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Utilization of carbide sludge and urine for sustainable biocement production

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ABSTRACT

Biocement is an emerging construction material via the microbially induced calcite precipitation (MICP) process for soil improvement, which could be produced using waste materials at ambient temperature. This paper investigates the possibility to produce sustainable biocement using industrial waste carbide sludge and urine. Urine was used as the growth medium for the cultivation of urease-producing bacteria (UPB). It was also used to provide urea to activate the MICP procedure together with the soluble calcium ions from carbide sludge and UPB to form calcium carbonate precipitation. The UPB produced in this way was urease active, capable of hydrolyzing urine-contained urea for calcium carbonate precipitation. The sand treated with biocement using the above ingredients achieved an unconfined compression strength of 1.2–1.7 MPa at 7.3–7.7% calcium carbonate content, whereas the permeability was reduced by two orders of magnitude. These results show the potential to use carbide sludge and urine for sustainable production of biocement.

1. Introduction

Biocement is a new cementitious product that can be used as a substitute for cement for soil improvement. It is made through microbial processes such as the microbially induced calcite precipitation (MICP) process and has been demonstrated to be effective in increasing the strength and reducing the permeability of soil [9,12,14,18,30]. During the MICP process, urease producing bacteria (UPB) decompose the urea to form carbonate ions (Eq. 1), where calcium carbonate precipitation occurs in the presence of soluble calcium ions (Eq. 2) [18,31]. When this process is taking place inside soil, the calcium carbonate generated will bond the soil particles or fill the pores between soil particles to improve the strength and reduce the permeability.

$$CO(NH_2)_2 + 2\,H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad (1)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3\,(\downarrow) \quad (2)$$

UPB, urea, and soluble calcium ions are the three main components of biocement. The cost of biocement can be reduced if waste materials can be used for making any of the three components. A cheaper UPB enrichment method was developed by Yang et al. [31] to isolate and enrich UPB from activated sludge under non-sterile conditions. Urease active bacteria could also be isolated from fertile soil [2] and calcareous site [10], while the costly protein-rich yeast extract medium could be replaced by cheaper commercial milk powder or lysed activated sludge [5] to minimize the cost. Although many researchers have studied the possibility to replace urea with urine [13,15] or find alternative calcium sources, such as calcium acetate, calcium nitrate, and calcium sulfate [1, 29], the cost is still a big obstacle of large-scale applications of MICP technique [4,11] Therefore, making MICP technique more sustainable and cheaper to facilitate large-scale application is prevailing.

This paper aims to reduce the cost of biocement production through the use of wastes. These include the use of urine as both the only growth medium for bacteria cultivation and also a replacement of urea, and the use of carbide sludge from an acetylene production process as a soluble calcium source for sustainable production of biocement. As such, both raw materials are in theory easy to obtain and have substantial availability. For example, urine-diverting dry toilets have been established and evaluated at a municipality scale to produce agricultural fertilizers from human urine in eThekwini, South Africa, which was supported by

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Table 1
The main species and concentration obtained from fresh urine.

Chemical concentration (mg/L)				
NH_4^+	P	Urea	Creatinine	Uric Acid
397	813	24,150	1481	375

the Bill and Melinda Gates Foundation [3,26]. Common chemical species and substances found in human urine are listed as follows: Na^+, K^+, Cl^-, P, NH_4^+ (inorganic), Urea (not exceed 0.4 M), Creatinine, Uric acid, protein (organics) [23]. It has been approved that the NH_4^+, P, Urea, and protein [5,31] are the main nutrients of growth medium for UPB cultivation. The method presented in this study provides a sustainable approach to use the MICP methods for soil improvement in a cost-effective manner.

2. Materials and methods

2.1. Carbide sludge

The carbide sludge was collected from a local acetylene production industry in Singapore, which contains 30% of $Ca(OH)_2$ in w/w. A total of oven-dried 100 g of carbide (greater than 30 g of $Ca(OH)_2$, roughly 0.4 moles of solid $Ca(OH)_2$) was dissolved in 1 L of 0.8 M of waste nitric acid from a local chemical plant. The mixture is well-mixed to allow complete reaction, the residue was then filtered using filter paper, leading to the obtained solution containing appropriately 0.4 M of soluble Ca^{2+}. This obtained solution after the acid-base neutralization reaction mainly contains calcium nitrate.

2.2. Pretreatment of urine and preparation of cementation solutions

Fresh urine was collected and pretreated using qualitative filter paper with a pore size of 6 μm (Grade 3 circle, diameter 23 mm), followed by 6 h of Ultraviolet (UV) irradiation for disinfection before safely use as growth culture. The main species and concentration obtained from fresh urine are listed in Table 1. The UV can destroy and deactivate all kinds of pathogens like viruses and bacteria but keep the urine composition unchanged. The activated sludge collected from Sembcorp Utilities, Integrated Wastewater Treatment Plant at Jurong Island in Singapore was used as the seeds for the cultivation of urease producing bacteria. After concentration, it has a total suspended solids of 13.3 ± 0.1 g/L, dissolved organic carbon (DOC) of 33 ± 0.1 mg/L, and pH= 6.5 [24]. During the cultivation, 10 ml of activated sludge was mixed with 90 ml of the pretreated urine and the initial pH of the mixture was adjusted to 9.2 using 1 M of NaOH, followed by stirring at a speed of 200 rpm for 48 h. The enriched bacteria culture was incubated at room temperature (25 ± 1 °C). The pH value and urease activity were constantly monitored every 6 h followed by the method mentioned by Cheng [4]. The time at which urease activity reaches the highest was selected as the harvest time of urease producing bacteria to produce biocement. A comparative study using 5 g/L of glucose addition for another batch of bacteria cultivation was prepared with the identical enrichment process as mentioned above.

To identify the prevalence of enriched bacterial culture, the nearly full-length 16 S rRNA gene was amplified by Polymerase Chain Reaction (PCR) with forward primer 27 F and reverse primer Universal 1492 R [28]. A pair-wise 16 S rRNA gene sequences were finally assembled to produce the full-length sequence, which was compared with all other sequences available in the NCBI Genbank database. The probable identity of the selected bacterial strain was thus confirmed.

The fresh urine was collected and mixed with previously obtained soluble calcium (calcium nitrate) to form a mixture consisting of approximately 0.2 M of equimolar urea and soluble calcium ions, termed as cementation solution.

2.3. Biocementation test of sand columns

Twelve transparent acrylic columns (50 mm inner diameter and 100 mm in length) were filled with 350 g dry silica Ottawa sand with D_{50} = 0.4 mm (conforms to ASTM C788) for biocementation. The flowchart of the current study for sustainable biocement production is presented in Fig. 1. Columns were divided into three groups and treated for 20, 30, and 40 times. Before treatment, the enriched bacteria culture harvested at the stationary phase (approximately 30 h) was centrifuged at 5000 rpm for 10 min to separate bacterial biomass. After centrifugation, the supernatant was removed and bottom bacterial biomass was re-suspended in 0.9% saline solution to prevent osmotic shock of bacterial cells before treatment [12,27]. For each treatment, 100 ml (1.1 times of void volume) of the enriched bacteria culture (urease activity =

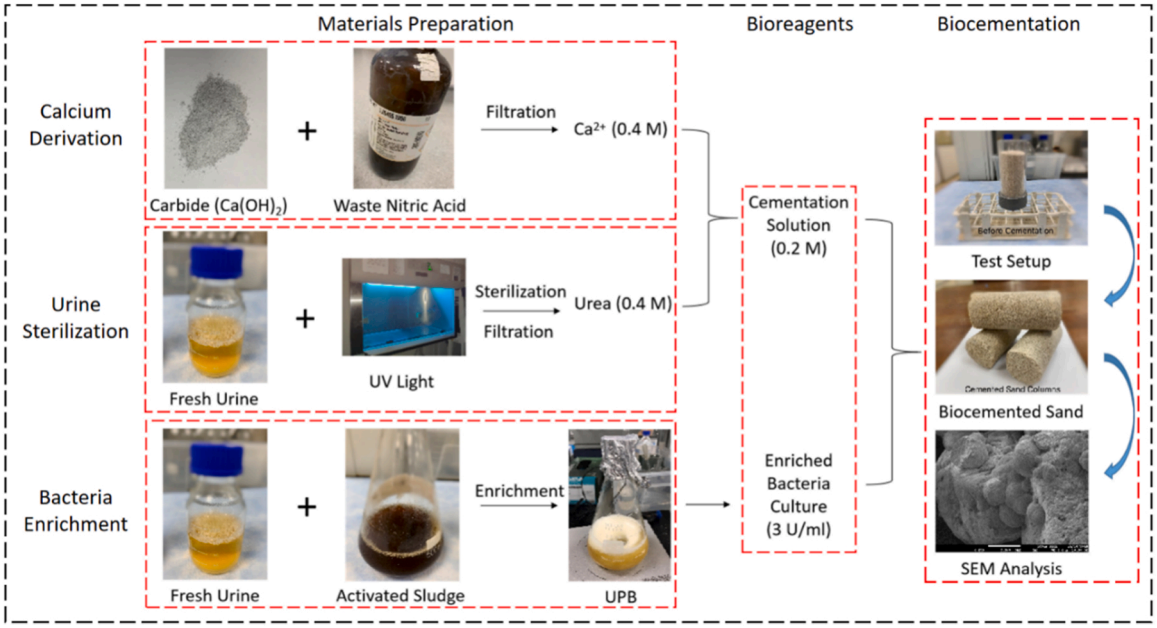


Fig. 1. Flowchart of the sustainable biocement production under this study.

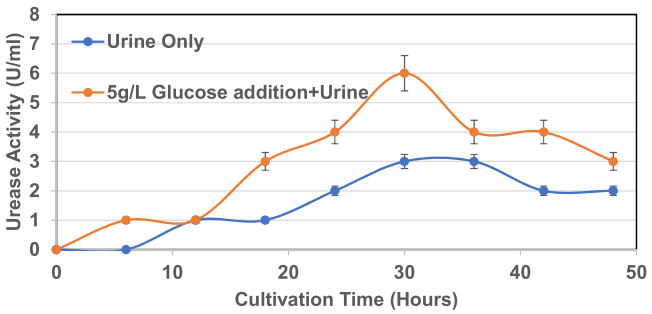


Fig. 2. Effect of different urine-based growth medium on the urease activity development at different cultivation time.

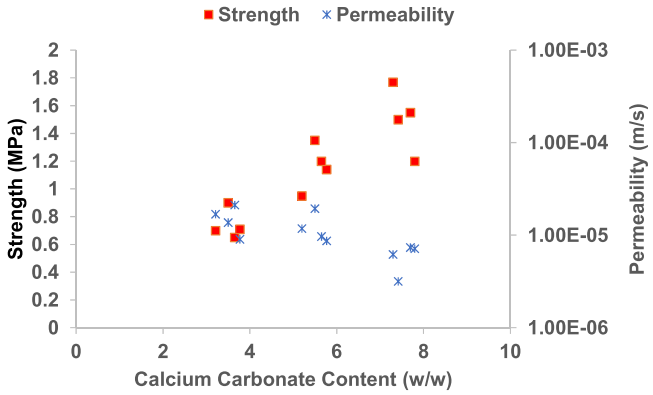


Fig. 3. Correlation of strength and permeability at various calcium carbonate content.

3 U/ml, pH = 8.2 ± 0.1) was flushed into the sand column, followed by flushing another 100 ml of 0.2 M of equimolar cementation solution with a half an hour time interval, allowing sufficient time for the bacteria attained on the sand surface to facilitate the subsequent precipitation process. Sand columns were kept at room temperature (25 ± 1 °C) for 12 h to allow complete reaction of each treatment prior to the next treatment. After treatment, permeability tests were conducted on the treated sand columns using a falling-head method in accordance with ASTM D5084, followed by unconfined compression test. The calcium carbonate content was determined by the acid-washing and rinsing method [7] to reveal the cementation level of biocemented sand. Fig. 1.

2.4. Microstructural analysis of biocemented sand and carbide sludge

The X-ray diffraction (XRD) analysis of carbide sludge and biocemented samples was conducted to confirm the chemical composition of carbide and precipitates. XRD analysis was conducted using Bruker Advance D8 equipment and reordered on a Philips PW 1800 spectrometer using Cu Kα radiation (40 kV, 40 mA) with a scanning rate of 2° 2θ/step from 5 to 80° 2θ. Scanning Electron Microscopy (SEM, Zeiss EV050, UK) observations and analysis were also carried out to image the morphologies of the precipitated calcium carbonate, which is important to study the mechanical properties of biocemented sand in detail. Microstructural specimens were obtained from crushed biocemented samples. All specimens were rinsed with tap water and oven-dried at 105 °C for 24 h prior to microstructural tests.

3. Results and discussion

3.1. Effect of urine and glucose addition on urease activity

Urine was used as the only growth medium for the cultivation of urease producing bacteria. During cultivation, the proliferation curve of urease producing bacteria is shown in Fig. 2. Evidently, the urease activity initially increased and then decreased along with cultivation time. The urease activity reached its peaks around 3 U/ml after 30 h of cultivation with urine only, whereas the urease activity increased to around 6 U/ml in 30 h with 5 g/L of glucose addition. It was noticed that the glucose addition in the urine-based growth medium could improve the urease activity [25]. This is because the amount of nutrients is limited in the urine solution, glucose addition can provide more nutrients for bacteria growth. Although glucose addition will increase the cost of cultivation, the proposed use of glucose is still cheaper than the other nutrients such as yeast extract or soya peptone, that needs to be used for the cultivation of bacteria. For example, the yeast extract was $18 per kg, while the current market price of glucose was only $1. Additionally, the industrial grade of urea and calcium chloride were both $0.2 per kg [22]. Thus, glucose addition is also considered as a cost-effective method to provide nutrients and improve urease activity, while urine-carbide system saves the cost of urea and calcium source, highlighting the sustainability of the proposed method. As such, it is believed that the urine solution can be a possible substitute for growth medium and the urease producing bacteria can be enriched in the urine-based growth medium from activated sludge. Although the obtained urease activity is relatively low, it is sufficient for the conversion of 0.2 M of cementation solution. For instance, [6] have demonstrated that the urease activity of 1.25 U/ml is capable to convert 60% of 1 M of cementation solution into calcium carbonate precipitates.

The enriched bacterial culture was identified via 16 S rDNA sequence analysis that belonged to 3 typical bacteria genera, including *Sporosarcina, Atopostipes, and Pseudomonas*. *Sporosarcina* was the most prevalent genus in the enriched bacterial culture with a frequency of 68.75%, followed by *Atopostipes* and *Pseudomonas* with a frequency of 18.75% and 12.5%, respectively.

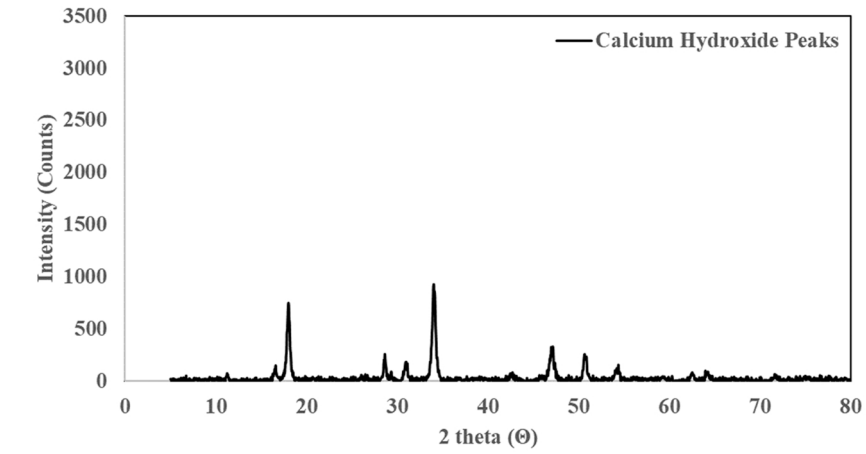
The preliminary results of 16 S rRNA gene sequence identity search against known species in NCBI Genebank database are as follows:

1. *Sporosarcina pasteurii* strain NCCB 48,021: Identity = 98%
2. *Atopostipes suicloacalis* strain PPC79: Identity = 96%
3. *Pseudomonas caeni* strain HY-14: Identity = 95%

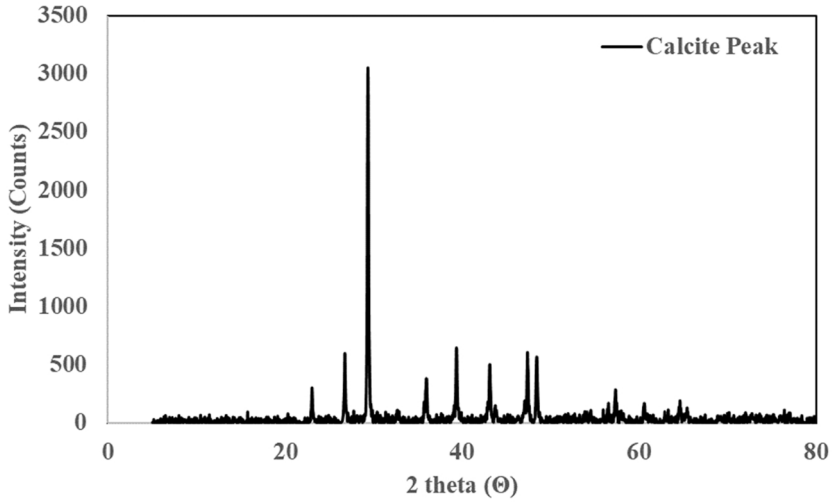
Other than the commonly used urease producing bacteria of *Sporosarcina pasteurii* [16], both *Atopostipes* and *Pseudomonas* were confirmed not pathogens or hazardous bacteria species. Therefore, the enriched bacteria culture is safe to be used for biocementation.

3.2. Shear strength and permeability

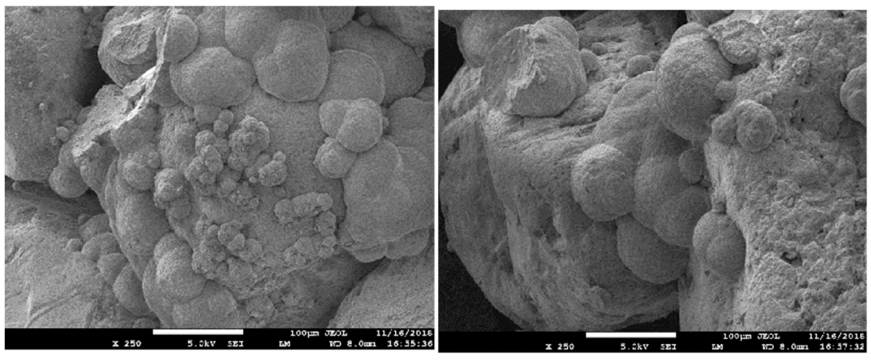
The effectiveness of the biocement produced using the proposed method in the treatment of sand was evaluated by measuring the strength improvement and permeability reduction for the samples with the different number of treatments. As shown in Fig. 3, the unconfined compressive strength increased while the permeability decreased with the increase in calcium carbonate content. The unconfined compressive strength of sand increased from zero to a value ranging from 0.6 to 1.7 MPa and the permeability has reduced from 4×10^{-4} m/s to 4.1×10^{-6} m/s after 20, 30, and 40 times of treatment. This was attributed to the increased amount of calcium carbonate precipitation at various cementation level, strengthening the sand particles and gradually filling out more pores among sand particles. However, it is interesting to find that the achieved strength was slightly higher than the previous results obtained by Lambert and Randall [15], who indicated a 0.2–0.7 MPa after 48 times treatments. This is also higher than a bio-column made from synthetic urine but similar to the compressive strengths obtained in other MICP studies by [21] (0.35 – 1.3 MPa) and [8] (0.88 – 1.1 MPa). The variation in compressive strength could be probably due to the different treatment methods (e.g. recirculation or two-phase injection method), the particle size of sand, and the type or the ability of bacteria in each test.



(a)



(b)



(c) (d)

Fig. 4. Microstructural results: (a) XRD pattern of carbide sludge; (b) XRD pattern of the precipitates from the urine-carbide based MICP process; and (c and d) SEM images of the biocemented sand with 7.7% of calcium carbonate content (w/w) after 40 times of treatments.

3.3. Microscopic observation

Fig. 4a shows the XRD pattern of the carbide sludge, which confirms that the carbide sludge mainly consists of calcium hydroxide. Thus soluble calcium could be derived by treating carbide sludge with nitric acid as mentioned in Section 2.1. The XRD pattern of the material precipitated from the MICP process using urine-carbide is shown in Fig. 4b. It matched perfectly with the calcite peaks and thus confirming the precipitated material was indeed calcium carbonate. Fig. 4c and d depict the SEM images of sand particles cemented with calcium carbonate crystals induced by the urine-carbide based biocement. The SEM shows numerous spherical crystals with various sizes ranging from 5 to 100 μm, forming large clusters of calcium carbonate crystals via agglomeration which are typically formed during the MICP process. It should be noted that the quite unique morphology and characteristics of precipitates induced by the current system were different from the most

previously published results of crystals shape and size [14]. For example, the maximum size of single calcium carbonate crystals was up to 100 μm, which was bigger than the normal one with a size of 20–30 μm [17]. This might be due to the disintegration and recrystallization process associated with the repeated treatment process, leading to the growth of crystal size. Similar observations were made by Ogino et al., [19] and Phua & Røyne, [20].

3.4. Practical applications

The proposed process involves several stages and the use of various chemical reagents for the involved wastes to be made harmless prior to use, which could reduce their possible economic advantages, however, this study was a proof-of-concept research to confirm the possibility of using urine-carbide system for sustainable biocement production. Further research will be conducted to simplify this system to enhance the economic advantages of this process, such as by integrating with the current urine collecting system.

It is also worthwhile mentioning that as there is a high risk that the ingredients found in drugs may release in our urine with concentrations varying depending on individuals and dosages [3], which could possibly have effects on bacterial activity or the action of urease on urea. Thus, it is necessary to ensure a treatment step is added as part of the resource recovery process configuration prior to engineering application, such as advanced oxidation or flocculation.

4. Conclusion

This note discussed a method to produce sustainable biocement from wastes. By utilizing urine as the growth medium, urease producing bacteria can be enriched from activated sludge and used for subsequent biocement production. Urine was also used to replace urea for the MICP process. Calcium ions extracted from carbide sludge were also used. The effectiveness of the biocement produced using the proposed method for soil improvement was evaluated using strength and permeability tests. The UC strength of the treated sand increased to 1.7 MPa and the permeability reduced to 4.1×10^{-6} m/s at a calcium carbonate content of 7.7%. Thus, biocement produced using the proposed method could potentially become a sustainable and cost-effective method for soil improvement.

CRediT authorship contribution statement

Yang Yang: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Jian Chu:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Funding acquisition. **Hanlong Liu:** Methodology, Investigation, Validation. **Liang Cheng:** Conceptualization, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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