http://journal.uir.ac.id/index.php/JGEET

E-ISSN : 2541-5794
P-ISSN : 2503-216X

Journal of Geoscience,
Engineering, Environment, and Technology
Vol 11 No 2 2026

RESEARCH ARTICLE

Effect of Sand Pretreatment on Bio-Stabilization Using *Rhizopus oligosporus*

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Received: Mar 5, 2026; Accepted: Jun 23, 2026.
DOI: 10.25299/jgeet.2026.11.02.27475

Abstract

Ottawa sand is a type of sand widely used in geotechnical research for its uniformity and high silica content. However, Ottawa sand is classified as a non-cohesive soil, lacking intergranular bonds, resulting in low shear strength. Therefore, a soil improvement method that enhances the material's mechanical stability is needed. This study aims to analyze the effect of Ottawa sand pretreatment on increasing soil shear strength through a bio-stabilization method using the fungus *Rhizopus oligosporus*. The research method used is a laboratory experimental method with an unconfined compression test. Ottawa sand samples were divided into three treatments: normal treatment (S-0), washed sand treatment (S-1), and washed and oven-dried treatment (S-2). Each sample was mixed with tempeh yeast containing *Rhizopus oligosporus* and distilled water, each at 5% of the sand weight. The curing process was conducted in a closed container, with temperature, humidity, and mycelium development monitored during incubation. The results showed that sand pretreatment increased the soil's unconfined compressive strength. The samples treated with washing and oven drying (S-2) had the highest unconfined compressive strength of 9.72 kPa and shear strength of 4.86 kPa at 12 days of curing. Meanwhile, the samples without pretreatment showed lower unconfined compressive strengths. Visually observed mycelial growth showed a more even distribution in the samples with sand pretreatment. The results of this study indicate that bio-stabilization using *Rhizopus oligosporus* has the potential to be a more environmentally friendly soil-improvement method for increasing the stability of sandy soil.

Keywords: Bio-stabilization, Fungal Mycelium, Ottawa Sand, *Rhizopus oligosporus*, Soil Stabilization, Unconfined Compression Test

1. Introduction

Ottawa sand is a granular material widely used in geotechnical research due to its highly uniform physical and chemical characteristics. Composed of over 99% silica, Ottawa sand has a relatively rounded grain shape and a uniform grain size distribution, making it frequently used as a standard sand or reference material in various laboratory tests (Guzzetti et al., 2004). This homogeneity control of experimental variables enables more precise measurement of the soil's mechanical response. However, despite its advantages as a test material, Ottawa sand has limitations in terms of mechanical behavior. As a non-cohesive soil, Ottawa sand lacks chemical bonds or natural cohesion between particles. Intergranular interactions rely entirely on frictional forces and interlocking mechanisms. This results in a near-zero effective cohesion value, and the soil shear strength is strongly influenced by the internal friction angle and relative density. In loose or under-compacted conditions, Ottawa sand exhibits low bearing capacity and is susceptible to excessive deformation under static and dynamic loads. Furthermore, granular materials with a loose structure are also prone to instability, including lateral shifting, settlement, and an increased risk of liquefaction under saturated conditions and cyclic loading (Lim et al., 2020). This problem is significant in civil engineering applications, such as the construction of shallow foundations, embankments, road pavements, and stabilization of sandy slopes. Soils with low shear strength are unable to withstand the shear stresses developed by structural loads, potentially leading to shear failure or differential settlement. Therefore, soil improvement efforts are needed to enhance interparticle bonds, increase apparent cohesion, and improve the overall mechanical characteristics of the soil (Lee and Gomez, 2024; Patil et al., 2023).

Conventional soil improvement is generally carried out using chemicals that can pollute groundwater and emit greenhouse gases (Bayat et al., 2021; Lim et al., 2020; Poudyal and Adhikari, 2021). One of the most used chemicals is cement. The addition of cement aims to increase soil cohesion and stiffness through hydration processes and pozzolanic reactions that produce bonds between particles. In fine-grained soils, this method has proven effective in increasing compressive strength, shear strength, and reducing compressibility. However, in coarse-grained soils such as sand, the effectiveness of chemical stabilization is highly dependent on grain distribution and initial density, as there are insufficient fine fractions to support optimal chemical reactions. Cement contains alkaline solutions that dissolve during hydration. The use of cement in soil can raise groundwater pH to 13.5, thereby increasing sodium levels and causing soil to become sodic, which can damage ecosystems (Djuwansah, 2013; Karolinoerita and Annisa, 2020; Sun et al., 2018). With increasing demand for sustainable, low-emission construction practices, alternative approaches are needed to improve soil mechanical properties without causing significant ecological impacts. In this context, microorganism-based bio-stabilization methods are being developed as a more environmentally friendly solution that utilizes natural biological processes to form stronger bonds between soil particles. Bio-mediated soil improvement methods have been shown to effectively enhance the mechanical properties of granular soils through various biological mechanisms (De Jong et al., 2009; Jacob et al., 2024; Jiang et al., 2017).

Fungi-Mycelium Treated Soil (FMTS) is a biotechnological approach that utilizes fungal mycelium in the soil to modify soil characteristics. This approach was developed as an alternative to synthetic materials commonly used in soil improvement techniques. The FMTS mechanism involves the growth of a mycelial network that can spread to form a structure between soil particles, resulting in a biofilm that acts as a biological matrix binding soil particles (Lazo et al., 2024). One potential microorganism is *Rhizopus oligosporus*, a fungus commonly used in tempeh fermentation. This fungus produces mycelium that can envelop and bind sand grains, thus forming a more stable soil structure. Soil improvement using fungi can increase resistance to wind- and water-induced erosion (Mardhiah et al., 2016; Park et al., 2026). Fungi have hyphae that form mycelium, which can reinforce soil particles through pores (Yang et al., 2021; Zhang et al., 2023). This is due to the strengthening of fungal hyphae and the hydrophobic nature of the fungal mycelium surface (Salifu and El Mountassir, 2021; Zhang et al., 2020). Furthermore, the addition of fungi to sandy soil can reduce permeability, increase shear strength, and increase shear modulus (Lim et al., 2023, 2020; Salifu et al., 2022). Fungal mycelium has also been shown to modify the dynamic shear modulus and damping characteristics of sand, suggesting broader applications beyond static strength improvement (Gou and Li, 2024). The hydraulic conductivity of sandy soil can also be modified by the addition of fungal mycelium (Lim et al., 2023; Park et al., 2023). Furthermore, a combination of Rhizopus oligosporus and Rhizopus oryzae has been shown to enhance the durability and hydraulic characteristics of fungal-treated silica sand (Lim et al., 2024).

The type of fungus used in this previous study was *Rhizopus Oligosporus*, which was mixed into loose sand with varying water content and curing time, resulting in the highest unconfined compressive strength when loose sand was added with 5.25% fungus and 5% water content with a curing period of 3 days (Lim et al., 2020). Meanwhile, another study examined the effect of varying sand gradation on sand strength, finding that the optimal composition was a 50% fine sand and 50% medium sand mixture, which produced the highest shear stress (Lim and Pianica, 2022). Based on this literature, *Rhizopus oligosporus* has the potential to increase soil strength. However, several factors need to be considered, including soil gradation, ambient temperature, and humidity during curing.

Previous studies have shown that applying *Rhizopus oligosporus* can increase the unconfined compressive strength and improve the mechanical properties of sandy soils. These studies focused on varying yeast and water content and short curing durations of up to 10 days. However, studies on the effect of sand pretreatment, such as washing or oven drying, on increasing the shear strength of Ottawa sand have not been systematically reviewed. This is despite the potential for material pretreatment to influence porosity, moisture distribution, and the effectiveness of mycelium growth during curing.

This study offers a review of the effect of material pretreatment on the bio-stabilization performance of Ottawa sand using *Rhizopus oligosporus*. To date, there has been little research specifically evaluating how the washing and oven-drying processes before mixing can affect mycelium growth and increase soil shear strength. Given that fungal growth is strongly influenced by environmental conditions, particularly temperature and humidity, pretreatment of the material and incubation control are thought to be key factors in the effectiveness of this method. Therefore, this research is expected to make a scientific contribution to the development of more effective, controlled, and sustainable methods for bio-stabilizing sandy soil.

2. Methodology

This research used an experimental laboratory approach to evaluate the effect of Ottawa sand pretreatment on increasing unconfined compressive strength through bio-stabilization with *Rhizopus oligosporus*. The experimental method was chosen to ensure control of the study variables, specifically the mixture composition, material pretreatment conditions, and environmental parameters during the curing process.

The research stages included material preparation, sand pretreatment, mixing with *Rhizopus oligosporus* and distilled water, molding test specimens, and curing. Testing was conducted to determine the sand's physical characteristics and unconfined compressive strength as a basis for calculating soil shear strength. Furthermore, visual observations of fungal growth during the curing period were conducted to identify the relationship between biological development and improvements in soil mechanical properties.

2.1 Materials

The material used in this study was Ottawa sand (Figure 1), a coarse-grained soil. Ottawa sand was chosen because it has a silica content of over 99% and a relatively uniform grain size distribution. Ottawa sand is a standard material frequently used in geotechnical laboratory testing, such as shear strength, soil improvement, liquefaction, sand cone, and many more (Guzzetti et al., 2004).

Fig. 1. Ottawa sand

The bio-stabilization agent used was a commercial tempeh yeast containing *Rhizopus oligosporus*, which thrives in warm, humid environments at temperatures between 7 °C and 45 °C, with an optimum temperature of around 37 °C (Sparringa et al., 2002). This fungus acts as a mycelial-forming agent, expected to bind sand particles through the growth of a hyphal network during curing. The yeast used in this study was the commercially available Raprima© brand (Figure 2), ensuring its easy accessibility and consistent composition.

Fig. 2. Yeast tempeh Raprima©

Rhizopus oligosporus has a distinctive, complex morphological structure that supports its role in fermentation processes, such as tempeh production. The primary structure is the hyphae, long threads that form a branching network, non-septate, and serve as the primary channel for nutrient absorption from the substrate. These hyphae then unite to form mycelium, a dense network that spreads across the surface of the medium, much like soybeans in fermented tempeh. The mycelium at the rear of the colony generally appears denser and darker in color, while the Growing Front region exhibits sparser mycelium but is active in colony growth and expansion (Jennessen et al., 2008).

Rhizopus oligosporus has sporangiophores, upright stem structures with vertically oriented hyphae. These sporangiophores support the sporangium, a spherical sac at the tip of the stem that typically functions as a place for spore formation and storage. Spores formed within the sporangium are released into the environment as part of the reproductive process. Root-like structures that grow through the substrate are called rhizoids; they function as attachment organs and absorb additional nutrients (Jennessen et al., 2008). The morphological structure of *Rhizopus oligosporus* is shown in Figure 3.

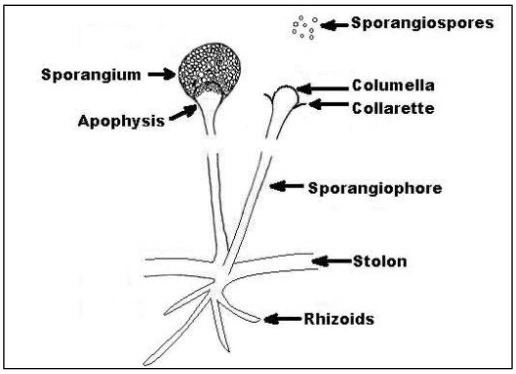
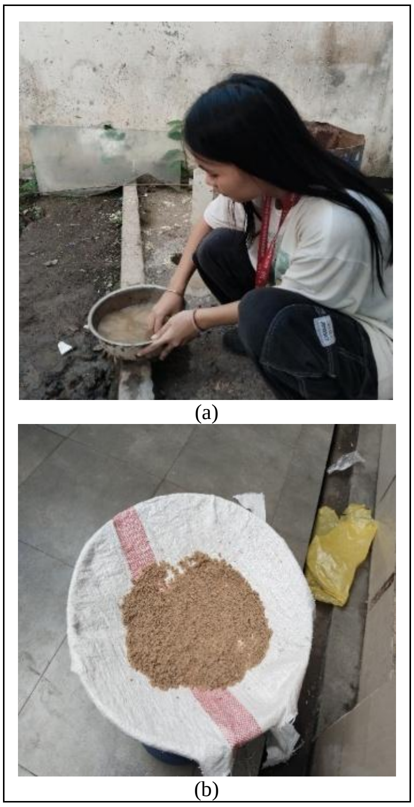


Fig. 3. Morphology of *Rhizopus oligosporus*


(a)

(b)

Fig. 4. Pre-treatment of Ottawa sand before bio-stabilization: (a) washing process to remove fine particles and dust from Ottawa sand, and (b) drying of washed sand to reduce moisture content before specimen preparation

2.2 Sand Pre-Treatment

Sand pretreatment was conducted to evaluate the effect of material conditions on the bio-stabilization process. In this study, Ottawa sand was divided into three pretreatment conditions: normal (S-0), washed (S-1), and washed and oven-dried (S-2). These variations were designed to identify how changes in the surface condition and initial moisture content of the sand affect mycelium growth and increase the soil shear strength.

In normal condition (S-0), the sand was used without additional treatment, which represents the material in its natural state as received from its source. In the washed condition (S-1), the sand was cleaned with water to remove fine dust or small particles that may still be attached to the grain surface. The washing process continued until the rinse water appeared clear, and the sand was then dried at room temperature before being used in the mixing process. The process of washing and drying Ottawa sand for this treatment is shown in Figure 4.


(a)

(b)

Fig. 5. Pre-treatment of Ottawa sand before bio-stabilization: (a) washing process to remove dust and fine particles from Ottawa sand, (b) oven drying process to reduce moisture content before specimen preparation

In the washed and oven-dried condition (S-2), the washed sand is then dried in an oven at 105 °C to 110 °C for 24 hours, until its weight is constant. The purpose of drying in the oven is as a sterilization process due to limitations in specialized sterilization equipment, such as autoclaves. The process is shown in Figure 5.

This pretreatment assumes that grain surface cleanliness, initial moisture distribution, and initial pore conditions can influence the mycelium's ability to grow, spread, and form bonds between sand particles. Therefore, evaluating these three conditions is expected to provide a more comprehensive understanding of the role of material pretreatment in enhancing the effectiveness of bio-stabilization methods.

2.3 Experimental Plans

The main objective of this study was to analyze the effect of Ottawa sand pretreatment on increasing soil shear strength using bio-stabilization with the fungus *Rhizopus oligosporus*. The fundamental factor influencing soil shear strength in this study was the Ottawa sand pretreatment. Table 1 presents an experimental plan for determining the soil shear strength of the treated samples.

Table 1. Experimental plans

Parameter	S-0	S-1	S-2
Ottawa sand mass (g)	300	300	300
Sand pretreatment	None	Washed + air-dried	Washed + oven-dried
Initial water content (%)	0,21	0,21	0,21
Inoculum (% dry wt.)	5 (15g)	5 (15g)	5 (15g)
Distilled water (% dry wt.)	5 (15mL)	5 (15mL)	5 (15mL)
Mixing method	Dry mix	Dry mix	Dry mix
Mold dimension (cm)	∅5cm × 10cm	∅5cm × 10cm	∅5cm × 10cm
Compaction	3 layers, 10 tamps/layer	3 layers, 10 tamps/layer	3 layers, 10 tamps/layer
Curing (days)	12	12	12
Objective	To serve as a baseline reference for evaluating the effect of bio-stabilization without any sand pretreatment	To evaluate the effect of surface cleaning through washing on mycelial growth and unconfined compressive strength	To evaluate the combined effect of washing and thermal sterilization on mycelial homogeneity and unconfined compressive strength

2.4 Specimen Preparation

The sample preparation process was conducted after the Ottawa sand underwent pretreatment. The prepared sand was then mixed with *Rhizopus oligosporus* in yeast at 5% of the sand's dry weight using a dry mix method. Dry mixing was performed first to ensure even distribution of the yeast among the sand particles before adding water (Lim et al., 2020).

Once the mixture was homogeneous, distilled water was added at a 5% ratio to the sand's dry weight, and the mixture was stirred. The use of distilled water aimed to minimize the influence of dissolved substances that could interfere with fungal growth. The mixture was then molded into a cylindrical PVC pipe with a 5 cm diameter and a 10 cm height. The molding process was carried out in stages, dividing the mold into 1/3-layer layers and pounding each layer 10 times to achieve a relatively uniform density distribution without disrupting the initial structure of the mixture (Lim et al., 2020).

After the molding process was complete, the samples were left in the mold for 2–3 days to allow initial mycelial growth, which binds the sand particles. After this initial period, the samples were removed from the molds and subsequently cured according to the research stages. The specimen preparation is shown in Figure 6.

2.5 Curing Procedure

After molding and removal, the samples were cured in a closed container with ventilation holes to allow limited air circulation. This curing setup was designed to maintain an adequate oxygen supply while controlling humidity, as *Rhizopus oligosporus* requires aerobic conditions with limited CO_2 accumulation for optimal mycelial development (Sparringa et al., 2002). Active mycelium formation began to be observed on the second to third day after mixing, so the curing time was calculated from the mixing process to the 28th day, according to the research plan.

To maintain a stable moisture content of the mixture, water was added during the curing period. The moisture content during curing should be maintained at around 5% to support the biological activity of the fungi (Šelo et al., 2023). Therefore, the total water addition over 28 days was calculated based on the weight of the mixture poured into the mold. Distilled water was sprayed every other day to maintain the moisture content close to the planned value.

During the curing process, the ambient temperature and humidity were monitored daily. Silica gel was placed inside the container to help control humidity in the curing chamber. Temperatures during incubation ranged from 23.9 °C to 28.5 °C, while the maximum relative humidity recorded was approximately 71%. Although the temperature remained within the growth tolerance range for *Rhizopus oligosporus*, the lower-than-optimal relative humidity is thought to limit mycelial growth. Fungal growth was visually observed during the curing process using a mobile phone camera to identify changes in color and surface texture, and to detect indicators of active and declining biological activity. The curing setup used in this study is illustrated in Figure 7.

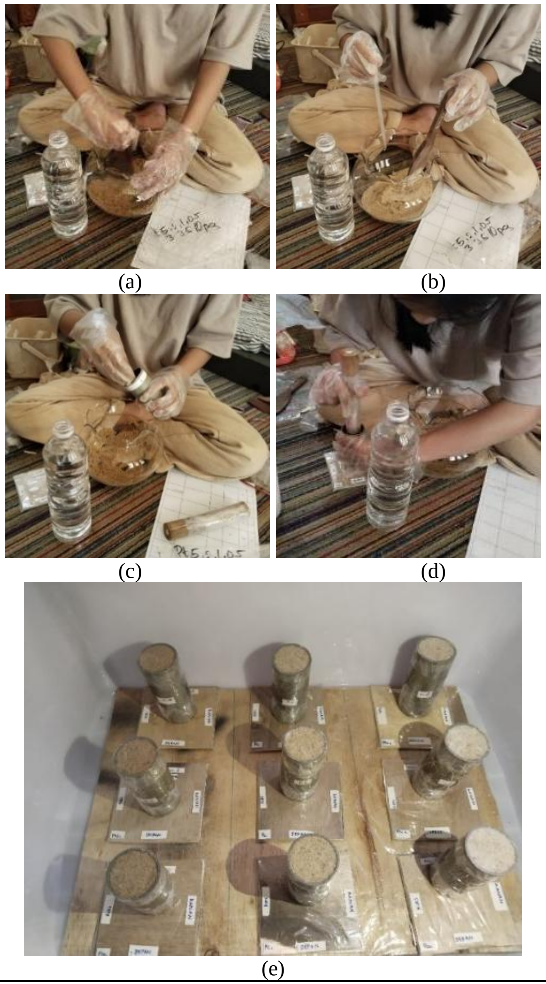


Fig. 6. Specimen preparation process: (a) mixing Ottawa sand with *Rhizopus oligosporus* yeast, (b) addition of distilled water into the mixture, (c) mixing to obtain a homogeneous mixture, placement of the mixture into the cylindrical mold, (d) layer-by-layer compaction during specimen molding, (e) curing process inside the mold

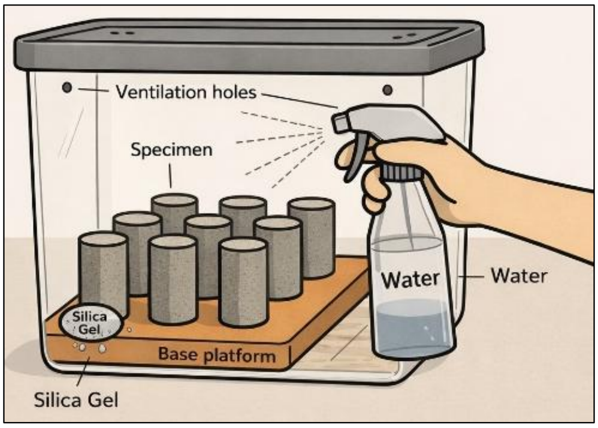


Fig. 7. Experimental curing setup for bio-stabilized Ottawa sand specimens using *Rhizopus oligosporus*

2.6 Unconfined Compression Test Procedures

The Unconfined Compression Test (UCT) was conducted to determine the unconfined compressive strength and calculate the shear strength of the bio-stabilized soil. This test was chosen because it is suitable for evaluating the apparent increase in soil cohesion resulting from biological binding treatment.

Cylindrical specimens were tested on a compression testing machine at a constant loading rate until failure. Before testing, the dimensions and weight of each specimen were measured to calculate the initial unit weight. Axial loads were applied vertically until the maximum stress was reached or significant deformation occurred in the specimen.

The unconfined compressive strength was calculated by dividing the maximum recorded load by the initial cross-sectional area of the specimen. Next, the shear strength (τ) was determined using an approximation assuming that the angle of internal friction (φ) approaches zero in the unconfined state, so the equivalent shear strength is obtained as half the unconfined compressive strength. This value was used as the primary parameter in comparing the effectiveness of each sand pretreatment on increasing mechanical strength.

The tests were conducted after the curing period. The test results were then analyzed to identify the relationships among the initial material treatment, curing conditions, and the increase in shear strength resulting from *Rhizopus oligosporus* mycelium growth. The Unconfined Compression Test (UCT) is shown in Figure 8.

Fig. 8. The Unconfined Compression Test (UCT)

3. Result and Discussion

This section presents the results of experimental testing on Ottawa sand samples pretreated and bio-stabilized with *Rhizopus oligosporus*. The results are analyzed to evaluate the effect of pretreatment variations on increasing the unconfined compressive strength. Furthermore, the discussion covers the relationships among curing conditions, mycelial growth, and changes in mechanical properties across sample variations.

A comparative analysis was conducted across treatment variations to identify patterns of strength increase and factors influencing the effectiveness of the bio-stabilization process. The test results are then compared with previous research findings to strengthen scientific interpretation and place this research's contribution in the context of developing microorganism-based soil improvement methods.

3.1 Physical Properties of Ottawa Sand

Before the bio-stabilization process, the physical characteristics of the Ottawa sand were tested. The results of the physical properties tests of Ottawa sand are presented in Table 2. These parameters serve as the basis for understanding the mechanical behavior of Ottawa sand as the test material in this study.

Table 2. Physical properties of Ottawa sand

Parameter	
Initial water content (%)	0,21
Specific gravity	2,65
% retained on sieve No. 200	2,88
D10 (mm)	0.629
D30 (mm)	0.730
D60 (mm)	0.924
Cu	1.47
Cc	1.86
Liquid Limit	NP
Plastic Limit	NP

Based on Table 2, the initial water content of 0.21% indicates a near-dry condition before mixing, enabling more precise moisture control during curing and minimizing the influence of initial moisture on mycelial growth. Specific gravity of 2.65 falls within the standard range for pure silica sand (ASTM D-854), consistent with its silica content exceeding 99%, indicating the absence of significant clay fractions or organic matter. The grain-size analysis shows that only 2.88% passing sieve No. 200, confirming the material's coarse-grained nature. The coefficient of uniformity (Cu = 1.47) and coefficient of gradation (Cc = 1.86) classify Ottawa sand as a poorly graded sand (SP) according to the Unified Soil Classification System (ASTM D-2487), indicating a narrow and uniform particle size distribution. This uniformity makes Ottawa sand a reliable and consistent medium for controlling experimental variables. The non-plastic nature of the material (LL = NP, PL = NP) further confirms the absence of cohesive behavior, so any increase in shear strength observed during testing can be directly attributed to the bio-stabilization process.

3.2 Visual Observation of Fungal Growth

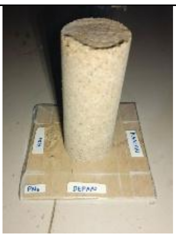
Temperature and humidity were monitored daily using a hygrometer thermometer throughout the curing process. Observation data showed that the incubation temperature inside the container ranged from 23.9 °C to 28.5 °C, while the maximum outside temperature recorded was 30.8 °C. It has not yet reached the optimal temperature of 35 °C (Wahyudi, 2018). The maximum relative humidity recorded was only 71%, which is still far below the optimal range for fungal growth of 90–97%

(Lim et al., 2020). Although distilled water was sprayed every two days to maintain the mixture's moisture content at ±5%, the curing environment could not create the ideal humidity for optimal mycelial growth. Ventilation in the container box is suspected to have contributed to humidity fluctuations and accelerated water evaporation from the sample surface. These less-than-optimal environmental conditions have the potential to limit mycelial development and directly affect the effectiveness of the bio-stabilization process.

Visual observations of fungal growth were conducted from the third day of mixing through the end of curing. During the curing process, visual observations were made of the development of the fungi on Ottawa sand specimens. These observations aimed to identify the dynamics of mycelial growth and changes in sample surface morphology that occurred during the bio-stabilization process.

Visual observations were conducted to identify differences in the development of *Rhizopus oligosporus* fungal colonies in specimens without pretreatment (S-0) and specimens with sand pretreatment (S-1 and S-2). This documentation aims to examine the effect of initial material conditions on the distribution and intensity of mycelial growth during the curing process. The results of visual observations are shown in Table 3.

Table 3. Visual observation

Day	S-0	S-1
3	 Fungal growth begins to appear on the surface of the specimen, indicated by a thin white layer.	 Fungal growth begins to appear on the surface of the specimen, indicated by a thin white layer.
4	 The white mycelium layer becomes more visible and starts spreading across the specimen surface.	 Initial fungal growth begins to appear on the specimen surface.
5	 Mycelium growth continues to expand, covering more areas of the specimen and becoming denser.	 The mycelial layer thickens and spreads across the upper surface of the specimen.
6	 The fungal appears thicker and more uniform, indicating active mycelium development.	 The mycelium network becomes more visible and thicker, indicating active fungal development.
7	 The mycelial layer remains present, but the growth rate begins to slow compared to previous growth rates.	 The specimen surface remains covered by white mycelium, but the growth rate begins to stabilize.
9	 The fungal colony shows reduced growth and appears less fluffy, indicating the onset of the decline phase.	 The fungal colony shows signs of reduced activity, and the mycelium appears less fluffy than in previous days.
11	 The specimen surface starts to change color slightly, becoming dull or grayish as fungal activity decreases.	 The fungal colony shows signs of reduced activity, and the mycelium appears less fluffy than in previous days.
12	 The fungal colony shows clear signs of reduced activity, with darker coloration and weaker mycelium structure.	 The fungal colony becomes duller and darker, suggesting that fungal activity has significantly decreased.

Based on visual observations in Table 1, differences in colony development patterns of *Rhizopus oligosporus* were observed between untreated specimens (S-0) and those with pretreatment (S-1 and S-2). In general, mycelial growth in the pretreated specimens (S-1 and S-2) showed a more even distribution and more stable colony development than in the untreated specimens.

In specimen S-0, mycelial growth tended to be uneven on some parts of the specimen surface. This condition is suspected to be caused by fine particles or dirt adhering to sand grains' surfaces, inhibiting direct contact between the mycelial network and soil particles. The presence of these impurities can affect fungal colonization and lead to a less homogeneous distribution of mycelial growth.

Conversely, in the sand pretreated specimens (S-1 and S-2), mycelial growth appeared more evenly distributed across the specimen surface. The sand-washing process is thought to have removed fine particles and dust adhering to the sand grains, thereby increasing contact between the fungal mycelium and the particle surfaces. Meanwhile, in the S-2 treatment, the combination of washing and oven drying provided cleaner particle surfaces and a more controlled initial moisture content, thereby supporting the development of more stable fungal colonies.

Environmental conditions, such as temperature and humidity, critically govern the colonization efficiency of fungal mycelium in sandy soils (Salifu et al., 2025). Thus, these visual observations support the hypothesis that environmental curing factors, particularly suboptimal humidity, are the primary limiting factor in the effectiveness of bio-stabilization and directly contribute to the mechanical test results.

3.3 Unconfined Compression Test Result

The Unconfined Compression Test (UCT) was used to assess changes in the mechanical behavior of Ottawa sand resulting from the bio-stabilization process. Given that Ottawa sand lacks natural cohesion, any increase in the unconfined compressive strength (qu) value can be directly associated with the contribution of mycelium formation as a particle binding agent. Biologically treated sand exhibited measurable increases in shear strength attributable to microbial binding activity, even under suboptimal curing conditions (Bayat et al., 2021). Therefore, UCT analysis is a key indicator in evaluating the effectiveness of pretreatment and curing conditions on increasing soil shear strength.

Testing was conducted over the twelve days. The test results showed differences in unconfined compressive strength values between the initial treatment variations. The unconfined compressive strength for the normal sample (S-0) is 5.59 kPa, the washed treatment (S-1) is 6.61 kPa, and the washed and oven-dried treatment (S-2) is 9.72 kPa. The shear strength (Su) for normal treatment (S-0) is 2.79 kPa, the washed treatment (S-1) is 3.30 kPa, and the washed and oven-dried treatment (S-2) is 4.86 kPa.

The washed and oven-dried treatment (S-2) showed the greatest increase in strength among the variations. Compared to normal treatment (S-0), the unconfined compressive strength increased by approximately 74% for the washed and oven-dried treatment (S-2), while PC increased by approximately 18% compared to the normal treatment (S-0).

This difference indicates that material pretreatment influences the effectiveness of bio-stabilization. The washing and oven-drying processes are thought to improve particle surface conditions and the initial moisture distribution, thereby supporting more effective mycelium formation and producing apparent cohesion. Conversely, under normal treatment (S-0) conditions, mycelium growth tends to be suboptimal, resulting in a lower increase in strength.

However, the unconfined compressive strength obtained was still significantly lower than that reported in previous research conducted under optimal temperature and humidity conditions (Lim et al., 2020). This discrepancy cannot be adequately explained by environmental factors alone and warrants a more critical examination of the methodological and material differences between the two studies.

First, the most critical difference lies in the type of sand used. (Lim et al., 2020) used Padang loose sand, which contains a more heterogeneous mineral composition including 14.17% Al_2O_3, 7.06% Fe_2O_3, and other compounds that may provide additional surface reactivity and nutrient availability for fungal growth. In contrast, Ottawa sand consists of over 99% SiO_2, an exceptionally pure and chemically inert material. The near-absence of organic matter and reactive mineral surfaces in Ottawa sand may limit *Rhizopus oligosporus* hyphae's ability to adhere to and colonize grain surfaces, thereby reducing the effectiveness of bio-cementation.

Second, the curing duration differs fundamentally. (Lim et al., 2020) reported peak qu at only 3 days of curing, after which strength declined sharply due to fungal die-off. In this study, curing was extended to 12 days, yet the qu values remained far below the reference. This suggests that, beyond the peak growth window, prolonged curing without adequate environmental control does not compensate for suboptimal fungal activity and may, in fact, reduce strength as mycelium degrades.

Third, the specimen dimensions differ between the two studies. (Lim et al., 2020) used a mold with dimensions Ø 38 mm × 76 mm, whereas this study used Ø 50 mm × 100 mm. The larger specimen volume in this study requires a more extensive and uniform mycelial network to achieve comparable strength, which is inherently more difficult to achieve under uncontrolled curing conditions. This dimensional effect on apparent qu is a factor that deserves acknowledgment.

Fourth, the relative density of the sand was explicitly controlled at $Dr \approx 40\%$ in (Lim et al., 2020), whereas the present study neither reported nor controlled for the relative density of Ottawa sand specimens. Differences in initial packing density directly influence the porosity available for mycelial growth and the degree of interparticle contact, both of which affect the final qu. This represents a methodological limitation that should be addressed in future studies.

Taken together, these factors suggest that the lower qu obtained in this study is not merely a consequence of suboptimal environmental conditions during curing but reflects a more fundamental incompatibility between Ottawa sand, as a chemically inert, uniform, and coarse-grained material, and the growth requirements of Rhizopus oligosporus under laboratory-ambient conditions. Future studies should control relative density, use nutrient-supplemented water, and conduct curing under regulated temperature (30°C–37°C) and humidity (>85% RH) to better approximate the conditions reported in the literature.

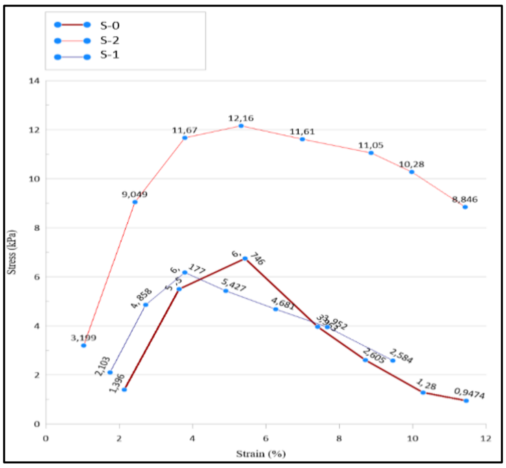


Fig. 9. Stress–strain curve for variations in initial Ottawa sand treatment (S-0, S-1, and S-2)

The relationship between axial stress and strain in Ottawa sand samples that have undergone bio-stabilization treatment, with variations in initial conditions: S-0 (normal treatment), S-1 (washed treatment), and S-2 (washed and oven-dried treatment), is shown in Figure 9. The stress–strain curves show significant differences in mechanical response between treatment variations.

The S-2 (washed and oven-dried treatment) showed the highest peak stress, indicating the greatest increase in unconfined compressive strength. Furthermore, the S-2 curve showed a more stable trend of increasing stress before reaching a peak, indicating the formation of pseudo-cohesion due to more effective mycelial growth.

The S-1 (washed treatment) showed a higher maximum stress value than the S-0 (normal treatment), but still lower than the S-2 (washed and oven-dried treatment). Meanwhile, the S-0 sample showed the lowest mechanical response, with low peak stress and a more rapid decline after reaching its maximum. This suggests that without additional pretreatment, the effectiveness of bio-stabilization tends to be more limited.

The differences in the curve shapes between the variations indicate that the material pretreatment affects the deformation mechanisms and stress distribution within the specimen. The variation in washing and oven-drying treatment (S-2) is thought to result in a more effective distribution of mycelium-binding sand particles, thereby increasing the capacity to withstand axial stress before failure.

This finding is consistent with the average unconfined compressive strength results, which showed that S-2 (washed and oven-dried treatment) had the highest qu value, followed by S-1 (washed treatment) and S-0 (normal treatment). Thus, the stress–strain curves confirm that material pretreatment significantly enhances the effectiveness of the bio-stabilization process.

To identify deformation mechanisms and failure patterns under axial loading, visual documentation was conducted on the specimens before and after testing (Figures 10-12). This observation aimed to evaluate the type of failure that occurred and to relate it to the effectiveness of pseudo-cohesion formation due to mycelial growth across sand pretreatment variations.

(a) (b)

Fig. 10. Specimen conditions before and after unconfined compressive test at S-0 (normal treatment): (a) specimen condition before loading, and (b) specimen failure pattern after testing

Visual documentation reveals differences in failure patterns between the initial treatment variations. In the S-0 (normal treatment), failure tends to occur with more diffuse deformation and without clear shear planes. This pattern indicates the characteristics of a loose granular material with limited apparent cohesion, leading to failure due to the loss of interparticle stability.

In the S-1 (washed treatment), local shear planes with more defined cracks began to appear compared to the S-0 (normal treatment). This indicates an increase in apparent cohesion due to mycelial growth, although its distribution is not yet completely homogeneous.

(a) (b)

Fig. 11. Specimen conditions before and after unconfined compressive test at S-1 (washed treatment): (a) specimen condition before loading, and (b) specimen failure pattern after testing

(a) (b)

Fig.12. Specimen conditions before and after unconfined compressive test at S-2 (washed and oven-dried treatment): (a) specimen condition before loading, and (b) specimen failure pattern after testing

The S-2 (washed and oven-dried treatment) exhibited a more pronounced failure pattern, with the formation of well-defined diagonal shear planes. This pattern resembles the shear failure of pseudo-cohesive materials, indicating that the washing and oven-drying pretreatments enhanced the effectiveness of biological bonding between sand particles. In general, the failure pattern in S-2 (washed and oven-dried treatment) exhibited a more controlled behavior before reaching peak stress, consistent with the highest unconfined compressive strength values in this variety. This difference in deformation patterns confirms that the material pretreatment influences the stress distribution mechanism and the development of pseudo-cohesion during the bio-stabilization process.

3.4 Unconfined Compression Test Result

The results of this study indicate that *Rhizopus oligosporus* can increase the mechanical strength of Ottawa sand by forming a mycelial network that acts as a binder between sand particles. Fungal mycelial growth can form a biological network structure that increases interlocking between sand particles. This mycelial network acts as a natural matrix that binds soil particles, thereby increasing apparent cohesion in inherently non-cohesive materials (Lim et al., 2020).

Fungal-based microorganisms have the potential to increase the stability of granular soils through bio-cementation and bio-binding mechanisms (Gou et al., 2025; Lim and Pianica, 2022). This study explained that fungal hyphal growth can form a net-like structure that strengthens the bonds between soil particles. The results of this study reinforce these findings, showing that samples with a more even mycelial distribution had a greater increase in unconfined compressive strength than those without pretreatment.

Mycelium development in soil media is significantly influenced by environmental conditions such as temperature and humidity during the curing process (Sunaryo et al., 2023). This is consistent with the results of this study, which showed that curing conditions with a relative humidity of 71% and a temperature range of 23.9 °C to 28.5 °C were still below the optimum conditions for *Rhizopus oligosporus* growth, potentially affecting optimal fungal colony development.

The main difference between this study and previous studies lies in the evaluation of Ottawa sand pretreatment on the effectiveness of the bio-stabilization process. The results showed that pretreatment, such as washing and oven drying, can increase the surface homogeneity of the sand particles and support more even mycelium growth. These conditions increase the unconfined compressive strength of the soil compared to samples without pretreatment.

4. Conclusion

This study evaluated the effect of Ottawa sand pretreatment on increasing soil shear strength through bio-stabilization with the fungus *Rhizopus oligosporus*, based on results from the Unconfined Compressive Test (UCT).

The following conclusions are drawn based on the experimental results:

1. Sand pretreatment significantly influenced the effectiveness of bio-stabilization. The washed and oven-dried treatment (S-2) produced the highest unconfined compressive strength of 9.72 kPa and undrained shear strength (Su) of 4.86 kPa, representing an increase of approximately 74% compared to the untreated series (S-0, qu = 5.59 kPa). The washed treatment (S-1) yielded an intermediate qu of 6.61 kPa, an increase of approximately 18% over S-0.
2. Visual observations confirmed that sand pretreatment promoted more uniform mycelial distribution across the specimen surface. Specimens in S-1 and S-2 exhibited more homogeneous fungal colonization compared to S-0, where uneven mycelial growth was attributed to fine particles and impurities adhering to grain surfaces that inhibited direct hypha-to-grain contact.
3. Mycelial growth in all series became visually active between days 5 and 6 of curing. Growth activity declined after day 8, as indicated by darkening coloration and reduced mycelium density, consistent with the measured curing environment of 71% relative humidity and temperatures ranging from 23.9°C to 30.8°C, conditions below the optimum growth range of Rhizopus oligosporus (30°C–40°C).
4. The failure patterns observed in UCT specimens were consistent with the strength results. S-2 exhibited well-defined diagonal shear planes characteristic of pseudo-cohesive behavior, while S-0 showed diffuse deformation with no clear shear plane, indicating limited mycelial bonding.

This study has several limitations. The curing environment was not controlled, with temperature and humidity remaining below the optimum conditions for Rhizopus oligosporus growth throughout the 12 days. The relative density of the specimens was neither measured nor controlled, and mycelial growth was assessed solely by visual observation, without microstructural analysis. Additionally, only a single specimen per series was tested, limiting the statistical reliability of the results.

Future studies should conduct curing under regulated temperature (30°C–40°C) and relative humidity (>85% RH) to maximize mycelial development. Controlling the specimen's relative density, increasing the number of test replicates, and incorporating SEM analysis to observe hyphal-to-grain bonding are also recommended. Extended curing durations beyond 12 days under control conditions should be explored to determine the optimal curing window for this bio-stabilization method.

Acknowledgement

The authors would like to thank the Laboratorium Teknik Sipil Institut Teknologi Garut for providing the facilities and equipment used throughout the experimental program.

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