4

ANALYSIS OF BIOLOGICAL GROWTH

4.1 INTRODUCTION

As can be noticed from existing literature, several studies have been carried out to assess the biological growth on cementitious and stone materials. Most of these experimental programs were carried out in laboratory conditions, where it was possible to control some of the parameters involved, such as temperature, relative humidity, day and night regime and the culture solution (Manso, 2014). In this way it is possible to isolate the variables that wanted to be analysed. Examples of these kind of experimental programs are described at the end of section 2.4.

However, it is also important to study the behavior of organisms and the colonization of the materials under environmental conditions, where the parameters related to climatic conditions and the presence of other pioneer organisms can not be controlled.

The variables that influence biological growth are many and still under study, but in general they can be divided in three main categories: the bioreceptivity of the materials, the type of organism and the climate conditions. A scheme of these parameters is presented in Figure 4.1.

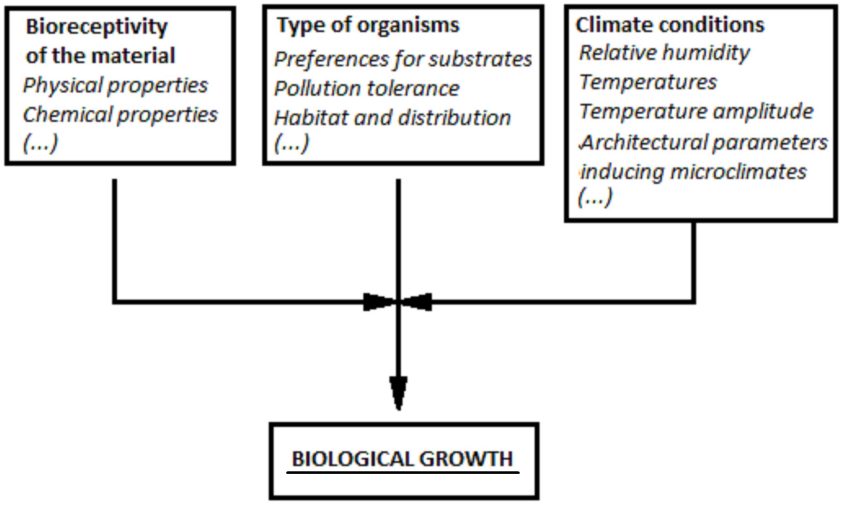

Figure 4.1: Scheme of the parameters involved in biological growth

In the experimental program presented in the following, the variables involved were evaluated appropriately while only controlling some of them to observe the effects on lichen growth.

Regarding the bioreceptivity of the material, the 15 materials selected and produced according to the previous chapter, were used for environmental exposure.

Concerning the type of organisms, lichens collected in two different locations were included in the test. Since it is impossible to control the presence of other organisms in the environment, which depends on the geographical situation and on other parameters, there is the possibility that other pioneer organisms colonize the substrates and compete with the lichens.

Also looking at climate conditions, all the specimens were placed in the same location, but 4 different wooden supporting structures were produced in order to assess the effects of exposure and orientation on the biological growth. Architectural parameter can influence the micro climatic conditions. Specimens exposed to North or under a shelter receive less direct solar radiation then South exposed specimens, therefore, it is possible that they take more time to dry after a rainfall, creating better growing conditions for the lichens.

The procedural steps followed in this part of the project are:

- the selection and construction of suitable substrates;
- the selection of the lichen species;
- the formulation of a suitable inoculation-suspension;
- allowing the propagules to attach to the substrate;
- the optimization of the growing conditions for the chosen species community.

The first step, the selection and construction of suitable substrates was described in the previous chapter. The other steps are presented in the following sections of this chapter.

4.2 MATERIALS AND METHODS

4.2.1 *Collection of Lichens*

In order to evaluate the effect of climate on different type of lichens, two locations were selected for the collection of the samples. Lichens were collected from Iceland so as to test their adaptability to slightly different climate, while other samples of lichens were collected in Denmark, because it was assumed that local varieties of lichens can grow easily if the climate conditions are the same of where they were taken from.

The species of lichens chosen for the experiment were *Xanthoria parietina* and *Physcia tenella*. This choice was based on the observation that these species of lichens are widespread and it is very common to find them growing on building materials. Furthermore, they were selected because of their high tolerance for pollutants, and the preference for slightly alkaline substrates. Another factor that influenced the selection of *Xanthoria parietina* was its appearance, with its yellow/orange colour it can add an aesthetic value to a building's façade. Around 1 kg (dry weight) of *Xanthoria parietina* was collected and exported from Iceland, with the permission of the Icelandic Institute of Natural History. The lichens were kept in paper bags, in order to accelerate the drying process, to keep the lichens alive during the transportation.

Another 1 kg (dry weight) of *Xanthoria parietina* and *Physcia tenella* were collected from the Copenhagen area.

The locations where the lichens were collected from, are shown in the maps in Figure 4.2.

Figure 4.2: Lichens' collection locations: 1. Hólavallagarður cemetery, Reykjavík; 2. Vesturhlíð, Reykjavík, Iceland; 3/4. Assistens Kirkegård, Copenhagen

The lichens were carefully removed from their substrates while they were moist, with a metal scraper. Often, part of the external surface of the substrates, where the lichens were growing was taken away, so as to be sure to do not damage the lichens. For the same reason, during the transportation, x-ray scanning was avoided, thanks to the collaboration of the Keflavík Airport custom.

4.2.2 *Lichens respiration test*

Since the time between the collection of the lichen samples and the inoculation process was less than one month, it was assumed that the lichens were still alive. In order to assess this, a respiration test with methylene blue was tried at the chemical laboratory of the National Food Institute at the Technical University of Denmark. The principle behind this test is that viable cells reduce the methylene blue staying unstained, while dead cells are not able to reduce the oxidized methylene blue and they become stained blue.

Small pieces of the different lichens collected and of a reference lichens collected immediately before the test were placed in a methylene blue solution. After stirring and waiting 10 minutes, some drops of the solution were poured on the glass slides for inspection under microscope. It was observed that several cells for the collected samples, as well for the reference case, remained unstained, suggesting that the lichens were alive.

Since lichens are symbiotic organisms, composed by a fungal part and a photobiont, there is the possibility that some algae or cyanobacteria, growing alone outside the fungal partner, were present in the solution. In this case, the algae in presence of light makes photosynthesis, producing oxygen, and therefore, influencing the results of the test. More sophisticated method to test viability can be made with Walz instruments, but since these tests are not very precise, it was chosen to skip them and presume that the lichens were alive.

4.2.3 *Growth media suspension*

Based on previous studies and research made on growth media and in vitro culture of lichens (Verma; Demaray, 2014), it was decided to use a suspension composed by casein peptone and water, with a ratio of 1:2. Peptone, in powder, and water were mixed together, obtaining a dense sticky solution. Then, the Icelandic and the local lichens were gently crushed in small pieces and mixed with the solution, with a lichen:solution ratio of 1:6. The two suspensions obtained were spread on the substrates. The suspension with the imported Icelandic lichens was spread on the left side of the specimens covering a surface area of about 80 cm^2, while the suspension with the local lichen was spread on the right side covering the same surface area. A stripe of 2x14 cm^2 between the two parts covered by the suspensions, was left clean (Figure 4.3). The specimens were left to dry on a table for 24 hours. Finally all the specimens were attached to the wooden structures through stainless steel clips, screws and dices.

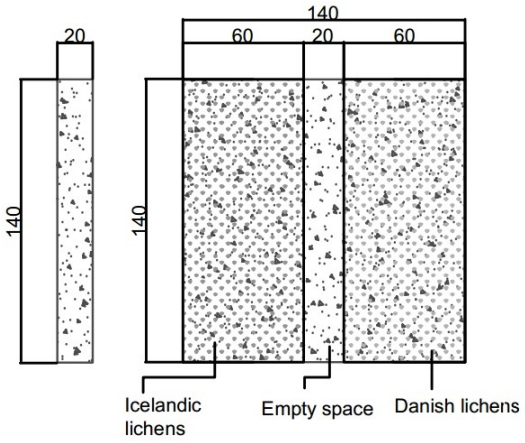

Figure 4.3: Drawing of the distribution of the different suspensions on the specimens' surface

In Figure 4.4, some of the pictures taken during the lichens' inoculation process are shown.

Figure 4.4: Lichens' inoculation process

4.2.4 *Location of the test*

The specimens were placed on four appositely made, different supporting structures, in the space reserved for the project, outside Building 121A, in the campus of the Technical University of Denmark, in Kongens Lynbgy, Denmark (Figure 4.5).

Figure 4.5: Location of the outdoor experiment

The location is in the oceanic climate zone, where the weather conditions are unstable because of the low-pressure systems from the Atlantic. Precipitation can be considered moderate, a part in the period between July to September, where slightly higher rainfall are registered. From late December to late April there is the possibility of snow, and exceptional weather conditions can bring up to 50 cm of snow in some days of the year. In winter the average hours of sunshine are one and a half per day, while in June the average is about eight hours. The average temperatures are between 17 to 20°C in July and around 0°C in January.

All the structures were placed in the test location between December 11th, 2015 and December 12th, 2015.

4.2.5 *Weather Data*

The climate data corresponding to the location of the experiment under environmental conditions were acquired and monitored by means of the climate station at DTU Civil Engineering department. The station is placed on the roof of building 119, at a distance of less than 200 m from the experiment setup. The measurements of weather data are related to the period between December 11th, 2015 and January 18th, 2016. The data related to the 22nd, the 26th and part of the 28th of December were missing, as well as the data related to the night between the 10th and the th of January. But this does not influence the project in a significant way as only a couple of days are missing from a longer period of time and will not influence the conclusions of the results.

Figure 4.6 shows the average ambient temperatures over the time period of the experiment. The maximum value was recorded on the 20th of December, reaching an average temperature of 11.7 °C. The minimum average temperature was -8.2 °C and was registered on the 16th of January.

Figure 4.6: Average ambient temperature [°C] at the location of the outdoor experiment

Figure 4.7 shows the relative humidity, the maximum of 91.4% RH was registered on the 17th of December, while the minimum of 44.8% RH was recorded on the 4th of January.

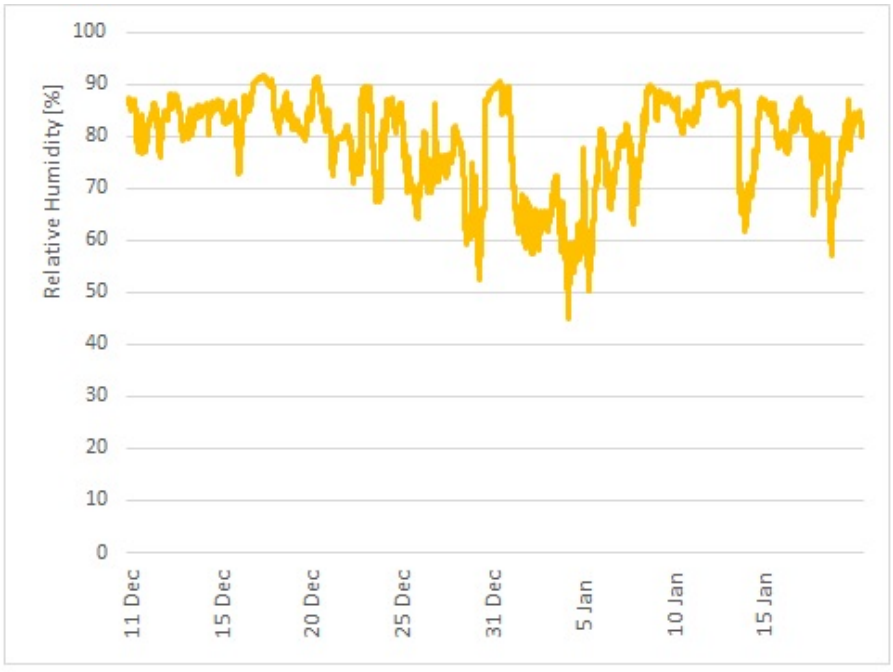

Figure 4.7: Relative humidity [%] measured at the location of the outdoor experiment

In Figure 4.8, the data regarding sunshine and total solar irradiance on the horizontal plane are presented. For the sunshine measurements, a value of 1 was attributed when the measured solar irradiance was greater than 300 W/m^2, and a value of 0 when it was equal or less than 300 W/m^2.

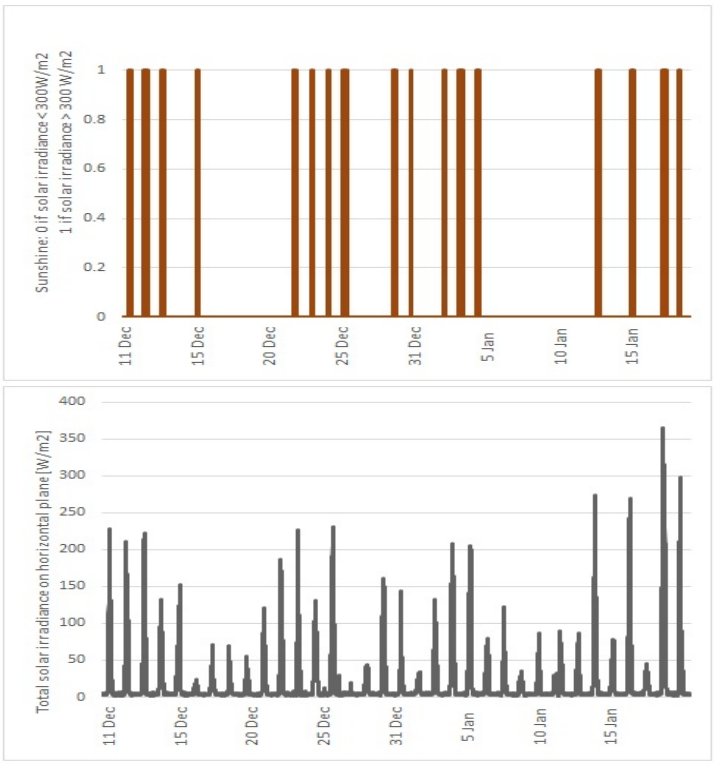

Figure 4.8: Sunshine and total solar irradiance on the horizontal plane, measured at the location of the outdoor experiment

Figure 4.9 shows the rain accumulation [mm] and the rain intensity [mm/h] over the test period. The maximum value of precipitation was 0.33 mm (or l/m^2), while the maximum rain intensity was 17.4 mm/h, both recorded on December, 24$^{\text{th}}$.

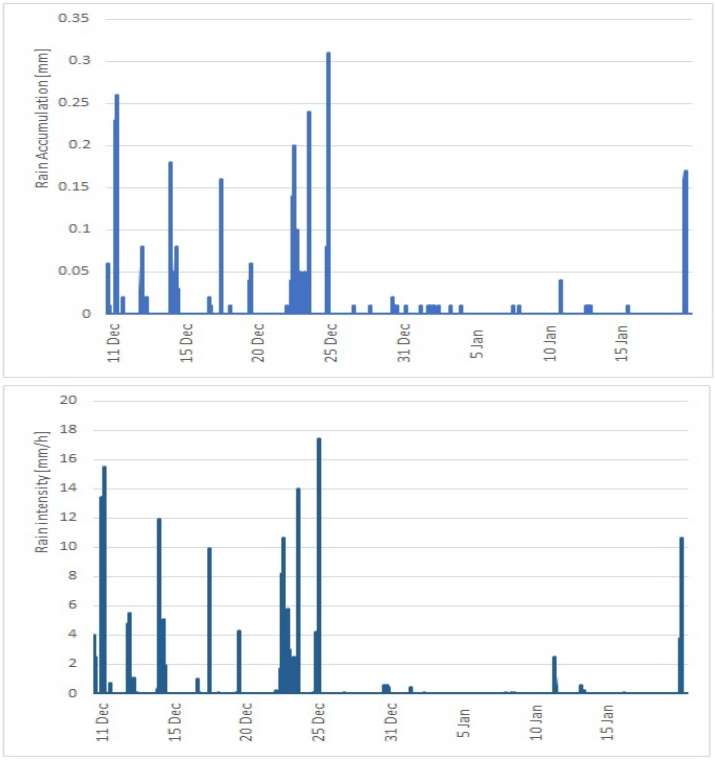

Figure 4.9: Rain accumulation [mm] and rain intensity [mm/h] measured at the location of the outdoor experiment over the test period

Finally, Figure 4.10 shows the wind rose related to the wind data measured at the location of the experiment over the test period. On the graph, the time fraction when wind was coming from a certain direction in a certain wind speed range, on the total time of the test, is plotted.

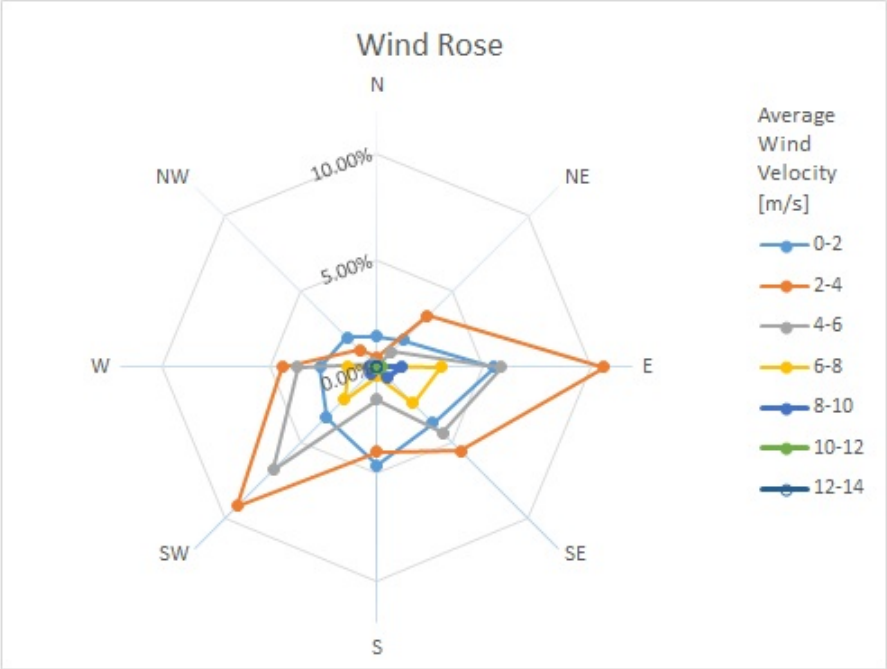

Figure 4.10: Wind rose showing the fraction of time, wind was coming from a certain direction at a certain speed, over the total time period of the outdoor experiment.

4.2.6 *Specimens and Setup*

Four wooden structure were constructed appositely to evaluate different expositions and orientations of the substrates, and their effects on the lichens' growth. Two of the structure were made to place the specimens horizontally, while the other two where made to place them vertically. One of the vertical structure and one of the horizontal were covered with a transparent Plexiglas shelter, to protect the suspension and the lichens from heavy rain and snow, but still to allow solar radiation to reach the specimens. The supporting structures and how the specimens are connected and placed on them are shown in Figures 4.11, 4.12, 4.13 and 4.14.

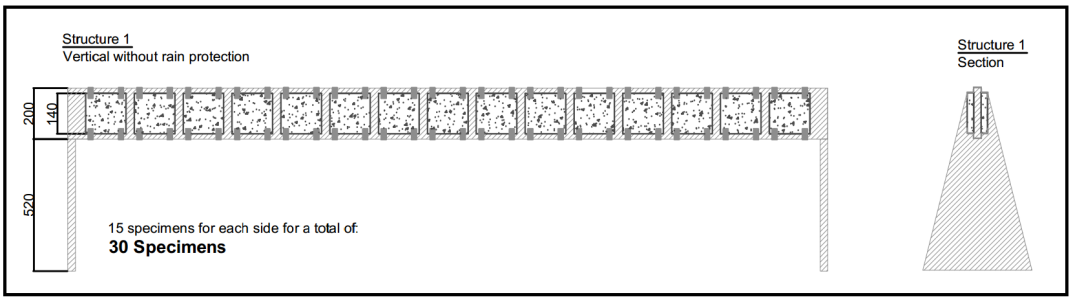

Figure 4.11: Structure 1 (V/S,V/N) setup

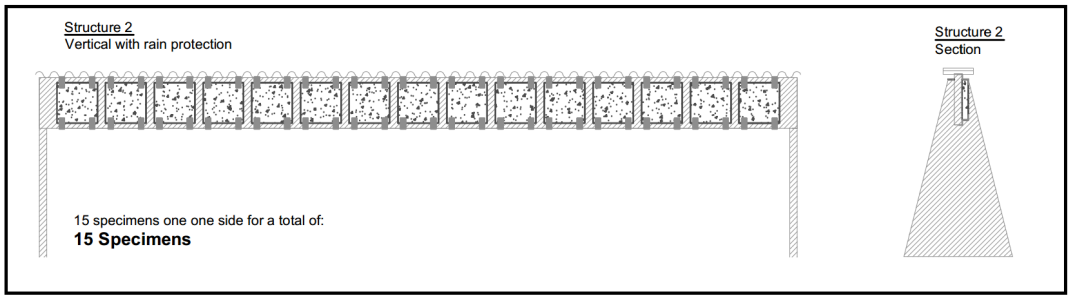

Figure 4.12: Structure 2 (V/C) setup

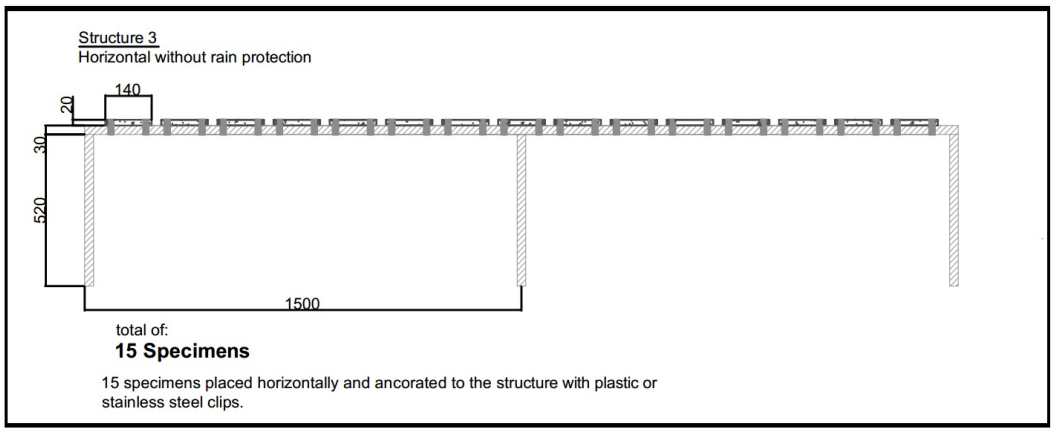

Figure 4.13: Structure 3 (H) setup

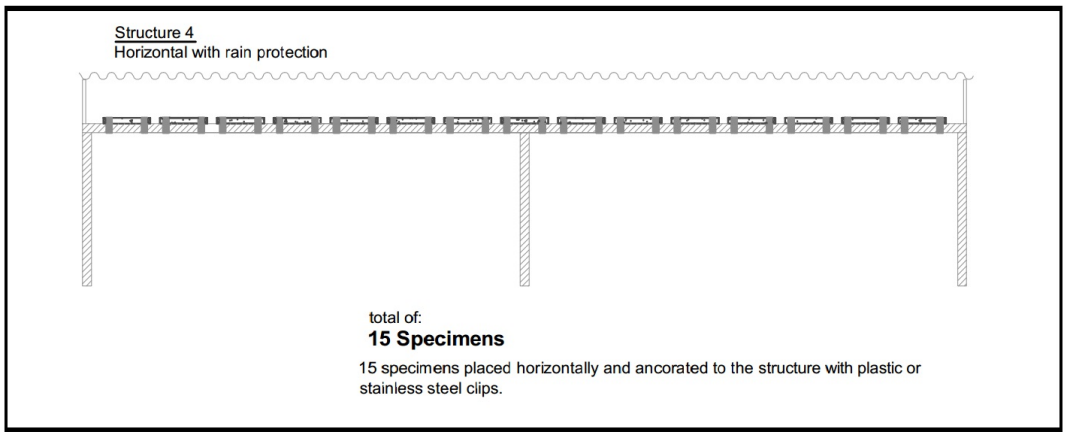

Figure 4.14: Structure 4 (HC) setup

A total of 75 specimens, 5 for each of the 15 different substrates presented in the previous chapter, were produced for the experiment under environmental conditions. The specimens were 14 cm x 14 cm with different thickness.

15 different specimens were connected and attached to each of the wooden support structures. On the vertical structure without the shelter, 15 specimens were attached to both sides of the structure facing

South and North, in order to compare the effects of different orientations on the lichen growth. A drawing of the setup is presented in Figure 4.15.

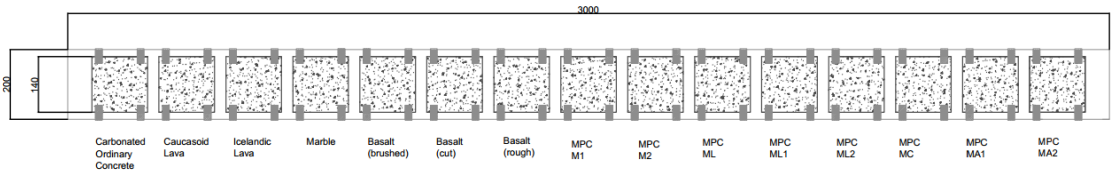

Figure 4.15: Setup of specimens

4.3 RESULTS AND ANALYSIS

Since lichen's growth rate is very slow, and this thesis is part of a project that will continue for two more years, it was decided that the experiment setup will be kept outside until the end of the Nordic built project, in 2017. The results presented in the current section are related to the observations made in the first 37 days of environmental exposure, from December 12th, 2015 to January 18th, 2016.

Periodical visual inspections were effectuated to evaluate the lichens adhesion to the substrates and their growth.

Due to the continuation of the experiment, it was not possible to remove the specimens from the setup to perform further analysis, but only visual inspections were possible. There are several methods that have been used in existing literature to evaluate the presence of pioneer organisms and biological growth on substrates. These methods can be destructive and non-destructive and include biomass quantification, colorimetric measurements, SEM, plate counting and biochemical and microscopic analysis (Manso, 2014).

In order to avoid evaluation methods that can have an influence on future results, for this test it was chosen to analyse the lichens adhesion and growth only through visual inspections. The last inspection was carried out on January 18th, 2016. The results presented are related to this last inspection.

In the first month it was observed that the peptone suspension was progressively washed out and taken away from the specimens, by rain and wind. The exposure and inclination of the samples, in addition to the presence or not of the protection shelter, influenced the duration of the period from the begin of the experiment to the moment when the peptone suspension was completely washed away. Therefore, in some cases the lichens had more time to adhere to the substrates.

4.3.1 *Results*

Monitoring of the lichens growth and their adhesion to the substrates started after 20 days from the beginning of the experiment. Visual inspection were carried out regularly, every 3 or 4 days. As mentioned before, the reported results are related to the last inspection made on January 18th, 2016, 37 days after the beginning of the test.

5 different exposure and orientation of the specimens were analysed, however it was impossible to obtain results for the specimens placed horizontally on the supporting structure, without the protective shelter, because of the presence of snow, that was completely covering the specimens.

In the next figures, a code is used to define the specimen orientation, exposure and the presence or not of the Plexiglas protective shelter. The first letter, "V" or "H" designates the orientation, corresponding to vertical and horizontal orientation respectively. The second letter, "S" or "N" indicates the exposition of the specimens to south or north respectively, while the "C" designates if the protective shelter was present. For instance, a supporting structure designated as H/C, means that the specimens were placed horizontally, and protected with a shelter, while a structure designated as V/S indicates that the specimens were placed vertically, facing South.

As mentioned before, lichens collected in Iceland were inoculated on the left side of the specimens, while the lichens collected in Denmark were placed on the right side.

Figure 4.16 shows the pictures, photographed at the same distance, of the specimens placed vertically and exposed to South, without any protective shelter (V/S).

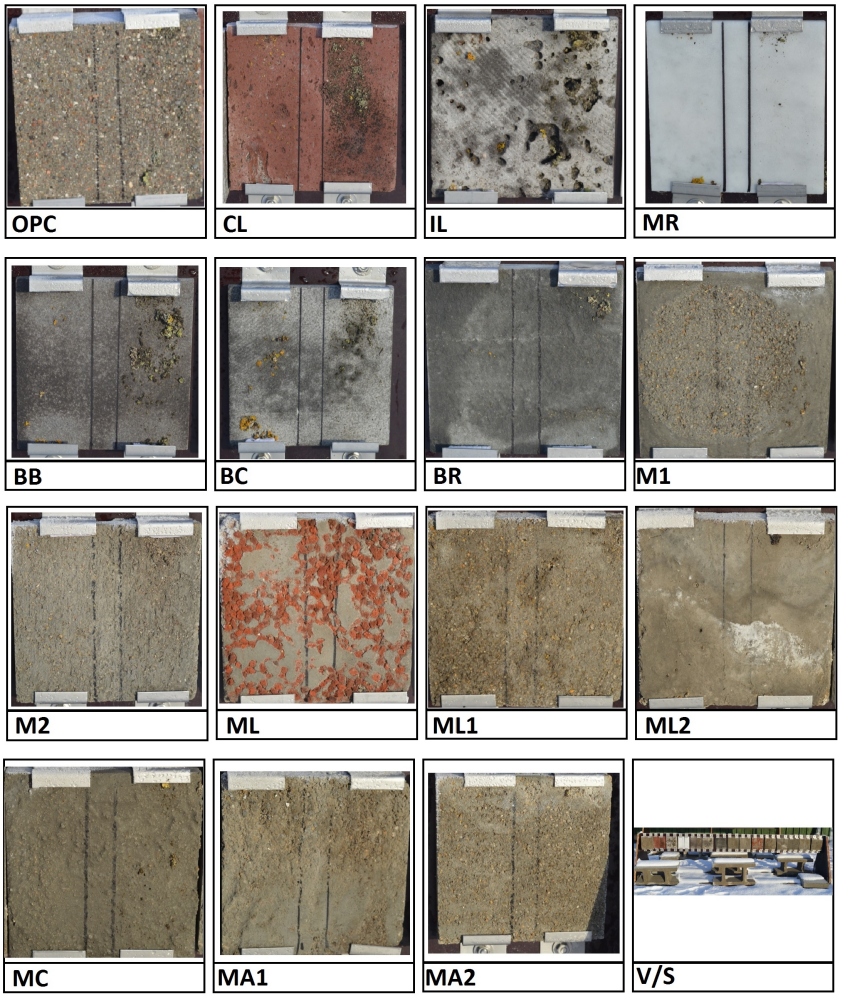

Figure 4.16: Pictures of the specimens, vertically oriented, South exposed, after 37 days subjected to environmental conditions

From the visual inspections, as can be observed from the pictures, it is possible to conclude that the lichens, both Danish and Icelandic, attached to several specimens. No visible colonization was observed on most of the MPC samples, with the exception of M1, M2, ML1 and MC, where only small parts of the surfaces were covered by lichens, mostly by the local one. The best results in terms of colonization were observed for two (BB, BC) of the three the basalt stones specimens, especially for the one with the cut surface. Almost no colonization was observed on the carbonated ordinary Portland concrete specimen (OPC), and on marble (MR), while adhesion between the local lichens and the substrate was high for the Caucasoid lava. Regarding the Icelandic lava, both Icelandic and Danish lichens were individuated especially inside the big superficial pores, suggesting that the pores acted as micro-refuges protecting the lichens from rain, wind and snow.

In the next figure (Figure 4.17), the specimens placed vertically and exposed to North (V/N), without the protective shelter are shown. It is important to point out that the specimens were shadowed for almost all the duration of the experiment, and no direct solar radiation reached the surface of the specimens. Furthermore, since the specimens faced the building where the structures were placed in front of, it can be assumed that a sort of protection from wind was also offered in comparison to the other exposition (V/S).

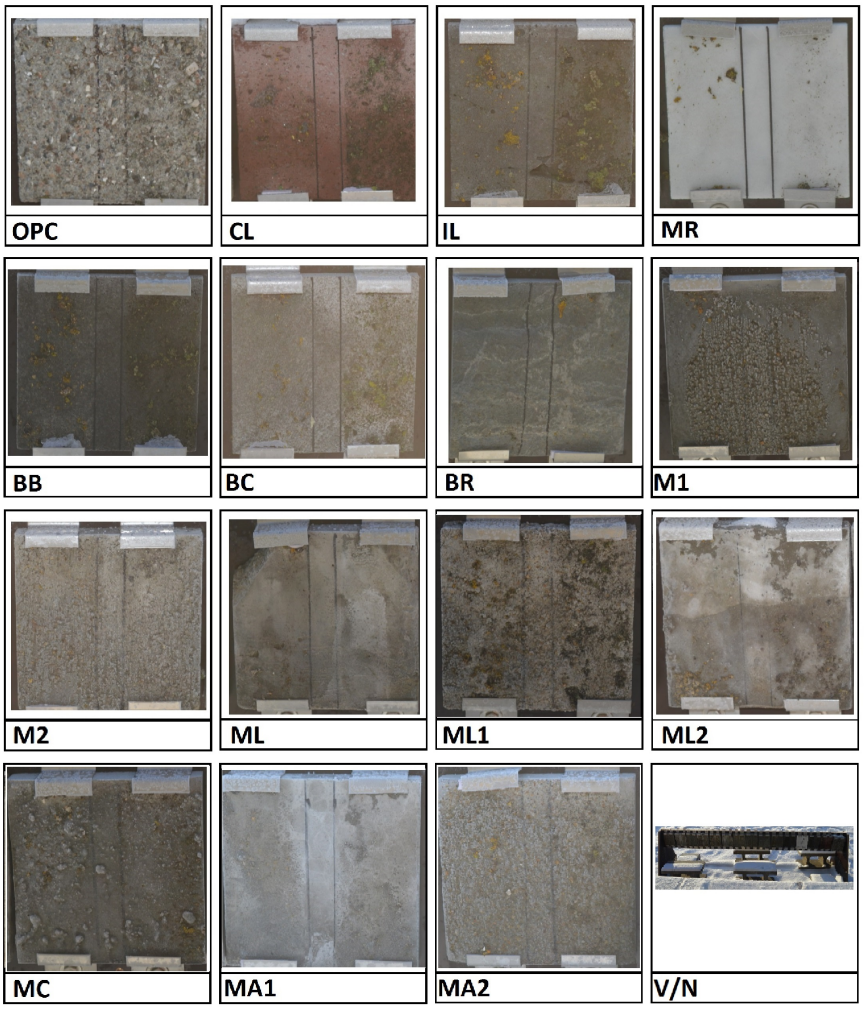

Figure 4.17: Picture of the specimens, vertically oriented, North exposed, after 37 days subjected to environmental conditions

A general increment in the total surface covered by the lichens was observed from the monitoring for the V/N orientation compared to the previous V/S. The best results were obtained again on the basalt specimens with brushed and cut surfaces (BB, BC) and for the lava stone specimens (CL, IL). From the pictures it can be noticed that on the left side of the CL specimen, the Danish lichens are distributed heterogeneously on the whole surface. Regarding the IL specimen, most

of the lichens were found again inside the large superficial pores. No signs of colonization were observed, from the visual inspection, on the OPC, on marble (MR) as well as on almost all of the MPC samples and the basalt specimen with rough surface (BR). From a deeper inspection, looking from a closer distance, it is possible to assess a relatively low presence of Danish lichens on the ML1 and MC samples, while presence of Icelandic lichens was observed on marble (MR), BR, M1, ML1, ML2, MC and MA1 specimens.

Figure 4.18 shows the pictures of the specimens vertically oriented, South exposed and protected from the Plexiglas shelter (V/C).

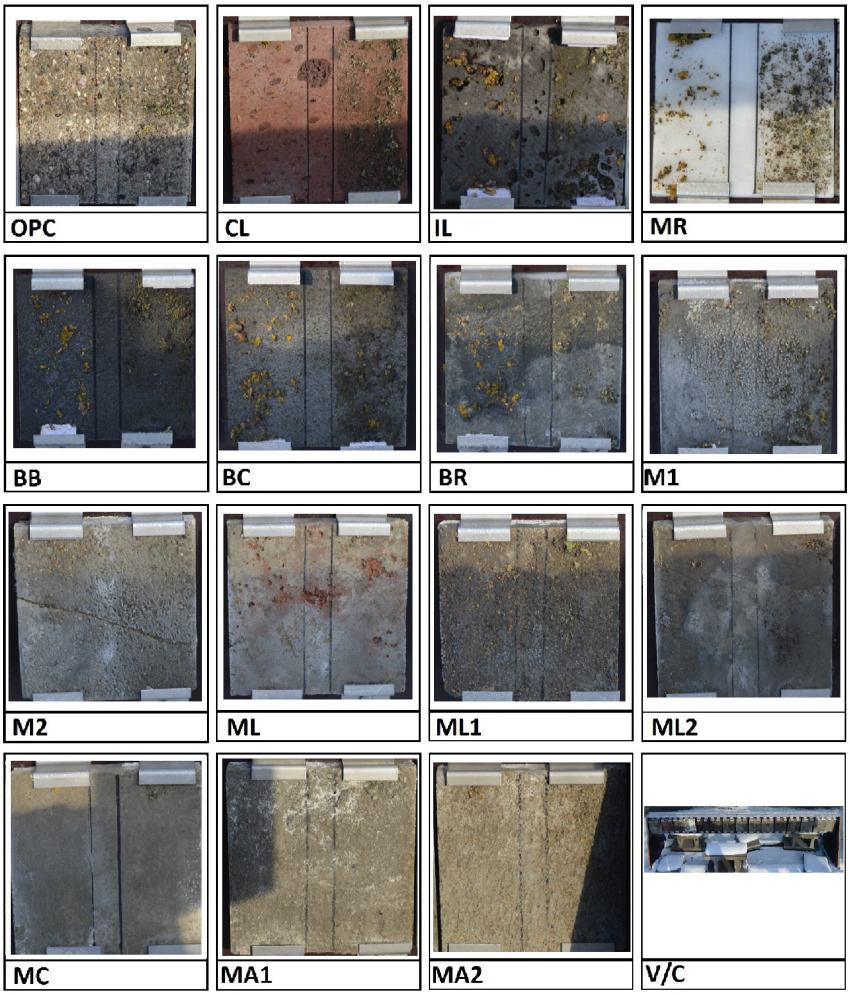

Figure 4.18: Picture of the specimens, vertically oriented, with shelter, after 37 days exposed to environmental conditions

In general, a higher percentage of the surfaces of all the specimens was covered by lichens, if compared to the two other expositions (V/S,V/N) previously analysed. This suggests that the shelter protected the specimens from heavy rain, giving more time to the lichens in order to adhere to the substrates, and favourite their growth, avoiding detach-

ment. From the visual inspection, the presence of lichens was clearly observed on the surface of specimens CL, IL, MR, BC, BB and BR. Also for this exposure, the Caucasoid lava shown a high bioreceptivity regarding the local (Danish) community of lichens, while just a few points were covered by Icelandic lichens on the left side of the specimen. Several lichens, both local and imported, were observed inside the large pores of the Icelandic lava, as for the replicates placed on the other supporting structures. Regarding the basalt samples, the presence of both communities of lichens was detected for all the different surfaces (BB, BC and BR). Very different results were registered, in this case, for the marble sample, when compared to the other marble specimens placed vertically (V/S and V/N). Lichens were found on both sides of the specimen's surface, and especially the local lichens on the right side were clearly visible from the naked eye inspection. On the OPC sample, only local lichens were found, partially covering the right side of the specimen. Regarding the MPC samples, biofouling was observed on the right side of M1, showing the presence of Danish lichens distributed heterogeneously on the surface, while a small presence of both Danish and Icelandic lichens were observed on ML1.

Figure 4.19, shows the samples oriented horizontally and covered with the shelter.

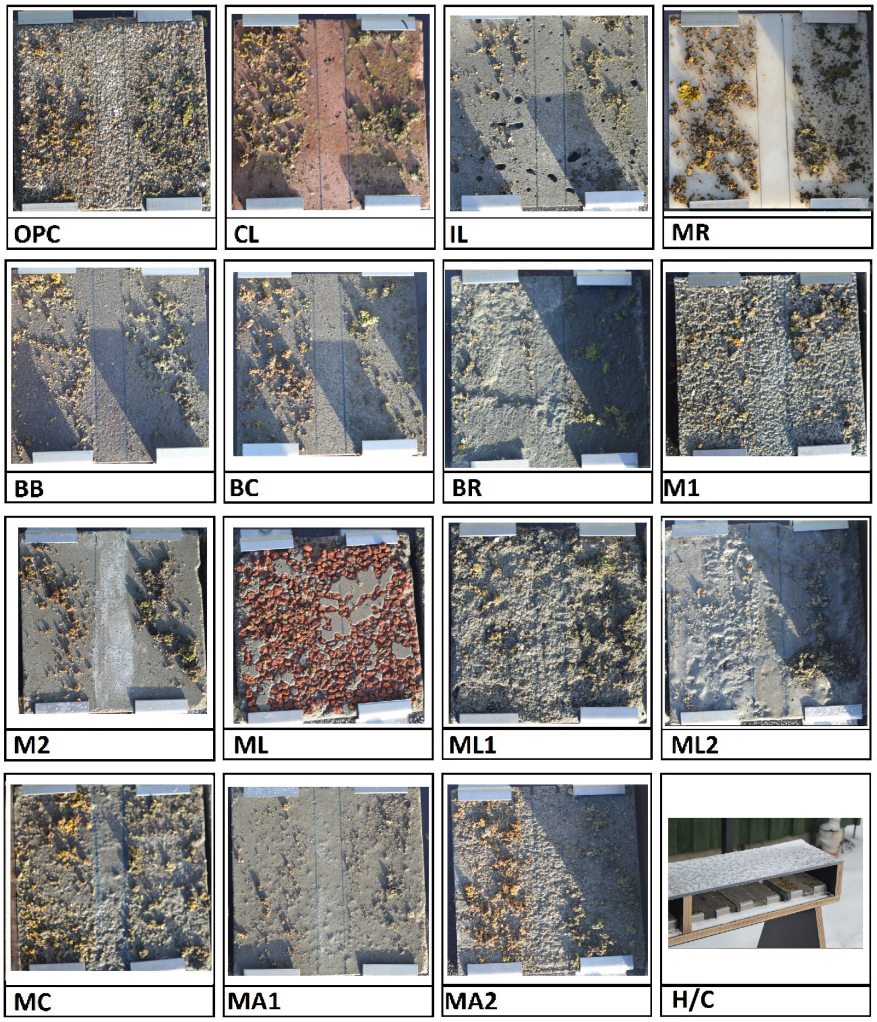

Figure 4.19: Picture of the specimens, horizontally oriented, with shelter, after 37 days of exposure to environmental conditions

Both imported and local lichens were largely found on all the specimens placed on this supporting structure. However, it is important to notice that only few of them seemed to be attached to the substrate, while most of the lichens appeared to just lay on the surface of the specimens. Thanks to the shelter and the horizontal orientation, was possible to observe that the Peptone solution was not completely removed from the surface of some specimens (CL, MR), by rain, wind and snow. A slightly lower quantity of lichens was observed on ML, MA1 and on the left side of ML2, when compared with the other materials.

As mentioned before, it was not possible to photograph and retrieve the results from the specimens oriented horizontally on the supporting structure without protective shelter (H), because they were completely covered by snow. It was decided to avoid the removal of the snow, since there was the risk of removing the lichens as well, during the operation.

From the last visual inspection before the snowfall it was observed that only few parts of the surfaces of the specimens were covered by lichens, and in general rainfall and wind removed almost all the organisms from the substrates. Figure 4.20 shows the supporting structure with the specimens covered by snow.

Figure 4.20: Picture of the specimens, horizontally oriented, without shelter, covered by snow

The general results from the last visual inspection can be seen in Figure 4.21. A colour code was used to report the results, a green dot means that the presence of lichens could be easily identified at first sight, yellow dot indicates that a closely inspection was necessary to observe the presence of lichens or that only few parts of the substrate's surface was colonized. Red dot means that no visible signs of colonization were observed on the specimen. In order to evaluate the differences due to material, orientation, exposition, presence or not of the protective shelter, and between local and imported lichens, a value of 1 was given for each of the green dots, 0.5 for the yellow dots and 0 for the red ones.

Specimen	Lichen	V/S	V/N	V/C	H/C	H/U	TOT			
OPC	*Icelandic*					-	1.0		3.0	
	Danish					-	2.0			
CL	*Icelandic*					-	2.5		6.5	
	Danish					-	4.0			
IL	*Icelandic*					-	3.5		7.5	
	Danish					-	4.0			
MR	*Icelandic*					-	2.5		4.5	
	Danish					-	2.0			
BB	*Icelandic*					-	3.5		7.5	
	Danish					-	4.0			
BC	*Icelandic*					-	3.5		7.5	
	Danish					-	4.0			
BR	*Icelandic*					-	2.5		4.5	
	Danish					-	2.0			
M1	*Icelandic*					-	1.5		4.0	
	Danish					-	2.5			
M2	*Icelandic*					-	1.5		2.5	
	Danish					-	1.0			
ML	*Icelandic*					-	0.5		1.0	
	Danish					-	0.5			
ML1	*Icelandic*					-	2.0		4.5	
	Danish					-	2.5			
ML2	*Icelandic*					-	1.5		2.5	
	Danish					-	1.0			
MC	*Icelandic*					-	1.5		3.5	
	Danish					-	2.0			
MA1	*Icelandic*					-	0.5		1.0	
	Danish					-	0.5			
MA2	*Icelandic*					-	1.5		2.5	
	Danish					-	1.0			
TOT	-	9.5	11.5	14	27.5	-				

Legend	
	High presence of microorganisms
	Low presence of microorganisms
	No visible signs of colonization

Figure 4.21: Results of the bilogical growth under environmental conditions obtained from the last visual inspection

From Figure 4.21, it can be noticed that, in general, the local lichens covered a larger fraction of the total surface independently from the type of substrate, orientation or exposition. The same observation can be done in particular for the Caucasoid lava, which shown a high degree of bioreceptivity, in all the studied positions, for local lichens, but it did not show the same behavior regarding the imported lichens.

Comparing the materials between each other, both Icelandic and Danish lichens were found on BB, BC and IL specimens, for all the orientation and exposures. While on the other basalt specimens, the ones with the rough surface (BR), less lichens were observed to adhere to the substrate.

Despite the initial expectations, MPC samples, in general, shown a low degree of bioreceptivity. Biofouling was observed only on few of the samples' surfaces. Between the MPC samples, those who shown the highest presence of organisms were M1, ML1 and MC.

Marble shown an high degree of bioreceptivity only when the samples were protected by the Plexiglas shelter.

Regarding positions and orientations, the supporting structure where most of the samples were covered by the lichens was the HC, where specimens were placed horizontally and covered by the shelter. However,

as already mentioned, most of the lichens seemed to lay on the surface and not to actually adhere to it.

Regarding the structures were the samples were placed vertically, the best results in terms of quantity of colonizing organisms, were obtained, as expected, for the one with the shelter (V/C). Furthermore, the specimens exposed to North (V/N) shown an higher degree of bioreceptivity, compared to the specimens facing South (V/S).

4.4 CONCLUSIONS

In general, a higher quantity of local lichens was found growing on the substrates than imported lichens. This is likely to be a consequence of the predilection of lichens for the climate and the environment of the location where they were growing first. Even though Danish and Icelandic climate is similar, air quality, temperatures, solar radiation, and many other parameters slightly differ between the two locations, therefore influencing the ability of a determined lichen community to colonize the substrates.

Basalt is the material that shows an higher bioreceptivity for both of the lichens communities taken into consideration in this experimental program. Biological growth and lichen adhesion was observed for all the analysed positions of the specimens, on BB and BC, while the other basalt sample, with rough surface (BR) did not show the same results. This may be a consequence of the physical properties related to the surfaces (roughness and superficial texture) of the materials, since BB, BC and BR have the same chemical composition and therefore the same chemical properties.

Regarding the local lichens colonization, the material that shown the highest bioreceptivity was the Caucasoid lava stone (CL), which is also the material that has the highest porosity of all samples. Further investigations are necessary in order to figure out the reasons of a low bioreceptivity regarding imported lichens. It was observed that the Icelandic lichens adhere and grown mostly on the materials imported from Iceland. Further studies should be carried out in order to assess if there is any possibility that lichens, collected in a specific location, prefer, as substrates, materials collected from the same location.

Concerning the placement and the orientation of the samples it was observed that the presence of lichens was higher on the specimens placed horizontally and covered with the Plexiglas shelter. However, not all the lichens were actually attached to the substrates and since the intended application is on building vertical facades, the results obtained for vertical orientation are more interesting in this sense.

The best results regarding vertical orientation of the samples, were obtained when the protective shelter was present. It seems that the shelter protected the lichens from rainfall and hydrodynamic forces, therefore reducing the removal of the lichen's cells. Furthermore, it is

possible that partial shading offered by the shelter, reduced the evaporation of water favouring the adhesion and the growth of lichens on the substrates. The same observation could be made in regards of the North exposure, where direct solar radiation does not reach the specimens' surface and the house placed in front of the structure protected the lichens from removal by wind.

Regarding the colonization on marble specimens, a great difference between the sheltered and not sheltered samples was found. Lichen adhesion was observed almost only on the specimens covered with the shelter. It may be a consequence of the longer period that rain and wind took to wash away the Peptone solution, leaving the time to the lichens' rhizines to grow, anchoring the lichens' thallus to the substrates.

Despite the good bioreceptivity shown by MPC samples for *Chlorella vulgaris* in the literature (Manso, 2014), the specimens produced with Magnesium phosphate cement for this experiment did not show a good bioreceptivity for lichens *Xanthoria parietina* and *Physcia tenella*. It is important to notice that the MPC specimens used for this experiment were produced using an already prepared mix, from Sika, called Sikaset 45, while Manso developed her own formulation starting from raw materials. Therefore both physical and chemical properties widely differ between the MPC used for this experiment and the one used by Manso.

OPC did not show a high degree of bioreceptivity, except in some cases for the local lichens. This is probably due to its chemical composition, and in particular the high pH value determined in the last session of measurements.

Finally, regarding the Icelandic lava, the macro pores present on the specimens' surface, acted as micro-refuges where the lichens were protected from hydrodynamic forces due to rainfall and wind and giving the final material a pleasant aspect, that can be important for an architectural point of view.

5

ANALYSIS OF CORRELATION BETWEEN PORE SIZE AND BIORECEPTIVITY UNDER LAB CONDITIONS

5.1 INTRODUCTION

In chapter 3, the physical parameters that have an influence on the bioreceptivity of the materials were described. In general porosity and superficial roughness are the two physical properties, that have been most studied in relation to bioreceptivity. From previous experiments and researches it can be seen that porosity influences the water content and the period of water retention of the substrate (Warscheid et al., 1991; Guillitte and Dreesen, 1995; Tomaselli et al., 2000). This can be both an advantage and a disadvantage for lichen's growth, because water availability can increase the growth rate, but can also allow other organisms to colonize the substrate, leading to a possible competition between the species. While porosity mainly influences the water content, roughness is important because of the irregularities present on the surface may form anchoring sites and micro-refuges for the attachment and settlement of biological colonization. Micro-refuges protect microorganisms from hydrodynamic forces, reduce cell removal and partially determine the availability of water, nutrients and thus the survival of microorganisms (Koestler and Salvadori, 1996; Scardino et al., 2008; Miller et al., 2012b). It has been demonstrated that the attachment of microorganisms is lower on surfaces with irregularities below the size of the cell, and higher on rough surfaces and when textures provides numerous attachment points. In fact, micro-refuges occur when the cell is smaller than the size of the pore (Scardino et al., 2008). Furthermore, the observation of a major presence of microorganisms on rough surfaces than on smooth surfaces, mainly due to the total surface area, which is higher for rougher surfaces(Morton et al., 1998; Miller et al., 2012b).

Despite the effects of roughness on biological colonization are important, it was decided to deeper study the direct relation between pore size and water content and retention, and, therefore, bioreceptivity.

In order to have an evaluation of the relation between pores diameters and bioreceptivity, an experimental program was carried out at the Building Materials Laboratory of the Technical University of Denmark.

Many researchers conducted experiments to investigate biofouling on construction materials, several evaluation methods have been proposed and used in these researches. These methods include the evaluation of bioreceptivity by means of evaluating the changes in the surface colour (De Muynck et al., 2009; Manso, 2014). For the experiment presented in the current chapter, the surface colour changes were detected by colorimetric measurements.

5.2 MATERIALS AND METHODS

5.2.1 *Ceramic specimens*

Ceramic porous discs, with different porosity and pore size were ordered from CoorsTek. A list of the discs and their properties are presented in Table 5.1. The ceramic discs used for the experiment, have a pH of 7.

Material designation	**Average pore diameter**	**Apparent porosity**	**Absorption**
-	$[\mu m]$	[%]	[%]
P-1/2-AC	<0.5	38.1	22.5
P-3-C	1.7	45.4	25.2
P-10-C	7	45.7	26.1
P-40-C	40	37.6	20.1
P-100-C	100	40.2	23.0

Table 5.1: Ceramic plates list and properties

Where, apparent porosity is defined as the volume relation of pore volume to total volume, calculated as:

$$\%ApparentPorosity = PoreVolume/TotalVolumex100 \quad (7)$$

and absorption is defined as the weight relation between the saturated pore weight to the dry weight of the piece, calculated as:

$$\%Absorption = SaturatedWt.\breve{}DryWt.x100 \quad (8)$$

5.2.2 *Specimens and Setup*

The ceramic discs were covered with a suspension composed by peptone, water and crushed *Xanthoria parietina* lichens collected in Denmark. The peptone to water ratio used for the culture media was 1:2, while the lichens to solution ratio was 1:6.

The plates were then placed in a climate room where temperature and relative humidity were controlled. The plates were also exposed to

light by means of a 90 W Growlights LED lamp placed at about 0.5 m above the setup. The lichens were grown at a temperature of 23±1°, with alternating photoperiod of 18 hours of light and 6 hours of dark and 85±5 % of relative humidity in the culture room for a period of 37 days. A picture of the setup can be seen in Figure 5.1.

Figure 5.1: Setup of ceramic specimens

5.2.3 *Evaluation*

The change of the visual aspect of a surface colonized by a community of microorganisms, can be directly related to its degree of bioreceptivity. Colorimetric analysis are now widely accepted in the scientific community for the monitoring of biological growth and biofouling on surfaces (De Muynck et al., 2009; Manso, 2014). The instrument used for the colorimetric measurements gives the results in CIELAB form, in which the values of L*, a* and b* are plotted in a Cartesian coordinate system. CIELAB colour space defines the colour of a surface based on the lightness factor L* (black-white component) and on two chromatic coordinates a* (green-red component) and b* (blue-yellow component). In order to evaluate the changes in the visual aspect, thus the degree of bioreceptivity of the samples, colorimetric measurements were performed. The instrument used for the analysis was a Minolta CR-200 colorimeter with a 10 mm aperture, borrowed from the the National Food Institute of the Technical University of Denmark. The measurements were taken every 3 or 4 days, starting 25 days after the beginning of the experiment. Five measurements per specimens were

taken at fixed position for each measurement session (one in the center, and four along the side of the specimen, as can be seen in Figure 5.2).

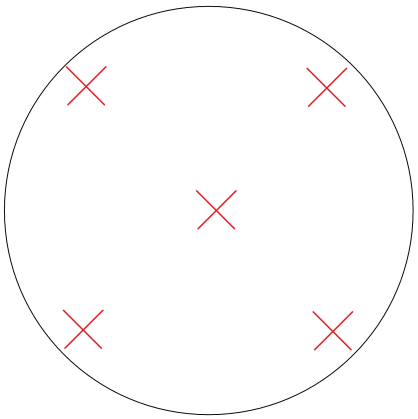

Figure 5.2: Colorimetric measurement points

Colorimetric measurements were also taken on the back side of the specimens in order to analyse the colour difference between the surface's colours before and after the experiment.

From the measurements data obtained, L*, a* and b*, it was possible to calculate the difference between the data taken at the beginning of the experiment and the data collected after colonization (ΔL*, Δa* and Δb*). Afterwards it was possible to calculate the total colour difference (ΔE*) with the equation:

$$\Delta E* = \sqrt[2]{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \tag{9}$$

It was also possible to calculate the chromatic variations (ΔC*) and the hue changes (ΔH*) respectively with the equations (De Munyk et al., 2009; Ferri et al., 2011, Manso,2014):

$$\Delta C* = \sqrt[2]{(a_{\mathrm{i}}*)^2 + (b_{\mathrm{i}}*)^2} - \sqrt[2]{(a_0*)^2 + (b_0*)^2} \tag{10}$$

$$\Delta H* = \sqrt[2]{(\Delta E*)^2 - (\Delta L*)^2 - (\Delta C*)^2} \tag{11}$$

The results obtained from the colorimetric analysis on the back of the specimens, therefore the representation of the colours of the specimens before starting the test, are presented in Table 5.2.

5.3 RESULTS AND ANALYSIS

5.3.1 *Colorimetric measurements and Analysis*

As already mentioned, the colorimetric analysis was carried out by means of a Minolta CR-200 colorimeter, in order to evaluate the biofouling intensity and therefore, the degree of bioreceptivity of the specimens. The total colour difference (ΔE*), the chromatic variations

Specimen designation	**L***	**a***	**b***
P-1/2-AC	92.49±0.29	-2.65±0.00	11.47±0.00
P-3-C	93.68±1.19	-4.11±2.04	12.32±1.10
P-10-C	94.06±0.98	-5.54±0.00	13.19±0.32
P-40-C	92.33±1.59	-5.56±0.04	12.40±1.32
P-100-C	91.26±0.08	-5.58±0.01	11.46±0.01

Table 5.2: Colorimetric analysis of specimens before the beginning of the test

(ΔC*) and the difference in hue (ΔH*) were calculated respectively with equations (7), (8) and (9). The measurements were taken, starting 25 days after that the lichens were inoculated on the ceramic plates and exposed to cycles of 18 hours light and 6 hours dark, at 80±5% RH and 23±0.5 °C. 5 different points were analysed for each specimen, as mentioned before. The arithmetic average of the 5 measurement and the standard deviation were calculated. The measurement were taken at 25, 29 and 32 day s from the beginning of the test. The values of ΔE*, ΔC* and ΔH* refer to the difference between the measurements taken on day 32, and the first measurements taken before the start of the experiment. The results obtained from the colorimetric measurements are presented in Table 5.3.

Specimen	**Day**	**L***	**a***	**b***	**ΔE***	**ΔC***	**ΔH***
P-1/2-AC	0	92.49±0.29	-2.65±0.00	11.47±0.00	31.99	0.25	0.19
	32	60.50± 10.64	-2.52± 0.72	11.75± 2.32			
P-3-C	0	93.68±1.19	-4.11±2.04	12.32±1.10	41.25	-1.86	0.20
	32	52.47±4.43	-3.34± 0.68	10.61± 3.30			
P-10-C	0	94.06±0.98	-5.54±0.00	13.19±0.32	44.81	-4.58	0.97
	32	49.50±1.90	-3.02± 0.67	9.25± 1.21			
P-40-C	0	92.33±1.59	-5.56±0.04	12.40±1.32	44.59	-4.76	0.67
	32	47.99± 1.39	-3.11± 0.31	8.26± 0.75			
P-100-C	0	91.26±0.08	-5.58±0.01	11.46±0.01	45.22	-3.50	0.54
	32	46.18± 1.81	-3.62± 0.68	8.50± 1.83			

Table 5.3: Colorimetric measurements

As can be seen from the table, the maximum total difference in colour (ΔE*) was observed for the specimen with the highest pore diameter. Since the chemical composition and so the chemical properties of the samples used, were the same, only the physical properties differ between the samples. As mentioned in the previous section, the main difference between the specimens was the pore diameter. Depending on the type of community used for colonization, the pore diameter appears to have an influence on the biofouling intensity and on the degree of bioreceptivity. It can be observed that if the pore size is bigger

than the cells size, they can work as micro-refuges for the attachment and settlement of biological colonization (Koestler and Salvadori, 1996; Scardino et al., 2008; Miller et al., 2012b). The relation between the total colour difference and the pore size is plotted in Figure 5.3.

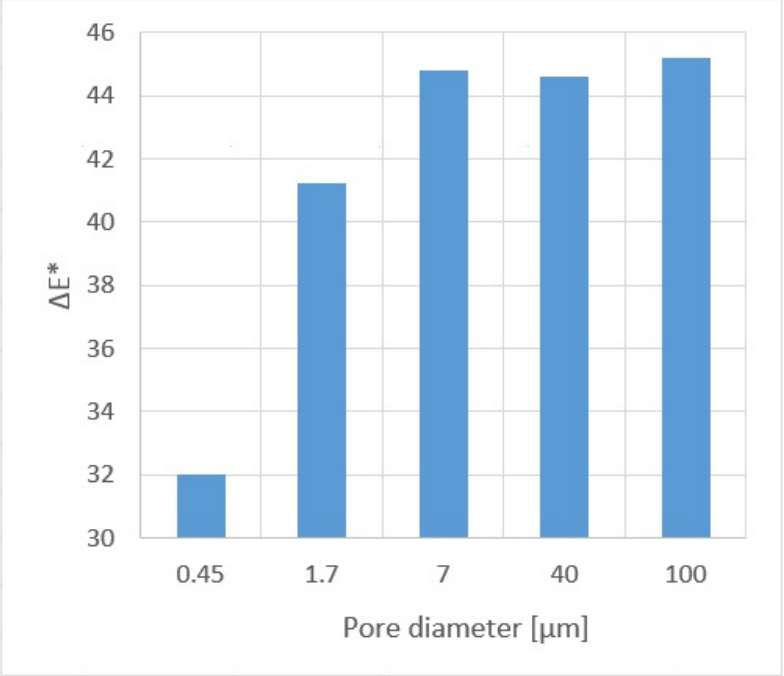

Figure 5.3: Correlation between pore size and total colour difference

The ΔC* value represents the chromatic variations, positive values means that the sample is more chromatic or more saturated, while the ΔH* value represents the difference in hue, meaning that a positive difference indicates that the surface is greener in the hue than it was before the experiment.

The difference in lightness (ΔL*) observed during the test period is plotted in Figure 5.4. The curves show how lightness L* decreases as the biofueling intensity on the samples surfaces increased during the experiment. Unfortunately there is not any data available for the first 25 days after starting the experiment, because of difficulties in finding the colorimeter.

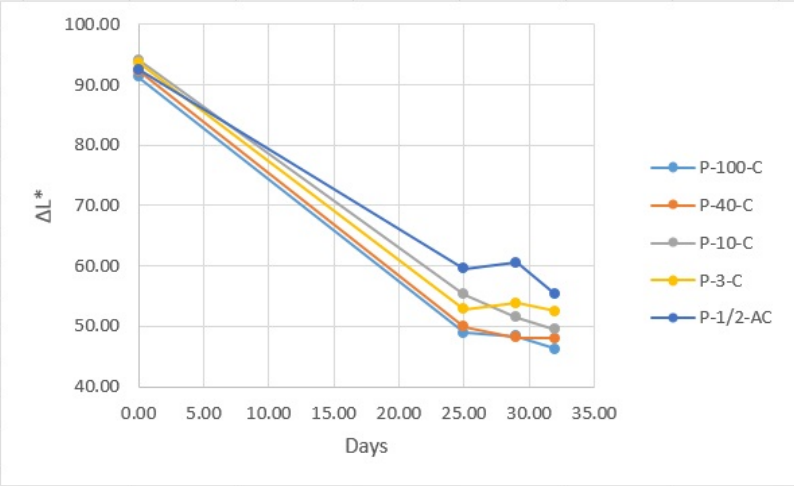

Figure 5.4: Change of lightness in the specimens in function of the number of days

5.4 CONCLUDING REMARKS

It is important to notice that not only the lichens *Xanthoria parietina* but also other organisms, such as moulds and bacterias, colonized the specimens. These organisms influenced the results, therefore, from this experiment, it is not possible to assess that higher pore size has a direct influence on lichens growth and adhesion to the substrates. However, from the obtained results it is clear that higher values in pores diameter lead to higher degrees of bioreceptivity for moulds and bacteria. There was not a lot of difference between the specimens with the higher pore sizes (P-40-C and P-100-C). This may be interpreted as an indicator that the cells size of the colonizing communities was lower than the P-40-C pores dimension.

Visual inspections revealed a complete fouling on P-100-C after 16 days from the beginning of the experiment, while for the other specimens, it took a longer time. The complete fouling of the surface was not reached for P-1/2-AC at the conclusion of the test.

6

CONCLUSIONS AND FUTURE STUDIES

6.1 INTRODUCTION

The work presented provides several information regarding the possibility of using building material as substrates for biological growth, focusing especially on two species of lichens, *Xanthoria parietina* and *Physcia tenella*. It is important to point out that the parameters that influence biological growth are many, and they should be carefully considered when similar applications are tried in different environments. Therefore, this project does not presume to be able to individuate which material is the most suitable in terms of biological growth in general, or to identify the most correct method of inoculation for these kinds of applications.

The results obtained from the experimental programs contained in this project should be considered as related to the specific conditions present during the experiments. Therefore, the same applications, if carried out in different locations or periods of the year with different lichen's species or with different materials may lead to very different results.

In the this chapter, the conclusions obtained from the current work as well as the suggested future studies are presented.

6.2 CONCLUSION

The benefits of including vegetation in urban areas and the lack of horizontal available ground surfaces lead to an increased interest in greening of building envelopes. The technologies for vertical green systems advanced substantially in the last years. However, they often have a low level of integration with the building envelope.

Biological growth directly on façade materials is a possible solutions to create green walls, decreasing the installation and maintenance costs, and the other problems due to the low integration of the existing systems.

In general, the results presented in the previous chapters showed that it was possible to let lichens adhere and grow on several of the materials tested under environmental conditions. Therefore, the general objective of this project was accomplished. However, due to the slow growth rate

of lichens, it is important that periodic monitoring of the specimens exposed to environmental conditions will be carried out in the next 2 years, in order to have more definitive results and to individuate the best materials in terms of bioreceptivity.

Regarding the specific objectives of the thesis, the most relevant conclusions are listed below.

- In view of the definition of the parameters that influence bioreceptivity, it was demonstrated, through the experimental program described in chapter 5, that pore size and bioreceptivity are directly correlated, and that specimens with higher pore sizes were colonized faster and more intensively. However, it was observed that, besides the inoculated lichens, other microorganisms colonized the specimens, therefore it can not be concluded that larger pores of the material lead to an higher bioreceptivity for lichens specifically

- Between the materials taken into consideration for the experimental program described in chapter 4, the specimens that showed a higher bioreceptivity for both Icelandic and Dansih lichens were the basalt samples with brushed and cut surfaces. Furthermore, both Danish and Icelandic lichens were also found, even though in lower quantities, to adhere on the Icelandic lava specimens for all the tested condtitions.

- Regarding the local lichens, the Caucasoid lava specimens were the ones whose surfaces were more heterogeneously covered. This may be due to the higher porosity and probably higher water retention capacity of the Caucasoid lava compared to the other materials

- Concerning the provenience of the lichens used for the test, the local lichens were found in higher quantities on most of the specimens' surfaces than the Icelandic ones, suggesting that lichens may prefer the climatic conditions of the location where they were taken from

- Both Danish and imported lichens were found in large quantities on all the specimens placed horizontally and protected from hydrodynamic forces by the shelter. However, it was observed that adhesion of the thallus to the substrates did not occour on several specimens.

- Regarding the specimens placed vertically, a major presence of lichens was observed on the samples that were covered with the shelter, confirming the hypothesis that the micro-climate induced by the shelter and the protection from heavy rainfall and wind favourites the adhesion of lichens to the substrates.

- The cementitious materials tested (MPC and OPC), showed a lower degree of bioreceptivity than the other materials. This result may be attributed to the chemical properties of these materials, whose pH values were slightly higher.

- Between the cementitious materials the ones that performed better for lichens colonization were the specimens designated as M1 and ML1. Colonization was observed also on MC. The water/mix ratio used in producing these specimens was half of the ratio used for most of the other specimens and consequently the open porosity values were lower. However, it is possible that the roughness of the surface, which, from a visual inspection, seems higher on these specimens compared to the others, had an great influence on lichens adhesion.

- The Peptone suspension used for the inoculation of the lichens seemed to favour the adhesion of the lichens thalli to the surfaces and to accelerate the colonization process. The increasing durations of the periods between the inoculation and the moment when the suspension is completely washed away from the specimens, appeared to have a positive effect on the quantity of lichens that were colonizing the specimens.

6.3 FUTURE STUDIES

As mentioned before, the experimental program under environmental conditions will be continued in the next two years.

It is important that periodical visual inspection, taking pictures of each specimen, will be carried out in order to observe the lichen growth.

Image analysis by means of dedicated software may be included in the evaluation process, to have quantified parameters, so as to better compare the specimens between each other and to observe variation of the lichens' size over time.

Further studies should be done regarding the suitability of cementitious materials for lichens' colonization. Other formulations characterizing the chemical and mainly the physical properties of the MPC specimens could be tested in the same conditions. Also fully carbonated OPC specimens may be included in future tests. The other physical properties influencing bioreceptivity such as roughness and surface texture should be measured in order to evaluate the relation between these properties and the lichens' presence on the substrate.

Furthermore, other materials chosen from the group of acid-base cements could be included in the experiment in order to compare their performances in terms of bioreceptivity with the results obtained for both cementitious and natural stone materials.

An element for façades could be designed based on the results obtained from the experiments, and also taking into consideration the

parameters that can induce micro-climates, favouring water retention and protecting the organisms from rain, wind and snow that may remove the lichens from the substrates.

Studies regarding the environmental benefits of lichen colonization of building envelopes should be carried out. These studies include the possibility of increasing thermal and noise reduction performances of building envelopes due to the presence of lichens.

Lastly, the effects of air pollution on lichens have been widely studied, but research on the effects that lichens may have on the reduction of pollutants present in the air may be studied further.

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