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DEVELOPMENT OF FIRED CLAY GRANULES AS ECO-FRIENDLY SUBSTANCES FOR NITROGEN-FIXING BACTERIAL CELLS

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ABSTRACT: Modified clay granules were used to promote *Azotobacter vinelandii* cell adhesion. The *A. vinelandii* cells in the clay granules were used as a biofertilizer and a plant material. The production process was carried out under variable temperatures. The raw ingredients consisted of clay, sawdust waste, and spent coffee grounds in different ratios. Scanning electron microscopy (SEM) was used to analyze the microstructure. The results of the study showed the addition of sawdust waste and spent coffee grounds had increased the water absorption of the fired clay granules based on their porosity. However, increasing the firing temperature in the range of 900°C - 1100°C decreased the water absorption and porosity and increased the bulk density of the fired clay granules. *A. vinelandii* was enriched to be used as a cell suspension. The fired clay granules were immersed in a cell suspension to immobilize the *A. vinelandii* cells for 48 h. The SEM-based investigations indicated that the fired clay granules were suitable for containing *A. vinelandii* cells. The results demonstrated high viability of bacterial cells fixed in the fired clay granules at 2.7×10^7 CFU/g. Furthermore, the test results of bacterial cells in the fired clay granules for marigold planting media revealed that it had effectively encouraged plant growth. The nitrogen-fixing bacterial cells in the clay granules obtained from this research were determined to be appropriate for use as an ecological soil replacement in the future.

Keywords: Fired clay granules, Sawdust waste, Spent coffee grounds, Nitrogen-fixing bacteria, Biofertilizer

1. INTRODUCTION

The use of fired clay has been widespread for centuries, because it is a reasonable construction material. Nevertheless, the firing process requires elevated firing temperatures, resulting in substantial energy consumption and heat pollution [1]. Fired clay is certainly fired at a range from 900°C to 1200°C since the phase transformation of clay at the changing temperatures constantly affects the physical-mechanical properties of the final product [2]. The technology of producing highly porous clay by adding waste materials, such as sawdust and coffee grounds, has resulted in innovations that can be applied in various fields. Moreover, it reduces the amount of waste and, thus, saves the cost of producing high porosity-fired clay. The indirect benefit represents a way to develop high porosity-fired clay as an alternative for the growing construction industry. Fired clay granules are commonly used in planting materials to increase ventilation. Fired clay granules are used as a cover material for ornamental flowerpots to enhance attractiveness and prevent dust from soil or other planting materials. It can absorb water, but it does not have the properties of ion exchange. Fired clay granules have high porosity and a strong and durable structure. These properties can be used as a plant material and a carrier for immobilized nitrogen-fixing bacteria, making it an environmentally friendly material. Although fired clay granules can be used as a soil substitute, there is a major problem in the lack of abundance for plant growth.

The application of plant growth-promoting rhizobacteria (PGPR) in farming is constantly developing since it proposes an adequate mechanism to displace the use of chemical fertilizers, pesticides, and other dangerous supplements [3, 4]. Growth-promoting substances are constructed in enormous portions by the activity of these rhizosphere microorganisms that directly or indirectly affect the general morphology and physiology of crops [4]. *Azotobacter* is a group of Gram-negative, free-living, nitrogen-fixing aerobic bacteria that inhabit the soil. They are oval or spherical in shape, and under unfavorable environmental conditions, they form thick-walled cysts [5].

A. vinelandii and *A. Chroococcum* can produce auxin-promoting hormones and plant growth regulators. These are microorganisms that improve crop yields via the process of fixing nitrogen from the atmosphere into plant nutrients [6]. *Azotobacter* is exposed to acidic pH, high salt concentration, and temperature [7]. They pose promising influences on crop growth and yield via the biosynthesis of biologically active substances, the stimulus of rhizosphere microorganisms, the construction of phytopathogenic inhibitors, the alteration of nutrient uptake, and ultimately, the stretching of the biological nitrogen fixation [8]. The use of nitrogen-fixing microorganisms enhances plant growth by increasing plant weight, as well as by decreasing soil salinity [9]. However, free-living nitrogen-fixing microorganisms in the soil cannot live for prolonged periods and drop rapidly as they are washed away by rain or irrigation water. Currently, procedures are being studied to sustain the nitrogen-fixing microorganisms inhabiting the soil. Natural materials are being used as substances to which nitrogen-fixing microorganisms can attach to promote plant growth [10]. Although there is some information, little investigation has been undertaken on the media that can be used to attach the nitrogen-fixing microorganisms. The use of microbial cell immobilization materials contains both natural and synthetic materials, which have various benefits and weaknesses depending on the properties of the material. However, there is a study that supports the use of natural materials and shows the benefits of including plant nutrients and being a material that can facilitate the growth of microbial cells. In addition, using natural materials is not toxic to microbial cells, and through natural mechanisms, these materials can disintegrate [11].

The purpose of this research was to develop an eco-friendly planting material so that the material's increased properties could serve as a carrier for the immobile bacterial cells. In this study, the modified clay granules, which were used as substances for the adhesion of bacterial cells, were created from high-porosity clay, given its status as a high-efficiency plant material. The supplementation of agricultural wastes created numerous porosities after the burning of the clay. The obtained clay granules are lightweight and have good air permeability. Initial characterization tests showed that the clay material can be used as a planting material. The fired clay material is a moisture-absorbent material that is insoluble, strong, clean, and reusable. It can be molded into various sizes depending on the application, such as a material for seedlings or root cuttings and as a regeneration material. It can be used to grow hydroponic plants from the seedling stage to the actual transplantation process. Moreover, this innovation is resistant to physical and chemical environments, in addition to being an eco-friendly growing material. These modified clay granules will result in cost savings. They will effectively reduce the use of chemicals and residues generated by growing crops in closed systems at present and in the future.

2. MATERIALS AND METHODS

2.1 Raw Materials and Physical Properties

The sawdust waste and spent coffee grounds were obtained from a small industry and a café, respectively, while the raw clay was sourced from Udon Thani, Thailand. All primary components were sun-dried and then sieved through an 80-mesh screen before use. Raw materials and microstructure images using scanning electron microscopy (SEM) are shown in Figure 1.

The X-ray fluorescence analysis of the raw materials was determined. The results of the chemical analysis of the clay, sawdust waste, and spent coffee grounds using XRF (X-ray fluorescence) are summarized in Table 1. The particle-size analysis of the individual clay, sawdust waste, and spent coffee grounds was analyzed using laser diffraction (Mastersizer). The results of the particle size analysis are shown in Figure 2. The average particle size D [4, 3] of the individual clay, sawdust waste, and spent coffee grounds were 53.84, 357.73, and 196.03 µm, respectively.

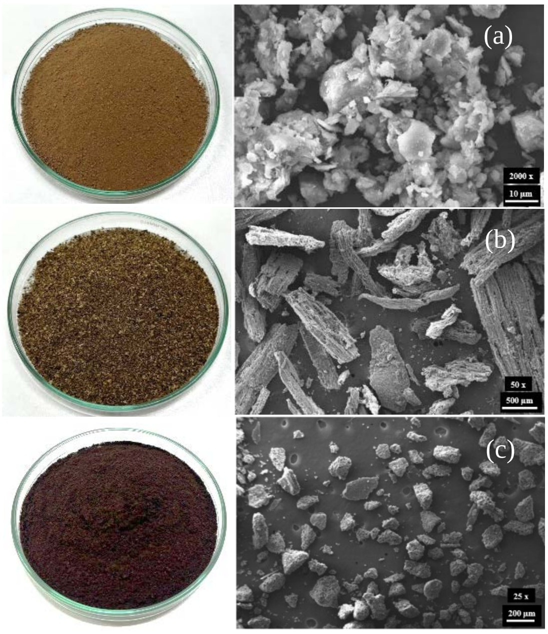

Fig.1. The raw materials and the SEM image of (a) individual clay, (b) sawdust waste, and (c) spent coffee grounds.

In addition, the X-ray diffractometer (XRD, Bruker D8 Advance) was used to qualitatively determine the predominant crystalline phases in the clay, sawdust waste, and spent coffee grounds. The

X-ray diffraction (XRD) analysis of the raw materials is presented in Figure 3. The XRD peaks of the raw clay consisted of kaolinite ($Al_2(Si_2O_5)(OH)_4$) and quartz (SiO_2) while sawdust waste consisted of cellulose ($(C_6H_{10}O_5)_n$) and quartz (SiO_2). The XRD patterns for the spent coffee grounds indicated that contained only amorphous phases.

Table 1 The X-ray fluorescence (XRF) analysis of the raw materials.

Oxide contents (% w/w)	Clay	Sawdust waste	Spent coffee grounds
SiO_2	64.33	55.52	21.66
Al_2O_3	14.42	10.40	-
Fe_2O_3	7.52	9.54	1.47
CaO	0.32	3.41	20.76
K_2O	1.19	1.73	15.61
Na_2O	0.73	-	-
P_2O_5	-	-	7.47
TiO_2	-	-	-
MnO	0.10	0.32	2.44
MgO	0.96	-	-
SO_3	-	1.22	10.34
LOI (1000°C)	10.43	17.86	20.25

Note: Loss on ignition (LOI) is the percentage of weight change caused by heating the sample to a high temperature and is used to give a measure of the extent of volatile substances.

2.2 Fired Clay Granules Preparation

In this study, the percentage of sawdust waste and spent coffee grounds added to the clay varied at the concentrations of 0, 5, 10, and 15% (w/w). For each sample, the materials were mixed in the ball mill to acquire a homogenous mixture. Table 2 provides a detailed breakdown of the raw clay, sawdust waste and spent coffee grounds ratios for each clay granules sequences, facilitating transparency and reproducibility in research protocol. Then, a water solution comprising 30–35% water was added to the mixture to enhance plasticity for shaping. The clay granules were formed by hand-clay soft-mud pavers to a diameter of 1.0 - 1.5 cm. The clay granules were then sun-dried for three days. Then, the clay granules were fired at 900, 1000, and 1100°C, as shown in Figure 4.

The assessment of the physical and mechanical properties of the fired clay samples was conducted following standardized testing procedures. Physical properties were determined in accordance with standard test methods for determination of water absorption and associated properties by vacuum method.

Table 2 The mixture material of the fired clay granules under the variation of firing temperatures at 900, 1000, and 1100 °C.

Treatment	The components of the fired clay granules (% w/w)		
	Clay	Sawdust waste	Spent coffee grounds
Control	100	0	0
1	95	5	0
2	90	10	0
3	85	15	0
4	95	0	5
5	90	0	10
6	85	0	15

2.3 Testing Method

The apparent porosity, bulk density, and water absorption properties of the fired clay samples were analyzed using the Archimedes method, following the guidelines outlined in the ASTM C373-18 (2018) standard [12]. To measure the firing shrinkage, the lengths of the fired clay before and after shrinkage were compared following the ASTM C326-09 (2018) standard [13]. The mechanical strength of the fired clay samples was assessed using a universal testing machine, following the ASTM C773-88 (2020) standard [14].

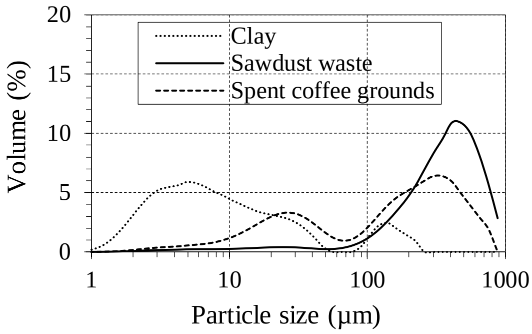

Fig.2 The particle-size analysis of the raw materials.

2.4 Microbial Preparations and Cell Adhesion in the Fired Clay Granules

The bacteria *A. vinelandii* was cultivated in nitrogen-free medium agar at 37°C for 5 days. The *A. vinelandii* cells were then transferred to an enriched medium for the cell suspension preparation. This procedure used a nitrogen-free liquid medium, which was carried out using 500-mL conical flasks containing 250 mL of medium at 37°C for 5 days using an incubator.

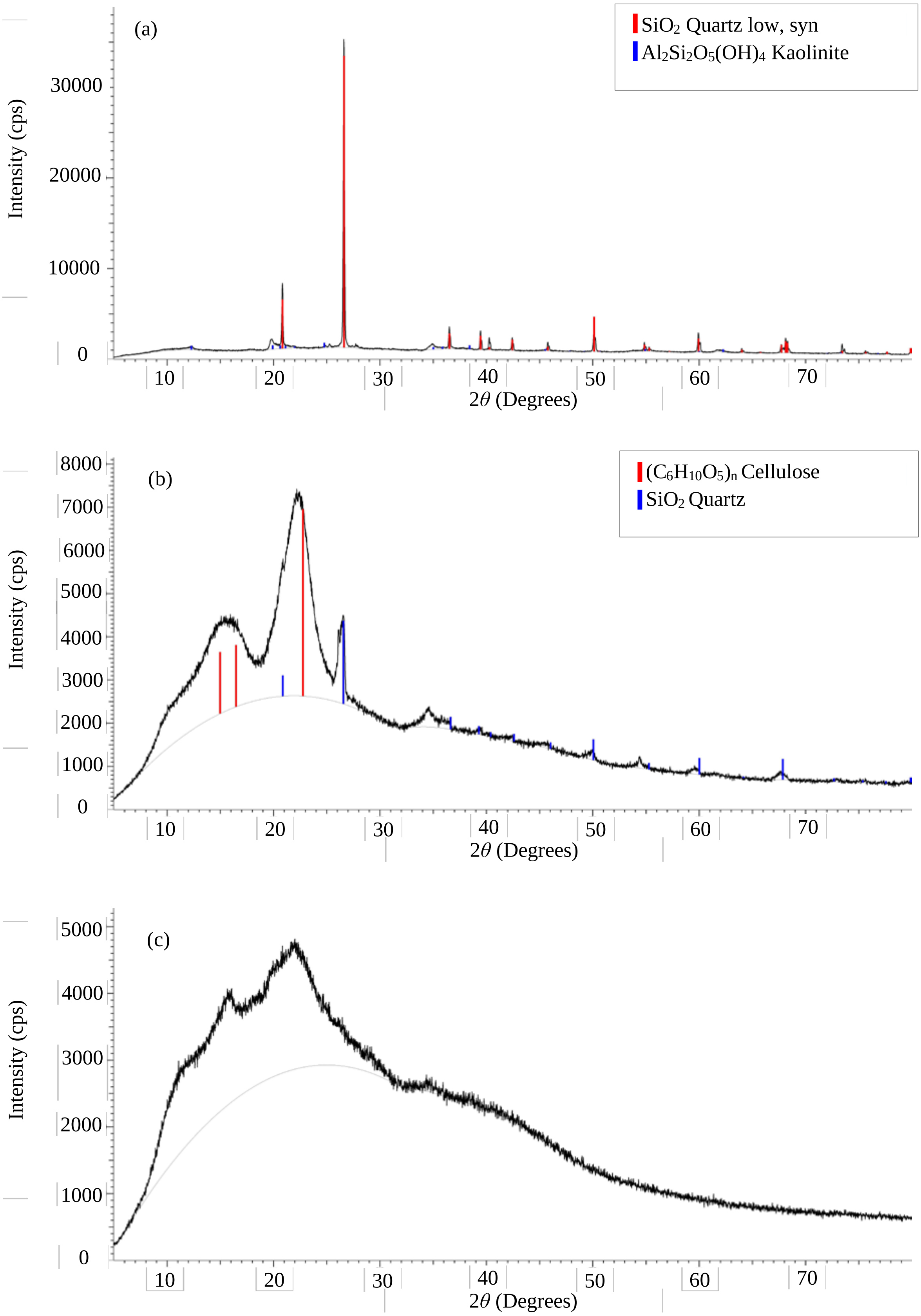

Fig.3 The crystalline phases analysis using X-ray diffractometer (XRD) of the raw materials: (a) individual clay, (b) sawdust waste, and (c) spent coffee grounds.

The *A. vinelandii* cells in the fired clay granules preparation are presented in Figure 5. In this study, the colony-forming units (CFU) of *A. vinelandii* in fired clay granules used as plant material were counted to analyze the properties of the nitrogen-fixing microbial cell adhesion substances. The colony forming of *A. vinelandii* in the porous fired clay was investigated using serial dilutions and plate count techniques. Ten grams of fired clay granules used as the plant material were finely ground under sterile conditions. The clay powder was then diluted in a 0.85% (w/v) sodium chloride solution until the bacteria were diluted enough to count accurately.

Fig.4 The clay granules process: (a) the fine grinding and sieving of the clay, (b) an ingredient mixing step, and (c) the clay granules molding step.

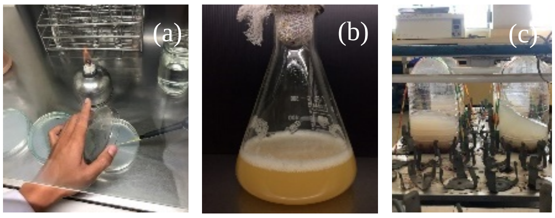

Fig.5 The microbial preparation process: (a) the pure culture preparation, (b) the microbial enrichment process, and (c) the microbial cells immobilization step.

The microbial preparation process consisted of a pure culture preparation, microbial enrichment using the synthetic medium, and the immobilization of the microbial cells in the fired clay granules. After the *A. vinelandii* cells had been fixed for 48 hours, the clay granules were air-dried until completely dry, as shown in Figure 6. The prepared clay pellets were used as plant material in the next step.

2.5 Scanning Electron Microscopy

The structure of the fired clay granules was determined and analyzed by using a scanning electron microscope Hitachi Tabletop SEM TM, 3000 (Hitachi High Technology Corp., Tokyo, Japan). The entire surface of each sample was treated with ethanol to remove any grease so that images of the highest quality could be obtained.

Fig.6 The air-dried fired clay granules with the immobilized microbial cells.

2.6 The Using of the Fired Clay Granules as a Planting Material on the Growth of Marigolds

After the microstructure analysis using scanning electron microscopy (SEM), the fired clay granules with the highest porosity were selected and used as the substrate for *A. vinelandii* cell adhesion. In this study, the intra-particle porosity and microstructure analysis with the addition of spent coffee grounds showed the highest porosity compared to the addition of individual clay and sawdust waste, respectively.

The fired clay granules, which had had spent coffee grounds added to them, were then prepared by immersion in a cellular suspension of *A. vinelandii*. The cultivations were suspended and were exposed to shaking at room temperature for 48 hours so that the bacterial cells could adhere to the fired clay granules before being used as the planting media for marigolds. Then the marigolds grown in fired clay granules were examined for 55 days. The parameters that indicate plant growth were measured, consisting of height, root length, number of leaves, flowers, and fresh and dry weights.

3. RESULTS AND DISCUSSION

At present, an increasing of population creates the request for food which in turn leads to the rise of agricultural produce. Widespread use of chemicals causes adverse effects on the health of humans and the environment and has also contributed to climate change. A potential substitute for agrochemicals is to exploit renewable sources that could help in achieving sustainability without conciliatory produces. In such cases, the microbes from the native agricultural ecosystem could support the issue.

Products established from microbes could support agriculture by growing the nutrient uptake from the soil, immunity against pathogens and insect pests, attractive the nutritional quality of the foods, and remediate the environment. Microbes can alleviate biotic and abiotic stresses thereby encouraging the growth of plants in drought, saline, or alkaline conditions. So as to provide the desired effect, inoculated microbes have to out-compete the native microflora. This can be achieved by the use of carrier materials that could provide longer shelf life and viability to the organism and can also create controlled-release systems that could provide a prolonged response. Microbes can also be genetically modified to support plant growth [15].

Consequently, this study focuses on the development of various formulations of microbial products or planting material that could aid in sustainability. Therefore, in order to achieve the results of this research, the results and discussion section of this study contains topics related to the development of environmentally friendly planting material production technology as follows:

3.1 The Properties of the Fired Clay Granules

The shrinkage of the fired clay granules during the firing process is depicted in Figure 7.

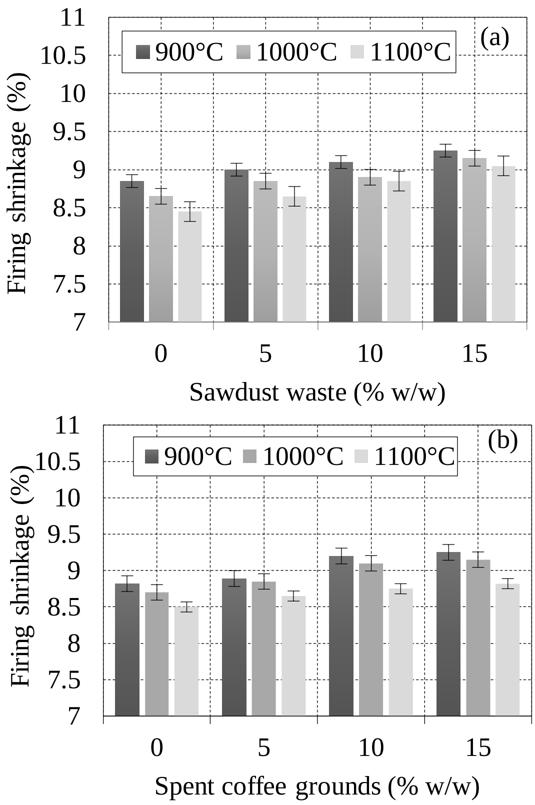

Fig.7 The firing shrinkage of the clay granules based on the different concentrations of (a) sawdust waste and (b) spent coffee grounds.

The firing shrinkage of the fired clay granules, which occurs during drying and firing, directly affects the dimensions of the fired clay and is an important consideration when designing molds for fired clay production. The clay particles merge as water is removed from the structure and body, resulting in contraction [16, 17]. The firing shrinkage of the clay granules and their behavior aligns with the sintering process [18, 19]. The results indicated that the shrinkage had increased with increasing temperatures. Figure 7 also found that the increased content of sawdust waste and spent coffee grounds used as additives had increased the firing shrinkage. Multiple factors affected the water absorption and bulk density, including the density of the material, the manufacturing method, and the firing temperature. Low-density clay bricks have the advantages of reducing the weight of structures and improving thermal performance [19].

Figure 8 shows that the water absorption had ranged from 5.0 to 24% and had increased as both the sawdust waste and the spent coffee grounds were increased. A strong correlation exists between the apparent porosity and the water absorption. The fired clay granules at 900°C without any additives displayed the highest bulk density.

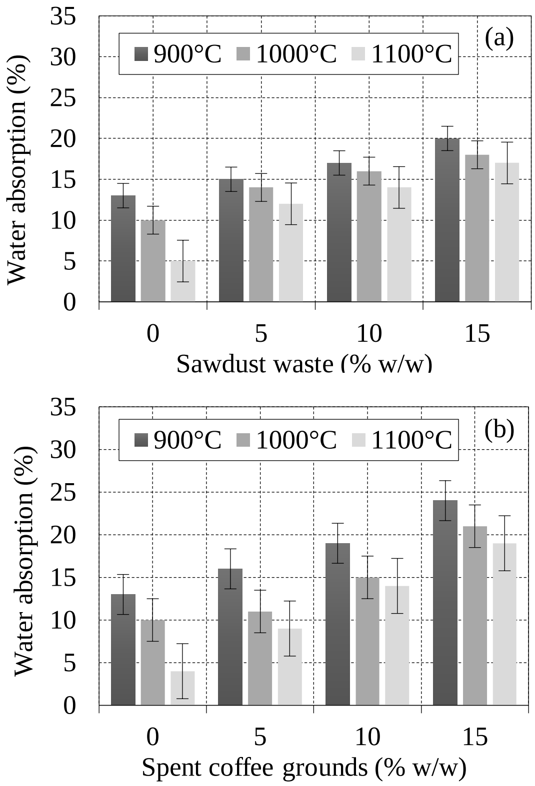

Fig.8 The water absorption of the clay granules at the different concentrations of (a) sawdust waste and (b) spent coffee grounds.

The degree of open porosity significantly affects the water absorption capacity of the fired clay granules. A decrease in water absorption was associated with an increase in the bulk density, which reduced the number of open pores, as shown in Figure 9.

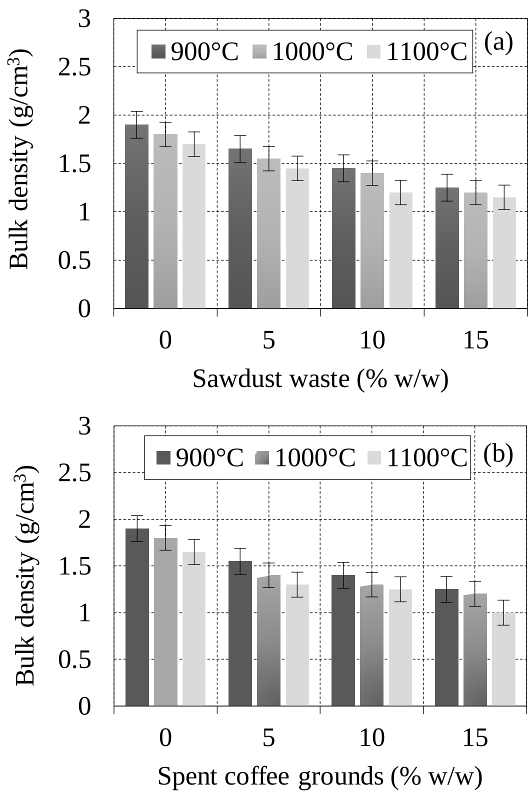

Fig.9 The bulk density of the clay granules based on the different concentrations of (a) sawdust waste and (b) spent coffee grounds.

3.2 The Mechanical Properties

Compressive strength is a mechanical property used in fired clay specifications. In this study, the compressive strength of the fired clay samples at temperatures of between 900°C and 1100°C depended on the content of the sawdust waste and the spent coffee grounds used in the fired clay mixture. Increases in the firing temperature had increased the compressive strength of the fired clay samples, resulting in a decrease in porosity, although there had been an increase in density. However, the addition of sawdust waste and spent coffee grounds increased the micropores in the fired clay granules, as shown in Figure 10.

3.3 Microstructural Analysis

The microstructures of the samples fired at 1100°C had been denser than those fired at 900°C given that higher temperatures encourage greater melting. Scanning electron microscopy (SEM) was employed to analyze the microstructure of the fired clay. Figure 10 presents the intra-particle porosity resulting from the addition of sawdust waste and spent coffee grounds and the non-addition of those two substances to the granular clay mixture. The SEM images of the fired clay filled with spent coffee grounds obtained at 900°C also showed considerable porosity in the microstructure. Consequently, the fired clay added with spent coffee grounds obtained at 900°C was used as the substance for *A. vinelandii* cell adhesion. The colony-forming units (CFU) of *A. vinelandii* in the porous fired clay granules used as the plant material were investigated using the plate count technique.

Figure 11 shows living cells of *A. vinelandii*, which can be observed in the porous structure of the clay granules under the 200 nm magnification of SEM analysis. The SEM-based investigations indicated that the fired clay granules were suitable for containing the *A. vinelandii* cells. The results demonstrated a high viability of bacterial cells fixed in the fired clay granules at 2.7×10^{7} colonies forming a unit per gram (CFU/g) carrier. Furthermore, the test results from the bacterial cells in the fired clay granules that were used as the marigold planting media revealed that it had effectively encouraged plant growth.

3.4 The Use of Fired Clay Granules as Planting Material

This study was conducted to produce high-porosity clay granules by adding biomass to the mixture. The analysis of the internal structure of the fired clay granules revealed that they are a material that is highly porous and can absorb moisture efficiently.

The process of reacting the fired clay granules was carried out adding 0-15% (w/w) of spent coffee grounds and sawdust waste at temperatures of between 900°C to 1100°C to obtain granules of high porosity, which could effectively absorb nitrogen-fixing bacterial cells. The characteristics of the fired clay granules used as the planting material for planting marigolds are shown in Table 3.

Table 3 The properties of the fired clay granules based on the different concentrations of sawdust waste and spent coffee grounds.

Physical properties	Measured value
The firing shrinkage (%)	8.64-9.28
The water absorption (%)	9.14-24.61
The apparent porosity (%)	16.81-31.73
The bulk density (g/cm^3)	1.68-2.05
The compressive strength (MPa)	12.76-48.90

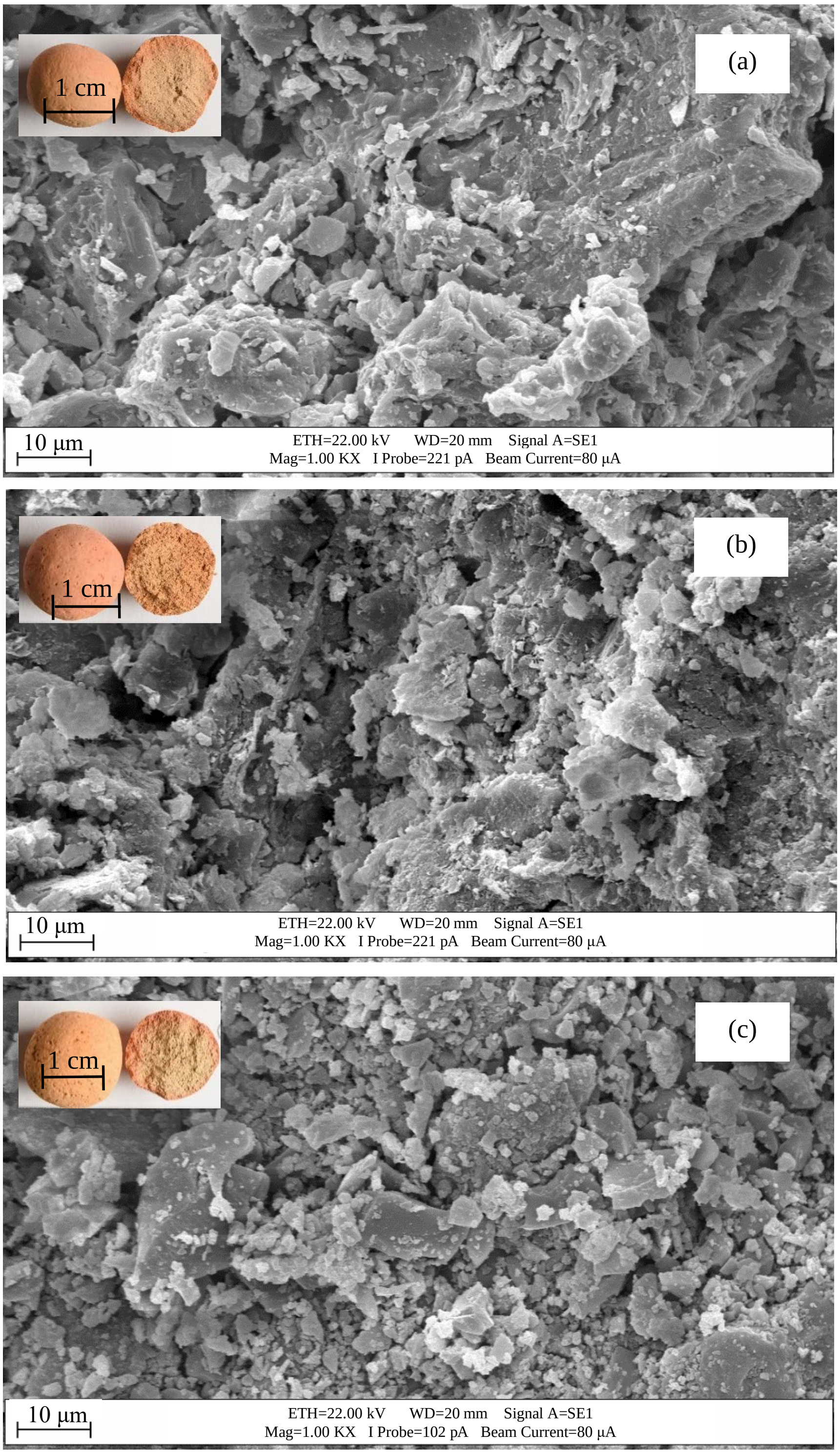

Fig.10 The intra-particle porosity and the microstructure analysis ensuing from the addition of (a) individual clay, (b) sawdust waste, and (c) spent coffee grounds.

This study revealed that agro-wastes can be used as an additive for the production of fired clay granules to reduce waste and production costs and increase the porosity of the fired clay granules. Agro-wastes like dry grass, corn cobs, and sawdust waste can be used as pore-forming additives to make fired clay [19-21]. The addition of the agro-wastes indicated a reduction effect on compressive strength [20-23].

The physical properties of the fired clay granules indicated that they are suitable for promoting plant growth. The results of this study showed that the fired clay granules can improve plant growth and accelerate the duration of marigold flowering. The high porosity of the created clay makes it lighter and more suitable for use as a decoration and plant material. This method turns normal clay into fertile granular clay by adding agricultural microorganisms. The performance tests of the prototype plant material showed that the plants grown in clay with fixed bacterial cells had been more than twice as productive as those plants grown in regular clay (Table 4).

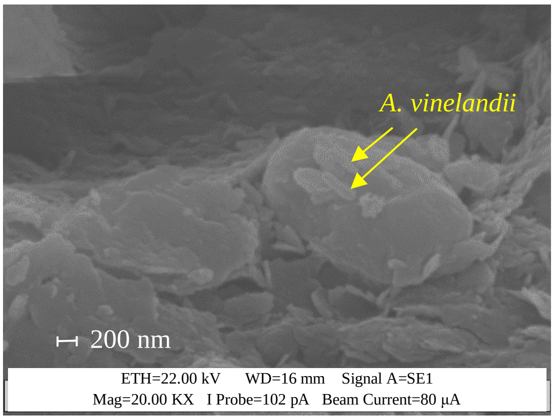

Fig.11 The *A. vinelandii* cells adhere to the porous in the clay granule under the 200 nm magnification of SEM analysis (yellow arrow).

Table 4 The growth of the marigolds planted in the bacterial cells in the fixed clay and in the regular clay.

The marigolds growth (55 days)	Test results	
	The clay fixed with bacterial cells	The regular clay
Height (cm.)	23.5±1.3	11.6±0.3
Root length (cm.)	11±0.3	10±1.0
Stem size (cm.)	1.4±0.1	0.9±0.1
Numbers of leaves	12±0.3	10±0.2
Flowering	Yes	No
Fresh weight (g)	4.2±0.7	0.9±0.1
Dry weight (g)	0.3±0.1	0.09±0.1

Figure 12 shows that "the bacterial cells fixed clay" is a highly effective planting material, which contains the main nutrient. This nitrogen fertilizer is derived from the activity of fixing nitrogen gas from the atmosphere and making it into a form that plants can use. The bacterial cells of the fixed clay contain micronutrients that promote plant growth through microbial activity. The use of this planting material resulted in strong, well-developed plants, accelerated flowering, and high yields. Moreover, there is no need to add additional chemical fertilizers and other plant growth promoters. In the future, this planting material will be recommended, given its ability to reduce the use of chemicals and to reduce residues from growing crops. Concerning agricultural practices, there are still challenges in meeting the nutritional needs of the world's population and in using chemical fertilizers. The application of valuable microbiomes as biofertilizers, which can be applied as a part of sustainable agricultural practices, has developed as an innovative technology for improving soil fertility and plant growth.

Among biological processes, nitrogen fixation is considerably important. It has been deemed to be an interesting microbial activity on the earth's surface since it affords a way to recycle nitrogen and to recreate an important function in nitrogen homeostasis in the biosphere [24]. Additionally, biological nitrogen fixation further sustains soil fertility and enhances crop productivity [25].

Fig.12 The comparison of marigold plants grown in pots with regular clay (a-1 to a-2) and in clay with fixed bacterial cells (b-1 to b-2).

Azotobacter is a useful organism. It can be used as a bioinoculant and to investigate the nitrogen fixation method. Its advantage lies in its capability to develop rapidly and to generate enormous quantities of nitrogen swiftly. Moreover, *Azotobacter* can transform atmospheric nitrogen into ammonia, which plants can use as an essential mineral [26]. These bacteria are particularly resistant to oxygen during nitrogen fixation due to the respiration protection of nitrogenase [27]. Also, in respiratory protection, uptake hydrogenase uptake exists, as well as mechanisms that can switch on and switch off to protect the nitrogenase enzyme from oxygen [28].

The uptake of hydrogenase is concerned with the metabolism of the hydrogen (H_2) that is discharged during the progression of nitrogen fixation [29]. The existence of optimum calcium levels is necessary for the enriched growth of *Azotobacter* and its capability of fixing nitrogen [30]. Meanwhile, improved nitrogen levels adversely affect the activity of *Azotobacter* [31]. Some reports have proposed that *Azotobacter* has the efficiency of fixing about 20 kg N/ha/per year and, as such, can be applied successfully in crop yield as an alternative for at least some portion of mineral nitrogen fertilizers [32, 33]. Different reports that are available have focused on the diminished need for nitrogen fertilizers in crop plants that have been inoculated with *Azotobacter* [34]. One report revealed that using a mixed culture of *Azotobacter* strains could decrease the condition for nitrogen fertilizers by about half of the total.

Since *Azotobacter* is a non-symbiotic microbe, its ultimate probability of enhancing plant productivity can be spent by co-inoculating it with other biofertilizers compared to applying it singly. In addition to directly satisfying the plants through improved mineral uptake, *Azotobacter* can also accelerate the valuable activities of other biofertilizers if used in consortium. Moreover, information on other microorganisms improving the plant growth activity of *Azotobacter* is obtainable. Presently, several investigations of *Azotobacter* being utilized along with other microbes are favorably sensual among researchers, as well as agriculturalists [6].

Azotobacter spp. has been indicated to be used as a biofertilizer to substitute the nitrogen level. When seeking to improve the nutritional properties of *Azotobacter* as a bio-fertilizer, it is important to evaluate a cost-effective strategy that can supply a more inexpensive source of biofertilizer to an agriculture enterprise [35]. Also, *Azotobacter* is one of the most suitable options, and it can be used as a biofertilizer for eco-friendly and sustainable crop production. The performance and operation of all these beneficial properties of *Azotobacter* may prove to be a key attraction for future crop improvement efforts [36].

For future research commendations and practical applications, researchers may consider optimizing the proportions of clay, sawdust waste and spent coffee grounds to balance enhanced properties and cost. Further exploring the microstructure at different compositions and firing temperatures could yield more profound insights into the underlying mechanisms. Other essential properties, viz., absorption capacity and salt resistance of clay granules, should also be studied. Additionally, considering the environmental impact, a life cycle assessment would be valuable in quantifying the sustainability benefits of combine sawdust waste and spent coffee grounds in clay granules as planting material production.

4. CONCLUSIONS

The conclusion of adding biomass additives to the clay material mixture is that they improve air and water permeability and retain moisture within the clay material. The conclusions obtained from this study can be classified as follows.

- The clay material is lighter after burning and indicated the nontoxic material for environment.
- The using of clay, sawdust waste, and spent coffee grounds as raw materials for fired clay granules is a sustainable approach that can help reduce waste and environmental impact.
- The high-temperature firing during the manufacturing process could mitigate or eliminate any potential toxicity of fired clay and transform into inert, non-toxic products suitable for various applications.
- The use of clay, sawdust waste and spent coffee grounds in the process could improve the physical properties i.e., water absorption, the porosity and the bulk density.
- The increase in the firing temperature had decreased the water absorption and the porosity, as well as increased the bulk density of the fired clay granules.
- The microstructure analysis indicated that the fired clay granules could be suitable substances for containing bacterial cells.
- The high porosity of the created clay granules makes it suitable as a plant material that indicated the high viability of bacterial cells fixed in the fired clay granules.
- The bacterial cells fixed in the fired clay granules were effectively stimulating plant growth more than 2-3-fold when compared to using regular clay.

In summary, agro-waste was used as an additive for granular clay production to reduce both waste and production costs, as well as to increase the porosity of granular clay. The high porosity of the created clay makes it lighter and suitable as both a decoration and a plant material. This method turns normal clay into fertile clay by introducing agricultural microorganisms. Not only can this innovation improve plant growth, but it can also accelerate the duration of flowering. This innovation has resulted in lowering costs and effectively reducing the use of chemicals and residues that are generated by growing crops in closed systems.

5. ACKNOWLEDGMENTS

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6. REFERENCES

[1] Lawanwadeekul S., Otsuru T., Tomiku R., and Nishiguchi H., Thermal-acoustic clay brick production with added charcoal for use in Thailand. Construction and Building Materials, Vol. 255, 2020, 119376.

[2] Phonphuak, N., and Thiansem, S., Effects of charcoal on physical and mechanical properties of fired test briquettes. Science Asia, Vol. 37, 2011, pp. 120-124.

[3] Chaudhary, P., Parveen, H., Gangola, S., Kumar, G., Bhatt, P., and Chaudhary, A., Plant growth-promoting rhizobacteria and their application in sustainable crop production. Microbial Technology for Sustainable Environment, 2021. pp. 217-234.

[4] Alori, E.T., Olaniyan, F.T., Adekiya, A.O., Ayorinde, B.B., Daramola, F. Y., Joseph, A., and Babalola, O.O., Response of soil microbial community (bacteria and fungi) to organic and inorganic amendments using tomato as a test crop. Air, Soil and Water Research, Vol. 16, 2023, 11786221231214063.

[5] Kozieł, M., Martyniuk, S., and Siebielec, G., Occurrence of *Azotobacter* spp. in cultivated soils in Poland. Polish Journal of Agronomy, Vol. 44, 2021. pp.3-8.

[6] Li, L., Tong, L., Lv, Y., Influence of Bio-Fertilizer Type and Amount Jointly on Microbial Community Composition, Crop Production and Soil Health. Agronomy, Vol. 13, No. 7, 2023, 1775.

[7] Shah, D., Khan, M. S., Aziz, S., Ali, H., and Pecoraro, L., Molecular and biochemical characterization, antimicrobial activity, stress tolerance, and plant growth-promoting effect of endophytic bacteria isolated from wheat varieties. Microorganisms, Vol. 10, No. 1, 2021, 21.

[8] Poria, V., Dębiec-Andrzejewska, K., Fiodor, A., Lyzohub, M., Ajijah, N., Singh, S., and Pranaw, K., Plant Growth-Promoting Bacteria (PGPB) integrated phytotechnology: A sustainable approach for remediation of marginal lands. Frontiers in Plant Science, Vol. 13, 2022, 999866.

[9] Kaur, T., Kour, D., Pericak, O., Olson, C., Mohan, R., Yadav, A., and Yadav, A.N., Structural and functional diversity of plant growth promoting microbiomes for agricultural sustainability. Journal of Applied Biology and Biotechnology, Vol. 10, No. 1, 2022, pp. 70-89.

[10] Suphan, S., Prajankett, P., and Pongsawat, S., Using Nitrogen Fixing Bacteria with Biological Supporting Media for Promoting the Plant Growth. Science and Technology RMUTT Journal, Vol. 6, 2016, pp. 17-28.

[11] Santos, J.C., Mussatto, S.I., Dragone, G., Converti, A., and Siva, S.S., Evaluation of porous glass and zeolite as cells carriers for xylitol production from sugarcane bagasse hydrolysate. Biochemistry Engineer Journal, Vol. 23, 2005, pp. 1-9.

[12] ASTM C373-18. Standard Test Method for Water Absorption, Bulk Density, Apparent Porosity, and Apparent Specific Gravity of Fired Whiteware Products, Ceramic Tiles, and Glass Tiles. West Conshohocken, Pennsylvania. ASTM Book of Standards 15.02., 2018, pp. 1-6.

[13] ASTM C326-09. Standard Test Method for Drying and Firing Shrinkages of Ceramic Whiteware Clays. West Conshohocken, Pennsylvania. ASTM Book of Standards 15.02., 2018, pp. 1-2.

[14] ASTM C773-88. Standard Test Method for Compressive (Crushing) Strength of Fired Whiteware Materials, West Conshohocken, Pennsylvania. ASTM Book of Standards. 15.02., 2020, pp. 1-4.

[15] Sowmeya, V.G., and Sathiavelu, M., Development of microbial biotechnology products for sustainable agriculture. In The Potential of Microbes for a Circular Economy, 2024, pp. 219-252. Academic Press.

[16] Phonphuak, N., Kanyakam, S., Chindaprasirt, P., Utilization of waste glass to enhance physical–mechanical properties of fired clay brick. Journal of Cleaner Production, Vol. 112, Part 4, 2016, pp. 3057-3062.

[17] Ahmed, M.M., Ali, S.A., Tarek, D., Maafa, I.M., Abutaleb, A., Yousef, A., and Fahmy, M. K., Development of Bio-based lightweight and thermally insulated bricks: Efficient energy performance, thermal comfort, and CO_2 emission of residential buildings in hot arid climates. Journal of Building Engineering, Vol. 91, 2024, 109667.

[18] Chindaprasirt, P., Srisuwan, A., Saengthong, C., Lawanwadeekul, S., and Phonphuak, N., Synergistic effect of fly ash and glass cullet additive on properties of fire clay bricks. Journal of Building Engineering, Vol. 44, 2021, 102942.

[19] Lawanwadeekul, S., Srisuwan, A., Phonphuak, N., and Chindaprasirt, P., Enhancement of porosity and strength of clay brick fired at reduced temperature with the aid of corn cob and waste glass. Construction and Building Materials, Vol. 369, 2023, 130547.

[20] Phonphuak, N., Application of Dry Grass for Clay Brick Manufacturing. Key Engineering Materials, Vol. 757, 2017, pp. 35-39.

[21] Phonphuak, N., Teerakun, M., Srisuwan, A., Ruenruangrit, P., and Saraphirom, P., The use of sawdust waste on physical properties and [thermal conductivity of fired clay brick. International Journal of GEOMATE, Vol. 18, No. 69, 2020, pp. 24- 29.

[22] Phonphuak, N., and Thiansem, S., Using charcoal to increase properties and durability of fired test briquettes. Construction and Building Materials, Vol. 29, 2012, pp. 612-618.

[23] Phonphuak, N., and Chindaprasirt, P., Utilization of sugarcane bagasse ash to improve properties of fired clay brick. Chiang Mai Journal of Science, Vol. 45, No. 4, 2018, pp. 1855-1862.

[24] Sumbul, A., Ansari, R.A., Rizvi, R., and Mahmood, I., *Azotobacter*: A potential bio-fertilizer for soil and plant health management. Saudi Journal of Biological Sciences, Vol. 27, No. 12, 2020, pp. 3634-3640.

[25] Soumare, A., Diedhiou, A.G., Thuita, M., Hafidi, M., Ouhdouch, Y., Gopalakrishnan, S., and Kouisni, L., Exploiting biological nitrogen fixation: a route towards a sustainable agriculture. Plants, Vol. 9, No. 8, 2020, 1011.

[26] Singh, S., Annapurna, K., Shrivastava, N., and Varma, A., Symbiotic interplay of Piriformospora indica and *Azotobacter chroococcum* augments crop productivity and biofortification of Zinc and Iron. Microbiological Research, Vol. 262, 2022, 127075.

[27] Song, W., Liu, X.Y., Wang, Y. L., Tong, Y.D., Bai, Z.P., and Liu, C.Q. Nitrogen isotope differences between atmospheric nitrate and corresponding nitrogen oxides: A new constraint using oxygen isotopes. Science of the total environment, Vol. 701, 2020, 134515.

[28] Chonkar, P.K., Pareek, R.K., Rao, D.L.N., and Adiya, T.K., Soil Biology and Biochemistry. Fundamentals of Soil Science, 2009, pp. 535-565.

[29] Natzke, J., Noar, J., and Bruno-Bárcena, J.M., Azotobacter vinelandii nitrogenase activity, hydrogen production, and response to oxygen exposure. Applied and environmental microbiology, Vol. 84, No. 16, 2018, e01208-18.

[30] Volli, V., and Singh, R.K., Production of bio-oil from mahua de-oiled cake by thermal pyrolysis. Journal of Renewable and Sustainable Energy, Vol. 4, No. 1, 2012, 013101.

[31] Hindersah, ., Kamaluddin, N. N., Samanta, S., Banerjee, S., and Sarkar, S., Role and perspective of *Azotobacter* in crops production. SAINS TANAH-Journal of Soil Science and Agroclimatology, Vol. 17, No. 2, 2020, pp. 170-179.

[32] Aasfar, A., Bargaz, A., Yaakoubi, K., Hilali, A., Bennis, I., Zeroual, Y., & Meftah Kadmiri, I., Nitrogen fixing *Azotobacter* species as potential soil biological enhancers for crop nutrition and yield stability. Frontiers in microbiology, Vol. 12, 2021, 628379.

[33] Esmailpour, A., Hassanzadehdelouei, M., and Madani, A., Impact of livestock manure, nitrogen and biofertilizer (*Azotobacter*) on yield and yield components wheat (*Triticum Aestivum* L.). Cercetari Agronomice in Moldova, Vol. 46, No. 2, 2013, pp. 5-15.

[34] Romero-Perdomo, F., Abril, J., Camelo, M., Moreno-Galván,A., Pastrana, I., Rojas-Tapias, D., and Bonilla, R., *Azotobacter chroococcum* as a potentially useful bacterial biofertilizer for cotton (*Gossypium hirsutum*): Effect in reducing N fertilization. Revista Argentina de Microbiología, Vol. 49, No. 4, 2017, pp. 377-383.

[35] Wang, Z., Shi, Q., Miao, B., Li, B., and Li, S., Exopolysaccharide (EPS) synthesised from *Azotobacter vinelandii* and its characterisation and application in bioflotation. Minerals Engineering, Vol. 211, 2024, 108693.

[36] Kyaw, E.P., Soe, M.M., San San Yu, Z.K.L., and Lynn, T., Study on plant growth promoting activities of *Azotobacter* isolates for sustainable agriculture in Myanmar. Journal of Biotechnology and Bioresearch, Vol. 1, No. 5, 2019, pp. 1-6.