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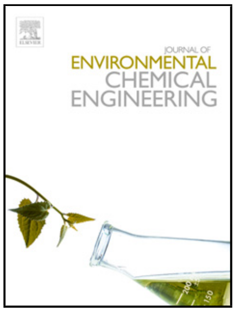

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Microbial induced calcium carbonate precipitation at elevated pH values (>11) using *Sporosarcina pasteurii*

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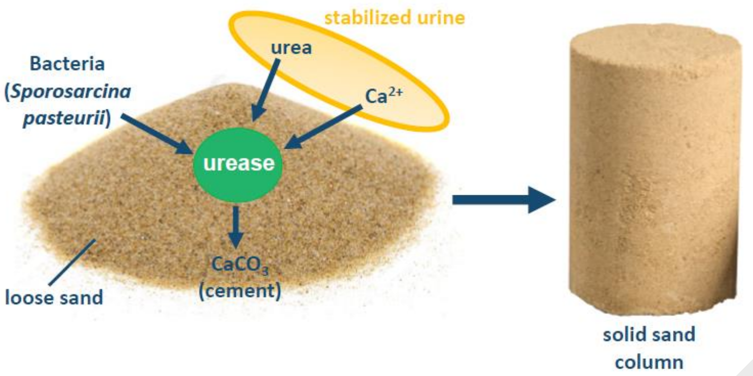
Graphical Abstract



Abstract:
We have shown, for the first time, that stabilized synthetic urine can be used to facilitate a microbial induced calcium carbonate precipitation reaction at elevated pH values. Stabilized urine is mixed with sand and urease-producing bacteria. The bacteria convert the urea present in the urine to carbonate ions, which then combine with excess calcium ions to form calcium carbonate. This calcium carbonate cements any sand particles together, thus forming a bio-solid of any shape. The resilience of *Sporosarcina pasteurii* bacteria to survive pH values of approximately 11.2 was not expected, but this finding is useful for using stabilized urine as a source of urea since the solution has a pH typically greater than 12. This process thus has the potential to create more sustainable building products from urine using natural processes.

Keywords: bio-solids; building products; calcium hydroxide; urine; urea hydrolysis

1. Introduction

Microbially-induced calcium carbonate precipitation (MICP) is a biomineralisation process, where specific microorganisms induce the precipitation of calcium carbonate ($CaCO_3$). These biological reactions are usually facilitated by either the enzymatic hydrolysis of urea (Gat et al., 2014) or the interconversion of CO_2 to HCO_3^- by the enzyme, carbonic anhydrase (Li et al., 2013). This leads to the supersaturation of calcium carbonate and its subsequent precipitation (De Muynck et al., 2010). The precipitation process of $CaCO_3$ is very slow, but these enzymes can significantly speed up these reactions. For example, the enzymatic hydrolysis of urea is approximately 10^{14} times faster than the chemical decomposition of urea (Jabri et al., 1995). The enzymatic hydrolysis of urea can be explained using the following reactions (Gat et al., 2014; Mobley and Hausinger, 1989):

$$CO(NH_2)_2 + H_2O \rightarrow NH_3 + NH_2COOH \qquad (1)$$

$$NH_2COOH + H_2O \leftrightarrow H_2CO_3 + NH_3 \qquad (2)$$

$$H_2CO_3 \leftrightarrow H_+ + HCO_3^- \qquad (3)$$

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \qquad (4)$$

$$Ca^{2+} + HCO_3^- + OH^- \leftrightarrow CaCO_3\downarrow + H_2O \qquad (5)$$

The urea is hydrolysed to carbonate and ammonia by the biological catalyst urease (1), followed by the spontaneous hydrolysis of carbamate to carbonic acid and ammonia (2) which is then further hydrolysed according to reactions (3) and (4) (Gat et al., 2014). This ultimately results in a pH increase and the precipitation of $CaCO_3$, as explained by reaction (5).

MICP has been extensively investigated for the modification of soils (Gat et al., 2014), the rehabilitation of structures (De Muynck et al., 2010) and even the production of bio-bricks (Bernardi et al., 2014). Bio-bricks or solids of any shape can be produced using MICP because the calcium carbonate can be used to cement an otherwise loose mass of particles together. Several studies have successfully used synthetic urea solutions (Bernardi et al., 2014; Eryürük et al., 2015; Rowshanbakht et al., 2016) for the MICP process. The issue with using synthetic urea for MICP is that the urea is produced by the Haber-Bosch process, an energy intensive process that produces N-based fertilizers from gaseous N_2, accounting for about 1% of the global energy demand (Chesworth, 2008). If MICP processes that utilise urea as feedstock are to be truly sustainable, alternative and environmentally friendly methods of producing urea are required.

Fortunately, urine has significant amounts of urea, accounting for up to 90% of the nitrogen present in human urine (Lentner, 1981). However, urine is often collected and stored which results in the urea being naturally converted to ammonia and carbonate ions by urease-producing bacteria (Udert et al., 2003b). The pH of the stored urine increases due to the formation of ammonia and bicarbonate ions (Udert et al., 2003c) reaching an approximate pH of 9.25 as a result of the equilibrium state of the NH_3-NH_4^+ ion pair. If the storage container is not sealed correctly and is in contact with air, significant amounts of nitrogen could be lost due to ammonia volatisation (see Figure 1). In addition, storing urine results in almost all the urea being converted to ammonia or ammonium ions and hence stored urine cannot be used for MICP. However, the urea in fresh urine could be used for MICP, provided the enzymatic urea hydrolysis is prevented during storage.

Recently, researchers have shown that enzymatic urea hydrolysis can be inhibited in urine by merely increasing the pH of the solution above 11 through the addition of calcium hydroxide (Randall et al., 2016). The use of calcium hydroxide to stabilise urine therefore offers an easy method for storing urine without any urea loss because the calcium hydroxide can be added as a powder and it dissolves as required, maintaining a constant high pH. This urine stabilisation process also produces calcium phosphate, which can be used as an inorganic fertiliser thus offering an additional advantage. The pH of urine stabilized with calcium hydroxide is 12.5 at 25°C (Randall et al., 2016) which could be an issue for urease-producing bacteria since optimal growth usually occurs near a pH of 9.

Sporosarcina pasteurii bacteria has commonly been used for MICP due to its ability to produce high amounts of the enzyme urease (De Muynck et al., 2010) and because it is a non-pathogenic species. To date, no researcher has investigated the applicability of *Sporosarcina pasteurii* bacteria to survive at elevated pH values. Not only would the bacteria's ability to survive at elevated pH values be useful for use in the novel urine stabilisation process with calcium hydroxide, but researchers have also shown that an elevated pH value (albeit for carbonic anhydrase) promotes the precipitation of $CaCO_3$ (Li et al., 2013).

This study therefore focuses on investigating the feasibility of a MICP process at elevated pH values, typical of stabilised urine, to determine if *Sporosarcina pasteurii* bacteria can survive and operate at these conditions. This would provide a first step for using urine in a MICP process, thus offering an alternative, more sustainable source of urea for MICP processes.

2. Material and Methods

2.1 Microbiological Culture

The non-pathogenic species *Sporosarcina pasteurii* (ATCC® 11859) was used as the urease-producing bacteria. For the culturing of *Sporosarcina pasteurii*, the Ammonia-Yeast media (ATCC® 1376) was used, which contains 20 g/L yeast extract and 10 g/L $(NH_4)_2SO_4$. A 0.13M Tris buffer was used for pH adjustment to 9. The yeast extract and ammonium sulfate were added separately to 150 mL of de-ionised water. A mass of 15.75 g of the Tris-base was 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.

2.2 Synthetic urine

Synthetic stabilised urine containing urea, nutrient broth and calcium hydroxide was used for the column experiments to obtain a solution of constant properties. 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 reported average values of 0.33 M (Pillai et al., 2014) and 0.19 M (Randall et al., 2016). It was therefore decided to work with a 0.3 M urea solution. Additionally, 3 g/L Nutrient Broth No. 1 (Sigma-Aldrich) was added to the synthetic urine. This concentration of nutrient broth was used in other studies where *Sporosarcina pasteurii* was utilised for MICP ((Stocks-Fischer et al., 1999); (Okwadha and Li, 2010); (Qiu et al., 2014)) to support 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 Whatman Grade 595 Filter paper to remove excess $Ca(OH)_2$. Hydrochloric acid with a concentration of 1 M was then used to decrease the pH to the desired value. The resulting solution was termed “cementation media”.

2.3 Analytical methods

A Hanna pH-Electrode (type HI1131B), in combination with a temperature probe, was used to measure the pH and temperature of the solution. 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 (Association, 2007). For example, at a measured temperature of 21° C, the calibration pH was 12.592. The proprietary Hanna pH-buffers are automatically temperature corrected by the software.

For calcium and ammonium concentrations, a colorimetric method was used. A Thermo Scientific Gallery (ThermoFisher Scientific, Massachusetts, United States) automated this process. The measurement range of this device for calcium is 10 mg/L to 200 mg/L and for ammonia 0.45 mg/L to 2 mg/L. Samples were diluted with deionised water when necessary and analysed within 30 min of sampling. Ammonium samples were acidified with 0.1M HCl to a pH of 3.5± 0.5. The acidification of the sample prevented volatilisation of ammonium and inhibited the hydrolysis of urea such that the sample composition remained stable (Hellström et al., 1999).

A spectrophotometer was used to determine the optical density of the cell culture. A spectrophotometer is a quick and convenient method to estimate the cell biomass in suspension. The measurement is based on the scattering of light at a wavelength of 600 nm directed 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, 2007). Culture samples were analysed with a Thermo Scientific

GENESYS 10 Photospectrometer. In order to obtain a zero-reference value, a sterile, non-inoculated sample of the media broth at the same dilution as the culture sample was used.

2.4 Investigation of the initial pH on the MICP process

First, we sought 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* during the experiment. The experiment was repeated three times. Using a glass pipette, 100 mL of the filtered, synthetic urine was transferred to five 100 mL Erlenmeyer flasks. The pH was then decreased by addition of 1M hydrochloric acid to reach the required value. The appropriate amount of acid was determined by titration. The targeted pH values and the amounts of acid required per 100 mL are shown in Table 1.

A 100 mL culture of *Sporosarcina pasteurii* was grown overnight until late-exponential or early-stationary phase. Using optical density measurements, the growth phase was determined before undertaking any further steps. The bacterial culture was 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 bacterial pellet, were remaining. Using the same pipette, the pellet was re-suspended 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 was then added to each of the five flasks containing the synthetic urine solution such that a calculated optical density of 0.2 was obtained. An optical density of 0.2 was experimentally determined to be the starting point of the exponential growth phase of *Sporosarcina pasteurii*. All five flasks were placed on a shaker platform at 160 rpm and at room temperature to keep the bacteria and calcium carbonate crystals in suspension. To measure dissolved calcium concentration, samples were taken every 15 min for 1.5 hrs using a syringe. Prior experiments showed that the experiments would have taken less than 90 min to reach >90% Ca^{2+} precipitated. The sampling frequency was limited by the preparation and measurement effort for sampling. A higher frequency would not have been possible with the described methodology, and a lower frequency would have resulted in a loss of valuable information. A sample volume of approximately 3-5 ml was taken and filtered using 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.

2.5 Experimental set-up of bio-solid column reactors

Three column reactors were constructed to study MICP with sand as the media. The column reactors kept 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. Each reactor had a volume of 255 mL with an inside diameter of 57 mm and a length of 100 mm. The bottom and top lid were identical and removable, both sealed with an O-ring. On the inside face of both lids, a radial depression distributed the incoming liquid over a large part of the sand body surface. This minimized 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. 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 cylinder wall was first greased with silicone paste (Herschell) in order to seal the gap between the plastic sheet and cylinder wall from liquid intrusion. This construction prevented the precipitate from bonding directly to the cylinder wall and simplified the later extraction of the solid sand column upon experiment completion. 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 the lid and the fabric mesh. When closing the reactor, the sponge was compressed to approximately 1 cm thickness and was used to place the sand column under a light compressive force and to evenly disperse the impulse of the incoming liquid.

Traditional sand from the Cape Flats in Cape Town was used for these experiments. The sand was first sieved to obtain a defined grain size range of 0.15 mm to 0.6 mm, which is similar to that of masonry sand. 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 shaken to allow the sand to settle and the volume adjusted to 100 mL. The sand content was subsequently poured into 250 ml of water in a 500 ml measuring cylinder. The resulting shift in volume (V_{Total}) was noted. The procedure was repeated five times. The porosity Φ was then calculated according to equation 6. A porosity of 0.376 ± 0.017 was determined for the sieved sand.

$$\Phi = \frac{V_{\mathrm{Total}} - 250\ \mathrm{mL}}{100\ \mathrm{mL}} \qquad (6)$$

For the inoculation of the sand with bacteria, an amount of sand slightly exceeding the volume of the reactors was mixed with *Sporosarcina pasteurii* in a beaker, such that the sand was saturated. The sand-bacteria mixture was then added to the reactor until 1 cm below the top, while the bottom outlet was kept closed. Liquid covering the sand was drained through the outlet such that the level of liquid matched the level of sand in the reactor. The reactor was closed and the bacteria were kept in the reactor for 4 hrs after which treatment with the first batch of cementation media started.

Samples of the effluent were taken as follows: the first 20 to 30 mL of effluent were collected in a beaker followed by a 30 mL sample. The first 20 to 30 mL of effluent were discarded to make sure that the sampled volume was taken from within the reactor volume and not the tube and connectors. The 100 mL beaker with the first fraction of effluent was then adjusted to 100 mL such that, in total, 130 mL of liquid was pumped through the reactor. The estimated pore volume of the reactor was 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 filter the suspension. Samples of the filtrate were then analyzed for dissolved calcium and ammonia.

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 not have been possible by manual operation. The timer switch regulated a peristaltic pump that was connected to each reactor by independent tubing. Liquid was injected from the bottom of the reactor and flowed out the 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 of 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 possibly caused by contamination with *Sporosarcina pasteurii*. This was important since any hydrolysis would cause a drop of the pH. Treatment was started with cementation media containing 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. The retention time was then increased to 6 hrs. After day 4, the calcium concentration was increased to 0.23 M with a retention time of 6 hours. Samples were taken twice a day. Calcium carbonate formation was confirmed using similar tests to Tang and co-workers whereby HCl was added to the solid product, producing CO_2 bubbles (Tang et al., 2017). In contrast, no bubbles were observed when adding HCl to the raw sand.

2.6 Calcium and urea usage efficiency

The process efficiency in the column reactor was assessed by means of two indicators: calcium usage efficiency ($Eff_{Calcium}$) and urea usage efficiency (Eff_{Urea}). For both calcium and urea, it is desirable that the amount in the inflow is completely utilized during the process. Therefore, the calcium usage efficiency is given by equation 7, with 100% being the best achievable value.

$$Eff_{Calcium}[\%] = \frac{Calcium_{IN} - Calcium_{OUT}}{Calcium_{IN}} \times 100\% \qquad (7)$$

From reactions (1) to (5), it can be concluded that one molecule of urea leads to the precipitation of one molecule of calcium carbonate, thus defining the urea usage efficiency (equation 8).

$$Eff_{Urea}[\%] = \frac{Calcium_{IN}\,[M] - Calcium_{OUT}[M]}{Urea_{IN}[M]} \times 100\% \qquad (8)$$

3. Results and Discussion

Based on Figure 2, we show that when the initial pH of the solution is decreased to below approximately 11.15, the calcium ion concentration in the solution begins to decrease, thus indicating the production of calcium carbonate. If the pH is kept above 12, the calcium ion concentration does not decrease because the urease-producing bacteria are inactivated at this higher pH. We also see that the initial calcium ion concentration is depleted faster at elevated pH values (11.15) when compared to low pH values (9.3), which is likely a result of a greater concentration of carbonate ions being present. However, both initial pH values result in the same calcium concentration after 90 minutes of operation. Overall, the MICP process is a strong function of pH and, surprisingly, the *Sporosarcina pasteurii* are able to survive at pH values greater than 9, which has been reported in the literature as optimal for growth (Anbu et al., 2016). This insight is beneficial for using stabilized urine as a source of urea for MICP since these findings suggest that the pH of the stabilised urine does not have to be decreased to these optimum values and, hence, less acid is required for the process.

Figure 3 shows two of the final solid columns that were formed from the process along with the reactor used to “grow” them.

Three column experiments were started with 0.025 M Ca^{2+} in the influent, which was then doubled twice until a concentration of 0.11 M (Figure 4A). Up to this concentration, almost complete utilisation of the influent calcium for precipitation could be observed at a retention time of 3 hrs. Approximately 0.18 M urea was hydrolysed (Figure 4B). 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 hours. However, this had no effect on the amount of urea being hydrolysed. Contrary to expectations, the level of ammonia, which was measured in the effluent, remained approximately constant. Regardless, the influent calcium concentration was then increased to 0.23 M (Figure 4A). 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 approximately 88% (Figure 4C). 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 for the three reactors increased. After two days, the calcium concentration was reduced to 0.11 M (Figure 4A). The amount of calcium that precipitated stabilised at a calcium usage efficiency of approximately 65% (Figure 4C). The hydrolysis of urea continued with a slight 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.

An increase in calcium concentration to 0.23 M was followed by a spike in the observed ureolytic activity and a subsequent drop to lower activity. The most probable reason for this change was the increase in the influent calcium concentration. However, it is unclear if the observed spike in ureolytic activity was the result of exceeding a threshold in calcium concentration. It could likely be the result of a stress reaction due to the sudden change of, for example, ionic strength, which is known to reduce the activity of bacteria (Hammes and Verstraete, 2002). The influent calcium concentration was lowered to 0.1 M two days after the increase to 0.23 M. The decrease in the influent calcium concentration stabilised the calcium efficiency at approximately 67%, as seen in Figure 4C. This was significantly lower compared to the value observed before at the same influent concentration. Additionally, a slight downward trend of the ureolytic activity was observed (the calcium efficiency was used as a proxy for the ureolytic activity). These observations seem to show that the increase in calcium concentration from 0.1 M to 0.23 M did, in fact, have a negative impact on the microbial community in the column. This finding warrants further investigation to better understand the process and the sudden decrease of microbial activity.

In addition, with only urea hydrolysis, the pH tends towards approx. 9.25 as a result of the equilibrium state of the NH^3-NH_4^+ ion pair (Udert et al., 2003c). This equilibrium pH is altered by the precipitation of calcium carbonate. It can be seen from Figure 4D, that the decreasing pH effluent trend correlates strongly to an increase in the amount of calcium carbonate precipitation.

These findings showed that an influent calcium concentration of up to 0.11 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 treatment cycles, each of the three reactors generated 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 $CaCl_2$ to produce bio-bricks with up to 84 treatment cycles. 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 of the columns produced in the experiments were tested for compressive strength, whereas the third was kept for demonstration purposes. While one column could withstand a maximum stress of 870 kPa, the second failed at 290 kPa. 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. An average compressive strength of 440 kPa with a maximum of 930 kPa and a minimum of 120 kPa were measured, while our findings 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 (Bernardi et al., 2014). The strength of a bio-brick is scalable and can reach values similar to that of a cement brick. However, this would require more treatment cycles than those needed to produce a brick of similar strength than a 40% lime brick (Bernardi et al., 2014).

These findings show that MICP, using synthetic urine at an elevated pH, can operate using a simple setup as demonstrated. 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 sufficient 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 0.11 M. However, the urea is not used efficiently at such a low calcium concentration, since only one third of the available urea is effectively utilized for the precipitation. 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 of urine close to the theoretical maximum value, which is the equimolar amount 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 the cementation media.

Following these findings, there are three major areas of focus for further research on the proposed process. First, since this study used synthetic, stabilised urine for MICP, further studies need to investigate the process using real, stabilized urine. Although it was shown that a solution similar to that of stabilised urine with regard to concentration of urea, calcium hydroxide as well as pH can be used in MICP, it is plausible that other properties, like ionic strength or specific components that are present in urine, might inhibit the activity of *Sporosarcina pasteurii*. Furthermore, in order to introduce a novel building material to the infrastructure sector, proof that it fulfils the building product requirements must be provided. It would be important to consider not only compressive strength, but other properties such as water absorption and chemical resistance. A first step is to investigate the relationship between the number of treatment cycles and the compressive strength at a constant influent calcium concentration. Lastly, 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 urea efficiency of 35% was observed for the experiments during stable bacterial activity conducted in this study. Higher urea efficiencies (~68%) were obtained during the experiment but these efficiencies were short-lived and, therefore, cannot be used as the baseline value. From these experiments, it was seen that a maximum influent calcium concentration of 0.11 M could not be exceeded in the long-term although 0.3 M of urea was available. Interestingly, our findings suggest that not all the urea was hydrolysed, even when the retention time was doubled to 6 hours. Decreasing the concentration of the urea by dilution with the cementation media, while keeping the calcium concentration constant, might help increase the urea usage efficiency, but this would not be ideal in water scarce areas as more water would be needed. However, this could also be a strategy to decrease the adverse effects of ionic strength (or specific components in urine) on the activity of *Sporosarcina pasteurii* that might emerge when the process is tested with real urine.

Conclusions

The high pH of stabilized urine delays the enzymatic urea hydrolysis process until the urine can be re-initiated for MICP. This suggests that fresh urine can be stored for later MICP applications, provided it is stabilised with calcium hydroxide. We have shown, for the first time, that stabilized synthetic urine can be used to facilitate a MICP reaction at elevated pH values, which can subsequently be used to cement loose sand particles into a solid shape. The resilience of *Sporosarcina pasteurii* bacteria to survive pH values of approximately 11.2 was not expected, but this finding is insightful for using stabilised urine as a source of urea. The next step would be to test MICP with real urine and test the integrity of the solid products formed as compared to conventionally-produced solids, such as bricks.

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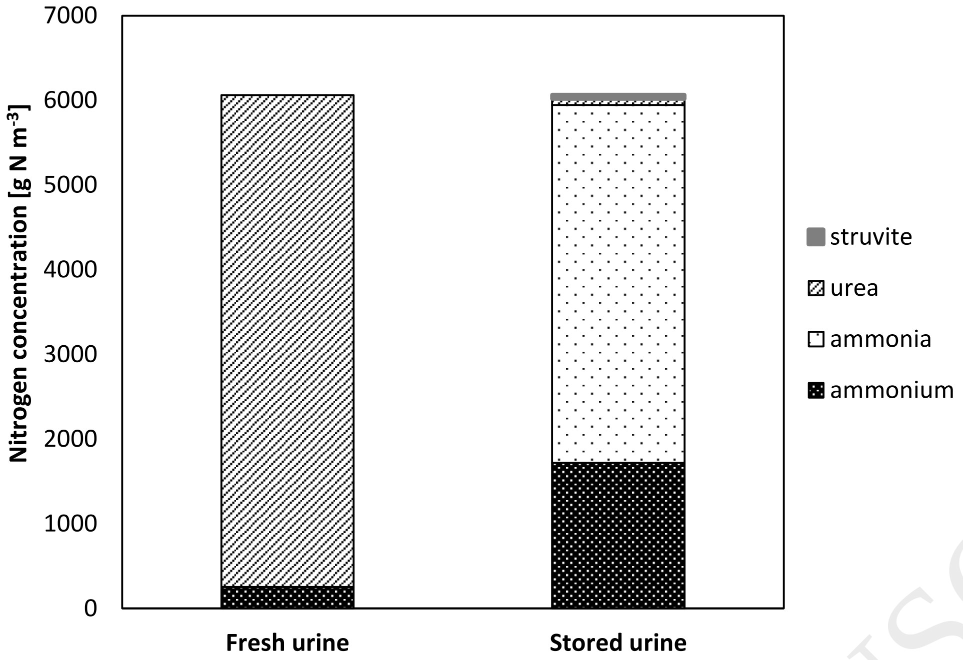

Figure 1: Nitrogen composition in fresh and stored urine based on data from Udert and co-workers (Udert et al., 2003a).

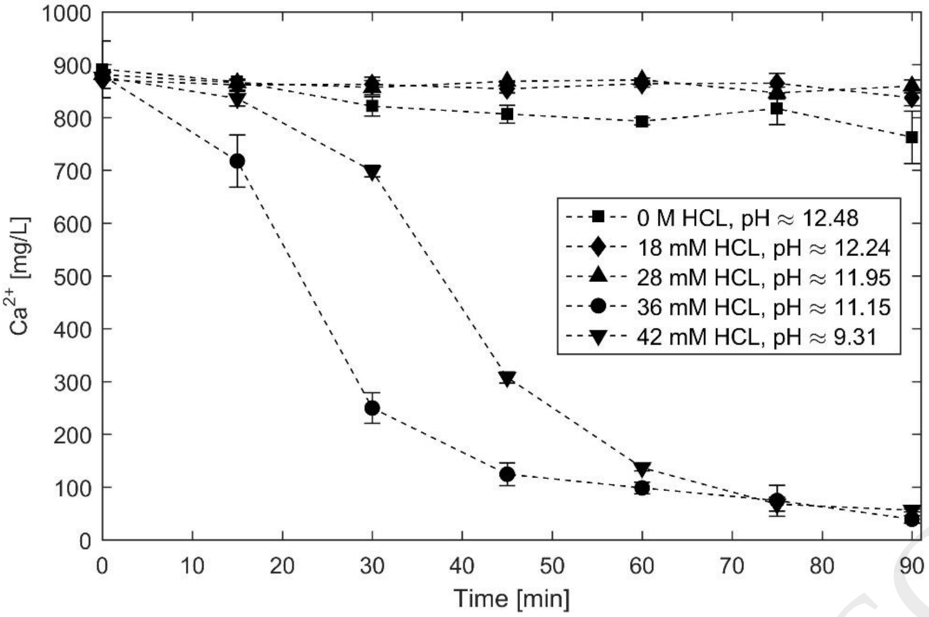

Figure 2: Calcium ion concentration as a function of acid added (pH) to a synthetic stabilized urine solution with Sporosarcina pasteurii bacteria present

Figure 3: The solid columns that were produced using MICP and synthetic, stabilized urine.

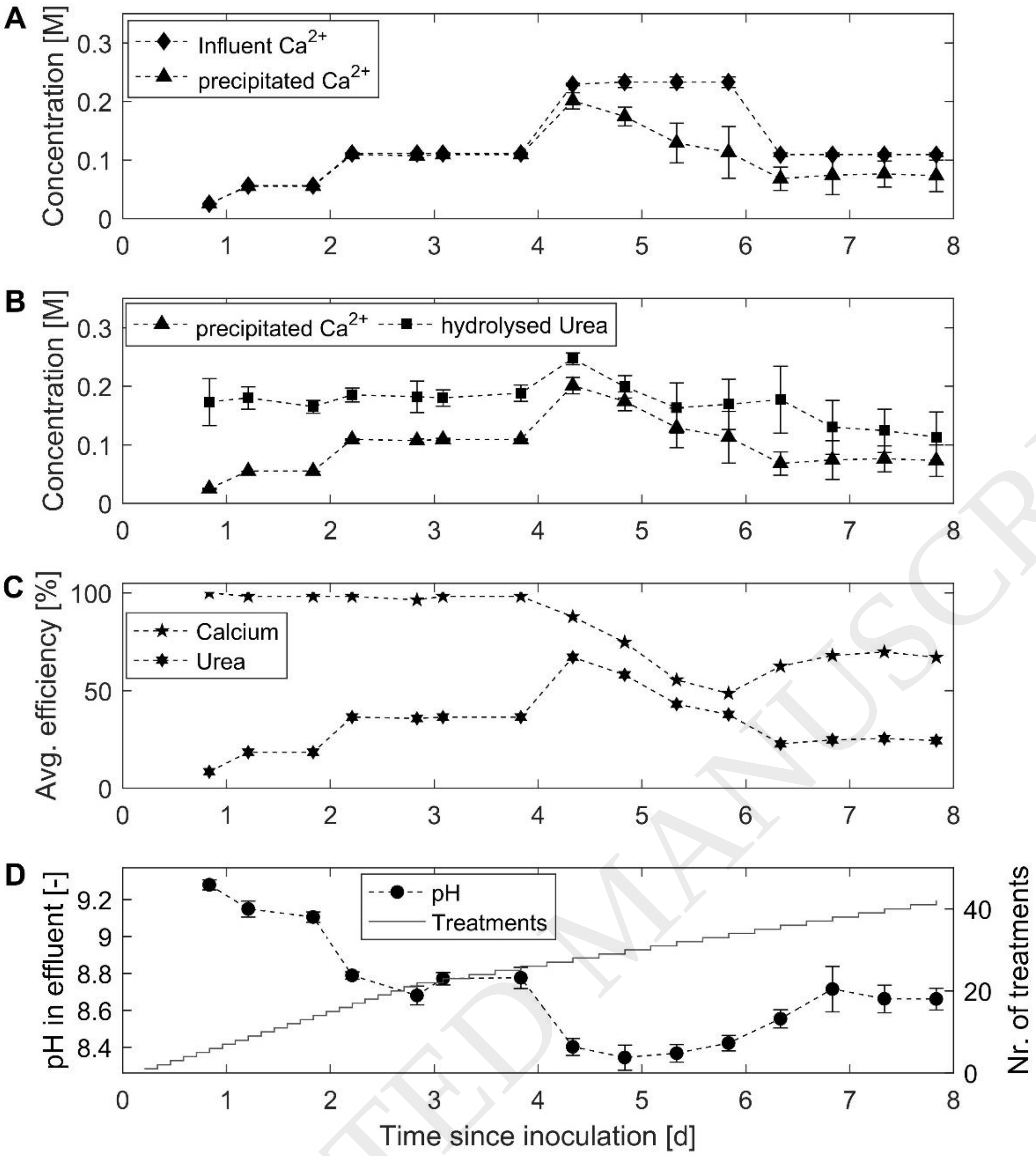

Figure 4: The course of the measured variables in the effluent and influent of the bio-solid production experiment. Samples were taken twice a day in between treatment cycles. A: The amount of calcium that precipitated in the column in comparison to the amount fed to the reactors. B: The relationship between the amount of calcium carbonate precipitated and the amount of urea that was hydrolysed. C: the average calcium and urea usage efficiency. D: 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.

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

	Flask Nr.				
	1	**2**	**3**	**4**	**5**
1 M HCl (mL/100 mL)	0	1.8	2.8	3.6	4.2
pH [-]	12.48	12.24	11.95	11.15	9.31