FACULTY OF ENGINEERING
AND ARCHITECTURE

Impact of bioagents on the cementitious matrix

Michiel Vandervoort
Student number: 01100534

Supervisors: Prof. dr. Nele De Belie, Dr. ir. Jianyun Wang

Master's dissertation submitted in order to obtain the academic degree of
Master of Science in de industriële wetenschappen: bouwkunde

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Vandervoort Michiel

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Ghent, January 2019

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Abstract

Cracking of concrete is one of the main factors that are adverse for the overall durability. These cracks transition into high repair and maintenance costs, reduction of the lifecycle of the construction and leads to an increased demand for concrete. The concrete industry is currently the second largest producer of carbon dioxide and is responsible for 5% of worldwide emission. Recent developments have been made to increase the durability of concrete by means of microbially induced calcium carbonate precipitation (MICP) which involves incorporating bacterial cells, urea, yeast extract and calcium nitrate into the concrete mix. Although the application of bio-concrete has shown to be feasible, the influence of these bioagents on the cementitious matrix remain ill-understood.

The influences of the three additives; urea, yeast extract and calcium nitrate, on the cementitious matrix were tested by performing 7 tests on 16 compositions, both fresh and cured mortar compositions. Additionally, the influence of these bioagents in combination with bacterial cells on the amount of precipitated $CaCO_3$ content was tested in 6 compositions.

It was found that both urea and calcium nitrate acted as an inert filler with positive effects on certain properties and overall durability. Additionally, the precipitation of calcium carbonate was increased when with bacterial cells were incorporated. However, yeast extract negatively affected the cementitious matrix on all off the properties tested in this work. This depreciates the durability of the cementitious matrix. Although yeast extract was found to increase the amount of precipitated calcium carbonate, it needs to be limited in its dosage, or preferably substituted to negate the effects brought forward as a result of its addition.

Keywords: bio-concrete, urea, yeast extract, calcium nitrate, *Bacillus sphaericus*, mortar properties, $CaCO_3$

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Abstract - **Cracking of concrete is one of the main factors that are adverse for the overall durability. These cracks transition into high repair and maintenance costs, reduction of the lifecycle of the construction and leads to an increased demand for concrete. The concrete industry is currently the second largest producer of carbon dioxide and is responsible for 5% of worldwide emission. Recent developments have been made to increase the durability of concrete by means of microbially induced calcium carbonate precipitation (MICP) which involves incorporating bacterial cells, urea, yeast extract and calcium nitrate into the concrete mix. Although the application of bio-concrete has shown to be feasible, the influence of these bioagents on the cementitious matrix remain ill-understood.**

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I. INTRODUCTION

The implementation of bacteria to achieve self-healing concrete is a hot topic in civil engineering. This healing is achieved by introducing calcium carbonate precipitating bacteria in combination with various bioagents. Much of the recent research is focused on the optimal implementation of bacteria in concrete to maximize the self-healing capabilities, which is done by screening different bacteria and carriers in which they are introduced into the concrete mix [1]. Another approach is to evaluate the potential of the bio-concrete is by determination of the healing capabilities and deriving an optimal mixture and bacterial compositions from this [2]. However, the individual influences of each of the bioagents commonly used in modern bio-concrete are under-documented and their mechanics are ill-understood.

The aim of this work was to lay out an accessible work that documented the main influences and characteristics of three commonly used bioagents, additionally, the influence of bacterial cells on the precipitated $CaCO_3$ content in the matrix was investigated. Beyond quantifying, each mechanism was given a plausible explanation on a molecular level. This way further research in the optimization of bio-concrete can fall back on this work to identify the main actors of specific characteristics when using these additives to compliment the bacteria in a composition.

In this work, 8 tests were performed to gain insight into the basic properties of both fresh and hardened mortar compositions. The compositions had 5 varying w/w% series for each of the 3 bioagents and one mixture that contained all three of the bioagents in the most commonly found concentrations. Additionally, 6 compositions were tested with bacterial cells included.

II. MATERIALS AND METHODS

All mortar compositions were produced in accordance to NBN B15-001 (2004) and stored in a moisture curing room with a temperature of 20 ± 2°C and >90% relative humidity until the required age was reached. The 17 bioagent compositions were coded with the letters, U, Y and Ca for urea, yeast extract and calcium nitrate respectively, followed by a number which signified the amount of weight per weight of cement percentage. For example, U4 stood for a mortar mix containing 4 w/w% of urea and Y0.85 defines a mortar mix that contained 0.85w/w% of yeast extract. Furthermore, the name MIX was used for a mortar mix containing U4, Y0.85 and Ca8. Additionally, 7 compositions consisted of a reference, 10^9 cells/mL, 10^9 cells/mL + U4, 10^9 cells/mL + U4 + Ca8, 10^9 cells/mL + U4 + Y0.85, 10^9 cells/mL + U4 + Y0.85 + Ca8 and one mix contained a grown culture with a cell concentration of 10^8 cells/mL. These mixes were coded REF, T1, T2, T3, T4, T5 and T6 respectively. A water-cement factor of 0.5 is used throughout this work.

Urea - also known as Carbamide (CH_4N_2O) of producer Carl Roth was used. It comes in the form of white pearls that easily dissolve in water.

Yeast extract - A dried yeast autolysate by Carl Roth was used, it is produced through aqueous extraction of autolysed brewer's yeast. It is highly soluble in water with a solubility of 410 g/L.

Calcium nitrate - The calcium nitrate was supplied as a tetrahydrate by Carl Roth. The fact that it is a tetrahydrate is important since every molecule will be surrounded by four molecules of water. This additional water will need to be considered when calculating the water-cement factor. The chemical formula is $Ca(NO_3)_2 . 4H_2O$.

Microorganisms - *Bacillus sphaericus* LMG 22257 was used in this study. The bacteria were cultured in a growth

Flow	Setting	Mechanical properties*	Water absorption	Freeze-thaw resistance	Carbonation	Cement hydration
		3*, 7*, 14*, 28* ,56d	28+7,14,28d	28d	28+14,28,56d	

Table 1. Summary of tests performed

medium consisting of urea, 22 g/L, and yeast extract, 20 g/L, also referred to as an UYE-medium. The cells were harvested by centrifugation at 7000 rpm for 7 minutes by Sorvall RC 6+, Thermo Scientific^{TM}. After draining off the excess UYE-medium the cells were resuspended in a sterile saline solution (8.5 g/L NaCl). The final concentration of the bacteria in suspension was approximately 10^9 cells/mL while that of the grown culture was approximately 10^8 cells/mL. The grown cultures and suspended cells were stored at 4°C in a fridge. Before usage, the cells were left to heat up until room temperature.

Table 1 gives a summary of all test performed in this work. An asterix (*) denotes that the test was also performed with bacterial mortar. Additionally, as mentioned later, the $CaCO_3$ content of bacterial mortar was tested at 3, 7, 14 and 28 days.

Flow – The flow of fresh mortar was tested by means of a flow table test. The testing method is described in NBN-EN-1015-3 (1999) and results in a percentage of diameter increase compared to the diameter of the conical mould after being jolted.

Setting - The setting of the mortar was tested with the Vicatronic E044N by Matest®, this is an automatic Vicat machine that executes the Vicat test in accordance with NBN EN 196-3 and NBN EN 480-2. Initial and final setting were determined which are respectively the moment when the needle does not penetrate the mortar completely and the moment when the needle does not penetrate the mortar at all.

Mechanical properties – After calculating the density of each mortar prism, a Walter+Bai DB 250/15 machine was used to perform flexural strength testing. The two resulting halves were then subjected to compressive strength testing. The testing method follows EN 196-1.

Water absorption - The water absorption, in accordance with NBN B 15-215, was tested on 40x40x160 mm prisms by fully submerging in water at a stable temperature of 20 ± 1°C. The weight increase was measured and the percentage of weight increase compared to dry weight was calculated.

Freeze-thaw resistance - The freeze-thaw resistance was performed in order with CEN/TS 12390-9. The mortar prisms were cut up into square blocks of approximately 40x40x40mm and subjected to 28 cycles of freeze and thaw with a temperature varying from -17.50 to 22.50°C. The machine used for this experiment was a WT3 – 1000/40 by Weiss Technik.

Carbonation - The carbonation depth was evaluated as described in ISO 1920-12:2015. The accelerated carbonation was evaluated on 14, 28 and 56 days of being subjected to 1% of CO_2 in a carbonation chamber, which was a WEKK 0130 by Weiss Technik. Each prism was coated twice with epoxy resin (SikaCor-277) with 24 hours of drying period between layers to ensure air sealing where one side was left blank so unidirectional infiltration of CO_2 was guaranteed when being subjected to a constant concentration of 1% CO_2 in the carbonation chamber. Afterwards, they were split into three pieces by means of a Brazilian split test, where a line load was introduced perpendicular to the length of the prism and sprayed with phenolphthalein.

Cement hydration - The cement hydration heat was measured by means of calorimetry, in this work, a TAM-air apparatus was used. This machine measured the energy required to keep the sample that is loaded in a capsule in an isolated chamber at 20°C, this energy was given in mV. The conversion factor, to convert mV to W, was calculated by calibrating the machine with empty chambers. Afterwards, these values were integrated to calculate the accumulative heat in J/g.

$CaCO_3$ content - While many methods are available to identify the presence of $CaCO_3$, many of these are complex, time-consuming or expensive. The most prominent methods are X-ray diffraction (TOPAS method) (XRD), thermo-gravimetric analysis method (TGA), ASTM, ICP-AES, titration and washing. The results of these methods vary with XRD, TGA and ASTM giving the most reliable and average values [3]. The main problem with these techniques is the sample size that is used, which resulted in varying results depending on where the sample was taken. Important points when considering a testing setup are; the price, repeatability, ability to test a sizeable sample and accuracy. The proposed testing method is based on the reaction of calcium carbonate with hydrochloric acid.

The conservation of mass law states that the mass of the reactants and products is equal, however, because carbon dioxide is a gas it will result in a decrease in mass of the aqueous products. And thus, from the mass loss, the amount of calcium carbonate can be calculated. Three weights were noted; M1and M2 which are the weight of the sample plus beaker with HCl before and after reaction respectively, and M3 which was calculated from the difference between M1 and M2, and therefore represents the mass of CO_2 produced. Hydrochloric acid was added in abundance so that the calcium carbonate was always the limiting reagent in the reaction.

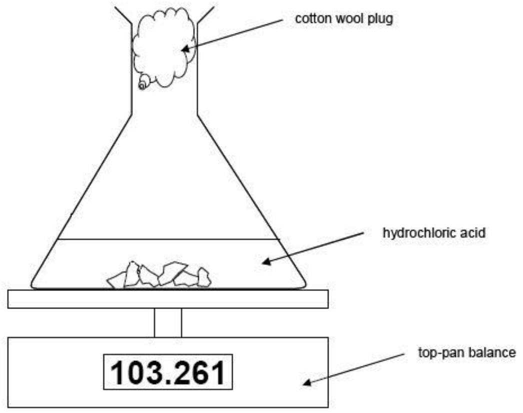

Figure 1. Test setup to measure $CaCO_3$ content

The prisms were subjected to a three-point flexural and compressive test, after which three ± 5 g samples were obtained. These samples were collected from the centre of the mortar to avoid any calcium carbonate formed due to carbonation. The samples were then mechanically crushed and grounded into a fine powder which resulted in 9 fine powder samples per composition. For each sample ± 2 g was weighed and ± 20 mL of 0.1 M HCl solution was weighed by means of a pipet helper. The combined weight of the sample and solution was calculated after which the sample was introduced to the solution and manually swirled for 10 s. After this, the solution was let to rest on a scale and the weight after 50 s, meaning one minute in total, was noted.

III. RESULTS AND DISCUSSION

Flow – The addition of urea resulted in an increase of 3, 19, 28, 34 and 41 % respectively for U1, U2, U3, U4 and U5 compared to the reference. This is explained by an increase of solution volume that occurs due to the fact that urea dissolves in water but does not hydrolyse [4].

Yeast extract brought forth an increase of 23 % when adding 0.34 w/w% to the mortar mix, this increase raised linearly through Y0.85, Y0.128 and Y1.27 that produced an increase of 29, 33 and 39% respectively. Lastly, for Y2.56 an increase of 55% was observed. Three paths of thoughts are laid out to declare these effects. The first has been discussed in the literature, the following two mechanisms are possible paths proposed by the author and will need to be verified in future research. Firstly, the highly soluble carbohydrate content, which is more known under the generic name of sugar, is known as a strong retarding agent [5, 6]. The main reason here is that the interaction of sugar with C_3A prevents the formation of the cubic phase C_3AH_6 (Tricalcium aluminate hydrate) by clogging the surface of C_3A on the cement particle. This aggressive reaction of water with C_3A is also known as “flash setting” as it produces a lot of heat and starts immediately when mixed. Secondly, yeast autolysate produces nitrogen compounds during its autolysis [7]. This formation of nitrogen gas links back to foam concrete, also known as cellular lightweight concrete. The pores in the yeast extract compositions had interconnecting airpockets that were verified visually after inspecting the obtained prism halves after destructive strength testing. Lastly, the other proposed path of thought is the production of air bubbles and formation of acidic products due to the degradation of the protein, this due to hydrolysis at one or more of its peptide bonds [8].

Calcium nitrate reduced the flow in linear correlation with the w/w% calcium nitrate introduced to the composition. When a dosage of 8 w/w% was reached the flowability dropped rapidly to the point where no change in diameter was observed when jolting. This last observation is a result of flash setting that occurred when adding dosages greater than 8 w/w%. The flow ratio was 0.93, 0.90, 0.78, 0.18 and 0.09% compared to reference for Ca2, Ca4, Ca6, Ca8 and Ca10 respectively.

Lastly, it was found that the MIX composition showed an increase of 33% in flow ratio. This is a combination of the results found for U4 (133%) and Y0.85 (129%), however, the flash setting of Ca8 (18%) did not show. This means that the final characteristics of MIX are not the superposition of the individual effects, but is rather dominated by the dosage of urea and yeast extract.

Setting – Reference mortar showed initial setting after 180 minutes and final setting after 270 minutes. The initial setting and final setting when adding urea both showed a strong linear correlation with respect to its w/w%, where an increase in urea lead to an increase in both the initial and final setting time. Initial/final setting times of 240/285, 255/330, 270/360, 285/390 and 315/400 minutes were found for increasing w/w% of urea. The mechanism for this delay is twofold; firstly, the internal covalent bonds will not split into ions in water, therefore the volume of the solution is increased, delaying setting [9, 10]. Secondly, the endothermic reaction of dissolving urea in water causes the hydration heat to lower with respect to the amount of urea added delaying the initial and final setting.

Yeast extract acted as a retarder, where both the initial and final setting times were delayed with increasing w/w% of yeast extract. However, when 2.56 w/w% of yeast extract was added, a decrease in the initial and final setting time was found. Therefore, it can be concluded that yeast extract can both function as an accelerator and retarder depending on the amount added. The initial/final setting for increasing w/w% were 300/420, 480/660, 600/840, 720/930 and 150/180 minutes. Yeast extract only acted as a retarder up to a dosage, which is to be determined more precisely, between 1.7 and 2.56 w/w%. At higher dosages than the aforementioned limit, it acted as an accelerator. When adding large quantities of sugar to the mortar mix most of the gypsum is combined fairly quickly, this leads to gypsum losing its effects as an inhibitor of flash setting, and causes the mortar to set quickly. However, the remaining C_3A does not hydrate for days, this can be seen in the lack of strength development for Y2.56.

Addition of calcium nitrate resulted in an acceleration of the setting time. This acceleration was linear with the w/w% of calcium nitrate that was added. Initial/final setting times of 150/195, 135/180, 135/165, 75/120 and 60/105 minutes were found. When calcium nitrate dissolves in water, the calcium ions Ca^{2+} become separated from the nitrate ions NO_3^-. This increases concentration of calcium ions and leads to a faster super-saturation of the water with respect to calcium hydroxide. Additionally, the formation of plate calcium aluminate hydrate (C_4AH_x) has been observed by XRD measurements [11]. This reaction is so rapid that when it occurs, it releases a large amount of heat which causes the paste to set within minutes, this phenomenon is called flash setting.

Again, the mix composition showed similar results to that of yeast extract, but not urea. An initial setting time of 450 and final setting time of 570 was found which almost perfectly matches the result of Y0.85.

Mechanical properties – (i) Bioagents - Urea showed a positive influence on the compressive strength. However, while U1, U2 and U3 showed greater compressive strength than the reference at all testing moment, U4 and U5 only achieved the same compressive strength as the reference at 56 days. The optimal compressive strength was achieved when incorporating 2 w/w% of urea in the composition where, after 56 days, a compressive strength of 71 MPa was achieved compared to 64 MPa for the reference. The fact that U1 and U2 had a higher flexural strength at ages between 14 and 28 days is explained by the low impact of the endothermic cooling of urea.

Yeast extract decreased the compressive strength at all ages with increasing w/w%. And, similar to its effects on the flexural strength, Y2.56 showed very low strength at 3 days. After 56 days 94, 51, 48, 47 and 30% of reference strength was found for Y0.34, Y0.85, Y1.28, Y1.7 and Y2.56 respectively. The low compressive strength of Y2.56 at 3 days is again the result of the accelerating effect on the setting due to a high dosage of sugar.

Lastly, for calcium nitrate, results showed a higher increase in early strength for Ca2, Ca4 and Ca6 compared to Ca8 and Ca10, while, for later ages, the opposite was true. In general, it can be stated that calcium nitrate improves the compressive strength at ages 28 days and after with increasing w/w%. The increase in compressive strength that is found is the result of the denser structure that is formed by the addition of calcium nitrate.

The MIX composition showed similar characteristics as Y0.85, but its strength did not increase as one would expect when averaging the effects of all bioagents. This again strengthens the author's case that because combination where yeast extract acts as an air-entrapment agent and calcium nitrate shows flash setting the porosity remains at such a high level that it inherits all characteristics of the yeast extract component.

(ii) Bacteria – For T1 the addition of bacteria had no effects on the compressive strength, the pathway where dead bacteria act as an organic fibre only has an effect on the tensile strength. For T2, when adding urea alongside bacteria, ureolysis takes place. It is this deteriorating mechanism that leads to the decrease of compressive strength at an early age. However, at later ages, strength gained more rapidly compared to reference, which lead to an overall small negative effect. In T3, where urea and calcium nitrate were added alongside bacterial cells, the early strength decreased even further. A combination of a high solution volume, as a result of urea, and the flash setting by means of calcium nitrate leads to a weak early strength with less chance of bacterial strength recovery due to its high density. Due to this high density, the bacterial cells face harsh survival conditions, leading to a low active cell count at later ages. Furthermore, the lack of nourishment, in the form of yeast extract, for the bacterial decreases the chance of survival. In both T4 and T5 yeast extract further decreased the compressive strength by increasing the porosity. The later composition further increases the porosity due to the addition of calcium nitrate, the mechanism for this has been explained above but requires future research to be confirmed. Lastly, a decreased compressive strength was also found for T6. Due to the strong ammonium smell that was perceived when mixing this composition, the explanation, as found above, is that the ammonium ions deteriorate the cementitious matrix [12].

It is important to note that the compressive strength after 28 days when incorporating bacterial cells was found to be the same as when just the bioagents were added.

Water absorption - Urea has little effect on water absorption, no difference in water absorption was observed. The author thinks that because of the aforementioned lack of hydrolysis, residual urea dissolves in the water that penetrates the mortar pores. This reduces that volume of water that can be absorbed even though the pores are more plentiful.

A strong correlation between the amount of yeast extract added, and the increase in water absorption was found. The same aforementioned porosity increasing mechanisms are responsible for this. Peculiar was the “grouped” results that were found, where Y0.85, Y0.85 and Y1.28 showed similar results.

Only calcium nitrate showed a decreased water absorption, however, this effect was not correlated with increasing w/w%, a positive effect was found when adding 2 w/w%, but, when increasing the dosage, limited further positive effects were found. It is the author’s opinion that during the formation of low-density C-S-H, which means before the end of the second hydration peak, some amount of calcium hydroxide will form a mixture with the low-density C-S-H gel which is a calcium silicate hydrate called jennite with the chemical formula $Ca_9Si_6O_{18}(OH)_6.8H_2O$ instead of 1.4 nm tobermorite $Ca_5Si_6O_{16}$ [13]. This would give reason to believe this can explain the decreased water absorption.

The MIX composition again showed a higher water absorption than the superposition of all its bioagents. The reason for this follows the same explanation where the combination of flash setting and air entrapment from calcium nitrate and yeast extract respectively increase the porosity.

Freeze-thaw resistance – In this test, no damage was found. However, rather similar results were found as found for water absorption. The difference with the results found in this test was that all weight loss, and therefore all evaporated water, is water that was present in the cementitious matrix and did not yet partake in hydration. Therefore, the weight loss was higher for urea compared to yeast extract and calcium nitrate.

Carbonation - The carbonation depth decreased with increasing w/w% of urea, increased with the time it is exposed to CO_2 and resulted in higher carbonation depths compared to reference when the dosage was lower than 2 w/w%. Furthermore, a very small carbonation depth was observed after being exposed for 7 days after which the carbonation depth noticeably increased. A mechanism that increases the calcium carbonate content while decreasing the calcium hydroxide content which increases the durability of the mortar by acting as a filler to decrease porosity was found [14].

In line with the results for yeast extract found above, the same “grouped” results were noticeable. The carbonation depth increased greatly with increasing w/w% of yeast extract, and this for all ages of exposure. Additionally, it should be noted that the magnitude of these results differed by a factor of 15 to even 35 compared to those of urea and calcium nitrate where the carbonation depth remained far under 1 mm.

The results for calcium nitrate, where an increase in carbonation depth as a result of addition of calcium nitrate was found could not be explained. However, it was found that the carbonation depth decreased with increasing w/w%.

Lastly, the carbonation depth of MIX was lower than that of Y0.85. This is the only characteristic in this work where yeast extract was not the dominant bioagent.

Cement hydration - Fresh mortar with urea had a delayed setting and increased flow-ratio, a delay of 0.21, 0.89, 1.86, 2.30, 3.35 and 4.44 hours was found for U1, U2, U3, U4 and U5 respectively. Additionally, the peak hydration heat was found to be 3.34, 3.13, 2.96, 2.92, 2.65 and 2.57 W/g for increasing w/w% of urea. The reason for this decrease in temperature is the endothermic nature of the reaction when dissolving urea in water.

A delay in setting and decrease in cumulative heat was observed when adding yeast extract. The other clear observation that could be made was the large amount of heat generated by Y2.56 in its first stage, this mechanism has been explained as a result of the fact that sugar has an accelerating effect when used in high dosages above 0.06 w/w%. Additionally, the total amount of cumulative heat of Y2.56 after this burst in its first few hours accounted for 50% of the total heat. This explains the lack of further strength development at high yeast extract dosages.

Calcium is a known setting accelerator. This was confirmed with the results found in this work where the peak hydration heat of Ca8 and Ca10 was far greater, and occurred earlier than those of Ca6, Ca4 and Ca2 and REF. A strong initial burst in cumulative heat for Ca8 and Ca10 was found which is the result of the flash setting mechanism which is discussed above.

Lastly, it was found that the hydration curve of MIX was similar to that of REF, however, the first hydration peak, which explains most of the early mechanisms, was not able to be visualised in this work.

$CaCO_3$ content - As can be seen in the results, not all weight loss was a result of the reaction of hydrochloric acid with calcium carbonate, most of the weight loss was a result of the acid reacting with other hydration products as could be seen by the high value for REF. The components of the cement paste are broken down during contact with the acid while aggregates stay intact [15]. Additionally, the microstructure changes which results in fewer products that react with HCl [16]. Lastly, calcium carbonate itself acts as both an inert filler and as an active participant in the hydration process, for which the ratio can be calculated [17]. For T1 the suspended bacterial cells were added to the composition which resulted in an increase of weight loss. Since there are no bioagents for the bacterial cells to react with this cannot be explained by bacterial activity with regards to bioagents. Therefore, there a reaction seems to occurs between the bacterial cells and the cement particles. This should be investigated further in future research by investigation of the microstructure. For T2 a lower amount of weight loss was observed. This goes against what one may think due to the addition of urea that is an essential agent for ureolytic activity. However, the density did not increase which meant that a harsher environment for the bacteria was not a valid explanation for this. However, in previous research, it has been proven that above a certain concentration, more urea does not result in more precipitation. This is due to the fact that the bacterial cells denature [18]. The results in this work state that not only does it not benefit ureolytic activity, it even obstructs the active activity, resulting in a lower amount of precipitated calcium carbonate. This statement, however, needs to be evaluated in future research. For T3 an increase in weight loss was found, and the results were similar to those of T1. As can be seen, the weight loss happened slower, this can be explained as a result of the bacterial surfaces being bound with calcium ions, creating a barrier which lowers the urea diffusion rate and thus slows down the precipitation rate [19]. It seemed that this slow precipitation was the reason that more weight loss was found compared to T2 and REF, however, future research should focus on the microscopic mechanism of these interactions. The addition of yeast extract for T4 resulted in a similar weight loss found for T3 as well as T1. The fact that no higher values were found can be explained by the fact that there is no source of calcium ions to form calcium carbonate, and therefore is the limiting reagent. This was also verified in the results for T5.

For T5, initially, a lower value of weight loss was recorded. However, at later ages, a higher value was found. This is a result of the aforementioned mechanism where calcium ions create a barrier with slows down ureolytic activity. However, unlike T4, calcium nitrate was no longer a limiting agent, which lead to the precipitation to continue and resulted in the higher weight loss that was found. When adding the grown culture, results equal to T5 were found. This is peculiar since no calcium nitrate was added to precipitate this high amount of calcium carbonate. However, the cells were added in a spent UYE medium, which, for some reason that needs to be investigated further, spread the bacterial cells less uniform in the cementitious matrix. This would explain the high standard deviation for T6 since more concentrated spots of $CaCO_3$ would get formed. When considering this high deviation, one can notice that the results were similar to those of T1 where the same number of cells are added.

Overall it can be stated that the addition of bioagents is necessary to increase the amount of precipitated $CaCO_3$. Lastly, it should be noted that overall, for all results, a high standard deviation was found.

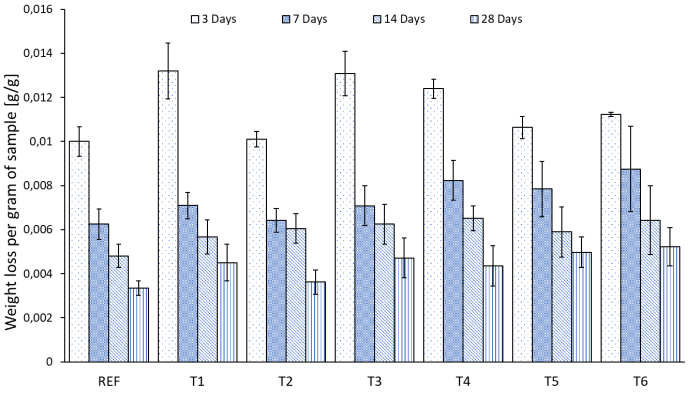

Figure 2. Weight loss per gram of sample for bacterial mortar at 3, 7, 14 and 28 days

IV. CONCLUSION

Urea is the most crucial bioagent since without ureolysis no bacterial calcium carbonate can be precipitated. In this work, it is found that urea is the most inert bioagent out of the three bioagents researched. Up to a dosage of 5 w/w%, no negative effects were observed. The effects of urea on the hydration of cement are observed where an endothermic reaction was proven. This promotes the use of urea in massive constructions. This also explained the optimal dosage of 2 w/w% that was found, the reason for this should be investigated further but is believed to be due to the endothermic balance found in this work.

Not only is it shown in this work that yeast extract has a negative effect almost all properties of both fresh and hardened mortar, but it also is responsible for 10 and up to 60 % of total operating costs of the UYE medium it is used in [20]. Therefore, it’s important to not only replace yeast extract with a cheaper, but also a more inert, and even positive, additive with similar characteristics for the bacterial cells. Recently corn steep liquor (CSL) has been proposed, which is an industrial by-product. A similar test should be executed as shown in this work to compare the effect of both these bioagents. Due to its negative effects, yeast extract should not be used. However, if required, the dosage should stay under 0.34 w/w% to ensure these negative effects remain limited. The main priority should be put on finding a viable alternative for yeast extract in future research. Lastly, the mechanism proposed in this work should be verified by specific research in future works. Although these mechanisms explain all of the changes in properties yeast extract bring forth, no proof can be given.

Calcium nitrate acted as an inert and mostly positive additive in the cementitious matrix. Due to the decrease in porosity it brought forth, the overall durability was increased by calcium nitrate. However, one should be careful when increasing the dosage of calcium nitrate, as shown in this work flash setting occurs on high dosages, which negatively influences the fresh properties of mortar. To prevent this its dosage should not exceed 8 w/w%. While most of the tests showed a positive result as a result of the addition of calcium nitrate, a worrisome result mechanism was discovered when combining yeast extract and calcium nitrate. The negative effects due to the air entrapping characteristic of yeast extract seem to get amplified with the addition of calcium nitrate. The previously researched and proposed optimal dosage of bioagents in combination with bacterial cells was confirmed in this work, where the largest $CaCO_3$ content was found. However, this is at the expensive of many of the characteristics as a result of the added yeast extract. As mentioned above yeast extract should be replaced or isolated from the cement particles to negate its negative influences.

Furthermore, a new testing method to test for $CaCO_3$ content was proposed. In this work similar results were found with literature, where more expensive, time-consuming and smaller sample size testing methods were used. It is the author’s opinion that the sample size should be increased further to obtain even more precise results. Due to the good results found in this work, it is strongly recommended to compare this testing method with the currently commonly used methods in future research.

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Invloed van biologische agentia op de cementgebonden matrix

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Abstract – **Het voorkomen van scheuren in beton is een van de belangrijkste factoren die de duurzaamheid van beton beperkt. Deze scheuren brengen dure herstellings- en onderhoudskosten met zich mee en verkorten de ontwerpsleeftijd. Hieruit volgt een verhoogde vraag naar beton. De betonindustrie is momenteel de tweede grootste producent van koolstofdioxide en is verantwoordelijk voor 5% van de globale uitstoot. Recente vorderingen zijn gemaakt om de duurzaamheid van beton te verhogen met behulp van microbiële geïnduceerde calciumcarbonaat neerslag die het inbrengen van bacteriële cellen, ureum, gistextract en calciumnitraat in de betonmix vereist. Ondanks dat het gebruik van bio-beton bewezen is, blijven de invloeden van de biologische agentia op de cementgebonden matrix slecht verstaan en onder gedocumenteerd.**

De invloed van drie additieven; ureum, gistextract en calciumnitraat op de cementgebonden matrix werden getest door het uitvoeren van 7 test op 16 samenstellingen, zowel op verse als verharde mortel. Verder werd de invloed van deze biologische agentia samen met bacteriële cellen op hun hoeveelheid geproduceerde $CaCO_3$ inhoud getest met 6 samenstellingen.

Er werd gevonden dat zowel ureum en calcium nitraat zich gedragen als een inerte vuller met positieve effecten op enkele eigenschappen en zo ook de duurzaamheid. Verder werd ook de hoeveelheid geproduceerde calciumcarbonaat vergroot wanneer bacteriële cellen toegevoegd werden bij deze twee biologische agentia. Daarentegen beïnvloedde gistextract de cementgebonden matrix negatief, en dit voor bijna alle geteste eigenschappen. Hierdoor verminderde de duurzaamheid van de cementgebonden matrix. Ondanks het feit dat gistextract de geproduceerde hoeveelheid calciumcarbonaat positieve beïnvloedde, dient het beperkt te worden, of met voorkeur vervangen te worden, om de negatieve effecten die gepaard gaan met de toevoegingen ervan te beperken.

Trefwoorden **- bio-beton, ureum, gistextract, calciumnitraat, *Bacillus sphaericus*, mortel eigenschappen, $CaCO_3$**

I. INLEIDING

De implementatie van bacteriën om zelfhelend beton te verkrijgen is momenteel een hot topic. De heling komt voorts uit het introduceren van calciumcarbonaat producerende bacteriën met verschillende biologische agentia. Momenteel legt veel onderzoek de nadruk op het maximaliseren van de hoeveelheid geproduceerde calciumcarbonaat, dit om het zelfhelende effect te optimaliseren. Dit wordt gedaan door het onderzoeken van verschillende bacteriën en de dragers in welke ze geïntroduceerd kunne worden in de betonmix [1]. Een andere gebruikte aanpak is het evalueren van het potentieel van bio-beton door het evalueren van de helende eigenschappen en zo een optimale samenstelling van bacteriën en biologische agentia te bekomen [2]. Hierdoor bleven echter de invloeden van deze biologische agentia onder gedocumenteerd en onvolledig verklaard.

Het doel van dit werk was een verstaanbaar en toegankelijk document te bekomen dat de voornaamste mechanismen van drie vaak gebruikte biologische agentia verklaard. Verder werd ook de invloed van bacteriële cellen op de hoeveelheid geproduceerde calciumcarbonaat in de matrix onderzocht. Naast kwantificeren werd er ook voor gezorgd dat elk mechanisme een plausibele verklaring kreeg. Op deze manier kan verder onderzoek staat terugvallen op dit werk om de belangrijkste biologische agentia voor bepaalde eigenschappen te identificeren, wanneer deze gebruikt worden in een betonsamenstelling.

In dit werk werden 8 testen uitgevoerd om inzicht te bekomen in de basiseigenschappen van zowel verse als verharde mortelsamenstellingen. De samenstellingen werden steeds gemaakt met 5 verschillende doseringen voor elk van de agentia als ook één samenstelling met alle agentia met hun meest gebruikte concentratie. Bovendien werden ook 6 samenstellingen getest waarin, naast biologische agentia, ook bacteriële cellen toegevoegd werden.

II. MATERIALEN EN METHODEN

Alle mortelsamenstellingen werden geproduceerd conform de norm NBN B15-001 (2004) en opgeslagen in een vochtige kamer met een temperatuur van 20 ± 2°C en een relatieve vochtigheid van >90% totdat de gewenste leeftijd werd bereikt. De 17 composities werden gecodeerd met de letters U, Y en Ca voor ureum, gistextract en calcium nitraat respectievelijk, gevolgd door een nummer dat de procentuele hoeveelheid agentia per gewicht van cement weergeeft. Voorts werd de naam MIX gegeven aan een mortelsamenstelling met U4, Y0.85 en Ca8. Alsook 7 samenstellingen, welke bestonden uit een referentie,10^9 cellen/mL, 10^9 cellen/mL + U4, 10^9 cellen/mL + U4 + Ca8, 10^9 cellen/mL + U4 + Y0.85, 10^9 cellen/mL + U4 + Y0.85 + Ca8 en een samenstelling waarin cellen in een ureum-gistextract medium toevoegt werd met een concentratie van 10^8 cellen/mL werden aangemaakt. Deze samenstellingen werden respectievelijk REF, T1, T2, T3, T4, T5 en T6 gecodeerd. Steeds werd een water/cement-factor van 0.5 aangehouden.

Ureum - (CH_4N_2O) van de producent Carl Roth werd gebruikt. Deze kwam in de vorm van witte parels die oplossen in water.

Gistextract – Een gedroogd autolysaat van de producent Carl Roth werd gebruikt. Deze is zeer oplosbaar met een oplosbaarheid van 410 g/L.

Vloei	Binding	Mechanische eigenschappen*	Water absorptie	Vorst-dooi weerstand	Carbonatatie	Cement hydratatie
		3*, 7*, 14*, 28* ,56d	28+7,14,28d	28d	28+14,28,56d	

Tabel 1. Samenvatting van de uitgevoerde testen

Calciumnitraat- Het calcium nitraat werd geleverd als een tetrahydraat van producent Carl Roth. Het feit dat deze geleverd werd tetrahydraat is belangrijk omdat elke molecule omringd is door vier watermoleculen. Dit extra water dient in rekening gebracht te worden wanneer men de water-cement factor bepaald. De chemische formule is $Ca(NO_3)_2 . 4H_2O$.

Micro-organisme - *Bacillus sphaericus* LMG 22257 werd gebruikt in dit werk. De bacteriën warden gekweekt in een medium bestaande uit ureum, 22 g/L, en gistextract, 20 g/L, ook wel een UYE-medium genoemd. De cellen werden geoogst na het centrifugeren aan 7000 tpm gedurende 7 minuten in een Sorvall RC 6+, Thermo Scientific^{TM}. Nadat het overschot aan UYE-medium afgegoten was werden de cellen opnieuw gesuspendeerd in een steriele zoutachtige oplossing (8.5 g/L NaCl). De finale concentratie van de bacteriën in de suspensie was ongeveer 10^9 cellen/mL terwijl dat van de cellen in UYE-medium ongeveer 10^8 cellen/mL was. Deze soluties werden in een koelkast op 4°C bewaard. Voor gebruik liet men de bacteriële soluties opwarmen tot kamer-temperatuur voor deze te gebruiken.

Tabel 1 geeft een samenvatting van alle testen die uitgevoerd werden in dit werk. Een asterisk (*) geeft aan dat de test ook uitgevoerd werd op bacteriële mortel. Alsook, werd de hoeveelheid calciumcarbonaat van deze bacteriële mortel getest na 3, 7, 14 and 28 dagen.

Vloeibaarheid – De vloeibaarheid van verse mortel werd getest met behulp van een schoktafel. De testmethode is beschreven in NBN-EN-1015-3 (1999) en bekomt als resultaat een percentage dat de diameter na schokken weergeeft ten opzichte van de referentie na schokken.

Binding – De binding van mortel werd getest met behulp van de Vicatronic E044N van Matest®, dit is een automatische Vicat machine die de Vicat test uitvoert, conform NBN EN 196-3 en NBN EN 480-2. Initiële en finale binding werden onderzocht, dit is respectievelijk het moment dat de naald de mortel niet meer volledig penetreert, en wanneer de naald de mortel niet meer kan penetreren.

Mechanische eigenschappen – Na het bepalen van de densiteit van de prisma's werd gebruikgemaakt van een Walter+Bai DB 250/15 machine om de buigsterkte te bepalen. De twee halve stukken prisma die hieruit volgden werden dan onderworpen aan een druksterktetest. De testen gebeurden conform EN 196-1.

Water absorptie – De water absorptie werd bepaald conform NBN B 15-215 op 40x40x160 mm prisma's welke volledig ondergedompeld werden in water met een temperatuur van 20 ± 1°C. De gewichtstoename werd gemeten en de percentuele toename van gewicht ten opzichte van het drooggewicht werd bepaald.

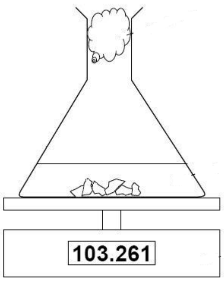
Vorst-dooi weerstand – De vorst-dooi weerstand werd bepaald conform CEN/TS 12390-9. De mortel prisma's werden verzaagd in vierkante blokken met afmetingen van 40x40x40 mm en onderworpen aan 28 vorst-dooi cycli waarin de temperatuur tussen -17.50 en 22.50°C schommelde. De machine die hiervoor gebruikt werd was een WT3 – 1000/40 van Weiss Technik.

Carbonatatie – De carbonatatiediepte werd onderzocht conform ISO 1920-12:2015 en dit na 14, 28 en 56 dagen waarin de prisma's onderworpen werden aan 1% CO_2 in een carbonatatiekast. De gebruikte kast was een WEKK 0130 van Weiss Technik. Elke prisma werd tweemaal bedekt een epoxyhars (SikaCor 277), waartussen steeds 24 uren gelaten werd zodat de laag volledig kon drogen en luchtdichtheid kon garanderen. Door één zijde niet te bedekken werd een eenzijdige indringing van CO_2 gegarandeerd. Naderhand werden de prisma's in drie gelijke delen gesplitst en bespoten met fenolftaleine.

Cement hydratatie – De cement hydratatie werd gemeten door middel van calorimetrie. In dit werk werd een TAM-air machine gebruikt. Deze machine meet de genoodzaakte energie om een monster op een constante temperatuur te houden. Deze energie werd gemeten in mV, welke dat na conversie omgezet werd naar W. Naderhand werden de bekomen resultaten geïntegreerd om de totale geaccumuleerde warmte te bepalen in J/g.

$CaCO_3$ inhoud – Ondanks het feit dat er veel methoden bestaan om de aanwezigheid van $CaCO_3$ te testen, blijken deze vaak; te complex, duur of tijdrovend. De meest gebruikte technieken zijn; X-Ray diffractie (TOPAS-methode) (XRD), thermogravimetrische analyse (TGA), ASTM, ICP-AES, titratie en wassen. De resultaten van deze methoden schommelen sterk, waarin XRD, TGA en ASTM de meest betrouwbare en gemiddelde waarden geven [3]. Het grootste probleem van deze technieken is de kleine monstermassa die getest kan worden, dit resulteert in sterk variërende resultaten, afhankelijk van waar het monster genomen is. De voorgestelde testmethode is gebaseerd op de reactie van calciumcarbonaat met zoutzuur.

De wet van behoud van massa stelt dat de massa van reactanten gelijk is aan deze van de producten. Echter, aangezien er een gas gevormd wordt, zal er een gewichts-afname vastgesteld worden. Aan de hand van dit massaverlies kan de hoeveelheid calciumcarbonaat bepaald worden. Drie gewichten, M1 en M2 worden genoteerd. Deze zijn respectievelijk het gewicht van het monster plus glaswerk met HCl voor en na reactie. M3 is het verschil van deze twee massa's, en stelt zodus de hoeveelheid produceerde CO_2 voor. De prisma's werden eerst getest op buigsterkte en nadien op druksterkte. Uit de gebroken prisma's werden drie monsters van ± 5 g genomen. Om calciumcarbonnat ten gevolge van carbonatatie te beperken werden deze uit het midden van de prisma's genomen. Deze monsters werden dan mechanisch verbrijzeld en vermalen tot een fijn poeder. Van elk monster werd ± 2 g afgewogen en ± 20 mL van een 0.1 M HCl oplossingen werd afgewogen met behulp van een pipet. Na het noteren van de massa's werden de twee gedurende 10 s gemengd. Na 50 seconden, en dus totaal 1 minuut na mengen, werd het finale gewicht bepaald. Zoutzuur werd in zulke mate toegevoegd dat steeds al het calciumcarbonaat kon reageren.



Figuur 1. Testopstelling meten van CaCO3

III. RESULTATEN EN DISCUSSIE

Vloeibaarheid – De toevoeging van ureum zorgde voor een toename in vloeibaarheid van 3, 19, 28, 34 en 41% ten opzichte van REF voor U1, U2, U3, U4 en U5 respectievelijk. Dit komt door de toename van het volume van de oplossing door het feit dat ureum niet hydrolyseert in water. [4].

Gistextract bracht een toename van 23% teweeg bij toevoeging van 0.34 w/w% aan de mortelsamenstelling. Deze toename verliep lineair met een toename van 29, 33 en 39% respectievelijk voor Y0.85, Y0.128 en Y1.27. De hoogste dosis, Y2.56, zorgde voor een toename van 55%. Drie mogelijke verklaringen worden voorgelegd om deze effecten te verklaren. Ten eerste is het hoge oplosbare koolhydraatgehalte, beter bekend als suiker, een gekend vertragingsmiddel [5, 6]. De belangrijkste reden hiervoor is dat de interactie van suiker met C_3A de vorming van kubische C_3AH_6 (Tricalciumaluminaathydraat) voorkomt door het oppervlak van C_3A op de cementkorrels te bedekken. Deze agressieve reactie van water met C_3A is ook bekend als “flash setting” met de productie van veel warmte als gevolg. Flash setting start zodra de mortel wordt gemengd. Ten tweede produceert gistautolysaat stikstofverbindingen gedurende zijn autolyse [7]. Deze vorming van stikstofgas gelijkt sterk op de productie van schuimbeton, ook bekend als lichtbeton. De poriën hiervan in de gistextract-samenstellingen waren onderling verbonden, wat visueel geverifieerd werd na destructieve compressietesten. Ten slotte is een tweede mogelijke oorzaak van deze luchtbellen de afbraak van eiwitten, dit door hydrolyse bij één of meer van zijn peptide-verbindingen [8].

Calcium nitraat verlaagde de vloeibaarheid in een lineaire correlatie met de hoeveelheid w/w% calciumnitraat toegevoegd. Bij een dosering van 8 w/w% daalde de vloeibaarheid snel tot een punt waar geen verandering in de diameter meer werd waargenomen. Dit door het optreden van flash setting. De vloeibaarheid was 0.93, 0.90, 0.78, 0.18 and 0.09% vergeleken met referentie voor Ca2, Ca4, Ca6, Ca8 en Ca10 respectievelijk

Tenslotte werd gevonden dat de MIX-samenstelling een toename van 33% in vloeibaarheid vertoonde. Dit is een gevolg van het gecombineerd effect van U4 (133%) en Y0.85 (129%). Bijzonder is dat de flash setting van Ca8 (18%) geen effect heeft op de vloeibaarheid van de MIX-samenstelling.

Binding – Referentiemortel startte initiële binding na 180 minuten en finale binding vond plaats na 270 minuten. De initiële en finale binding door toevoeging van ureum vertoonde beide een sterkte lineaire correlatie met een toename van het gewichtspercentage, waarbij een verhoging van ureum leidde tot een toename in zowel de initiële als finale binding. Initiële/finale bindingstijden waren 240/285, 255/330, 270/360, 285/390 and 315/400 minuten voor U1, U2, U3, U4 en U5 respectievelijk. Het mechanisme hiervoor is tweevoudig; ten eerste worden de interne covalente binding niet gesplitst in ionen in water. Hierdoor wordt het volume van de oplossing verhoogt wat de binding vertraagt [9, 10]. Ten tweede zorgt de endotherme reactie van het oplossen van ureum in water ervoor dat de hydratatiewarmte lager wordt met betrekking tot de toegevoegde hoeveelheid ureum, wat de initiële en finale binding vertraagt.

Gistextract werkte als een vertrager, waarbij zowel de initiële als de finale bindingstijden werden vertraagd met toenemende w/w% gistextract. Wanneer 2,56 w/w% gistextract werd toegevoegd, werd een afname van de begin- en finale bindingstijd gevonden. Daarom kan worden geconcludeerd dat gistextract zowel een versneller als een vertrager kan zijn, afhankelijk van de toegevoegde hoeveelheid. De initiële/finale binding bij stijgende w/w% gistextract was 300/420, 480/660, 600/840, 720/930 en 150/180 minuten. Gistextract fungeerde alleen als vertrager tot een dosering, die nog nauwkeuriger moet worden bepaald, tussen 1,7 en 2,56 w/w%. Bij hogere doseringen dan de bovengenoemde limiet fungeerde het als een versneller. Wanneer grote hoeveelheden suiker aan de mortelmengeling wordt toegevoegd, reageert deze vrij snel met gips, deze reactie leidt ertoe dat gips zijn effect verliest dat flash setting verhinderd, en zorgt ervoor dat de mortel snel bindt. De resterende C_3A hydrateert echter niet in de eerste dagen, waardoor een gebrek aan sterkteontwikkeling voor Y2.56 plaatsvindt.

Toevoeging van calciumnitraat resulteerde in een versnelling van de bindingstijd. Deze versnelling was lineair met de w/w% calciumnitraat die werd toegevoegd. Initiële/finale bindingstijden van 150/195, 135/180, 135/165, 75/120 en 60/105 minuten werden gevonden. Wanneer calciumnitraat oplost in water, worden de calciumionen Ca^{2+} gescheiden van de nitraationen NO_3-. Dit verhoogt de concentratie van calciumionen en leidt tot een snellere superverzadiging van het water met betrekking tot calciumhydroxide. Bovendien is de vorming van calciumaluminaathydraat (C_4AH_x) waargenomen door XRD-metingen [11]. Deze reactie verloopt zo snel, dat wanneer deze optreedt, deze zulke grote hoeveelheid warmte afgeeft waardoor de mortel binnen enkele minuten bindt, dit fenomeen wordt flash setting genoemd.

Wederom toonde de mixsamenstelling vergelijkbare resultaten met die van gistextract, maar niet van ureum. Een initiële bindingstijd van 450 en een finale bindingstijd van 570 minuten werd gevonden, deze kwam sterk overeen met het resultaat van Y0.85.

Mechanische eigenschappen – (i) Biologische agentia – Ureum toonde een positieve invloed op de druksterkte. Ondanks het feit dat U1, U2 en U3 een grotere druksterkte vertoonde dan de referentie, bereikten U4 en U5 slechts gelijke druksterkte als referentie na 56 dagen. De maximale druksterkte werkt bereikt bij een dosering van 2 w/w% ureum na 56, dagen, waar een druksterkte van 71 MPa, in vergelijking met 64 MPa voor referentie werd gevonden. Het feit dat U1 en U2 een hogere buigsterkte hadden tussen 14 en 28 dagen, wordt verklaard door de lage impact van de endothermische koeling die plaatsvond.

Gistextract verlaagde de druksterkte op alle leeftijden met toenemende w/w%. En, parallel aan de buigsterkte, vertoonde Y2.56 een zeer lage sterkte na 3 dagen. Na 56 dagen werden 94, 51, 48, 47 en 30% van de referentiesterkte gevonden voor respectievelijk Y0.34, Y0.85, Y1.28, Y1.7 en Y2.56. De lage druksterkte van Y2.56 na 3 dagen is opnieuw het resultaat van het versnellende effect op de binding door de hoge dosering van suiker in gistextract.

Ten slotte vertoonden de resultaten voor calciumnitraat een hogere toename in vroege sterkte voor Ca2, Ca4 en Ca6 in vergelijking met Ca8 en Ca10 terwijl, voor latere leeftijden, het tegenovergestelde waar was. In het algemeen kan worden gesteld dat calciumnitraat de druksterkte verbetert na 28 dagen en dit me toenemende w/w%. De toename in compressie die werd gevonden, is het resultaat van de dichtere structuur die werd gevormd door de toevoeging van calciumnitraat.

De MIX-samenstelling vertoonde vergelijkbare resultaten als Y0,85, maar de sterkte nam niet toe zoals men zou verwachten bij het middelen van de effecten van alle biologische agentia. Dit versterkt opnieuw het argument van de auteur waar gistextract werkt als een luchtinsluitingsmiddel en calciumnitraat flash setting veroorzaakt, waardoor de porositeit zo hoog blijft dat het alle kenmerken van de gistextractcomponent erft.

(ii) Bacteriën – Voor T1 had de toevoeging van bacteriën geen effect op de druksterkte, één verklaring, waar dode bacteriën fungeren als een organische vezel, had echter enkel een effect op de buigsterkte. Voor T2 echter, wanneer ureum naast bacteriën werd toegevoegd, vindt ureolyse plaats. Het is dit mechanisme dat op jonge leeftijd leidt tot afname van de druksterkte. Op latere leeftijd nam de kracht echter sneller toe in vergelijking met referentie, wat leidde tot een negatief effect. In T3, waar naast bacteriële cellen ureum en calciumnitraat werden toegevoegd, nam de kracht op jonge leeftijd nog verder af. Een combinatie van een hoog oplossingsvolume, als gevolg van ureum, en de flash setting door middel van calciumnitraat leidde tot een zwakke vroege sterkte met minder kans op bacteriële krachtterugwinning vanwege de hoge dichtheid. Vanwege deze hoge dichtheid krijgen de bacteriecellen te maken met moeilijkere overlevingscondities, wat op latere leeftijd tot een laag aantal levende cellen in de prisma’s leidt. Bovendien vermindert het gebrek aan voeding, in de vorm van gistextract, de overlevingskans van de bacterie. Voor zowel T4 als T5 verminderde gistextract de druksterkte verder door de porositeit te verhogen. De latere samenstelling verhoogde de porositeit door de toevoeging van calciumnitraat. Het mechanisme hiervoor is hierboven uitgelegd maar dient door toekomstig onderzoek te worden bevestigd. Ten slotte werd ook een verminderde druksterkte gevonden voor T6. Vanwege de sterke ammoniumgeur die werd waargenomen bij het mengen van deze samenstelling, is de verklaring, zoals hierboven weergegeven, dat de ammoniumionen de cementgebonden matrix aantasten [12].

Het is belangrijk op te merken dat de compressiesterkte na 28 dagen bij het toevoegen van bacteriële cellen even groot bleek te zijn als wanneer alleen de biologische agentia werden toegevoegd.

Water absorptie - Ureum had geen effect op de water absorptie. De auteur denkt dat vanwege het eerdergenoemde gebrek aan hydrolyse resterend ureum in oplossing gaat met het water dat de mortelporiën binnendringt. Dit vermindert het volume water dat kan worden opgenomen, ook al zijn er meer poriën.

Er werd een sterke correlatie gevonden tussen de toegevoegde hoeveelheid gistextract en de toename in absorptie van water. Dezelfde voorgaande genoemde porositeit verhogende mechanismen zijn hiervoor verantwoordelijk. Eigenaardig was de "gegroepeerde" resultaten die werden gevonden, waarbij Y0.85, Y0.85 en Y1.28 vergelijkbare resultaten hadden.

Alleen calciumnitraat vertoonde een verminderde water absorptie, dit effect was echter niet gecorreleerd met toenemende w/w%. Een positief effect werd waargenomen bij toevoeging van 2 w/w%, maar bij het verhogen van de dosering werden beperkte verdere positieve effecten waargenomen. Het is de mening van de auteur dat tijdens de vorming van CSH met lage dichtheid, wat betekent vóór het einde van de tweede hydratatiepiek, een hoeveelheid calciumhydroxide een mengsel zal vormen met de CSH-gel met lage dichtheid, een calciumsilicaathydraat genaamd jennite met de chemische formule $Ca_9Si_6O_{18}(OH)_6.8H_2O$ wordt gevormd, in plaats van 1,4 nm tobermorite $Ca_5Si_6O_{16}$ [13]. Deze formatie geeft reden om aan te nemen dat dit de verminderde wateropname verklaart.

De MIX-samenstelling vertoonde wederom een hogere water absorptie dan de superpositie van alle biologische agentia. De reden hiervoor volgt dezelfde verklaring waarbij de combinatie van flash setting en luchtinsluiting ten gevolge van calciumnitraat en gistextract respectievelijk de porositeit verhoogt.

Vorst-dooi weerstand – In deze test werd geen schade gevonden, echter werden er soortgelijke resultaten gevonden zoals besproken voor de water absorptie. Het verschil met de resultaten gevonden in deze test was dat al het gewichtsverlies afkomstig was van het water dat reeds aanwezig was in de prisma’s en nog niet had deelgenomen aan hydratatie. Daarom was het gewichtsverlies hoger voor ureum in vergelijking met gistextract en calciumnitraat.

Carbonatatie - De carbonatatiediepte nam af met toenemende w/w% ureum, nam toe met de tijd dat het werd blootgesteld aan CO_2 en resulteerde in hogere carbonatatiediepten vergeleken met referentie wanneer de dosering lager was dan 2 w/w%. Verder werd een zeer kleine carbonatiediepte waargenomen na 7 dagen te zijn blootgesteld, waarna de carbonatatiediepte merkbaar toenam. Een mechanisme dat het calciumcarbonaatgehalte verhoogt en zodus het calciumhydroxidegehalte wordt verlaagd, waardoor de duurzaamheid van de mortel wordt verhoogd, werd gevonden [14]. De duurzaamheid verbeterd doordat $CaCO_3$ als vulmiddel werkt om de porositeit te verminderen

In overeenstemming met de hierboven gevonden resultaten voor gistextract, waren dezelfde "gegroepeerde" resultaten merkbaar. De carbonatiediepte nam sterk toe met een toenemend gewichtspercentage gistextract, en dit voor alle blootstellingsleeftijden. Bovendien moet worden opgemerkt dat de grootte van deze resultaten verschilde met een factor 15 tot zelfs 35 in vergelijking met die van ureum en calciumnitraat, waarbij de carbonatiediepte ver onder 1 mm bleef.

De resultaten voor calciumnitraat, waar een toename in carbonatatiediepte als gevolg van toevoeging van calciumnitraat werd gevonden, konden niet worden verklaard. Er werd echter wel gevonden dat de carbonatiediepte afnam met toenemende w/w%.

Ten slotte was de carbonatatiediepte van MIX lager dan die van Y0.85. Dit is het enige kenmerk in dit werk waarbij gistextract niet het bepalende biologische agens was.

Cement hydratatie - Verse mortel met ureum had een vertraagde uitharding en verhoogde vloei-verhouding. Een vertraging van 0,21, 0,89, 1,86, 2,30, 3,35 en 4,44 uur werd gevonden voor respectievelijk U1, U2, U3, U4 en U5. Bovendien bleek de piek hydratatiewarmte 3,34, 3,13, 2,96, 2,92, 2,65 en 2,57 W/g te zijn bij het verhogen van de w/w% van ureum. De reden voor deze temperatuurdaling is de endotherme aard van de reactie bij het oplossen van ureum in water.

Een vertraging in binding en verlaging van de cumulatieve warmte werd waargenomen bij het toevoegen van gistextract. De andere duidelijke waarneming die gemaakt kon worden was de grote hoeveelheid warmte die gegenereerd werd door Y2.56 in zijn eerste fase, dit mechanisme wordt verklaard als gevolg van het feit dat suiker een versnellend effect heeft bij gebruik in doseringen boven 0,06 w/w%. Bovendien was de totale hoeveelheid cumulatieve warmte van Y2.56 na deze vlaag in de eerste paar uren goed voor 50% van de totale warmte. Dit verklaart het gebrek aan verdere sterkte-ontwikkeling bij van hoge doseringen gistextract.

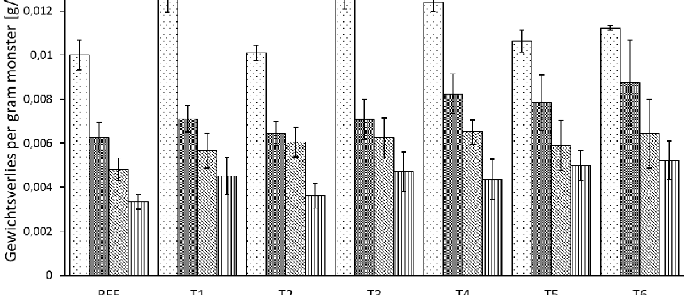
Calciumnitraat is een bekende bindingsversneller. Dit werd bevestigd met de resultaten gevonden in dit werk, waar de piek van de hydratatiewarmte voor Ca8 en Ca10 veel groter was, en eerder optrad dan die voor Ca6, Ca4 en Ca2 en REF. Een sterke initiële vlaag in cumulatieve warmte voor Ca8 en Ca10 werd gevonden, hetgeen het resultaat is van flash setting dat hierboven is besproken.

Ten slotte werd gevonden dat de hydratatiekromme van MIX vergelijkbaar was met die van REF, maar de eerste hydratatiepiek, die de meeste vroege mechanismen verklaart, kon in dit werk niet worden gevisualiseerd.

$CaCO_3$ inhoud - Zoals te zien is in de resultaten, was niet al het gewichtsverlies een gevolg van de reactie van zoutzuur met calciumcarbonaat. Het grootste deel van het gewichtsverlies was het gevolg van de reacties met andere hydratatieproducten, zoals te zien was aan de hoge waarde voor REF. De componenten van de cementpasta worden afgebroken tijdens contact hun met het zuur, terwijl aggregaten intact blijven [15]. Bovendien verandert de microstructuur, wat resulteert in minder producten die reageren met HCl [16]. Ten slotte werkt calciumcarbonaat zelf zowel als een inerte vulstof als een actieve deelnemer aan het hydratatieproces, waarvoor de verhouding kan worden berekend [17]. Voor T1 werden de gesuspendeerde bacteriecellen aan de samenstelling toegevoegd, wat resulteerde in een toename van gewichtsverlies. Aangezien er geen biologische agentia zijn waarmee de bacteriecellen kunnen reageren, kan dit niet worden verklaard door bacteriële activiteit met betrekking tot biologische agentia. Daarom lijkt het dat er een reactie plaatsvindt tussen de bacteriecellen en de cementdeeltjes. Dit moet verder worden onderzocht in toekomstig onderzoek door onderzoek van de microstructuur. Voor T2 werd een lagere hoeveelheid gewichtsverlies waargenomen. Dit druist in tegen wat men denkt als gevolg van de toevoeging van ureum, dat een essentieel agens is voor ureolytische activiteit. De dichtheid nam echter niet toe, wat betekende dat zwaardere omgevingsfactoren voor de bacteriën geen geldige verklaring hiervoor was. In eerder onderzoek is echter bewezen dat boven een bepaalde concentratie van ureum, een toename niet in meer neerslag resulteert. Dit komt door het feit dat de bacteriecellen denatureren [18]. De resultaten in dit werk geven aan dat het niet alleen de ureolytische activiteit niet ten goede komt, het belemmert zelfs de actieve activiteit, wat resulteert in een lagere hoeveelheid neergeslagen calciumcarbonaat. Deze verklaring moet echter worden geëvalueerd in toekomstig onderzoek. Voor T3 werd een toename van gewichtsverlies gevonden, alsook waren de resultaten vergelijkbaar met die van T1. Zoals te zien is, gebeurde het gewichtsverlies langzamer. Dit kan worden verklaard doordat de bacteriële oppervlakken gebonden zijn met calciumionen, waardoor een barrière wordt gecreëerd die de ureum-diffusiesnelheid verlaagt en dus zo de neerslagsnelheid vertraagt [19]. Het leek er ook op dat deze langzame neerslag de reden was dat er meer gewichtsverlies werd gevonden in vergelijking met T2 en REF, maar toekomstig onderzoek zou zich moeten concentreren op het microscopische mechanisme van deze interacties. De toevoeging van gistextract voor T4 resulteerde in een vergelijkbaar gewichtsverlies gevonden voor zowel T3 als T1. Het feit dat geen hogere waarden werden gevonden, kan worden verklaard door het feit dat er geen calciumionenbron is om calciumcarbonaat te vormen en daarom het beperkende reagens is. Dit werd ook geverifieerd in de resultaten voor T5.

Voor T5 werd aanvankelijk een lagere waarde van gewichtsverlies geregistreerd. Op latere leeftijd werd echter een hogere waarde gevonden. Dit is het resultaat van het eerdergenoemde mechanisme waarbij calciumionen een barrière vormen met een vertragende ureolytische activiteit. In tegenstelling tot T4 was calciumnitraat echter niet langer het beperkend agens, wat ertoe leidde dat de neerslag doorging en resulteerde in een hoger gewichtsverlies dat werd geconstateerd. Bij het toevoegen van de gegroeide cultuur werden resultaten gevonden die gelijk waren aan T5. Dit is eigenaardig omdat er geen calciumnitraat werd toegevoegd om deze grote hoeveelheid calciumcarbonaat te doen neerslaan. De cellen werden echter toegevoegd in een gebruikt UYE-medium die, door een in de toekomst te bepalen mechanisme, de bacteriële cellen minder uniform in de cementgebonden matrix verspreidt. Dit zou de hoge standaardafwijking voor T6 verklaren omdat er meer geconcentreerde plekken van $CaCO_3$ zouden worden gevormd. Bij het beschouwen van deze hoge afwijking, kan men opmerken dat de resultaten vergelijkbaar waren met die van T1 waar hetzelfde aantal cellen werd toegevoegd.

Over het algemeen kan worden gesteld dat de toevoeging van biologische agentia noodzakelijk is om de hoeveelheid geprecipiteerde $CaCO_3$ te verhogen. Tenslotte moet worden opgemerkt dat over het algemeen voor alle resultaten een hoge standaardafwijking werd gevonden.



Figuur 2. Gewichtsverlies per gram monster voor bacteriële mortel na 3, 7, 14 en 28 dagen

IV. CONCLUSIES

Ureum is de meest cruciale biologische agens omdat zonder ureolyse geen bacterieel calciumcarbonaat kan worden geprecipiteerd. In dit werk werd gevonden dat ureum de meest inerte agens is uit de drie onderzochte agentia. Tot een dosering van 5 w/w% werden geen negatieve effecten waargenomen. De effecten van ureum op de hydratatie van cement worden verklaard door de endotherme reactie die plaatsvindt. Dit promoot het gebruik van ureum in massieve constructies en verklaarde ook de een optimale dosering van 2 w/w% die gevonden werd.

In dit werk werd aangetoond dat gistextract bijna alle eigenschappen van zowel verse als verharde mortel negatief beïnvloedde, alsook is het verantwoordelijk voor 10 en tot 60% van de totale bedrijfskosten van het UYE-medium waarin het gebruikt wordt [20]. Daarom is het belangrijk om gistextract niet alleen te vervangen door een goedkoper, maar ook een meer inert of zelfs positief additief met vergelijkbare kenmerken voor de bacteriecellen te vinden. Recent is maisweekvloeistof (CSL) voorgesteld. Dit is een industrieel bijproduct. Soortgelijke testen dienen uitgevoerd te worden om het effect van beide agentia te vergelijken. Vanwege de negatieve effecten mag gistextract niet worden gebruikt. Indien nodig, moet de dosering lager blijven dan 0,34 w/w% om ervoor te zorgen dat deze negatieve effecten beperkt blijven. De belangrijkste prioriteit moet worden gelegd op het vinden van een vatbaar alternatief voor gistextract in toekomstig onderzoek. Ten slotte moeten de mechanismen die in dit werk werden voorgesteld worden geverifieerd door verder onderzoek. Hoewel deze mechanismen alle veranderingen in eigenschappen die gistextract voortbrengt verklaren, kan hiervoor geen bewijs worden gegeven.

Calciumnitraat werkt als een inert of positief additief in de cementgebonden matrix. Vanwege de verminderde porositeit in voortbrengsels wordt de algehele duurzaamheid verhoogd met calciumnitraat. Men dient echter voorzichtig te zijn bij het verhogen van de dosering van calciumnitraat, dit door de flash setting die plaatsvindt die de verse eigenschappen van mortel negatief beïnvloeden. Om dit te voorkomen, mag de dosering niet hoger zijn dan 8 w/w%. Hoewel het grootste deel van de testen een positief resultaat vertoonde door de toevoeging van calciumnitraat, werd een zorgwekkend mechanisme ontdekt bij het combineren van gistextract en calciumnitraat. De negatieve effecten als gevolg van de toename in porositeit, lijken te worden versterkt door de toevoeging van calciumnitraat. De eerder onderzochte en voorgestelde optimale dosering van deze agentia in combinatie met bacteriële cellen werd bevestigd in dit werk, waarvoor de grootste $CaCO_3$ inhoud werd gevonden. Deze toevoeging gaat echter gepaard met alle negatieve effecten van het gistextract. Zoals hierboven vermeld, moet gistextract worden vervangen of geëncapsuleerd worden om de negatieve invloeden ervan te neutraliseren.

Verder werd een nieuwe testmethode voorgesteld om de $CaCO_3$ inhoud te testen. In dit werk werden vergelijkbare resultaten gevonden met literatuur, waarbij duurdere en tijdrovende methoden met kleinere monsters werden gebruikt. De auteur is van mening dat de monstermassa verder moet worden verhoogd om preciezere resultaten te verkrijgen. Vanwege de goede resultaten die in dit werk werden gevonden, wordt sterk aangeraden om deze testmethode te vergelijken met de momenteel gangbare methoden in toekomstig onderzoek.

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II

Nomenclature

Abbreviations

AN	Amino nitrogen
CEN	Comité Européen de Normalisation
DE	Diatomaceous earth
FEM	Finite element analysis
MICP	Microbially induced carbonate precipitation
OD	Optical density
SCC	Self-compacting-concrete
TLC	Thin-layer chromatography
TN	Total nitrogen
UCSL	Urea-corn steep liquor
UYE	Urea-yeast extract

Roman symbols

F	Force	kN
K	Carbonation rate constant	mm/year$^{0.5}$
M	Mass	kg
R	Resistance	kN
T	Time	s
V	Volume	m^3
x	Depth	mm

Greek symbols

ρ	Density	kg/m³

Subscripts

c	Compressive
ca	Carbonation
d	Dry
f	Flexural
u	Urea
w	Water

Cement chemist notation

C	Calcium oxide – Lime	CaO
S	Silicon dioxide - Silica	SiO_2
A	Aluminium oxide – Alumina	Al_2O_3
F	Iron oxide – Rust	Fe_2O_3
T	Titanium dioxide – Titania	TiO_2
M	Magnesium oxide – Periclase	MgO
K	Potassium oxide	K_2O
N	Sodium oxide	Na_2O
H	Water	H_2O
$\bar{C}$	Carbon dioxide	CO_2
$\bar{S}$	Sulfur trioxide	SO_3
P	Phosphorus hermi-pentoxide	P_2O_5
C_3S	Tricalcium silicate – Alite	$3CaO.SiO_2$
C_2S	Dicalcium silicate – Belite	$2CaO.SiO_2$
C_3A	Tricalcium aluminate – Aluminate / Celite	$3CaO.Al_2O_3$
C_4AF	Tetracalcium alumino ferrite - Ferrite	$4Ca.Al_2O_3.Fe_2O_3$
CH	Calcium hydroxide	$Ca(OH)_2$
CSH	Calcium silicate hydrate	-
CAH	Calcium aluminate hydrate	-
AFt	Calcium trisulfoaluminate hydrate – Ettringite	-
AFm	Calcium monosulfoaluminate	-
C_3AH_6	Hydrogarnet	$3CaO.Al_2O_3.6H_2O$

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1

Introduction

1.1. Introduction

In today's modern society, concrete is the most widely produced human-made material. Its origin dates all the way back to the prehistory where Nabataean traders discovered the advantages of hydraulic lime. Later, in Ancient Egyptian and Roman eras, builders discovered the usage of additives in the concrete mix. More specifically they discovered that adding volcanic ash to the mix would allow the concrete to set underwater. The main influence of concrete during the Roman era can be found in their architecture, for the reason that concrete does not have the restrictions that stones or bricks bring. It can be freely shaped in arches, vaults and domes without having to worry about internal thrusts and strains that brickwork suffered from. Modern concrete has changed a lot since then, countless compositions have been developed to provide a tailored solution for each specific application and developments continue rapidly as a result of active research in the field. However, with the cement industry being responsible for 5% of the global carbon dioxide (CO_2) emission, durability is of critical importance to limit the emission of this greenhouse gas [1].

Concrete is bound to display cracks at some point, Eurocode 2 provides a method to calculate the maximum width of a crack and limits these in accordance to their exposure class. Not only does the manual repairing of these cracks come with high maintenance and repair costs, but more importantly, these cracks are detrimental to the durability of the concrete structure since gasses and liquids will be able to intrude the structure. Due to the fact that cracks will eventually appear at some point during concrete's lifetime, researchers have moved away from prevention and started looking at healing the cracks when they appear. Recently, promising research has been produced by implementing bacteria in the concrete mix, through microbially induced carbonate precipitation (MICP). Bacteria present at the crack surface will precipitate calcium carbonate ($CaCO_3$) to heal the cracks, the overall reactions are shown below;

$$CO(NH_2)_2 + 2H_2O \xrightarrow{Bacterial\ urease} 2{NH_4}^{+} + {CO_3}^{2-} \tag{1}$$

$$Ca^{2+} + {CO_3}^{2-} \rightarrow CaCO_3 \tag{2}$$

Since the amount and rate of biogenic $CaCO_3$ precipitation are directly related to the efficiency of crack repair, the concentration of urea ($CO(NH_2)_2$) and calcium nitrate ($Ca(NO_3)_2$) must be optimized to maximize the amount of precipitated calcium carbonate. Additionally, a supplement of extra bacterial nutrient, yeast extract, is added to stimulate bacterial growth. Although optimal concentrations to maximize the precipitation of calcium carbonate have been researched, little attention has been given to the influences of these individually added bioagents [2].

1.2. Problem statement

The implementation of bacteria to achieve self-healing concrete is a hot topic in civil engineering. This healing is achieved by introducing calcium carbonate precipitating bacteria in combination with various bioagents. Much of the recent research is focused on the optimal implementation of bacteria in concrete to maximize the self-healing capabilities, which was done by screening different bacteria and carriers in which they were introduced into the concrete mix [3]. Another approach is to evaluate the potential of the bio-concrete by determination of the healing capabilities and deriving an optimal mixture and bacterial compositions [2]. However, the individual influences of each of the bioagents commonly used in modern bio-concrete are under-documented and their mechanisms are ill-understood.

The aim of this work is to lay out an accessible work that documents the main influences and characteristics of three commonly used bioagents. Additionally, the influence of bacterial cells on the precipitated $CaCO_3$ content in the matrix was investigated. Beyond quantifying, each mechanism will be given a plausible explanation on a molecular level. In this way, further research in the optimization of bio-concrete can fall back on current work to identify the main actors of specific characteristics when using these additives to facilitate the bacteria in the mix.

1.3. Research plan

In this study the impact of each bioagent on the characteristics of mortar was studied individually. Later the combinations of different bioagents were tested to quantify any positive or negative interdependent relations. Additionally, the availability of precipitated calcium carbonate was tested by incorporating bacterial cells in combination with bioagents. In the second chapter, a literature review was conducted on all of the currently researched characteristics. Afterwards, an overview of the selected material and testing methods which were used are given. In the fourth chapter, the tests were executed, and the results were noted and explained. Afterwards, the results from this work were critically compared to the literature and discussed. Lastly, in the fifth chapter, a conclusion with the all significant results was given after which the most crucial focus points for further future research were listed.

Eight tests were performed to gain insight into the basic properties of both fresh and hardened mortar compositions as shown in Table 1-1, an X-sign signifies the test is performed. The compositions have 5 varying w/w% series for each of the 3 bioagents and one mixture that contains all three of the bioagents in the most commonly found concentration. Additionally, 6 compositions were tested with bacterial cells included.

Table 1-1. Research plan

	Flow	Setting	Mechanical strength	Water absorption	Freeze-thaw resistance	Carbonation	Cement hydration	$CaCO_3$ content
Reference	X	X	X	X	X	X	X	X
Urea	X	X	X	X	X	X	X	
Yeast extract	X	X	X	X	X	X	X	
Calcium nitrate	X	X	X	X	X	X	X	
MIX	X	X	X	X	X	X	X	
Bacterial concrete			X					X

2

Literature review

2.1. Flow

The workability of concrete is one of its most important characteristics. A low workability results in; problematic mixing and casting, increased labour costs and increased building time. However, on the other side of the spectrum, if workability is too high, it will lead to segregation and weak mechanical properties. An ideal composition has great mechanical strength and is easily workable by the operator. The flow test is inexpensive, fast and can be performed in situ for a quick analysis.

In a study of from 2007, it is shown that mortar flow is increased with the increase in the mix ratio of urea. This directly relates to the flow of the fresh mortar which increased by approximately 22 to 26% compared to the reference composition and this for every 5% increase in the volume of mixed urea [4]. This increase in flow was explained due to the increase in the solution volume as a result of the added volume of urea which dissolved in water. This conclusion was backed up by a paper where the mechanical properties of cement-soil were improved using urea [5]. Furthermore, it is proven the fluidity of a soil-cement can be maintained and construction is feasible if the W/C-factor is between 45% and 60% provided the (W+U)/C-factor remains above 60%. This proves that urea can be used as a partial replacement for water. Wang et al reported a suitable concentration for urea to be 0.5 M when looking for optimal self-healing properties [2].

Contradictory to the importance of yeast extract which is "… considered to be necessary for substantial growth of 'contaminating' microorganisms to occur ", little research has been done to its effects [6]. A recurring dosage of 20 $g.L^{-1}$ can be found throughout literature when being used in an urea-yeast medium [7, 8]. However, yeast fermentation waste has been proven to be viable as a plasticizer to concrete mixtures [9].

In a journal publication about the influence of calcium nitrate as antifreeze admixture, a slump flow test was executed. An increase in the slump was obtained when using 6 w/w% calcium nitrate [10]. It is important to note that in this research the water content of the mixture was not decreased to compensate for the tetrahydrate that comes with the calcium nitrate. Therefore, it cannot be confirmed if calcium nitrate has a positive effect on the slump flow ratio. In a 2011 research on the effect of calcium nitrate on concrete containing volcanic ash, it was briefly mentioned that the workability decreased as the percentage of calcium nitrate increased, this with a slump value ranging between 40 and 60 mm [11]. However, it is not clear if the authors modified the water content for the reasons mentioned above. When calcium nitrate is implemented at a water-cement factor of 0.35, results show that dosages of $Ca(NO_3)_2$ higher than 3 w/w% are not recommended due to the excessive increase in viscosity of the mixtures after mixing [12].

The workability of self-healing concrete suffers from unfavourable effects due to the addition of high carbon contents, which lead to having adverse effects on the workability [13]. Moreover, the chosen encapsulating material of the bacteria also has an effect on the workability. When encapsulated in a modified-alginate based hydrogel the workability was found to decrease slightly. When using diatomaceous earth (DE) as the carrier for bacterial cells, it was found that the workability decreased significantly when the concentration was higher than 50% [7, 14, 15].

2.2. Setting

The setting time of a mixture is detrimental for the practicality of a good mortar. A short setting time puts the casting process under pressure, which could lead to mistakes and non-homogenous casts when casting in more than one layer. A long setting time, however, will delay the construction by delaying the time suitable for demoulding. Setting signifies the end of workability, after the initial setting the composition cannot be mixed anymore; after the final setting, the mix is not workable anymore [16].

In a 1997 study, it was shown that urea slowed down the setting time of concrete, the reason brought forward for this is the presence of calcium hydroxide in the mortar [17]. In a more recent publication, in 2017, the same conclusion was made. However, the delay in the setting was attributed to the lower peak hydration temperature of cement [4, 18]. The increase in setting was also confirmed when urea was combined with self-compacting concrete (SCC) [19].

Little literature is found on the influence on the setting of yeast extract due to the reason that it is mostly used in combination with urea to form a Urea-Yeast Extract (UYE) medium for the bacteria to grow in. Noticeable delay in initial and final setting times of cement mixes were observed [13]. Due to the low amount of research, it raises the question whether yeast-extract accelerates, is inert or delays the setting. This question will be answered in this study.

Calcium nitrate functioned as a setting accelerator and reduced both the initial and final setting times by 20% when adding 1 w/w% [20, 21]. A set accelerator is defined as an admixture that decreases the initial setting for the transition of the mix from the plastic to the rigid state [21]. However, reports show that the effect of calcium nitrate is dependent on the cement type as well as the temperature [22]. The addition of calcium nitrate is therefore beneficial in environments where the concrete is exposed to low temperatures [10]. Additionally, it was found that the efficiency of calcium nitrate depends on the type of cement that was used or, more specific, the chemical composition of the cement [21].

When adding bacteria along the bioagents, multiple factors are responsible for the setting time. The setting time is decreased with the addition of diatomaceous earth or metakaolin. Furthermore, the strain also influences the setting, for unprotected *Bacillus sphaericus* a forty-minute delays in both initial and final setting times were observed. However, for *Diaphorobacter nitroreducens,* no delay in initial setting is reported [3, 13]. *Sporosarcina pasteurii* was used in combination with a urea-corn steep liquor (UCSL) medium to limit the negative effects of yeast in a UYE-medium where a negative effect was found on the setting times, however, this delay was significantly lower [23].

2.3. Mechanical properties

One of the most important requirements of cementitious materials is its mechanical properties. Compressive and flexural strength are crucial for withstanding mobile and permanent loads

In a recent study granular urea was used in self-consolidating concrete together with untreated rice husk ash. As a result, a 20% decrease in the compressive strength was found after 28 days [19]. Similar results were found where urea was incorporated under cold weather conditions. The maximum compressive strength was observed at -5°C with 6% urea where the strength was found to be 80% after 28 days compared to the reference sample [24]. These results harmonize with the results where urea was used as an additive material under ideal circumstances. Urea was included at 3 and 5% of cement by weight, where the water-cement factor was kept even using following the equation:

$$\frac{M_u}{1320} + \frac{M_w}{1000} = \frac{M}{1000} \qquad (3)$$

Where M_u denotes the mass of urea and M_w the mass of water. In a study, a strength loss of 18.66% and 23.94% was found after 7 days, 23.59% and 27.8% after 28 days but a strength gain was found after 91 days of 6.44% and a strength loss of 1.534% each time for 3 and 5 w/w% respectively [25]. These results are also backed up where urea was added up to 15% mass ratio to concrete. However, an increase in strength was found at 15% at 3, 7 and 28 days [4]. Strength recovery was found to appear as curing period increased, this led to a demand for additional research after 56 and 91 days [26].

Yeast extract and urea are usually both introduced to the cement as a mixture. In a 2013 study, the concentration of yeast extract was increased from 5 g/L to 15 g/L, which resulted in a strength decrease from 27% to 76% [15]. Additionally, in a 2015 study, a severe negative effect on the compressive strength was attributed to the presence of yeast extract [3]. In this same paper, it was proposed to encapsulate the yeast extract to avoid these negative effects. In an earlier study, compressive strength values were reported to half compared to those of the reference [27]. This negative effect of adding yeast extract is however limited when added in combination with bacteria [13].

In cold environments, calcium nitrate has been proposed as an antifreeze admixture. It was found that the compressive strength of calcium nitrate concrete decreased with decreasing deep freeze temperatures, but the decrease was lower than those of the reference concrete. Nonetheless, a significant increase in the compressive strength was observed due to the setting accelerating properties of calcium nitrate, these helped achieve the concrete sufficient strength to develop resistance to internal freezing [10]. Additionally, a long-term strength increase was found by the addition of calcium nitrate, this gain increased until a dosage of 2% calcium nitrate and flattened out for higher dosages [20]. Contradictory to previous research, no strength gain at an early age was found when calcium nitrate was used in a cement paste mixture, though it improved the 28 days strength by 20% [21]. When used in combination with volcanic ash, a significant strength increase was noted which was linearly correlated with the amount of calcium nitrate added [11]. It was also confirmed that the optimum dosage of accelerator depends on the ambient temperature. An initial hypothesis that the accelerating mechanism is based on interactions between nitrate and the aluminate phase has been discredited [22]. The influence on the cement with CEM I R and CEM II R was tested, which resulted in an increase of 30% in the early strength of the concrete after 2 days compared to the reference [28].

The general trend in the literature shows that bio-concrete provides a status quo and, in some situations, even an increase in compressive strength compared to neat concrete. This is due to the plugging of the pores inside the concrete and is due to bacterial induced calcium carbonate precipitation [29-31]. However, this strongly depends on the admixtures and bacteria used. When using bio-based admixtures, the nutrient mortar that contained an UCSL-medium resulted in greater compressive strengths compared to the bacterial, *Sporosarcina pasteurii*, mortar at 3, 7 and 28 days [32]. When introducing *S. pasteurii* cells in a vegetative state and as killed cells by autoclaving, both with an UYE-medium, the vegetative cells showed an increase of the compressive strength while the killed cells produced an adverse effect [33]. This was confirmed in another research which showed that the optimal cell concentration lays around 10^4-10^5 cells/mL of water, with a decrease in compressive strength at lower and higher concentrations [34, 35]. Extensive research evaluating the influence of the carriers effect on the self-healing system including bacterial spores, the carriers Argex, microcapsules and hydrogel was done. This showed a decrease in compressive strength of 12, 27 and 70% respectively while using lava as a carrier results in a 10% strength increase [7, 14, 36]. Furthermore, the bacteria can also be applied from the outside, an increase when applying B. *Sphaericus* through a spray twice a day and as a submersion medium, an increase of compressive straight is examined [13, 35].

It is important to note that the compressive strength does not represent the healing characteristic of bio-concrete. Strength regain is the characteristic that can be tested by reloading a specimen after cracks have been healed by means of MICP.

2.4. Water absorption

Little has been written about the absorption properties of mortar with urea as an additive material. A sorption test has been executed on cubes with 0 to 7 w/w% of urea added, of which the results showed a decrease in sorptivity, measured in mm/min when increasing the amount of urea [25]. However, clear results showing the impact of urea on the absorption rate are lacking.

For the influence of yeast extract on the absorption of water no literature can be found. This is for the same reason as mentioned above. Yeast extract and urea were mostly used as a liquid medium (UYE-medium) to cultivate the cultures in or as a submersion medium which leads to bio-deposition on the surface of the concrete [2, 14]. These combined usages do not allow to fully identify the influence on the cementitious matrix which further stresses the necessity of this work.

Due to the anti-freeze applications of calcium nitrate in concrete, tests have been performed to validate the capillary suction and water diffusion properties. No change in the capillary porosity was found within a series of various calcium nitrate dosages. However, the temperature at which the samples were dried was reported to have an influence [20].

When incorporating bacteria into the concrete mix, a decrease in water absorption is observed. This is an aftereffect of the formation of calcite by converting $Ca(OH)_2$ which helps to fill the voids within the concrete [14, 29, 31]. This positive effect was observed up to 1 optical density (OD) [8]. The carrier of the bacteria also plays a big role in the water absorption, a decrease of 50 up to 70% compared to reference was found when adding diatomaceous earth as a carrier [14].

These results are directly translatable to the sorptivity, which is the unidirectional absorption of water due to capillary rise [13]. It is important to note that the most favourable effect on sorptivity was obtained when applying the bacteria on a surface for biodeposition treatment, this creates a barrier which strongly reduced the water absorbed by a factor of 5 [6, 34]. Research investigating the influence of the cell concentration concluded a maximal cumulative water absorption at 10^7 cells/mL while a minimal value was reached at 10^9 cells/mL [37]. In a series where only nutrients, nutrients with pure microcapsules and nutrients with loaded microcapsules were tested, a decrease of 14, 42 and 48% respectively in water sorptivity compared to reference was concluded. However, this decrease was mentioned to mainly be the result of the microcapsules [38].

2.5. Freeze-thaw resistance

No literature is found for both urea and yeast extract on their effects when being subjected to freeze-thaw attacks.

Calcium nitrate, however, is a known anti-freeze admixture [10]. In this study the concrete was subjected to up to 14 days of exposure of -10°C after which was concluded that calcium nitrate can be used as an anti-freeze admixture under these circumstances.

However, more importantly, an increased resistance towards carbonation, chloride penetration and freezing and thawing was found for mortar specimen. This was concluded in a study where limestone prisms were treated with an ureolytic biodeposition treatment to investigate the effect of the surface treatment [39, 40].

2.6. Carbonation

Carbonation of concrete provokes the corrosion of steel reinforcement and shrinkage of the concrete. When corrosion of the reinforcing steel happens, the concrete cover can subsequently start to chip off. Many models have been proposed to predict the carbonation of concrete [41-43]. The problem with these models is that they do not predict the influence of modern additives.

The influence of carbonation was measured on cylindrical specimens containing urea that were placed in a chamber with 20% CO_2 for 7, 28 and 91 days. The carbonated thickness reduced where the amount of urea in concrete increased from 0 to 120 kg/m^3, and the rate of reduction was greater where the cement content was decreased [17]. The explanation that was given for this was that the amount of calcium hydroxide, which normally combines with CO_2 reduced as a result of the change of characteristics that urea brought forth in the cementitious matrix.

No meaningful research describes the role of yeast extract on the carbonation process was found at the time of writing.

The influence of calcium nitrate on carbonation depth is unclear. Results show that the carbonation depth of concrete prepared with CEM I or CEM II/A-V (fly ash) can be reduced up to 40%. However, concrete produced with CEM II/A-LL (limestone) do not benefit from the addition of calcium nitrate [44]. It was mentioned that calcium nitrate is a simple and effective corrosion inhibitor when compared to complicated systems like sealers or coatings applied to the reinforcing bars [45].

The pH inside the concrete matrix can raise to be as high as 12 to 13. However, in crack zones, the pH can drop due to carbonation. Therefore suitable, alkali-tolerant or alkaliphilic, strains need to be incorporated [36]. The carbonation rate constant K was found to drop 25 to 30% compared to the untreated specimen as a result of the effects of biodeposition treatment [31, 34, 46]. Meanwhile, when applying bacterial cell walls into the mortar, an increase in the carbonation rate was found [14].

2.7. Cement hydration

Addition of urea and its effects on the hydration heat have been tested by analysing the adiabatic temperature rise. With this data, a practical structure sized model was analysed by finite element analysis (FEM). A decrease in the initial and maximum temperature, as well as a delay in the time to reach this maximum temperature, was found [26]. A study that to validate the temperature decrease found that this decrease in solution temperature happened instantaneously and that this was proportional to the used volume of urea. The cumulative generated heat showed similar levels to that of the control mix when less than 15 w/w% urea was used, this indicated that the cement hydration progressed sufficiently [4]. The same results were found when adding granular urea to self-consolidating concrete [19].

When studying the hydration behaviour of 0.67% yeast extract, a retarding effect was observed with the peak of the second hydration at 20 hours after mixing, comparable to that of the nutrient paste [33]. Similar observations were made by Wang et al. [38]. Additionally, due to the foaming effects of yeast extract, the heat of hydration cannot dissipate easily which leads to an increase of temperature inside the concrete [47].

Calcium nitrate has been proven to accelerate cement hydration when added as 8 w/w%. Additionally, a positive effect on the hydration degree was found where the heat production after 7 days was 9% higher than that for the reference [38]. The research concluded that accelerated cement hydration took place because an increase in compressive strength at an early age took place [10]. The same conclusion is reached as a result of an earlier setting up to a dosage of 3,86%. However when the dosage was higher than 7.79% CN a delay in setting and thus delay in hydration was noted [22]. The reason for this is explained due to an increase in calcium concentration which leads to a faster super-saturation of the fluid with respect to calcium hydroxide [48, 49].

The delaying effect on the second hydration peak was directly correlated with an increased concentration of bacterial cells between 10^5 and 10^7 CFU/mL (Colony forming unit per millilitre) [33].

2.8. $CaCO_3$ content

Hydrolysis of urea in the presence of urease leads to the formation of NH_4^+ and CO_3^{2-}, this carbonate ion is the base for the formation of $CaCO_3$ in combination with calcium ions [50, 51]. Intuitively, this would lead to think that an ever increasing concentration of urea, and correspondingly calcium ions and urease, however, this is not the case [14, 52]. Rather, it was found that, due to their negative charge, Ca^{2+} will bind to the bacterial surface until through densification, no more urea can reach the cell. Therefore an optimal concentration was calculatable, and this primarily in function of the number of cells and the number of calcium ions [14].

In this extensive dissertation, the influence of the yeast extract concentration was also examined. When adjusting the concentration of bacteria, urea and yeast extract it was found that the amount of urea decomposed was positively influenced by the amount of yeast extract added [14]. In this situation, the optimal concentration is limited by the negative influences on the cement matrix as mentioned above.

The surface of bacteria is negatively charged [53]. This negative charge facilitates the adhesion of Ca^{2+} which acts as nucleation sites [52]. Similarly, to urea, after concentrations above 0.5 M, the calcite precipitation efficiency reduced. Optimal concentrations were reported for *B. sphaericus* LMG 22257 to be 0.5 M [36, 54]. The amount of calcium that is present in concrete during mixing, however, is not only a result of its addition, the concrete matrix itself is also a source of calcium ions.

The effect of the concentration of spores was found out to only affect the early decomposition of urea after 1 day. Although an increased decomposition of urea was noticed when increasing the concentration of spores, this was only true at early ages. After 3 days, the concentration of urea dominated the amount of decomposition. Therefore, it was concluded that for early decomposition, the cell concentration is most the important while afterwards the concentration of urea takes over this role [14]. This is due to the importance of urease in the hydrolysis of urea that is directly related to the bacterial biomass [55].

Additionally, more influence factors control the eventual amount of precipitated $CaCO_3$. Since the precipitation of calcium carbonate is only activated at a specific pH range between 7.0 and 9.5 where urea hydrolysis is activated, the pH will influence the amount of precipitated $CaCO_3$ [55-57]. This is due to the fact that at low pH levels, carbonate tends to dissolve and is not precipitated [55]. The temperature also influenced enzymatic urea hydrolysis, this was tested for the temperature ranging. from 20 to 37°C. An increase in activity up to 5 times was reported when the temperature increased from 15 to 20°C. When increasing the temperature from 10 to 20°C, an increase up to 10 was found [58, 59]. Moreover, it was found that urease was stable until 35°C. When the temperature rose to 55°C, its enzymatic activity was reduced by 53% [53]

3

Test methods and materials

3.1. Materials

3.1.1. Used materials

Cement

All compositions were produced with the same type of cement, this is a CEM I 52,5 N produced by Holcim. In which I stands for “Portland cement”, 52.5 is the compression strength at 28 days in MPa and N indicates an ordinary early strength. The average values of the chemical compositions are shown in Table 3-1.

Table 3-1. Chemical composition of the used cement

	%
CaO	63,0
SiO_2	18,9
Al_2O_3	5,8
Fe_2O_3	4,2
MgO	1,1
Na_2O	0,44
K_2O	0,6
SO_3	3,5
Cl^-	0,06
Ignition losses	1,2

Sand

The sand used is prepacked CEN-standard sand from Normensand according to EN 196-1. It contains sand with a specific grain size distribution between 0.08 and 2.00 mm and has a maximum moisture content of 0.2%. The specific grain distribution is displayed in Figure 3-1.

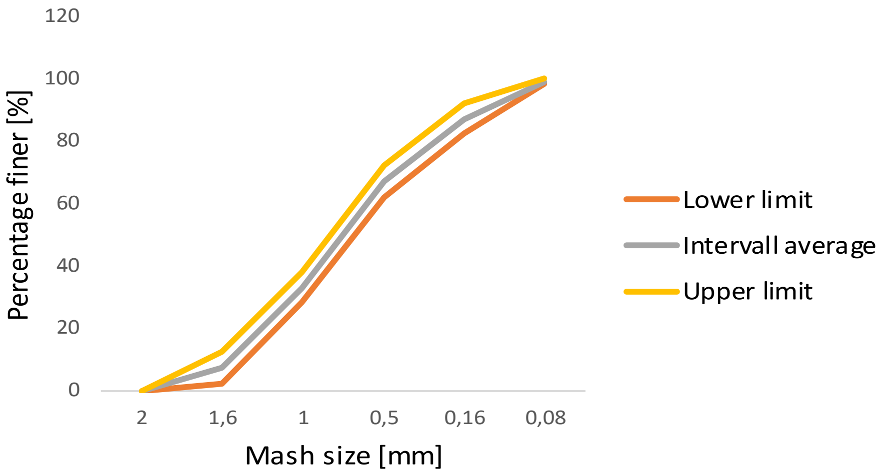

Figure 3-1. Specific grain distribution of Normensand

Urea

Urea, also known as Carbamide (CH_4N_2O) of producer Carl Roth was used. It comes in the form of white pearls that easily dissolve in water. Its guarantee analysis is given in Table 3-2.

Table 3-2. Guarantee analysis of urea

Assay (TLC)	≥99,5%
Chloride (Cl)	≤0,001%
Sulphate (SO_4)	≤0,001%
Melting point (TLC)	132-135°C
Heavy metals (as Pb)	≤0,001%
Appearance	White pearls

Yeast extract

A dried yeast autolysate by Carl Roth was used, it is produced through aqueous extraction of autolysed brewer's yeast. Its chemical analysis is given in Table 3-3. It is highly soluble in water with a solubility of 410 g/L.

Table 3-3. Chemical analysis of yeast extract

Total nitrogen	≥8%
Amino nitrogen	4,8-6,3%
Ratio AN/TN	41-60
Loss on drying	≤6%
Ash	≤18%
pH (5% in H_2O)	6,4-7,4
Solubility	clear

Calcium nitrate tetrahydrate

The calcium nitrate was supplied as a tetrahydrate by Carl Roth. The fact that it is a tetrahydrate is important since every molecule will be surrounded by four molecules of water. This additional water will need to be considered when calculating the water-cement factor. The chemical formula is $Ca(NO_3)_2 \cdot 4H_2O$.

To calculate the additional amount of water brought in by the calcium nitrate tetrahydrate the weight percentage of the water must be calculated. The producer defined the molar mass as 236.15 g/mol and the molar mass of water is known as 18,0152 g/mol. With four moles of water per mole of calcium nitrate tetrahydrate, this calculates to 30.52 w%. For each composition, the amount of water for that specific concentration is calculated and subtracted from the water put in, this way the water cement-factor is stable. Its type analysis, as given by the producer, is given in Table 3-4.

Table 3-4. Guarantee analysis of calcium nitrate tetrahydrate

Assay (TLC)	≥98,0%
Chloride (Cl)	≤0,01%
Sulphate (SO_4)	≤0,01%
pH-value (5% solution)	5,0-7,5
Heavy metals (as Pb)	≤0,002%
Iron (Fe)	≤0,001%

Hydrochloric acid

A 35 w/w% solution of hydrochloric acid (HCl) is used. This solution is manufactured by VWR and has a density of 1.18 kg/l and a molar mass of 36.46 g/mol. This solution is diluted down to 0.1 M for the purpose of this research.

Phenolphthalein indicator (1%) in isopropanol

Phenolphthalein, $C_{20}H_{14}O_4$, is a colourless indicator that made into a solution with isopropanol due to its limiting dissolvability in water. The organic phenolphthalein is used as an acid-base indicator, it has a visual transition interval that is colourless at pH 8.0, fuchsia at pH 8.5 at red at pH 10.0 and higher.

Bacillus sphaericus

Bacillus sphaericus LMG 22257 was used in this study. The bacteria were cultured in a growth medium consisting of urea, 22 g/L, and yeast extract, 20 g/L, also referred to as an UYE-medium. The cells were harvested by centrifugation at 7000 rpm for 7 minutes by Sorvall RC 6+, Thermo Scientific^{TM}. After draining off the excess UYE-medium, the cells were resuspended in a sterile saline solution (8.5 g/L NaCl). The final concentration of the bacteria in suspension was approximately 10^9 cells/mL while that of the grown culture was approximately 10^8 cells/mL. The grown cultures and suspended cells were stored at 4°C in a fridge. Before usage, the cells were left to heat up until room temperature.

Coating material

SikaCor-227 was used to coat the concrete with a protective coating against chemically aggressive media. It is an epoxy resin-based coating material which is mixed in a 4:1 ratio.

The epoxy is always applied in two layers which require a 24-hour drying cycle between each coating and is applied by means of a brush.

3.1.2. Tested compositions

To study the impact of the three bioagents, a total of 17 compositions were produced. A large portion of these compositions are single-additives compositions with various concentrations. The aim is to provide an insight into the impact of that particular bioagent on the cementitious matrix. When the exact impact of these different products is understood, a composition with multiple additives will show the interactions between the different bioagents. Additionally, a total of 6 compositions with bacterial cells incorporated were produced to study the possibility of $CaCO_3$ precipitation.

All mortar compositions were produced in accordance to NBN B15-001 (2004) and were stored in a moisture curing room with a temperature of 20 ± 2°C and >90% relative humidity until the required age was reached. Each composition is made with 450 g of cement, 1350 g of sand and a water-cement factor of 0.5. As stated before, all compositions containing calcium nitrate tetrahydrate have reduced water weight to compensate for the water that is added from the tetrahydrate, in all other cases 225 g of water is used.

Table 3-5 gives a summary of all tests executed on the mortar compositions that solely include bioagents.

Table 3-5. Summary of tests performed on mortar with bioagents

Flow	Setting	Mechanical properties	Water absorption	Freeze-thaw resistance	Carbonation	Cement hydration
		3, 7, 14, 28 ,56d	28+(7,14,28)d	28d	28+(14,28,56d)	

The 17 bioagent compositions were coded with the letters, U, Y and Ca for urea, yeast extract and calcium nitrate respectively, followed by a number which signifies the amount of weight per weight of cement percentage. For example, U4 stood for a mortar mix containing 4 w/w% of urea and Y0.85 defined a mortar mix that contained 0.85 w/w% of yeast extract. Furthermore, the name MIX was used for a mortar mix that contained U4, Y0.85 and Ca8. This way the following 17 compositions were defined, a reference mix coded REF, 15 compositions containing a single bioagent coded, U1-2-3-4-5, Y0.34-0.85-1.28-1.7-2.56, Ca2-4-6-8-10 respectively and a final MIX which contained U4, Y0.85 and Ca8 as mentioned above. When the test is noted with 28+x it means the specimen is cured for 28 days and tested after being subjected to x days of the specific conditions for that test. This means that for the carbonation test, specimens were cured for 28 days and then were tested after being subjected to CO_2 for 14, 28 and 56 days. Additionally, the following tests, as shown in Table 3-6, were performed on 6 compositions that include bacterial cells.

Table 3-6. Summary of tests performed on mortar with bacterial cells

Mechanical properties	$CaCO_3$ content
3, 7, 14, 28d	3,7,14,28d

The total 7 compositions consist of a reference, 10^9 cells/mL, 10^9 cells/mL + U4, 10^9 cells/mL + U4 + Ca8, 10^9 cells/mL + U4 + Y0.85, 10^9 cells/mL + U4 + Y0.85 + Ca8 and one mix containing a grown culture with a cell concentration of 10^8 cells/mL. These mixes were coded REF, T1, T2, T3, T4, T5 and T6 respectively.

An overview of all compositions and their respective contents is given in Table 3-7.

Table 3-7. Compositions with code and contents

	C	W	S	U	YE	CN	Cells	Culture
	g	g	g	g	g	g	mL	mL
REF	450	225	1350					
U1	450	225	1350	4,50				
U2	450	225	1350	9,0				
U3	450	225	1350	13,50				
U4	450	225	1350	18,00				
U5	450	225	1350	22,50				
Y0,34	450	225	1350		1,53			
Y0,85	450	225	1350		3,25			
Y1,28	450	225	1350		5,75			
Y1,7	450	225	1350		7,65			
Y2,56	450	225	1350		11,52			
Ca2	450	222,25	1350			9		
Ca4	450	219,51	1350			18		
Ca6	450	216,76	1350			27		
Ca8	450	214,01	1350			36		
Ca10	450	211,27	1350			45		
MIX	450	214,01	1350					
T1	450	205,00	1350				20	
T2	450	205,00	1350	18,00			20	
T3	450	194,00	1350	18,00		36,00	20	
T4	450	205,00	1350	18,00	3,83		20	
T5	450	194,00	1350	18,00	3,83	36,00	20	
T6	450	25,00	1350					200

3.2. Performed tests

In total, eight tests were executed as shown in Table 3-5 and Table 3-6. All tests were executed in triplicate unless stated otherwise to compensate for any variations in the obtained results. In what follows a brief overview of all tests is given, a more detailed explanation is given in their respective chapters.

3.2.1. Mortar preparation

The norm “EN 196-7 Methods of Testing Cement - Part 7: Methods of Taking and Preparing Samples Of Cement” was used. The norm defines the preparation of 450 ± 2 g of CEM I 52.5 cement with a water-cement ratio of 0.5, 1350 ± 1 g of Normensand and addition of various bioagents. The cement and water were added to the mixing bowl and mixed on low speed for 30 s, afterwards the sand was evenly added over a period of 30 s while mixing at low speed. When the sand was added it was mixed for another 30 s on high speed. After this the sides and bottom of the bowl were manually scraped so there was no unhydrated cement stuck in places the mixing blade couldn’t reach. To ensure a good filling of the mould, it was first filled a little over halfway and jolted with a vibrating apparatus. After 60 jolts the mould was completely filled, and the jolting process was repeated. Once finished the excessive mortar paste was disposed of by striking it in a sawing motion to ensure a flat top surface for all prisms.

3.2.2. Flow

The flow table test, as described in NBN EN 1015-3 (1999) in the norm “tests for mortar paste – determining the consistency of fresh mortar with the flow table”, measured the increase in the diameter of a fresh mortar test sample. A conic mould, 60 ± 5 mm in height, with an internal diameter of 100 ± 0.5 mm at the bottom and 70 ± 0.5 mm at the top was placed on the flow table. Before testing the flow-table was washed with a damp cloth, the mortar was then introduced in two layers with each layer being compacted by 10 short strokes by means of a tamper, this to ensure uniform filling of the mould. Excess mortar was skimmed with a palette knife, and any spilt mortar on the flow table was cleaned with a damp cloth. After removing the mould, the table was jolted 10 times at a rate of one jolt per second. The spread-out mortar was measured by means of callipers at its largest diameters and perpendicular to this measurement. A mean value of these two measurements was calculated. Finally, the flow ratio was calculated as;

$$\frac{\emptyset_{flow}-\emptyset_{mould}}{\emptyset_{mould}} * 100 \quad [\%] \qquad (4)$$

With $\emptyset_{mould}$, the diameter of the mould, being 100 ± 0.5 mm, the flow ratio was calculated. The result was expressed in a ratio that is larger than 100%. It is clear that the workability of the concrete increases with the flow diameter. This being said, a good flow-ratio is only a requirement, but not a guarantee for a good concrete mixture. For this reason, the norm defines 4 slump classes to classify concrete and mortar mixtures, this way a certain consistency can be demanded when ordering a composition based on its properties contrary to ordering based on a specific composition. It is clear that flow class S4 is to be avoided to prevent segregation of aggregates during casting. Because the test is prone to human errors, it is executed three times for each composition.

Table 3-8. Slump flow classes according to EN 1015-03

Flow class	Flow range [mm]
S1	10 - 40
S2	50 - 90
S3	100 - 150
S4	160 - 210
S4	210 -

3.2.3. Setting

The setting of the mortar was tested with the Vicatronic E044N by Matest®. This is an automatic Vicat machine that executes the Vicat test in accordance with NBN EN 196-3 and NBN EN 480-2. Initial and final setting were determined which are respectively the moment when the needle does not penetrate the mortar completely and the moment when the needle does not penetrate the mortar at all. The Vicat apparatus contains a plunger made of non-corrodible metal needle with a length of 45mm, a surface area of 1mm^2 at its tip and a total mass of 300 ± 1 mm. The mould is truncated conical in shape and made of hard rubber, it is 45 ± 0.01 mm deep and has an internal diameter of 65 ± 0.01 mm at the top and 75 ± 0.01 mm at the bottom. This mould was placed inside of a hollow cylinder pipe that was sealed with epoxy on one side to avoid any spilling during the casting.

3.2.1. Mechanical strength

Prior to testing of the specimen, the density was calculated, the dimensions were measured by means of a Vernier calliper after which the specimen was weighed. The was done with the following formula:

$$\rho = \frac{M}{V} \quad [\text{kg/m}^3] \tag{5}$$

The norm EN 196-1 “Methods of testing cement – Part 1: Determination of strength” describes the testing of mortar strength. The Walter+Bai DB 250/15 machine was used, it is able to apply a load of 10 kN with an accuracy of ± 1.0%. The flexure device incorporates two steel supporting rollers with a diameter of 10.0 ± 0.5 mm spaced apart 100.0 ± 0.5 mm. A third steel loading roller was placed centrally between the two above the specimen. The three vertical planes through the axes of the rollers need to remain parallel and remain equidistant during the test. To ensure a uniform distribution of the load one of the bottom rollers should be capable of tilting without subjecting the specimen to any torsional stresses. The specimen was then subjected to an increasing load at a rate of 50 ± 10 N/s until the prism broke. The two resulting halves were placed in the compressive testing device that has an accuracy of ± 1.0% of the record load in accordance with EN ISO 7500-1 and provided its load at a rate increasing at 2400 ± 200 N/s. The testing machine was provided with the platens made out of hardened steel with a Vickers hardness of at least HV 600 and a thickness of at least 10 mm and 40.0 ± 0.1 mm wide and long. The flexural strength, R_f, in megapascals was calculated from;

$$R_f = \frac{1.5 * F_f * l}{b^3} \quad [\text{MPa}] \tag{6}$$

In this formula; b is the side of the square section of the prism in millimetres, F_f the applied load in Newton and l the distance between the supports in millimetres. For the compressive test, the compressive strength R_c was calculated by:

$$R_c = \frac{F_c}{1600} \quad [\text{MPa}] \tag{7}$$

In this, F_c is the maximum load at fracture in newtons and 1600 resembles the area of the auxiliary plates (40 x 40 mm) in square millimetres. The mean of the results was calculated and any specimens that vary more than ± 10% from their mean was discarded.

3.2.2. Water absorption

The water absorption, in accordance with NBN B 15-215, was tested on 40x40x160 mm prisms by fully submerging in water at a stable temperature of 20 ± 1°C. Firstly, the specimens were left to cure for 28 days, after which their weight was noted. When noted, the specimens were placed in a basin of water at 20 ± 2°C on supports so the whole surface was in contact with the water. After 7, 14 and 28 days the specimens were taken from the water, excessive water was dried off, and the prisms was measured. After the weight of the specimen after 28 days of submersion were noted, the specimens were dried in an oven at 50°C for at least 72 hours until a constant mass was reached. This constant mass is reached when weight loss for the specimens, weighted with 24 hours in between, a difference smaller than 0.1% of the mass of the specimen is obtained. The results were then expressed as the percentage of weight gain over the dry mass of the specimen given by equation (8)

$$\Delta m\% = \frac{M_x - M_d}{M_d} \quad [\text{w/w\%}] \qquad (8)$$

In which M_d stands for the dry weight and M_x for the weight of the prism after x days of full submersion.

3.2.3. Freeze-thaw resistance

The freeze-thaw resistance was performed in order with CEN/TS 12390-9. The mortar prisms were cut up into square blocks of approximately 40x40x40mm, as can be seen in Figure 3-2, and were subjected to 28 cycles of freeze and thaw with a temperature varying from -17.50 to 22.50°C. The machine used for this experiment was WT3 – 1000/40 by Weiss Technik

Figure 3-2. Testing samples for freeze-thaw resistance

3.2.4. Carbonation

The carbonation depth was evaluated as described in ISO 1920-12:2015, The accelerated carbonation was evaluated on 14, 28 and 56 days of being subjected to 1% of CO_2 in a carbonation chamber, which was a WEKK 0130 by Weiss Technik. Each prism was double coated with epoxy resin SikaCor-277 with 24 hours of drying period between layers to ensure air sealing. By being subjected to a constant concentration of 1% CO_2 in the carbonation chamber, this epoxy layer ensured that carbon dioxide infiltrated the mortar unidirectional. After the prism was subjected to the CO_2 in the carbonation chamber for their respective duration, they were split into three pieces by means of a Brazilian split test where a line load is introduced perpendicular on the length of the prism.

Three of the four created internal faces were then sprayed with phenolphthalein, the colour gradient and their corresponding pH values can be seen in Figure 3-3.

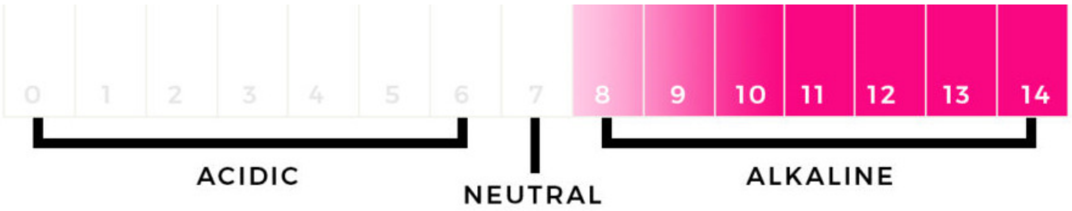

Figure 3-3. Colour gradient of phenolphthalein in function of pH

In fresh mortar the pH level lies above 12.5 while the pH of carbonated mortar drops to a value around 7, this value is below the passivation threshold of steel. The carbonation constant was calculated as seen in (9) [60, 61].

$$k_{ca} = \frac{x_{ca}}{\sqrt{t}} \quad [\text{mm/years}^{0.5}] \tag{9}$$

In which t is the time in years, and x_{ca} is the carbonation depth in concrete after a certain time a.

3.2.5. Hydration

The cement hydration heat was measured by means of calorimetry, in this work, a TAM-air apparatus was used. This machine measured the energy required to keep the sample that is loaded in a glass vial in an isolated chamber at 20 °C. This energy is given in mV. The conversion factor, to convert mV to W, was calculated by calibrating the machine with empty chambers. Afterwards, these values were integrated to calculate the accumulative heat in J/g.

A program that recorded hydration heat every 20 s was programmed and ran for 100 h from the point of when the chamber was loaded with the sample. Seventeen samples, as shown in Table 3-9, are prepared and tested.

As shown in Figure 3-4, the hydration process consists of four discernible stages. Stage 1, the initial hydrolysis, generates great amounts of heat but only lasts around 15 minutes and starts as soon as water and cement are mixed. In this stage, an amorphous layer of hydration products around the cement particles is formed. This layer prevents further dissolution. This rapid heat evolution is thus followed by rapid cooling which transits into stage 2.

Table 3-9. Summary of compositions used to test hydration heat

	Cement	Water	Urea	Yeast	Ca-nitrate
REF	5	2,5			
U1	5	2,5	0,05		
U2	5	2,5	0,10		
U3	5	2,5	0,15		
U4	5	2,5	0,20		
U5	5	2,5	0,25		
Y0,34	5	2,5		0,017	
Y0,85	5	2,5		0,043	
Y1,28	5	2,5		0,064	
Y1,7	5	2,5		0,085	
Y2,56	5	2,5		0,128	
Ca2	5	2,47			0,10
Ca4	5	2,44			0,20
Ca6	5	2,41			0,30
Ca8	5	2,38			0,40
Ca10	5	2,35			0,50
MIX	5	2,38	0,20	0,04	0,40

Stage 2 is the dormant period which can last from one to three hours, the reason why this stage ends is still up to debate and is the reason for much research. However, at the end of stage 2, as discussed in 4.1.2, the initial setting takes places. Therefore, stage 3 is called the hydration acceleration period, and even though the hydration heat is not as high as found in stage 1 it takes place much longer and therefore is the biggest factor in the total hydration heat and much of the strength development takes place here. A bit after the final setting takes place, at the top of the second hydration peak, the hydration heat stagnates and starts to decelerate.

The start of stage 4, therefore, is defined at the moment the second hydration peak reaches his maximum value and is named the diffusion-limited reaction period. After this stage the hydration period proceeds at a very slow rate, this remainder hydration is sometimes called the steady state and can go on for years.

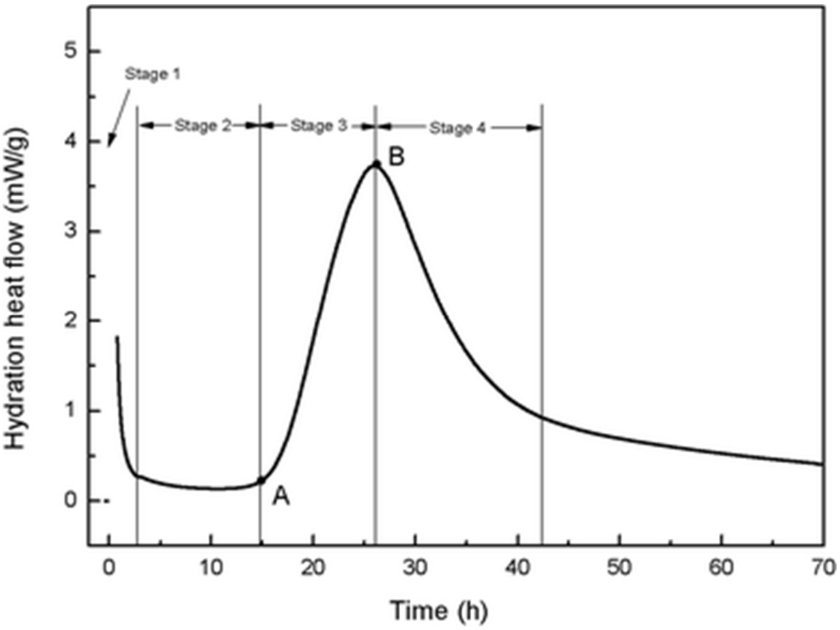

Figure 3-4. Typical hydration curve of cement [62]

3.2.6. $CaCO_3$ content

While many methods are available to identify the presence of $CaCO_3$, many of these are complex, time-consuming or expensive. The most prominent methods are X-ray diffraction TOPAS method (XRD), thermogravimetric analysis method (TGA), ASTM, ICP-AES, titration and washing. The results of these methods vary with XRD, TGA and ASTM giving the most reliable and average values [63]. The main problem with these techniques was the sample size used which produced varying results depending on the location of where the sample was taken. Important points when considering a testing setup are the price, repeatability, ability to test a sizeable sample and accuracy. The proposed testing method is based on the reaction of calcium carbonate with hydrochloric acid in the following reaction.

$$CaCO_{3(s)} + 2HCL_{(aq)} \rightarrow CaCl_{2(aq)} + CO_{2(g)} + H_2O_{(l)} \qquad (10)$$

The conservation of mass law states that the mass of the reactants and products is equal, however, because carbon dioxide is a gas it will result in a decrease in mass of the aqueous products. And thus, from the mass loss, the amount of calcium carbonate can be calculated. Three weights are noted; M1and M2 which are the weights of the sample plus beaker with HCl before reaction and after reaction respectively, and M3 which is calculated from the difference between M1 and M2 and therefore represents the mass of CO_2. Hydrochloric acid is added in abundance so that the calcium carbonate is always the limiting reagent in the reaction.

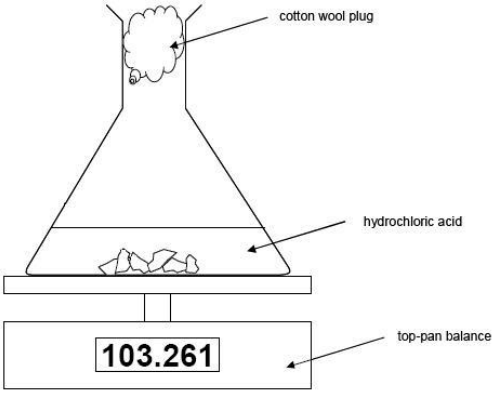

Figure 3-5. Test setup to measure $CaCO_3$ content

A primary test was executed where pure calcium carbonate reacted with hydrochloric acid, from the chemical reaction, as shown above, the theoretical mass loss was calculated. The test was executed for a sample of 0,4 and 0,3 g of $CaCO_3$ reaction with 10 mL of 1 M HCl, which resulted in a theoretical CO_2 mass of 0.1759 and 0.1319 g respectively. The measured weight loss, as shown in Table 3-10 was 0.1769 and 0.1390 g respectively which is an error of less than 0,007 g.

Table 3-10. Test results of pure calcium carbonate

Results (all results are in grams)														
1					2					3				
CaCO3	HCL	M1	M2	M3	CaCO3	HCL	M1	M2	M3	CaCO3	HCL	M1	M2	M3
0,4007	9,88	10,2807	10,11	0,1707	0,4001	10,32	10,7201	10,54	0,1801	0,3998	10,12	10,5198	10,34	0,1798
0,2987	10,07	10,3687	10,23	0,1387	0,2983	10,05	10,3483	10,21	0,1383	0,3001	9,92	10,2201	10,08	0,1401
Average:	stddev:	Difference with theoretical												
0,1769	0,005343	-0,0010	g											
0,1390	0,000945	-0,0071	g											

This proved the viability of this test to not only detect but also quantitatively predict the mass of $CaCO_3$ in a sample.

The prisms were subjected to the three-point flexural and compressive test, of which the results are shown in 4.1.3, afterwards, three ± 5 g samples were obtained. These samples are from the centre of the mortar to avoid any calcium carbonate formed due to carbonation, Figure 3-6 shows the positions where the samples were approximately obtained from. The samples were then mechanically crushed and grounded into a fine powder which resulted in 9 fine powder samples per composition.

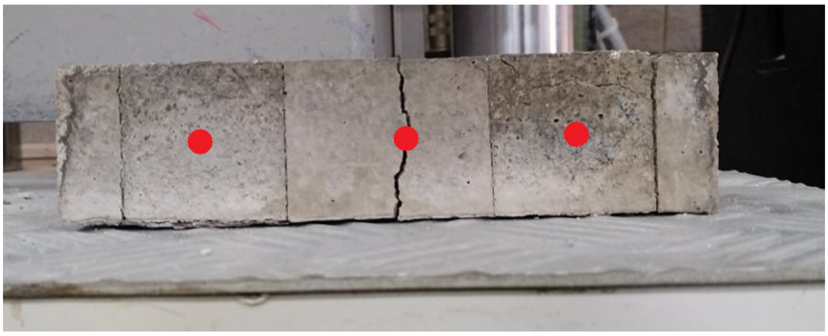

Figure 3-6. Location of the three samples obtained for testing

For each sample ± 2 g was weighed and ± 20 mL of 0.1 M HCl solution was weighed by means of a pipet helper. The combined weight of the sample and solution was calculated after which the sample was introduced to the solution and manually swirled for 10 s. After this, the solution was let to rest on a scale 50 s, that is one minute in total, after which the weight was noted. The reaction during calibration as stated above occurred rapidly, and therefore it was chosen to only let the reaction go on for 60 seconds, this also allows the different samples to be compared and checked for human errors.

3.2.7. Statistical analysis

All data is shown with their respective standard deviants to display the amount of dispersion within a data point. Additionally, within series linear trendlines are formulated with their respective R^2 value to demonstrate a possible linear correlation.

4

Results and discussion

4.1. Results

4.1.1. Flow

The increase in flow-ratio for urea was found to be strongly linearly correlated with the weight to cement weight ratio. The flow ratio of the mortar increased 3, 19, 28, 34 and 41% respectively for U1, U2, U3, U4 and U5 compared to the reference. A linear trendline, as can be seen in (11), was constructed with an R square value of 0.9759.

$$y = 0.0156x + 0.9984 \qquad (11)$$

The reason for this increase in flowability can be attributed to the increase of the solution volume due to the dissolving of urea. Due to its high solubility, urea expands the volume of the residual water for the mortar to use and thereby artificially affects the “water-cement factor” of the composition. This excess of water does not participate in the hydration of cement and leads to an increase in flowability [64]. Additionally, the presence of this extra solution volume leads to an increase in porosity, which is visible in the water absorption test results that were found in 4.2.4. Due to this strong linear correlation, the following formula has been proposed and used in literature as an alternative to the water/cement ratio [4, 17, 26]. Urea does not react with cement, and therefore the solution acts as free water which increase the flowability.

$$\frac{(W + U)}{C} \qquad (12)$$

In which W denotes the weight of water, U that of urea and C that of cement respectively. However, the limit and extent of this formula still have to be tested in future research.

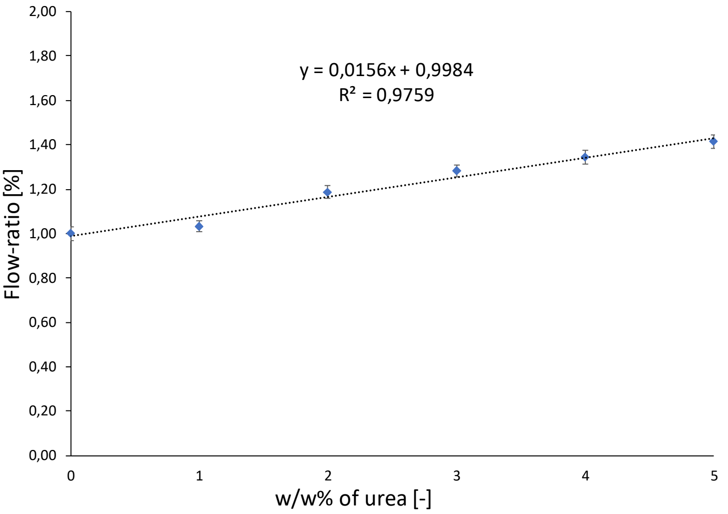

Figure 4-1. Influence of urea on the flow of mortar

This increase in flow with increasing dosage of urea has previously been wrongly explained by a decrease of calcium hydroxide, which crystallizes and partakes in the strength gain in the mortar [4]. The decrease of calcium hydroxide is explained by the formatting of carbonic acid which comes forth out of the hydrolysis of urea.

$$CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3 \quad (13)$$

$$CO_2 + H_2O \rightarrow H_2CO_3 \quad (14)$$

However, this theory is not always applicable with regards to the usage of urea with cement since the hydrolysis of urea into bicarbonate and ammonia requires the enzyme urease [18]. Therefore, urea will, endothermically, dissolve in water but not undergo hydrolysis. This is shown in SEM micrographs that were made of cement pastes as shown in Figure 4-2.

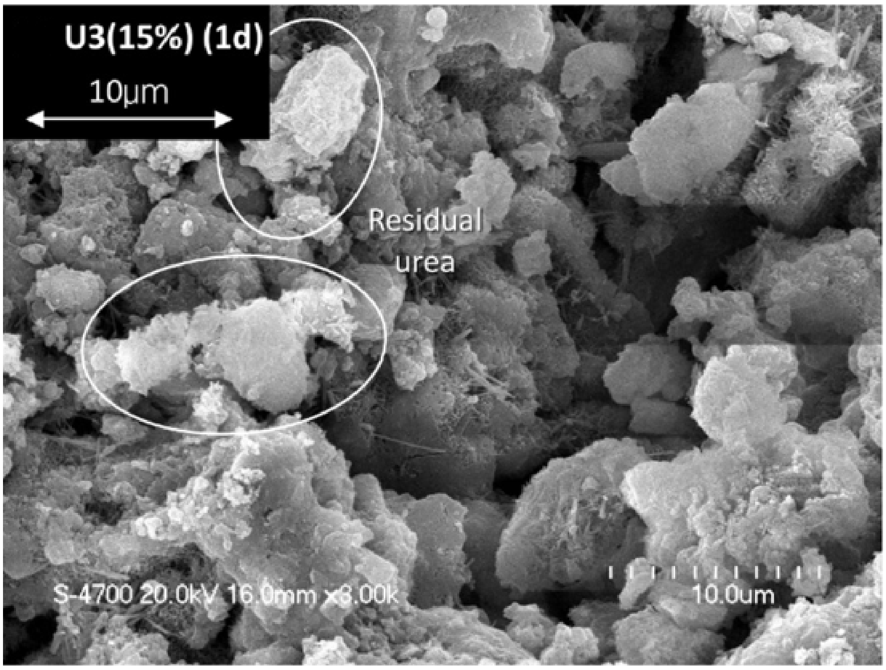

Figure 4-2. SEM micrographs of cement pastes (x3000) [24]

As can be seen in Figure 4-3, yeast extract brought forth an increase of 23% when adding 0.34 w/w% to the mortar mix, and this increase raised linearly through Y0.85, Y0.128 and Y1.27 that caused an increase of 29, 33 and 39% respectively. Lastly, for Y2.56 an increase of 55% was observed. The low R^2 value was a consequence of the big gap in flow-ratio between the reference and Y0.34, additionally, Y2.56 seemed to have a stronger effect as expected. The linear trendline is given by (15).

$$y = 0.0448x + 1.1122 \quad (15)$$

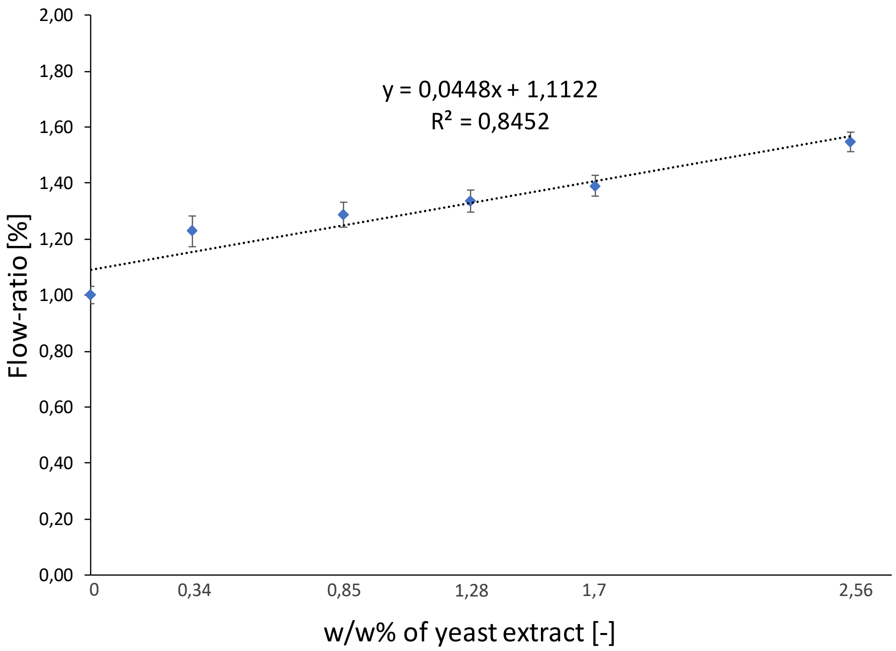

Figure 4-3. Influence of yeast extract on the flow of mortar

Although yeast extract did not extensively increase the water volume due to its high solubility and low dosage, the flow-ratio still increased in a linear correlation to the amount of yeast extract added as can be seen in Figure 4-3. Three paths of thoughts are laid out to declare these effects. The first has been discussed in the literature, the following two mechanisms are possible paths proposed by the author and will need to be verified in future research Firstly, the high soluble carbohydrate content, which is more known under the generic name of sugar, is known as a strong retarding agent [65, 66]. The main reason here is that the interaction of sugar with C_3A prevents the formation of the cubic phase C_3AH_6 (Tricalcium aluminate hydrate) by clogging the surface of C_3A on the cement particle. This aggressive reaction of water with C_3A is also known as “flash setting” as it produces a lot of heat and starts immediately when mixed. However, in 4.1.2, this is challenged. Secondly, yeast autolysate produces nitrogen compounds during its autolysis [67]. This formation of nitrogen gas links back to foam concrete, also known as cellular lightweight concrete. Nitrogen gas is used due to the fact that it is an inert gas that does not modify or react with any of the cement-hydration-products. However, when producing foamed concrete, the input of nitrogen is controlled which guarantees tiny air pockets that do not coalesce or migrate. When yeast extract is added, and autolysis occurs, the process is not controlled. This leads to a non-uniform distribution of pore sizes which can even interconnect which leads to different pathways for gasses and liquids to intrude the mortar matrix. The pores in the yeast extract compositions had interconnecting airpockets that could be verified visually after inspecting the obtained prism halves after destructive strength testing. Lastly, the other proposed path of thought is the production of air bubbles and formation of acidic products due to the degradation of the protein. This is a result of hydrolysis at one or more of its peptide bonds [68]. This chemical process is visualized in Figure 4-4.

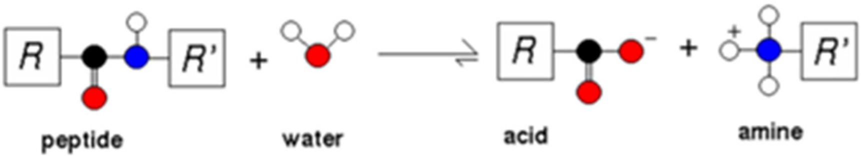

Figure 4-4. Hydrolysis of peptide bonds in an alkaline environment

The flow test does however not accurately describe the flowability of this foamed concrete. An explanation for this can be found on the micro-scale level with the analytical investigation. During the flow table test the height of the pile decreases after every drop, which means that the potential energy E_p decreases as well. This loss in potential energy is consumed in the deformation of the pile and overcoming the interfacial shear at the base of the pile. Firstly, we need to assume that the wet mixture preserves its volume during each drop. The potential energy for each drop is then given by equation (16) [69].

$$(\mathrm{E_p})_\mathrm{i} = \mathrm{w}\left(\mathrm{h_d} + \frac{\mathrm{h_{i-1}} - \mathrm{h_i}}{2}\right) \tag{16}$$

Where w is the weight of the pile, h_d is the height of the drop and h_i is the height of the pile after the i-th drop. This equation shows the linear correlation between the weight of the pile and the potential energy available for that drop.

The flow ratio decreased in linear correlation with the w/w% calcium nitrate introduced to the composition. When a dosage of 8 w/w% was reached the flowability dropped rapidly to the point where no more change in diameter was seen when jolting.

When calcium nitrate dissolves in water, calcium and nitrate ions are made as shown below.

$$Ca(NO_3)_2 + H_2O \rightarrow Ca^{2+} + 2NO_3^- \tag{17}$$

Both the anion and cation show accelerating effects on tri-calcium silicate (C_3S) which is responsible for the early strength gain and first hydration peak [22, 28, 70]. This phenomenon is called “flash setting” and is explained in depth in 4.1.2. The flow-ratio stays relatively even compared to reference up to 6 w/w% calcium nitrate. However, at higher dosages, here tested at 8 and 10 w/w%, the flow-ratio drops drastically up to the point where the pile does no longer spread as can been seen in Figure 4-5.

Figure 4-5. Result of flow test for Ca8

Due to this turnover point at 8 w/w%, it is hard to accurately predict the flow characteristics linearly. Even though a good R² value is reached the linear equation does not adequately predict the flow ratio.

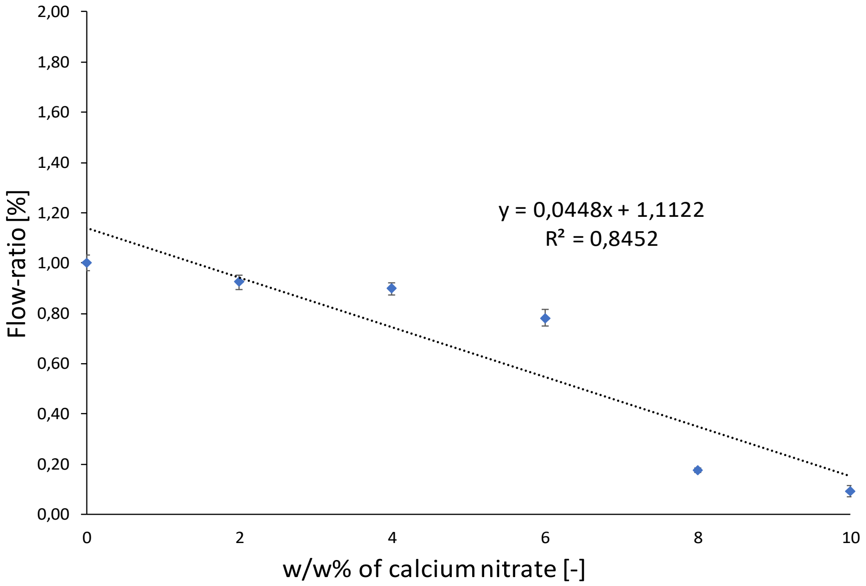

Figure 4-6. Influence of calcium nitrate on the flow of mortar

Allthough the research has shown that some retarders and accelerators can be combined, this is not the case for yeast extract, being the retarder, and calcium nitrate, being the accelerator. [71]. The flow for a young mortar incorporating all bioagents was almost completely inherited from the amount of yeast extract added to the composition. Where Y0.85 has an increase of 29%, MIX increased 33% compared to the reference, this small increase can be explained by the 34% increase that was found when testing U4. This means that the flow of MIX is a superposition of the effects brought forward by yeast extract and urea It is clear that the calcium nitrate mechanism, as explained above, is easily disrupted through the addition of additional bioagents.

As can be seen in the summary of the results in Figure 4-7, both urea and yeast extract show a linear increase in the flow-ratio compares to that of the reference. Calcium nitrate, however, decreases with increasing dosage and at 8 w/w% a strong decline is observed. As can be seen in Figure 4-5, the pile no longer spread out as a result of jolting because of flash setting. Additionally, it is observed that the mix composition, for fresh mortar, is dominated by the characteristic of yeast extract, which means that the flash setting that was observed for Ca8 is delayed by the combined characteristics of urea and yeast extract, with the last being the more predominant one.

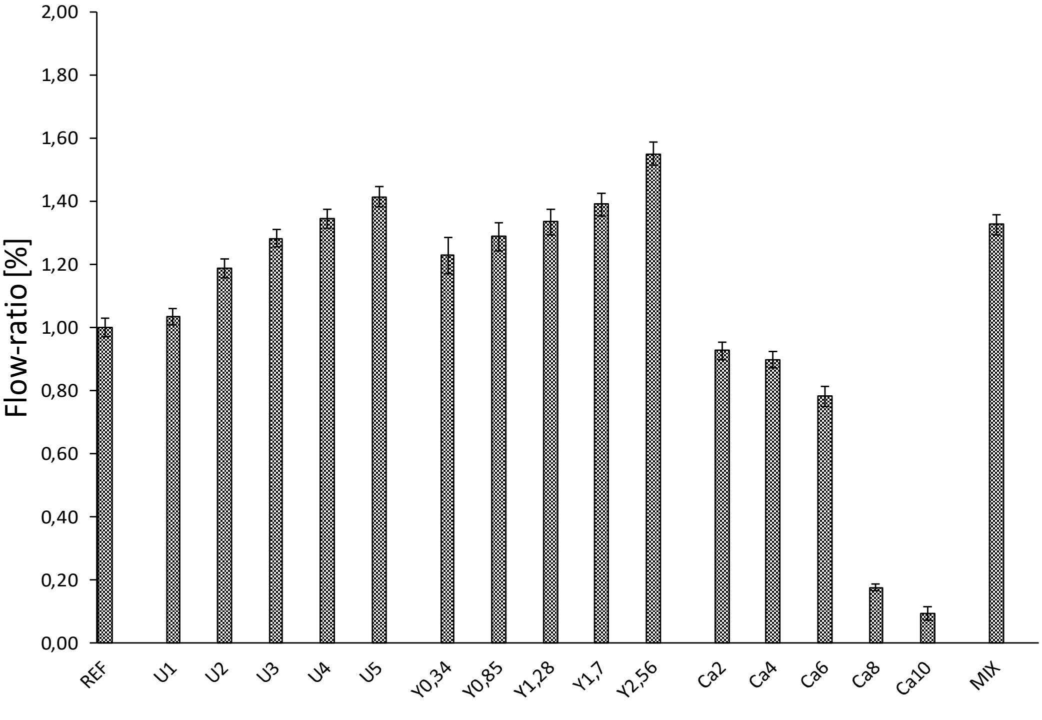

Figure 4-7. Influence of bioagents on the flow of mortar

4.1.2. Setting

The initial setting takes places at the end of the dormant period in the hydration process as can be seen in Figure 4-8. At this time, often referred to as stage 3, the accelerated reaction of alite, which is impure form of tricalcium silicate C_3S, occurs. Thiss leads to the formation of outer calcium silicate hydrate (C-S-H), ettringite rods and portlandite. This outer C-S-H, including its internal gel pores, occupy a greater volume than the original C_3S mineral it forms from. It is due to this outwards expansion that the gel interconnects into a continuous phase which results in initial setting. The aforementioned “outer” C-S-H can also be found in literature as “early” or “low-density” C-S-H gel.

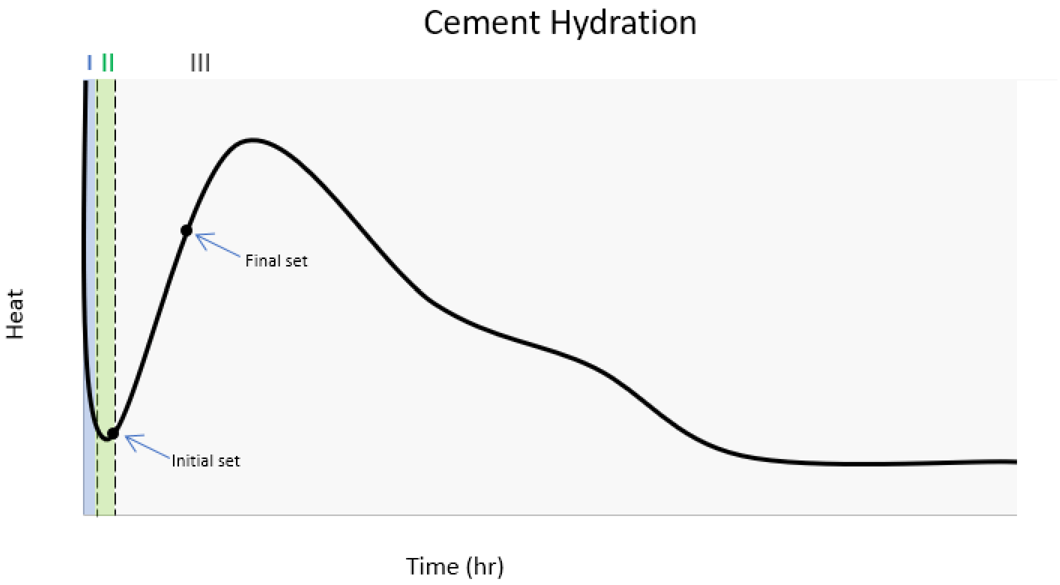

Figure 4-8. Initial and final setting of cement on hydration curve

As can be seen in Figure 4-8, the final setting takes places before the maximum second hydration peak. This is due to the fact that final setting is defined as the time where the Vicat needle can no longer penetrate the concrete surface. The concrete, however, will continue to harden and perform second hydration of tricalcium silicate, which produces long ettringite. This hydration produces “inner”, “late” or “high-density” C-S-H, which forms inside the shell whereas the “early” C-S-H gel formed in the space that was originally filled with water. Due to the densification of the formed hydrate layer, this reaction eventually slows down as can be seen in the gradual decrease of heat in time in Figure 4-8.

As explained above, the hydration reactions at the microscopic level are responsible for these times, therefore the microscopic factors will be discussed and visualised in 4.1.7 while in this section the results are solely explained.

The initial setting and final setting when adding urea are plotted in Figure 4-9 and both showed a strong linear correlation with respect to its w/w%, where an increase in urea lead to an increase in both the initial and final setting time.

The mechanism for this delay is twofold. Firstly, the internal covalent bonds will not split into ions in water. Therefore, the volume of the solution is increased, this increased volume functions as a solution which leads to the formulation of equation (12). A previous research shows that with increased water-cement factor a delay in stage 3 of the hydration curve is found [72, 73]. Secondly, the endothermic reaction of dissolving urea in water causes the hydration heat to decrease with respect to the amount of urea added, therefore, the second hydration peak is not only lowered but also spread out in time which causes a less steep stage 3 and eventually delays the initial and final setting of the concrete.

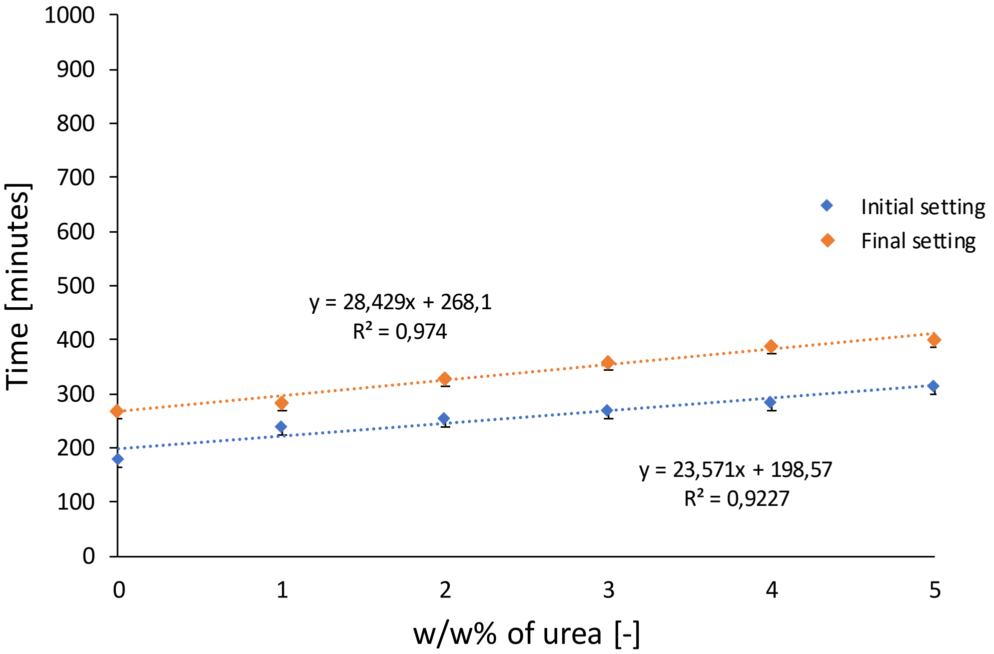

Figure 4-9. Influence of urea on the setting of mortar

Yeast extract acted as a retarder, where both the initial and final setting times were delayed with increasing w/w% of yeast extract. However, when adding 2.56 w/w% of yeast extract a decrease in the initial and final setting time was found. Therefore, it was concluded that yeast extract can both be an accelerator and retarder depending on the amount added.

Yeast extract has been described by its manufacturer as: "… a mixture of amino acids, peptides, water soluble vitamins and carbohydrates". It is its last classification that characterises the effects of yeast extract on the cementitious matrix. Carbohydrates take the form of sugar or starches, with starches being a long molecule of sugars that require digestive enzymes to be broken up. For the longest time, the retarding effect of sugar was explained as a result of the prevention of sugar on the hydration reaction between water and cement. As can be seen however in Figure 4-10, yeast extract only acted as a retarder up to a dosage, which is to be determined more precisely, between 1.7 and 2.56 w/w%. At higher dosages than the aforementioned limit, it acted as an accelerator. These results are confirmed in research where plain sugar was used, the limits they found where this reversed effect occurs are 0.06 w/w% and 0.10 w/w% respectively [74, 75]. This would mean that assuming the limit is 0.10 w/w%, yeast extract consists of 3.91% sugar. Therefore, new mechanisms were proposed to explain and expand the effects of sugar on the cementitious matrix. The four principal ones being; the adsorption theory, which explains retardation as a result of the absorption of sugar on the hydration products, and therefore inhibiting its contact with water. Precipitation theory, which states that the retarder reacts with calcium, hydroxyl or both at the same time in the aqueous phase, this leads to the formation of an insoluble and impermeable layer around the cement grains. The nucleation theory proposes that sugar adsorbs on the nuclei of hydration products, which would poison their future growth [76, 77]. Lastly is the complexation theory. In this theory it is suggested that the calcium ions are chelated by the retarded, which then prevents the formation of nuclei. However, all these theories are plausible, and no research has currently been to interdict any of them. Therefore, it could also be that a combination of the theories mentioned above occurs. The problem is that all these theories predict and explain the retarding effects that occur between 0 and 1.7 w/w% of yeast extract, it is only when a greater amount of yeast extract is added that the theories fall short.

Portland cement consists principally of four compounds; tricalcium silicate (C_3S), dicalcium silicate (C_2S), tricalcium aluminate (C_3A) and tetracalcium aluminoferrite (C_4AF). It is the aluminate phases, in specific C_3A, that reacts rapidly, it is this mechanism, as explained in 4.1.1, that is responsible for flash setting. When adding large quantities of sugar to the mortar mix, most of the gypsum is combined fairly quickly, this leads to gypsum losing its effects as an inhibitor of flash setting, and causes the mortar to set quickly. However, the remaining C_3A does not hydrate for days, this can be seen in the lack of strength development for Y2.56 in Figure 4-24. This means that the rapid setting which occurs due to high amounts of yeast extract is not caused by the acceleration of hydration of C_3S, it is caused exclusively by the reaction of the phases that contain aluminate and the strength is a result of the formation of ettringite. The theory for this is that the high amounts of sugar affect the solubility and rate of solution of C_3A following the reduction of calcium ions in the solution by the presence of the organic admixture [78, 79]. Further research is required to fully understand why sugar acts as a retarded at low dosages, and accelerates setting at high dosages.

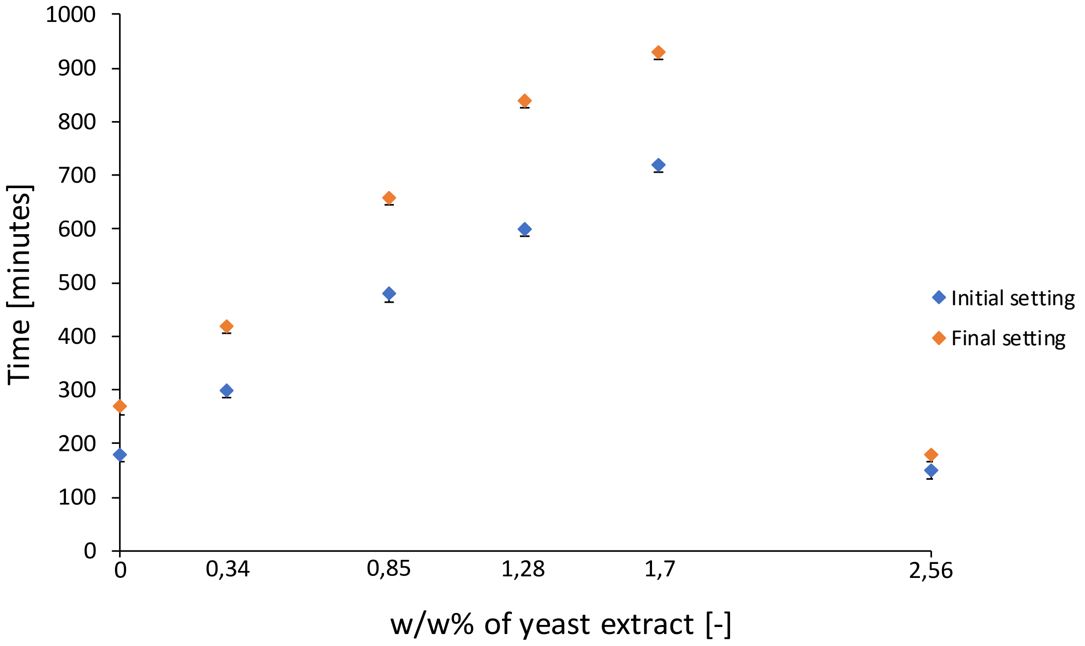

Figure 4-10. Influence of yeast extract on the setting of mortar

It is the author's opinion that this mechanism further provoked a "grouped" effect that was mentioned later for hardened mortar for compositions Y0.85, Y1,28 and Y1.7 as a result of the underlying mechanisms counteracting each other

Addition of calcium nitrate resulted in an acceleration of the setting time. This acceleration was linear with the w/w% of calcium nitrate added.

When calcium nitrate dissolve in water, the calcium ions Ca^{2+} become separated from the nitrate ions NO_3^-. This increased concentration of calcium ions leads to a faster super-saturation of the water with respect to calcium hydroxide. Additionally, the formation of plate calcium aluminate hydrate (C_4AH_x) was observed by XRD measurements [80]. This is the same product that is formed from the hydration of C_3A in pure water as shown in the following reaction.

$$2C_3A \quad + 21H \rightarrow C_4AH_{19} \tag{18}$$

This reaction is so rapid that when it occurs it releases a large amount of heat, which causes the paste to set within minutes. This phenomenon is called "flash setting", not to be confused with "false setting" that can be remedied by mixing again [80, 81]. The reaction shown in (18) is prevented with the addition of gypsum to the Portland cement, the gypsum dissolves and releases sulphate ions into the pore solution, this causes the C_3A to undergo the following reaction.

$$C_3A \quad + 3C\bar{S}H_2 + 26H \rightarrow C_6A\bar{S}_3H_{32} \qquad (19)$$

This last product is mineral ettringite. However, if all gypsum is used, monosulfoaluminate will be created. To summarize, gypsum prevents flash setting by retarding the hydration of the aluminate phases that are present in the concrete. However, when additional aluminate phases are introduced to the mix as a result of adding calcium nitrate, additional calcium aluminate hydrate phases will be formed. Because of this, all the available gypsum gets completely used and monosulfoaluminate is still formed which leads to “flash setting”.

As can be seen in Figure 4-11, a steep decline in initial setting was observed for Ca8 and Ca10, which strongly resembled the strong decline on the flow-ratio for the same compositions as seen in Figure 4-6. Therefore, it can be concluded that the flash setting, as explained above, was prominent starting at dosages of 8 w/w% and was responsible for the decline in both flow-ratio and initial setting.

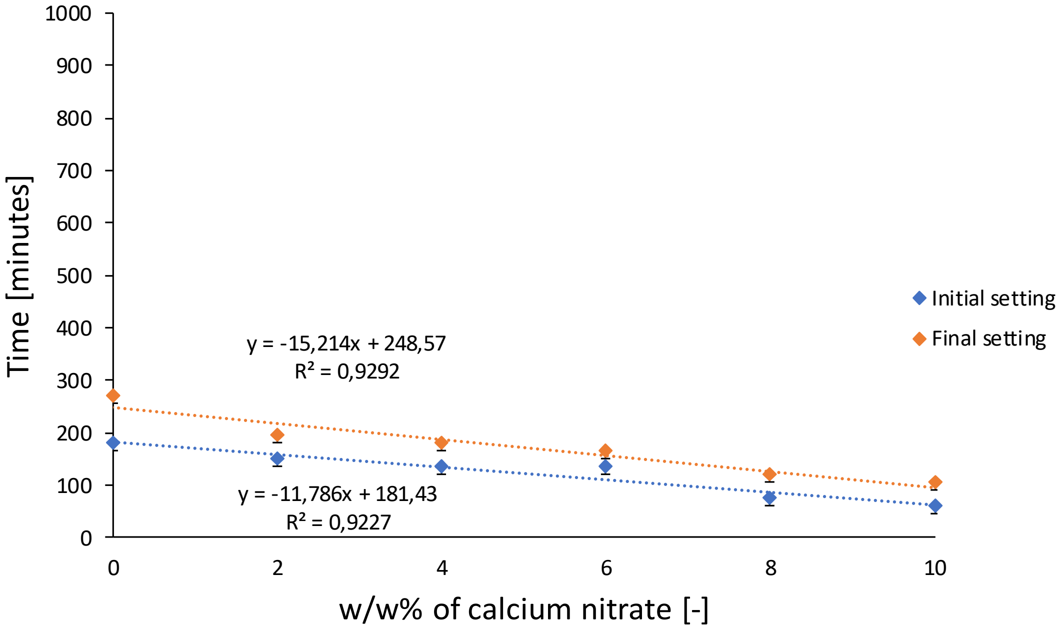

Figure 4-11. Influence of calcium nitrate on the setting of mortar

As Figure 4-12 shows, the setting time of the mix is almost an exact copy of that of Y0.85. This reaffirms that conclusion made in 4.1.2 that yeast extract is the dominant bioagent in the fresh mortar and is the reason for the characteristics found at this early age. The effects of flash setting that comes forth when using 8 w/w% calcium nitrate are completely negated.

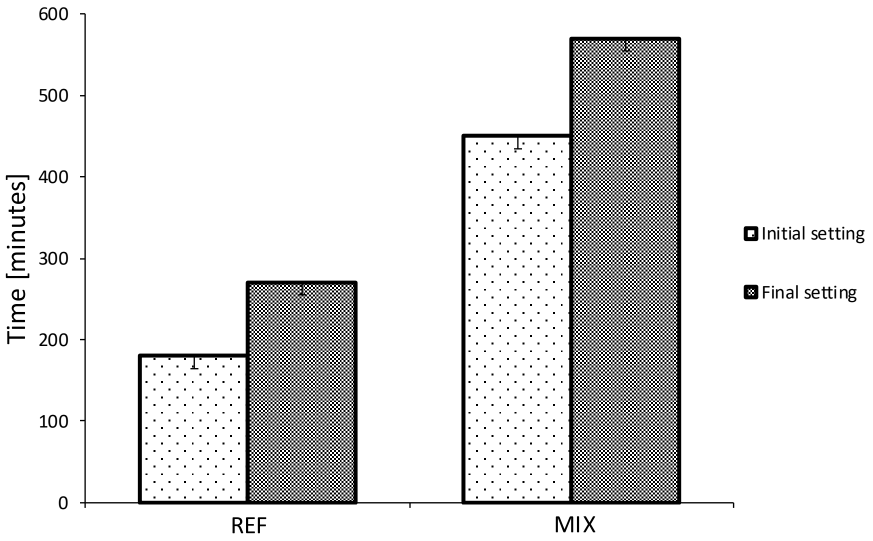

Figure 4-12. Influence of mix on the setting of mortar

The norm mentions that initial setting time should be at least 30 minutes, while final setting should not exceed 600 minutes. This eliminates dosages of solely yeast extract higher than 0.85 w/w% being used, since its final setting takes places much later than 600 minutes after casting. Furthermore, all the effects are visualized in Figure 4-13; the turnover point of yeast extract where it turned from a retarder to accelerator at dosages higher than 1.7 w/w% and the flash setting where initial setting strongly decreased for calcium nitrate dosages greater than 8 w/w%. Urea had a, albeit little, retarding effect due to the increased volume of free solution-water and its endothermic dissolution which is discussed in 4.1.7.

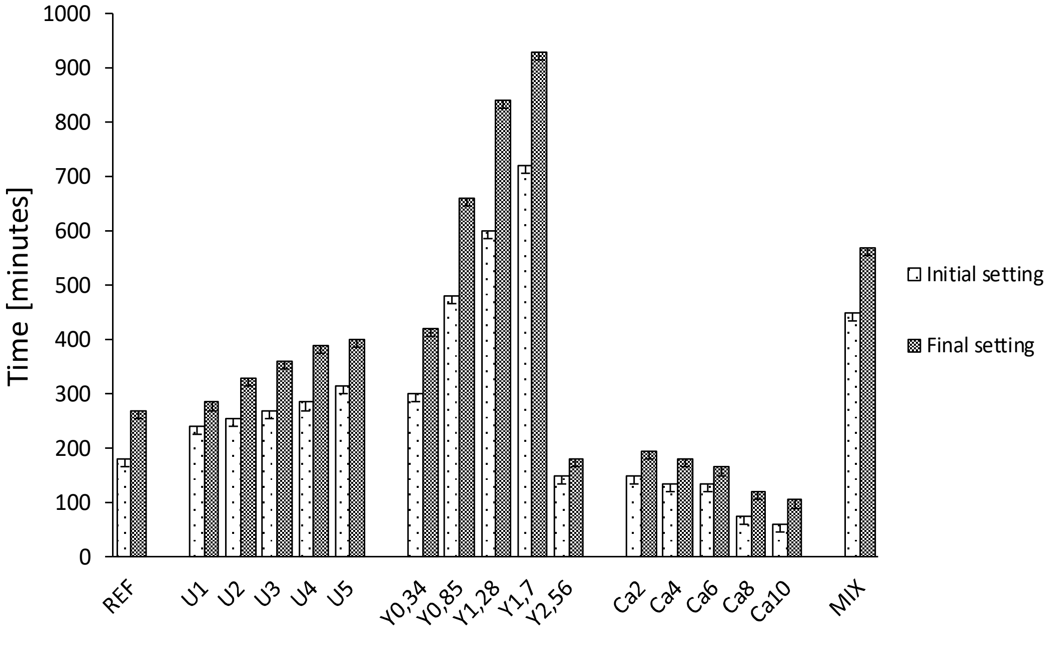

Figure 4-13. Influence of bioagents on the setting time

4.1.3. Mechanical properties

The mortar prisms were demoulded and stored in a curing chamber until their testing age was reached. This curing chamber had a temperature of 20 ± 2 °C and >90% relative humidity. To test for the mechanical properties throughout its hardening process, the prisms were tested at 3, 7, 14, 28 and 56 days respectively after casting. Firstly, the density was calculated from its dimensions and weight, afterwards a three-point bending test was executed to calculate its flexural strength and lastly a compressive test was performed on both of the resulting halves from the flexural bending test. Each bioagent is first discussed separately after which a summary is given that shows the general trends of each bioagent.

An important notice is that during testing the supplier, Holcim N.V., changed their product and, although the composition of the product remained the same, a strong change in strength development was discerned at the age of 7 days. The three overview graphs for all bioagent are also given in addendum for enhanced readability.

4.1.3.1 Density – Bioagents

In Figure 4-14, the influence of urea on the density is plotted. As can be seen, the density decreased in the first 14 days after casting, after which it remained relatively constant. Additionally, while the density stayed relatively equal to reference at a young age, it did not decrease as much as the reference, which lead to the density of mortar incorporating urea being higher than the reference by 100 kg/m³.

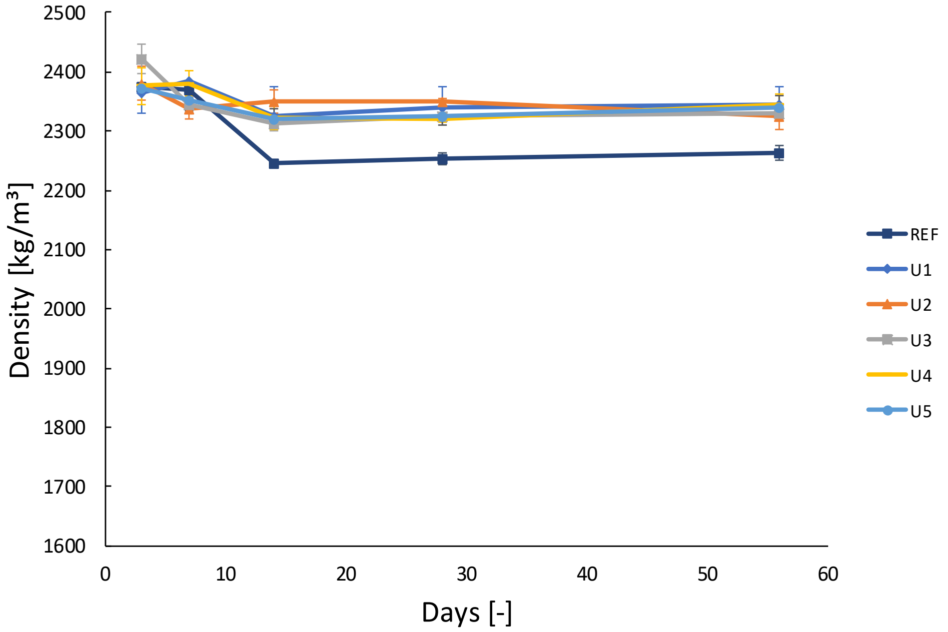

Figure 4-14. Influence of urea on the density – 3, 7, 14, 28 and 56 days

This decrease in density follows from the evaporation of some of the water that did not partake in the hydration process. However, when urea is added, the solution evaporates but leaves behind the urea which results in a higher density. This mechanism does not explain why little difference is found with various w/w% of urea added, it's the author's opinion that this can be explained by the results found in 4.1.7 where is shown that the amount of urea added has a varying effect on the peak hydration temperature.

A strong linear decrease in density when increasing the amount of added yeast extract was observed. Additionally, it was also found that the first drying phase resulted in a much larger weight decrease when compared to when adding urea. Lastly, when adding 2.56 w/w% of yeast extract the density was found to drop to around 1650 kg/m³.

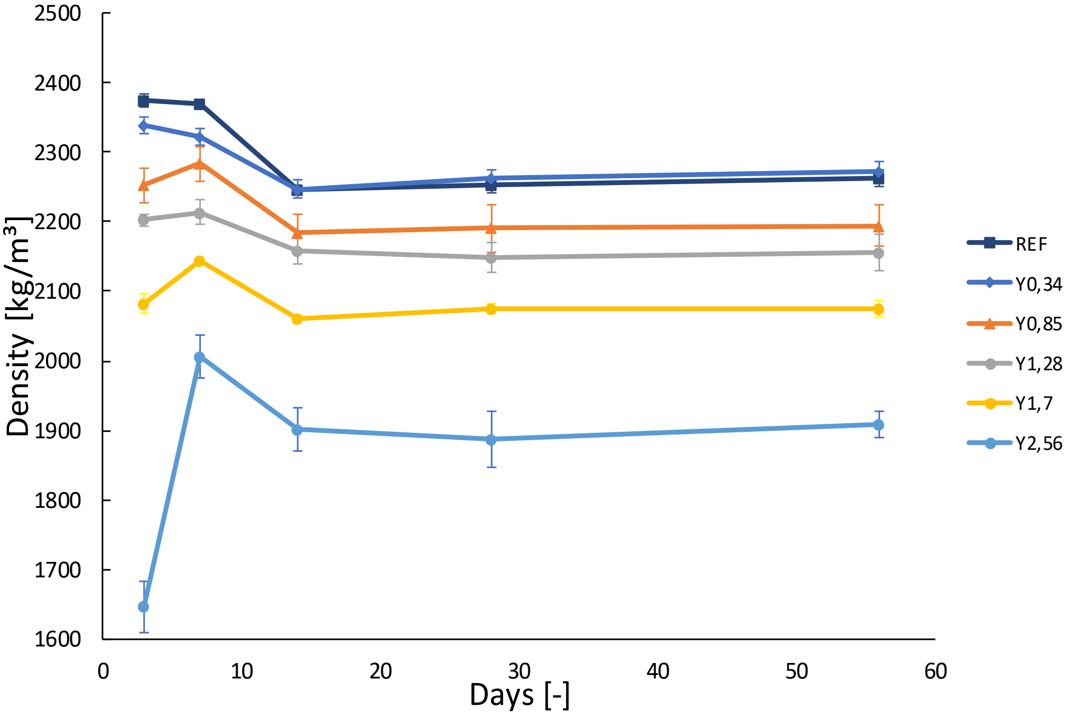

Figure 4-15. Influence of yeast extract on the density – 3, 7, 14, 28 and 56 days

The decrease in density can be explained due to the high protein-content of yeast extract. Protein works as an air-entrapment facilitator and increases the porosity with increasing w/w% of yeast extract as explained in 4.1.4. Furthermore, the steep decrease of Y2.56 at 3 days is a result of the flash setting that occurs due to the high dosage of sugar as explained in 4.1.2. and 4.1.7. In these sections, it is explained that the composition sets fast but has very little strength gain, due to this low strength gain the prisms stick to the mould and break the prisms, as can be seen in Figure 4-16, resulting in a much lower density since it is calculated as a prism with dimension 40x40x160 mm.

Figure 4-16. Damage to Y2.56 when demoulding after 2 days

Lastly, calcium nitrate increased the density of mortar, and this was linearly with increasing w/w% added. As can be seen in Figure 4-17, Both Ca2 and Ca4 had identical density evolutions as the reference, while the density for Ca6, Ca8 and Ca10 increased linearly.

This increase in density is explained as a result of the decrease in porosity which is explained in 4.1.4. Furthermore, the fact that the density decreased much less between casting and 14 days of curing is a result of the flash setting discussed in 4.1.2 and further proven in 4.1.7.

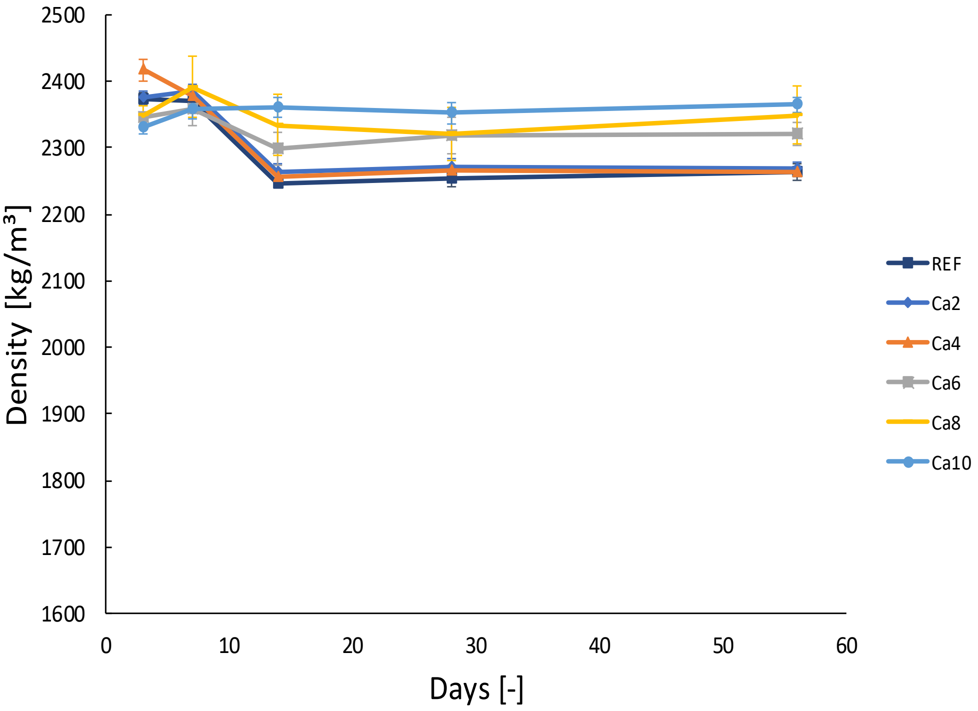

Figure 4-17. Influence of calcium nitrate on the density – 3, 7, 14, 28 and 56 days

A summary of all results is given in Figure 4-18, where each individual influence can be seen. Urea slightly increased the density but remained constant with increasing dosage up to 5 w/w%. Yeast extract strongly decreased the density due to their characteristic of acting as an air-entrapment agent, and calcium nitrate linearly increased density with increasing w/w%. Additionally, the density of MIX was also lower than that of Y0.34, which was the only bioagent that had a negative effect on the density. As mentioned above, it is the author's opinion that this is due to the combined effect of flash setting by Ca8 and the air-entrapment effect of Y0.85 that causes this to happen, however, this still needs to be confirmed in future research.

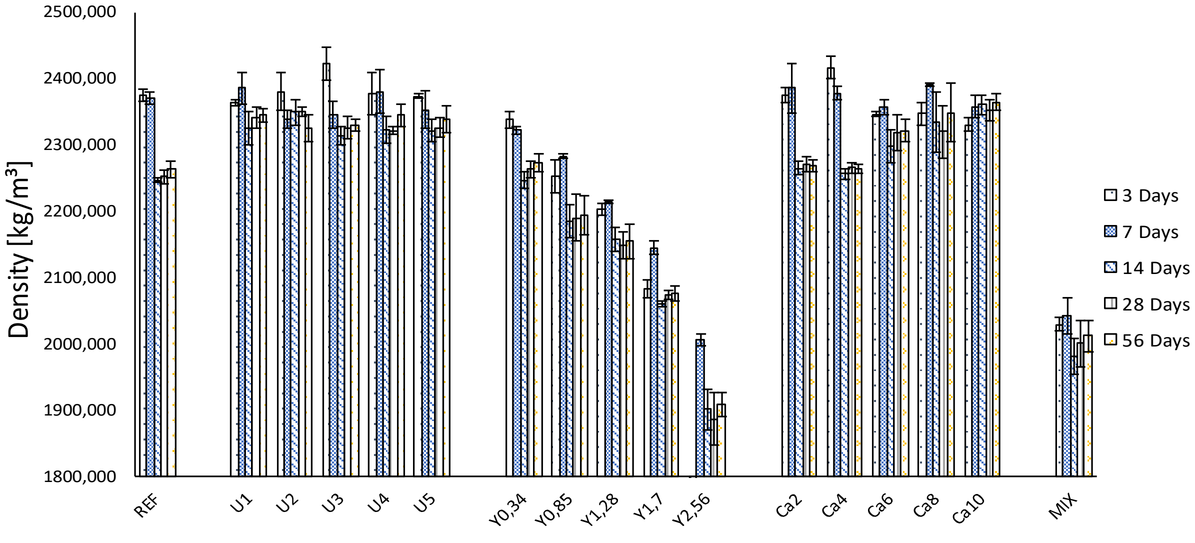

Figure 4-18. Influence of bioagents on the density – 3, 7, 14, 28 and 56 days

In what follows the results of both the flexural and compressive strength tests are given. During the casting of prisms, the manufacturer of cement changed their product, and even though the same characteristics were promised, a clear difference can be observed. Therefore, results of samples tested at 3 and 7 days should be viewed separately from the results after 14, 28 and 56 days. It was found that the strength of the cement used for testing at 3 and 7 days gained straight much faster, and thus, results will be discussed as if these values follow the general expected density and strength development. Additionally, the results should not be seen as exact values but should be used to investigate the effects of a certain bioagent.

4.1.3.2 *Flexural strength – bioagents*

In Figure 4-19 the influence of urea on the flexural strength is shown. The flexural strength increased rapidly after casting and slowed down at 3 days, after which the flexural strength gradually increased to a maximum value after 28 days. Further, the strength slightly decreased. U1 and U2 showed a slightly higher flexural strength at early ages, but after 56 days all compositions showed comparable strength to that of REF.

The fact that the above-mentioned change in cement has an impact on the results, is observed due to the fact that the prisms with urea had a higher strength than REF. This should not be true, as shown in 4.1.7 and 4.1.2, where respectively a lower cumulative hydration heat and higher solution to cement factor were found. This lowers the flexural strength at early age. The same conclusion was also found in the literature [4]. The fact that U1 and U2 had a higher flexural strength at ages between 14 and 28 days is explained by the low impact of the endothermic cooling of urea as shown in 4.1.7. It can be concluded that urea has an impactful effect where it lowers the flexural strength at early ages. However, due to the comparable cumulative heat after 56 days, the flexural strength was comparable to that of REF.

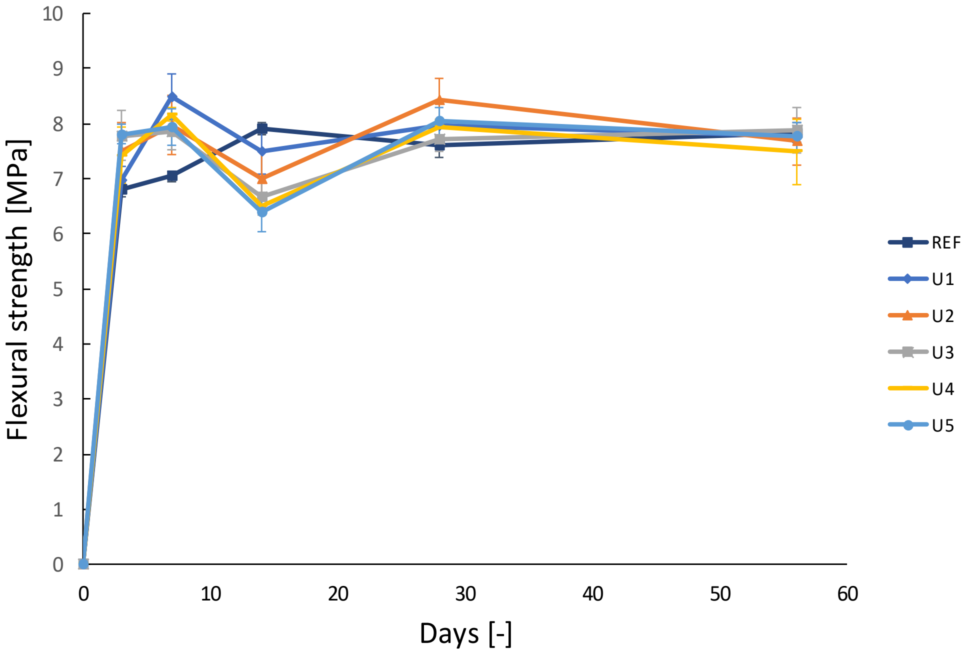

Figure 4-19. Influence of urea on the flexural strength – 3, 7, 14, 28 and 56 days

The flexural strength when incorporating yeast extract, which can be seen in Figure 4-20, showed strong similarities to that of its density, where the flexural strength decreased with increasing w/w% of yeast extract. Two peculiar results were observed, which were the low flexural strength of Y2.56 at 3 days and a higher flexural strength of Y0.34 than REF at 56 days.

The strong decrease in early strength is a result of the retarding effect of yeast extract as explained in 4.2.3. Although the flexural strength still gradually increases after 14 days, it is unable to make up for the lost hydration heat at the early stage. The fact that Y2.56 shows almost no flexural strength at 3 d is the result of the flash setting that a high dosage of sugar causes as explained in 4.1.2. Furthermore, a peculiar observation is found for the flexural strength of Y0.34 at 56 days, where a value higher than that of reference is found. However, as indicated by the high standard deviation this is more likely a one-off result and a more realistic value would be 7 MPa. At 56 days the flexural strengths are 108, 75, 68, 58 and 45% of Y0.3, Y0.85, Y1.28, Y1.7 and Y2.56 respectively compared to the reference.

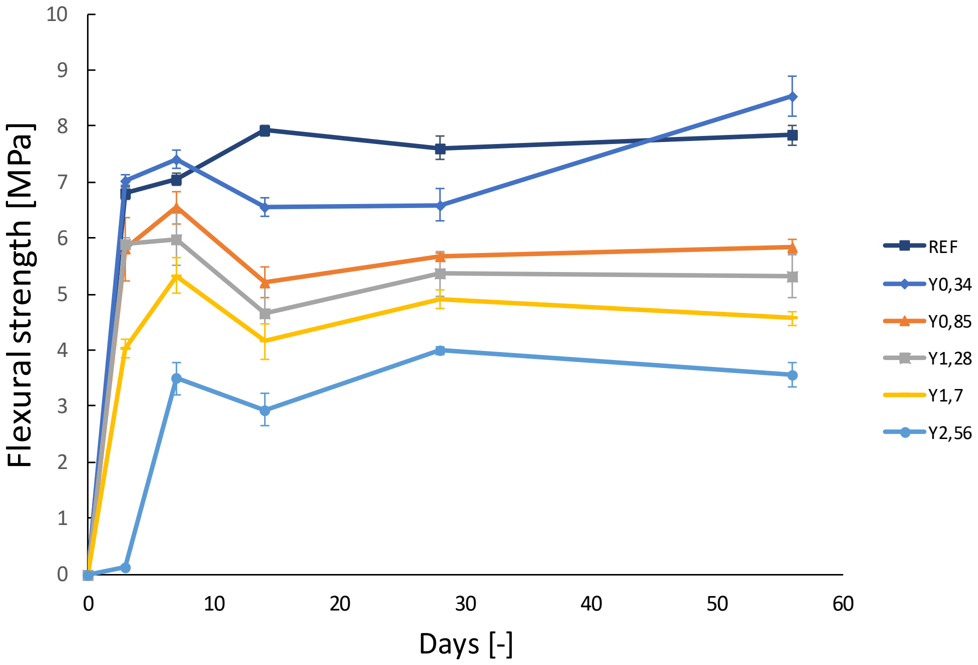

Figure 4-20. Influence of yeast extract on the flexural strength – 3, 7, 14, 28 and 56 days

Shown in Figure 4-21 is the flexural strength for mortar with calcium nitrate. At the early age of 3 and 7 days, Ca8 and Ca10 showed much lower strengths than that of REF and low w/w% dosages of calcium nitrate. However, at the later age, this observation reversed and Ca8 and Ca10 showed higher flexural strengths than REF while Ca2, Ca4 and Ca6 showed strengths lower than that of REF.

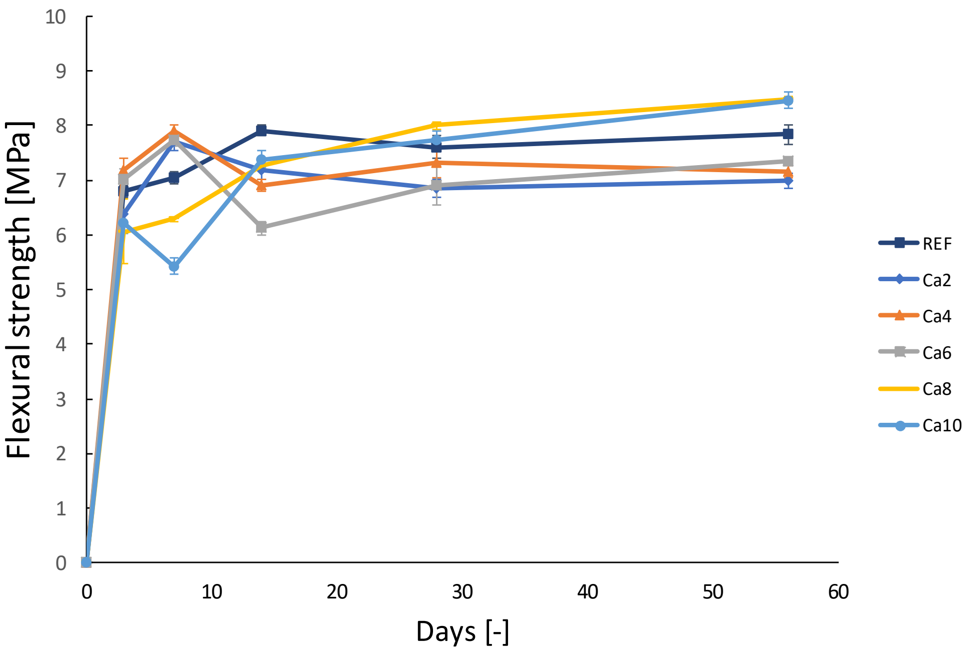

Figure 4-21. Influence of calcium nitrate on the flexural strength – 3, 7, 14, 28 and 56 days

As shown and explained in 4.1.8, the flash setting that occurred at a dosage of 8 and 10 w/w% produced a small but high hydration peak that in comparison to REF and lower dosages leads to less cumulative heat at an early age. However, as time progressed and hydration continued, these two compositions produced far more heat which lead to a higher strength. At dosages lower than 8 w/w% however, a lower strength was found, which leads to the conclusion that calcium nitrate is not a hardening accelerator. This is also found in the literature [82].

Figure 4-22 summarizes the results found in this subsection. As can be seen, urea had an almost inert, slightly improving effects, yeast extract decreased the flexural strength with increasing w/w% and calcium nitrate improved the flexural strength at dosages of 8 w/w% and higher. The results found for MIX were lower than the superposition of all the effects, the author follows the same reasoning for this as mentioned before, which explains the combined effect of yeast extract and calcium nitrate.

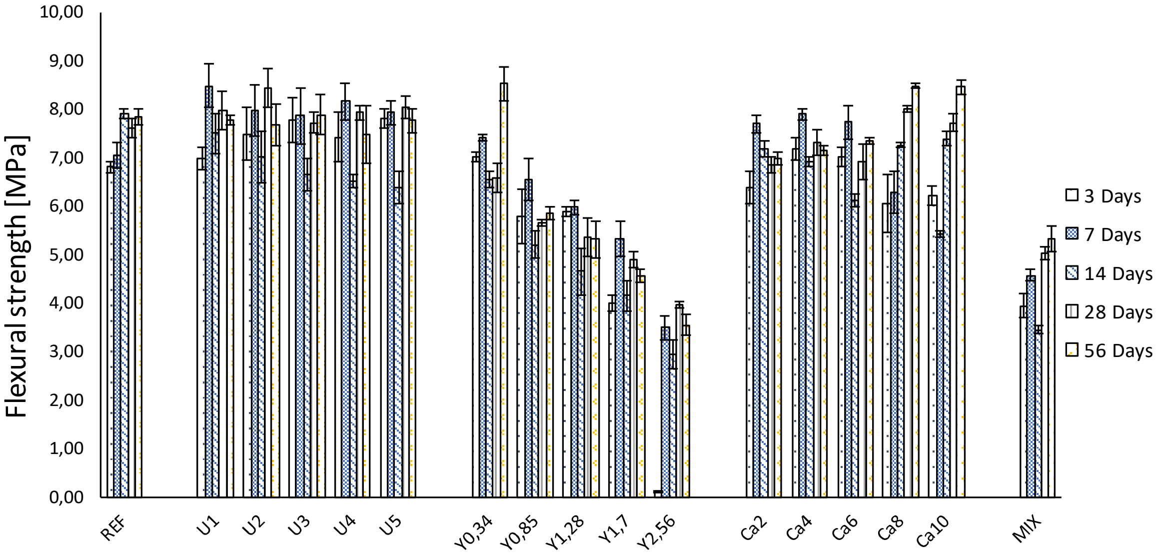

Figure 4-22. Influence of bioagents on the flexural strength – 3, 7, 14, 28 and 56 days

4.1.3.3 *Compressive strength – Bioagents*

With regards to the compressive strength, urea, as shown in Figure 4-23, shows a positive influence. While U1, U2 and U3 show greater compressive strength than the reference at all testing moment, U4 and U5 only achieve the same compressive strength as the reference at 56 days. The optimal compressive strength is achieved when incorporating 2 w/w% of urea in the composition;

The fact that U1, U2 and U3 showed the most positive effect on the compressive strength is identical to that explained in the subsection of flexural strength and was thus fully explained in 4.1.7. The endothermic reaction was not able to influence the hydration heat at dosages lower than 4 w/w%. Due to this greater hydration heat the compressive strength was higher. Additionally, the lower porosity, as described in 4.1.4 helped with the compressive strength. In other research it was often concluded that urea solely has a negative effect on the compressive strength [19]. However, this is because these works use dosages of 0, 5 and 10 w/w%. In this work it was found that the maximum compressive strength was reached at 2 w/w%, and therefore it can be concluded that urea does have a positive effect on the compressive, however its w/w% should be limited, which was not done in other works, to find this maximum.

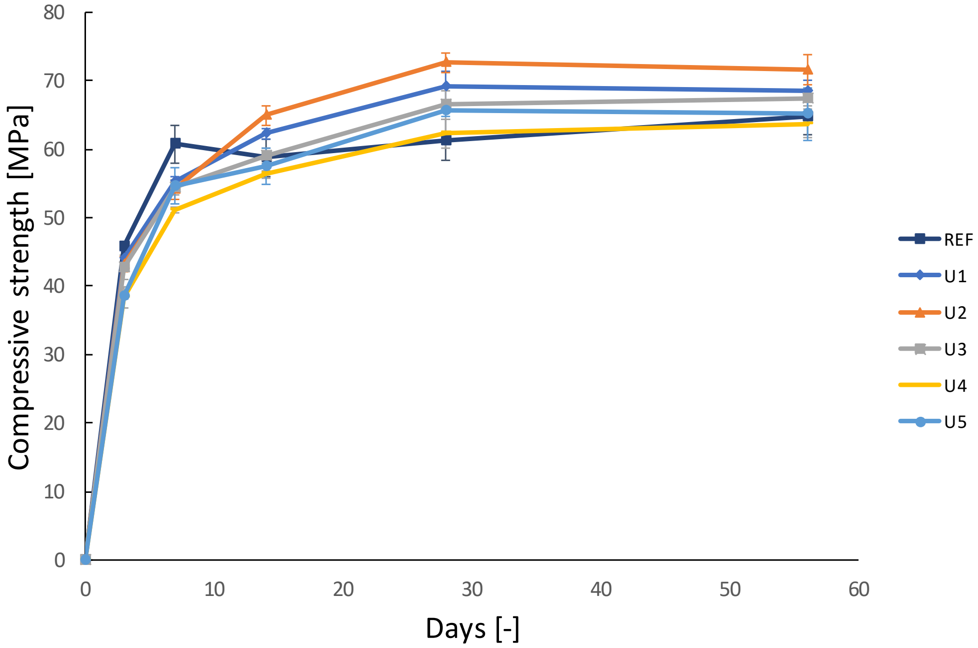

Figure 4-23. Influence of urea on the compressive strength – 3, 7, 14, 28 and 56 days

In Figure 4-24, the effect of yeast extract on the compressive strength is shown. As can be seen, yeast extract decreased the compressive strength at all ages with increasing w/w%. And, similarly to its effects on the flexural strength, Y2.56 showed very low strength at 3 days.

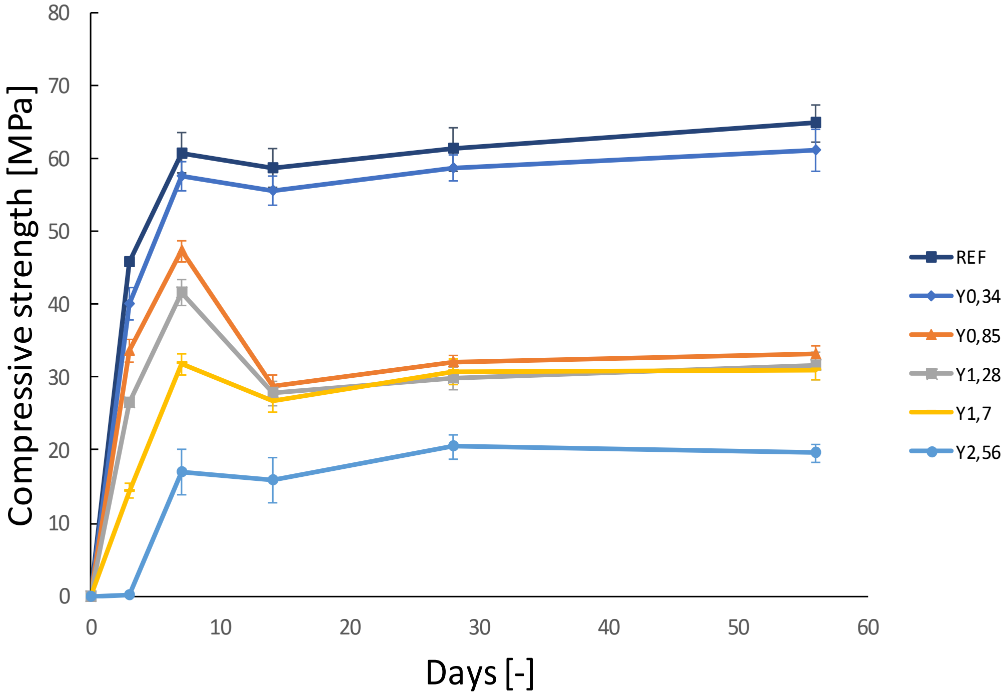

Figure 4-24. Influence of yeast extract on the compressive strength – 3, 7, 14, 28 and 56 days

As expected, in line with the results found in 4.1.4 and 4.1.7, yeast extract heavily decreased the compressive strength as a result of the mechanisms discussed in the aforementioned subsections. Peculiar here again is the “grouped” characteristics of Y0.85, Y1.28 and Y1.7 that almost showed identical strengths at ages beyond 14 days. These effects seem to make a recurring appearance throughout this work and almost indicates as if there are certain “classes” of w/w% that propagate identical results. However, at the time of writing, no viable explanation for this could be found and thus this is a base for future research. The low compressive strength of Y2.56 at 3 days was again the result of the accelerating effect on the setting due to a high dosage of sugar as discusses in 4.1.2.

Lastly, for calcium nitrate, the results for the compressive tests are found in Figure 4-25. These results showed a higher increase in early strength for Ca2, Ca4 and Ca6 compared to Ca8 and Ca10, while, for later ages, the opposite was true. In general, it can be stated that calcium nitrate improved the compressive strength at ages 28 days and after with increasing w/w%.

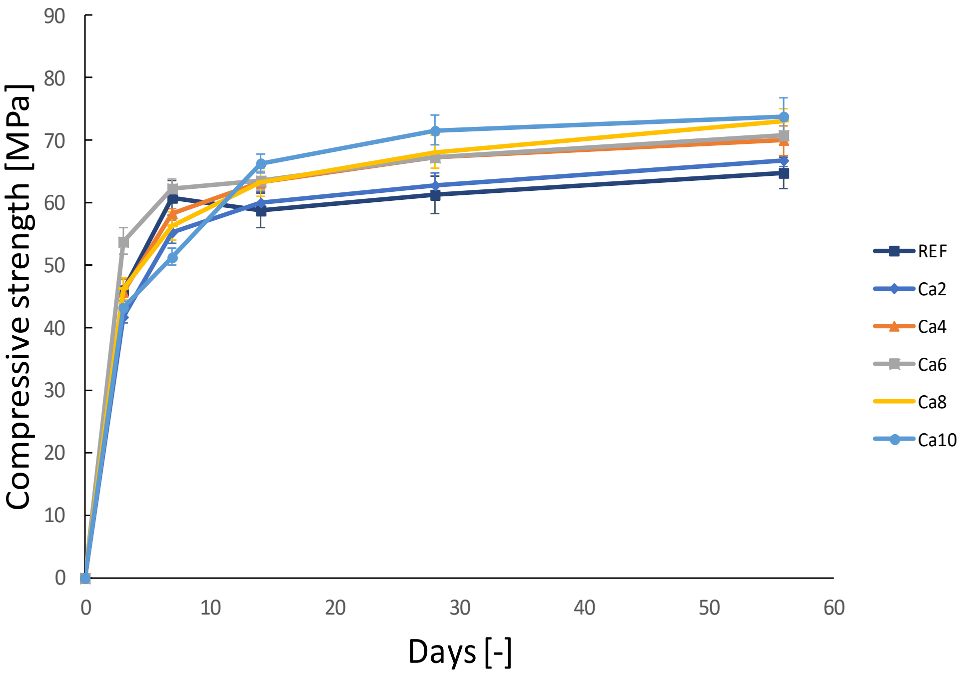

Figure 4-25. Influence of calcium nitrate on the compressive strength – 3, 7, 14, 28 and 56 days

The increase in compressive that is found is the result of the denser structure that is formed by the addition of calcium nitrate, the mechanism for this densification is discussed in 4.1.4. Similar to the results found for the effects of calcium nitrate on the flexural strength, Ca8 and Ca10 showed lower early strength compares to other, lower, dosages. The reason for this is the short burst of heat that occurred directly after casting and results in the flash setting as discussed in 4.1.2 and verified by hydration testing in 4.1.7.

The summary of results is shown in Figure 4-26, it is clear that for all bioagents the compressive strength increased with increased curing age, with the exception of yeast extract that showed the higher strength due to the problem stated at the start of this section. Urea showed both positive and negative effects depending on the w/w% of urea used, the maximal compressive strength was found for U2. Yeast extract showed a decrease in compressive strength with increasing w/w%, however, the “grouped” effect of Y0.85, Y1.28 and Y1.7 is found again. The addition of calcium nitrate hindered the strength development at early ages, however, more importantly, increased the compressive strength at later ages with increasing w/w%. The MIX composition showed similar characteristics as Y0.85, but the strength did not increase as one would expect when averaging the effects of all bioagents. This again strengthens the author's case that because combination where yeast extract acts as an air-entrapment agent and calcium nitrate shows flash setting the porosity remains at such a high level that it inherits all characteristics of the yeast extract component.

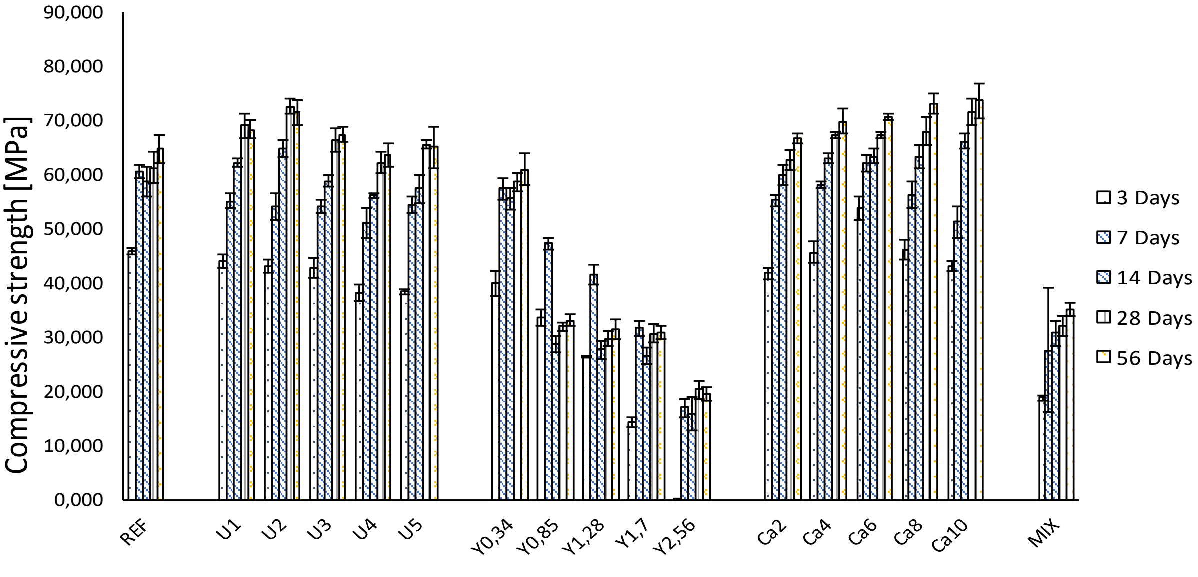

Figure 4-26. Influence of bioagents on the compressive strength – 3, 7, 14, 28 and 56 days

4.1.3.4 *Density - Bacterial*

As shown in Figure 4-27, it can be concluded that the addition of yeast extract (T4 and T5), and the addition of a grown culture in UYE medium (T6) have a negative, impact on the density of mortar at all ages. While the density for all other compositions stays relatively constant.

Peculiar, for T2, the positive impact on the density that urea brings, as seen in 4.1.3.1, was not found when adding bacterial cells. This is because the urease enzyme is present in the bacterial solution results in ureolysis by adding urea and therefore the density remains constant. However, to confirm this theory, future research should test if urea gets decomposed.

It was noticeable that only for T4, T5 and T6, a decrease in density was found. For T4 and T5 this corresponds to the findings mentioned earlier and is explained due to the addition of yeast extract.

Furthermore, by incorporating the bacterial cells in a spent UYE medium, the density also dropped. In T6 the grown culture was introduced in a spent UYE medium that consisted of 20 $g.L^{-1}$ yeast extract and 22 $g.L^{-1}$ urea with a bacterial concentration of 10^8 cells/mL, strong ammonia smell was perceived which is a result of the following reaction taking place;

$$CO(NH_2)_2 + 2H_2O \rightarrow H_2CO_3 + 2NH_3 \quad (20)$$

$$H_2CO_3 + Ca(OH)_2 \rightarrow CaCO_3 + 2H_2O \quad (21)$$

It is because of this second reaction that, in 4.1.8, this composition is also tested for $CaCO_3$ content.

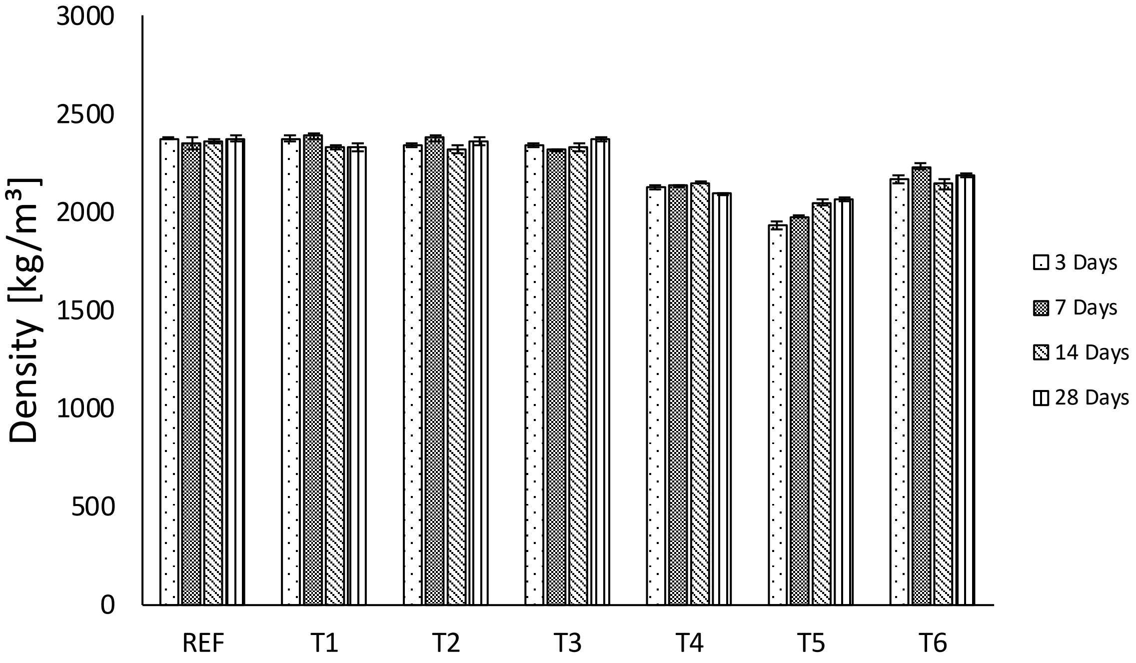

Figure 4-27. Influence of bio-concrete on the density – 3, 7, 14 and 28 days

4.1.3.5 Flexural strength – Bacterial

Figure 4-28 shows the impact of bacterial cells combined with bioagents on the flexural strength of mortar prisms. While T1 and T2 showed relatively similar results compared to the reference, T3, T4, T5 and T6 all showed a strong decrease in flexural strength.

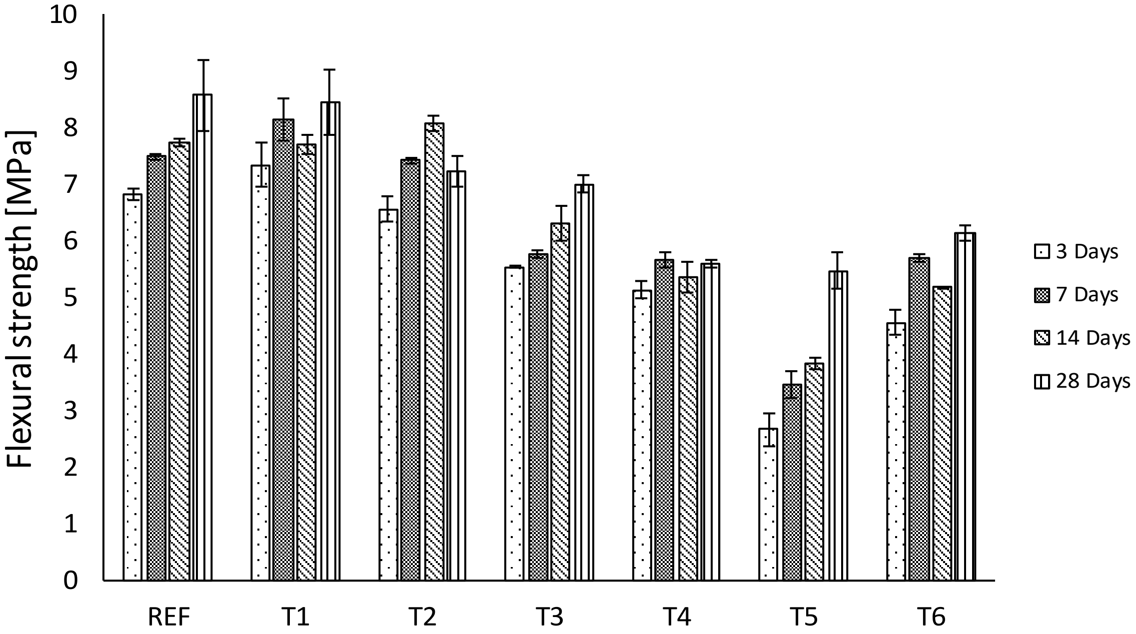

Figure 4-28. Influence of bio-concrete on the flexural strength – 3, 7, 14 and 28 days

The flexural strength of T1 increased with respect to the reference, however, this gain is not significant. An explanation for the increase in strength might the harsh survival for the bacteria by densification of the mortar as a result of the hydration process and the lack of nourishment, causing the bacteria to die and remain in the mortar mix acting as an organic fibre that slightly increases the flexural strength at early ages [83]. This would explain the ability of the organic fibres to help with the negative moment that is induced in the three-point bending test by acting as organic reinforcement.

For T2 both for early and late flexural strength, a negative effect was observed. As seen above, urea has a negligible effect on the flexural strength but a positive effect on the compressive strength. However, when including bacteria, ureolysis, which forms ammonia and carbonic acid, happens. It is this carbonic acid that will aggravate the cementitious matrix. One may except the formation of $CaCO_3$ here, however, no strength gain, nor increase in calcium carbonate, as can be seen in 4.1.8, was found. It is the author's opinion that this could be caused by the lack of calcium ions. Two reactions can occur [84];

$$CaCO_3 + CO_3^{2-} \rightarrow Ca(HCO_3)_2 \qquad (22)$$

$$CSH\ gel + H^{+} \rightarrow Si(OH)_4 + H_2O + Ca^{2+} \quad (23)$$

It is this deterioration of the cementitious matrix that lowers both the flexural strength at an early age. Additionally, the increased solution volume, as discussed before, aids in the lowering of mechanical properties.

Although calcium nitrate has a positive effect on both strengths at 8 w/w%, as shown above, the compressive and flexural strength further decreased for T3. Primarily, one can think that due to the increase of calcium ions additional calcium carbonate can be formed by reaction with carbonate ions, however, it’s the author's opinion that as a result of the accelerated setting a more porous material is obtained that provides an easier pathway for carbonic acid to affect the cementitious matrix. Therefore, in future research, it should be priority to investigate the microstructure.

Similar effects when adding yeast extract to the neat mortar were found in bacterial concrete, where the compressive strength decreases rapidly. As can be seen by the decrease in density in Figure 4-27, the same mechanisms as when adding yeast extract to neat mortar are responsible for its effects. When also adding calcium nitrate to these compositions, as seen in T5, the mechanical properties decrease even further to the lowest values of the series, it is the author’s opinion that the same mechanisms are at work as proposed earlier, where the flash setting causing a large porosity as a result of the air entrapment function of yeast extract.

T6 showed similar flexural strength as T4, this because the same mechanism takes places. The only difference is that for T6, the grown culture was not resuspended. The bacterial cells can undergo ureolysis before being mixed, this way the negative effects of the yeast extract are somewhat negated. However, this seems to have minimal impact since the strengths are comparable.

4.1.3.6 Compressive strength - Bacterial

As can be seen in Figure 4-29, the compressive strength seems to be closely related to the flexural strength. T2, T3, T4, T5 and T6 all showed a decrease in compressive strength, where T1 showed similar strength compared to the reference.

For T1 the addition of bacteria had no effects on the compressive strength, the pathway where dead bacteria acted as an organic fibre only has an effect on the tensile strength.

For T2, when adding urea alongside bacteria, however, ureolysis takes place. It is the same deteriorating mechanism, as discussed above for flexural strength, that leads to the decrease of compressive strength at an early age. However, at later ages, strength gains more rapidly to reference, which leads to an overall small negative effect.

In T3, where urea and calcium nitrate were added alongside bacterial cells, the early strength decreased even further. A combination of a high solution volume, as a result of urea, and the flash setting by means of calcium nitrate leads to a weak early strength with less chance of bacterial strength recovery due to its high density. Due to this high density, the bacterial cells face harsh survival conditions, leading to a low alive cell count at later ages. Furthermore, the lack of nourishment, in the form of yeast extract, for the bacterial decreases the chance of survival.

In both T4 and T5, yeast extract further decreased the compressive strength by increasing the porosity as described above and in 4.1.1 as well as 4.1.2. The later composition further increased the porosity due to the addition of calcium nitrate, the mechanism for this has been explained above but requires future research to be confirmed.

Lastly, a decreased compressive strength was also found for T6. Due to the strong ammonium smell that was perceived when mixing this composition, the explanation, as found above, is that the ammonium ions deteriorates the cementitious matrix [85].

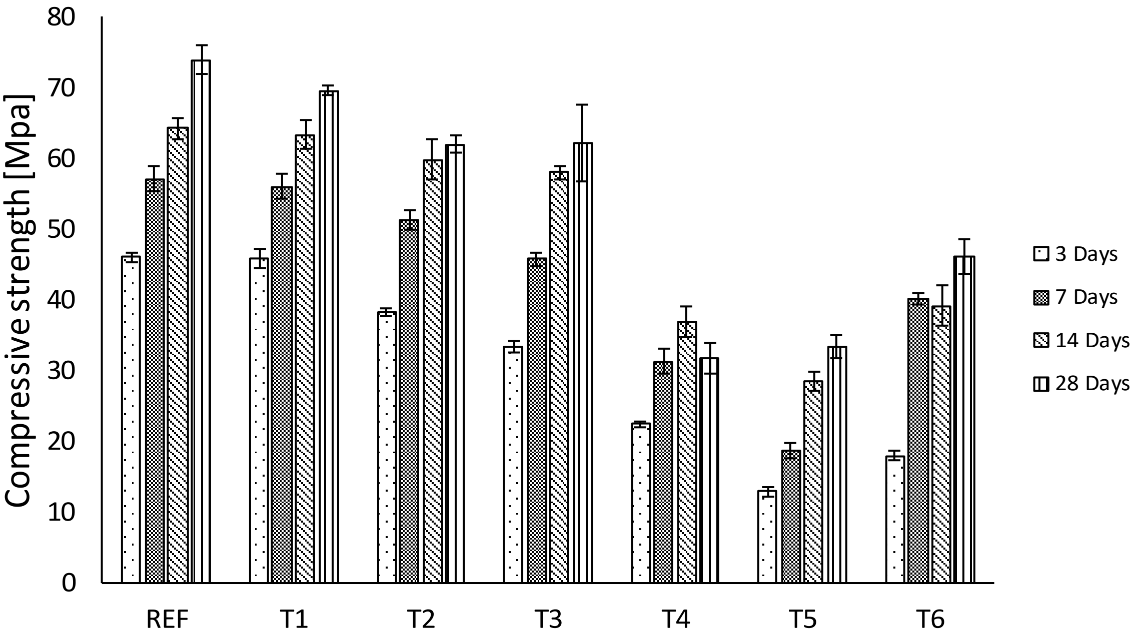

Figure 4-29. Influence of bio-concrete on the compressive strength – 3, 7, 14 and 28 days

It is important to note that the compressive strength after 28 days when incorporating bacterial cells was the same as one where just the bioagents were added.

4.1.4. Water absorption

As can be seen in Figure 4-30, urea had little effect on water absorption, no visible difference in water absorption was observed.

Due to the fact that urea, $H_2N\text{-}CO\text{-}NH_2$, has internal covalent bonds, it will not split into ions in a water medium [86]. This hydrolysis only happens in presence of urease [87, 88]. However due to (12), which adjusts the water-cement factor by including the volume of urea, one may think the water absorption would increase due to the increased porosity that results from an increase water-cement factor [89]. This is not the case, as can be seen in Figure 4-30, where it shows that there is no correlation between the water absorption and the amount of urea added to the composition. The author thinks that because of the aforementioned lack of hydrolysis, residual urea, as can be seen in Figure 4-2, dissolves due to the water that penetrates the mortar pores. This reduces that volume of water that can be absorbed even though the pores are more plentiful.

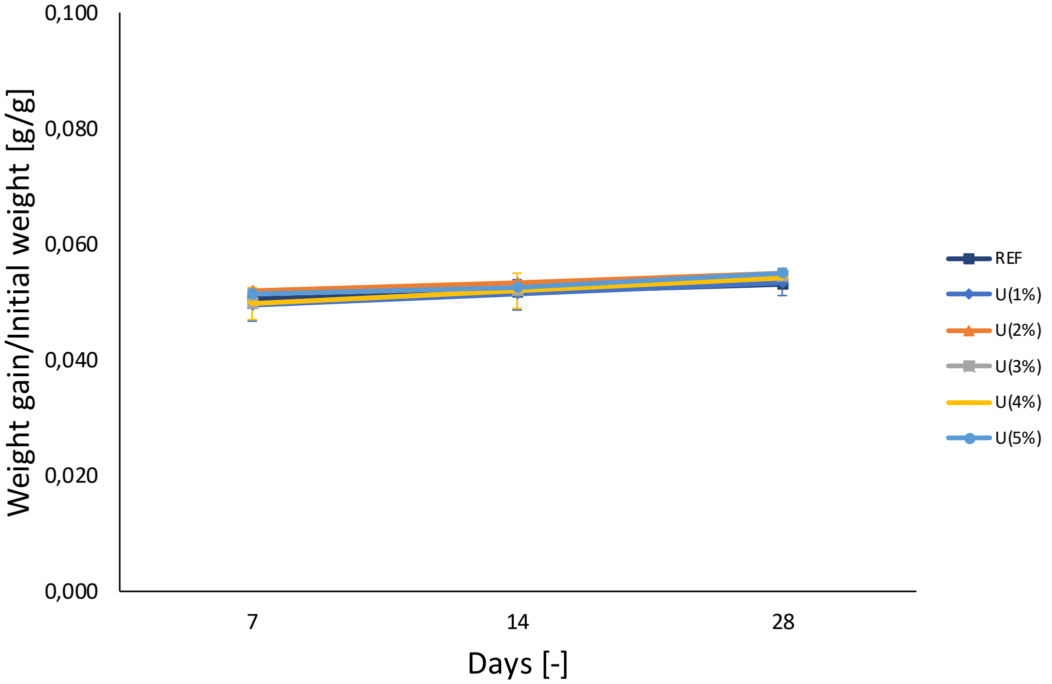

Figure 4-30. Influence of urea on the water absorption after 7, 14 and 28 days

In Figure 4-31, the effect of yeast extract on the water absorption is visualized. A strong correlation between the amount of yeast extract added, and an increase in water absorption was found.

The influence of yeast extract on water absorption of the cementitious matrix is highly correlated to the w/w% of the added additive. However, the same "grouped" effect occurs which is similar to the results of the density tests, where Y0.34 showed similar characteristics to the REF, where Y0.85, Y1.28 and Y1.7 strongly differed from the REF but are reasonably similar and Y2.56 which again doubled the characteristics value difference between REF and Y1.28. The author confirms that the same mechanisms as discussed in 4.1.2 are responsible for this negative effect. However, additional research is required to investigate the “grouped” effect that corresponds to the different amounts of yeast extract added.

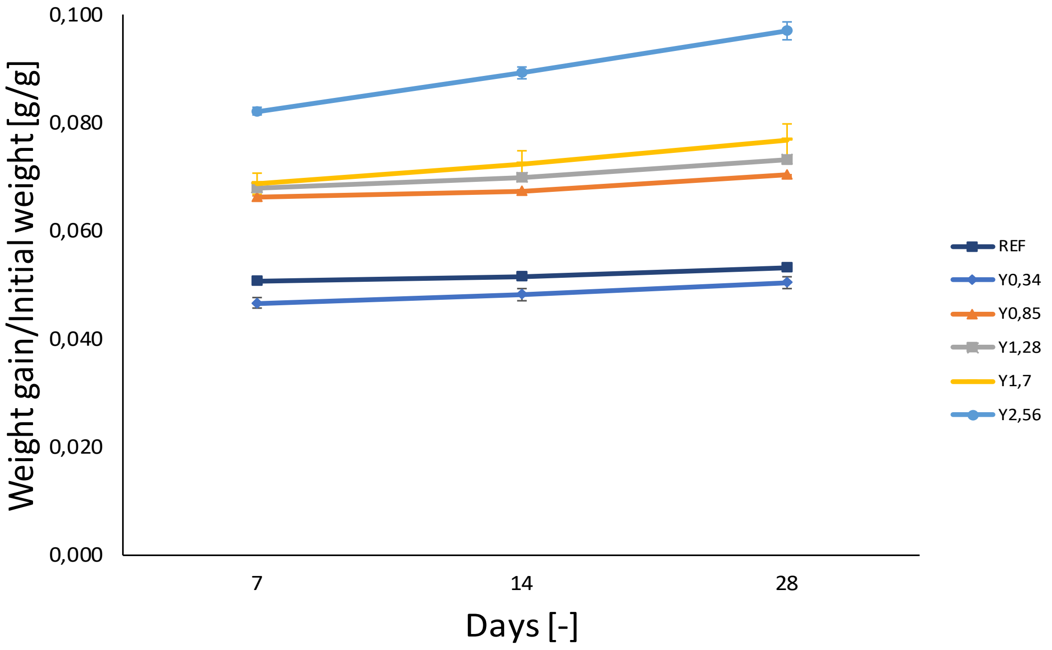

Figure 4-31. Influence of yeast extract on the water absorption after 7, 14 and 28 days

Only calcium nitrate showed a decreased water absorption. As can be seen in Figure 4-32, the water absorption decreased when adding 2 w/w% of calcium nitrate. However, with higher dosages, the additional calcium did not contribute as much to decreasing the water absorption even further.

The flash setting effects as discussed in 4.1.1, is not responsible for this characteristic due to the fact that the first test is executed when the mortar already has reached an age of 28 days. Two paths of thought need to be considered here. Firstly, due to the addition of calcium nitrate plenty of calcium ions, Ca^{2+}, are present in the solution, allowed calcium hydroxide $Ca(OH)_2$, or CH in cement chemist notation, to be formed. This reduced the total pore volume since some of the liquid water gets transformed into a solid form. It should be noted that this CH is the most soluble of the hydration products, and thus acts as a weak link for the durability when exposed to fresh water. When exposed the CH will dissolve and leach out, increasing the porosity and making it vulnerable to chemical attacks once more [90, 91]. It is important to note here already that a higher concentration of calcium hydroxide will also, under suitable conditions, lead to a greater amount of formed calcium carbonate when reacting with carbon dioxide (CO_2) [92]. This mechanism is further explained in 4.1.6. Secondly, during the formation of low-density C-S-H, which means before the end of the second hydration peak, some amount of calcium hydroxide will form a mixture with the low-density C-S-H gel which is a calcium silicate hydrate called jennite with the chemical formula $Ca_9Si_6O_{18}(OH)_6.8H_2O$ instead of 1.4 nm tobermorite $Ca_5Si_6O_{16}$ [93]. This second path of thought could also be seen as an explanation, although the rich in calcium jennite has a higher porosity than tobermorite [94]. It is, however, not specified in the paper if jennite is formed alongside tobermorite or is additionally formed, which would give reason to believe this can explain the decreased water absorption. This could, however, not be confirmed and should the composition and microstructure of the C-S-H gel should be investigated in future research. The fact that an increasing w/w% of calcium nitrate does not linearly decrease the absorption further, as can be seen in Figure 4-32, does support the explanation that jennite is additionally formed alongside the cement surface where tobermorite does not form and a saturation of additional calcium ions is reached at the low dosage of 2w/w%.

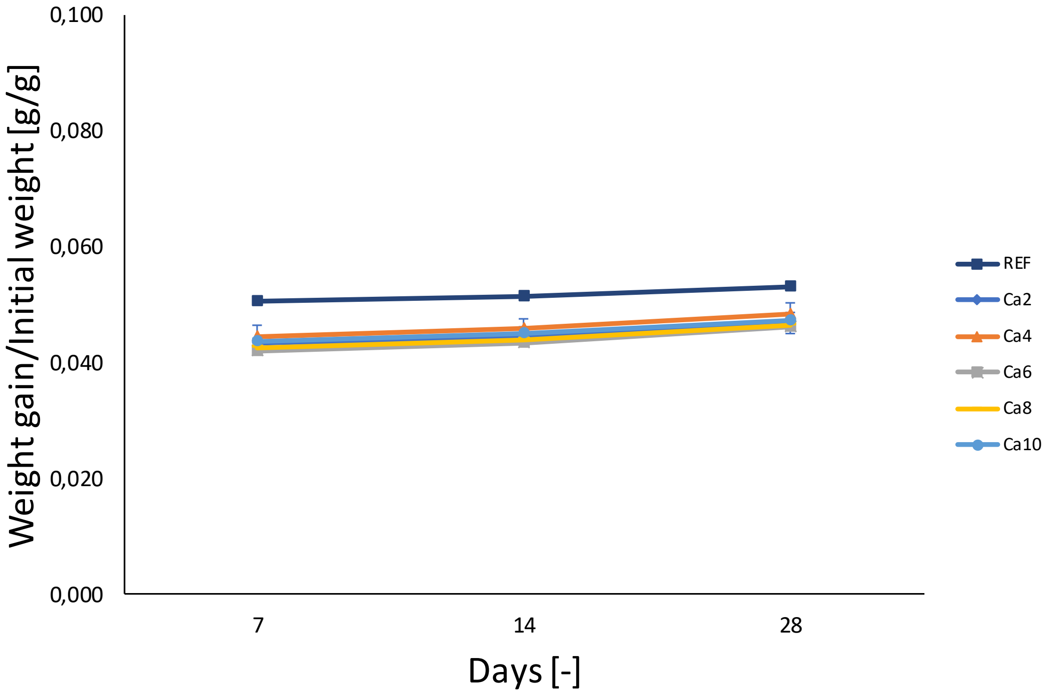

Figure 4-32. Influence of calcium nitrate on the water absorption after 7, 14 and 28 days

The water absorption of the mixed composition was much higher than that of Y0.85 as can be seen in Figure 4-33, which is peculiar due to calcium nitrate having a positive effect and urea's effects being nugatory. This effect is reason for future research to investigate if the water absorption is a result of the microstructure and on a larger scale, the porosity. An explanation for this could be the fact that the air bubble formation, as explained in 4.1.2, as a result of yeast extract, happens after the quick initial setting due to calcium nitrate, which leads to a higher porosity. This thought process is backed up with the results of the lower than Y0.85 density and strength of the mix, as can be seen in 4.1.4, but should still be investigated further in future works. However, this high water absorption property for the mix composition is possibly a good thing when incorporating bacteria. The survivability of bacteria is highly impacted by porosity since bacteria would get squeezed, in an already harsh environment, causing the survivability to drop [7]. However, the mechanical strength of mortar is negatively correlated with its porosity as can be seen in 4.1.4 [95].

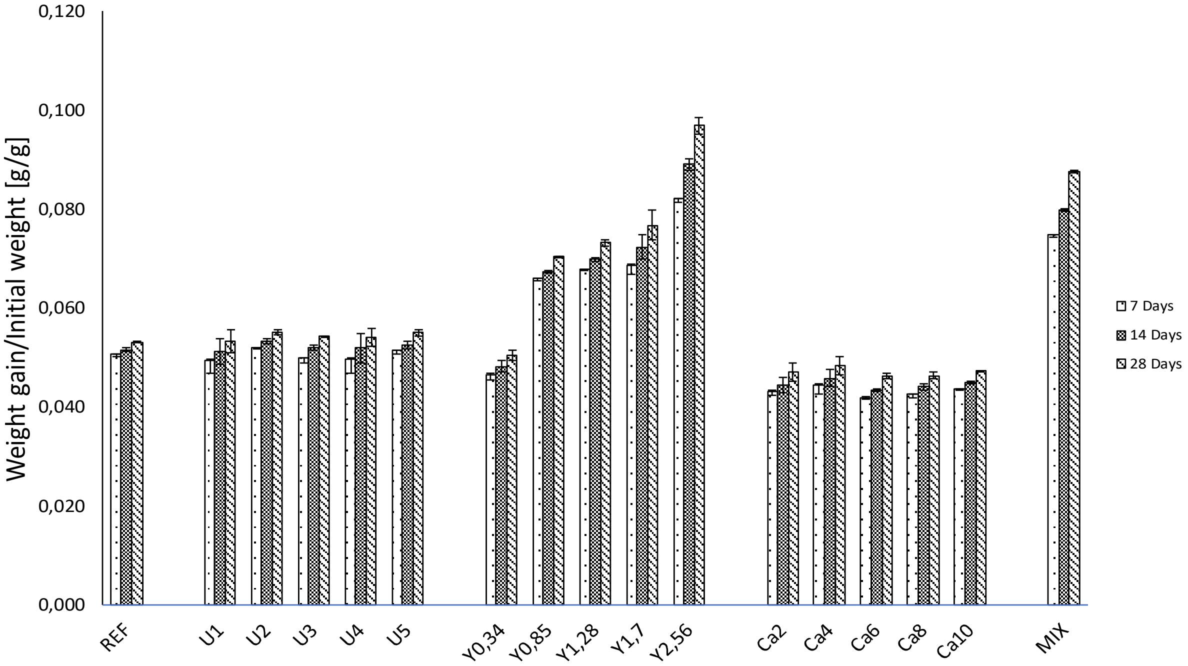

Figure 4-33. Influence of bioagents on the water absorption after 7, 14 and 28 days

4.1.5. Freeze-thaw resistance

The effect of 28 days of freezing and thawing is shown in Figure 4-34. As can be seen, the strongly resembled these of the water absorption as shown in 4.1.4, this due to the fact that no damage was found. This means that all the weight loss is the result of the evaporation of free water in the mortar prisms, this evaporation is facilitated by a more porous material.

The fact that the yeast extract series had a lower weight loss than the urea series does not mean the latter is more porous, this is because of the reason that the cubic specimens were not saturated with water. The water that was available for evaporation was solely the water that did not, yet, partake in the hydration process. One can reason that due to the retarding effects of yeast extract, as show in 4.1.2, however, since these tests were performed after 28 days, most of the water has already been used for hydration. Furthermore, this means that the higher weight loss value for urea confirms the explanation made in 4.1.1, where it was said that incorporating urea generates a higher volume of free solution. Additionally, because urea does not decrease the porosity the weight loss is still relatively small compared to MIX, where due to the high porosity of yeast extract and additional free solution volume the evaporation is facilitated.

Although these results support the observations made in 4.1.4 and 4.1.3.1, the expected results were structural damage due to the freeze-thaw cycles. To investigate this further the prisms should be saturated with water beforehand or be subjected to a freeze-thaw salt attack.

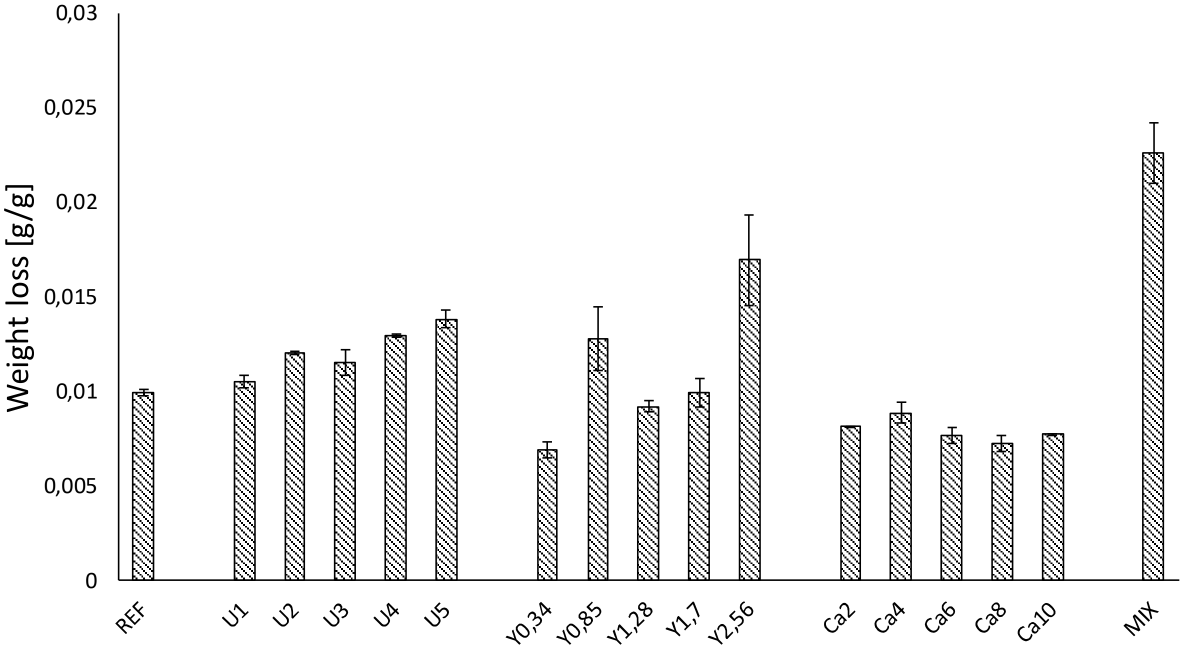

Figure 4-34. Weight loss as a result of 28 freeze-thaw cycles

4.1.6. Carbonation

As a result of the great difference in the order of magnitude of the y-axis, the graphs concerning yeast extract were formatted using a different y-axis scale than those of urea and calcium nitrate. The magnitude of difference will be visualized in the final graph that includes all the compositions.

4.1.6.1 *Carbonation depth*

As can be seen in Figure 4-35, the carbonation depth decreased with increasing w/w% of urea, increased with the time it is exposed to CO_2 and resulted in higher carbonation depths compared to reference when the dosage was lower than 2 w/w%. Furthermore, a very small carbonation depth was observed after being exposed for 7 days after which the carbonation depth noticeably increased.

When keeping in mind, the empirical formula stating that the water-cement factor of compositions should be altered by including the weight of urea added one would think this would mean a more porous mortar and thus more susceptibility to carbonation. However, as results in 4.1.3.1 and 4.1.4 show, the density increases and water absorption decreases respectively. These characteristics can, however, be explained by the increasing water plus urea-cement factor. When CO_2 finds its way in the mortar and it will dissolve in the solution causing the formation of carbonic acid. This carbonic acid will then react with the hydration product calcium hydroxide to form calcium carbonate, this leads to a mechanism that increases the calcium carbonate content while decreasing the calcium hydroxide content which also increases the durability of the mortar by acting as a filler to decrease porosity [4].

$$H_2O + CO_2 \rightarrow H_2CO_3 \qquad (24)$$

$$H_2CO_3 + Ca(OH)_2 \rightarrow CaCO_3 + 2H_2O \qquad (21)$$

This explains the decreasing carbonation depth with increasing w/w% of urea. However, as can be seen in Figure 4-35, this mechanism is overcome by the increased solution volume at low w/w% of urea, which leads to an increased carbonation depth. Additionally, it is speculated by the author that as a result of the high solution volume, the relatively wet mortar will prevent CO_2 from entering the pores, which leads to the low carbonation depth at an early age. The reason for this thought was the visual observation of the sections obtained after the three-point bending test that looked “wet” when compared to other section. However, this needs to be verified in future research.

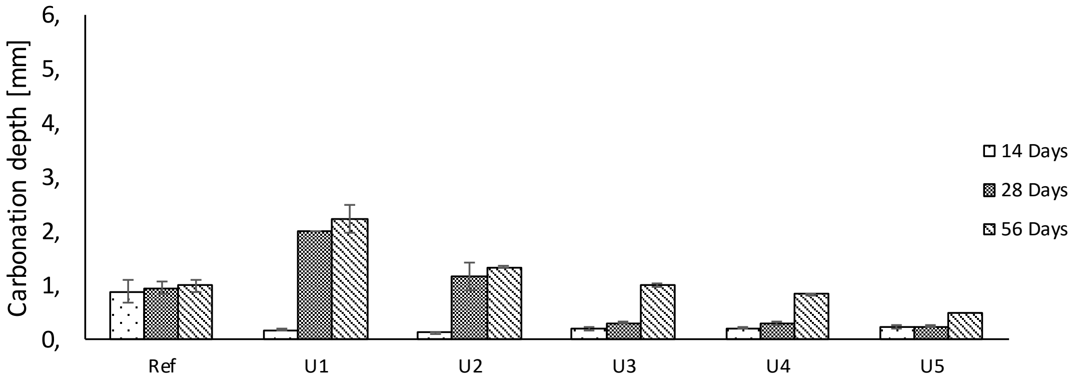

Figure 4-35. Influence of urea on the carbonation depth after 14, 28 and 56 days

In line with the results for yeast extract found in 4.1.4, the same "grouped" results are noticeable. In Figure 4-36 it can be seen that the carbonation depth increased greatly with increasing w/w% of yeast extract, and this for all ages of exposure. Additionally, it should be noted that the magnitude of these results differed by a factor of 15 to even 35 compared to those of urea and calcium nitrate where the carbonation depth remained far under 1 mm.

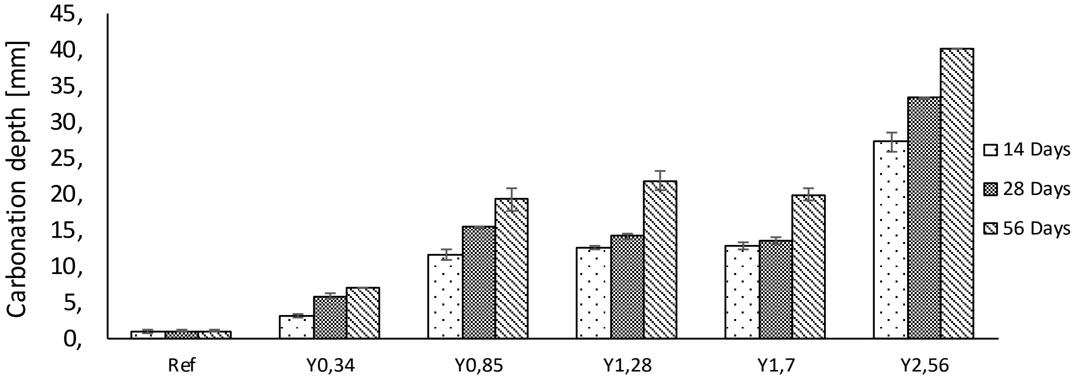

Figure 4-36. Influence of yeast extract on the carbonation depth after 14, 28 and 56 days

It is also noteworthy that a large "gradient" surface was observed when testing the mortars incorporating yeast extract at 7 days due to the rapid intrusion of CO_2. As can be seen in Figure 4-37, a double carbonation front is observed. A first front, light pink, and a second deeper front that is dark purple. The explanation for this phenomenon is that when slow diffusion occurs, the C-S-H will consume all inward diffusion carbonate ions before reaction with CH, that is further inside, occurs, this creates a distinct carbonation front. However, when rapid carbonation occurs, a double carbonation front can be observed as a result of the release of CH from the C-S-H being too slow to consume all the carbonate ions. Hence, a number of carbonate ions will pass the only partly altered C-S-H and react with CH that is further inside the prism. This reaction clearly visualises the difference between the transport and reaction rate of carbonation [96].

Figure 4-37. Double carbonation front due to yeast extract

The main negative effect, however, follows from the strongly increased porosity, which can be confirmed by the low density and high water absorption found in 4.1.3.1 and 4.1.4 respectively.

The results found for calcium nitrate, shown in Figure 4-38, are counter-intuitive considering the results found 4.1.3 and 4.1.4, where an increase in density and decrease in water absorption is observed since a more dense structure usually leads to a decrease in carbonation depth [97]. Nonetheless, the carbonation depth does decrease similarly to the increase of density, however, the carbonation depth for Ca2 compared to REF is increased three to five-fold. Because of these peculiar results, it seems more likely that the carbonation depth of the reference sample is extremely low.

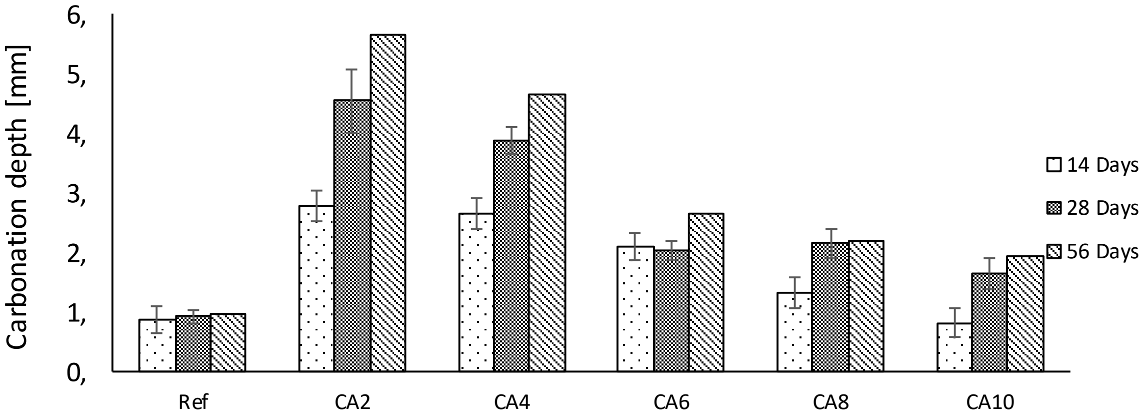

Figure 4-38. Influence of calcium nitrate on the carbonation depth after 14, 28 and 56 days

The formation of calcium carbonate due to carbonation is clearly noticeable due to the high calcium concentration as can be seen in Figure 4-39 where the carbonated surface layer, marked with a red line for the outer specimen, is clearly visible due to its light grey colour. This layer will be responsible for delaying further carbonation.

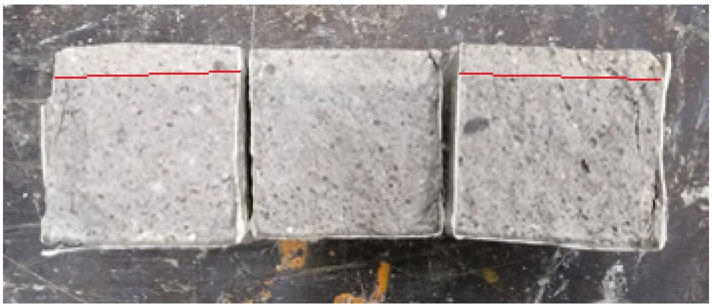

Figure 4-39. Carbonated surface layer for Ca2

Figure 4-40 again shows that the effects of the combined bioagents are equal to the superposition of the individual effects of the additives. While the carbonation depth reached values up to 20 mm for yeast extract, the mixed compositions remained far below 7 mm. This is an important assessment since this means that the negative effect of yeast extract is moderated by both urea and calcium nitrate. This observation is for example not true for the water absorption.

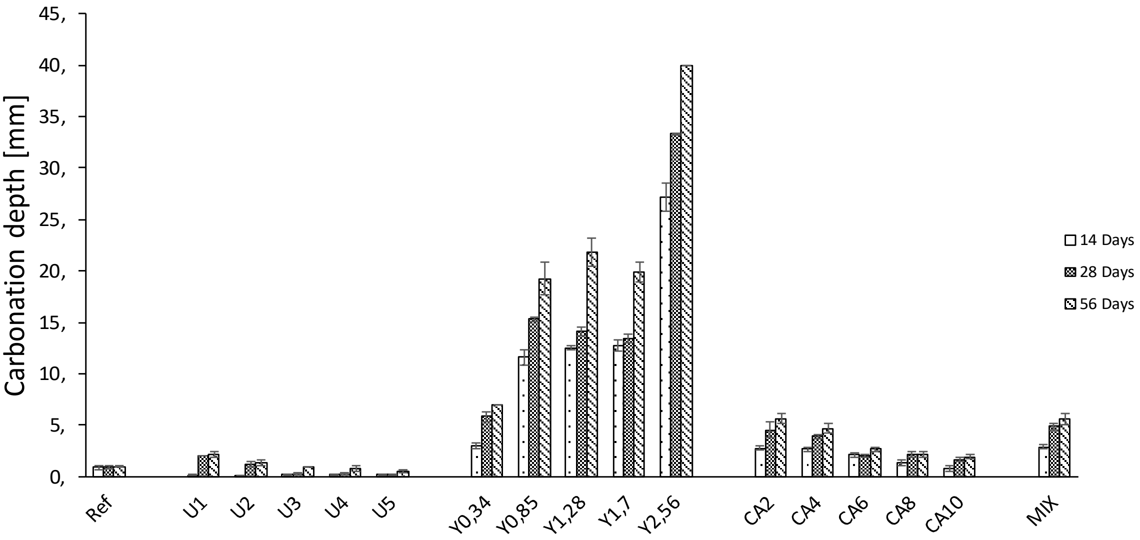

Figure 4-40. Influence of bioagents on the carbonation depth after 14, 28 and 56 days

4.1.6.2 *Carbonation constant*

In this section, the carbonation constant as a result of the addition of each bioagent is analyzed. As mentioned above the carbonation rate drops due to the formation of a carbonated layer that is dense as a result of the formation of calcium carbonate in the pores. However, urea was the only bioagent that resulted in a lower carbonation after 14 days compared to the carbonation rate at 28 days, this could be explained by understanding that as a result of the increased liquid solution that was introduced to the composition the pores were filled and thus the CO_2 could not enter, however, this still needs future research.

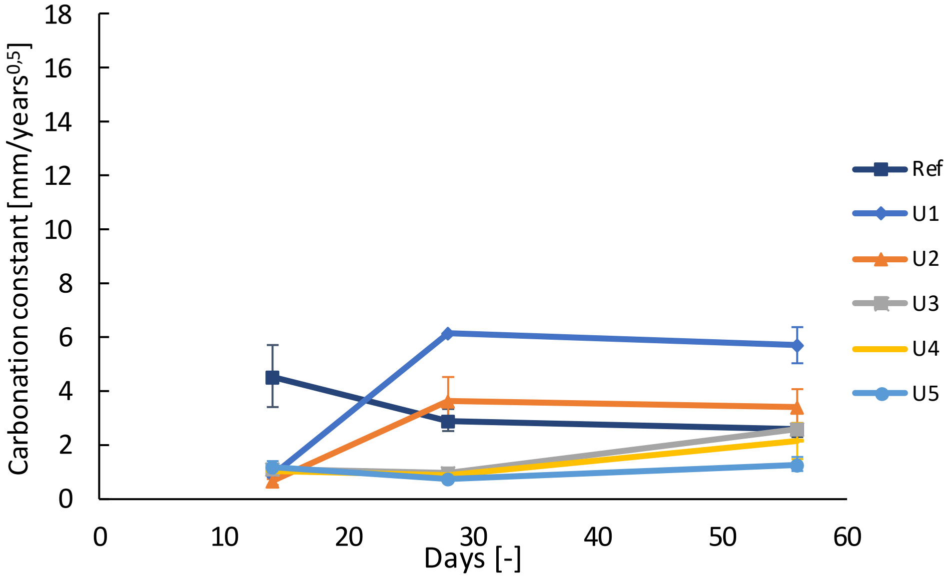

Figure 4-41. Influence of urea on the carbonation constant after 14, 28 and 56 days

Since yeast extract brought forth such a high porosity, even the formation of calcium carbonate to fill some of these pores did not slow down the carbonation rate as can be seen in Figure 4-42.

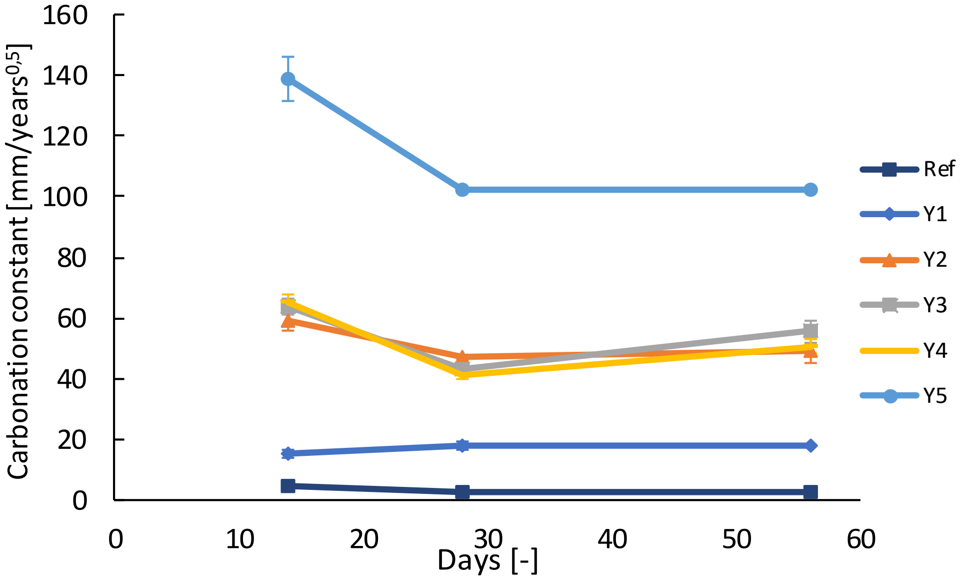

Figure 4-42. Influence of yeast extract on the carbonation constant after 14, 28 and 56 days

An, albeit, small decrease in the carbonation rate was observed when adding calcium nitrate to the mortar composition. This “barrier” that caused this is visualised in Figure 4-39.

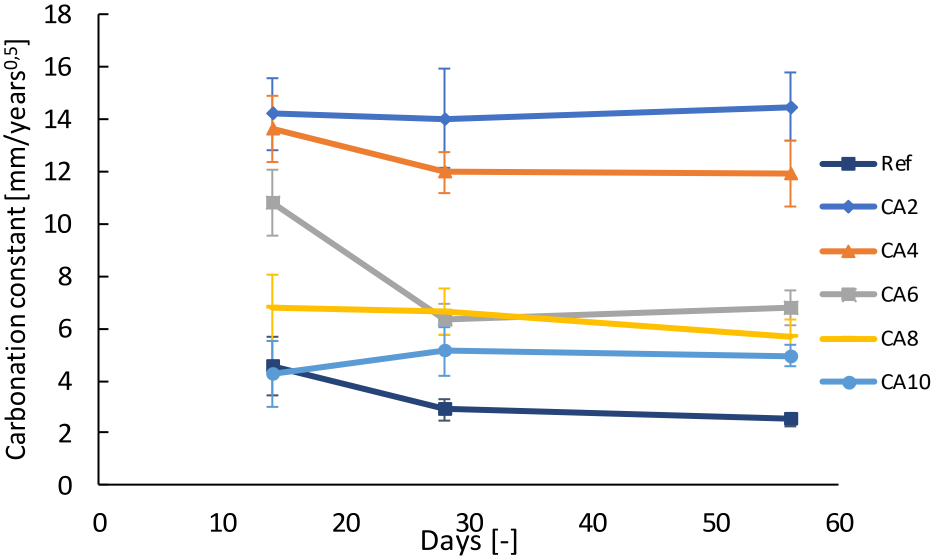

Figure 4-43. Influence of calcium nitrate on the carbonation constant after 14, 28 and 56 days

4.1.7. Cement hydration

As discussed in 4.1.2 and 4.1.1, fresh mortar with urea has a delayed setting and increased flow-ratio. This retarding effect is visualized in Figure 4-45 where a delay of 0.21, 0.89, 1.86, 2.30, 3.35 and 4.44 hours was found for U1, U2, U3, U4 and U5 respectively. Additionally, the peak hydration heat was found to be 3.34, 3.13, 2.96, 2.92, 2.65 and 2.57 W/g for increasing w/w% of urea. The reason for this decrease in temperature is the endothermic nature of the reaction when dissolving urea in water, which is explained in the aforementioned sections. To further study this endothermic effect of urea, an additional test was run where 5 g of water was mixed with 1, 2, 3, 4 and 5 w/w% of urea, these results are given in Figure 4-44. As can be seen, U1, U2 and U3 did require some cooling to reach the temperature of 20°C, however, U4 and U5 required heating to reach this same temperature. This explains the jump in hydration heat between U3 and U4.

The delay of stage 3 has been explained before. The twofold mechanism first undergoes an increase in solution volume which leads to a delay in setting and secondly the endothermic reaction as explained above will cause the dormant period to be extended.

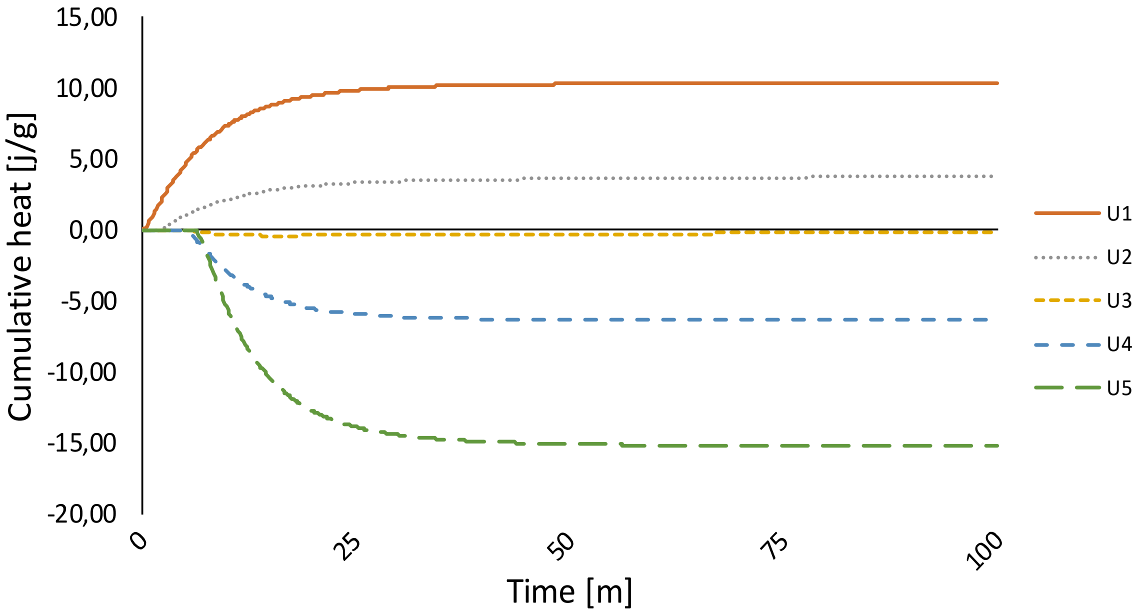

Figure 4-44. Cumulative heat of urea mixed in water

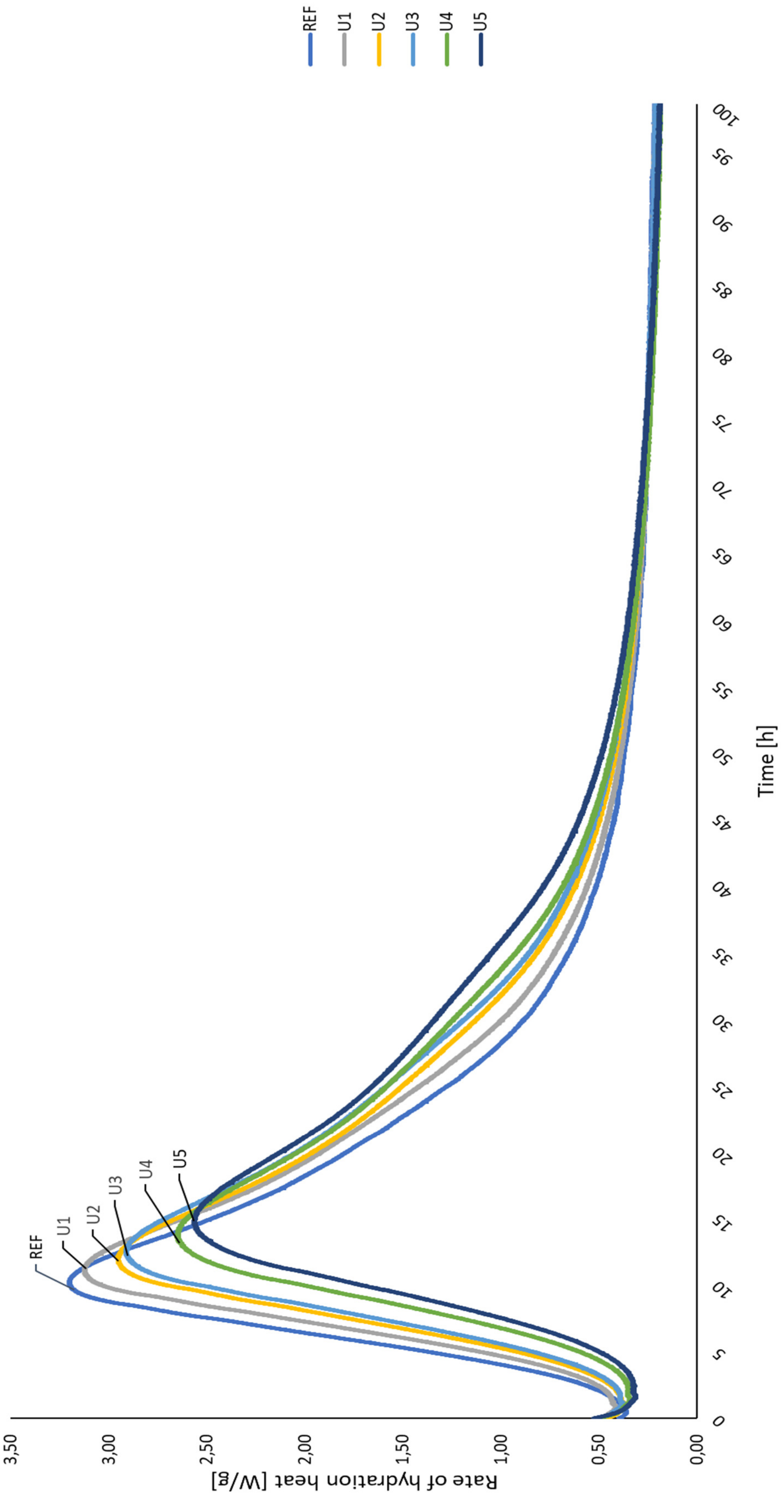

Figure 4-45. Effect of urea on the rate of hydration of mortar

The cumulative heat, as shown in Figure 4-46, clearly showed the gap between U3 and U4 as a result of this endothermic effect. However, it can also be observed that the cumulative heat after 100 hours was comparable for all series, with a slight decrease with increase w/w% urea. This confirmed the observation that urea has similar strengths compared to reference at ages over 3 days, as observed in 4.1.3.

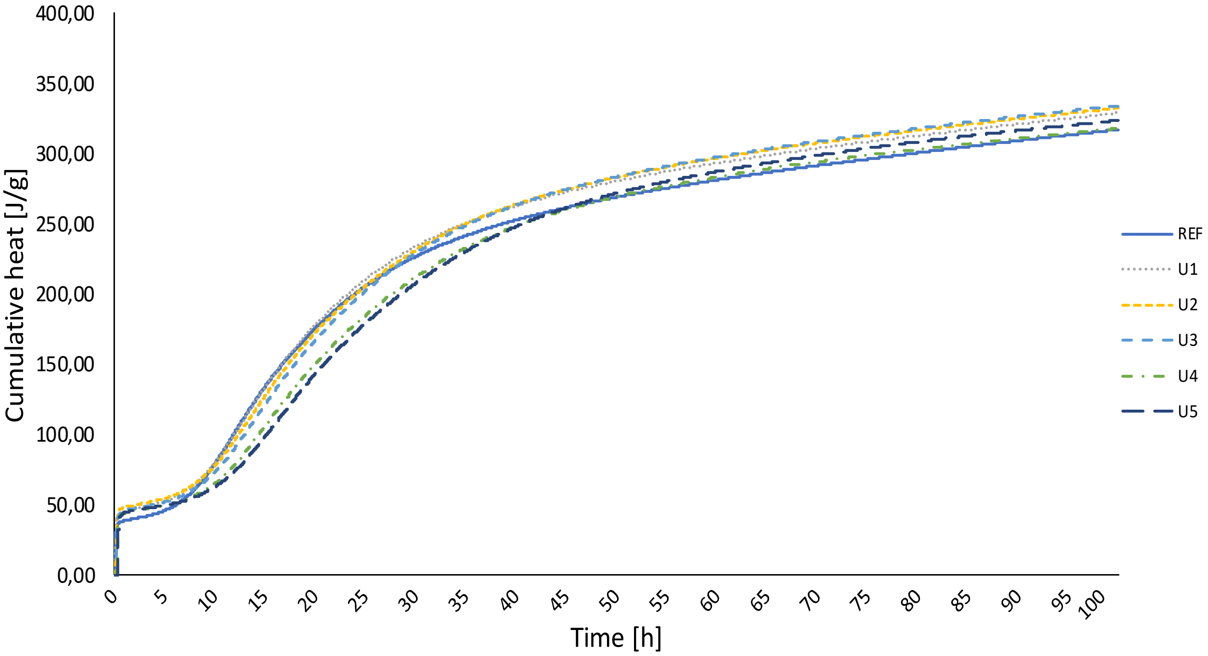

Figure 4-46. Effect of urea on the cumulative heat of mortar

The effects of yeast extract on setting and strength development are perfectly explicable by Figure 4-47 and Figure 4-48. For the compositions Y0.34 through Y1.7 both a delay in setting and decrease in cumulative heat was observed, and these effects were explained in their respective chapters. The other clear observation that could be made is the large amount of heat generated by Y2.56 in its first stage. This mechanism has been explained in 4.1.2 where it was found that sugar has an accelerating effect when used in high dosages above 0.06 w/w%. Additionally, the total amount of cumulative heat of Y2.56 after this burst in its first few hours accounts for 50% of the total heat. This explains the lack of further strength development at high yeast extract dosages.

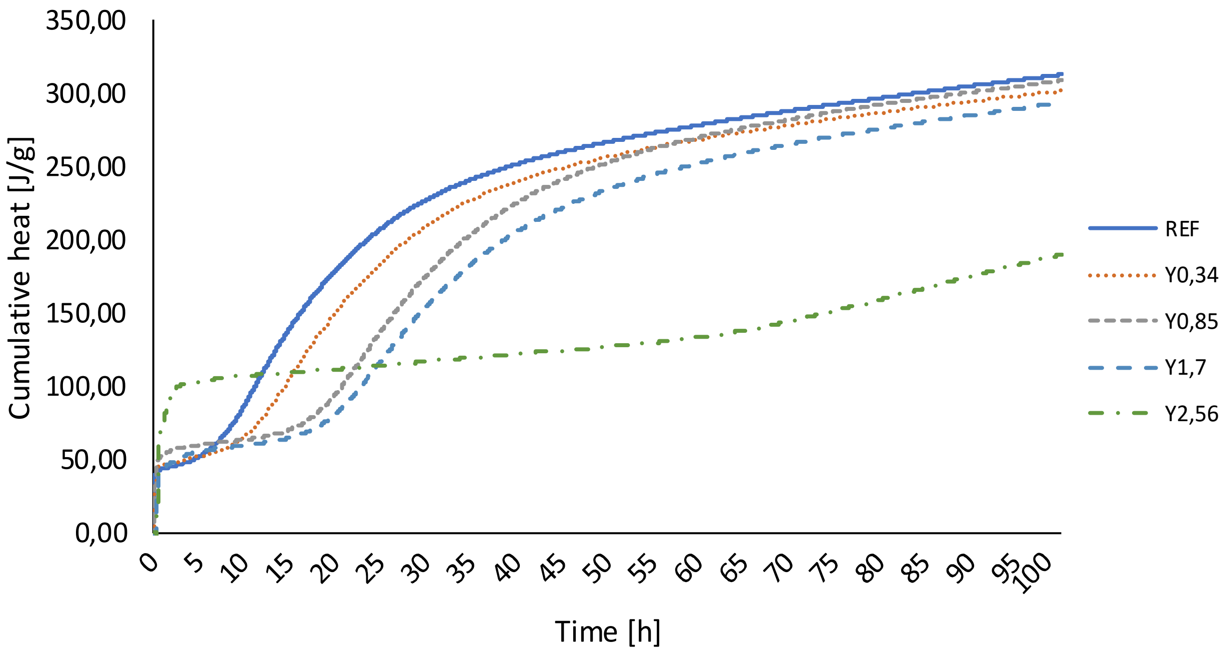

Figure 4-47. Effect of yeast extract on the cumulative heat of mortar

The series Y1.28 has been removed from the graphs as a result of a malfunction of its chamber. However, if one imagines the results, which is appropriate to assume considering the previous results, of this series to fall between Y0.85 and Y1.7 the same grouped effect shows up which has been recurring throughout this work. The maximal heats are 2.69, 2.48, 2.22 W/g and their delays 0.42, 1.22 and 1.36 hours for Y0.34, Y0.85 and Y1.7 respectively.

Taken into account the sudden rise of heat for Y2.56 after 65 hours, the aforementioned barrier that restricts hydration might still be able to get penetrated after this time, however, more research is required in this specific direction.

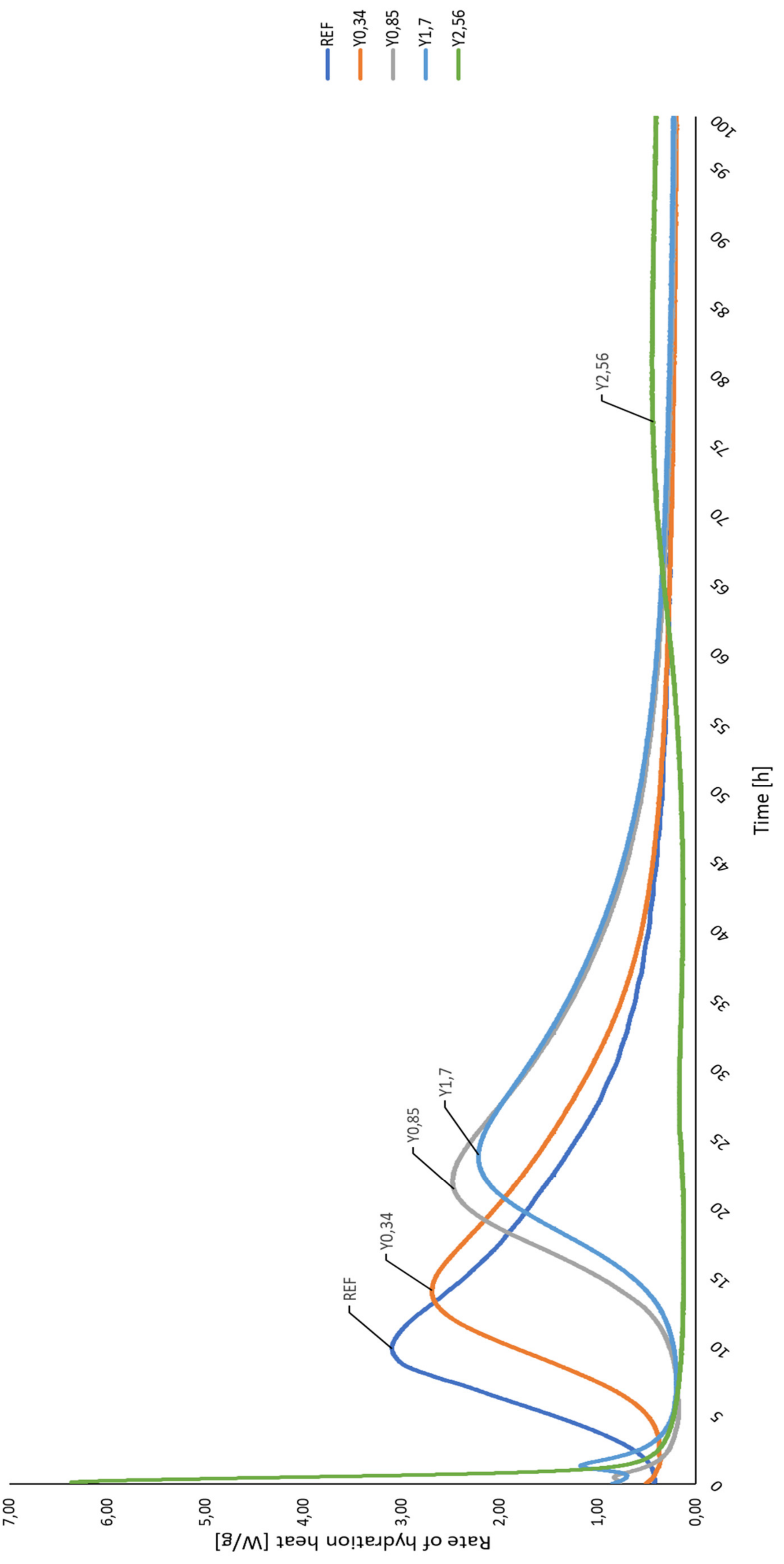

Figure 4-48. Effect of yeast extract on the rate of hydration of mortar

Calcium nitrate is a known setting accelerator, which is confirmed in Figure 4-49 and Figure 4-50 where its hydration curve was shifted to the left. Likewise, the flash setting of Ca8 and Ca10 was characterized by a high initial burst of hydration heat as seen in Figure 4-49. However, the lower hydration heat is often characterized by a lower mechanical strength, which is especially true for Portland cement [98]. With the results from 4.1.3, where it shows that Ca10 has a higher compressive strength than REF, it leads to asking the question whether any other influences impacted the strength. However, the more likely explanation is that as a result of the instantaneousness of the stage 1 heat, some of it has already dissipated in the air before loading the capsule in the chamber. This is also why Ca8 shows a relatively low burst, even though it was already proven that it shows flash setting in 4.1.2.

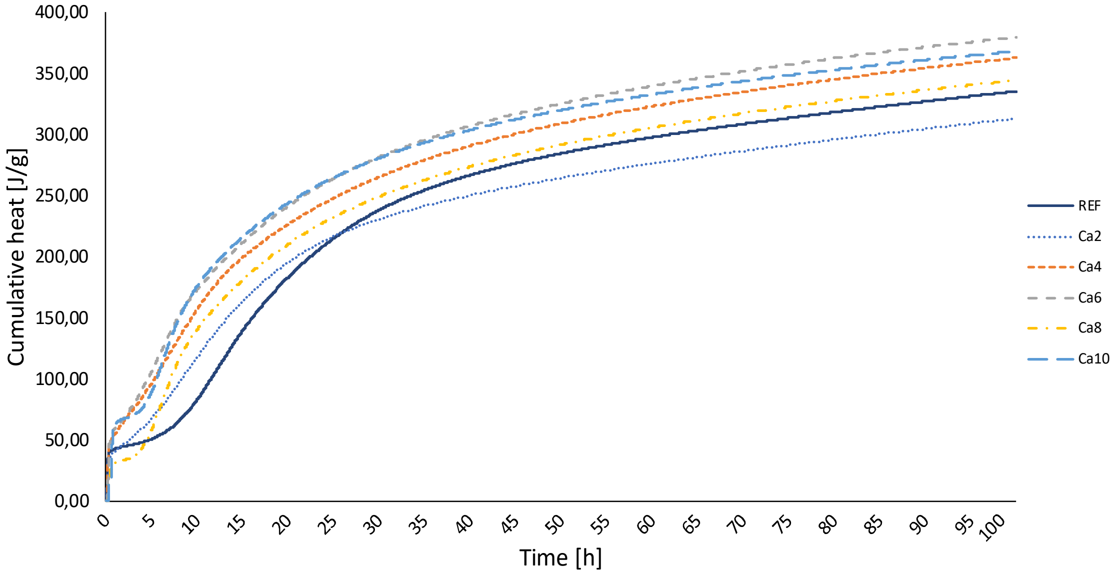

Figure 4-49. Effect of calcium nitrate on the cumulative heat of mortar

Furthermore, the second hydration peak becomes narrower with increase w/w% of calcium nitrate, which would cause the initial and final setting to happen in quick succession. However, this does not seem to be the case considering the results in 4.1.2. The explanation for this found in Figure 4-50 where the dormant phase occur so quickly that the stage 1 heat has not even decelerated before stage 3 start, this causes the acceleration phase to raise less to reach final setting, which compensates for the less narrow second hydration peak.

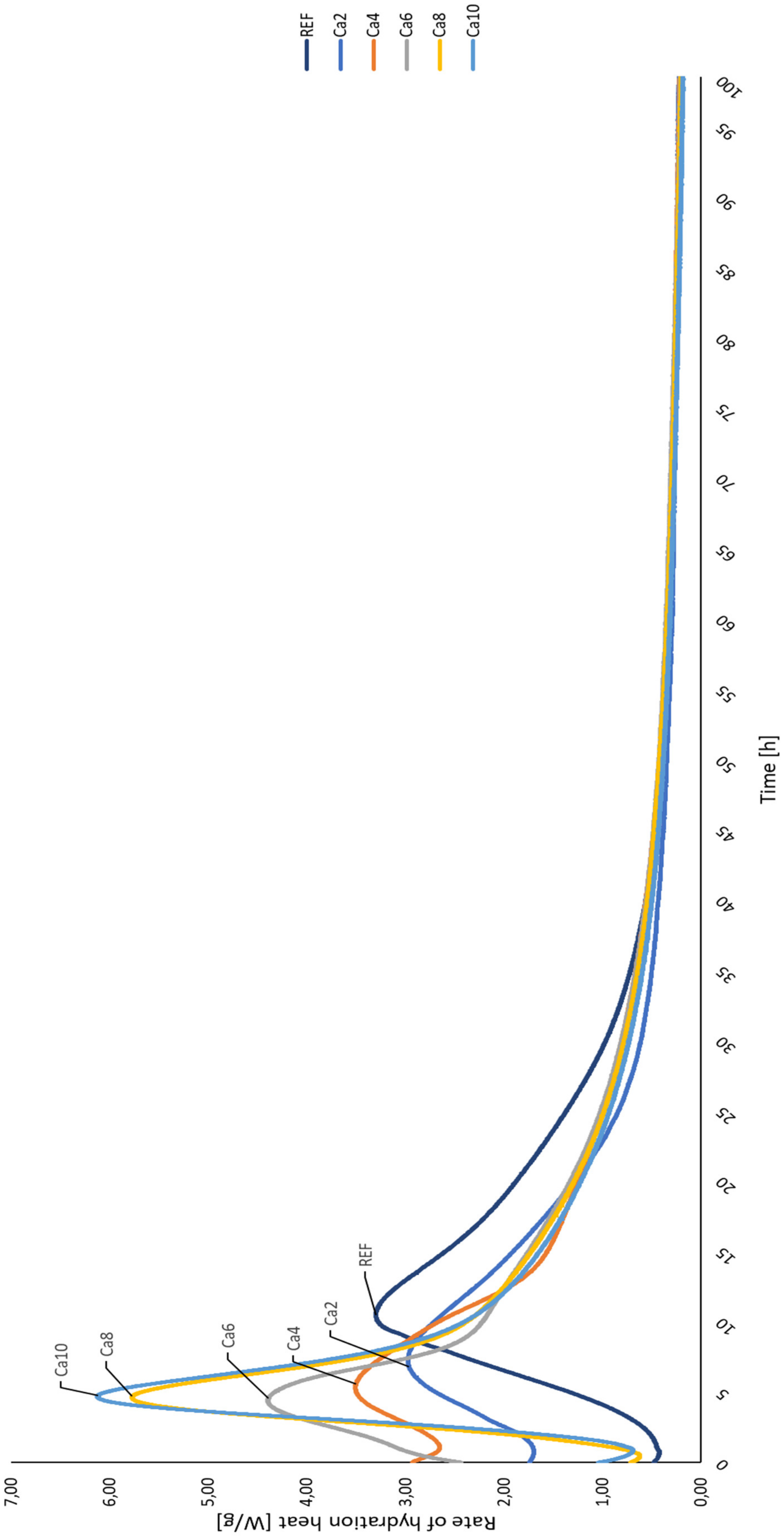

Figure 4-50. Effect of calcium nitrate on the rate of hydration of mortar

Lastly, hydration lines for reference, the individual hydration lines for all bioagents of which the MIX consists and the hydration lines of MIX are plotted. Although, when combining all individual effects, a hydration curve similar to that of reference was obtained, the MIX composition showed very different characteristics, as was proven in previous chapters. However, this could also be the fault of the operator, since each test was only executed once. Peculiar is the high first hydration peak, which shows as a rapid rise in the first five hours of the cumulative heat line, this could also be explained due to the high porosity at the start where yeast extract functions as an air entrapment agent while calcium nitrate is already producing hydration products that harden the cementitious matrix.

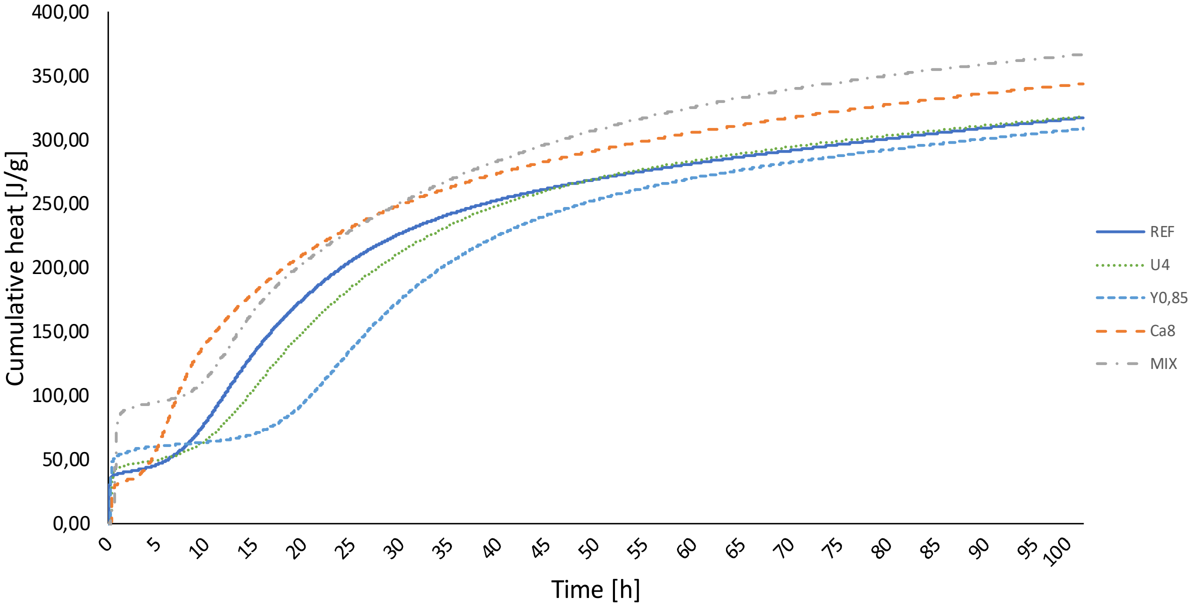

Figure 4-51. Effect of combined bioagents on the rate of hydration of mortar

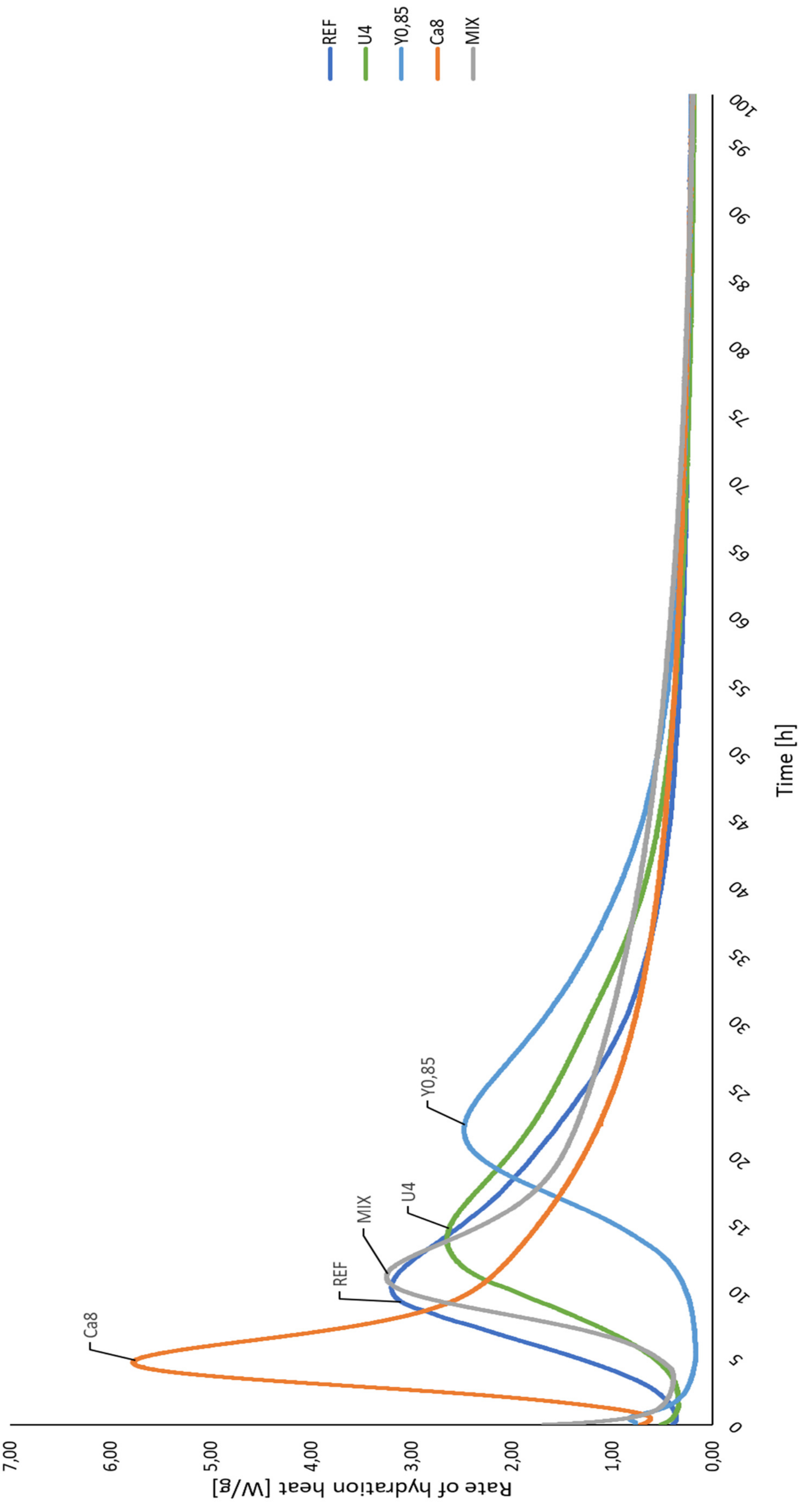

Figure 4-52. Effect of combined bioagents on the rate of hydration of mortar

4.1.8. $CaCO_3$ content

The calcium carbonate content per gram of sample is visualized in Figure 4-53. As can be seen, the weight loss decreased with curing age. Additionally, high values are spotted at all ages for the mortar incorporating bacterial cells compared to the reference. Furthermore, the addition of yeast (T4) seemed to have little influence, while the addition of calcium nitrate (T3) seemed to have a positive effect. While the combined effect (T5) seemed to be the better than both bioagents separate.

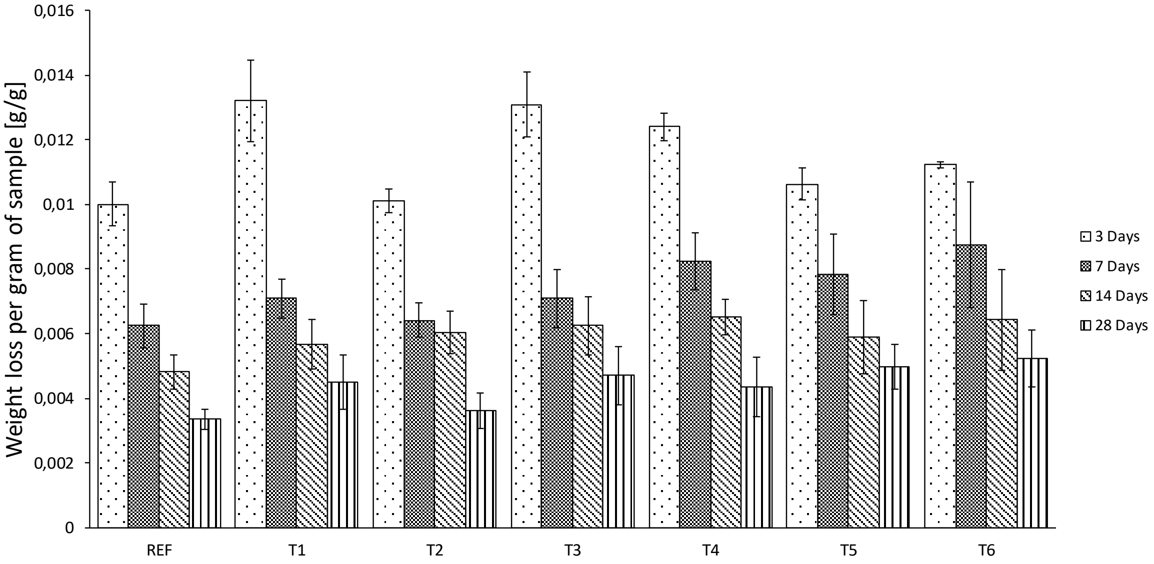

Figure 4-53. Weight loss per gram of sample for bacterial mortar at 3, 7, 14 and 28 days

As can be seen in the results, not all weight loss was a result of the reaction of hydrochloric acid with calcium carbonate, most of the weight loss was a result of the acid reacting with other hydration products as can be seen by the high value for REF. The components of the cement paste are broken down during contact with the acid while aggregates stay intact [99]. Additionally, the microstructure changes which results in fewer products that react with HCl [100]. Lastly, calcium carbonate itself acts both as an inert filler and an active participant in the hydration process, for which the ratio can be calculated [101]. It should be noted that it is possible that reference also contains $CaCO_3$, however, what is measured here is the additional amount of $CaCO_3$.

For T1 the suspended bacterial cells were added to the composition which resulted in an increase of weight loss. Since there are no bioagents for the bacterial cells to react with this can not be explained by bacterial activity with regards to bioagents. Therefore, there a reaction seems to occurs between the bacterial cells and the cement particles. This should be investigated further in future research by investigation of the microstructure.

For T2 a lower amount of weight loss was observed. This goes against what one may think due to the addition of urea that is an essential agent for ureolytic activity. Additionally, as seen in 4.1.3.4, the density did not increase. This means that a harsher environment for the bacteria is not a valid explanation for this. However, in previous research, it has been proven that above a certain concentration, more urea does not result in more precipitation. This is due to the fact that the bacterial cells denature [87]. The results in this work state that not only does it not benefit ureolytic activity, it even obstructs the active activity, resulting in a lower amount of precipitated calcium carbonate. This statement, however, needs to be evaluated in future research.

For T3 an increase in weight loss was found, and the results were similar to those of T1. As can be seen, the weight loss happened slower, this can be explained as a result of the bacterial surfaces being bound with calcium ions, creating a barrier which lowers the urea diffusion rate and thus slows down the precipitation rate [38]. It seems that this slow precipitation was the reason that more weight loss was found compared to T2 and REF, however, future research should focus on the microscopic mechanism of these interactions.

The addition of yeast extract for T4 resulted in a similar weight loss found for T3 as well as T1. The fact that no higher values were found can be explained by the fact that there is no source of calcium ions to form calcium carbonate, and therefore was the limiting reagent. This is also verified in the results for T5.

For T5, initially, a lower value of weight loss is recorded. However, at later ages, a higher value is found. This is a result of the aforementioned mechanism where calcium ions create a barrier with slows down ureolytic activity. However, unlike T4, calcium nitrate is no longer a limiting agent, which leads to the precipitation to continue and results in the higher weight loss that is found.

When adding the grown culture, results equal to T5 were found. This is peculiar since no calcium nitrate was added to precipitate this high amount of calcium carbonate. However, the cells were added in a spent UYE medium, which, for some reason that needs to be investigated further, spreads the bacterial cells less uniform in the cementitious matrix. This would explain the high standard deviation for T6 since more concentrated spots of $CaCO_3$ would get formed. When considering this high deviation, one can notice that the results are similar to those of T1 where the same number of cells are added. However, unlike T5, there is no lack of calcium ions here, which should lead to a higher value. This needs to be investigated further.

Overall, it can be stated that the addition of bioagents is necessary to increase the amount of precipitated $CaCO_3$. Lastly, it should be noted that overall, for all results, a high standard deviation is found. This begs the question if using a bigger sample size would solve this deviation. However, considering that in this test a sample size of 2 grams is used, a more representative result is already found compared to other techniques.

4.2. Discussion

4.2.1. Flow

The flow-ratio of mortar is influenced by many factors due to its fresh state. The test is used as a first indication to validate the prospect of incorporating a new additive to the composition. Usually, to influence the consistency of mortar, chemical additives, also referred to as superplasticisers, are added to the mix. These have the properties of increasing the workability while keeping the water-cement factor constant or lowering the water-cement factor while keeping the workability constant [102]. The type, shape, and fineness of the aggregates chosen to be incorporated in the mortar also influence the flow. By increasing the number of fine aggregates the voids between larger aggregate particles were filled which led to lower friction between large aggregates that results in lower viscosity and thus a higher flow [103, 104]. Additionally, the water/cement factor is directly correlated to the resulting flow of the mortar mix [105]. Due to these reasons, many have looked to incorporate fine recycled aggregates and, albeit chemical, additives to adjust the workability of mortar and concrete mixtures.

In the flow test the workability was tested and the influence of the bioagents on this characteristic of the fresh mortar. For urea, a linear correlation was found that could predict the flow given by (5).

$$y = 0.0156x + 0.9984 \qquad (5)$$

These same results were found in a study where 5, 10 and 15% of urea was tested [4]. In the same study, an increase of 22 to 26% was found per 5 w/w% increase of urea while in this study an average of 8.2% increase was found per 1 w/w% which leads to an increase of 41% per 5 w/w% of urea. However, this difference can be explained by the fact that in the mentioned research the water volume slightly decreased and not kept constant over the series like was done in this work.

An explanation was found in the increased solution volume as a result of the dissolving, but not hydrolysis, of urea in water. The increased solution volume leads to similar results as increasing the water-cement factor. This theory was also supported in the literature [64]. In addition to this, a formula was introduced in literature to support this observation which states that the volume of urea added has a direct effect on the water-cement factor [4, 17, 26]

$$\frac{(W+U)}{C} \qquad (12)$$

Yeast extract was found to have a great influence on the consistency of the mortar. A linear trendline given by (15) was found.

$$y = 0.0448x + 1.1122 \tag{15}$$

This relation is also found in a work where the viability of yeast fermentation waste as a plasticizer for concrete was evaluated [9]. The flow-ratio of mortar incorporating yeast extract was found to be underestimating the consistency, due to the high drop in density, as can be seen in 4.1.3.1, which reduces the potential energy that is paired with each jolt of the flow table [69].

Calcium nitrate has a decreasing effect on the flow-ratio as a result of the calcium ions and nitrate ions that are formed from its hydrolysis. Because of the high, supersaturated, concentration of calcium ions plate calcium aluminate hydrate is formed [80]. This reaction is rapid and produces large amounts of heat which causes the paste to set much faster. At a concentration above 8 w/w% of calcium nitrate, this mechanism would cause the mortar to set within minutes, also known as “flash setting”. Although this is often characterised as a negative effect, the flash setting has found its use in three-dimensional printing [106].

It is important to note that when using calcium nitrate tetrahydrate water is added to the mortar mix, therefore the water content should be reduced with respect to the amount of calcium nitrate added. This is the reason why an increase in viscosity for both mortar and pastes are reported [10, 12]. However, when incorporating calcium nitrate in combination with volcanic ash a decrease in workability is observed [11].

4.2.2. Setting

The setting is heavily influenced by a multitude of factors, the most apparent and variable are listed. A first influence factor is the composition of the cement that is used, for example, the amount of C_3A will increase the heat of hydration and therefore accelerate the hydration process. A second factor is the addition of gypsum. Gypsum eliminates flash setting, which would occur seconds after addition of water, by reacting with C_3A and generate calcium sulfoaluminate hydrate which forms a protective film on the cement particles that hinders the hydration of C_3A and delays setting time of cement. However, a high dosage of gypsum could have the opposite effect due to the fact that gypsum can generate a clotting agent itself, therefore, SO_3 is limited to 4.0% by norms. A third factor is the fineness of the cement particles, by decreasing the particle size the total surface area will increase which results in a bigger contact area with water. This bigger contact area will result in accelerated hydration which accelerates the initial setting and early hardening. Fourthly, the curing conditions also have a great impact on the setting of mortar due to the fact that at freezing temperatures the hydration can slow down and even be terminated [107]. In the same manner, hot weather can evaporate the water in the cement which will halt the hydration process. A fifth influence is the one studied here, which is the impact of admixtures. The hydration of cement is constrained by C_3S and C_3A, however so-called acceleration or retarding agents can chemically interact with C_3S and C_3A to respectively accelerate and delay the initial setting which will both affect the development of early strength. The norms prescribe a minimum of 45 minutes before initial setting can occur.

For urea a strong linear relation was found to predict the initial and final setting times accurately, these were respectively;

$$y = 28.429x + 268.1 \tag{25}$$

$$y = 23.571x + 198.57 \tag{26}$$

In which x is the w/w% of urea and y the time in minutes. As can be seen from the formulas a linear increase in the setting time was found, which was also found in the literature. Two proposed mechanisms for this were found, the first one pointed out that the decreased amount of calcium hydroxide causing the setting time to be delayed, while the other proposed mechanism attributed the delay to lower peak hydration. The author follows the second reasoning and confirms it by the additional testing of urea on the hydration heat when incorporating yeast extract, as shown in 4.1.8. Additionally, the mechanism where the urea increases the solution volume as a result of not undergoing hydrolysis led to a higher solution to cement factor, which is a known parameter that increases the setting time [108].

Yeast extract can act both as a retarder as an accelerator, this depending on the w/w% of yeast extract that is used. One, and the only, literature that was found regarding the setting of mortar incorporating yeast extract found a delay [13]. However, it was quickly discovered that the large percentage of carbohydrates, better known as sugar or starch was responsible for the effects. The findings in this work were supported by two works where sugar was incorporated, and the value where sugar went from a retarder to an accelerator was determined to be 0.06 and 0.10 w/w% [74, 75]. Four theories are currently under consideration to explain the delaying effect, namely, absorption, precipitation, nucleation and complexation theory. However, none of them is complete since they only explain the results found for sugar additions lower than the critical value. The acceleration and flash setting with higher dosages, that occurred was explained by the formation of ettringite, however, after this initial setting no strength development happened, which led to very low mechanical properties [78, 79].

Calcium nitrate is a known setting accelerator and has been incorporated in many designs for this specific reason, setting time accelerations of up to 20% for 1 w/w% are found in the literature. Similar results were found in this work, where an acceleration of 20% was found for 2 w/w% of calcium nitrate, and even 50% for 8 w/w%. Although good linear trendlines can be constructed they would give a correct insight in the mechanism, a linear relation between 2 and 4 w/w% can be constructed. However, at 8 w/w% and more flash setting occurred. This flash setting occurred as a result of the formation of plate calcium aluminate hydrate, which was observed by XRD measurements [80]. The accelerated setting that comes forth when using calcium nitrate under 8 w/w% was due to the super-saturation of the wat with respect to calcium hydroxide. However, this is not a general characteristic of calcium nitrate, since its effectiveness to accelerate depends on the type of cement that is used [21]

4.2.3. Mechanical properties

Although being first stated in 1896, Ferret's law still holds true for the most part [60]. The formula states the following:

$$f_c = K\left(\frac{c}{c+w+a}\right) \tag{27}$$

In this formula, f_c is the strength of concrete and c, w and a are absolute volumes of cement, water and air respectively. K is a constant which mainly depends on the type of aggregates that are used. This leads to the first conclusion that a low water/cement-factor that still has practical workability should be maintained. A second conclusion that follows from this equation is the fact that the volume of voids and pores should be kept as low as possible. This translates to a dense, and well compacted in situ, concrete mixture. Even with the recent development of chemical additives this formula still contemporary despite its simplicity, Abram later stated the 'Abram's water-cement ratio law' which states that the strength of a concrete mix is inversely related to the mass ratio of water to cement. This, however, is a simplified version of Ferret's more broad formula.

Furthermore, the strength of concrete is highly dependent on the age on which it is tested. Many models have been proposed to predict the strength evolution [109, 110]. However, due to the broad market of accelerators, retarders, foaming agents, air entrapment agents and more, a practical universal formula is not yet been formulated. Therefore, it is common for producers to test their compositions at various ages and provide these values.

4.2.3.1 Bioagents

In a paper that tested 5, 10 and 20 w/w% addition of urea to self-compacting concrete a decrease in compressive strength was found [19]. Decreases in compressive strength were found up to 15 and 25% for U3 and U5 respectively at 7 days, but this lowered when curing time increased [26]. The same results were found in this work where all dosages resulted in a decrease in compressive strength at 7 days. However, at 56 days, urea seemed to have a positive effect on the compressive strength, with the optimum dosage being 2 w/w%. This was confirmed by a work where an increase in compressive strength was found after 91 days for U3 [25]. This optimum dosage was confirmed by its hydration heat, which harmonized with a work that found sufficient hydration heat for U5 and U10 [4]. This leads to say that urea can be used as a strength improver when used in the right dosage. However, it will most likely not have an influence on compressive strength with current commonly used dosages of 4 w/w%.

When yeast extract was incorporated, and increased from 5 g/L to 15 g/L, a decrease in strength of 27 to 76% was found [15]. This conforms to the results found in this work where yeast extract strongly decreases the compressive strength with increasing w/w%. These findings are repeated in works where a severe negative on the compressive strength is mentioned [3]. As a result of the 75% decrease in compressive strength found for Y2.56, one should be very cautious when incorporating yeast extract in a cementitious mix.

Calcium nitrate is commonly used as an antifreeze admixture, however, strength increasing characteristics were observed due to its setting accelerating properties [10]. Furthermore, long-term strength increase was found as a result of the addition of calcium nitrate, this gain increased until a dosage of 2 w/w% calcium nitrate and flattened out for higher dosages [20]. Contradictory to previous research, no strength gain at an early age was found when calcium nitrate was used in a cement paste mixture, though it improved the 28 days strength by 20% [21]. Additionally, when used in combination with volcanic ash a significant strength increase was noted which was linearly correlated with the amount of calcium nitrate added [11]. It was also confirmed that the optimum dosage of accelerator depends on the ambient temperature. An initial hypothesis that the accelerating mechanism is based on interactions between nitrate and the aluminate phase has been discredited [22]. In this work, an optimum strength gain after 56 days was found for dosages of 8 and 10 w/w%, this was due to the flash setting that occurred. A positive effect was also noted for 2, 4 and 6 w/w%. The influence on the cement with CEM I R and CEM II R was tested which resulted in an increase of 30% in the early strength of the concrete after 2 days compared to the reference [28].

4.2.3.2 Bacteria

In a 2014 work where the impact of bacterial cells on hydration was studied, it was found that the nutrient mortar achieved similar strength to that of the bacterial mortar after 28 days [33]. The same observations were made in this work where both achieved compressive strength of about 32 MPa after 28 days. These results are also confirmed in a work where baghouse filter dust is added to bacterial concrete [29].

Interestingly, while other works speak about an increase in strength when adding bacterial cells, this work shows a small decrease in strength [54]. However, this strength loss is negligible compared to that which follows from the addition of bioagents, most importantly yeast extract. This opinion is shared in a work where the influence of urea and calcium dosage is investigated on its effectiveness of MICP [6]. This brings forwards the trade-off that was made between the properties of the hardened mortar and the effectiveness of the MICP.

Strives to negate the effects of bioagents on the cementitious matrix have been made by encapsulating these in microcapsules, however, compressive strength was reported to drastically decrease by incorporating these [38]. This was also the case when using hydrogels [31].

4.2.4. Water absorption

After evaporation of the excess water that did not take part in the hydration process voids, which act capillaries, are created. These capillaries are directly related to the porosity of permeability of the mixture. Therefore, a high water-cement factor leads to a mixture that will evaporate more water and eventually leave a less dense structure that will absorb water more easily [60]. Additionally, when concrete is compactly poorly more airpockets are created. Therefore, proper vibration of the concrete, to remove any excess air is not only important for the mechanical strength but also to ensure good durability by constructing a compact concrete.

Even though liquids and gasses can infiltrate and reach the surface of steel reinforcements bars embed in the concrete, it is not automatically guaranteed that corrosion will take place. This is due to the passive film that is formed around the surface of the steel by the alkaline solution film of concrete [111, 112]. The environment plays a crucial role, as it was found that marine environments are one of the most challenging to amend due to its chloride diffusion and penetration of contaminated water [113].

Current solutions include coating the concrete, using hydrophobic concrete and the usage of additive materials [114, 115]. However, due to the negative side-effects of some of the researches have, in light of previous research on this field, proposed a surface treatment based on bacterial carbonate precipitation [116].

The influence of urea on the water absorption of mortar is under-documented. However, one research showed a decrease in sorption when incorporating 0 to 7 w/w% of urea in mortar cubes [25]. Similar results were found in this work for urea, where urea, varying from 0 to 5 w/w% showed a small increase of water absorption and thus had a negative effect on the durability of the mortar. These results, however, seem counterintuitive considering urea increases the porosity of the mortar [4]. However, as a result of the greater volume of residual urea in the pores, less water can enter the pores since its pores can only hold the solution volume, which was greatly increased by the dissolving of residual urea. This all leads to a reasonably steady increased water absorption over the series with reference to the control.

No research on the effects of yeast extract on the absorption is found. However, negative effects as a result of its presence are not to be neglected. A doubling of the water absorption was observed when adding 2.56 w/w% of yeast extract in the mortar mix. This is future proof to support the arguments presented in 4.1.1 of the mechanisms that yeast extract introduces. The high porosity presented itself clearly in the results found. Yet, the grouping effect between

YE0.85, YE1.28 and YE1.7 could not be explained. Even at 28 days, the curves were still steep enough to assume that the mortars were not yet fully saturated with water.

The only positive effect for the durability, which was a decrease in water absorption, was found when incorporating calcium nitrate tetrahydrate in the composition. No significant porosity increase was reported when adding 0 to 5 w/w% of calcium nitrate [20]. This supports the author’s proposition which states that this steady absorption amount over the series is a result of jennite that is formed from low-density C-S-H with calcium hydroxide alongside 1.4 nm tobermorite. However, this theory, as explained more in-depth in 4.1.4 is purely theoretical and will need to be confirmed in future research.

4.2.5. Freeze-thaw resistance

As a result of the specific execution of the test performed in this work, no literature is found. The motivation for this test was a study where limestone prisms were treated with an ureolytic biodeposition treatment to investigate the effect of the surface treatment, an increased resistance towards carbonation, chloride penetration and freezing and thawing was found for mortar specimens [39, 40].

Because there was no damage observed for the specimen, the trend of the results corresponds to the results found for the water absorption. The sole difference is that the amount of weight lost equalled the amount of water that was added to the mixing process, and therefore no additional water was forced into the pores. This caused the mortar with yeast extract to have a lower weight loss since it is more porous than the mortar with urea. Additionally, with the mechanisms mentioned above, urea brings in a greater volume of solution water, which caused its high weight loss.

No literature is found for both urea and yeast extract on their effects when being subjected to freeze-thaw attacks.

Calcium nitrate, however, is a known anti-freeze admixture [10]. In this study the concrete was subjected to up to 14 days of exposure of -10°C after which was concluded that calcium nitrate can be used as an anti-freeze admixture in these circumstances.

4.2.6. Carbonation

Calcium carbonation occurs in concrete due to calcium-bearing phases that are present in the concrete. These phases get attacked by carbon dioxide that is present in the air or water after which the chemical conversion of calcium hydroxide with carbon dioxide to calcium carbonate and water takes place. This conversion can be seen in (28).

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O \qquad (28)$$

However, when all calcium hydroxide turned into calcium carbonate most of the calcium oxide from the hydrated C-S-H gel will also form calcium carbonate and the AFm (alumina, ferric oxide, monosulfate phase), as well as the Aft (alumina, ferric oxide, tri-sulfate) phases, will be decomposed [96]. The carbonation process requires the presence of water, this will allow CO_2 to be dissolved in water forming carbonic acid. This means that if the concrete is too dry, CO_2 will not be able to dissolve. Moreover, if the concrete is too wet, CO_2 will not be able to enter its pores and no carbonation will occur.

Carbonation is a process that starts from the surface and therefore the amount of carbonation material is directly related to the exposed surface and the length of exposure. It is clear that the speed of transport has a direct relation to the porosity of the concrete. However, due to the carbonation, and thus the precipitation of $CaCO_3$ in the pores of the concrete, a small decrease in the permeability occurs. This slows down the speed of transport [117].

The carbonation constant factor k_{ca} as given in (9) depends on the permeability of concrete, the quality of cement, the presence of additives and environmental conditions [61, 118].

Similarly to results found in this work, the literature shows that the carbonation depth decreases with increasing amount of urea added to the mortar composition [17]. However, little explanation for these results or even additional experiments were found. In this work, two main mechanisms were proposed that characterise the effect urea has on the cementitious matrix with regard to carbonation. Firstly, as a result of the high solution volume, since urea does not hydrolyse, CO_2 is unable to enter the pores, this is the reason for the low carbonation depth at early ages. Secondly, because of the presence of water, CO_2 reacts to form carbonic, which in its turn reacts with the hydration product calcium hydroxide to create calcium carbonate. This calcium carbonate acts as a filler and decreases porosity, leading to a decrease in carbonation depth [4].

No research has been found regarding the effects of yeast extract on carbonation. Therefore, this work serves to provide a first insight into the mechanisms that take place. The increased porosity, which was concluded from the increase water absorption and decreased density simplified the diffusion of CO_2 in and throughout its pores. This led to a rapidly increasing carbonation depth up to a factor of 40 when comparing to reference. Moreover, a double carbonation front was observed and this was explained as a result of the rapid intrusion of CO_2 where the rate of transport was much faster than the reaction rate of the carbonation. Similar results for this mechanism was found in very porous materials [96]. Although this might be seen as a mechanism where CO_2 of the concrete production lifecycle can be recovered, one has to be careful when using yeast extract in combination with reinforced concrete.

Calcium nitrate has been used commonly as an effective corrosion inhibiting system in reinforced concrete. This is for the increased density that follows from incorporating calcium nitrate, and therefore lowers the carbonation rate [45]. However, research also showed that the effectiveness strongly depends on the type of cement used, as for CEM I, a reduction of up to 40% was found [44]. In this work, a clear reduction in carbonation depth was found, notwithstanding that for Ca2 a carbonation depth greater by a factor of 5 was found. It is the author's opinion that the carbonation depth values of reference are a one-off and calcium nitrate should have a decrease in carbonation, as a result, starting at a dosage of 2 w/w%. This shows that mortar and even concrete do not always react to carbonation as expected, which underlines the importance of regularly monitoring of constructions that are susceptible to carbonation.

4.2.7. Cement hydration

A great multitude of factors have an influence on the development of the hydration heat of mortar in time. The paramount factors are discussed. Firstly, the composition of the cement is one of the main factors that determines the setting and hardening rate, by increasing the amount of C3A, the setting will occur faster and hydration heat will rise [119]. Secondly, as discussed before, gypsum is added to prevent flash setting. Therefore, gypsum is a retarding agent, when adding an amount of gypsum that is too little the retarding effect will not be sufficient. On the other hand, when adding too much gypsum it will act as an accelerator itself [120, 121]. Thirdly, the fineness of the cement will have an impact on both the setting and hardening. Fine cement particles have a larger total surface area and therefore the area that is in contact with water is bigger. This will result in quicker hydration and thus faster setting and hardening which leads to a higher early strength [122, 123]. An example of this is low heat cement that not only limits the weight of fine particles but also changes the chemical composition to be used in massive construction like dams. Fourthly, both the curing age and conditions have an influence. The curing conditions are kept optimal in a lab environment by controlling the temperature and relative humidity. Notwithstanding, in practical situations, a dry environment could cause the water to evaporate which leads to an abrupt stop of hydration that could lead to cracking. On the contrary, a temperature under the freezing point the hydration will not only stop, but the structure of the cement will be destroyed [124]. Fifthly, the impact of the water content, or generally expressed as the water-cement factor, influences the setting. Since with increasing water-cement factor, an increase of capillary porosities, lower strength and delayed setting times found. Lastly, and most important in this work, is the addition of additives. These additives influence the hydration of C_3S and C_3A, which affect the hydration.

The hydration of cement incorporating was reported to have both a decrease in initial and maximum temperature, as well as a delay in the second hydration peak [26]. The same results were found when incorporating urea in this work, additionally, further tests were performed to visualize the effect of the endothermic dissolving of urea in water, there it was found that starting from 4 w/w% of urea, it was able to cool water down significantly. The cumulative heat was found to show similar levels with respect to the reference, this for up to 5 w/w%. Furthermore, in literature this same conclusion was found up to 15 w/w% [4]. However, not only this endothermic reaction was found to be responsible for the retarding effect, additionally the increase of solution volume, which is equal to the volume of urea added, as a result of urea not undergoing hydrolysis resulted in the conclusion that it showed similar effects to raising the water-cement factor. A decrease up to 13°C at peak hydration temperature was found when incorporating 20 w/w% urea

All previous results found in this work were able to get validated by its hydration heat curve. Most importantly, the reversed effect on the setting, from retarder to accelerator, was clearly shown. The retarding effect, which was found in two other works, was found to be linear up to a dosage of 1.7 w/w% of yeast extract [33, 38]. Dosages higher than this exhibit an accelerating effect similar to this of the flash setting that occurred at high dosages of calcium nitrate. Also, at this dosage, the cumulative heat was found to be low which validates the low mechanical strengths it exhibits. However, on multiple occasions in this work, the connection between yeast extract mortar and foamed concrete was made, this was proven not to be correct for all characteristics. Literature shows that as a result of this foaming the hydration heat increase since its high porosity acts as an insulator, however, in this work it was found that the peak hydration heat decreased with increasing w/w% of yeast extract [47].

Calcium nitrate showed, as expected, a hydration line of which the second hydration peak moves to the left with increasing w/w% of calcium nitrate which is in line with the literature [10]. However, for dosages higher than 6 w/w% the second hydration peak no longer occurred earlier, but rather its maximum hydration heat raised while the peak got narrower. Additionally, the dormant period was less distinguishable for Ca2, Ca4 and Ca6 which leads to the initial and final setting occurred in the same period as Ca8, and Ca10 even though the peak was narrower and therefore the stage 3 acceleration was steeper. This accelerating effect happened up to 10 w/w% while literature speaks of a delay occurring when incorporating more than 7.79% CN [22].

4.2.8. $CaCO_3$ content

Calcium carbonate is introduced to the cementitious matrix in two ways. Firstly, as discussed in 4.1.6, calcium-bearing phases can be attacked by carbon dioxide that is present in the air and reaction together to form calcium carbonate [125]. In concrete, this calcium-bearing phase is usually calcium hydroxide. This mechanism is visually noticeable when water leaks through concrete and dissolves calcium hydroxide from the matrix, upon contact with the atmosphere. It then reacts with carbon dioxide to form calcium carbonate, which is also known as “efflorescence”. Furthermore, after removing all calcium hydroxide from the matrix, additional calcium oxide can be freed which will also carbonate.

Secondly, *B. sphaericus* can precipitate $CaCO_3$ by means of urea hydrolysis [55].

$$CO(NH_2)_2 + 2H_2O \xrightarrow{Bacterial\ urease} 2NH_4{}^{+} + CO_3{}^{2-} \tag{1}$$

$$Ca^{2+} + CO_3{}^{2-} \rightarrow CaCO_3 \tag{2}$$

However, this only occurs when certain bacterial strains, that are capable of precipitating calcium carbonate, are introduced to the composition. In this mechanism the most important influence factors are the type of bacteria [126], bacterial cell concentration [14], temperature [57, 58, 127], urea and calcium concentration [14, 52, 127], and the pH of the medium [14, 52].

In this work, the influence of bioagents and the influence of the number of cells was tested with regards to their ability to precipitate $CaCO_3$. What was found is that the presence of bioagents increased the amount of weight loss per gram. Moreover, it was found that the bacterial solution was able to precipitate calcium carbonate without the presence of bioagents. However, this was only true for T6, where the bacterial cells were not centrifuged, but remaint in the spent UYE-medium. In this medium, cells decompose urea and this results in the presence of carbonate ions, that when combined with calcium ions forms calcium carbonate. These results correspond to the findings in a previous work where the same conclusion was made [38]. Furthermore, the addition of urea, yeast extract and calcium nitrate had a positive effect on the amount of precipitated calcium carbonate, for which a maximum amount of weight loss is found when all three bioagents were introduced to the mix, with the exception where T1 shows a larger decrease after 3 days of casting. This again corresponds to previous research on this topic [2].

Lastly, it was verified, that when incorporating bacterial cells in a spent UYE medium, an increase in precipitated calcium carbonate was found that resulted in a greater weight loss compared to that found when using the optimal, in terms of calcium carbonate precipitation, the concentration of bioagents and a low cell concentration. This conclusion is supported by results found in the literature [33, 126, 128].

Lastly, it should be noted that still a high standard deviation is found in this test. Therefore, in future research, it is advised to increase the sample size and increasing the molarity or volume of the hydrochloric acid solution to get more uniform results with less deviation.

5

Conclusion and future work

Bacterial concrete has been the topic of much research lately. Its viability has been proven many times in research and promising results have been found regarding the crack-repair and strength regain. However, a clear lack of knowledge of the fundamental influences of the added bioagents to obtain the optimal aforementioned characteristics is also observed. In this work, a critical evaluation of mortar incorporating these bioagents has been made. Additionally, the influence of these bioagents on the precipitation in mortar prisms was evaluated. In the following sections, the most important influences of each individual bioagents are given. Before that, a few general concerns are given.

Firstly, the labour intensive and time-consuming task of preparing bacterial solutions as well as the cautious task of avoiding contamination should not be underestimated when trying to upscale the production of bio-concrete from lab-scale to industry-scale. Secondly, due to the biological aspect of bio-concrete, a peculiar smell was perceived that is mainly the result of yeast extract and ureolysis, although this might be less of a problem in industrial projects, the influence thereof in commercial or even residential projects should not be overlooked. Lastly, the influences that the addition of these bioagents brings forth should be evaluated on a case to case basis, meaning that the negative effects should be weighed off against the positives before deciding whether or not to incorporate bacterial concrete in a project.

However, with continuous research in this field, it is the author's opinion that bacterial concrete will play a major role in future projects.

5.1. Urea

Urea is the most crucial bioagent since without ureolysis, no bacterial calcium carbonate can be precipitated. In this work, it was found that urea was the most inert bioagent out of the three bioagents researched. Up to a dosage of 5 w/w%, no negative effects were observed.

The effects of urea on the hydration of cement were observed where an endothermic reaction was proven. This promotes the use of urea in massive constructions. This also caused an optimal dosage of 2 w/w% to be found. The reason for this should be investigated further. However, it is believed to be due to the endothermic balance found in this work.

5.2. Yeast extract

Not only in this work was it shown that yeast extract had a negative effect almost all properties of both fresh and hardened mortar, it was also responsible for 10 and up to 60% of total operating costs of the UYE medium it was used in [129]. Therefore, it is important to not only replace yeast extract with a cheaper, but also a more inert, and even positive, additive with similar characteristics for the bacterial cells. Recently corn steep liquor (CSL) has been proposed, which is an industrial by-product. A similar test should be executed as shown in this work to compare the effect of both these bioagents.

Due to its negative effects, yeast extract should not be used. However, if required, the dosage should stay under 0.34 w/w% to ensure these negative effects remain limited. The main priority should be put on finding a viable alternative for yeast extract in future research.

Lastly, the mechanism proposed in this work should be verified by specific research in future works. Although these mechanisms explain all of the changes in properties yeast extract bring forth, no proof can be given.

5.3. Calcium nitrate

Calcium nitrate acts as an inert and mostly positive additive in the cementitious matrix. Due to the decrease in porosity in brings forth, the overall durability was increased by calcium nitrate. However, one should be careful when increasing the dosage of calcium nitrate, as shown in this work flash setting occurs on high dosages, which negatively influences the fresh properties of mortar. To prevent this its dosage should not exceed 8 w/w%.

While most of the test showed a positive result as a result of the addition of calcium nitrate, a worrisome result mechanism was discovered when combining yeast extract and calcium nitrate. The negative effects due to the air entrapping characteristic of yeast extract seem to get amplified with the addition of calcium nitrate. As mentioned in this work, it is the author’s opinion that the flash setting of calcium nitrate causes these negative effects.

5.4. $CaCO_3$ content

The previously researched and proposed optimal dosage of bioagents in combination with bacterial cells was confirmed in this work, where the largest amount of precipitated $CaCO_3$ content was found. However, this was at the expense of many of the characteristics as a result of the added yeast extract. As mentioned above, yeast extract should be replaced or isolated from the cement particles to negate its negative influences.

Furthermore, a new testing method to test $CaCO_3$ content was proposed. In this work, similar results were found with literature, where more expensive, time-consuming and smaller sample sized testing methods were used. It is the author’s opinion that the sample size should be increased further to obtain even more precise results. Due to the good results found in this work, it is strongly recommended to compare this testing method with the currently commonly used methods in future research.

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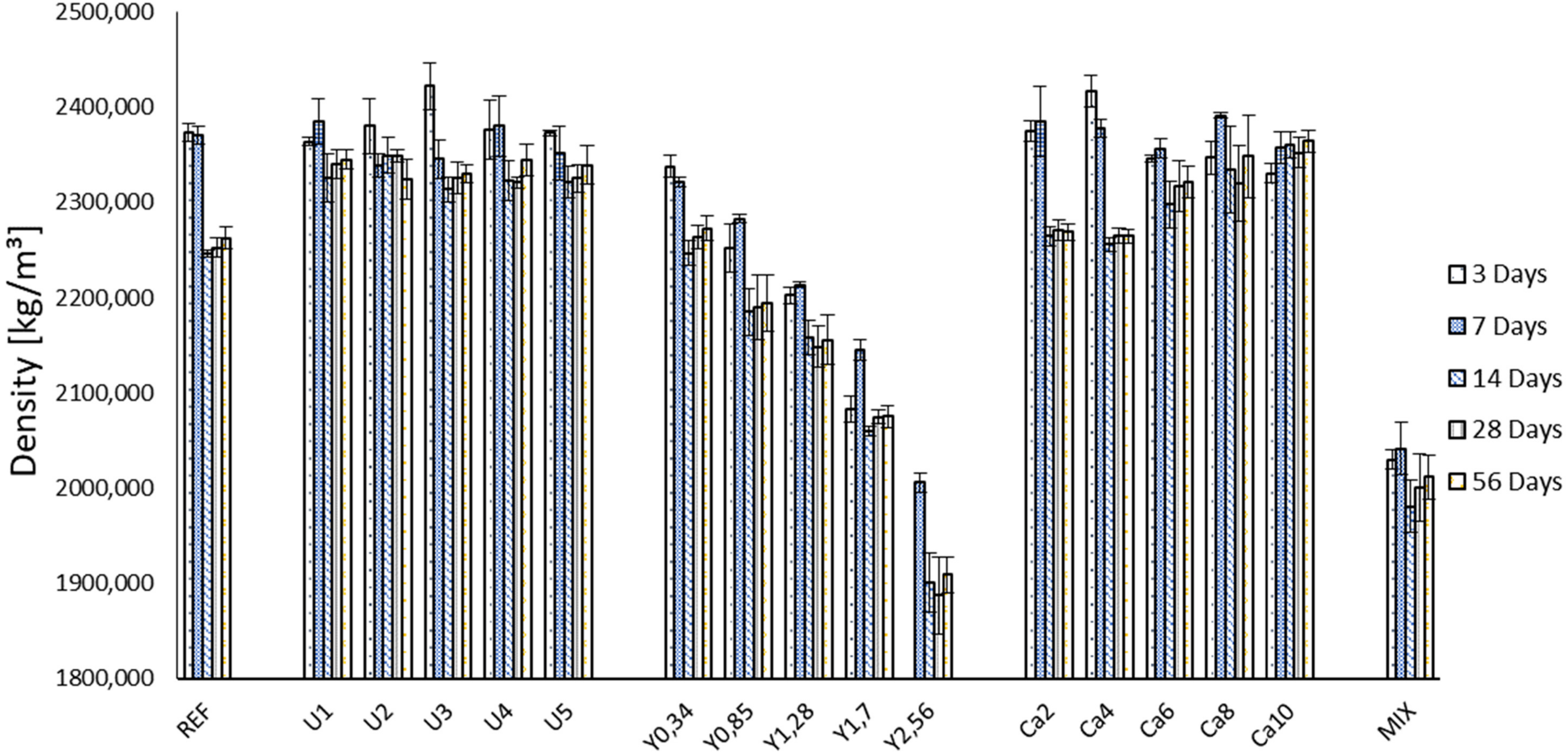
Addendum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U4
U5
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Y0,85
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Ca10
MIX
3 Days
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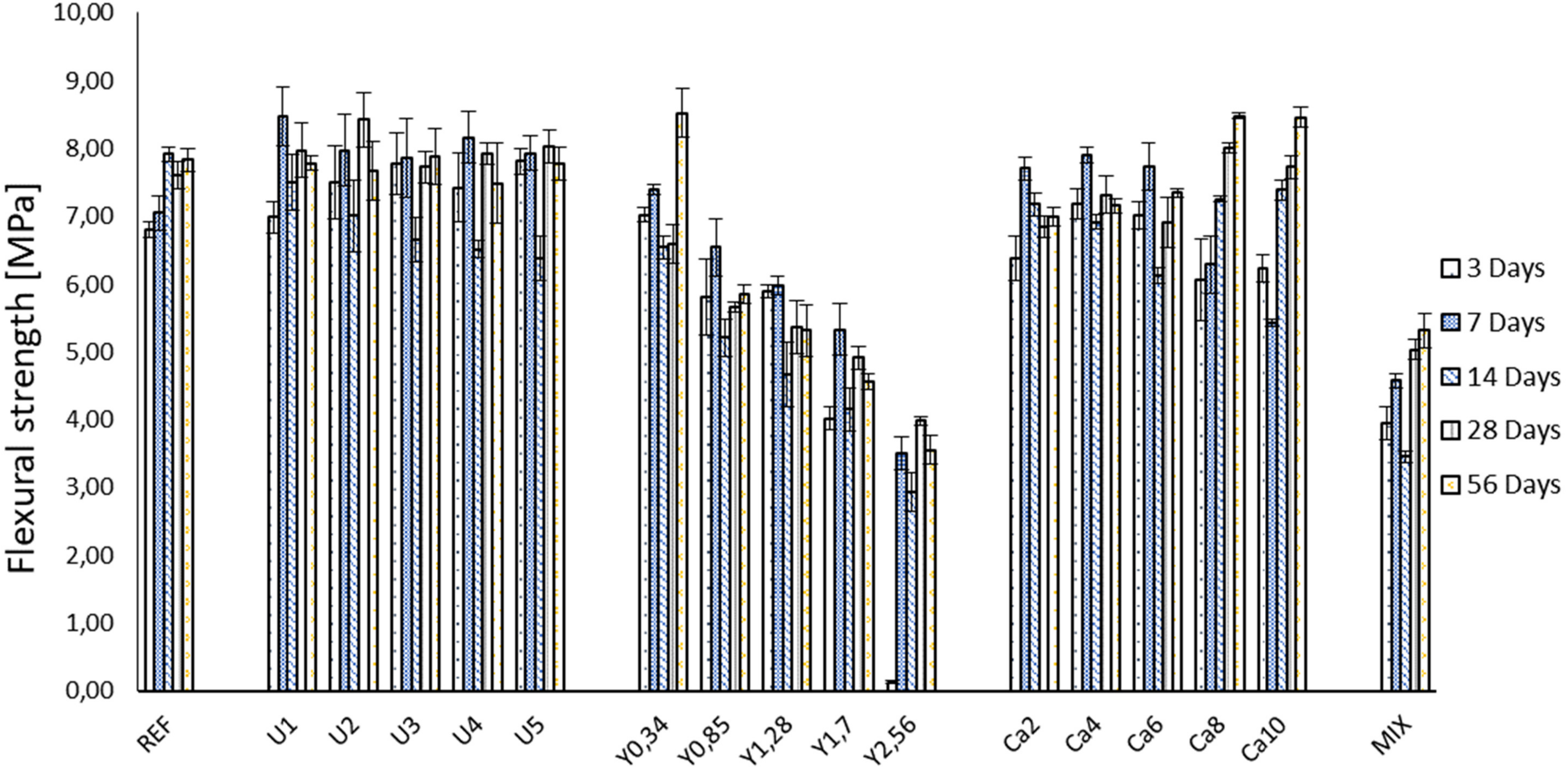
28 Days
56 Days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0,00
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U3
U4
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Y0,34
Y0,85
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Ca2
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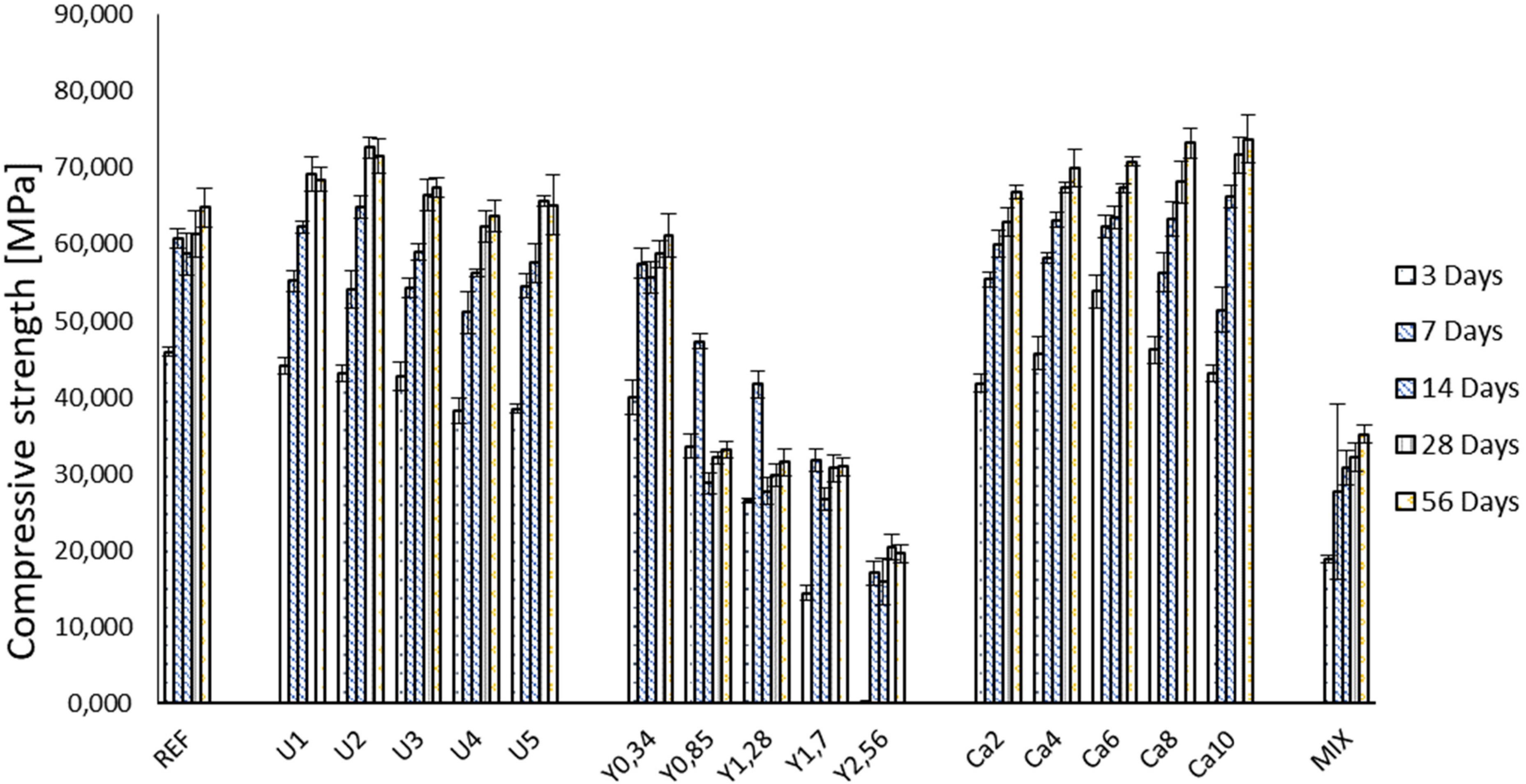
Ca6
Ca8
Ca10
MIX
3 Days
7 Days
14 Days
28 Days
56 Days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U2
U3
U4
U5
Y0,34
Y0,85
Y1,28
Y1,7
Y2,56
Ca2
Ca4
Ca6
Ca8
Ca10
MIX
3 Days
7 Days
14 Days
28 Days
56 Days