

# Investigation of the frost resistance in bacteria treated self-healing concrete

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# Preface

This master thesis of 30 credits is the final part of the Master's Program in Civil Engineering. The work was carried out at the Division of Building Materials and the Division of Biotechnology at LTH during the spring of 2016, in cooperation with the R&D laboratories at Vattenfall AB.

I would like to thank my supervisor Katja Fridh and my assistant supervisors Martin Rosenqvist and Gashaw Mamo for all the support, commitment and interesting discussions.

I would also like to thank Bo Mattiasson for the help in the start and my examiner Lars Wadsö, the laboratory technicians Stefan Backe and Bengt Nilsson and also Martin Strand for all the help during the laboratory work.

Thank you all for a great time and a fun project!

Lund, 2016  
Karin Persson



# Explanations

## *Dictionary English - Swedish*

Frost resistance - Frostbeständighet

Frost damage - Frostskada

Calcite - Kalcit

Limestone - Kalksten

Calcium carbonate - Kalciumkarbonat [CaCO<sub>3</sub>]

Lactate - Mjölksyra [C<sub>3</sub>H<sub>6</sub>O<sub>3</sub>]

Precipitate - Fälla ut

Novel - Ny, av senaste

Immersed - Nedsänkt

Hydrochloric acid - Saltsyra [HCl]

Viable - Livsduglig, livskraftig

SEM (Scanning electron microscope) - Svepelektronmikroskop

AEA (Air entraining agent) - Luftporbildare

CEM I 42,5 N SR 3 MH/LA - *Anläggningscement Degerhamn* (in this thesis)

SCMs (Supplementary cementitious materials) - Tillsatsmaterial

## *Abbreviations in material science - Concrete*

C - CaO

S - SiO<sub>2</sub>

H - H<sub>2</sub>O

CH - Ca(OH)<sub>2</sub>

C<sub>3</sub>S - 3CaO · SiO<sub>2</sub>

C<sub>2</sub>S - 2CaO · SiO<sub>2</sub>

CSH - C<sub>3</sub>S<sub>2</sub>H<sub>3</sub>



# Abstract

Half of the electricity needed in Sweden today is produced by hydro power [26]. Most structure parts in hydro power stations are made of concrete and as many of other concrete structures they contain cracks. Cracking through dams are a big problem for hydro power since they create leakage.

To prevent cracking a new method is under investigation. It consists of incorporating calcium carbonate precipitating bacterial spores in the cement matrix. Upon cracking the spores get activated by water and oxygen and precipitate calcium carbonate that will fill the cracks. The method is considered to be environmental friendly and the precipitation is the same material that the cement has its origin from. The filling then obtains the same modulus of elasticity and coefficient of thermal expansion as concrete. The method is also considered to be cost efficient since the bacterial mix is relatively inexpensive, but most of all the costs for inspection and repairs can be held to a minimum, since the bacteria repair the cracks spontaneously without human intervention.

In a laboratory environment crack widths up to 0.5 mm has been totally repaired by this method [30]. This value can be compared with the maximum allowed crack widths from different loading cases that are between 0.2-0.4 mm. The method to incorporate bacteria in the cement matrix has never been tested in a full scale project, not that has been verified in articles at least. To be able to test the method in full scale there has to be a certainty that some parameters work as well in bacteria-treated concrete as it does in the normal concrete, e.g. hydration, strength development and frost resistance.

Three different concrete recipes has been used in this study, all based on CEM I cement. One recipe had bacteria, lactate and air entrainment agent (AEA), one recipe had only AEA and the last one did not have any AEA which created a concrete that is not frost resistant.

To test hydration and strength development two isothermal calorimetry tests, one compressive strength test and one tensile strength test was preformed. The first calorimetry test showed small differences in hydration between the bacteria treated concrete and other two with about one hour delay of the start of the main reaction and a small increase in the thermal power. The second test where the bacteria treated concrete was divided in three groups (one with lactate, one with bacteria

and one with both bacteria and lactate) showed that the bacteria probably was responsible for the delay in main reaction and that the lactate for the increase in thermal power. The differences were small though and the accumulated heat was in the normal range for the CEM I cement.

The tensile and compressive strength were not effected by the addition of bacteria.

To receive a good frost resistance the added air voids need to be empty. To see whether the bacteria had created precipitations in the air voids pictures were taken with a scanning electron microscope (SEM). They showed nothing that would effect the frost resistance. A freeze-thaw test was also preformed, both with fresh water and salt water. The test with fresh water showed only small differences in scaling between the concrete with both bacteria and added air voids and the concrete with only added air voids. This indicates that the air voids are unaffected by the added bacteria, as the SEM-analysis also showed. In salt water the differences in scaling are larger.

To sum it up, with the concentration of bacteria solution used no negative effects was seen in this study. However, higher concentrations should be tested and also the crack healing efficiency. This study was also limited in time and the long term effects are still unknown.

**Keywords:** Concrete, Hydraulic Structures, Hydro Power, Frost Resistance, Self-healing, Bacteria, Precipitation, Calcium Carbonate



# Sammanfattning

Vattenkraften står för hälften av vårt elbehov i Sverige idag [26]. Merparten av vattenkraftverken är gjutna i betong och som många andra betongkonstruktioner så drabbas betongen av sprickbildning av olika orsaker. För vattenkraften är genomgående sprickor ett problem då dessa skapar läckage.

För att förhindra dessa sprickor har en ny metod börjat undersökas där bakterier som kan avsätta kalciumkarbonat blandas in i betongen i sporform. När sedan en spricka uppstår aktiveras sporer av vatten och syre och avsätter kalciumkarbonat som fyller igen sprickorna. Metoden anses miljövänlig och kalciumkarbonat är samma material som cementet har sitt ursprung från och man får samma elasticitetsmodul och värmeutvidgningskoefficient på lagningsmaterialet. Metoden anses också kostnadseffektiv eftersom bakterieblandningen är relativt billig och framför allt för att kostnader för inspektion och lagning kan hållas till ett minimum, då bakterierna spontant lagar sprickorna utan mänsklig inblandning.

I laborativ miljö har sprickor med sprickvidder uppemot 0,5 mm lyckats lagas helt [30], detta att jämföra med maximalt godkända sprickvidder på grund av last som ligger i spannet 0,2-0,4 mm. Metoden att gjuta in bakterierna i betong har dock aldrig testats i ett fullskaligt projekt, i alla fall inte vad som framgår av publicerade artiklar. För att metoden ska kunna testas måste dock några parametrar fungera lika bra för självläkande bakterie-betong som för vanlig betong, bland annat hydratationsförlopp, hållfasthet och frostbeständighet, vilket studeras i denna studie.

Tre olika betongrecept användes i denna studie, alla baserade på Anläggningscement. Ett innehöll bakterier, kalciumlaktat och luftporstillsats och ett recept hade luftporstillsats. Det sista receptet hade ingen luftporstillsats vilket då skapar en dålig betong frostmässigt.

För att testa hydratationsförlopp och hållfasthet genomfördes två test med isoterm kalorimetri, ett tryckhållfasthetstest och ett draghållfasthetstest. Det första kalorimetertestet visade tendenser på skillnader i hydratationsförlopp mellan bakteriebetongen och de två övriga med cirka en timmes retardation för huvudreaktionen och en liten förhöjning av effekten. Ett nytt test där bakteriebetongen delades upp i tre olika grupper (en med laktat, en med bakterier och en med bakterier och laktat) visade att bakterierna troligtvis var ansvariga för retardationen och laktatet för den

förhöjda effekten. Skillnaderna är dock små och den ackumulerade värmeutvecklingen var inom den normala spridningen för Anläggningscement.

I draghållfasthet och tryckhållfasthet kunde inga skillnader upptäckas.

För god frostbeständighet krävs tomma luftporer. För att se så att bakterierna inte hade skapat utfällningar i luftporerna gjordes en SEM-analys. Den visade inget som ansågs påverka frostbeständigheten. I ett frystest med sötvatten påvisades även att skillnaderna mellan betong med både tillsatta luftporer och bakterier och betong med enbart tillsatta luftporer är små. Detta tyder också på att luftporerna är intakta och tomma, precis som SEM-analysen visade. I saltvatten är skillnaderna lite större.

Sammanfattningsvis, med den använda koncentrationen av bakterier kunde ingen negativ påverkan påvisas. Förslagsvis bör dock fler och då högre koncentrationer testas samt bakteriernas spricklagande förmåga. Dessutom är denna studie mycket begränsad i tid och eventuella långtidseffekter av bakterietillsatsen fortfarande okända.

**Nyckelord:** Betong, Vattenbyggnader, Vattenkraft, Frostbeständighet, Självläkning, Bakterier, Utfällning, Kalciumkarbonat

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# Chapter 1

## Introduction

Many of Sweden's hydro power plants today are built during the beginning and middle of the 20th century, and most of the parts are made of concrete. During these decades, concrete was assumed to be a so durable material that the structures did not need any maintenance [9]. During the 80's the question about the concrete's durability was raised and today a lot of research takes place concerning concrete's durability and degradation mechanisms. This is not only an important question for hydraulic concrete structures but for all outdoor constructions made of concrete, but especially for those in contact with water. Concrete are exposed to many degradation mechanisms, e.g. chemical attacks, erosion, reinforcement corrosion and frost attacks and many of them need water to occur and create cracks.

Hydro power is our primary renewable energy resource in Sweden today and about half of our need of electricity is produced by hydro power [26]. No new hydro power plants will probably be built in Sweden but the ones running are in need of maintenance and replacing of parts. Even though the main costs for hydro power is in the construction phase these structures are in need of maintenance as well. For hydro power, one of the main challenges is to solve the problem with cracks in concrete, and especially leaking cracks.

A new method for repairing cracks, that has proven to work in a laboratory environment is to incorporate calcium carbonate precipitating bacteria in the concrete matrix, either in the mix or apply them in cracks when they appear [25][30][29]. The bacteria precipitate calcium carbonates through metabolism. They are aerobic which means they need oxygen to be active and in return they produce calcium carbonate ( $\text{CaCO}_3$ ) and carbon dioxide ( $\text{CO}_2$ ).  $\text{CO}_2$  might increase the rate of carbonation which increase the risk of reinforcement corrosion.

The most efficient way in a crack healing perspective would be to add them to the mixture from the beginning where they hopefully will repair cracks without human intervention when the concrete has hardened and cracks appear. Otherwise inspection of the structure and man-made application of a bacteria solution is needed.

But since they precipitate calcium carbonate in the cracks, will they also precipitate it in other cavities in the concrete? In the air voids for examples, which play an important role when it comes to the frost resistance?

Sweden has a climate where the temperature fluctuates from degrees above to degrees below zero in the whole country. In addition to the fluctuating temperatures in Sweden, most of Vattenfalls hydro power plants are located in the northern part, see figure 1.1. It is not uncommon that the temperature will drop below  $-25^{\circ}\text{C}$  at least one time every winter and the mean temperatures in January are below zero in almost the whole country, see figure 1.2. That means that the frost resistance has to be taken into account during the planning process for outdoor concrete structures. This is why all outdoor concrete constructions contain "added" air voids. They work as expansion vessels where ice can form without creating any tension in the material (when water freezes it increases its volume by about 9% [10]). In order for the air voids to work as an expansion vessel it is very important that they are empty.

In this thesis the main focus will be on the frost resistance of a pure Portland cement (CEM I 42,5 N - SR 3 MH/LA, called *Anläggningscement* in Swedish) with about 95% Portland clinker components [18]. Both concrete with and without added bacteria and air entrainment agent will be tested with a freeze-thaw testing method that is developed at the Division of Building Materials at LTH. To verify that the hydration of the cement is unaffected by the added bacteria isothermal calorimetry tests and strength tests will be performed. Also a pH-indicator test will be done to see if any changes of the pH can be detected due to carbonation.



Figure 1.1: Left: Vattenfall's hydro power plants in Sweden [26]. Right: Hydro power plant in Älvkarleby during winter (Photo: Martin Rosenqvist)

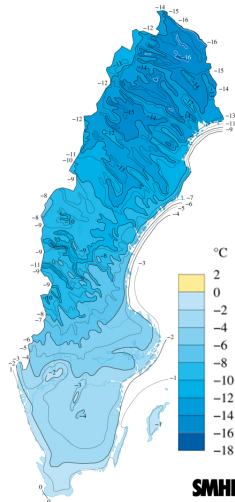


Figure 1.2: Mean temperatures in January. It is below zero in almost the whole country (Source: [www.smhi.se](http://www.smhi.se))

## 1.1 Research questions

- How does calcium carbonate precipitating bacteria affect the frost resistance of concrete?
- Can precipitation be detected in the air voids?
- Are the hydration and/or strength affected by the bacteria?
- Can changes in the pH be detected in concrete with bacteria in comparison with concrete without bacteria?

## 1.2 Limitations

The focus is restricted to cement type CEM I without any supplementary cementitious materials (SCMs) and the frost resistance of concrete made of this cement. Since the method of adding bacteria is new, there will be some tests so that the bacteria does not affect the hydration process and the strength development but there will be no control of the actual efficiency of this method in terms of crack healing. The freeze-thaw test method used is not the standard method in Sweden for testing frost resistance in concrete, why no comparisons can be made with specimens tested with the standard method or with the limit values connected with that method.





# Chapter 2

## Theory

### 2.1 Concrete

Concrete might be thought of as one homogeneous material but actually the material is built up by several different components. There are also so many reactions going on in the cement paste, especially in the beginning. It is those reactions that gives the concrete many of its properties, e.g. watertightness, strength, frost resistance and/or resistance against ingress of chemical substances. To understand the material this thesis begins with an explanation of the initial reactions in the cement, from when the calcite is heated to the hydration of the concrete is almost complete. It shall also be noted that concrete is a hydraulic material, which means that it react with water to become a material that is resistant against water [2]. This makes it a good material to use in outdoor hydraulic constructions like bridges, hydro power plants etc. However, some of the degradation mechanisms has its origin when the structure is in contact with water, e.g. frost attacks [8]. The degradation might lead to cracking of the concrete.

As this thesis is about larger outdoor constructions the focus is on structures that uses a pure Portland cement as a binder, called *Anläggningscement*. It has about 95% Portland clinker [18], and it has been a tradition in Sweden to use this pure Portland cement without SCMs for hydraulic structures. It has moderate strength development and in that case also moderate generation of heat, and is suited for medium to large constructions [14].

The chemical reactions and the generation of heat is important for the hydration and strength development of the concrete but there are other important parameters that affects the properties of the hardened concrete as well. Porosity and air void content are two of the more important ones and they greatly affect the frost resistance of concrete.

Cracking of concrete is normal. There are a lot of reasons for cracking but all has

their origin in the low tensile strength of concrete. The most important reasons for cracking in hydro power stations will be discussed in section 2.1.2.

### 2.1.1 Microstructure of the concrete

Concrete consists of two phases, cement paste and aggregate. Concrete is a porous material with gel pores, capillary pores and air voids, that have impact on the properties of the concrete [9]. The final properties of the concrete is highly dependent on the different amount of ingredients that is added [2], and one ratio that affects many of the final properties is the w/c-ratio that is defined as

$$w/c - ratio = \frac{W}{C} \quad (2.1)$$

$W$  and  $C$  is the amount of water and the amount of cement added in  $\text{kg}/\text{m}^3$ . With a low w/c-ratio the cement particles are closer to each other when suspended in water and the concrete gets denser faster than a concrete with high w/c-ratio during hydration, see figure 2.1.

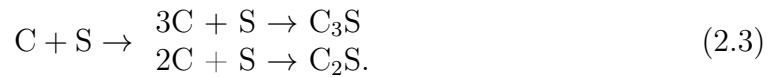
As CEM I cement mostly consists of Portland clinker, the reactions described below is limited to the silicate reaction.

#### Chemical reactions

The origin of the cement is limestone and in the limestone there is calcite, that is a form of calcium carbonate with chemical name  $\text{CaCO}_3$ . The calcite is granulated and heated in a rotation kiln, carbon dioxide depart at about  $750^\circ\text{C}$  from the calcite and only quicklime ( $\text{CaO}$ ) is left. This process is called calcination and can be seen in equation 2.2.



The quicklime is a highly reactive substance and not only calcite is added to the rotation kiln but also some clay. Clay contains quartzite also called silicon dioxide ( $\text{SiO}_2=\text{S}$ ) in different amount that react with the  $\text{CaO}=\text{C}$  at about  $1450^\circ\text{C}$  to form a clinker grain containing both  $\text{C}_3\text{S}$  and  $\text{C}_2\text{S}$  and can be seen in equation 2.3.



These two components are also called calcium silicates. These two calcium silicate components together with two other clinker components,  $\text{C}_3\text{A}$  (tricalcium aluminate)

and  $C_4AF$  (tetracalcium aluminoferrite), are grinded into a fine powder together with some gypsum (circa 5%) [2] [14] and this is basically what CEM I cement consists of ( $A=Al_2O_3$  and  $F=Fe_2O_3$ ).

The reaction when cement react with water  $H_2O=H$  is called the hydration of the cement (see equation 2.4 and 2.5).



and



It is in this reaction the strength of the concrete is built up by the reaction product of CSH-gel.

The residual product is  $CH=Ca(OH)_2$  and is of great importance when it comes to keeping the alkalinity in the concrete. The alkalinity protects the reinforcement from corrosion.

### Porosity and air void content

In addition to the CSH-gel that builds up the strength there are some cavities that also give the concrete some of its characteristics. Normal hardened concrete consists off about 12-20 percent of total porosity [2], located mostly in the cement paste [7] because of the non-porous aggregates used in Sweden [10]. The porosity in the cement paste can be as high as 47% for a w/c-ratio of 0.6 when it is fully hydrated [18].

The porosity consists of both capillary pores (about 1  $\mu m$  in diameter), gel pores (1-100 nm in diameter) and compaction pores (0.22-0.30 mm in diameter) [18], see figure 2.2. The size of the pores are of importance when it comes to the frost resistance of concrete [7], and will be explained more in detail in section 2.1.3.

The *capillary pores* are the leftover space between cement grains and their hydration products after hydration, see the white areas in figure 2.1. The amount of capillary pores is dependent on the w/c-ratio, degree of hydration and particle size distribution of the aggregate [7]. Depending on the w/c-ratio and whether more water could enter during hydration the capillary pores can be more or less filled with water [18].

The *gel pores* are found in the CSH-gel, and this hydration product is larger than the original cement particle. So when the capillary porosity decreases with higher degree of hydration the gel porosity increases [7], see the gray areas in figure 2.1.

A higher degree of hydration also means a more dense concrete with higher resistance against substance ingress. There is also a w/c-ratio dependency. A low w/c-ratio means that the cement particles are close to each other from the beginning, and that the CSH-gel might bridge the distance between the particles completely, and

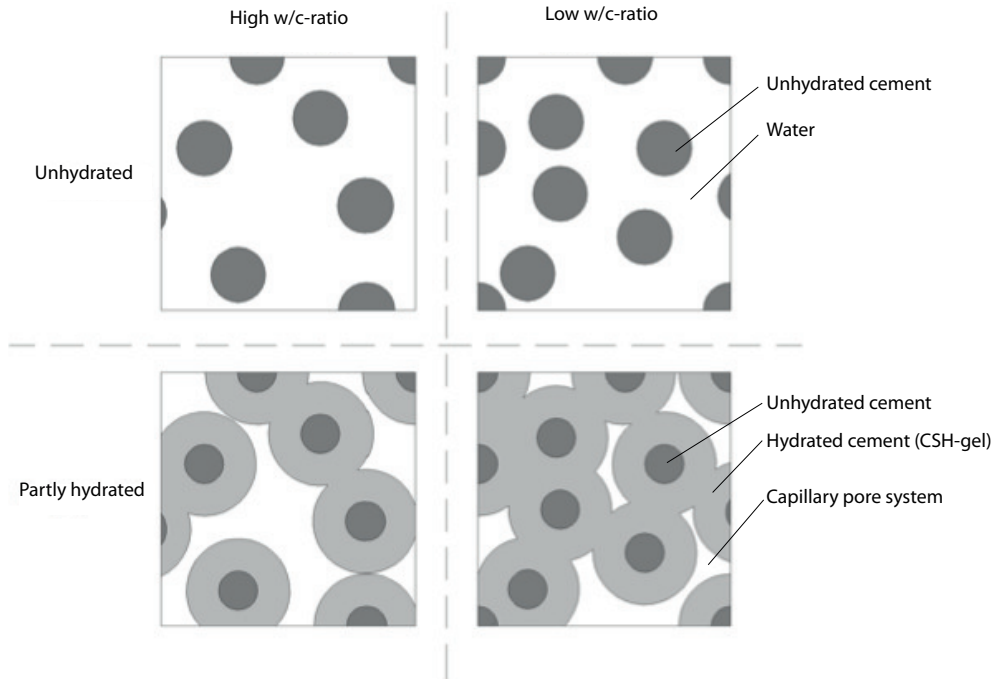


Figure 2.1: Cement particles suspended in water with a high  $w/c$ -ratio to the left and a low  $w/c$ -ratio to the right.

separate the capillary pores from each other, see left of figure 2.1. A too low ratio though, will lead to incomplete hydration because of lack of space to host the hydration products. The theoretical lower limit is set to 0.36 [18], but for practical use the lower limit is 0.39 [14].

*Air voids* can arise both from the natural air content from incomplete compaction and/or from the addition of air entrainment agents (AEA). The natural air voids are bigger than the air voids that the AEA creates, and they are also further apart from each other. The natural air content is about 1.5-2.5% and with AEA a normal air content gets about 4-7% [9] depending on how much AEA that is added. A higher air void content than 7% is usually not accepted due to large strength losses and that the air void system might be connective and then easily filled with water[9].

The AEA used today is made of surface active agents with one hydrophilic side and one hydrophobic side on the molecule. They arrange themselves in the interface of air and water so that the hydrophobic side from many of the molecules captures air between them, see figure 2.3. In this case small stable bubbles if left in the cement matrix[7].

Addition of air entrainment agents will decrease both compressive and tensile strength due to less material/volume unit [18] but air voids are important when it comes to the frost resistance and will be described more in section 2.1.3.

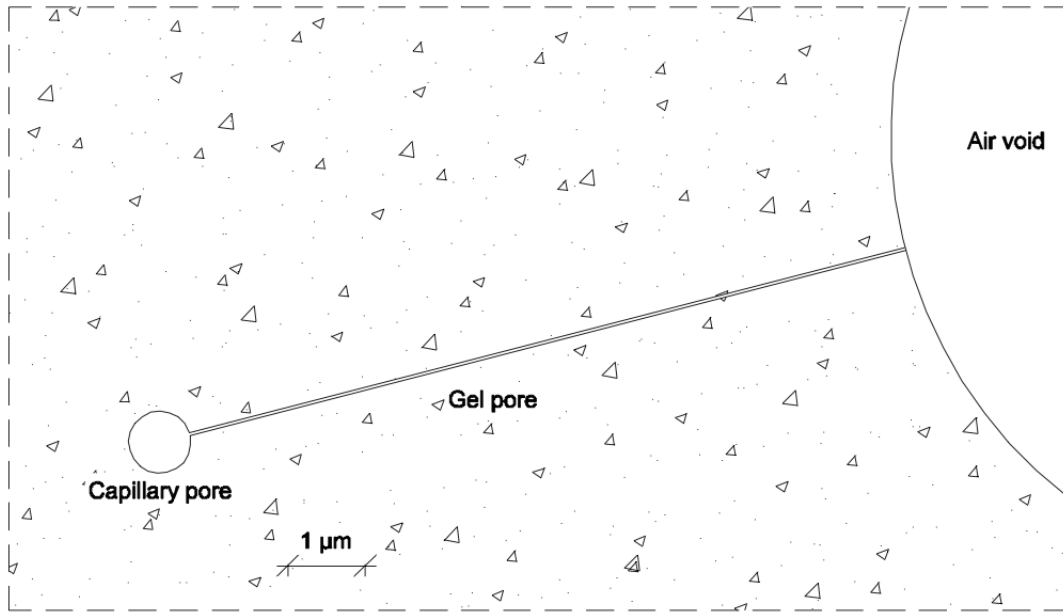


Figure 2.2: Schematic picture of the different sizes of the pores in the cement matrix.

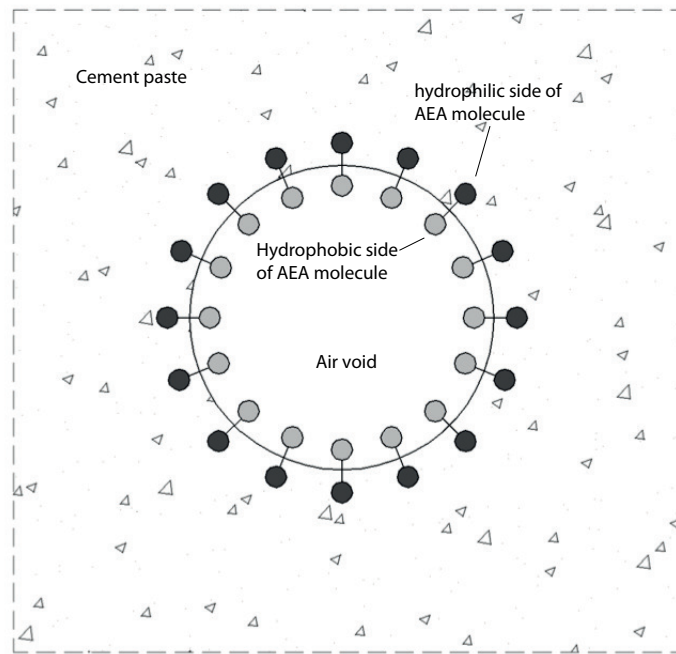


Figure 2.3: Surface active molecules build up the air voids

## Heat production

The hydration process is an exothermic reaction that means the reaction produces heat. A great way to evaluate the hydration process is to measure the heat production rate and the accumulated heat developed. This can be done by a isothermal calorimetry test.

There are some different phases of the hydration process. The different phases correspond to four different rates of heat production, see figure 2.4. First is the initial reactions that take place during a short period of time and are most of the time already done when the concrete arrives at the construction site. The next phase is the dormant, where nothing happens that are related to the strength development and the thermal power is very low. This means that it is possible to work with the concrete and place it in the mould. This period lasts for about 3 hours. The next period is the main reaction where a very clear peak in figure 2.4 can be seen. This peak is found 3-24 hours after mixing and here the concrete builds up most of its strength. The last period is a period (again) with low thermal power where some later reactions take place [27].

To evaluate the heat production rate through an isothermal calorimetry test is a good way to see whether any additional materials effect the hydration process.

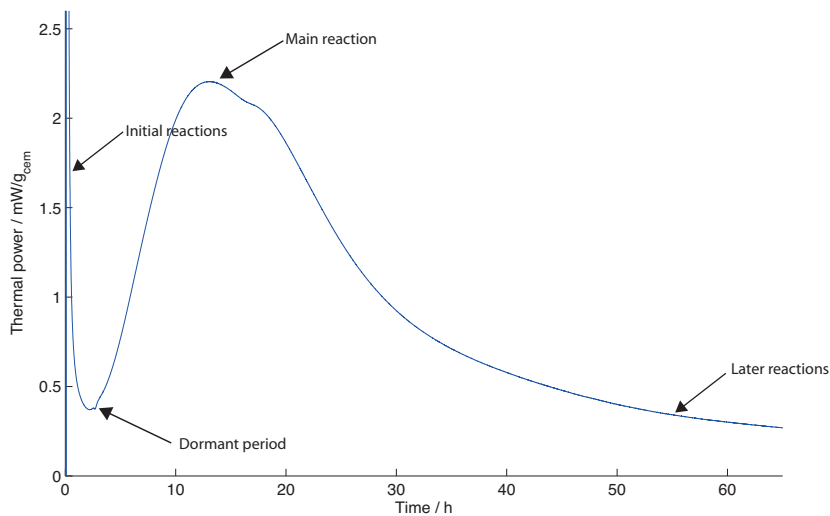


Figure 2.4: Schematic diagram over heat development during beginning of hydration

The cement used in this study has an accumulated heat of hydration for 1 day of  $170 \pm 20$  kJ/kg cement [3] and this value can be used to detect changes in hydration.

## 2.1.2 Formations of cracks

Cracking is a big problem for concrete structures. There are a lot of reasons for formations of cracks but most of them can be related to the low tensile strength of the concrete. Some of the reasons for cracking in addition to loading are shrinkage due to loss of water, thermal expansion/shrinkage due to volume changes and/or chemical reactions due to e.g reinforcement corrosion [17]. Cracking of concrete are almost impossible to avoid.

For hydro-power stations leakage through the construction can be a big problem. Cracking through a water retaining structure though might lead to leakage that could leach the concrete and small cracks could increase the ingress of harmful substances.

### Cracking in hydro power stations

The most important reasons for cracking in hydro-power stations are cracking due to drying shrinkage due to loss of water, temperature gradients and loads.

*Drying shrinkage:* When the structure has reached its full strength it can change in volume due to moisture content. Different moisture content internal and external or restraint from the subgrade might cause cracking when the resulting tensile stresses exceed the tensile strength of the concrete [11]. The shrinkage/expansion occur in the cement paste and can be up to 1% in the paste but is limited in the whole structure to about 0.06% due to the strength of the aggregate [11]. The amount of drying shrinkage is affected mostly by the aggregate and the w/c-ratio [11].

*Autogenous shrinkage* also qualifies as drying shrinkage but is an internal loss of moisture content due to the hydration of the cement. This shrinkage becomes significant when the w/c-ratio goes below 0.42 [11]. To avoid this kind of cracking concrete dams has been built with a higher w/c-ratio, between 1940-1970 normally 0.5 [20] and before 1940 it was even higher [10].

Another reason for cracking is *thermal cracking*. This cracking has its origin in temperature differences that cause *thermal stresses* due to the coefficient of thermal expansion. The differences might occur from sun radiation on the surface or from the cement hydration, where the surface loses the heat faster than the internal parts. This cracking phenomena due to heat development during hydration normally shows up in larger structures like dams, footings, large beams etc. where the temperature gradient can be large.

Thermal cracking can cause two different types of cracks, surface cracking or through-crack [8]. Both can be harmful to the structures. The through-crack because they lead to leakage and leaching of the structure if it is subjected to one-sided water pressure. The surface cracking might cause a general loss of durability due to increased ingress of water and/or harmful substances [8].

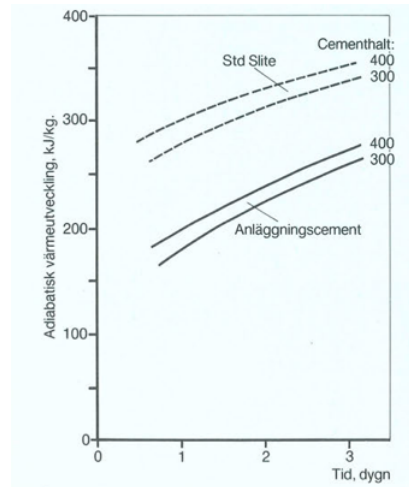


Figure 2.5: Heat production for two Swedish cements, *Std Slite* and *Anläggningscement* [10].

Thermal cracks can arise at as small temperature differences as  $16^{\circ}\text{C}$ , where *Anläggningscement* with moderate generation of heat is preferable to thick structures because of its slower heat production compared to for example *Std Slite*, as seen in figure 2.5.

The last main reason for cracking is due to *loads*. It is natural that concrete cracks from being exposed to tensile forces and there are few structures that only have compressive forces acting on it. That is why most of concrete structures have reinforcement, so in case of tensile cracking the reinforcement takes the load instead. Therefore these cracks occur close to reinforcement and makes it easier for substances to reach the reinforcement bars. But the bars are also there to spread out the cracks and limit their width [11] so that less ingress of substances will occur. For different environmental exposures there are different maximum crack widths allowed due to loading according to Swedish standard SS-EN 1992-1-1:2005, where 0.2-0.4 mm are accepted. In figure 2.6 can repaired cracks in hydro power station be seen.



Figure 2.6: Repaired cracks in hydro power stations (Photo: Martin Rosenqvist)



### 2.1.3 Frost attacks on concrete

Frost damage to concrete structures is a known phenomenon in cold countries. It is also one of the worst degradation mechanisms for outdoor structures in Sweden [9]. In hydraulic structures with especially hydro power stations in mind frost damage can be both internal or external [10].

#### Internal and external frost damage

*Internal frost damage* leads to a general loss of strength and modulus of elasticity due to inner expansion of water when freezing [10]. It can be hard to detect internal frost damage to structures because it is not always that the damage will reach the surface. The reason for this is that the surface of water exposed structures surrounded by air, e.g. dams with one-sided water pressure or foundations in constant contact with ground-water, are almost always dryer than its interior. That means it will be more space at the surface to take care of the expansion than the inside of the structure will have [10]. An internal frost damage can be seen in figure 2.7.



Figure 2.7: Internal frost damage causing a corner in a hydro power station to fail (Photo: Martin Rosenqvist)

*External frost damage* appears often in the form of surface scaling. Scaling as a frost damage phenomenon is the most common, especially when the structure freezes in contact with salt water. The scaling is in the form of small cementitious flakes which detach from the surface [23]. The scaling, if very bad, may expose the reinforcement to both liquids and air. This might cause corrosion of the reinforcement and/or a higher degree of saturation inside the concrete [23]. An example of external frost damage can be seen in figure 2.8.

There are four proposed mechanisms for the internal frost damage, *closed container mechanism*, *hydraulic pressure mechanism*, *osmotic micro ice body growth* and *macroscopic ice lens growth*.



Figure 2.8: Example of surface scaling at the waterline (Photo: Martin Rosenqvist).

### Frost damage mechanisms

A normal hardened concrete specimen consists off about 12-20 percent of total porosity [2]. The general cause for frost damage is that water inside the capillary pores freezes and when water freezes it expands about 9% and creates high pressures that cause cracking [2].

There could be water in many of the capillary pores and also in the gel pores but the water in the gel pores isn't freezable at normal outdoor temperatures in Sweden. This is due to the small size of the pores and with that the water inside those pores are subjected to capillary underpressure [18]. With decreasing pore size the freezing temperature decreases, the water is supercooled. The water isn't pure (it contains different kinds of ions which lower the freezing point) and the pore water freezes part by part during a temperature range below zero [18], both due to the impurities and the pore size distribution. Valenza and Scherer [23] stated that no scaling occurred when the lowest temperature was above  $-10^{\circ}\text{C}$  for freezing in salt water.

The amount of freezable water versus the amount of space available for this water is called effective degree of saturation:

$$S = \frac{w_f}{w_f + a} \quad (2.6)$$

where  $w_f$  is the amount of freezable water and  $a$  is the volume of air filled pores.

The first mechanism, called *closed container mechanism*, can be explained with the degree of saturation in mind. Since the water expands when freezing it means that the maximal water content can be 91,7% of the volume of the capillary pore if it is seen as a closed container where no water can escape through the pore walls and the ice wouldn't exert a pressure on the pore walls. If the degree of saturation is higher than 0.917 the expanding ice would exert a pressure on the walls, as seen in figure 2.9 and there is risk of frost damage. The critical degree of saturation ( $S_{cr}$ ) would

in this case be 0.917, which means that no damage would arise if  $S < S_{cr}=0.917$  [13].

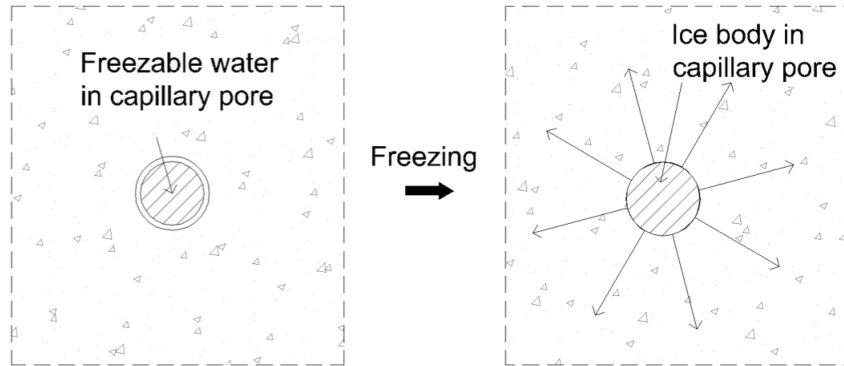


Figure 2.9: Closed container mechanism. When water freezes it expands about 9% and if there is lack of space for the expansion the ice will exert a pressure on the wall of the pore that will create damage.

In experiments it has been shown though that the degree of saturation was lower than 0.917 and frost damage still occurred, which means that there has to be another explanation. The second mechanism introduces hydraulic pressure, that water can escape through the pore wall.

The *hydraulic pressure* arises when ice forms in the capillary pores and wants to expand [13]. This expansion wants to move the non-frozen water and since the water can not freeze in the small gel pores they work as a drain pipe for this pressure, but need a bigger reservoir of air in the other end to be able to completely release the pressure, see figure 2.10. This is why the air void content is of importance when it comes to the frost resistance, air voids are the reservoirs needed for the expansion but they need to be empty.

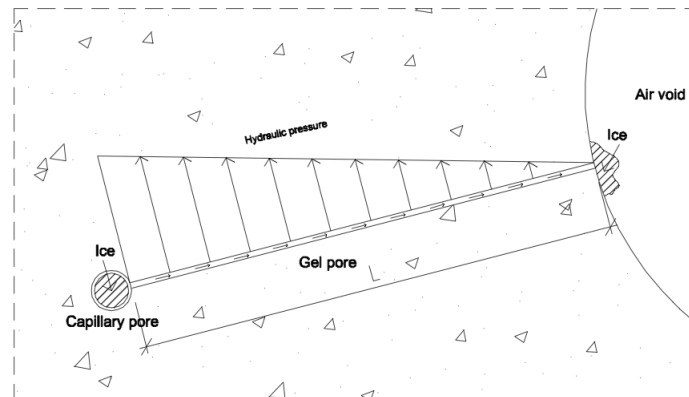


Figure 2.10: Pores in the cement matrix and the hydraulic pressure that can arise when water freezes.

There is a maximum distance of  $L_{cr}$  to prevent the pressure to get to high in the gel pores that is half of the mean distance between the air voids. This means that  $L$  have to be less than  $L_{cr}$ , that is called the critical spacing factor.  $L_{cr}$  is about 0.22-0.25 mm when freezing in fresh water and about 0.18 mm when freezing in salt water [9].

The third mechanism is based on *osmotic micro ice body growth*. A water transport starts since non-frozen water has a higher energy content than ice at the same temperature. This makes the unfrozen water to move towards the ice. The ice can exist both in the air voids and in the capillary pores at this point as seen from the discussion about hydraulic pressure. The unfrozen water freezes when it reaches the capillary pore and the air void and increases the amount of ice, as seen in top of figure 2.11. When the capillary pores are filled completely with ice the pore walls exerts a pressure on the ice and increases the ice body's energy. The ice in the air void is in this case the one with lowest energy since the ice can expand without any resistance and the water starts to transport against the air void instead [13]. The further distance it is between the air voids the longer it takes before the process turns and the bigger pressure can be built up in the capillary pores. This means that the maximum critical spacing factor also can be applied here.

The fourth mechanism is called *macroscopic ice lens growth* and is closely related to frost heaves in soils. Frost heaves in soils occur when an ice lens is built up somewhere below the surface, in the interface between frozen and un-frozen soil. Below the ice lens in the un-frozen soil water can transport up to the ice due to capillary suction and high enough permeability for the transport. This mechanism also requires long periods of temperatures below zero and all this can happen in thin concrete dams during winter, exposed to one-sided water pressure. This causes large damage and can even create totally "rotten" concrete below the waterline on the water side of the dam [19]. The mechanism can be seen in figure 2.12.

The external damage gets worse in a salt concentration of about 3% [24], and this worst concentration is independent of what kind of salt that is used (e.g. sodium chloride [NaCl] or calcium chloride [CaCl]). It has been shown that it is when this quite low concentration acts on the exterior of the structure the worst scaling occur, the internal salt concentration is not as important [13]. The mechanism behind this worst concentration is under investigation but is still not totally clear.

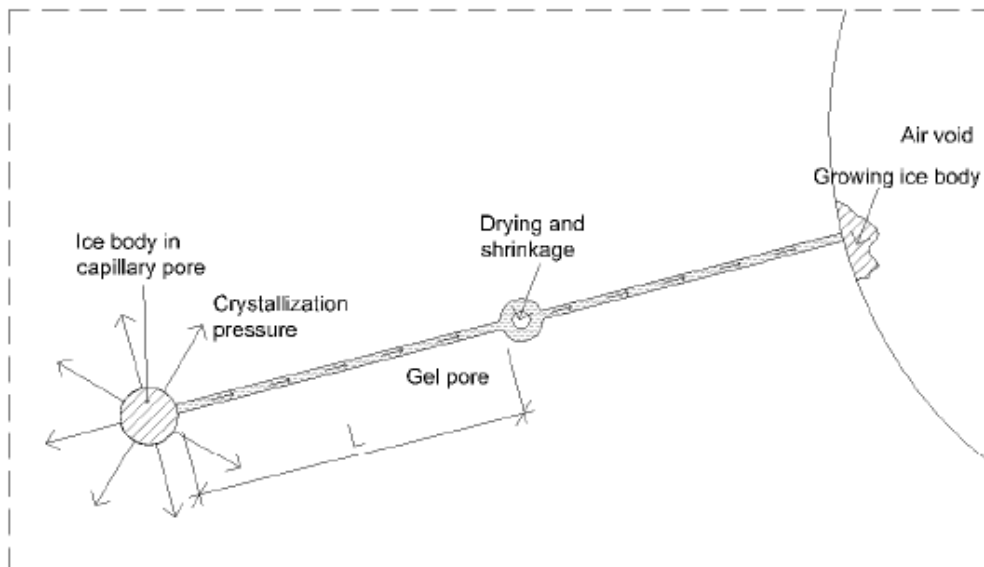
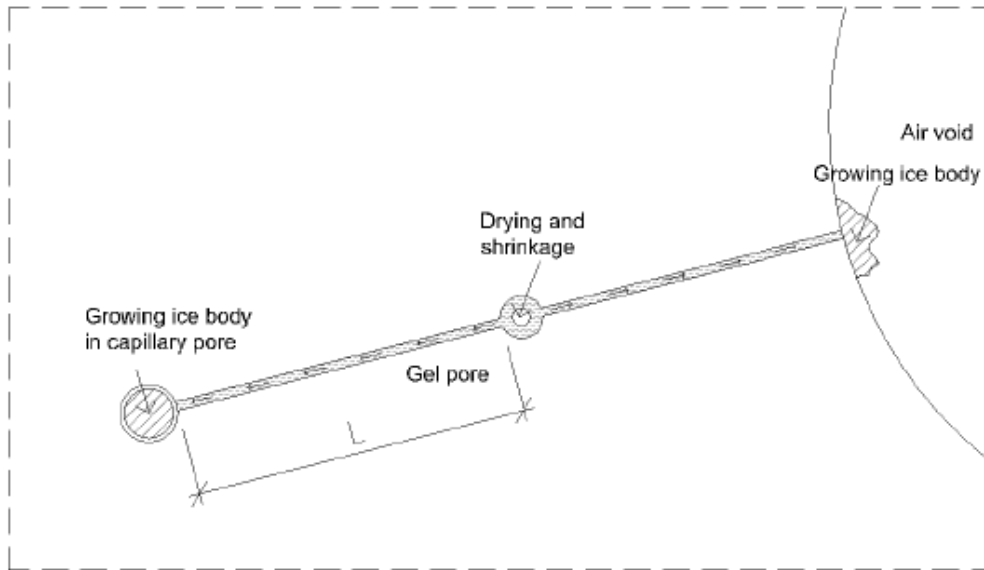


Figure 2.11: Osmotic micro ice body growth. Top: Both ice bodies attract non-frozen water due to higher energy content. Bottom: The ice in the capillary pore has stopped to attract non-frozen water.

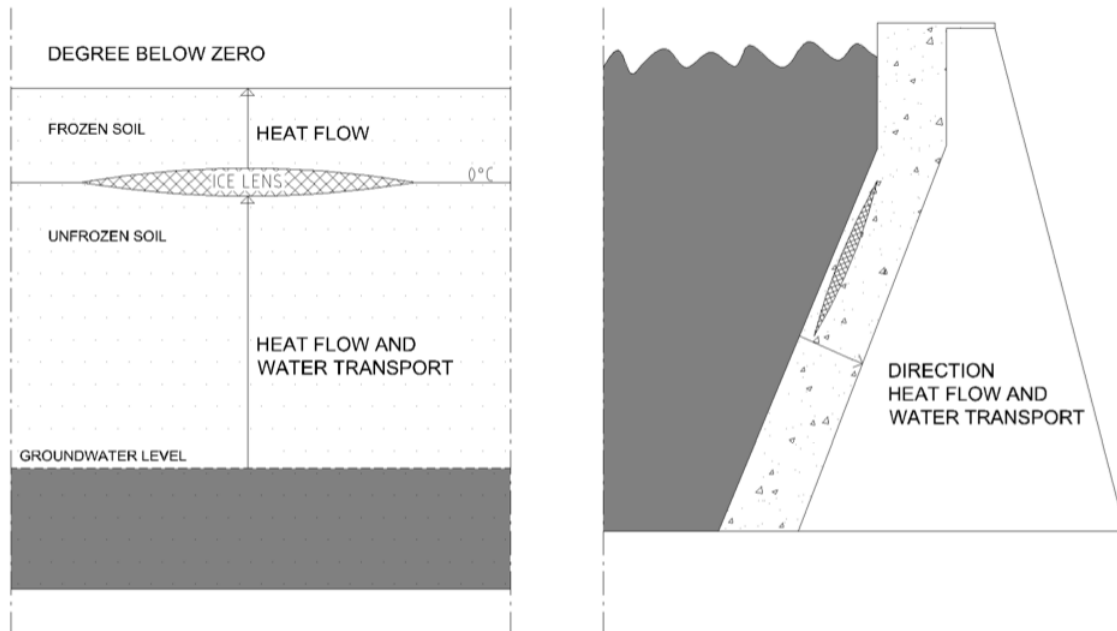


Figure 2.12: To the left: Frost heave in soil with ice lens growth in the interface between frozen and un-frozen soil. To the right: Macroscopic ice lens growth in a thin concrete dam.

### How to create good frost resistance

The freezing water needs volume to expand into. Air entraining agents (AEA) can create that volume and a good frost resistance could be accomplished. But the air void system needs to have many small air voids (that are empty) with small distances between each air void. There is a natural air void volume that is about 1.5-2.5% [9] (the compaction pores) but this volume is not enough to protect the concrete from frost damage. The natural air voids are also in general big with a long distance between them. At least 3.5% air content is needed when freezing occurs in contact with fresh water and at least 5% when freezing occurs in contact with salt water [9]. But for freezing in salt water the frost resistance is much more dependent on factors such as water-cement-ratio, compact method and transportation of the fresh concrete (since the transportation can change the air void content), so a frost resistance test should always be done on concrete recipe for outdoor structures in a salt water environment [9]. And for structures in contact with an aggressive environment, e.g. salt water, the w/c-ratio shall be kept low. This because a low w/c-ratio creates a denser cement matrix and prevents water movements.

If the concrete has entrained air and if the aggregates are non-porous the concrete shows great resistance against internal frost damage. In Sweden the aggregate are of good quality in general and often non-porous and since the use of air entraining

agents started in the 1960's concrete structures of later age manage this kind of degradation mechanism very well [10]. This means that as long as the air void system is kept in good condition (not filled with water or precipitations etc.) concrete dams show good resistance against internal frost damage. Still problem with internal frost damage has been seen, why is under investigation. But empty air voids are a necessity for good frost resistance of concrete.

## 2.2 Microbial induced carbonate precipitation

A new biological method for repairing cracks in concrete has been found [30] [17] [21]. The method is based on the addition of calcium carbonate precipitating bacteria in the concrete matrix, that in case of cracking precipitate calcite and repair the crack.

Bacteria are essential for us to be able to live on earth. We have on average 2-2.5 kg bacteria in our body [15] and they are also present in buildings. But if most of the bacteria in the body are good for you, the building bacteria have so far been seen as something not so nice, e.g. *Legionella pneumophila*, that is a bacteria that can cause death among elderly people. It can grow in water tap systems with a temperature range of 25-45°C. When the water is used the shower fog spread the bacteria and we can breath it. The symptoms are pneumonia-like, but the disease can be more fatal [28]. In Sweden there are regulations about the temperature in the tap water systems, it should always be warmer than 50°C to avoid growth of microorganisms [1]. It is not so common with bacteria that have their origin in buildings and if bacteria should be added it should be of a good sort, both for the building and for the humans and animals in contact with it.

The discovery of a bacteria that might precipitate calcium carbonate in a crystal form has proven efficient when tested in-situ to protect ornamental stone. This method got patented in 1990 [5]. After that more and more research has been done to verify whether this bacteria can be used in more applications when it comes to building materials ([5]). So since this discovery it might be possible to actually add bacteria to our most used building material to make the concrete more independent of man-made repairs of cracks.

### 2.2.1 The bacteria

The bacteria used in most of the earlier studies that studied crack healing of bacteria treated concrete belongs to the genus *Bacillus* [30][29] and they can be both soil bacteria or water bacteria. This bacteria is aerobic which means they need oxygen to reproduce and be able to precipitate calcium carbonate. The bacteria also need an external calcium-source (a substrate). Why is not completely clear since there are calcium in the binder. Perhaps there are not enough calcium or that the calcium is to closely bound in their respectively molecules. The bacteria also need some protection from the harsh environment in the concrete or from the mixing process, it could be in sporulated form, in microcapsules or in expanded clay.

The *Bacillus* is a bacteria that is able to form spores, which means that they can go into a dormant or inactive mode. Sporulated the bacteria can be able to resist temperatures of a least -80°C [15], which means they will be able to survive the harsh Swedish winters. Their activity, once germinated, decrease in sub-zero temperatures though. In sporulated form they can also resist the mechanical impact the mixing



of the concrete will have on them [15]. Theoretically, this kind of alkali-resistant bacteria can be viable for up to 200 years sporulated in an dry environment [21]. But bacterial spores directly added to the cement matrix were only viable for about 4 month according to Jonkers et al. [12]. The reason they suggested was that the spores would have more and more problems to survive as the pore size decreases below  $1\ \mu\text{m}$  during hydration, and the typical size of a spore is  $1\ \mu\text{m}$ . They concluded that the spores got crushed. In order to avoid crushing of the spores Jonkers et al. reasoned that addition of AEA could create isolated micropores where the spores could survive.

*Substrate:* The bacteria need a calcium-source as substrate. Different substrates and calcium-sources have been used in the earlier investigations. Everything from yeast extracts, urea, calcium nitrate, calcium chloride, and calcium lactate in different amount and combinations [25][29]. Calcium chloride is not recommended because the chloride ions might be harmful to the reinforcement [25]. Calcium nitrate on the other hand accelerated the cement hydration and had a positive effect on the overall degree of hydration [29]. The positive effect on the total degree of hydration was good but some careful measurements has to be made if calcium nitrate would be used in larger structures, since thermal cracking might occur, see section 2.1.2. The use of calcium lactate was verified by Wiktors et al. [30] with good results.

*Protection of the bacteria:* Both the mechanical impact from the mixing process and the alkalinity might be harsh for the bacteria.

The crack healing efficiency when *expanded clay particles* was impregnated with spores and calcium lactate was verified by Wiktors et al. [30] in 2011. The research group had earlier tested without any protection and saw that the amount of viable spores dropped with aging of the concrete [12]. In their next attempt they impregnated expanded clay particles to protect the spores from crushing and with stereomicroscopic observation and measurements of initial and healing crack widths the crack healing efficiency could be established. After 70 days of immersion in tap water the bacteria still healed cracks in the concrete [30], compered with their earlier study where the number of viable spores dropped significantly between 9 days of curing to 22 days of curing [12].

*Microcapsules* are a sort of shells which in a wet state are flexible but when dried very brittle. The microcapsules were used when there was an uncertainty whether the bacteria would withstand the alkalinity in the concrete [29], and even if they would, the study suggested that the crack-healing efficiency would be better if the spores were immobilized in the capsules and only activated when the capsules cracked and the spores got in contact with oxygen and water [29]. In this study they verified the cracking of the microcapsules with SEM and evaluated the crack healing efficiency both with light microscope and a water permeability test. The light microscope revealed visually that the cracks healed and the water permeability test proved it for all of the study's incubation conditions except the specimens incubated in air with RH 95% [29]. It also showed crack healing in the control specimens that can

be related to autogenous self-healing which will be describes more in section 2.2.2. The specimens with bacteria showed a higher self-healing ratio, about 30% higher than the non-treated specimens [29].

Luo et al. [17] verified a crack-healing efficiency without any protective capsules or clay particles in 2015 with only the *sporulation* of the bacteria. They studied different factors that affected the crack healing efficiency in terms of crack age, crack width and curing conditions. In accordance with Jonkers et al. [12] the crack healing rate dropped when the crack age increased [17]. Luo et al. [17] suggested that either the survival of the bacteria was a problem in the harsh environment or that the transportation of bacteria and/or substrate decreased as pore sizes decreased with increasing hydration.

*Heat:* All living creatures produce heat and energy when they metabolize food [15], and so also the bacteria. This means that both the chemical reaction between water and cement and the biological process when the bacteria precipitate calcium carbonate produces heat. This means that the hydration process might get affected by the addition of the bacteria.

Other types of bacteria has been used in building material applications as well. The use of microorganisms to improve compressive strength has also been investigated by Mandal et al. [16] with good results. The microorganism used in that study was an anaerobic bacteria that would decrease the porosity in the matrix and probably close a lot of the pores. That would be devastating for the frost resistance why for crack healing purposes aerobic bacteria was used in this study.

## 2.2.2 Crack healing efficiency

In many of the articles studied the crack healing effect has been proven in a controlled laboratory environment [29][30], with some different values of the actual crack healing capacity. Wiktor and Jonkers [30] stated that their *Bacillus alkalinitricilus* together with calcium lactate could bridge 0.46 mm-wide cracks completely in 100 days immersed in water, while the control specimens without bacteria only healed cracks with a width of 0.18 mm completely. As mentioned the maximal cracks allowed due to loading was 0.2-0.4 mm which means that this type of cracking could be completely repaired by this method, if the circumstances are right.

### Favorable conditions

Tittelboom et al. [25] and Wang et al. [29] summarized the circumstances needed as:

- Access to liquid water
- A calcium source and presence of nucleation site

- High/right pH

The need for liquid water has been established by the Wang et al. [29] and Luo et al. [17]. Wang et al. tested both in liquid water, liquid medium, wet-dry cycles and 95% RH. Self-healing occurred in all of the conditions except the 95% RH-condition, both in bacteria treated concrete and in non-treated concrete. The reason for this need was related to transportation issues according to Luo et al. [17]. The liquid water transported bacteria and substrate to the crack due to concentration differences.

The nucleation site can be the cell wall of the bacteria [25], that is negatively charged and attract the positively charged calcium ions.

And since the bacteria used is alkali-resistant the pH preferably has to stay in the same range as their natural environment, which is alkali-rich soils or water.

### **Self-healing with or without bacteria**

Self-healing occurs in non-treated concrete as well. This phenomena is called autogenous self-healing, and has its origin in that upon cracking unhydrated cement gets exposed to ingress water and can hydrate and fill the crack with hydration products. This is why most of the articles also test control specimens to verify what crack-filling material that has its origin in autogenous self-healing and what has its origin in the calcium carbonate precipitation. But in water retaining structures like hydro-power plants there are a few more explanations for the autogenous self-healing according to Schlangen et al. [21]. In addition to the previously mentioned the reason could be sedimentation of particles, formation of calcium hydroxide or calcium carbonate or swelling of the cement matrix.

### **Benefits of this method**

This method is being considered environmentally friendly [29] and since the precipitation is of the same material as the original material (and also the material that the carbonation process creates) the modulus of elasticity is probably in the same range and also the thermal expansion/shrinkage.

This method could also be cost effective since the materials used is not expensive and if the bacteria can be integrated in the cement matrix from the beginning the need for man-made examinations and repairs can be held to a minimum. Since there will be no new major hydro power stations in Sweden [26], this method might be used in bigger renovations or when replacing parts of the structures. The gain might then be that these parts has an extended service life.

Other applications can be to protect ornamental stone [6] or stabilizing loose particles, like sand or soil [29].

## In-situ testing and freeze-thaw testing

There have been a few in-situ tests and freeze-thaw tests with this method. One of them was in an existing parking garage where they combined the two [31]. Though they used a spray-on system and they did not incorporate the bacteria in the cement paste. The results were good, the water permeability through a crack decreased and the freeze-thaw test made on drilled cores, three bacteria-treated (sprayed with two solutions containing anaerobic bacteria) and 3 non-treated, showed less scaling in the bacteria-treated. The concrete turned out to have a very bad frost resistance and the test was stopped only after 7 cycles. But the result was that the treated concrete scaled off  $1.9 \pm 0.3 \text{ kg/m}^2$  while the non-treated scaled off  $3.6 \pm 1.3 \text{ kg/m}^2$  on average. This is a lot of scaling but nevertheless the treated deteriorated less.

### 2.2.3 Carbonates

The main task for the bacteria in the concrete will be to precipitate  $\text{CaCO}_3$ , which belongs to the carbonates. Both crystals named calcite and aragonite has the chemical name  $\text{CaCO}_3$  [4].

The anionic complex,  $\text{CO}_3^{-2}$  is a triangular strongly bond molecule as seen to the left in figure 2.13. But the bond can be broken by acid in the reaction

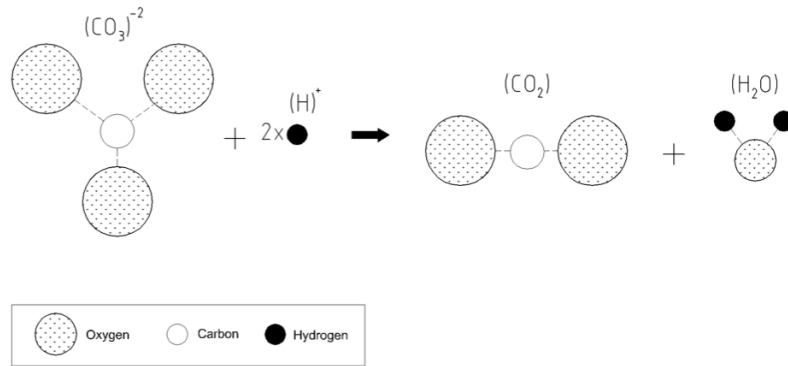
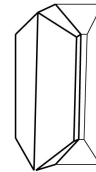
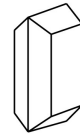
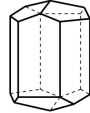
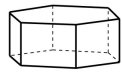


Figure 2.13: Carbonate molecule reacts with acid and creates water and carbon dioxide.

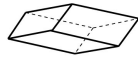
to create carbon dioxide and water. There are a lot of different mineral shapes of both calcite and aragonite. Calcite had over 300 shapes defined in 1985 [4], but three important shapes are prismatic, rhombohedral and scalenohedral. A few of the most important shapes from both calcite and aragonite can be seen in figure 2.14, with calcite to the left and aragonite to the right.



Prismatic



Rhombohedral



Scalenohedral

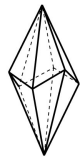


Figure 2.14: Both crystals and their schematic shapes. Calcite to the left and aragonite to the right (Sources: Photos from <https://www.goodfreephotos.com/> and shapes drawn from Dana (1995) [4]).

## 2.3 Strength parameters and the effect of the bacteria, the choice of protection and/or the choice of substrate

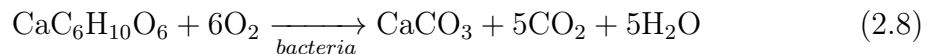
The strength is an important parameter for concrete structures. With calcium lactate the compressive strength could increase according to Jonkers et al. [12]. With other "food" (yeast extract, peptone or calcium acetate) the compressive strength decreased dramatically according to the same study.

The use of microcapsules did not have an effect on the volume density but anyhow it affected the strength in a negative way, mainly the compressive strength. It decreased with every added percent of microcapsules. The tensile strength was clearly affected when more than 3% of the cement weight was replaced with microcapsules [29]. They also tested the nutrients and capsules separately to see which of the two that was responsible for the decrease and the conclusion was that it was the microcapsules. The nutrients used in this study were calcium nitrate, yeast extract and urea.

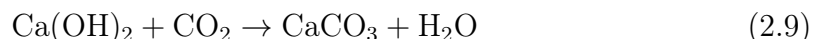
There have been studies on a bacterial strain that actually would increase the strength parameters [16]. The method used was to incorporate an anaerobic *Shewanella* bacteria that would precipitate minerals in the pores. The pore volume decreased and the compressive strength increased with addition of bacteria spores up to a specific limit in their study. But since the plugging of the pores isn't wanted in cold climates this strength improvement method should be avoided.

The bacteria used in this study belongs to the genus *Bacillus* and has its origin from an alkaline lake. The choice of calcium-source was calcium lactate, since it didn't contained any chloride ions. It didn't seem to affect the compressive strength parameters as much as the others in the application used by Jonkers et al. [12] either. That could both mean that the porosity remained more or less intact and that the hydration of the cement was unaffected by the additions made. This will be verified in the following chapter.

The chemical reaction when the bacteria produces calcium carbonate with calcium lactate as the calcium source [30] is



This reaction produces carbon dioxide ( $\text{CO}_2$ ) that can react with the calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ) that is a residual chemical compound from the hydration reaction, especially from Portland cement clinker components.



This reaction builds even more of the calcium carbonate but it lowers the amount of  $\text{OH}^-$  that means that the pH might decrease. A decrease of pH might be harmful to the reinforcement that is protected by the alkaline environment, see section 2.1.1. This reaction is actually the same as happens in all concrete structures with or without added bacteria, called carbonation. It happens spontaneously with the ingress of carbon dioxide from air and is a slow reaction. The addition of bacteria might increase the rate that the carbonation has, since the bacteria itself produces the carbon dioxide needed for this reaction inside the concrete. A change in pH can be detected by a pH indicator sprayed on the concrete surface, and this will be tested in this study.

## 2.4 This study

In this study 10.787 kg bacteria solution/ $\text{m}^3$  of fresh concrete was added, in accordance with Mian et al. [17]. The bacteria solution has a concentration of 5.8 million cells/ml.

The concrete recipe is based on CEM I cement with 380 kg of cement/ $\text{m}^3$  of fresh concrete, a normal amount of cement for hydro power stations in Sweden [20]. The amount of added AEA is chosen so that the air void content will be about 5%, so that a good frost resistance will be achieved both when the concrete is in contact with fresh water and with salt water.

If it shall be possible to test this method in a bigger scale some parameters has to behave for bacteria treated concrete as they do for a normal CEM I concrete. The hydration is the first parameter, where an isothermal calorimetry will be used to see if any changes in hydration could be seen with the addition of bacteria and lactate. This test will be supported with two strength tests, where changes in hydration can be seen as differences in the strength parameters. Strength tests are normally conducted at day 28 from casting so this tests can reveal changes in later or overall hydration and the isothermal calorimetry reveals changes in the early hydration.

Since this study is short in time and used a bacteria from an alkaline environment the need of protection was mostly from the mechanical impact [15], the sporulated form was chosen. This form of protection was also chosen since this study aims to verify whether the bacteria get activated spontaneously and precipitate carbonates in the air voids without any crack present. The air voids are of great importance when it comes to the frost resistance as seen in section 2.1.3. So that no precipitations will be found there will be verified with a scanning electron microscope (SEM). The frost resistance will also be verified by a freeze-thaw test to support the results from the SEM analysis.





# Chapter 3

## Materials and Methods

### 3.1 Casting of concrete

Three different batches of concrete were cast. One without air entraining agent (#C), one with AEA (#CA) and one with both bacteria and AEA (#CAB). AEA was used to obtain an air-content close to 5%, earlier stated as the lower limit for frost resistance when freezing in salt water, and is enough for freezing in fresh water.

#### 3.1.1 Concrete recipe

The concrete recipe chosen is outlined in table 3.1. The water-cement ratio was 0.5 with a cement content of 380 kg/m<sup>3</sup>. Due to very stiff texture in the concrete without AEA, super plasticizer was added. The interaction between the bacteria and the admixtures is unsure and the use was held to a minimum and was controlled with isothermal calorimetry.

#### 3.1.2 Mixing

Casting procedure started by determining the moisture content in the aggregate 0-2 mm to be able to balance the amount of water. This was done by mixing the aggregate to make the moisture content homogeneous. About 300 g of aggregate was weighted ( $w_m$ ) and placed in a drying cabinet (105°C) over night and then weighed it again ( $w_d$ ).

The moisture content was calculated according to:

$$u = \frac{w_m - w_d}{w_d} \quad (3.1)$$

where

$u$  : moisture content

$w_m$  : weight of aggregate before drying cabinet

$w_d$  : dry weight of aggregate after drying cabinet

Cement, water and aggregate was weighed on a scale with an accuracy of 1 g. AEA, bacteria solution, calcium lactate and super plasticizer was weighed on a scale with an accuracy of 0.1 g before added to the mixing water. The mixing water with the additions was poured into a 100 litre concrete mixer where the aggregate and the cement already were mixed for a short time. Batch #C was mixed 3 minutes and batch #CA and batch #CAB were mixed 1 minute and 15 seconds after the addition of the water.

Table 3.1: Concrete recipe

Batch	#C*	#CA**	#CAB***
Water-cement-ratio	0.5	0.5	0.5
Cement <sup>1</sup> [kg/m <sup>3</sup> ]	380	380	380
Water [kg/m <sup>3</sup> ]	190	190	190
Aggregate 0-2 mm [kg/m <sup>3</sup> ]	824.3 <sup>5</sup>	765 <sup>5</sup>	765 <sup>5</sup>
Aggregate 8-12 mm [kg/m <sup>3</sup> ]	1007.5	935	935
Air Entraining Agent (AEA) <sup>2</sup> [kg/m <sup>3</sup> ]	-	0.574	0.579
Super plasticizer <sup>4</sup> [kg/m <sup>3</sup> ]	1,916	-	-
Bacterial preparation [kg/m <sup>3</sup> ]	-	-	10.787
Calcium lactate solution CaC <sub>3</sub> H <sub>6</sub> O <sub>3</sub> [kg/m <sup>3</sup> ]	-	-	10.787
Air content <sup>3</sup> [%]	1.4	4.6	4.4
Slump test measurement <sup>3</sup> [mm]	51	18	23
Density <sup>3</sup> [kg/m <sup>3</sup> ]	2401	2352	2362

\* Concrete without AEA

\*\* Concrete with AEA

\*\*\* Concrete with AEA, spores  
and calcium lactate

<sup>1</sup> Anl gningscement Degerhamn CEM I 42,5  
density 3200 kg/m<sup>3</sup> [3]

<sup>2</sup> Sika AirPro

<sup>3</sup> Measured after mixing

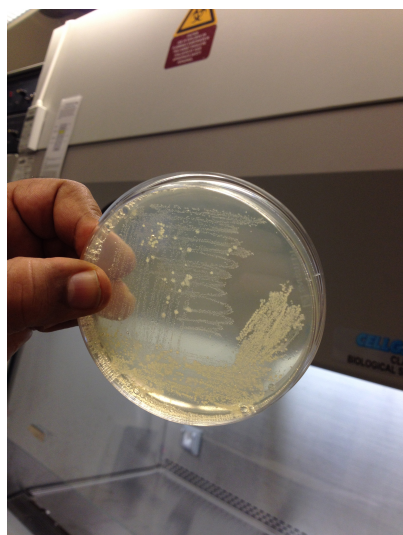
<sup>4</sup> Sika Evo-26

<sup>5</sup> Dry weight

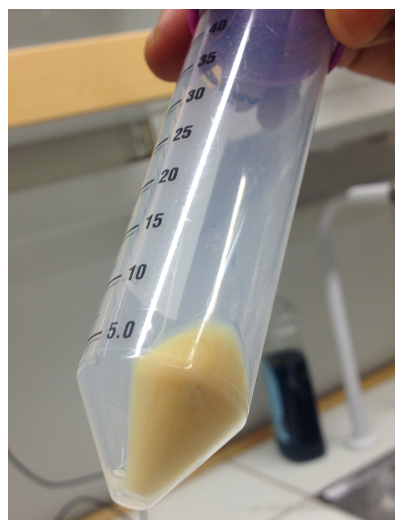
### 3.1.3 The addition of bacteria

#### Preparation of bacteria

Preparations of the bacteria solution took 3 days. The first day a Petri dish with solid nutrient medium was prepared (autoclaved in 120°C for 20 minutes). The bacteria was transferred to the Petri dish and the plate was incubated at 30°C overnight. The following day bacteria growth was confirmed, see figure 3.1a. A liquid medium was prepared and autoclaved and the bacteria was transferred to the liquid medium. Liquid medium with bacteria was placed on a shaker at 30°C and 150 rpm for about 24h. On the third day bacteria were harvested by centrifugation at 10 000g for 10 minutes. The concentrated bacteria, see figure 3.1b, could either be freeze-dried into a powder or re-suspended in a solution. The re-suspension method was used in this study.



(a) Solid medium with bacteria



(b) Bacteria after harvesting

Figure 3.1: Preparation of the bacteria

#### Adding the bacteria

The bacteria solution, see figure 3.2a, had a concentration of  $5.8 \cdot 10^6$  cells/ml, and was mixed with the calcium lactate solution (concentration 100 mM) with a ratio 1:1 (figure 3.2b), the same amount of bacteria solution and calcium lactate solution. Some reaction happened instantly, see figure 3.2c. The precipitations was probably  $\text{Na}_2\text{CO}_3$  or  $\text{CaCO}_3$  but not in a crystal form.

The mixture was then added to the mixing water where the AEA already had been added, figure 3.2d.



(a) Solution with bacteria



(b) Solution with calcium lactate



(c) Bacteria and calcium lactate mixed



(d) Bacteria, calcium lactate, AEA and water

Figure 3.2: The bacteria solution, the calcium lactate and the mix of the two. Bottom right is the mixture solved in the mixing water.

### 3.1.4 Testing of the fresh concrete and preparation of samples

After mixing the air content was measured using an 8 litre air entrainment meter. The same container was used to determine the density of the fresh concrete. The results of the measurements can be seen in table 3.1. A concrete slump test was conducted to assess the workability of the concrete. It was performed according to SS-EN 12350-2:2009, that is a standard method for concrete with stiff textures with slumps between 10-210 mm. The result from this test can also be seen in table 3.1.

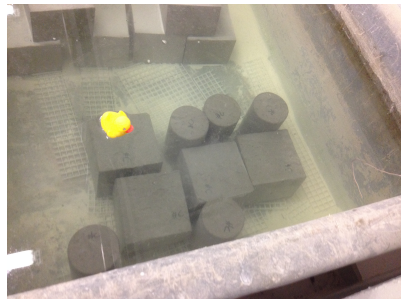
Four cubes with size  $150 \times 150 \times 150 \text{ mm}^3$  and five cylinders with diameter 100 mm and height 200 mm were cast in moulds. They were filled with 1/3 at a time and then vibrated on a vibration table. The samples were covered and left at room temperature over night, see figure 3.3b. After 24 hours samples were de-moulded and put in a water bath.



(a) Moulds for casting



(b) The cover of the samples



(c) Water bath



(d) Samples to freeze thaw test

Figure 3.3: Preparations of the specimens

## 3.2 Strength tests

Both the compressive strength and the tensile strength were determined. The curing was performed according to SS-EN 12390-2:2009, which means that the specimens were kept in moulds for 24 h and after de-molding, kept in a water bath until testing. The strength tests were performed at day 28 from casting. The samples were removed from the water and all the surfaces were wiped off of excessive water. For the length measurement of the specimens a slide gauge was used, accuracy 0.1 mm.

### 3.2.1 Compressive strength test

The compressive strength test was performed according to SS-EN 12390-3:2009. It is a hands-on machine where loading rate was manually governed. According to SS-EN 12390-3:2009 the stress increase should be  $0.6 \pm 0.2$  MPa/s. 0.6 MPa/s was chosen for this study. For a 150x150 mm<sup>2</sup> specimen the force rate used was 13.5 kN/s. The highest load reached was noted and the failure type was examined according to the standard.

### 3.2.2 Tensile strength test

The tensile strength was evaluated with the help of SS-EN 12390-6:2009 *Testing hardened concrete - Part 6: Tensile splitting strength of test specimens*. The test setup can be seen in figure 3.4. A thin slice of fiberboard (10x3x200 mm<sup>3</sup>, density 950kg/m<sup>3</sup>) was used as a pressure equalizer between the steel plates and the specimen. The loading rate was set to 1.57 kN/s as the standard recommended between 0.04-0.06 MPa/s. The highest load reached was noted.



Figure 3.4: Test setup for testing the tensile strength of a cylinder specimen

### 3.3 Verification of live bacteria and their cooperation with the cement

#### 3.3.1 Test with HCl

When de-moulding batch #CAB it was a gap between the plastic cover in two of the samples. At those spots some white salty-like crystals had developed, see figure 3.5. This was scraped off and together with some pieces from batch #CAB and #CA were tested with cold diluted hydrochloride acid.

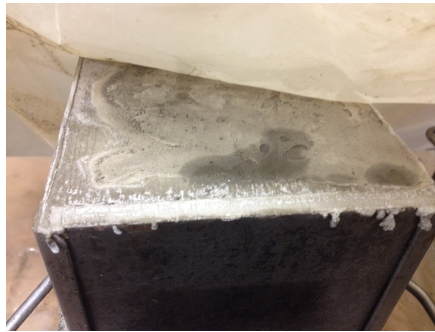


Figure 3.5: White salty-like crystals

#### 3.3.2 Isothermal calorimetry

Two isothermal calorimetry tests were performed on 8 different samples each time. The first test was done with 8 samples that would correspond to the three different batches. Two samples correspond to the batch #C, and three samples each for batches #CA and #CAB. In the next test the batch #CAB was divided into 2 samples with cement, AEA, water, bacteria and lactate. Three had only cement, AEA, water and bacteria and the last three had cement, AEA, water and lactate.

Each sample contained 4 g of cement (*AnlÄggningscement Degerhamn*) and about 2 g of liquid. The water was prepared with 0.3 % of AEA that correspond to 0.15 % of the cement weight. Also the bacteria solution and the calcium lactate were prepared with the same percent of AEA. Samples were mixed for 20 seconds in a rotating machine before placed into the isothermal calorimeter that had a constant temperature of 20.1°C.

### **3.3.3 SEM**

To verify whether any crystals were formed on the surface of the concrete or in the air voids some samples were studied in a scanning electron microscope (SEM). The samples were from the same specimens that were used for the compressive strength test. After the strength test small pieces from both the surface and the interior were placed in a drying cabinet for 3 days and then stored in an airtight jar. Before the samples were investigated under the microscope they were covered by a thin layer of gold, called sputtering deposition.

### **3.3.4 Repairing of crack**

After the tensile splitting test the specimens was taped together and immersed in tap water. After 50 days the tape was cut and the old cracks from the tensile splitting test was examined.

### **3.3.5 Testing with pH indicator**

One cube from each batch was immersed in water for 77 days after casting, then 2 days drying in room temperature. The cubes were split into two halves and the split surface was sprayed with StoDivers Carbondioxidtester, an pH indicator liquid.



## 3.4 Freeze thaw-test

A freeze-thaw test was performed to determine whether there was any differences in the frost resistance of the three different batches of concrete. This freeze-thaw method is new so a comparison between earlier tests with the same freeze-thaw test will also be made.

### 3.4.1 Preparation of concrete specimens

Two cylinders from each batch was taken out of the mould after 24 h and then kept in a water bath for 6 days. When taken out of the water the cylinders were placed in a plastic bag that was properly sealed and placed in a plastic box with some water, see figure 3.3d. The box was placed in a climate chamber with  $20\pm 2^{\circ}\text{C}$  and a relative humidity of 60% for 17 days.

After the climate chamber the cylinders were divided into two  $\Phi 100$  mm and  $h=100$  mm cylinders. The 1/2 cylinders were then dried for two hours and after that the side surfaces (not the top and bottom) was brushed with a steel brush to make the surface rougher. Glue (Casco XtremFix) was applied on the cylinders and two EPDM-rubber sheet ( $40\times 320$  mm<sup>2</sup>) was attached to each 1/2-cylinder, see figure 3.6. The gap between the sheets was filled with more glue. The specimens were left for three days at room temperature and then (1 day before the start of the tests) the specimens were sawn in the middle of the glued gap. In total 24 samples were prepared, these were placed in fresh water over night with the cut surface in free contact with water.

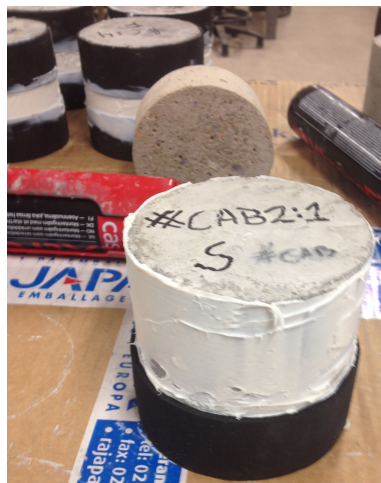


Figure 3.6: Preparation of the specimens to freeze-thaw tests. Two black rubber sheets was attached to the specimens with glue and the gap between the sheets was filled with more glue, as seen in the background

## Test setup

Every sample was squeezed into an insulation foam form in the shape of an octagon, with 10 mm left outside. In the bottom there was a plastic cup with a diameter of 118 mm, also with insulation foam around it. The test setup used can be seen in figure 3.7 and figure 3.8.

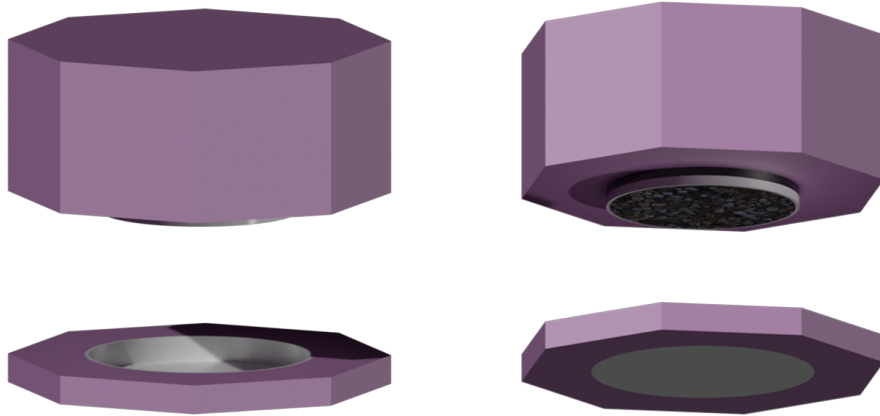


Figure 3.7: Test setup in 3D

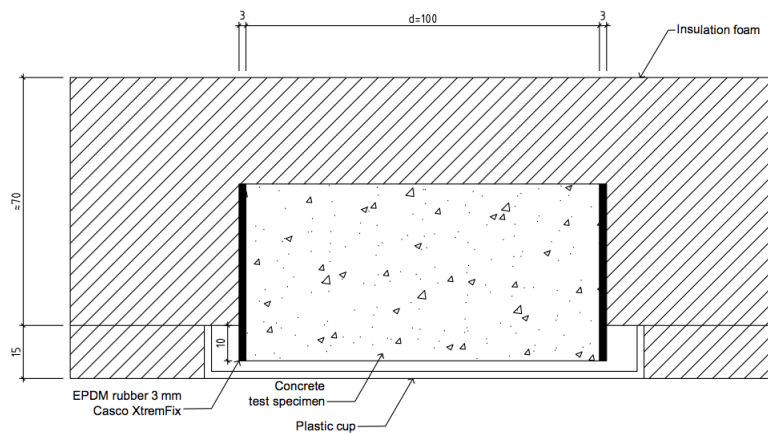


Figure 3.8: Drawing of test setup

The plastic cups were filled with 100 ml liquid, 12 sample cups (four from each concrete batch) were filled with salt water (de-ionized water with 3 w-% salt) and the other 12 were filled with de-ionized water. The samples were placed in a climate chamber with cycling temperature. 12 hours with  $-22^{\circ}\text{C}$  and 12 hours with  $+21^{\circ}\text{C}$ . Air was the thermal conductor and the temperature cycle and the temperatures in the fresh water solution and the salt water solution can be seen in figure 3.9.

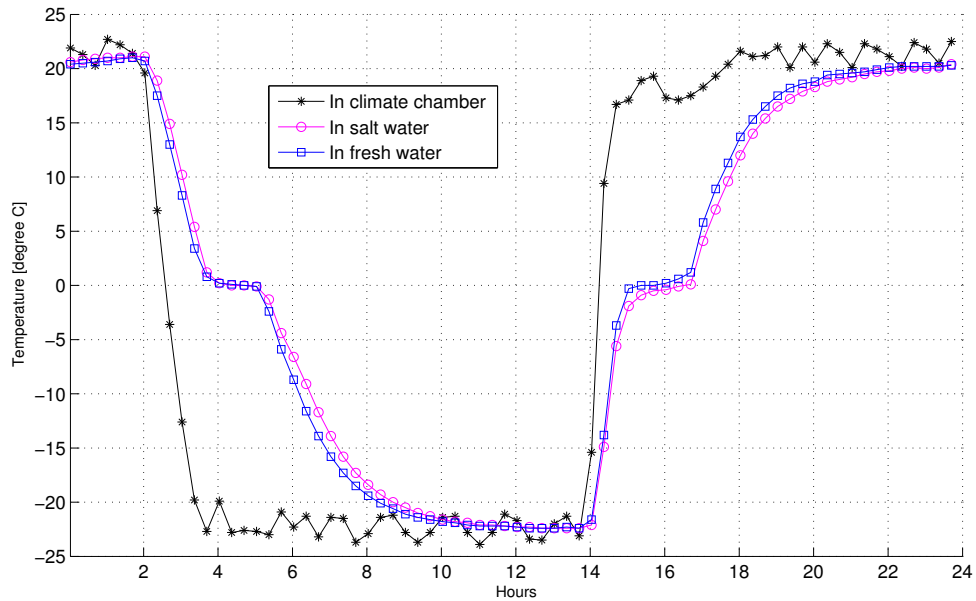


Figure 3.9: Temperature graphs for one freeze-thaw cycle (24h).

### 3.4.2 Collecting scaling of the samples

Every 7th day the samples were taken out of the climate chamber and brushed with a stiff brush. 20 strokes in four perpendicular directions. The scaling was collected in a filter and then dried overnight at 105°C. The scaling and filter were weighed on a scale with 0.001 g accuracy.



# Chapter 4

## Results

### 4.1 Testing of the fresh concrete

The results from testing of the fresh concrete can be seen in table 3.1 in chapter 3.

### 4.2 Strength test

For the strength tests three specimens were used for each test from each batch. In table 4.1 the results from the compressive strength test and the tensile splitting test can be seen.

Table 4.1: Results from strength test

Batch	#C	#CA	#CAB
Compressive strength $f_c$ [MPa]	$60.6 \pm 1.7$	$40.7 \pm 0.2$	$40.1 \pm 0.6$
Tensile strength $f_{ct}$ [MPa]	$3.95 \pm 0.62$	$3.60 \pm 0.30$	$3.25 \pm 0.51$

### 4.3 Verification of live bacteria and their cooperation with the cement

#### 4.3.1 Test with HCl

The pieces from batch #CAB effervesced when HCl was dropped onto the surfaces and on the white salty-like scrape off. This was not seen when dropped onto the surface of batch #CA.

### 4.3.2 Isothermal calorimetry

The results from the two calorimetry tests can be seen in figure 4.1 to figure 4.4, where the two first figures belong to the first test and the two last belong to the second test. Figure 4.1 and figure 4.3 show the thermal power and figure 4.2 and figure 4.4 show the accumulated energy production.

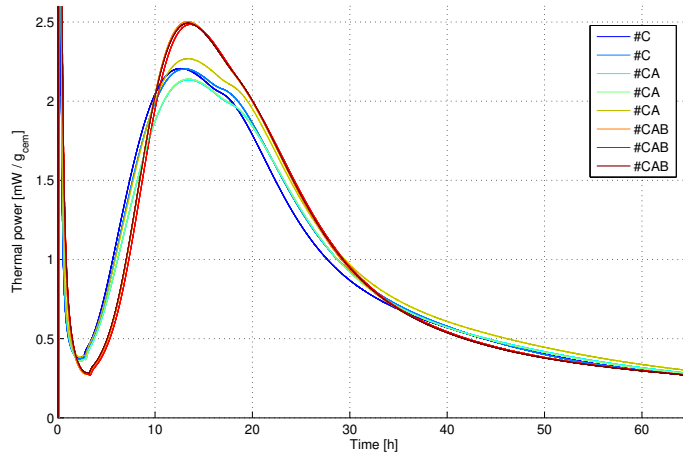


Figure 4.1: The results from the first isothermal calorimetry test with the three different concrete recipes showing energy production/time unit. #C is the mixture without AEA, #CA is the mixture with AEA and #CAB is the mixture with AEA, bacteria and lactate.

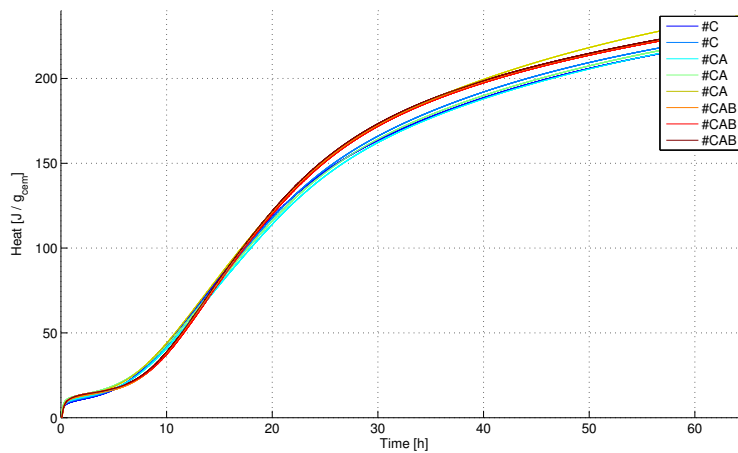


Figure 4.2: The results from the first isothermal calorimetry test showing the accumulated energy production. #C is the mixture without AEA, #CA is the mixture with AEA and #CAB is the mixture with AEA, bacteria and lactate.

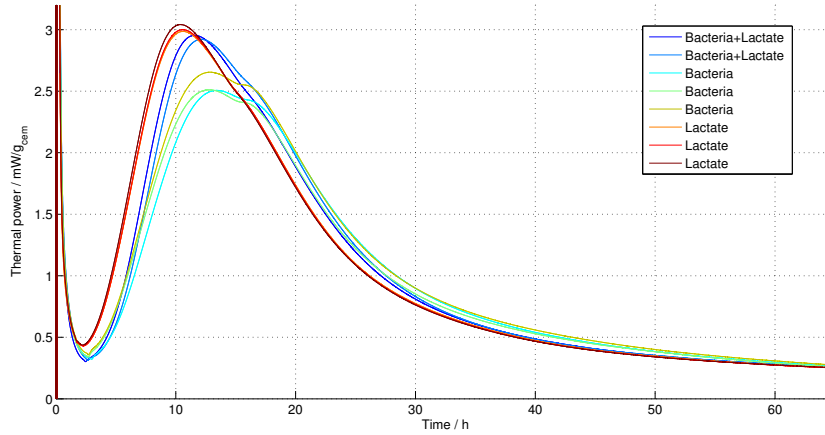


Figure 4.3: The results from the second isothermal calorimetry test where the #CAB batch was divided in three different scenarios, one with only bacteria, one with lactate and one with both bacteria and lactate. All samples contained AEA, cement and water. (Energy production/time unit).

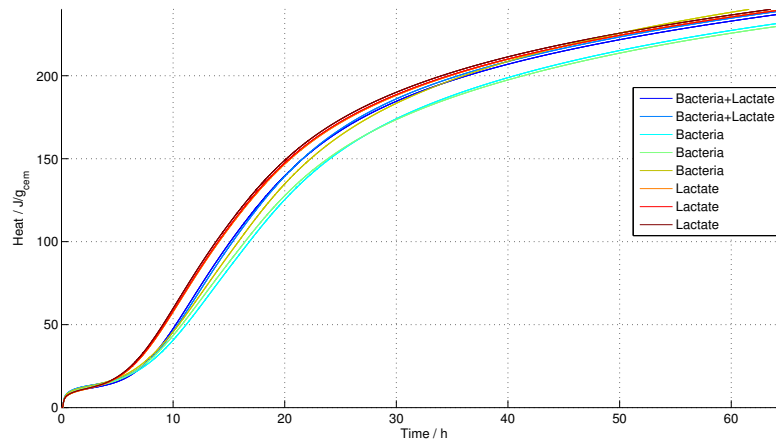


Figure 4.4: The results from the second isothermal calorimetry test showing accumulated energy production. Here the #CAB batch was divided in three different scenarios, one with only bacteria, one with lactate and one with both bacteria and lactate. All samples contained AEA, cement and water.

### 4.3.3 SEM

In figure 4.5 the surface can be seen, both from the bacteria treated concrete and the non-treated. In figure 4.6 air voids can be seen.

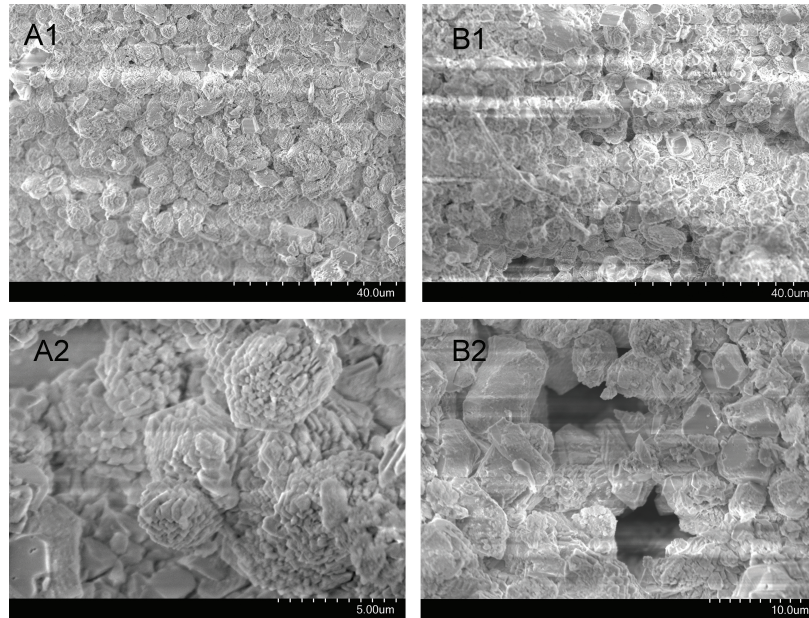


Figure 4.5: SEM picture of the surface, with different scales (marked in each picture). A1 and A2 is the non-treated concrete and B1 and B2 is bacteria-treated concrete.

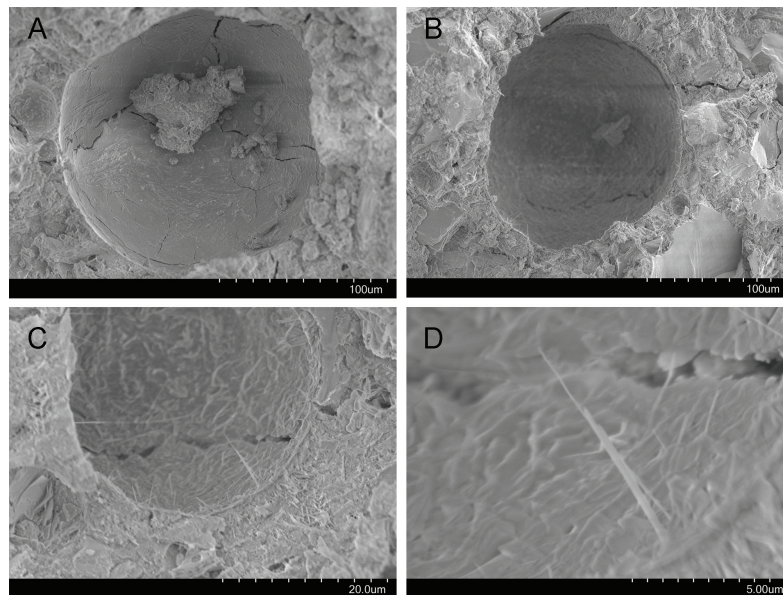


Figure 4.6: SEM picture of air voids, with different scales (marked in each picture). A is untreated concrete and B-D is bacteria-treated concrete. Note the needle-like structures in the bacteria-treated concrete.



#### 4.3.4 Evaluation of cracks

In figure 4.7 can the cracked specimens that was kept in water for 50 days after splitting be seen.

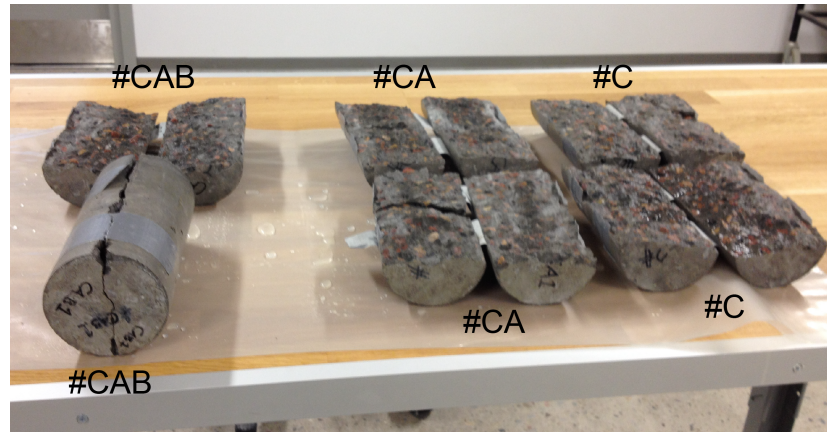


Figure 4.7: Specimens kept in water bath after splitting test. Note that one of the bacteria treated specimens (#CAB) stays together after the tape was cut.

#### 4.3.5 pH evaluation

In figure 4.8 can the evaluation of the pH in the concrete be seen.

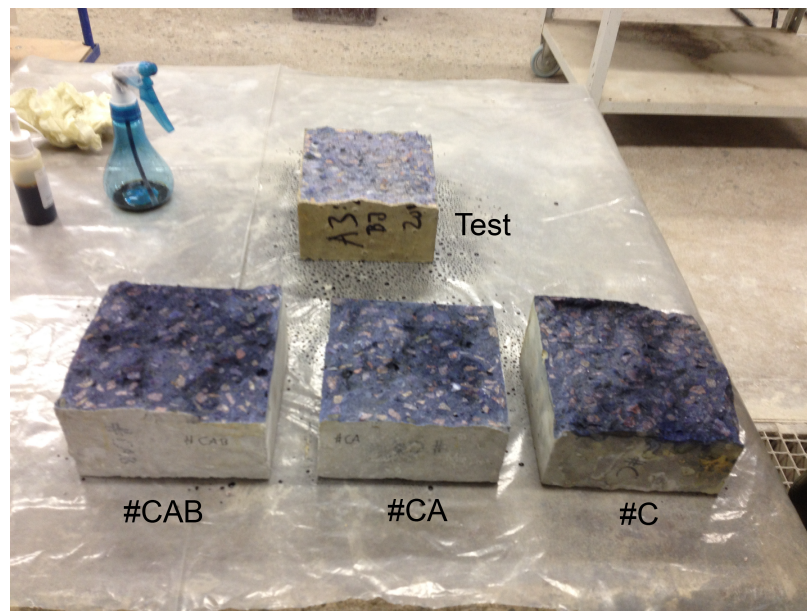


Figure 4.8: Indication of pH in the concrete. Blue indicates a  $\text{pH} > 10$ . The test specimen was an old one with carbonation close to the surface.

## 4.4 Freeze-thaw test

### 4.4.1 Freezing in fresh water

The results from the freeze-thaw test when tested with fresh water can be seen in figure 4.9 and figure 4.10. F=fresh water, C=sample from #C, CA=sample from batch #CA and CAB=sample from #CAB.

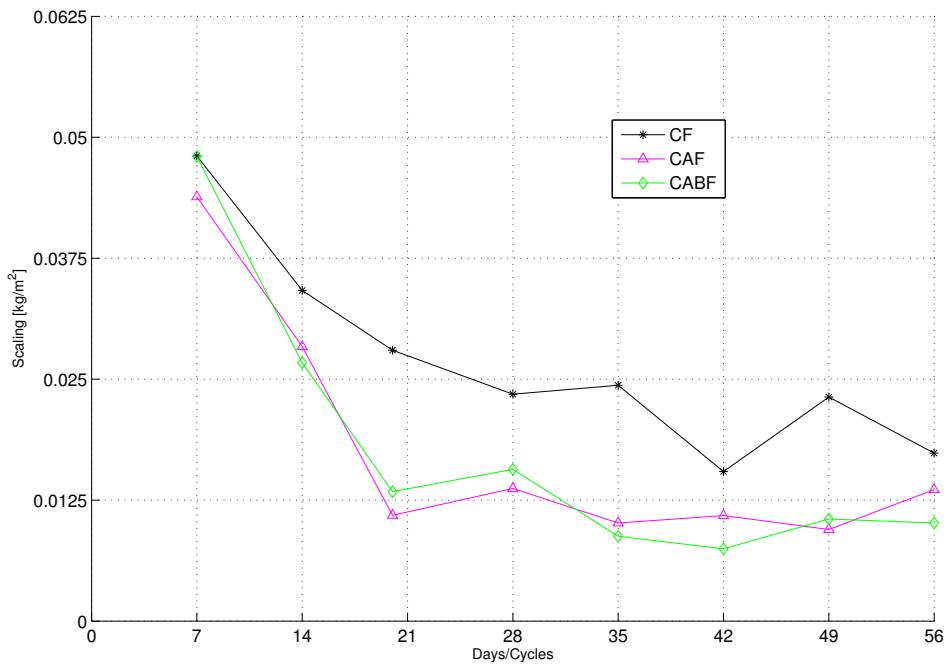


Figure 4.9: Mean values from three specimens of scaling/measurement in kg/m<sup>2</sup> when tested in fresh water. The measurements were conducted every seventh day, except one measurement that was conducted at day 20 instead.

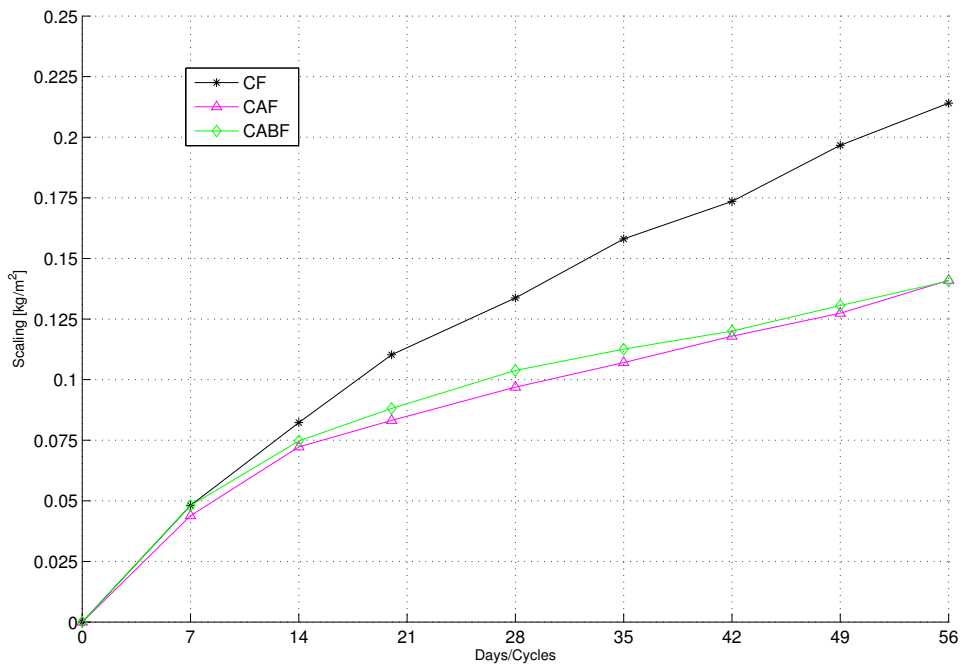


Figure 4.10: Mean values from three specimens of accumulated scaling in kg/m<sup>2</sup> when tested in fresh water.

## 4.4.2 Freezing in salt water

The results from the freeze-thaw test when tested with salt water can be seen in figure 4.11 and figure 4.12. S=salt water, C=sample from #C, CA=sample from batch #CA and CAB=sample from #CAB.

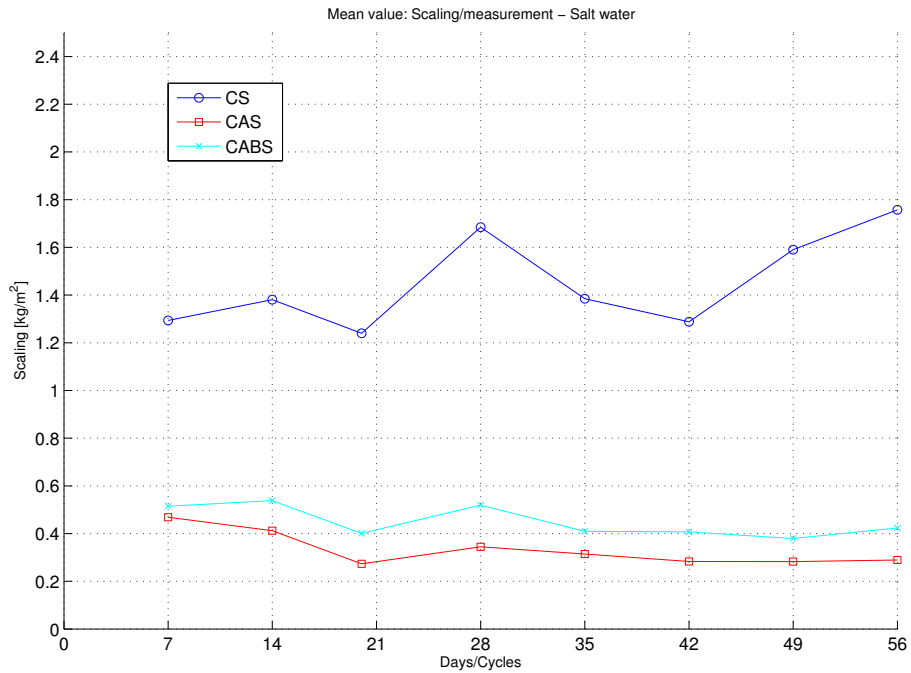


Figure 4.11: Mean values from three specimens of scaling/measurement in kg/m<sup>2</sup> when tested in salt water. The measurements were conducted every seventh day, except one measurement that was conducted at day 20 instead.

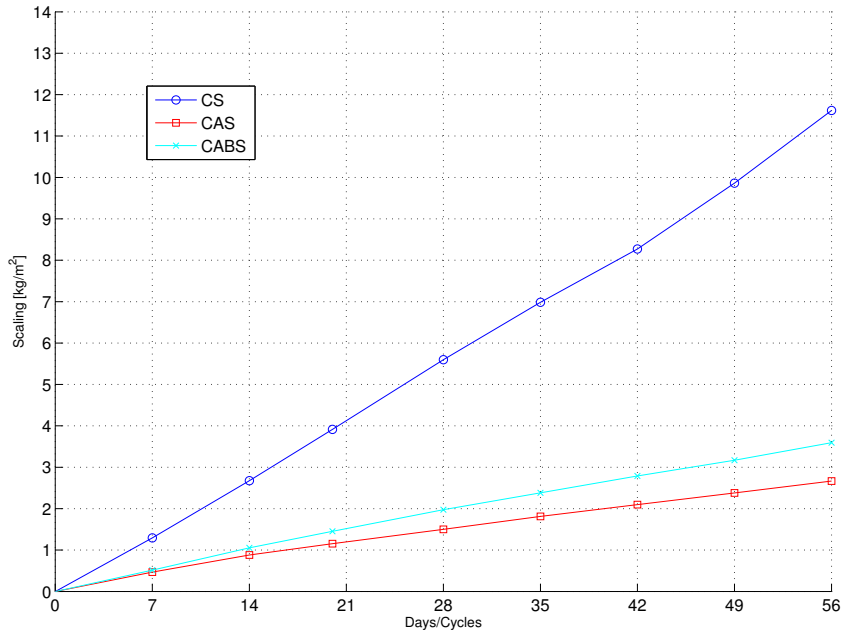


Figure 4.12: Mean values from three specimens of accumulated scaling in  $\text{kg/m}^2$  when tested in salt water.

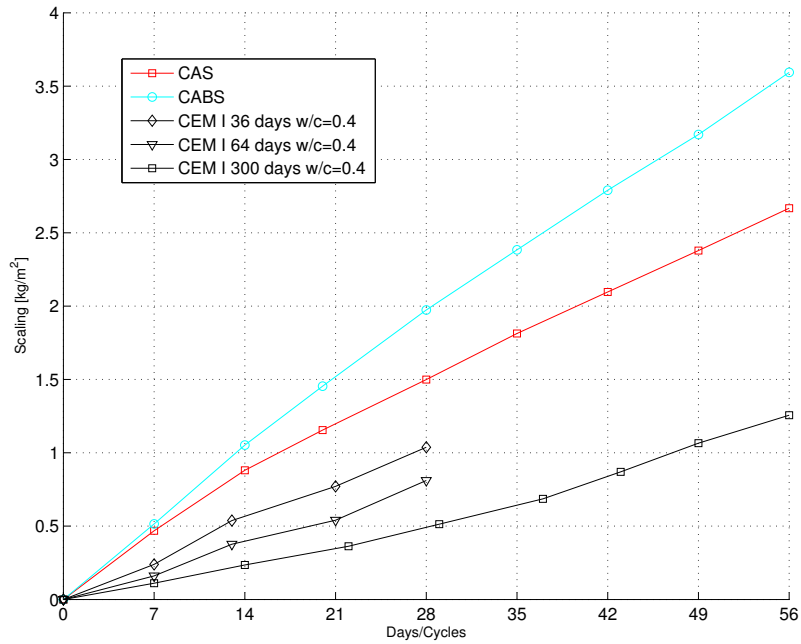


Figure 4.13: The results from this study compared with other specimens tested with the same method [22].



# Chapter 5

## Discussion and Conclusions

### 5.1 Discussion

To verify that the bacteria would work in case of cracking the splitted specimens was taped together and immersed in tap water. As seen in figure 4.7 one of the specimens from batch #CAB stayed together when the tape was cut while the rest of the specimens fell apart (even one from the bacteria treated batch, this could be due to that the crack widths was too big in some cases after the splitting test). This together with the small salty-like crystals that reacted with the hydrochloride acid is taken as proof that the bacteria was active. It would have been better to verify that in another way, like creating artificial cracks and then study the crack healing with permeability tests or through microscopic analysis of the crack.

The amount of bacteria (cells/ml) added was lower than other tests made where the articles gave a cell count. The aims here was to have a higher concentration but the bacteria did not grow as expected.

#### *Frost resistance*

The trend for the bacteria treated concrete (#CAB) follow the concrete with only AEA (#CA) very well, both when tested in salt water and in fresh water. They are no way near the concrete specimens without entrained air (#C) in terms of scaling, which means that they show quite good resistance against frost damage. There is an initial difference between the batches #CA and #CAB and that difference stays quite even through the experiment (when looking at scaling/measurement) when tested in salt water and decreases slightly when tested in fresh water, see figure 4.9 and 4.11. The initial difference might be explained by the initial difference in the air void content, small but present, as seen in table 4.1.

The test in salt water showed that #CABS has a total accumulated scaling of 3.6 kg/m<sup>2</sup> and #CAS has 2.7 kg/m<sup>2</sup>, see figure 4.12. The difference is about 0.9 kg/m<sup>2</sup> (33% more for #CAB compared to #CA) and can not be explained just with the

difference in initial air content though. The initial difference between #CABF and #CAF had completely disappeared when the tests were completed after 56 days.

When the results from this study are compared with other tests made with this method the results seems to be logical. As seen in figure 4.13 the batch #CAB and #CA gives more scaling than the other three. The other three have a lower w/c-ratio (0.4), which means a denser cement paste. The degree of hydration can also be assumed to be higher in those since they have been exposed to the same hydration conditions in terms of temperature (and also moisture condition) as the specimens from this study but for a longer period of time. This also means a more dense cement paste where water transport is more difficult than in the specimens from this study, and water transport is important in the frost mechanisms.

The pictures from the scanning electron microscope show that there is something in the air void of the bacteria-treated concrete, see figure 4.6 (B-D), These needle-like structures are probably ettringite and in that case not related to the addition of bacteria solution and are so small and can not explain the differences in scaling all by them selves. The bacteria-treated concrete isn't as bad as the one without any additional entrained air (#C), and the SEM-pictures also show that the bacteria definitely haven't plugged any of the air voids completely.

Worth mention here is that the scaling when the specimens were tested in fresh water are in the order of 0.1-0.2 kg/m<sup>2</sup> and when tested in salt water 2.5-12 kg/m<sup>2</sup>, which means 25-60 times higher. Salt water is more aggressive to the concrete than fresh water. Since the focus is on hydro power stations that are exposed to fresh water the addition seems to have no negative effect on the frost resistance. Also, if the bacteria had the right circumstances and wanted to precipitate calcium carbonate in the air voids they would probably have done it right away. It would then have been revealed in the SEM-pictures since the concrete was 56 days old. So for fresh water the addition of bacteria seems to work well but in salt water the result is more uncertain.

#### *Strength development, hydration and pH*

Small difference in the hydration could be detected as seen in figure 4.1. A delay for about one hour in the start of the main reaction for the bacteria-treated concrete but also a higher peak in the main reaction. As seen in the second test, in figure 4.3, the main reason for the delay in batch #CAB seems to be the bacteria and the reason for the higher peak seems to be the lactate added alone. Not the combination itself between the lactate and the bacteria which could be expected due to the metabolism of the bacteria and therefore a heat production from them. But none of the changes were big and the overall heat production and degree of hydration are in the end more or less the same as seen in figure 4.2 and figure 4.4. And the heat production after 24 hours is as expected about 170±20 kJ/kg of cement as stated by the manufacturer of the cement. Therefor no changes in hydration because of the bacteria or the lactate could be established.



No changes in the compressive or the tensile strength can be statistically established between the #CA and the #CAB either. This is in line with the measurements of the density made as seen in appendix B and the pictures from the SEM-analysis. The big difference between the #C-batch and the other two in the strength were expected due to the difference in air content.

No changes in pH could be detected that could be harmful to potential reinforcement, as seen in figure 4.8. The blue color indicates that pH in the concrete is higher than 10 and all three specimens kept the blue color over the whole parted surface. Contrary to the test specimen (a old specimen kept in the laboratory for some years) that got a 3 mm yellow stripe around the edge that indicates that the carbonation visually could be seen in that specimen.

Due to the literature review about this bacteria there seems to be an agreement between different research groups that the need of liquid water is essential for the bacteria to be able to precipitate calcium carbonate why water retaining structures like hydro power stations would work quite well with the bacteria. Or any other outdoor structures in contact with water.

Even though the tests shows good results according to both strength development and frost resistance, the crack healing efficiency with this amount of bacteria added and also higher concentrations should be investigated. This study also took place during only about 20 weeks which means that no conclusions about the long term effects can be made.

## 5.2 Conclusions

With CEM I cement, AEA and this concentration of bacteria and lactate added following conclusions could be made:

- No changes in hydration nor in the pH because of the bacteria or the lactate could be established.
- No changes in the strength development could be detected that could not be explained by the different air content in the fresh concrete.
- The frost resistance were not affected by the addition of bacteria and calcium lactate when tested in fresh water.

## 5.3 Continuous work

All continuous work should include a verification of the crack healing efficiency either through permeability tests or microscopic analysis of the cracks with the bacteria and substrate used. Preferably also with a test with different concentrations.

Since the literature review indicated that the long terms effect in viability of the spores could be a problem when adding them without microcapsules or in expanded clay it would be interesting to do long term tests with bacteria and AEA and see if they survive longer with AEA added compared to if no AEA is added.

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# Appendices





# Appendix A

## Freeze thaw tests

### A.1 Freezing in fresh water

The result from the freeze-thaw test when tested with fresh water can be seen in figure A.1 and figure A.2.

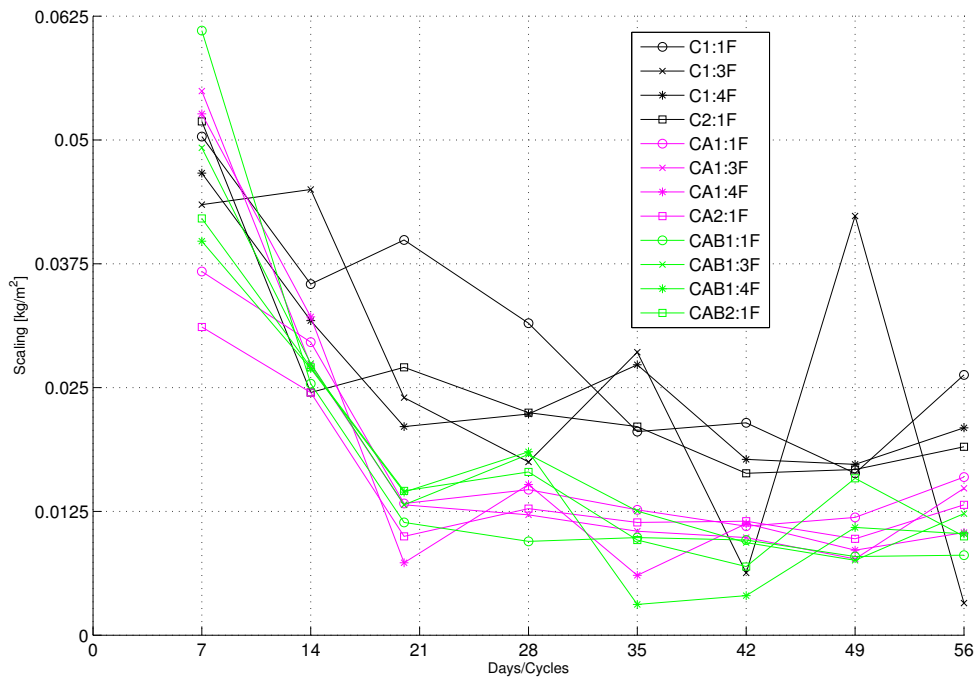


Figure A.1: All values from the freeze-thaw tests. Scaling/measurement in  $\text{kg}/\text{m}^2$  when tested in fresh water

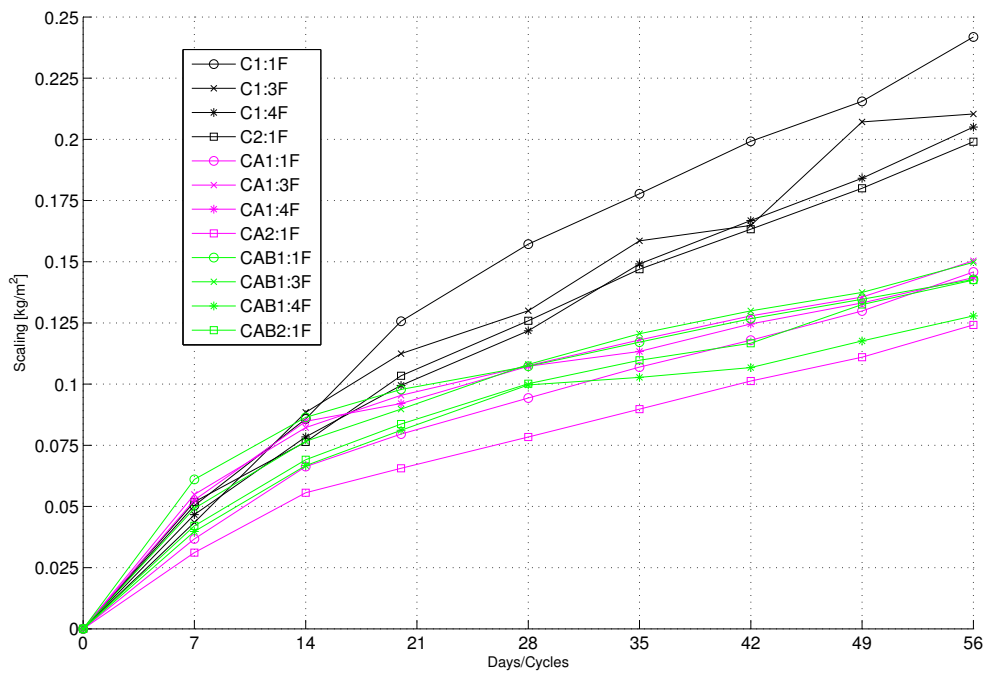


Figure A.2: All values from the freeze-thaw tests. Accumulated scaling in  $\text{kg/m}^2$  when tested in fresh water

## A.2 Freezing in salt water

The result from the freeze-thaw test when tested with salt water can be seen in figure A.3 and figure A.4.

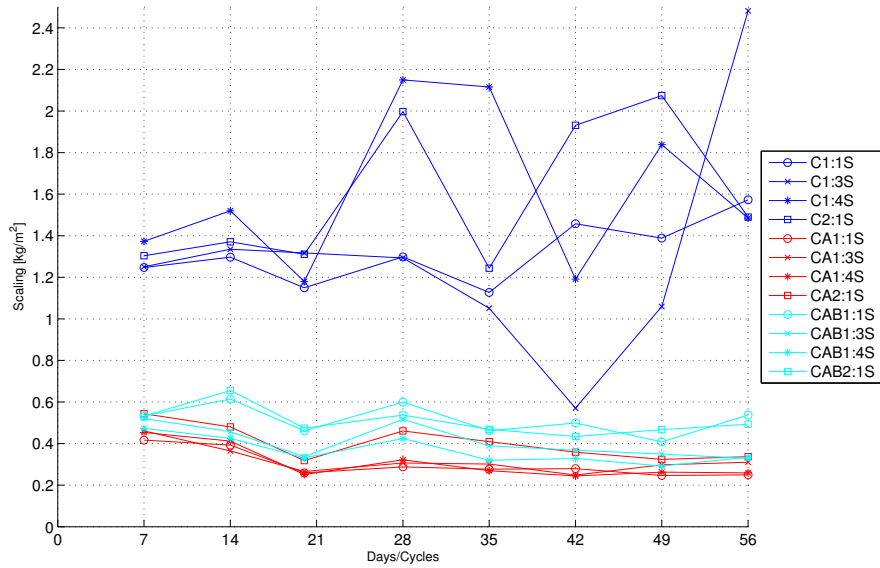


Figure A.3: All values from the freeze-thaw tests. Scaling/measurement in  $\text{kg}/\text{m}^2$  when tested in salt water

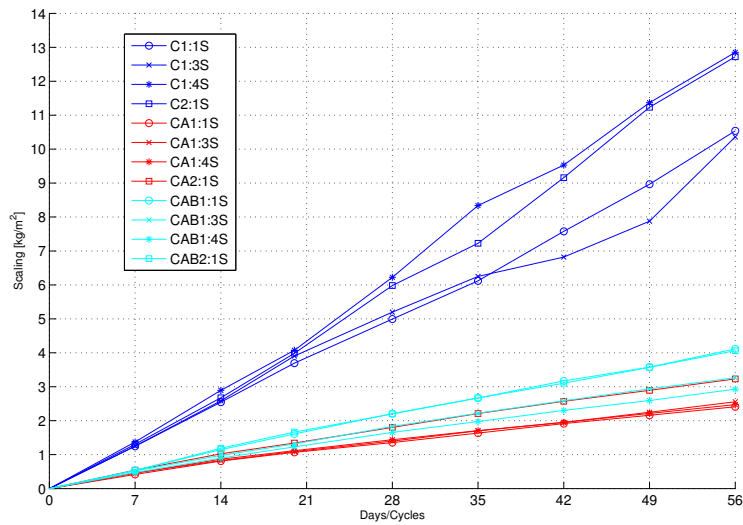


Figure A.4: All values from the freeze-thaw tests. Accumulated scaling in  $\text{kg}/\text{m}^2$  when tested in salt water



# Appendix B

## Strength Tests

<b>Compressive strength</b>								
	<b>F<sub>max</sub> [kN]</b>	<b>Mean width [mm]</b>	<b>Mean height [mm]</b>	<b>Area [m<sup>2</sup>]</b>	<b>f<sub>c</sub> [MPa]</b>	<b>Weight [kg]</b>	<b>Volume[m<sup>3</sup>]</b>	<b>Density [kg/m<sup>3</sup>]</b>
C1	1415	150,15	150,75	0,0226	62,5		0,00340	[kg/m <sup>3</sup> ]
C2	1340	150,8	149,5	0,0225	59,4	8,32	0,00340	2447
C3	1355	150,1	150,9	0,0227	59,8	8,38	0,00340	2466
CA1	905	149,9	149,05	0,0223	40,5	8,08	0,00335	2411
CA2	920	150,7	150,15	0,0226	40,7	8,07	0,00341	2365
CA3	925	149,8	151	0,0226	40,9	8,14	0,00339	2401
CAB1	905	152,5	150,2	0,0229	39,5	8,13	0,00349	2326
CAB2	920	150,85	149,9	0,0226	40,7	8,00	0,00341	2346
CAB3	905	151,1	149,75	0,0226	40,0	8,03	0,00342	2347

n=	3	<b>Standard deviation:</b>	<b>f<sub>ct</sub> [MPa]</b>	<b>Standard deviation:</b>
C	60,6	1,676	3,97	0,615
CA	40,7	0,195	3,61	0,295
CAB	40,1	0,591	3,27	0,508

<b>Tensile strength</b>					
	<b>F<sub>max</sub> [kN]</b>	<b>Mean diameter [mm]</b>	<b>Mean height [mm]</b>	<b>f<sub>ct</sub> [MPa]</b>	<b>Vikt [kg]</b>
C1	136,2	100,3	200,8	4,31	3,87
C2	135,8	99,8	199,95	4,33	3,866
C3	103,1	100,3	201,05	3,26	3,884
CA1	120,5	100,05	201,1	3,81	3,775
CA2	117,7	100,05	200,65	3,73	3,763
CA3	102,7	99,9	200,3	3,27	3,748
CAB1	94,6	99,95	200,25	3,01	3,756
CAB2	102,2	100,15	200,4	3,24	3,762
CAB3	112,3	100,85	199,75	3,55	3,747

Figure B.1: All values from compressive strength test and tensile splitting test