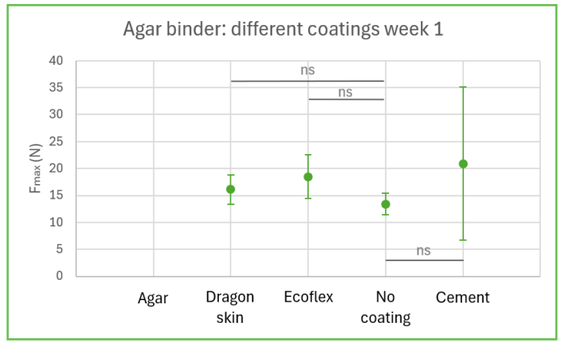


Image 46.1

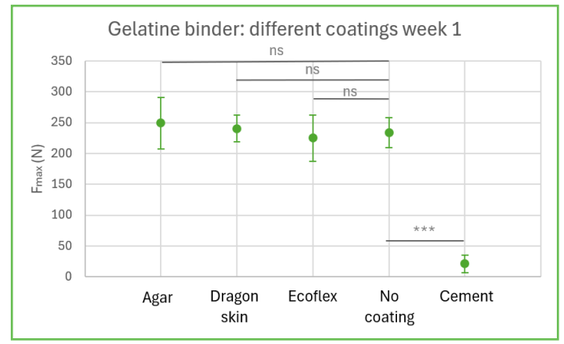


Image 46.2

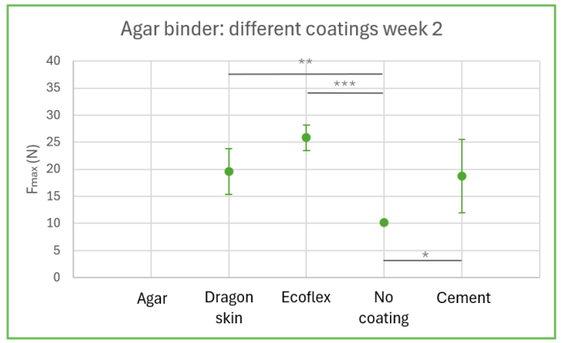


Image 46.3

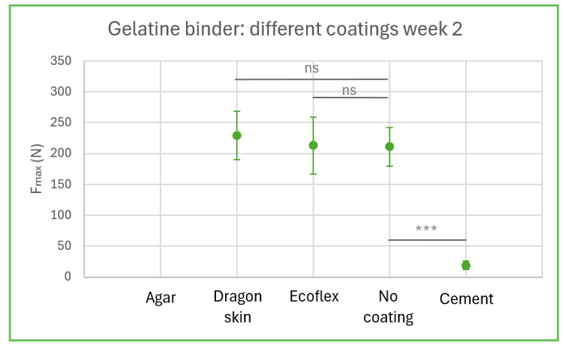


Image 46.4

Compression tests for under water use

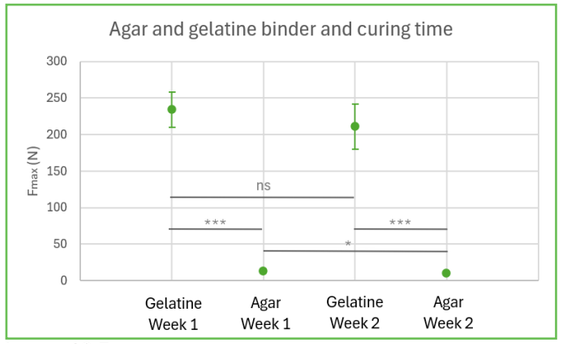


Image 46.5

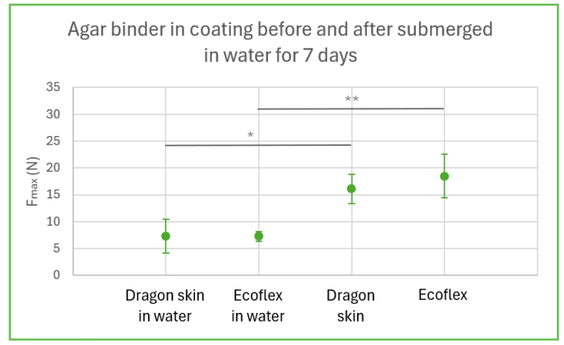


Image 46.6

Image 46, Compression test results

Conclusion

From the water test, it can be concluded that the cubes with a gelatine binder are not suitable for under water use, adding a silicone rubber or agar coating does not prevent the material from dissolving in seawater, the material still completely dissolves in two days. When a coating is applied to the material, the original shape of the material will be maintained, but the material itself will dissolve within the coating. The dissolving of the material within the rubber silicone coating suggests that there is an exchange of substances through the coating, which can be ideal for keeping living materials alive like the living composite of Li et al. but is not ideal for our material (Li et al., 2023).

The agar bonded cubes with no coating on the other hand, stayed together in seawater and maintained their shape for 7 days but were very fragile and could not be taken out of the seawater. The cubes with silicone rubber coatings stayed sturdy and were compression tested. It can be concluded that agar is a more suitable binder for under water use. Adding a silicone rubber coating to the agar bonded material improves its durability when put in seawater, which is beneficial for temporary structure use and under water application. But the material is not strong enough, after a week in seawater the material can only handle 7N. Furthermore, agar as a binder is not suitable for above water use if the load will be more than 16N on 1mm³, gelatine as a binder is more suitable because it can handle more weight.

When the gelatine bonded cubes without a coating are compared with the gelatine bonded cubes with a coating, it can be concluded that there is no significant difference in the load they can withstand under compression. Therefore, the coating does not have an influence on the strength of the material and therefore should not be added to improve strength. Also for the agar bonded cubes the coating does not have a significant influence on the strength of the material, only after two weeks of curing a significant difference is detected. After being submerged in seawater, the agar bonded cubes with a silicone rubber coating are significantly stronger than the cubes with no coating, so to improve the agar bonded material, the material first needs to cure for 3 days, after which a silicone rubber coating needs to be added, followed by a 4 day cure.

For above water use a gelatine binder is most suitable because of its strength. Adding a coating does not add benefits.
For under water use an agar binder is preferred, adding a silicone rubber coating of Dragon skin 30 or Ecoflex 00-10 prolongs the survivability.
For temporary construction under water an agar binder with a rubber silicone coating is the most suitable. For temporary construction above water, a gelatine and agar binder are suitable as long as the agar bonded material does not need to handle a load more than 16N.

8.3 Biomineralization time

The last design change that has been implemented was changing the biomineralization time. The choice to focus on this aspect of the process is based on the biomineralization process, this process needs time to happen, the longer the bacteria have to biomineralize, the more crystals will be present in the medium and potentially strengthen the material. Combined with including the sand in the medium during biomineralization, there is a chance that the crystals will attach to the sand particles and therefore also potentially strengthen the material.
It was decided to use two different biomineralization times to compare: 2 days and 7 days (see image 47).

To allow the medium, sand and bacteria to be mixed properly during their biomineralization time, the flasks with the mixture are placed on a shaker, 130 rpm. The gelatine is excluded form the mixture during biomineralization and is added after that phase because it would solidify and interrupt the shaking motion of the mixture which would hinder the binding of the crystals with the sand particles. To allow for even better mixing, the ratio of medium and sand was changed to 500ml/1000gr, leading to a more fluid mixture.

The cubes were made following the protocol in image 48 and appendix C.
The cubes that were developed are shown in image 47 and 48.

Material appearance and behaviour

When placed on the shaker the adjusted medium/sand ratio and the exclusion of gelatine allowed for enough movement for the medium, the mixture could therefore mix properly and provided access to all components. When the material was being put in the moulds, a part of the medium surfaced and had therefore to be discarded, this was due to the adjusted medium/sand ratio. It is therefore not possible to know what the final medium/sand ratio in the cubes was.

Next to this the adjusted medium/sand ratio allowed for more consistently cast cubes, the liquidity of the mixture led to better distribution of the mixture in the moulds, the material therefore was better shaped and had no gabs, leading to easier demoulding and no deformation of the cube during the curing process.

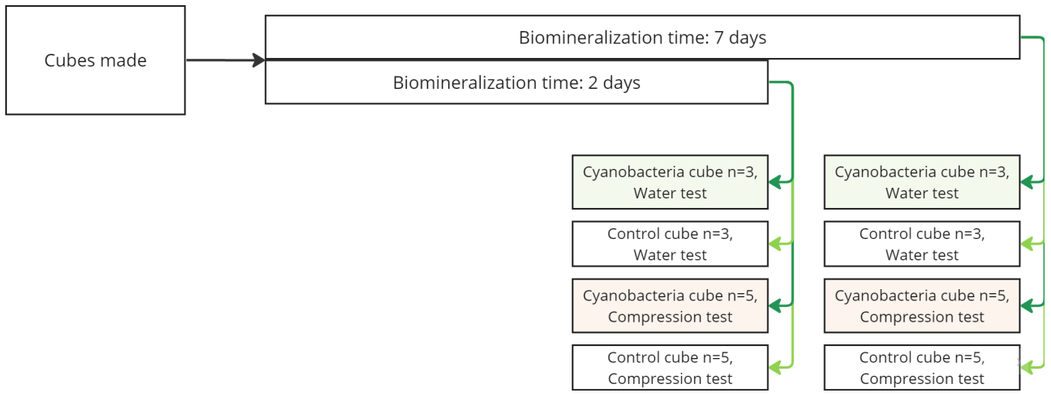


Image 47, Overview of developed cubes

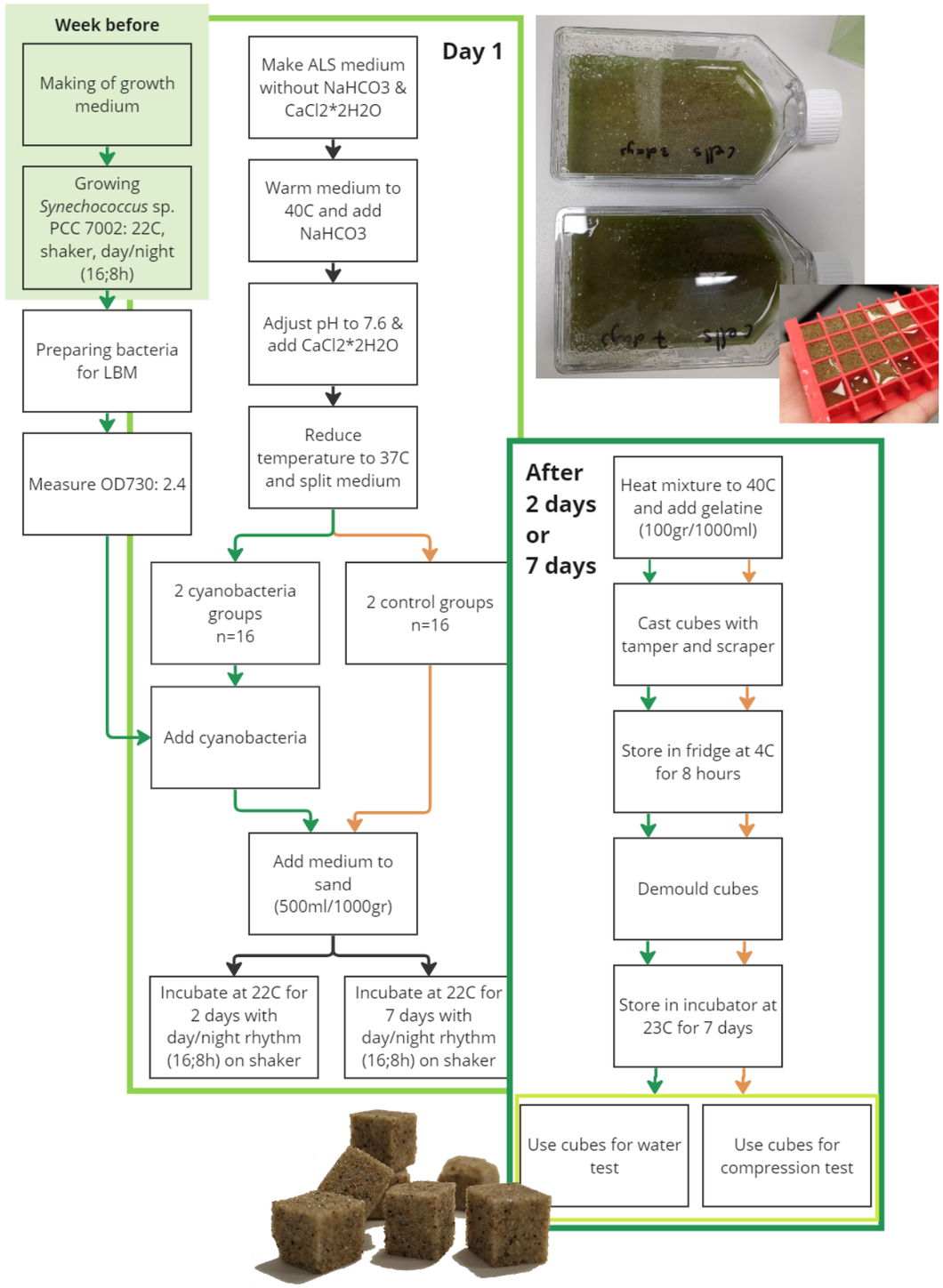


Image 48, Overview of preparation of gelatine binded samples, adapted from Heveran et al., 2020

Results and discussion

Water test
Once the cubes with a biomineralization time of 2 days were demoulded and had cured for 7 days, they were submerged in seawater (see image 49). On day zero the cubes maintained their integrity. The next day all the control cubes and cyanobacteria cubes were partially dissolved, the same that was observed in chapter 8.1 and 8.2. On day two all the cubes were completely dissolved.

The cubes with a biomineralization time of 7 days were also submerged in seawater after 7 days of curing (see image 49). Also here the cubes maintained their integrity on day zero. The next day all the cubes were partially dissolved and the second day all the cubes had dissolved completely.

Compression test
The compression test of the material that biomineralized for 2 days (see image 50.2) showed that the material with cyanobacteria could withstand an average maximum force of 1025N and the control material could withstand an average maximum force of 1063N, there is no significant difference between these two materials.

The compression test of the material that biomineralized for 7 days (see image 50.2 and appendix B) showed that there is a significant difference between the material with and without cyanobacteria. With cyanobacteria the material could withstand an average maximum force of 766N and without cyanobacteria it could withstand 1251N.

When the material with cyanobacteria and a biomineralization time of 2 and 7 days is compared (see image 50.1), it can be seen that the material with a biomineralization time of 7 days lost significant strength, but was still significantly stronger than the material used in previous experiments with a biomineralization time of 18 hours. When the control material is compared after 2 and 7 days of biomineralization (see image 50.2) no significant difference can be detected, this group does not change over time and is therefore more stable than the material with cyanobacteria.

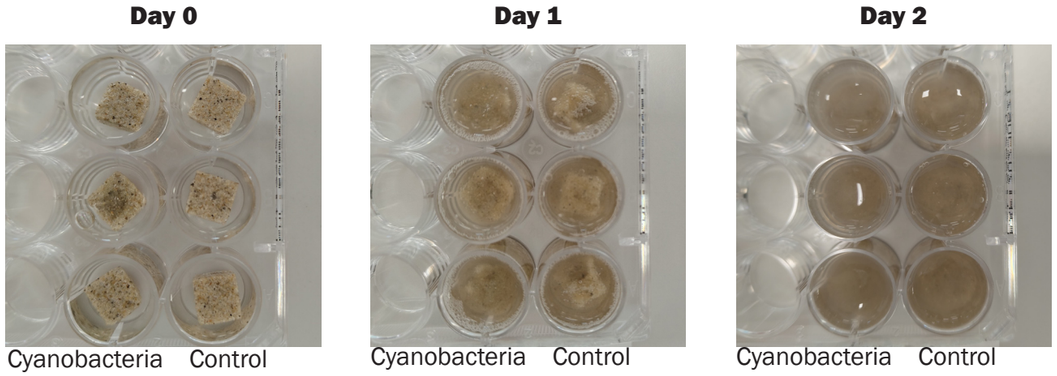


Image 49, Overview of cubes in water after 7 days of biomineralization. It also represents the results after 2 days of biomineralization.

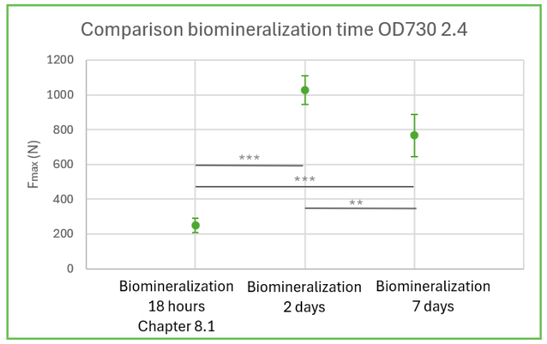


Image 50.1

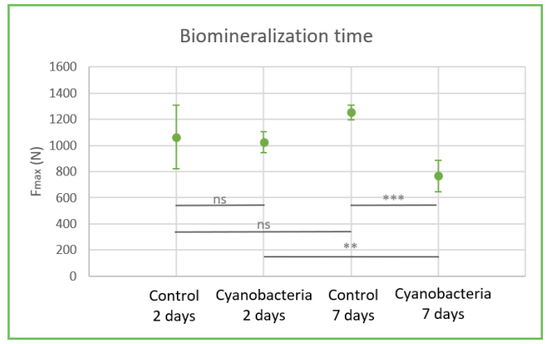


Image 50.2

Image 50,Compression test results

Conclusion

Even though the cubes with longer biomineralization time are significantly stronger than the cubes with a shorter biomineralization time from previous tests, they still dissolve in seawater in the same amount of time, two days. This does not make the material suitable for the under water use.

The cubes with a longer biomineralization time of 2 or 7 days are significantly stronger than the cubes with a shorter biomineralization time of 18 hours from previous tests, but it cannot be concluded if the increase in strength comes from the different sand/medium ratio or from the increased biomineralization time since they were adjusted simultaneously. But the improved strenght provides more application possibilities for the material. Despite the strength of the material, the bacteria add no significant extra strength after a biomineralization time of 2 days, they even weaken the material compared to the control material after a biomineralization time of 7 days.

To really know if the cyanobacteria have a significant influence on the overall development of the material, an option is to test if the material improves with bacteria compared to the material without bacteria when the environment does not have a lot of $CaCl_2 *2H_2O$. This way the calcification reaction cannot happen spontaneously and the control material will have less crystals compared to the material with bacteria which will speed up the calcification reaction.

For above water use a biomineralization time of 2 days is most suitable since it strengthens the material the most.
For under water use none of the biomineralization times improve the material, since it dissolves.
For temporary construction the biomineralization time also has no usefull influence when applied under water, but above water the biomineralization time of 2 days is most suitable because of the strength.

Chapter 9

Material suitable for offshore applications

Conclusion

The goal of this project was to design a material that caters to the requirements of offshore applications to contribute to a more sustainable practice. Three offshore applications have been selected for the appliance of this material: Under water use, Above water use, Temporary construction. The material can also have applications where it can be used in a combination of the applications, being partially submerged or being applied in the splash zone of for example bridges.

With no alterations the cyanobacteria biomineralized material can be recreated from other studies. The cyanobacteria biomineralization has been proven with SEM observations, still if this submerged in seawater it dissolves within days. The speed of the dissolving process depends on the size of the material, cubes of 35mm take three days to completely dissolve whilst cubes of 10mm completely dissolve within two days. This leads to suspect that when the material is applied with large dimensions the material will be able to withstand the offshore conditions for a longer period of time, which could be beneficial for temporary construction.

When the recipe of the material is altered, which resulted in a design space, (see image 51) application possibilities arise. The first adaptation that had been made is changing the cyanobacteria density. A cyanobacteria optical density of 2.4 turned out to be the strongest compared to all the other tested optical densities. The control material will eventually gain around the same strength but takes longer to get there, the density of 2.4 therefore speeds up the biomineralization process, making it possible to speed up the construction time needed before the material can be applied. On the other hand the survivability of the material submerged in seawater with different cyanobacteria densities did not improve, it completely dissolved within two days.

The next change to the material was changing the binder and adding coatings. The chosen binder was agar because it is water resistant. Putting this agar bonded material in seawater resulted in the material maintaining its shape for at least 7 days, but the material was very weak and collapsed when touched. Adding a silicone rubber coating of Dragon skin 30 or Ecoflex 00-10 improved its strength when applied in water but goes against the sustainable quality of the material. On the contrary testing the material for above water use resulted in low strength of the agar bonded material, making the agar not a preferred binder for above water use when large loads will be applied.
Adding a silicone rubber coating to the gelatine bonded cubes had no influence on the dissolvability of the material, it maintained its shape but the material itself still dissolved within the coating.

Changing the biomineralization time was the next adaptation to the material. Prolonging the biomineralization time, and therefore keeping the cyanobacteria alive for a longer period of time, gives the cyanobacteria more time to biomineralize and thus probably improving the material's qualities. The material was significantly stronger than the material with a shorter biomineralization time. Because the biomineralization time had been prolonged the sand/medium ratio was simultaneously adjusted in the protocol, it is therefore not possible to know if the change in strength comes from the biomineralization time or the ratio.
The significant difference in strength of the material did not improve its dissolvability in seawater.
Next to this there was no significant difference between the control material and the material with cyanobacteria, this is probably due to the presence of big amounts of $CaCl_2*2H_2O$,

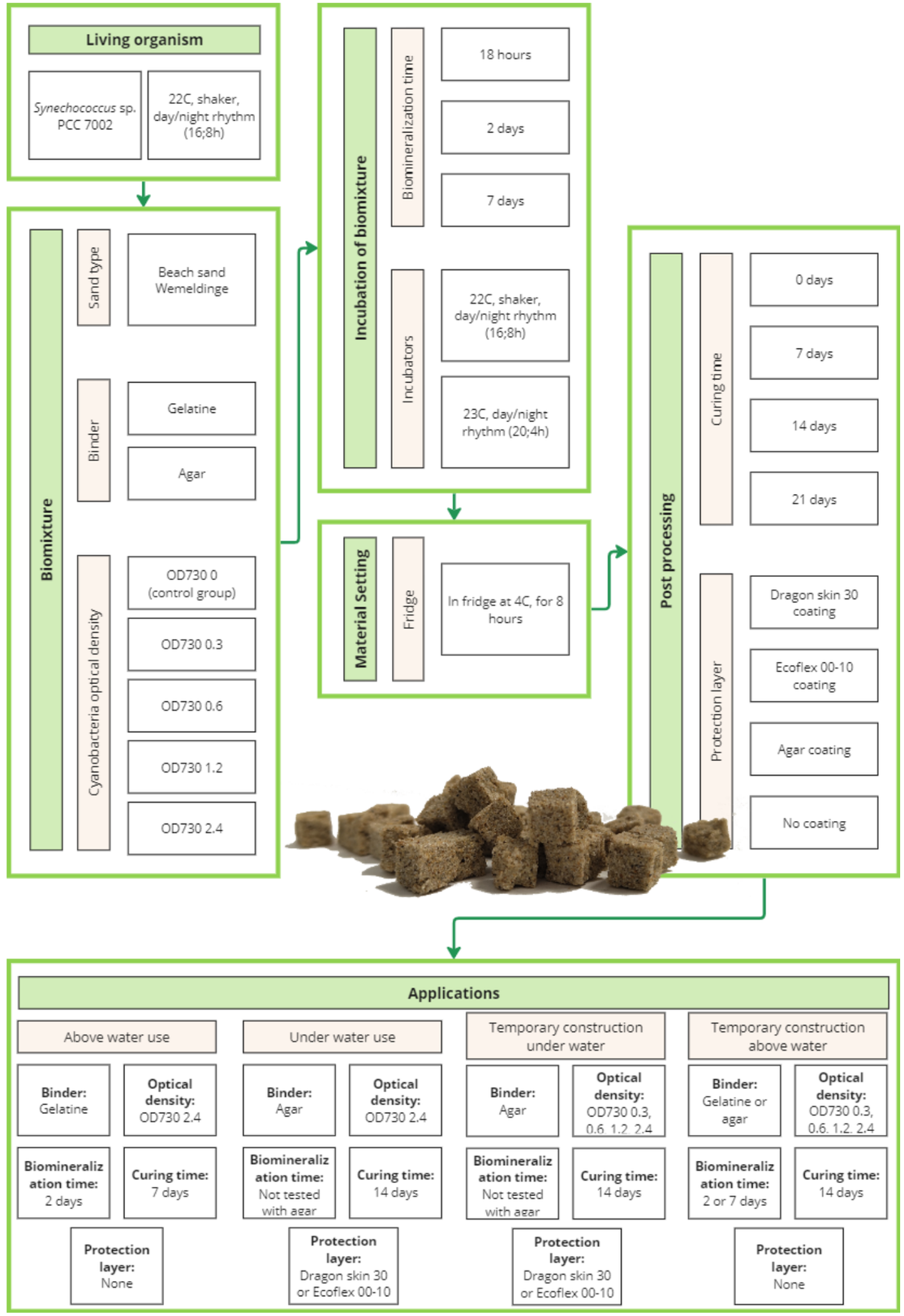


Image 51, Design space of the cyanobacteria biomineralized material

leading to the calcification reaction to also occur spontaneously in big amounts leading to the creation of many crystals. This way it is not possible to see the influence of the cyanobacteria, it is therefore beneficial to do more experiments with lower $CaCl_2*2H_2O$ levels so the cyanobacteria can biomineralize but the spontaneous calcification reaction cannot create so many crystals.

One of the potentials for this material is that it contains a living component, when kept viable this component can add beneficial functions to the material, like regeneration (Heveran et al., 2020) or potentially self-healing abilities. Because the cyanobacteria biomineralized material needs time to cure to gain integrity, it should first be created above water and given time to cure before it can be applied. In the curing conditions used in this thesis the material was not viable. When the material is kept at at least 50% RH, the viability of the material improves (Heveran et al., 2020; Delesky et al., 2023). Also adding a desiccation protectant improves the materials viability (Qui et al., 2021). It is therefore beneficial to also test the materials viability when the optimal relative humidity conditions and protectant are applied.

Adding the findings of all the experiments to the chosen application options results in a different recipe for each offshore application, more research is needed to prove whether these combinations are the most optimal:

For above water use a biomineralization time of 2 days is most beneficial together with using a gelatine binder and a cyanobacteria density of 2.4. All these components resulted in the strongest material, and could therefore eventually be applied in several construction applications like the non-submerged parts of bridges.

For under water use an agar binder is most suitable, together with a silicone rubber coating of Dragon skin 30 or Ecoflex 00-10 and a cyanobacteria optical density of 2.4. The agar makes the material seawater resistant and the coating prolongs its survivability whilst the optical density of 2.4 strenghtens the material. This material still needs to be improved to be applied for under water construction like attachments to the seabed.

Temporary construction used above water needs the same components as the material for above water use but a longer biomineralization time, lower cyanobacteria optical densities (OD_{730} 0.3, 0.6, 1.2) and an agar binder can be used if the material has to resist lower forces. For underwater temporary construction this material with an agar binder is suitable when low loads are applied. Adding a coating helps maintain its shape and durability in seawater. It could therefore be applied as a protector for nature or sea animals as temporary protection layers.

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Acknowledgements

First of all, I would like to thank my supervisory team Joana Martins and Jovana Jovanova for their support and knowledge assistance, which definitely took the project to the next level. I also found the enthusiasm with which the team was involved in this project very motivating, which made for a very nice and productive working atmosphere.

Furthermore, I would like to thank Hao Shi for his additional support and assistance to the supervisory team. I would also like to thank Dr. Marie-Eve Aubin-Tam and her postdocs for the support with the SEM analysis at the Faculty of Applied Sciences, TU Delft.

Furthermore, I would like to thank Joren Wierenga for the incredibly good, pleasant and patient help in the biolab. I learned a lot there and discovered that I really have a passion for this kind of work.

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Chapter 11

Appendix

Content

Appendix A: Calculation sheet

Calculation sheet for material

To be able to make the material, a spreadsheet was made to be able to calculate all the amounts of ingredients that are needed. This Excel sheet calculates everything automatically. This sheet made the process and preparation much easier and faster. So more experiments could be done. The sheet is divided into three segments.

Ratio segment

The first segment is the ratio segment, this contains all the ratio's that are used for the making of the cubes (Heveran et al., 2020). The light green segments can be changed, this is the cube size.

Ratios 300 ml mixture / 1 kg sand
100 gr gel / 1000 ml medium

Sand ml	10	A
Sand gr	16	1000

Sand ml	10	625
Sand gr	16	1000

A= 625

Calculation tables

Amount of sand and mix

Sand ml	625	Y
Mix ml	300	X
Total ml (volume cube)	925	1,00

Sand ml	625	Y
Mix ml	300	0,32
Total ml (volume cube)	925	1,00

Sand ml	625	0,68
Mix ml	300	0,32
Total ml (volume cube)	925	1,00

X= 0,32 Y= 0,68

Amount of gelatine and mix

Gelatine gr	100	Z
Mix ml total	1000	0,32

Gelatine gr	100	0,0324
Mix ml	1000	0,32

Z= 0,03

Group segment

The second segment is the group segment. This is an automatically calculated overview of all the ingredients needed to make the different types of cubes. One group of cubes with bacteria and one group of cubes without bacteria. The light green segments can be changed.

Amount of blocks **with cells**	60
Amounts with calculated **extra material** with cells **(+1 block)**	
Total amount of sand needed	41,22 ml
Total amount of mix needed (gel+medium)	19,78 ml
Total amount of gelatine needed	1,98 gr
Total amount of medium needed	19,78 ml

Amount of blocks **without cells**	52
Amounts with calculated **extra material** without cells **(+30%)**	
Total amount of sand needed	46,85 ml
Total amount of mix needed (gel+medium)	22,49 ml
Total amount of gelatine needed	2,25 gr
Total amount of medium needed	22,49 ml

Amount of medium	0,020 L		
	ml	**ul**	**gr**
Monopotassium phosphate		4,946	0,001
Magnesium sulfate	0,405		0,099
Sodium nitrate	0,028		0,020
Ferric ammonium citrate + EDTA		19,784	
Potassium chloride	0,237		0,012
Tris HCl			0,020
Calcium chloride	1,939		0,291
Sodium bicarbonate			0,166
Gelatine			2,0

Amount of medium	0,022 L		
	ml	**ul**	**gr**
Monopotassium phosphate		5,621	0,001
Magnesium sulfate	0,461		0,112
Sodium nitrate	0,032		0,022
Ferric ammonium citrate + EDTA		22,486	
Potassium chloride	0,270		0,013
Tris HCl			0,022
Calcium chloride	2,204		0,331
Sodium bicarbonate			0,189
Gelatine			2,25

Overview segment

The last segment is an overview of all the ingredients needed for the two groups of cubes that need to be made. Because the medium needed to be made all at once and was then split into the bacteria and no bacteria groups.

Total amount of blocks	112
Amounts with calculated **extra material**	
Total amount of sand needed	88,06 ml
Total amount of mix needed (gel+medium)	42,27 ml
Total amount of gelatine needed	4,23 gr
Total amount of medium needed	42,27 ml

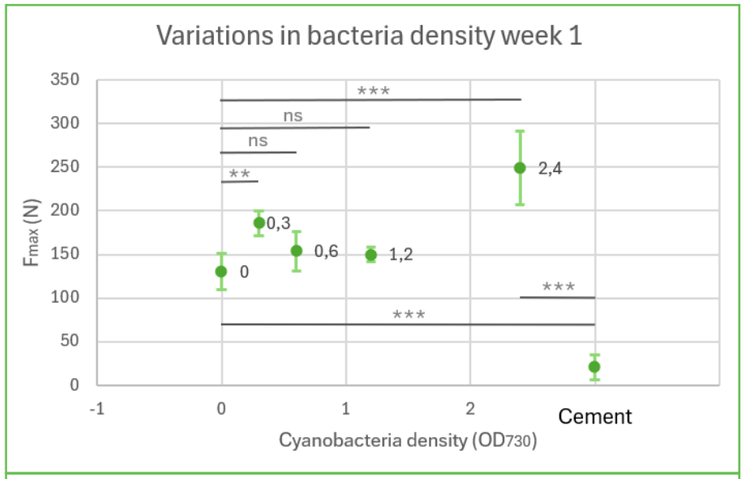
Amount of medium	0,042 L		
	ml	**ul**	**gr**
Monopotassium phosphate		10,567	0,002
Magnesium sulfate	0,866		0,211
Sodium nitrate	0,060		0,042
Ferric ammonium citrate + EDTA		42,270	
Potassium chloride	0,507		0,025
Tris HCl			0,042
Calcium chloride	4,142		0,621
Sodium bicarbonate			0,355
Gelatine			4,23

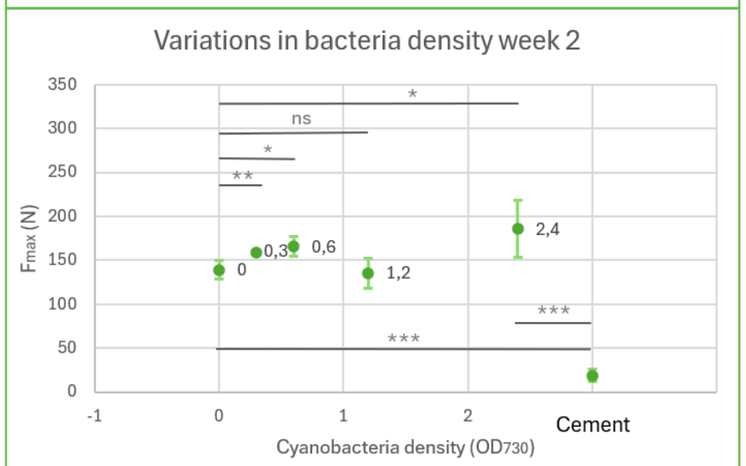
Appendix B: Compression tests

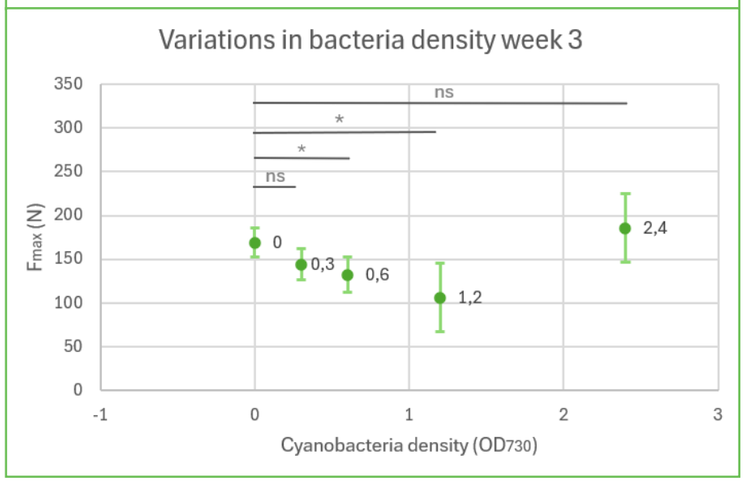
Variations in bacteria density

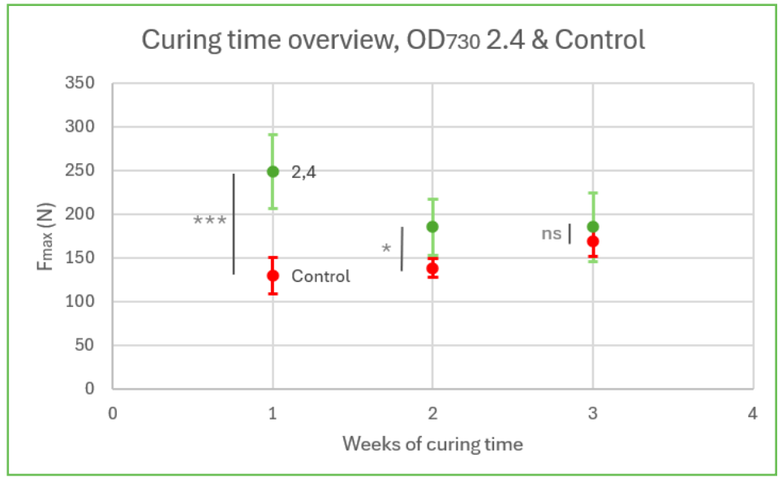
The results of compression tests that have been conducted with cubes of 1cm by 1cm by 1cm with different densities of cyanobacteria are shown in the images below.

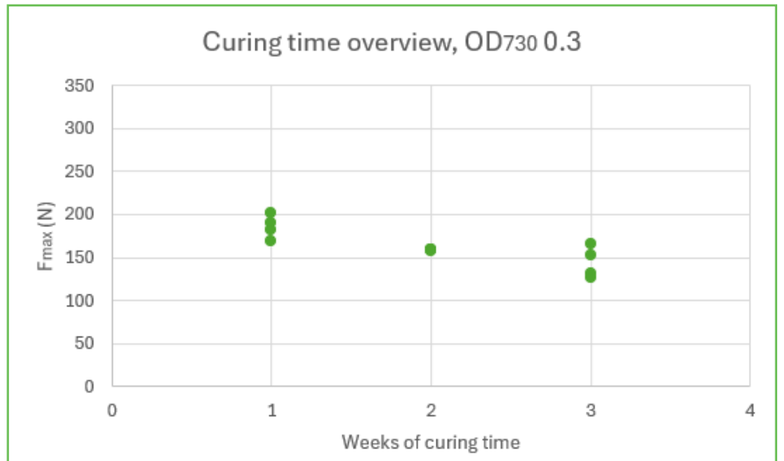
Five groups of cubes were made: one a control group without cyanobacteria (0), and four groups of cubes with either a bacteria density of 0.3, 0.6, 1.2 or 2.4.
The five groups of cubes were set to cure for 7 days, 14 days or 21 days.

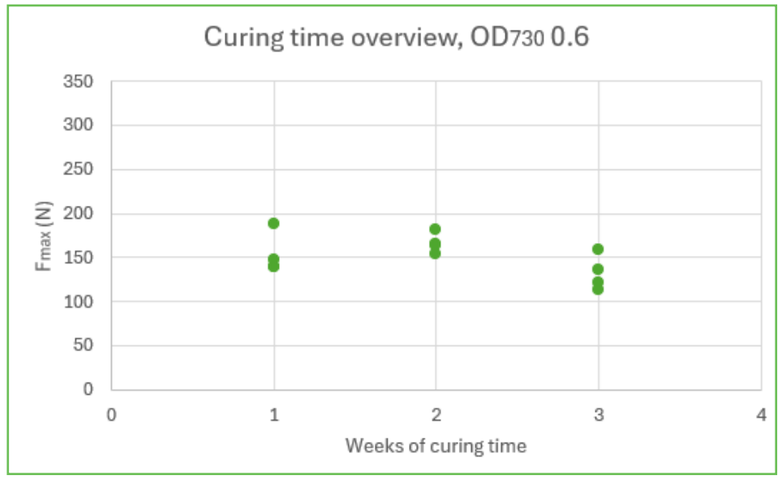


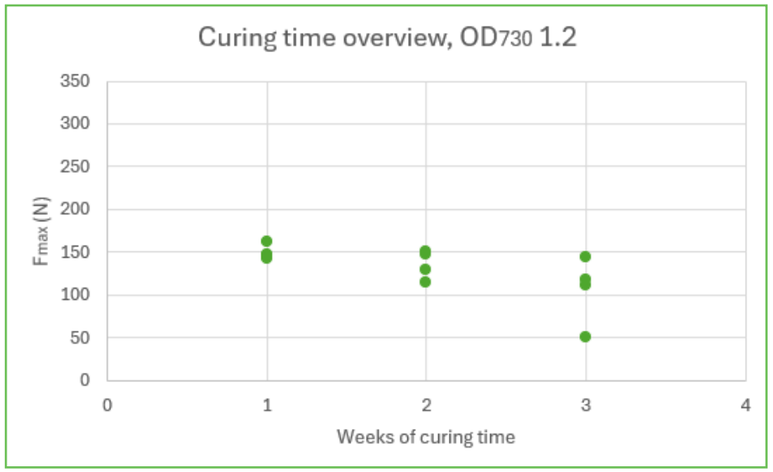


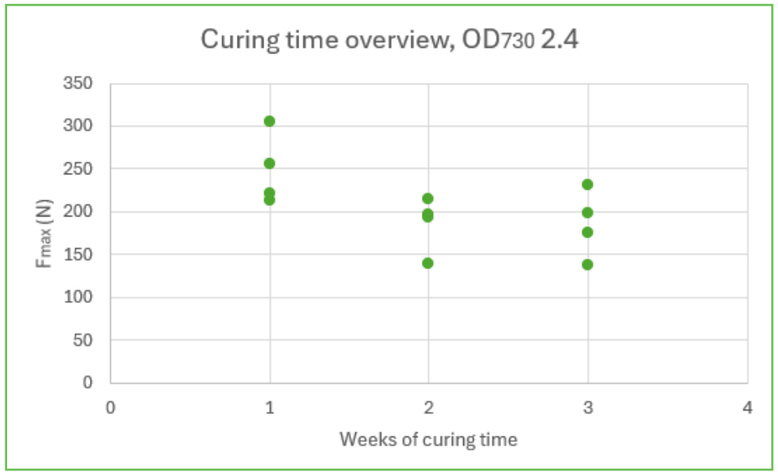


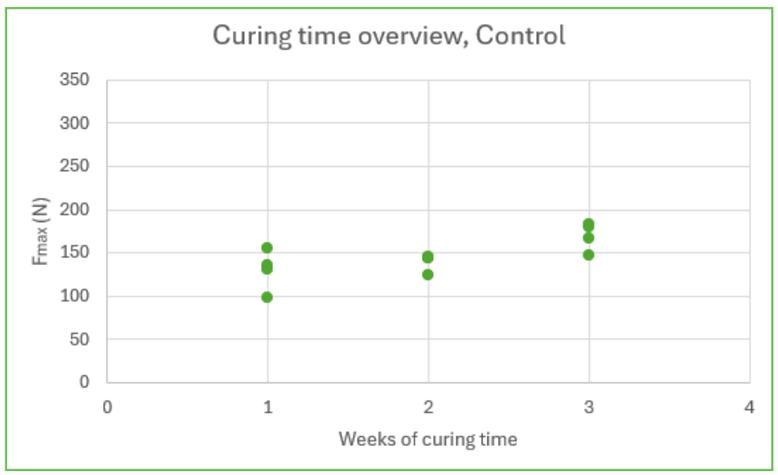






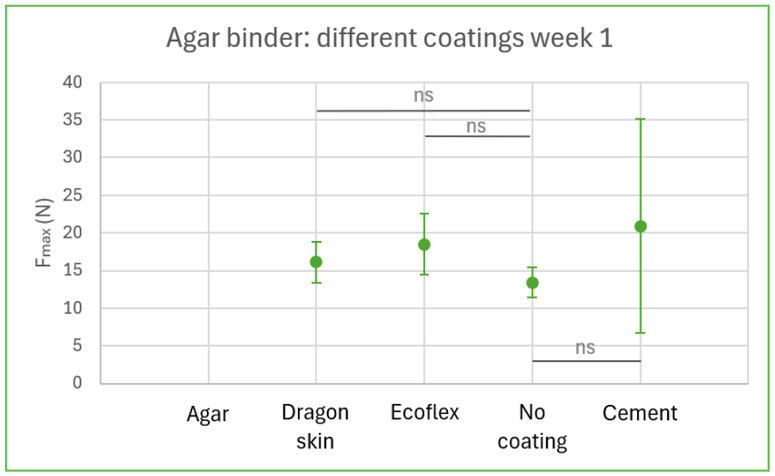


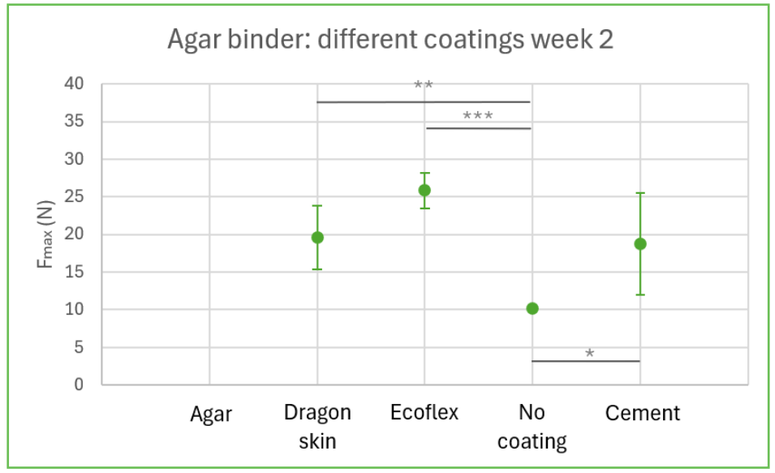


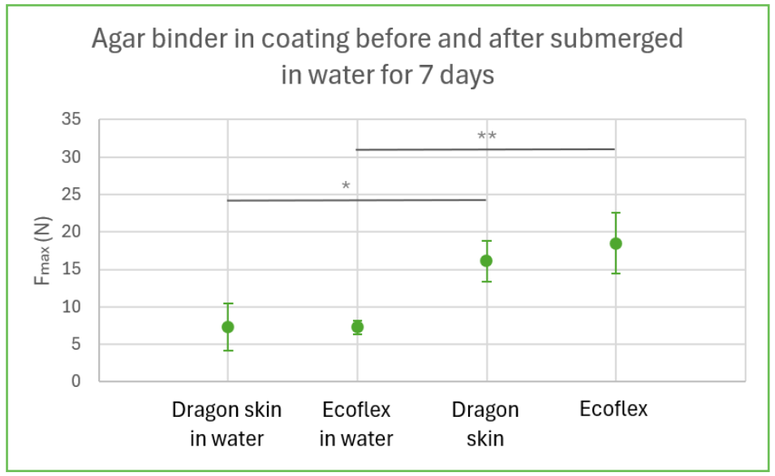


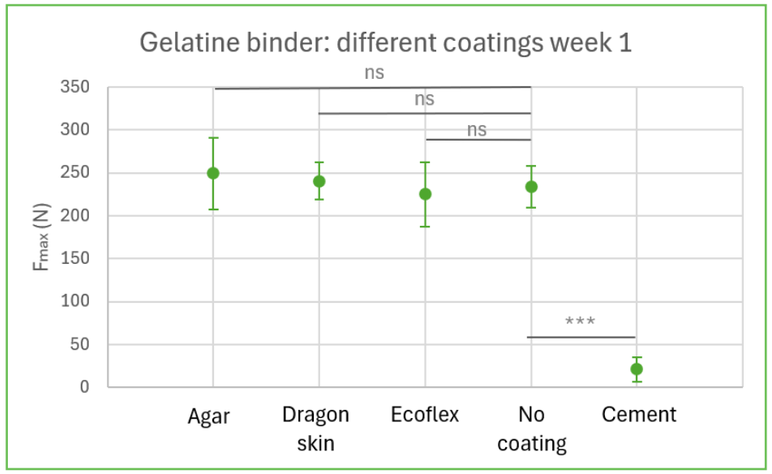
Different binders and coatings

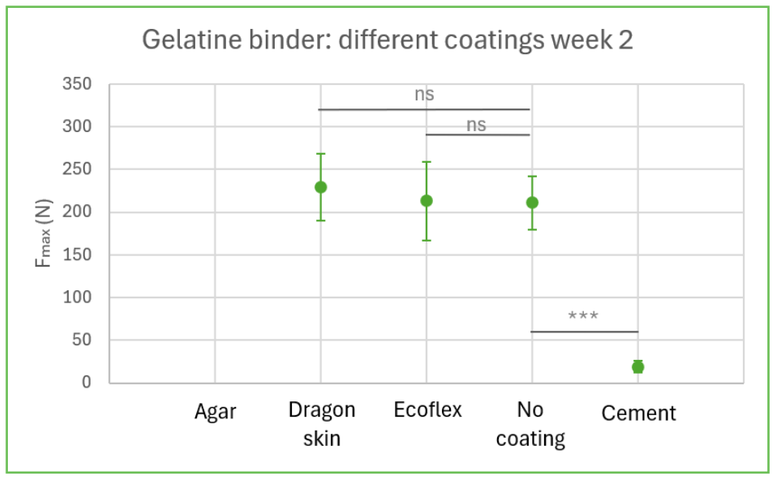
A different binder than gelatine, agar, was compression tested. Also the cubes were given coatings. The results of these compression tests can be found in the images below.

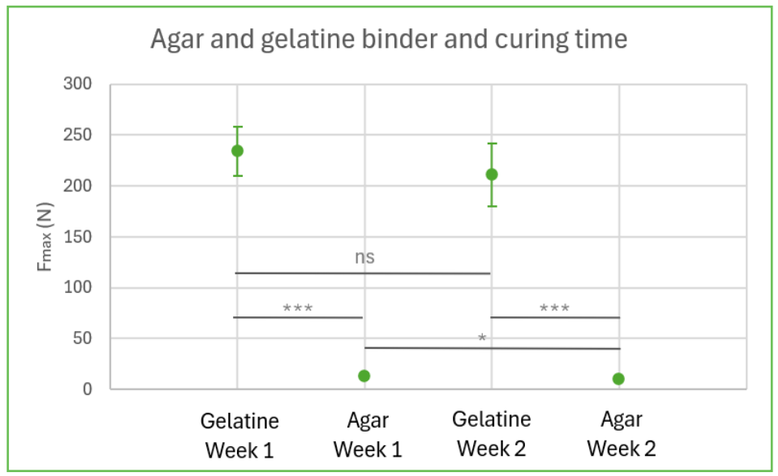






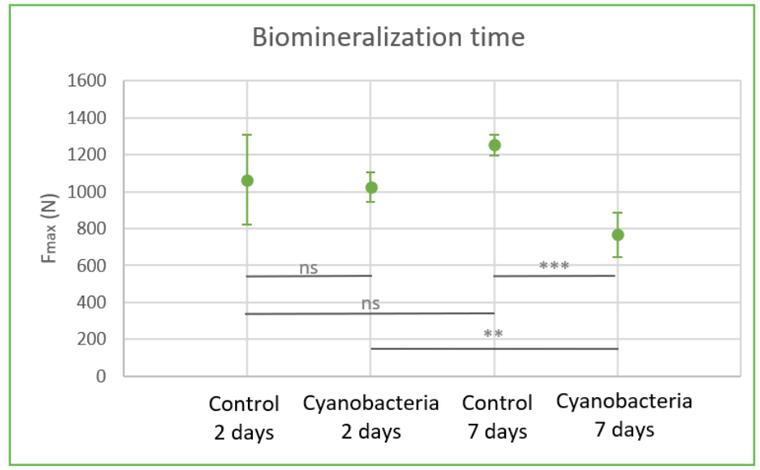


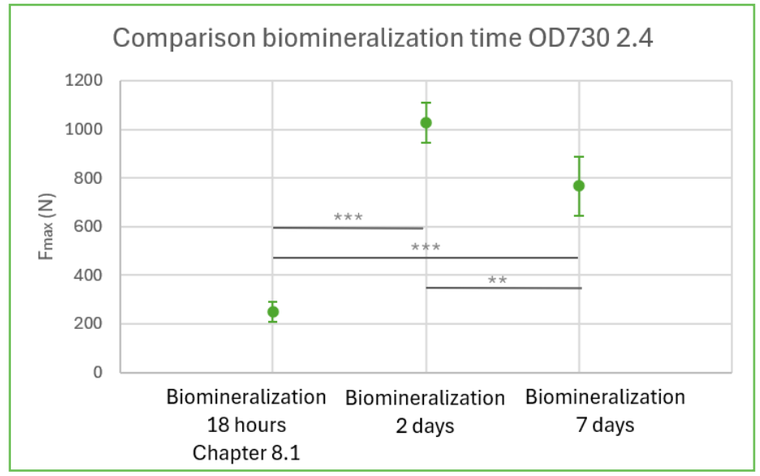




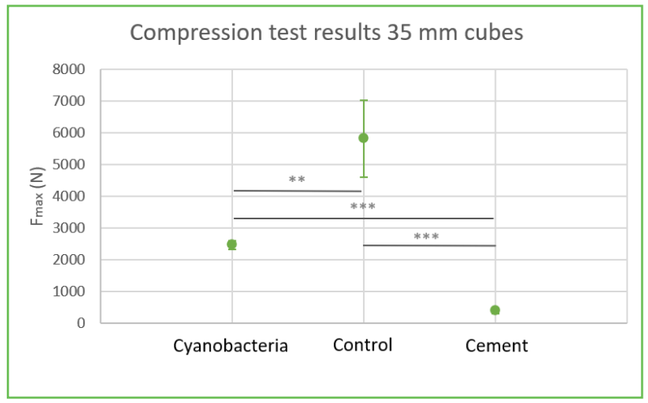
Biomineralization time

A longer biomineralization time is tested. Instead of 22 hours of biomineralization time the time was changed to 2 days and 7 days. They were compression tested and the results can be found in the images below.





Material performance in submerged conditions



Appendix C: Ingredients overview

Material performance in submerged conditions

Amount of blocks **without cells**	9			
Amounts with calculated **extra material** without cells **(+30%)**				
Total amount of sand needed	395,99	ml		
Total amount of mix needed (gel+medium)	118,80	ml		
Total amount of gelatine needed	11,88	gr		
Total amount of medium needed	106,92	ml		
Amount of medium	0,107	L		
	ml	**ul**	**gr**	
Monopotassium phosphate		26,729	0,005	
Magnesium sulfate	2,190		0,535	
Sodium nitrate	0,152		0,107	
Ferric ammonium citrate + EDTA		106,917		
Potassium chloride	1,283		0,064	
Tris HCl			0,107	
Calcium chloride	10,478		1,572	Seperate in distilled water
Sodium bicarbonate			0,898	Add last
Gelatine			11,88	

Take distilled water amount of total volume - Calcium chloride volume!!

Amount of blocks **with cells**	6			
Amounts with calculated **extra material** with cells **(+1 block)**				
Total amount of sand needed	231,00	ml		
Total amount of mix needed (gel+medium)	69,30	ml		
Total amount of gelatine needed	6,93	gr		
Total amount of medium needed	62,37	ml		
Amount of medium	0,062	L		
	ml	**ul**	**gr**	
Monopotassium phosphate		15,593	0,003	
Magnesium sulfate	1,278		0,312	
Sodium nitrate	0,089		0,062	
Ferric ammonium citrate + EDTA		62,370		
Potassium chloride	0,748		0,037	
Tris HCl			0,062	
Calcium chloride	6,112		0,917	Seperate in distilled wate
Sodium bicarbonate			0,524	Add last
Gelatine			6,9	

Take distilled water amount of total volume - Calcium chloride volume!!

Amount of blocks	15			
Amounts with calculated extra material				
Total amount of sand needed	626,99	ml		
Total amount of mix needed (gel+medium)	188,10	ml		
Total amount of gelatine needed	18,81	gr		
Total amount of medium needed	169,29	ml		
Amount of medium	0,169	L		
	ml	**ul**	**gr**	
Monopotassium phosphate		42,322	0,008	
Magnesium sulfate	3,468		0,846	
Sodium nitrate	0,241		0,169	
Ferric ammonium citrate + EDTA		169,287		
Potassium chloride	2,031		0,102	
Tris HCl			0,169	
Calcium chloride	16,590		2,489	Seperate in distilled water
Sodium bicarbonate			1,422	Add last
Gelatine			18,81	

Take distilled water amount of total volume - Calcium chloride volume!!

Cyanobacteria optical density and curing time

Amount of blocks **without cells**	18	24 blocks for extra material	
Amounts with calculated **extra material** without cells **(+1/3)**			
Total amount of sand needed	16,22	ml	
Total amount of mix needed (gel+mediu	7,78	ml	
Total amount of gelatine needed	0,78	gr	
Total amount of medium needed	7,78	ml	
Amount of medium	0,00778	L	
	ml	**ul**	**gr**
Monopotassium phosphate		1,946	0,00039
Magnesium sulfate	0,159		0,039
Sodium nitrate	0,011		0,008
Ferric ammonium citrate + EDTA		7,784	
Potassium chloride	0,093		0,005
Tris HCl			0,008
Calcium chloride	0,763		0,114
Sodium bicarbonate			0,065
Gelatine			0,78
Take distilled water amount of total volume - Calcium chloride volume!!			

Amount of blocks **with cells**			60	
Amounts with calculated **extra material** with cells **(+1 block)**				
Total amount of sand needed			41,22	ml
Total amount of mix needed (gel+medium)			19,78	ml
Total amount of gelatine needed			1,98	gr
Total amount of medium needed			19,78	ml
Amount of medium	0,020	L		
	ml	**ul**	**gr**	
Monopotassium phosphate		4,946	0,0010	
Magnesium sulfate	0,405		0,099	
Sodium nitrate	0,028		0,020	
Ferric ammonium citrate + EDTA		19,784		
Potassium chloride	0,237		0,012	
Tris HCl			0,020	
Calcium chloride	1,939		0,291	Seperate in distilled w
Sodium bicarbonate			0,166	Add last
Gelatine			2,0	
Take distilled water amount of total volume - Calcium chloride volume!!				

Amount of blocks	78			
Amounts with calculated extra material				
Total amount of sand needed	57,43	ml		
Total amount of mix needed (gel+mediu	27,57	ml		
Total amount of gelatine needed	2,76	gr		
Total amount of medium needed	27,57	ml		
Amount of medium	0,0276	L		
	ml	**ul**	**gr**	
Monopotassium phosphate		6,892	0,0014	
Magnesium sulfate	0,565		0,138	
Sodium nitrate	0,039		0,028	
Ferric ammonium citrate + EDTA		27,567		
Potassium chloride	0,331		0,017	
Tris HCl			0,028	
Calcium chloride	2,702		0,405	Seperate in distilled water
Sodium bicarbonate			0,232	Add last
Gelatine			2,76	
Take distilled water amount of total volume - Calcium chloride volume!!				

Coating, binder and curing time

Amount of blocks **with cells in AGAR**	60				
Amounts with calculated **extra material** with cells **(+1 block)**					
Total amount of sand needed	41,22	ml			
Total amount of mix needed (gel+mediu	19,78	ml			
Total amount of gelatine needed	1,98	gr			
Total amount of medium needed	19,78	ml			
Amount of medium	0,020	L			
	ml		**ul**	**gr**	
Monopotassium phosphate					
Magnesium sulfate					
Sodium nitrate					
Ferric ammonium citrate + EDTA					
Potassium chloride					
Tris HCl					
Calcium chloride	**1,939**			**0,291**	Seperate in distilled water
Sodium bicarbonate				**0,166**	Add last
Gelatine					

Take distilled water amount of total volume - Calcium chloride volume!!

Amount of blocks **with cells**	60				
Amounts with calculated **extra material** with cells **(+1 block)**					
Total amount of sand needed	41,22	ml			
Total amount of mix needed (gel+medium)	19,78	ml			
Total amount of gelatine needed	1,98	gr			
Total amount of medium needed	19,78	ml			
Amount of medium	0,020	L			
	ml		**ul**	**gr**	
Monopotassium phosphate			4,946	0,001	
Magnesium sulfate	0,405			0,099	
Sodium nitrate	0,028			0,020	
Ferric ammonium citrate + EDTA			19,784		
Potassium chloride	0,237			0,012	
Tris HCl				0,020	
Calcium chloride	1,939			0,291	Seperate in distilled wa
Sodium bicarbonate				0,166	Add last
Gelatine				2,0	

Take distilled water amount of total volume - Calcium chloride volume!!

Amount of blocks	120				
Amounts with calculated extra material					
Total amount of sand needed	82,43	ml			
Total amount of mix needed (gel+mediu	39,57	ml			
Total amount of gelatine needed	3,96	gr			
Total amount of medium needed	39,57	ml			
Amount of medium	0,040	L			
	ml		**ul**	**gr**	
Monopotassium phosphate			4,946	0,001	
Magnesium sulfate	0,405			0,099	
Sodium nitrate	0,028			0,020	
Ferric ammonium citrate + EDTA			19,784		
Potassium chloride	0,237			0,012	
Tris HCl				0,020	
Calcium chloride	3,878			0,582	Seperate in distilled water
Sodium bicarbonate				0,332	Add last
Gelatine				3,96	

Take distilled water amount of total volume - Calcium chloride volume!!

Biomineralization time

Amount of blocks **without cells**	40		
Amounts with calculated **extra material** without cells **(+30%)**			
Total amount of sand needed	29,63	ml	
Total amount of mix needed (gel+medium)	23,70	ml	
Total amount of gelatine needed	2,37	gr	
Total amount of medium needed	23,70	ml	

Amount of medium	0,024	L	
	ml	**ul**	**gr**
Monopotassium phosphate		5,926	0,001
Magnesium sulfate	0,486		0,119
Sodium nitrate	0,034		0,024
Ferric ammonium citrate + EDTA		23,703	
Potassium chloride	0,284		0,014
Tris HCl			0,024
Calcium chloride	2,323		0,348
Sodium bicarbonate			0,199
Gelatine			2,37

Amount of blocks **with cells**	40		
Amounts with calculated **extra material** with cells **(+1 block)**			
Total amount of sand needed	22,78	ml	
Total amount of mix needed (gel+medium)	18,22	ml	
Total amount of gelatine needed	1,82	gr	
Total amount of medium needed	18,22	ml	

Amount of medium	0,018	L	
	ml	**ul**	**gr**
Monopotassium phosphate		4,556	0,001
Magnesium sulfate	0,373		0,091
Sodium nitrate	0,026		0,018
Ferric ammonium citrate + EDTA		18,222	
Potassium chloride	0,219		0,011
Tris HCl			0,018
Calcium chloride	1,786		0,268
Sodium bicarbonate			0,153
Gelatine			1,8

Total amount of blocks	80		
Amounts with calculated **extra material**			
Total amount of sand needed	52,41	ml	
Total amount of mix needed (gel+medium)	41,93	ml	
Total amount of gelatine needed	4,19	gr	
Total amount of medium needed	41,93	ml	

Amount of medium	0,042	L	
	ml	**ul**	**gr**
Monopotassium phosphate		10,481	0,002
Magnesium sulfate	0,859		0,210
Sodium nitrate	0,060		0,042
Ferric ammonium citrate + EDTA		41,925	
Potassium chloride	0,503		0,025
Tris HCl			0,042
Calcium chloride	4,109		0,616
Sodium bicarbonate			0,352
Gelatine			4,19

IDE Master Graduation Project

Project team, procedural checks and Personal Project Brief

In this document the agreements made between student and supervisory team about the student's IDE Master Graduation Project are set out. This document may also include involvement of an external client, however does not cover any legal matters student and client (might) agree upon. Next to that, this document facilitates the required procedural checks:

- Student defines the team, what the student is going to do/deliver and how that will come about
- Chair of the supervisory team signs, to formally approve the project's setup / Project brief
- SSC E&SA (Shared Service Centre, Education & Student Affairs) report on the student's registration and study progress
- IDE's Board of Examiners confirms the proposed supervisory team on their eligibility, and whether the student is allowed to start the Graduation Project

STUDENT DATA & MASTER PROGRAMME

Complete all fields and indicate which master(s) you are in

Family name	Goense	6844	
Initials	DJR		
Given name	Daniëlle		
Student number	4860098		

IDE master(s) IPD ✔ DfI ☐ SPD ☐

2nd non-IDE master

Individual programme *(date of approval)*

Medisign ☐

HPM ☐

SUPERVISORY TEAM

Fill in he required information of supervisory team members. If applicable, company mentor is added as 2nd mentor

Chair	Joana Martins	dept./section	SDE / MD
mentor	Jovana Jovanova	dept./section	3ME / TEL
2nd mentor			
client:			
city:		country:	
optional comments			

! Ensure a heterogeneous team. In case you wish to include team members from the same section, explain why.

! Chair should request the IDE Board of Examiners for approval when a non-IDE mentor is proposed. Include CV and motivation letter.

! 2nd mentor only applies when a client is involved.

APPROVAL OF CHAIR on PROJECT PROPOSAL / PROJECT BRIEF -> to be filled in **by the Chair** of the supervisory team

Sign for approval (Chair)

Name Joana Martins Date 16 Oct 2023 Signature

CHECK ON STUDY PROGRESS

To be filled in **by SSC E&SA** (Shared Service Centre, Education & Student Affairs), after approval of the project brief by the chair. The study progress will be checked for a 2nd time just before the green light meeting.

Master electives no. of EC accumulated in total _____ EC

Of which, taking conditional requirements into account, can be part of the exam programme _____ EC

★	**YES**	all 1st year master courses passed
	NO	missing 1st year courses

Comments:

Sign for approval (SSC E&SA)

Name K. Veldman Date 20 Oct 2023 Signature K. Veldman

APPROVAL OF BOARD OF EXAMINERS IDE on SUPERVISORY TEAM -> to be checked and filled in by IDE's Board of Examiners

Does the composition of the Supervisory Team comply with regulations?

YES	★	Supervisory Team approved
NO		Supervisory Team not approved

Comments:

- approved for 3mE mentor

Based on study progress, students is …

★	**ALLOWED** to start the graduation project
	NOT allowed to start the graduation project

Comments:

Sign for approval (BoEx)

Name Monique von Morgen Date 31 Oct 2023 Signature

Personal Project Brief – IDE Master Graduation Project

Name student Daniëlle Goense **Student number** 4,860,098

PROJECT TITLE, INTRODUCTION, PROBLEM DEFINITION and ASSIGNMENT

Complete all fields, keep information clear, specific and concise

Project title Cyanobacteria biomineralized materials for offshore applications

Please state the title of your graduation project (above). Keep the title compact and simple. Do not use abbreviations. The remainder of this document allows you to define and clarify your graduation project.

Introduction

Describe the context of your project here; What is the domain in which your project takes place? Who are the main stakeholders and what interests are at stake? Describe the opportunities (and limitations) in this domain to better serve the stakeholder interests. (max 250 words)

Cyanobacteria are bacteria that can be found in water bodies. Fossils of 2.8 billion years ago have been found containing cyanobacteria. Their ability to perform oxygenic photosynthesis leads to the presence of oxygen on earth, being therefore, considered the initiators of life on earth (Reinhardt et al., 2023).Recently, cyanobacteria, in particular the strain Synechococcus sp. PCC 7002, were used for the development of a new living material (LBM) with possible applications within the built environment (Heveran et al., 2020). The combination of cyanobacteria with sand, gelatine, and nutrients leads to the development of a cement-like material through the biomineralization process (see figure 1). This material is also able to regenerate itself, see image 1 (Heveran et al., 2020), and absorb greenhouse gasses instead of emitting them (Bushwick, 2020). The current research has so far focused on the necessary conditions for the development of this material, still its possible applications and/or further development of it for offshore structures remains to be explored. Cyanobacteria LBMs can be very interesting for the offshore industry which deals with problems of harsh environmental conditions for materials, and CO2 emissions due to the use of equipments and concrete. The building sector is responsible for 39% of global carbon dioxide emissions, the used concrete is responsible for 11% CO2 emissions and is therefore a big contributor to climate change (Reinhardt et al., 2023; Architecture, 2022). Concrete is used a lot in the offshore industry, for only 44 oil platforms, 2.966.357 m3 concrete is used (Skinner, 2023; Fernandes et al., 2008). The making of concrete emits large amounts of CO2, if this is replaced by the LBM, the offshore industry will have less CO2 emissions and will be less impactful for the environment. Cyanobacteria are naturally found in water, which is a an added value for the development of LBM for offshore habitats. Next to this, we plan to understand if the LBM has the potential to be used for crack repairs, which will lower the effort to be made when something is broken (see figure 2), as well as lowering their CO2 emissions. Building upon that, we propose to research if these novel materials can cater to the requirements of offshore applications, thereby contributing to a more sustainable practice. This graduation project will open up new possibilities for the applications of this LBM for the offshore industry e.g., tunnels, sewers, oil rigs, dyke stones, protection for cables on the seabed, artificial corals. These structures can be used as temporary structures during assembly or growing structures at the seabed.

➔ *space available for images / figures on next page*

introduction (continued): space for images

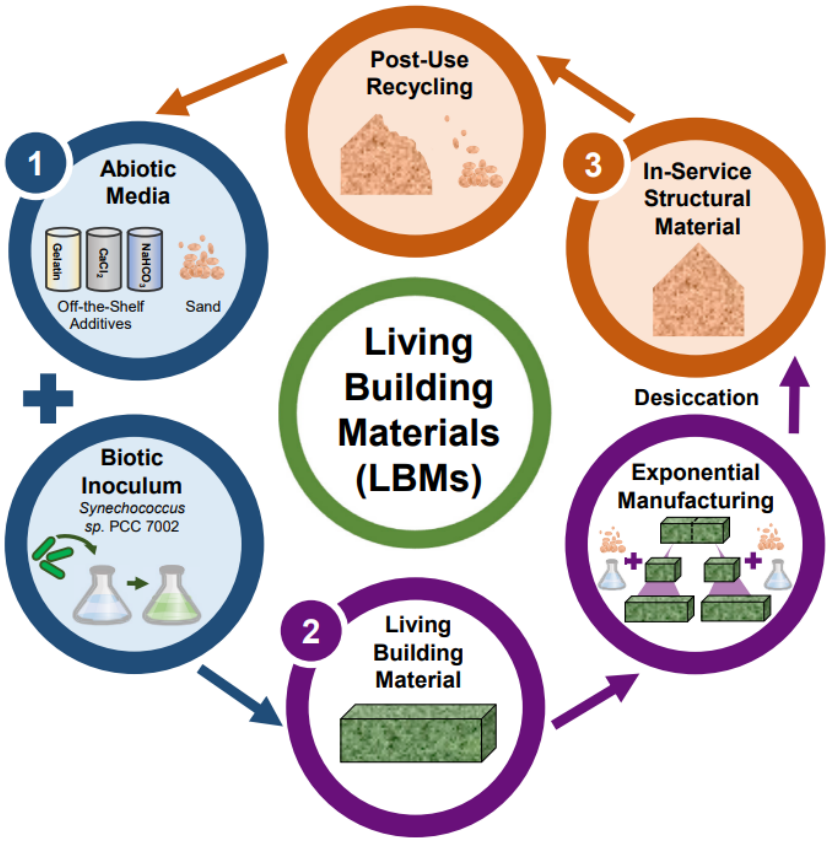


image / figure 1 How the cyano LBM is made (Heveran et al., 2020)

image / figure 2 A lot of effort needs to be made to place or replace materials for offshore projects.

Personal Project Brief – IDE Master Graduation Project

Problem Definition

What problem do you want to solve in the context described in the introduction, and within the available time frame of 100 working days? (= Master Graduation Project of 30 EC). What opportunities do you see to create added value for the described stakeholders? Substantiate your choice.
(max 200 words)

There is an urgent need to develop novel materials and solutions for the offshore industry.
The first step of this project is validating the replication of cyanobacterial biomineralization for the development of LBM using the same conditions applied by Heveran et al. Secondly, new LBM will be designed through a combination of different sand types, temperature conditions (to simulate natural conditions), and hydrogels. Lastly, the materials will be tested simulating offshore conditions. Mechanical tests will be performed, and cyanobacterial viability will be determined.
To provide the most suitable shape of this material for the offshore industry, the material will be grown in different shapes and submitted to mechanical tests (tensile strength tests and strength tests). In addition, the ability of this material to interact with common offshore materials (e.g., steel) and/or to be grown underwater will be also tested in order to understand their possible applications as healing materials.

The final result of this graduation project will be a technical file and the design space, with all the results to make this new material suitable for the offshore industry. This will also be presented in the form of a demonstrator that represents all these findings (e.g., an example piece of the right sand, hydrogel, and nutrient combination) in the most optimal shape for this industry.

Assignment

This is the most important part of the project brief because it will give a clear direction of what you are heading for. Formulate an assignment to yourself regarding what you expect to deliver as result at the end of your project. (1 sentence) As you graduate as an industrial design engineer, your assignment will start with a verb (Design/Investigate/Validate/Create), and you may use the green text format:

A technical file, design space, and demonstrator of an LBM with cyanobacteria for the offshore industry.

Then explain your project approach to carrying out your graduation project and what research and design methods you plan to use to generate your design solution (max 150 words)

The goal is to make a new cement like material of cyanobacteria that eventually can be used in the offshore industry to make the industry more durable and environmentally friendly. This will be done by finding the right sand type, finding the right shape, and discovering how the new material interacts with the other materials in use in the offshore industry. The wish is to show the results in data form and in the form of demonstrator of the new material.

This will be achieved by following three stages: “ Research ”, “ Development, experiment and lab work ”, and “ Demonstrating and documenting ”.

The “ Research ” stage will give the right knowledge through literature research to be able to make test setups in the lab. Then the “ Development, experiment and lab work ” stage will start. This stage includes experiments in the lab, material tests and processing of the data. This stage needs a lot of time for iteration, because experiments in the lab are never linear. The last stage is “ Demonstrating and documenting ”. Here, the technical file, design space and the demonstrator will be made. The focus will also be on the conclusion and discussion in the report.

Project planning and key moments

*To make visible how you plan to spend your time, you must make a planning for the full project. You are advised to use a Gantt chart format to show the different phases of your project, deliverables you have in mind, meetings and in-between deadlines. Keep in mind that all activities should fit within the given run time of 100 working days. Your planning should include a **kick-off meeting**, **mid-term evaluation meeting**, **green light meeting** and **graduation ceremony**. Please indicate periods of part-time activities and/or periods of not spending time on your graduation project, if any (for instance because of holidays or parallel course activities).*

Make sure to attach the full plan to this project brief.
The four key moment dates must be filled in below

Kick off meeting	16 Oct 2023
Mid-term evaluation	19 Dec 2023
Green light meeting	7 Mar 2024
Graduation ceremony	23 Apr 2024

In exceptional cases (part of) the Graduation Project may need to be scheduled part-time. Indicate here if such applies to your project

Part of project scheduled part-time	✔
For how many project weeks	25
Number of project days per week	4,0

Comments:
Working part time will help me to stay motivated.

Motivation and personal ambitions

Explain why you wish to start this project, what competencies you want to prove or develop (e.g. competencies acquired in your MSc programme, electives, extra-curricular activities or other).

Optionally, describe whether you have some personal learning ambitions which you explicitly want to address in this project, on top of the learning objectives of the Graduation Project itself. You might think of e.g. acquiring in depth knowledge on a specific subject, broadening your competencies or experimenting with a specific tool or methodology. Personal learning ambitions are limited to a maximum number of five.
(200 words max)

During my studies at IDE, I found during the elective Designing Living Artefacts that I like working in the lab and discovering new things by doing instead of learning from books. I also have had an interest in Biology my whole life.

During the course AED I found that I also really like prototyping, working in the lab is also a form of prototyping. Also, working with living things has my interest because you can see what your effect is on things by seeing how they behave; it is less predictable. During Designing Living Artefacts, I also did a lab project, I made my own plates and grew my own mycelium. I have some lab skills and knowledge, during this project I hope to further develop those.

The skills that I need to learn during this project are the skills related to material testing and the calculations behind them. The material and shape testing in this project will provide the gaining of this knowledge.