

DEVELOPMENT OF SOLIDIFIED CULTIVATING SOIL BLENDED WITH WASTE WOODY BIOMASS PREPARED BY THE CEMENTATION TECHNIQUES

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ABSTRACT: Bio-cementation is well known as an environmentally friendly technique. It is studied for mitigating liquefaction, reducing dust, and increasing ground strength. This technique has been considered for use in the ground, but it is important to reduce ammonia (NH₃) and ammonium ions (NH₄⁺) generation. On the other hand, these by-products have the potential to act as beneficial nutrient sources in agricultural applications. We aim to develop a solidified cultivating soil using generated ammonium ions (NH₄⁺). Additionally, the importation of peat moss, a principal material of solidified cultivating soil, has declined due to the depletion of natural resources, prompting the search for environmentally sustainable alternative substrate materials. In this study, we conducted solidification and germination tests using EICP as a solidification method, which, unlike other bio-cementation techniques, does not require microbial cultivation, and using waste woody biomass such as bamboo powder and coco peat. Additionally, we investigated the collapse index of solidified cultivating soil, as well as the effects of ammonium nitrogen on solidification and germination. The results show that the solidification strength, represented by the collapse index, was enhanced with increased injection frequency at lower cementation concentrations (0.1 M and 0.25 M). At a concentration of 0.5 M, the solidification was sufficiently strong even with four injections. The germination rate increased as the molar concentration of cementation solution decreased. These results suggest that CaCO₃ cementation, when optimized in terms of concentration and injection frequency, holds promise for developing sustainable solidified cultivating soil.

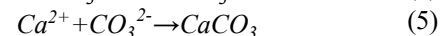
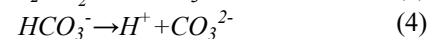
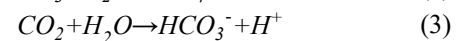
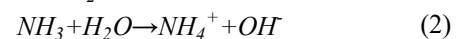
Keywords: Bio-cementation, EICP, NH₄⁺, Bamboo powder, Solidified cultivating soil

1. INTRODUCTION

The labor force in Japanese agriculture continues to decline due to an aging population and a decreasing birthrate. Various countermeasures have been implemented across different sectors to address these issues. In the agricultural sector, smart agriculture has emerged, with the aim of enhancing efficiency and productivity through the integration of advanced technologies [1,2]. These innovations include hydroponic systems and mechanized transplanting, which use growth substrates such as solidified cultivating soil and rock wool. Solidified cultivating soil is primarily composed of organic materials, such as peat moss, and is solidified using thermal bonding fibers [3] or chemical binders. Rock wool is a fibrous material manufactured by melting slag at high temperatures [4]. These substrates exhibit three characteristics: high water permeability and retention capacity, ease of handling and transportation, and low weight. However, this process raises environmental concerns due to substantial CO₂ emissions. Additionally, peat moss, a principal material of solidified cultivating soil, is imported in smaller quantities due to limited natural resources, prompting the need for environmentally sustainable alternative substrate materials.

In our research, we aim to develop a sustainable

form of solidified cultivation soil with minimal environmental impact. Specifically, we focus on a cementation method that utilizes calcium carbonate (CaCO₃) and incorporates waste woody biomass, and the cementation process relies on microbially induced carbonate precipitation (MICP) and enzyme-induced carbonate precipitation (EICP), which are established techniques used in applications such as erosion control [5,6], liquefaction mitigation [7], and slope stabilization [8]. These processes employ microbial or enzymatic activity to promote CaCO₃ precipitation through the following biochemical reactions [9]:



The MICP method is a technique that utilizes specific ureolytic microorganisms, primarily bacteria such as *Sporosarcina pasteurii* [10,11]. The advantages of MICP include high precipitation efficiency and long-term sustainability. However, its primary disadvantage lies in the complexity of the management process, as it requires stringent environmental control over factors such as pH,

temperature, and nutrient sources (e.g., organic matter) [12]. In contrast, The EICP method directly utilizes the urease enzyme extracted and purified from plants, such as jack beans, rather than the microorganisms themselves. The advantages of EICP include easier standardization and simplification of operations compared to MICP, as it eliminates the need for complex equipment and processes for culturing microbes or managing their survival and growth on-site [11]. Furthermore, the rate and amount of CaCO_3 precipitation can be controlled relatively easily by adjusting the concentration of the injected enzyme. Additionally, since no microorganisms are used, EICP reduces secondary environmental risks, such as clogging due to excessive microbial growth or the persistence of residual microbes after treatment. Conversely, the primary disadvantage of EICP is the high cost associated with the extraction and purification of high-purity urease enzymes.

This study selected EICP as the solidification method, which is a cementation technique based on enzyme reactions, for two reasons: it does not require cultivation, and it can utilize waste. Urease is generally extracted from beans such as jack beans and soybeans [11]. Recently, researchers have studied extracting urease from waste materials such as watermelon seeds and pumpkin seeds [13,14]. In the future, we aim to reduce costs by using EICP with urease derived from waste materials.

Regarding waste woody biomass, we used wood materials sourced from Coco peat and Moso bamboo powder to constitute the cultivating soil. Coco peat is a wood material produced by finely crushing coconut husks (Fig.1). It is widely used as a soil conditioner for fields and as a substrate for hydroponic culture. Bamboo powder is produced by chipping felled bamboo and then fermenting it in a fiberizing machine (Fig.2). This serves as a domestically sourced and waste-derived wood biomass. In recent years, damage caused by the invasive spread of bamboo has become a serious environmental issue, creating a demand for effective utilization methods. Consequently, its use as a component in growing media is currently being investigated [15].

One concern associated with this technology is the potential generation of ammonia NH_3 and ammonium ions NH_4^+ , which can lead to nitrogen pollution. To address these issues, a solidification treatment method using calcium phosphate compounds (CPC) has been proposed; it has been reported that this method can reduce ammonium emissions by over 90% [16]. Furthermore, recognizing that ammonia also functions as a beneficial nutrient for plants, several studies have investigated the interactions between MICP/EICP, plant growth, and soil properties [17,18]. However, since the materials studied to date have primarily been sand and soil, there are no reported cases where cementation technology has been applied to woody biomass such

as coco peat or bamboo powder. Therefore, the applicability of this technology to such materials remains unclear.

This study reports the results of an analysis of wood biomass treated with a solidification solution composed of urea, calcium nitrate, and urease. We investigated the effects of varying solution concentrations on the collapse index (solidification strength), germination rate, and ammonium nitrogen content.



Fig.1 Coco peat



Fig.2 Bamboo powder

2. RESEARCH SIGNIFICANCE

Solidified cultivating soil represents a promising material for the Japanese agricultural and construction markets, both of which currently face significant labor shortages. Compared to the MICP method, the EICP method offers distinct advantages, such as greater ease of handling. The solidified medium developed in this study provides several superior features over existing solidified substrates: environmental friendliness (as it requires no thermal energy), simultaneous incorporation of solidification and fertilizing components (NH_4^+), and the effective utilization of waste materials. Furthermore, this study offers valuable insights into expanding the

applications of cementation technology and the efficient utilization of generated ammonia. Therefore, the research and development conducted in this study are of great importance.

3. MATERIALS AND METHODS

3.1 Waste Woody Biomass

Table 1 summarizes their physical properties. We used wood materials sourced from Sri Lankan Coco peat (Hirota Horticultural Farm Co., Ltd.) and Moso bamboo powder from Gifu Prefecture (Kamo Forestry Association) to constitute the Cultivating soil. Bulk density was measured as the mass of biomass filling 100 mL in a graduated cylinder. Ignition loss was measured in accordance with JIS A1226:2020. The pH and electrical conductivity (EC) were measured using a compact meter (LAQUAtwin, HORIBA, Ltd.).

Table 1 Characteristics of wood materials

	Bamboo powder	Coco peat
Bulk density (g/cm ³)	0.195	0.154
Ignition loss (%)	88.5	90.9
pH	5.3	5.4
EC (dS/m)	1.83	4.8

3.2 Solidification Test

The solidification test was conducted to evaluate

the relationship among the collapse index of the substrate, the amount of rinse water, and the frequency of solidification solution injections. Fig. 3 shows the solidification test flowchart. The details of the experimental procedure are as follows:

Sample preparation: Coco peat and bamboo powder were air-dried for 24 hours, sieved through a 2 mm screen, and mixed in a 1:1 volumetric ratio. The mixture was homogenized using a high-speed mixer (Braun MultiQuick9, De'Longhi Japan Corp.) operating at 1500 rpm.

Solution preparation: A urease solution (0.1 g/L, 2.1 U/mL) was prepared using urease reagent (Fujifilm Holdings Corp.). Urea and calcium nitrate [Ca(NO₃)₂] solutions with concentrations of 0.1 M, 0.25 M, and 0.5 M were prepared using urea reagent and calcium nitrate reagent (Fujifilm Holdings Corp.). The urease solution and the urea-calcium nitrate solutions were mixed in a 1:1 volumetric ratio to form the solidification solution.

Cultivating Soil Preparation: A 200 mL sample of the biomass mixture was combined with 160 mL of the solidification solution and packed into a commercial cell tray (Meiwa Co., Ltd.) with a filling density of approximately 0.44~0.71 g/cm³. In addition, the number of cells was twelve.

Injection and rinsing: Solidification solution (10 mL) was injected at 3-hour intervals, either 3 or 7 times. The reason for setting this interval at three hours is that the decrease in calcium ions plateaus three hours after the solution is injected, and no further precipitation of calcium carbonate is expected thereafter. Subsequently, the samples were rinsed with 50 mL or 250 mL of deionized water. Control samples received only deionized water. The experimental variables were: i) concentration of the solidification solution, ii) number of solution injections, and iii) rinsing volume. The test was

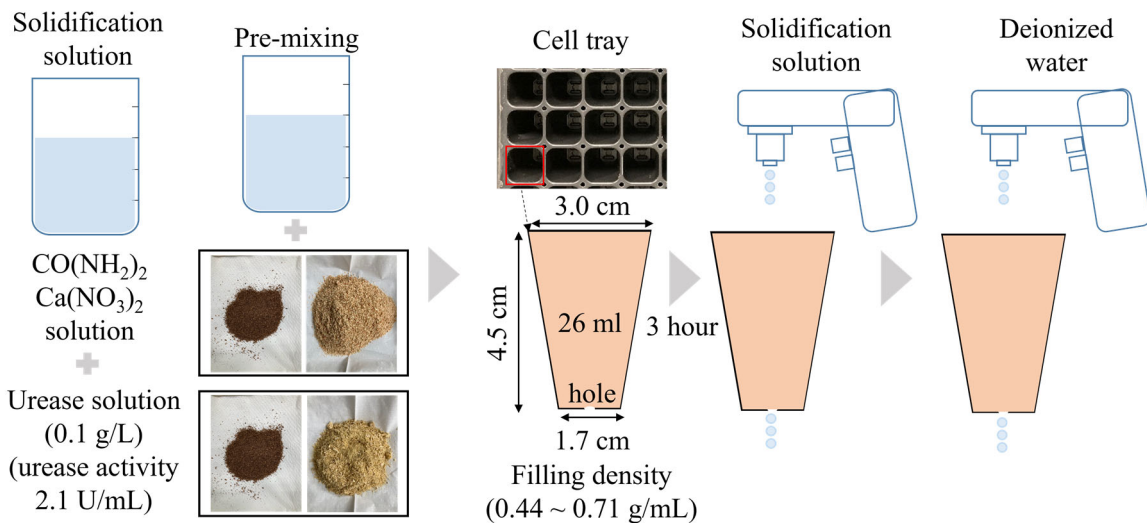


Fig. 3 Flow of solidification test

repeated twice.

3.3 Germination Test

The germination test was conducted to evaluate the relationship between seed germination rate and solidification solution concentration. The samples used for the germination test were prepared using the same method as those used for the collapse measurement, but they were different samples. Seeds of Japanese mustard spinach (komatsuna) were placed in 3 mm-deep holes at the center of each substrate surface (see Table 2 for test conditions). Germination was defined as the emergence of cotyledons. The germination rate was calculated as:

$$\text{Germination Rate (\%)} = \left(\frac{\text{Number of Germinated Seeds}}{\text{Total Seeds}} \right) \times 100 \quad (6)$$

In this experiment, total seeds were twelve, the test was repeated twice.

Table 2 Conditions of germination test

condition	
Temperature (°C)	25
Term (day)	7
Watering (times/day)	1

3.4 Measurement of Collapse Index

Previous bio-cementation studies have typically used a needle penetrometer to assess physical strength [19]. However, due to the fragile nature of the solidified substrates in this study, an alternative collapse measurement method (illustrated in Fig. 4) was employed, as proposed in [3]. By pushing the

solidified cultivating soil out from the bottom, collapse index was estimated based on the collapse state of the extracted solidified soil. The collapse index increases as the degree of collapse decreases. The collapse index shows how strong the solidified soil is, and it also shows how easily it breaks when taken out of the cell tray.

3.5 Chemical Analysis

After the germination tests, the pH and EC of the substrates were measured using the compact meter (LAQUAtwin, HORIBA, Ltd.). Ammonium nitrogen concentrations were measured in accordance with the indophenol blue absorptiometry method. Specifically, for 20 g of sample, 100 mL of 2 M KCl was added and stirred for 30 minutes to prepare the extract. For each 1 mL of extract, 14 mL of pure water, 2 mL of Nitroprusside Phenol Solution, and 3 mL of Sodium Hypochlorite Solution were added, and the mixture was measured using a spectrophotometer. Note that ammonium nitrogen was measured only once during the two trials, using a combined sample from all twelve cells.

4. RESULTS AND DISCUSSION

Table 3 presents a summary of the experimental results. In this experiment, Case 1 represents the control group. The number of injections refers to the frequency of solidification solution application, while the rinse indicates the amount of deionized water used. The detailed findings from the solidification and germination tests are discussed below. The value of the collapse index shows the average of the 12 cells.

4.1 Solidification Test

Fig. 5 shows the relationship between the collapse index and the concentration of the solidification solution. It is evident that Case 2 (0.1 M, 4 injections) exhibited insufficient strength. This is attributed to

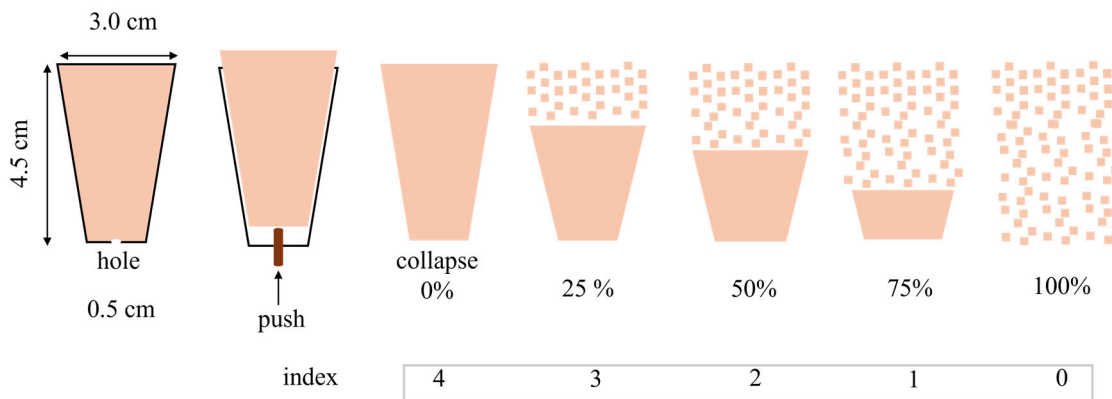


Fig. 4 Evaluation of collapse index [3]

Table 3 Summary of experiments results

	Urease (g/L)	Urea and Ca(NO ₃) ₂ (mol/L)	Injections (times)	Rinse (mL)	Germination rate (%)	pH	EC (mS/cm)	Ammonium nitrogen (mg/kg)	Collapse index
Case 1	-	-	-	50	92	7.1	0.1	98.2	0
Case 2	0.1	0.1	4	50	92	6.3	0.4	1240	0
Case 3	0.1	0.25	4	50	92	7	0.4	2810	1.4
Case 4	0.1	0.5	4	50	0	8.1	0.5	2240	3
Case 5	0.1	0.1	8	50	83	7.5	0.2	1660	2.4
Case 6	0.1	0.25	8	50	50	7.9	0.3	3070	2.9
Case 7	0.1	0.5	8	50	0	8.1	0.5	3190	3
Case 8	0.1	0.1	8	250	83	7.6	0.2	1160	2.3
Case 9	0.1	0.25	8	250	33	7.7	0.2	2640	3
Case 10	0.1	0.5	8	250	58	8	0.2	2620	3

both the low concentration of the cementation solution and the limited number of injections. Additionally, Case 6 (0.25 M, 8 injections) exhibited less collapse than Case 3 (0.25 M, 4 injections), and Case 4 (0.5 M, 4 injections) showed similar strength to Case 7 (0.5 M, 8 injections). These results suggest that, at concentrations of 0.1 M and 0.25 M, a minimum of 8 injections is necessary to achieve sufficient strength. On the other hand, at a concentration of 0.5 M, the number of injections (4 or 8) did not significantly influence the collapse index, indicating that regardless of the number of injections, sufficient solidification can be achieved.

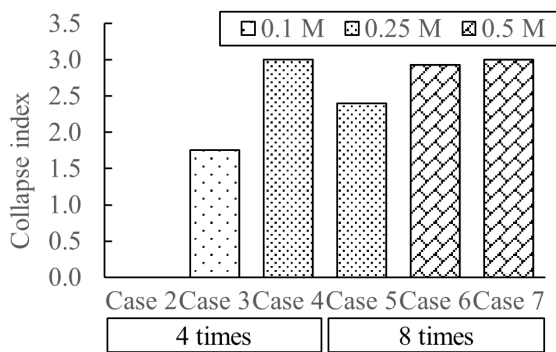


Fig. 5 Relation of collapse index and concentration of solidification solution

Fig. 6 shows the relationship between the collapse index and the amount of rinsing. This result is consistent with the chemical properties of CaCO₃, which is relatively insoluble in water. Thus, CaCO₃ maintains its solidification role even after rinsing.

Fig.7 and 8 present visual comparisons of the solidified cultivating soil.

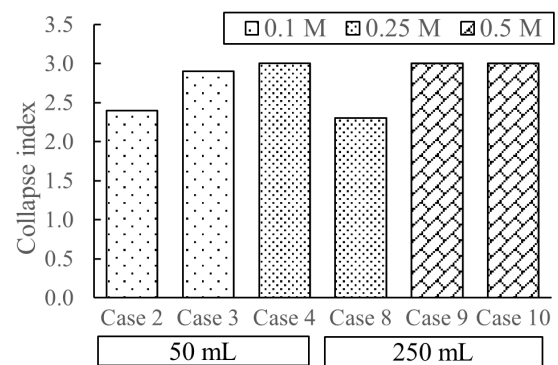


Fig. 6 Relation of collapse index and rinse amount



Fig. 7 Photograph of solidified cultivating soil (Case 8, collapse index 2.3)



Fig. 8 Photograph of solidified cultivating soil (Case 10, collapse index 3)

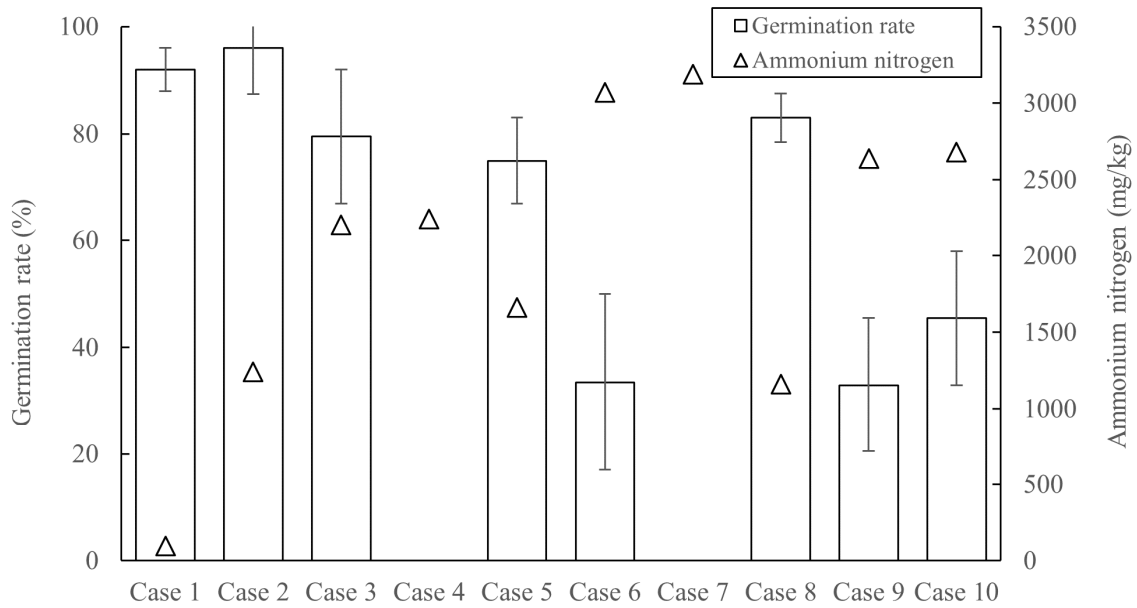


Fig. 12 Relation of Germination rate and ammonium nitrogen

It is evident that Case 10 exhibited a higher collapse index (i.e., greater strength) than Case 8, supporting the numerical results.

4.2 Germination Test

Fig. 9 shows the inverse relationship between germination rate and the concentration of the solidification solution. Specifically, higher germination rates were observed in substrates treated with lower concentrations. This phenomenon is likely due to increased osmotic pressure in substrates solidified with higher concentrations, which may hinder seed water uptake and thus suppress germination. Also, past studies showed that root development is restricted as soil hardness increases. When the concentration of solidification solution is higher, the collapse index becomes higher. This may mean that germination and strength are related.

Fig. 10 and 11 show images of the germination

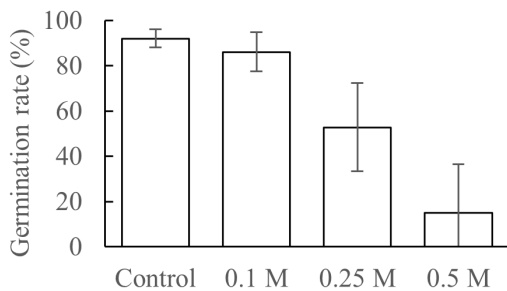


Fig. 9 Relation of Germination rate and concentration

test for Case 8 and Case 10, respectively. In Case 8, komatsuna germination was confirmed at 83%, whereas in Case 10, komatsuna germination was 58%, lower than Case 8, and plant growth was also poor.

Fig. 12 shows the relationship between germination rate and ammonium nitrogen concentration. A higher germination rate was associated with lower ammonium nitrogen levels. In Cases 8 through 10, the solidified substrates were still



Fig. 10 images of the germination test (Case 8)

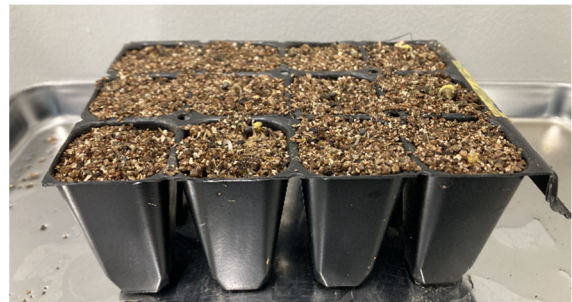


Fig. 11 images of the germination test (Case 10)

appeared to negatively affect plant growth. These rinsed to remove excess ammonium nitrogen. Although the initial concentration of ammonium nitrogen was reduced by approximately 15–30%, the findings of high residual concentrations are consistent with earlier studies indicating the phytotoxic effects of excessive ammonium nitrogen [20]. However, after germination, nitrogen becomes an essential element for plant growth. In particular, if nitrogen is deficient during the transition from cotyledon to true leaf, the leaves may turn yellow and wither. Future research will investigate the amount of ammonium nitrogen that does not inhibit germination and can serve as a nutrient afterward.

Woody biomass materials, such as coco peat and bamboo powder, can adsorb ammonium ions through ion exchange and micropore interactions. This experiment confirmed that rinsing alone was insufficient to reduce nitrogen concentrations to safe levels. Therefore, additional mitigation strategies such as the use of nitrifying bacteria may be required to minimize nitrogen-related stress during germination.

5. CONCLUSION

In this study, we aimed to develop a sustainable form of solidified cultivation soil with minimal environmental impact. We conducted solidification and germination tests using EICP as a solidification method, which, unlike other bio-cementation techniques, does not require microbial cultivation. We also used as substrate waste woody biomass such as bamboo powder and coco peat. Additionally, we investigated the collapse index of solidified cultivating soil, as well as the effects of ammonium nitrogen on germination. The findings are summarized as follows:

- Collapse index, represented as the solidification strength, increased with more frequent injections, especially at lower cementation concentrations (0.1 M and 0.25 M). At 0.5 M, sufficient strength was achieved even with fewer injections. Rinsing with deionized water did not have a significant impact on strength, as CaCO_3 is minimally soluble in water.
- Germination rate was inversely related to the concentration of the solidification solution. Solidified cultivation soil treated with 0.1 M solution showed the highest germination rates. This phenomenon is likely due to increased osmotic pressure in substrates solidified with higher concentrations, which may hinder seed water uptake and thus suppress germination.
- High concentrations of ammonium nitrogen negatively affected germination. Although rinsing reduced ammonium levels by 15–30%, it was not sufficient to eliminate phytotoxic effects. Additional methods, such as biological

nitrification, may be necessary to improve the substrate's growing conditions.

These results suggest that CaCO_3 cementation, when optimized in terms of concentration and injection frequency, holds promise for developing sustainable cultivation substrates using waste woody biomass.

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