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Original article

Fertilizer-Grade Urea and Calcium Enable Cost-Effective $CaCO_3$ Precipitation by *Sporosarcina pasteurii* without Compromising Performance

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Abstract

In laboratory scale studies, analytical-grade urea and calcium sources are used to induce microbial $CaCO_3$ precipitation. However, their application at industrial scale becomes economically impractical due to high material costs. This study evaluated the feasibility of using economical fertilizer-grade urea and calcium sources as substitution of analytical-grade chemicals for $CaCO_3$ precipitation by *S. pasteurii*. Experimental approaches included bacterial subculture, $CaCO_3$ precipitation tests using different combinations of urea and calcium sources, and using various concentrations of fertilizer-grade urea by measuring $CaCO_3$ weight, pH, and ammonium concentration. The results demonstrated that fertilizer-derived materials produced comparable, and in some cases higher, $CaCO_3$ precipitate weights (0.552 g/50 mL) relative to analytical-grade treatments. Increasing fertilizer-grade urea concentration resulted in proportional increases in $CaCO_3$ formation, accompanied by similar pH values and elevated ammonium production. These findings suggest that fertilizer-grade materials can serve as cost-effective alternatives for MICP and may support more scalable industrial applications.

Keywords: ureolytic bacteria, $CaCO_3$ precipitation, fertilizer-grade materials

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Introduction

Microbially induced calcium carbonate precipitation (MICP) is a biologically mediated mineralization process in which microorganisms promote the formation of calcium carbonate ($CaCO_3$). This technology has gained increasing attention because of its potential applications in soil stabilization, biocementation, crack repair, self-healing concrete, and environmental remediation (Attri *et al.*, 2021; Fouladi *et al.*, 2024; Jogi & Lakshmi, 2021). In ureolytic MICP, $CaCO_3$ formation is mainly driven by microbial urease activity, which hydrolyzes urea and generates alkaline conditions favorable for carbonate formation and subsequent precipitation in the presence of calcium ions (Novanti *et al.*, 2019; Omoregie *et al.*, 2021; Su *et al.*, 2021).

Among ureolytic bacteria, *Sporosarcina pasteurii* is one of the most widely studied microorganisms for MICP because of its strong urease activity and ability to induce $CaCO_3$ precipitation. During urea hydrolysis, ammonia and carbonate-related compounds are produced, leading to an increase in pH and carbonate availability. The carbonate compounds then react with Ca^{2+} to form $CaCO_3$ precipitates. Therefore, urea and calcium sources are key substrates that directly influence the efficiency of the MICP process (Kim *et al.*, 2025; Omoregie *et al.*, 2021).

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Most laboratory-scale MICP studies rely on analytical-grade urea and calcium salts because these chemicals provide consistent purity and reproducible experimental conditions (Maulas *et al.*, 2024; Mauludin *et al.*, 2024; Okwadha & Li., 2010). However, this practice becomes less practical when MICP is projected for larger-scale applications, where large volumes of cementation solution are required. In the Indonesian market, fertilizer-grade urea and calcium sources may cost up to approximately 100 times less than their analytical-grade counterparts. This substantial price difference highlights the need to explore lower-cost substrates that can maintain $CaCO_3$ precipitation performance while improving the economic feasibility of MICP. Therefore, this study aimed to evaluate the use of alternative fertilizer-grade materials as substitutes for analytical-grade urea and calcium sources by assessing $CaCO_3$ precipitation, pH values, and ammonium concentrations. The results are expected to provide a more economical and technically feasible approach to support industrial-scale applications of MICP.

Material and Methods

Bacterial Subculture and Purity Confirmation

This study used *Sporosarcina pasteurii* InaCC B342 obtained from the National Research and Innovation Agency of Indonesia (BRIN), which is capable of producing urease and precipitating $CaCO_3$. Bacterial subculture was performed on Urea Mineral Agar (UMA) medium consisting of (g/L): 0.2 $MgSO_4.7H_2O$ (Merck); 0.02 $CaCl_2$ (Merck); 5 NaCl (Merck); 1 yeast extract (Oxoid), 20 urea (Merck), 0.012 phenol red, and 15 agar bacteriological (Oxoid). The medium preparation was

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carried out in two stages (Jonkers *et al.*, 2010; Phang *et al.*, 2018). The basal components were dissolved, pH adjusted to 6.9-7.0, and autoclaved at 121°C for 15 minutes, while urea was sterilized separately through a 0.2 µm membrane filter to prevent thermal degradation (Dhami *et al.*, 2013). Both solutions were mixed aseptically in a laminar airflow cabinet just before the agar solidifies and were then poured into sterile Petri dishes. The bacterial isolate was thawed from a -80°C glycerol stock, transferred onto UMA plates, and incubated for four days at room temperature (25-27 °C). Ureolytic activity was monitored by the color change of phenol red from red (neutral pH) to magenta (alkaline pH) (Subramaniyan *et al.*, 2023). Isolate purity was confirmed through serial dilution up to 10^{-5}, spread plate culture, Gram staining, and Schaeffer-Fulton endospore staining (Jamshad *et al.*, 2024). Microscopic observation at 1000x magnification was performed to confirm cell morphology and isolate purity.

$CaCO_3$ Precipitation Test with Different Urea and Calcium Sources

This test was conducted to evaluate the potential of fertilizer-grade urea and calcium sources as alternatives to analytical-grade chemicals in $CaCO_3$ precipitation. Urea Mineral Broth medium (UMB) was prepared similarly to UMA, but without the addition of phenol red and bacteriological agar. Starter culture was made by inoculating bacterial colony into 20 mL of UMB and incubated for four days. The entire culture volume was then transfered into 180 mL of UMB and incubated for another four days. Cell density was measured at OD_{600} and adjusted to 10^6 CFU/mL based on a previously prepared standard curve. The precipitation test was performed by inoculating 5 mL of the adjusted bacterial culture into 45 mL of UMB containing a calcium source in a 100 mL glass bottle. In this stage, the UMB medium was prepared using two different urea sources: analytical-grade urea (Merck) and fertilizer-grade urea (Petrokimia, 97-98% purity). The calcium sources used were analytical-grade calcium acetate (Merck) and fertilizer-grade calcium nitrate (Meroke-CALNIT, 80-90% purity). The amount of each calcium source added per liter of UMB medium was 28.5 g. Based on these materials, four treatment combinations were prepared: U-C (analytical-grade urea – analytical-grade calcium), UF-C (fertilizer-grade urea – analytical-grade calcium), U-CF (analytical-grade urea – fertilizer-grade calcium), and UF-CF (fertilizer-grade urea – fertilizer-grade calcium), each with three replicates.

After a 7-day incubation at room temperature (25–27 °C), $CaCO_3$ precipitates observed as white deposits were harvested. Precipitates that strongly adhered to the inner surface of the glass bottles was detached using a sonicator (Bandelin) for 30 minutes. The resulting suspension was then filtered through Whatman No.42 filter paper and dried at 60 °C for 3 hours (Krishnapriya *et al.*, 2015). The weight of dried precipitate retained on the filter paper was measured. Subsequently, the liquid filtrate passing through the filter paper was analyzed for pH and ammonium concentration. The pH was measured using a pH meter, whereas ammonium concentration was determined using a Sera Ammonium Test Kit based on the indophenol blue reaction principle, with absorbance measured at 700 nm using a spectrophotometer. Prior to analysis, the samples were diluted 1:100, and the final ammonium concentration was calculated using a standard curve. The treatment producing the highest precipitate yield was selected for further analysis. To assess economic advantage of using fertilizer-grade urea and calcium source over analytical-grade chemicals, the cost per gram of $CaCO_3$ produced was calculated by dividing the total material cost used in 50 mL of medium by the mass of $CaCO_3$ precipitated in each treatment.

$CaCO_3$ Precipitation Test at Various Concentrations of Urea Fertilizer

This test was conducted to evaluate the effect of fertilizer-grade urea concentration on ureolytic activity. The assay was performed using bacterial culture in UMB medium, following the procedure described in the previous $CaCO_3$ precipitation test. In this stage, Petrokimia fertilizer-grade urea was used as the sole urea source and was tested at concentrations of 0, 10, 15, 20, 25, and 30 g/L. MerokeCALNIT calcium nitrate was used as the calcium source at a fixed concentration of 28.5 g/L. Each treatment was performed in triplicate. The weight of the formed $CaCO_3$ precipitate, as well as the pH and ammonium concentration, were measured.

Data Analysis

The data were processed using Microsoft Excel 2021, and statistical analyses were performed via R software (v4.4.0) and RStudio (v2024.12.1). Following normality (Kolmogorov-Smirnov) and homogeneity (Levene's) tests, data were subjected to ANOVA. Significant treatment differences, illustrated by letter groupings in the figures, were isolated using Tukey's HSD or Games-Howell post-hoc tests for homogenous and non-homogenous variances, respectively. Graphical results include error bars representing standard deviation (SD).

Results and Discussion

Purity Confirmation of *S. pasteurii* Isolate

S. pasteurii obtained from glycerol stock was subcultured on Urea Mineral Agar (UMA) medium and exhibited visible colony growth after 48 hours of incubation (Figure 1). Its ureolytic activity, indicated by a color change of the medium to magenta, was observed within 24 hours. This rapid response reflects strong urea hydrolysis, leading to the release of alkaline compounds. The alkaline condition occurs due to the accumulation of hydroxide ions (OH^-) when ammonia (NH_3), released during urea hydrolysis, reacts with water molecules to form ammonium (NH_4^+) and (OH^-) (Maulas *et al.*, 2024).

Gram staining revealed that *S. pasteurii* cells were bacillus-shaped and appeared purple under 1000× microscopic magnification (Figure 2), confirming that the isolate is a Gram-positive bacterium as previously reported (Fouladi *et al.*, 2024). The retention of the purple stain is attributed to the thick peptidoglycan layer in the cell wall, which effectively retains the crystal violet-

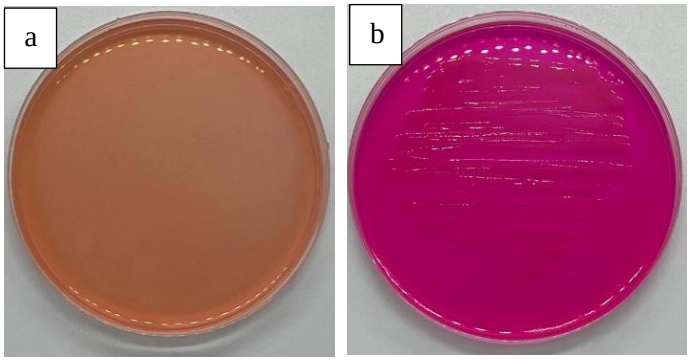


Fig. 1. Confirmation of color change in UMA medium: (a) uninoculated medium and (b) medium inoculated with *S. pasteurii*

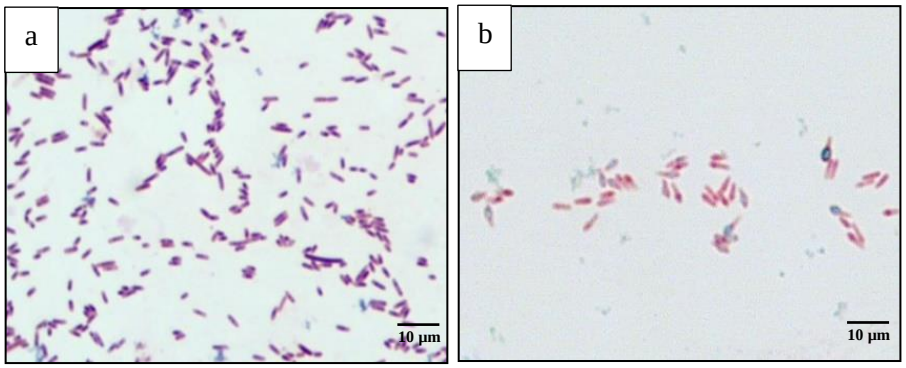


Fig. 2. Staining results of *S. pasteurii*: (a) Gram staining and (b) endospore staining

iodine complex during the staining (Ningsih *et al.*, 2018). Furthermore, endospore staining revealed green-colored endospores located at terminal positions within red-stained vegetative cells, indicating the bacterium's ability to form endospores. This characteristic is associated with increased survival under unfavorable environmental conditions and supports its potential application in MICP-based self-healing concrete systems.

$CaCO_3$ Formation in Response to Different Urea and Calcium Sources

Two types of urea and two calcium sources were evaluated as key components in a ureolytic bacterial system using UMB medium to assess $CaCO_3$ precipitation. All treatments exhibited no visible $CaCO_3$ precipitate on day 0. After 72 h, visible deposits began to form and adhere to the inner surface of the glass bottles. A greater amount of precipitate was observed on day 7, when the $CaCO_3$ precipitates were harvested (Figure 3). In all treatments, most of the $CaCO_3$ were suspended in the medium, while a significant portion adhered strongly to the glass surface and required sonication for detachment. It suggests that $CaCO_3$ formation likely occurred through heterogeneous nucleation on the substrate surface rather than solely in the bulk solution. Surface-mediated nucleation is commonly reported in MICP systems, where mineral formation is facilitated by physicochemical interactions between Ca^{2+} ions and negatively charged surfaces, as well as by microbial cells and extracellular polymeric substances (EPS) that serve as nucleation templates and promote crystal attachment (Stocks-Fischer *et al.*, 1999).

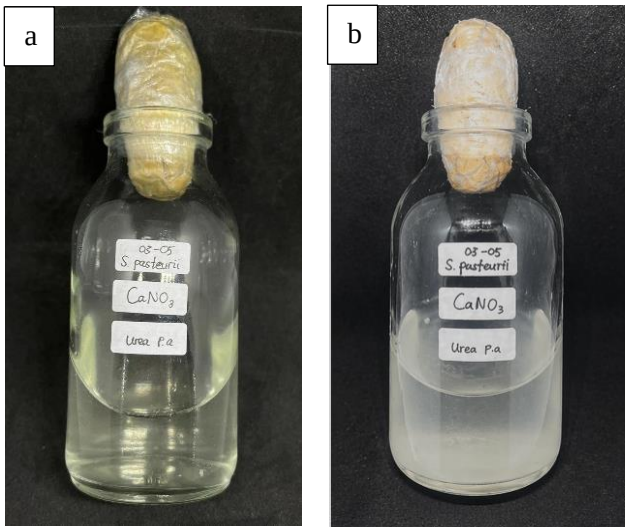


Fig. 3. $CaCO_3$ precipitation in the fertilizer-grade urea and calcium treatment (UF–CF): (a) day 0 and (b) day 7

As shown in figure 4, the amount of $CaCO_3$ precipitate formed varied among treatments. The highest $CaCO_3$ weight was obtained from the combination of fertilizer-grade urea and fertilizer-grade calcium (UF-CF), reaching 0.552 g/50 mL. In contrast, the lowest precipitate production was observed in the combination of fertilizer-grade urea and analytical-grade calcium (UF-C), with a value of 0.200 g/50 mL. These results indicate that the use of fertilizer-grade calcium sources, particularly calcium nitrate, can achieve precipitation performance comparable to or even exceeding that of analytical-grade reagents. The enhanced $CaCO_3$ precipitation observed in the fertilizer-based treatment may be related to additional

compounds or trace minerals present in fertilizer-grade materials, which could influence microbial biomineralization processes. Mineral composition and ionic interactions have been reported to affect carbonate mineral formation in microbial systems (Kim *et al.*, 2025; Pradhan *et al.*, 2025).

The pH measurement at the end of the incubation period showed values ranging from 7.29 to 7.42 (Figure 5). These values were higher than the initial pH, which ranged from 6.9 to 7.0. In ureolytic processes, an increase in pH is expected due to the release of hydroxide ions (OH^-), and pH values resulting from ureolytic activity typically exceed 8.0 (Zhao *et al.*, 2022). However, in calcium-containing systems, part of OH^- ions is consumed during $CaCO_3$ precipitation. The ions promotes the conversion of dissolved CO_2 into bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}), which subsequently react with Ca^{2+} to form $CaCO_3$, thereby limiting further pH increase (Stocks-Fischer *et al.*, 1999).

This interaction between alkalinity generation and carbonate precipitation may explain the pH variation observed among treatments. Based on the results, the fertilizer-based combination (UF–CF) showed the lowest pH value (7.29), whereas the analytical-grade combination (U–C) exhibited the highest pH value (7.42). When compared with the $CaCO_3$ precipitate weight data, the pH values appeared to show an inverse relationship with precipitate formation, where treatments producing higher amounts of $CaCO_3$ tended to show lower final pH values. In the carbonate system, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions contribute to alkalinity and help maintain alkaline conditions (Hammes & Verstraete, 2002). During active $CaCO_3$ precipitation, part of these carbonate species and the associated alkalinity are consumed for mineral formation, which can suppress further pH increase even though the final pH remains above neutral conditions.

The ammonium concentration in the fertilizer-based combination (UF–CF) was nearly comparable to that in the analytical-grade combination (U–C) (Figure 6). From a bacterial perspective, ammonium is required as a nitrogen source for the synthesis of essential cellular components. However, excess ammonium that is not assimilated by bacterial cells may become an environmental concern when produced at large scales (Omoregie *et al.*, 2021). In this study, the ammonium concentration produced in the UF–CF treatment did not exceed that of the analytical-grade combination. These results suggest that the use of fertilizer-grade urea and calcium sources may not increase environmental risks associated with ammonium accumulation burden related to ammonium accumulation compared with analytical-grade materials.

The results of economic assessment in order to compare the cost of using fertilizer-grade chemicals and analytical-grade chemicals in MICP are presented in Table 1. It showed a cost advantage of fertilizer-grade chemicals over analytical-grade chemicals. Although the total material cost for the analytical-grade treatment was IDR 5,854 per 50 mL medium, the fertilizer-grade treatment required only IDR 62 for the same volume. Despite this substantial reduction in material cost, the

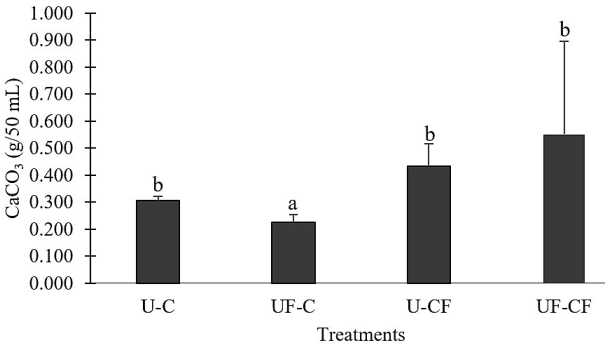


Fig. 4. Weight of $CaCO_3$ precipitated by *S. pasteurii* under different urea and calcium source treatments. Error bars represent the standard deviation (SD) of three replicates. Different letters above the histogram bars indicate significant differences among treatments according to the Games-Howell post hoc test ($p < 0.05$)

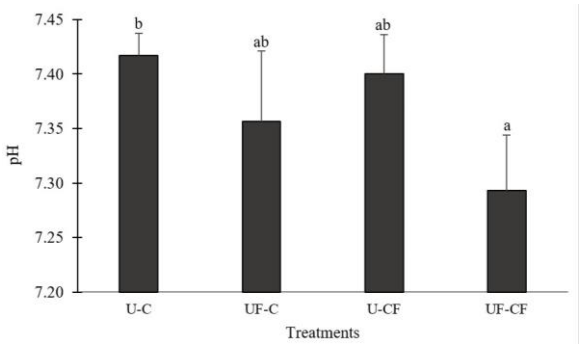


Fig. 5. pH values of the medium after 7 days of incubation under different urea and calcium source treatments. Error bars represent the standard deviation (SD) of three replicates. Different letters above the histogram bars indicate significant differences among treatments according to the Tukey's HSD post hoc test ($p < 0.05$)

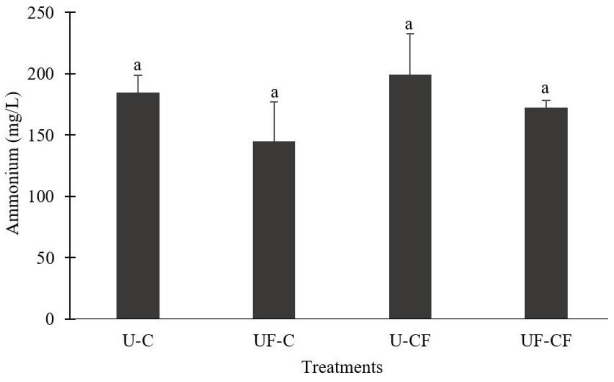


Fig. 6. Ammonium concentrations in the medium after 7 days of incubation under different urea and calcium source treatments. Error bars represent the standard deviation (SD) of three replicates. The same letters above the histogram bars indicate significant differences among treatments according to the Tukey's HSD post hoc test ($p > 0.05$)

Table 1. Cost efficiency and performance of $CaCO_3$ production from analytical-grade and fertilizer-grade chemicals in MICP by *S. pasteurii*

Treatment	Cost (urea + calcium/50 mL)	$CaCO_3$ Yield (g/50 mL)	Cost per g $CaCO_3$
Analytical-grade	IDR 5,854	0.306	IDR 19,129
Fertilizer-grade	IDR 62	0.552	IDR 112

fertilizer-grade treatment produced a higher $CaCO_3$ yield (0.552 g/50 mL) than the analytical-grade treatment (0.306 g/50 mL). This indicates that the lower-cost fertilizer-grade substrates did not compromise $CaCO_3$ precipitation performance.

$CaCO_3$ Formation in Response to Various Concentration of Fertilizer-Grade Urea

Based on the results, the weight of $CaCO_3$ precipitate increased with increasing fertilizer-grade urea concentration. The highest precipitate weight (0.606 g/50 mL) was obtained at the highest urea concentration of 30 g/L (Figure 7). This result indicates that fertilizer-grade urea availability may still act as a limiting factor for $CaCO_3$ formation under the tested conditions. Previous studies using analytical-grade substrates have also shown that urea concentration is one of the important factors controlling ureolysis and microbial carbonate precipitation. Other research reported optimum conditions of 666 mM urea (40 g/L) for microbial carbonate precipitation by *S. pasteurii* (Okwadha & Li, 2010). However, further addition of urea should be carefully considered because they found that increasing urea concentrations beyond certain levels did not necessarily increase $CaCO_3$ yield.

In contrast, pH and ammonium concentrations did not differ significantly among fertilizer-grade urea concentrations, although both showed an increasing tendency (Figure 8 and 9). Alkaline conditions are important in MICP because ureolysis produces ammonium and hydroxide ions, increasing pH and promoting carbonate formation in calcium-rich environments. At pH values below 7, $CaCO_3$ precipitation is generally limited because carbonate ions are less available, with dissolved inorganic carbon predominantly existing as CO_2 and HCO_3^-. Under these conditions, the formation of $CaCO_3$ becomes less favorable, and ureolytic activity may also be reduced. Meanwhile, although ammonium serves as a nitrogen source for bacterial growth, excessive ammonium accumulation may inhibit microbial activity through increased osmolarity, ionic strength, and free ammonia stress, depending on pH and bacterial tolerance (Rajagopal *et al.*, 2013).

Conclusion

The results of this study indicate that fertilizer-grade urea and calcium sources can produce $CaCO_3$ precipitate weights comparable to those obtained using analytical-grade chemicals. The final pH values remained above neutral, supporting alkaline conditions required for $CaCO_3$ precipitation, and the ammonium concentrations were not higher than those produced in the analytical-grade treatment. In addition, fertilizer-grade urea concentration was shown to be a limiting factor in $CaCO_3$ production by *S. pasteurii*. These findings suggest that fertilizer-grade urea and calcium sources have potential as alternative materials for cost-effective MICP applications.

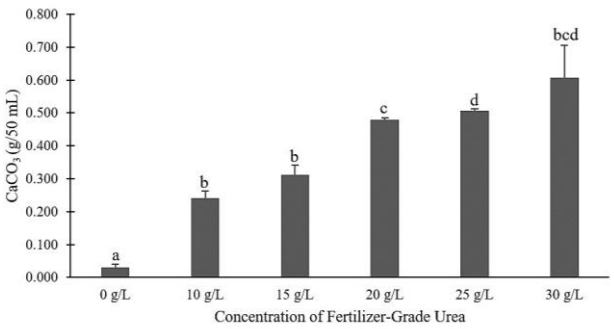


Fig. 7. Weight of $CaCO_3$ precipitated by *S. pasteurii* under different urea concentration treatments. Error bars represent the standard deviation (SD) of three replicates. Different letters above the histogram bars indicate significant differences among treatments according to the Tukey's HSD post hoc test ($p < 0.05$).

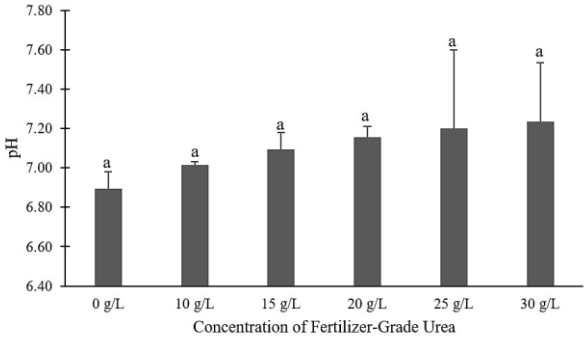


Fig. 8. Medium pH values after 7 days of incubation under different urea concentration treatments. Error bars represent the standard deviation (SD) of three replicates. The same letters above the histogram bars indicate significant differences among treatments according to the Tukey's HSD post hoc test ($p > 0.05$).

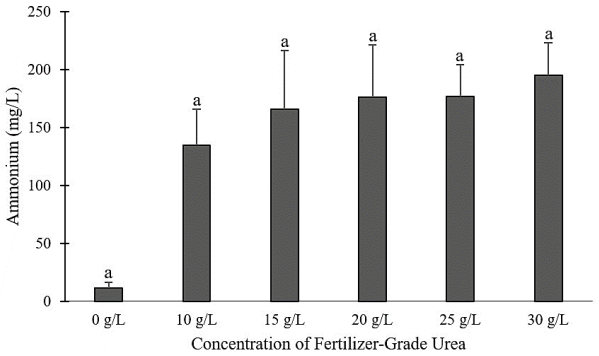


Fig 9. Ammonium levels in the medium after 7 days of incubation under different urea concentration treatments. Error bars represent the standard deviation (SD) of three replicates. The same letters above the histogram bars indicate significant differences among treatments according to the Tukey's HSD post hoc test ($p > 0.05$).

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