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# Low-cost cultivation of *Sporosarcina pasteurii* strain in food-grade yeast extract medium for microbially induced carbonate precipitation (MICP) application

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ABSTRACT

*Sporosarcina pasteurii* is a well-known ureolytic microbial species that proficiently induces the deposition of calcium carbonate through microbially induced carbonate precipitation (MICP) process for various biotechnological and engineering purposes. In view to resolving the concern on high-cost bacterial cultivation due to the conventional use of laboratory-grade growth medium for MICP studies, an inexpensive food-grade yeast medium was investigated in this current study for its feasibility to serve as a suitable alternative media for bacterial growth, urease activity and calcium carbonate precipitation. The effect of different media concentration and initial pH medium on biomass production and urease activity were determined. The performance of this low-cost media was also compared with eight laboratory-grade media (nutrient broth, yeast extract, tryptic soy broth, luria broth, fluid thioglycollate medium, cooked meat medium, lactose broth and marine broth). Results in this current study showed cultivation in low-cost media at 15 g $L^{-1}$ (w/v) and initial pH 8.5 of the food-grade yeast media both constituted the highest biomass concentration and urease activity when supplemented with urea (4%, w/v). Comparison of the food-grade media with laboratory-grade media indicated that bacterial cultivation cost was significantly reduced to 99.80%. After the biomineralization test, X-ray diffraction (XRD) analysis was used to confirm the elemental composition of $CaCO_3$ and polymorphs which were identified as calcite and vaterite. These findings suggest the food-grade yeast extract can serve as a potential candidate for bacterial cultivation in MICP application from the perspective of cost reduction.

## 1. Introduction

Microbially induced carbonate precipitation (MICP) refers to the precipitation of calcium carbonate ($CaCO_3$) which involves the combination of microbial and various biochemical activities (Bosak, 2011). MICP process via ureolysis (urea hydrolysis) utilizes microorganisms such as *Sporosarcina pasteurii* (*S. pasteurii*) to produce urease enzyme that facilitates the breakdown of urea ($CO[NH_2]_2$) for the generation of ammonium ($NH_4^+$) and carbonate ($CO_3^{-2}$) ions (Hammes and Verstraete, 2002). In the presence of calcium ions ($Ca^{+2}$) such as calcium chloride ($CaCl_2$), $CO_3^{-2}$ reacts with $Ca^{+2}$ to form $CaCO_3$ (Stocks-Fischer et al., 1999).

In recent decades, microbial urease for MICP process has fervently seen an increased relevance as a catalyst for the precipitation of calcium carbonate ($CaCO_3$) for various applications in biotechnological and engineering disciplines. Investigations on use of ureolysis-driven MICP process has been widely studied for the soil strengthening and stabilization, remediation of concrete cracks, restoration of limestone surfaces, mitigation of soil erosion, treatment of industrial wastewater and remove heavy metals (Burbank et al., 2013; De Muynck et al., 2010; Fu and Wang, 2011; Işik et al., 2010; Sarda et al., 2009; Van Tittelboom et al., 2010; Whiffin et al., 2007). MICP via ureolysis process can also be used for biosensor, agriculture and healthcare applications (Phang et al., 2018). Nonetheless, MICP is frequently employed to produce biocalcifying agent ($CaCO_3$) which is used as a construction and building material to improve the geotechnical properties of soils.

Despite numerous attainable outcomes of this technology at laboratory-scale, MICP is still considered expensive for commercial implementation or field-scale applications (Mujah et al., 2017). One of the reasons is due to the nutrient medium used in the biotechnological process for bacteria cultivation (Achal et al., 2009). The use of laboratory-grade growth media (i.e. nutrient broth, yeast extract and

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tryptic soy broth) are a major cost factor for bacterial cultivation due to their high cost that can reach up to 60% of the total production cost (Kristiansen, 2006).

To date, only limited studies in the literature have reported the use of alternative nutritional sources to cultivate ureolytic bacteria for MICP application. The use of waste materials such as chicken manure effluent, corn steep liquor, lactose mother liquor and activated sludge from agricultural, dairy and bakery industries have been the focus of alternative nutritional source (Achal et al., 2010, 2009; Cuzman et al., 2015a; Joshi et al., 2018; Whiffin, 2004; Yoosathaporn et al., 2016). These attempts are mostly performed to limit the economic challenges faced for cultivating ureolytic bacterial in large volume (Anbu et al., 2016). Irrespective of the promising findings which have been reported in the aforementioned studies for bacterial cultivation and also help minimize accumulative environmental wastes through recycling (Yoosathaporn et al., 2016). Unfortunately, most recent contemporary studies on MICP still make use of conventional growth media to grow various ureolytic bacterial species.

There are some suggestive drawbacks that can hinder the use of waste materials for MICP applications which includes: (i) limited quality control and processing (Williams et al., 2016); (ii) unavailability in localized regions; (iii) insufficient bulk quantity (i.e. large volume) and (iv) transportation to site. Hence, there is a need to explore other types of nutrition sources which are easily accessible at low or no cost suitable for MICP application. The aim of this present study was to determine the feasibility of cultivating *S. pasteurii* strain in an economical food-grade yeast extract (FG-YE) medium and investigate its effect on bacterial production, urease activity and biocalcification. The performance of FG-YE was compared with eight laboratory-grade growth media. XRD analysis was further used to determine the elemental compositions and identify the polymorphs of $CaCO_3$ precipitates after biocalcification test.

## 2. Materials and methods

### 2.1. Ureolytic bacteria and cultivation

Ureolytic bacterial *S. pasteurii* NB28(SUTS) (GenBank accession no. **KX212192.1**) isolated by Omoregie et al. (2017) from limestone cave sample was used in this study due to its high urease activity. The bacterium was revived on Petri dishes containing sterile tryptone soya agar (40 g $L^{-1}$, HiMedia, Laboratories Pvt. Ltd) and urea (4% w/v, Merck, Shd. Bhd). The Petri dishes were incubated (MMM incucell 55) at 30 °C for 48 h under aerobic conditions and the grown colonies were subsequently sub-cultured until pure isolates were obtained. A loopful of the bacterial colony was then taken and inoculated into a universal bottle containing sterile 10 mL tryptic soy broth (30 g $L^{-1}$ Becton Dickinson Shd. Bhd) and urea (4%, w/v). The broth cultures were incubated (CERTOMAT® CT plus – Sartorius) at 30 °C for 24 h with shaking (130 rpm). The bacterial cultures were then temporarily preserved in the fridge (4 °C) at the end of the incubation until when needed. All the standard bacterial inoculum used for subsequent experiments were not stored for more than a month (Cuzman et al., 2015b).

### 2.2. Evaluation of FG-YE medium

The ability of *S. pasteurii* NB28(SUTS) to grow in alternative low-cost media was tested by cultivating it in FG-YE manufactured by Angel Yeast Co., Ltd. (Supplementary materials, Fig. A1) which is habitually used for baking and cooking purposes. The nutrition contents of the media and physiochemical properties are both shown in Supplementary materials, Table A1-A2. The ureolytic bacterium was grown in standard 250 mL Erlenmeyer culture flasks containing 125 mL of FG-YE (20 g $L^{-1}$) and supplemented with urea (4%, w/v). The initial pH of the growth medium was adjusted to 8.0 using 0.1 M NaOH or 0.1 M HCl (Reyes et al., 2009). Similarly, laboratory-grade yeast extract (LG-YE) media (20 g $L^{-1}$, BD BACTO™) and urea (4%, w/v) were prepared alongside the FG-YE media to serve as a comparative growth media for test on bacterial growth profile, pH profile and urease activity. The bacterial cultures were aerobically incubated at 30 °C for 60 h with shaking (130 rpm) and monitored regularly (12 h interval) using different parameters (optical density, pH and conductivity). The optical density (OD) and pH were both measured hourly until the end of the incubation in order to evaluate the bacterial development (biomass) while the conductivity was only measured at the end of the incubation to determine the urease activity.

### 2.3. Growth and pH profiles

Bacterial growth was assessed by measuring the OD which served as a biomass concentration indicator for ureolytic bacteria (Harkes et al., 2010). The OD was measured by using a spectrophotometer (Thermo Scientific™ GENESYS™ 20) at a wavelength of 600 nm. Before measurement of the OD, un-inoculated growth medium which served as blanks were used to calibrate the spectrophotometer. Three millilitre (3 mL) of the aliquot was sampled from the bacterial cultures and placed into clean 10 mm cuvettes and the OD values were recorded. Additionally, a pH meter (SevenEasy™–Mettler Toledo) was used to measure the acidity or alkalinity of the bacterial culture in relation to the number of hydrogen ions ($H^+$) or hydroxyl ions ($OH^-$). The pH electrode was first calibrated with pH 4, 7 and 10 buffer solutions (Sigma-Aldrich) before immersing into the medium to obtain the respective pH values of the bacterial cultures after incubation.

### 2.4. Urease activity

Urease activity (mM urea hydrolysed·$min^{-1}$) of the bacteria cultures were determined immediately after sampling through electric conductivity measurement (Whiffin, 2004). The urease reaction involves the hydrolysis of non-ionic substrate urea to ionic products that lead to an increasing proportionate conductivity under standard conditions (Al-Thawadi, 2008). For the urease assay, 10 mL of overnight grown bacterial cultures were inoculated into sterile beakers (250 mL capacity) containing 90 mL of 1.5 M urea solution. The respective conductivity values (mS.$cm^{-1}$) from the bacterial-urea solution were recorded for a duration of 5 min at 25 ± 2 °C (Whiffin et al., 2007). The probe of the conductivity meter (Milwaukee, MI806) was immersed into the beakers containing the solution to obtain the conductivity readings. At the end of the assay, the conductivity variation rate (mS·$cm^{-1}$·$min^{-1}$) was then determined from a slope of the plotted graph using values of conductivity measurement against time (Omoregie et al., 2017). In the measured range of activities, 1 mS $min^{-1}$ corresponds with a hydrolysis activity of 11 mM urea $min^{-1}$ (Harkes et al., 2010).

### 2.5. Effect of media concentration

Different concentrations of the FG-YE media were evaluated to establish the appropriate amount needed for maximum bacterial growth and urease production. The concentration of the media studied ranged from 5 to 25 g $L^{-1}$ (w/v). In order to understand the influence of urea addition in the inexpensive FG-YE, cultivation medium with aforementioned concentration was prepared and grouped into two; (i) medium containing urea (4%, w/v) and (ii) medium without urea. When the nutrient medium was sterilized, only then was urea added in the selected culture flasks after the medium had cool down. The cultivation medium was therefore inoculated with overnight grown bacterial culture and incubated at 30 °C for 24 h with shaking (130 rpm). The conductivity and OD were measured at the end of the cultivation period. The optimum media concentration that promoted the highest enzyme activity and OD was selected then used for subsequent

experiments unless where stated otherwise.

### 2.6. Effect of initial pH medium

The influence of initial pH medium for the FG-YE on the performance of the ureolytic bacteria was studied ranging from 5.0 to 11.0 with an interval of 0.5. The bacteria cultures were incubated at conditions previously mentioned (subsection 2.5. Effect of media concentration). The OD, pH and urease activity of the bacterial cultures in various medium were measured at the end of the incubation period.

### 2.7. Comparison with various laboratory-grade growth medium

A variety of eight laboratory-grade cultivation media were selected and used in this study for comparison with the inexpensive FG-YE on the basis of bacterial growth and urease activity performances. The standard media selected in this experiment which are typically used for a wide range of bacterial cultivation are: LG-YE (20 g $L^{-1}$, BD BACTO™), nutrient broth (NB) (13 g $L^{-1}$, Merck), luria broth (LUB) (25 g $L^{-1}$, Merck), tryptic soy broth (TSB) (30 g $L^{-1}$, BD DIFCO™), fluid thioglycollate medium (FTM) (29.5 g $L^{-1}$, BD BBL™), cooked meat medium (CMM) (125.0 g $L^{-1}$, BD DIFCO™), lactose broth (LB) (13 g $L^{-1}$, Merck) and marine broth (MB) (37.4 g $L^{-1}$, BD DIFCO™). Alongside these high-cost media, the FG-YE medium (15 g $L^{-1}$, Angel Yeast Co., Ltd.) was also prepared and each of the growth media was supplemented with urea (4%, w/v). The bacteria were cultivated in Erlenmeyer flasks (125 mL working volume) incubated at 30 °C for 24 h with shaking condition (130 rpm). The optical density, pH and urease activity of the bacteria in all nine growth media was determined as described in the monitoring parameters sub sections. The characteristic of the ingredients and cost analysis for all the growth media used in this study are presented in Table 1.

### 2.8. Biomineralization test

A modified biomineralization method of Arias et al. (2017) was used in this experiment to test the bio-calcifying effect of the selected growth media with the use of the ureolytic bacteria. The overnight grown bacterial cultures were inoculated in falcon tubes containing cementation solution which consisted of urea (20 g $L^{-1}$, Merck, Shd. Bhd) and calcium chloride (20 g $L^{-1}$, HiMedia). To constitute bacterial growth during the MICP process, the cultivation media (5 g $L^{-1}$) mentioned in the aforementioned subsection (2.7. Comparison with various laboratory-grade growth medium). The cementation reagents were prepared in deionized water before transferring to beakers (500 mL). The growth media were sterilized with the autoclave machine while the urea and calcium chloride were ultraviolet light-sterilized. These chemicals were added into the growth medium after being cooled down in order to avoid degradation of the chemicals. These cementation solutions (45 mL) were then transferred into sterile falcon tubes and inoculated with 5 mL of overnight grown bacterial cultures. The assay was performed in triplicate and the falcon tubes were incubated for 72 h at 32 °C with no shaking. At the end of the incubation period, the tubes were kept in a centrifuge machine (Eppendorf, 5804R) 10,000*g* for 5 min to separate the precipitates from the effluents. The effluents were transferred into separate sterile falcon tubes (50 mL) and their respective pH values measured. The precipitates were washed with distilled water, dried at 60 °C for 4 h and then weighed.

### 2.9. XRD analysis

For determination of elemental composition and crystalline phase of precipitated samples after biocalcification test, four sets of precipitated samples which were induced by ureolysis process and separate treatment cementation solutions containing FG-YE, LG-YE, TSB and NB were respectively selected for XRD analysis. The dried precipitated samples

**Table 1**
Growth media and cost analysis.

| Growth media | Composition | Media acronym | [a]Quantity (g·$L^{-1}$) | [b]Cost (US$) |
|---|---|---|---|---|
| Yeast extract (Angel Yeast/FB00) | yeast extract, maltodextrin food addictive (monosodium glutamate and disodium 5′-ribonucleotide) and sodium chloride. | FG-YE | 15 | 0.27 |
| Yeast extract (BD BACTO™/ #212750) | peptides, free amino acids, purine base, pyrimidine base and hydro-soluble vitamin B group. | LG-YE | 20 | 3.56 |
| Nutrient broth (Merck®/#70149) | peptone (5 g $L^{-1}$), sodium chloride (5 g $L^{-1}$), beef extract (1.5 g $L^{-1}$) and yeast extract (1.5 g $L^{-1}$). | NB | 13 | 1.59 |
| Luria broth (Merck®/#L3522) | tryptone (10 g $L^{-1}$), sodium chloride (10 g $L^{-1}$) and yeast extract (5 g $L^{-1}$). | LUB | 25 | 4.09 |
| Tryptic Soy Broth (BD DIFCO™ / #211825) | pancreatic digest of casein (17 g $L^{-1}$), peptic digest of soybean (3 g $L^{-1}$), glucose (2.5 g $L^{-1}$), sodium chloride (5 g $L^{-1}$) and dipotassium phosphate (2.5 g $L^{-1}$). | TSB | 30 | 3.68 |
| Fluid Thioglycollate Medium (BD BBL™/ #211260) | pancreatic digest of casein (15 g $L^{-1}$), L-cystine (0.5 g $L^{-1}$), anhydrous dextrose (5 g $L^{-1}$), sodium chloride (2.5 g $L^{-1}$), yeast extract (5 g $L^{-1}$), sodium thioglycolate (0.5 g $L^{-1}$), resazurin (5 g $L^{-1}$) and agar (0.75 g $L^{-1}$). | FTM | 29.5 | 5.42 |
| Cooked Meat Medium (BD DIFCO™/ #226730) | heart tissue granules (98 g $L^{-1}$), peptic digest of animal tissue (20 g $L^{-1}$), dextrose (2 g $L^{-1}$) and sodium chloride (2.5 g $L^{-1}$). | CMM | 125 | 134.06 |
| Lactose Broth (Merck®/#70142) | tryptose (20 g $L^{-1}$), lactose (5 g $L^{-1}$), dipotassium phosphate (2.75 g $L^{-1}$), monopotassium phosphate (2.75 g $L^{-1}$), sodium chloride (5 g $L^{-1}$) and sodium lauryl sulfate (0.1 g $L^{-1}$). | LB | 13 | 2.19 |
| Marine Broth (BD DIFCO™/ #2216) | peptone (5 g $L^{-1}$), yeast extract (1 g $L^{-1}$), ferric citrate (0.1 g $L^{-1}$), sodium chloride (19.45 g $L^{-1}$), magnesium chloride (5.9 g $L^{-1}$), magnesium sulfate (3.24 g $L^{-1}$), calcium chloride (1.8 g $L^{-1}$), potassium chloride (0.55 g $L^{-1}$), sodium bicarbonate (0.16 g $L^{-1}$), potassium bromide (0.08 g $L^{-1}$), strontium chloride (0.034 g $L^{-1}$), boric acid (0.022 g $L^{-1}$), sodium silicate (0.004 g $L^{-1}$), sodium fluoride (0.0024 g $L^{-1}$), ammonium nitrate (0.0016 g $L^{-1}$) and disodium phosphate (0.008 g $L^{-1}$). | MB | 37.4 | 7.83 |

[a] Quantity of all the media added except for FG-YE were based on manufacturer's instruction.
[b] All growth media used in this study were produced in Malaysia. Exchange rate MYR 4.15 = US$ 1.0.

were first transferred into newly sterilized falcon test tubes (50 mL) before they were sent to Quasi-S Sdn. Bhd. Malaysia for XRD analytical service. The XRD spectra were obtained using X-ray Diffractometer X'Pert$^3$ Powder XRD equipment (Panalytical) scanning the fine-powdered samples from 5° to 80° 2θ with Cu anode having 40 kV and 35 mA running parameters. The analysed components of the precipitated samples were identified by comparing them with standards established by the International Centre for Diffraction Data (Al-Salloum et al., 2017; Navdeep Kaur Dhami et al., 2017a).

### 2.10. Statistical analysis

All the experiments in this work were carried out in triplicates. The experimental results were analysed using the statistical analysis software GraphPad Prism® (version 7) to determine the significance of differences between the means 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. Results and discussion

### 3.1. Evaluating FG-YE medium

The growth and pH profiles of *S. pasteurii* NB28(SUTS) which was studied up to 60 h at 30 °C in both FG-YE and LG-YE medium yielded similar trends in relation to their respective OD (Fig. 1). LG-YE had a constant growth rate of 0.04 $h^{-1}$ and a doubling time of 16.96, while

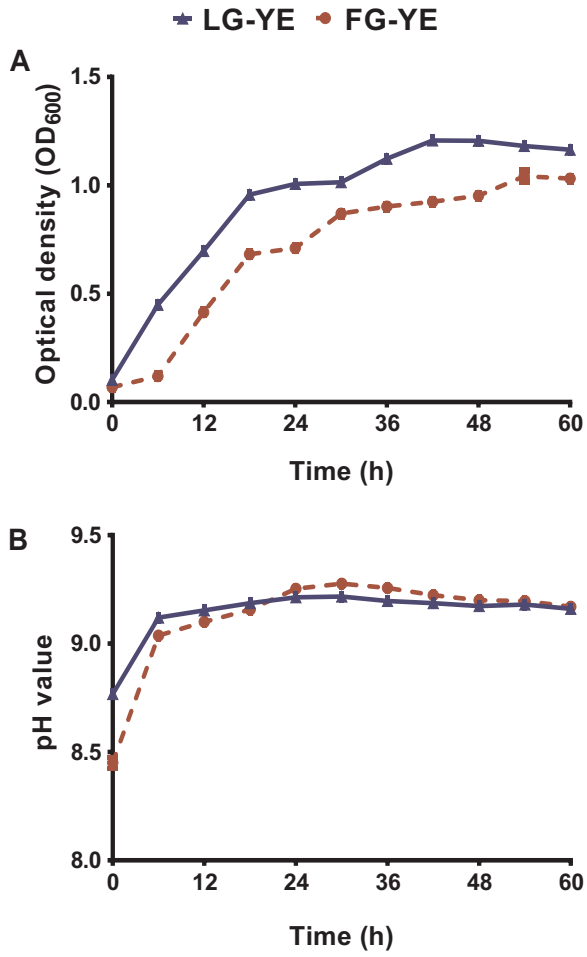


**Fig. 1.** The growth profile (A) and pH profile (B) of *S. pasteurii* NB28(SUTS) cultivated in LG-YE (20 g $L^{-1}$) and FG-YE (20 g $L^{-1}$).

FG-YE a constant growth rate of 0.05 $h^{-1}$ and a doubling time of 14.23 h at the end of the growth study. Noteworthily, the maximum OD value (1.21) for LG-YE occurred at 42 h incubation period, while that of FG-YE occurred at 54 h incubation period with OD of 1.04. The pH profile of the ureolytic bacteria in both media showed a simultaneous increase towards alkaline level as the growth profile increased which is an indication of ureolysis process. The final pH values of the bacterial culture in LG-YE and FG-YE medium were 9.16 and 9.17, respectively. There were noticeable slight differences between the growth profile of the ureolytic bacteria when grown LG-YE and FG-YE, it is possible this could be influenced by the yeast extract components such as maltodextrin food additive and salt contents present in the FG-YE medium. Yeast extract typically contains abundant of amino acids, peptides, carbohydrates, salts, vitamins and minerals which are necessary for microbial cell cultivation (Li et al., 2011). Commercially manufactured yeast extract for bakery and cooking purposes often contains other ingredients such as flavouring agent in soup, snacks and canned foods for food industry containing (Milić et al., 2007). Nevertheless, the growth and pH profile showed that the ureolytic bacteria could adapt in the low-cost media.

### 3.2. Effect of media concentration

For mass production of ureolytic bacteria, it is important to use inexpensive media which can support bacterial growth and high urease activity (Cuzman et al., 2015b). In order to optimize biomass and urease production in the inexpensive FG-YE to favour cost reduction, several cultures of the ureolytic bacteria were grown with different concentrations (5–25 g $L^{-1}$). It is important to note that some low-cost media are often subjected to lower quality control and reproductivity, hence its effect on urease production should be considered (Cuzman et al., 2015b). The results in Fig. 2**A** and Fig. 3 showed that the respective maximum OD (1.02) and urease activity (20.16 mM urea hydrolysed.$min^{-1}$) for the ureolytic bacteria in the FG-YE medium containing urea was produced in 15 g $L^{-1}$ media concentration, while 5 g $L^{-1}$ produced the lowest OD (0.87) and urease activity (10.43 mM urea hydrolysed.$min^{-1}$). On the other hand, the maximum and lowest OD (1.56 and 1.15) for the bacteria in the FG-YE medium without urea (Fig. 2**B**) were respectively produced in 10 g $L^{-1}$ and 5 g $L^{-1}$ media concentrations, while the maximum and lowest urease activity (8.72 and 6.92 mM urea hydrolysed $min^{-1}$) were respectively produced in 20 g $L^{-1}$ and 5 g $L^{-1}$ as shown in Fig. 3. ANOVA and Tukey-Kramer post hoc test showed there were significant differences on the effect of media concentration among the group means when the bacteria were grown in FG-YE medium containing urea (P-value = 3.49E-4) and without urea (P-value = 2.13E-9). Additionally, the statistical analysis for urease activity also showed significant differences among the group means when cultured in FG-YE medium with urea (P-value is 1.26E-5) and no urea (P-value = 3.41E-6).

### 3.3. Effect of initial pH medium

The growth, pH and urease activity of the ureolytic bacteria studied in an FG-YE medium having different initial pH medium is shown in Fig. 4. pH 8.5 had the highest OD (1.43) and urease activity (22.84 mM urea hydrolysed.$min^{-1}$), but when the initial pH medium was at 11, it had the highest pH value (9.41) which occurred at the end of the incubation period. On the other hand, the lowest pH values (9.14) and urease activity (14.78 mM urea hydrolysed $min^{-1}$) were observed when the initial pH medium was at pH 5.0 with the lowest OD of 1.15. The initial pH of the culture media has a huge effect on the growth of microbes, for most bacteria there is a correlation as the growth rate increases, the pH approaches its optimum value (Basu et al., 2015). These results showed that *S. pasteurii* NB28(SUTS) strain could grow and produce urease when FG-YE was adjusted to acidic or alkaline conditions but for maximum biomass and urease activity, the initial pH

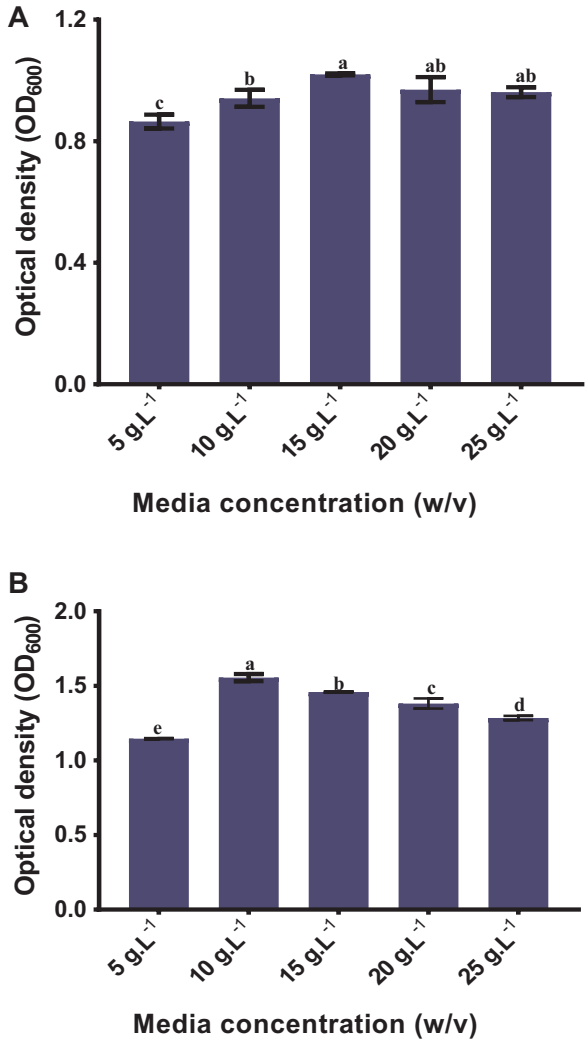


**Fig. 2.** Influence of the low-cost media concentration with urea (A) and without urea (B) on biomass production. Vertical error bars indicate the means ± SE of three independent repetitions. The alphabets indicate statistically significances ($P < 0.05$) as determined by one-way analysis of variance (ANOVA) with means comparison using Tukey-Kramer post hoc test.

medium has to be set at 8.5. This finding is supported by other studies which have indicated that pH 8–9 are the optimum conditions to produce good levels of urease activity (Bang et al., 2001; Stocks-Fischer et al., 1999). It was also noticed that at the end of each incubation period, despite adjusting the initial pH medium of the broths between 5 and 11, the bacteria were able to regulate the micro-environment to its near preferred optimum pH value. It is possible that the ureolytic bacteria was able to secrete different compounds which allowed it to alter the growth medium's pH, hence to a much-favoured pH state. Bacteria are able to manipulate their environment to maintain a certain cytoplasmic pH which correlates with their optimal functional and structural integrity (Padan et al., 2005). The statistical analysis for biomass production (P-value is = 0.00), pH value (P-value is = 0.00) and urease activity (P-value is = 1.51E-16) on the effect of different initial pH medium showed that there were significant differences between the group means.

### 3.4. Comparison with laboratory-grade media

To further determine the suitability of using the FG-YE media as an alternative nutrient source, eight standard growth media were selected and tested for comparison with the low-cost FG-YE media. The result in

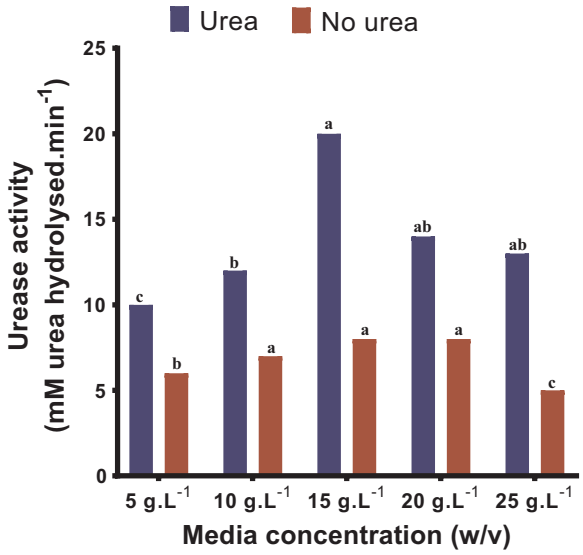


**Fig. 3.** Influence of the low-cost media concentration with and without urea on urease activity. The alphabets indicate statistically significances ($P < 0.05$) as determined by one-way analysis of variance (ANOVA) with means comparison using Tukey-Kramer post hoc test.

Fig. 5 showed that among all the growth media used to cultivate the bacteria, TSB has the highest OD (1.07) for biomass production while MB had the lowest OD (0.81). In addition, CMM was shown to have the highest pH value (9.25), while MB had the lowest pH value (9.08). The result also showed that TSB had the highest urease activity (18.71 mM urea hydrolysed.min$^{-1}$), while LB had the lowest urease activity (10.85 mM urea hydrolysed.min$^{-1}$). FG-YE had comparable biomass (0.99), pH (9.20) and urease activity (14.77 mM urea hydrolysed.min$^{-1}$) with these eight selected standard media under the same growth conditions. The statistical analyses showed there were significant differences for biomass production (P-value is = 1.118E-8), pH values (P-value is = 8.806E-6) and urease activity (P-value is = 2.175E-12) when the group means were compared between each other.

In terms of cost as shown in Table 1, FG-YE media was the cheapest (0.29 US$ per litre) media, while CMM was the most expensive (143.43 US$ per litre) media. By using the FG-YE media for the cultivation of *S. pasteurii* as a replacement to conventional microbiological media, the bacterial production cost which was significantly reduced ranged between 82.80% and 99.80%. Although the cost of laboratory-grade growth media is excessively expensive when considered for large-scale cultivation of ureolytic bacteria (Cheng and Cord-Ruwisch, 2013). The low-cost FG-YE media which is commercially available and sold for US $18.00 per kilogram makes it remarkably affordable considering that the laboratory-grade growth media used in this current study cost between US$122.00–1073.00 per kilogram. Clearly, the use of MB media to cultivate ureolytic bacteria for MICP applications is impracticable due to its extreme high cost, but the use of other laboratory-grade media is often common, particularly TSB, NB and AG-YE media. For mass production of ureolytic bacteria, it is important to use inexpensive media which can support bacterial growth and high urease activity (Cuzman et al., 2015b). In order to perform biocalcification treatment of building materials, the price of growth medium and nutrients to cultivate ureolytic bacteria for the concentration of 2–3 g per meter square have been estimated to cost up to US$240.00 per kilogram (De Muynck et al., 2010). Hence, the use of low-cost growth media at a concentration of 15 g L$^{-1}$, high level of urease activity and bacterial production are feasible with the prospect of replacing laboratory-grade growth media.

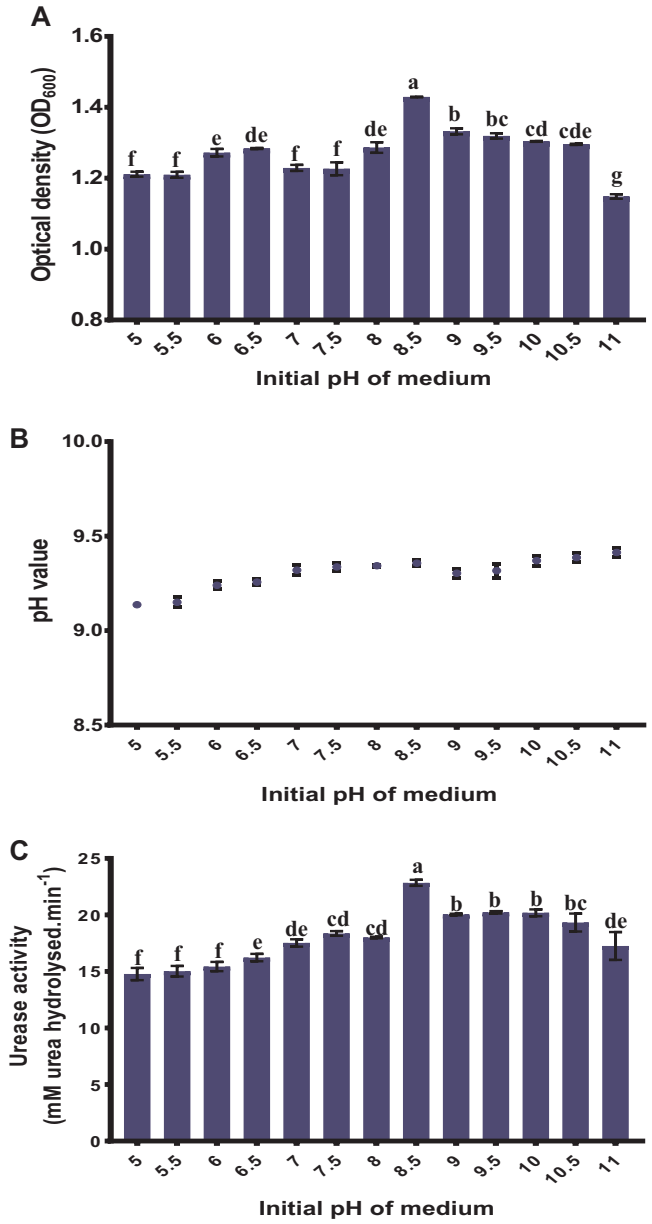


**Fig. 4.** Influence of various initial pH medium on the biomass production (A); pH (B) and urease activity (C) for S. pasteurii NB28(SUTS). Vertical error bars indicate the means ± SE of three independent repetitions. The alphabets indicate statistically significances ($P < 0.05$) as determined by one-way analysis of variance (ANOVA) with means comparison using Tukey-Kramer post hoc test.

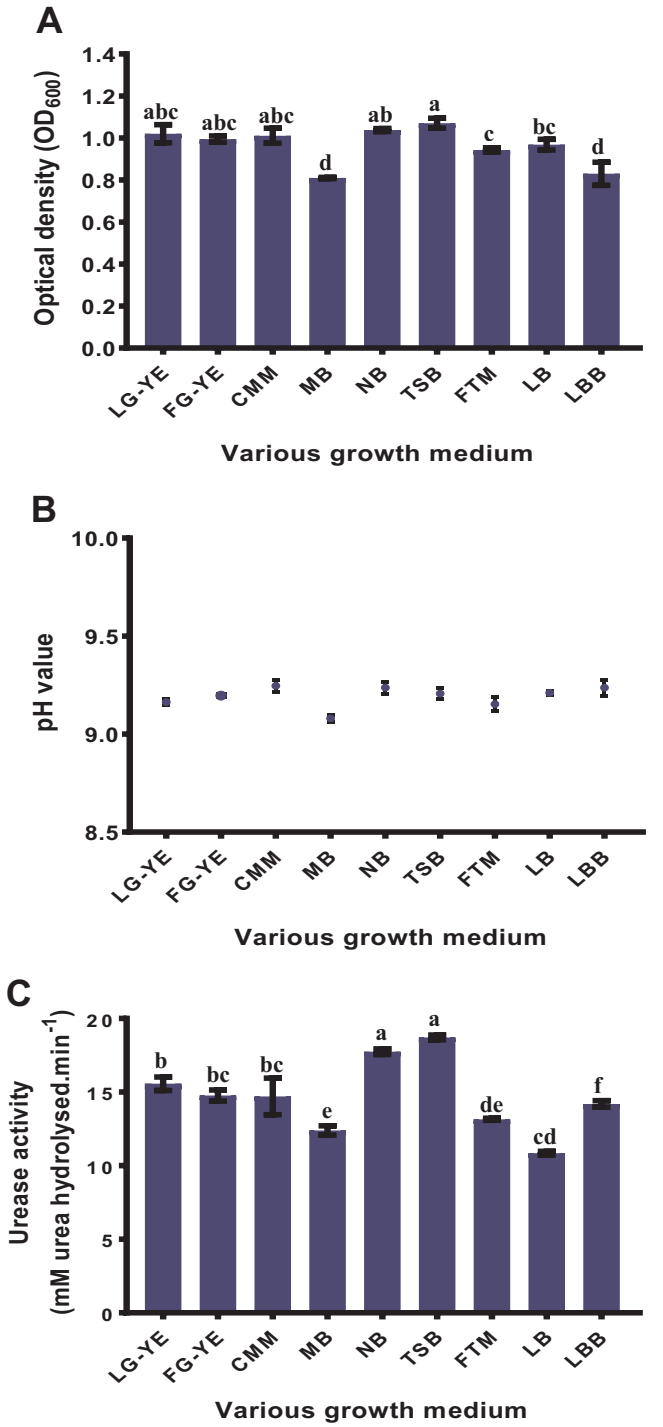


**Fig. 5.** Comparison of different selected laboratory-grade growth media with the food-grade yeast extract media for their respective performances on growth (A); pH (B) and urease activity (C). Vertical error bars indicate the means ± SE of three independent repetitions. The alphabets indicate statistically significances ($P < 0.05$) as determined by one-way analysis of variance (ANOVA) with means comparison using Tukey-Kramer post hoc test.

### *3.5. Biomineralization test*

Carbonate crystals were precipitated by the ureolytic bacteria in all the cementation solutions which contained different growth media as part of essential cementation reagents required to induced calcification after 72 h at 32 °C. As shown in Fig. 6, despite the similarity of the weight contents of $CaCO_3$ constituted by the different cementation solution, MB (6.76 g $L^{-1}$) produced the highest, while LB (2.80 g $L^{-1}$) produced the lowest precipitate. The effluents were collected and measured for pH readings showed that TSB (8.06) had the highest pH value after the biocalcification process, while NB (6.84) showed to have the lowest pH value. In addition, prior to the biocalcification test, the measured pH values for the cementation solutions showed that TSB (5.30) also had the lowest reading, while CMM (7.09) had the highest reading. The FG-YE had comparable results with the rest of the samples having 5.79 g $L^{-1}$ of the estimated $CaCO_3$ precipitates and effluent with pH 7.61. The statistical analysis showed significant differences (P-value is = 1.536E-12) among the group means.

MICP via urea hydrolysis is a straightforward biochemical process which is regulated by the concentration of calcium ion, the concentration of dissolved inorganic carbon, the pH of the

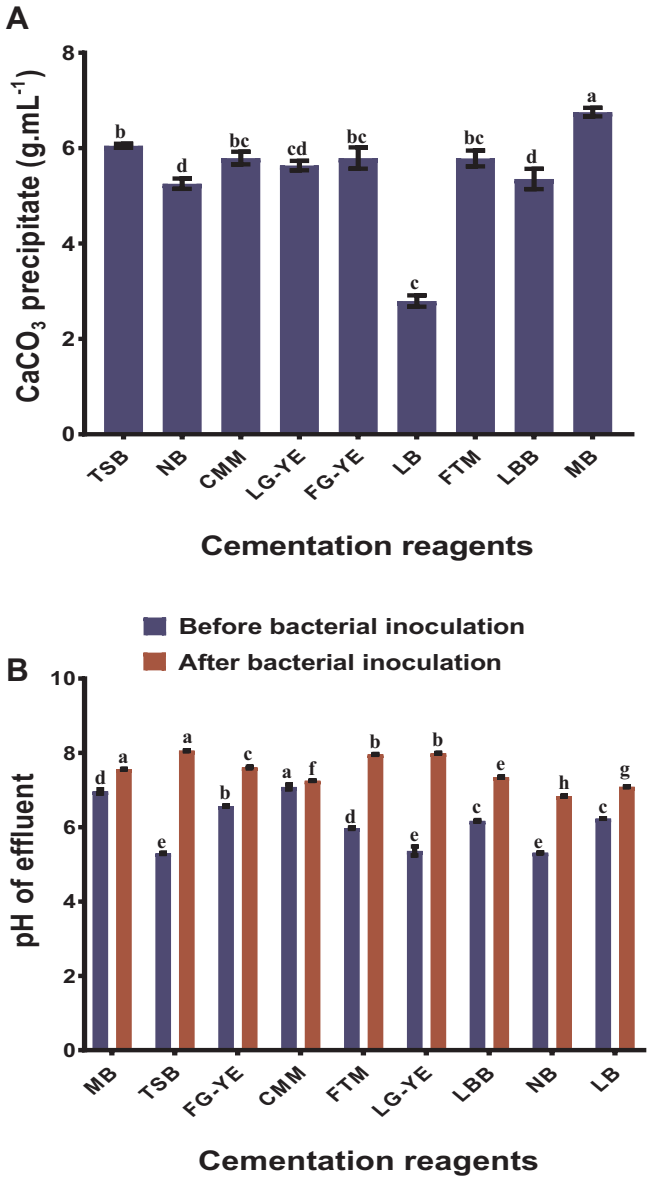


**Fig. 6.** The measurement of calcium carbonate contents (A) and pH of effluents (B). The biocalcification test was performed with cementation solutions containing cementation solutions supplemented with different growth media and were incubated at 32 °C for 24 h incubation without shaking. Vertical error bars indicate the means ± SE of three independent repetitions. The alphabets indicate statistically significances ($P < 0.05$) as determined by one-way analysis of variance (ANOVA) with means comparison using Tukey-Kramer post hoc test.

microenvironment and availability of nucleation sites (Hammes and Verstraete, 2002). These factors play essential roles which favour $CO_3^{-2}$ during the ureolysis-driven process. High bacterial cells concentration (0.8–1.2 OD) helps to enhance calcite deposits during the MICP process because the higher cells increase urease concentration for urea hydrolysis (Langdon et al., 2000). In this study, the $CaCO_3$ induced by the ureolytic bacteria in the cementation solution containing FG-YE was similar to other standard media which indicates that the low-cost media can serve as alternative reagent growth media in the cementation solution. The results showed that none of the growth media affected carbonate precipitation because they all allowed the precipitation of $CaCO_3$. The measured effluents indicated the presence of urease activity after the biocalcification test because there was an increase in the pH of all cementation solution from acidic to an alkaline level. The pH levels of ureolytic bacteria's microenvironment are often increased due to the release of ammonium ions during urea hydrolysis.

### 3.6. XRD analysis

The four-powder crystal precipitated samples which were selected and tested for XRD analysis showed that the most intensive peaks were clearly observed at the angle of 29.8° 2θ as shown in Fig. 7. Calcite was the only $CaCO_3$ polymorph detected in each of the precipitated samples tested except for FG-YE which indicated the precipitates had calcite (94.9%) and vaterite (5.1%) polymorphs of $CaCO_3$. The XRD analysis also indicated that the intensity of the peaks for the minerals differed for each sample *at* the angle of 29.8° 2θ. TSB sample had the highest XRD spectrum with an intensity reaching up to 3400, while NB sample showed the lowest spectrum having an intensity of 1200. It's been suggested that different morphological $CaCO_3$ crystals formed are often influenced by urease activity by strain-specific microorganisms and alternatively the extracellular polymeric substance (EPS) protein produced by different microbial strains (Hammes et al., 2003; Park et al., 2010). This is because EPS is known to specifically bind with $Ca^{2+}$ and induce the type of $CaCO_3$ polymorphs crystals which will the precipitated (Dhami et al., 2013a, 2013b).

The XRD analysis for crystal minerals precipitated by ureolytic bacteria in cementation solutions separately containing TSB, NB and AG-YE were selected alongside low-cost media because they are often used as part of cementation reagents for MICP studies (Al-Salloum et al., 2017). Initially, the current authors hypothesized that $CaCO_3$ polymorphism might be affected when the FG-YE is utilized as a cementation reagent. Thus, it was interesting that our result showed that the low-cost media induced both calcite (94.9%) and vaterite (5.1%) polymorphs, while all the standard growth media all had only calcite. The result also showed that irrespective of the growth media used, calcification will still occur and the formation of stabilized calcite might not significantly be affected if food-grade media is used. Previous studies have demonstrated that $CaCO_3$ polymorphs can be influenced by the composition of growth medium, substrate, pH and type of microbes (Dhami et al., 2013a, 2013b; Rodriguez-Navarro et al., 2012). This is because the ionic strength of different growth media affects ureolytic bacteria in manners that supports or distress formation of monohydrocalcite (Bansal et al., 2016). The results in this present study thus strongly indicate that the FG-FE is capable of cultivating ureolytic bacteria and can be used to induce $CaCO_3$ precipitates for biocement applications.

It is desirable to precipitate calcite mineral as it is the most thermodynamically stable polymorph of $CaCO_3$ when compared to other less stable and less abundant polymorphic forms of $CaCO_3$ (aragonite and vaterite) (Boulos et al., 2014). The formation of vaterite is very rare but elevated temperature or water rich in NaCl can accelerate its transformation (Skinner and Jahren, 2007). Our current research study intended to achieve high calcite formation using FG-YE as part of cementation reagents used for MICP treatment. XRD analysis showed that low percentage of vaterite were performed alongside calcite when FG-YE is used as part of cementation reagents for biomineralization test. It is suggested that this trace amount of vaterite could have been stimulated by the salt contents present in the FG-YE media. Carbonate polymorphs have a high significance on the proficiency of biocementation due to their varying characteristics of the polymorphs such as stability and durability (Dhami et al., 2017a). Calcite enhances the mechanical strength properties of soils via MICP process better than aragonite and vaterite (Dhami et al., 2016). Hence, it is often preferred to have calcite contents for soil stabilization. However, the presence of low vaterite amount with high calcite contents after biocementation might not have a significant effect on strength improvement of soils. A recent study by Dhami et al. (2017b) have shown vaterite precipitation alongside calcite after MICP treatment on loose soils and the presence of vaterite formations did not impede soil solidification. Thus, the use of

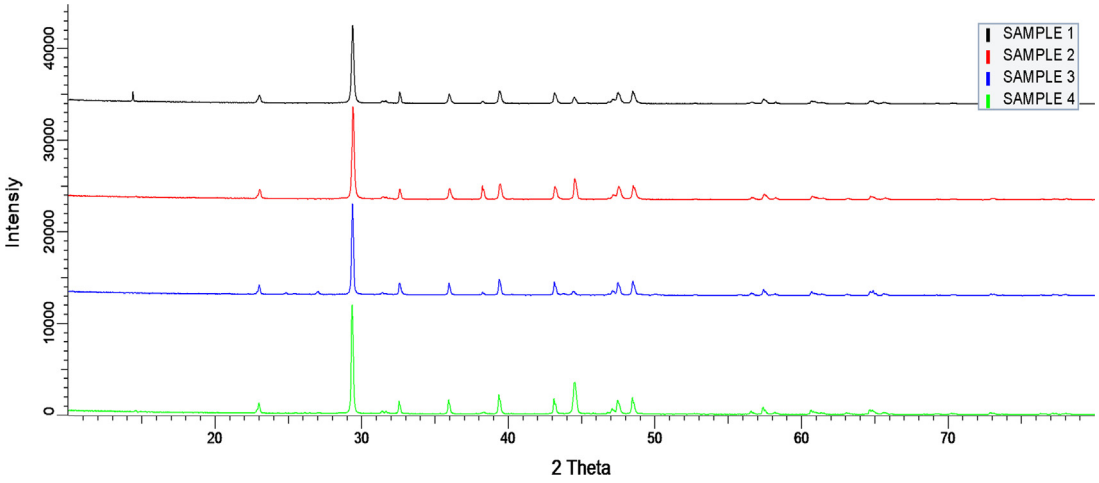


**Fig. 7.** X-Ray Diffraction analysis of four selected precipitated samples obtained after ureolysis process. Sample 1 (TSB); Sample 2 (AG-YE); Sample 3 (FG-YE) and Sample 4 (NB).

FG-YE media for bacterial cultivation and $CaCO_3$ precipitation might not be disadvantageous at upscaling stage of MICP application.

## 4. Conclusions

In this present study, the feasibility of cultivating *S. pasteurii* NB28(SUTS) strain in food-grade yeast extract medium was investigated. The results obtained from this report showed the food-grade yeast media was a good alternative source of nutrients for low-cost bacterial cultivation in a growth medium which can support the biomass, urease activity and biomineralization of $CaCO_3$. For the promotion of maximum biomass and urease activity in the inexpensive media, the effect of different media concentration and initial pH medium were examined. The concentration of 15 g $L^{-1}$ and initial pH 8.5 produced the highest biomass and urease activity when the bacterial was cultivated in the media supplemented with urea (4%, w/v). The urease activity was significantly better when the food-grade yeast extract medium was supplemented with urea (4%, w/v) than without urea. When the low-cost medium was compared with laboratory-grade growth medium which is commonly used for bacterial cultivation, tryptic soy broth had the highest values while marine broth and luria broth both had the lowest values for the obtained biomass and urease activity, respectively. The media comparison as a key reagent in cementation solution for calcification showed that marine broth had the highest estimated carbonate precipitate, while luria broth had the lowest value. The food-grade growth medium showed comparable results to the laboratory-grade media for biomass production, pH, urease activity and $CaCO_3$ precipitates. In addition, the results also showed that food-grade yeast extract medium could help reduce the bacterial cultivation cost up to 99.80% when compared with the conventional growth media. This can tremendously be useful for large-scale cultivation of ureolytic bacteria. XRD analysis used to identify the polymorphs of $CaCO_3$ showed that the tested precipitated samples from the three laboratory-grade media were calcite, while that of the food-grade media were calcite (94.9%) and vaterite (5.1%).

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bcab.2018.11.030.

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