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Research paper



Vege-Grout: a Potential Bio-Grout Material from Vegetable Waste for Bio-Cementation

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Abstract

Studies have reported that the calcite precipitation induced by ureolytic bacteria through the hydrolysis of urea was influenced by several important factors including the concentration of calcium ions, the surrounding pH and temperature. Recently, the microbial induced calcite precipitations (MICP) were further explored using natural elements and microorganisms from the environment. Vegetable waste provides a proper substrate for microorganism's growth and activities. In this study, the calcite forming ability of indigenous bacteria in the vegetable waste was investigated by mixing the extract of vegetable waste known as vege-grout with sandy soil. The vege-grout optimum content was determined by unconfined compression test to find the suitable ratio of vege-grout content. The results showed that there was an increase of compressive strength after 28 days of curing with vege-grout and significant improvement in soil shear strength. SEM and EDX analysis showed aggregation of soil particles and formation of calcium carbonate ($CaCO_3$). Microbiological analysis of vege-grout extract indicated the presence of ureolytic bacteria that could be responsible for the bio-cementation process. ICP-MS analysis showed that the vege-grout contained a rich source of carbon, nitrogen and calcium elements. The findings have demonstrated the potential application of vegetable waste for microbial cementation of soil particles.

Keywords: *Vege-grout; vegetable waste; bio-cementation;$CaCO_3$; compressive strength.*

1. Introduction

Bio-mediated cementation of soil using microbial technology has attracted many researchers in the field of geotechnical engineering. This bio-mediated process is known as microbial induced calcium carbonate precipitation (MICP) that focused on 'bio-clogging' and 'bio-cementation' of soil particles using bacterial strains [1]. Bio-clogging refers to the process of filling soil void by the bio-mechanism products of microorganisms such as biomass and exopolymeric substances that can reduce the permeability of soil by restricting the water flow in the soil. Bio-cementation refers to the binding of soil particles through the mineralization or precipitation process of microbial activities that help improve the shear strength of soil [2]. The application of this new microbial technology has been explored in many areas such as the improvement of soil strength and impermeability, mitigation of soil liquefaction, concrete repair and lately, the making of zero cement bricks [3-4].

There are many factors that affect the performance of MICP. Among them are nutrients such as chemical elements that include N, C, Mg, Fe, Ca etc which are very important for the microorganisms to live and survive [5-6]. Without these, the microorganisms will die or consume each other, and the bio-mechanism process will stop that subsequently affects the process of bio-mineralization. For an effective calcite precipitation, the type of bacteria also plays a major role in the bio-cementation process [7]. Many studies have focused on the use of ureolytic bacteria such as *Sporosarcina pasteruii* and *B. megaterium* to induce MICP based on urease activities through urea hydrolysis which yield ammonium ions and carbonate ions [8-9]. Bio-mineralization can also be achieved by other bio-chemical pathways such as denitrification, iron reduction and sulphate reduction [10-11]. However, it was discovered that the production of calcite through urea hydrolysis was higher and faster compared to the other bio-mechanism processes [12]. Particularly for urea hydrolysis and urease activity, pH and temperature also influence the MICP process. Alkaline environment is necessary to activate the urease enzyme. It was demonstrated that the urease activity was optimized at pH value ranged from 7.5 to 8.0. Optimum temperature for MICP application is also essential for bacterial growth. One study mentioned the optimum temperature for urease activity ranged from 10°C and 60°C [13].

Previous studies utilized specific bacteria and specialized growth medium to induce the bio-cementation process [14]. However, these methods are not practical and economical for large scale implementation. Therefore, the usage of natural resources that have all the factors for bio-cementation process would be an advantage. Our research on vegetable waste or vege-grout has proved that substrate from vegetable waste can also replace the role of nutrient media and broth for bacterial growth by using the fermentation process. Fermentation actually increases the nutrient content of the vegetable waste and also promotes growth of variety of microorganisms [15]. The concept of utilizing vegetable waste as a replacement will encourage recycling of waste products that can be converted to other useful resources. One study used pig urine as a substitute for industrial urea to induce calcite formation [16]. In this study, substrate of vegetable waste will be used as an alternative to synthetic urea to induce $CaCO_3$ precipitation through MICP. The effectiveness of this method was determined by measuring the shear strength of the treated soil samples. The formation of calcite or calcium carbonate was evaluated by Scanning Elec-

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tron Microscope and energy dispersive X-Ray spectroscopy analysis (SEM-EDX). The chemical elements such as Silica, Ferum and Calcium in the vege-grout were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

2. Experimental

2.1. Vege-Grout Preparation and Analysis

Vegetable waste such as cabbage, long bean, cucumber and spinach were collected from various sources like the wet market and farmer market around the area and brought back to the laboratory. All the vegetables were washed and rinsed thoroughly using distilled water to avoid cross-contamination. Then the vegetables were cut into small pieces and kept in a clean container for fermentation process. The containers were tightly closed and kept for one month at room temperature. The pH of the vegetable was monitored once a week with a pH meter. The alkalinity of the substrate was maintained by adding urea throughout the fermentation process to promote the growth of ureolyic bacteria.

The vegetable substrate gave a rotten smell and the color appeared brownish that was generated by microbial activities. After one month, the substrate was filtered, collected and transferred to another clean container for further analysis. Figure 1 showed the process of vege-grout preparation. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to measure the amount of silica, iron and calcium elements in the vegetable substrate. These minerals play a major role for the bio-clogging and bio-cementation of soil particles through various bio-chemical pathways induced by the microorganisms [17]. The substrate sample was also sent to a certified microbiology lab to screen for pathogenic bacteria.



Fig. 1: Vege-grout preparation

2.2. Determination of Vege-Grout Content by UCS

The optimum content of vege-grout has to be determined first before any test for engineering soil properties is carried out. This was necessary to estimate the percentage of the vege-grout used in the soil mixture. First, the basic soil properties of the soil were determined through several tests in accordance to BS1377:1990 (Table 2). The experiments were done by unconfined compression test (UCS). Four remoulded samples of different vege-grout mixture consisting of 15%, 17.5%, 20%, and 25% vege-grout were cured for 10 days and tested using the UCS test (Figure 2). The optimum vege-grout content will be determined from the highest compressive strength obtained. The UCS test followed the procedure in BS 1377-7: 1990. The soil in this study was classified as sandy SILT with intermediate plasticity. (data not included). The procedure of the research work is illustrated in the flow chart in Figure 3.

Table 2: Basic soil properties

Properties	Value
Soil type	Sandy Silt
Gravel fraction (%)	2.82
Sand fraction (%)	33.45
Silt fraction (%)	63.74
Coefficient of uniformity	19.74
Coefficient of gradation	2.75
Specific gravity	2.71
Liquid Limit (%)	38.38
Plastic Limit (%)	30.33
Plasticity index	7.5
Liquidity index	-0.13
Moisture content (%)	29.9

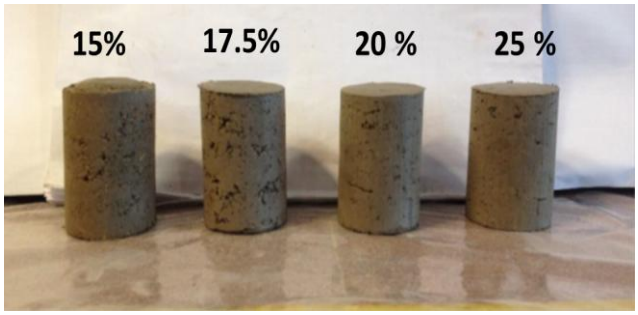


Fig.2: Remoulded samples containing vege-grout mixtures.

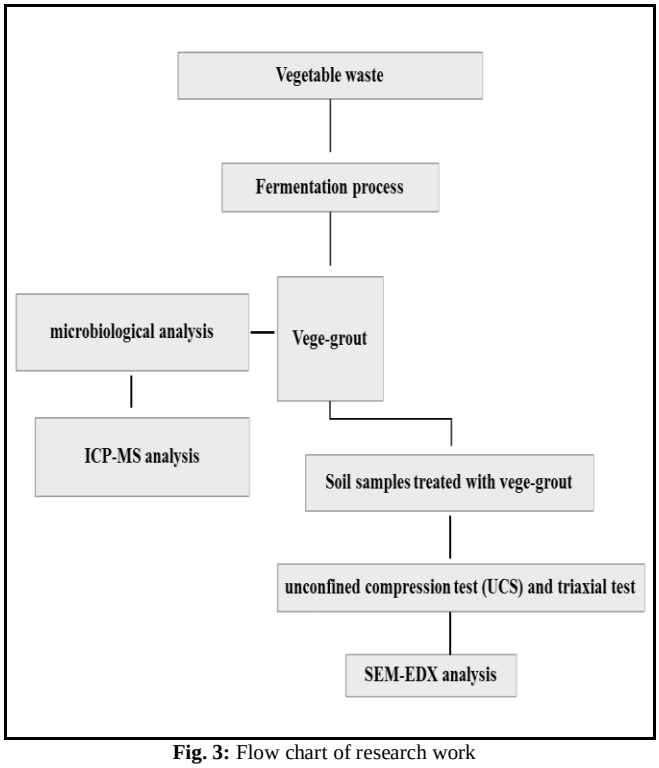


Fig. 3: Flow chart of research work

2.3. Determination of Shear Strength by Triaxial Test

Triaxial test was conducted by Consolidated Drained (CD) method to determine the shear strength. 3 trials were carried out to establish the Mohr-Coulomb circle. The Mohr Circle and failure envelope will be interpolated to attain the shear strength; friction angle and the cohesion values. The soil shear strength was tested following BS 1377-7:1990 Clause 5.5 guideline. Six series of triaxial tests were carried out comprising of original soil and treated soils based on curing period. In this work, 100, 200, and 300 kPa of cell pressures were used for every set of experiment. The analysis was focused on the comparison between original and treated soil with respect to cohesion, friction angle and shear strength of the soils based on curing time elapsed of the vege-grout treatment.

3. Results and Discussion

3.1. IC-MS and Microbiological Analysis

Analysis of IC-MS on the vege-grout showed that the vege-grout contained a high amount of chemical elements such as Si, Fe and Ca which are involved in the bio-mineralization process [18-19]. Apart of that, the substrate from vegetable waste was also rich in Carbon and Nitrogen which are essential for bacterial growth [20] Table 1 showed the total chemical elements in the vege-grout.

Table 1: Total chemical elements in vege-grout

Sample	Silica (Si), iron (Fe) and calcium (Ca) (mg/L)	Carbon (C), (mg/mL)	Nitrogen (N), (mg/mL)
Vege-grout extract	42.63	253.4	9236.27

Microbiological analysis of the vege-grout showed a variety of bacterial species which can be found from the environment such as soil and water. Pathogenic bacteria such as *E.Coli* and Salmonella was absent or present in the least amount indicating that the vege-grout extract is suitable and safe for MICP application. The presence of coliform bacteria could be due to cross-contamination during the handling process. However, the amount of colony units was relatively small.

Table 2: Microbiological analysis of vege-grout

Test Parameter	Unit	Vege-grout
Escherichia coli	CFU/100mL	<1
Salmonella	Present/Absent	Absent
Staphylococcus aureus	CFU/100mL	<1
Clostridium perfringens	CFU/100mL	<1
Pseudomonas aeruginosa	CFU/100mL	1.3 x 10^4
Enterococcus/Streptococcus Groups	CFU/100mL	2.3 x 10^3

3.2. Determination of Bio-Grout Content from UCS Test

From the experiment, the 15% of vege- grout content was found not suitable for sandy silt soil since it had more percentage of fine grains. Figure 4 shows the failure of the soil samples by shearing. The stress-strain relationship of vege-grout treated samples in variable mixtures is shown in Figure 5. Experiment showed that the strength of treated soil increased as the vege-grout content increased. As the vege-grout content exceeded 20%, the strength dropped from 56.83 kPa to 49.28 kPa. Therefore, it can be concluded that the vege-grout optimum content for sandy silt soil was 20%.

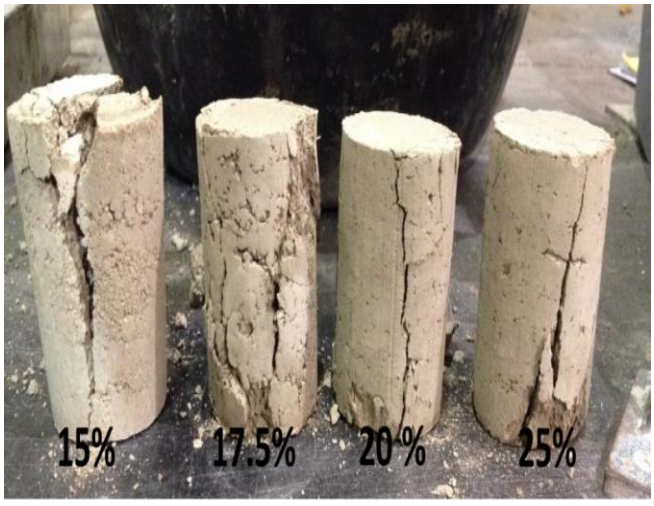


Fig.4: Failure of treated soil samples by shearing

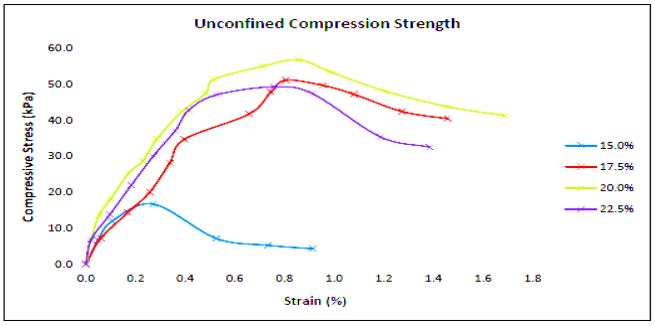


Fig.5: UCS of vege-grout treated soil

3.3. Soil Shear Strength

The shear strength is a function of cohesion between the soil particles and frictional resistance between soil particles. In the triaxial test analysis, two parameters are obtained from Mohr's circles that is generated based on minor and major principal stresses which are in term of effective stresses. The effective stress is carried by the soil solids. Thus, the c' and ϕ' are the effective stress cohesion and effective angle of friction. Figure 6 showed the improvement of soil strength of treated samples by the increment of cohesion and friction angle values. The increment of these values resulted in the improvement of the shear strength of the treated soil. Results showed that the cohesion and the friction angle of the treated soil have increased by 117.77% and 31.53% respectively as the curing period increased, which lead to the improvement of soil consistency and density (see Figure 7).

All the results showed that there are significant improvement in soil shear strength with respect to effective stress cohesion, effective angle of friction and shear stress of the soil. Based on this consolidated drained triaxial test, the soil shear strength has showed significant improvement as curing period increased. This indicates that the aggregation of soil particles have somehow increased through microbial activities in the vege-grout treated soil samples. The same findings were also reported in previous studies of microbial bio-cementation of sandy soils that showed an increase on compressive strength when treated with ureolytic bacteria [21-22].

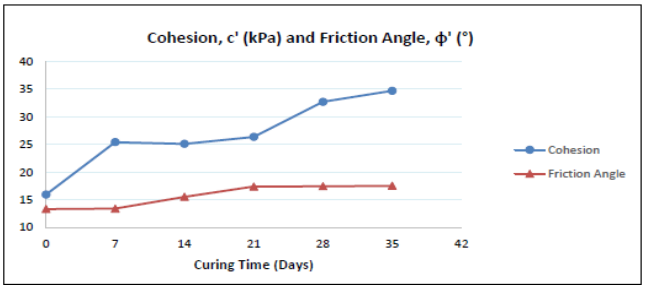


Fig.6: Improvement of shear strength parameters

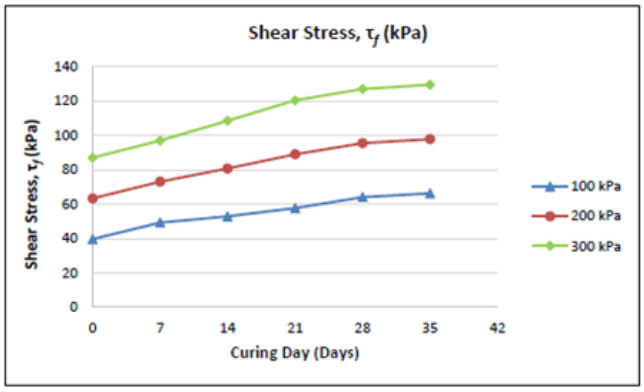


Fig. 7: Improvement of shear stress based on curing period

3.4. SEM and EDX Analysis

For SEM-EDX analysis, the dried samples were coated with gold and analysed at a voltage of 10.00 kv (12,000 X magnification).
The results of the SEM-EDX analysis showed the presence of elevated amount of $CaCO_3$ in the vege-grout treated soil samples. This indicates that the bio-cementation process based on precipitation of calcium carbonate ($CaCO^3$) has taken place through bio-mediated activities of microorganisms from vege-grout. Figure 8 showed the presence of calcium element in the treated sample. Calcium carbonate crystals were detected in the SEM image. The existence of bacterial cells can be observed among the carbonate crystals which look like rod shaped bacteria which suggest that the bacteria could belong to the bacillus type group. Further test will be carried out in the next study to identify the bacteria that were involved in the bio-cementation process that produce calcite precipitation.

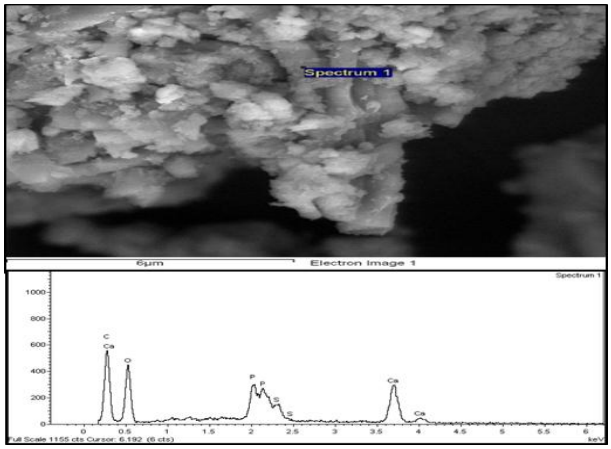


Fig. 8: SEM-EDX profile of vege-grout treated soil. (12000X Mag)

4. Conclusion

Most research studies used cultivated bacteria, urea and nutrient media or broths to induce MICP via hydrolysis of urea which were quite expensive for large scale application. In this study, vegetable waste was processed using fermentation method to obtain a variety of microorganisms communities to induce MICP. Vege-grout substrate can be used as an alternative substitute for urea. Apart of that, the vege-grout abstract was also rich in nutrients to ensure the survival and growth of the microorganisms. Here, it was demonstrated that vege-grout from vegetable waste was able to induce MICP by the evidence of calcite formation shown in the SEM-EDX analysis. The bio-cementation process has occurred resulting in the improvement of the soil strength. It can be concluded that vege-grout from vegetable waste can be used as a replacement for cultivated bacterial culture and nutrient media. This method is economical, promotes the usage of waste products and it is suitable for large-scale application for soil improvement. The results demonstrated that vege-grout could be an effective substrate for bio-cementation process of soil particles.

Acknowledgement

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References

[1] Chu J, Ivanov V, Naeimi M, Stabnikov V and Liu HL (2014) "Optimization of calcium-based bioclogging and biocementation of sand,"*Acta Geotechnica*, vol.9, pp. 277-285.

[2] Ivanov V and Stabnikov V (2017) "Bioclogging and biogrouts," *Construction Biotechnology*, pp.139-178.

[3] Wang Z, Zhang N, Cai G, Jin Y, Ding N and Shen D (2017), "Review of ground improvement using microbial induced carbonate precipitation (MICP)," *Marine Georesources & Geotechnology*, vol.35, pp. 1135-1146.

[4] Achal V and Mukherjee A (2015) "A review of microbial precipitation for sustainable construction," *Construction and Building Materials*, vol.93, pp. 1224-1235.

[5] Joshi S, Goyal S and Reddy MS (2018) "Influence of nutrient components of media on structural properties of concrete during biocementation," *Construction and Building Materials*, vol.158, pp. 601-613.

[6] Fujita M, Nakashima K, Achal V and Kawasaki S (2017) "Whole-cell evaluation of urease activity of Pararhodobacter sp. isolated from peripheral beachrock," *Biochemical Engineering Journal*, vol.124, 1-5.

[7] Graddy CM, Gomez MG, Kline LM, Morrill SR, DeJong JT and Nelson DC (2018) "Diversity of Sporosarcina-like Bacterial Strains Obtained from Meter-Scale Augmented and Stimulated Biocementation Experiments," *Environmental science & technology*, vol. 52, pp.3997-4005.

[8] Zhao Q, Li L, Li C, Li M, Amini F and Zhang, H. (2014) "Factors affecting improvement of engineering properties of MICP-treated soil catalyzed by bacteria and urease," *Journal of Materials in Civil Engineering*, pp. 26

[9] Soon, N. W., Lee, L. M., Khun, T C and Ling, H. S. (2014) "Factors affecting improvement in engineering properties of residual soil through microbial-induced calcite precipitation," *Journal of Geotechnical and Geoenvironmental Engineering*, vol 140.

[10] Shashank BS, Sharma S, Sowmya S, Latha RA, Meenu PS and Singh DN (2016) "State-of-the-art on geotechnical engineering perspective on bio-mediated processes," *Environmental Earth Sciences*, vol.75, pp. 270.

[11] Basha S, Lingamgunta LK, Kannali J, Gajula SK, Bandikari R, Dasari S and Balaji VK (2018) "Subsurface Endospore-Forming Bacteria Possess Bio-Sealant Properties," Scientific reports, pp.8

[12] Omorogie AI, Khoshdelnezamiha G, Senian N, Ong DEI and Nissom PM (2017) "Experimental optimisation of various cultural conditions on urease activity for isolated Sporosarcina pasteurii strains and evaluation of their biocement potentials," *Ecological Engineering*, vol. 109, pp. 65-75.

[13] Harkes MP, Van Paassen LA, Booster JL, Whiffin VS and van Loosdrecht MC (2010) "Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement," *Ecological Engineering*, vol.36, pp.112-117.

[14] Dhami, N. K., Alsubhi, W. R., Watkin, E and Mukherjee, A. (2017). "Bacterial Community Dynamics and Biocement Formation during Stimulation and Augmentation: Implications for Soil Consolidation," *Frontiers in Microbiology*, pp. 8

[15] Khan MNH, GGNN A, Shimazaki S and Kawasaki S (2015) "Coral sand solidification test based on microbially induced carbonate precipitation using ureolytic bacteria," *Materials transactions*, vol.56, pp. 1725-1732.

[16] Chen HJ, Huang YH, Chen, CC, Maity JP and Chen CY (2018) "Microbial Induced Calcium Carbonate Precipitation (MICP) Using Pig Urine as an Alternative to Industrial Urea," *Waste and Biomass Valorization*, pp.1-9.

[17] Tamang JP, Watanabe K and Holzapfel WH (2016) "Diversity of microorganisms in global fermented foods and beverages," *Frontiers in microbiology*, vol.7, pp. 377.

[18] Zeth K, Hoiczyk E & Okuda M (2016) "Ferroxidase-mediated iron oxide biomineralization: novel pathways to multifunctional nanoparticles," *Trends in biochemical sciences*, vol.41, pp.190-203.

[19] Seifan M, Ebrahiminezhad A, Ghasemi Y, Samani AK and Berenjian A (2018) "Amine-modified magnetic iron oxide nanoparticle as a promising carrier for application in bio self-healing concrete," *Applied microbiology and biotechnology*, vol.102, pp.175-184.

[20] Klumpp S and Hwa T (2014) "Bacterial growth: global effects on gene expression, growth feedback and proteome partition," *Current opinion in biotechnology*, *vol.*28, pp.96-102.

[21] Azadi M, Ghayoomi M, Shamskia N and Kalantari H (2017) "Physical and mechanical properties of reconstructed bio-cemented sand," *Soils and Foundations*, vol.57, pp.698-706.

[22] Canakci H, Sidik W and Kilic IH (2015) "Effect of bacterial calcium carbonate precipitation on compressibility and shear strength of organic soil," *Soils and Foundations*, vol.55, pp. 1211-1221.