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Biocementation of sand by *Sporosarcina pasteurii* strain and technical-grade cementation reagents through surface percolation treatment method

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Highlights

- Low-cost growth medium was used to cultivate *Sporosarcina pasteurii* prepared in deionized water and tap water.
- Soil biocementation treatment was performed through surface percolation method.
- Analytical-grade cementation reagents can be replaced with technical-grade cementation reagents.
- Utilizing technical-grade reagents for sand biocementation resulted in 47 to 51-fold cost reduction.

ABSTRACT

The use of microbially induced carbonate precipitation (MICP) to produce biocementitious material for soil stabilization has emerged in recent decades as a sustainable alternative approach to conventional methods. However, the use of standard analytical-grade reagents for various MICP studies makes this technology very expensive and unsuitable for field-scale consideration. In this present study, the feasibility of using commercially available and inexpensive technical-grade reagents for the cultivation of ureolytic bacteria and enhancement of soil stabilization was investigated. Low-cost growth media prepared in deionized water and tap water were used to cultivate *Sporosarcina pasteurii* as a replacement to standard laboratory-grade medium. Biocement treatment was carried out on sand columns using different concentrations (0.25-1.0 M) of technical-grade and analytical-grade cementation solutions via surface percolation method. After 92 h of treatment, the columns were cured for 3 weeks at room temperature ($26 \pm 2^\circ\text{C}$) before analysing their respective surface strengths, CaCO_3 content, pH of effluents and sand microscopic structures. The results indicated that the growth of bacteria in low-cost cultivation medium was similar to that observed in the standard cultivation medium. Surface strengths and CaCO_3 contents of the consolidated samples were in the ranges of 11448.00 ± 69.00 - 4826.00 ± 00 kPa and 5.56 ± 1.15 - $33.24 \pm 0.59\%$, respectively. Overall, the obtained results of the current study encourage future MICP studies to utilize commercially available technical-grade reagents for economical MICP field-scale trials.

Keywords: *Sporosarcina pasteurii*; biocementation; crystal morphology; surface strength; calcium carbonate; urease enzyme; nutrient; low-cost reagents

1.0. Introduction

Cement is one of the most important binding agents used globally for construction and infrastructure purposes. Its production from calcining limestone generates about 0.814 tons of carbon dioxide (CO_2) per one ton of limestone and singularly accounts for 6-8% of global anthropogenic CO_2 emissions [1,2]. Greater awareness on the environmental and human impacts of conventional cement production has inspired the need to investigate alternatives binders. Biocement (insoluble crystal) has recently emerged as a new binding material which is produced through microbially induced carbonate precipitation (MICP) for soil stabilization [3]. It has the ability to bind porous materials together and enhance their mechanical properties (i.e. strength and impermeability).

Soil improvement by biocement treatment methods often involves the addition or injection of ureolytic bacteria such as *Sporosarcina pasteurii* and cementation reagents (i.e. urea, calcium chloride and growth nutrients) into the soil [4]. Many microorganisms can precipitate calcium carbonate (CaCO_3) but are not suitable for MICP due to biosafety concerns related to

their pathogenic capacity. Ureolytic bacteria such as *Staphylococcus aureus*, *Helicobacter pylori* and *Pseudomonas* sp. are known to cause gastritis, ulcers, urinary tract infections, respiratory tract infections and nosocomial infections [5]. However, *S. pasteurii* strains are often used in MICP studies because they are unlikely to cause human diseases. This bacterium is also often selected for biocement investigations due to its highly active urease production and crystallization of CaCO_3 [6].

Biocement materials are able to bridge soil particles together when the biocement materials are properly precipitated in the soil matrix, eventually enhancing the mechanical properties [7,8]. Unlike most established soil stabilization methods, biocementation can occur at ambient conditions with minimal emission of CO_2 [9]. Conventional soil stabilization practices which use mechanical (i.e. soil compaction) or chemical (i.e. liquid silica) methods require considerable labour, involve high viscosity materials with low soil penetrability and are hazardous to the environment and humans [10,11]. On the other hand, biocementation is durable, less hazardous and suitable for deep penetration treatment with fine pores [5]. Over the past two decades, biocementation via urea hydrolysis has been widely studied for numerous MICP applications which includes concrete and crack repair, soil consolidation and stabilization, and erosion control [12–17].

Despite numerous laboratory-scale reports on soil biocalcification, the practical implementation of such methods is currently limited [6]. It is crucial that the next phase of soil biocalcification development should focus on the efficacy of scaling to field-scale treatments [8]. Notwithstanding the promising results of the MICP process from the literature, it is still considered expensive for practical or commercial purposes. The feasibility of biocementation does not only rely on technical aspects for successful implementation, but it is also accompanied by economic challenges [7]. For example, the nutrient media (i.e. yeast extract) and synthetic chemicals (urea and calcium chloride) used for bacterial cultivation and treatment process are usually of high-grade. The use of these high-purity reagents is essential for fundamental laboratory-scale investigations, but their high cost makes their utilization extremely costly for industrial applications [18]. In fact, the use of conventional nutrient media for bacterial cultivation can contribute up to 60% of the total operating costs [19]. Additionally, the amount for these cementation reagents can reach up to US\$9/m³, while the total cost for soil treatment ranges between US\$25/m³ and US\$500/m³ [16,20,21].

To date, there are limited studies in the literature that have explored the use of alternative cementation reagents for MICP. Seawater, eggshells, limestone from aggregate quarries, calcareous sand, feed and cask powders have been investigated for their suitability as calcium sources [1,22–25]. Cuzman et al. [26] and Fahmi et al. [27] recently reported the use of fertilizer urea as a replacement for synthetic analytical-grade urea, while Kuar et al. [28] and Chen et al. [29] showed that CO_2 and pig urine could serve as suitable substitutes for synthetic analytical-grade urea. Waste materials such as corn steep liquor have also been studied as a possible replacement for the standard growth medium used to cultivate ureolytic bacteria [12,27,30]. Unfortunately, most contemporary biocalcification studies still make use of costly analytical-grade chemicals and microbiological media. It is crucial that future investigations explore other cementation reagents which are either of low or no cost and can be easily accessible [31]. The aim of this current study was to evaluate the feasibility of using commercially available and inexpensive technical-grade reagents as replacements to the costly analytical-grade reagents for bacterial cultivation and soil biocalcification. Through surface percolation treatment, different concentrations (0.25-1.0 M) of cementation reagents prepared in tap water and deionized water were used to calcify soil samples. The performance of technical-grade reagents was compared with that of analytical-grade reagents.

2.0. Materials and methods

2.1. Ureolytic bacteria and cultivation medium

The ureolytic bacterial strain *S. pasteurii* NB28(SUTS) (GenBank accession no. KX212192.1) previously isolated by Omoregie et al. [32] from a local limestone cave sample was used throughout this current study due to its high urease activity, biocalcifying ability and non-pathogenicity. The bacterium was revived on Petri plates containing tryptone soya agar (40 g.L⁻¹, HiMedia, Laboratories Pvt. Ltd.) and urea (4% w/v, Merck, Shd. Bhd.). The bacteria were then incubated (MMM incucell 55) at 30°C for 48 h under aerobic conditions and sub-cultured until a pure culture was obtained. The ureolytic bacterial cultures were stored at 4°C and retrieved for use when needed [33]. All bacterial cultures used for the subsequent experiments were stored not more than a month [26]. Two different cultivation medium designated Growth Medium-1 (GM-1) and Growth Medium-2 (GM-2) were used throughout this current study to grow the ureolytic bacteria. GM-1 contained commercially available, inexpensive ingredients and was selected as an alternative nutrient source to cultivate *S. pasteurii*, while GM-2 contained standard and costlier ingredients typically used for laboratory experimentations to cultivate a wide range of microorganisms. The compositions of both growth media and their respective cost analysis are presented in **Table 1** and **Table 2**, respectively. Both media were prepared in Schott bottles (1 L) containing deionized water and tap water. Physicochemical properties of the tap water (**Table 3**) were analysed by EnviC Laboratory Sdn. Bhd Malaysia using the American Public Health Association standard method for examination of water and waste water samples. Sterile urea (by 0.45 µm filter sterilization) was added aseptically to the Schott bottles after autoclaving to prevent chemical decomposition under autoclave condition [32]. The initial pH was adjusted to 7.00 using 0.1 M sodium hydroxide or 0.1 M hydrochloride acid after sterilization [34]. 12.5 mL of overnight cultures were inoculated into separate 250 mL culture flasks containing 112.5 mL growth medium and incubated at 32°C for 72 h with shaking (130 rpm).

Table 1: The two nutrient media used to cultivate *S. pasteurii* NB28(SUTS)

Culture medium	Ingredients (concentration, supplier)				Initial pH
^a GM-1	Yeast extract (15 g.L ⁻¹ , Angel Yeast Co. Ltd.)	Urea (40 g.L ⁻¹ , Petronas Chemical Company Bhd.)	Ammonium sulphate (10 g.L ⁻¹ , Petronas Chemical Company Bhd.)	Sodium acetate (4.1 g.L ⁻¹ , Petronas Chemical Company Bhd.)	^c 6.53 and ^d 7.02
^b GM-2	Yeast extract (15 g.L ⁻¹ , Oxoid™)	Urea (40 g.L ⁻¹ , Merck, Shd. Bhd.)	Ammonium sulphate (10 g.L ⁻¹ , Merck, Shd. Bhd.)	Sodium acetate (4.1 g.L ⁻¹ , Merck, Shd. Bhd.)	^c 5.93 and ^d 6.36

^aTechnical-grade reagents are not entirely of high purity but are commercially sold for industrial purposes.

^bAnalytical-grade reagents are of high purity and suitable for numerous laboratory applications.

^cCultivation media prepared in deionized water.

^dCultivation media prepared in tap water.

Table 2: Cost analysis of the ingredients used in this study for bacterial cultivation

Medium component	*Cost (US\$/1kg) for GM-1	*Cost (US\$/1kg) for GM-2
Yeast extract	18.67	180.00
Urea	1.03	111.25
Ammonium sulphate	3.80	176.90
Sodium acetate	4.15	19.51
Total	27.65	487.66

*The reagents were procured in Malaysia (the exchange rate is MYR 4.11 = USD\$ 1.0)

Table 3: Physiochemical properties of tap water used in this study

Parameter	Unit	Result
Dissolved oxygen	mg.L ⁻¹	6.42
Biochemical oxygen demand	mg.L ⁻¹	1.90
Chemical oxygen demand	mg O ₂ .L ⁻¹	38.7
Total suspended solids	mg.L ⁻¹	1.00
Total dissolved solids	mg.L ⁻¹	51.20
Salinity	%	0.20
pH	-	7.42
Chloride	mg.L ⁻¹	5.10
Fluoride	mg.L ⁻¹	0.01
Sodium	mg.L ⁻¹	3.46
Magnesium	mg.L ⁻¹	1.05
Iron	mg.L ⁻¹	0.01
Aluminium	mg.L ⁻¹	3.83
Nitrate	mg.L ⁻¹	1.26
Ammonium nitrogen	mg.L ⁻¹	0.01

2.2. Optical density and pH

The performance of *S. pasteurii* NB28(SUTS) in both GM-1 and GM-2 was monitored (OD and pH profiles) during cultivation. The changes in cell density (biomass concentration) were monitored by absorbance (optical density) using a spectrophotometer (Thermo Scientific™ GENESYS™ 20) at a wavelength of 600 nm (OD₆₀₀). Three millilitres of the culture was sampled and placed into clean 10 mm cuvettes before reading the values [35]. The spectrophotometer was calibrated using un-inoculated growth media (GM-1 and GM-2) as blanks before the optical density of bacterial cultures grown in these two respective medium was measured. A pH meter (SevenEasy™–Mettler Toledo) was used to measure the pH of the bacterial cultures. Prior to measuring the pH values, the pH electrode was calibrated with pH 4.01, 7.00 and 10.30 buffer solutions (Sigma-Aldrich) [35]. The optical density and pH values were both monitored regularly (6 h interval) until the end of the incubation period (48 h).

2.3. Urease activity

Urease activity (mM urea hydrolysed.min⁻¹) of the bacterial cultures was measured at the end of the incubation period using conductivity measurement method [13]. Urease hydrolyses the urea substrate in the solution to produce ammonium and carbonate ions and increases the conductivity under standard conditions. Whiffin [13] determined that urease activity could be measured by assessing the electrical conductivity changes per minute of the bacterial-urea

solution. For the conductivity measurement, 10 mL of bacterial culture was mixed with 90 mL of urea solution (1.5 M) and the relative conductivity change was measured for 5 min at $25 \pm 2^\circ\text{C}$. The gradient, also known as the conductivity variation rate ($\text{mS}\cdot\text{cm}^{-1}\cdot\text{min}^{-1}$), was obtained from the plotted graph slope, multiplied by the dilution factor (10) and converted to urease activity. The urease activity was calculated by taking into account the dilution to provide the undiluted value [36]. Specific urease activity ($\text{mM urea hydrolysed}\cdot\text{min}^{-1}\cdot\text{OD}^{-1}$) was determined by dividing the obtained urease activity by the biomass value (optical density) [13]. In the measured range of activities, one unit of urease activity is determined as the amount of enzyme which degrades 1 mM urea $\cdot\text{min}^{-1}$ at $25 \pm 2^\circ\text{C}$ [37].

2.4. Biomineralization test

For the biomineralization test (**Fig. 1**), two cementation solutions were prepared each containing technical-grade and analytical-grade cementation reagents, respectively. The major difference between technical-grade and analytical-grade cementation reagents is purity. Hence, biomineralization test was essential to evaluate how the purity level affects the biomineralization process when ureolytic bacteria are inoculated into these two cementation solutions. The physicochemical properties of the commercially available technical-grade cementation reagents are shown in the **supplementary materials, Table OA1**. Technical-grade cementation solution contained 15 $\text{g}\cdot\text{L}^{-1}$ of food-grade yeast extract (Angel Yeast Co. Ltd.), 1 M of urea (Petronas Chemical Company Bhd.) and 1 M of calcium chloride (Lianyungang Longyi Industry Co. Ltd). The analytical-grade cementation solution contained laboratory-grade yeast extract (15 $\text{g}\cdot\text{L}^{-1}$, Oxoid TM), urea (1 M, Merck, Shd. Bhd.), calcium chloride (1 M, Merck, Shd. Bhd.). Both solutions were prepared by first autoclaving the yeast extract placed in Schott bottles containing 1 L tap water. After cooling, urea and calcium chloride were added and carefully shaken until the reagents were totally dissolved. The cementation solutions were further sterilized using ultraviolet radiation in a biological safety cabinet (Escoglobal) before transferring them into sterile beakers (**Fig. 1**). 5 mL of overnight *S. pasteurii* cultures in GM-1 and GM-2 were inoculated into beakers containing 95 mL of the cementation solutions and incubated aerobically for 72 h at 32°C with no shaking. At the end of the incubation period, the precipitates were collected in a centrifuge tube (50 mL) as described by Omoregie et al. [35], oven-dried at 60°C for 4 h and kept at room temperature ($26 \pm 2^\circ\text{C}$) for further analysis.

Fig. 1: The two cementation solutions containing technical-grade (A) and analytical-grade (B) reagents before being inoculated with *S. pasteurii*.

2.5. XRD analysis

X-ray diffraction (XRD) is a rapid analytical technique regularly used to determine the mineralogical composition and crystalline phase of the CaCO_3 precipitates after the biomineralization test. XRD analysis was performed by Quasi-S Sdn. Bhd. Malaysia. Powdered samples were arranged on a carbon tape and covered with gold via sputter-coating prior to microscopic analysis. A Panalytical system (X'Pert³ Powder) with Cu $\text{K}\alpha$ tube anode having 40 KV and 35 mA running parameters was used to scan the fine-powdered samples. The angle of diffraction (2θ) of the X-ray was set from 5° to 80° , with a step size of 0.05° and a scanning speed of 5° per minute. The analyzed components were identified by comparing them with standards established by the International Centre for Diffraction Data.

2.6. Biocement treatment

Sandy soil was used for biocement experiments presented herein. Particle sizes of the sand ranged from fine sand (0.075 mm) to fine gravel (4.75 mm) and are classified as poorly graded in accordance with Unified Soil Classification System (USCS). Key properties of the poorly graded soil were 0.22 mm, 0.27 mm, 0.35 mm, 1.60 and 0.95 for D_{10} , D_{30} , D_{60} , C_u and C_c , respectively. The dry density and pH value of the sand were 1.67 g/cm^3 and 7.10, respectively. Polyvinyl chloride (PVC) tubing with an internal length of 11.8 cm in length and diameter of 5.8 cm served as a column in this experiment. The PVC tubes were carefully vertically cut into halves and tightly held together with plastic seal strips. The columns were then placed on polypropylene sheets and the spot where the columns were to be placed was perforated with holes to allow discharge of effluent during treatment. The holes were covered with Whatman[®] cellulose filter papers to act as filters before the columns were then placed upon the filter papers. 350 g of dry sterile sand was placed into each column before being subjected to biocement treatment (Fig. 2). To enable the collection of effluents during treatment, a plastic container was placed below the polypropylene sheets in a fume hood (Lab Craft Basix).

The following acronyms AG (DW), AG (TW), TG (DW) and TG (TW) represented analytical-grade deionized water, analytical-grade tap water, technical-grade deionized water and technical-grade tap water, respectively. The sand columns were treated using four different concentrations of cementation solutions of 0.25 M, 0.50 M, 0.75 M and 1.0 M. Equal molar of urea and calcium chloride concentrations were prepared in deionized water and tap water. For comparison purposes, a total of four treatment groups (i.e. different concentrations) were formed and each group was sub-grouped into two: a) AG (DW) AG (TW); and b) TG (DW) and TG (TW). For each cementation solution used, the urea and calcium chloride were added into Schott bottles containing sterilized 5 g.L^{-1} of yeast extract. The cementation solutions were further subjected to UV-sterilization before carrying out the sand treatment. The ureolytic bacterial cultures used for the biocement treatment were cultivated in GM-1 and GM-2 prepared in deionized water and tap water. The cultures were added to sand samples which were to be treated with corresponding reagents. For example, a sand column containing *S. pasteurii* cultivated in GM-1 (tap water) will likewise be treated with technical-grade cementation solution prepared in tap water. To achieve cementation, surface percolation was used to introduce cultures and cementation solutions. All the solutions were applied from the top of the columns and allowed to slowly percolate by gravity and capillary forces [37].

The sand columns were treated with 195 mL of bacterial cultures and cementation solutions in triplicate. Columns were first percolated with bacterial cultures and allowed to settle for 6 h before the cementation solutions were added. The treatment was repeated for three more days and reaction time was 24 h. For each day of biocement treatment upon the soil samples, the bacterial cultures were added prior to each application of cementation solution. The treatment

was performed at room temperature ($26 \pm 2^\circ\text{C}$). After the treatments, 30 mL of deionized water and tap water were respectively used to flush the sand columns to wash out any soluble salts which might have formed as by-products [3]. The biocemented sand samples were carefully dismantled from their respective columns and allowed to cure for 3 weeks at room temperature. After completion of the curing period, each sample was subjected to a surface strength test, CaCO_3 content test and SEM analysis. For pH analysis of effluents from samples during biocement treatment, 20 mL of the effluents from the columns were extracted and retained for further analysis. The effluents were placed in sterile plastic centrifuge tubes (50 mL capacity) and their respective pH were examined for detection of urease activity after treatment. The initial pH of all cementation solutions was measured before the soil biocement treatment (supplementary materials, Table OA2) for comparison purpose.

Fig. 2: Setup of sand columns prior to biocement treatment.

2.7. EDXRF analysis

Energy dispersive X-ray fluorescence (ED-XRF) spectrometry is a non-destructive analytical technique used to analyse and quantify the relative concentration of samples by measuring the wavelength and intensity generated through fluorescent X-rays. The analysis was performed by Quasi-S Sdn. Bhd. Malaysia using an X-ray fluorescence spectrometer (Thermo Electron /Model ARL Quant'X). ED-XRF analysis was only performed on technical-grade cementation reagents (yeast extract, urea and calcium chloride) to verify the elements and concentration of each reagent since they are not of highest purity grades. ED-XRF spectrometry was used to analyse the samples because it is cost-effective, fast and provides accurate results. X-ray fluorescence analysis was performed using the following parameters; filter (cellulose), voltage (7 kV), Maximum Energy (40 keV), emission current (1 mA), power (1 mA) and lifetime (50 sec). The collected dried samples were placed into the sample holder section of the spectrophotometer. Bulk analysis of the sample was performed under vacuum and the X-ray fluorescence data were obtained.

2.8. Surface strength and CaCO_3 content

A simple, lightweight hand-held pocket penetrometer (ELE International, 38-2695) was used to measure the strength of the treated sand samples. The pocket penetrometer is commonly used in geotechnical engineering to rapidly estimate the surface strength of cohesive soils after biocement treatment [37,38]. The penetration piston (6.4 mm) was manually pushed into the

treated soil until the penetration resistance value from the calibrated spring was obtained on the scale values engraved onto the penetrometer barrel. The penetration piston was pushed from the top section of all treated sand samples. The penetrometer used in this study had a scale which ranged from 0-4826 kPa [32]. After a strength test of each biocemented samples, CaCO₃ content was measured using the gravimetric acid washing technique [39]. Portions (100 g) of the treated samples were oven-dried at 60°C for 24 h before subjecting to acid wash. The samples were weighed before and after being digested in 1 M hydrochloride acid under continuous stirring [3]. The dissolved CaCO₃-acid washed solutions were rinsed several times, filtered using Whatman® cellulose filter paper, allowing the dissolved CaCO₃ to be rinsed off while retaining the sand grains. The difference in the two mass of the sand before and after acid wash was determined [39]. The CaCO₃ content (%) was expressed as the ratio of CaCO₃ weighed prior and after acid wash.

2.9. SEM-EDX analysis

The surface topography, morphology and mineralogical compositions of samples subjected to biocement treatment with 1 M cementation solution were investigated at the Biotechnology Research Institute, Universiti Malaysia, Sabah using scanning electron microscope (SEM). SEM micrographs were obtained using Hitachi S-3400N attached with energy dispersive X-ray (EDX) unit, with accelerating voltage of 15 kV, magnification 5x up to 300,000x and resolution of 3.0-4.0 nm. The crushed samples were properly arranged on carbon tape, sputter-coated with a thin layer of gold and placed onto the stub before being analysed. EDX spectroscopy was used examine the elemental compositions of the technical-grade and analytical grade reagents (yeast, urea and calcium chloride) used in this study.

2.10. Statistical analysis

All the experiments in this work were carried out in triplicate. The experimental results were analysed using the statistical analysis software GraphPad Prism® (version 8) to determine the significance of differences between the means of groups obtained in this study. One-way analysis of variance (ANOVA) and Tukey-Kramer post hoc test were performed using Minitab® (version 18). The significance level was set at P<0.05.

3.0. Results

3.1. Growth performance of *S. pasteurii*

The growth profiles of *S. pasteurii* after being incubated aerobically for 72 h at 32°C under shaking conditions (130 rpm) in different cultivation media (supplementary materials, Fig. OA1) which were prepared using deionized water and tap water are presented in Fig. 3A. Cultivation of the bacteria from single colonies showed it could grow well in GM-1 similarly to GM-2. Following a lag phase of 6 h, the bacteria displayed logarithmic phase growth in all media until approximately 30 h of incubation period. The cultures remained at stationary phase until the end of the cultivation period (72 h). At the end of the experiment, the maximum OD for the ureolytic bacteria all the growth medium was 1.2 ± 0.01 . The kinetics parameters which includes the growth rate and doubling time for the cultivation medium used to study the performance of *S. pasteurii* can be seen in Table 4.

Since alkalinity is one of the essential factors of MICP, pH profiles of ureolytic bacteria in the cultivation medium were also determined. In Fig. 3B, it was observed that the pH profiles were similar to growth profiles of the ureolytic bacteria. As bacterial culture densities increased during incubation, the pH values increased simultaneously. Immediately after inoculation of

the bacterial starter cultures, the pH values increased from 7.0 (initial pH medium) to 7.5 ± 0.01 - 7.9 ± 0.02 , serving as an indication of urea hydrolysis. At the end of the incubation period, the maximum pH of all culture medium were between 9.2 ± 0.01 - 9.3 ± 0.01 . Following the procedures described by Whiffin [13], the urease activities of *S. pasteurii* cultures were determined by measuring their electrical conductivities at the end of the incubation period. The maximum urease activity and specific urease activity were 21.1 ± 0.06 mM urea hydrolysed.min⁻¹ and 18.6 ± 0.10 mM urea hydrolysed.min⁻¹.OD⁻¹ from GM-2 (tap water), while the lowest urease activity (17.4 ± 0.19 mM urea hydrolysed.min⁻¹) and specific urease activity (14.9 ± 0.19 mM urea hydrolysed.min⁻¹.OD⁻¹) were found in GM-1 (deionized water).

Fig. 3: Monitored (A) growth and (B) pH profiles of *S. pasteurii* strain when cultivated in Growth Medium-1 (GM-1) and Growth Medium-2 (GM-2). The vertical error bars represent mean $\pm$ SD (n=3).

Table 4: Growth kinetics of *S. pasteurii* NB28 (SUTS) in different cultivation media

Kinetics parameters	GM-1 (deionized water)	GM-1 (tap water)	GM-2 (deionized water)	GM-2 (tap water)
Specific growth rate (h^{-1})	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Doubling time (h)	18.19 ± 0.08	17.28 ± 0.21	18.95 ± 0.12	18.02 ± 0.20
Minimum OD ₆₀₀	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00
Maximum OD ₆₀₀	1.2 ± 0.01	1.2 ± 0.01	1.2 ± 0.00	1.2 ± 0.01
Minimum pH	7.6 ± 0.02	7.9 ± 0.02	7.7 ± 0.04	7.5 ± 0.01
Maximum pH	9.2 ± 0.01	9.3 ± 0.01	9.2 ± 0.01	9.3 ± 0.01
Urease activity (mM urea hydrolysed. min^{-1})	17.4 ± 0.19	19.2 ± 0.03	19.1 ± 0.75	21.1 ± 0.06
Specific urease activity (mM urea hydrolysed. $\text{min}^{-1}.\text{OD}^{-1}$)	14.9 ± 0.19	16.6 ± 0.05	16.3 ± 0.72	18.6 ± 0.10

The data are given as mean $\pm$ SD (n = 3).

3.2. Biomineralization and XRD analysis

For biomineralization test of cementation solutions prepared using technical-grade and analytical-grade reagents in tap water (**supplementary materials, Fig. OA1**), ureolytic bacteria previously cultivated in GM-1 and GM-2 were inoculated, respectively into these solutions. Upon addition of the bacterial cultures, slurry precipitations (**Fig. 4**) formed almost immediately. There were noticeable visual differences in the rate at which the insoluble precipitations occurred. The precipitates formed in the technical-grade cementation solution and quickly settled at the bottom of the conical flask after approximately 1 h of ureolysis reaction period while the precipitates formed in the analytical-grade cementation solution moderately settled at the bottom of the conical flask after approximately 4 h of ureolysis reaction period.

Fig. 4: Ureolysis-driven CaCO_3 formation in beakers containing (A) technical-grade cementation solution and solution (B) analytical-grade cementation solution after addition of *S. pasteurii* cultures.

Fig. 5: XRD pattern of CaCO_3 polymorphs obtained from (A) analytical-grade and (B) technical-grade samples.

After the biomineralization test, the collected precipitate samples (**supplementary materials, Fig. OA2**) were oven-dried before characterization by XRD analysis to identify the precipitates. XRD analysis provides direct qualitative and quantitative evidence of CaCO_3 precipitation from MICP by determining the number of different phases in the selected samples [40]. The XRD analysis (**Fig. 5**) showed that the precipitates induced by *S. pasteurii* in analytical-grade and technical-grade cementation solutions were identified as CaCO_3 having almost undistinguishable polymorphs peaks. The precipitates obtained from analytical-grade solution produced calcite (93%) and vaterite (7%), while that of technical-grade solution produced calcite (92.6%) and vaterite (7.4%).

3.3. Soil biocementation, ED-XRF and EDX analyses

Four different concentrations of cementation solutions ranging from 0.5-1.0 M were used in the current study. The sand samples which were treated with ureolytic bacterial cultures and cementation solution (1 M) through surface percolation are presented in **supplementary materials, Fig. OA3**. Surface percolation was used in his experiment due to its ease of operation. This method does not require heavy machinery and expensive treatment techniques [41]. However, it is limited with treatment depth as it is most suitable for solution penetration into the soil to a depth of 2 m [42]. Hence, the use of the surface percolation method was suitable for this current study since the depth of the PVC columns was less than 1 m. The use of technical-grade cementation solution for soil biocalcification has an enormous economic advantage over analytical-grade solution.

Prior to biocement treatment, the pH of each cementation solution used was measured as shown in **supplementary materials, Table OA2**, indicating the solutions were acidic. The initial pH of AG (DW) ranged between 5.05 ± 0.04 - 5.42 ± 0.12 , while AG (TW) ranged between 5.23 ± 0.02 - 5.40 ± 0.03 for the cementation solutions (0.25-1.0 M). On the other hand, the initial pH of TG (DW) ranged between 4.36 ± 0.03 - 4.53 ± 0.03 , while AG (TW) ranged between 4.58 ± 0.05 - 4.88 ± 0.03 for the cementation solutions (0.25-1.0 M). The results for pH effluents in **Fig. 6** illustrated fluctuating pH values in different concentrations for all the samples treated with analytical-grade and technical-grade cementation solutions. In all the collected effluent samples (0.25-1.0 M), it was observed that the measured pH for AG (DW) and AG (TW) were in the range of 7.44 ± 0.03 - 9.31 ± 0.02 and 7.39 ± 0.01 - 9.16 ± 0.01 , respectively. Additionally, the pH effluents for TG (DW) and TG (TW) were in the ranges of 7.44 ± 0.05 - 9.26 ± 0.01 and 7.67 ± 0.12 - 9.26 ± 0.02 , respectively.

The ED-XRF analysis (**Fig. 7A**) carried out on yeast extract, a food-grade material commonly used for baking and cooking purposes, showed that phosphorus (3.73%), sulphur (3.84%), chlorine (43.03%) and potassium (49.40%) were present. The ED-XRF analysis (**Fig. 7B**) of calcium chloride, widely used industrially as a de-icing agent on winter roads, showed that sodium (13.70%), magnesium (0.65%), calcium (30.77%), chlorine (54.64%) and sulphur (0.243%) were present. The ED-XRF analysis (**Fig. 7C**) was also performed on urea, widely used in the agriculture industry as a nitrogen fertilizer for soil nourishment. The spectrophotometer could only detect the presence of calcium (82.90%) and chloride (17.10%) in the samples.

For a comparative analysis of the technical-grade and analytical grade reagents used in this study, EDX was used to determine elemental compositions and concentrations of the reagents (**Table 5**). Based on the analysis for analytical-grade and technical-grade yeast extract media, carbon had the highest values ($51.44 \pm 1.89\%$ and $46.51 \pm 1.53\%$) among all the elements detected, while sodium ($0.18 \pm 0.03\%$) and sulphur ($0.29 \pm 0.06\%$) had the lowest values for the high-purity and low-purity reagents, respectively. Additionally, a trace amount of aluminium,

($0.43 \pm 0.07\%$) was detected only in technical-grade yeast extract media. The EDX analysis for analytical-grade and technical-grade urea showed that nitrogen had the highest values ($38.62 \pm 0.48\%$ and $39.74 \pm 0.16\%$) among all the elements detected, while calcium had the lowest values ($0.01 \pm 0.00\%$ and $0.32 \pm 0.42\%$) for the high-purity and low-purity reagents. The EDX analysis for analytical-grade and technical-grade calcium chloride showed that chlorine had the highest values ($51.5 \pm 2.11\%$ and $47.80 \pm 1.44\%$) among all the elements detected, while oxygen ($10.84 \pm 1.08\%$) and magnesium ($0.97 \pm 0.48\%$) both had the lowest values. Additionally, sodium ($1.36 \pm 0.31\%$) alongside magnesium were only detected in the technical-grade reagent.

Fig. 6: pH of the effluents collected from treated sand columns. The vertical error bars represent mean $\pm$ SD ($n=3$).

Fig. 7: ED-XRF spectrum results of the commercially available technical-grade reagents used for biocement treatment. (A) yeast extract, (B) calcium chloride and (C) urea.

Fig. 7 continued.

Table 5: Comparison of technical-grade and analytical-grade elemental compositions

Grade type	Cementation reagents and elemental compositions (atomic %)		
	Yeast extract	Urea	Calcium chloride
Analytical-grade	carbon (51.4 ± 1.89), nitrogen (7.4 ± 0.38), oxygen (32.0 ± 1.70), sodium (0.2 ± 0.03), phosphorous (1.9 ± 0.33), sulphur (1.2 ± 0.11), chlorine (0.2 ± 0.11) and potassium (5.7 ± 1.24).	carbon (23.9 ± 0.75), nitrogen (38.6 ± 0.48), oxygen (34.5 ± 0.74), aluminium, (2.8 ± 0.19), chlorine (0.1 ± 0.02) and calcium (0.1 ± 0.00).	carbon (4.6 ± 0.27), nitrogen (11.0 ± 1.35), oxygen (10.8 ± 1.08), chlorine (51.5 ± 2.11) and calcium (22.5 ± 0.51).
Technical-grade	carbon (46.5 ± 1.53), nitrogen (8.9 ± 0.50), oxygen (33.6 ± 2.49), sodium (5.3 ± 0.73), aluminium, (0.4 ± 0.07), phosphorous (0.8 ± 0.09), sulphur (0.3 ± 0.06), chlorine (2.7 ± 0.41) and potassium (1.4 ± 0.14).	carbon (21.8 ± 0.69), nitrogen (39.7 ± 0.16), oxygen (37.4 ± 1.01), aluminium, (0.5 ± 0.09), chlorine (0.3 ± 0.02) and calcium (0.3 ± 0.42).	carbon (8.6 ± 0.69), nitrogen (7.6 ± 1.20), oxygen (13.7 ± 1.89), sodium (1.4 ± 0.31), magnesium (0.9 ± 0.48), chlorine (47.8 ± 1.44) and calcium (22.0 ± 0.93).

The data are given as mean $\pm$ SD (n = 3).

3.4. Surface strength of the biocemented sand samples

Fig. 8 shows surface strength of the biocemented samples after treatment with ureolytic bacterial cultures and different concentrations (0.25-1.0 M) of analytical-grade and technical-grade cementation reagents in deionized water and tap water for 96 h. It can be seen that the results of treated samples with high-grade and low-grade cementation solutions were comparable. The result showed that for the sample group treated with cementation reagents at a concentration of 0.25 M, AG (TW) had highest surface strength (1793.00 ± 138 kPa) while TG(DW) had lowest surface strength (1448.00 ± 69.00 kPa). For the samples which were treated with cementation reagents at a concentration of 0.50 M, TG (DW) showed the highest strength (3861.00 ± 365.11 kPa) while AG (TW) had the lowest strength (2941.67 ± 443.04 kPa). Additionally, when the sand samples were treated with cementation reagents at a concentration of 0.75 M, AG (DW) had the highest strength (4734.34 ± 158.77 kPa) while TG (TW) had the lowest strength (4274.67 ± 497.01 kPa). Contrary to the rest of the groups, when the sand columns were treated with 1.0 M of cementation solutions, they all had same strength values (4826.00 ± 00 kPa). Statistical analysis using one-way ANOVA showed that there were significant differences (P-value is $1.63E-19$) among the group means of the analysed results.

Fig. 8: Surface strength measurement of the biocemented sand samples using pocket penetrometer. Treatments were carried out using ureolytic bacterial cultures with analytical-grade and technical-grade solutions. The acronyms AG (DW), AG (TW), TG (DW) and TG (TW) represent analytical-grade deionized water, analytical-grade tap water, technical-grade deionized water and technical-grade tap water, respectively. To determine whether the differences between group means are statistically significant at $P < 0.05$, one-way ANOVA with Tukey-Kramer post hoc analyses were used. The rectangular bars that share a letter (i.e. a, b, c, d, e, f) indicates there are no statistical significant differences between their means. The vertical error bars represent mean $\pm$ SD (n=3).

3.5. CaCO_3 contents of the biocemented sand samples

CaCO_3 content measurements are often performed to determine increases in CaCO_3 following biocement treatment of soil samples [14]. **Fig. 9** shows the CaCO_3 content of the biocemented sand specimens treated with ureolytic bacterial cultures and different concentrations of analytical-grade and technical-grade cementation reagents. The sample group treated with cementation reagents at a concentration of 0.25 M, AG (DW) had the highest CaCO_3 content ($7.1 \pm 1.72\%$) while TG (DW) had the least CaCO_3 content ($5.6 \pm 1.15\%$). For the samples which were treated with cementation reagents at a concentration of 0.50 M, TG (TW) had the highest CaCO_3 content ($14.7 \pm 1.84\%$) while AG (TW) had the least CaCO_3 content ($12.2 \pm 0.47\%$). Additionally, samples with cementation reagents at a concentration of 0.75 M showed that AG (DW) had the highest CaCO_3 content ($23.7 \pm 1.32\%$) while TG (TW) had the least CaCO_3 content ($21.7 \pm 4.10\%$). Finally, results from the samples with cementation reagents at a concentration of 1.0 M showed that TG (TW) had the highest CaCO_3 content ($33.2 \pm 0.59\%$) while AG (TW) had the least CaCO_3 content ($31.2 \pm 1.97\%$). Statistical analysis showed that there were significant differences (P-value is 0.0) among the group means.

Fig. 9: CaCO_3 contents of the biocemented sand samples. Treatments were carried out using ureolytic bacterial cultures with analytical-grade and technical-grade solutions. The acronyms AG (DW), AG (TW), TG (DW) and TG (TW) represent analytical-grade deionized water, analytical-grade tap water, technical-grade deionized water and technical-grade tap water, respectively. To determine whether the differences between group means are statistically significant at $P < 0.05$, one-way ANOVA with Tukey-Kramer post hoc analyses were used. The rectangular bars that share a letter (i.e. a, b, c, d, e, f) indicates there are no statistical significant differences between their means. The vertical error bars represent mean $\pm$ SD (n=3).

3.6. SEM analysis

In order to visualise the microscopic structure of the sand columns after biocement treatment, SEM analysis was performed and the samples treated with 1 M cementation solutions were compared with an untreated sand sample. The SEM image in **supplementary materials, Fig. OA5** clearly illustrates the absence of crystals in the untreated sand while **Fig. 10** shows abundant crystal formation on surface of the treated soil particles. The crystal morphology of CaCO_3 precipitates showed large abundant spheroidal shapes with rough surfaces. The SEM observations suggested there are no significant differences among the morphological structures of the CaCO_3 precipitates formed on the surface of the treated sand particles. The high magnification SEM images in **Fig. 11** clearly show different topographical features of CaCO_3 crystal precipitates formed on the sand particles when treated with different cementation solution. **Fig. 11A** shows detailed flower-like spherical structures in analytical-grade cementation solution (1 M) prepared in deionized water. The topography of the crystal structures indicates bundles of cubes clustered together. In **Fig. 11B**, SEM results for crystal treated with technical-grade cementation solution (1 M) prepared in deionized water illustrate well-projected fibre-like hexagonal structures. The topography of the crystal seems like the structures bundle of fibre are tangled together to form a block. **Fig. 11C** shows rhombohedral-like structures in crystals formed after treatment with analytical-grade cementation solution (1 M) prepared in tap water. The topography of this crystal suggests the structures are assembled to form an irregular ball shape. **Fig. 11D** shows blurry, wrinkled-like crystal structures with technical-grade cementation solution (1 M) prepared in tap water. In contrast to other SEM images shown in **Fig. 11A- Fig. 11C**, the topography of the crystals shown in **Fig. 11D** does not display vivid features, but instead the presence small cubic crystals clustered together.

Fig. 10: SEM analysis showing crystal morphologies of CaCO₃ on surfaces of the sand samples treated with *S. pasteurii* and 1.0 M of different cementation solutions. Analytical-grade (A) and technical-grade (B) reagents in deionized water; analytical-grade (C) and technical-grade (D) reagents in tap water.

Fig. 11: SEM analysis showing the surface topography of CaCO_3 after soil biocementation was performed with *S. pasteurii* and 1.0 M of different cementation solutions. Analytical-grade (A) and technical-grade (B) reagents in deionized water; analytical-grade (C) and technical-grade (D) reagents in tap water.

4.0. Discussion

4.1. Bacterial growth in GM-1 and GM-2

The observed results showed that all *S. pasteurii* cultures had similar biomass, growth rates, doubling time, pH profiles and urease activities (**Table 4**). Nutritive molecules in culture media often differ and can influence the growth of microorganism [30]. Typically, nutrients with small peptides and amino acids will easily be absorbed by the microorganism for quicker growth. This could be the reason there were noticeable differences in the growth patterns of the bacteria in the growth medium (**Fig. 3A**). The pH profiles (**Fig. 3B**) of all cultivation medium increased steadily until the end of the incubation period. The escalation of pH was due to urea hydrolysis by microbial urease which is essential for CaCO_3 precipitation [43].

Ureolysis allows the changes of pH to alkaline in the presence of ammonia and carbonate ions. From 36 h until the end of incubation, the pH values stabilized which could be a result of depletion in biomass production and urease activity. The hydrolysis of urea by urease occurs more at a higher bacterial concentration which, in turn, leads to high pH values but when bacterial concentration is low, pH of the culture medium correspondingly declines [44]. The cultivation of ureolytic bacteria in deionized water and tap water was performed to determine if the presence or absence of inorganic materials in water would influence the bacterial growth performance or urease activity. Typically, tap water is treated through water purification systems prior to removal of elements or heavy metals. However, the results in **Fig. 3** suggested *S. pasteurii* was not noticeably affected when cultivated in growth medium prepared tap water. Tap water can be used for biocalcification because the concentration of nutrients or cementation reagents exceeds the concentration of inorganics present in tap water by one or two orders of magnitude [25]. However, it was observed that *S. pasteurii* had higher biomass production when cultivated in GM-1 and GM-2 prepared with deionized water. On the other hand, the urease activities were higher when *S. pasteurii* was cultivated in GM-1 and GM-2 prepared with tap water. Deionized water is commonly used for the preparation of cultivation medium to avoid the influence of contaminating ions during bacterial growth [17], but it seemed the use of tap water favoured the urease activity of *S. pasteurii*, probably due to the presence of trace elements. This might be influenced by the electronegativity of bacterial cell walls, thus allowing adsorption of cations from the trace elements and enhancing urease activity [43].

The ability of *S. pasteurii* to grow and produce high urease activity in GM-1 suggests that this low-cost medium could serve as a replacement substrate to GM-2 which is prohibitively expensive. The reason the ureolytic bacterial growth in GM-1 was similar to that in GM-2 might be because of the nutrient components in GM-1. The food-grade medium comprises yeast extract (50%), maltodextrin (40%), monosodium glutamate (8.8%) and disodium 5'-ribonucleotide (1.2%). The high concentrations of yeast extract and maltodextrin provide proteins and carbon sources needed for bacterial growth, since *S. pasteurii* is known to grow well in protein-rich or carbon-rich culture media [13,42]. Although maltodextrins are commonly used in a wide spectrum of food products, such as nutritional beverages, they have also been reported as suitable alternative carbon and energy sources for ureolytic bacteria (i.e. *Bacillus subtilis*) [45,46]. Interestingly, the trace amount of monosodium glutamate and disodium 5'-ribonucleotide in the technical-grade yeast extract, which serves as a flavour enhancer for the food-grade medium, did not inhibit biomass production and urease activity of *S. pasteurii*.

4.2. Biomineralization

The precipitation (**Fig. 4**) which rapidly occurred simultaneously in both cementation solutions after addition of the bacterial culture showed a strong depletion rate up to 4 hours until no more precipitates formed. The visual observation noticed during the ureolysis-driven reaction period corresponded with findings reported in the literature [3,24,47]. Lu et al. [24] reported that when calcareous sand samples were dissolved into acetic acid, fast occurring carbonate precipitates were observed in the first half hour and quickly showed a decline in the rate of precipitation. In another study, Joshi et al. [47] observed significant delay in precipitate formation during the paste mixture period in nutrient broth paste mix which influenced the setting characteristic of cement when compared with other paste mixtures (i.e. corn steep liquor). However, in one of the corn steep liquor paste mix samples, an increase setting or reaction time was observed. It was suggested that the presence of high carbohydrate content might have resulted in inhibition of the calcification process. In a recent study by Phua et al [3], carbonate precipitates were obtained by dissolving powdered chalk in lactic acid via an enzyme-induced carbonate precipitation process with Jack Bean urease. Rapid crystal formation was observed within the first 10 min after addition of the reagent into the CaCl_2 -solution and significantly slowed after 4 h.

Ureolytic bacteria play two key roles in the biomineralization test, first to generate urease for urea hydrolysis which then produces a suitable alkaline environment for carbonate precipitation and, second, to provide nucleation sites essential for the formation of carbonate crystallization, morphology and yield of the precipitation [44]. Typically, urea and calcium chloride are the two common chemical reagents used for the biomineralization test but nutrients such as yeast extract are often incorporated into cementation solutions to enhance biomass production and urease activity which subsequently triggers greater CaCO_3 content [12]. The additional ingredients such as salts (i.e. monosodium glutamate) and maltodextrin present in the technical-grade nutrient media used in this current study might have influenced the reaction rate of CaCO_3 precipitation. Well investigated factors such as chemical reagents, biomass concentration, the composition of nutrient, pH, salinity and reaction medium are known to influence CaCO_3 precipitation and polymorphs [12,48–50]. Other synthesis factors such as reaction time, stirring rate and impurities (e.g., Mg) are also known to influence the formation of CaCO_3 polymorphs by influencing the dissolution and recrystallization of crystals [51].

4.3. XRD analysis

The XRD spectra showed that the maximum peak intensities were clearly observed at $29.4^\circ 2\theta$ for calcite. Based on the result illustrated in **Fig. 5**, the values for analytical-grade and technical-grade samples are identical within the margin of error (92.6 and 7.4 is identical to 93 and 7). The XRD spectra showed that the intensity peak values for the identified calcite obtained from the analytical-grade cementation solution and technical-grade cementation solution were 1400 and 1280, respectively. Several studies have also reported identifying calcite as the major CaCO_3 polymorphs with a trace amount of vaterite [17,28,52,53]. CaCO_3 has three natural polymorphs (i.e. calcite, aragonite and vaterite); calcite is the most thermodynamic stable form under the ambient conditions [51]. In our previous studies [35], XRD analysis of precipitated samples obtained after biomineralization test was performed in analytical-grade and technical-grade cementation solutions both prepared in deionized water showed that the precipitates from analytical-grade solutions were identified as calcite (100%). On the other hand, technical-grade cementation solution was identified as calcite (94.9%) and vaterite (5.1%) polymorphs. Irrespective of whether analytical-grade or technical-grade cementation solutions were prepared in deionized water or tap water, it appears that when

biomineralization is performed under laboratory conditions, calcite is mostly formed as the dominant CaCO_3 polymorphs while vaterite is formed in trace amounts. In most MICP studies, calcite crystal precipitates are often reported as the dominant CaCO_3 polymorph and this might be because most biocement treatments are typically performed at ambient conditions. Calcite formation is the most direct manifestation of MICP since CaCO_3 is the final product of the process [40]. However, other MICP studies that have reported vaterite and aragonites as the dominant crystals upon XRD analysis of their precipitated samples [29,49].

4.4. MICP treatment of sand columns

MICP is often regulated by the concentration of ions, concentration of organic carbon, pH of the microenvironment and availability of nucleation site [43]. Therefore, the effect of various conditions (i.e. cultivation medium, concentration of cementation reagents, water source for cementation solution, surface percolation treatment method) on soil biocalcification were investigated. In addition, a comparison was carried out to determine if technical-grade cementation reagents of different concentrations can replace the standard analytical-grade reagents. Since these low-grade chemicals are inexpensive and less harmful to the geo-environment than pure chemicals, it was necessary to investigate their potential feasibility for MICP applications [7].

During the soil treatment, there were comparable outcomes from the samples treated with analytical-grade and technical-grade cementation solutions. Some of the samples treated with higher solution concentrations (i.e. 0.75 M and 1.0 M) showed the formation of precipitates on the surface of the sand. Clogging formation is a common problem during the biocement treatment when using percolation or injection treatment [42,54,55]. This is also often influenced by using a higher concentration of cementation solutions or when multiple treatments are used for soil biocementation. The issue with using multiple cementation solution treatments involves the formation of bio-clogging especially at the site of entry during treatment (**supplementary materials, Fig. OA3**). This formation inhibits further penetration of cementation solution into the soil particles and, instead, allows accumulation of the CaCO_3 on the surface of the soil samples (treatment point of entry). The reaction of the ureolytic bacteria and cementation solution around the treatment point of entry limits penetration into the soil [41]. Repeated injection or addition of the bacterial culture and cementation solution eventually leads to less CaCO_3 formation inside the soil matrix and uneven CaCO_3 distribution [42]. Hence, proper treatment interval between the bacterial culture and cementation solution should be taken into consideration [48].

Surface clogging can also lead to self-enhancement of strength due to the increase in crystal and decreased porosity [42]. During the treatment, it was noticed that samples treated with 0.75 M and 1.0 M concentrations of the cementation solutions showed decreased soil permeability after 48 h. The reduction of permeability allows an increase in the hydraulic retention time of the cementation solution during infiltration and more crystal formation at the treatment regions [42]. However, it has been highlighted that excess soil clogging of CaCO_3 formation at the treatment site can result in improper attachment and retention of the bacterial culture in the soil matrix, thus affecting the homogeneity of CaCO_3 distribution and strength of the soil [41]. Thus, after the biocement test, the strength of the soil and CaCO_3 content were analysed (see sections 3.7 and 3.8). Early microbial activity upon inoculation of the ureolytic bacterial culture alongside the cementation solution initiates premature formation of CaCO_3 precipitates which clog the injection points [38]. This clogging inhibits free flow of the cementitious material between soil particles, hence resulting in un-even CaCO_3 distribution [56]. Obtaining complete uniformity may be very challenging but researchers are exploring ways to resolve this

biocement limitation through different techniques such as multiple injection, surface percolation, bioslurry, one-phase low-pH injection and temperature reduction [42,56–59]. Results from the aforementioned techniques showed that initial clogging formation at the injection points were reduced, allowing an improvement in the distribution of the cementation solution, enhanced strengths of the treated soil samples and homogenous contents of CaCO_3 precipitate formed between the soil particles.

4.5. pH of effluent samples

pH is a key parameter used to measure urease activity of microorganism during MICP process, hence the pH of effluent samples was analyzed which ranged between 7.39 ± 0.01 – 9.26 ± 0.02 . The results are similar pH of effluent samples previously reported (8.5–9.3) [9,60,61]. It was suggested that the reason pH effluents varied was due to different bacterial cell concentration. The rise in pH values was due to greater urease activities produced by a higher concentration of ureolytic bacteria [60]. The analysis of the pH effluents illustrates that urea hydrolysis occurred from the first day of biocement treatment until the final treatment on the fourth day. This situation was also reported by Dhami et al. [9] for effluents studied up to ten days. It was also noted that throughout this treatment, the pH of the effluents remained alkaline which indicated active urease activity of the bacteria in all samples irrespective of treatment with analytical-grade or technical-grade cementation solutions. In Fig. 6, the pH effluent profiles of samples collected and measured after biocement treatment on sand columns using technical-grade and analytical-grade cementation solutions shows depletion of the pH values. For all concentrations (0.25–1.0 M) of analytical-grade samples, a much more pronounced fall in pH was observed when compared with the technical-grade samples. This was especially noticeable in 0.5 M and 0.75 M samples after the 48 h incubation period. The increase or decrease of pH during MICP is mostly attributed to urease activity. Hence, the fall of urease activities as the incubation period progressed from 24–96 h occurred in all measured samples. There are several possibilities to explain the observed decline in the urease activities of the effluent samples, including depletion of necessary nutrients, accumulation of ammonia or carbonate ions, and production of extracellular metabolites [47]. The fall of pH in the analytical-grade and technical-grade samples might be attributed to the purity of the reagents used for soil biocementation. The EDX analysis (Table 5) indicated there were noticeable differences in the concentrations of elements present in the cementation reagents. For example, the technical-grade yeast extract needed for microbial growth contained trace amounts of aluminum and sulphur, while technical-grade calcium chloride showed trace amounts of sodium and magnesium which were absent in the analytical-grade reagents. It was previously suggested that the concentration of magnesium has a remarkable effect on MICP, CaCO_3 deposition rate and polymorphs [62]. Hence, it is possible that the presence of these elements (aluminum, sulphur, sodium and magnesium) could affect urease activities and, thus, might be the reason for the less pronounced fall in pH observed for the technical-grade samples.

4.6. Cost analysis

The use of technical-grade cementation solution for soil biocalcification has an enormous economic advantage over analytical-grade solution. The calculated cost for the technical-grade cementation reagents of 0.25–1.0 M concentrations ranged between 0.07–0.26 US\$/L which is considerably lower than the cost of for the analytical-grade cementation reagents for the same concentrations (3.33–13.29 US\$/L). This shows a cost difference of 47 to 51-fold between the analytical-grade solution and technical-grade based on the market price of the reagents procured in Malaysia. The use of the commercially available technical-grade reagents was able to tremendously reduce treatment cost, which is a considerable advantage over the costs of

standard reagents typically used in biocement studies. Utilization of low-cost cementation reagents would certainly be suitable for future MICP field-scale advancements [7].

All the growth media and chemicals prepared in this study were sterilised by autoclaving before use to ensure control and reproducibility, although this is not viable for large-scale or field-scale implementation. The use of sterile equipment is also not practical or feasible due to the sheer size of the construction machinery. Additionally, outsourcing sterilization services from biotechnological companies is possible but this would also drive up the MICP construction costs. Recognising this challenge, future large-scale MICP investigations should explore the use of non-sterile reagents for bacterial cultivation and biocement treatment. It is understandable that other microorganisms (urease-producers or non-urease producers) might influence the MICP process. In addition, the growth of non-desired microbes during bacterial cultivation and treatment using non-sterile reagents could result in competition for nutrients with the introduced microbe (i.e. *S. pasteurii*) and, thus, affect bacterial growth, urease activity or CaCO_3 precipitation. To limit this occurrence, two approaches could be adopted as a low-cost method of preparing growth media solutions and chemicals, namely, filtration and steam. The potential outcome of performing MICP studies under non-sterile conditions might address the economic challenges of supplying large-scale volumes of ureolytic bacterial cultures for future *in situ* MICP studies or construction applications.

4.7. Surface strength, CaCO_3 contents and SEM analysis

The concentration of cementation solution is one of the key factors which affect CaCO_3 crystal formation [7]. Hence, sand biocement formation using different solution concentrations was assessed with technical-grade reagents compared with analytical-grade reagents under the same conditions. The results of the penetrometer tests (Fig. 8) showed that the biocement treatment process based on ureolytic bacteria and cementation solutions was able to improve the surface strength of the sand samples. The result also showed that the increase in concentration of cementation solutions correlated with increased strength of the treated samples since higher amounts of calcium can induce greater CaCO_3 formation and improve soil strength. From an economic point of view, lower concentrations might be preferred but cementation treatment needs to be completed rapidly, hence higher concentrations are recommended [63]. To minimize the cost of using a high concentration of the cementation reagents, the technical-grade reagents will serve as a suitable choice. It is important to note that the cementation reaction might not only be influenced by reagent concentrations; other factors such as temperature, injection speed, treatment interval and treatment duration play essential roles [40,41,64]. For the biocementation, the samples were treated and allowed to cure at room temperature. The treatment solutions were treated once a day for a duration of 92 hr. This might have supported the proper formation of crystals or bonding in the sand matrix especially for samples treated with higher concentrations resulting in higher compressive strengths.

The most common unconfined compression tests are typically carried out on a compression machine to describe the compressive strength of biocemented soils as reported by several researchers [3,22,24,28,30,53,63], but available reports in the literature have shown that pocket penetrometers can also be used to evaluate the surface (top) strength of treated samples [37,38,42]. Typically, many local strength points are selected, hence in this study, several sets of triplicate samples were prepared and each set contained three replicates to enhance repeatability of the treatment and support the strength test. The improvement or changes of strength values for the samples differed due to the deposition of CaCO_3 by the ureolytic bacteria within the pores of the sand matrix [54]. The formation of CaCO_3 crystal and its contents within the sand particles are essential for soil solidification and improvement of soil strength. It was

also observed that the addition of yeast extract as an ingredient of the cementation solution did not inhibit the formation of CaCO_3 crystals despite repeated addition of the treatment solution. Addition of media to the cementation solution stimulates the growth of bacterial cells during biocement treatment due to the richness of free amino acid, protein vitamins, carbohydrates and minerals [53].

The reduction of soil permeability or porosity depends on the extent of CaCO_3 crystal formation and the distribution during biocement treatment [29]. The biocemented samples were analysed to determine if there was any correlation between CaCO_3 contents and compressive strength. Generally, MICP is a biogeochemical process that utilizes microbiological methods to mediate the improvement of geological materials used in engineering [24]. The findings of this study showed that the use of surface percolation treatment of soil samples through an ureolysis-driven MICP process was able to enhance the CaCO_3 content. The biocemented samples (supplementary materials, Fig. OA4) were analysed to determine if there was any correlation between CaCO_3 content (Fig. 9) and surface strength. Overall, a correlation between the surface strength and CaCO_3 content of the samples was observed with the use of difference concentrations of cementation solutions. The results show improvement in surface strength and CaCO_3 content as the concentration of cementation solutions increased. However, when comparisons were made between groups, it was observed that there were no correlations between the results from surface strength and CaCO_3 content. For example, in samples treated with 0.25 M, AG (TW) had the highest strength value, while AG (DW) had the highest CaCO_3 contents. For samples treated with 0.5 M, TG (DW) had the highest strength value, while TG (DW) had the highest CaCO_3 contents. For the sample group treated with 0.75 M, AG (DW) had the highest values for surface strength and CaCO_3 contents. However, it was noticed that for samples treated with 1.0 M, all AG and TG samples had equal strength values which is understandable because of the scale for the penetrometer used in this study to measure the surface strength of treated samples. The maximum surface strength reading obtained for samples treated with 1.0 M of cementation solution was 4826.00 ± 00 kPa, reaching the maximum level on the scale of the pocket penetrometer. There is a significant possibility that the surface strength of these biocemented sand sample would vary if a different penetrometer with measuring scales exceeding the range of 4826.00 ± 00 kPa was used. However, CaCO_3 contents did not show similar result as the strength results for this group. Instead, the CaCO_3 contents result showed that TG (TW) had the highest value for samples treated with 1.0 M.

SEM analysis (Fig. 10 and Fig. 11) of the CaCO_3 crystals formed by *S. pasteurii* after soil biocementation showed that with the use of technical-grade cementation reagents, similar morphological characteristics were obtained to that of analytical-grade cementation reagents. CaCO_3 crystals with different morphologies in the treated sand samples have been reported in previous MICP studies [3,44,49]. The morphological characteristics of CaCO_3 crystal can be influenced by various conditions such as type of bacterial strains, urease activity and nucleation site [43]. Alternatively, microbial extracellular polymeric substances mainly composed of polysaccharides, proteins and DNA, are known to improve the aggregation of soil particles and contributes to production of different CaCO_3 polymorphs or crystal morphologies [54]. Based on the SEM analysis, it seems technical-grade cementation reagents can serve as suitable alternatives to analytical-grade cementation reagents since they both precipitate CaCO_3 crystals having similar morphological structure.

5.0. Conclusions

This study was performed to investigate the feasibility of using low-cost commercially procurable technical-grade reagents for large-scale MICP applications. The technical reagents

served as alternatives to analytical-grade reagents typically used in most small-scale MICP experiments. The performance of *S. pasteurii* in technical-grade reagents used for cultivation and sand biocementation was compared those of analytical-grade reagents. The following conclusions can be drawn:

- *S. pasteurii* showed similar performance when cultivated in GM-1 and GM-2, indicating that technical-grade cultivation media can serve as a replacement to standard laboratory cultivation medium typically used to grow a wide range of microorganisms.
- The efficacy of using technical-grade cementation solutions (0.25-1.0 M) to improve soil strength showed comparable outcomes, with respect to surface strength and CaCO₃ content, with those treated with analytical-grade solutions.
- Cost analysis indicated that soil biocementation through surface percolation treatment favoured the use of technical-grade cementation reagents (0.07-0.26 US\$/L) over analytical-grade reagents (3.33-13.29 US\$/L) with a cost difference of 47-51 fold.

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Supplementary Materials

Table OA1: Physicochemical compositions of the technical-grade cementation reagents

Reagents	Brand name	Properties	Application
Yeast extract	FD00-A (previously known as FB00)	yeast extract (50%), maltodextrin (40%), monosodium glutamate (8.8%), disodium 5'-ribonucleotide (1.2%), moisture (3.90%), Sodium chloride (4%), pH (5.72), canary yellow colour and very soluble in water.	used in food industry for seasoning of meat products, flavouring of biscuits and baking of cakes.
Urea	N46	nitrogen (46%), biuret (1%), moisture (0.50%) purity as urea (98%), pH (7.1), granular form, pure white colour and very soluble in water.	used in agriculture industry as nitrogen fertilizer and chemical industry as raw material for production of plastic and resins.
Calcium chloride	LONGYI	calcium chloride (77%), sulphate (0.2%), sodium chloride (6%), magnesium chloride (0.5%), calcium hydroxide (0.2%), pH (8.2), flake form, pure white colour and very soluble in water.	used in chemical and construction industry for de-icing, dust control, concrete acceleration and tire-weighting.

Table OA2: Initial pH of cementation solutions containing different concentration reagents

Treatment solution	Concentration of cementation reagents			
	0.25 M	0.50 M	0.75 M	1.0 M
^a AG (DW)	5.42 ±0.12	5.22 ±0.13	5.10 ±0.20	5.05 ±0.04
^b AG (TW)	5.40 ±0.03	5.28 ±0.09	5.24 ±0.08	5.23 ±0.02
^c TG (DW)	4.53 ±0.03	4.50 ±0.04	4.44 ±0.04	4.36 ±0.03
^d TG (TW)	4.62 ±0.15	4.65 ±0.14	4.58 ±0.05	4.88 ±0.03

^aAnalytical-grade cementation reagents in deionized water.

^bAnalytical-grade cementation reagents in tap water.

^cTechnical-grade cementation reagents in deionized water.

^dTechnical-grade cementation reagents in tap water.

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1134 **Fig. OA1:** Different cultivation medium used to grow ureolytic bacterial strain. (A) Growth
1135 Medium-1 contained low-cost ingredients serving as an alternative nutrient medium while (B)
1136 Growth Medium-2 contained more expensive microbiological-grade ingredients typically used
1137 for cultivation of microorganisms.

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1141 **Fig. OA2:** CaCO_3 precipitates after treatment with technical-grade (left) and analytical-grade
1142 (right) cementation solutions.

Fig. OA3: Samples after treatment with *S. pasteurii* cultures and 1 M of cementation solutions by surface percolation.

Fig. OA4: Biocemented samples treated with ureolytic bacterial cultures and cementation solutions.

Fig. OA5: SEM analysis of the untreated sand sample.