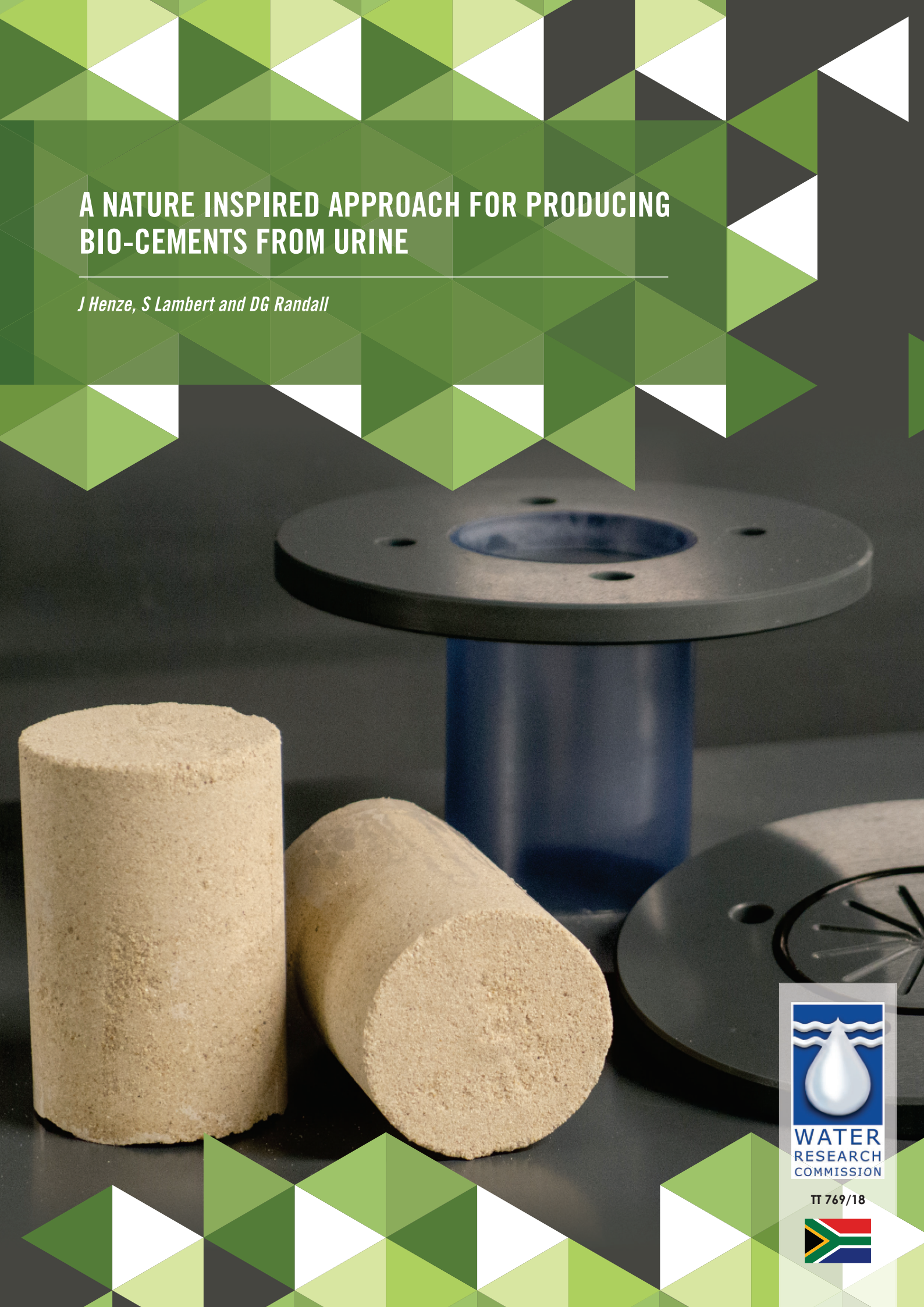
A NATURE INSPIRED APPROACH FOR PRODUCING BIO-CEMENTS FROM URINE

J Henze, S Lambert and DG Randall



WATER RESEARCH COMMISSION

TT 769/18

A nature inspired approach for producing bio-cements from urine

Report to the
Water Research Commission

by

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WRC Report No. TT 769/18

October 2018

Obtainable from

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South Africa

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ISBN 978-0-6392-0051-4

Printed in the Republic of South Africa

EXECUTIVE SUMMARY

The aim of this work was to produce bio-solids using microbial precipitation and the urea present in human urine. The reason for engaging with such a project was to find an alternative use for urine. Removing nitrogen and phosphorus from a wastewater treatment plant requires large amounts of energy, separating urine and excreta at the source would negate this energy requirement. This work seeks to use urine as an alternative source of urea for bio-brick production in a more energy and water efficient brick-making process. In addition, the urine is sourced from urinals which also stabilize the urine (prevention of urea hydrolysis) while producing calcium phosphate, an inorganic fertilizer.

Microbially Induced Calcium Carbonate Precipitation (MICP) was investigated for the production of these bio-solids using synthetic urine and real urine. This was done using the bacteria *Sporosarcina Pasteurii*. First, the stabilized urine was titrated to investigate its buffer capacity. Thereafter, tests on the effect of the alkalinity of the urine on MICP and ureolytic activity was performed. These tests indicated that real urine could be used as cementation media for MICP provided the pH was decreased to 10.8 or below. Next, ordinary masonry sand was added to customized column reactors. The sand was inoculated with the bacteria and then the cementation media was pumped through the column to fill the pore volume. The media was retained for three hours. Every three hours new cementation media was pumped through the column. The pH of the media was adjusted with 0.1 M HCl to reach a pH of 10.8. Additional calcium was added in the form of calcium chloride to increase the amount of calcium carbonate that could precipitate between the loose sand particles.

To fill a pore volume of 12.5% it would theoretically take 8 days with 56 treatments for a column to solidify, assuming the calcium usage efficiency is 85%. To produce bio-solids with the same volume as a standard brick, the following inputs would be required: 1.72 dm^3 masonry sand, 36 L of urine, 433 g of calcium chloride dihydrate, 133 mL of 32% HCl and 850 mL of bacteria culture. The process could produce a profit of R24 per brick, provided calcium phosphate and ammonium sulfate are also produced.

The proposed integrated system combines the production of bio-solids with waterless urinals and phosphate and ammonia fertilizer production. The process could produce products that have low embodied energy and little impact on the environment, while simultaneously being economically viable.

ACKNOWLEDGEMENTS

We are grateful to the people of the Water Quality Lab at UCT, especially to Njabulo Thela and Hector Mafungwa, who gave technical support for all the experiments. The same appreciation goes to the staff of the CeBER Laboratory who supported us with their expertise and equipment to grow the required bacteria. Here, we want to particularly thank Sharon Rademeyer for patiently tutoring us in the methods of cultivation. Without her guidance, this project would not have been possible. We also wish to thank Prof Eberhard Morgenroth for his valuable input and comments regarding this work. Finally, the financial support from the Water Research Commission is gratefully acknowledged.

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Chapter 1 Introduction

1.1 Motivation

The increased awareness of limited natural resources and the need to reduce energy demand in the future is placing significant pressure on engineers to find more innovative solutions to these challenges. In recent years the term *Sustainable Infrastructure* has become more important in the field of Civil Engineering and Architecture. According to Thome et al. (2016) the number of publications in the field of *sustainable infrastructure* has grown exponentially since 2003. It is estimated that approximately 75% of all infrastructure that will be in place in 2050 has not been built yet. Considering the long lifespan of infrastructure, this represents a significant opportunity to reduce the environmental footprint of our future societies. However, it poses a risk as well when the issue of sustainability is not appropriately addressed (Egler and Frazao, 2016). One possible leverage point for architects and civil engineers to enhance the sustainability of infrastructure can be achieved through the careful selection of building material. The traditional brick has always been an import part of infrastructure. Various production processes exist differing in achieved strength, chemicals required and energy consumption. An alternative production process for a solid material that could be used to make more sustainable bricks is the core subject of this report. When assessing the environmental impact of a material or product, in general, three different life cycle stages should be considered: (i) production, (ii) use, (iii) end-of-use. Although these stages seem to indicate a linear process, advances are being made to transfer material or energy from end-of-use to the production phase of the same or another product instead of disposal. This idea is the core to the concept of a "Circular Economy", where, in an ideal case, no disposal should take place anymore (EMF, 2014).

Conventional waste water treatment plants have mainly been designed to avoid adverse effects on receiving water bodies from inorganic and organic pollutants (Grundmann and Maass, 2017). However, the recovery of nutrients from waste water becomes more important in view of unsustainable practices by the fertiliser industry. For example, the production of nitrogen fertiliser from gaseous N_2 by means of the Haber-Bosch process was estimated to account for about 1.2% of the worldwide energy demand in 1998 (UNEP and IFA, 1998). Also, the production of phosphate fertilisers involves the depletion of finite phosphate containing minerals. Because of the reliance on limited natural resources, a peak-phosphorous is expected, although opinions about its timing are deeply divided (Neset and Cordell, 2012). In fact, nutrients or the energy required to concentrate, convert or extract them from a natural resource are undergoing a linear usage process that ends at the conventional treatment of wastewater. Here, nitrogen is either lost as gas during denitrification or together with phosphorous through incineration of sludge, in a landfill or in the effluent of the treatment plant (Gujer, 2006). The recovery of these nutrients from wastewater could partially close their usage cycle whereby the depletion of natural mineral deposits or the extensive use of energy becomes more and more obsolete. The recovery

of nutrients such as phosphorous and nitrogen from source-separated urine is an integral part of this alternative production process that is also the subject of this report.

1.2 Background

Two concepts are the key to understanding how the recovery of nutrients from urine can be integrated into the production process of an alternative building material:

- Source-separation of urine & urine stabilisation
- Microbiologically induced calcium carbonate precipitation (MICP)

A urine-diversion toilet enables the source-separation of urine from other wastewater components. The separation facilitates the recovery of nutrients that the human body excretes. Urine contributes over 50% of the phosphorus and over 75% of the nitrogen contained in domestic waste water (Larsen and Gujer, 1996). Approximately 90% of the nitrogen in urine is present as urea (Udert et al., 2003). In conventional wastewater collection and treatment urea is naturally converted to ammonia by certain bacteria which produce the enzyme urease, a catalyst for this reaction. Randall et al. (2016) described a method to stabilise urine through the addition of calcium hydroxide such that enzymatic urea hydrolysis is inhibited. The inhibition of enzymatic urea hydrolysis prevents the loss of urea in urine and the characteristic ammonia smell. In addition, the process also produces calcium phosphate which can be used as an inorganic fertiliser. The urea and residual calcium can be further used to drive the microbiologically induced calcium carbonate precipitation (MICP). MICP is a biomineralisation process, where specific microorganisms induce the precipitation of calcium carbonate ($CaCO_3$), e.g. through the decomposition of urea to ammonia (NH_3) and carbonate ions. This leads to a supersaturation of calcium carbonate and its subsequent precipitation (Muynck et al., 2010).

MICP has recently been used to produce so-called bio-bricks as an alternative to conventional bricks (Bernardi et al., 2014). The formation of calcium carbonate is used to cement an otherwise loose mass of particles together, such as sand or waste building material. As a first manufacturer on the market, BioMason is in the process of upscaling their brick production with MICP, stating that the strength of their products is comparable to traditional masonry (BioMason, 2017). BioMason uses synthetic urea solution for their production though and therefore competes with urea for fertilisers on the market. The process being the subject of this report aims to use an otherwise lost source of urea instead – the urea found in urine. Additionally, it provides the opportunity to recover phosphate and ammonia as an important nutrient resource from the urine. The combined process might create high value products, such that urine is no longer seen as a pollutant, but rather as a valuable resource.

Chapter 2 Scope & Objectives

The production of bio-solids (e.g. in the shape of building bricks) from stabilised urine using MICP is a new approach for which no data about its feasibility currently exists[1]. In contrast, several studies have applied MICP with synthetic urea solutions (Bernardi et al., 2014; Rowshanbakht et al., 2016; Eryürük et al., 2015). Stabilised urine has a pH around 12.5, which is required to inhibit the natural degradation of urea. As a first step, this project investigated the feasibility of using a highly alkaline synthetic solution that was saturated with calcium hydroxide. Thereafter, real urine was used.

The proposed process to produce bio-solids from sand and urine consists of the following steps:

1. Collection and stabilisation of fresh urine using calcium hydroxide.
2. Separation of calcium phosphate and other solids from the stabilised urine.
3. Preparation of the cementation media: lowering the pH of the stabilised urine.
4. Inoculation of sand with urea degrading bacteria.
5. Saturating the sand with the cementation media.
6. Rinsing and drying of the consolidated brick

The alkaline pH of stabilised urine inhibits the enzymatic hydrolysis of urea (Randall et al., 2016). For this reason, the pH must be decreased before MICP can be initiated. The exact pH is unknown and will be subject of the first part of this study. The non-pathogen species *Sporosarcina pasteurii* is a well-suited organism for the proposed process. Its optimal pH for growing in alkaline conditions is around a pH of 9. It has commonly been used for MICP due to its ability to produce high amounts of the enzyme urease (Muynck et al., 2010).

In the second part of this study, the bio-cement and MICP process is investigated using a sealed sand column. Key operating parameters need to be found while also testing the feasibility of the MICP process. A brief material and cost calculation for the proposed process is also given. The two major aims of this project are therefore to:

1. Determine the ideal bio-cement process and ‘recipe’
2. Manufacture bio-cement from urine

[1] The use of urine in bio-brick production was mentioned in several web articles, but confuses urine with urea solution, which was actually used in the mentioned experiments (Zhang, 2014).

Chapter 3 Theory

The precipitation of solids through a biomineralisation process can occur through two mechanisms: microbiologically controlled or microbiologically induced. In a controlled mechanism, the microorganism synthesizes the mineral particles independently of the environmental conditions in a form that is unique for it. In contrast, an organism that induces the precipitation of a mineral alters the environment of a solution in such a way that the soluble phase of the mineral is supersaturated. Microbiologically induced calcium carbonate precipitation is such a mechanism. The precipitation of calcium carbonate is mainly controlled by 4 environmental variables (Muynck et al., 2010): (i) Ca^{2+} concentration, (ii) the concentration of dissolved inorganic carbon (DIC), (iii) the pH and (iv) the availability of nucleation sites for crystallisation.

Precipitation of calcium carbonate occurs when the solubility limit of the dissolved species Ca^{2+} and CO_2^{3-} in solution is reached resp. when the ion activity product of the precipitation reaction described by Equation 3.1 exceeds its solubility product Ksp (4.8×10^{-9} at 25°C).

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \downarrow \qquad (3.1)$$

The concentration of carbonate ions is a function of the total dissolved inorganic carbon and the pH. It increases with an increasing, more alkaline pH as well as with the DIC. In Figure 3.1A this behaviour is modelled in a system that is closed to the atmosphere and thus no gas exchange will occur. The DIC in such a system is independent of the pH and can be changed through chemical reactions. The concentration of carbonate ions is thus controlled by both environmental variables. In an open system however, as shown in Figure 3.1B, the DIC is only controlled by the pH and by the equilibrium with CO_2 in the atmosphere. Any deviation of the DIC from the equilibrium will lead to the volatilisation or dissolution of CO_2 until equilibrium is restored. The DIC is thus not controllable and the concentration of CO_3^{2-} can only be changed through the pH. In this project, a closed column reactor is used which ideally limits gas exchange to negligible values. In such a closed system, the DIC can be increased by biological activity. A well-known mechanism is the microbiological hydrolysis of urea to bicarbonate and ammonia. The enzyme urease plays a key role in the hydrolysis process by increasing the rate of this reaction by factor 10^{14} in comparison to the naturally occurring rate (Jabri et al., 1995). In the presence of microorganisms that are capable of synthesizing this enzyme, urea is quickly degraded resulting in an increase of DIC as well as a change of pH. The rate of precipitation is thus a combination of the kinetics of urea hydrolysis and the kinetics of calcium carbonate precipitation. Both mechanisms are rather complex in nature and depend on various factors.

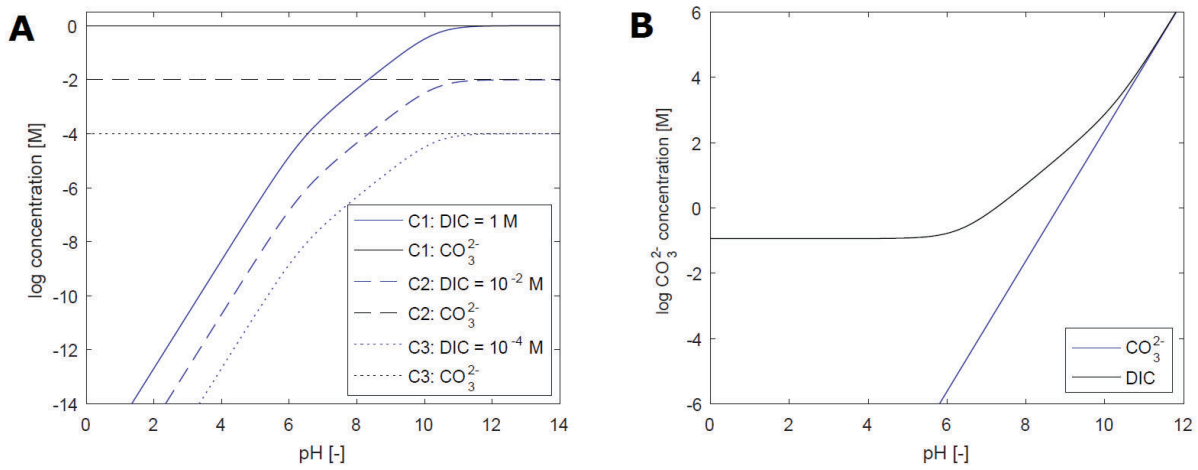


Figure 3-1: The modelled concentrations of CO_3^{2-} as a function of DIC (dissolved inorganic carbon) and the pH at 25°C. In an open system (B) the DIC is a function of the pH and the atmospheric CO_2 pressure and thus not controllable. Therefore, the CO_3^{2-} concentration can only be increased by increasing the pH. In a closed system (A) the DIC can be increased independently from the pH, e.g. through a chemical reaction.

A simplified model for both reaction kinetics is described by Equation 3.2 (Lauchnor et al., 2015) and Equation 3.3 (Tobler et al., 2011).

$$\frac{dS_{urea}}{dt} = V_{max} \cdot \frac{S_{urea}}{K_{m,urease} + S_{urea}} \tag{3.2}$$

The enzymatic hydrolysis of urea can be described by the Michaelis-Menten kinetics, where the substrate – here urea – binds reversibly to an enzyme. As the substrate increases the reaction rate approaches V_{max}. This parameter is on one side dependant on the enzyme concentration, which is again a function of the activity of urease synthesizing microorganisms. But it is also influenced by the enzyme activity which is strongly affected by the pH.

$$\frac{dX_{CaCO_3}}{dt} = k_{prec} \cdot \left[\frac{\{Ca^{2+}\} \cdot \{CO_3^{2-}\}}{K_{s,calciumcarbonate}} - 1\right]^{n_{prec}} \tag{3.3}$$

The rate of precipitation or dissolution of calcium carbonate is mainly affected by the activity of its ions. The higher the quotient of the ion activity product (IAP) and the solubility product constant $K_{s,calciumcarbonate}$ the faster precipitation will take place. The absolute value depends on the rate constants k_{prec} and n_{prec} which are a function of crystal growth mechanism, mineral type, crystal surface area and number of crystals. Many of these factors cannot be sufficiently monitored or controlled, yet the equations are useful in helping to understand how these processes work.

Chapter 4 General Material & Methods

4.1 Micro-biological culture

The non-pathogen species *Sporosarcina pasteurii* has commonly been used for MICP due to its ability to produce high amounts of the enzyme urease and its optimal growth in alkaline conditions at pH 9 (Muynck et al., 2010). It is a facultative-anaerobe, halotolerant organism and can therefore survive at low-oxygen concentration and withstand high levels of salinity. For these reasons, it was the microorganism of choice for this work. For the propagation of *Sporosarcina pasteurii* the Ammonia-Yeast media (ATCC® 1376) was used. It contains: yeast extract 20 g/L, $(NH_4)_2SO_4$ 10 g/L, 0.13 M Tris buffer, adjusted to pH 9. Approximately 20 g of yeast extract and 10 g ammonium sulphate were each prepared in 150 mL of deionised water. 15.75 g of tris-base were prepared in 700 ml of deionised water and its pH was adjusted with 32% HCl. All ingredients were autoclaved separately and then aseptically mixed at room temperature. The growth curve of *Sporosarcina pasteurii* was determined in a separate experiment and is described in Appendix A.

4.2 Synthetic urine

Synthetic stabilised urine containing urea, nutrient broth and calcium hydroxide was used in the following experiments to obtain a solution of constant properties and sufficient supply. The concentration of urea in urine was reported to be in the range of 0.15 M to 0.39 M (Putnam, 1971). Other literature report average values of 0.33 M (Pillai et al., 2014) or 0.19 M (Randall et al., 2016). It was decided to work with a 0.3 M urea solution to imitate urine. Additionally, 3 g/L Nutrient Broth No. 1 obtained from Sigma-Aldrich were added. This concentration of nutrient broth was used by other researchers that used *Sporosarcina pasteurii* in for MICP processes (Stocks-Fischer et al. (1999); Okwadha and Li (2010); Qiu et al. (2014) to support the bacterial metabolism during the experiment. The solution was then stabilised with 2 g/L calcium hydroxide for storage. Before any application in a MICP process, the stabilised, synthetic urine was filtered with a Whatman Grade 595 Filter paper to remove excess $Ca(OH)_2$. 1 M hydrochloric acid was then used to decrease the pH to the desired value. The resulting solution was called *Cementation Media*.

4.3 Analytical methods

4.3.1 pH measurement

A Hanna pH-Electrode type HI1131B in combination with a temperature probe was used for measuring the pH of solutions. The device was calibrated before any experimental run, using a 4-point calibration with Hanna pH-buffers at 4.01, 7.01, 10.01 and a saturated calcium hydroxide solution. The calibration pH of the latter was calculated as a function of the measured temperature according to data given by the National Lime Association (2007). At a measured temperature of e.g. 21°C, the calibration pH was thus 12.592. The proprietary Hanna pH-buffers are automatically temperature corrected by the software.

4.3.2 Dissolved calcium and ammonium concentration

For both calcium and ammonium concentrations, a colorimetric method was used. A Thermo Scientific Gallery automated this process. The measurement range of this device for calcium is 10 mg/L to 200 mg/L and for ammonia it is 0.45-2 mg/L. Samples were diluted with deionised water when necessary and analysed within 30 min of sampling.

Ammonium samples were acidified with 0.1 M HCl to a pH of 3.5 ± 0.5. The acidification prevents volatilisation of ammonium and inhibits the hydrolysis of urea, such that the sample composition is stable (Hellström et al., 1999).

4.3.3 Detection of bacteria viability and ureolytic activity

In order to be able to distinguish between dead and viable cells of *Sporosarcina pasteurii* as well as other species probably contaminating the culture stock, Christensen's Urea Agar was used. This media is commonly applied to rapidly detect ureolytic activity. It makes use of the pH increase at neutral pH when urea is degraded and contains phenol red as an indicator (Christensen, 1946). Urea Agar Base (Christensen) from Sigma-Aldrich was adjusted to a pH of 6.8 +/-0.2 and then autoclaved. 20 g/L urea was filter sterilised with a 0.2 µm filter and aseptically added to the autoclaved agar solution at a temperature of 50°C to 55°C. Petri dishes were then prepared with the mixture.

4.3.4 Detection of bacteria viability and ureolytic activity

The determination of optical density of a cell culture in a spectrophotometer is a quick and convenient method to estimate the cell biomass in suspension. The measurement is based on the scattering of a light beam at a wavelength of 600 nm sent through the sample returning a value of absorbance. The absorbance is approximately proportional to the optical density at low concentration (< 0.4 AU resp. OD) (Widdel, 2010). Culture samples were analysed with a

Thermo Scientific GENESYS 10 Photospectrometer. In order to obtain a zero-value reference, the absorbance of the sample matrix had to be measured as well. For the application here, a sterile, non-inoculated sample of the media broth at the same dilution as the culture sample was used.

Chapter 5 MICP in highly alkaline environment

5.1 Materials & Method

5.1.1 Titration

In order to obtain a titration curve of the stabilised, synthetic urine, 100 mL of the filtered solution was pipetted into a beaker and stirred. 1 M HCl was then added with a micropipette in steps of 100 µm. The pH was recorded each step until it reached a value of below 9.

5.1.2 Effect of the initial pH

The goal of this experiment was to investigate the effect of the initial pH on the rate and total amount of calcium carbonate precipitation as well as the viability of *Sporosarcina pasteurii* after the experiment. The experiment was repeated three times. Each repeat was conducted according to the description below.

Using a glass pipette, five times 100 mL of the filtered, synthetic urine were filled into 100 mL Erlenmeyer flasks. The pH was then decreased by the addition of 1 M hydrochloric acid. The appropriate amount of acid was determined according to the results of the titration described in section 5.1.1. The targeted pH values and the amounts of acid required per 100 mL are listed in Table 5.1.

Table 5-1: The amount of hydrochloric acid added to each flask of the experiment and the expected pH.

	Flask Nr.				
	1	2	3	4	5
1M HCL [mL per 100mL]	0	1.8	2.8	3.6	4.2
pH [-]	12.55	12.26	11.95	11.28	9.25

A 100 mL culture of *Sporosarcina pasteurii* was grown overnight until late-exponential/early stationary phase. With the measurement of optical density, the growth phase was determined before undertaking any further steps. The bacteria were then centrifuged in two 50 mL tubes at a relative centrifugal force (RCF) of 2570 g for 20 min. The supernatant was carefully removed with a pipette until 2-3 mL of liquid, including the bacteria pellet were left. Using the same pipette, the pellet was resuspended in the remaining fluid by trituration and the contents of both tubes were mixed. A 40 µL sample was taken for a subsequent measurement of optical density. The appropriate volume of bacteria depending on the optical density was added to each of the five flasks with the synthetic urine solution such that a calculated optical density of 0.2 was obtained. All five flasks were placed on a shaker platform at 160 rpm to keep the bacteria and calcium carbonate crystals in suspension. A picture of the setup can be found in Appendix C. Samples

for the measurement of dissolved calcium concentration were taken every 15 min for 1.5 hrs. Using a syringe, a sample of about 3-5 ml was taken and filtered with a syringe filter with a pore size of 0.2 µm. The filtered sample was then analyzed at a dilution of 5. The dilution factor was progressively decreased to 4.2 and 3 on the basis of the preceding measurement results.

After 1.5 hrs, the shaker was turned off in order for the particles and bacteria to settle. The supernatant was decanted. A sterile loop-wire was inserted into the sediment and aseptically streaked onto Christensen's Urea Agar. The agar plates were incubated at 30°C for 24 hours.

5.2 Materials & Method

5.2.1 Titration

The titration curve of the synthetic urine solution has a distinctive shape as shown in Figure 5.1. While the pH is well buffered in the range of 11.5 to 12.5, it quickly drops to below a pH of 9 after the addition of a small amount of acid. Adjusting this solution to a specific pH therefore becomes more difficult below a pH of 11, where the addition of a small amount of acid added causes a large change in the pH. Figure 5.1 also shows the position of the contents of the five flasks (red diamonds), that were prepared for the experiment described in Section 5.1.2. The measured values of the titration are summarized in Table C.1 in the Appendix.

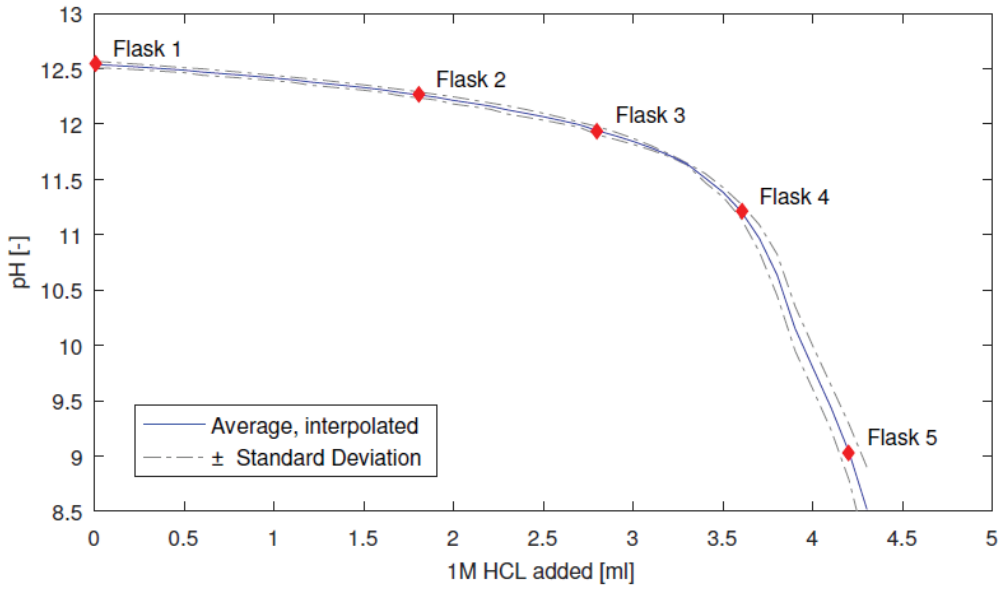


Figure 5-1: The titration curve of the stabilised, synthetic urine after filtration. The red diamonds indicate the position on the curve of the content in the five flasks prepared for this experiment.

5.2.2 Effect of the initial pH

In each of the five flasks, the pH was adjusted to values of 12.55, 12.26, 11.95, 11.28, 9.25 respectively. Figure 5.1 shows the target pH for each flask in the experiment on the titration curve of the stabilised, synthetic urine after filtration. The measured pH after the addition of the previously determined amount of acid deviated slightly from these target values with an average deviation of 0.03 and a maximum of 0.2.

For all three repeats, the bacteria *Sporosarcina pasteurii* were harvested at the end of the exponential growth phase with optical densities of 1.31, 1.34 and 1.44. Through centrifuging the microbial culture, the optical density could be increased by an average factor of 34. The viability of the bacteria after centrifuging was positively tested on Christensen's Urea Agar. In order to reach the target optical density in the experiment of 0.2, about 0.3 to 0.4 mL of concentrated culture per 100 mL of the prepared cementation media were required. The dilution as a result of the added liquid can therefore be neglected. Table C.2 in the Appendix contains the summarized data.

Figure 5.2A shows the course of the dissolved calcium in all five flasks for the repeated experiment. A rapid decrease of dissolved calcium due to calcium carbonate precipitation can be observed in flasks with an initial pH of 9.25 and 11.28. The decrease starts earlier and occurs with a slightly higher rate in the flask with an initial pH of 11.28. After 75 min the differences between these two flasks is negligible and the precipitation process significantly slows down. After 90 min, more than 90% of the calcium has precipitated. In contrast, no significant precipitation is observed at an initial pH of 11.95 and 12.26. However, at the initial pH of 12.55 – which is the saturation pH of calcium hydroxide – a slight decrease in dissolved calcium can be seen. The measured pH after 90 min in flask 4 and 5 dropped in average from 11.15 and 9.32 to 9.25 and 9.15 respectively. In flask 1, 2 and 3 a slight decrease of 0.19, 0.25 and 0.48 was observed in average. The course of the pH was logged once for flask 3, 4 and 5 and can be found in Figure 5.2B.

Figure C.1 in Appendix C shows the inoculated Christensen's Urea Agar plate after approx. 2 hours (left) and 24 hours (right) for one experimental run. Each of the sections corresponds to a sample from the sediment in the flask with the indicated number. No colour change was observed after 2h for flasks 1 and 2, thus indicating that the viability of the bacteria were strongly affected by the high pH. Flask 3, 4 and 5 contained viable cells after the experiment, although a comparably weak colour change is seen for flask 3. Although the sediment contained viable cells, their concentration was supposedly much smaller than in the sediments of flask 4 and 5. After 24 hours the complete agar plate turned purple due to the diffusion of the products from hydrolysis. As such, the difference in uerolytic activity for each section is no longer visible. However, it can be seen in Figure C.1 on the right that bacterial growth only occurred for flask 3, 4 and 5, with only a few colonies forming from flask 3. The second and third experiment run

resulted in similar observation. The numeric results are summarized in Table C.3 and Table C.4 in Appendix C.

Figure C.3 in Appendix C shows a liquid sample from flask 5 after the experiment under the microscope at 40x magnification. The precipitated calcium carbonate crystals can clearly be seen. Along the edges of some of the crystals, the rod-shaped Sporosarcina pasteurii is observed.

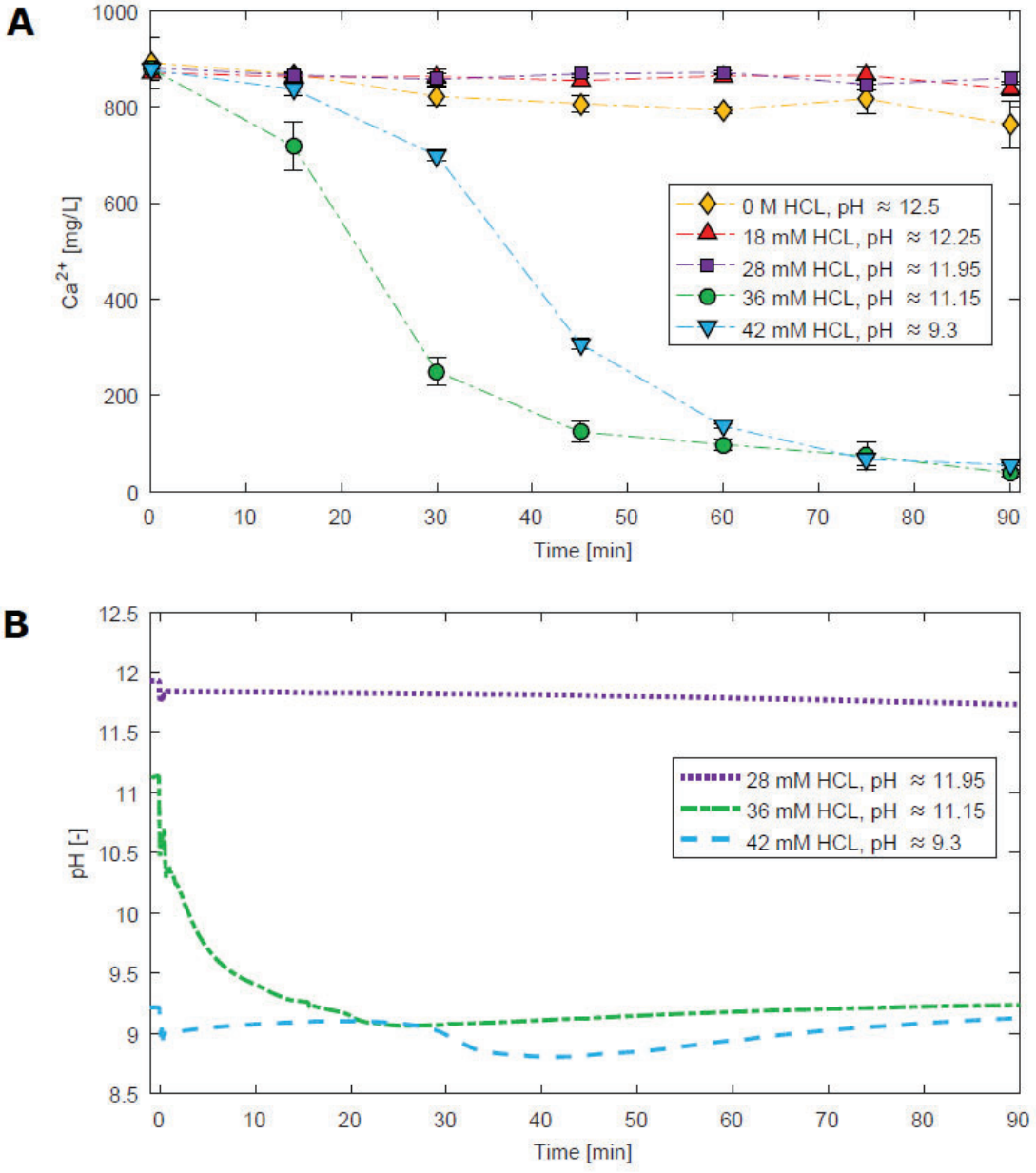


Figure 5-2: The course of the dissolved calcium concentration and pH after adding *Sporosarcina pasteurii* to cementation media at different initial pH. The experiment was repeated three times. The error bar gives the standard deviation of the results. A very high initial pH inactivates the bacteria, while a rapid calcium carbonate precipitation could be observed at a pH below 11.15. Due to the higher concentration carbonate ions, saturation of calcium carbonate occurs earlier in the upper part of the feasible pH range.

5.3 Discussion

This experiment has shown that alkaline urea-calcium solutions can be used in MICP. However, this is limited to a maximum pH of 11.15 for the conditions assessed in this experiment. Interestingly, precipitation started earlier with a high initial pH although other literature suggests that the urease activity of *Sporosarcina pasteurii* has its optimum near a pH of 9 and decreases strongly with increasing pH (Lauchnor et al., 2015). The reason the precipitation process starts earlier at higher pH values can be explained by the higher initial concentration of carbonate ions at a pH of 11.15 compared to a lower pH. When looking at the rate equation 3.3 in Chapter 3, this is the only factor which might deviate in the flasks at initial conditions. Although the hydrolysis of urea might be slower at a pH of 11.15 than at the lower pH of 9.25, the saturation of calcium carbonate is exceeded earlier. From the course of the pH in flask 5 with an initial pH of 9.25, it can be seen that after an initial drop – due to the added volume of bacteria – the pH increases towards the kPa of ammonia (9.25) indicating the release of ammonia by urea hydrolysis. Only after 10 min, when enough urea had been hydrolysed, is the saturation and subsequent precipitation of calcium carbonate observed.

The pH alone is not sufficient to assess if a highly alkaline cementation media can be used in a MICP process. Similar significance must be given to the buffer capacity of a potential cementation media. Any calcium carbonate precipitation and urea hydrolysis causes a highly alkaline pH to decrease. When a weakly buffered cementation media comes in contact with *Sporosarcina pasteurii* or other urea degrading bacteria better conditions for the urease enzyme are then soon established. No significant disadvantage in regard to an experiment with a lower initial pH would be observed. This is probably the case in flask 4, where the pH has been adjusted close to the edge of the titration curve. From the course of the pH during the experiment it can be seen that a high pH above 10 only lasts the first 5 min after adding the bacteria. It is soon within the range of the pH in flask 5 and close to the optimal pH for the urease enzyme. Would the solution have a larger buffer capacity, the precipitation rate in flask 4 would probably fall behind the rate of the one in flask 5 due to the permanently reduced urease activity.

The effect of the high pH on *Sporosarcina pasteurii* complies well with results obtained by Cuzman et al. (2015a). They state that the enzymatic activity was clearly inhibited in the highest alkaline conditions at pH 11 and pH 12. A decrease of enzymatic activity to 18% of its maximum value was observed at a pH of 12. From the decreasing trend of the pH in flask 3, initially adjusted to 11.95 it can be anticipated that a very small amount of urea is being hydrolysed despite the high pH. However, because no calcium carbonate precipitation is observed, saturation is not reached. From the examination of the Christensen's Urea Agar, a high pH affects the viability of *Sporosarcina pasteurii* negatively. The critical value is certainly below a pH of 12 and could even be below 11. Cuzman et al. (2015a) showed that the critical pH-value was greater than or equal to 10. Due to the rapid decrease in pH in flask 3 (with an initial

pH of 11.15) the bacteria were probably not sufficiently exposed to the high pH such that a possible negative impact could be observed.

This again supports the conclusion that the initial pH is not the only factor determining the feasibility of using alkaline media for MICP. The same significance must be given to the buffer capacity of that media. If any initial urea hydrolysis or calcium carbonate precipitation drops the pH to a region where *Sporosarcina pasteurii* can survive, MICP will happen almost unaffected and even start sooner. If the buffer capacity is sufficiently high and *Sporosarcina pasteurii* bacteria are exposed long enough to extreme alkaline conditions, it is expected that the urease activity will decrease and eventually become negligible. The titration curve is therefore a necessity when assessing the applicability of a highly alkaline media for the MICP process.

Chapter 6 Column Reactor Experiment

6.1 Material & Methods

The methods described in this section were enhanced in an iterative process after the pilot run using synthetic urine. Deviations to the original methodology are mentioned in the results and discussion when relevant. The following paragraphs describe the final version of the methodology used for these experiments.

6.1.1 Column Reactor Design & Preparation

A column reactor was constructed to study MICP with sand as the media. The column reactor keeps the loose mass in shape while allowing flow of a liquid through it. A closed design was chosen to minimize the loss of nitrogen by ammonia volatilisation. The constructed column reactor is shown in Figure 6.1. The technical drawings can be found in Appendix B. It has a volume of ca. 255 mL with an inside diameter of 57 mm and a length of 100 mm. The bottom and top lid are identical and removable, both sealed with an O-Ring. On the inside face of both lids, a radial depression distributes the incoming liquid over a large part of the sand body surface. This minimizes the chances of clogging. In order to keep the sand grains from leaving the reactor through the effluent, a fabric mesh was placed between the sand and lid. The mesh size is not specified.

Before the reactor columns were filled with sand, a transparent plastic sheet was cut and placed along the inside cylinder wall. The space between the transparency and the wall was greased with silicone paste (Manufacturer: Herschell) beforehand. The purpose of the paste was to seal the gap between the plastic sheet and cylinder wall from liquid intrusion. This construction prevents the precipitate from bonding directly to the cylinder wall and simplifies the later extraction of the sand column after the process. Additionally, a 4 cm thick scrub sponge was cut into a cylindrical shape with a diameter of 57 mm and placed on the inflow side of the reactor in-between lid and fabric mesh. When closing the reactor, the sponge was compressed to about 1 cm. The purpose of the sponge was to put the sand column under a light compressive force and to disperse the impulse of the incoming liquid evenly.

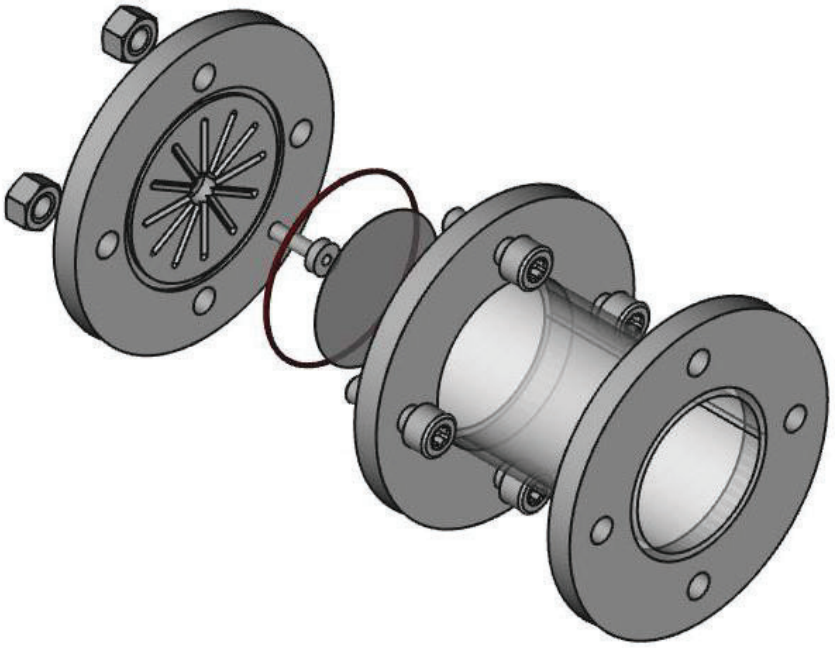


Figure 6-1: A CAD drawing of the constructed reactor. The reactor is symmetrical and the lid on the left fits on the right as well. A fine-grained mesh keeps the sand particles from leaving the reactor through the effluent.

6.1.2 Cementation Media

The target value for the pH was 11.2. This value was chosen on the basis of the results discussed in the previous Chapter 5. Calcium hydroxide has a very low solubility in water. In order to elevate the calcium concentration in the cementation media, various amounts of calcium chloride (as $CaCl_2 \cdot 2H_2O$) were added to the filtered, synthetic urine before the pH was adjusted.

6.1.3 Bacteria

Sporosarcina pasteurii was grown from a glycerol stock culture, as described in Appendix A.1.3. The bacteria were then subcultured once in 100 mL media in a 500 mL shake flask, from which two 1 L shake flasks with each 200 mL of media (ATCC® 1376) were inoculated. The culture was grown overnight until late-exponential/early-stationary phase and the optical density was measured to reassure the growth phase. Both cultures were then mixed and diluted with fresh media to an optical density of 1.0 ± 0.05.

6.1.4 Sand

Ordinary Sand from the Cape Flats was obtained from the Civil Engineering Laboratory at the University of Cape Town. The sand was first sieved in order to obtain a defined grain size range of

0.15 mm to 0.6 mm, which is similar to that of masonry sand (Premier Equestrian, 2013). It was then sterilised in an autoclave at 121°C for 30 min.

An estimate of the porosity of the sand was obtained by measuring 100 ml of sand in a measuring cylinder. The cylinder was rattled such that the sand could settle and then topped off to 100 mL. Its content was subsequently poured into 250 ml of water in a 500 mL measuring cylinder. The resulting volume V_{Total} was noted. The procedure was repeated five times. The porosity Φ was then calculated according to Equation 6.1.

$$\Phi = \frac{V_{Total} - 250mL}{100mL} \tag{6.1}$$

A porosity of 0.376 ± 0.017 was determined for the sieved sand.

6.1.5 Inoculation

Two different methods were used to inoculate the sand with a culture of *Sporosarcina pasteurii*:

Method 1 (*isolated inoculation*): The reactor was filled with dry sand mass and closed. Deionised water was pumped through the column to remove any dust and to check for leaks. The flow rate is set to approximately 100 mL/min. A culture of *Sporosarcina pasteurii* is subsequently pumped through the reactor. Five times the columns pore volume of culture is recycled back through the reactor. The pump is then switched off. The culture is kept in the reactor for 4 hrs after which treatment with cementation media begins.

Method 2 (*open inoculation*): An amount of sand slightly exceeding the volume of the reactors is mixed with the overnight culture of *Sporosarcina pasteurii* in a beaker, such that the sand is saturated. The sand-bacteria mixture is added to the reactor with the bottom outlet shut. Liquid covering the sand is drained through the outlet such that the level of liquid matches the level of sand in the reactor. If the level of liquid is too low additional culture is added. The reactor is closed and the bacteria are kept in the reactor for 4 hrs after which treatment with cementation media begins.

Method 1 allows for a clean and aseptic preparation of the reactor before treatment. The flow rate can easily be adjusted with water before inoculation, while in Method 2 the flow rate must be measured and adjusted during the treatment process. There is less chance of contamination with bacteria from the environment in Method 1. On the other hand, Method 2 guarantees that the sand is inoculated homogeneously while Method 1 is prone to the effects of preferential flow during inoculation.

6.1.6 Sampling

Samples of the effluent were taken as follows: The first 20 to 30 mL of effluent were collected in a beaker. Then, a 30 mL sample was taken. The 100 mL beaker with the first fraction of the effluent

was then topped up to 100 mL, such that in total 130 mL of liquid were pumped through the reactor. The estimated pore volume of the reactor is 95 mL. First the pH of the sample was measured, after which a syringe filter with a pore size of 0.2 µm was used to filtrate the suspension. Samples of the filtrate were then analyzed for dissolved calcium and ammonia. Samples of the influent were directly taken from the storage tank and analyzed for dissolved calcium without filtration.

Apart from directly interpreting the measurement values, the following characteristic values were estimated: Calcium Usage Efficiency, Urea Usage Efficiency, the volume of calcium carbonate precipitate. The required Equations D.1 to D.5 can be found in Appendix D.

6.1.7 Pilot Run

The purpose of this experiment was to verify that MICP can occur in the constructed reactors and at elevated pH. It was also used to explore the range of possible influent concentrations of calcium and to get an estimate for the timescales of the process. Because the conditions within the closed reactor column are different, especially in regard to gas exchange compared to the experiment in Chapter 5, feasibility of the process must first be reassured. Additionally, the amount of calcium carbonate required to solidify the sand can be assessed.

Isolated Inoculation (Method 1) was used to prepare three columns for the treatment process. The pump was manually operated. Around 4 to 5 five treatment cycles were achieved per day with retention times ranging from 2 hrs to up to 18 hrs. The exact data can be found in Appendix D in Table D.1 to D.3 together with the measurement results. Cementation media without additional $CaCl_2$ was used during the first 10 cycles, after which the calcium concentration was then elevated to values in a range of 0.19 to 0.37 M with $CaCl_2$.

6.1.8 Experiment 1

The results from the pilot run were analyzed and used to improve the methodology. Instead of manually refilling the column reactor with cementation media a timer switch was used to achieve more uniform retention times and a longer overall treatment duration than what would be possible by manual operation. The timer switch regulated a peristaltic pump that was connected to each reactor by independent tubing. Liquid was injected from bottom to top. The cementation media was stored in a 5 L container with its outlet connected to each of the three pump inlets via Y-connectors. The air inlet of the container was equipped with a filter of pore size 0.45 µm. The media was freshly prepared every 24 hrs for a retention time of 3 hrs or every 48 hrs for a retention time of 6 hrs. The pH of the cementation media was controlled twice a day in order to detect unwanted urea hydrolysis, e.g. due to contamination by Sporosarcina pasteurii. Any hydrolysis would cause a drop of the pH. A picture of the setup can be found in Figure D.1 of the Appendix.

Isolated Inoculation was used to prepare the columns for the treatment process. Treatment was started with cementation media at a calcium concentration of 0.025 M and a retention time of 3 hrs.

The calcium concentration was approximately doubled every 24 hrs up to 0.11 M. Then the retention time was increased to 6 hrs. Samples were taken twice a day.

6.1.9 Experiment 2

This experiment was conducted similarly to the one described in section 6.1.8, except for two variations: Open inoculation was used in this experiment (Method 2). Additionally, the calcium concentration was further increased up to 0.23 M on the 4th day of treatment at a retention time of 6 hrs.

6.2 Results

6.2.1 Pilot Run

During the first 10 cycles, the reactor columns were treated with 0.8 g/L (0.02 M Ca^{2+}) on average. Within one to three cycles after inoculation, the undiluted effluent sample of the column reactors reached a residual calcium concentration below the determination limit of 10 mg/L. The course of the calculated calcium usage efficiency[2] and the estimated amount of precipitate is shown in Figure 6.2. The pH of the effluent was within the range of 9.30 to 9.45. After these 10 cycles the first reactor column was opened to assess the progress of solidification. No sign of solidification could be found. An estimate of the amount of calcium carbonate that precipitated within the pore volume of the column resulted in 1.28 g. Such an amount of calcium carbonate corresponds to 0.69% of the pore volume. Based on these results the experiment was continued with an increased calcium ion concentration through the addition of $CaCl_2$ to the cementation media. The second and third reactor were opened after 24 and 28 cycles respectively. A hammer was required to remove the sand from the reactor. Only a few pieces of solidified sand were recovered from the column. According to the measured mass balance of dissolved calcium in the influent and effluent of each reactor approximately 7% to 9% of the pore volume was filled up with calcium carbonate. All data obtained for this experiment can be found in Appendix D in Table D.1 to D.3. The precipitation of calcium carbonate was less efficient in relative terms than during the first 10 cycles ranging from 13% to 96% (depending on the retention time, influent calcium concentration and probably also the preceding calcium load).

Figure 6.3 compares the pH measured in the effluent to the estimated amount of calcium that precipitated in the column. It can clearly be seen that the pH decreases with an increasing amount of precipitation.

[2] ($Ca_{IN} - Ca_{IN Effluent}$): indicates how much calcium is effectively precipitated in the column

6.2.2 *Experiment 1*

Similar to the results of the pilot run in Section 6.2.1, an efficient removal of calcium from the solution could be observed at an influent concentration of around 0.025 M. The amount of urea being hydrolysed – measured as ammonia in the effluent – stayed constant at a level of around 0.083 M. The data for the duration of the experiment duration can be seen in Figure 6.4. The exact values and derived data can be found in Table D.4 in the Appendix. The increase of the calcium concentration to 0.054 M caused a peak in urea hydrolysis, followed by a decline to levels below the previously measured amount of 0.083 M. The amount of calcite precipitate decreased as well. The calcium concentration was further increased to 0.107 M during which the urease activity slightly increased as well as the absolute amount of precipitates formed.

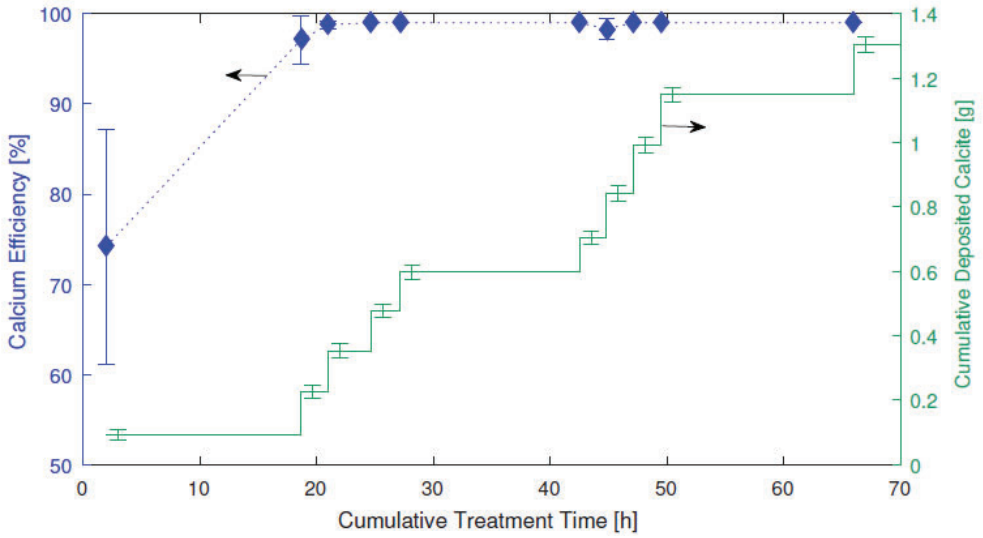


Figure 6-2: The course of the calcium usage efficiency and the amount of calcium carbonate precipitate during the first 10 cycles. The process is in fact a discrete process with individual treatment cycles. Each step indicates the injection of fresh cementation media and an update of the current mass balance for the reactor column. For this reason, neither a direct nor a stepwise connection of the data points can represent the true course of its state.

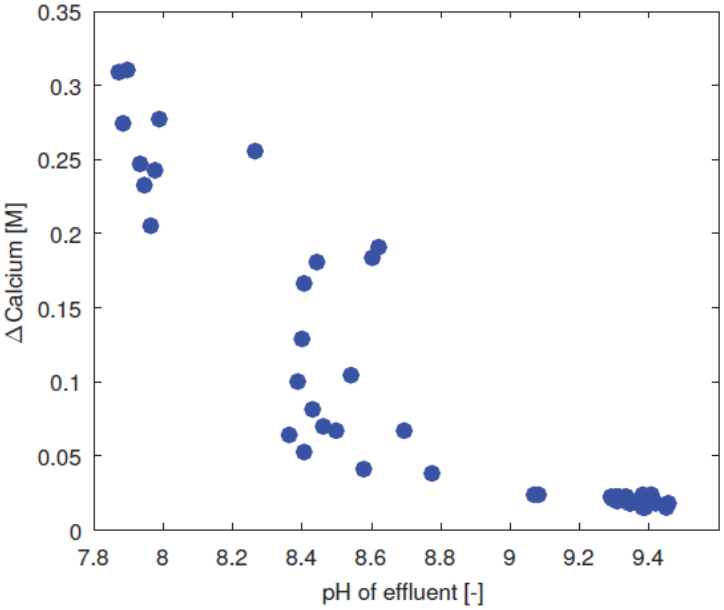


Figure 6-3: A scatter plot of the effluent pH vs. the amount of calcium that precipitated.

However, the calcium usage efficiency was low at a level of around 50% to 70%. A doubling of the retention time to 6 hrs did not cause the expected increase in efficiency. To the contrary, both the amount of urea being hydrolyzed as well as the calcium precipitation decreased further. Eventually the pH of the effluent was above 10 after 36 treatments indicating that MICP had stopped. The first reactor was subsequently opened and a small piece of solidified sand could be recovered. The surrounding sand did not show any sign of solidification. The second reactor revealed a similar piece of solidified sand as shown in Figure D.2 in the Appendix, giving indication for the presence of a preferential flow path during inoculation. The injection of methylene blue into the third reactor before opening seemed to confirm that preferential flow paths in the reactor column existed.

6.2.3 Experiment 2

On the basis of the observation from Experiment 2 another method of inoculation was tested. Here the sand was thoroughly mixed with bacteria broth before being filled into the reactor. The course of the experiment is shown in Figure 6.5. The numeric values can be found in Table D.5 in the Appendix. The treatment process was started with 0.025 M Ca^{2+} in the influent, which was then doubled twice until a concentration of 0.11 M. Up to this concentration almost complete utilisation of the influent calcium for precipitation could be observed at a retention time of 3 hrs. Around 0.18 M urea was hydrolysed. Before increasing the influent calcium concentration above a level of 0.18 M the hydrolysis of additional urea was required. Consequently, the retention time was increased to 6 hrs. This had no effect on the amount of urea being hydrolysed though. Contrary to the expectation, the level of ammonia which was measured in the effluent stayed approximately constant. Regardless, the influent calcium concentration was then increased to 0.23 M. Instead of detecting excess dissolved calcium in the effluent, an increase of urea hydrolysis was observed. Approximately 0.25 M urea was degraded in the first treatment cycle after increasing the calcium

concentration. Although not all of the 0.23 M calcium precipitated, the calcium efficiency reached a value of about 88%. In the subsequent treatment cycles, a decrease in both the amount of urea hydrolysis and the amount of precipitate formed was observed. Additionally, the experimental error in between the three reactors increased. After two days, the calcium concentration was reduced to 0.11 M. The amount of calcium that precipitated stabilised at a calcium usage efficiency of approx. 65%. The hydrolysis of urea continued with a slightly decreasing trend and was below the level that was observed at the same influent calcium concentration before the increase to 0.23 M for 2 days.

All three reactors were opened after 42 treatment cycles. In each reactor, the whole sand column had been solidified and could be handled as one piece as seen in Figure 6.6. An estimate of the volume of deposited calcium carbonate showed that it made up 12.5% of the pore volume of the sand column. Two columns were tested for compressive strength. The third was kept for demonstration purposes. While one column could withstand a maximum stress of 870 kPa, the other one failed already at 290 kPa. The stress-strain curve can be seen in Figure D.3 in the Appendix.

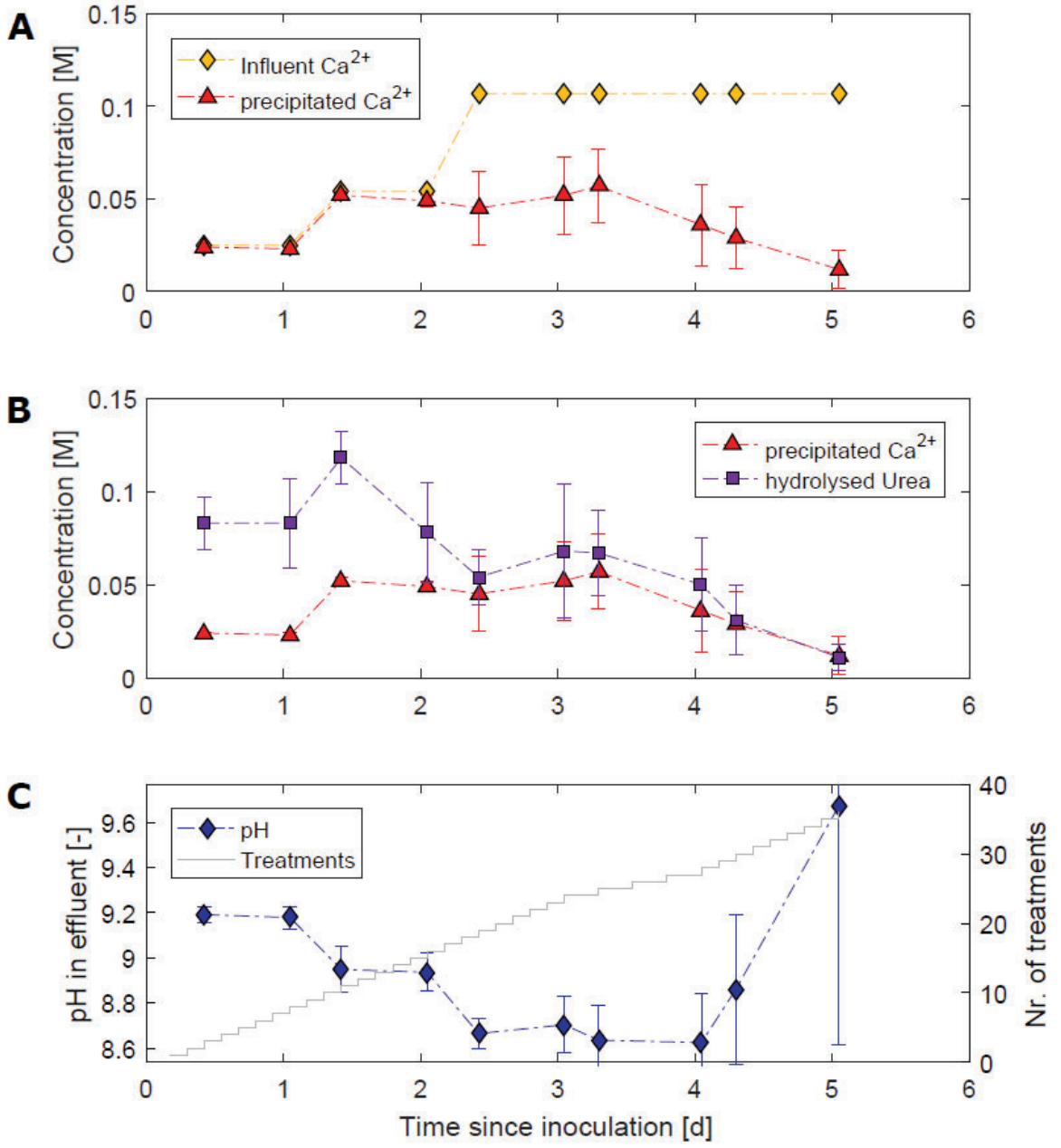


Figure 6-4: The course of the measured variables in the effluent and influent of the Column Reactor Experiment 1. Samples were taken twice daily in-between treatment cycles. The experiment was conducted in triplicate. The error bar gives the standard deviation of the results. A: The amount of calcium that precipitated in the column in comparison to the amount being fed to the reactors. B: The relation between the amount of calcium carbonate precipitate and the amount of urea that was hydrolysed. C: The pH measured in the effluent and the cumulative amount of treatment cycles over time. The retention time was increased from 3 hrs to 6 hrs after 3 days and decreased to 3 hrs again after 4 days.

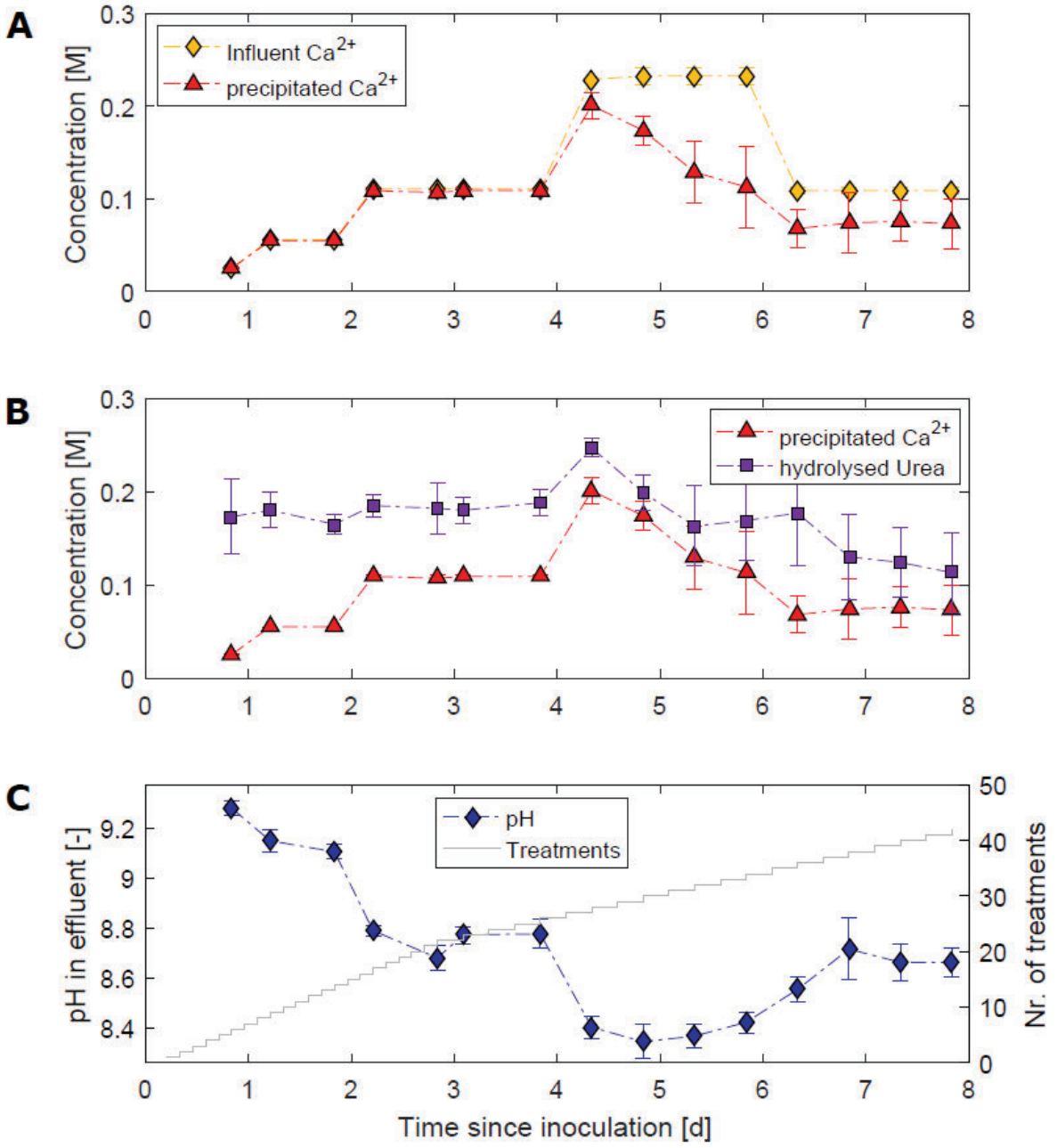


Figure 6-5: The course of the measured variables in the effluent and influent of the Column Reactor Experiment 2. Samples were taken twice a day in between treatment cycles. A: The amount of calcium that precipitated in the column in comparison to the amount being fed to the reactors. After increasing the calcium concentration in the influent to 0.23 M a sudden decrease in the calcium usage efficiency was observed. After decreasing the influent concentration to the previous value the efficiency stabilised at a lower level than observed before. B: The relation between the amount of calcium carbonate precipitate and the amount of urea that was hydrolysed. The increase of the retention time from 3 hrs to 6 hrs did not cause the expected increase in the amount of urea being hydrolysed. C: The pH measured in the effluent and the cumulative amount of treatment cycles over time. The retention time was increased from 3 hrs to 6 hrs after 3 days.

6.3 Discussion

The results of these three experiments show that MICP in a sand volume with synthetic urine that had been stabilised by increasing its pH can work in a simple setup like the one used here. In principle, adding a culture of *Sporosarcina pasteurii* to loose sand, followed by saturation with a urea-calcium media at concentrations that are typical for stabilised urine and a slightly lower pH (~11) is already enough to trigger this process. However, an increase of the calcium concentration in stabilised urine is necessary for an efficient process. Experimental results show that the process is robust and repeatable when operated at an influent calcium concentration of up to 0.025 M. However, the urea is not used efficiently at such a low calcium concentrations. Only a twelfth of the available urea is effectively used. Subsequently, the volume of urine required to produce one unit volume of solid would be higher than necessary. It is desirable to increase the amount of calcium carbonate precipitate per litre urine close to the theoretical maximal value which is the equimolar amount to that of the urea concentration. In addition, operating conditions in which the required microbial community can survive and reproduce need to be maintained to enable repeated injection of cementation media.

Various calcium usage efficiencies were observed in the pilot run ranging from 13% to 96% when the influent calcium concentration was increased up to 0.37 M. This high variance was mainly the result of deviating conditions in between treatment cycles with differing treatment durations and influent concentrations. It was observed, that the pH in the effluent decreases with an increasing amount of calcium carbonate precipitate. The pH ranged from 7.3 to 9.4 in the pilot run. This observation agrees with observation made by Brison (2016). Although the hydrolysis of urea causes the pH to trend towards its equilibrium pH around 9.25, calcium carbonate precipitation counters this trend. The resulting pH depends on the amount of precipitate that formed. In order to get more interpretable data an enhanced methodology was used to observe the effects of various influent calcium concentrations and the effect of retention time. Starting from a low calcium concentration (known to be feasible) it was increased step by step while the concentrations of calcium and ammonia in the effluent were monitored.

In the first experiment run, a robust MICP process was only observed up to a concentration of 0.025 M Ca^{2+}. Although the calcium usage efficiency stayed above 90% after increasing the influent calcium concentration to 0.05 M, a sudden peak of urea hydrolysis followed by a steep decrease was observed. In the following course of the experiment the amount of urea being hydrolysed never recovered to the level before the peak and eventually completely deceased after 3.5 days. Examining the column with methylene blue before opening revealed the existence of a preferential flow path as well as pieces of solidified sand. Solidification of only a fraction of the column was most probably caused by partial inoculation of the column. Assuming that only the initial preferential flow path might have been inoculated with bacteria, the subsequent calcite precipitation and constriction of pore space in this volume causes the redirection of the liquid flow around it. As a result, less and less cementation media flows through the initial volume but through the part of the column which

has not been inoculated. Subsequently a decrease of microbial activity is monitored. This, however, does not explain why the increase of Ca^{2+} causes a peak in the observed ureolytic activity.

The aim of the second experiment was to proof the hypothesis of partial inoculation and deliver better data on the effect of elevated calcium concentrations. Sand was submerged in bacteria broth and then filled into the reactor thus making sure that the complete volume of sand was inoculated. In fact, this does not solve the issue of preferential flow of the cementation media. However, it is expected that preferential flow will eventually balance itself under these conditions. A volume that is initially more conductive will receive more cementation media. This leads to a quicker decrease of conductivity until it is in equilibrium with the surrounding volume. For this reason, it is essential that the complete sand volume is equally inoculated. In case that one part of the sand column has not or only been weakly inoculated, the cementation media will eventually flow only through that region because its conductivity stays high.

In the second experiment run the first obvious difference was the higher concentration of ammonia in the effluent equivalent to 0.18 M urea compared to 0.08 M that was measured in the preceding experiment at the same influent calcium concentration. A probable reason is a larger microbial community because the column was more thoroughly inoculated. This indicates a higher ureolytic activity. Consequently, high calcium usage efficiencies above 95% were observed for this experiment. Surprisingly the increase of the influent calcium concentration to 0.1 M did not cause the expected drop in calcium efficiency that was seen in the previous experiment. Only the following increase to 0.2 M led to a similar observation. The increase was also followed by a spike in the observed ureolytic activity and a subsequent drop to levels below the ones observed previously. This reaction is caused by the increase of the influent calcium concentration. Because the reaction occurred at two different concentration changes, it cannot be ascribed to the absolute concentration. The observed spike in ureolytic activity might rather be the result of a stress reaction due to the sudden change of e.g. ionic strength which is known to affect bacteria (Hammes and Verstraete, 2002).

The influent calcium concentration was lowered to 0.1 M two days after the increase to 0.2 M. The decrease in the influent calcium concentration stabilised the calcium efficiency around a value of 67%, significantly lower compared to the value of 97% observed before. Additionally, a slight downward trend of the ureolytic activity was observed. These observations show that the increase of the calcium concentration from 0.1 M to 0.2 M did in fact have a negative impact on the microbial community in the column. The phenomena should be further investigated to understand the process and the sudden decrease of microbial activity.

However, this second experiment showed that an influent calcium concentration of up to 0.1 M can be applied repeatedly provided that the reactor is homogeneously inoculated. A calcium usage efficiency of more than 95% as well as a urea usage efficiency of more than 35% were achieved. After 42 treatments cycles each of the three reactor revealed a completely solidified column. A treatment scheme with such a concentration agrees with Bernardi et al. (2014), who used a cementation media with 0.1 M CaCl2 to produce bio-bricks with up to 84 treatment cycles. The

compressive strength obtained in this experiment is also comparable with the results achieved by Bernardi et al. (2014) for the same number of treatments. They measured an average compressive strength of 440 kPa with a maximum of 930 kPa and a minimum of 120 kPa, while this experiment resulted in a compressive strength of 870 kPa and a minimum of 290 kPa. The test results are comparable to that of a 40% lime brick but lower than that of a 20% cement brick. The latter can obtain a compressive strength of up to 2000 kPa. Such values were obtained for a bio-brick process after a total of 84 treatment cycles by Bernardi et al. (2014). They conclude that the strength of a bio-brick is scalable and can reach values similar to that of a cement brick but require more treatment cycles than those needed to produce a brick of similar strength than a 40% lime brick.

Following this research there are two mayor areas of focus for further research on the proposed process. Firstly, to introduce a new building material to the infrastructure sector, proof that it fulfils the requirements must be given. Not only compressive strength but also other properties such as water absorption and chemical resistance are important properties in possible applications. A first step is to investigate the relation between the number of treatment cycles and the compressive strength at a constant influent calcium concentration.

Secondly, an increase of the urea usage efficiency is desirable to minimize the required volume of stabilised urine to produce one bio-solid unit. A maximum efficiency of 67% was observed for the experiments conducted in this study. From these experiments, it was seen that a maximum influent calcium concentration of ca. 0.1 M could not be exceeded in the long-term although 0.3 M of urea were available. Interestingly not all of the urea was hydrolysed in this experiment. Even when the retention time was doubled to 6 hrs no increased hydrolysis was observed. Decreasing the concentration of the urea by dilution of the cementation media while keeping the calcium concentration constant might increase the urea usage efficiency, but would not be ideal in water scarce areas. However, this could also be strategy to decrease adverse effects of e.g. ionic strength that might emerge when the process is tested with real urine.

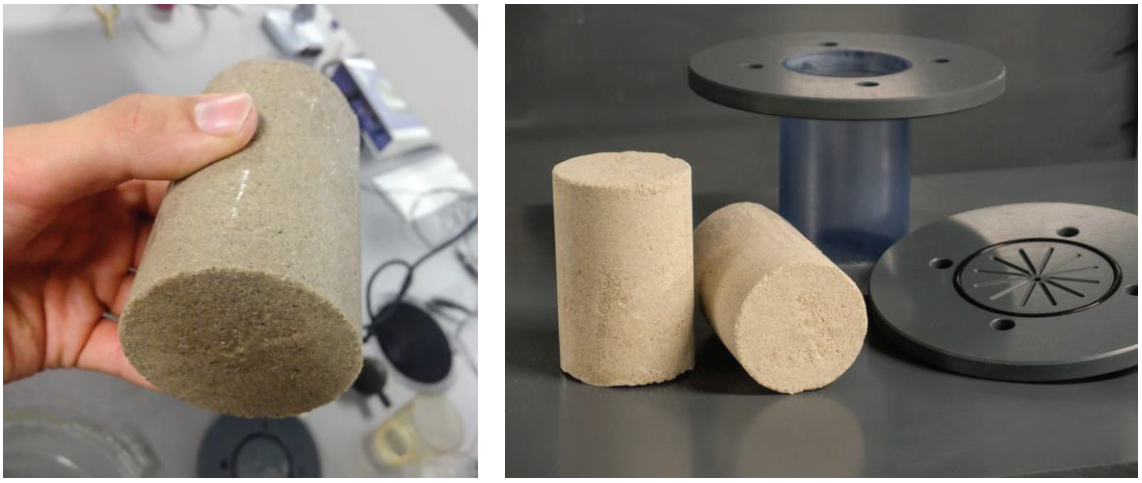


Figure 6-6: Photographs of the produced bio-solids. The columns could withstand a load of 75 to 226 kg on their circular surface with a radius of 2.8 cm.

Chapter 7 Real urine experiments

7.1 Materials & Methods

The same procedure used for the synthetic experiments were followed for these experiments. Urine was collected by a separate project which aimed to produce fertilizers from urine. The urine was collected from waterless urinals. The urine was stabilized on site with 10 g calcium hydroxide per litre of urine, in 25 L urinal containers. As the urinals filled up, calcium phosphate precipitated in the urinal container. Once full, the urinal container was left for a day to allow time for the precipitates to settle. Subsequently, the supernatant was decanted and filtered through grade 595 filter paper. The filtered liquid was used for all the experiments done in this project.

7.2 Results

7.2.1 Pilot run

The pilot run using real urine attempted to mirror the synthetic urine experiments. This was performed to see if the if MICP process could be performed using real urine. On the seventh day, calcium chloride was added. It was added sporadically to observe the ureolytic activity and precipitation of calcium carbonate. The reactors leaked through the sealing between the PVC pipe and the base lid. An attempt to seal the leaks from the outside was made using a sealant, but reactor 1 and 2 continued to leak to some degree for the duration of the run.

The leaking caused only the bottom portion of the column to be inundated with the cementation media for the full retention time. As a result, only the bottom portion of the sand column solidified. This effect was magnified when the retention time was increased to 6 hours. Solidification occurred, indicating that MICP could be replicated with real urine. Figure 7.1 shows the solidification in one of the columns during the pilot run.

Figure 7-1: A bio-solid produce in the pilot run using real urine.

On day 13 the ureolytic activity appeared to decrease due to the increase in Ca^{2+} concentration in the influent to 0.17 M (see Figure 7.2A). It must be noted, that this is less than the limit of 0.2 M found for the synthetic urine experiments. The peak in ureolytic activity was at an influent Ca^{2+} concentration of 0.6 M and 0.8 M. An increase in influent Ca^{2+} concentration caused an increase in ureolytic activity. However, no record of the added calcium and the expected concentration of the influent were recorded for the trial run. From the graphs, the sporadic nature of the replenishment of the media and calcium chloride addition can be seen by the changing of influent concentration. This was reflected in the ureolytic activity and deposition of $CaCO_3$, which varies with the varying influent concentration.

Figure 7.2A shows that when the concentration of influent Ca^{2+} moves above 1.4 M a deviation between the influent and effluent concentration occurs and the calcium efficiency decreases from what was previously around 100% to 89% in the next sample (Figure 7.2B). After raising the concentration to above 0.17 M the calcium usage efficiency decreased down to 40%. A levelling off of the hydrolyzed urea can be observed after the 0.17 M spike in the influent Ca^{2+} concentration. In contrast, the calcium usage efficiency continues to decrease.

A portion of Ca^{2+} ions in the influent were lost due to the deposition of calcium phosphate which saturates at a pH of above 12.5 (Randall et al., 2016). This happens in replenishing process and in the stagnant stored urine. Scouring of the precipitate was found on the walls of the influent 5 L bottle. In addition to the influent bottles, the effluent bottles showed sign of scouring, although this was due to the deposition of $CaCO_3$.

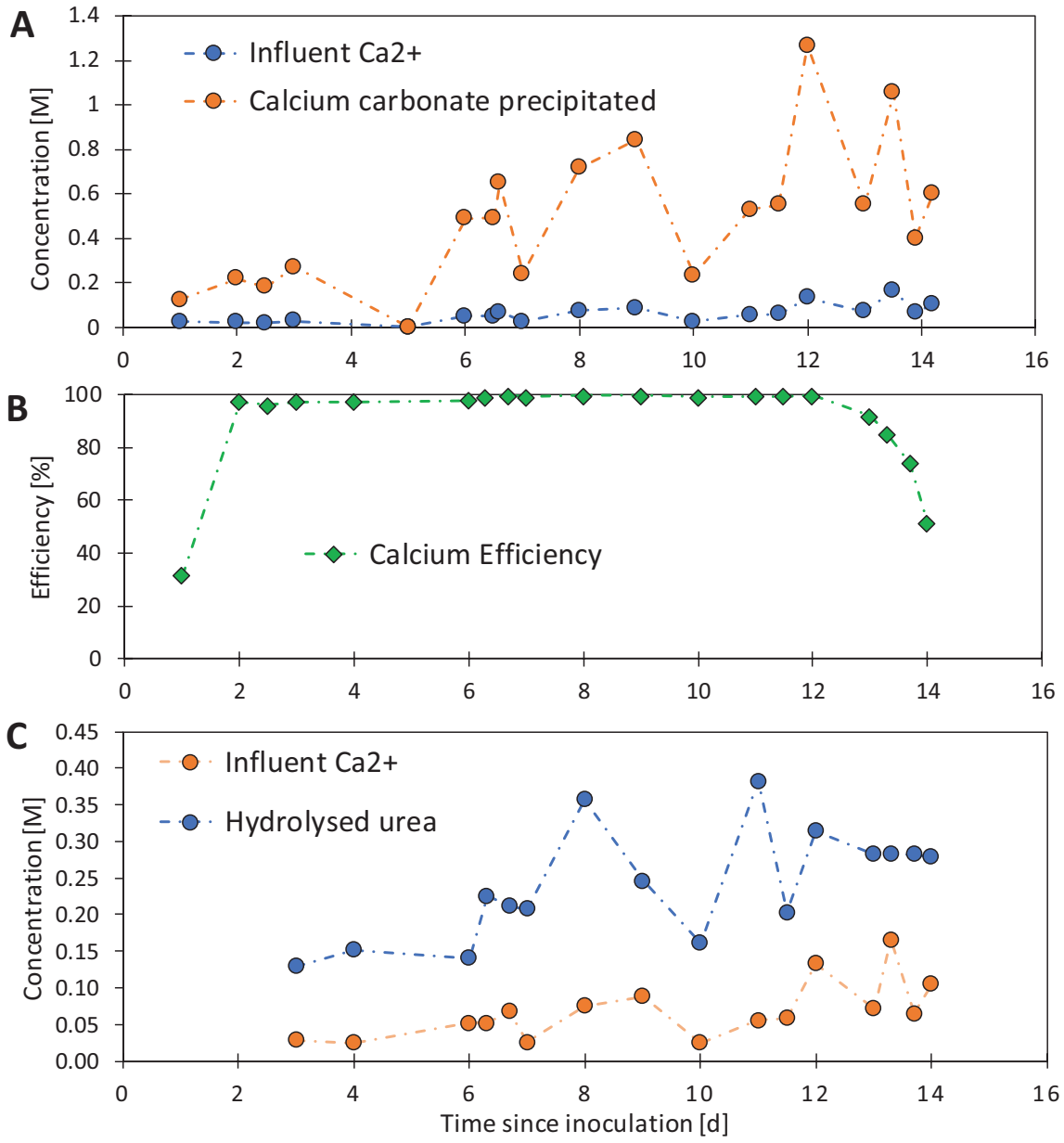


Figure 7-2. Pilot run experiment. A: shows the relation to the influent calcium concentration and the precipitated calcium carbonate in the columns over the treatment period. B: the percentage of calcium from the influent precipitated in calcium carbonate within the reactor over the treatment period (calcium usage efficiency). C: the amount of urea hydrolyzed in the reactor with the influent calcium concentration.

7.2.2 Experiment 3

To ensure a more accurate calculation of the theoretical $CaCO_3$ deposited, the effluent bottles were replaced by glass beakers preceding sampling. Effluent was pumped out into the glass beakers and samples were taken immediately. After which, the effluent bottles were cleaned and attached back to the reactor system.

A method to monitor the influent Ca^{2+} ion concentration and expected concentration was formulated for the subsequent experiments. To ensure reliability of results, rather than taking one sample reading for each effluent bottle and the influent, 3 readings were taken for each.

From Figure 7.3 it was observed that at the beginning of the experiment when no calcium chloride was added to the influent the conversion into calcium carbonate was 90-100% but the amount of urea hydrolyzed was small at about 0.09 M. When the calcium concentration was increased to 0.11 M, the amount of calcium precipitated increased by about 0.7 M. However, calcium efficiency decreased to within the range of 75-85%. In addition, the ureolytic activity increased as the amount of urea hydrolyzed increased. The Ca^{2+} concentration was increased to 0.16 M on the fourth day of treatment. This resulted in a significant drop in the amount of calcium precipitated, the calcium usage efficiency and the amount of urea hydrolyzed. On day 5 the influent Ca^{2+} concentration was lowered to 0.09 M. The amount of precipitated calcium increased but the calcium efficiency plateaued at 46% and remained around this value until the end of the experiment. The amount of hydrolyzed urea stabilized and stayed at 0.09 M. The change in the pH followed a pattern corresponding to ureolytic activity. The effluent pH was lowest when the most urea was hydrolyzed. The change in pH increased with increasing Ca^{2+} influent concentration omitting the peak at 0.16 M. At the peak, the pH shift negligibly from the influent pH and stayed at a pH 10.4. On the 9th day of treatment the reactor was opened and a portion of solidified sand could be recovered, but the column had not solidified into a usable bio-solid.

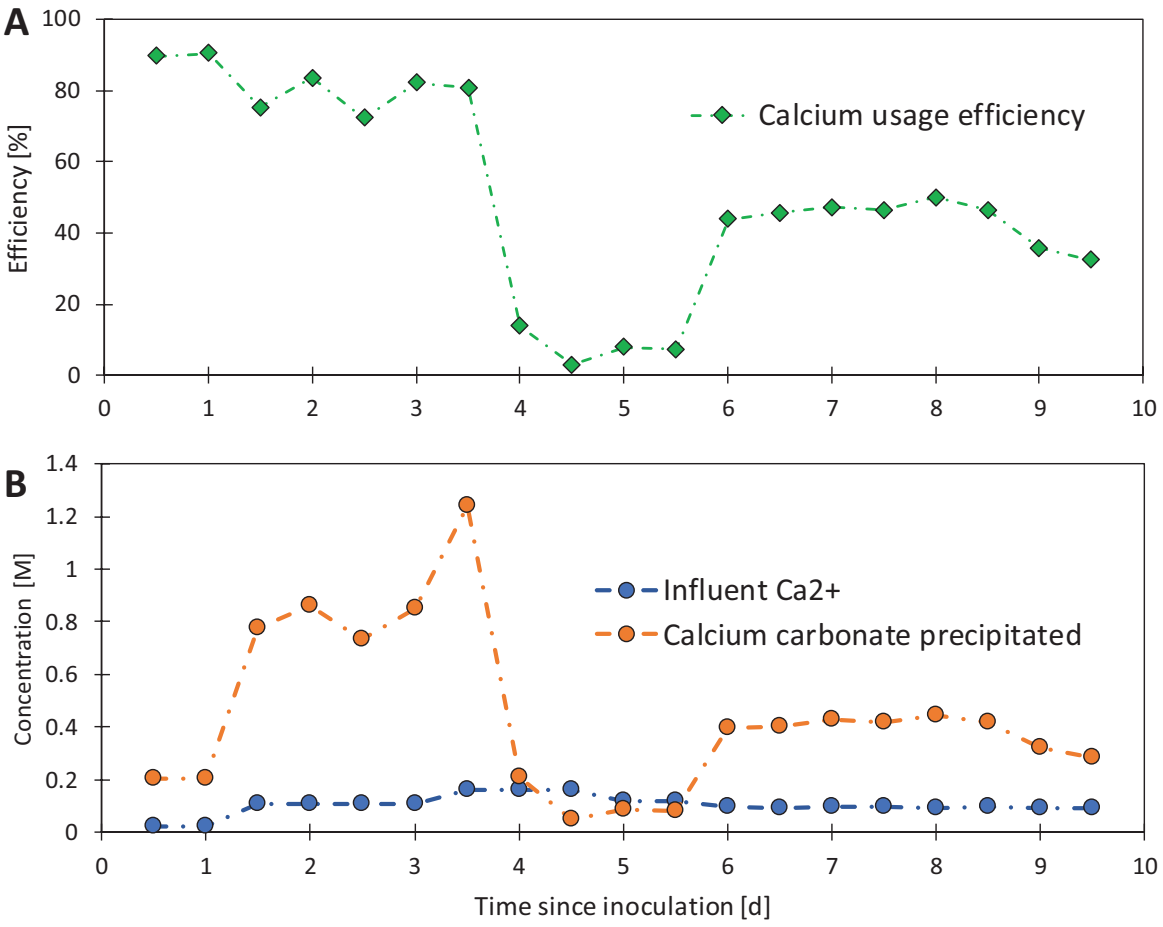


Figure 7-3: Experiment 3: A: the percentage of calcium from the influent precipitated in calcium carbonate within the reactor over the treatment period (calcium usage efficiency). B: the relationship between the influent calcium concentration and the precipitated calcium carbonate in the columns over the treatment period.

7.3 Discussion

Many of the issues experienced during the pilot run were resolved before carrying out experiment 3. However, the maximum Ca^{2+} influent concentration where the bacteria had the capacity to tolerate its ionic strength and continue to promote MICP was still unidentified. In addition, it was still unknown whether the bacteria could revive after its ionic strength capacity was surpassed.

From experiment 3, it was apparent that the ureolytic activity was dependent on the ionic strength of the calcium. If the influent concentration surpasses 0.16 M then the bacteria was affected and its efficiency to hydrolyze urea decreases but at an influent concentration of 0.11 M the calcium efficiency was still high and the amount of urea utilized is at its highest.

Hammes and Verstraete (2002) discuss how the combination of an extracellular alkaline pH and calcium ions pose a stressful environment for bacteria. Passive calcium influx as a result of the

complementary $Ca^{2+}/2H^{+}$ electrochemical gradients lead to intracellular calcium build-up and excessive proton expulsion. At the cellular level, this event can be detrimental due to the disruption of intracellular calcium-regulated signal processes, alkalisation of intracellular pH and depletion of the proton pool required for numerous other physiological processes (Herbaud et al., 1998). A low effluent pH indicates that the bacteria was able to shift the pH by a large degree. This signifies a considerable amount of ureolytic activity. Monitoring the pH can give a simpler self-contained assessment of the conditions within the reactors. In a separate trial of the first experiment the pH of the influent media was not decreased to 10.8 and subsequently the urea hydrolyzed decreased drastically and the bacteria did not recover. Thereafter, the reactor was opened and the experiment was re-started. This again shows the importance of maintaining an environment with which the bacteria can survive.

The scouring on the effluent bottles meant that the calculation of the $CaCO_3$ deposited within the mould was subject to inaccuracies and overestimation. This was possibly the result of the pH change or temperature change to the saturation point in the effluent bottle environment.

It was clear from the experiments that the process is repeatable when using a calcium concentration of 0.025 M however only a small portion of the urea is used in this. It is desirable to use less urine to produce the bio-solids and utilize an amount of urea that is equi-molar to the amount of calcium carbonate precipitated. In both the experiments the maximum urea usage is 45.5%. This is as a result of sustaining the bacteria by maintaining an environment with which they can survive. From both experiments an influent concentration of 0.11 M of calcium appears to work without significantly detrimental effects to the bacteria. From this we can deduce how many treatments are needed to form a solid. To fill a pore volume of 12.5% it would take 8 days with 56 treatments. This is assuming the calcium usage efficiency is 85%.

Chapter 8 Material & Cost Calculation

8.1 An integrated system

The production of bio-solids relies on inhibiting urea hydrolysis. This system therefore needs to be an integrated one. Figure 8.1 below shows the integrated recovery process.

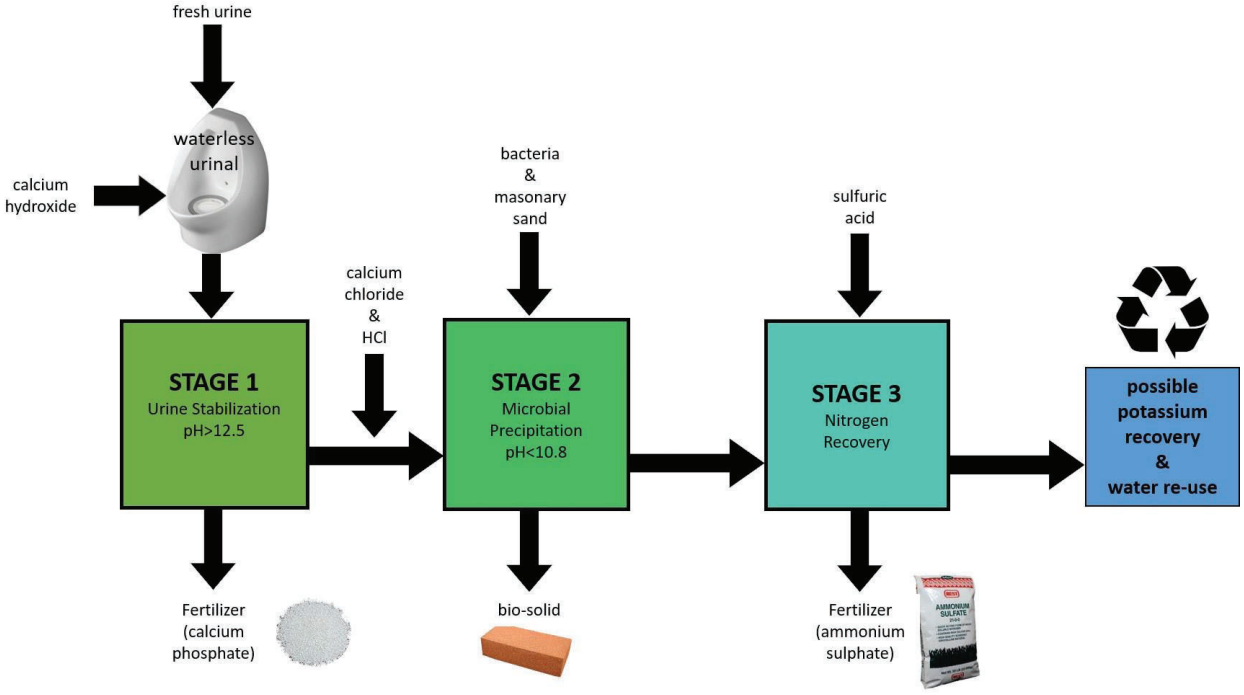


Figure 8-1: The process flow of the theoretical integrated resource recovery system.

Stage 1 uses a novel, on-site urinal to collect and stabilize urine producing calcium phosphate, an inorganic fertilizer. This process uses a waterless system, so no water is required to flush the urine away. It is beneficial in that water is avoided as it would dilute nutrient concentrations making nutrient recovery less efficient. In addition, the use of a waterless system is pertinent for a water stressed country such as South Africa. A separate study has shown that the average urinal at UCT is used about 75 times a day (Chipako, 2017). This would equate to a water savings of 165 litres/day from using just one of these urinals. Scaling of solids with urinals is often perceived as a maintenance issue, but the solids deposited are often phosphates which this system seeks to recover altering the perception of the solid as a maintenance issue to a valuable product. The supernatant liquid from stage 1 still has a pH of above 12.5 and the nitrogen is present in the form of urea. The liquid is subsequently filtered and used to produce bio-bricks or other building material (stage 2). This stage requires lowering the pH to where the bacteria can operate and survive. Sand or other potential recycled material can be used in the process. Stage 2 can essentially produce solid material of any shape at an ambient temperature.

The liquid effluent leaving stage 2 to has a high concentration of ammonium ions. This has the potential to become an additional nutrient recovery stream. The recovery of ammonium sulphate,

another alternative fertilizer, can be done with a sulfuric acid scrubber. Further fertilizers could also be produced from the effluent stream of stage 3. For example, potassium based fertilizers could be crystallized from this stream. 95% of urine is composed of water (Guyton and Hall, 2006) so the waste stream could be utilized for secondary purposes such as toilet flushing, or it could be recovered as pure water in an additional process.

8.2 Financial implications

The cost inputs for operating a bio-solid production are relatively small (see Table 8.1). Ignoring possible transport costs of the urine to the resource recovery plants, and assuming the urine is obtained free of charge, the largest input would be the nutrient media required for culturing the bacteria (about 75% percent of the total operating cost). Research has shown that alternative nutrient sources such as industrial wastes from dairies and brewery industries can be used to grow bacteria (Cuzman et al., 2015b), and thus could be obtained with minimal cost. For the purpose of costing the waste system was used and the bacteria culture incurred no cost.

Table 8-1: The costing calculation done for the integrated system. Investment and labor costs were not included in this calculation

	Amount	Revenue per unit (R)	Total Revenue (R)	Reference
INPUTS				
urine (L)	36.00	0.00	0.00	
CaOH (Kg)	0.36	-2.02	-0.73	www.kemcore.com
growth media (mL)	850.00	0.00	0.00	
$CaCl_2$ (Kg)	0.43	-4.25	-1.84	www.ics.com
32%HCl (L)	0.13	-3.65	-0.48	www.kemcore.com
masonry sand (dm^3)	1.72	-0.12	-0.21	www.Afrisam.co.za
sulfuric acid (kg)	0.21	-4.90	-1.03	Pradhan et al.,2017
		Total input	-4.29	
OUTPUTS				
calcium phosphate (kg)	0.40	23.01	9.20	Pradhan et al., 2017
bio-solid (brick)	1.00	10.50	10.50	
ammonium sulphate (kg)	1.10	7.76	8.54	Pradhan et al., 2017
		Total output	28.24	
		Total revenue	23.96	

For the economical evaluation, it was assumed that the bio-solids are produced in the shape of a standard brick. The volume of a standard brick is 1.72 dm^3. It is assumed that a bio-solid can be made from real urine using 56 treatment cycle. Therefore, to make a 1.72 dm^3 bio-solid, the following inputs are required: 36 L of urine, 360 g of calcium hydroxide, 433 g of calcium chloride dihydrate, 133 mL of 32% HCl, 850 mL of bacteria culture and 1.72 dm^3 of sand.

The amount of calcium phosphate recovered in Stage 1 was taken from Flanagan (2017). This amounted to 280 g per 25 L waterless urinal container. In addition, it is possible to recover ammonium sulphate using a sulfuric acid scrubber from the effluent of the bio-solid production. It was assumed that all the urea is hydrolyzed in the effluent stream and that the process yields 80%. Equation 8.1 shows the reaction to produce ammonium sulphate. The stoichiometry of the reaction was used to calculate the amount of sulfuric acid needed and amount of ammonium sulphate produced (Pradhan et al., 2017).

$$2NH + H_2SO_4 \rightarrow (NH_4)_2SO_4 \quad (8.1)$$

8.3 Discussion

This calculation serves as rough calculation of revenues, costs and volumes involve in the proposed integrated system of recovering nutrients and producing bio-solids. The calculation resulted in a positive margin. This profit could be further maximized by recycling waste material from construction sites to be used as the sand for the process while, simultaneously negating the impact of mining sand on the environment. In addition, including the recovery of ammonium sulphate raises the economic value of the bio-solid and calcium phosphate production.

Compared to conventional production of fertilizers and bricks, the integrated system's use of energy and its impact on the environment is significantly less. Utilizing waterless urinals would reduce water usage, especially pertinent for water scarce area such as Cape Town. In addition, they reduce energy and cost at wastewater treatment plants associated with removal of ammonia and decrease the influent sewage load on receiving plants. The ammonia recovery system has the potential to replace the energy intensive Haber-Bosch process which relies heavily on fossil fuels. The proposed phosphate recovery would reduce the global demand for mined phosphorous. The implementation of urine source-separation also has a positive effect on the energy requirements of conventional wastewater treatment. The removal of ammonia from wastewater requires aeration, which is the biggest loss of energy in wastewater treatment. Wilsenach and van Loosdrecht (2006) showed that, with urine-separation wastewater treatment plants can become net-energy producers, while the effluent quality is acceptably low.

The bio-brick production would negate the need for energy intensive kilns. In conclusion, the embodied energy of the products is lower than their conventional counterparts and could be economically viable.

Chapter 9 Conclusion

Two different experimental setups were used to investigate the feasibility of producing bio-solids from stabilised urine with a MICP process. In a first batch experiment, it was shown that a high pH greater or equal 11.95 inhibited the enzymatic hydrolysis of urea and thus the precipitation of calcium carbonate. The bacteria *Sporosarcina pasteurii* was negatively affected by the high pH and its viability was reduced. However, when the pH of the media is reduced to values smaller than or equal to 11.15, MICP occurs. However, when assessing the feasibility of using a highly alkaline media for MICP the buffer capacity must be considered as well. The hydrolysis of urea and the subsequent calcium carbonate precipitation reduce a high pH. If *Sporosarcina pasteurii* is added to a weakly buffered media, the pH will quickly decrease to lower values and the microbial community might not or only marginally be affected. This was probably the case in this experiment, where the titration curve showed a distinct drop around a pH of 11. Subsequently, the media that was adjusted to a pH of 11.15 dropped to a pH below 10 within 5 minutes. It is therefore possible that *Sporosarcina pasteurii* might already be negatively affected by a pH below 11.95 or even below 11 as other literature suggests. The results from this experiment have given an indication of how high the pH can be maintained for effective MICP.

In the second experimental setup, a column reactor was used to study the behaviour of the MICP process in sand. Two different kind of inoculation methods were applied. It was shown that mixing the sand with the bacteria culture prior to filling the reactor column (method 2) is preferable as it guarantees a homogeneous inoculation. The first method, where the culture is circulated several times through the saturated sand column is prone to the effects of preferential flow where bacteria only colonise sections of it. A steady hydrolysis of urea and precipitation of calcium carbonate within the sand column was observed at an influent calcium concentration of up to 0.025 M. When the reactor was inoculated with method 2, a maximum calcium concentration of 0.1 M could be injected into the reactors. An increase to 0.2 M negatively affected the microbial community and a reduction in both urea hydrolysis and calcium carbonate formation was observed. This is probably a stress reaction by the microbial community due to an increased ionic strength of the cementation media. Both experiments differ in the number of bacteria present, which might explain why the stress reaction was observed at two different calcium concentrations. Further investigation is required to fully understand what caused the decrease in microbial activity. Making use of all the available urea in urine is desirable as it would decrease the volumes required for the production of a bio-solid. This can only be achieved by either decreasing the amount of urea or increasing the amount of dissolved calcium.

After 42 treatment cycles, an estimated formation of calcium carbonate equal to 12.5% of the pore space, solid sand columns were recovered from the reactors. A compressive strength of 0.87 MPa and 0.29 MPa was achieved. These values in average are similar to that of a 40% lime brick. The treatment of the columns with additional cementation media would lead to higher strength. A more detailed study on the relationship between treatment duration and strength needs to be conducted in the future. Although this study focused on MICP, the process is not complete without taking the formation of valuable calcium phosphate and ammonium sulphate as fertiliser into account, especially when using urine as the source of urea. Urine contains most of the phosphorous and nitrogen the human body excretes. Recovering these resources is an integral part when urine separation is considered. Calcium phosphate already precipitates during the stabilisation of urine with calcium hydroxide. Further studies should investigate the solid produced in stabilised urine, which will be a combination of calcium phosphate, residual calcium hydroxide and other minor components. Additionally, the type of calcium phosphate that precipitates at a pH of 12.5 should be assessed, because this determines its usability as a fertiliser. Nitrogen in the form of ammonium ions can be recovered from the effluent of the proposed process with a sulphuric acid scrubber, producing the common fertiliser ammonium sulphate.

For experiments using real urine, an influent concentration of 0.025 M calcium the efficiency of calcium carbonate precipitation was consistent at 90% or higher. Contrastingly, when the calcium in the influent was raised to 0.16 M, the bacteria experienced detrimental effects and their ability to induce urea hydrolysis diminished. The process showed to be most effective when it was conducted with an influent calcium concentration between 0.08 M and 0.11 M. Nonetheless, the experiments have shown that real urine can be used to facilitate a MICP and cement sand particles together. To fill a pore volume of 12.5% it would take 8 days with 56 treatments for a column to solidify, assuming the calcium usage efficiency is 85%. To produce a bio-solid with the volume of a standard brick the inputs amount to: 1.72 dm^3 masonry sand, 36 L of urine, 433 g of calcium chloride dihydrate, 133 mL of 32% HCl and 850 mL of bacteria culture. The process could produce a profit of R24 per brick, provided calcium phosphate and ammonium sulfate are also produced.

References

American Type Culture Collection (2013). ATCC 11859. Product Sheet.

Bernardi, D., DeJong, J., Montoya, B. and Martinez, B. (2014). Bio-bricks: Biologically cemented sandstone bricks. *Construction and Building Materials*, 55:462-469.

bioMASON (2017). http://www-biomason.com. Retrieved: 2017-02-03.

Brison, A. (2016). Understanding the processes involved during the stabilization of urine with calcium hydroxide. *Project Thesis, ETH Zürich*.

Bundur, Z.B., Kirisits, M.J. and Ferron, R.D. (2015). Biomineralized cement-based materials: Impact of inoculating vegetative bacterial cells on hydration and strength. *Cement and Concrete Research*, 67:237-245.

Chipako, T. (2017). Implementing waterless urinals at UCT. Bachelor thesis, Dept. of Civil Engineering, University of Cape Town, November 2017.

Christensen, W.B. (1946). Urea decomposition as a means of differentiating proteus and paracolon cultures from each other and from salmonella and shigella types. *Journal of Bacteriology*, 52(4):461-466.

Cuzman, O.A., Rescic, S., Richter, K., Wittig, L. and Tiano, P. (2015a). Sporosarcina pasteurii use in extreme alkaline conditions for recycling solid industrial wastes. *Journal of Biotechnology*, 214:49-56.

Cuzman, O.A., Richter, K., Wittig, L. and Tiano, P. (2015b). Alternative nutrient sources for biotechnological use of sporosarcina pasteurii. *World Journal of Microbiology and Biotechnology*, 31(6):897-906.

Egler, H.-P. and Frazao, R. (2016). *Sustainable Infrastructure and Finance: How to contribute to a sustainable future*. UNEP Inquiry, Global Infrastructure Basel (GIB).

EMF (2014). *Towards the Circular Economy: Economic and business rationale for an accelerated transition*. Ellen MacArthur Foundation.

Eryürük, K., Yang, S., Suzuki, D., Sakaguchi, I., Akatsuka, T., Tsuchiya, T. and Katayama, A. (2015). Reducing hydraulic conductivity of porous media using caco3 precipitation induced by sporosarcina pasteurii. *Journal of Bioscience and Bioengineering*, 119(3):331-336.

Flanagan, C. Investigating the feasibility of harvesting phosphorus from public urinals at UCT. Bachelor thesis, Dept. of Civil Engineering, University of Cape Town, November 2017.

Grundmann, P. and Maaß, O. (2017). Wastewater reuse to cope with water and nutrient scarcity in agriculture – a case study for braunschweig in Germany. In *Competition for Water Resources*, pages 352-365. Elsevier.

Gujer, W. (2006). *Siedlungswasserwirtschaft*, pages 396-397. Springer Berlin Heidelberg.

Guyton, A. and Hall, J. (2006). Textbook of Medical Physiology. Elsevier Saunders, ISBN 9780721602400. URL https://books.google.co.za/books?id=sTZHAQAAIAAJ.

Hammes, F. and Verstraete, W. (2002). Key roles of pH and calcium metabolism in microbial carbonate precipitation. *Reviews in Environmental Science and Biotechnology*, 1(1):3-7.

Hellström, D., Johansson, E. and Grennberg, K. (1999). Storage of human urine: acidification as a method to inhibit decomposition of urea. *Ecological Engineering*, 12(3-4):253-269.

Herbaud, M.L., Guiseppi, A., Denizot, F., Haiech, J. and Kilhoffer, M.C. (1998). Calcium signalling in bacillus subtilis. *Biochimica et Biophysica Acta – Molecular Cell Research*, 1448(2):212-226.

Jabri, E., Carr, M.B., Hausinger, R.P., and Karplus, P.A. (1995). The crystal structure of urease from klebsiella aerogenes. *Science*, 268 5213:998-1004.

Larsen, T.A. and Gujer, W. (1996). Separate management of anthropogenic nutrient solutions (human urine). *Water Science and Technology*, 34(3-4):87-94.

Lauchnor, E., Topp, D., Parker, A. and Gerlach, R. (2015). Whole cell kinetics of ureolysis by sporosarcina pasteurii. *Journal of Applied Microbiology*, 118(6):1321-1332.

Muynck, W.D., Belie, N.D. and Verstraete, W. (2010). Microbial carbonate precipitation in construction materials: A review. *Ecological Engineering*, 36(2):118-136.

National Lime Association (2007). Lime, the versatile chemical. Fact Sheet.

Neset, T.-S.S. and Cordell, D. (2012). Global phosphorus scarcity: identifying synergies for a sustainable future. *Journal of the Science of Food and Agriculture*, 92(1):2-6.

Okwadha, G.D. and Li, J. (2010). Optimum conditions for microbial carbonate precipitation. *Chemosphere*, 81(9):1143-1148.

Pillai, M.G., Simha, P. and Gugalia, A. (2014). Recovering urea from human urine by bio-sorption onto microwave activated carbonized coconut shells: Equilibrium, kinetics, optimization and field studies. *Journal of Environmental Chemical Engineering*, 2(1):46-55.

Pradhan, S.K., Mikola, A. and Vahala, R. (2017). Nitrogen and phosphorus harvesting from human urine using a stripping, absorption, and precipitation process. *Environmental Science & Technology*, 51(9):5165-5171.

Premier Equestrian (2013). Arena sand specification. Sand Specification Booklet.

Putnam, D.F. (1971). Composition and concentrative properties of human urine. Technical report, National Aeronautics and Space Administration.

Qiu, J., Tng, D.Q.S. and Yang, E.-H. (2014). Surface treatment of recycled concrete aggregates through microbial carbonate precipitation. *Construction and Building Materials*, 57:144-150.

Randall, D.G., Krähenbühl, M., Köpping, I., Larsen, T.A. and Udert, K.M. (2016). A novel approach for stabilizing fresh urine by calcium hydroxide addition. *Water Research*, 95:361-369.

Rowshanbakht, K., Khamehchiyan, M., Sajedi, R.H. and Nikudel, M.R. (2016). Effect of injected bacterial suspension volume and relative density on carbonate precipitation resulting from microbial treatment. *Ecological Engineering*, 89:49-55.

Stocks-Fischer, S., Galinat, J.K. and Bang, S.S. (1999). Microbiological precipitation of caco3. *Soil Biology and Biochemistry*, 31(11):1563-1571.

Thome, A.M.T., Ceryno, P.S., Scavarda, A. and Remmen, A. (2016). Sustainable infrastructure: A review and a research agenda. *Journal of Environmental Management*, 184, Part 2:143-156.

Tobler, D.J., Cuthbert, M.O., Greswell, R.B., Riley, M.S., Renshaw, J.C., HandleySidhu, S. and Phoenix, V.R. (2011). Comparison of rates of ureolysis between sporosarcina pasteurii and an indigenous groundwater community under conditions required to precipitate large volumes of calcite. *Geochimica et Cosmochimica Acta*, 75(11):3290-3301.

Udert, K.M., Larsen, T.A., Biebow, M. and Gujer, W. (2003). Urea hydrolysis and precipitation dynamics in a urine-collecting system. *Water Research*, 37(11):2571-2582.

UNEP and IFA (1998). *The Fertilizer Industry, World Food Supplies and the Environment*.

Widdel, F. (2010). Grundpraktikum Mikrobiologie, 4. Sem. (B.Sc.). Universität Bremen.

Wilsenach, J.A. and Van Loosdrecht, M.C. (2006). Integration of processes to treat wastewater and source-separated urine. *Journal of Environmental Engineering*, 132(3):331-341.

Zhang, S. (2014). Would you live in a house of bricks made from pee and bacteria? January 2014. www.gizmodo.com.

Appendix A: Growth of Sporosarcina Pasteurii

A.1. Material & Methods

A.1.1. Reviving a freeze-dried strain of Sporosarcina pasteurii

A freeze dried strain of *Sporosarcina pasteurii* (ATCC® 11859™) was rehydrated with 1 mL of sterile culture media (ATCC® 1376) according to the protocol given by the American Type Culture Collection (2013). This aliquot was then transferred to a sterile tube with 5 mL of the same media and mixed well. From this broth 5 times 1 mL were transferred to additional broth tubes containing 5 mL media, which were then incubated at 30°C for 48 hrs. Three of these broth tubes were used to inoculate 100 ml of media each in 500 ml Erlenmeyer flasks. Subcultures of these flasks were prepared and incubated at 30°C and finally used for the determination of the growth curve and for storing stock cultures on Christensen's Urea Agar in the fridge at 4°C.

A.1.2. Growth Curve

In order to delineate the growth stages of *Sporosarcina pasteurii* in (ATCC® 1376) the growth curve had to be determined. The experiment was conducted in triplicates. An overnight culture of *Sporosarcina pasteurii* was used to inoculate 100 mL of ATCC® 1376 media in 500 mL shake flasks. The optical density of the overnight culture was measured. The appropriate volume of inoculate was added to the shake flasks, so that the calculated, initial OD was 0.05. The flasks were placed on a shaker at 120 rpm and 25°C. During the first 14 hrs, a 2 mL sample was taken every 2 hours from each shake flask and the optical density was measured.

A.1.3. Glycerol Stocks

Glycerol Stocks of *Sporosarcina pasteurii* were prepared after the growth curve had been determined. One 500 ml flasks with 100 ml media was inoculated with 5 mL of a liquid culture in stationary phase (resulting in an OD of about 0.1) and incubated until mid-exponential phase. After confirming the phase of bacterial growth through a measurement of optical density, 1 mL of that liquid culture was aseptically mixed with 1 mL of sterile 50 vol% glycerol and filled into sterile, cryogenic vials with a volume of 2 mL. These vials were then stored at -50°C for later use. Glycerol Stocks can easily be revived. Approximately 1 ml of the thawed glycerol stock is transferred to 100 mL of ATCC® 1376 media in a 500 mL shake flask and incubated at 30°C on a shaker. Growth should be observed after 2 to 4 days. These revived cultures where subcultured twice before being used in experiments. Alternatively, a heated loop wire can be used to melt a fraction of the frozen glycerol stock and then be streaked onto ATCC® 1376 agar. The remaining, frozen content can then be returned to the freezer.

A.2. Results

A.2.1. Growth Curve

The collected data are shown in Figure A.1. Approx. 3 to 4 hrs after inoculation the lag phase is over and the cell biomass increases exponentially. Under the assumption that the optical density is a good approximation of the cell concentration during that growth phase a cell doubling time of ca. 2.02 ± 0.09 hrs is calculated according to Equation A.1 with the data from $t_1 = 4h$ and $t_2 = 8h$.

$$T_d = \frac{t_2 - t_1}{lnOD_{t_2} - lnOD_{t_1}} \cdot \ln(2) \qquad \text{(A.1)}$$

After 8 hrs, around an OD of 1 growth slows down and reaches stationary phase around an optical density of 1.9 to 2. Figure A.2 shows Sporosarcina pasteurii under the microscope with a 100x magnification. It is rod-shaped with a length of ca. 2.5 to 5 μm

A.3 Discussion

Sporosarcina pasteurii shows the typical stages of growth with a distinctive lag-phase, exponential growth and stationary phase at an OD of about 2. The timescale of the obtained growth curve is roughly similar to the one observed by Bundur et al. (2015), but differ especially in the duration of the lag-phase and the maximum growth rate. This is no surprise, as both growth curves were obtained under different conditions. The data in Bundur et al. (2015) were obtained by growing Sporosarcina pasteurii at 30°C with urea-yeast media while the data here where obtained at 25°C with ammonia-yeast media. In general, growth curves cannot simply be compared unless the experiment has been conducted under the very same conditions. Nevertheless, the growth curve obtained here facilitates the scheduling of the experiments and estimation of the growth stage before the culture is used, as long as the same growth conditions apply.

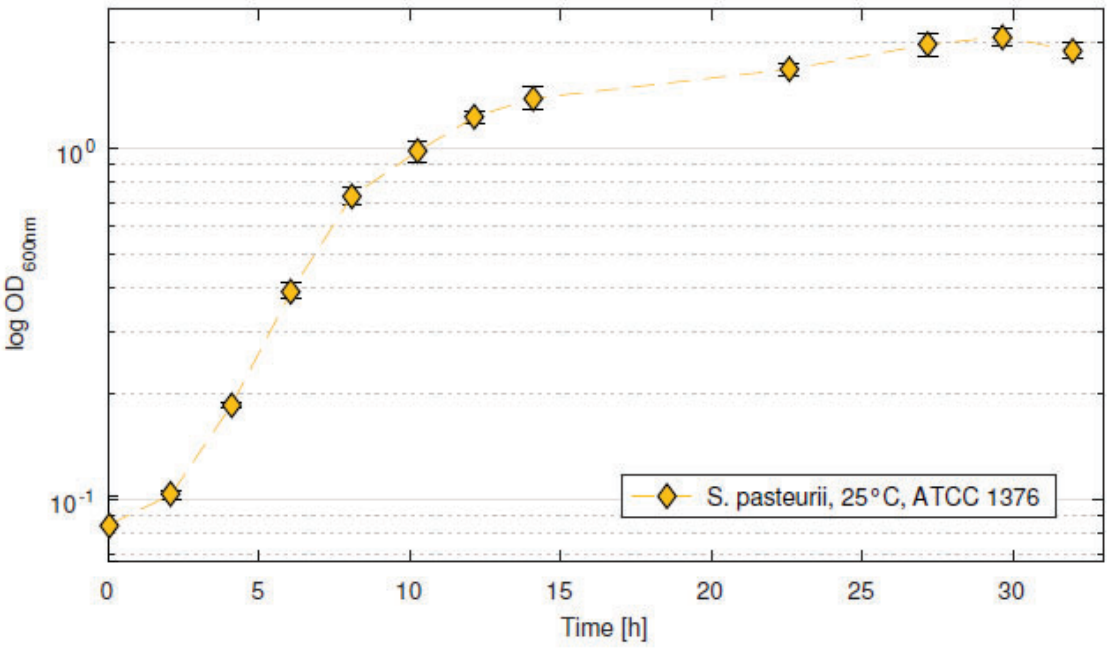


Figure A.1: The growth curve of Sporosarcina pasteurii in Ammonia-Yeast media (ATCC 1376) at 25°C.

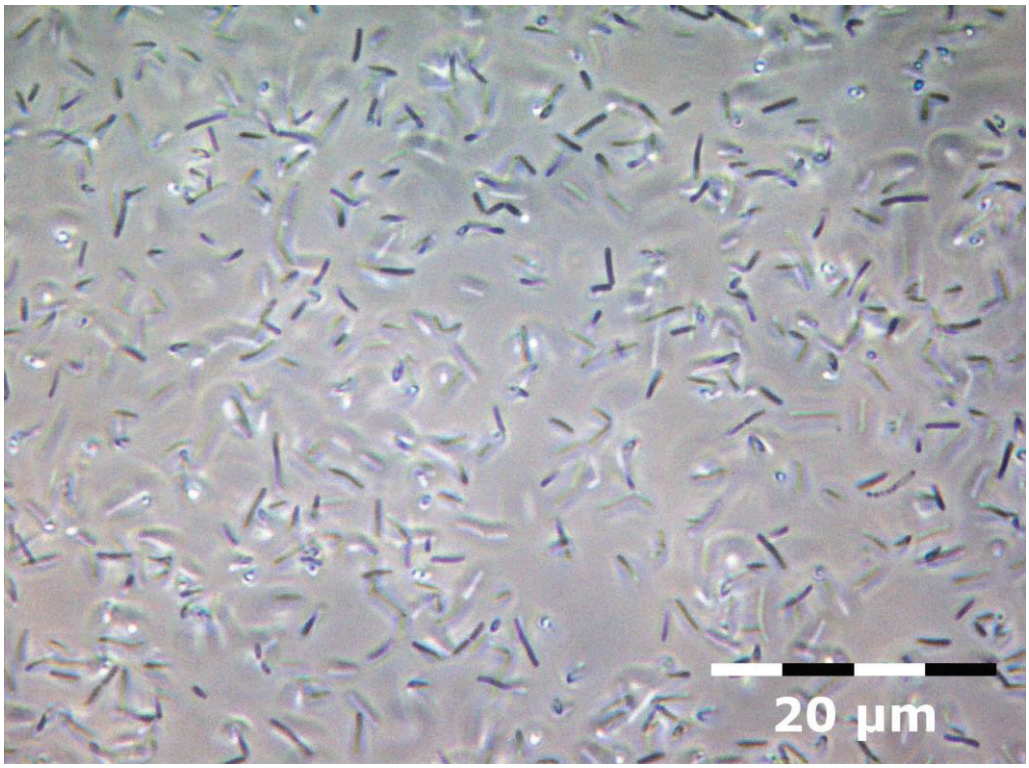
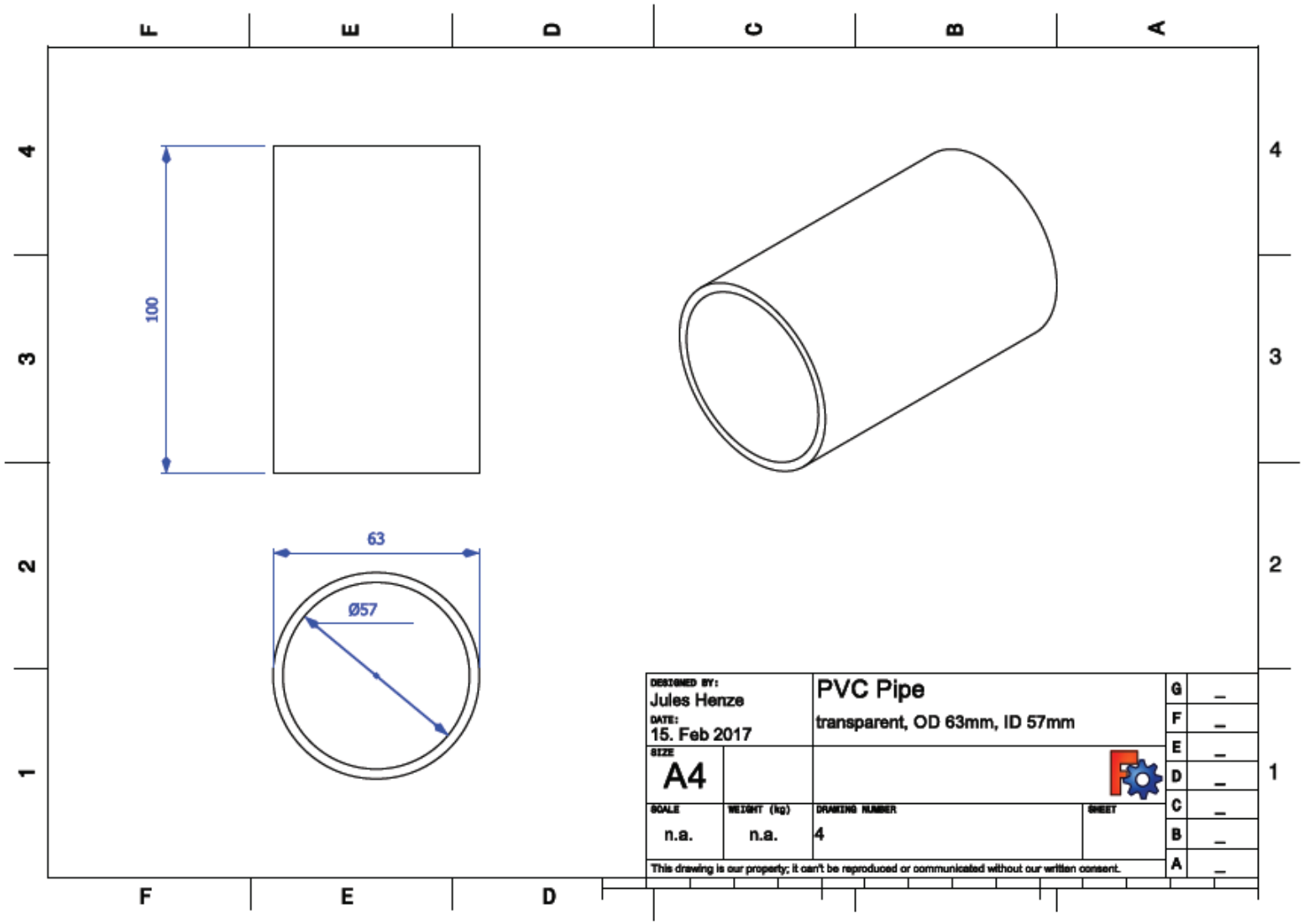


Figure A.2: Sporosarcina pasteurii magnified with the help of an optical microscope.

Appendix B: Column reactor CAD drawings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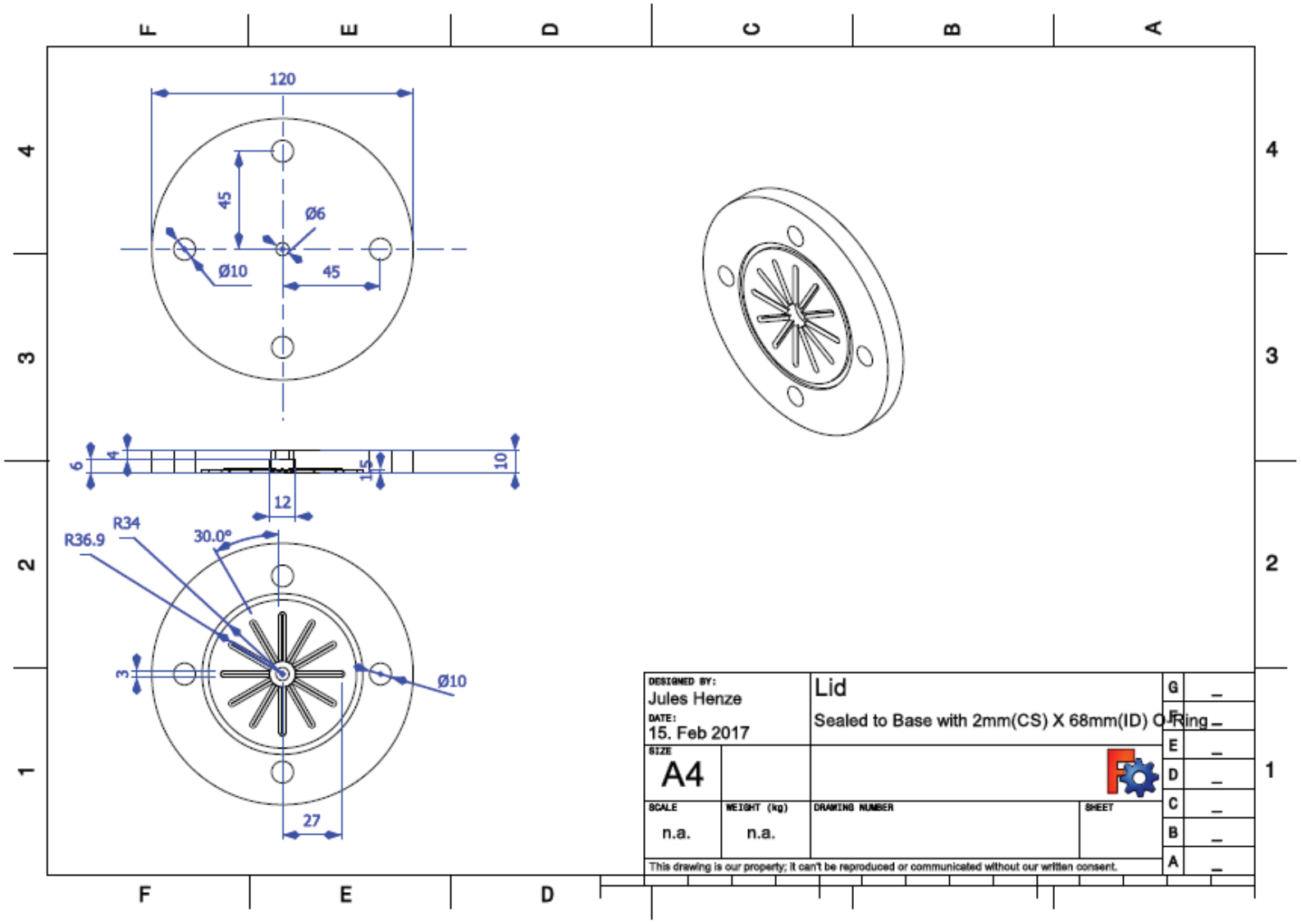

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SCALE n.a.	WEIGHT (kg) n.a.	DRAWING NUMBER	SHEET

G	_
F	_
E	_
D	_
C	_
B	_
A	_

Hose Connector
Is glued to Lid, for OD8mm/ID6mm Tube
DESIGNED BY:
Jules Henze
DATE:
15. Feb 2017
SIZE
A4
SCALE
n.a.
WEIGHT (kg)
n.a.
DRAWING NUMBER
3
SHEET
This drawing is our property; it can't be reproduced or communicated without our written consent.
12
4
4
6
24

Cylinder Base
is glued to PVC Cylinder
DESIGNED BY:
Jules Henze
DATE:
15. Feb 2017
SIZE
A4
SCALE
n.a.
WEIGHT (kg)
n.a.
DRAWING NUMBER
2
SHEET
This drawing is our property; it can't be reproduced or communicated without our written consent.
10
Ø10
45
63
120
45

Appendix C: MICP in highly alkaline environment

Table C.1: Titration results for the synthetic, stabilised urine after filtration of excess calcium hydroxide. 1 M hydrochloric acid was used. In total 4.3 mL of acid were added to 100 ml of the titrated solution.

HCl	pH		HCl	pH		HCl	pH		HCl	pH	
[mM]	∅	σ	[mM]	∅	σ	[mM]	∅	σ	[mM]	∅	σ
0	12.54	0.03	11	12.40	0.02	22	12.16	0.03	33	11.64	0.01
1	12.53	0.03	12	12.38	0.03	23	12.13	0.04	34	11.51	0.04
2	12.52	0.03	13	12.36	0.03	24	12.10	0.03	35	11.38	0.04
3	12.51	0.03	14	12.35	0.03	25	12.07	0.03	36	11.21	0.07
4	12.50	0.02	15	12.33	0.02	26	12.03	0.03	37	10.97	0.12
5	12.49	0.02	16	12.31	0.02	27	11.99	0.02	38	10.64	0.19
6	12.47	0.03	17	12.29	0.03	28	11.94	0.04	39	10.15	0.20
7	12.46	0.03	18	12.26	0.03	29	11.89	0.03	40	9.79	0.20
8	12.44	0.03	19	12.24	0.03	30	11.84	0.03	41	9.44	0.20
9	12.43	0.03	20	12.21	0.03	31	11.78	0.02	42	9.04	0.25
10	12.42	0.02	21	12.19	0.03	32	11.72	0.01	43	8.52	0.38

Table C.2: A description of the concentrated culture that was added to the prepared cementation media. All experiments were conducted at room temperature

	Triplicate Run		
	1	2	3
Culture OD	1.34	1.31	1.44
Concentrated OD	42.84	52.42	45.65
Concentrate added [ml/100ml]	0.47	0.38	0.44
T Experiment [°C]	21 ± 1	21 ± 1	21 ± 1

Table C.3: The numeric results of the analyzed calcium samples used in Figure 5.2. The pH shown here has been manually measured in the beginning and at the end of each experiment. The pH data used in Figure 5.2 are data logged once for Flask 3, 4 and 5 in 5 s intervals.

Repeated Experiments	HCL [mM]	pH 0 min	pH 90 min	Ca2+ conc. [mg/L] after given time [min]						
				0 min	15 min	30	45	60	75	90
Flask Nr. 1/1	0	-	12.28	953	862	839	817	801	782	729
Flask Nr. 1/2	0	12.46	12.41	852	868	802	787	791	833	740
Flask Nr. 1/3	0	12.50	12.18	870	872	824	815	787	835	820
Average	0	12.48	12.29	892	868	821	807	793	817	763
SD		0.03	0.12	54	5	18	17	7	30	49
Flask Nr. 2/1	18	12.24	11.99	876	852	879	850	870	851	823
Flask Nr. 2/2	18	12.23	12.16	884	872	854	851	864	887	836
Flask Nr. 2/3	18	12.27	11.84	853	862	855	862	858	856	853
Average	18	12.24	12.00	871	862	863	854	864	865	837
SD		0.02	0.16	16	10	14	7	6	19	15
Flask Nr. 3/1	28	11.95	11.53	875	866	867	870	868	853	858
Flask Nr. 3/2	28	11.94	11.72	892	870	862	868	875	834	849
Flask Nr. 3/3	28	11.96	11.17	876	861	842	867	870	854	872
Average	28	11.95	11.47	881	866	857	868	871	847	860
SD		0.01	0.28	10	4	13	1	4	11	12
Flask Nr. 4/1	36	11.13	9.27	884	704	257	143	109	100	42
Flask Nr. 4/2	36	11.08	9.42	886	676	218	101	87	42	31
Flask Nr. 4/3	36	11.21	9.23	869	773	275	129	99	81	45
Average	36	11.14	9.30	880	718	250	124	98	74	39
SD		0.06	0.10	9	50	29	22	11	29	7
Flask Nr. 5/1	42	9.28	9.24	877	834	689	317	132	80	55
Flask Nr. 5/2	42	9.40	9.30	877	823	711	310	135	70	54
Flask Nr. 5/3	42	9.27	9.15	876	851	697	296	143	54	60
Average	42	9.31	9.23	877	836	699	308	137	68	56
SD		0.07	0.08	1	14	11	10	6	13	3

Table C.4: A qualitative assessment of viability after the experiment on Christensen's Urea Agar

Flask Nr.	Result for repeats 1 / 2 / 3	
	Urease activity (2h)	Colonies (24h)
1	no	0
2	no	0 / 24 / 5
3	no / little / little	4 / 32 / 38
4	yes	many
5	yes	many

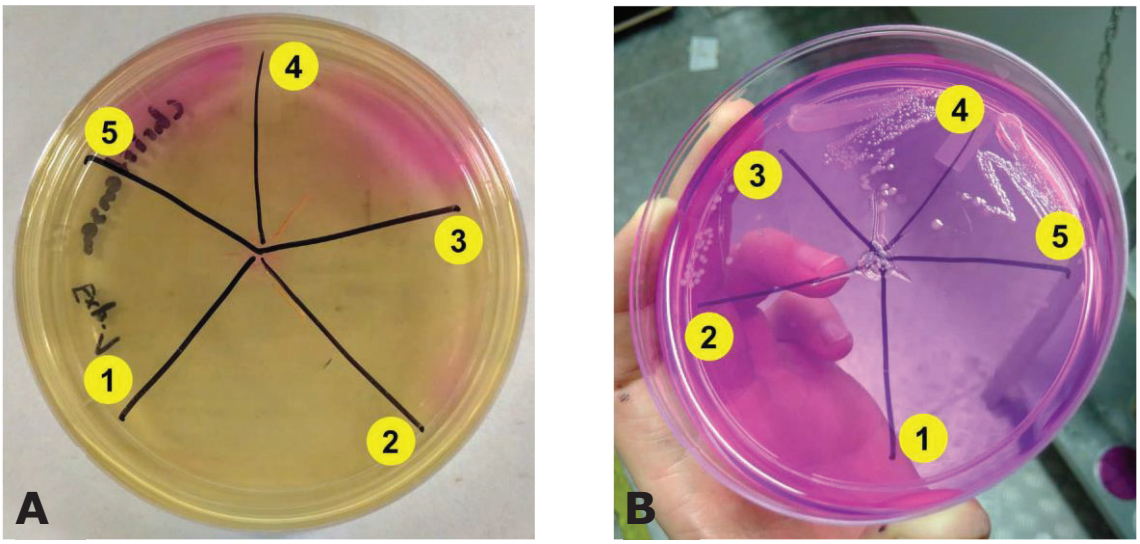

Figure C.1: Christensen's Urea Agar was used to detect ureolytic activity. Within a few hours a colour change from yellow to pink/purple indicates the microbial hydrolysis of urea. After a longer incubation time, the colour cannot be used to differentiate between differently inoculated sections due to diffusion, but the number of grown colonies indicate the viability of the streaked cells. Flask 3, 4 and 5 showed microbial activity after the experiment, with flask 3 showing a reduced number of viable cells.

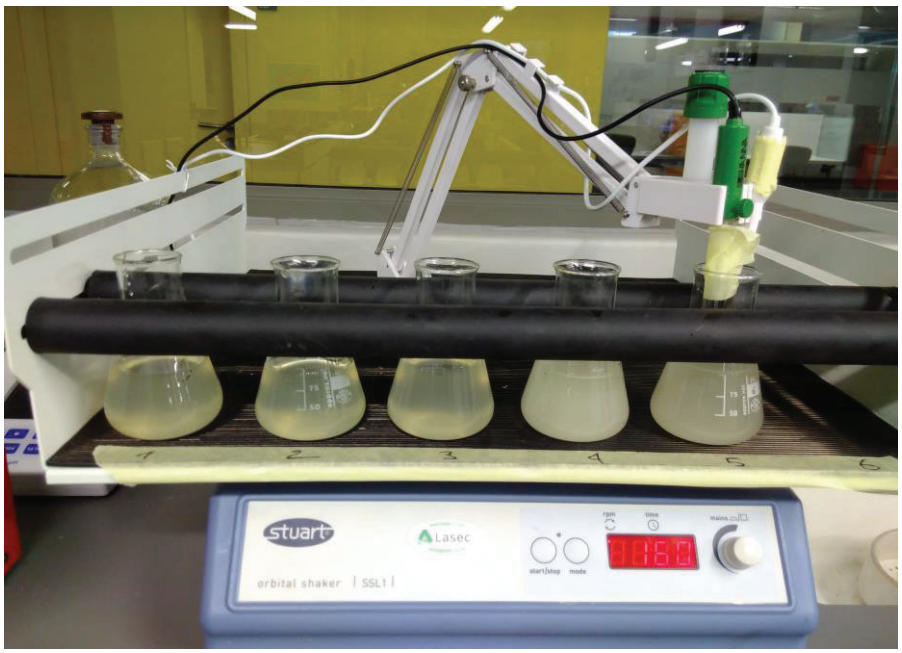

Figure C.2: The experimental setup used to determine the effect of the initial pH. The second flask from the right shows the highest amount of precipitate in suspension. A pH electrode with a logger was used to collect data with a 5s interval.

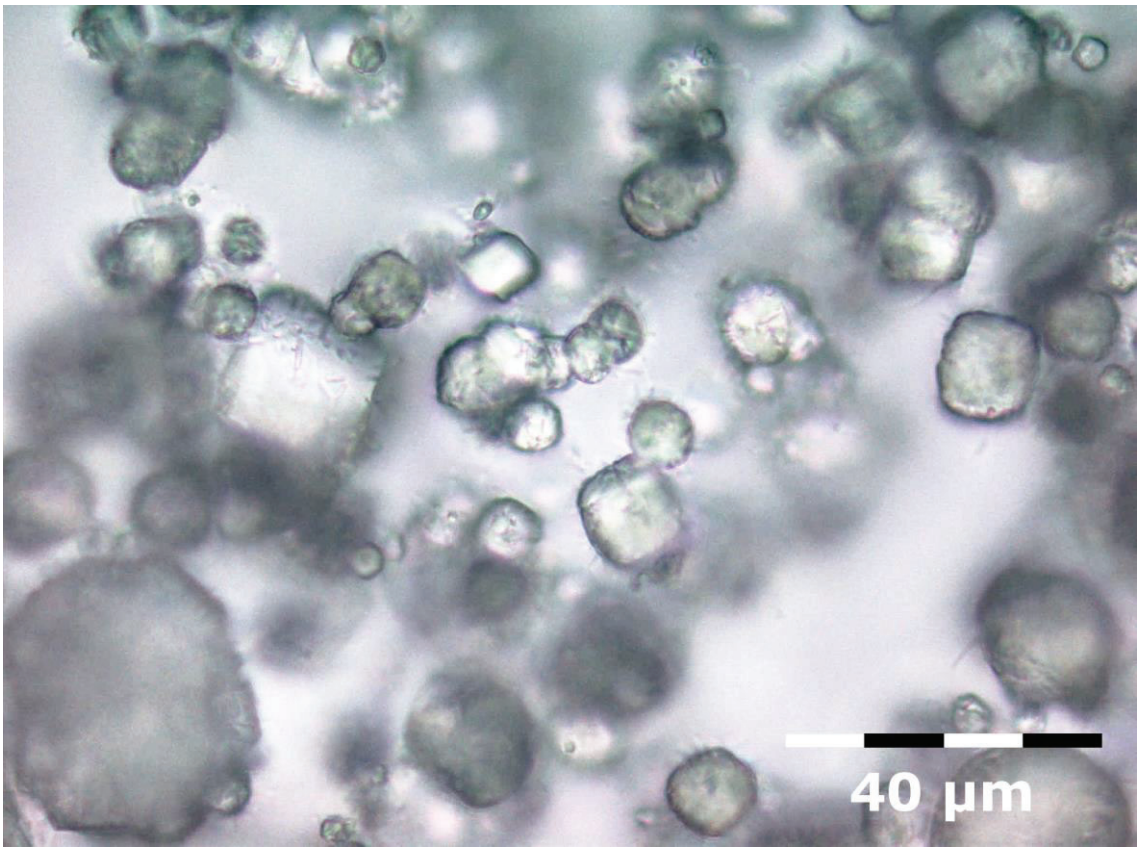

Figure C.3: A sample of flask 5 under the microscope shows the rhombohedral shaped calcite crystals. On closer examination *Sporosarcina pasteurii* can be seen around the crystals.

Appendix D: Column Reactor Experiment

$$\Delta Calcium\ [g/L] = Ca_{Influent} - Ca_{Effluent} \tag{D.1}$$

$$\Delta CaCO_3\ [g] = \frac{\Delta Calcium[g/L]}{M_{Ca}} * M_{CaCO_3} * V_{Reactor}[L] * \Phi_{Sand} \tag{D.2}$$

$$void\ filled\ [\%] = \frac{100}{V_{Reactor}[mL] * \Phi_{Sand}} * \frac{\Delta CaCO_3[g]}{\rho_{calciumcarbonate}[g/mL]} \tag{D.3}$$

$$Calcium\ Efficiency\ [\%] = \frac{100}{Ca_{Influent}[g/L]} * \Delta Calcium[g/L] \tag{D.4}$$

$$Urea\ Efficiency\ [\%] = \frac{100}{Urea_{Influent}[M]} * \frac{\Delta Calcium[g/L]}{M_{Ca}} \tag{D.5}$$

Table D.1: The Results from Pilot Run, Reactor Column 1

Nr.	Date	Time	Retention Time [h]	pH meas.		Calcium meas. [mg/L]			CaCO3 Deposition calc. [g]			Efficiencies	
				Influent	Effluent	Influent	Effluent	Δ	Δ	cum.	Void filled [%]	Calcium [%]	Urea [%]
1	5.4.17	14:00	2.00	9.77	-	745	302.85	442	0.08	0.08	0.04	59	3.68
2	5.4.17	16:00	16.67	11.01	9.42	798	47.46	750	0.13	0.20	0.11	94	6.24
3	6.4.17	8:40	2.33	10.87	9.38	753	12.81	740	0.13	0.33	0.18	98	6.16
4	6.4.17	11:00	3.67	10.94	9.35	738	<10	738	0.13	0.45	0.25	100	6.14
5	6.4.17	14:40	2.50	10.88	9.45	706	<10	706	0.12	0.57	0.31	100	5.88
6	6.4.17	17:10	15.33	11.03	9.45	630	<10	630	0.11	0.68	0.37	100	5.24
7	7.4.17	8:30	2.33	11.07	9.37	823	24.65	798	0.14	0.82	0.44	97	6.64
8	7.4.17	10:50	2.33	11.00	9.33	881	<10	881	0.15	0.97	0.52	100	7.32
9	7.4.17	13:10	2.33	11.18	9.38	916	<10	916	0.16	1.12	0.61	100	7.61
10	7.4.17	15:30		11.18	-	921	<10	921	0.16	1.28	0.69	100	7.66

Table D.2: The Results from Pilot Run, Reactor Column 2

Nr.	Date	Time	Retention Time [h]	pH meas.		Calcium meas. [mg/L]			CaCO3 Deposition calc. [g]			Efficiencies	
				Influent	Effluent	Influent	Effluent	Δ	Δ	cum.	Void filled [%]	Calcium [%]	Urea [%]
1	5.4.17	14:10	2.00	9.77	-	745	152	592	0.10	0.10	0.05	80	4.9
2	5.4.17	16:10	16.58	11.01	9.37	798	<10	798	0.14	0.24	0.13	100	6.6
3	6.4.17	8:45	2.42	10.87	9.33	753	<10	753	0.13	0.36	0.20	100	6.3
4	6.4.17	11:10	3.33	10.94	9.34	738	<10	738	0.13	0.49	0.27	100	6.1
5	6.4.17	14:30	2.83	10.88	9.38	706	<10	706	0.12	0.61	0.33	100	5.9
6	6.4.17	17:20	15.33	11.03	9.39	630	<10	630	0.11	0.72	0.39	100	5.2
7	7.4.17	8:40	2.42	11.07	9.30	823	<10	823	0.14	0.86	0.46	100	6.8
8	7.4.17	11:05	2.25	11.00	9.31	881	<10	881	0.15	1.01	0.55	100	7.3
9	7.4.17	13:20	2.33	11.18	9.33	916	<10	916	0.16	1.16	0.63	100	7.6
10	7.4.17	15:40	16.33	11.18	9.32	921	<10	921	0.16	1.32	0.71	100	7.7
11	8.4.17	8:00	2.00	11.08	-	897	<10	897	0.15	1.47	0.80	100	7.5
12	8.4.17	10:00	3.25	10.34	7.99	13765	2665	11100	1.88	3.35	1.82	81	92.3
13	8.4.17	13:15	2.75	10.55	7.95	14733	5403	9330	1.58	4.94	2.68	63	77.6
14	8.4.17	16:00	4.42	10.84	7.97	14982	5279	9703	1.65	6.58	3.57	65	80.7
15	8.4.17	20:25	12.42	10.82	7.90	14750	2283	12467	2.12	8.70	4.72	85	103.7
16	9.4.17	8:50	2.50	10.87	8.43	11906	8616	3290	0.56	9.26	5.02	28	27.4
17	9.4.17	11:20	3.83	11.19	8.77	12075	10523	1552	0.26	9.52	5.17	13	12.9
18	9.4.17	15:10	3.33	10.71	9.08	995	34	961	0.16	9.69	5.26	97	8.0
19	9.4.17	18:30	13.83	11.96	9.41	954	<10	954	0.16	9.85	5.34	100	7.9
20	10.4.17	08:20	2.00	11.06	8.70	12281	9587	2695	0.46	10.31	5.59	22	22.4
21	10.4.17	10:20	8.33	10.75	8.41	8316	1661	6654	1.13	11.44	6.21	80	55.3
22	10.4.17	18:40	2.33	11.07	8.41	8033	5946	2087	0.35	11.79	6.40	26	17.4
23	10.4.17	21:00	11.50	10.96	8.60	7984	606	7377	1.25	13.04	7.08	92	61.4
24	11.4.17	08:30	-	-	8.49	-	-	-	-	-	-	-	-

Table D.3: The Results from Pilot Run, Reactor Column 3

Nr.	Date	Time	Retention Time [h]	pH meas.		Calcium meas. [mg/L]			CaCO3 Deposition calc. [g]			Efficiencies	
				Influent	Effluent	Influent	Effluent	Δ	Δ	cum.	Void filled [%]	Calcium [%]	Urea [%]
1	5.4.17	14:20	2	9.77	-	745	122	623	0.11	0.11	0.06	84	5.2
2	5.4.17	16:20	16.58	11.01	9.37	798	13	784	0.13	0.24	0.13	98	6.5
3	6.4.17	8:55	2.42	10.87	9.31	753	<10	753	0.13	0.37	0.20	100	6.3
4	6.4.17	11:20	3.17	10.94	9.39	738	<10	738	0.13	0.49	0.27	100	6.1
5	6.4.17	14:30	3.00	10.88	9.37	706	<10	706	0.12	0.61	0.33	100	5.9
6	6.4.17	17:30	15.33	11.03	9.38	630	<10	630	0.11	0.72	0.39	100	5.2
7	7.4.17	8:50	2.50	11.07	9.30	823	<10	823	0.14	0.86	0.47	100	6.8
8	7.4.17	11:20	2.17	11.00	9.31	881	<10	881	0.15	1.01	0.55	100	7.3
9	7.4.17	13:30	2.33	11.18	9.33	916	<10	916	0.16	1.16	0.63	100	7.6
10	7.4.17	15:50	16.33	11.18	9.29	921	<10	921	0.16	1.32	0.72	100	7.7
11	8.4.17	8:10	2.00	11.08	-	897	<10	897	0.15	1.47	0.80	100	7.5
12	8.4.17	10:10	3.33	10.34	7.88	13765	2788	10977	1.86	3.34	1.81	80	91.3
13	8.4.17	13:30	2.67	10.55	7.96	14733	6512	8221	1.40	4.73	2.57	56	68.4
14	8.4.17	16:10	4.33	10.84	7.93	14982	5085	9897	1.68	6.41	3.48	66	82.3
15	8.4.17	20:30	12.50	10.82	7.87	14750	2342	12408	2.11	8.52	4.62	84	103.2
16	9.4.17	9:00	2.50	10.87	8.36	11906	9316	2590	0.44	8.96	4.86	22	21.5
17	9.4.17	11:30	3.83	11.19	8.50	12075	9405	2670	0.45	9.41	5.11	22	22.2
18	9.4.17	15:20	3.33	10.71	9.07	995	23	972	0.17	9.58	5.20	98	8.1
19	9.4.17	18:40	13.83	11.96	9.38	954	<10	954	0.16	9.74	5.28	100	7.9
20	10.4.17	08:30	2.00	11.06	8.54	12281	8123	4158	0.71	10.44	5.67	34	34.6
21	10.4.17	10:30	8.33	10.75	8.44	8316	1056	7260	1.23	11.68	6.34	87	60.4
22	10.4.17	18:50	2.17	11.07	8.46	8033	5247	2786	0.47	12.15	6.59	35	23.2
23	10.4.17	21:00	11.50	10.96	8.62	7984	347.5	7636	1.30	13.45	7.30	96	63.5
24	11.4.17	8:30	5.75	10.97	8.39	7665	3664	4001	0.68	14.13	7.66	52	33.3
25	11.4.17	14:15	3.58	11.10	8.58	8482	6838	1644	0.28	14.40	7.82	19	13.7
26	11.4.17	17:50	15.33	11.18	8.26	11359	1127	10232	1.74	16.14	8.76	90	85.1
27	12.4.17	9:10	4.92	11.16	8.40	12673	7478	5195	0.88	17.02	9.24	41	43.2
28	12.4.17	14:05	-	-	-	-	-	-	-	-	-	-	-

Table D.4: The numeric results of Experiment 1 discussed in Section 6.3.

Nr.	Treatment Date	Time	RT[1] [h]	Influent Ca [M]	Effluent pH ∅ [2]	σ [3]	ΔCa^{2+} [M] ∅	σ	Δ Urea [M] ∅	σ	Calcit [% PV] Δ	Cum.	Efficiency [%] Calcium	Urea
3	12.05.2017	18:00	3	0.025	9.19	0.03	0.024	0.000	0.083	0.014	0.27	0.27	98.3	8.1
8	13.05.2017	9:00	3	0.025	9.18	0.05	0.023	0.001	0.083	0.024	0.44	0.71	94.3	7.8
11	13.05.2017	18:00	3	0.054	8.95	0.10	0.052	0.002	0.118	0.014	0.41	1.12	96.6	17.3
16	14.05.2017	9:00	3	0.054	8.94	0.08	0.049	0.003	0.078	0.027	0.92	2.04	91.7	16.4
19	14.05.2017	18:00	3	0.107	8.66	0.07	0.045	0.020	0.054	0.015	0.51	2.55	42.2	15.1
24	15.05.2017	9:00	3	0.107	8.70	0.13	0.052	0.021	0.068	0.036	0.88	3.43	48.9	17.5
25	15.05.2017	15:00	6	0.107	8.64	0.15	0.057	0.020	0.067	0.023	0.20	3.63	53.4	19.0
28	16.05.2017	9:00	6	0.107	8.62	0.22	0.036	0.022	0.056	0.014	0.49	4.12	33.2	11.8
30	16.05.2017	15:00	3	0.107	8.86	0.33	0.029	0.017	0.031	0.019	0.23	4.35	26.7	9.5
36	17.05.2017	9:00	3	0.107	9.67	1.06	0.012	0.010	0.011	0.007	0.43	4.77	10.9	3.9

[1] Retention Time
[2] Average
[3] Standard Deviation

Table D.5: The numeric results from Experiment 2. After 42 treatment cycles solid sand columns could be recovered from the reactor.

	Treatment			Influent	Effluent pH		ΔCa^{2+} [M]		Δ Urea [M]		Calcit [% PV]		Efficiency [%]	
Nr.	Date	Time	RT[4] [h]	Ca [M]	∅ [5]	σ [6]	∅	σ	∅	σ	Δ	Cum.	Calcium	Urea
6	25.05.2017	9:00	3	0.025	9.28	0.03	0.025	0.001	0.173	0.040	0.55	0.55	98.3	8.3
9	25.05.2017	18:00	3	0.056	9.15	0.04	0.055	0.000	0.180	0.019	0.44	0.99	99.2	18.4
14	26.05.2017	9:00	3	0.056	9.11	0.03	0.055	0.000	0.165	0.011	1.01	2.00	98.5	18.3
17	26.05.2017	18:00	3	0.111	8.79	0.02	0.109	0.001	0.185	0.012	0.89	2.88	97.7	36.2
22	27.05.2017	9:00	3	0.111	8.68	0.05	0.107	0.003	0.182	0.027	1.93	4.81	96.0	35.5
23	27.05.2017	15:00	6	0.111	8.77	0.03	0.109	0.002	0.180	0.014	0.38	5.19	98.0	36.3
26	28.05.2017	9:00	6	0.111	8.78	0.06	0.109	0.001	0.188	0.014	1.14	6.33	97.7	36.2
28	28.05.2017	21:00	6	0.229	8.40	0.05	0.201	0.014	0.247	0.010	1.07	7.40	87.7	67.2
30	29.05.2017	9:00	6	0.233	8.34	0.07	0.174	0.016	0.199	0.019	1.28	8.68	74.3	56.9
32	29.05.2017	21:00	6	0.233	8.37	0.05	0.129	0.034	0.163	0.043	1.02	9.70	54.7	41.9
34	30.05.2017	9:00	6	0.233	8.42	0.04	0.113	0.044	0.169	0.043	0.81	10.51	47.7	36.6
36	30.05.2017	21:00	6	0.109	8.55	0.05	0.068	0.020	0.177	0.057	0.60	11.11	62.8	22.8
38	31.05.2017	9:00	6	0.109	8.72	0.12	0.074	0.033	0.130	0.046	0.47	11.58	67.5	24.5
40	31.05.2017	21:00	6	0.109	8.66	0.08	0.076	0.022	0.124	0.037	0.49	12.06	69.3	25.2
42	01.06.2017	9:00	6	0.109	8.66	0.06	0.073	0.027	0.113	0.043	0.48	12.55	67.2	24.4

[4] Retention Time
[5] Average
[6] Standard Deviation

Figure D. 1: The reactor setup used in Experiment 1 & 2. Top: Cementation media is injected from the bottom to the top and the effluent is collected in 1 L bottles. The approximate setting of the flow rate could be monitored by the collected volume of effluent. Samples were manually captured in separate glassware. Bottom: The peristaltic pump (bottom, center) was controlled by a timer switch. Each reactor had its own, independent tubing. The influent container on the right has a volume of 5 L and the air inlet on top was equipped with a 0.45µm filter.

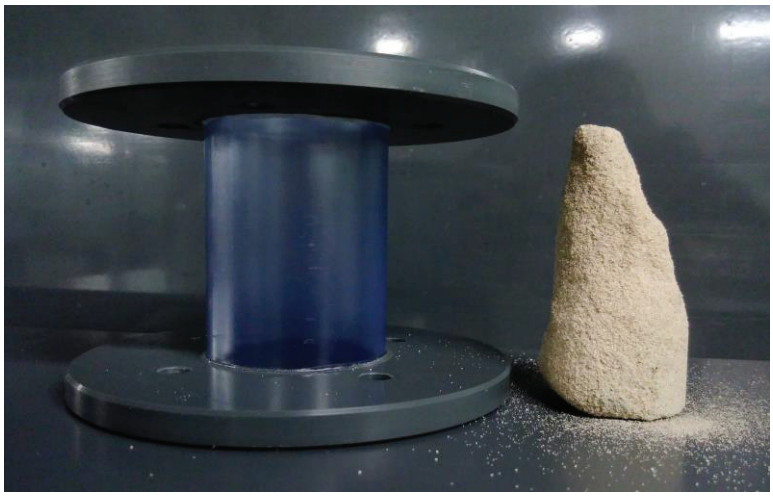

Figure D.2: The solidified part recovered from one reactor in experiment 2. The surrounding sand did not show any sign of solidification. This indicates the effect of a preferential flow path during inoculation.

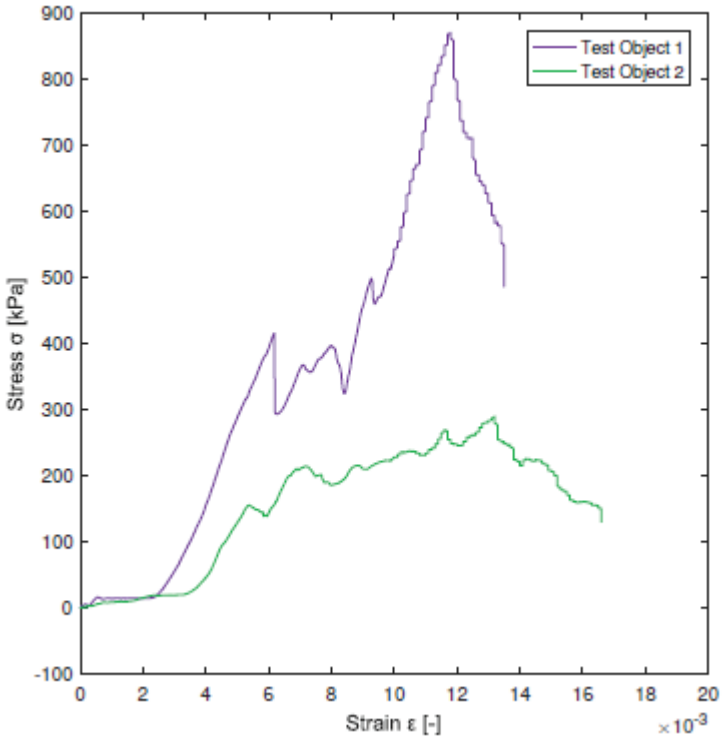

Figure D. 3: The stress-strain curve of two solidified sand column from experiment 2. Pressure was applied until failure of the columns. A compression testing machine from Zwick Roell was used. A maximum compression stress of 870 kPa could be applied to Test Object 1. Because both test objects did not feature perfectly aligned and flat bottom and top surfaces, pressure could not be applied homogeneously. This explains the jagged stress-strain curve for both objects. Small protruding sections failed individually before the whole test object was under load.

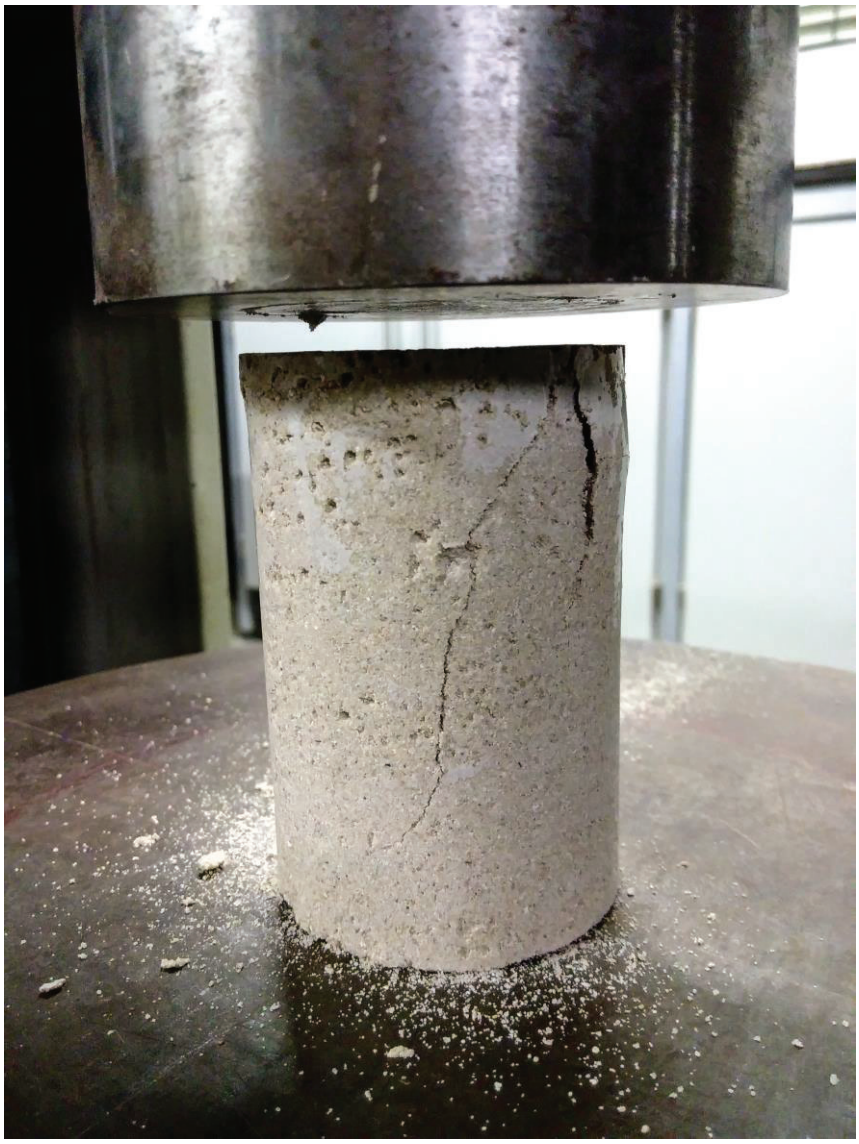

Figure D.4: The Test Object 1 from Figure D.3 after failure.


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