FACULTY OF SCIENCES

EVALUATING THE IMPACT OF WETTABILITY ON THE BIORECEPTIVITY OF SEDIMENTARY BUILDING STONES

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After blood, sweat and many tears my master's thesis now finally lies before me. Before I start talking about the scientific stuff, I first want to thank some of the people who helped me get this far.

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Contents

1. Introduction

In nature rocks are weathered by physical, chemical and/or biological processes. This is no different in the building environment. Building materials can be colonised by many different micro-organisms. This can cause changes in their colour and in their physical and chemical properties (figure 1).

Figure 1: Images showing the effect of biodeterioration on an angel statue from the cathedral of Cologne, (a) shows the statue in 1880, (b) shows the statue in 1993 (Warscheid & Braams, 2000).

Micro-organisms growing on rocks (and thus on building stones) form biofilms that consist of extracellular polymeric substances (EPS) together with the cells of those organisms. These EPS consist of polysaccharides, lipids, proteins and enzymes (Warscheid & Braams, 2000). The EPS are there to keep the micro-organisms attached to each other and to the rock surface. It also protects the micro-organisms from drying out and can catch gasses and particulates from the atmosphere (Gorbushina, 2007). These particulates form nutrients for the organisms and the gasses are necessary components for photosynthesis. Biofilms are often complex ecosystems with many different micro-organisms, such as cyanobacteria, algae, heterotrophic bacteria and fungi. It is believed that this colonisation starts with phototrophic organisms like cyanobacteria or eukaryotic algae. These organisms only need light, water and some minerals to be able to grow. This allows cyanobacteria to grow on nutrient-poor stone surfaces. Cyanobacteria colonies are one of the most studied biofilms (Gorbushina, 2007; Miller et al., 2012). Due to their adaptions to desiccation and solar radiation, these bacteria can occur in a wide range of habitats (from deserts to rain forests, to polar regions). In the built environment, many different genera of cyanobacteria have been reported to occur on differing stone types (limestone, marble, sandstone, granite, travertine and dolomite in Macedo et al. (2009)). Cyanobacteria, along with other primary

producers produce nutrients so that, eventually heterotrophic organism such as organotrophic fungi and bacteria can also colonise the stone (Gorbushina, 2007; Warscheid & Braams, 2000).

Biofilms growing on stones can cause changes in the characteristics of these stones. The most visible of these changes, is a change in colour. This is due to pigments produced by the micro-organisms in the biofilm (e.g. green chlorophyl, brown to black melanin, yellowish to red carotenoids) (Warscheid, 2000). Biofilms also can catch soot and other pollutants, which further leads to the discolouration of the stone. The micro-organisms in biofilms can produce inorganic and organic acids which causes chemical degradation. These acids can dissolve the matrix of the stone and cause pitting (Gorbushina, 2007). Biofilms shrink and expand depending on the amount of water that they contain. This can cause mechanical stress in the stones (Warscheid & Braams, 2000). Because water is so important for organisms, biofilms modify water transport in stones. This changes stone properties such as the capillarity and the water-vapour diffusion. It is assumed that biofilms will cause the stone surface to stay wetter for a longer time (Schröer et al., 2021). When stones stay wet for a longer period, they might be more susceptible to freeze-thaw weathering. Microbial colonisation not only has negative effects, since biofilms reduce the capillarity in building stones. This means that less water will be absorbed by the stone, giving the biofilm a more protective role (Schröer et al., 2022).

This protective role of the biofilm can be referred to by the term bioprotection. Organisms, mainly flora, can form a bioprotective barrier on soils and rocks, which can protect the stone from erosion due to wind and rain. This barrier provides thermal blanketing and keeps the temperature and humidity of surfaces constant (Carter & Viles, 2005). For micro-organisms, this bioprotective effect has been observed in lichens and cyanobacteria-containing biofilms (Carter & Viles, 2005; Ramírez et al., 2010). Any negative changes caused by the colonisation of micro-organisms can be called by the terms 'biodeterioration' or 'biodegradation'. The first term has a negative connotation and groups any unwanted changes caused by the colonisation of living organisms. The second term views the changes as part of the natural process of soil formation and thus has a more useful connotation (Miller et al., 2012). Although soil formation is a natural process, it still leads to loss of material which means that both terms have a negative view towards the colonisation by micro-organisms.

To study microbial colonisation in a more objective way, it might be better to consider the characteristics that allow colonisation to occur. Instead of only focussing on the positive and negative effects of microbial growth. For this reason, the term 'bioreceptivity' was created. The concept of bioreceptivity was created by Guillitte (1995) as *"the aptitude of a material (or any other inanimate object) to be colonised by one or several groups of living organisms"*. Bioreceptivity can be considered as a sum of the properties that a material has that support the establishment and the development of organisms. If we consider this for building stones, this contains properties like porosity, surface roughness, capillarity and chemical composition. It is important to note that the colonisation of organisms is not only dependent on material properties. It also depends on the properties of the organisms itself (how they disperse, what they need to grow), and on the environmental conditions (the surrounding climate, the amount of sunlight or shade, the water available) (Sanmartín et al., 2021). To study the bioreceptivity of a building material without these factors, Guillette (1995) proposed to artificially inoculate the material with

an organism, and then putting this material in the ideal environment for the organism to grow (e.g. a growth chamber with a constant temperature and relative humidity).

Over the years much research has been done to better understand, which material properties are the most important for microbial growth. Since all organisms need water, it is fair to assume that properties linked to water movement in the material will have an effect on the bioreceptivity (like the open porosity, capillarity or water vapour permeability) (Miller et al., 2012). Next to these, the surface roughness is also important since it provides anchoring sites where organisms can attach to. Still once anchored these organisms are again dependent on the water availability (Guillitte & Dreesen, 1995). The chemical composition and the pH also have an effect on the bioreceptivity. Carbonates, such as $CaCO_3$, have a buffering effect. This means that stones containing calcite will have a constant pH that is suitable bacterial growth. Stones that contain weathering prone minerals, such as clays, feldspars and ferruginous minerals, are also prone to colonisation. The weathering minerals provide a nutrient source for the growing bacteria. According to Miller et al. (2012) there is no real consensus as to which stone properties are the most essential for bacterial colonisation. Meaning that more research is necessary.

Although the presence of biofilms may have some positive effects, today it is generally preferred to remove this colonisation from building materials. This is done to prevent possible biological weathering and to create a cleaner look of the building (by reducing staining) (Sanmartín et al., 2021). Reducing the growth of biofilms can be done by reducing the growth conditions for the bacteria in it. Warscheid (2000) calls this process 'good housekeeping'. By drainage, ventilation and climate control the amount of moisture in a building material can be reduced, thus reducing the bacteria. If the climatic conditions cannot be controlled, a water repellent can be applied to reduce the moisture content. This combination of 'good housekeeping' and water repellents (or other protective treatments) is generally preferred over using biocides that may be toxic to humans or the environment (Warscheid & Braams, 2000). The most commonly used water repellents consist of silicone compounds dissolved in an organic solvent (Chen et al., 2023). When these are applied to a stone, they form a hydrophobic silicone film on the surface and on the pore walls. After water repellents are applied, they reduce surface energy and the capillarity of the stone, which will slow down water absorption (Urzì & De Leo, 2007).

1.1 Objectives

In this thesis the bioreceptivity of three sedimentary building stones with different properties will be tested using cyanobacteria. To do this, first the properties of the stones will be determined. To research how these properties effect the bacterial growth. A water repellent will be applied to some of the stones to compare the bioreceptivity between the normal and the hydrophobic stones. After this the samples will be placed in a climatic chamber with a constant temperature and simulated rains to see how this effects the bacterial colonisation.

2. Materials

2.1 Stones

Three different sedimentary building stones were used in this study: the Belgian blue stone, the Bentheimer sandstone and the Savonnières limestone. These specific stones were chosen because they were/are often used as building stones or replacement stones in Belgium. The stones have different porosities and pore structures. The Belgian blue stone and the Savonnières stone mainly consist of calcite (>96%). The Bentheimer mainly consists of Quartz.

2.1.1 Belgian blue stone

The Belgian blue stone is a dark-coloured grey blueish crinoidal limestone. It was deposited during the middle to upper Tournaisian (early Carboniferous) and can be found in four different regions: the Soignies-Ecaussinnes-Neufvilles basin, the Condroz basin, the Bocq basin and the Molignée basin (WTCB, 2001). The samples from this thesis came from the Carrières du Hainaut in Soignies, Belgium.

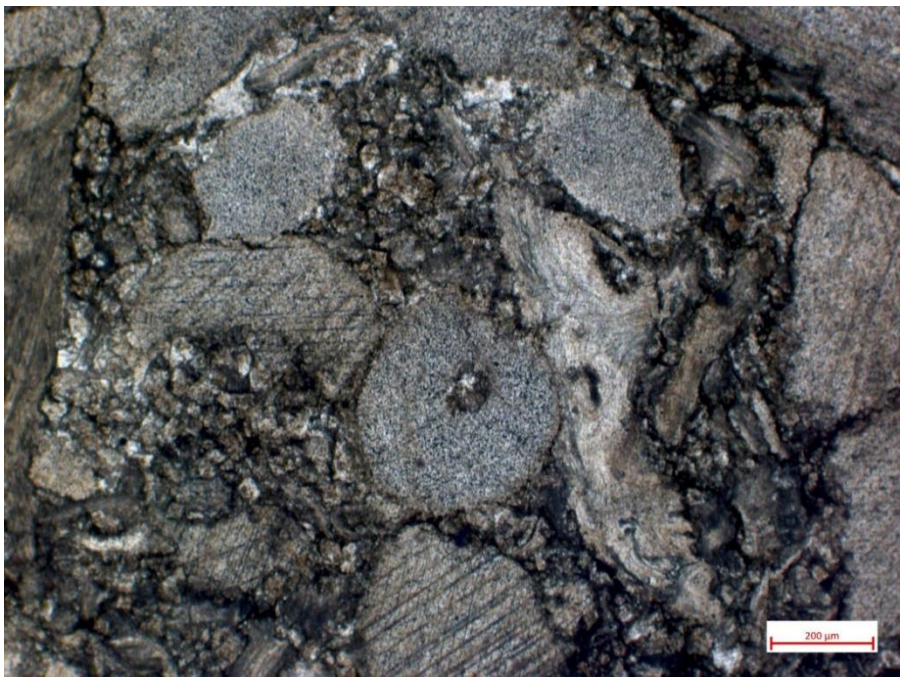

Figure 2: Microscopic image of the Belgian blue stone under crossed polarizers, a crinoid fossil can be seen in the middle of the image, the black surrounding the grains is organic matter.

This stone can be classified as a bioclastic packstone or grainstone (Dunham, 1962). Next to the crinoid fossils (visible in figure 2), it also contains fragments of brachiopods, bryozoans and rugose corals (Dreesen et al., 2007). The stone mainly consists of calcium carbonate (96-99%) (Pereira et al., 2015). It also contains a small amount of quartz, dolomite, iron sulphides, clay minerals and a small amount of organic matter which causes the dark colour of the stone (Guillitte & Dreesen, 1995). The Belgian blue stone has a low average porosity of 0.28% (WTCB, 2001). It is used often in Belgium for window and door sills, kerbs and tiles, it is also used in many buildings e.g. the arcade in the Parc du Cinquantenaire in Brussels (Pereira et al., 2015).

2.1.2 Bentheimer sandstone

The Bentheimer sandstone is a beige, pale yellow to ochre-coloured sandstone. It is a German building stone from the early Valangian (Lower Cretaceous). This stone is found in the southwestern part of the Lower Saxony basin and has outcrops close to the Dutch-German border in the region of Bad Bentheim and Gildehaus (Dubelaar & Nijland, 2015).

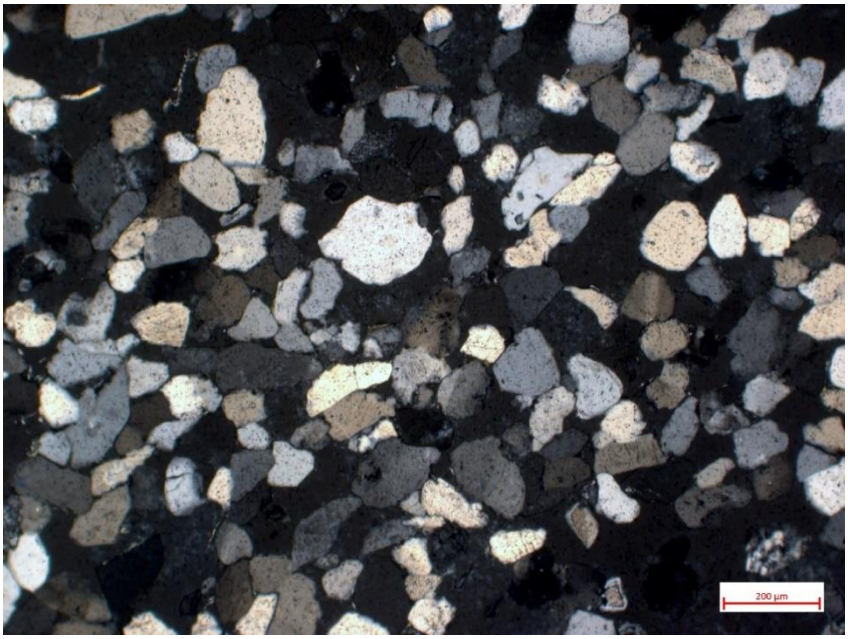

Figure 3: Microscopic image of Bentheimer sandstone under crossed polarizers, it mainly contains angular to more rounded quartz grains.

The Bentheimer sandstone contains mostly quartz (figure 3), with some feldspars, iron hydroxides, heavy minerals and a small amount of clay minerals <1% (Dubelaar & Nijland, 2015). These iron hydroxides and heavy minerals are visible in the samples as red and black spots. The samples also contain some lighter coloured whitish layers. The Bentheimer is a well-sorted medium grained sandstone with grains between 180 and 300 µm (Dubelaar & Nijland, 2015). It has a porosity between 23 and 27% with most pores having a diameter between 0.02 and 0.18 mm (Peksa et al., 2015). This building stone became popular in the Netherlands from the 16th century, and to a lesser extent in Flanders e.g. the Saint Rumbold's cathedral in Mechelen (Dubelaar & Nijland, 2016). It was used as replacement stone in the 19th and 20th century for the Lede stone (a sandy limestone from Belgium) (Chen et al., 2023; De Kock et al., 2014).

2.1.3 Savonnières limestone

The Savonnières limestone is an off-white, crème coloured layered oolithic limestone. This is a French building stone from the Tithonian (Upper Jurassic). This stone is found in the eastern part of the Paris basin in the Meuse department (Dreesen et al., 2007). It has outcrops close to the boundaries of the Champagne and Lorraine regions (Fronteau et al., 2010).

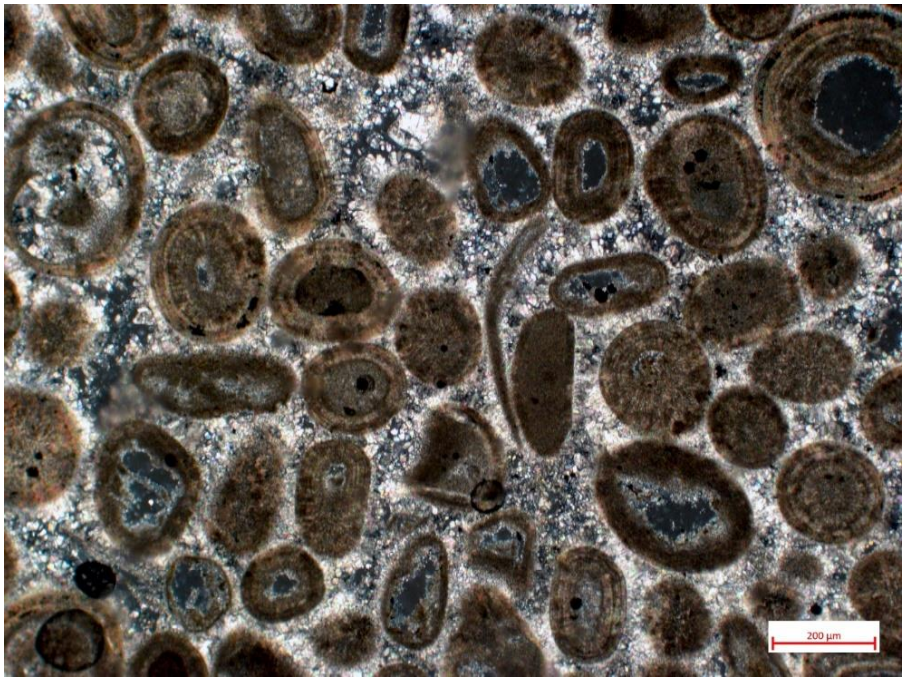

Figure 4: Microscopic image of Savonnières limestone under crossed polarizers, the concentric structure of the ooids is visible here along with some hollow ooids, there is a shell fragment in the middle of the image, between the grains is the dog tooth sparite.

This limestone can be classified as an oolithic grainstone (Dunham, 1962). It consists almost entirely of calcium carbonate (98%) with some occasional dolomite minerals (Fronteau et al., 2010). This limestone is composed of round to elliptical ooids with an average diameter of 0.5 mm (Dewanckele et al., 2014). These ooids consist of concentric layers that are visible under the microscope (figure 4), in many of the ooids the inside layers are dissolved creating cavities. Next to the ooids, the stone can contain some shell fragments, which are visible in the samples. These grains are cemented together with sparite calcite crystals, which form triangular pores between the grains (Derluyn, Dewanckele, et al., 2014). The Savonnières has an open porosity between 20 and 40%. This porosity consists of the cavities in the ooids, the triangular pores between the grains and micropores inside and between the grains (Derluyn, Dewanckele, et al., 2014; Roels et al., 2001). The Savonnières was often used as a building stone in the 19th and early 20th centuries (e.g. the Jozef-Plateau building in Ghent), and as a replacement stone for other limestones (Chen et al., 2023; Dreesen et al., 2012).

2.2 Bacteria

The bacteria used in this thesis come from the Belgian Coordinated Collection of Microorganisms (BCCM). In this study two different types of cyanobacteria were used: Phormidium autumnale ULC086 and Pseudanabaena epilithica ULC0788. Cyanobacteria were chosen for this study, because they are important primary producers, and they are often one of the first organisms to colonise building stones.

2.2.1 *Phormidium autumnale*

Phormidium autumnale forms a complex of different species with different morphotypes in many different habitats all over the world. It can be found in freshwater habitats like streams, springs, river and lake shores, close to waterfalls and on wet rocks (Strunecký et al., 2012). It forms biofilms where the filaments are more or less parallel. These green to brown-coloured filaments have a width between 3 and 8 µm (figure 5) and are simple, unbranched and cylindrical (Strunecký et al., 2013).

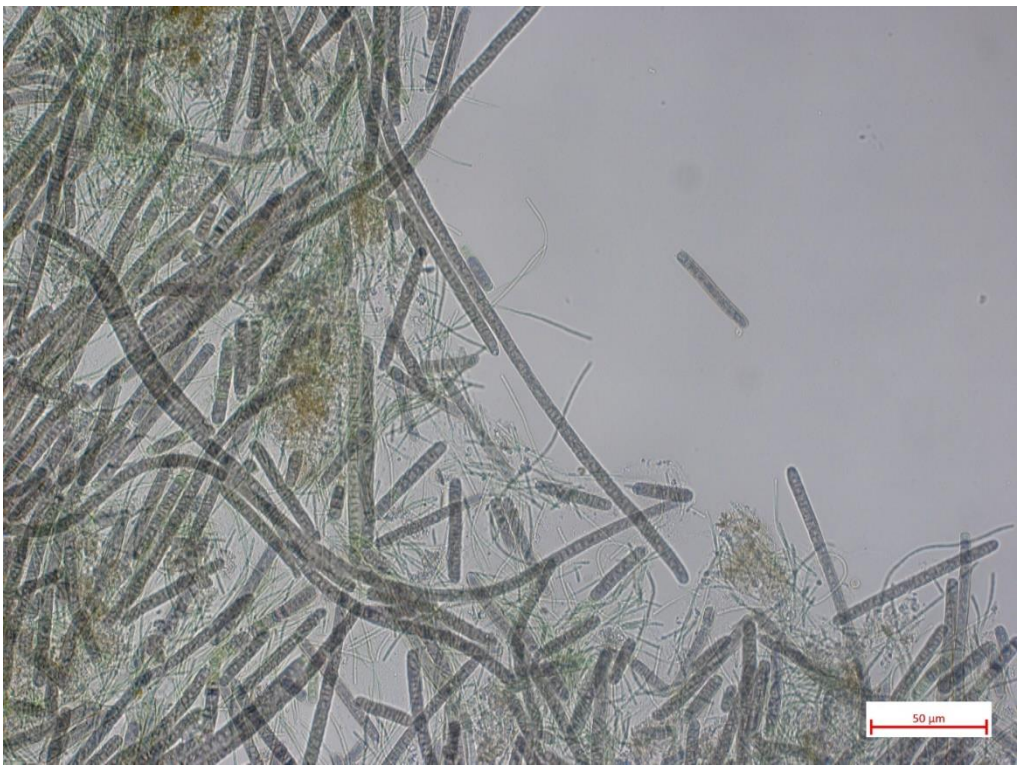

Figure 5: Microscopic image of Phormidium autumnale.

P. autumnale is part of the order Oscillatoriales. The specific strain used in this system was ULC086 (CCALA 697 in the culture collection of autotrophic organisms), it came from Ellesmere Island in the Arctic in Canada, from the bottom of a glacial stream (Strunecký et al., 2010). It forms a dark blueish-green biofilm that sticks to surfaces and a dark green blob-shaped biofilm that floats in the Erlenmeyer. These bacteria were chosen for this study since it was found to naturally occur on building stones (Macedo et al., 2009); it was also used in studies that are similar to my master thesis (Schröer et al., 2022). In 2013, the taxonomy of *Phormidium autumnale* was revised, and its name changed to *Microleus autumnalis*. In this thesis the name *P. autumnale* will still be used since that is the name of the strain on the website of the BCCM.

2.2.2 *Pseudanabaena epilithica*

This bacterium forms a purple, reddish-brown biofilm that sticks to substrates. This biofilm consists of small clusters with densely arranged coiled and curved filaments (figure 6). These purplish brown filaments are 1.7 to 1.9 µm wide and can be recognised by a refractive granule at their apical cells (Christodoulou et al., 2023). When looked at under the microscope, the filaments visibly move.

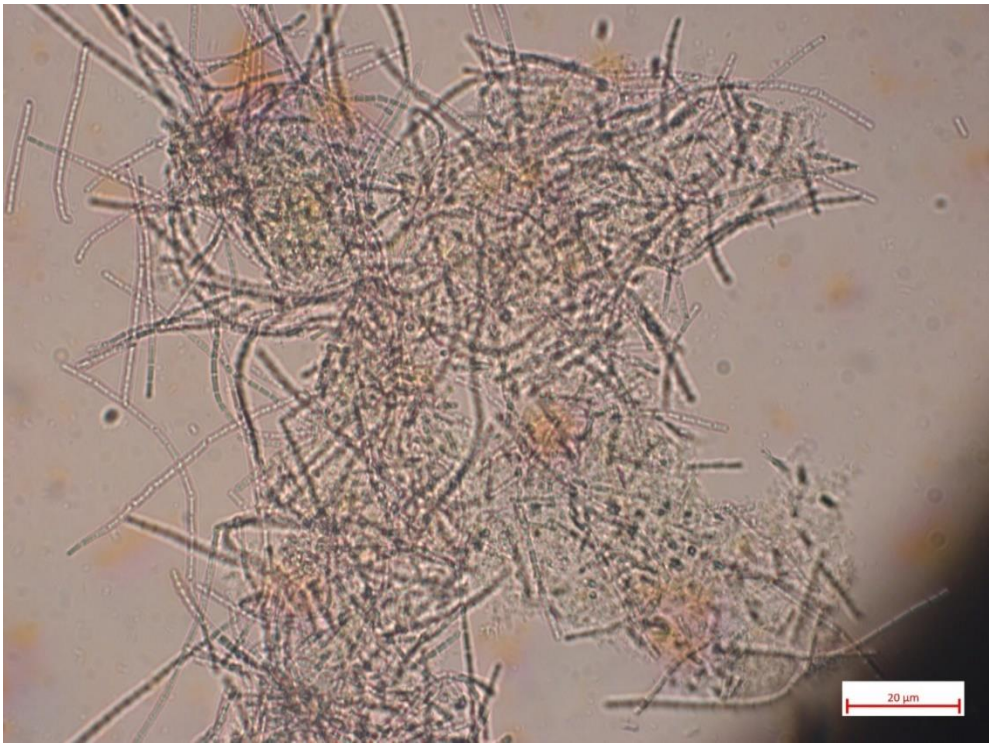

Figure 6: Microscopic image of Pseudanabaena epilithica.

P. epilithica was collected next to Kuhakoski waterfall (Southern Finland), where it was growing on wet rocks in a man-made cavity without direct sunlight (Christodoulou et al., 2023). This bacterium was chosen for this study since it grows on rocks in its natural habitat. It was also chosen because it is easier to homogenize than the other bacteria, which makes it easier to inoculate the stones.

2.3 Redisil S

The water repellent used in this study is called Redisil S from Rewah. The active ingredient in this product are modified oligomeric siloxanes (methyl-ethoxy type). Redisil S contains 10% of these siloxanes dissolved in aliphatic solvents (white spirit). This water repellent stops moisture infiltration in the stone without reducing the water vapour permeability. This means that when this is applied to a wall, the wall can still allow water vapour transport. When the water repellent is applied to a stone, the siloxanes will polymerise and form a hydrophobic film on the stone surface and the pore walls. Redisil S is only used on porous materials, because the polymerising siloxanes can cause staining on non-porous stones (Rewah, technical data sheet 2024). This means that the water repellent may cause stains on the Belgian blue stone used in this thesis. Few studies have been done about the effectivity of this water repellent (Chen et al, 2023), but it can be assumed that this water repellent will be more effective when applied on the Bentheimer sandstone than the Savonnières limestone (Chen et al., 2023).

3. Methods

3.1 Stone properties

For each building stone, 14 cubic samples of 5x5x5 cm were used. Some samples of the Bentheimer sandstone and the Savonnières limestone were layered. For these stones, the top surface was chosen to be more or less parallel to the stratification of the samples. But some variation is possible for the Bentheimer samples. Since the layers were not always very clear the see, and sometimes even ran diagonally through the samples. These samples were used for all the following property tests except for the contact angle measurement.

3.1.1 Porosity

The porosity measurements were done according to the European standard EN1936 (2006). The samples were dried in an oven at 40 °C. Before the measurement started, the samples were put in a desiccator for at least half an hour, for the stones to cool down. Then the dry weight m_d was measured. The samples were put in a vacuum to remove all the air from the pores. After two hours under vacuum, demineralized water was slowly added from the bottom of the samples. When the samples were fully immersed, the pressure was returned to atmospheric pressure. After leaving the samples under water for 24 hours, the saturated samples were weighed first under water m_h and then above water m_s. With these three weights, the open porosity p_o (%) was calculated using the following formula:

$$p_o = \frac{m_s - m_d}{m_s - m_h} * 100$$

The apparent density ρ_b (kg/m^3) was also be calculated from these weights:

$$\rho_b = \frac{m_d}{m_s - m_h} * \rho_{rh}$$

With ρ_{rh} being the density of water in kg/m^3.

The porosity measurement was only done for one Belgian blue stone sample, since it had a very low porosity. But based on the literature the porosity should be around 0.28 ± 0.13 %, and the density 2687 ± 15 kg/m^3 (WTCB, 2001).

3.1.2 Capillarity

The capillarity measurements were done according to the European standard (EN1925, (1999)). The drying and weighing of the dry samples followed the same procedure as the porosity tests. The area of the base of the samples was also measured. The samples were placed in a box on a mesh, and then immersed in 3 mm of demineralised water. When the samples were immersed, they were weighed after 1, 3, 5, 7, 10, 15, 20, 30, 45, 60, 240, 420 and 1440 minutes. After weighing all samples, the box was closed so that water would not evaporate of the stones. Since the absorption went a bit slower in the Savonnières limestones some extra steps were added at 120,

180, 300 and 360 minutes. For some Bentheimer samples the absorption was very fast. For these samples shorter timesteps were used (0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 minutes). For every time step the water absorption y_i (g/m^2) was calculated using the following formula:

$$y_i = \frac{m_i - m_d}{A}$$

With m_d (g) being the dry mass, m_i (g) the mass weighed at a certain time step and A (m^2) the area of the submerged plane.

These values were then plotted, with the square root of time on the x-axis and the water absorbed on the y-axis. This graph could generally be approximated by two straight lines. The coefficient of water absorption by capillarity C_1 (g/m^2. s$^{0.5}$) was equal to the slope of this first line. C_1 could then be calculated at any timestep using the following formula:

$$C_1 = \frac{m_i - m_d}{A \cdot \sqrt{t_i}}$$

To remain consistent, this formula was always applied at the third time step (at 5 minutes). The capillarity coefficient calculated here is C_1, because this capillarity flows roughly perpendicular to the layers of the stone (planes of anisotropy in EN1925 (1999)). The capillarity was not measured for the Belgian blue stone since its porosity was too low.

3.1.3 Ultrasonic pulse velocity (UPV)

Ultrasonic pulse velocity (UPV) measurements were done with the Consonic G2-GS from Geotron Elektronik, using the Lighthouse DW software. This machine sent out ultrasonic waves with a frequency of 80 kHz (Rozgonyi-Boissinot et al., 2021), the arrival of these waves was measured, and the P and S wave velocity was calculated. Measurements of these velocities were done perpendicular to the layers in the sample. The orientation of the samples can be seen in figure 7. The length, width and dry weight of the sample were put into the software. Then the velocity of the P and S waves (V_P and V_S in km/s) and the elastic modulus (E in kN/mm^2) were measured. The measurements were repeated 8 times and then averaged.

Figure 7: Image showing the orientation used for the UPV measurements, the two transducers that send out the waves can also be seen next to the sample.

The UPV used is designed to measure samples that are longer than they are wide, but the samples measured were square-shaped. This caused errors during some measurements, especially for the Belgian blue stone.

3.1.4 TinyPerm

Permeability measurements were done using the TinyPerm from New England research. This air permeameter consists of a rubber nozzle that is connected to cylindrical chamber. The volume of this chamber can be changed by moving the syringe. The nozzle is pressed against the sample and the syringe is pulled. This pulls air from the sample surface. When the syringe is pushed back towards the sample it creates a vacuum, so that the nozzle stays connected to the sample. During this process, the controller that is connected to the TinyPerm monitors the volume of the chamber and the pressure at the surface of the sample. The controller then calculates the response function of the sample/instrument system and shows a characteristic value of this function on its display. This characteristic TinyPerm value T can then be used to determine the matrix permeability K (in mD) using the following formula:

$$T = -0.8206 \log_{10}(K) + 12.8737$$

The TinyPerm was kept upright using a stand (figure 8). Per sample three measurements were done to create an average value. No measurements were done on the Belgian blue stone due to its low permeability.

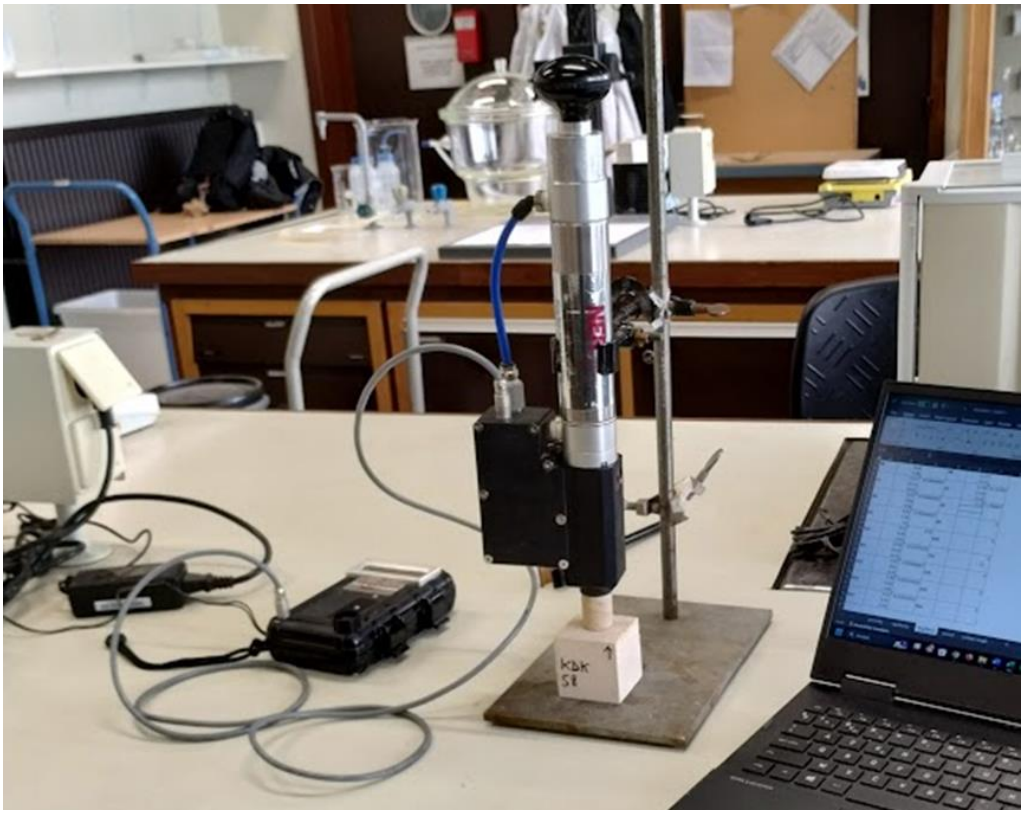

Figure 8: Image showing the setup used for the permeability measurements, it also shows the TinyPerm and the console connected to it.

3.1.5 Contact angle

Contact angle measurements were done using the FTA2000 from First Ten Ångstroms. Since the table of this machine could not move, the usual samples of 5x5x5cm could not be used since they were too big. So, for the contact angle measurement three smaller samples (one for each stone type) were used that were 2.5x5x5 cm. The machine works by dropping a droplet of demineralised water on the sample, while pictures were taken at a rate of 1 picture/second. In the FTA32 software, the contact angle can then be calculated for every picture (figure 9). The contact angle was measured in five different locations on the samples. For the Belgian blue stone, the static contact angle was measured according to the European standard (EN 15802, 2009). This is the contact angle 10 seconds after a drop was deposited. This measurement was not possible for the Savonnières and the Bentheimer, since these stones absorbed the drop too fast. For the Bentheimer sandstone, the contact angle was determined at first contact. Even this was not possible for the Savonnières since the drop absorbed too fast. However, we know from literature that the contact angle at first contact for the Savonnières limestone is approximately 47° (Schröer, De Kock, et al., 2022).

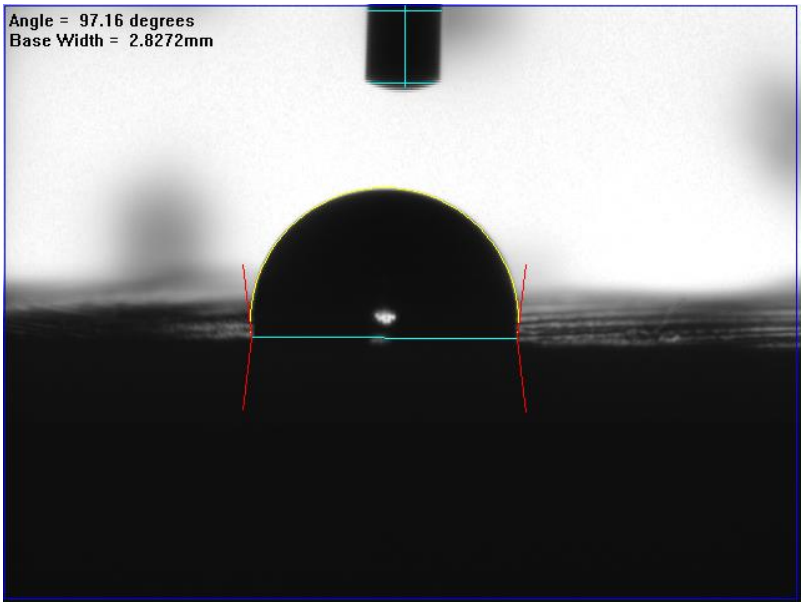

Figure 9: Example of an output from the contact angle measurement, the red lines show the contact angle calculated by the FTA32 software.

3.2 Photos & colour measurements

Since photos and colour measurements were always taken together in this thesis (first the samples were photographed then measured with the spectrophotometer), they will be discussed together here. This procedure of photographing and colour measuring was used throughout the experiments in this thesis. Photos were taken using a Canon Eos 550D. The samples were always put in the same orientation when taking photos. When the images were taken, the camera was installed at a height of 30 cm. The pictures that were taken, were calibrated using a colour checker classic mini from X-rite.

Colour measurements were done to monitor bacterial growth. These measurements were done using a CM-600d spectrophotometer from Konica Minolta (aperture of 8mm) using the Spectramagic NX software (figure 10).

Figure 10: Image of the CM-600d Spectrophotometer used in this thesis.

On every sample, five measurements were taken to calculate an average value of the sample. These measurements were always taken in the same order, at about the same location. At every spot the spectrum of visible light was measured (400-700 nm) and the colour was measured using the CIEL*a*b* system. Here the L* is the lightness value from 0 (black) to 100 (white), a* is the green-red part of the colour (negative is green, positive red) and b* the blue-yellow part of the colour (negative is blue, positive yellow). To estimate the colour difference in an objective way the following formula was used:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

The spectrum of visible light was measured to see how the reflection changed over time. This change happens because the chlorophyll and the carotenoids in the cyanobacteria absorb certain wavelengths of light. This causes two clear minima in the reflection spectrum, one around 670 nm and a smaller one around 620 nm. To quantify the effect of chlorophyll (the Chlorophyll Discolouration CD), the following formula was used from Schröer et al. (2022).

$$CD = Reflectance_{700\ nm} - Reflectance_{local\ minimum\ (between\ 650-670\ nm)}$$

When there were bacteria on a sample, measurements were taken a couple of millimetres away from the stone instead of right on the surface. This is so that the biofilm would not come loose from the sample. This distance led to a difference in the L value of about 6% and a smaller difference of 0.2 and 1% for the a and b values respectively. This difference is based on a colour measurement that was done on the surface, and then a couple of millimetres away from the surface of a dry Bentheimer sample.

3.3 Bacterial growth

The cyanobacteria were grown in autoclaved Erlenmeyer flasks closed off with cotton wool and aluminium foil, using BG11+ medium from Sigma-Aldrich. This medium contains 367 µg/L calcium chloride dihydrate, 56 µg/L citric acid, 314 µg/L dipotassium hydrogen phosphate, 10 µg/L disodium magnesium ETDA, 60 µg/L ferric ammonium citrate, 360 µg/L magnesium sulphate, 200 µg/L sodium carbonate and 150 mg/L sodium nitrate. The pH of this medium had to be increased to a pH of 7.2 using NaOH, to be suitable for bacterial growth.

The Erlenmeyer flasks with the bacteria were kept in a lab at room temperature. The flasks were put under led strips, so that the bacteria were continuously illuminated with approximately 15 - 25 µmol m^{-2} s^{-1} light. Every couple of weeks some of the bacteria were transferred to a new Erlenmeyer flask with new medium so that they could continue growing. This transfer was a way to keep track of the age of the bacteria, so that we knew how old bacteria were when they were used to inoculate stones. At the beginning of this thesis, 100 ml of the old medium with the bacteria was added to about 100 ml of new medium. Further along the thesis (around the second preliminary test) this process changed. After that point only ± 3 ml of the old bacteria was added to about 100 ml of new medium. This bacterial transfer was done by my promotor Laurenz Schroër.

3.4 Preliminary set-up tests

Preliminary tests were done to decide which bacteria would be chosen, and to decide the best set-up for the main experiment. The test was also a way to practice working with the camera and the spectrophotometer. For the preliminary tests, six spare stone samples were used (two per stone type). The following names were given to these samples: ATR, BTR, STR, ATG, BTG and STG. The first letter of this name (A, B, S) refers to the stone type of the sample: Arduin (A), Bentheimer (B), Savonnières (S). The second letter T refers to the fact that it were test samples and not main samples. And the last letter (R or G) refers to the bacteria used: the Red bacteria *Pseudanabaena epilithica* and the Green bacteria *Phormidium autumnale*. Before the tests were started, photos and colour measurements were taken of the stone when they were dry and when they were wet. This was to have a background value of each stone sample.

3.4.1 First preliminary test

In this test, one set of every stone type was inoculated with *P. autumnale*, another set was inoculated with *P. epilithica*. The bacteria were initially disentangled by vortexing and homogenized so that the bacteria were equally spread out across the solution. From this homogenized medium 5 ml was taken and added on top of the stone using a pipette. These stones were then put in a dish of demineralized water to absorb water via capillarity. The two sets of stones were kept separated to avoid cross-contamination. These dishes were then put under the same LED strip as the Erlenmeyer flasks, so that the cyanobacteria had light to grow. The setup can be seen in figure 11. At the start and the end of the week, 5 ml of BG11+ medium was added on top of the stones. (Sometimes a bit less than 5 ml on the Bentheimer and the Savonnière samples so that it would not overtop). The medium was added on top of the stones, to avoid bacteria growing in the water dish. Before the medium was added, pictures were taken of the stones to see how they changed throughout the experiment. The colours were also measured

using the spectrophotometer. On days of the week when no medium was added, tap water was added on the surface of the blue stone, since it has almost no capillarity.

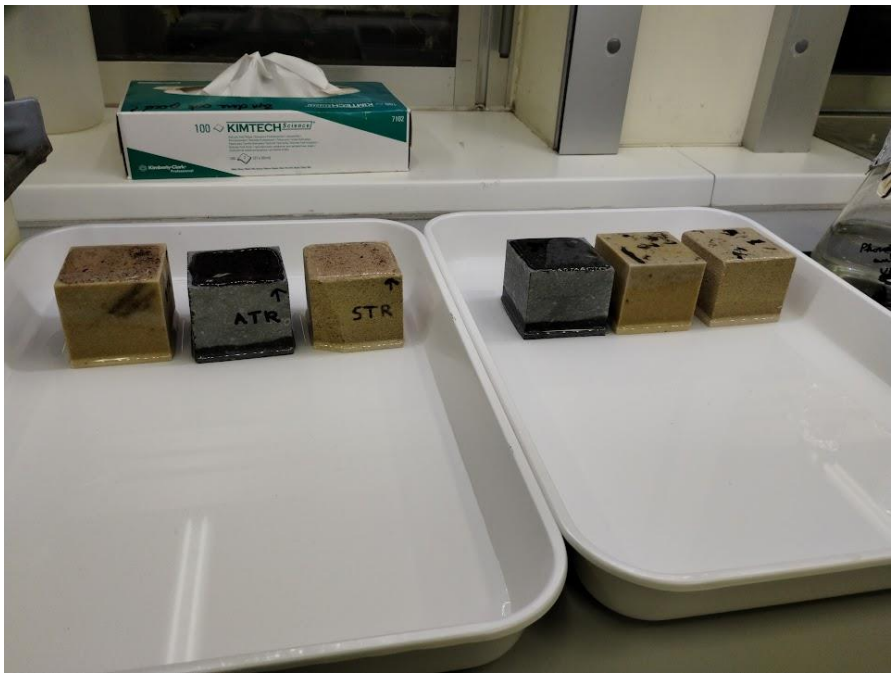

Figure 11: Set-up used for the preliminary tests, the samples were illuminated with a LED strip. The red samples are in the left dish, and the green samples are on the right. During the second test the samples were turned on a different side.

3.4.2 Second preliminary test

A second preliminary test was deemed necessary during the research. For this test the same samples were used as the previous preliminary test, but the bacteria were applied on a different side. The biofilms from the previous test were scraped off with a brush. The samples were also submerged in demineralised water so that any deposited salts from the medium from the previous test could dissolve. During this test, instead of putting the medium directly on the surface of the samples, the medium was put in the dish with the demineralised water. Before inoculating the samples, they were put in the dish with 400 ml of water and 40 ml medium. This means that when the bacteria were added, there was already water and medium available for them. The dishes were set up in the same way as in figure 11.

At the start of the test, only three stones (one of each stone type) were inoculated with *P. epilithica*. These bacteria were only five days old. This was done to make sure that the bacteria used to inoculate the stones, would be still active and actively growing. Since these bacteria grew on the bottom of the Erlenmeyer flasks, the top layer of medium was first pipetted off. This was to concentrate the amount of bacteria in the medium. The remaining medium was shaken lightly and some of the biofilm was scraped of the bottom of the Erlenmeyer with a pipette. This was done to homogenize the medium (a lot more gently then in the first test). As was done in the previous test, 5 ml was taken from this homogenized mixture and put on the surface of the samples. The pictures and colour measurements followed the same procedure as the previous test. No more medium or tap water was added to the sample.

After two weeks all six stones (three already inoculated with *P. epilithica* and the other three from the previous test) were inoculated with *P. autumnale*. These bacteria were 18 days old. The inoculation followed the same procedure as for the *P. epilithica*. Since the bacteria of *P. autumnale* are stuck together more tightly, some of the biofilm had to be take apart with tweezers. After this the test continued for another two weeks.

3.5 Assessing the effect of wettability

For this test the same cubic samples were used as for the stone property tests. This way the properties of the stones can be linked to the bacterial growth. Per stone type, seven samples were made hydrophobic. This was done to test the effect of the wettability on biological growth. From those hydrophobic stones, six were inoculated with bacteria together with six untreated stones. The remaining two samples, one hydrophobic and one untreated, were left blank to compare it to the treated stones. Before the water repellent was applied, photos and colour measurements were taken of the samples (when wet and when dry). This was to have a background value for the stones, to compare to when the stones were hydrophobic and/or inoculated with bacteria.

3.5.1 Application of water repellent

Redisil S was applied to the stones to hydrophobize the surface, using a syringe. The manufacturer recommended using a knapsack sprayer or an electric pump to apply the water repellent, but this is meant for application on an entire wall. In this study every sample was treated separately resulting in very small treatment areas. The syringe also made it possible to measure the exact amount of product that was applied to a sample (up until 0.5 ml).

Before the product was applied the samples were wrapped in parafilm. On the samples of the Bentheimer sandstone and the Savonnières limestone 2.5 ml of product was added. This amount was calculated based on the recommendation of the manufacturer, which states that per square meter of stone between 0.25 to 1 L of product is used depending on the porosity. On the Belgian blue stone only 1 ml or less was added, due to its low porosity. After the first application, the samples were left at least half an hour. After this, the product was re-applied when the stones were still wet. The samples were then dried under the fume hood for two days, after which the parafilm was removed.

Six days after the water repellent was applied, photographs were taken, and the colours were measured. This was to know the colour difference caused by the water repellent. Smaller samples (2.5x5cm) were also treated with the Redisil S. These samples were for contact angle measurements, to see the difference in value between the treated and untreated stones.

3.5.2 Inoculation with bacteria

The inoculation of the stones was done similarly to the second preliminary test. Only *P. epilithica* was used in this test. The bacteria used were six days old. Since a lot of samples had to be inoculated at the same time (12 per stone type), the contents of multiple Erlenmeyer flasks were poured into a larger container, which was then homogenized. Once again 5 ml of this mixture was put on the samples. The inoculated stones were put in a dish with 400 ml of demineralised water

and 40 ml of medium (one dish per stone type) (figure 12). The samples without bacteria were put in a separate dish without the medium. These samples were left to grow in the lab for 10 days. On the last day, photographs and colour measurements were taken to see how much the bacteria had grown.

Figure 12: Example of one of the dishes used in the lab setup, these are the Bentheimer samples a couple of days after inoculation.

3.5.3 Further growth using a climatic chamber

After the colour measurements from the previous experiment, the samples were put in a climatic chamber. The chamber used was the SunEvent 600 from Weiss-Technik. The climatic chamber can simulate the effect of sunlight and solar radiation. It also has a sprinkler system to simulate rain with demineralised water. The climatic chamber provides a constant temperature and relative humidity for the samples to be put in. The lamp in the climatic chamber was too bright for the bacteria to grow. That is why a construction was made with two LED light strips (the same as the ones used in the lab setup), to be put in the climatic chamber (figure 13). These lights provided a light intensity of 390 to 425 lux. The set-up of the samples can be seen in figure 13.

Figure 13: Image showing the position of the samples in the climatic chamber, the installed light strips can be seen at the top of the image.

In the climatic chamber, the temperature was installed to be 18 °C. Due to the rain from the sprinkler system, the humidity could not be controlled in the climatic chamber. It rained 4 times a day (every 6 hours). After 5 days, the samples were taken out for photographs and colour measurements and then put back in the climatic chamber. This was done for 14 samples at a time (one stone type at a time). A week later this process was repeated. Afterwards, all the samples were taken out of the climatic chamber and the experiment was ended.

4. Results

4.1 Properties

4.1.1 Open porosity

The results of the open porosity measurements can be seen in table 1. The values of every sample can be found in appendix 1. The Savonnières limestone has a higher average porosity of 31% compared to the Bentheimer sandstone. The measurements on the Savonnières show a higher standard deviation than the Bentheimer sandstone. The one measured Belgian blue stone sample has an open porosity of 0.28% with an apparent density of 2686.97 kg/m^3. Based on this one measurement, the Belgian blue has a much lower porosity and is denser than the other two stone types in this thesis.

Table 1: Average calculated open porosity and apparent density, together with the standard deviation.

Stone type	**p_o (%)**	**ρ_b (kg/m^3)**
Bentheimer sandstone	23.08 ± 0.26	2023.67 ± 6.39
Savonnières limestone	30.98 ± 1.00	1868.99 ± 26.95

4.1.2 Capillarity

The results of the capillarity measurements can be seen in table 2. The values of every sample can be found in appendix 2. The Bentheimer sandstone has a higher average capillary absorption coefficient than the Savonnières limestone. The standard deviation for the Bentheimer samples is very high, this is because some of the Bentheimer samples absorbed water very fast and some very slow. Based on the capillarity, the Bentheimers can be grouped in three groups. The first group of samples was saturated after a couple of minutes. This group with samples B3, B4, B5, B6, B7, B9 and B13 had an average capillarity coefficient of 563.64 ± 28.22 g/m^2. s$^{0.5}$. The second group of samples was saturated after 30 to 50 minutes. This group with samples B1, B2, B10, B11 and B12 had an average capillarity coefficient of 310.39 ± 68.33 g/m^2. s$^{0.5}$. The last group of samples never seemed entirely saturated. When these samples were submerged under water, a part of the samples seemed to stay dry (figure 14). This group with samples B8 and B14 had an average capillarity coefficient of 72.88 ± 12 g/m^2. s$^{0.5}$.

Table 2: Average calculated capillarity coefficient, with the standard deviation.

Stone type	**C_1 (g/m^2. s$^{0.5}$)**
Bentheimer sandstone	403.09 ± 183.36
Savonnières limestone	112.90 ± 23.62

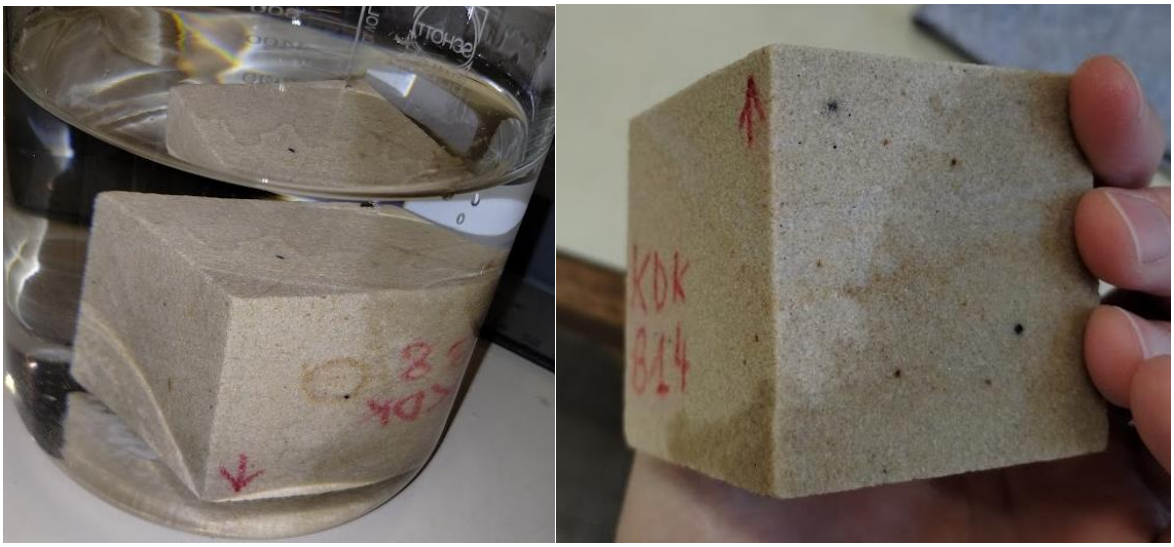

Figure 14: Images of the Bentheimer samples B8 and B14, half of the samples seemed to stay dry even when under water.

The Savonnières samples absorbed water much slower compared to the Bentheimer samples. Many of the samples took multiple hours before they were completely saturated. During the measurements, as the Savonnières stones absorbed water, the flow of water would slow down every time it reached a layer in the stone, where the ooids were less densely packed. These layers visibly have a higher amount of bigger pores compared to other layers in the of stone surface

4.1.3 UPV

The results of the UPV measurements can be seen in table 3. The values of every sample can be found in appendix 3. The values of the Belgian blue stone are only based on 7 samples: A1, A3, A4, A6, A8, A12 and A13 (A stands for Arduin). For the other Belgian blue samples, the runtime of the P-wave could not be determined by the UPV. Of the stone types, the Belgian blue has the highest average E-modulus of 19.57 kN/mm^2 and the highest average P-wave velocity of 3.34 km/s. The Savonnières has the highest average S-wave velocity of 1.81 km/s. Since the UPV is designed for longer samples instead of cubes, the machine often gave a warning that the measurement was maybe incorrect for the selected geometry. However, the standard deviations for the measurements were all relatively small.

Table 3: Average calculated P- and S wave velocity and the E-modulus, with standard deviations. The values for the Belgian blue stone (*) are based on only 7 samples.

Stone type	**VP (km/s)**	**VS (km/s)**	**E-mod (kN/mm^2)**
Belgian blue stone	3.34 ± 0.20 *	1.69 ± 0.03 *	19.57 ± 0.62 *
Bentheimer sandstone	2.84 ± 0.06	1.70 ± 0.05	14.27 ± 0.73
Savonnières limestone	3.24 ± 0.06	1.81 ± 0.05	15.41 ± 1.06

4.1.4 Permeability

The results of the permeability measurements can be seen in table 4. The values for every sample can be found in appendix 4. The Bentheimer sandstone has a higher permeability then the Savonnières limestone. This was noticeable during the measurements, because for the Savonnières samples, it was more difficult to push the syringe down and the measurement took longer to calculate on the TinyPerm console. Although the variation on the TinyPerm value is very small, the standard deviations for the permeability are very big.

Table 4: Average TinyPerm value and the calculated permeability, with standard deviations.

Stone type	**TinyPerm value**	**K (mD)**
Bentheimer sandstone	9.96 ± 0.07	3574.15 ± 679.32
Savonnières limestone	11.20 ± 0.20	131.89 ± 100.24

4.1.5 Contact angle

The results of the contact angle measurements can be seen in table 5. The values for every sample can be found in appendix 5. No static contact angle was measured for the Bentheimer sandstone since the drop was absorbed faster than 10 seconds. An attempt was made to make a contact angle measurement on the Savonnières limestone. But the drop disappeared so quickly that no clear photos could be taken to make a measurement (figure 15).

Table 5: Average contact angle at first contact and static contact angle (10 sec), with standard deviations.

Stone type	**First contact (°)**	**After 10 sec (°)**
Belgian blue stone	68.73 ± 6.69	60.42 ± 5.15
Bentheimer sandstone	83.27 ± 7.51	-

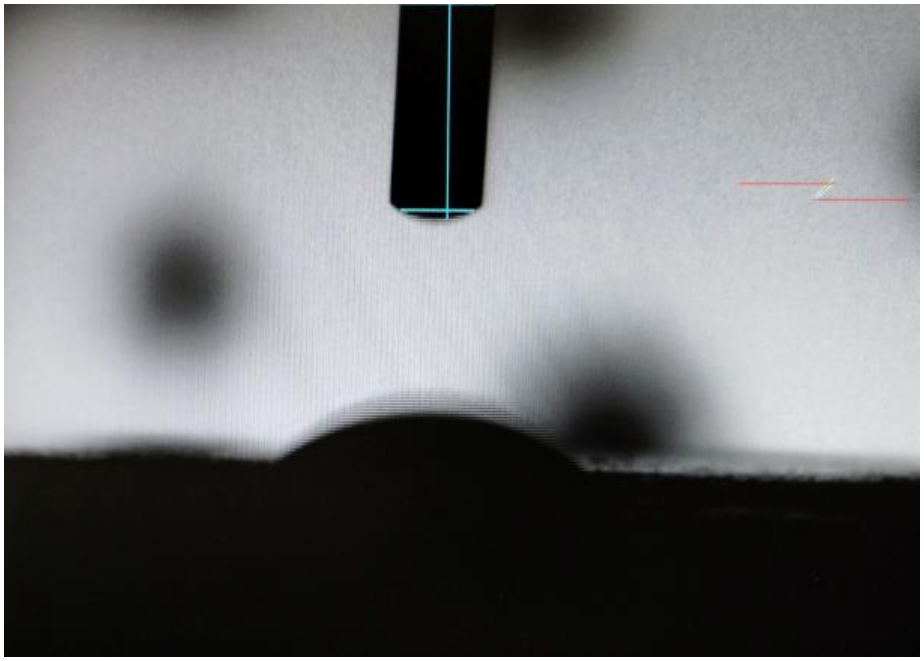

Figure 15: Output of the attempted contact angle measurement on the Savonnières limestone, because the camera can only take one picture per second, no clear picture of the drop could be taken.

The Bentheimer sandstone has a bigger contact angle at first contact of 83.27 degrees, then the Belgian blue stone with an average contact angle of 68.73 degrees at first contact. This means that the Bentheimer is more hydrophobic than the Belgian blue stone. Since none of the contact angles are bigger then 90°, these stone types are not hydrophobic. There is some variation on the contact angle measurements, due to the low framerate of the camera. Therefore, the timing of the image of the first contact could be slightly different between each measurement.

4.2 Preliminary set-up tests

4.2.1 First test

During the first preliminary test, the samples inoculated with *P. autumnale,* did not change very much. For all three of the samples, the biofilm on the stone surface became lighter green over time (figure 16). On the samples inoculated with *P. epilithica*, more change was visible. On ATR, the bacteria quickly changed colour from red to green (figure 17). Over the course of the experiment, the biofilm seemed less and less visible. The biofilm on BTR also turned green and less visible, but much more gradually then compared to the Belgian blue sample. STR was the only sample where the biofilm increased, but the biofilm also slowly turned from a dark red to a dark green colour (figure 18).

Except for the STR sample, no real pattern could be recognised in the colour differences ΔE over the course of the experiment (table 6). The ΔE for ATR starts at 4.63 then decreases to 2.48 and then increases again in the last two measurements to 4.32 at the end. A similar decrease and increase can be seen in the ΔE values for ATG and BTR. On the other hand, the ΔE values for BTG first increase from 20.22 to 20.30 to then decrease to 14.54. This increase and then decrease is also seen in sample STG. For the most part (except for ATG and STR) the samples show a lower

colour difference at the end of the test compared to the beginning of the test. Only for STR, the ΔE increased over the course of the test: from 17 at the start to 23.02 at the end of the test.

Table 6: Measured colour differences over the course of the test.

ΔE	**ATR**	**BTR**	**STR**	**ATG**	**BTG**	**STG**
27 February	4.63	16.87	17.00	8.65	20.22	16.04
1 March	2.48	13.68	17.83	5.87	20.30	20.49
5 March	4.27	11.74	20.78	4.87	14.54	18.09
8 March	4.32	13.66	23.02	9.12	18.14	13.58

No real pattern could be recognised in the evolution of the Chlorophyll discolouration (CD) either (table 7). The CD seemed to hover around the same value throughout the test, for every sample. ATR varied around 0.71, BTR around 4.48, STR around 8.08, ATG around 0.99, BTG around 2.99 and STG around 5.45.

Table 7: Chlorophyll discolouration over the course of the test.

CD	**ATR**	**BTR**	**STR**	**ATG**	**BTG**	**STG**
27 February	0.60	4.92	8.49	0.99	3.09	5.47
1 March	0.86	4.39	7.43	1.00	3.19	4.76
5 March	0.74	4.42	7.99	1.02	2.80	6.04
8 March	0.65	4.20	8.39	0.95	2.86	5.54

A white layer formed on the surface of the Belgian blue samples. This patina can be seen in figure 17.

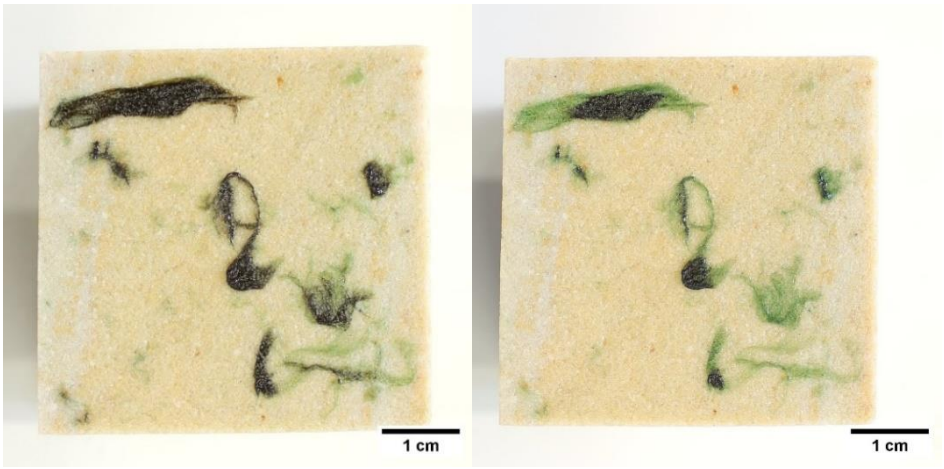

Figure 16: Image of BTG at the start (5 days after inoculation) and at the end of the first preliminary test. The Phormidium autumnale biofilm turned from a dark brownish looking green to a lighter green.

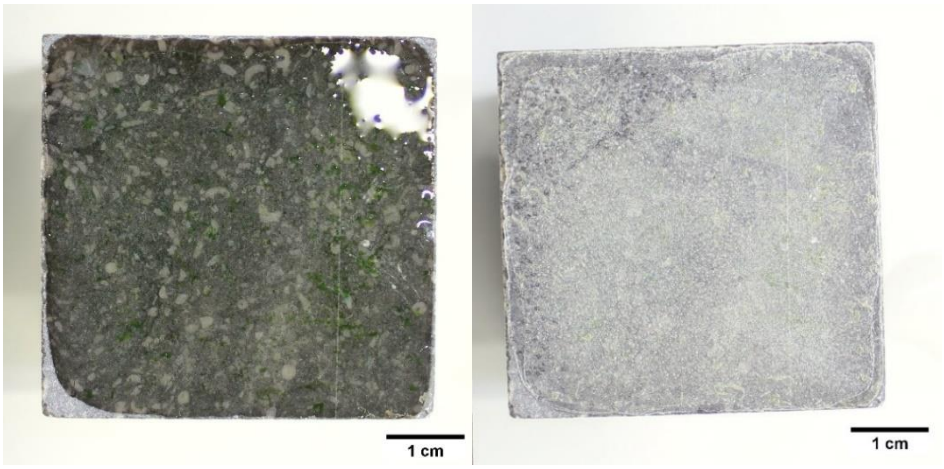

Figure 17: Image of ATR at the start and end of the first preliminary test. In the first image, Pseudanabaena epilithica had already turned green. At the end of the test, the biofilm is a lot less visible, and the sample is covered with a white patina.

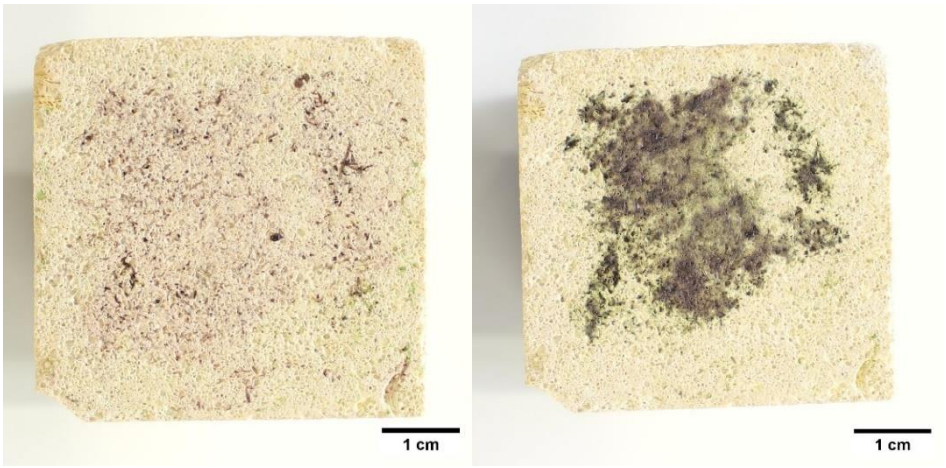

Figure 18: Image of STR at the start and end of the first preliminary test. The Pseudanabaena epilithica biofilm increased over the course of the experiment, with the bacteria changing in colour from dark reddish purple to dark green.

4.2.2 Second test

Similar to the first test, the samples that were only inoculated with *P. autumnale* did not change much. The *P. autumnale* biofilm turned lighter green on sample STG, this colour change was less visible on the other samples.

P. epilithica grew quite slow on the BTR and STR the first two weeks of the test. In the beginning it even seemed like nothing was changing on the surface of STR. After the *P. autumnale* was added to these samples, *P. epilithica* seemed to grow a lot faster (from 19 April) (figure 20 & 21). The biofilm on the BTR had a different colour then the one on the STR. The Bentheimer biofilm was more reddish purple, while the Savonnières looked more dark brown to dark green in colour. At the end of the test, the biofilm on the BTR got smaller. The corners and edges first turned green and then faded. The *P. epilithica* biofilm on STR also was less dark at the end of the test.

The *P. autumnale* biofilm itself did not change on these samples either (figure 19), except for a lighter green colour on sample STR. No bacteria seemed to grow well on the Belgian blue samples.

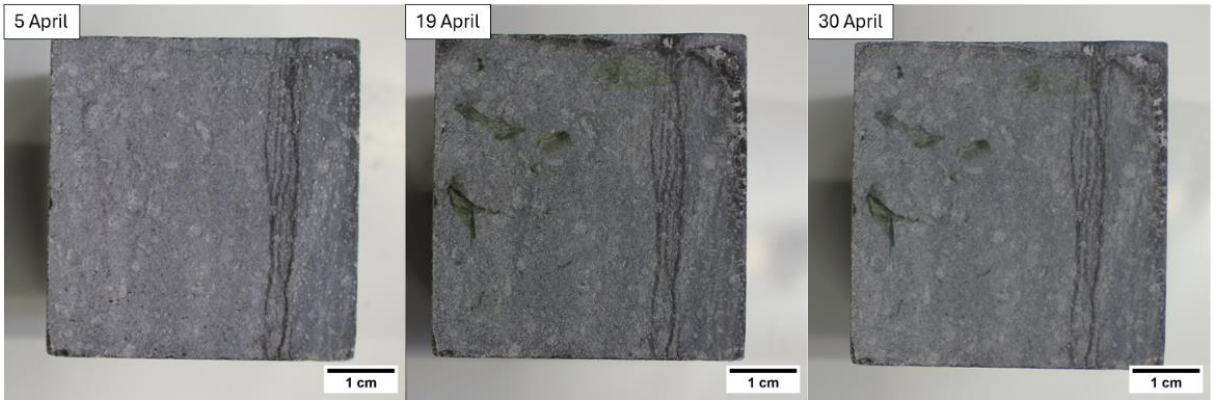

Figure 19: Images of the ATR after inoculation with P. epilithica, after inoculation with P. autumnale *and close to the end of the test. The surfaces of the samples were dry, and no bacteria seemed to grow on the surface.*

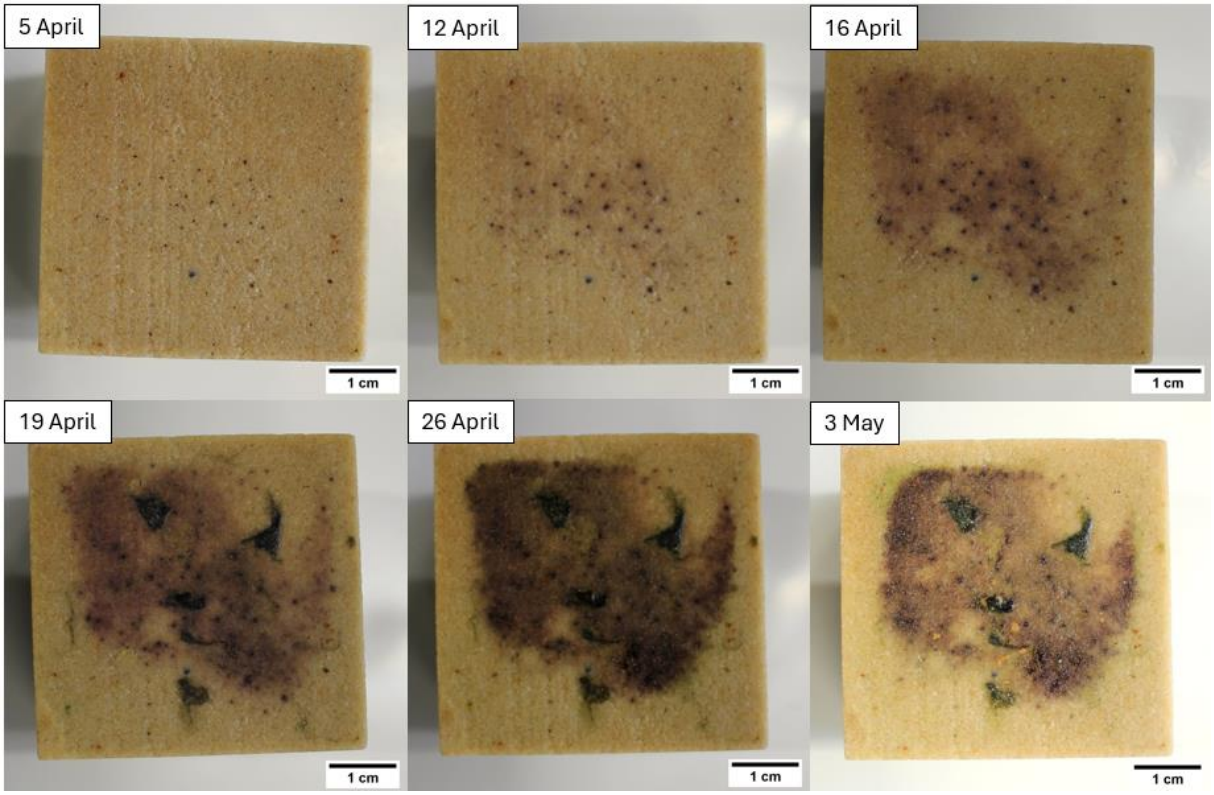

Figure 20: Images of sample BTR throughout the second preliminary test, growth seems to occur faster after the 19th of April. On the last image, the purple biofilm is smaller and the border turned green. Orange specks can be seen in the middle of the sample.

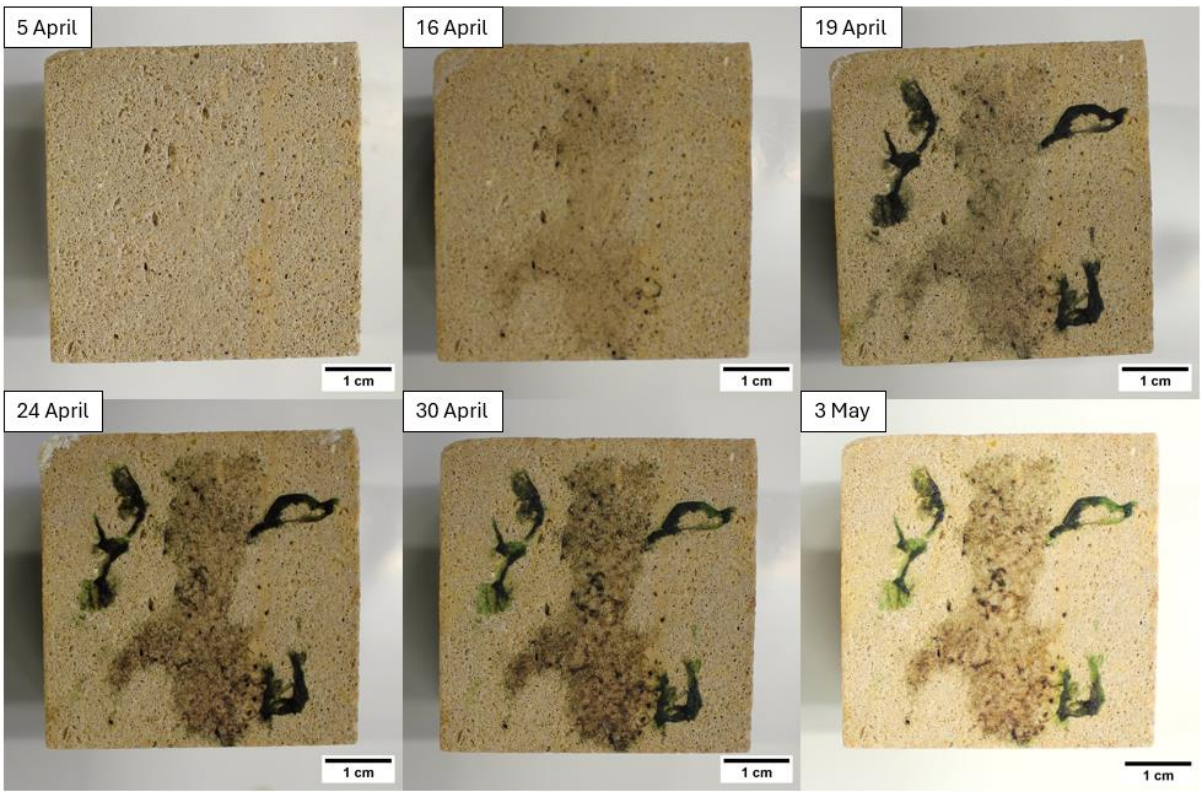

Figure 21: Images of sample STR throughout the second preliminary test, bacterial growth seems very slow at the start and speeds up after the 19th of April. The salt that appears on the 16th of April disappears on the image of the 30th of April.

The growth pattern of the biofilms can be recognised in the colour measurements (table 8). BTR and STR first show a slow increase in ΔE. When the samples were inoculated with *P. autumnale*, there is a jump in the ΔE values, which then decreases at the end of the test. This jump is also visible in ATR, ΔE decreases directly after this point. For the samples that were only inoculated with *P. autumnale* no pattern is visible. The ΔE of STG increases a bit, but not as much as c the other Savonnières sample. The ATG and BTG show an increase in ΔE at first, but then it decreases towards the end of the test.

Table 8: Measured colour differences throughout the second test, the empty quadrant were days when the green samples were not inoculated yet.

ΔE	**ATR**	**BTR**	**STR**	**ATG**	**BTG**	**STG**
5 April	4.56	5.50	0.86			
9 April	2.72	7.48	1.14			
12 April	2.07	6.29	2.26			
16 April	3.94	11.54	3.43			
19 April	7.87	14.86	10.55	5.36	3.98	1.90
24 April	6.07	15.07	9.31	5.91	4.19	1.84
26 April	5.89	15.06	11.30	5.63	6.99	1.90
30 April	5.52	17.10	10.55	1.15	4.63	2.89
3 May	2.19	14.62	10.54	1.20	3.45	2.95

Looking at the chlorophyll discolouration (table 9), the values increase over the course of the test for both Bentheimer samples and STR. The CD for ATR consistently decreased, apart from the jump after it was inoculated with *P. autumnale*. For ATG, the CD increased at first, and then there was no visible minimum in the absorption spectrum anymore.

Table 9: Measured chlorophyll discolouration over the course of the second test, areas with a (-) show no visible minimum in the measured reflection spectrum.

CD	**ATR**	**BTR**	**STR**	**ATG**	**BTG**	**STG**
5 April	0.50	2.39	-			
9 April	0.30	2.21	-			
12 April	0.19	3.02	-			
16 April	0.09	3.81	-			
19 April	0.57	5.14	5.03	0.33	2.44	-
24 April	0.40	5.58	4.64	0.43	2.23	-
26 April	0.45	6.22	5.31	0.52	2.31	-
30 April	0.45	6.42	5.66	-	2.98	-
3 May	0.34	8.11	7.05	-	3.04	-

About two weeks into the test, salt started growing on the upper two corners of the Savonnières samples (first on STR a couple of days later on STG) (figure 21). Two weeks later this salt had disappeared. At the end of the test, some sort of orange blobs also appeared on the surface of the BTR (figure 20). This is assumed to be some sort of mould.

Since the water that the samples were in, contained medium in this test, organisms started to grow on the bottom of the dishes as well. The water in the dish containing the stones inoculated with *P. epilithica* turned reddish purple and then reddish brown a few days later (figure 22). The water in the other dish turned yellowish green, with green flecks floating in it (figure 23). When looking at these biofilms under the microscope, the red dish contained *Pseudanabaena epilithica* the same bacteria that were growing on the stones (figure 22). The green dish contained organisms that did not look like *P. autumnale* (figure 23) and were most likely different types of algae or cyanobacteria.

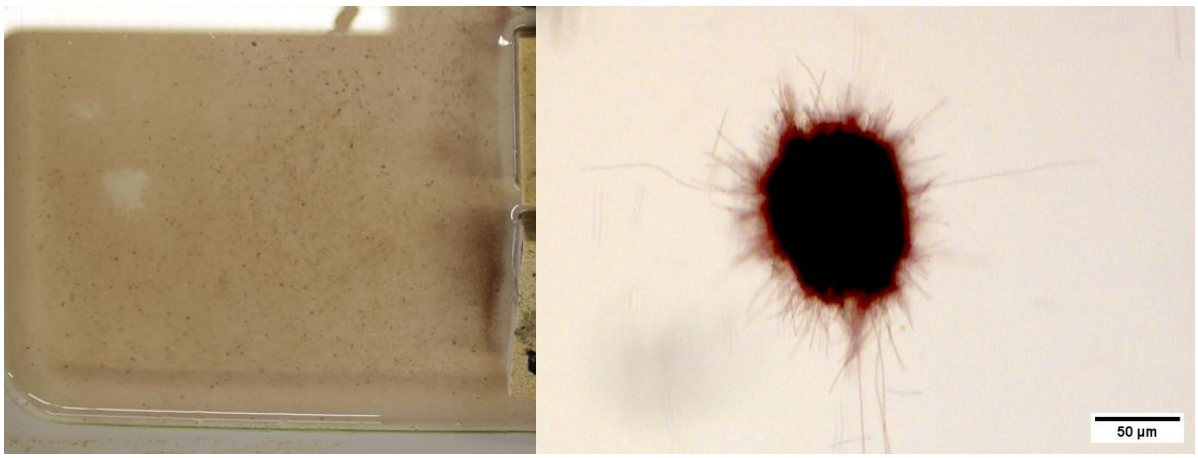

Figure 22: (left) Image of the red dish with P. epilithica growing at the bottom of the dish, (right) image of what the bacteria growing in the dish looked like under the microscope.

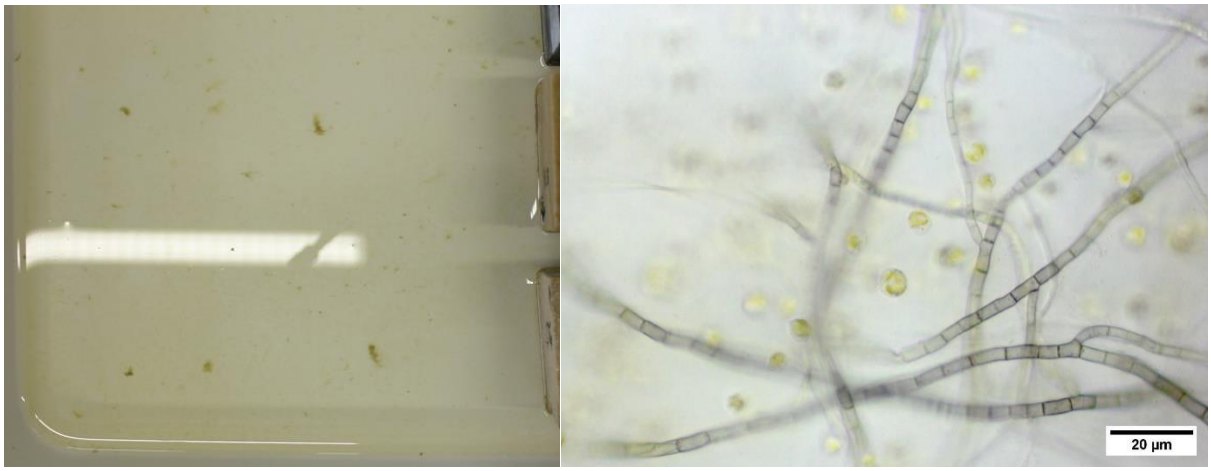

Figure 23: (left) Image of the green dish, the organisms growing at the bottom of the dish and floating through the dish are not P. autumnale. (Right) image of the organisms growing in the dish under the microscope.

4.2.3 Spectral differences

When comparing the measured reflection spectra from the preliminary tests, the background measurements for the Bentheimer and Savonnières samples are a lot higher in the first test when compared to the second test (figure 24). In the first preliminary test the Bentheimer spectrum is 8 to 23 percent lower when compared to the background spectrum, for the Savonnières spectrum this is 8 to 25 % lower. When looking to the spectra for the second preliminary test this difference is only 2 to 3 percent at most for the Bentheimer and Savonnières spectra.

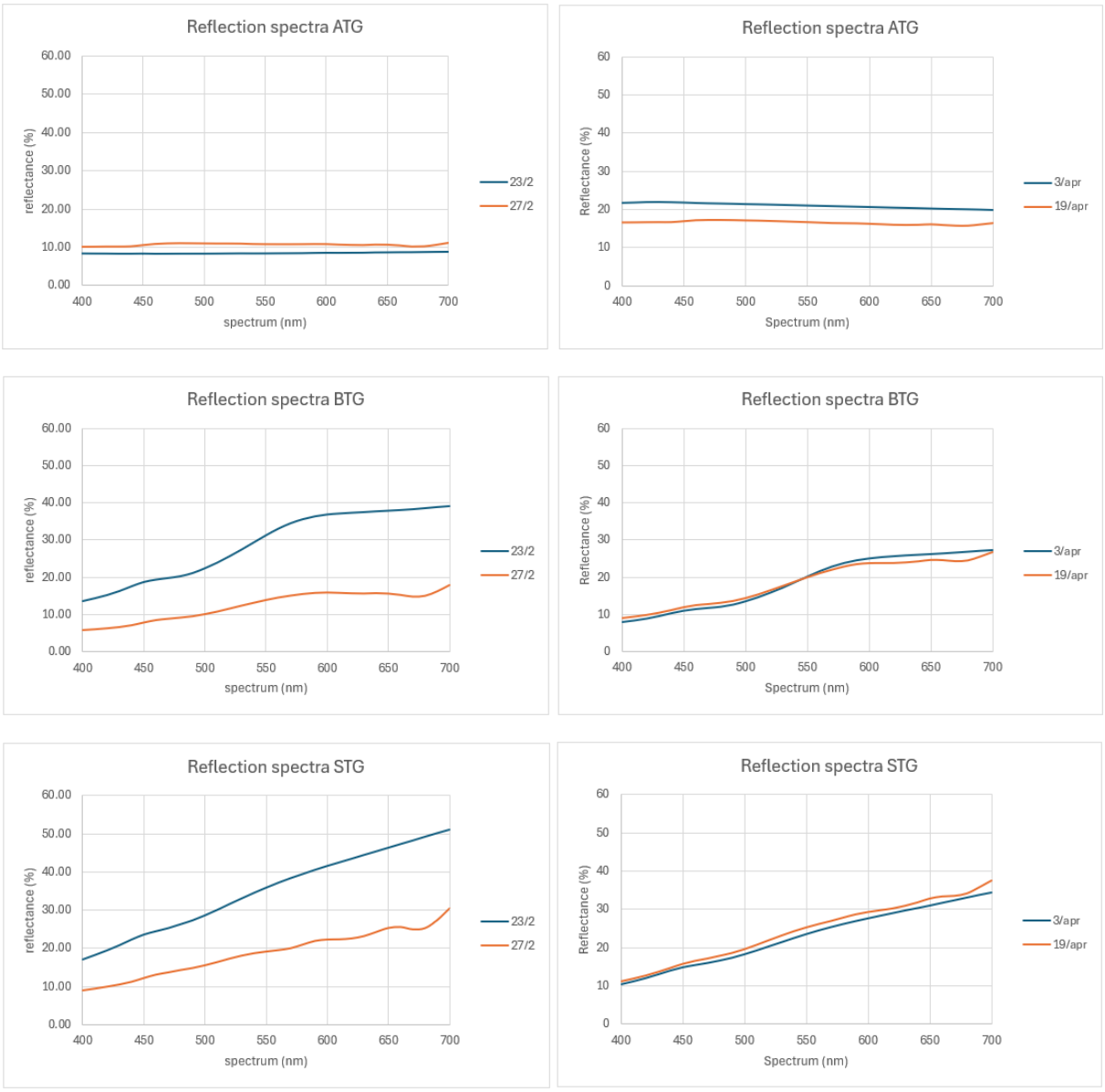

Figure 24: Graphs showing the measured background value and the first measurement after inoculation for the first and second preliminary test. All the graphs on the left are from the first preliminary test, the graphs on the right are from the second preliminary test. The blue line shows the measured background value (before inoculation), and the orange line shows the measurement after inoculation.

The background values for the first preliminary test were measured with the spectrophotometer touching the stone surface. The background values for the second test were measured with the

spectrophotometer a few milimeters of the surface, the same procedure as if there were bacteria on the sample.

4.3 Main test

4.3.1 Colour differences

When looking at the difference between the wet and dry samples, the Belgian blue stone showed the biggest average colour difference of 21.08. The Savonnières had an average colour difference of 13.72, and the Bentheimer sandstone showed the smallest colour difference between wet and dry samples of 12.11. The calculated standard deviations for the samples can be seen in table 10, and the specific colour change for all the samples can be found in appendix 6.

Table 10: Average measured colour differences between wet and dry samples, with the standard deviation.

Stone type	**ΔE**
Belgian blue stone	21.08 ± 1.04
Bentheimer sandstone	12.11 ± 2.04
Savonnières limestone	13.72 ± 0.77

The colour of the Belgian blue stone changed the most due the application of water repellent. This big colour difference could be staining that is referred to in the technical data sheet for the Redisil S (Rewah, 2024). The product caused an average colour difference of 24.34 and the stone went from a light grey to blueish colour to a dark grey to black colour. The Bentheimer had an average colour change of 6.68 due to the water repellent. It became a bit more orange-rusty in colour. The average colour change in the Savonnières limestone was 2.48. This colour change was less visible on the samples, then for the other two stone types. The calculated colour differences for all the samples can be seen in table 11.

Table 11: Colour differences calculated between the dry samples before treatment and the dry samples after treatment.

	ΔE*ab
A8	24.92
A9	25.52
A10	22.07
A11	25.57
A12	24.60
A13	22.70
A14	25.00
Average	24.34
Stdev	1.28

	ΔE*ab
B8	6.69
B9	6.10
B10	5.69
B11	6.97
B12	6.77
B13	6.94
B14	7.58
Average	6.68
Stdev	0.57

	ΔE*ab
S8	2.14
S9	2.71
S10	2.65
S11	2.24
S12	1.87
S13	3.41
S14	2.37
Average	2.48
Stdev	0.46

During the application of the water repellent some of the parafilm around the samples was torn and even melted partly. This made it difficult to remove the parafilm once the samples were dry, especially for the Belgian blue stone.

4.3.2 Hydrophobized contact angle

Due to application of the water repellent, the drops did not get absorbed immediately anymore. This means that a static contact angle could be measured for the Bentheimer sandstone and the Savonnières limestone (table 12). The contact angle at first contact and the static contact angle for the Belgian blue stone increased with ± 28 degrees after being made hydrophobic, from 68.37° to 96.25° at first contact and from 60.42° to 88.87° after ten seconds. For the Bentheimer, the contact angle at first contact increased with ± 33 degrees, from 85.22° to 117.86°. The average contact angle for the treated Savonnières limestone was 116.58 at first contact and 102.04° after ten seconds. For both the Bentheimer sandstone and the Belgian blue stone, the standard deviation on the results got smaller compared to the ones calculated for the non hydrophobic measurements (Table 5). The standard deviation for the Belgian blue stone decreased from 6.69 to 3.00 degrees for the contact angle at first contact and from 5.15 to 3.68 for the static contact angle. The standard deviation for the Bentheimer sandstone decreased from 7.17 to 2.94 degrees for the static contact angle.

Table 12: Contact angle measurements of the hydrophobized Belgian blue stone (A), Bentheimer (B) and Savonnières (S) samples, both at first contact and after 10 sec (static contact angle).

	first contact (°)	**10 sec (°)**
A1	97.16	89.41
A2	93.2	87.35
A3	101.42	95.75
A4	93.38	85.6
A5	96.08	86.23
Avg.	96.25	88.87
Stdev	3.00	3.68

	first contact (°)	**10 sec (°)**
B1	117.32	112
B2	114.17	106.88
B3	123.13	121.46
B4	118.02	115.62
B5	116.68	112.22
Avg.	117.86	113.64
Stdev	2.94	4.81

	first contact (°)	**10 sec (°)**
S1	120.62	110.12
S2	117.14	101.53
S3	121.95	102.52
S4	110.52	93.58
S5	112.69	102.47
Avg.	116.58	102.04
Stdev	4.41	5.24

Before the application none the stone types could be considered hydrophobic since the Bentheimer and the Belgian blue stone both had an average contact angle <90°. After the treatment all of the stone types had a contact angle above 90°, meaning that all three stone types became hydrophobic after treatment with the water repellent.

4.3.3 Bacterial growth

4.3.3.1 Savonnières samples

After growing for 10 days in the lab a reddish-purple biofilm was visible on all untreated Savonnières samples. Biofilms were also forming on all hydrophobic samples, except for S12. These biofilms on the hydrophobic samples were a lot smaller than the biofilms on the non-treated samples. The biofilms were also spread out differently: on the non-treated samples the bacteria formed one big colonisation that covered most of the stone surface. On the hydrophobic samples, the bacteria formed separate smaller spots that did not seem to be connected to each other (figure 25). Similar to the second preliminary test, bacteria started growing in the dish itself (figure 25).

Figure 25: Dish containing the Savonnières samples. The six stones on the right are the hydrophobic samples, the one empty sample is sample S12. P. epilithica can also be seen growing in between the samples and on the sides of the dish.

4.3.3.2 Bentheimer samples

The Bentheimer samples showed less visible bacterial growth on the surface of the samples then the Savonnières samples. The only clear growth happened on sample B4 and B6 (figure 26). The biofilm on B4 only covered about half of the surface with some other spread-out spots. The colonisation on B6 was even smaller, covering a small area in the upper right corner of the sample. On B1 some small purple dots were visible, that had not grown very much yet. On the other non-hydrophobic samples (B2, B3, B5), the only thing left from the bacteria were a few green dots that were only visible when looking very closely. On the hydrophobic samples, no bacteria were visible. The surfaces were all dry, and the only thing still visible from inoculation was an outline where the liquid had been.

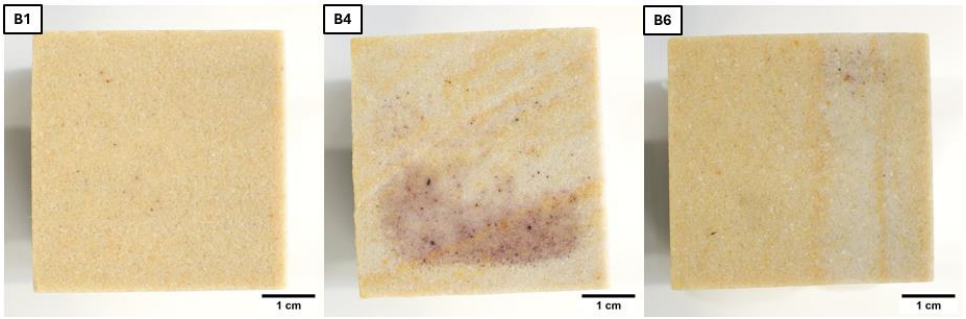

Figure 26: Images of the Bentheimer samples that showed visible bacterial colonisation.

4.3.3.3 Belgian blue samples

After 10 days, the surface of all the Belgian blue stones was dry. At some places on the non hydrophobic samples some reddish-purple pieces of dried-up biofilm could be seen (figure 27). Similar to the hydrophobic Bentheimer samples, an outline could be seen where the medium had been applied. This outline contained many small white-looking crystals, which were most likely from the salts in the medium. On the hydrophobic samples, this outline had a greenish yellow sheen (figure 27).

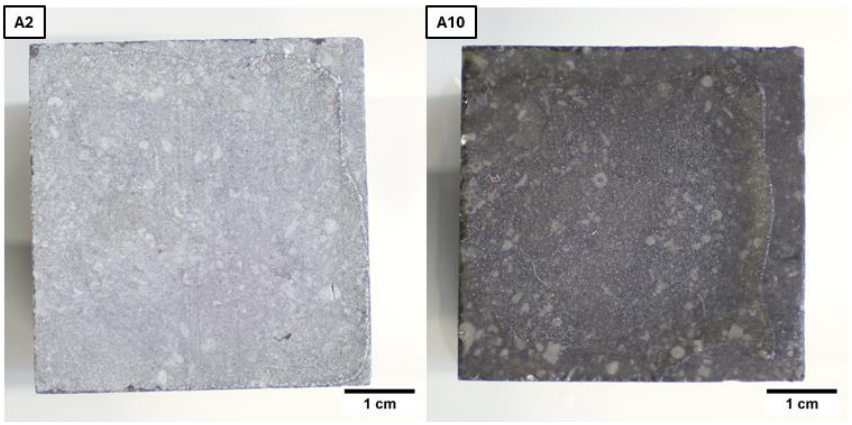

Figure 27: Images of two Belgian blue samples, both the hydrophobic and the non-hydrophobic sample show an outline where inoculation occurred. At the bottom right corner of A2, biofilm can be seen. Sample A10 shows the greenish colour on the surface.

4.3.3.4 Colour measurements

The calculated colour differences and the chlorophyll discolorations can be seen in table 13. The colours of A1-A7 were compared to background measurements on the dry Belgian blue samples. The colours of B1-B7 and S1-S14 were compared to background measurements on wet Bentheimer and wet Savonnières samples respectively. A8-A14 and B8-B14 were compared to the background measurements on the hydrophobic Belgian blue stone and Bentheimer samples.

The measured colour differences seemed very high for the Savonnières, Bentheimer and the non-treated samples of Belgian blue with an average ΔE of 14.98, 9.54 and 8.92 respectively. These averages were calculated from the values of S1-S6 and S8-S13, from the values of B1-B6 and B8-B13 and from the values of A1-A6. But the blank samples (7 and 14 for all the stone types), also seem to have very high values. If we take this into consideration, for the Belgian blue stone only samples A1-A4 and sample A6 show a big ΔE, with an average value of 9.51. For the Bentheimer sandstone only samples B3-B6 show a big ΔE, with an average value of 12.10. Almost all the Savonnières samples show a big ΔE when compared to the blank samples.

When looking at the chlorophyll discolouration, all the non-hydrophobic Belgian blue samples show a small CD. The hydrophobic blank sample A14 also shows a small CD, but looking at the graph this value is not caused due to a local minimum. Of the Bentheimer samples only B4 shows a clear CD, the spectrum of B6 also shows a small dip when compared to the other samples. All the non hydrophobic Savonnières show CD values together with sample S10. When looking at the reflection spectrums S9, S11 and S13 also show a small dip, but these dips are a lot smaller when compared to the other samples.

Table 13: Calculated colour differences and chlorophyll discolorations for the Belgian blue samples, the Bentheimer samples and the Savonnières samples. Samples 8 to 14 are hydrophobic, sample 7 and 14 are blank samples. Areas with a (-) show no visible minimum in the measured reflection spectrum.

	ΔE	CD
A1	12.14	0.18
A2	8.13	0.18
A3	9.30	0.14
A4	8.64	0.13
A5	5.96	0.10
A6	9.35	0.09
A7	6.64	-
A8	2.55	-
A9	3.20	-
A10	1.76	-
A11	1.99	-
A12	1.48	-
A13	1.89	-
A14	2.12	0.17

	ΔE	CD
B1	9.39	-
B2	8.01	-
B3	11.27	-
B4	16.71	4.69
B5	10.19	-
B6	10.25	-
B7	8.94	-
B8	8.05	-
B9	8.74	-
B10	7.71	-
B11	7.76	-
B12	8.03	-
B13	8.41	-
B14	8.20	-

	ΔE	CD
S1	14.27	5.71
S2	17.71	7.67
S3	20.91	10.68
S4	18.99	9.43
S5	24.55	11.05
S6	20.39	8.06
S7	6.94	-
S8	6.74	-
S9	12.32	-
S10	13.35	5.97
S11	7.80	-
S12	9.89	-
S13	12.84	-
S14	4.65	-

4.3.4 Climatic chamber

After the initial growth in the lab, the samples were put in the climatic chamber. After five days in the climatic chamber, almost all of the biofilms on the samples had disappeared. No visible bacteria could be seen on the Bentheimer and Belgian blue samples anymore. Some black spots were left on the Savonnières samples (figure 28), but it was difficult to see if these spots were small remaining biofilms or shaded pores between the ooids, since some of the pores have a purplish looking hue. Sample S10 showed a green stain where the biofilm used to be (figure 28). The outline from inoculation that was visible on untreated Belgian blue stones and hydrophobic Bentheimer had disappeared. While on the hydrophobic Belgian blue stone these outlines seemed to be more visible, showing clear white staining on the samples (figure 29).

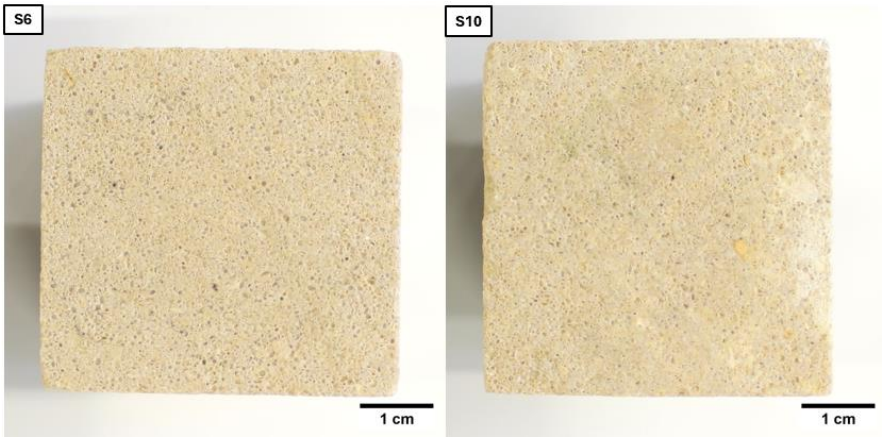

Figure 28: Samples showing what remains of the P. epilithica biofilms after 5 days in the climatic chamber. B6 shows some very small reddish-purple spots spread out over the surface, together with the purple hued pores. In the left corner of B10 there is a vague green stain.

After five more days in the climatic chamber nothing really changed, the hydrophobic Belgian blue samples seemed whiter than before (figure 29) and some of the black spots on sample S6 and the green stain on sample S10 had gone away. All the other samples seemed to look the same. When the Belgian blue samples were removed from the climate chamber for measuring, the non-treated samples dried up faster when compared to the hydrophobic samples.

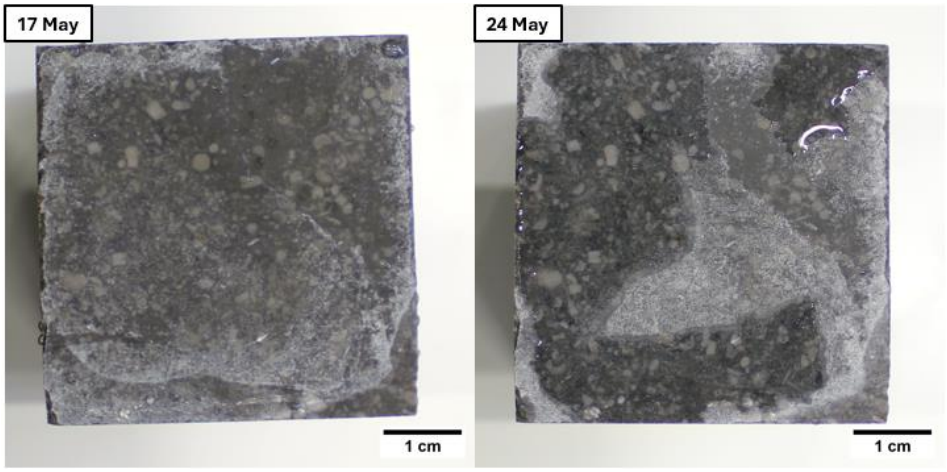

Figure 29: Sample A13 after five days and after 10 days in the climatic chamber, the outline from inoculation becomes more visible over time. And the white staining on the surface of the sample becomes more visible.

When looking at the calculated colour differences (table 14), the values decrease for most of the samples. But increase for the hydrophobic Belgian blue samples, this increase is quite high for samples A9, A10 and A11. When looking at the later colour measurements from the 17th and the 24th of May, most of the CD values disappear, with only B6 still having a, admittedly lower CD of 1.91. The spectra for the Savonnières samples S1-S6 and S10 still show a dip, when compared to the other samples. However, this dip is a lot smaller when compared to earlier measurements.

Table 14: Colour differences calculated for the Belgian blue, the Bentheimer and the Savonnières samples. The measurements on the 13th of May 2024 were taken before the samples were put in the climatic chamber (these measurements of the 13th are the same data as in table 13, but were added here for comparison).

ΔE	13 May	17 May	24 May
A1	12.14	2.28	1.57
A2	8.13	0.86	2.18
A3	9.30	0.93	3.06
A4	8.64	0.43	3.67
A5	5.96	1.40	5.41
A6	9.35	2.65	3.08
A7	6.64	0.58	4.73
A8	2.55	5.00	7.59
A9	3.20	11.43	9.69
A10	1.76	9.84	11.46
A11	1.99	6.28	9.46
A12	1.48	2.02	7.89
A13	1.89	2.76	6.62
A14	2.12	0.60	2.79

ΔE	13 May	17 May	24 May
B1	9.39	7.05	6.74
B2	8.01	9.40	5.64
B3	11.27	8.53	6.96
B4	16.71	8.37	5.64
B5	10.19	7.86	6.62
B6	10.25	8.46	6.96
B7	8.94	7.87	5.56
B8	8.05	4.98	4.96
B9	8.74	6.86	5.51
B10	7.71	9.61	7.21
B11	7.76	5.55	4.19
B12	8.03	7.54	5.59
B13	8.41	5.08	7.30
B14	8.20	5.02	4.77

ΔE	13 May	17 May	24 May
S1	14.27	6.25	5.66
S2	17.71	7.62	5.55
S3	20.91	8.95	5.82
S4	18.99	5.68	4.43
S5	24.55	8.13	5.26
S6	20.39	6.00	4.85
S7	6.94	7.23	5.08
S8	6.74	7.16	6.03
S9	12.32	7.46	5.45
S10	13.35	7.19	6.36
S11	7.80	7.32	5.49
S12	9.89	5.46	5.34
S13	12.84	4.83	4.82
S14	4.65	2.72	2.34

5. Discussion

5.1 Properties

The results of the porosity tests are in line with the expected values from literature (Derluyn et al., 2014; WTCB, 2001; Wim Dubelaar & Nijland, 2015). The larger variation on the calculated average porosity for the Savonnières samples (table 1) can be explained by the more heterogeneous pore space of the Savonnières compared to the Bentheimer sandstone.

The measured capillarity values for the Savonnières limestone are a bit lower than the values in Chen et al. (2023); the calculated standard deviation is also higher. When looking at the Bentheimer sandstone the absorption by capillarity value from Chen et al. (2023) is the most comparable to the capillarity values of the 'fast' Bentheimers that were described in the results of this thesis. These Bentheimer samples absorbed water much faster compared to the other Bentheimer samples, the average capillary absorption coefficient for these fast samples is about 560 $g/m^2 . s^{0.5}$. Some of the Bentheimer samples absorbed water much slower comparted to the other samples. These 'slow' samples had a much lower average capillarity absorption coefficient of 72.88 $g/m^2 . s^{0.5}$. This lower capillarity absorption coefficient could be caused by the presence of clay minerals in the samples. These clay layers can make the samples a bit more hydrophobic, but it is doubtful that these can have such a dramatic effect as visible in figure 14 where entire parts of the stone remain dry even when submerged under water.

According to (WTCB, 2001) the E-modulus of the Belgian blue stone is supposed to be a lot higher than what was measured in this thesis (77.6 compared to 19.6 kN/mm^2). The P-wave velocity (speed of sound in WTCB (2001)) is also a lot faster than what was measured. During the measurements sometimes the P-wave could not be measured for the Belgian blue stone; the signal could have gotten lost due to the high density of the stone. The UPV used in this thesis, is also mostly used for mortars and cements that are a lot lighter, compared to the heavy Belgian blue stone. The E-modulus of the Bentheimer sandstone and the Savonnières limestone is higher when compared to the values from Wim Dubelaar & Nijland (2015) and Derluyn, Moonen, et al. (2014). Since the UPV used, is also designed for longer samples instead of square samples, this could explain some of the difference between the measured values and the values from the literature.

The permeability values for the Bentheimer sandstone from Peksa et al. (2015) (measured with a gas permeameter) are lower than the average permeability found in this thesis. Due to the high standard deviation on the results, this value from literature still falls within the range of the measurements. The measured permeability for the Savonnières was a lot lower when compared to permeability from Schröer, De Kock, et al. (2022), even though this value was also measured with the TinyPerm. Similar to the results from this thesis, the results from Schröer, De Kock, et al. (2022) show very high standard deviations. Meaning that the results from the TinyPerm might not be as accurate. The results from the TinyPerm do show that the Bentheimer sandstone has a much higher permeability than the Savonnières limestone. This is due to the more heterogeneous pores in the Savonnières limestone and the smaller pore throats.

The standard deviation on the contact angles can be explained due to the low framerate of the camera during the contact angle measurements. This is also the reason why the contact angle of the Savonnières limestone could not be measured.

A summary of the stone properties that were used in this study can be found in table 15. Most of the values in this table are based on the average values that were calculated throughout this thesis. Due to its low porosity, there is no value for the coefficient of absorption by capillarity C1, and no permeability value K for the Belgian blue stone. The capillary coefficient of the Bentheimer sandstone is based on the average value of the fast Bentheimers in this study. The contact angle value is based on the value from Schröer, De Kock, et al. (2022).

Table 15: summary of the stone properties used in this study.

Stone type	**P_o (%)**	**ρ_b (kg/m3)**	**C1 ($g/m^2.s^{0.5}$)**	**VP (km/s)**	**VS (km/s)**	**E-mod (kN/mm^2)**	**K (mD)**	**contact angle (°)**
Belgian blue stone	0.28	2686.97	-	3.34	1.69	19.57	-	68.73
Bentheimer sandstone	23.08	2023.67	560	2.84	1.7	14.27	3574.2	83.27
Savonnières limestone	30.98	1868.99	112.9	3.24	1.81	15.41	131.89	47*

5.2 Preliminary tests

When bacteria grow, it would be expected that the colour differences and the chlorophyll discolouration increase over the course of the test. This is not what happened for most of the test samples. The only clearly increasing colour difference occurred in the Savonnières sample inoculated with *Pseudanabaena epilithica*. Even for this sample, the CD stayed mostly constant during the study. The *Phormidium autumnale* biofilm turning lighter green was also not a good sign, since this meant that the bacteria were not healthy. *P. epilithica* changing colour from red to green could be a response to the amount of light it was exposed to. But since the biofilm also got smaller this colour change was most likely because the bacteria died. Comparing the results from the preliminary test to (Schröer, De Kock, et al., 2022), makes it clear that *P. autumnale* was not growing. In that study, the surface of Savonnières limestone inoculated with *P. autumnale*, was entirely covered in biofilm after 1-2 weeks. This caused ΔE values of up to 60, while the maximum ΔE on STG in this thesis was only 20.49. And the amount of biofilm never seemed to increase during the tests.

There could be many reasons why the bacteria did not grow on the samples during the first preliminary test. The tap water added onto the Belgian blue samples was not boiled first, so this could possibly contain some chlorine which could be harmful for the bacteria. This explanation only works for the Belgian blue stones. Another reason why the bacteria were not growing could be because of the salt in the medium. When the medium was added on the surfaces of the samples, some of it could dry up causing too high of a salt concentration for the bacteria. The light intensity from the LED strips could also be too high for the bacteria to grow. The vortexing to homogenize the bacteria, could have damaged some of the bacteria and that was why they did not grow. In the first test, less consideration was given to the age of the bacteria. This means that

the bacteria were older at the time of inoculation, and there were a lot more bacteria added to the surface off the samples. Since the bacteria were older, some of the bacteria added might not have been able to grow anymore.

Since there were several reasons why the first preliminary test could have failed, a second preliminary test took place. To remove some of the reasons why the first test possibly failed, the medium was now added to the water of the samples instead of on top of the samples, no more tap water was added, and the bacteria were homogenized more gently. During this test, the bacteria used to inoculate the samples were younger than in the previous test. The bacteria had only been growing for 5 days in the Erlenmeyer before they were put on the samples. This was done make sure that the bacteria were still growing. This approach seemed successful, since *P. epilithica* now seemed to grow on both the Bentheimer sandstone and the Savonnières limestone. This growth did seem a lot slower in the second preliminary test when compared to the first one (figure 30). But this could be because the samples were inoculated with less bacteria then in the first test, because the bacteria were younger. The bacterial growth in the second test seemed to increase after the samples were inoculated with *P. autumnale*. Since *P. autumnale* did not seem to grow itself, this increase in bacterial growth was most likely due to the medium that was added to the surface during the inoculation process.

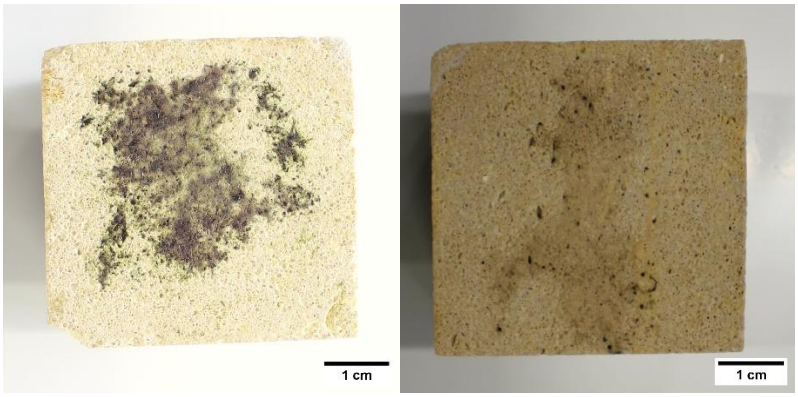

Figure 30: Bacterial growth on the STR sample after two weeks during the first preliminary test (left) and during the second preliminary test (right).

No bacteria grew on the Belgian blue stone. Since no more tap water was added to the surface, the surface stayed dry meaning that there was no water available for the bacteria. This lack of water is also because the Belgian blue stone cannot take up water via capillarity like the Bentheimer sandstone and Savonnières limestone. In the second test no white layer appeared on the surface of the Belgian blue stone, which did appear in the first test. This means this white layer could be caused by lime from the tap water, but no chemical tests have been done to verify this.

5.3 Main test

Since the bacterial growth was most successful in the second preliminary test, this setup was used for the main test, where the bioreceptivity of the normal samples was compared to the samples treated with the water repellent Redisil S. After allowing the bacteria to grow on the samples for 10 days. *Pseudanabaena epilithica* clearly grew on the surface of almost all the Savonnières samples. The growth on the non-hydrophobic samples seemed faster than the growth on sample STR in the second preliminary test. When comparing sample STR on 16 April to the main Savonnières samples there is a lot more biofilm on the surface of the main samples

(figure 31), even though both these samples had been growing for 10 days. Bacteria were also growing on all the hydrophobic samples except for S12. There was clearly a lot less biofilm on the hydrophobic samples then compared to the non-treated samples. The surfaces of the hydrophobic samples were all wet, meaning that the water repellent had lost its effectiveness. This is similar to what happened in the study of (Chen et al., 2023), where the treatment with Redisil S was less effective on the Savonnières limestone.

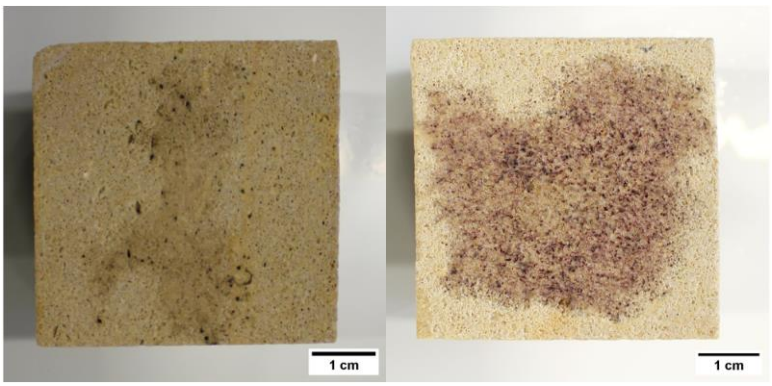

Figure 31: (left) Image of sample STR during the second preliminary test, (right) image of sample S5. Both stones had been growing for about 10 days, but the sample on the right shows a lot more biofilm on the sample.

Although *P. epilithica* grew well on the Bentheimer samples during the second preliminary test, this is not what happened in the main experiment. The bacteria only seemed to have grown on three of the non-hydrophobic samples and only covered a small part of the stone surface. On the other non-treated samples, the biofilm had turned green and the bacteria most likely died. When comparing the samples to the test sample BTR after 10 days of growing during the second preliminary test, the bacteria had also not grown all that much on that sample (figure 32). The bacteria on sample BTR had grown about the same amount when compared to sample B4 but had grown more then sample B1 and B6. This could mean that *P. epilithica* would have grown a bit more on the samples, if it had had more time to grow in the lab setting (longer then 10 days). The surfaces of the hydrophobic samples were all dry, so since the bacteria on the surface did not have a water source they did not grow on these samples.

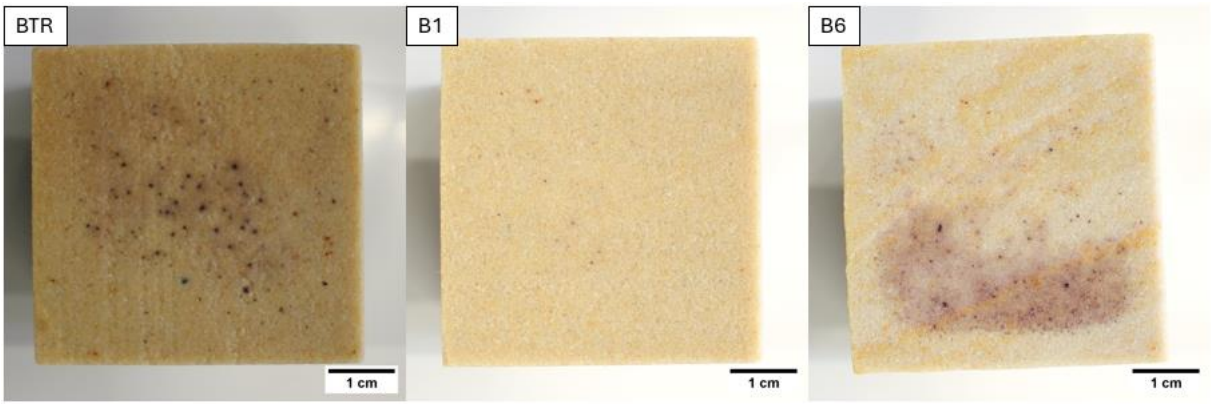

Figure 32: Images of sample the Bentheimer sample during the second preliminary test, and during the main experiment after 10 days of growing. There is about as much biofilm on sample BTR as sample B6, but there is a lot less biofilm on sample B1.

Similar to the preliminary tests, no bacteria grew on any of the Belgium blue stone samples. The samples were dry and the bacteria most likely dried out as well. The green sheen on the hydrophobic Belgian blue stones, was most likely caused by *P. epilithica* dying. The sheen

appeared only on the hydrophobic samples, but an outline where the stone was inoculated was visible on the non-hydrophobic samples as well. This means that the green sheen could be more visible on the hydrophobic samples due to their dark colour.

5.3.1 Climatic chamber

In the lab setup used in the second preliminary test and the main lab test, the surface of the Belgian blue stone stayed dry. This is because the Belgian blue stone cannot take up water via capillarity. This means that the water could not reach the surface of the samples, where the bacteria were. Without a source of water, the bacteria had no chance to grow. For this reason the samples were put into the climatic chamber with the artificial rain, to give the bacteria on the Belgian blue stone a source of water and a chance to grow. But five days after the samples were put in the climatic chamber almost all the biofilms had disappeared. The biofilms most likely had all been washed away by the rain in the chamber. The rain might have been too aggressive or too much for the bacteria to stick to the surfaces of the samples. The fact that the rain consisted of demineralised water was also not ideal for the bacteria to grow.

The only other thing that changed in the climatic chamber next to the bacteria disappearing was the colour of the hydrophobic Belgian blue samples (figure 29). The shape of the discolouration of the samples follows the outline where the samples were inoculated with medium, and it became more pronounced the longer the samples stayed in the climatic chamber. This was different for the non-treated Belgian blue samples, where the outline disappeared, after they were put in the climatic chamber. This outline could be caused due to the salts that were in the medium used to inoculate the samples, but these salts would have most likely dissolved due to the demineralized water in the rains. The hydrophobic stones turned from a dark grey to black colour to a lighter grey colour. This colour is more similar to the colour of the samples before they were made hydrophobic (figure 33). No tests were done to see if the samples were less hydrophobic at the end of the test with the staining, when compared to the start of the test. This discolouration could be the staining that is referenced in the technical data sheet for Redisil S (Rewah, 2024), which states that the water repellent may cause staining on stones with a low porosity (such as the Belgian blue stone).

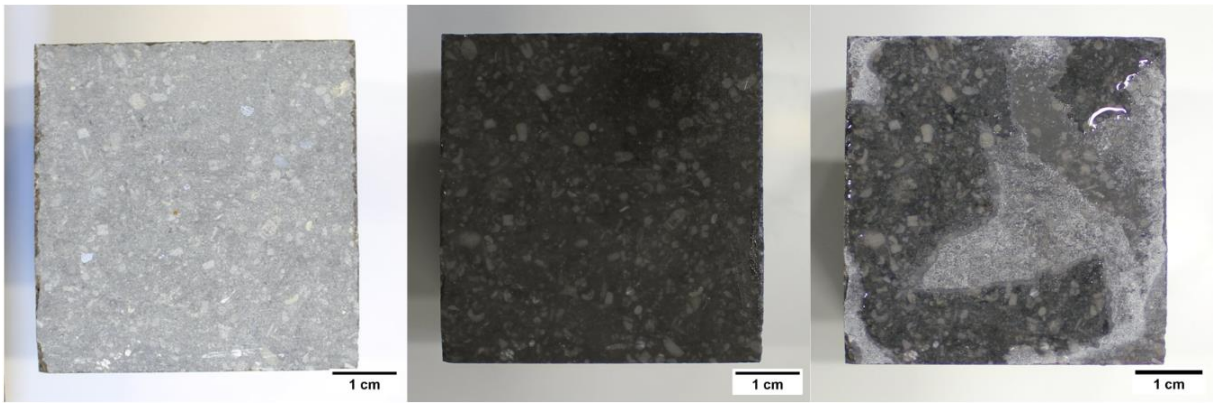

Figure 33: Image of Belgian blue sample A13, the image on the left is the dry untreated sample, the image in the centre is the dry sample after treatment with Redisil S, and the image on the right is the hydrophobized sample after 10 days in the climatic chamber.

5.3.2 Spectrophotometer data

When looking at the data from the spectrophotometer (table 14), the calculated colour differences varied quite a lot over time for the blank samples. This while the blank samples were untreated and thus should not have changed all that much. The ΔE measurements were also quite high for the blank samples (e.g. 8.94 for sample B7). Since these stones were not treated, the measured colour changes are then most likely caused because the background measurement was taken on the surface of the stone, and the main measurement was taken a couple of millimetres away from the stone. This small distance away from the stone was not constant and most likely varied a bit throughout this thesis which could explain some variation. Due to the small distance from the stone, the amount of light in the lab could have also affected the measurements. The fact that there was sunlight in the lab or not could affect the lightness value L*, which also affects the calculated colour differences.

6.Conclusion

During this thesis the bioreceptivity of three building stones was tested, the Belgian blue stone, the Bentheimer sandstone and the Savonnières limestone using two different types of cyanobacteria: *Phormidium autumnale* and *Pseudanabaena epilithica*. Of these bacteria only *P. epilithica* grew successfully on both the Bentheimer sandstone and the Savonnières limestone. Of these two stones the Savonnières limestone has the highest bioreceptivity, since the bacteria grew on this stone type successfully in all the tests, while the bacteria failed to grow on the Bentheimer in one of the tests. The Savonnières limestone has the highest porosity and the lowest contact angle of all the stone types used in this study. It has a lower capillarity coefficient and permeability when compared to the Bentheimer sandstone, even though these properties were assumed to be very important for bacterial growth. It may be possible that *P. epilithica* prefers growing on limestones, but this is difficult to say since the other limestone used in this thesis has such a low porosity. There have also been no previous studies growing *P. epilithica* on stone samples in a lab environment. This makes it difficult to put the results of this thesis in a broader scientific context.

The application of a water repellent negatively effected the bacterial growth. It completely stopped colonisation on the Bentheimer sandstone, and it decreased colonisation on the Savonnières limestone. This means that, although the water repellent was less effective on the limestone, it still caused a decrease in bacterial colonisation. However, it is difficult to say how this decrease occurred since no capillarity measurements took place on the treated Savonnières samples. The water repellent did not really affect the bioreceptivity of the Belgian blue stone, since no bacteria were growing on the stones before treatment, and no bacteria were growing on them after treatment.

The climatic chamber with the artificial rain did not have a good effect on the bacterial growth, since the demineralised water rain washed away all the bacteria in only 5 days. However, the use of a climatic chamber can still be valuable for bioreceptivity testing, since it has been used before for this purpose (Guillitte & Dreesen, 1995). During this thesis no measurements were made of the humidity and the temperature in the lab, while this could have had an effect on the bacterial growth. Further research with the climatic chamber but with a changed setup, such as softer or lesser rain, or maybe a higher relative humidity could be used to test the bioreceptivity of the Belgian blue stone in a better way. This way, the surface of the stone can stay humid for the bacteria growing on the surface.

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8.Appendix

Appendix 1: results of the porosity measurements for the Bentheimer sandstone samples (B) and the Savonnières limestone samples (S)

	porosity (%)	density (kg/m3)
B1	23.01	2034.10
B2	22.86	2037.87
B3	23.33	2026.52
B4	23.52	2021.27
B5	23.25	2028.27
B6	23.02	2034.38
B7	22.76	2040.70
B8	22.91	2035.05
B9	22.96	2035.89
B10	23.45	2023.26
B11	22.77	2041.79
B12	23.40	2026.19
B13	22.77	2040.31
B14	23.11	2031.76
Average	23.08	2032.67
Stdev	0.26	6.39

	porosity (%)	density (kg/m3)
S1	29.80	1901.22
S2	30.65	1877.75
S3	32.36	1832.53
S4	31.95	1842.54
S5	33.14	1811.23
S6	30.49	1882.69
S7	31.19	1863.53
S8	31.17	1864.42
S9	31.94	1842.36
S10	30.53	1879.58
S11	29.78	1899.73
S12	29.97	1897.51
S13	29.93	1898.34
S14	30.87	1872.45
Average	30.98	1868.99
Stdev	1.00	26.95

Appendix 2: results of the capillarity tests for the Bentheimer sandstone samples (B) and the Savonnières limestone samples (S)

	C1 ($g/m^2.s^{0.5}$)
B1	339.07
B2	224.54
B3	517.48
B4	608.30
B5	558.74
B6	579.40
B7	532.95
B8	60.04
B9	576.43
B10	232.63
B11	376.92
B12	378.81
B13	572.16
B14	85.72
Average	403.09
Stdev	183.37

	C1 ($g/m^2.s^{0.5}$)
S1	101.98
S2	155.20
S3	93.08
S4	105.45
S5	87.34
S6	119.18
S7	160.64
S8	126.80
S9	88.57
S10	112.47
S11	0.00
S12	121.84
S13	124.91
S14	108.29
Average	107.55
Stdev	23.62

Appendix 3: results of the UPV measurements for the Belgian blue stone samples (A), the Bentheimer sandstone samples (B) and the Savonnières limestone samples (S). The Belgian blue stones with an (-) could not be measured with the UPV.

	Vp (km/s)	Vs (km/s)	E-mod (kN/mm2)
A1	3.25	1.69	19.40
A2	-	-	-
A3	3.25	1.68	19.04
A4	3.23	1.69	19.15
A5	-	-	-
A6	3.31	1.69	20.94
A7	-	-	-
A8	3.82	1.62	19.14
A9	-	-	-
A10	-	-	-
A11	-	-	-
A12	3.26	1.72	19.91
A13	3.25	1.71	19.40
A14	-	-	-
Average	3.34	1.69	19.57
Stdev	0.20	0.03	0.62

	Vp (km/s)	Vs (km/s)	E-mod (kN/mm2)
B1	2.79	1.74	14.17
B2	2.76	1.71	14.80
B3	2.87	1.64	13.99
B4	2.99	1.70	13.90
B5	2.88	1.66	13.30
B6	2.81	1.74	15.06
B7	2.86	1.78	14.90
B8	2.84	1.75	15.03
B9	2.75	1.71	14.93
B10	2.87	1.62	13.45
B11	2.81	1.74	14.65
B12	2.85	1.62	13.68
B13	2.84	1.63	12.84
B14	2.81	1.75	15.15
Average	2.84	1.70	14.27
Stdev	0.06	0.05	0.73

	Vp (km/s)	Vs (km/s)	E-mod (kN/mm2)
S1	3.26	1.83	16.49
S2	3.33	1.86	16.55
S3	3.24	1.80	15.42
S4	3.22	1.76	14.09
S5	3.16	1.78	14.21
S6	3.35	1.93	17.65
S7	3.25	1.75	14.82
S8	3.14	1.85	15.22
S9	3.17	1.81	15.07
S10	3.21	1.80	14.88
S11	3.22	1.85	15.60
S12	3.30	1.81	15.31
S13	3.31	1.85	16.68
S14	3.17	1.73	13.74
Average	3.24	1.81	15.41
Stdev	0.06	0.05	1.06

Appendix 4: results of the permeability measurements for the Bentheimer sandstone samples (B) and the Savonnières limestone samples (S)

	T	K (mD)
B1	10.04	2839.26
B2	10.05	2760.70
B3	9.93	3829.93
B4	9.86	4749.18
B5	10.01	3088.61
B6	9.96	3587.21
B7	10.09	2490.78
B8	9.96	3587.21
B9	9.86	4749.18
B10	9.93	3865.92
B11	10.04	2812.83
B12	9.93	3902.25
B13	9.94	3723.96
B14	9.91	4051.01
Average	9.96	3574.15
Stdev	0.07	679.32

	T	K (mD)
S1	11.34	73.27
S2	10.93	235.88
S3	10.70	445.57
S4	11.40	61.92
S5	11.12	137.12
S6	11.08	151.98
S7	11.41	60.21
S8	11.32	77.50
S9	11.35	71.24
S10	11.30	81.97
S11	11.28	88.34
S12	11.02	179.84
S13	11.36	69.92
S14	11.19	111.62
Average	11.20	131.89
Stdev	0.20	100.24

Appendix 5: results of the contact angle measurements for the Belgian blue stone samples (A) and the Bentheimer sandstone samples (B).

	first contact (°)	10 sec (°)
A1	69.82	64.73
A2	74.85	66.49
A3	59.19	55.16
A4	75.88	62.12
A5	62.12	53.59
Average	68.37	60.42
Stdev	6.69	5.15

	first contact (°)
B1	86.11
B2	75.22
B3	84.14
B4	95.41
B5	75.45
Average	85.22
Stdev	7.17

Appendix 6: Calculated colour differences between the wet and dry samples of the Belgian blue stone (A), the Bentheimer sandstone (B) and the Savonnières limestone (S).

	ΔE*ab
A1	21.81
A2	22.35
A3	20.25
A4	20.08
A5	20.28
A6	21.10
A7	19.40
A8	20.73
A9	22.37
A10	20.45
A11	23.03
A12	21.73
A13	20.01
A14	21.57
Average	21.08
Stdev	1.04

	ΔE*ab
B1	13.35
B2	11.85
B3	11.21
B4	12.15
B5	12.78
B6	13.58
B7	15.45
B8	10.91
B9	11.12
B10	8.47
B11	13.75
B12	14.72
B13	13.29
B14	8.15
Average	12.11
Stdev	2.04

	ΔE*ab
S1	14.23
S2	14.41
S3	12.02
S4	13.97
S5	13.21
S6	13.11
S7	13.55
S8	13.58
S9	13.85
S10	14.47
S11	12.85
S12	13.37
S13	15.09
S14	14.44
Average	13.72
Stdev	0.77